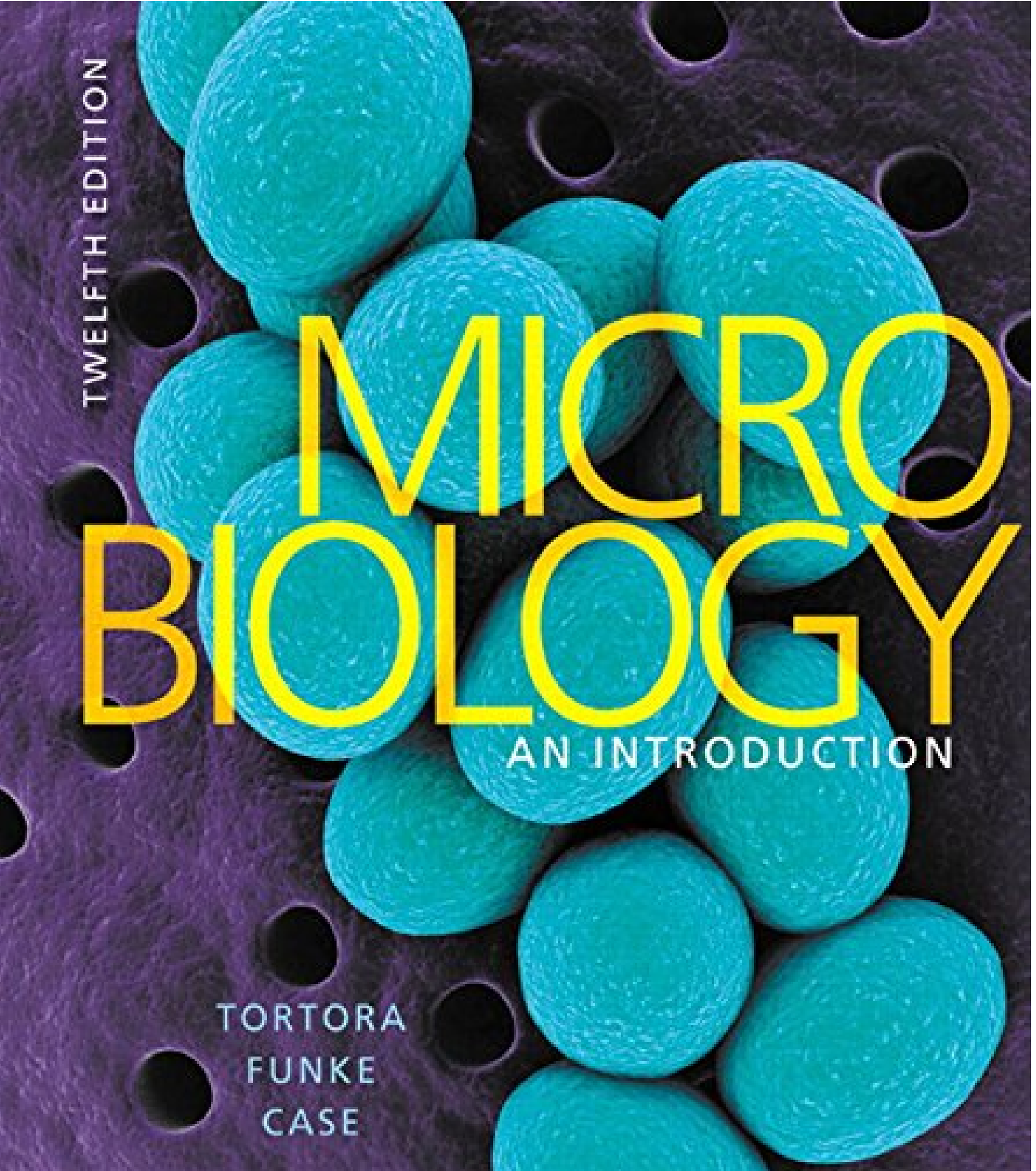
TWELFTH EDITION
MICRO
BIOLOGY
AN INTRODUCTION
TORTORA
FUNKE
CASE

Master Microbiology Where it Matters…

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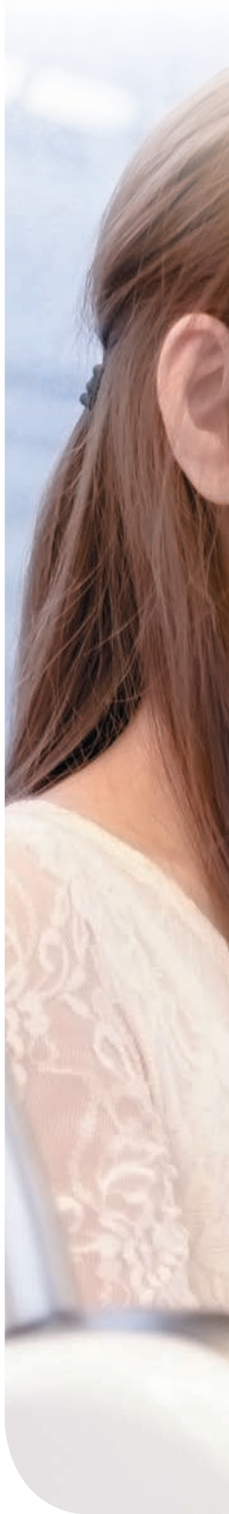
All chapter content is tagged to ASM Curriculum Guidelines for Undergraduate Microbiology



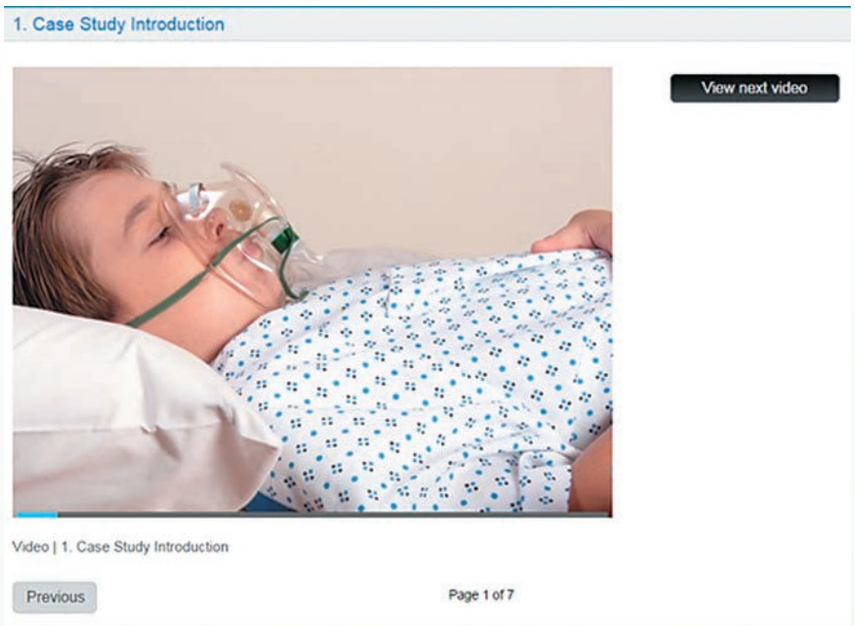
…Everywhere

AT&T
5:48 PM
Modules
Chapter 24: Microbial Diseases of the Second Group
A 35-year-old woman presents with violent, uncontrollable coughing fits and reports that she is just getting over a cold. Her sputum does NOT contain blood. Which of the following is the most likely diagnosis?
tuberculosis
walking pneumonia
the flu
pertussis
I DON'T KNOW YET

Explore and Apply Key Concepts with Interactive Microbiology!

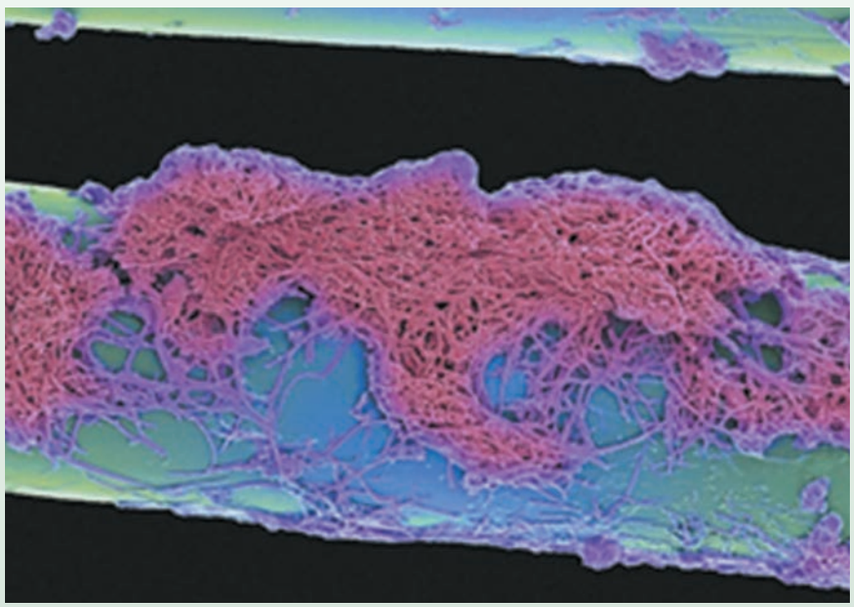
NEW!

interactivemicrobiology is a dynamic suite of interactive tutorials and animations that teach key concepts in microbiology. Students actively engage with each topic and learn from manipulating variables, predicting outcomes, and answering formative and summative assessment questions.

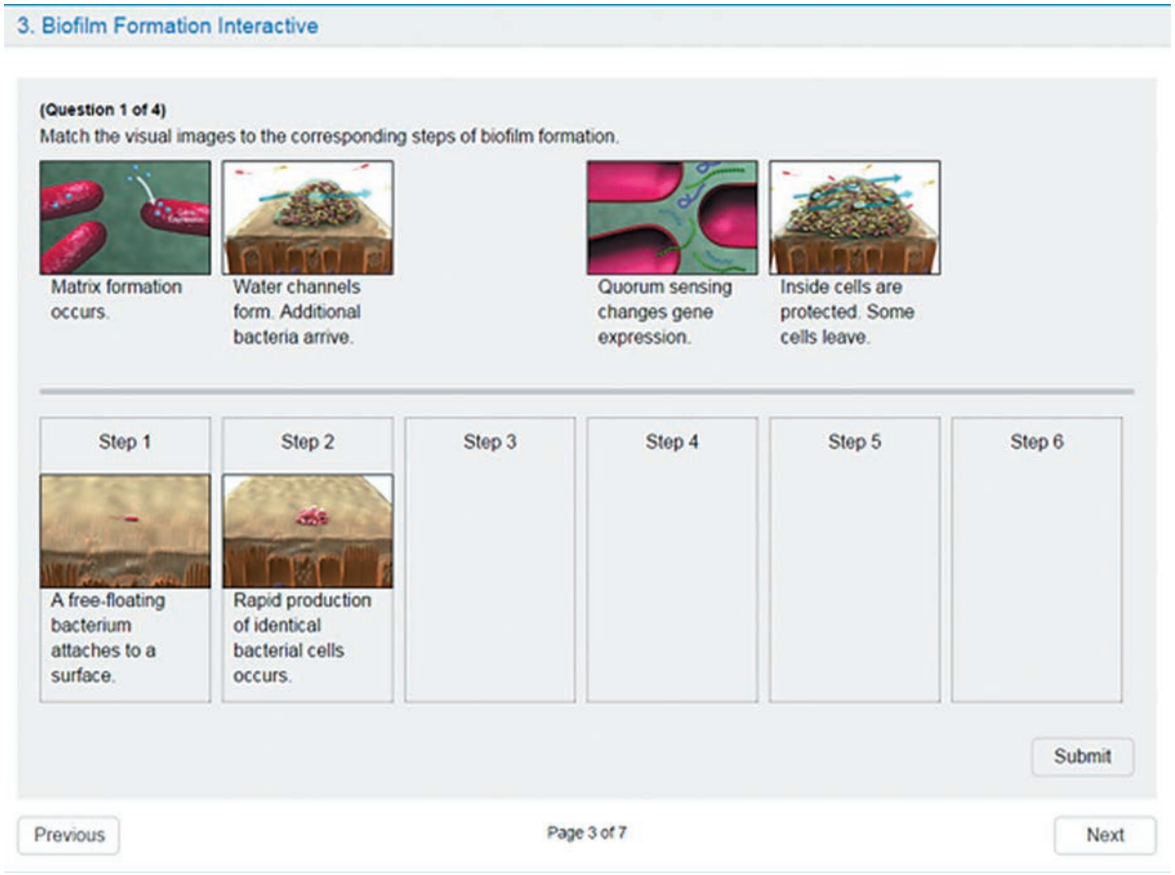


Each Interactive Microbiology tutorial begins with a clinical case scenario, allowing you, the learner, to explore different real world health care situations.

Experience and learn microbiology principles by engaging with interactive animations.



Each Interactive Microbiology module is supported by interactive tutorials that reinforce concepts presented in the animations.



Accessible in the Study Area for on-the-go studying with mobile devices and assignable through MasteringMicrobiology®, Interactive Microbiology explores challenging and important topics including Operons, Biofilms and Quorum Sensing, Aerobic Respiration in Bacteria, Complement, and more.

Focus on the Big Picture

NEW!
Big Picture spreads have been added to the Twelfth Edition, integrating text and illustrations to help students gain a broad, "big picture" understanding of important course topics.

Seven Big Picture spreads focus on **a particular disease with an application to a related real-world challenge.** Many of the featured diseases explore public health issues:

Human Microbiome and IBD pp. 518–519
Fungal Keratitis pp. 600–601
Neglected Tropical Diseases pp. 622–623
Climate Change and Disease pp. 658–659
Pertussis pp. 682–683
Cholera After Natural Disasters pp. 720–721
STI Home Test Kits pp. 752–753

BIG PICTURE

Human Microbiome and IBD

The Human Microbiome Project uses genetic sequencing to study correlations between changes in the microbiome and inflammatory bowel disease.

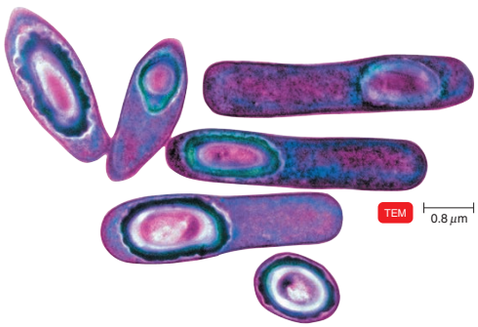
Our bodies are complex sets of ecosystems, with segments that come into contact with the outer world, each having its own microbial population. Our relationship with gut microbiota is usually commensal or mutualistic. However, a change in microbiota can result in dysbiosis, an imbalance that causes adverse effects in the human. For example, *Clostridium difficile,* or C-diff, is usually a minor component of the normal gut microbiota. But when antibiotic therapy kills normal microbiota, C-diff proliferates, producing two toxins that create significant inflammation and gas production in the intestines.

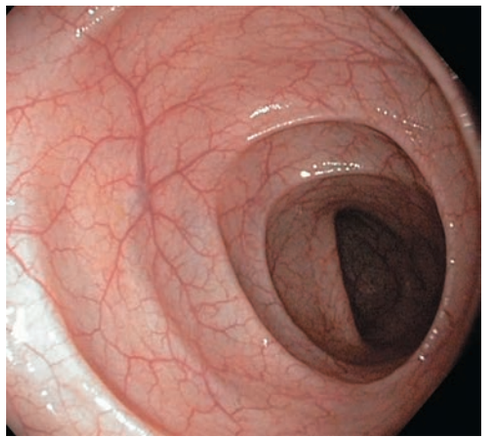
Could Dysbiosis Be the Cause of Inflammatory Bowel Diseases (IBD)?

Dysbiosis is now being closely studied as a possible cause for inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. Rationale for this hypothesis hinges on the fact that some metabolic products of normal microbiota, such as butyrates, exert an antiinflammatory effect on the body.

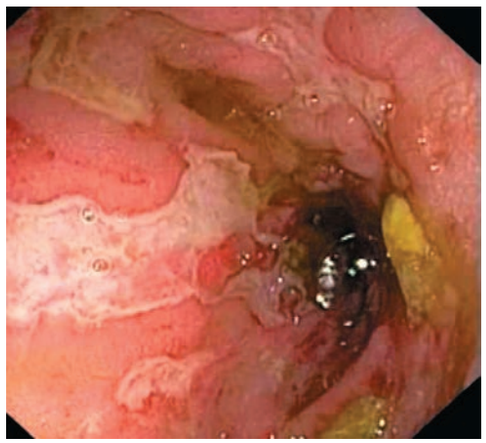
Crohn's disease, whose symptoms include swelling of the GI tract, is often characterized by excessive amounts of the cytokines tumor necrosis factor alpha (TNF-α) and interleukin-12 (IL-12). Researchers hypothesize that this excess could result from a disruption in the balance of normal microbiota that would usually help keep inflammatory cytokines under control.

Another clue being investigated regarding the link between IBD and microbiota is that these diseases are more common in developed countries than less-developed countries. Antibiotic usage tends to be higher in developed countries. Studies have demonstrated that the microbiome may not recover its full diversity after antibiotic treatment, which may lead to loss of organisms that would keep inflammation under control.





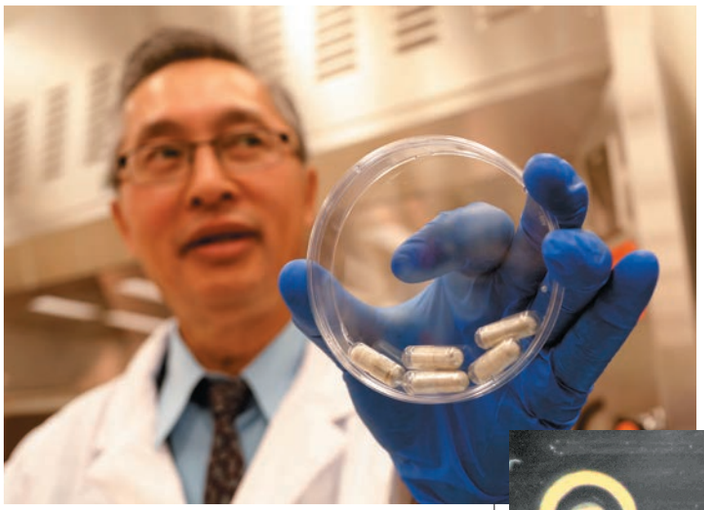
Endoscope view of a healthy colon



Endoscope view of an inflamed and ulcerated colon of a patient with Crohn's disease

Left: *Clostridium difficile,* or C-diff, can proliferate when antibiotics kill normal microbiota, leading to inflammation of the intestines.

Harnessing Microbes to Fight Inflammatory Bowel Diseases



Dr. Thomas Louie at the University of Calgary holds a dish of "poop pills" used for fecal transplantation. Photo credit: Associated Press

Below, eggs of *Trichuris suis*, the pig whipworm used to treat Crohn's disease

Fecal Transplants Shown to Successfully Treat *Clostridium difficile* Infections

Scientists have found success treating C-diff infections and some IBD with fecal microbiota transplants. Fecal transplants involve taking gut microbiota from a healthy individual (usually a family member) and then transplanting it into the patient via an enema, gastroscope, or nasojejunal tube, which is placed through the nose and runs down to the small intestine. Because this technique has been much more effective than antibiotic treatment, the FDA recently relaxed the restrictions it had placed on this procedure.

Researchers are working on ways to transplant microbiota in a more palatable fashion. Dr. Thomas Louie, an infectious disease specialist at the University of Calgary, has developed a method to deliver the microbiota in pills surrounded by a triple layer of gel, to prevent breakdown in the stomach. These "poop pills" have been successful in treating his patients with C-diff, and it is hoped that the process can also be used for IBD.

Treating Crohn's Disease with Worms

Hypotheses of how normal microbiota may assist our immune systems have led to some unusual treatments. One clinical study at the University of Iowa, where Crohn's patients were treated with pig whipworm eggs, found a 73% remission rate. Helminths, such as the whipworm, suppress certain T helper cell pathways – the exact pathways that are overactive in Crohn's disease. Since the worms don't take up residence in humans, the treatment must be repeated periodically to maintain the effect.

KEY CONCEPTS

- Normal microbiota are important in maintaining a healthy immune system. **(See Chapter 14, "Relationships Between Normal Microbiota and the Host," pages 391–393.)**
- The Human Microbiome Project is sequencing the genes for 16S ribosomal RNA to help scientists to catalogue normal microbiota that are difficult to culture and identify in the laboratory **(See Chapter 9, "Genome Projects," page 252.)**
- *Trichuris suis* is a roundworm related to *T. trichiura*. **(See Chapter 12, "Nematodes," page 349.)**
- Inflammatory diseases are characterized by increased amounts of cytokines produced by T helper cells, including tumor necrosis factor alpha and interleukins. **(See Chapter 16, "Inflammation," pages 452–455.)**

3

Each Big Picture spread is paired with a coaching activity and assessment questions within MasteringMicrobiology®.

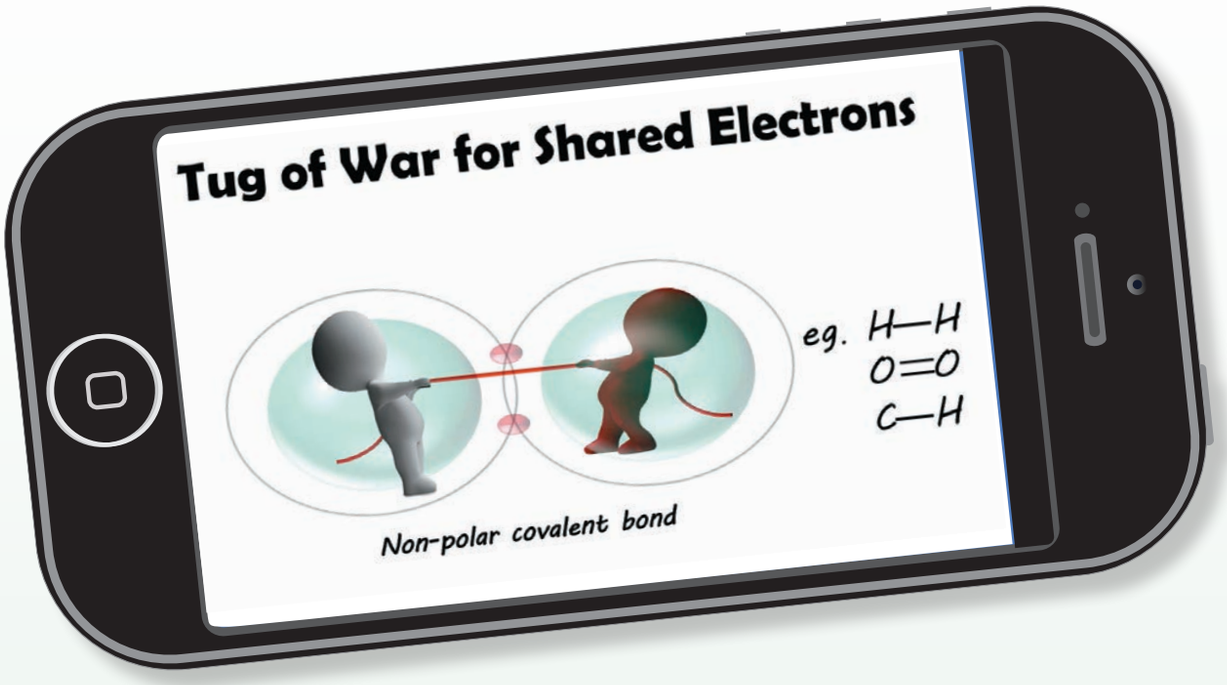
Big Picture spreads include Key Concepts that encourage students to make the connection between the presented topic and previously learned microbiology principles.

Three Big Picture spreads focus on the **most complex microbiology topics** and include an easy-to-reference overview that breaks down important concepts into manageable steps and gives students a clear learning framework for the related chapters:

Metabolism pp. 108–109
Genetics pp. 202–203
Immunity pp. 440–441

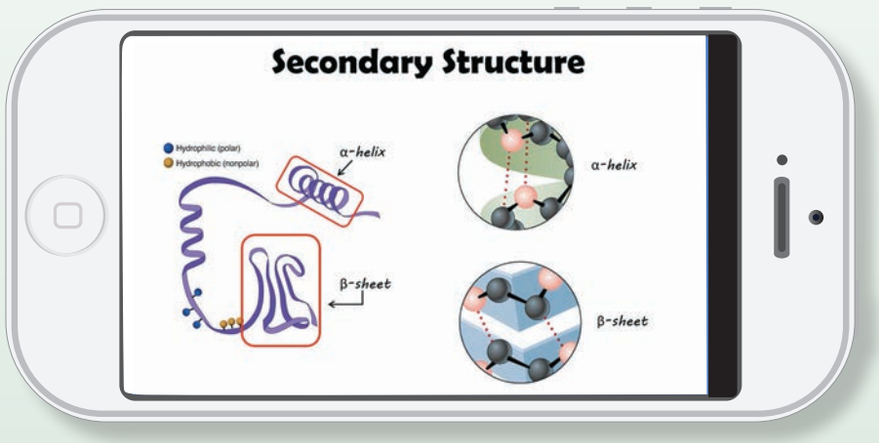
Access Study Tools Whenever and

MasteringMicrobiology® is now more mobile-friendly, allowing instructors to easily create 100% mobile-ready assignments that students can access using smartphones, tablets, and computers.

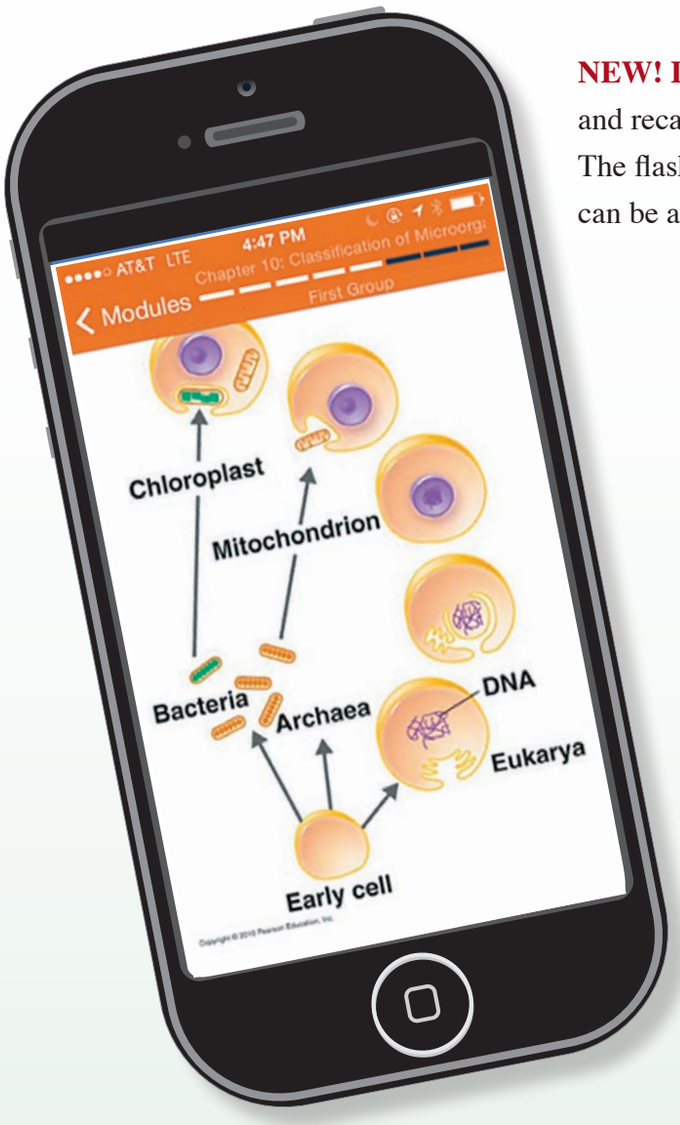


NEW! MicroBoosters are a suite of brief video tutorials that cover key concepts that some students may need to review or re-learn, including Study Skills, Math, Scientific Terminology, Basic Chemistry, Cell Biology, and Basic Biology.

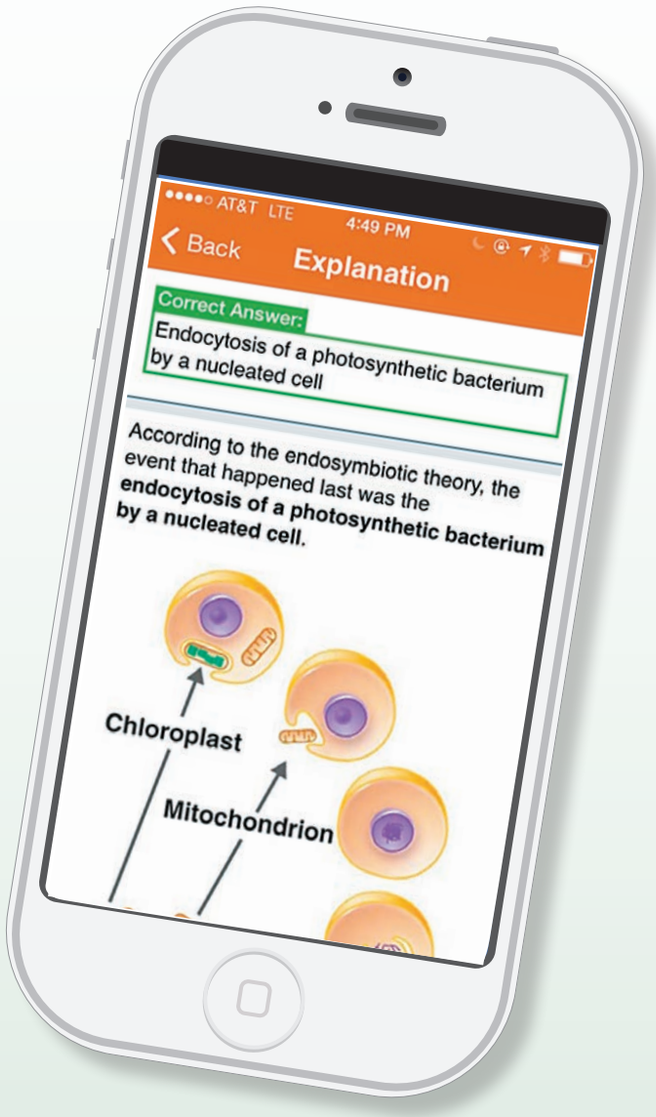
MicroBoosters can be assigned in the MasteringMicrobiology® Item Library or as Dynamic Study Modules, and are also available for student self-study in the Mastering Study Area.



Wherever You Need Them



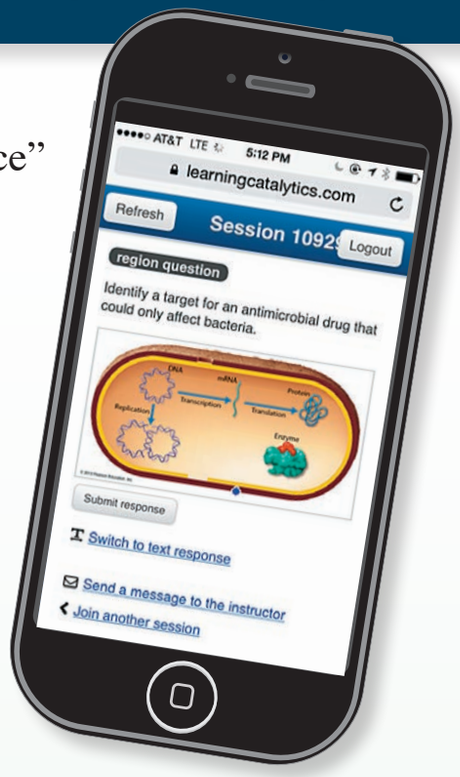
NEW! Dynamic Study Modules help students acquire, retain, and recall information faster and more efficiently than ever before. The flashcard-style modules are available as a self-study tool or can be assigned by the instructor.



NEW! Adaptive Follow-Up Assignments can be optionally assigned based on each student's performance on the original homework assignment and provide additional coaching and practice as needed. Exclusively available with ***Microbiology: An Introduction***, these question sets continuously adapt to each student's needs, making efficient use of study time.

Classroom Resources for Active Learning

NEW! Learning Catalytics is a "bring your own device" (laptop, smartphone, or tablet) student engagement, assessment, and classroom intelligence system. With Learning Catalytics, instructors can assess students in real time using open-ended tasks to probe student understanding. MasteringMicrobiology® users may select from Pearson's new library of questions designed especially for use with Learning Catalytics.



Instructor's Resource DVD for Microbiology: An Introduction
0-13-390553-5 / 978-0-13-390553-3

The Instructor's Resource DVD (IR-DVD) organizes all instructor media resources by chapter into one convenient and easy-to-use package. It contains:

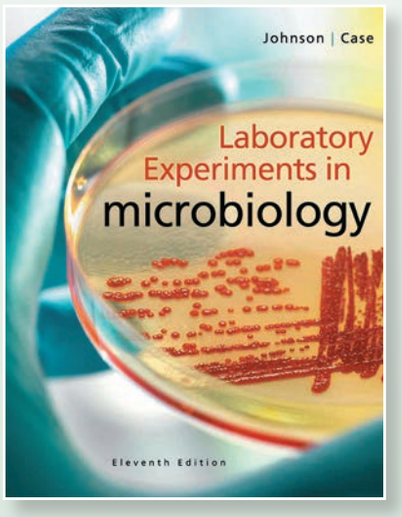
- All figures, photos, and tables from the textbook in both labeled and unlabeled formats
- TestGen Test Bank
- MicroFlix animations
- Instructor's Guide

A wealth of additional classroom resources can be downloaded from the "Instructor Resources" area of MasteringMicrobiology®.

Laboratory Experiments in Microbiology 11e
Johnson/Case
0-321-99493-0 / 978-0-321-99493-6

Engaging, comprehensive and customizable, ***Laboratory Experiments in Microbiology*** is the perfect companion lab manual for ***Microbiology: An Introduction***, Twelfth Edition.

Fourteen New Part-opening Case Studies show how the exercises are medically relevant to the students' future careers.



Lab and Lecture: Putting it All Together. Each exercise contains a feature in MasteringMicrobiology® that prompts students to connect the lab experiment to what they have learned in lecture.

Updates include new ASM biosafety protocols. Updates to the ASM BSL guidelines have changed how and what instructors do in lab. The authors revised each exercise to show which procedural steps and alternate bacteria are more appropriate for BSL-2 facilities.

MICRO BIOLOGY

AN INTRODUCTION

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MICROBIOLOGY

AN INTRODUCTION

TWELFTH EDITION

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About the Authors

Courtesy of Rev. Dr. James F. Tortora

Gerard J. Tortora Jerry Tortora is a professor of biology and teaches microbiology, human anatomy and physiology at Bergen Community College in Paramus, New Jersey. He received his M.A. in Biology from Montclair State College in 1965. He belongs to a number of biology/microbiology organizations, such as the American Society for Microbiology (ASM), Human Anatomy and Physiology Society (HAPS), American Association for the Advancement of Science (AAAS), National Education Association (NEA), New Jersey Educational Association (NJEA), and the Metropolitan Association of College and University Biologists (MACUB). Jerry is the author of numerous biological science textbooks. In 1995, he was selected as one of the finest faculty scholars of Bergen Community College and was named Distinguished Faculty Scholar. In 1996, Jerry received a National Institute for Staff and Organizational Development (NISOD) excellence award from the University of Texas and was selected to represent Bergen Community College in a campaign to increase awareness of the contributions of community colleges to higher education.

Berdell R. Funke Bert Funke received his Ph.D., M.S., and B.S. in microbiology from Kansas State University. He has spent his professional years as a professor of microbiology at North Dakota State University. He taught introductory microbiology, including laboratory sections, general microbiology, food microbiology, soil microbiology, clinical parasitology, and pathogenic microbiology. As a research scientist in the Experiment Station at North Dakota State, he has published numerous papers in soil microbiology and food microbiology.

Christine L. Case Chris Case is a registered microbiologist and a professor of microbiology at Skyline College in San Bruno, California, where she has taught for the past 44 years. She received her Ed.D. in curriculum and instruction from Nova Southeastern University and her M.A. in microbiology from San Francisco State University. She was Director for the Society for Industrial Microbiology (SIM) and is an active member of the ASM and Northern California SIM. She received the ASM and California Hayward outstanding educator awards. In 2008, Chris received the SACNAS Distinguished Community/Tribal College Mentor Award for her commitment to her students, several of whom have presented at undergraduate research conferences and won awards. In addition to teaching, Chris contributes regularly to the professional literature, develops innovative educational methodologies, and maintains a personal and professional commitment to conservation and the importance of science in society. Chris is also an avid photographer, and many of her photographs appear in this book.

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Preface

Since the publication of the first edition nearly 30 years ago, well over 1 million students have used *Microbiology: An Introduction* at colleges and universities around the world, making it the leading textbook for non-majors microbiology. The twelfth edition continues to be a comprehensive beginning text, assuming no previous study of biology or chemistry. The text is appropriate for students in a wide variety of programs, including the allied health sciences, biological sciences environmental science, animal science, forestry, agriculture, home economics, and the liberal arts.

The twelfth edition has retained the features that have made this book so popular:

- **An appropriate balance between microbiological fundamentals and applications, and between medical applications and other applied areas of microbiology.** Basic microbiological principles are given greater emphasis, and health-related applications are featured.
- **Straightforward presentation of complex topics.** Each section of the text is written with the student in mind.
- **Clear, accurate, and pedagogically effective illustrations and photos.** Step-by-step diagrams that closely coordinate with narrative descriptions aid student comprehension of concepts.
- **Flexible organization.** We have organized the book in what we think is a useful fashion while recognizing that the material might be effectively presented in other sequences. For instructors who wish to use a different order, we have made each chapter as independent as possible and have included numerous cross-references. The Instructor's Guide provides detailed guidelines for organizing the material in several other ways.

NEW TO THE TWELFTH EDITION

The twelfth edition focuses on big-picture concepts and themes in microbiology, encouraging students to visualize and synthesize more difficult topics such as microbial metabolism, immunology, and microbial genetics.

The twelfth edition meets all students at their respective levels of skill and understanding while addressing the biggest challenges that instructors face. Updates to the twelfth edition enhance the book's consistent pedagogy and clear explanations. Some of the highlights follow.

- **Cutting-edge media integration.** MasteringMicrobiology (www.masteringmicrobiology.com) provides unprecedented, cutting-edge assessment resources for instructors as well as self-study tools for students. Big Picture Coaching Activities are paired with the book's new Big Picture: Tough Topics and Big Picture: Disease features; Interactive Microbiology is a dynamic suite of interactive tutorials and animations that teach key concepts in microbiology; and MicroBoosters are brief video tutorials that cover key concepts that some students need to review or re-learn.
- **Big Picture "tough topic" features.** These two-page spreads focus on the most challenging topics for students to master: metabolism (Chapter 5), genetics (Chapter 8), and immunology (Chapter 16). Each spread breaks down these important concepts into manageable steps and gives students a clear learning framework for the related chapters. Each includes a quick-reference (QR) code that allows students to link to related MicroFlix videos with their smartphones.
- **Big Picture Disease features.** These two-page spreads appear within each organ-system disease chapter (Chapters 21–26) as well as Chapter 19 (Disorders of the Immune System). Each spread focuses on a particular disease and applies it to a related real-world challenge, many dealing with public health issues.
- **Reworked complement section in Chapter 16 (Innate Immunity: Nonspecific Defenses of the Host).** New art and more straightforward discussions make this challenging and critical material easier for students to understand and retain.
- **In the Clinic.** This new feature, appearing at the start of every chapter, includes critical thinking questions that encourage students to think as health care professionals would in various clinical scenarios and spark student interest in the forthcoming chapter content.
- **ASM guidelines.** The American Society of Microbiology has released six underlying concepts and 22 related topics to provide a framework for key microbiological topics deemed to be of lasting importance beyond the classroom. The twelfth edition explains the themes and competencies at the beginning of the book and incorporates callouts when chapter content matches one of these 22 topics. Doing so addresses two key challenges: it helps students and instructors focus on the enduring principles of the course, and it provides another pedagogical tool for instructors to assess students' understanding and encourage critical thinking.

CHAPTER-BY-CHAPTER REVISIONS

Every chapter in this edition has been thoroughly revised, and data in the text, tables, and figures have been updated. The main changes to each chapter are summarized below.

Chapter 1

- New sections on Middle East respiratory syndrome (MERS), coronavirus, and severe acute respiratory syndrome (SARS) have been added.
- A new table, Table 1.2, addresses representative discoveries of the Golden Age of Microbiology.

Chapter 2

- The section on activation energy has been revised.

Chapter 3

- Foundation Figure 3.2, Microscopes and Magnification, has been revised.

Chapter 4

- The discussion of facilitated diffusion has been revised.
- The cell art has been revised.

Chapter 5

- A new Big Picture feature, addressing metabolism, has been added.
- The discussion of enzyme specificity has been revised.
- Figure 5.25, showing photophosphorylation, has been revised.
- The discussion of chemoheterotrophs has been revised.

Chapter 8

- A new Big Picture feature, addressing genetics, has been added.
- The central dogma of genetics is described.
- Mutation and gene transfers are now included in a new section.

Chapter 9

- Vectors are defined.

Chapter 10

- Figure 10.9, showing the new EnteroPluri-test, is revised.

Chapter 11

- The order Thiotrichales is now included.
- Discussion of the new genus *Cronobacter* has been added.
- Several of the figures have been replaced with improved illustrations.
- The tables have been revised and simplified.
- Nomenclature has been updated.

Chapter 12

- The discussion of algal and protozoan taxonomy is updated.

Chapter 13

- A discussion of the use of oncolytic viruses to treat cancer has been added.
- The discussion of viral enzymes has been revised.

Chapter 14

- The chapter has been updated to reflect the use of the term *healthcare-associated infection*.

Chapter 16

- A new Big Picture feature, addressing immunity, has been added.
- A new figure and discussion of hematopoiesis have been added.
- Figure 16.14 has been revised.
- The discussions of the complement system and interferons have been extensively revised.

Chapter 17

- The introductory material has been revised.
- Several figures have been revised.

Chapter 18

- The tables showing vaccination schedules have been updated.
- A discussion of virus-like particle (VLP) vaccines has been added.
- Clinical Focus box has been rewritten and updated.
- The discussions of vaccination technologies and monoclonal antibodies have been updated.

Chapter 19

- A new Big Picture Disease feature, Human Microbiome and IBD, has been added.
- The discussion of HIV/AIDS has been updated with new, informative maps.
- The chemotherapy of AIDS section has been completely revised, including new figures depicting the action of HIV therapies.

Chapter 20

- The discussion of antiviral drugs has been updated.
- The discussion of antibiotics effective against dormant cells has been expanded.

Chapter 21

- A new Big Picture Disease feature, Fungal Keratitis, has been added.
- A discussion of hand-foot-and-mouth disease is now included.

Chapter 22

- A new Big Picture Disease feature, Neglected Tropical Diseases, has been added.
- The discussion of developments in testing for leprosy has been updated.

Chapter 23

- A new Big Picture Disease feature, Climate Change and Disease, has been added.
- Several of the maps have been updated.
- The discussion of sepsis and septic shock has been revised.
- The discussion of Lyme disease has been revised to include the topic of immunity to reinfection.
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ASM Recommended Curriculum Guidelines for Undergraduate Microbiology

The American Society for Microbiology (ASM) endorses a concept-based curriculum for introductory microbiology, emphasizing skills and concepts that remain important long after students exit the course. The ASM *Curriculum Guidelines for Undergraduate Microbiology Education* provide a framework for key microbiological topics and agree with scientific literacy reports from the American Association for the Advancement of Science and Howard Hughes Medical Institute. This textbook references part one of curriculum guidelines throughout chapters. When a discussion touches on one of the concepts, readers will see the ASM icon, along with a summary of the relevant statement.

ASM GUIDELINE CONCEPTS AND STATEMENTS

Evolution:

- Cells, organelles (e.g., mitochondria and chloroplasts), and all major metabolic pathways evolved from early prokaryotic cells.
- Mutations and horizontal gene transfer, with the immense variety of microenvironments, have selected for a huge diversity of microorganisms.
- Human impact on the environment influences the evolution of microorganisms (e.g., emerging diseases and the selection of antibiotic resistance).
- Traditional concept of species is not readily applicable to microbes due to asexual reproduction and the frequent occurrence of horizontal gene transfer.
- Evolutionary relatedness of organisms is best reflected in phylogenetic trees.

Cell Structure and Function:

- Structure and function of microorganisms have been revealed by the use of microscopy (including brightfield, phase contrast, fluorescent, and electron).
- Bacteria have unique cell structures that can be targets for antibiotics, immunity, and phage infection.
- Bacteria and Archaea have specialized structures (e.g. flagella, endospores, and pili) that often confer critical capabilities.
- While microscopic eukaryotes (for example, fungi, protozoa, and algae) carry out some of the same processes as bacteria, many of the cellular properties are fundamentally different.
- Replication cycles of viruses (lytic and lysogenic) differ among viruses and are determined by their unique structures and genomes.

Metabolic Pathways:

- Bacteria and Archaea exhibit extensive, and often unique, metabolic diversity (e.g. nitrogen fixation, methane production, anoxygenic photosynthesis).
- Interactions of microorganisms among themselves and with their environment are determined by their metabolic abilities (e.g., quorum sensing, oxygen consumption, nitrogen transformations).
- Survival and growth of any microorganism in a given environment depends on its metabolic characteristics.
- Growth of microorganisms can be controlled by physical, chemical, mechanical, or biological means.

Information Flow and Genetics:

- Genetic variations can impact microbial functions (e.g. in biofilm formation, pathogenicity, and drug resistance).
- Although the central dogma is universal in all cells, the processes of replication, transcription, and translation differ in Bacteria, Archaea, and Eukaryotes.
- Regulation of gene expression is influenced by external and internal molecular cues and/or signals.
- Synthesis of viral genetic material and proteins is dependent on host cells.
- Cell genomes can be manipulated to alter cell function.

Microbial Systems:

- Microorganisms are ubiquitous and live in diverse and dynamic ecosystems.
- Most bacteria in nature live in biofilm communities.
- Microorganisms and their environment interact with and modify each other.
- Microorganisms, cellular and viral, can interact with both human and nonhuman hosts in beneficial, neutral, or detrimental ways.

Impact of Microorganisms:

- Microbes are essential for life as we know it and the processes that support life (e.g., in biogeochemical cycles and plant and/or animal microbiota).
- Microorganisms provide essential models that give us fundamental knowledge about life processes.
- Humans utilize and harness microorganisms and their products.
- Because the true diversity of microbial life is largely unknown, its effects and potential benefits have not been fully explored.

In the Clinic

As the nurse practitioner in a rural hospital, you are reviewing a microscope slide of a skin scraping from a 12-year-old girl. The slide shows branched, intertwined nucleated hyphae. The girl has dry, scaly, itchy patches on her arms.

What is causing her skin problem?

Hint: Read about types of microorganisms (pages 3–5).

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

1 The Microbial World and You

The overall theme of this textbook is the relationship between microbes—very small organisms that usually require a microscope to be seen—and our lives. This relationship involves not only the familiar harmful effects of certain microorganisms, such as disease and food spoilage, but also their many beneficial effects. In this chapter we introduce you to some of the many ways microbes affect our lives. We begin by discussing how organisms are named and classified, followed by a short history of microbiology that reveals how much we have learned in just a few hundred years. Then we discuss the incredible diversity of microorganisms and their ecological importance, noting how they maintain balance in the environment by recycling chemical elements such as carbon and nitrogen among the soil, organisms, and the atmosphere. We also examine how microbes are used in commercial and industrial applications to produce foods, chemicals, and drugs (such as antibiotics); and to treat sewage, control pests, and clean up pollutants. We will discuss microbes as the cause of such diseases as avian (bird) flu, West Nile encephalitis, mad cow disease, diarrhea, hemorrhagic fever, and AIDS, and we examine the growing public health problem of antibiotic-resistant bacteria.

ASM: Microorganisms provide essential models that give us fundamental knowledge about life processes.

Staphylococcus aureus (STAF-i-lō-kok'kus OR-ē-us) bacteria on human nasal epithelial cells are shown in the photograph. These bacteria live harmlessly on skin or inside the nose. Misuse of antibiotics allows the survival of bacteria with antibiotic-resistance genes, such as methicillin-resistant *S. aureus* (MRSA). As illustrated in the Clinical Case, an infection caused by these bacteria is resistant to antibiotic treatment.

Staphylococcus aureus **bacteria on human nasal epithelial cells.**

Microbes in Our Lives

LEARNING OBJECTIVE

1-1 List several ways in which microbes affect our lives.

For many people, the words *germ* and *microbe* bring to mind a group of tiny creatures that do not quite fit into any of the categories in that old question, "Is it animal, vegetable, or mineral?" **Microbes,** also called **microorganisms,** are minute living things that individually are usually too small to be seen with the unaided eye. The group includes bacteria, fungi (yeasts and molds), protozoa, and microscopic algae. It also includes viruses, those noncellular entities sometimes regarded as straddling the border between life and nonlife (Chapters 11, 12, and 13, respectively).

We tend to associate these small organisms only with uncomfortable infections, with common inconveniences such as spoiled food, or with major diseases such as AIDS. However, the majority of microorganisms actually help maintain the balance of life in our environment. Marine and freshwater microorganisms form the basis of the food chain in oceans, lakes, and rivers. Soil microbes help break down wastes and incorporate nitrogen gas from the air into organic compounds, thereby recycling chemical elements among soil, water, living organisms, and air. Certain microbes play important roles in *photosynthesis,* a food- and oxygen-generating process that is critical to life on Earth. Humans and many other animals depend on the microbes in their intestines for digestion and the synthesis of some vitamins that their bodies require, including some B vitamins for metabolism and vitamin K for blood clotting.

Microorganisms also have many commercial applications. They are used in the synthesis of such chemical products as vitamins, organic acids, enzymes, alcohols, and many drugs. For example, microbes are used to produce acetone and butanol, and the vitamins B_2 (riboflavin) and B_{12} (cobalamin) are made biochemically. The process by which microbes produce acetone and butanol was discovered in 1914 by Chaim Weizmann, a Russian-born chemist working in England. With the outbreak of World War I in August of that year, the production of acetone became very important for making cordite (a smokeless form of gunpowder used in munitions). Weizmann's discovery played a significant role in determining the outcome of the war.

The food industry also uses microbes in producing, for example, vinegar, sauerkraut, pickles, soy sauce, cheese, yogurt, bread, and alcoholic beverages. In addition, enzymes from microbes can now be manipulated to cause the microbes to produce substances they normally don't synthesize, including cellulose, digestive aids, and drain cleaner, plus important therapeutic substances such as insulin. Microbial enzymes may even have helped produce your favorite pair of jeans (see the Applications of Microbiology box).

Though only a minority of microorganisms are **pathogenic** (disease-producing), practical knowledge of microbes is necessary for medicine and the related health sciences. For example, hospital workers must be able to protect patients from common microbes that are normally harmless but pose a threat to the sick and injured.

Today we understand that microorganisms are found almost everywhere. Yet not long ago, before the invention of the microscope, microbes were unknown to scientists. Thousands of people died in devastating epidemics, the causes and transmission of which were not understood. Entire families died because vaccinations and antibiotics were not available to fight infections.

We can get an idea of how our current concepts of microbiology developed by looking at a few historic milestones in microbiology that have changed our lives. First, however, we will look at the major groups of microbes and how they are named and classified.

CHECK YOUR UNDERSTANDING

- Describe some of the destructive and beneficial actions of microbes. **1-1***

Naming and Classifying Microorganisms

LEARNING OBJECTIVES

1-2 Recognize the system of scientific nomenclature that uses two names: a genus and a specific epithet.

1-3 Differentiate the major characteristics of each group of microorganisms.

1-4 List the three domains.

Nomenclature

The system of nomenclature (naming) for organisms in use today was established in 1735 by Carolus Linnaeus. Scientific names are latinized because Latin was the language traditionally used by scholars. Scientific nomenclature assigns each organism two names—the **genus** (plural: *genera*) is the first name and is always capitalized; the **specific epithet** (**species** name) follows and is not capitalized. The organism is referred to by both the genus and the specific epithet, and both names are underlined or italicized. By custom, after a scientific name has been mentioned once, it can be abbreviated with the initial of the genus followed by the specific epithet.

Scientific names can, among other things, describe an organism, honor a researcher, or identify the habitat of a species. For example, consider *Staphylococcus aureus,* a bacterium commonly found on human skin. *Staphylo-* describes the clustered arrangement of the cells; *-coccus* indicates that they are shaped like spheres. The specific epithet, *aureus,* is Latin for golden,

*The numbers following Check Your Understanding questions refer to the corresponding Learning Objectives.

Designer Jeans: Made by Microbes?

Denim blue jeans have been popular ever since Levi Strauss and Jacob Davis first made them for California gold miners in 1873. Now, companies that manufacture blue jeans are turning to microbiology to develop environmentally sound production methods that minimize toxic wastes and the associated costs.

Soft, Faded Jeans

A softer, faded denim is made with enzymes called *cellulases* from *Trichoderma* fungus. They digest some of the cellulose in the cotton. Unlike many chemical reactions, enzymes usually operate at safe temperatures and pH. Moreover, enzymes are proteins, so they are readily degraded for removal from wastewater.

Fabric

Cotton production requires large tracts of land, pesticides, and fertilizer, and the crop yield depends on the weather. However, bacteria can produce both cotton and polyester with less environmental impact. *Gluconacetobacter xylinus* bacteria make cellulose by attaching glucose units to simple chains in the outer membrane of the bacterial cell wall. The cellulose microfibrils are extruded through pores in the outer membrane, and bundles of microfibrils then twist into ribbons.

Bleaching

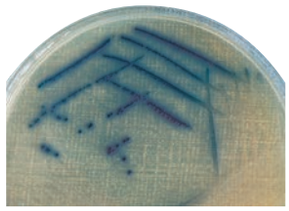
Peroxide is a safer bleaching agent than chlorine and can be easily removed from fabric and wastewater by enzymes. Researchers at Novo Nordisk Biotech cloned a mushroom peroxidase gene in yeast and grew the yeasts in washing machine conditions. The yeast that survived the washing machine were selected as the peroxidase producers.

Indigo

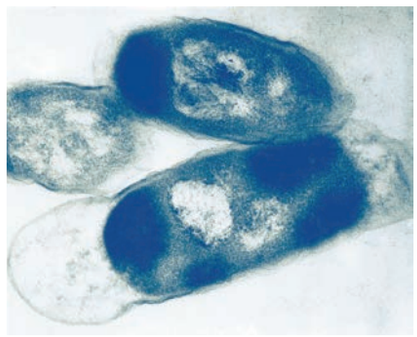
Chemical synthesis of indigo requires a high pH and produces waste that explodes on contact with air. However, a California biotechnology company, Genencor, has developed a method to produce indigo by using bacteria. Researchers identified a gene from a soil bacterium, *Pseudomonas putida*, that converts the bacterial by-product indole to indigo. This gene was put into *Escherichia coli* bacteria, which then turned blue.

Bioplastic

Microbes can even make plastic zippers and packaging material for the jeans. Over 25 bacteria make polyhydroxyalkanoate (PHA) inclusion granules as a food reserve. PHAs are similar to common plastics, and because they are made by bacteria, they are also readily degraded by many bacteria. PHAs could provide a biodegradable alternative to conventional plastic, which is made from petroleum.



***E. coli* bacteria produce indigo from tryptophan.**



Indigo-producing *E. coli* bacteria.

0.3 μm

TEM

the color of many colonies of this bacterium. The genus of the bacterium *Escherichia coli* (esh′er-IK-ē-ah KŌ-lī, or KŌ-lē) is named for a scientist, Theodor Escherich, whereas its specific epithet, *coli,* reminds us that *E. coli* live in the colon, or large intestine. Table 1.1 contains more examples.

CHECK YOUR UNDERSTANDING

Distinguish a genus from a specific epithet. 1-2

Types of Microorganisms

Here is an overview of the main types of microorganisms. (The classification and identification of microorganisms are discussed in Chapter 10.)

Bacteria

Bacteria (singular: **bacterium**) are relatively simple, single-celled (unicellular) organisms. Because their genetic material is not enclosed in a special nuclear membrane, bacterial cells are called **prokaryotes** (prō-KAR-e-ōts), from Greek words meaning pre-nucleus. Prokaryotes include both bacteria and archaea.

Clinical Case: A Simple Spider Bite?

Andrea is a normally healthy 22-year-old college student who lives at home with her mother and younger sister, a high school gymnast. She is trying to work on a paper for her psychology class but is having a hard time because a red, swollen sore on her right wrist is making typing difficult. "Why won't this spider bite heal?" she wonders. "It's been there for days!" She makes an appointment with her doctor so she can show him the painful lesion. Although Andrea does not have a fever, she does have an elevated white blood cell count that indicates a bacterial infection. Andrea's doctor suspects that this isn't a spider bite at all, but a staph infection. He prescribes a β-lactam antibiotic, cephalosporin. Learn more about the development of Andrea's illness on the following pages.

What is staph? Read on to find out.

TABLE 1.1 Making Scientific Names Familiar

Use the word roots guide to find out what the name means. The name will not seem so strange if you translate it. When you encounter a new name, practice saying it out loud (guidelines for pronunciation are given in Appendix D). The exact pronunciation is not as important as the familiarity you will gain.

Following are some examples of microbial names you may encounter in the popular press as well as in the lab.

	Pronunciation	Source of Genus Name	Source of Specific Epithet
Salmonella enterica (bacterium)	sal'mōn-EL-lah en-TER-i-kah	Honors public health microbiologist Daniel Salmon	Found in the intestines (*entero-*)
Streptococcus pyogenes (bacterium)	strep'tō-KOK-kus pī-AH-jen-ēz	Appearance of cells in chains (*strepto-*)	Forms pus (*pyo-*)
Saccharomyces cerevisiae (yeast)	sak'kar-ō-MĪ-sēz se-ri-VIS-ē-ī	Fungus (*-myces*) that uses sugar (*saccharo-*)	Makes beer (*cerevisia*)
Penicillium chrysogenum (fungus)	pen'i-SIL-lē-um krī-SO-jen-um	Tuftlike or paintbrush (*penicill-*) appearance microscopically	Produces a yellow (*chryso-*) pigment
Trypanosoma cruzi (protozoan)	TRI-pa-nō-sō-mah KROOZ-ē	Corkscrew- (*trypano-*, borer; *soma-*, body)	Honors epidemiologist Oswaldo Cruz

Bacterial cells generally appear in one of several shapes. *Bacillus* (bah-SIL-lus) (rodlike), illustrated in **Figure 1.1a**, *coccus* (KOK-kus) (spherical or ovoid), and *spiral* (corkscrew or curved) are among the most common shapes, but some bacteria are star-shaped or square (see Figures 4.1 through 4.5, pages 74–75). Individual bacteria may form pairs, chains, clusters, or other groupings; such formations are usually characteristic of a particular genus or species of bacteria.

Bacteria are enclosed in cell walls that are largely composed of a carbohydrate and protein complex called *peptidoglycan.* (By contrast, cellulose is the main substance of plant and algal cell walls.) Bacteria generally reproduce by dividing into two equal cells; this process is called *binary fission.* For nutrition, most bacteria use organic chemicals, which in nature can be derived from either dead or living organisms. Some bacteria can manufacture their own food by photosynthesis, and some can derive nutrition from inorganic substances. Many bacteria can "swim" by using moving appendages called *flagella.* (For a complete discussion of bacteria, see Chapter 11.)

Archaea

Like bacteria, **archaea** (AR-kē-ah) consist of prokaryotic cells, but if they have cell walls, the walls lack peptidoglycan. Archaea, often found in extreme environments, are divided into three main groups. The *methanogens* produce methane as a waste product from respiration. The *extreme halophiles* (*halo* = salt; *philic* = loving) live in extremely salty environments such as the Great Salt Lake and the Dead Sea. The *extreme thermophiles* (*therm* = heat) live in hot sulfurous water, such as hot springs at Yellowstone National Park. Archaea are not known to cause disease in humans.

Fungi

Fungi (singular: **fungus**) are **eukaryotes** (ū-KAR-ē-ōts), organisms whose cells have a distinct nucleus containing the cell's genetic material (DNA), surrounded by a special envelope called the nuclear membrane. Organisms in the Kingdom Fungi may be unicellular or multicellular (see Chapter 12, page 320). Large multicellular fungi, such as mushrooms, may look somewhat like plants, but unlike most plants, fungi cannot carry out photosynthesis. True fungi have cell walls composed primarily of a substance called *chitin.* The unicellular forms of fungi, *yeasts,* are oval microorganisms that are larger than bacteria. The most typical fungi are *molds* (Figure 1.1b). Molds form visible masses called *mycelia,* which are composed of long filaments (*hyphae*) that branch and intertwine. The cottony growths sometimes found on bread and fruit are mold mycelia. Fungi can reproduce sexually or asexually. They obtain nourishment by absorbing solutions of organic material from their environment—whether soil, seawater, freshwater, or an animal or plant host. Organisms called *slime molds* have characteristics of both fungi and amebae (see Chapter 12).

Protozoa

Protozoa (singular: **protozoan**) are unicellular eukaryotic microbes (see Chapter 12, page 337). Protozoa move by pseudopods, flagella, or cilia. Amebae (Figure 1.1c) move by using extensions of their cytoplasm called *pseudopods* (false feet). Other protozoa have long *flagella* or numerous shorter appendages for locomotion called *cilia.* Protozoa have a variety of shapes and live either as free entities or as *parasites* (organisms that derive nutrients from living hosts) that absorb or ingest organic compounds from their environment. Some protozoa, such as *Euglena* (ū-GLĒ-nah), are photosynthetic. They use light as a source of energy and carbon

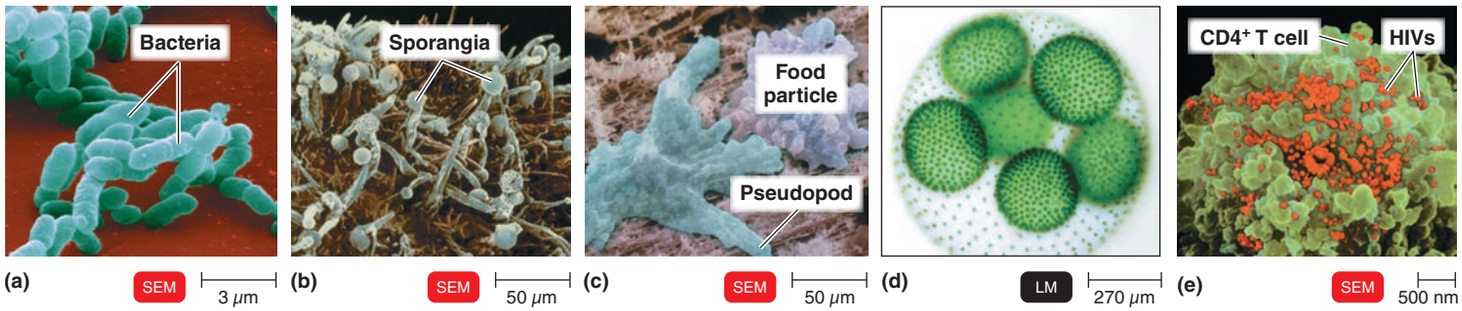


Figure 1.1 Types of microorganisms. **(a)** The rod-shaped bacterium *Haemophilus influenzae,* one of the bacterial causes of pneumonia. **(b)** *Mucor,* a common bread mold, is a type of fungus. When released from sporangia, spores that land on a favorable surface germinate into a network of hyphae (filaments) that absorb nutrients. **(c)** An ameba, a protozoan, approaching a food particle. **(d)** The pond alga *Volvox.* **(e)** Human immunodeficiency viruses (HIVs), the causative agent of AIDS, budding from a $CD4^+$ T cell.

Q How are bacteria, archaea, fungi, protozoa, algae, and viruses distinguished on the basis of cellular structure?

NOTE: Throughout the book, a red icon under a micrograph indicates that the micrograph has been artificially colored. SEM (scanning electron microscope) and LM (light microscope) scales are discussed in detail in Chapter 3.

dioxide as their chief source of carbon to produce sugars. Protozoa can reproduce sexually or asexually.

Algae

Algae (singular: **alga**) are photosynthetic eukaryotes with a wide variety of shapes and both sexual and asexual reproductive forms (Figure 1.1d). The algae of interest to microbiologists are usually unicellular (see Chapter 12, page 332). The cell walls of many algae are composed of a carbohydrate called *cellulose.* Algae are abundant in freshwater and saltwater, in soil, and in association with plants. As photosynthesizers, algae need light, water, and carbon dioxide for food production and growth, but they do not generally require organic compounds from the environment. As a result of photosynthesis, algae produce oxygen and carbohydrates that are then utilized by other organisms, including animals. Thus, they play an important role in the balance of nature.

Viruses

Viruses (Figure 1.1e) are very different from the other microbial groups mentioned here. They are so small that most can be seen only with an electron microscope, and they are acellular (not cellular). Structurally very simple, a virus particle contains a core made of only one type of nucleic acid, either DNA or RNA. This core is surrounded by a protein coat, which is sometimes encased by a lipid membrane called an envelope. All living cells have RNA *and* DNA, can carry out chemical reactions, and can reproduce as self-sufficient units. Viruses can reproduce only by using the cellular machinery of other organisms. Thus, on the one hand, viruses are considered to be living only when they multiply within host cells they infect. In this sense, viruses are parasites of other forms of life. On the other hand, viruses are not considered to be living because they are inert outside living hosts. (Viruses will be discussed in detail in Chapter 13.)

Multicellular Animal Parasites

Although multicellular animal parasites are not strictly microorganisms, they are of medical importance and therefore will be discussed in this text. Animal parasites are eukaryotes. The two major groups of parasitic worms are the flatworms and the roundworms, collectively called **helminths** (see Chapter 12, page 343). During some stages of their life cycle, helminths are microscopic in size. Laboratory identification of these organisms includes many of the same techniques used for identifying microbes.

CHECK YOUR UNDERSTANDING

Which groups of microbes are prokaryotes? Which are eukaryotes? **1-3**

Classification of Microorganisms

Before the existence of microbes was known, all organisms were grouped into either the animal kingdom or the plant kingdom. When microscopic organisms with characteristics of animals and plants were discovered late in the seventeenth century, a new system of classification was needed. Still, biologists couldn't agree on the criteria for classifying these new organisms until the late 1970s.

In 1978, Carl Woese devised a system of classification based on the cellular organization of organisms. It groups all organisms in three domains as follows:

1. Bacteria (cell walls contain a protein–carbohydrate complex called peptidoglycan)
2. Archaea (cell walls, if present, lack peptidoglycan)
3. Eukarya, which includes the following:
   - Protists (slime molds, protozoa, and algae)
   - Fungi (unicellular yeasts, multicellular molds, and mushrooms)

- Plants (mosses, ferns, conifers, and flowering plants)
- Animals (sponges, worms, insects, and vertebrates)

Classification will be discussed in more detail in Chapters 10 through 12.

CHECK YOUR UNDERSTANDING

What are the three domains? **1-4**

A Brief History of Microbiology

LEARNING OBJECTIVES

1-5 Explain the importance of observations made by Hooke and van Leeuwenhoek.
1-6 Compare spontaneous generation and biogenesis.
1-7 Identify the contributions to microbiology made by Needham, Spallanzani, Virchow, and Pasteur.
1-8 Explain how Pasteur's work influenced Lister and Koch.
1-9 Identify the importance of Koch's postulates.
1-10 Identify the importance of Jenner's work.
1-11 Identify the contributions to microbiology made by Ehrlich and Fleming.
1-12 Define *bacteriology, mycology, parasitology, immunology,* and *virology*.
1-13 Explain the importance of microbial genetics and molecular biology.

Bacterial ancestors were the first living cells to appear on Earth. For most of human history, people knew little about the true causes, transmission, and effective treatment of disease. Let's look now at some key developments in microbiology that have spurred the field to its current technological state.

The First Observations

In 1665, after observing a thin slice of cork through a crude microscope, Englishman Robert Hooke reported that life's smallest structural units were "little boxes," or "cells." Using his improved microscope, Hooke later saw individual cells. Hooke's discovery marked the beginning of the **cell theory**—the theory that *all living things are composed of cells.*

Though Hooke's microscope was capable of showing large cells, it lacked the resolution that would have allowed him to see microbes clearly. Dutch merchant and amateur scientist Anton van Leeuwenhoek was probably the first to observe live microorganisms through the magnifying lenses of the more than 400 microscopes he constructed. Between 1673 and 1723, he wrote about the "animalcules" he saw through his simple, single-lens microscopes. Van Leeuwenhoek made detailed drawings of organisms he found in rainwater, feces, and material scraped from teeth. These drawings have since been identified as representations of bacteria and protozoa (**Figure 1.2**).

CHECK YOUR UNDERSTANDING

What is the cell theory? **1-5**

The Debate over Spontaneous Generation

After van Leeuwenhoek discovered the previously "invisible" world of microorganisms, the scientific community became interested in the origins of these tiny living things. Until the second half of the nineteenth century, many scientists and philosophers believed that some forms of life could arise spontaneously from nonliving matter; they called this hypothetical process **spontaneous generation.** Not much more than 100 years ago, people commonly believed that toads, snakes, and mice could be born of moist soil; that flies could emerge from manure; and that maggots (which we now know are the larvae of flies) could arise from decaying corpses.

Physician Francesco Redi set out in 1668 to demonstrate that maggots did not generate spontaneously. Redi filled two jars with decaying meat. The first was left unsealed, allowing flies to lay eggs on the meat, which developed into larvae. The second jar was sealed, and because the flies could not get inside, no maggots appeared. Still, Redi's antagonists were not convinced; they claimed that fresh air was needed for spontaneous generation. So Redi set up a second experiment, in which he covered a jar with a fine net instead of sealing it. No larvae appeared in the gauze-covered jar, even though air was present.

Redi's results were a serious blow to the long-held belief that large forms of life could arise from nonlife. However, many scientists still believed that small organisms, such as van Leeuwenhoek's "animalcules," were simple enough to generate from nonliving materials.

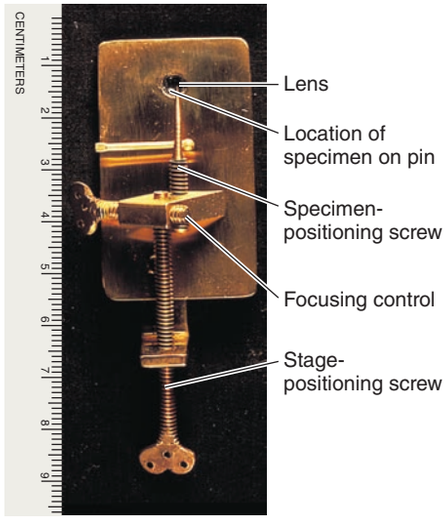
The case for spontaneous generation of microorganisms seemed to be strengthened in 1745, when John Needham found that even after he heated chicken broth and corn broth before pouring them into covered flasks, the cooled solutions were soon teeming with microorganisms. Needham claimed that microbes developed spontaneously from the fluids. Twenty years later, Lazzaro Spallanzani suggested that microorganisms from the air probably entered Needham's solutions after they were boiled. Spallanzani showed that nutrient fluids heated *after* being sealed in a flask did not develop microbial growth. Needham responded by claiming the "vital force" necessary for spontaneous generation had been destroyed by the heat and was kept out of the flasks by the seals.

Spallanzani's observations were also criticized on the grounds that there was not enough oxygen in the sealed flasks to support microbial life.

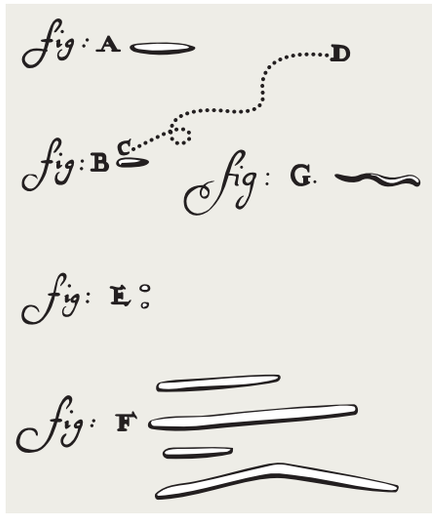
The Theory of Biogenesis

In 1858 Rudolf Virchow challenged the case for spontaneous generation with the concept of **biogenesis,** hypothesizing that living cells arise only from preexisting living cells. Because he could offer no scientific proof, arguments about spontaneous generation

(a) Van Leeuwenhoek using his microscope



(b) Microscope replica



(c) Drawings of bacteria

Figure 1.2 Anton van Leeuwenhoek's microscopic observations. **(a)** By holding his brass microscope toward a source of light, van Leeuwenhoek was able to observe living organisms too small to be seen with the unaided eye. **(b)** The specimen was placed on the tip of the adjustable point and viewed from the other side through the tiny, nearly spherical lens. The highest magnification possible with his microscopes was about 300× (times). **(c)** Some of van Leeuwenhoek's drawings of bacteria, made in 1683. The letters represent various shapes of bacteria. C–D represents a path of motion he observed.

Q Why was van Leeuwenhoek's discovery so important?

continued until 1861, when the issue was finally resolved by the French scientist Louis Pasteur.

Pasteur demonstrated that microorganisms are present in the air and can contaminate sterile solutions, but that air itself does not create microbes. He filled several short-necked flasks with beef broth and then boiled their contents. Some were then left open and allowed to cool. In a few days, these flasks were found to be contaminated with microbes. The other flasks, sealed after boiling, were free of microorganisms. From these results, Pasteur reasoned that microbes in the air were the agents responsible for contaminating nonliving matter.

Pasteur next placed broth in open-ended, long-necked flasks and bent the necks into S-shaped curves (**Figure 1.3**). The contents of these flasks were then boiled and cooled. The broth in the flasks did not decay and showed no signs of life, even after months. Pasteur's unique design allowed air to pass into the flask, but the curved neck trapped any airborne microorganisms that might contaminate the broth. (Some of these original vessels are still on display at the Pasteur Institute in Paris. They have been sealed but, like the flask in Figure 1.3, show no sign of contamination more than 100 years later.)

Pasteur showed that microorganisms can be present in nonliving matter—on solids, in liquids, and in the air. Furthermore, he demonstrated conclusively that microbial life can be destroyed by heat and that methods can be devised to block the access of airborne microorganisms to nutrient environments. These discoveries form the basis of **aseptic techniques,** procedures that prevent contamination by unwanted microorganisms, which are now the standard practice in laboratory and many medical procedures. Modern aseptic techniques are among the first and most important concepts that a beginning microbiologist learns.

Pasteur's work provided evidence that microorganisms cannot originate from mystical forces present in nonliving materials. Rather, any appearance of "spontaneous" life in nonliving solutions can be attributed to microorganisms that were already present in the air or in the fluids themselves. Scientists now believe that a form of spontaneous generation probably did occur on the primitive Earth when life first began, but they agree that this does not happen under today's environmental conditions.

CHECK YOUR UNDERSTANDING

- What evidence supported spontaneous generation? **1-6**
- How was spontaneous generation disproved? **1-7**

The Golden Age of Microbiology

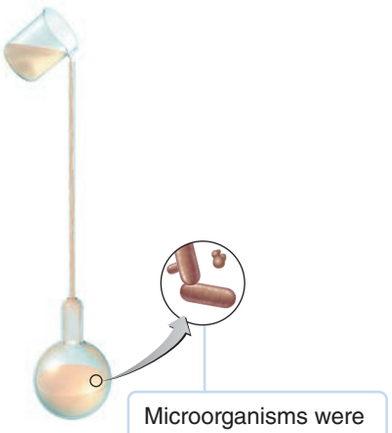
The period from 1857 to 1914 has been appropriately named the Golden Age of Microbiology. Rapid advances, spearheaded mainly by Pasteur and Robert Koch, led to the establishment of microbiology. Discoveries included both the agents of many diseases and the role of immunity in preventing and curing disease. During this

FOUNDATION FIGURE 1.3

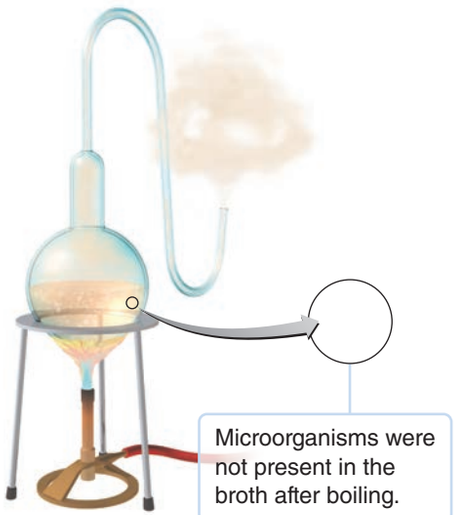
Disproving the Theory of Spontaneous Generation

According to the theory of spontaneous generation, life can arise spontaneously from nonliving matter, such as dead corpses and soil. Pasteur's experiment, described below, demonstrated that microbes are present in nonliving matter—air, liquids, and solids.

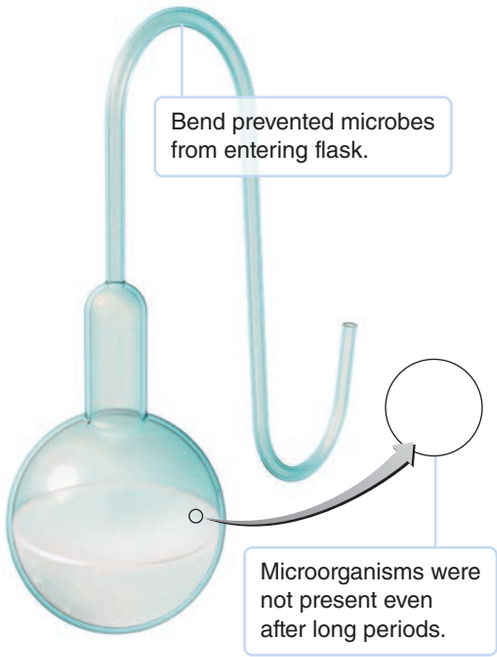
1 Pasteur first poured beef broth into a long-necked flask.



2 Next he heated the neck of the flask and bent it into an S-shape; then he boiled the broth for several minutes.



3 Microorganisms did not appear in the cooled solution, even after long periods.



Some of these original vessels are still on display at the Pasteur Institute in Paris. They have been sealed but show no sign of contamination more than 100 years later.

KEY CONCEPTS

- Pasteur demonstrated that microbes are responsible for food spoilage, leading researchers to the connection between microbes and disease.
- His experiments and observations provided the basis of aseptic techniques, which are used to prevent microbial contamination, as shown in the photo at right.

productive period, microbiologists studied the chemical activities of microorganisms, improved the techniques for performing microscopy and culturing microorganisms, and developed vaccines and surgical techniques. Some of the major events that occurred during the Golden Age of Microbiology are listed in Figure 1.4.

Fermentation and Pasteurization

One of the key steps that established the relationship between microorganisms and disease occurred when a group of French merchants asked Pasteur to find out why wine and beer soured. They hoped to develop a method that would prevent spoilage when those beverages were shipped long distances. At the time, many scientists believed that air converted the sugars in these fluids into alcohol. Pasteur found instead that microorganisms called yeasts convert the sugars to alcohol in the absence of air. This process, called **fermentation** (see Chapter 5, page 127), is used to make wine and beer. Souring and spoilage are caused by different microorganisms, called bacteria. In the presence of air, bacteria change the alcohol into vinegar (acetic acid).

Pasteur's solution to the spoilage problem was to heat the beer and wine just enough to kill most of the bacteria that caused the spoilage. The process, called **pasteurization,** is now commonly used to reduce spoilage and kill potentially harmful bacteria in milk as well as in some alcoholic drinks.

The Germ Theory of Disease

Before the time of Pasteur, effective treatments for many diseases were discovered by trial and error, but the causes of the diseases were unknown. The realization that yeasts play a crucial role in fermentation was the first link between the activity of a microorganism and

Golden Age of MICROBIOLOGY

1857	**Pasteur**—Fermentation
1861	**Pasteur**—Disproved spontaneous generation
1864	**Pasteur**—Pasteurization
1867	**Lister**—Aseptic surgery
1876	**Koch***—Germ theory of disease
1879	**Neisser**—*Neisseria gonorrhoeae*
1881	**Koch***—Pure cultures
	Finlay—Yellow fever
1882	**Koch***—*Mycobacterium tuberculosis*
	Hess—Agar (solid) media
1883	**Koch***—*Vibrio cholerae*
1884	**Metchnikoff***—Phagocytosis
	Gram—Gram-staining procedure
	Escherich—*Escherichia coli*
1887	**Petri**—Petri dish
1889	**Kitasato**—*Clostridium tetani*
1890	**von Bering***—Diphtheria antitoxin
	Ehrlich*—Theory of immunity
1892	**Winogradsky**—Sulfur cycle
1898	**Shiga**—*Shigella dysenteriae*
1908	**Ehrlich***—Syphilis treatment
1910	**Chagas**—*Trypanosoma cruzi*
1911	**Rous***—Tumor-causing virus (1966 Nobel Prize)

Louis Pasteur (1822–1895)
Demonstrated that life did not arise spontaneously from nonliving matter.

Joseph Lister (1827–1912)
Performed surgery under aseptic conditions using phenol. Proved that microbes caused surgical wound infections.

Robert Koch (1843–1910)
Established experimental steps for directly linking a specific microbe to a specific disease.

Figure 1.4 Milestones in the Golden Age of Microbiology. An asterisk (*) indicates a Nobel laureate.

Q Why do you think the Golden Age of Microbiology occurred when it did?

physical and chemical changes in organic materials. This discovery alerted scientists to the possibility that microorganisms might have similar relationships with plants and animals—specifically, that microorganisms might cause disease. This idea was known as the **germ theory of disease.**

The germ theory met great resistance at first because for centuries disease was believed to be punishment for an individual's crimes or misdeeds. When the inhabitants of an entire village became ill, people often blamed the disease on demons appearing as foul odors from sewage or on poisonous vapors from swamps. Most people born in Pasteur's time found it inconceivable that "invisible" microbes could travel through the air to infect plants and animals or remain on clothing and bedding to be transmitted from one person to another. Despite these doubts, scientists gradually accumulated the information needed to support the new germ theory.

In 1865, Pasteur was called upon to help fight silkworm disease, which was ruining the silk industry in Europe. Decades earlier amateur microscopist Agostino Bassi had proved that another silkworm disease was caused by a fungus. Using data provided by Bassi, Pasteur found that the more recent infection was caused by a protozoan, and he developed a method for recognizing afflicted silkworm moths.

In the 1860s, Joseph Lister, an English surgeon, applied the germ theory to medical procedures. Lister was aware that in the 1840s, the Hungarian physician Ignaz Semmelweis had demonstrated that physicians, who at the time did not disinfect their hands, routinely transmitted infections (puerperal, or childbirth, fever) from one obstetrical patient to another. Lister had also heard of Pasteur's work connecting microbes to animal diseases. Disinfectants were not used at the time, but Lister knew that phenol (carbolic acid) kills bacteria, so he began treating surgical wounds with a phenol solution. The practice so reduced the incidence of infections and deaths that other surgeons quickly adopted it. His findings proved that microorganisms cause surgical wound infections.

The first proof that bacteria actually cause disease came from Robert Koch (kōk) in 1876. Koch, a German physician, was Pasteur's rival in the race to discover the cause of anthrax, a disease that was destroying cattle and sheep in Europe. Koch discovered

rod-shaped bacteria now known as *Bacillus anthracis* (bah-SIL-lus an-THRĀ-sis) in the blood of cattle that had died of anthrax. He cultured the bacteria on nutrients and then injected samples of the culture into healthy animals. When these animals became sick and died, Koch isolated the bacteria in their blood and compared them with the originally isolated bacteria. He found that the two sets of blood cultures contained the same bacteria.

Koch thus established **Koch's postulates,** a sequence of experimental steps for directly relating a specific microbe to a specific disease (see Figure 14.3, page 395). During the past 100 years, these same criteria have been invaluable in investigations proving that specific microorganisms cause many diseases. Koch's postulates, their limitations, and their application to disease will be discussed in greater detail in Chapter 14.

Vaccination

Often a treatment or preventive procedure is developed before scientists know why it works. The smallpox vaccine is an example. Almost 70 years before Koch established that a specific microorganism causes anthrax, Edward Jenner, a young British physician, embarked on an experiment to find a way to protect people from smallpox. The disease periodically swept through Europe, killing thousands, and it wiped out 90% of the Native Americans on the East Coast when European settlers first brought the infection to the New World.

When a young milkmaid informed Jenner that she couldn't get smallpox because she already had been sick from cowpox—a much milder disease—he decided to put the girl's story to the test. First Jenner collected scrapings from cowpox blisters. Then he inoculated a healthy 8-year-old volunteer with the cowpox material by scratching the person's arm with a pox-contaminated needle. The scratch turned into a raised bump. In a few days, the volunteer became mildly sick but recovered and never again contracted either cowpox or smallpox. The protection from disease provided by vaccination (or by recovery from the disease itself) is called **immunity.** We will discuss the mechanisms of immunity in Chapter 17.

Years after Jenner's experiment, Pasteur discovered why vaccinations work. He found that the bacterium that causes fowl cholera lost its ability to cause disease (lost its *virulence,* or became *avirulent*) after it was grown in the laboratory for long periods. However, it—and other microorganisms with decreased virulence—was able to induce immunity against subsequent infections by its virulent counterparts. The discovery of this phenomenon provided a clue to Jenner's successful experiment with cowpox. Both cowpox and smallpox are caused by viruses. Even though cowpox virus is not a laboratory-produced derivative of smallpox virus, it is so closely related to the smallpox virus that it can induce immunity to both viruses. Pasteur used the term *vaccine* for cultures of avirulent microorganisms used for preventive inoculation. (The Latin word *vacca* means cow—thus, the term *vaccine* honored Jenner's earlier cowpox inoculation work.)

Jenner's experiment was actually not the first time a living viral agent—in this case, the cowpox virus—was used to produce immunity. Starting in the 1500s, physicians in China had immunized patients from smallpox by removing scales from drying pustules of a person suffering from a mild case of smallpox, grinding the scales to a fine powder, and inserting the powder into the nose of the person to be protected.

Some vaccines are still produced from avirulent microbial strains that stimulate immunity to the related virulent strain. Other vaccines are made from killed virulent microbes, from isolated components of virulent microorganisms, or by genetic engineering techniques.

CHECK YOUR UNDERSTANDING

- Summarize in your own words the germ theory of disease. **1-8**
- What is the importance of Koch's postulates? **1-9**
- What is the significance of Jenner's discovery? **1-10**

The Birth of Modern Chemotherapy: Dreams of a "Magic Bullet"

After the relationship between microorganisms and disease was established, medical microbiologists next focused on the search for substances that could destroy pathogenic microorganisms without damaging the infected animal or human. Treatment of disease by using chemical substances is called **chemotherapy.** (The term also commonly refers to chemical treatment of noninfectious diseases, such as cancer.) Chemicals produced naturally by bacteria and fungi to act against other microorganisms are called **antibiotics.** Chemotherapeutic agents prepared from chemicals in the laboratory are called **synthetic drugs.** The success of chemotherapy is based on the fact that some chemicals are more poisonous to microorganisms than to the hosts infected by the microbes. Antimicrobial therapy will be discussed in further detail in Chapter 20.

The First Synthetic Drugs

Paul Ehrlich was the imaginative thinker who fired the first shot in the chemotherapy revolution. As a medical student, Ehrlich speculated about a "magic bullet" that could hunt down and destroy a pathogen without harming the infected host. In 1910, after testing hundreds of substances, he found a chemotherapeutic agent called *salvarsan,* an arsenic derivative effective against syphilis. The agent was named *salvarsan* because it was considered to offer salvation from syphilis and it contained arsenic. Before this discovery, the only known chemical in Europe's medical arsenal was an extract from the bark of a South American tree, *quinine,* which had been used by Spanish conquistadors to treat malaria.

By the late 1930s, researchers had developed several other synthetic drugs that could destroy microorganisms. Most of these drugs were derivatives of dyes. This came about because the dyes synthesized and manufactured for fabrics were rou-

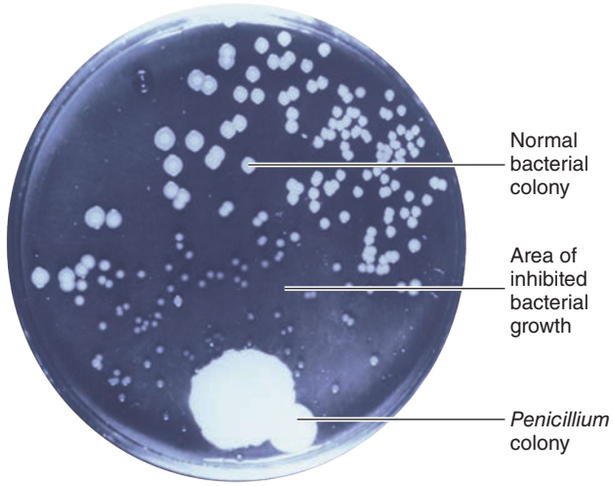


Figure 1.5 The discovery of penicillin. Alexander Fleming took this photograph in 1928. The colony of *Penicillium* mold accidentally contaminated the plate and inhibited nearby bacterial growth.

Q Why do you think penicillin is no longer as effective as it once was?

tinely tested for antimicrobial qualities by microbiologists looking for a "magic bullet." In addition, *sulfonamides* (sulfa drugs) were synthesized at about the same time.

A Fortunate Accident—Antibiotics

The first antibiotic was discovered by accident. Alexander Fleming, a Scottish physician and bacteriologist, almost tossed out some culture plates that had been contaminated by mold. Fortunately, he noticed the curious pattern of growth on the plates—a clear area where bacterial growth had been inhibited encircled the mold (**Figure 1.5**). Fleming was looking at a mold that inhibited growth of a bacterium. The mold became known as *Penicillium chrysogenum* (pen′i-SIL-lē-um krĪ-SO-jen-um), and the mold's active inhibitor was called *penicillin*. Thus, penicillin is an antibiotic produced by a fungus. The enormous usefulness of penicillin was not apparent until the 1940s, when it was finally tested clinically and mass produced.

Since these early discoveries, thousands of other antibiotics have been discovered. Unfortunately, antibiotics and other chemotherapeutic drugs are not without problems. Many antimicrobial chemicals kill pathogenic microbes but also damage the infected host. For reasons we will discuss later, toxicity to humans is a particular problem in the development of drugs for treating viral diseases. Viral growth depends on life processes of normal host cells. Thus, there are very few successful antiviral drugs, because a drug that would interfere with viral reproduction would also likely affect uninfected cells of the body.

Over the years, more and more microbes also developed resistance to antibiotics that were once very effective against them. Drug resistance results from genetic changes in microbes that enables them to tolerate a certain amount of an antibiotic that would normally inhibit them (see the box in Chapter 26, page 756). For example, a microbe might produce enzymes that inactivate antibiotics, or a microbe might undergo changes to its surface that prevent an antibiotic from attaching to it or entering it.

The recent appearance of vancomycin-resistant *Staphylococcus aureus* and *Enterococcus faecalis* (en′ter-ō-KOK-kus fē-KĀ-lis) has alarmed health care professionals because it indicates that some previously treatable bacterial infections may soon be impossible to treat with antibiotics.

CHECK YOUR UNDERSTANDING

✓ What was Ehrlich's "magic bullet"? **1-11**

Modern Developments in Microbiology

The quest to solve drug resistance, identify viruses, and develop vaccines requires sophisticated research techniques and correlated studies that were never dreamed of in the days of Koch and Pasteur.

The groundwork laid during the Golden Age of Microbiology provided the basis for several monumental achievements in the years following (**Table 1.2**). New branches of microbiology were developed, including immunology and virology. Most recently, the development of a set of new methods called *recombinant DNA technology* has revolutionized research and practical applications in all areas of microbiology.

TABLE 1.2 Nobel Prizes Awarded for Discoveries in Microbiology

Decade	Discoveries
1950s	Streptomycin Chemical steps of the Krebs cycle Poliovirus cultured in cell cultures Genetic control of biochemical reactions
1960s	Acquired immune tolerance
1980s	Technique for producing monoclonal (single pure) antibodies Genetics of antibody production Cancer-causing genes (oncogenes)
1990s	First successful transplants using immunosuppressive drugs Enzymes that regulate plant growth (protein kinases) Separation of genes onto different segments of DNA Polymerase chain reactions that amplify (make multiple copies of) DNA T cytotoxic cell recognition of virus-infected cells prior to their destruction
2000s	Water and ion channels in plasma membranes *Helicobacter pylori* as the cause of peptic ulcers Mechanism of RNA interference (RNAi), or gene silencing by RNA
2010s	Detailed structure and function of ribosomes

(a) A parasitic guinea worm (*Dracunculus medinensis*) is removed from the subcutaneous tissue of a patient by winding it onto a stick. This procedure may have been used for the design of the symbol in part (b).

(b) Rod of Asclepius, symbol of the medical profession.

Figure 1.6 Parasitology: the study of protozoa and parasitic worms.

Q How do you think parasitic worms survive and live off a human host?

Bacteriology, Mycology, and Parasitology

Bacteriology, the study of bacteria, began with van Leeuwenhoek's first examination of tooth scrapings. New pathogenic bacteria are still discovered regularly. Many bacteriologists, like Pasteur, look at the roles of bacteria in food and the environment. One intriguing discovery came in 1997, when Heide Schulz discovered a bacterium large enough to be seen with the unaided eye (0.2 mm wide). This bacterium, named *Thiomargarita namibiensis* (THĪ-ō-mar-gar′ē-tah nah′mib-ē-EN-sis), lives in the mud on the African coast. *Thiomargarita* is unusual because of its size and its ecological niche. The bacterium consumes hydrogen sulfide, which would be toxic to mud-dwelling animals (Figure 11.28, page 315).

Mycology, the study of fungi, includes medical, agricultural, and ecological branches. Fungal infection rates have been rising during the past decade, accounting for 10% of hospital-acquired infections. Climatic and environmental changes (severe drought) are thought to account for the tenfold increase in *Coccidioides immitis* (KOK-sid-ē-oi-dēz IM-mi-tis) infections in California. New techniques for diagnosing and treating fungal infections are currently being investigated.

Parasitology is the study of protozoa and parasitic worms. Because many parasitic worms are large enough to be seen with the unaided eye, they have been known for thousands of years. It has been speculated that the medical symbol, the rod of Asclepius, represents the removal of parasitic guinea worms (Figure 1.6). Asclepius was a Greek physician who practiced about 1200 B.C. and was deified as the god of medicine.

The clearing of rain forests has exposed laborers to previously undiscovered parasites. Parasitic diseases unknown until recently are also being found in patients whose immune systems have been suppressed by organ transplants, cancer chemotherapy, or AIDS.

Bacteriology, mycology, and parasitology are currently going through a "golden age" of classification. Recent advances in **genomics,** the study of all of an organism's genes, have allowed scientists to classify bacteria and fungi according to their genetic relationships with other bacteria, fungi, and protozoa. These microorganisms were originally classified according to a limited number of visible characteristics.

Immunology

Immunology is the study of immunity. Knowledge about the immune system has accumulated steadily and expanded rapidly. Vaccines are now available for numerous diseases, including measles, rubella (German measles), mumps, chickenpox, pneumococcal pneumonia, tetanus, tuberculosis, influenza, whooping cough, polio, and hepatitis B. The smallpox vaccine was so effective that the disease has been eliminated. Public health officials estimate that polio will be eradicated within a few years because of the polio vaccine.

A major advance in immunology occurred in 1933, when Rebecca Lancefield (Figure 1.7) proposed that streptococci be classified according to serotypes (variants within a species) based on certain components in the cell walls of the bacteria. Streptococci are responsible for a variety of diseases, such as sore throat (strep throat), streptococcal toxic shock, and septicemia (blood poisoning).

In 1960, interferons, substances generated by the body's own immune system, were discovered. Interferons inhibit replication of viruses and have triggered considerable research related to

Figure 1.7 Rebecca Lancefield (1895–1981), who discovered differences in the chemical composition of a polysaccharide in the cell walls of many pathogenic streptococci. Rapid laboratory tests using immunologic techniques now identify and classify streptococci into Lancefield groups based on this carbohydrate.

Q Why is it important to identify streptococci quickly?

the treatment of viral diseases and cancer. One of today's biggest challenges for immunologists is learning how the immune system might be stimulated to ward off the virus responsible for AIDS, a disease that destroys the immune system.

Virology

The study of viruses, **virology,** originated during the Golden Age of Microbiology. In 1892, Dmitri Iwanowski reported that the organism that caused mosaic disease of tobacco was so small that it passed through filters fine enough to stop all known bacteria. At the time, Iwanowski was not aware that the organism in question was a virus. In 1935, Wendell Stanley demonstrated that the organism, called tobacco mosaic virus (TMV), was fundamentally different from other microbes and so simple and homogeneous that it could be crystallized like a chemical compound. Stanley's work facilitated the study of viral structure and chemistry. Since the development of the electron microscope in the 1940s, microbiologists have been able to observe the structure of viruses in detail, and today much is known about their structure and activity.

Recombinant DNA Technology

Microorganisms can now be genetically modified to manufacture large amounts of human hormones and other urgently needed medical substances. In the late 1960s, Paul Berg showed that fragments of human or animal DNA (genes) that code for important proteins can be attached to bacterial DNA. The resulting hybrid was the first example of **recombinant DNA. Recombinant DNA (rDNA) technology** inserts recombinant DNA into bacteria (or other microbes) to make large quantities of a desired protein. This field combines elements from two other areas of study, including **microbial genetics,** which studies the mechanisms by which microorganisms inherit traits, and **molecular biology,** which looks at how genetic information is carried in molecules of DNA and how DNA directs the synthesis of proteins.

Although molecular biology encompasses all organisms, much of our knowledge of how genes determine specific traits has been revealed through experiments with bacteria. Unicellular organisms, primarily bacteria, have several advantages for genetic and biochemical research. Bacteria are less complex than plants and animals, and the life cycles of many bacteria last less than an hour, so scientists can cultivate very large numbers of bacteria for study in a relatively short time.

Once science turned to the study of unicellular life, rapid progress was made in genetics. In the 1940s, George W. Beadle and Edward L. Tatum demonstrated the relationship between genes and enzymes; DNA was established as the hereditary material by Oswald Avery, Colin MacLeod, and Maclyn McCarty; and Joshua Lederberg and Edward L. Tatum discovered that genetic material could be transferred from one bacterium to another by a process called conjugation. Then in the 1950s, James Watson and Francis Crick proposed a model for the structure and replication of DNA. The early 1960s also witnessed a further explosion of discoveries relating to the way DNA controls protein synthesis. François Jacob and Jacques Monod discovered messenger RNA (ribonucleic acid), a chemical involved in protein synthesis, and later they made the first major discoveries about the regulation of gene function in bacteria. During the same period, scientists were able to break the genetic code and thus understand how the information for protein synthesis in messenger RNA is translated into the amino acid sequence for making proteins.

CHECK YOUR UNDERSTANDING

- Define *bacteriology, mycology, parasitology, immunology,* and *virology.* **1-12**
- Differentiate microbial genetics from molecular biology. **1-13**

Microbes and Human Welfare

LEARNING OBJECTIVES

1-14 List at least four beneficial activities of microorganisms.

1-15 Name two examples of biotechnology that use recombinant DNA technology and two examples that do not.

As mentioned earlier, only a minority of all microorganisms are pathogenic. Microbes that cause food spoilage, such as soft spots on fruits and vegetables, decomposition of meats, and rancidity of fats and oils, are also a minority. The vast majority of microbes benefit humans, other animals, and plants in many ways. For example, microbes produce methane and ethanol that can be used as alternative fuels to generate electricity and power vehicles. Biotechnology companies are using bacterial enzymes to break down plant cellulose so that yeast can metabolize the resulting simple sugars and produce ethanol. The following sections outline some of these beneficial activities. In later chapters, we will discuss these activities in greater detail.

ASM: Microbes are essential for life as we know it and the processes that support life.

ASM: Humans utilize and harness microorganisms and their products.

Recycling Vital Elements

Discoveries made by two microbiologists in the 1880s have formed the basis for today's understanding of the biogeochemical cycles that support life on Earth. Martinus Beijerinck and Sergei Winogradsky were the first to show how bacteria help recycle vital elements between the soil and the atmosphere. **Microbial ecology,** the study of the relationship between microorganisms and their environment, originated with the work of these scientists. Today, microbial ecology has branched out and includes the study of how microbial populations interact with plants and animals in various environments. Among

the concerns of microbial ecologists are water pollution and toxic chemicals in the environment.

The chemical elements carbon, nitrogen, oxygen, sulfur, and phosphorus are essential for life and abundant, but not necessarily in forms that organisms can use. Microorganisms are primarily responsible for converting these elements into forms that plants and animals can use. Microorganisms, especially bacteria and fungi, return carbon dioxide to the atmosphere when they decompose organic wastes and dead plants and animals. Algae, cyanobacteria, and higher plants use the carbon dioxide during photosynthesis to produce carbohydrates for animals, fungi, and bacteria. Nitrogen is abundant in the atmosphere but in that form is not usable by plants and animals. Only bacteria can naturally convert atmospheric nitrogen to a form available to plants and animals.

Sewage Treatment: Using Microbes to Recycle Water

Our society's growing awareness of the need to preserve the environment has made people more conscious of the responsibility to recycle precious water and prevent the pollution of rivers and oceans. One major pollutant is sewage, which consists of human excrement, waste water, industrial wastes, and surface runoff. Sewage is about 99.9% water, with a few hundredths of 1% suspended solids. The remainder is a variety of dissolved materials.

Sewage treatment plants remove the undesirable materials and harmful microorganisms. Treatments combine various physical processes with the action of beneficial microbes. Large solids such as paper, wood, glass, gravel, and plastic are removed from sewage; left behind are liquid and organic materials that bacteria convert into such by-products as carbon dioxide, nitrates, phosphates, sulfates, ammonia, hydrogen sulfide, and methane. (We will discuss sewage treatment in detail in Chapter 27.)

Bioremediation: Using Microbes to Clean Up Pollutants

In 1988, scientists began using microbes to clean up pollutants and toxic wastes produced by various industrial processes. For example, some bacteria can actually use pollutants as energy sources; others produce enzymes that break down toxins into less harmful substances. By using bacteria in these ways—a process known as **bioremediation**—toxins can be removed from underground wells, chemical spills, toxic waste sites, and oil spills, such as the massive oil spill from a British Petroleum offshore drilling rig in the Gulf of Mexico in 2010 (see also the Applications of Microbiology box in Chapter 2, page 31). In addition, bacterial enzymes are used in drain cleaners to remove clogs without adding harmful chemicals to the environment. In some cases, microorganisms indigenous to the environment are used; in others, genetically modified microbes are used. Among the most commonly used microbes are certain species of bacteria of the genera *Pseudomonas* and *Bacillus*. *Bacillus* enzymes are also used in household detergents to remove spots from clothing.

Insect Pest Control by Microorganisms

Besides spreading diseases, insects can cause devastating crop damage. Insect pest control is therefore important for both agriculture and the prevention of human disease.

The bacterium *Bacillus thuringiensis* (ther-IN-jē-en-sis) has been used extensively in the United States to control such pests as alfalfa caterpillars, bollworms, corn borers, cabbageworms, tobacco budworms, and fruit tree leaf rollers. It is incorporated into a dusting powder that is applied to the crops these insects eat. The bacteria produce protein crystals that are toxic to the digestive systems of the insects. The toxin gene also has been inserted into some plants to make them insect resistant.

By using microbial rather than chemical insect control, farmers can avoid harming the environment. Many chemical insecticides, such as DDT, remain in the soil as toxic pollutants and are eventually incorporated into the food chain.

Modern Biotechnology and Recombinant DNA Technology

Earlier, we touched on the commercial use of microorganisms to produce some common foods and chemicals. Such practical applications of microbiology are called **biotechnology.** Although biotechnology has been used in some form for centuries, techniques have become much more sophisticated in the past few decades. In the last several years, biotechnology has undergone a revolution through the advent of recombinant DNA technology to expand the potential of bacteria, viruses, and yeast and other fungi as miniature biochemical factories. Cultured plant and animal cells, as well as intact plants and animals, are also used as recombinant cells and organisms.

The applications of recombinant DNA technology are increasing with each passing year. Recombinant DNA techniques have been used thus far to produce a number of natural proteins, vaccines, and enzymes. Such substances have great potential for medical use; some of them are described in Table 9.1 on page 242.

A very exciting and important outcome of recombinant DNA techniques is **gene therapy**—inserting a missing gene or replacing a defective one in human cells. This technique uses a harmless virus to carry the missing or new gene into certain host cells, where the gene is picked up and inserted into the appropriate chromosome. Since 1990, gene therapy has been used to treat patients with adenosine deaminase (ADA) deficiency, a cause of severe combined immunodeficiency disease (SCID), in which cells of the immune system are inactive or missing; Duchenne's muscular dystrophy, a muscle-destroying disease; cystic fibrosis, a disease of the secreting portions of

the respiratory passages, pancreas, salivary glands, and sweat glands; and LDL-receptor deficiency, a condition in which low-density lipoprotein (LDL) receptors are defective and LDL cannot enter cells. The LDL remains in the blood in high concentrations and leads to fatty plaque formation in blood vessels, increasing the risk of atherosclerosis and coronary heart disease. Results of gene therapy are still being evaluated. Other genetic diseases may also be treatable by gene therapy in the future, including hemophilia, an inability of the blood to clot normally; diabetes, elevated blood sugar levels; and sickle cell disease, an abnormal kind of hemoglobin.

Beyond medical applications, recombinant DNA techniques have also been applied to agriculture. For example, genetically altered strains of bacteria have been developed to protect fruit against frost damage, and bacteria are being modified to control insects that damage crops. Recombinant DNA has also been used to improve the appearance, flavor, and shelf life of fruits and vegetables. Potential agricultural uses of recombinant DNA include drought resistance, resistance to insects and microbial diseases, and increased temperature tolerance in crops.

CHECK YOUR UNDERSTANDING

- Name two beneficial uses of bacteria. **1-14**
- Differentiate biotechnology from recombinant DNA technology. **1-15**

Microbes and Human Disease

LEARNING OBJECTIVES

1-16 Define *normal microbiota* and *resistance.*

1-17 Define *biofilm.*

1-18 Define *emerging infectious disease.*

Normal Microbiota

We all live from birth until death in a world filled with microbes, and we all have a variety of microorganisms on and inside our bodies. These microorganisms make up our **normal microbiota,** or *flora** (**Figure 1.8**). The normal microbiota not only do us no harm, but in some cases can actually benefit us. For example, some normal microbiota protect us against disease by preventing the overgrowth of harmful microbes, and others produce useful substances such as vitamin K and some B vitamins. Unfortunately, under some circumstances normal microbiota can make us sick or infect people we contact. For instance, when some normal microbiota leave their habitat, they can cause disease.

When is a microbe a welcome part of a healthy human, and when is it a harbinger of disease? The distinction between

*At one time, bacteria and fungi were thought to be plants, and thus the term *flora* was used.

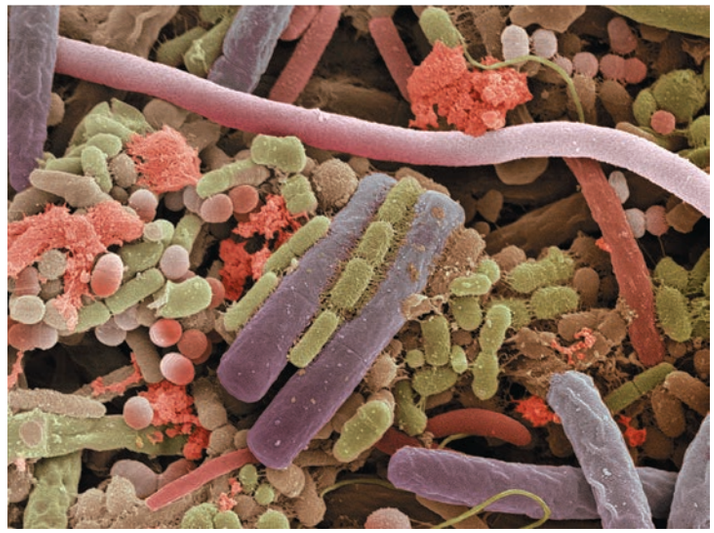


Figure 1.8 Several types of bacteria found as part of the normal microbiota on the surface of the human tongue.

Q How do we benefit from the production of vitamin K by microbes?

health and disease is in large part a balance between the natural defenses of the body and the disease-producing properties of microorganisms. Whether our bodies overcome the offensive tactics of a particular microbe depends on our **resistance**—the ability to ward off diseases. Important resistance is provided by the barrier of the skin, mucous membranes, cilia, stomach acid, and antimicrobial chemicals such as interferons. Microbes can be destroyed by white blood cells, by the inflammatory response, by fever, and by specific responses of our immune system. Sometimes, when our natural defenses are not strong enough to overcome an invader, they have to be supplemented by antibiotics or other drugs.

Clinical Case

Staph is the common name for *Staphylococcus aureus* bacteria, which are carried on the skin of about 30% of the human population. Although Andrea is diligent about taking her antibiotic as prescribed, she doesn't seem to be improving. After 3 days, the lesion on her wrist is even larger than before and is now draining yellow pus. Andrea also develops a fever. Her mother insists that she call her doctor to tell him about the latest developments.

Why does Andrea's infection persist after treatment?

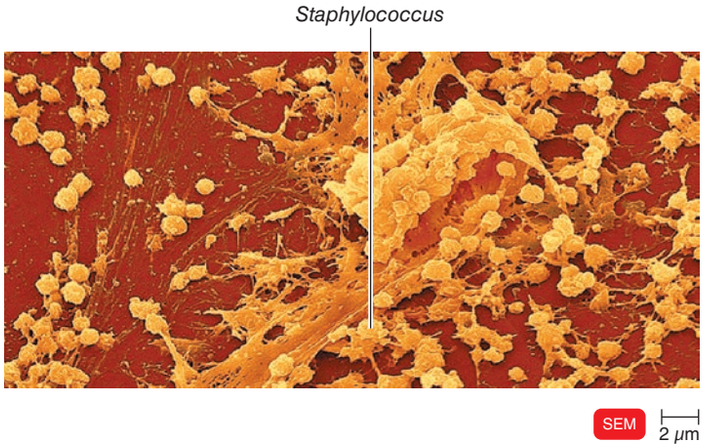


Figure 1.9 Biofilm on a catheter. *Staphylococcus* bacteria stick to solid surfaces, forming a slimy layer. Bacteria that break away from this biofilm can cause infections.

How does a biofilm's protective barrier make it resistant to antibiotics?

Biofilms

In nature, microorganisms may exist as single cells that float or swim independently in a liquid, or they may attach to each other and/or some usually solid surface. This latter mode of behavior is called a **biofilm,** a complex aggregation of microbes. The slime covering a rock in a lake is a biofilm. Use your tongue to feel the biofilm on your teeth. Biofilms can be beneficial. They protect your mucous membranes from harmful microbes, and biofilms in lakes are an important food for aquatic animals. Biofilms can also be harmful. They can clog water pipes, and on medical implants such as joint prostheses and catheters (Figure 1.9), they can cause such infections as endocarditis (inflammation of the heart). Bacteria in biofilms are often resistant to antibiotics because the biofilm offers a protective barrier. See the box in Chapter 3 on page 54. Biofilms will be discussed in Chapter 6.

Infectious Diseases

An **infectious disease** is a disease in which pathogens invade a susceptible host, such as a human or an animal. In the process, the pathogen carries out at least part of its life cycle inside the host, and disease frequently results. By the end of World War II, many people believed that infectious diseases were under control. They thought malaria would be eradicated through the use of the insecticide DDT to kill mosquitoes, that a vaccine would prevent diphtheria, and that improved sanitation measures would help prevent cholera transmission. Malaria is far from eliminated. Since 1986, local outbreaks have been identified in New Jersey, California, Florida, New York, and Texas, and the disease infects 300 million people worldwide. In 1994, diphtheria appeared in the United States, brought by travelers from the newly independent states of the former Soviet Union, which were experiencing a massive diphtheria epidemic. The epidemic was brought under control in 1998. Cholera outbreaks still occur in less-developed parts of the world.

Emerging Infectious Diseases

These recent outbreaks point to the fact that infectious diseases are not disappearing, but rather seem to be reemerging and increasing. In addition, a number of new diseases—**emerging infectious diseases (EIDs)**—have cropped up in recent years. These are diseases that are new or changing and are increasing or have the potential to increase in incidence in the near future. Some of the factors that have contributed to the development of EIDs are evolutionary changes in existing organisms (e.g., *Vibrio cholerae;* VIB-rē-ō KOL-er-ī); the spread of known diseases to new geographic regions or populations by modern transportation (e.g., West Nile virus); and increased human exposure to new, unusual infectious agents in areas that are undergoing ecologic changes such as deforestation and construction (e.g., Venezuelan hemorrhagic virus). EIDs also develop as a result of antimicrobial resistance (e.g., vancomycin-resistant *S. aureus*). An increasing number of incidents in recent years highlights the extent of the problem.

Between April 2012 and June 2014, there were 339 confirmed cases and 100 deaths in humans caused by a new virus called *Middle East respiratory syndrome coronavirus (MERS-CoV).* The virus belongs to the same family that causes illnesses from the common cold to severe acute respiratory syndrome (SARS), to be described shortly. Because all reported cases are linked to the Middle East, this latest emerging infectious disease is called **Middle East respiratory syndrome (MERS).**

Severe acute respiratory syndrome (SARS) is an emerging infectious disease that first appeared in China in 2002. It is a viral infection caused by the *SARS-associated coronavirus* (*SARS-CoV*).

H1N1 influenza (flu), also known as swine flu, is a type of influenza caused by a new virus called *influenza H1N1.* H1N1 was first detected in the United States in 2009, and that same year the World Health Organization declared H1N1 flu to be a pandemic disease (a disease that affects large numbers of individuals in a short period of time and occurs worldwide).

Avian influenza A (H5N1), or **bird flu,** caught the attention of the public in 2003, when it killed millions of poultry and 24 people in southeast Asia. Avian influenza viruses occur in birds worldwide. In 2013, a different avian influenza, H7N9, sickened 131 people in China.

Influenza A viruses are found in many different animals, including ducks, chickens, pigs, whales, horses, and seals. Normally, each subtype of influenza A virus is specific to certain species. However, influenza A viruses normally seen in one species sometimes can cross over and cause illness in another species, and all subtypes of influenza A virus can infect pigs. Although it is unusual for people to get influenza infections directly from animals, sporadic human infections and outbreaks caused by certain avian influenza A viruses and pig influenza viruses have been reported.

As of 2008, avian influenza had sickened 242 people, and about half of them died. Fortunately, the virus has not yet evolved to be transmitted successfully among humans.

Human infections with avian influenza viruses detected since 1997 have not resulted in sustained human-to-human transmission. However, because influenza viruses have the potential to change and gain the ability to spread easily between people, monitoring for human infection and person-to-person transmission is important (see the box in Chapter 13 on page 363).

Antibiotics are critical in treating bacterial infections. However, years of overuse and misuse of these drugs have created environments in which antibiotic-resistant bacteria thrive. Random mutations in bacterial genes can make a bacterium resistant to an antibiotic. In the presence of that antibiotic, this bacterium has an advantage over other, susceptible bacteria and is able to proliferate. Antibiotic-resistant bacteria have become a global health crisis.

Staphylococcus aureus causes a wide range of human infections from pimples and boils to pneumonia, food poisoning, and surgical wound infections, and it is a significant cause of hospital-associated infections. After penicillin's initial success in treating *S. aureus* infection, penicillin-resistant *S. aureus* became a major threat in hospitals in the 1950s, requiring the use of methicillin. In the 1980s, **methicillin-resistant *S. aureus,*** called **MRSA,** emerged and became endemic in many hospitals, leading to increasing use of vancomycin. In the late 1990s, *S. aureus* infections that were less sensitive to vancomycin (**vancomycin-intermediate *S. aureus,*** or **VISA**) were reported. In 2002, the first infection caused by **vancomycin-resistant *S. aureus* (VRSA)** in a patient in the United States was reported.

In 2010, the World Health Organization (WHO) reported that in some parts of the world (such as northwestern Russia) about 28% of all individuals with tuberculosis (TB) had the multidrug-resistant form of the disease (MDR-TB). Multidrug-resistant TB is caused by bacteria that are resistant to at least the antibiotics isoniazid and rifampicin, the most effective drugs against tuberculosis.

The antibacterial substances added to various household cleaning products are similar to antibiotics in many ways. When used correctly, they inhibit bacterial growth. However, wiping every household surface with these antibacterial agents creates an environment in which the resistant bacteria survive. Unfortunately, when you really need to disinfect your homes and hands—for example, when a family member comes home from a hospital and is still vulnerable to infection—you may encounter mainly resistant bacteria.

Routine housecleaning and handwashing are necessary, but standard soaps and detergents (without added antibacterials) are fine for these tasks. In addition, quickly evaporating chemicals, such as chlorine bleach, alcohol, ammonia, and hydrogen peroxide, remove potentially pathogenic bacteria but do not leave residues that encourage the growth of resistant bacteria.

Clinical Case

The *S. aureus* bacterium responsible for Andrea's infection is resistant to the β-lactam antibiotic prescribed by Andrea's doctor. Concerned about what his patient is telling him, Andrea's doctor calls the local hospital to let them know he is sending a patient over. In the emergency department, a nurse swabs Andrea's wound and sends it to the hospital lab for culturing. The culture shows that Andrea's infection is caused by methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA produces β-lactamase, an enzyme that destroys β-lactam antibiotics. The attending physician surgically drains the pus from the sore on Andrea's wrist.

How does antibiotic resistance develop?

3 15 17 18 19

West Nile encephalitis (WNE) is inflammation of the brain caused by West Nile virus (see the Clinical Focus box on page 215). WNE was first diagnosed in the West Nile region of Uganda in 1937. In 1999 the virus made its first North American appearance in humans in New York City. In 2007, West Nile virus infected over 3600 people in 43 states. West Nile virus is now established in nonmigratory birds in 48 states. The virus, which is carried by birds, is transmitted between birds—and to horses and humans—by mosquitoes. West Nile virus may have arrived in the United States in an infected traveler or in migratory birds.

In 1996, countries worldwide were refusing to import beef from the United Kingdom, where hundreds of thousands of cattle born after 1988 had to be killed because of an epidemic of **bovine spongiform encephalopathy** (en-sef-a-LOP-a-thē), also called **BSE** or **mad cow disease.** BSE first came to the attention of microbiologists in 1986 as one of a handful of diseases caused by an infectious protein called a *prion.* Studies suggest that the source of disease was cattle feed prepared from sheep infected with their own version of the disease. Cattle are herbivores (plant eaters), but adding protein to their feed improves their growth and health. **Creutzfeldt-Jakob disease** (KROITS-felt YA-kob), or **CJD,** is a human disease also caused by a prion. The incidence of CJD in the United Kingdom is similar to the incidence in other countries. However, by 2005 the United Kingdom reported 154 human cases of CJD caused by a new variant related to the bovine disease (see Chapter 22).

Escherichia coli is a normal inhabitant of the large intestine of vertebrates, including humans, and its presence is beneficial because it helps produce certain vitamins and breaks down otherwise undigestible foodstuffs (see Chapter 25). However, a strain called ***E. coli* O157:H7** causes bloody diarrhea when it grows in the intestines. This strain was first recognized in 1982

and since then has emerged as a public health problem. It is now one of the leading causes of diarrhea worldwide. In 1996, some 9000 people in Japan became ill, and 7 died, as a result of infection by *E. coli* O157:H7. The recent outbreaks of *E. coli* O157:H7 in the United States, associated with contamination of undercooked meat and unpasteurized beverages, have led public health officials to call for the development of new methods of testing for bacteria in food.

In 2004, emergence of a new epidemic strain of *Clostridium difficile* (klos-TRID-ē-um DIF-fi-sē-il) was reported. The epidemic strain produces more toxins than others and is more resistant to antibiotics. In the United States, *C. difficile* infections kill nearly 14,000 people a year. Nearly all of the *C. difficile* infections occur in health care settings, where the infection is frequently transmitted between patients via health care personnel whose hands are contaminated after contact with infected patients or their surrounding environment.

In 1995, a hospital laboratory technician in Democratic Republic of Congo (DROC) who had fever and bloody diarrhea underwent surgery for a suspected perforated bowel. Afterward he started hemorrhaging, and his blood began clotting in his blood vessels. A few days later, health care workers in the hospital where he was staying developed similar symptoms. One of them was transferred to a hospital in a different city; personnel in the second hospital who cared for this patient also developed symptoms. By the time the epidemic was over, 315 people had contracted **Ebola hemorrhagic fever** (hem-or-RAJ-ik), or **EHF,** and over 75% of them died. The epidemic was controlled when microbiologists instituted training on the use of protective equipment and educational measures in the community. Close personal contact with infectious blood or other body fluids or tissue (see Chapter 23) leads to human-to-human transmission.

Microbiologists first isolated Ebola viruses from humans during earlier outbreaks in DROC in 1976. (The virus is named after the Democratic Republic of the Congo's Ebola River.) In 2014, the World Health organization declared, an Ebola virus outbreak in West Africa. In 1989 and 1996, outbreaks among monkeys imported into the United States from the Philippines were caused by another Ebola virus but were not associated with human disease.

Recorded cases of **Marburg virus,** another hemorrhagic fever virus, are rare. The first cases were laboratory workers in Europe who handled African green monkeys from Uganda. Four outbreaks were identified in Africa between 1975 and 1998, involving 2 to 154 people with 56% mortality. In 2004, an outbreak killed 227 people. African fruit bats are the natural reservoir for the Marburg virus, and microbiologists suspect that bats are the reservoir for EHF.

In 1993, an outbreak of **cryptosporidiosis** (KRIP-tō-spor-i′dē-Ō-sis) transmitted through the public water supply in Milwaukee, Wisconsin, resulted in diarrheal illness in an estimated 403,000 persons. The microorganism responsible for this outbreak was the protozoan *Cryptosporidium* (KRIP-tō-spor-i′dē-um). First reported as a cause of human disease in 1976, it is responsible for up to 30% of the diarrheal illness in developing countries. In the United States, transmission has occurred via drinking water, swimming pools, and contaminated hospital supplies.

AIDS (acquired immunodeficiency syndrome) first came to public attention in 1981 with reports from Los Angeles that a few young homosexual men had died of a previously rare type of pneumonia known as *Pneumocystis* (noo-mō-SIS-tis) pneumonia. These men had experienced a severe weakening of the immune system, which normally fights infectious diseases. Soon these cases were correlated with an unusual number of occurrences of a rare form of cancer, Kaposi's sarcoma, among young homosexual men. Similar increases in such rare diseases were found among hemophiliacs and intravenous drug users.

Researchers quickly discovered that the cause of AIDS was a previously unknown virus (see Figure 1.1e). The virus, now called **human immunodeficiency virus (HIV),** destroys $CD4^+$ T cells, one type of white blood cell important to immune system defenses. Sickness and death result from microorganisms or cancerous cells that might otherwise have been defeated by the body's natural defenses. So far, the disease has been inevitably fatal once symptoms develop.

By studying disease patterns, medical researchers found that HIV could be spread through sexual intercourse, by contaminated needles, from infected mothers to their newborns

Clinical Case

Mutations develop randomly in bacteria; some mutations are lethal, some have no effect, and some may be beneficial. Once these mutations develop, the offspring of the mutated parent cell also carries the same mutation. Because they have an advantage in the presence of the antibiotic, bacteria that are resistant to antibiotics soon outnumber those that are susceptible to antibiotic therapy. The widespread use of antibiotics selectively allows the resistant bacteria to grow, whereas the susceptible bacteria are killed. Eventually, almost the entire population of bacteria is resistant to the antibiotic.

The emergency department physician prescribes a different antibiotic, vancomycin, which will kill the MRSA in Andrea's wrist. She also explains to Andrea what MRSA is and why it's important they find out where Andrea acquired the potentially lethal bacteria.

What can the emergency department physician tell Andrea about MRSA?

3 15 17 18 19

via breast milk, and by blood transfusions—in short, by the transmission from one person to another. Since 1985, blood used for transfusions has been carefully checked for the presence of HIV, and it is now quite unlikely that the virus can be spread by this means.

By the end of 2013, over 1 million people in the United States were living with AIDS. About 50,000 Americans become infected and 18,000 die each year. As of 2011, health officials estimated that 1.8 million Americans have HIV infection. In 2013, the World Health Organization (WHO) estimated that over 35 million people worldwide are living with HIV/AIDS and that 6000 new infections occur every day.

Since 1994, new treatments have extended the life span of people with AIDS. The majority of individuals with AIDS are in the sexually active age group. Because heterosexual partners of AIDS sufferers are at high risk of infection, public health officials are concerned that even more women and minorities will contract AIDS. In 1997, HIV diagnoses began increasing among women and minorities. Among the AIDS cases reported in 2009, 26% were women, and 49% were African American.

In the months and years to come, scientists will continue to apply microbiological techniques to help them learn more about the structure of the deadly HIV, how it is transmitted, how it grows in cells and causes disease, how drugs can be directed against it, and whether an effective vaccine can be developed. Public health officials have also focused on prevention through education.

AIDS poses one of this century's most formidable health threats, but it is not the first serious epidemic of a sexually transmitted infection. Syphilis was also once a fatal epidemic disease. As recently as 1941, syphilis caused an estimated 14,000 deaths per year in the United States. With few drugs available for treatment and no vaccines to prevent it, efforts to control the disease focused mainly on altering sexual behavior and on the use of condoms. The eventual development of drugs to treat syphilis contributed significantly to preventing the spread of the disease. According to the Centers for Disease Control and Prevention (CDC), reported cases of syphilis dropped from a record high of 575,000 in 1943 to an all-time low of 5979 cases in 2004. Since then, however, the number of cases has been increasing.

Just as microbiological techniques helped researchers in the fight against syphilis and smallpox, they will help scientists discover the causes of new emerging infectious diseases in the twenty-first century. Undoubtedly there will be new diseases. Ebola virus and *Influenzavirus* are examples of viruses that may be changing their abilities to infect different host species. Emerging infectious diseases will be discussed further in Chapter 14 on page 405.

Infectious diseases may reemerge because of antibiotic resistance (see the Clinical Focus box in Chapter 26 on page 756) and through the use of microorganisms as weapons. (See the Clinical Focus box in Chapter 23 on page 645.) The breakdown of public health measures for previously controlled infections has resulted in unexpected cases of tuberculosis, whooping cough, and diphtheria (see Chapter 24).

CHECK YOUR UNDERSTANDING

- Differentiate normal microbiota and infectious disease. **1-16**
- Why are biofilms important? **1-17**
- What factors contribute to the emergence of an infectious disease? **1-18**

* * *

The diseases we have mentioned are caused by viruses, bacteria, protozoa, and prions—types of microorganisms. This book introduces you to the enormous variety of microscopic organisms. It shows you how microbiologists use specific techniques and procedures to study the microbes that cause such diseases as AIDS and diarrhea—and diseases that have yet to be discovered. You will also learn how the body responds to microbial infection and how certain drugs combat microbial diseases. Finally, you will learn about the many beneficial roles that microbes play in the world around us.

Clinical Case Resolved

The first MRSA was health care–associated MRSA (HA-MRSA), transmitted between staff and patients in health care settings. In the 1990s, infections by a genetically different strain, community-associated MRSA (CA-MRSA), emerged as a major cause of skin disease in the United States. CA-MRSA enters skin abrasions from environmental surfaces or other people. Andrea has never been hospitalized before now, so they are able to rule out the hospital as the source of infection. Her college courses are all online, so she didn't contract MRSA at the university, either. The local health department sends someone to her family home to swab for the bacteria there.

MRSA is isolated from Andrea's living room sofa, but how did it get there? A representative from the health department, knowing that clusters of CA-MRSA infections have been seen among athletes, suggests swabbing the mats used by the gymnasts at the school Andrea's sister attends. The cultures come back positive for MRSA. Andrea's sister, although not infected, transferred the bacteria from her skin to the sofa, where Andrea laid her arm. (A person can carry MRSA on the skin without becoming infected.) The bacteria entered through a scratch on Andrea's wrist.

Study Outline

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Check out the Study Area of MasteringMicrobiology to explore Interactive Microbiology. Study on-the-go with Dynamic Study Modules. Check your understanding with chapter quizzes and animations—and much more!

Microbes in Our Lives (p. 2)

1. Living things too small to be seen with the unaided eye are called microorganisms.
2. Microorganisms are important in maintaining Earth's ecological balance.
3. Some microorganisms live in humans and other animals and are needed to maintain good health.
4. Some microorganisms are used to produce foods and chemicals.
5. Some microorganisms cause disease.

Naming and Classifying Microorganisms (pp. 2–6)

Nomenclature (p. 3)

1. In a nomenclature system designed by Carolus Linnaeus (1735), each living organism is assigned two names.
2. The two names consist of a genus and a specific epithet, both of which are underlined or italicized.

Types of Microorganisms (pp. 3–5)

3. Bacteria are unicellular organisms. Because they have no nucleus, the cells are described as prokaryotic.
4. Most bacteria have a peptidoglycan cell wall; they divide by binary fission, and they may possess flagella.
5. Bacteria can use a wide range of chemical substances for their nutrition.
6. Archaea consist of prokaryotic cells; they lack peptidoglycan in their cell walls.
7. Archaea include methanogens, extreme halophiles, and extreme thermophiles.
8. Fungi (mushrooms, molds, and yeasts) have eukaryotic cells (cells with a true nucleus). Most fungi are multicellular.
9. Fungi obtain nutrients by absorbing organic material from their environment.
10. Protozoa are unicellular eukaryotes.
11. Protozoa obtain nourishment by absorption or ingestion through specialized structures.
12. Algae are unicellular or multicellular eukaryotes that obtain nourishment by photosynthesis.
13. Algae produce oxygen and carbohydrates that are used by other organisms.
14. Viruses are noncellular entities that are parasites of cells.
15. Viruses consist of a nucleic acid core (DNA or RNA) surrounded by a protein coat. An envelope may surround the coat.
16. The principal groups of multicellular animal parasites are flatworms and roundworms, collectively called helminths.
17. The microscopic stages in the life cycle of helminths are identified by traditional microbiological procedures.

Classification of Microorganisms (pp. 5–6)

18. All organisms are classified into Bacteria, Archaea, and Eukarya. Eukarya include protists, fungi, plants, and animals.

A Brief History of Microbiology (pp. 6–13)

The First Observations (p. 6)

1. Hooke's observations laid the groundwork for development of the cell theory, the concept that all living things are composed of cells.
2. Anton van Leeuwenhoek, using a simple microscope, was the first to observe microorganisms (1673).

The Debate over Spontaneous Generation (pp. 6–7)

3. Until the mid-1880s, many people believed in spontaneous generation, the idea that living organisms could arise from nonliving matter.
4. Francesco Redi demonstrated that maggots appear on decaying meat only when flies are able to lay eggs on the meat (1668).
5. John Needham claimed that microorganisms could arise spontaneously from heated nutrient broth (1745).
6. Lazzaro Spallanzani repeated Needham's experiments and suggested that Needham's results were due to microorganisms in the air entering his broth (1765).
7. Rudolf Virchow introduced the concept of biogenesis: living cells can arise only from preexisting cells (1858).
8. Louis Pasteur demonstrated that microorganisms are in the air everywhere and offered proof of biogenesis (1861).
9. Pasteur's discoveries led to the development of aseptic techniques used in laboratory and medical procedures to prevent contamination by microorganisms.

The Golden Age of Microbiology (pp. 7–10)

10. The science of microbiology advanced rapidly between 1857 and 1914.
11. Pasteur found that yeasts ferment sugars to alcohol and that bacteria can oxidize the alcohol to acetic acid.
12. A heating process called pasteurization is used to kill bacteria in some alcoholic beverages and milk.
13. Agostino Bassi (1835) and Pasteur (1865) showed a causal relationship between microorganisms and disease.
14. Joseph Lister introduced the use of a disinfectant to clean surgical wounds in order to control infections in humans (1860s).
15. Robert Koch proved that microorganisms cause disease. He used a sequence of procedures, now called Koch's postulates (1876), that are used today to prove that a particular microorganism causes a particular disease.
16. In 1798, Edward Jenner demonstrated that inoculation with cowpox material provides humans with immunity to smallpox.

17. About 1880, Pasteur discovered that avirulent bacteria could be used as a vaccine for fowl cholera.
18. Modern vaccines are prepared from living avirulent microorganisms or killed pathogens, from isolated components of pathogens, and by recombinant DNA techniques.

The Birth of Modern Chemotherapy: Dreams of a "Magic Bullet" (pp. 10–11)

19. Chemotherapy is the chemical treatment of a disease.
20. Two types of chemotherapeutic agents are synthetic drugs (chemically prepared in the laboratory) and antibiotics (substances produced naturally by bacteria and fungi that inhibit the growth of bacteria).
21. Paul Ehrlich introduced an arsenic-containing chemical called salvarsan to treat syphilis (1910).
22. Alexander Fleming observed that the *Penicillium* fungus inhibited the growth of a bacterial culture. He named the active ingredient penicillin (1928).
23. Researchers are tackling the problem of drug-resistant microbes.

Modern Developments in Microbiology (pp. 11–13)

24. Bacteriology is the study of bacteria, mycology is the study of fungi, and parasitology is the study of parasitic protozoa and worms.
25. Microbiologists are using genomics, the study of all of an organism's genes, to classify bacteria, fungi, and protozoa.
26. The study of AIDS, analysis of the action of interferons, and the development of new vaccines are among the current research interests in immunology.
27. New techniques in molecular biology and electron microscopy have provided tools for advancing our knowledge of virology.
28. The development of recombinant DNA technology has helped advance all areas of microbiology.

Microbes and Human Welfare (pp. 13–15)

1. Microorganisms degrade dead plants and animals and recycle chemical elements to be used by living plants and animals.
2. Bacteria are used to decompose organic matter in sewage.
3. Bioremediation processes use bacteria to clean up toxic wastes.
4. Bacteria that cause diseases in insects are being used as biological controls of insect pests. Biological controls are specific for the pest and do not harm the environment.
5. Using microbes to make products such as foods and chemicals is called biotechnology.
6. Using recombinant DNA, bacteria can produce important substances such as proteins, vaccines, and enzymes.
7. In gene therapy, viruses are used to carry replacements for defective or missing genes into human cells.
8. Genetically modified bacteria are used in agriculture to protect plants from frost and insects and to improve the shelf life of produce.

Microbes and Human Disease (pp. 15–19)

1. Everyone has microorganisms in and on the body; these make up the normal microbiota, or flora.
2. The disease-producing properties of a species of microbe and the host's resistance are important factors in determining whether a person will contract a disease.
3. Bacterial communities that form slimy layers on surfaces are called biofilms.
4. An infectious disease is one in which pathogens invade a susceptible host.
5. An emerging infectious disease (EID) is a new or changing disease showing an increase in incidence in the recent past or a potential to increase in the near future.

Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. How did the idea of spontaneous generation come about?
2. Briefly state the role microorganisms play in each of the following:
   a. biological control of pests
   b. recycling of elements
   c. normal microbiota
   d. sewage treatment
   e. human insulin production
   f. vaccine production
   g. biofilms
3. Into which field of microbiology would the following scientists best fit?

Researcher Who	Field
_____ **a.** Studies biodegradation of toxic wastes	**1.** Biotechnology
_____ **b.** Studies the causative agent of Ebola hemorrhagic fever	**2.** Immunology
_____ **c.** Studies the production of human proteins by bacteria	**3.** Microbial ecology
_____ **d.** Studies the symptoms of AIDS	**4.** Microbial genetics
_____ **e.** Studies the production of toxin by *E. coli*	**5.** Microbial physiology
_____ **f.** Studies the life cycle of *Cryptosporidium*	**6.** Molecular biology
_____ **g.** Develops gene therapy for a disease	**7.** Mycology
_____ **h.** Studies the fungus *Candida albicans*	**8.** Virology

4. Match the microorganisms in column A to their descriptions in column B.

Column A	Column B
_____ **a.** Archaea	**1.** Not composed of cells
_____ **b.** Algae	**2.** Cell wall made of chitin
_____ **c.** Bacteria	**3.** Cell wall made of peptidoglycan
_____ **d.** Fungi	**4.** Cell wall made of cellulose; photosynthetic
_____ **e.** Helminths	**5.** Unicellular, complex cell structure lacking a cell wall
_____ **f.** Protozoa	**6.** Multicellular animals
_____ **g.** Viruses	**7.** Prokaryote without peptidoglycan cell wall

5. Match the people in column A to their contribution toward the advancement of microbiology, in column B.

Column A	Column B
_____ **a.** Avery, MacLeod, and McCarty	**1.** Developed vaccine against smallpox
_____ **b.** Beadle and Tatum	**2.** Discovered how DNA controls protein synthesis in a cell
_____ **c.** Berg	**3.** Discovered penicillin
_____ **d.** Ehrlich	**4.** Discovered that DNA can be transferred from one bacterium to another
_____ **e.** Fleming	**5.** Disproved spontaneous generation
_____ **f.** Hooke	**6.** First to characterize a virus
_____ **g.** Iwanowski	**7.** First to use disinfectants in surgical procedures
_____ **h.** Jacob and Monod	**8.** First to observe bacteria
_____ **i.** Jenner	**9.** First to observe cells in plant material and name them
_____ **j.** Koch	**10.** Observed that viruses are filterable
_____ **k.** Lancefield	**11.** Proved that DNA is the hereditary material
_____ **l.** Lederberg and Tatum	**12.** Proved that microorganisms can cause disease
_____ **m.** Lister	**13.** Said living cells arise from preexisting living cells
_____ **n.** Pasteur	**14.** Showed that genes code for enzymes
_____ **o.** Stanley	**15.** Spliced animal DNA to bacterial DNA
_____ **p.** van Leeuwenhoek	**16.** Used bacteria to produce acetone
_____ **q.** Virchow	**17.** Used the first synthetic chemotherapeutic agent
_____ **r.** Weizmann	**18.** Proposed a classification system for streptococci based on antigens in their cell walls

6. It is possible to purchase the following microorganisms in a retail store. Provide a reason for buying each.
   a. *Bacillus thuringiensis*
   b. *Saccharomyces*

7. NAME IT What type of microorganism has a peptidoglycan cell wall, has DNA that is not contained in a nucleus, and has flagella?

8. DRAW IT Show where airborne microbes ended up in Pasteur's experiment.

Multiple Choice

1. Which of the following is a scientific name?
   a. *Mycobacterium tuberculosis*
   b. Tubercle bacillus
2. Which of the following is *not* a characteristic of bacteria?
   a. are prokaryotic
   b. have peptidoglycan cell walls
   c. have the same shape
   d. grow by binary fission
   e. have the ability to move
3. Which of the following is the most important element of Koch's germ theory of disease? The animal shows disease symptoms when
   a. the animal has been in contact with a sick animal.
   b. the animal has a lowered resistance.
   c. a microorganism is observed in the animal.
   d. a microorganism is inoculated into the animal.
   e. microorganisms can be cultured from the animal.
4. Recombinant DNA is
   a. DNA in bacteria.
   b. the study of how genes work.
   c. the DNA resulting when genes of two different organisms are mixed.
   d. the use of bacteria in the production of foods.
   e. the production of proteins by genes.
5. Which of the following statements is the best definition of *biogenesis*?
   a. Nonliving matter gives rise to living organisms.
   b. Living cells can only arise from preexisting cells.

c. A vital force is necessary for life.
d. Air is necessary for living organisms.
e. Microorganisms can be generated from nonliving matter.

6. Which of the following is a beneficial activity of microorganisms?
a. Some microorganisms are used as food for humans.
b. Some microorganisms use carbon dioxide.
c. Some microorganisms provide nitrogen for plant growth.
d. Some microorganisms are used in sewage treatment processes.
e. all of the above

7. It has been said that bacteria are essential for the existence of life on Earth. Which of the following is the essential function performed by bacteria?
a. control insect populations
b. directly provide food for humans
c. decompose organic material and recycle elements
d. cause disease
e. produce human hormones such as insulin

8. Which of the following is an example of bioremediation?
a. application of oil-degrading bacteria to an oil spill
b. application of bacteria to a crop to prevent frost damage
c. fixation of gaseous nitrogen into usable nitrogen
d. production by bacteria of a human protein such as interferon
e. all of the above

9. Spallanzani's conclusion about spontaneous generation was challenged because Lavoisier had just shown that oxygen was the vital component of air. Which of the following statements is true?
a. All life requires air.
b. Only disease-causing organisms require air.
c. Some microbes do not require air.
d. Pasteur kept air out of his biogenesis experiments.
e. Lavoisier was mistaken.

10. Which of the following statements about *E. coli* is *false*?
a. *E. coli* was the first disease-causing bacterium identified by Koch.
b. *E. coli* is part of the normal microbiota of humans.
c. *E. coli* is beneficial in human intestines.
d. A disease-causing strain of *E. coli* causes bloody diarrhea.
e. none of the above

Analysis

1. How did the theory of biogenesis lead the way for the germ theory of disease?

2. Even though the germ theory of disease was not demonstrated until 1876, why did Semmelweis (1840) and Lister (1867) argue for the use of aseptic techniques?

3. The genus name of a bacterium is "erwinia," and the specific epithet is "amylovora." Write the scientific name of this organism correctly. Using this name as an example, explain how scientific names are chosen.

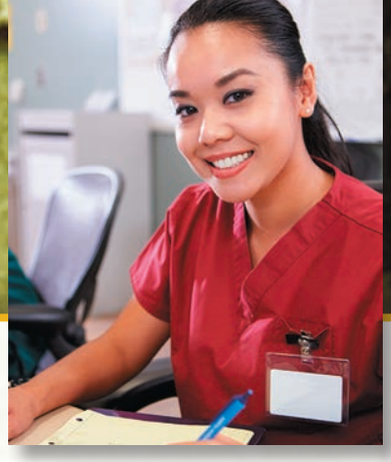
4. Find at least three supermarket products made by microorganisms. (*Hint:* The label will state the scientific name of the organism or include the word *culture, fermented,* or *brewed.*)

5. People once believed all microbial diseases would be controlled by the twenty-first century. Name one emerging infectious disease. List three reasons why we are identifying new diseases now.

Clinical Applications and Evaluation

1. The prevalence of arthritis in the United States is 1 in 100,000 children. However, 1 in 10 children in Lyme, Connecticut, developed arthritis between June and September 1973. Allen Steere, a rheumatologist at Yale University, investigated the cases in Lyme and found that 25% of the patients remembered having a skin rash during their arthritic episode and that the disease was treatable with penicillin. Steere concluded that this was a new infectious disease and did not have an environmental, genetic, or immunologic cause.
a. What was the factor that caused Steere to reach his conclusion?
b. What is the disease?
c. Why was the disease more prevalent between June and September?

2. In 1864, Lister observed that patients recovered completely from simple fractures, but that compound fractures had "disastrous consequences." He knew that the application of phenol (carbolic acid) to fields in the town of Carlisle prevented cattle disease. Lister treated compound fractures with phenol, and his patients recovered without complications. How was Lister influenced by Pasteur's work? Why was Koch's work still needed?



In the Clinic

As the health advisory nurse at a health service company, you receive a call from a man who is concerned that his supermarket does not carry organic sugar, and he says he eats only organic foods. **How would you respond to the man?**

Hint: Read about important biological molecules later in this chapter on pages 31–46.

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

2 Chemical Principles

Like all organisms, microorganisms use nutrients to make chemical building blocks for growth and other functions essential to life. For most microorganisms, synthesizing these building blocks requires them to break down nutrient substances and use the energy released to assemble the resulting molecular fragments into new substances.

We see evidence of these microbial chemical reactions in the world routinely, from a fallen tree rotting on the forest floor to milk going sour in the refrigerator. Although most people give little thought to the causes of such things, the chemistry of microbes is one of the most important concerns of microbiologists. Knowledge of chemistry is essential to understanding what roles microorganisms play in nature, how they cause disease, how methods for diagnosing disease are developed, how the body's defenses combat infection, and how antibiotics and vaccines are produced to combat the harmful effects of microbes.

The *Bacillus anthracis* (bah-SIL-lus an-THRĀ-sis) bacteria in the photograph make a capsule that is not readily digested by animal cells. As discussed in the Clinical Case, these bacteria can grow in mammals by avoiding host defenses. Researchers are investigating ways to identify unique chemicals made by *B. anthracis* and other potential biological weapons in order to detect bioterrorism. To understand the changes that occur in microorganisms and the changes microbes make in the world around us, we need to know how molecules are formed and how they interact.

Bacillus anthracis **bacteria (shown in red).**

The Structure of Atoms

LEARNING OBJECTIVE

2-1 Describe the structure of an atom and its relation to the physical properties of elements.

All matter—whether air, rock, or a living organism—is made up of small units called atoms. An **atom** is the smallest component of a substance, and it cannot be subdivided into smaller substances without losing its properties. Atoms combine to form **molecules.** Living cells are made up of molecules, some of which are very complex. The science of the interaction between atoms and molecules is called **chemistry.**

Atoms are the smallest units of matter that enter into chemical reactions. Every atom has a centrally located **nucleus** and negatively (−) charged particles called **electrons** that move around the nucleus in regions called *electron shells* (**Figure 2.1**). The nucleus is made up of positively (+) charged particles called **protons** and uncharged (neutral) particles called **neutrons.** The nucleus, therefore, bears a net positive charge. All atoms contain an equal number of electrons and protons. Because the total positive charge of the nucleus equals the total negative charge of the electrons, each atom is electrically neutral.

The nuclei of most atoms are stable—that is, they do not change spontaneously—and nuclei do not participate in chemical reactions. The number of protons in an atomic nucleus ranges from one (in a hydrogen atom) to more than 100 (in the largest atoms known). Atoms are often listed by their **atomic number,** the number of protons in the nucleus. Protons and neutrons are approximately the same weight, which is about 1840 times that of an electron, and the total number of protons and neutrons in an atom is its approximate **atomic weight.**

Chemical Elements

All atoms with the same number of protons behave the same way chemically and are classified as the same **chemical element**. Each element has its own name and a one- or two-letter symbol, usually derived from the English or Latin name for the element. For example, the symbol for the element hydrogen is H, and the symbol for carbon is C. The symbol for sodium is Na—the first two letters of its Latin name, *natrium*—to distinguish it from nitrogen, N, and from sulfur, S. There are 92 naturally occurring elements. However, only about 26 elements are commonly found in living things. **Table 2.1** lists some of the chemical elements found in living organisms.

Most elements have several **isotopes**—atoms with different numbers of neutrons in their nuclei. All isotopes of an element have the same number of protons in their nuclei, but their atomic weights differ because of the difference in the number of neutrons. For example, in a natural sample of oxygen, all the atoms contain eight protons. However, 99.76% of the atoms have eight neutrons, 0.04% contain nine neutrons, and the remaining 0.2% contain ten neutrons. Therefore, the three isotopes composing a natural sample of oxygen have atomic weights of 16, 17, and 18, although all will have the atomic number 8. Atomic numbers are written as a subscript to the left of an element's chemical symbol. Atomic weights are written as a superscript above the atomic number. Thus, natural oxygen isotopes are represented as ${}^{16}_{8}O$, ${}^{17}_{8}O$, and ${}^{18}_{8}O$. Isotopes of certain elements are extremely useful in biological research, medical diagnosis, the treatment of some disorders, and some forms of sterilization.

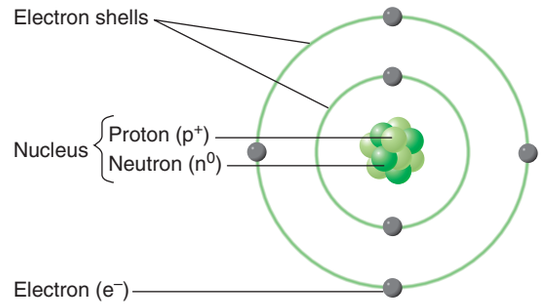


Figure 2.1 The structure of an atom. In this simplified diagram of a carbon atom, note the central location of the nucleus. The nucleus contains six neutrons and six protons, although not all the neutrons are visible in this view. The six electrons move about the nucleus in regions called electron shells, shown here as circles.

Q What is the atomic number of this atom?

Clinical Case: Drumming Up Dust

Jonathan, a 52-year-old drummer, is doing his best to ignore the cold sweat that is breaking out all over his body. He and his bandmates are performing in a local Philadelphia nightclub, and they are just about finished with the second set of the evening. Jonathan hasn't been feeling well for a while, actually; he has been feeling weak and short of breath for the last 3 days or so. Jonathan makes it to the end of the song, but the noise from the clapping and cheering audience seems to come from far away. He stands up to bow and collapses. Jonathan is admitted to a local emergency department with a mild fever and severe shaking. He is able to tell the admitting nurse that he also has had a dry cough for the last few days. The attending physician orders a chest X-ray exam and sputum culture. Jonathan is diagnosed with bilateral pneumonia caused by *Bacillus anthracis*. The attending physician is astonished by this diagnosis.

How did Jonathan become infected by *B. anthracis*? Read on to find out.

25 40 42 46

Table 2.1 The Elements of Life*

Element	Symbol	Atomic Number	Approximate Atomic Weight
Hydrogen	H	1	1
Carbon	C	6	12
Nitrogen	N	7	14
Oxygen	O	8	16
Sodium	Na	11	23
Magnesium	Mg	12	24
Phosphorus	P	15	31
Sulfur	S	16	32
Chlorine	Cl	17	35
Potassium	K	19	39
Calcium	Ca	20	40
Iron	Fe	26	56
Iodine	I	53	127

*Hydrogen, carbon, nitrogen, and oxygen are the most abundant chemical elements in living organisms.

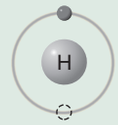
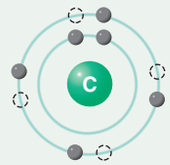
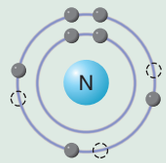
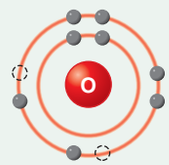
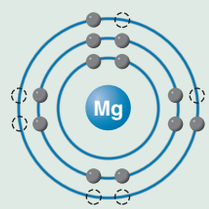
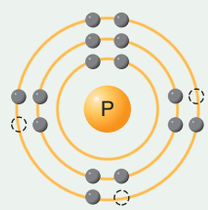
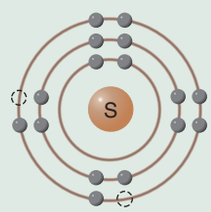
Electronic Configurations

In an atom, electrons are arranged in **electron shells,** which are regions corresponding to different **energy levels.** The arrangement is called an **electronic configuration.** Shells are layered outward from the nucleus, and each shell can hold a characteristic maximum number of electrons—two electrons in the innermost shell (lowest energy level), eight electrons in the second shell, and eight electrons in the third shell, if it is the atom's outermost (valence) shell. The fourth, fifth, and sixth electron shells can each accommodate 18 electrons, although there are some exceptions to this generalization. Table 2.2 shows the electronic configurations for atoms of some elements found in living organisms.

The outermost shell tends to be filled with the maximum number of electrons. An atom can give up, accept, or share electrons with other atoms to fill the outermost shell. The chemical properties of atoms are largely a function of the number of electrons in the outermost electron shell. When its outer shell is filled, the atom is chemically stable, or inert: it does not tend to react with other atoms. Helium (atomic number 2) and neon (atomic number 10) are examples of atoms of inert gases whose outer shells are filled.

When an atom's outer electron shell is only partially filled, the atom is chemically unstable. These unstable atoms react with other atoms, depending, in part, on the degree to which the outer energy levels are filled. Notice the number of electrons in the outer energy levels of the atoms in Table 2.2. We will see later how the number correlates with the chemical reactivity of the elements.

Table 2.2 Electronic Configurations for the Atoms of Some Elements Found in Living Organisms

Element	Diagram	Number of Valence (Outermost) Shell Electrons	Number of Unfilled Spaces	Maximum Number of Bonds Formed
Hydrogen		1	1	1
Carbon		4	4	4
Nitrogen		5	3	5
Oxygen		6	2	2
Magnesium		2	6	2
Phosphorus		5	3	5
Sulfur		6	2	6

CHECK YOUR UNDERSTANDING

How does $^{14}_{6}C$ differ from $^{12}_{6}C$? What is the atomic number of each carbon atom? The atomic weight? 2-1

How Atoms Form Molecules: Chemical Bonds

LEARNING OBJECTIVE

2-2 Define *ionic bond, covalent bond, hydrogen bond, molecular weight,* and *mole.*

When the outermost energy level of an atom is not completely filled by electrons, you can think of it as having either unfilled spaces or extra electrons in that energy level, depending on whether it is easier for the atom to gain or lose electrons. For example, an atom of oxygen, with two electrons in the first energy level and six in the second, has two unfilled spaces in the second electron shell; an atom of magnesium has two extra electrons in its outermost shell. The most chemically stable configuration for any atom is to have its outermost shell filled. Therefore, for these two atoms to attain that state, oxygen must gain two electrons, and magnesium must lose two electrons. Because all atoms tend to combine so that the extra electrons in the outermost shell of one atom fill the spaces of the outermost shell of the other atom, oxygen and magnesium combine so that the outermost shell of each atom has the full complement of eight electrons.

The **valence,** or combining capacity, of an atom is the number of extra or missing electrons in its outermost electron shell. For example, hydrogen has a valence of 1 (one unfilled space, or one extra electron), oxygen has a valence of 2 (two unfilled spaces), carbon has a valence of 4 (four unfilled spaces, or four extra electrons), and magnesium has a valence of 2 (two extra electrons).

Basically, atoms achieve the full complement of electrons in their outermost energy shells by combining to form molecules, which are made up of atoms of one or more elements. A molecule that contains at least two different kinds of atoms, such as H_2O (the water molecule), is called a **compound.** In H_2O, the subscript 2 indicates that there are two atoms of hydrogen; the absence of a subscript indicates that there is only one atom of oxygen. Molecules hold together because the valence electrons of the combining atoms form attractive forces, called **chemical bonds,** between the atomic nuclei. Therefore, valence may also be viewed as the bonding capacity of an element. Because energy is required for chemical bond formation, each chemical bond possesses a certain amount of potential chemical energy.

In general, atoms form bonds in one of two ways: by either gaining or losing electrons from their outer electron shell, or by sharing outer electrons. When atoms have gained or lost outer electrons, the chemical bond is called an *ionic bond*. When outer electrons are shared, the bond is called a *covalent bond*. Although we will discuss ionic and covalent bonds separately, the kinds of bonds actually found in molecules do not belong entirely to either category. Instead, bonds range from the highly ionic to the highly covalent.

Ionic Bonds

Atoms are electrically neutral when the number of positive charges (protons) equals the number of negative charges (electrons). But when an isolated atom gains or loses electrons, this balance is upset. If the atom gains electrons, it acquires an overall negative charge; if the atom loses electrons, it acquires an overall positive charge. Such a negatively or positively charged atom (or group of atoms) is called an **ion.**

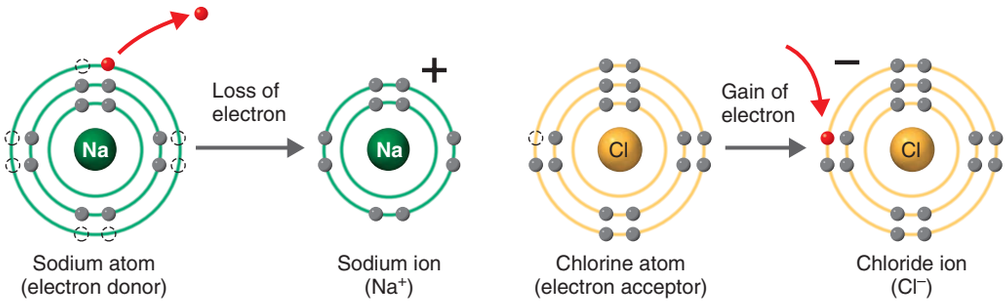
Consider the following examples. Sodium (Na) has 11 protons and 11 electrons, with one electron in its outer electron shell. Sodium tends to lose the single outer electron; it is an *electron donor* (**Figure 2.2a**). When sodium donates an electron to another atom, it is left with 11 protons and only 10 electrons and so has an overall charge of +1. This positively charged sodium atom is called a sodium ion and is written as Na^+. Chlorine (Cl) has a total of 17 electrons, seven of them in the outer electron shell. Because this outer shell can hold eight electrons, chlorine tends to pick up an electron that has been lost by another atom; it is an *electron acceptor* (see Figure 2.2a). By accepting an electron, chlorine totals 18 electrons. However, it still has only 17 protons in its nucleus. The chloride ion therefore has a charge of −1 and is written as Cl^-.

The opposite charges of the sodium ion (Na^+) and chloride ion (Cl^-) attract each other. The attraction, an ionic bond, holds the two atoms together, and a molecule is formed (Figure 2.2b). The formation of this molecule, called sodium chloride (NaCl) or table salt, is a common example of ionic bonding. Thus, an **ionic bond** is an attraction between ions of opposite charge that holds them together to form a stable molecule. Put another way, an ionic bond is an attraction between atoms in which one atom loses electrons and another atom gains electrons. Strong ionic bonds, such as those that hold Na^+ and Cl^- together in salt crystals, have limited importance in living cells. But the weaker ionic bonds formed in aqueous (water) solutions are important in biochemical reactions in microbes and other organisms. For example, weaker ionic bonds assume a role in certain antigen–antibody reactions—that is, reactions in which molecules produced by the immune system (antibodies) combine with foreign substances (antigens) to combat infection.

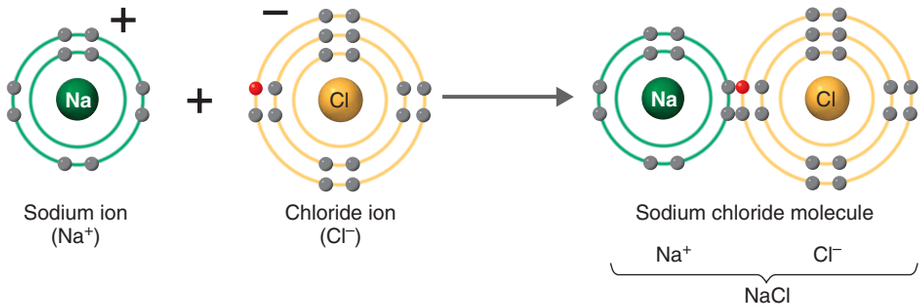
In general, an atom whose outer electron shell is less than half-filled will lose electrons and form positively charged ions, called **cations.** Examples of cations are the potassium ion (K^+), calcium ion (Ca^{2+}), and sodium ion (Na^+). When an atom's outer electron shell is more than half-filled, the atom will gain electrons and form negatively charged ions, called **anions.** Examples are the iodide ion (I^-), chloride ion (Cl^-), and sulfide ion (S^{2-}).

Covalent Bonds

A **covalent bond** is a chemical bond formed by two atoms sharing one or more pairs of electrons. Covalent bonds are stronger and far more common in organisms than are true ionic bonds. In



(a) A sodium atom (Na) loses one electron to an electron acceptor and forms a sodium ion (Na^+). A chlorine atom (Cl) accepts one electron from an electron donor to become a chloride ion (Cl^-).



(b) The sodium and chloride ions are attracted because of their opposite charges and are held together by an ionic bond to form a molecule of sodium chloride.

Figure 2.2 Ionic bond formation.

Q What is an ionic bond?

the hydrogen molecule, H_2, two hydrogen atoms share a pair of electrons. Each hydrogen atom has its own electron plus one electron from the other atom (Figure 2.3a). The shared pair of electrons actually orbits the nuclei of both atoms. Therefore, the outer electron shells of both atoms are filled. Atoms that share only one pair of electrons form a *single covalent bond*. For simplicity, a single covalent bond is expressed as a single line between the atoms (H—H). Atoms that share two pairs of electrons form a *double covalent bond,* expressed as two single lines (=). A *triple covalent bond,* expressed as three single lines (≡), occurs when atoms share three pairs of electrons.

The principles of covalent bonding that apply to atoms of the same element also apply to atoms of different elements. Methane (CH_4) is an example of covalent bonding between atoms of different elements (Figure 2.3b). The outer electron shell of the carbon atom can hold eight electrons but has only four; each hydrogen atom can hold two electrons but has only one. Consequently, in the methane molecule the carbon atom gains four hydrogen electrons to complete its outer shell, and each hydrogen atom completes its pair by sharing one electron from the carbon atom. Each outer electron of the carbon atom orbits both the carbon nucleus and a hydrogen nucleus. Each hydrogen electron orbits both its own nucleus and the carbon nucleus.

Elements such as hydrogen and carbon, whose outer electron shells are half-filled, form covalent bonds quite easily. In fact, in living organisms, carbon almost always forms covalent bonds; it almost never becomes an ion. *Remember:* Covalent bonds are formed by the *sharing* of electrons between atoms. Ionic bonds are formed by *attraction* between atoms that have lost or gained electrons and are therefore positively or negatively charged.

Hydrogen Bonds

Another chemical bond of special importance to all organisms is the **hydrogen bond,** in which a hydrogen atom that is covalently bonded to one oxygen or nitrogen atom is attracted to another oxygen or nitrogen atom. Such bonds are weak and do not bind atoms into molecules. However, they do serve as bridges between different molecules or between various portions of the same molecule.

When hydrogen combines with atoms of oxygen or nitrogen, the relatively large nucleus of these larger oxygen or nitrogen atoms has more protons and attracts the hydrogen electron more strongly than does the small hydrogen nucleus. Thus, in a molecule of water (H_2O), all the electrons tend to be closer to the oxygen nucleus than to the hydrogen nuclei. As a result, the oxygen portion of the molecule has a slightly negative charge, and the hydrogen portion of the molecule has a slightly positive charge (Figure 2.4a). When the positively charged end of one molecule

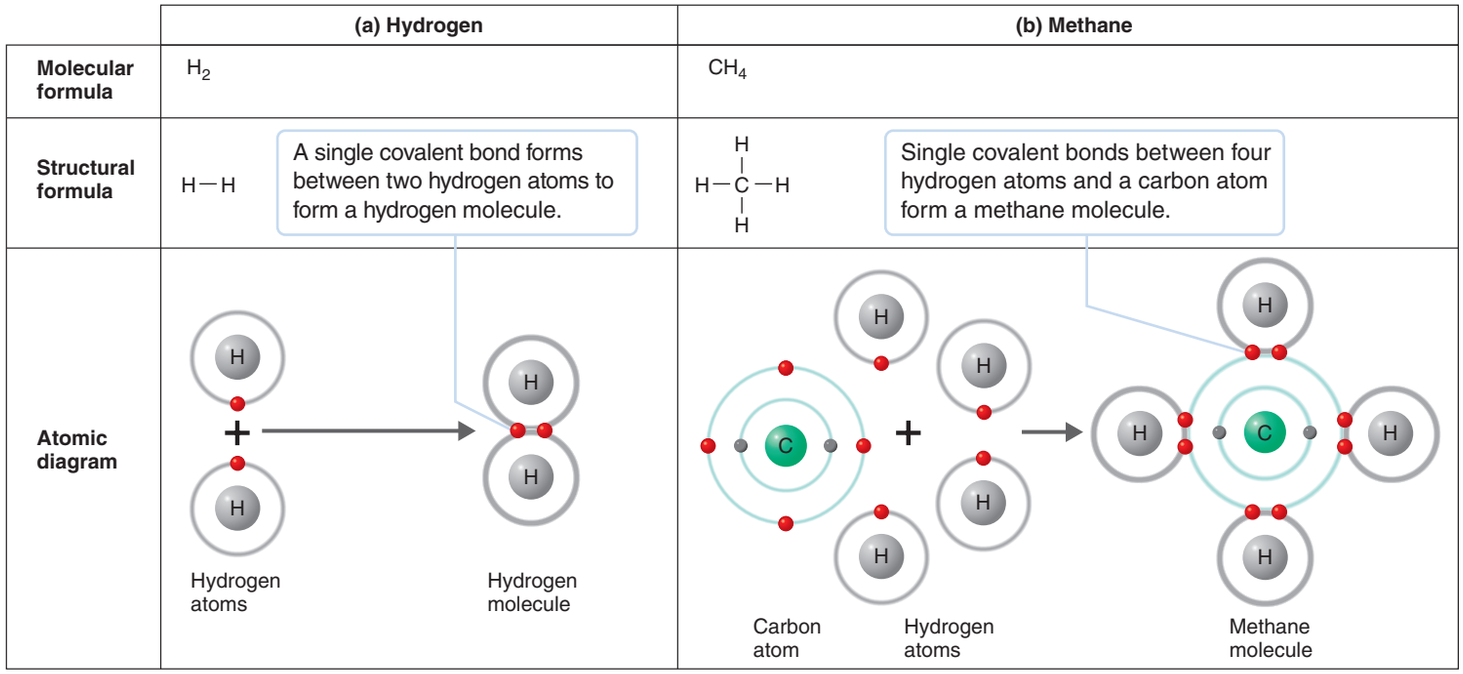


Figure 2.3 Covalent bond formation. The molecular formula shows the number and types of atoms in a molecule. In structural formulas, each covalent bond is written as a straight line between the symbols for two atoms. In molecular formulas, the number of atoms in each molecule is noted by subscripts.

Q What is a covalent bond?

is attracted to the negatively charged end of another molecule, a hydrogen bond is formed (Figure 2.4b). This attraction can also occur between hydrogen and other atoms of the same molecule, especially in large molecules, but oxygen and nitrogen are the elements most frequently involved in hydrogen bonding.

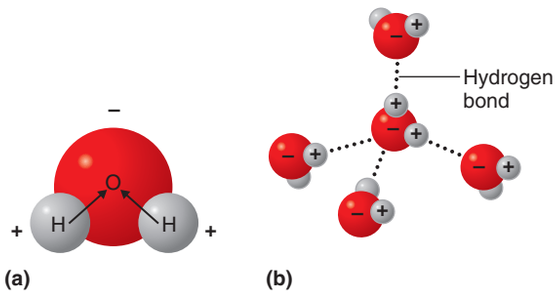


Figure 2.4 Hydrogen bond formation in water. (a) In a water molecule, the electrons of the hydrogen atoms are strongly attracted to the oxygen atom. Therefore, the part of the water molecule containing the oxygen atom has a slightly negative charge, and the part containing hydrogen atoms has a slightly positive charge. **(b)** In a hydrogen bond between water molecules, the hydrogen of one water molecule is attracted to the oxygen of another water molecule. Many water molecules may be attracted to each other by hydrogen bonds (black dots).

Q Which chemical elements are usually involved in hydrogen bonding?

Hydrogen bonds are considerably weaker than either ionic or covalent bonds; they have only about 5% of the strength of covalent bonds. Consequently, hydrogen bonds are formed and broken relatively easily. This property accounts for the temporary bonding that occurs between certain atoms of large and complex molecules, such as proteins and nucleic acids. Even though hydrogen bonds are relatively weak, large molecules containing several hundred of these bonds have considerable strength and stability. A summary of ionic, covalent, and hydrogen bonds is shown in **Table 2.3**.

Molecular Weight and Moles

You have seen that bond formation usually results in the creation of molecules. Molecules are often discussed in terms of units of measure called molecular weight and moles. The **molecular weight** of a molecule is the sum of the atomic weights of all its atoms. To relate the molecular level to the laboratory level, we use a unit called the mole. One **mole** of a substance is its molecular weight expressed in grams. For example, 1 mole of water weighs 18 grams because the molecular weight of H_2O is 18, or $[(2 \times 1) + 16]$.

CHECK YOUR UNDERSTANDING

Differentiate an ionic bond from a covalent bond. **2-2**

TABLE 2.3 Comparison among Ionic, Covalent, and Hydrogen Bonds

Type of Bond	Definition and Importance
Ionic	An *attraction* between ions of opposite charge that holds them together to form a stable molecule. Weaker ionic bonds are important in biochemical reactions such as antigen–antibody reactions.
Covalent	A bond formed by two atoms that *share* one or more pairs of electrons. Covalent bonds are the most common type of chemical bond in organisms and are responsible for holding together the atoms of most molecules in organisms.
Hydrogen	A relatively weak bond in which a hydrogen atom that is covalently bonded to one oxygen or nitrogen atom is attracted to another oxygen or nitrogen atom. Hydrogen bonds do not bind atoms into molecules, but serve as *bridges between different molecules* or different portions of the same molecule, for example, within proteins and nucleic acids.

Chemical Reactions

LEARNING OBJECTIVE

2-3 Diagram three basic types of chemical reactions.

As we said earlier, **chemical reactions** involve the making or breaking of bonds between atoms. After a chemical reaction, the total number of atoms remains the same, but there are new molecules with new properties because the atoms have been rearranged.

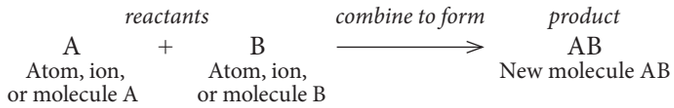
Energy in Chemical Reactions

All chemical bonds require energy to form or break. It is important to note that initially, *activation energy* is needed to break a bond (see page 111). In the chemical reactions of metabolism, energy is released when new bonds are formed after the original bonds break; this is the energy cells use to do work. A chemical reaction that absorbs more energy than it releases is called an **endergonic reaction** (*endo* = within), meaning that energy is directed inward. A chemical reaction that releases more energy than it absorbs is called an **exergonic reaction** (*exo* = out), meaning that energy is directed outward.

In this section we will look at three basic types of chemical reactions common to all living cells. By becoming familiar with these reactions, you will be able to understand the specific chemical reactions we will discuss later (particularly in Chapter 5).

Synthesis Reactions

When two or more atoms, ions, or molecules combine to form new and larger molecules, the reaction is called a **synthesis reaction.** To synthesize means to put together, and a synthesis reaction *forms new bonds*. Synthesis reactions can be expressed in the following way:

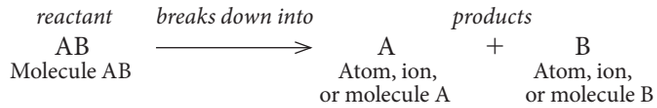


The combining substances, A and B, are called the *reactants;* the substance formed by the combination, AB, is the *product*. The *arrow* indicates the direction in which the reaction proceeds.

Pathways of synthesis reactions in living organisms are collectively called anabolic reactions, or simply **anabolism** (an-AB-ō-liz-um). The combining of sugar molecules to form starch and of amino acids to form proteins are two examples of anabolism.

Decomposition Reactions

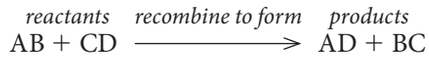
The reverse of a synthesis reaction is a **decomposition reaction.** To decompose means to break down into smaller parts, and in a decomposition reaction *bonds are broken*. Typically, decomposition reactions split large molecules into smaller molecules, ions, or atoms. A decomposition reaction occurs in the following way:



Decomposition reactions that occur in living organisms are collectively called catabolic reactions, or simply **catabolism** (ka-TAB-ō-liz-um). An example of catabolism is the breakdown of sucrose (table sugar) into simpler sugars, glucose and fructose, during digestion. Bacterial decomposition of petroleum is discussed in the Applications of Microbiology box.

Exchange Reactions

All chemical reactions are based on synthesis and decomposition. Many reactions, such as **exchange reactions,** are actually part synthesis and part decomposition. An exchange reaction works in the following way:



First, the bonds between A and B and between C and D are broken in a decomposition process. New bonds are then formed between A and D and between B and C in a synthesis process. For example, an exchange reaction occurs when sodium hydroxide (NaOH) and hydrochloric acid (HCl) react to form table salt (NaCl) and water (H_2O), as follows:

The Reversibility of Chemical Reactions

All chemical reactions are, in theory, reversible; that is, they can occur in either direction. In practice, however, some reactions do this more easily than others. A chemical reaction that is readily

Bioremediation—Bacteria Clean Up Pollution

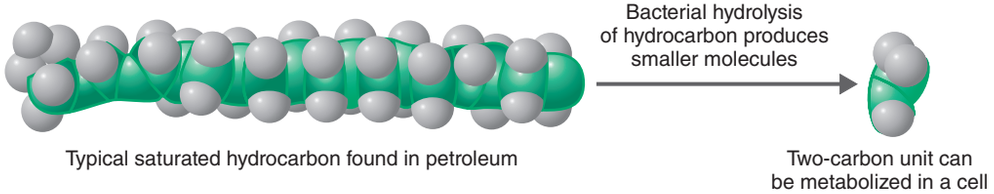
Although many bacteria have dietary requirements similar to ours—that's why they cause food spoilage—others metabolize (or chemically process) substances that are toxic to most plants and animals: heavy metals, sulfur, petroleum, and mercury.

Oil in the environment can come from natural oil that seeps from petroleum deposits, and it can also come from oil spills. Although there are oil-degrading bacteria in soil and sediments, these bacteria are in such small numbers that they cannot deal with large-scale contamination efficiently. Scientists are now working to improve the efficiency of natural pollution fighters. Using bacteria to degrade pollutants is called *bioremediation*.

One of the most promising successes for bioremediation occurred on an Alaskan beach following the *Exxon Valdez* oil spill in 1989. Several naturally occurring *Pseudomonas* bacteria are able to degrade oil for their carbon and energy requirements. In the presence of air, they remove two carbon atoms at a time from a large petroleum molecule (see the figure).

The bacteria degrade the oil too slowly to clean up an oil spill. However, scientists hit on a very simple way to speed up the process: they simply dumped ordinary nitrogen and phosphorus plant fertilizers (bioenhancers) onto a test beach. The number of oil-degrading bacteria increased compared with that on unfertilized control beaches, and oil was quickly cleared from the test beach.

This technique works on land but has not been studied in open water. A number of questions need to be addressed: Will the fertilizer stay near the oil? Will the fertilizers stimulate toxic algae?



reversible (when the end product can revert to the original molecules) is termed a **reversible reaction** and is indicated by two arrows, as shown here:

$$\underset{\text{A + B}}{\textit{reactants}} \quad \underset{\text{break down into}}{\overset{\text{combine to form}}{\rightleftharpoons}} \quad \underset{\text{AB}}{\textit{product}}$$

Some reversible reactions occur because neither the reactants nor the end products are very stable. Other reactions reverse only under special conditions:

$$\underset{\text{A + B}}{\textit{reactants}} \quad \underset{\text{water}}{\overset{\text{heat}}{\rightleftharpoons}} \quad \underset{\text{AB}}{\textit{product}}$$

Whatever is written above or below the arrows indicates the special condition under which the reaction in that direction occurs. In this case, A and B react to produce AB only when heat is applied, and AB breaks down into A and B only in the presence of water. See Figure 2.8 on page 36 for another example.

In Chapter 5 we will examine the various factors that affect chemical reactions.

CHECK YOUR UNDERSTANDING

This chemical reaction below is used to remove chlorine from water. What type of reaction is it? **2-3**

$$HClO + Na_2SO_3 \longrightarrow Na_2SO_4 + HCl$$

Important Biological Molecules

Biologists and chemists divide compounds into two principal classes: inorganic and organic. **Inorganic compounds** are defined as molecules, usually small and structurally simple, which typically lack carbon and in which ionic bonds may play an important role. Inorganic compounds include water, molecular oxygen (O_2), carbon dioxide, and many salts, acids, and bases.

Organic compounds always contain carbon and hydrogen and typically are structurally complex. Carbon is a unique element because it has four electrons in its outer shell and four unfilled spaces. It can combine with a variety of atoms, including other carbon atoms, to form straight or branched chains and rings. Carbon chains form the basis of many organic compounds in living cells, including sugars, amino acids, and vitamins. Organic compounds are held together mostly or entirely by covalent bonds. Some organic molecules, such as polysaccharides, proteins, and nucleic acids, are very large and usually

contain thousands of atoms. Such giant molecules are called *macromolecules*. In the following section we will discuss inorganic and organic compounds that are essential for cells.

Inorganic Compounds

LEARNING OBJECTIVES

2-4 List several properties of water that are important to living systems.

2-5 Define *acid, base, salt,* and *pH*.

Water

All living organisms require a wide variety of inorganic compounds for growth, repair, maintenance, and reproduction. Water is one of the most important, as well as one of the most abundant, of these compounds, and it is particularly vital to microorganisms. Outside the cell, nutrients are dissolved in water, which facilitates their passage through cell membranes. And inside the cell, water is the medium for most chemical reactions. In fact, water is by far the most abundant component of almost all living cells. Water makes up between 65% and 75% of every cell on average. Simply stated, no organism can survive without water.

Water has structural and chemical properties that make it particularly suitable for its role in living cells. As we discussed, the total charge on the water molecule is neutral, but the oxygen region of the molecule has a slightly negative charge, and the hydrogen region has a slightly positive charge (see Figure 2.4a). Any molecule having such an unequal distribution of charges is called a **polar molecule.** The polar nature of water gives it four characteristics that make it a useful medium for living cells.

First, every water molecule is capable of forming four hydrogen bonds with nearby water molecules (see Figure 2.4b). This property results in a strong attraction between water molecules and makes water an excellent temperature buffer. Because of this strong attraction, a great deal of heat is required to separate water molecules from each other to form water vapor; thus, water has a relatively high boiling point (100°C, 212°F). Because water has such a high boiling point, it exists in the liquid state on most of the Earth's surface. Conversely, water temperatures must drop significantly in order for it to freeze. Secondly, the hydrogen bonding between water molecules affects the density of water, depending on whether it occurs as ice or a liquid. For example, the hydrogen bonds in the crystalline structure of water (ice) make ice take up more space. As a result, ice has fewer molecules than an equal volume of liquid water. This makes its crystalline structure less dense than liquid water. For this reason, ice floats and can serve as an insulating layer on the surfaces of lakes and streams that harbor living organisms.

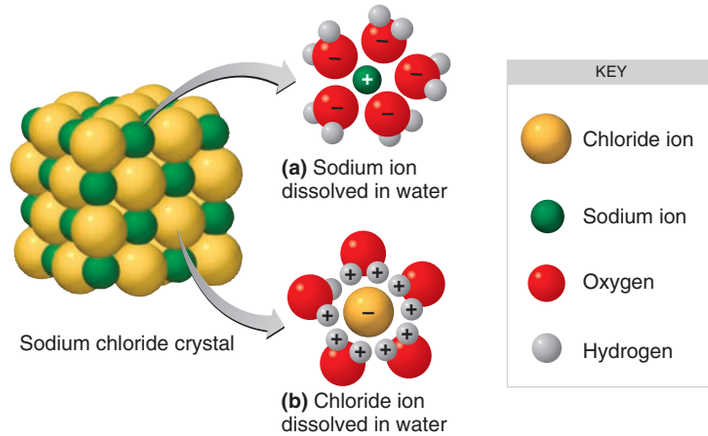


Figure 2.5 How water acts as a solvent for sodium chloride (NaCl). (a) The positively charged sodium ion (Na^+) is attracted to the negative part of the water molecule. **(b)** The negatively charged chloride ion (Cl^-) is attracted to the positive part of the water molecule. In the presence of water molecules, the bonds between the Na^+ and Cl^- are disrupted, and the NaCl dissolves in the water.

Q What happens during ionization?

Third, the polarity of water makes it an excellent dissolving medium, or **solvent.** Many polar substances undergo **dissociation,** or separation, into individual molecules in water—that is, they dissolve. The negative part of the water molecules is attracted to the positive part of the molecules in the **solute,** or dissolving substance, and the positive part of the water molecules is attracted to the negative part of the solute molecules. Substances (such as salts) that are composed of atoms (or groups of atoms) held together by ionic bonds tend to dissociate into separate cations and anions in water. Thus, the polarity of water allows molecules of many different substances to separate and become surrounded by water molecules (Figure 2.5).

Fourth, polarity accounts for water's characteristic role as a reactant or product in many chemical reactions. Its polarity facilitates the splitting and rejoining of hydrogen ions (H^+) and hydroxide ions (OH^-). Water is a key reactant in the digestive processes of organisms, whereby larger molecules are broken down into smaller ones. Water molecules are also involved in synthetic reactions; water is an important source of the hydrogen and oxygen that are incorporated into numerous organic compounds in living cells.

Acids, Bases, and Salts

As we saw in Figure 2.5, when inorganic salts such as sodium chloride (NaCl) are dissolved in water, they undergo **ionization** or *dissociation;* that is, they break apart into ions. Substances called acids and bases show similar behavior.

An **acid** can be defined as a substance that dissociates into one or more hydrogen ions (H^+) and one or more negative ions (anions). Thus, an acid can also be defined as a proton (H^+) donor. A **base** dissociates into one or more negatively charged

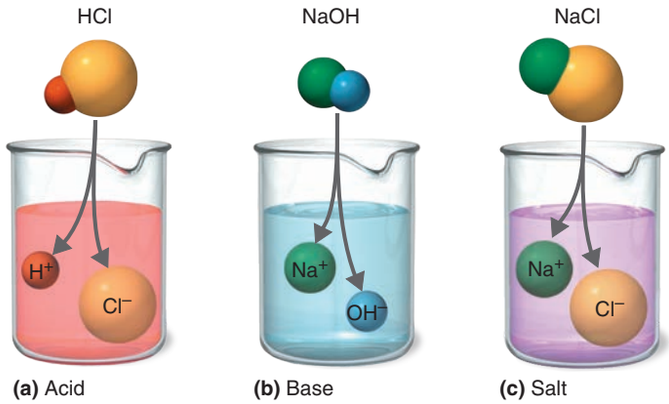


Figure 2.6 Acids, bases, and salts. **(a)** In water, hydrochloric acid (HCl) dissociates into H^+ and Cl^-. **(b)** Sodium hydroxide (NaOH), a base, dissociates into OH^- and Na^+ in water. **(c)** In water, table salt (NaCl) dissociates into positive ions (Na^+) and negative ions (Cl^-), neither of which are H^+ or OH^-.

Q How do acids and bases differ?

hydroxide ions (OH^-) that can accept, or combine with, protons, and one or more positive ions (cations). Thus, sodium hydroxide (NaOH) is a base because it dissociates to release OH^-, which has a strong attraction for protons and is among the most important proton acceptors. A **salt** is a substance that dissociates in water into cations and anions, neither of which is H^+ or OH^-. Figure 2.6 shows common examples of each type of compound and how they dissociate in water.

Acid–Base Balance: The Concept of pH

An organism must maintain a fairly constant balance of acids and bases to remain healthy. For example, if a particular acid or base concentration is too high or too low, enzymes change in shape and no longer effectively promote chemical reactions in a cell. In the aqueous environment within organisms, acids dissociate into hydrogen ions (H^+) and anions. Bases, in contrast, dissociate into hydroxide ions (OH^-) and cations. The more hydrogen ions that are free in a solution, the more acidic the solution is. Conversely, the more hydroxide ions that are free in a solution, the more basic, or alkaline, it is.

Biochemical reactions—that is, chemical reactions in living systems—are extremely sensitive to even small changes in the acidity or alkalinity of the environments in which they occur. In fact, H^+ and OH^- are involved in almost all biochemical processes, and any deviation from a cell's narrow band of normal H^+ and OH^- concentrations can dramatically modify the cell's functions. For this reason, the acids and bases that are continually formed in an organism must be kept in balance.

It is convenient to express the amount of H^+ in a solution by a logarithmic **pH** scale, which ranges from 0 to 14 (Figure 2.7). The term *pH* means potential of hydrogen. On a logarithmic scale, a change of one whole number represents a *tenfold* change from the previous concentration. Thus, a solution of pH 1 has ten times more hydrogen ions than a solution of pH 2 and has 100 times more hydrogen ions than a solution of pH 3.

A solution's pH is calculated as $-\log_{10}[H^+]$, the negative logarithm to the base 10 of the hydrogen ion concentration (denoted by brackets), determined in moles per liter $[H^+]$. For example, if the H^+ concentration of a solution is 1.0×10^{-4} moles/liter, or 10^{-4}, its pH equals $-\log_{10}10^{-4} = -(-4) = 4$; this is about the pH value of wine (see Appendix B). The pH values of some human body fluids and other common substances are also shown in Figure 2.7. In the laboratory, you will usually measure the pH of a solution with a pH meter or with chemical test papers.

Acidic solutions contain more H^+ than OH^- and have a pH lower than 7. If a solution has more OH^- than H^+, it is a basic, or alkaline, solution. In pure water, a small percentage of the

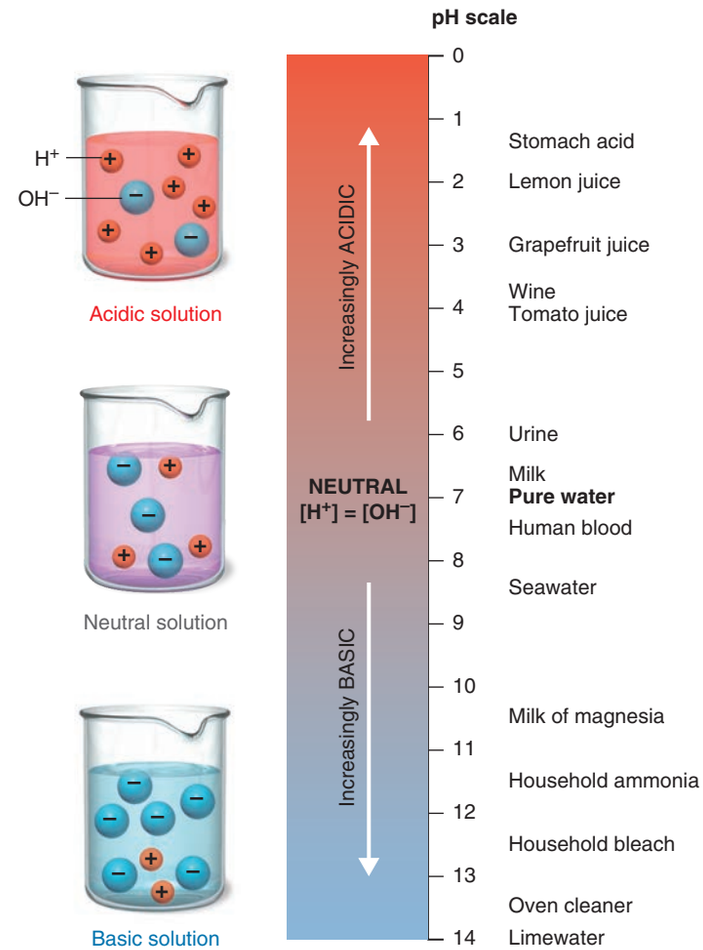


Figure 2.7 The pH scale. As pH values decrease from 14 to 0, the H^+ concentration increases. Thus, the lower the pH, the more acidic the solution; the higher the pH, the more basic the solution. If the pH value of a solution is below 7, the solution is acidic; if the pH is above 7, the solution is basic (alkaline). The approximate pH values of some human body fluids and common substances are shown next to the pH scale.

Q At what pH are the concentrations of H^+ and OH^- equal?

molecules are dissociated into H^+ and OH^-, so it has a pH of 7. Because the concentrations of H^+ and OH^- are equal, this pH is said to be the pH of a neutral solution.

Keep in mind that the pH of a solution can be changed. We can increase its acidity by adding substances that will increase the concentration of hydrogen ions. As a living organism takes up nutrients, carries out chemical reactions, and excretes wastes, its balance of acids and bases tends to change, and the pH fluctuates. Fortunately, organisms possess natural pH **buffers,** compounds that help keep the pH from changing drastically. But the pH in our environment's water and soil can be altered by waste products from organisms, pollutants from industry, or fertilizers used in agricultural fields or gardens. When bacteria are grown in a laboratory medium, they excrete waste products such as acids that can alter the pH of the medium. If this effect were to continue, the medium would become acidic enough to inhibit bacterial enzymes and kill the bacteria. To prevent this problem, pH buffers are added to the culture medium. One very effective pH buffer for some culture media uses a mixture of K_2HPO_4 and KH_2PO_4 (see Table 6.3, page 158).

Different microbes function best within different pH ranges, but most organisms grow best in environments with a pH value between 6.5 and 8.5. Among microbes, fungi are best able to tolerate acidic conditions, whereas the prokaryotes called cyanobacteria tend to do well in alkaline habitats. *Propionibacterium acnes* (prō-pē′on-ē-bak-TI-rē-um AK-nēz), a bacterium that causes acne, has as its natural environment human skin, which tends to be slightly acidic, with a pH of about 4. *Acidithiobacillus ferrooxidans* (a′sid-ē-thi′ō-bah-SIL-lus fer′rō-OKS-i-danz) is a bacterium that metabolizes elemental sulfur and produces sulfuric acid (H_2SO_4). Its pH range for optimum growth is from 1 to 3.5. The sulfuric acid produced by this bacterium in mine water is important in dissolving uranium and copper from low-grade ore (see Chapter 28).

CHECK YOUR UNDERSTANDING

- Why is the polarity of a water molecule important? **2-4**
- Antacids neutralize acid by the following reaction.

  $Mg(OH)_2 + 2HCl \rightarrow MgCl_2 + H_2O$

  Identify the acid, base, and salt. **2-5**

Organic Compounds

LEARNING OBJECTIVES

2-6 Distinguish organic and inorganic compounds.
2-7 Define *functional group*.
2-8 Identify the building blocks of carbohydrates.
2-9 Differentiate simple lipids, complex lipids, and steroids.
2-10 Identify the building blocks and structure of proteins.
2-11 Identify the building blocks of nucleic acids.
2-12 Describe the role of ATP in cellular activities.

Inorganic compounds, excluding water, constitute about 1–1.5% of living cells. These relatively simple components, whose molecules have only a few atoms, cannot be used by cells to perform complex biological functions. Organic molecules, whose carbon atoms can combine in an enormous variety of ways with other carbon atoms and with atoms of other elements, are relatively complex and thus are capable of more complex biological functions.

Structure and Chemistry

In the formation of organic molecules, carbon's four outer electrons can participate in up to four covalent bonds, and carbon atoms can bond to each other to form straight-chain, branched-chain, or ring structures.

In addition to carbon, the most common elements in organic compounds are hydrogen (which can form one bond), oxygen (two bonds), and nitrogen (three bonds). Sulfur (two bonds) and phosphorus (five bonds) appear less often. Other elements are found, but only in relatively few organic compounds. The elements that are most abundant in living organisms are the same as those that are most abundant in organic compounds (see Table 2.1).

The chain of carbon atoms in an organic molecule is called the **carbon skeleton;** a huge number of combinations is possible for carbon skeletons. Most of these carbons are bonded to hydrogen atoms. The bonding of other elements with carbon and hydrogen forms characteristic **functional groups,** specific groups of atoms that are most commonly involved in chemical reactions and are responsible for most of the characteristic chemical properties and many of the physical properties of a particular organic compound (**Table 2.4**).

Different functional groups confer different properties on organic molecules. For example, the hydroxyl group of alcohols is hydrophilic (water-loving) and thus attracts water molecules to it. This attraction helps dissolve organic molecules containing hydroxyl groups. Because the carboxyl group is a source of hydrogen ions, molecules containing it have acidic properties. Amino groups, by contrast, function as bases because they readily accept hydrogen ions. The sulfhydryl group helps stabilize the intricate structure of many proteins.

Functional groups help us classify organic compounds. For example, the —OH group is present in each of the following molecules:

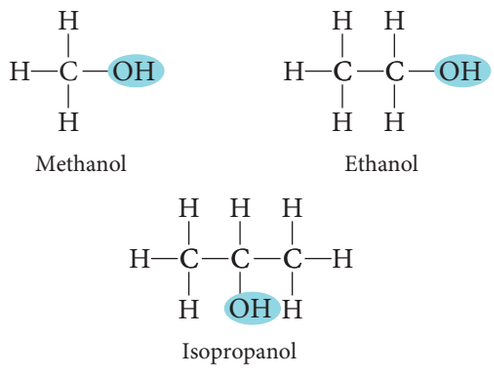
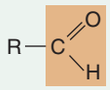
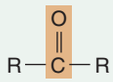
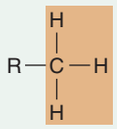
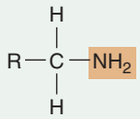
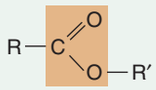
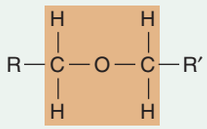
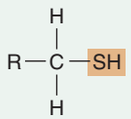
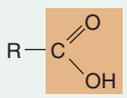
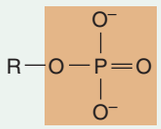


TABLE 2.4 Representative Functional Groups and the Compounds in Which They Are Found

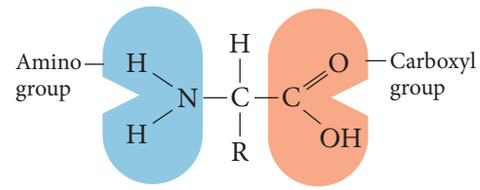
Structure	Name of Group	Biological Importance
R—O—H	Alcohol	Lipids, carbohydates
	Aldehyde*	Reducing sugars such as glucose; polysaccharides
	Ketone*	Metabolic intermediates
	Methyl	DNA; energy metabolism
	Amino	Proteins
	Ester	Bacterial and eukaryotic plasma membranes
	Ether	Archaeal plasma membranes
	Sulfhydryl	Energy metabolism; protein structure
	Carboxyl	Organic acids, lipids, proteins
	Phosphate	ATP, DNA

*In an aldehyde, a C═O is at the end of a molecule, in contrast to the internal C═O in a ketone.

Because the characteristic reactivity of the molecules is based on the —OH group, they are grouped together in a class called alcohols. The —OH group is called the *hydroxyl group* and is not to be confused with the *hydroxide ion* (OH^-) of bases. The hydroxyl group of alcohols does not ionize at neutral pH; it is covalently bonded to a carbon atom.

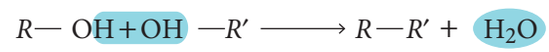
When a class of compounds is characterized by a certain functional group, the letter *R* can be used to stand for the remainder of the molecule. For example, alcohols in general may be written R—OH.

Frequently, more than one functional group is found in a single molecule. For example, an amino acid molecule contains both amino and carboxyl groups. The amino acid glycine has the following structure:



Most of the organic compounds found in living organisms are quite complex; a large number of carbon atoms form the skeleton, and many functional groups are attached. In organic molecules, it is important that each of the four bonds of carbon be satisfied (attached to another atom) and that each of the attaching atoms have its characteristic number of bonds satisfied. Because of this, such molecules are chemically stable.

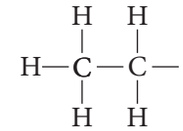
Small organic molecules can be combined into very large molecules called **macromolecules** (*macro* = large). Macromolecules are usually **polymers** (*poly* = many; *mers* = parts): polymers are formed by covalent bonding of many repeating small molecules called **monomers** (*mono* = one). When two monomers join together, the reaction usually involves the elimination of a hydrogen atom from one monomer and a hydroxyl group from the other; the hydrogen atom and the hydroxyl group combine to produce water:



This type of exchange reaction is called **dehydration synthesis** (*de* = from; *hydro* = water), or a **condensation reaction,** because a molecule of water is released (Figure 2.8a). Such macromolecules as carbohydrates, lipids, proteins, and nucleic acids are assembled in the cell, essentially by dehydration synthesis. However, other molecules must also participate to provide energy for bond formation. ATP, the cell's chief energy provider, is discussed at the end of this chapter.

CHECK YOUR UNDERSTANDING

- Define *organic*. **2-6**
- Add the appropriate functional group(s) to the ethyl group below to produce each of the following compounds: ethanol, acetic acid, acetaldehyde, ethanolamine, diethyl ether. **2-7**



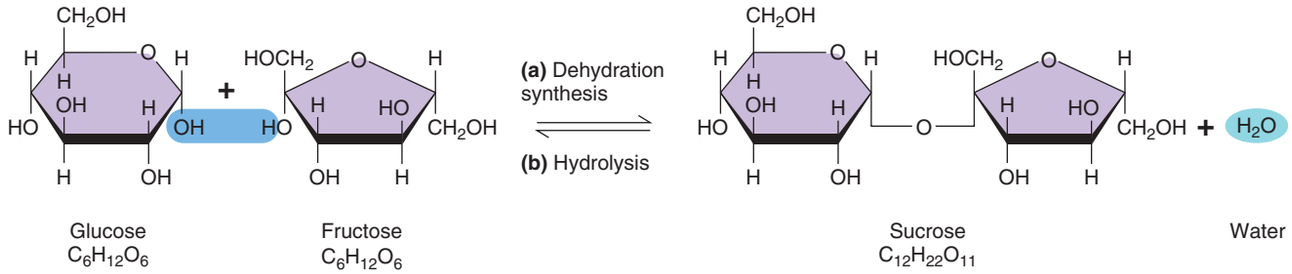


Figure 2.8 Dehydration synthesis and hydrolysis. (a) In dehydration synthesis (left to right), the monosaccharides glucose and fructose combine to form a molecule of the disaccharide sucrose. A molecule of water is released in the reaction. **(b)** In hydrolysis (right to left), the sucrose molecule breaks down into the smaller molecules glucose and fructose. For the hydrolysis reaction to proceed, water must be added to the sucrose.

Q What is the difference between a polymer and a monomer?

Carbohydrates

The **carbohydrates** are a large and diverse group of organic compounds that includes sugars and starches. Carbohydrates perform a number of major functions in living systems. For instance, one type of sugar (deoxyribose) is a building block of deoxyribonucleic acid (DNA), the molecule that carries hereditary information. Other sugars are needed for the cell walls. Simple carbohydrates are used in the synthesis of amino acids and fats or fatlike substances, which are used to build cell membranes and other structures. Macromolecular carbohydrates function as food reserves. The principal function of carbohydrates, however, is to fuel cell activities with a ready source of energy.

Carbohydrates are made up of carbon, hydrogen, and oxygen atoms. The ratio of hydrogen to oxygen atoms is always 2:1 in simple carbohydrates. This ratio can be seen in the formulas for the carbohydrates ribose ($C_5H_{10}O_5$), glucose ($C_6H_{12}O_6$), and sucrose ($C_{12}H_{22}O_{11}$). Although there are exceptions, the general formula for carbohydrates is $(CH_2O)_n$, where n indicates that there are three or more CH_2O units. Carbohydrates can be classified into three major groups on the basis of size: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides

Simple sugars are called **monosaccharides** (*sacchar* = sugar); each molecule contains three to seven carbon atoms. The number of carbon atoms in the molecule of a simple sugar is indicated by the prefix in its name. For example, simple sugars with three carbons are called trioses. There are also tetroses (four-carbon sugars), pentoses (five-carbon sugars), hexoses (six-carbon sugars), and heptoses (seven-carbon sugars). Pentoses and hexoses are extremely important to living organisms. Deoxyribose is a pentose found in DNA. Glucose, a very common hexose, is the main energy-supplying molecule of living cells.

Disaccharides

Disaccharides (*di* = two) are formed when two monosaccharides bond in a dehydration synthesis reaction.* For example, molecules of two monosaccharides, glucose and fructose, combine to form a molecule of the disaccharide sucrose (table sugar) and a molecule of water (see Figure 2.8a). Similarly, the dehydration synthesis of the monosaccharides glucose and galactose forms the disaccharide lactose (milk sugar).

It may seem odd that glucose and fructose have the same chemical formula (see Figure 2.8), even though they are different monosaccharides. The positions of the oxygens and carbons differ in the two different molecules; consequently, the molecules have different physical and chemical properties. Two molecules with the same chemical formula but different structures and properties are called **isomers** (*iso* = same).

Disaccharides can be broken down into smaller, simpler molecules when water is added. This chemical reaction, the reverse of dehydration synthesis, is called **hydrolysis** (*hydro* = water; *lysis* = to loosen) (Figure 2.8b). A molecule of sucrose, for example, may be hydrolyzed (digested) into its components of glucose and fructose by reacting with the H^+ and OH^- of water.

As you will see in Chapter 4, the cell walls of bacterial cells are composed of disaccharides and proteins, which together are called peptidoglycan.

Polysaccharides

Carbohydrates in the third major group, the **polysaccharides,** consist of tens or hundreds of monosaccharides joined through dehydration synthesis. Polysaccharides often have side chains branching off the main structure and are classified as macromolecules. Like disaccharides, polysaccharides can be split apart into their constituent sugars through hydrolysis. Unlike monosaccharides and disaccharides, however, they usually lack the characteristic sweetness of sugars such as fructose and sucrose and usually are not soluble in water.

One important polysaccharide is *glycogen,* which is composed of glucose subunits and is synthesized as a storage material by animals and some bacteria. *Cellulose,* another important

*Carbohydrates composed of 2 to about 20 monosaccharides are called **oligosaccharides** (*oligo* = few). Disaccharides are the most common oligosaccharides.

glucose polymer, is the main component of the cell walls of plants and most algae. Although cellulose is the most abundant carbohydrate on Earth, it can be digested by only a few organisms that have the appropriate enzyme. The polysaccharide *dextran,* which is produced as a sugary slime by certain bacteria, is used in a blood plasma substitute. *Chitin* is a polysaccharide that makes up part of the cell wall of most fungi and the exoskeletons of lobsters, crabs, and insects. *Starch* is a polymer of glucose produced by plants and used as food by humans.

Many animals, including humans, produce enzymes called *amylases* that can break the bonds between the glucose molecules in glycogen. However, this enzyme cannot break the bonds in cellulose. Bacteria and fungi that produce enzymes called *cellulases* can digest cellulose. Cellulases from the fungus *Trichoderma* (TRIK-ō-der-mah) are used for a variety of industrial purposes. One of the more unusual uses is producing stone-washed denim. Because washing the fabric with rocks would damage washing machines, cellulase is used to digest, and therefore soften, the cotton. (See the Applications box in Chapter 1, page 3.)

CHECK YOUR UNDERSTANDING

Give an example of a monosaccharide, a disaccharide, and a polysaccharide. **2-8**

Lipids

If lipids were suddenly to disappear from the Earth, all living cells would collapse in a pool of fluid, because lipids are essential to the structure and function of membranes that separate living cells from their environment. **Lipids** (*lip* = fat) are a second major group of organic compounds found in living matter. Like carbohydrates, they are composed of atoms of carbon, hydrogen, and oxygen, but lipids lack the 2:1 ratio between hydrogen and oxygen atoms. Even though lipids are a very diverse group of compounds, they share one common characteristic: they are *nonpolar* molecules so, unlike water, do not have a positive and a negative end (pole). Therefore, most lipids are insoluble in water but dissolve readily in nonpolar solvents, such as ether and chloroform. Lipids provide the structure of membranes and some cell walls and function in energy storage.

Simple Lipids

Simple lipids, called *fats* or *triglycerides,* contain an alcohol called *glycerol* and a group of compounds known as *fatty acids*. Glycerol molecules have three carbon atoms to which are attached three hydroxyl (—OH) groups (**Figure 2.9a**). Fatty acids consist of long hydrocarbon chains (composed only of carbon and hydrogen atoms) ending in a carboxyl (—COOH, organic acid)

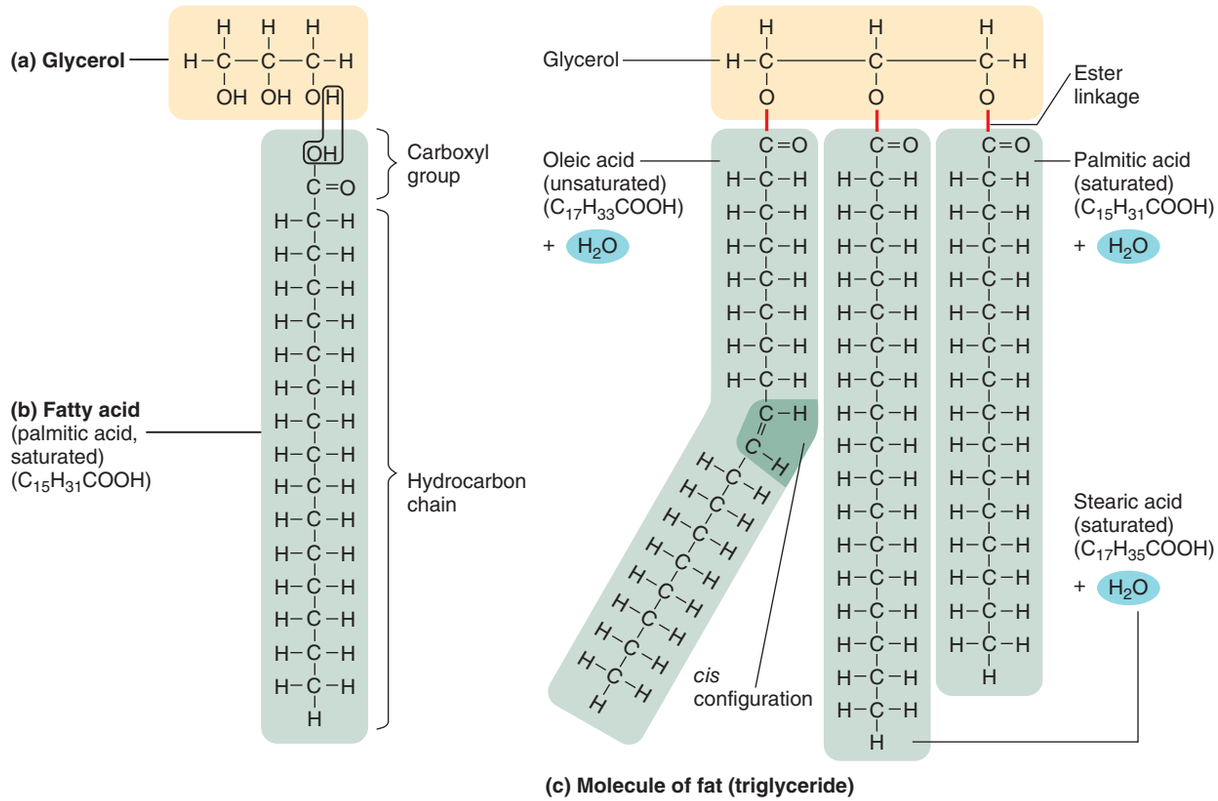


Figure 2.9 Structural formulas of simple lipids. **(a)** Glycerol. **(b)** Palmitic acid, a saturated fatty acid. **(c)** The chemical combination of a molecule of glycerol and three fatty acid molecules (palmitic, stearic, and oleic in this example) forms one molecule of fat (triglyceride) and three molecules of water in a dehydration synthesis reaction. Oleic acid is a *cis* fatty acid. The bond between glycerol and each fatty acid is called an ester linkage. The addition of three water molecules to a fat forms glycerol and three fatty acid molecules in a hydrolysis reaction.

Q How do saturated and unsaturated fatty acids differ?

group (Figure 2.9b). Most common fatty acids contain an even number of carbon atoms.

A molecule of fat is formed when a molecule of glycerol combines with one to three fatty acid molecules. The number of fatty acid molecules determines whether the fat molecule is a monoglyceride, diglyceride, or triglyceride (Figure 2.9c). In the reaction, one to three molecules of water are formed (dehydration), depending on the number of fatty acid molecules reacting. The chemical bond formed where the water molecule is removed is called an *ester linkage*. In the reverse reaction, hydrolysis, a fat molecule is broken down into its component fatty acid and glycerol molecules.

Because the fatty acids that form lipids have different structures, there is a wide variety of lipids. For example, three molecules of fatty acid A might combine with a glycerol molecule. Or one molecule each of fatty acids A, B, and C might unite with a glycerol molecule (see Figure 2.9c).

The primary function of lipids is to form plasma membranes that enclose cells. A plasma membrane supports the cell and allows nutrients and wastes to pass in and out; therefore, the lipids must maintain the same viscosity, regardless of the surrounding temperature. The membrane must be about as viscous as olive oil, without getting too fluid when warmed or too thick when cooled. As everyone who has ever cooked a meal knows, animal fats (such as butter) are usually solid at room temperature, whereas vegetable oils are usually liquid at room temperature. The difference in their respective melting points is due to the degrees of saturation of the fatty acid chains. A fatty acid is said to be *saturated* when it has no double bonds, in which case the carbon skeleton contains the maximum number of hydrogen atoms (see Figure 2.9c and **Figure 2.10a**). Saturated chains become solid more easily because they are relatively straight and are thus able to pack together more closely than unsaturated chains. The double bonds of *unsaturated* chains create kinks in the chain, which

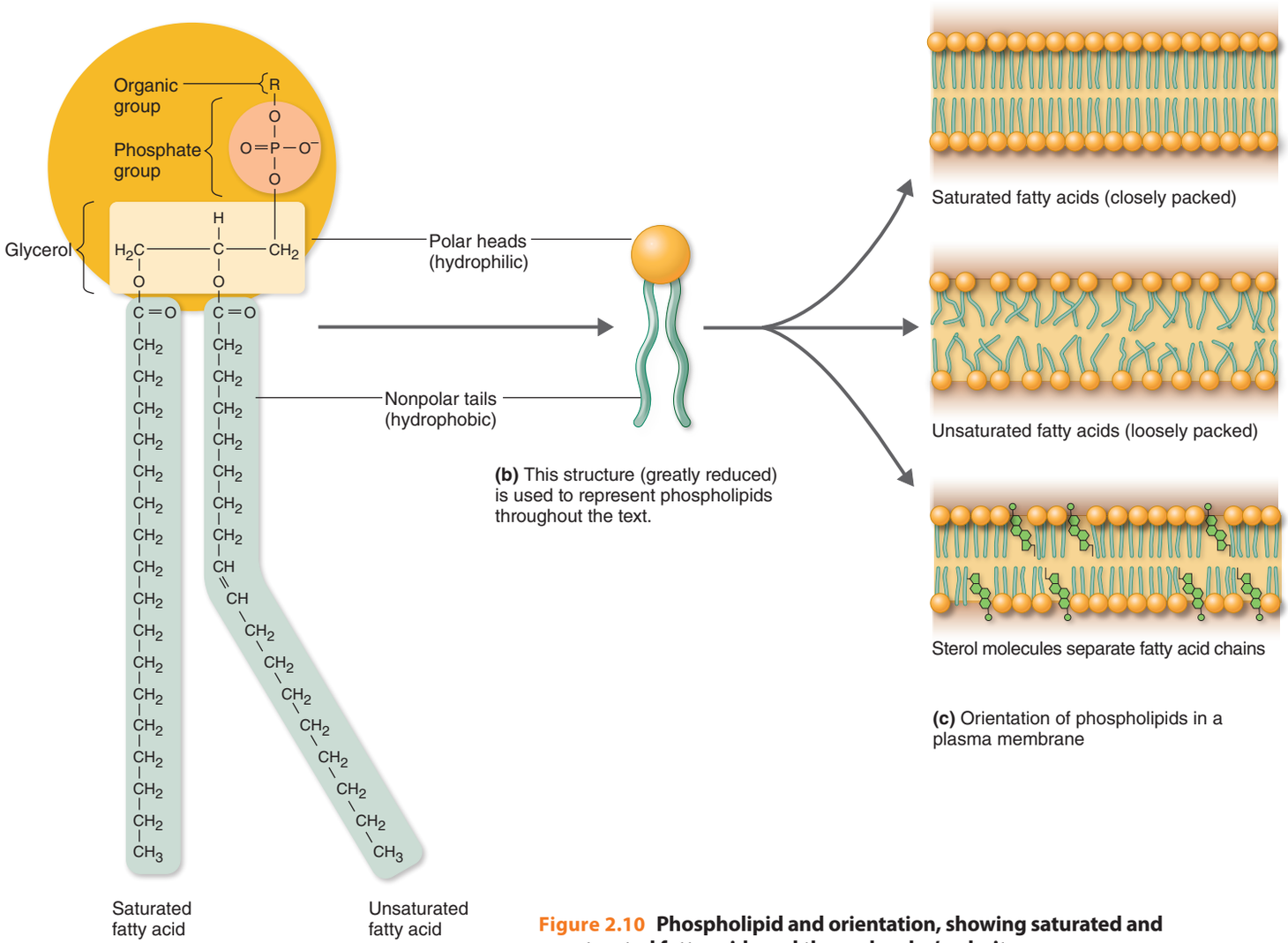


Figure 2.10 Phospholipid and orientation, showing saturated and unsaturated fatty acids and the molecules' polarity.

Q Where are phospholipids found in cells?

keep the chains apart from one another (Figure 2.10b). Note in Figure 2.9c that the H atoms on either side of the double bond in oleic acid are on the same side of the unsaturated fatty acid. Such an unsaturated fatty acid is called a *cis* fatty acid. If, instead, the H atoms are on opposite sides of the double bond, the unsaturated acid is called a *trans* fatty acid.

Complex Lipids

Complex lipids contain such elements as phosphorus, nitrogen, and sulfur, in addition to the carbon, hydrogen, and oxygen found in simple lipids. The complex lipids called *phospholipids* are made up of glycerol, two fatty acids, and, in place of a third fatty acid, a phosphate group bonded to one of several organic groups (see Figure 2.10a). Phospholipids are the lipids that build membranes; they are essential to a cell's survival. Phospholipids have polar as well as nonpolar regions (Figure 2.10a and b; see also Figure 4.14, page 86). When placed in water, phospholipid molecules twist themselves in such a way that all polar (hydrophilic) portions orient themselves toward the polar water molecules, with which they then form hydrogen bonds. (Recall that *hydrophilic* means water-loving.) This forms the basic structure of a plasma membrane (Figure 2.10c). Polar portions consist of a phosphate group and glycerol. In contrast to the polar regions, all nonpolar (hydrophobic) parts of the phospholipid make contact only with the nonpolar portions of neighboring molecules. (*Hydrophobic* means water-fearing.) Nonpolar portions consist of fatty acids. This characteristic behavior makes phospholipids particularly suitable for their role as a major component of the membranes that enclose cells. Phospholipids enable the membrane to act as a barrier that separates the contents of the cell from the water-based environment in which it lives.

Some complex lipids are useful in identifying certain bacteria. For example, the cell wall of *Mycobacterium tuberculosis* (mī′kō-bak-TI-rē-um too′ber-kū-LŌ-sis), the bacterium that causes tuberculosis, is distinguished by its lipid-rich content. The cell wall contains complex lipids such as waxes and glycolipids (lipids with carbohydrates attached) that give the bacterium distinctive staining characteristics. Cell walls rich in such complex lipids are characteristic of all members of the genus *Mycobacterium*.

Steroids

Steroids are structurally very different from lipids. **Figure 2.11** shows the structure of the steroid cholesterol, with the four interconnected carbon rings that are characteristic of steroids. When an —OH group is attached to one of the rings, the steroid is called a *sterol* (an alcohol). Sterols are important constituents of the plasma membranes of animal cells and of one group of bacteria (mycoplasmas), and they are also found in fungi and plants. The sterols separate the fatty acid chains and thus prevent the packing that would harden the plasma membrane at low temperatures (see Figure 2.10c).

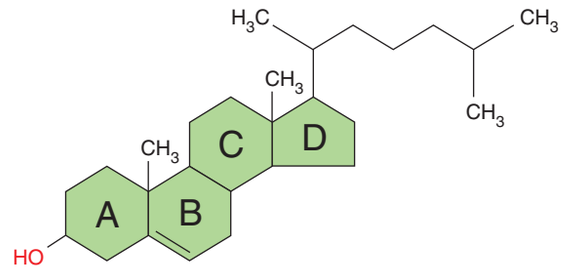


Figure 2.11 Cholesterol, a steroid. Note the four "fused" carbon rings (labeled A–D), which are characteristic of steroid molecules. The hydrogen atoms attached to the carbons at the corners of the rings have been omitted. The —OH group (colored red) makes this molecule a sterol.

Q Where are sterols found in cells?

CHECK YOUR UNDERSTANDING

How do simple lipids differ from complex lipids? **2-9**

Proteins

Proteins are organic molecules that contain carbon, hydrogen, oxygen, and nitrogen. Some also contain sulfur. If you were to separate and weigh all the groups of organic compounds in a living cell, the proteins would tip the scale. Hundreds of different proteins can be found in any single cell, and together they make up 50% or more of a cell's dry weight.

Proteins are essential ingredients in all aspects of cell structure and function. *Enzymes* are the proteins that speed up biochemical reactions. But proteins have other functions as well. *Transporter proteins* help transport certain chemicals into and out of cells. Other proteins, such as the *bacteriocins* produced by many bacteria, kill other bacteria. Certain *toxins,* called exotoxins, produced by some disease-causing microorganisms are also proteins. Some proteins play a role in the *contraction* of animal muscle cells and the *movement* of microbial and other types of cells. Other proteins are integral parts of *cell structures* such as walls, membranes, and cytoplasmic components. Still others, such as the *hormones* of certain organisms, have regulatory functions. As we will see in Chapter 17, proteins called *antibodies* play a role in vertebrate immune systems.

Amino Acids

Just as monosaccharides are the building blocks of larger carbohydrate molecules, and just as fatty acids and glycerol are the building blocks of fats, **amino acids** are the building blocks of proteins. Amino acids contain at least one carboxyl (—COOH) group and one amino ($—NH_2$) group attached to the same carbon atom, called an alpha-carbon (written C_α) (**Figure 2.12a**). Such amino acids are called *alpha-amino acids*. Also attached to the alpha-carbon is a side group (R group), which is the amino acid's distinguishing feature. The side group can be a hydrogen atom, an unbranched or branched

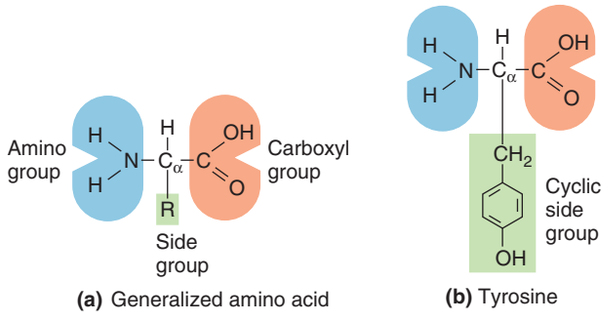


Figure 2.12 Amino acid structure. **(a)** The general structural formula for an amino acid. The alpha-carbon (C_α) is shown in the center. Different amino acids have different R groups, also called side groups. **(b)** Structural formula for the amino acid tyrosine, which has a cyclic side group.

Q What distinguishes one amino acid from another?

chain of atoms, or a ring structure that is cyclic (all carbon) or heterocyclic (when an atom other than carbon is included in the ring). Figure 2.12b shows the structural formula of tyrosine, an amino acid that has a cyclic side group. The side group can contain functional groups, such as the sulfhydryl group (—SH), the hydroxyl group (—OH), or additional carboxyl or amino groups. These side groups and the carboxyl and alpha-amino groups affect the total structure of a protein, described later. The structures and standard abbreviations of the 20 amino acids found in proteins are shown in **Table 2.5**.

Most amino acids exist in either of two configurations called **stereoisomers,** designated by D and L. These configurations are mirror images, corresponding to "right-handed" (D) and "left-handed" (L) three-dimensional shapes (**Figure 2.13**). The amino acids found in proteins are always the L-isomers (except for glycine, the simplest amino acid, which does not have stereoisomers). However, D-amino acids occasionally occur in nature—for example, in certain bacterial cell walls and antibiotics. (Many other kinds of organic molecules also can exist in D and L forms. One example is the sugar glucose, which occurs in nature as D-glucose.)

Although only 20 different amino acids occur naturally in proteins, a single protein molecule can contain from 50 to hundreds of amino acid molecules, which can be arranged in an almost infinite number of ways to make proteins of different lengths, compositions, and structures. The number of proteins is practically endless, and every living cell produces many different proteins.

Peptide Bonds

Amino acids bond between the carbon atom of the carboxyl (—COOH) group of one amino acid and the nitrogen atom of

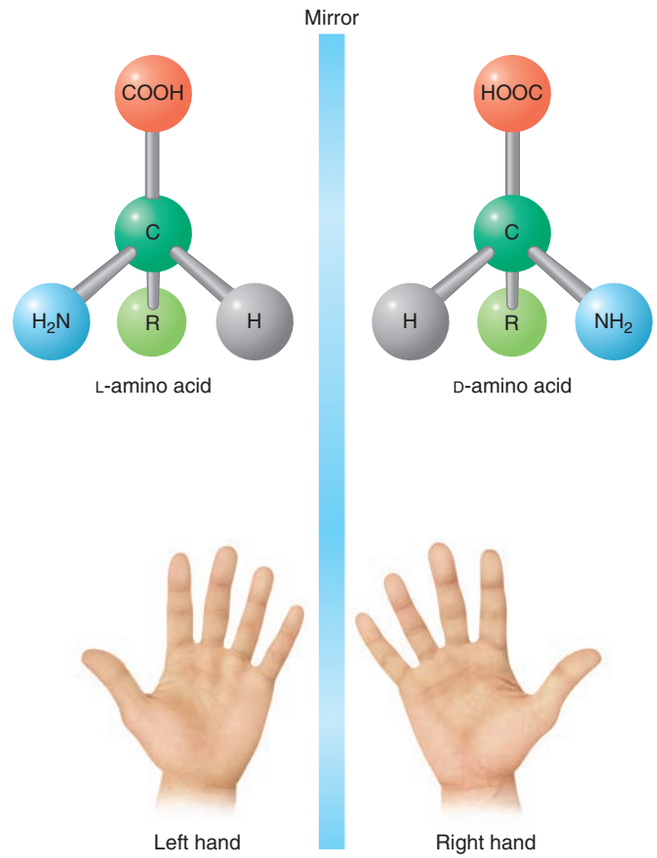


Figure 2.13 The L- and D-isomers of an amino acid, shown with ball-and-stick models. The two isomers, like left and right hands, are mirror images of each other and cannot be superimposed on one another. (Try it!)

Q Which isomer is always found in proteins?

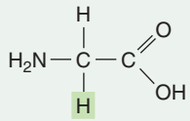
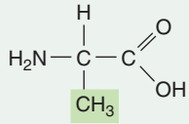
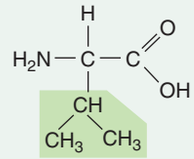
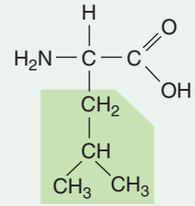
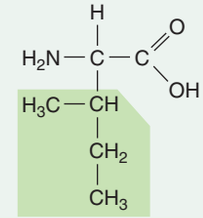
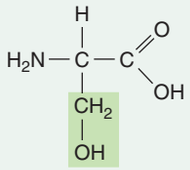
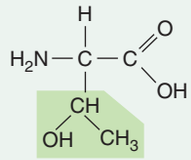
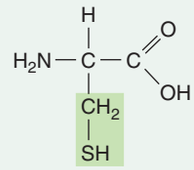
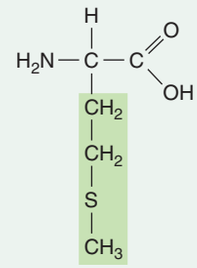
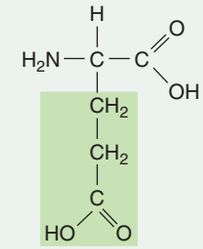
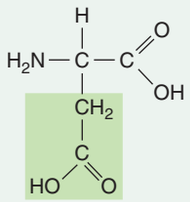
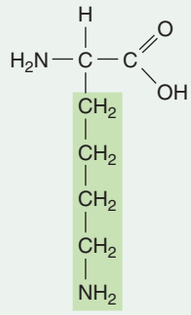
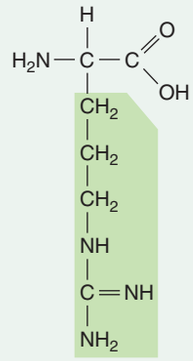
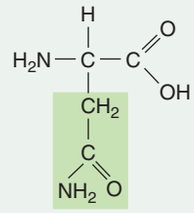
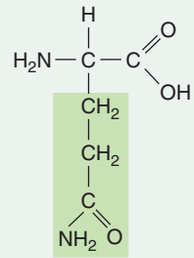
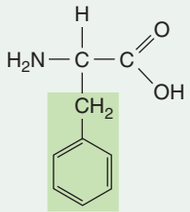
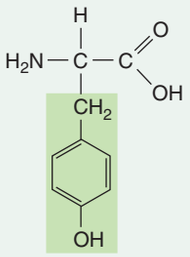
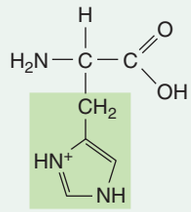
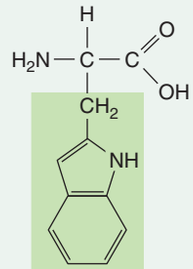
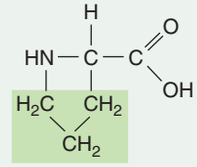
Clinical Case

While Jonathan is in intensive care, his wife, DeeAnn, and adult daughter talk with his physician and an investigator from the Centers for Disease Control and Prevention (CDC) to find the source of Jonathan's *B. anthracis* infection. Environmental investigations uncover *B. anthracis* at Jonathan's home, in his van, and in his workplace, but neither his wife nor children show signs of infection. His bandmates are also tested; they are all negative for *B. anthracis*. The CDC investigator explains to Jonathan's family that *B. anthracis* forms endospores that can survive in soil for up to 60 years. It is rare in humans; however, grazing animals and people who handle their hides or other by-products can become infected. *B. anthracis* cells have capsules that are composed of poly-D-glutamic acid.

Why are the capsules resistant to digestion by phagocytes? (Phagocytes are white blood cells that engulf and destroy bacteria.)

25 40 42 46

Table 2.5 The 20 Amino Acids Found in Proteins*

Glycine (Gly)	Alanine (Ala)	Valine (Val)	Leucine (Leu)	Isoleucine (Ile)
				
Hydrogen atom	Unbranched chain	Branched chain	Branched chain	Branched chain
Serine (Ser)	**Threonine (Thr)**	**Cysteine (Cys)**	**Methionine (Met)**	**Glutamic acid (Glu)**
				
Hydroxyl (—OH) group	Hydroxyl (—OH) group	Sulphur-containing (—SH) group	Thioether (SC) group	Additional carboxyl (—COOH) group, acidic
Aspartic acid (Asp)	**Lysine (Lys)**	**Arginine (Arg)**	**Asparagine (Asn)**	**Glutamine (Gln)**
				
Additional Carboxyl (—COOH) group, acidic	Additional amino (—NH_2) group, basic	Additional amino (—NH_2) group, basic	Additional amino (—NH_2) group, basic	Additional amino (—NH_2) group, basic
Phenylalanine (Phe)	**Tyrosine (Tyr)**	**Histidine (His)**	**Tryptophan (Trp)**	**Proline (Pro)**
				
Cyclic	Cyclic	Heterocyclic	Heterocyclic	Heterocyclic

*Shown are the amino acid names, including the three-letter abbreviation in parentheses (above), their structural formulas (center), and characteristic R group (in green). Note that cysteine and methionine are the only amino acids that contain sulfur.

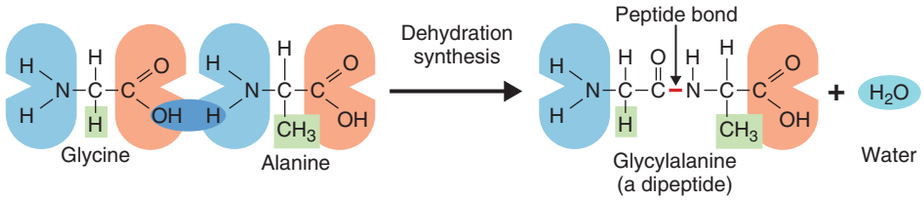


Figure 2.14 Peptide bond formation by dehydration synthesis. The amino acids glycine and alanine combine to form a dipeptide. The newly formed bond between the carbon atom of glycine and the nitrogen atom of alanine is called a peptide bond.

Q How are amino acids related to proteins?

the amino ($—NH_2$) group of another (Figure 2.14). The bonds between amino acids are called **peptide bonds.** For every peptide bond formed between two amino acids, one water molecule is released; thus, peptide bonds are formed by dehydration synthesis. The resulting compound in Figure 2.14 is called a *dipeptide* because it consists of two amino acids joined by a peptide bond. Adding another amino acid to a dipeptide would form a *tripeptide*. Further additions of amino acids would produce a long, chainlike molecule called a *peptide* (4–9 amino acids) or *polypeptide* (10–2000 or more amino acids).

Levels of Protein Structure

Proteins vary tremendously in structure. Different proteins have different architectures and different three-dimensional shapes. This variation in structure is directly related to their diverse functions.

When a cell makes a protein, the polypeptide chain folds spontaneously to assume a certain shape. One reason for folding of the polypeptide is that some parts of a protein are attracted to water and other parts are repelled by it. In practically every case, the function of a protein depends on its ability to recognize and bind to some other molecule. For example, an enzyme binds specifically with its substrate. A hormonal protein binds to a receptor on a cell whose function it will alter. An antibody binds to an antigen (foreign substance) that has invaded the body. The unique shape of each protein permits it to interact with specific other molecules in order to carry out specific functions.

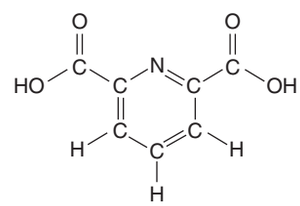
Proteins are described in terms of four levels of organization: primary, secondary, tertiary, and quaternary. The *primary structure* is the unique sequence in which the amino acids are linked together to form a polypeptide chain (Figure 2.15a). This sequence is genetically determined. Alterations in sequence can have profound metabolic effects. For example, a single incorrect amino acid in a blood protein can produce the deformed hemoglobin molecule characteristic of sickle cell disease. But proteins do not exist as long, straight chains. Each polypeptide chain folds and coils in specific ways into a relatively compact structure with a characteristic three-dimensional shape.

A protein's *secondary structure* is the localized, repetitious twisting or folding of the polypeptide chain. This aspect of a protein's shape results from hydrogen bonds joining the atoms of peptide bonds at different locations along the polypeptide chain. The two types of secondary protein structures are clockwise spirals called *helices* (singular: *helix*) and pleated sheets, which form from roughly parallel portions of the chain (Figure 2.15b). Both structures are held together by hydrogen bonds between oxygen or nitrogen atoms that are part of the polypeptide's backbone.

Tertiary structure refers to the overall three-dimensional structure of a polypeptide chain (Figure 2.15c). The folding is not repetitive or predictable, as in secondary structure. Whereas secondary structure involves hydrogen bonding between atoms of the amino and carboxyl groups involved in the peptide bonds, tertiary structure involves several interactions between various amino acid side groups in the polypeptide chain. For example, amino acids with nonpolar (hydrophobic) side groups usually interact at the core of the protein, out of contact with water. This *hydrophobic interaction* helps contribute to tertiary structure. Hydrogen bonds between side groups, and ionic bonds between oppositely charged side groups, also contribute to tertiary

Clinical Case

The host's phagocytes cannot easily digest D-forms of amino acids, such as D-glutamic acid found in the capsules of *B. anthracis*. Therefore, infection can develop. The CDC investigator's mention of animal hides gives DeeAnn an idea. Jonathan plays West African drums called *djembe*; the drum skins are made from dried imported goat hides from West Africa. Although most of these hides are legally imported, some slip through the cracks. It's possible that the hides on Jonathan's drums have been illegally imported and therefore have not been inspected by the U.S. Department of Agriculture. To create *djembe* drums, the hides are soaked in water, stretched over the drum body, and then scraped and sanded. The scraping and sanding generates a large amount of aerosolized dust as the hides dry. Sometimes this dust contains *B. anthracis* endospores, which contain dipicolinic acid.



What is the functional group in dipicolinic acid? See the figure above.

25 40 42 46

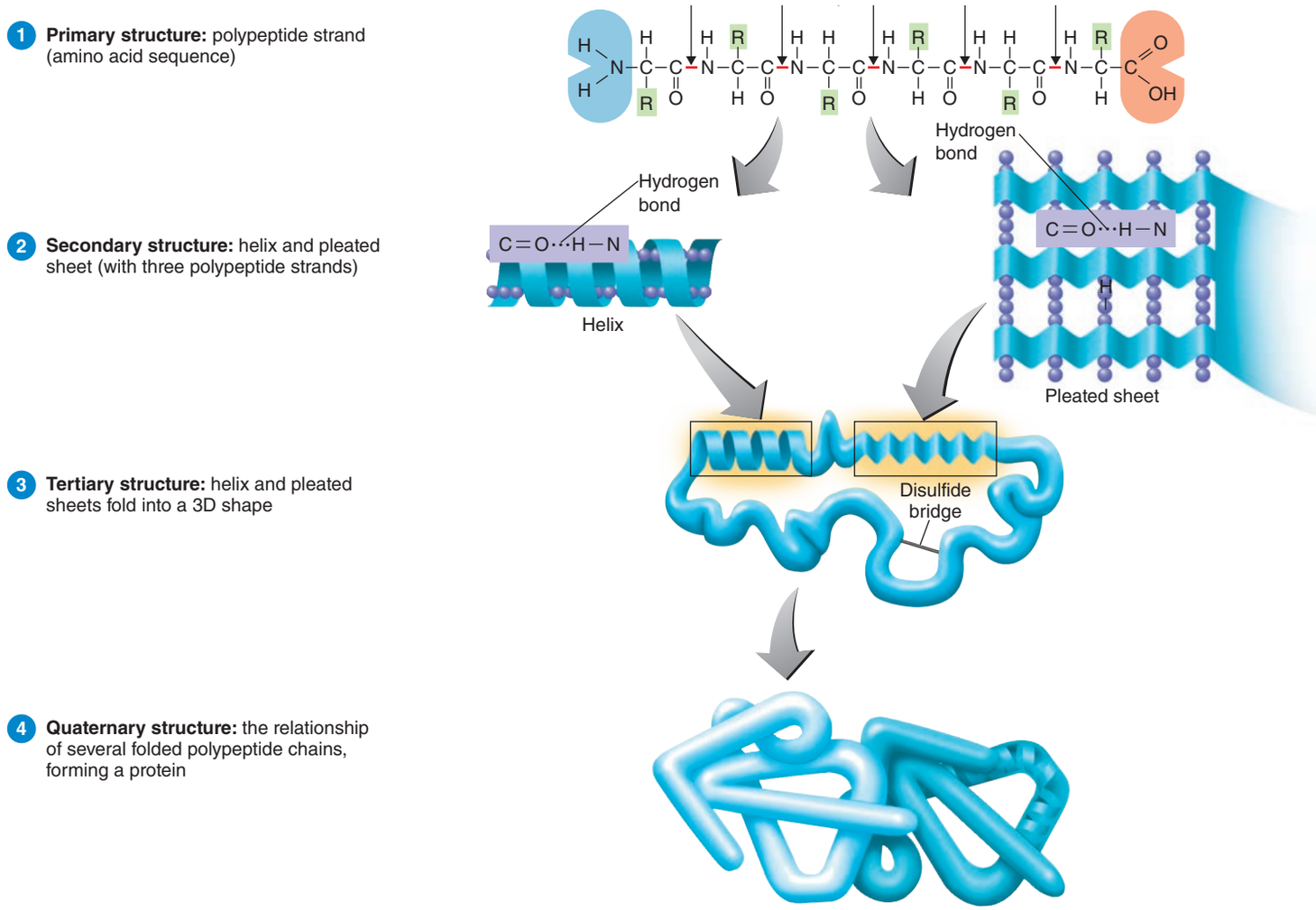


Figure 2.15 Protein structure. 1 Primary structure, the amino acid sequence. 2 Secondary structures: helix and pleated sheet. 3 Tertiary structure, the overall three-dimensional folding of a polypeptide chain. 4 Quaternary structure, the relationship between several polypeptide chains that make up a protein. Shown here is the quaternary structure of a hypothetical protein composed of two polypeptide chains.

Q What property of a protein enables it to carry out specific functions?

structure. Proteins that contain the amino acid cysteine form strong covalent bonds called *disulfide bridges*. These bridges form when two cysteine molecules are brought close together by the folding of the protein. Cysteine molecules contain sulfhydryl groups (—SH), and the sulfur of one cysteine molecule bonds to the sulfur on another, forming (by the removal of hydrogen atoms) a disulfide bridge (S—S) that holds parts of the protein together.

Some proteins have a *quaternary structure,* which consists of an aggregation of two or more individual polypeptide chains (subunits) that operate as a single functional unit. Figure 2.15d shows a hypothetical protein consisting of two polypeptide chains. More commonly, proteins have two or more kinds of polypeptide subunits. The bonds that hold a quaternary structure together are basically the same as those that maintain tertiary structure. The overall shape of a protein may be globular (compact and roughly spherical) or fibrous (threadlike).

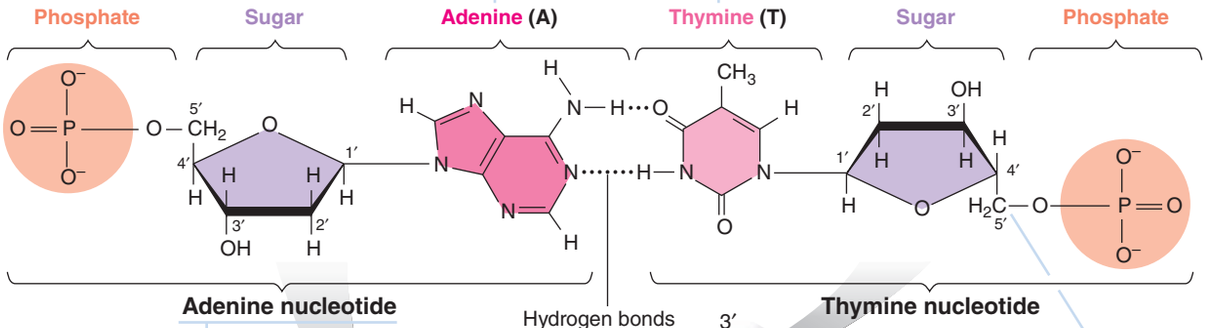
If a protein encounters a hostile environment in terms of temperature, pH, or salt concentrations, it may unravel and lose its characteristic shape. This process is called **denaturation** (see Figure 5.6, page 115). As a result of denaturation, the protein is no longer functional. This process will be discussed in more detail in Chapter 5 with regard to denaturation of enzymes.

The proteins we have been discussing are *simple proteins,* which contain only amino acids. *Conjugated proteins* are combinations of amino acids with other organic or inorganic components. Conjugated proteins are named by their non–amino acid component. Thus, glycoproteins contain sugars, nucleoproteins contain nucleic acids, metalloproteins contain metal atoms, lipoproteins contain lipids, and phosphoproteins

FOUNDATION FIGURE 2.16

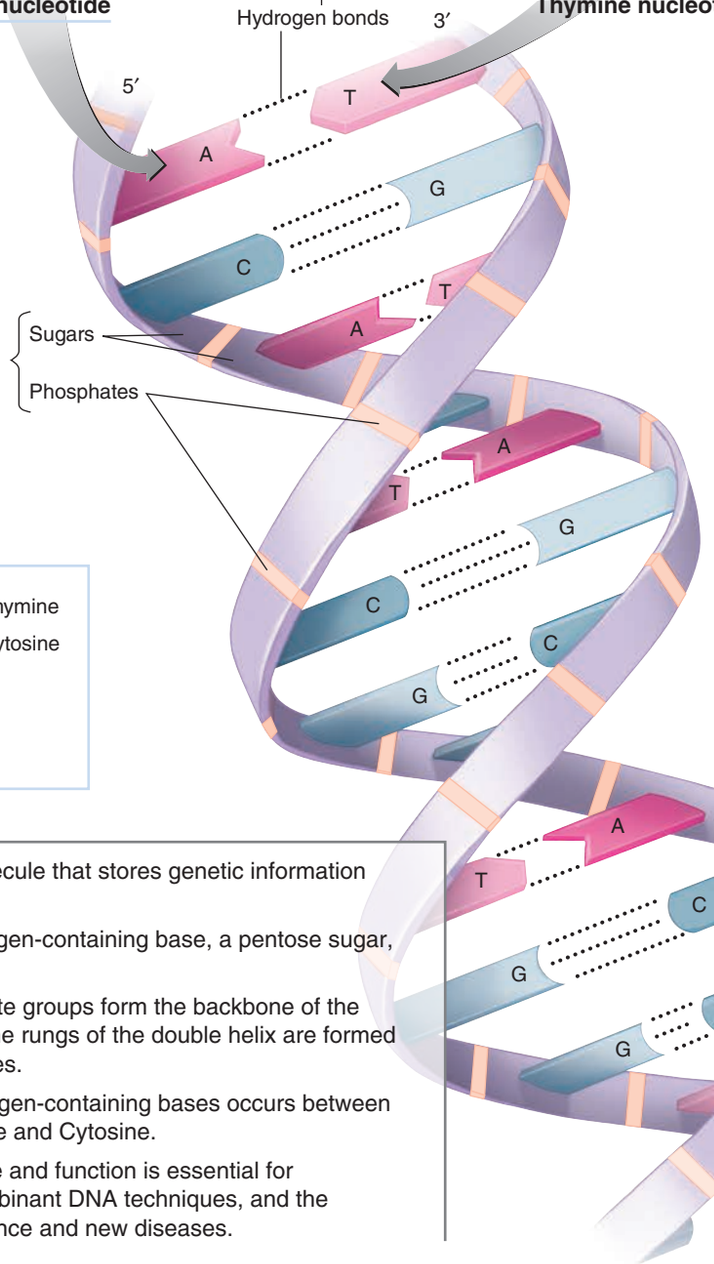
The Structure of DNA

Adenine and Thymine (as well as Cytosine and Guanine, not shown here) are nitrogenous bases, or nucleobases.



Individual DNA nucleotides are composed of a deoxyribose sugar molecule covalently bonded to a phosphate group at the 5′ carbon, and to a nitrogen-containing base at the 1′ carbon. The two nucleotides shown here are held together by hydrogen bonds.

The carbon atoms in the sugars are identified by adding a marker, ′ (for example, 5′, pronounced "5-prime"). This differentiates them from the carbon atoms in the nucleobases, such as Thymine.



Sugar-phosphate backbone

Sugars

Phosphates

The sugar-phosphate backbone of one strand is upside down, or antiparallel, relative to the backbone of the other strand.

DNA double helix

DNA's double-helical, ladderlike form is made up of many nucleotide base pairs, forming the rungs; and the repeating sugar-phosphate combination, forming the backbone.

Key

Adenine	A	T	Thymine
Guanine	G	C	Cytosine
Deoxyribose sugar			
Phosphate			
Hydrogen bond			

KEY CONCEPTS

- DNA is a double-stranded molecule that stores genetic information in all cells.
- A nucleotide consists of a nitrogen-containing base, a pentose sugar, and a phosphate group.
- Alternating sugar and phosphate groups form the backbone of the double helix (twisted ladder); the rungs of the double helix are formed by the nitrogen-containing bases.
- Complementary pairing of nitrogen-containing bases occurs between Adenine and Thymine; Guanine and Cytosine.
- Familiarity with DNA's structure and function is essential for understanding genetics, recombinant DNA techniques, and the emergence of antibiotic resistance and new diseases.

contain phosphate groups. Phosphoproteins are important regulators of activity in eukaryotic cells. Bacterial synthesis of phosphoproteins may be important for the survival of bacteria such as *Legionella pneumophila* that grow inside host cells.

CHECK YOUR UNDERSTANDING

What two functional groups are in all amino acids? **2-10**

Nucleic Acids

In 1944, three American microbiologists—Oswald Avery, Colin MacLeod, and Maclyn McCarty—discovered that a substance called **deoxyribonucleic acid (DNA)** is the substance of which genes are made. Nine years later, James Watson and Francis Crick, working with molecular models and X-ray information supplied by Maurice Wilkins and Rosalind Franklin, identified the physical structure of DNA. In addition, Crick suggested a mechanism for DNA replication and how it works as the hereditary material. DNA and another substance called **ribonucleic acid (RNA)** are together referred to as **nucleic acids** because they were first discovered in the nuclei of cells. Just as amino acids are the structural units of proteins, nucleotides are the structural units of nucleic acids.

Each **nucleotide** has three parts: a nitrogen-containing base, a pentose (five-carbon) sugar (either **deoxyribose** or **ribose**), and a phosphate group (phosphoric acid). The nitrogen-containing bases are cyclic compounds made up of carbon, hydrogen, oxygen, and nitrogen atoms. The bases are named adenine (A), thymine (T), cytosine (C), guanine (G), and uracil (U). A and G are double-ring structures called **purines,** whereas T, C, and U are single-ring structures referred to as **pyrimidines.**

Nucleotides are named according to their nitrogen-containing base. Thus, a nucleotide containing thymine is a *thymine nucleotide,* one containing adenine is an *adenine nucleotide,* and so on. The term **nucleoside** refers to the combination of a purine or pyrimidine plus a pentose sugar; it does not contain a phosphate group.

DNA

According to the model proposed by Watson and Crick, a DNA molecule consists of two long strands wrapped around each other to form a **double helix** (Figure 2.16). The double helix looks like a twisted ladder, and each strand is composed of many nucleotides.

Every strand of DNA composing the double helix has a "backbone" consisting of alternating deoxyribose sugar and phosphate groups. The deoxyribose of one nucleotide is joined to the phosphate group of the next. (Refer to Figure 8.3, page 207, to see how nucleotides are bonded.) The nitrogen-containing bases make up the rungs of the ladder. Note that the purine A is always paired with the pyrimidine T and that the purine G is always paired with the pyrimidine C. The bases are held together by hydrogen bonds; A and T are held by two hydrogen bonds, and G and C by three. DNA does not contain uracil (U).

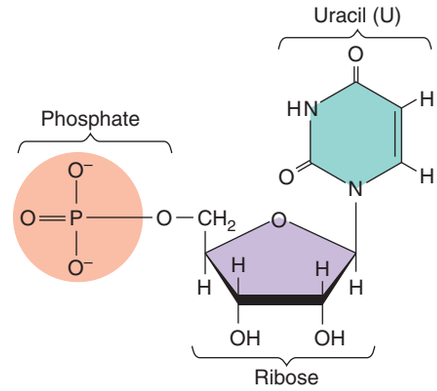


Figure 2.17 A uracil nucleotide of RNA.

How are DNA and RNA similar in structure?

The order in which the nitrogen base pairs occur along the backbone is extremely specific and in fact contains the genetic instructions for the organism. Nucleotides form genes, and a single DNA molecule may contain thousands of genes. Genes determine all hereditary traits, and they control all the activities that take place within cells.

One very important consequence of nitrogen-containing base pairing is that if the sequence of bases of one strand is known, then the sequence of the other strand is also known. For example, if one strand has the sequence . . . ATGC . . . , then the other strand has the sequence . . . TACG . . . . Because the sequence of bases of one strand is determined by the sequence of bases of the other, the bases are said to be *complementary*. The actual transfer of information becomes possible because of DNA's unique structure and will be discussed further in Chapter 8.

RNA

RNA, the second principal kind of nucleic acid, differs from DNA in several respects. Whereas DNA is double-stranded, RNA is usually single-stranded. The five-carbon sugar in the RNA nucleotide is ribose, which has one more oxygen atom than deoxyribose. Also, one of RNA's bases is uracil (U) instead of thymine (Figure 2.17). The other three bases (A, G, C) are the same as DNA. Three major kinds of RNA have been identified in cells. They are **messenger RNA (mRNA), ribosomal RNA (rRNA),** and **transfer RNA (tRNA),** each of which has a specific role in protein synthesis (see Chapter 8).

A comparison between DNA and RNA is presented in Table 2.6.

CHECK YOUR UNDERSTANDING

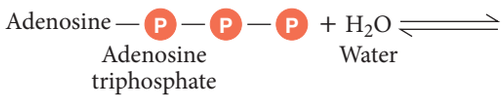
How do DNA and RNA differ? **2-11**

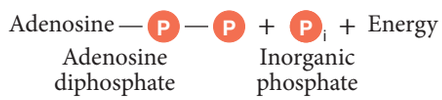
TABLE 2.6 Comparison between DNA and RNA

Backbone	DNA	RNA
Strands	Double-stranded in cells and most DNA viruses to form a double helix; single-stranded in some viruses (parvoviruses).	Single-stranded in celis and most RNA viruses; double-stranded in some viruses (reoviruses).
Composition	The sugar is deoxyribose.	The sugar is ribose.
	The nitrogen-containing bases are cytosine (C), guanine (G), adenine (A), and thymine (T).	The nitrogen-containing bases are cytosine (C), guanine (G), adenine (A), and uracil (U).
Function	Determines all hereditary traits.	Protein synthesis.

Adenosine Triphosphate (ATP)

Adenosine triphosphate (ATP) is the principal energy-carrying molecule of all cells and is indispensable to the life of the cell. It stores the chemical energy released by some chemical reactions, and it provides the energy for reactions that require energy. ATP consists of an adenosine unit, composed of adenine and ribose, with three phosphate groups (Ⓟ) attached (**Figure 2.18**). In other words, it is an adenine nucleotide (also called adenosine monophosphate, or AMP) with two extra phosphate groups. ATP is called a high-energy molecule because it releases a large amount of usable energy when the third phosphate group is hydrolyzed to become **adenosine diphosphate (ADP).** This reaction can be represented as follows:





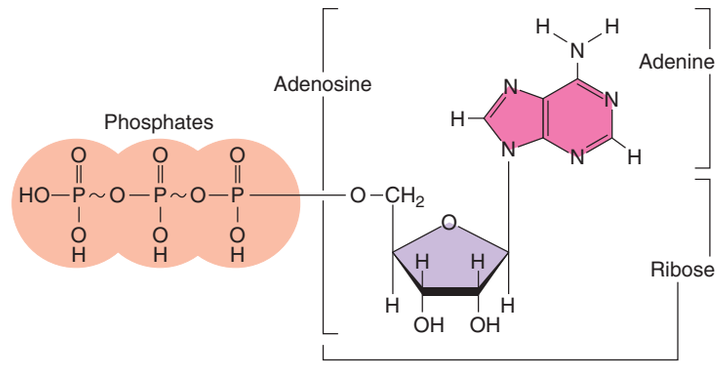


Figure 2.18 The structure of ATP. High-energy phosphate bonds are indicated by wavy lines. When ATP breaks down to ADP and inorganic phosphate, a large amount of chemical energy is released for use in other chemical reactions.

Q How is ATP similar to a nucleotide in RNA? In DNA?

A cell's supply of ATP at any particular time is limited. Whenever the supply needs replenishing, the reaction goes in the reverse direction; the addition of a phosphate group to ADP and the input of energy produces more ATP. The energy required to attach the terminal phosphate group to ADP is supplied by the cell's various oxidation reactions, particularly the oxidation of glucose. ATP can be made in every cell, where its potential energy is released when needed.

CHECK YOUR UNDERSTANDING

✓ Which can provide more energy for a cell and why: ATP or ADP? **2-12**

Clinical Case Resolved

The functional group in dipicolinic acid is carboxyl. *B. anthracis* infection is contracted by contact, ingestion, or inhalation of the endospores. In Jonathan's case, the process of stretching, scraping, and sanding the goat hides had created dust that settled on the drum skin and any surrounding crevices. *B. anthracis* endospores became airborne, or aerosolized, whenever Jonathan beat on the drum. He makes a full recovery, and from now on he makes certain that all parts of any drum he purchases have been legally imported.

Study Outline

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Study microbiology where it matters: everywhere.
Check out the Study Area of MasteringMicrobiology to explore Interactive Microbiology. Study on-the-go with Dynamic Study Modules. Check your understanding with chapter quizzes and animations—and much more!

Introduction (p. 24)

1. The science of the interaction between atoms and molecules is called chemistry.
2. The metabolic activities of microorganisms involve complex chemical reactions.
3. Microbes break down nutrients to obtain energy and to make new cells.

The Structure of Atoms (pp. 25–26)

1. An atom is the smallest unit of a chemical element that exhibits the properties of that element.
2. Atoms consist of a nucleus, which contains protons and neutrons, and electrons, which move around the nucleus.
3. The atomic number is the number of protons in the nucleus; the total number of protons and neutrons is the atomic weight.

Chemical Elements (pp. 25–26)

4. Atoms with the same number of protons and the same chemical behavior are classified as the same chemical element.
5. Chemical elements are designated by abbreviations called chemical symbols.
6. About 26 elements are commonly found in living cells.
7. Atoms that have the same atomic number (are of the same element) but different atomic weights are called isotopes.

Electronic Configurations (p. 26)

8. In an atom, electrons are arranged around the nucleus in electron shells.
9. Each shell can hold a characteristic maximum number of electrons.
10. The chemical properties of an atom are due largely to the number of electrons in its outermost shell.

How Atoms Form Molecules: Chemical Bonds (pp. 27–30)

1. Molecules are made up of two or more atoms; molecules consisting of at least two different kinds of atoms are called compounds.
2. Atoms form molecules in order to fill their outermost electron shells.
3. Attractive forces that bind two atoms together are called chemical bonds.
4. The combining capacity of an atom—the number of chemical bonds the atom can form with other atoms—is its valence.

Ionic Bonds (p. 27)

5. A positively or negatively charged atom or group of atoms is called an ion.
6. A chemical attraction between ions of opposite charge is called an ionic bond.
7. To form an ionic bond, one ion is an electron donor, and the other ion is an electron acceptor.

Covalent Bonds (pp. 27–28)

8. In a covalent bond, atoms share pairs of electrons.
9. Covalent bonds are stronger than ionic bonds and are far more common in organic molecules.

Hydrogen Bonds (pp. 28–29)

10. A hydrogen bond exists when a hydrogen atom covalently bonded to one oxygen or nitrogen atom is attracted to another oxygen or nitrogen atom.
11. Hydrogen bonds form weak links between different molecules or between parts of the same large molecule.

Molecular Weight and Moles (pp. 29–30)

12. The molecular weight is the sum of the atomic weights of all the atoms in a molecule.
13. A mole of an atom, ion, or molecule is equal to its atomic or molecular weight expressed in grams.

Chemical Reactions (pp. 30–31)

1. Chemical reactions are the making or breaking of chemical bonds between atoms.
2. A change of energy occurs during chemical reactions.
3. Endergonic reactions require more energy than they release; exergonic reactions release more energy.
4. In a synthesis reaction, atoms, ions, or molecules are combined to form a larger molecule.
5. In a decomposition reaction, a larger molecule is broken down into its component molecules, ions, or atoms.
6. In an exchange reaction, two molecules are decomposed, and their subunits are used to synthesize two new molecules.
7. The products of reversible reactions can readily revert to form the original reactants.

Important Biological Molecules (pp. 31–46)

Inorganic Compounds (pp. 32–34)

1. Inorganic compounds are usually small, ionically bonded molecules.

Water (p. 32)

2. Water is the most abundant substance in cells.
3. Because water is a polar molecule, it is an excellent solvent.
4. Water is a reactant in many of the decomposition reactions of digestion.
5. Water is an excellent temperature buffer.

Acids, Bases, and Salts (pp. 32–33)

6. An acid dissociates into H^+ and anions.
7. A base dissociates into OH^- and cations.
8. A salt dissociates into negative and positive ions, neither of which is H^+ or OH^-.

Acid–Base Balance: The Concept of pH (pp. 33–34)

9. The term *pH* refers to the concentration of H^+ in a solution.

10. A solution of pH 7 is neutral; a pH value below 7 indicates acidity; pH above 7 indicates alkalinity.
11. The pH inside a cell and in culture media is stabilized with pH buffers.

Organic Compounds (pp. 34–46)

1. Organic compounds always contain carbon and hydrogen.
2. Carbon atoms form up to four bonds with other atoms.
3. Organic compounds are mostly or entirely covalently bonded.

Structure and Chemistry (pp. 34–36)

4. A chain of carbon atoms forms a carbon skeleton.
5. Functional groups of atoms are responsible for most of the properties of organic molecules.
6. The letter *R* may be used to denote the remainder of an organic molecule.
7. Frequently encountered classes of molecules are R—OH (alcohols) and R—COOH (organic acids).
8. Small organic molecules may combine into very large molecules called macromolecules.
9. Monomers usually bond together by dehydration synthesis, or condensation reactions, that form water and a polymer.
10. Organic molecules may be broken down by hydrolysis, a reaction involving the splitting of water molecules.

Carbohydrates (pp. 36–37)

11. Carbohydrates are compounds consisting of atoms of carbon, hydrogen, and oxygen, with hydrogen and oxygen in a 2:1 ratio.
12. Monosaccharides contain from three to seven carbon atoms.
13. Isomers are two molecules with the same chemical formula but different structures and properties—for example, glucose ($C_6H_{12}O_6$) and fructose ($C_6H_{12}O_6$).
14. Monosaccharides may form disaccharides and polysaccharides by dehydration synthesis.

Lipids (pp. 37–39)

15. Lipids are a diverse group of compounds distinguished by their insolubility in water.
16. Simple lipids (fats) consist of a molecule of glycerol and three molecules of fatty acids.
17. A saturated lipid has no double bonds between carbon atoms in the fatty acids; an unsaturated lipid has one or more double bonds. Saturated lipids have higher melting points than unsaturated lipids.
18. Phospholipids are complex lipids consisting of glycerol, two fatty acids, and a phosphate group.
19. Steroids have carbon ring structures; sterols have a functional hydroxyl group.

Proteins (pp. 39–45)

20. Amino acids are the building blocks of proteins.
21. Amino acids consist of carbon, hydrogen, oxygen, nitrogen, and sometimes sulfur.
22. Twenty amino acids occur naturally in proteins.
23. By linking amino acids, peptide bonds (formed by dehydration synthesis) allow the formation of polypeptide chains.
24. Proteins have four levels of structure: primary (sequence of amino acids), secondary (helices or pleated), tertiary (overall three-dimensional structure of a polypeptide), and quaternary (two or more polypeptide chains).
25. Conjugated proteins consist of amino acids combined with inorganic or other organic compounds.

Nucleic Acids (pp. 45–46)

26. Nucleic acids—DNA and RNA—are macromolecules consisting of repeating nucleotides.
27. A nucleotide is composed of a pentose, a phosphate group, and a nitrogen-containing base. A nucleoside is composed of a pentose and a nitrogen-containing base.
28. A DNA nucleotide consists of deoxyribose (a pentose) and one of the following nitrogen-containing bases: thymine or cytosine (pyrimidines) or adenine or guanine (purines).
29. DNA consists of two strands of nucleotides wound in a double helix. The strands are held together by hydrogen bonds between purine and pyrimidine nucleotides: AT and GC.
30. Genes consist of sequences of nucleotides.
31. An RNA nucleotide consists of ribose (a pentose) and one of the following nitrogen-containing bases: cytosine, guanine, adenine, or uracil.

Adenosine Triphosphate (ATP) (p. 46)

32. ATP stores chemical energy for various cellular activities.
33. When the bond to ATP's terminal phosphate group is hydrolyzed, energy is released.
34. The energy from oxidation reactions is used to regenerate ATP from ADP and inorganic phosphate.

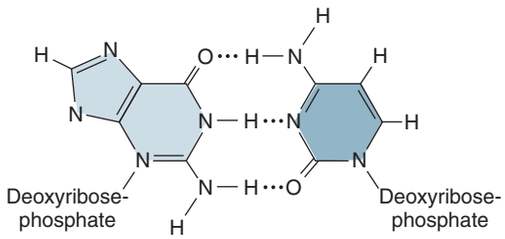
Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

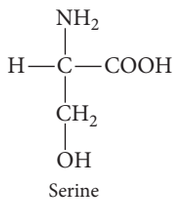
Review

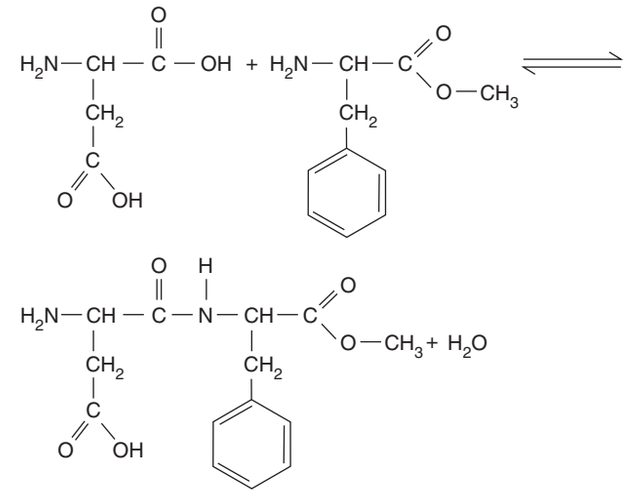
1. What is a chemical element?
2. DRAW IT Diagram the electronic configuration of a carbon atom.
3. What type of bond holds the following atoms together?
   - **a.** Li^+ and Cl^- in LiCl
   - **b.** carbon and oxygen atoms in methanol
   - **c.** oxygen atoms in O_2
   - **d.** a hydrogen atom of one nucleotide to a nitrogen or oxygen atom of another nucleotide in:



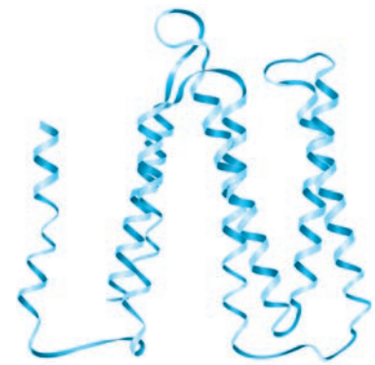
4. Classify the following types of chemical reactions.
   - **a.** glucose + fructose → sucrose + H_2O
   - **b.** lactose → glucose + galactose
   - **c.** $NH_4Cl + H_2O \rightarrow NH_4OH + HCl$
   - **d.** ATP ⇌ ADP + P_i
5. Bacteria use the enzyme urease to obtain nitrogen in a form they can use from urea in the following reaction:

$$\underset{\text{Urea}}{CO(NH_2)_2} + H_2O \rightarrow \underset{\text{Ammonia}}{2NH_3} + \underset{\text{Carbon dioxide}}{CO_2}$$

   What purpose does the enzyme serve in this reaction? What type of reaction is this?
6. Classify the following as subunits of either a carbohydrate, lipid, protein, or nucleic acid.
   - **a.** $CH3—(CH_2)_7—CH{=}CH—(CH_2)_7—COOH$ (Oleic acid)
   - **b.**  (Serine)
   - **c.** $C_6H_{12}O_6$
   - **d.** Thymine nucleotide
7. DRAW IT The artificial sweetener aspartame, or NutraSweet, is made by joining aspartic acid to methylated phenylalanine, as shown below.



   - **a.** What types of molecules are aspartic acid and phenylalanine?
   - **b.** What direction is the hydrolysis reaction (left to right or right to left)?
   - **c.** What direction is the dehydration synthesis reaction?
   - **d.** Circle the atoms involved in the formation of water.
   - **e.** Identify the peptide bond.
8. DRAW IT The following diagram shows the bacteriorhodopsin protein. Indicate the regions of primary, secondary, and tertiary structure. Does this protein have quaternary structure?



9. DRAW IT Draw a simple lipid, and show how it could be modified to a phospholipid.
10. NAME IT What type of microorganism has a chitin cell wall, has DNA that is contained in a nucleus, and has ergosterol in its plasma membrane?

Multiple Choice

Radioisotopes are frequently used to label molecules in a cell. The fate of atoms and molecules in a cell can then be followed. This process is the basis for questions 1–3.

1. Assume *E. coli* bacteria are grown in a nutrient medium containing the radioisotope ^{16}N. After a 48-hour incubation period, the ^{16}N would most likely be found in the *E. coli*'s
   a. carbohydrates.
   b. lipids.
   c. proteins.
   d. water.
   e. none of the above
2. If *Pseudomonas* bacteria are supplied with radioactively labeled cytosine, after a 24-hour incubation period this cytosine would most likely be found in the cells'
   a. carbohydrates.
   b. DNA.
   c. lipids.
   d. water.
   e. proteins.
3. If *E. coli* were grown in a medium containing the radioactive isotope ^{32}P, the ^{32}P would be found in all of the following molecules of the cell *except*
   a. ATP.
   b. carbohydrates.
   c. DNA.
   d. plasma membrane.
   e. none of the above
4. The optimum pH of *Acidithiobacillus* bacteria (pH 3,) is _____ times more acid than blood (pH 7).
   a. 4
   b. 10
   c. 100
   d. 1000
   e. 10,000
5. The best definition of ATP is that it is
   a. a molecule stored for food use.
   b. a molecule that supplies energy to do work.
   c. a molecule stored for an energy reserve.
   d. a molecule used as a source of phosphate.
6. Which of the following is an organic molecule?
   a. H_2O (water)
   b. O_2 (oxygen)
   c. $C_{18}H_{29}SO_3$ (Styrofoam)
   d. FeO (iron oxide)
   e. $F_2C{=}CF_2$ (Teflon)

Classify each of the molecules on the left as an acid, base, or salt. The dissociation products of the molecules are shown to help you.

7. $HNO_3 \rightarrow H^+ + NO_3^-$
8. $H_2SO_4 \rightarrow 2H^+ + SO_4^{2-}$
9. $NaOH \rightarrow Na^+ + OH^-$
10. $MgSO_4 \rightarrow Mg^{2+} + SO_4^{2-}$

a. acid
b. base
c. salt

Analysis

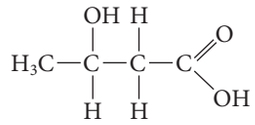
1. When you blow bubbles into a glass of water, the following reactions take place:

$$H_2O + CO_2 \xrightarrow{A} H_2CO_3 \xrightarrow{B} H^+ + HCO_3^-$$

   a. What type of reaction is *A*?
   b. What does reaction *B* tell you about the type of molecule H_2CO_3 is?
2. What are the common structural characteristics of ATP and DNA molecules?
3. What happens to the relative amount of unsaturated lipids in the plasma membrane when *E. coli* bacteria grown at 25°C are then grown at 37°C?
4. Giraffes, termites, and koalas eat only plant matter. Because animals cannot digest cellulose, how do you suppose these animals get nutrition from the leaves and wood they eat?

Clinical Applications and Evaluation

1. *Ralstonia* bacteria make poly-β-hydroxybutyrate (PHB), which is used to make a biodegradable plastic. PHB consists of many of the monomers shown below. What type of molecule is PHB? What is the most likely reason a cell would store this molecule?



2. *Acidithiobacillus ferrooxidans* was responsible for destroying buildings in the Midwest by causing changes in the earth. The original rock, which contained lime ($CaCO_3$) and pyrite (FeS_2), expanded as bacterial metabolism caused gypsum ($CaSO_4$) crystals to form. How did *A. ferrooxidans* bring about the change from lime to gypsum?
3. Newborn babies are tested for phenylketonuria (PKU), an inherited disease. Individuals with this disease are missing an enzyme to convert phenylalanine (phe) to tyrosine; the resulting accumulation of phe can cause mental retardation, brain damage, and seizures. The Guthrie test for PKU involves culturing *Bacillus subtilis,* which requires phe to grow. The bacteria are grown on media with a drop of the baby's blood.
   a. What type of chemical is phenylalanine?
   b. What does "no growth" in the Guthrie test mean?
   c. Why must individuals with PKU avoid the sweetener aspartame?
4. The antibiotic amphotericin B causes leaks into cells by combining with sterols in the plasma membrane. Would you expect to use amphotericin B against a bacterial infection? A fungal infection? Offer a reason why amphotericin B has severe side effects in humans.
5. You can smell sulfur when boiling eggs. What amino acids do you expect in the egg?

In the Clinic

Mike is one of your regular patients at the homeless clinic where you are a volunteer nurse. He has a severe cough and is quite thin. Last week you sent Mike's sputum sample to the laboratory and requested a Gram stain and acid-fast stain. The stain results are now posted in his chart: It says "acid-fast +." **What infection does Mike have?**

Hint: Read the acid-fast stain section on page 66.

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

3 Observing Microorganisms Through a Microscope

Microorganisms are much too small to be seen with the unaided eye; they must be observed with a microscope. The word *microscope* is derived from the Latin word *micro* (small) and the Greek word *skopos* (to look at). Modern microbiologists use microscopes that produce, with great clarity, magnifications that range from ten to thousands of times greater than those of van Leeuwenhoek's single lens (see Figure 1.2b on page 7). This chapter describes how different types of microscopes function and why one type might be used in preference to another. *Helicobacter pylori* (HĒ-lik-ō-bak'ter PĪ-lor-ē), shown in the photograph, is a spiral-shaped bacterium that was first seen in cadaver stomachs in 1886. The bacterium was largely ignored until the resolving ability of microscopes was improved. Microscopic examination of these bacteria is described in the Clinical Case.

Some microbes are more readily visible than others because of their larger size or more easily observable features. Many microbes, however, must undergo several staining procedures before their cell walls, capsules, and other structures lose their colorless natural state. The last part of this chapter explains some of the more commonly used methods of preparing specimens for examination through a light microscope.

You may wonder how we are going to sort, count, and measure the specimens we will study. To answer these questions, this chapter opens with a discussion of how to use the metric system for measuring microbes.

Helicobacter pylori **bacteria, a cause of ulcers in humans.**

Units of Measurement

LEARNING OBJECTIVE

3-1 List the units used to measure microorganisms.

When measuring microorganisms, we use the metric system. A major advantage of the metric system is that units relate to each other by factors of 10. Thus, 1 meter (m) equals 10 decimeters (dm) or 100 centimeters (cm) or 1000 millimeters (mm). Units in the U.S. system of measure do not have the advantage of easy conversion by a single factor of 10. For example, 3 feet, or 36 inches, equals 1 yard.

Microorganisms are measured in even smaller units, such as micrometers and nanometers. A **micrometer (μm)** equals 0.000001 m (10^{-6} m). The prefix *micro* indicates the unit following it should be divided by 1 million, or 10^6 (see the "Exponential Notation" section in Appendix B). A **nanometer (nm)** equals 0.000000001 m (10^{-9} m). Angstrom (Å) was previously used for 10^{-10} m, or 0.1 nm.

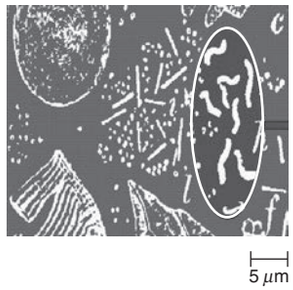
Table 3.1 presents the basic metric units of length and some U.S. equivalents.

CHECK YOUR UNDERSTANDING

How many nanometers is 10 μm? **3-1**

Clinical Case: Microscopic Mayhem

Maryanne, a 42-year-old marketing executive and mother of three, has been experiencing recurrent stomach pain, which seems to be getting worse. She jokes her husband should buy stock in Pepto-Bismol, because she buys so much of it.



At her husband's urging, she makes an appointment to see her primary care physician. After hearing that Maryanne feels better immediately after taking Pepto-Bismol, the doctor suspects Maryanne may have a peptic ulcer associated with *Helicobacter pylori*.

What is *Helicobacter pylori*? Read on to find out.

52 61 66 67

Microscopy: The Instruments

LEARNING OBJECTIVES

3-2 Diagram the path of light through a compound microscope.

3-3 Define *total magnification* and *resolution*.

3-4 Identify a use for darkfield, phase-contrast, differential interference contrast, fluorescence, confocal, two-photon, and scanning acoustic microscopy, and compare each with brightfield illumination.

3-5 Explain how electron microscopy differs from light microscopy.

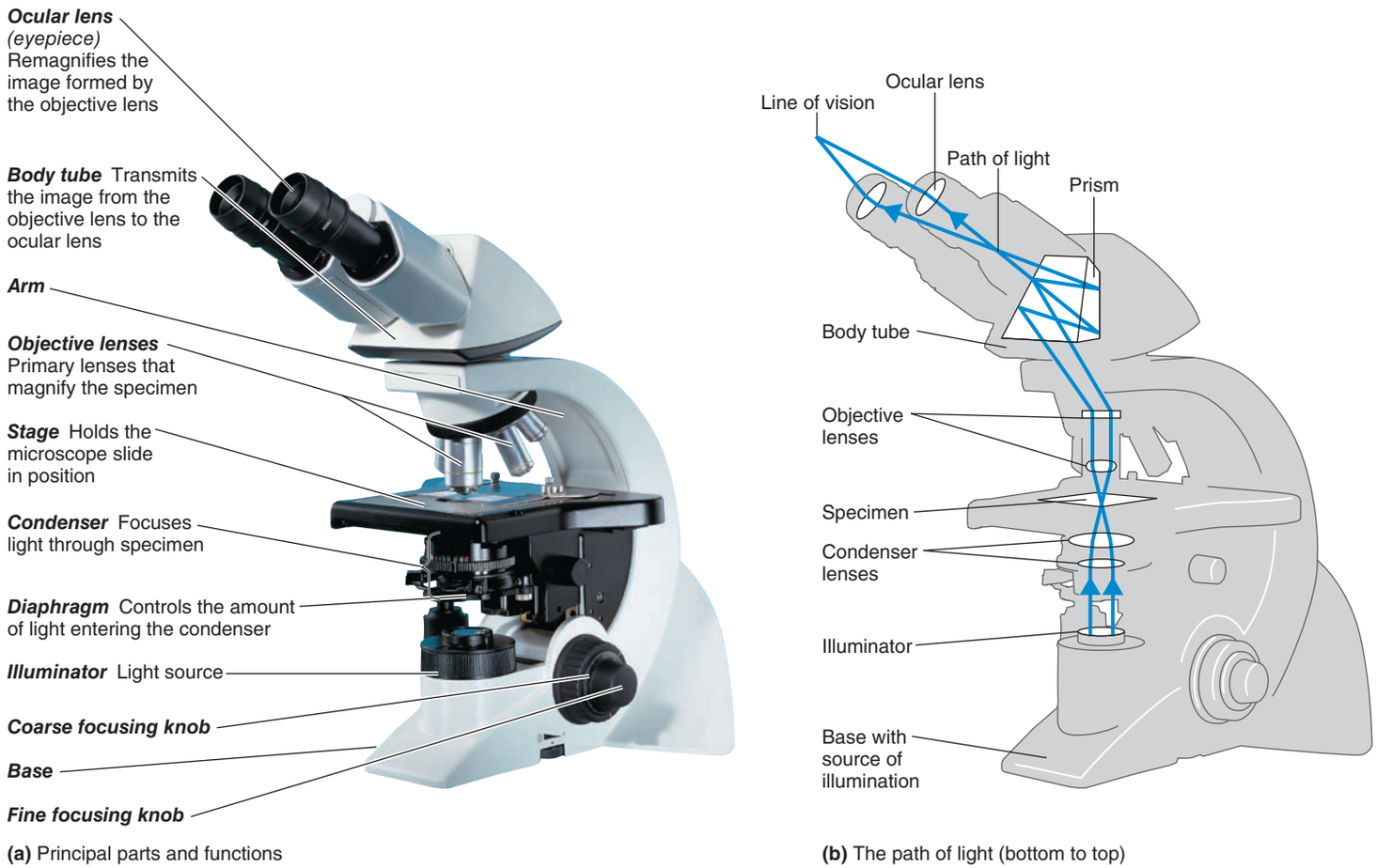
3-6 Identify uses for the transmission electron microscope (TEM), scanning electron microscope (SEM), and scanned-probe microscopes.

The simple microscope used by van Leeuwenhoek in the seventeenth century had only one lens and was similar to a magnifying glass. However, van Leeuwenhoek was the best lens grinder in the world in his day. His lenses were ground with such precision that a single lens could magnify a microbe 300×. His simple microscopes enabled him to be the first person to see bacteria (see Figure 1.2, page 7).

Contemporaries of van Leeuwenhoek, such as Robert Hooke, built compound microscopes, which have multiple lenses. In fact, a Dutch spectacle maker, Zaccharias Janssen, is credited with making the first compound microscope around 1600. However, these early compound microscopes were of poor quality and could not be used to see bacteria. It was not until about 1830 that a significantly better microscope was developed by Joseph Jackson Lister (the father of Joseph Lister).

TABLE 3.1 Metric Units of Length and U.S. Equivalents

Metric Unit	Meaning of Prefix	Metric Equivalent	U.S. Equivalent
1 kilometer (km)	*kilo* = 1000	1000 m = 10^3 m	3280.84 ft or 0.62 mi; 1 mi = 1.61 km
1 meter (m)		Standard unit of length	39.37 in or 3.28 ft or 1.09 yd
1 decimeter (dm)	*deci* = 1/10	0.1 m = 10^{-1} m	3.94 in
1 centimeter (cm)	*centi* = 1/100	0.01 m = 10^{-2} m	0.394 in; 1 in = 2.54 cm
1 millimeter (mm)	*milli* = 1/1000	0.001 m = 10^{-3} m	
1 micrometer (μm)	*micro* = 1/1,000,000	0.000001 m = 10^{-6} m	
1 nanometer (nm)	*nano* = 1/1,000,000,000	0.000000001 m = 10^{-9} m	
1 picometer (pm)	*pico* = 1/1,000,000,000,000	0.000000000001 m = 10^{-12} m	



(a) Principal parts and functions

(b) The path of light (bottom to top)

Figure 3.1 The compound light microscope.

Q What is the total magnification of a compound light microscope with objective lens magnification of 40× and ocular lens of 10×?

Various improvements to Lister's microscope resulted in the development of the modern compound microscope, the kind used in microbiology laboratories today. Microscopic studies of live specimens have revealed dramatic interactions between microbes (see the Applications of Microbiology box on page 54).

MM **Animation** Microscopy and Staining: Overview

Light Microscopy

Light microscopy refers to the use of any kind of microscope that uses visible light to observe specimens. Here we examine several types of light microscopy.

Compound Light Microscopy

A modern **compound light microscope (LM)** has a series of lenses and uses visible light as its source of illumination (**Figure 3.1a**). With a compound light microscope, we can examine very small specimens as well as some of their fine detail. A series of finely ground lenses (Figure 3.1b) forms a clearly focused image that is many times larger than the specimen itself. This magnification is achieved when light rays from an **illuminator,** the light source, pass through a **condenser,** which has lenses that direct the light rays through the specimen. From here, light rays pass into the **objective lenses,** the lenses closest to the specimen. The image of the specimen is magnified again by the **ocular lens,** or *eyepiece.*

ASM: The structure and function of microorganisms have been revealed by the use of microscopy (including brightfield phase-contrast, fluorescent, and electron).

We can calculate the **total magnification** of a specimen by multiplying the objective lens magnification (power) by the ocular lens magnification (power). Most microscopes used in microbiology have several objective lenses, including 10× (low power), 40× (high power), and 100× (oil immersion). Most ocular lenses magnify specimens by a factor of 10. Multiplying the magnification of a specific objective lens with that of the ocular, we see that the total magnifications would be 100× for low power, 400× for high power, and 1000× for oil immersion. Some compound light microscopes can achieve a total magnification of 2000× with the oil immersion lens.

What Is That Slime?

When bacteria grow, they often stay together in packs called biofilms. This can result in a slimy film on rocks, on food, inside pipes, and on implanted medical devices. Bacterial cells interact and exhibit multicellular organization (**Figure A**).

Pseudomonas aeruginosa can grow within a human without causing disease until the bacteria form a biofilm that overcomes the host's immune system. Biofilm-forming *P. aeruginosa* bacteria colonize the lungs of cystic fibrosis patients and are a leading cause of death in these patients (**Figure B**). Perhaps biofilms that lead to disease can be prevented by new drugs that destroy the inducer (discussed shortly).

Figure A *Paenibacillus*. As one small colony moves away from the parent colony, other groups of cells follow the first colony. Soon, all of the other bacteria join the relocation to form this spiraling colony.

Myxobacteria

Myxobacteria are found in decaying organic material and freshwater throughout the world. Although they are bacteria, many myxobacteria never exist as individual cells. *Myxococcus xanthus* cells appear to hunt in packs. In their natural aqueous habitat, *M. xanthus* cells form spherical colonies that surround prey bacteria, where they can secrete digestive enzymes and absorb the nutrients. On solid substrates, other myxobacterial cells glide over a solid surface, leaving slime trails that are followed by other cells. When food is scarce, the cells aggregate to form a mass. Cells within the mass differentiate into a fruiting body that consists of a slime stalk and clusters of spores, as shown in **Figure C**.

Vibrio

Aliivibrio fischeri is a bioluminescent bacterium that lives as a symbiont in the light-producing organ of squid and certain fish. When free-living, the bacteria are at a low concentration and do not give off light. However, when they grow in their host, they are highly concentrated, and each cell is induced to produce the enzyme luciferase, which is used in the chemical pathway of bioluminescence.

How Bacterial Group Behavior Works

Cell density alters gene expression in bacterial cells in a process called quorum sensing. In law, a quorum is the minimum number of members necessary to conduct business. *Quorum sensing* is the ability of bacteria to communicate and coordinate behavior. Bacteria that use quorum sensing produce and secrete a signaling chemical called an *inducer*. As the inducer diffuses into the surrounding medium, other bacterial cells move toward the source and begin producing inducer. The concentration of inducer increases with increasing cell numbers. This, in turn, attracts more cells and initiates synthesis of more inducer.

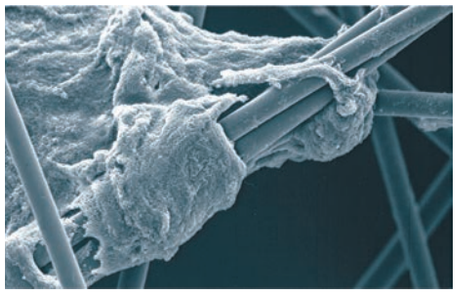
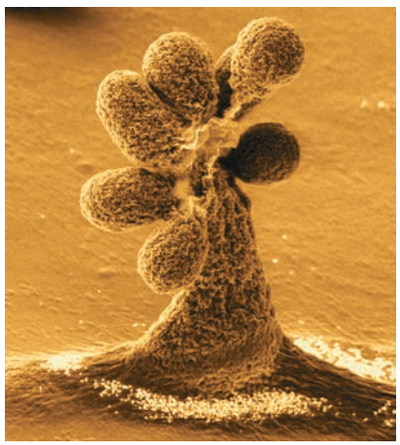


Figure B *Pseudomonas aeruginosa* biofilm.

Figure C A fruiting body of a myxobacterium.

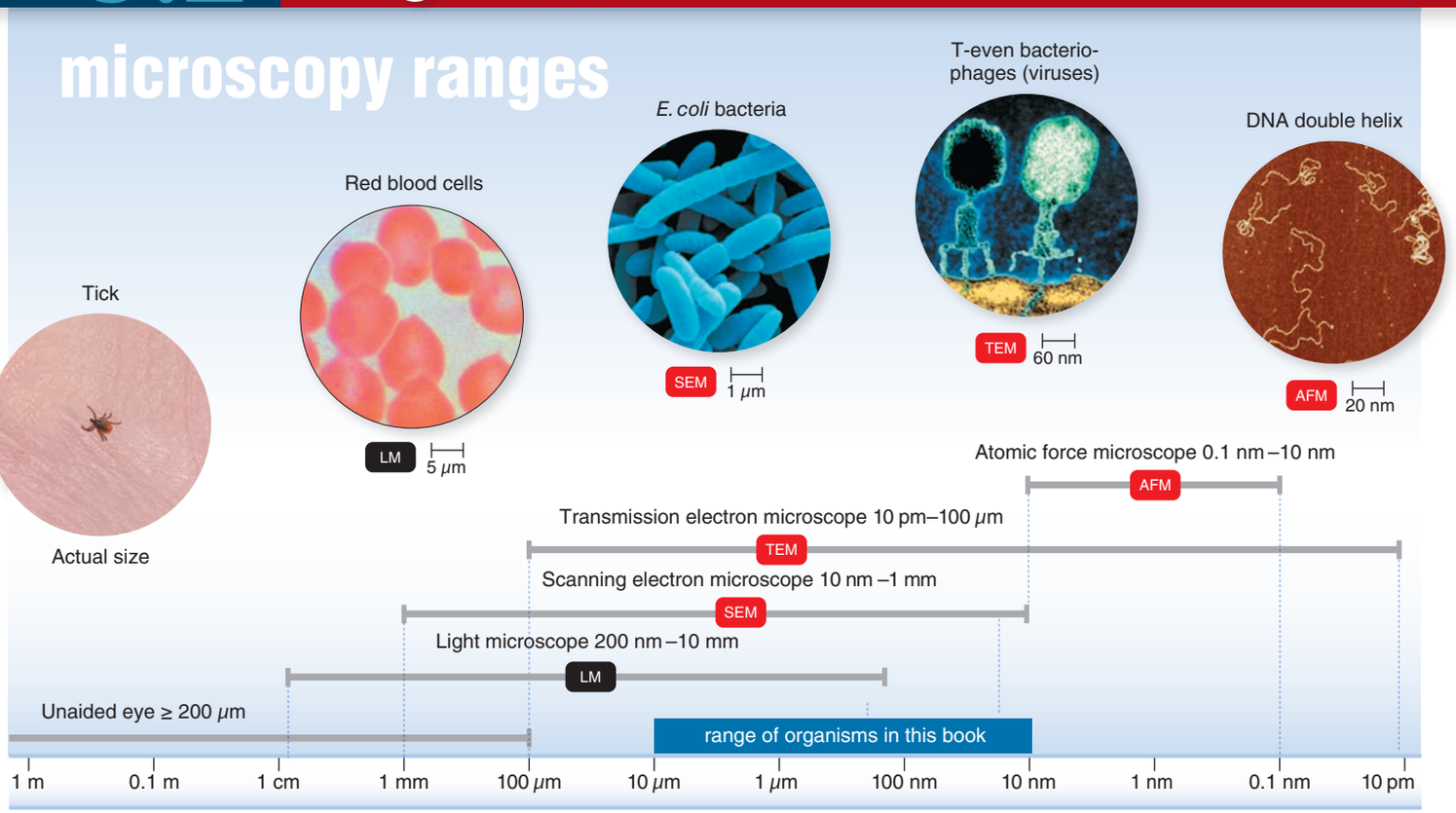


Resolution (also called *resolving power*) is the ability of the lenses to distinguish fine detail and structure. Specifically, it refers to the ability of the lenses to distinguish two points that are a specified distance apart. For example, if a microscope has a resolving power of 0.4 nm, it can distinguish two points if they are at least 0.4 nm apart. A general principle of microscopy is that the shorter the wavelength of light used in the instrument, the greater the resolution. The white light used in a compound light microscope has a relatively long wavelength and cannot resolve structures smaller than about 0.2 μm. This and other considerations limit the magnification achieved by the best compound light microscopes to about 2000×. By comparison, van Leeuwenhoek's microscopes had a resolution of 1 μm. **Figure 3.2** shows various specimens that can be resolved by the human eye and microscopes.

To obtain a clear, finely detailed image under a compound light microscope, specimens must contrast sharply with their *medium* (the substance in which they are suspended). To attain such contrast, we must change the refractive index of specimens from that of their medium. The **refractive index** is a measure of the light-bending ability of a medium. We change the refractive index of specimens by staining them, a procedure we will

FOUNDATION FIGURE 3.2

Microscopes and Magnification



KEY CONCEPTS

- Microscopes are used to magnify small objects.
- Because different microscopes have different resolution ranges, the size of a specimen determines which microscopes can be used to view the specimen effectively.
- Most micrographs shown in this textbook (like the ones above) have size bars and symbols to help you identify the actual size of the specimen and the type of microscope used for that image.
- A red icon indicates that a micrograph has been artificially colorized.
- Resolution increases with decreasing wavelength.

micro tip

If a bacterium is 1 micrometer long and your index finger is 6.5 cm long, how many of the bacteria can you place end-to-end on your finger? Answer: 32,500.

discuss shortly. Light rays move in a straight line through a single medium. After the specimen is stained, the specimen and its medium have different refractive indexes. When light rays pass through the two materials (the specimen and its medium), the rays change direction (refract) from a straight path by bending or changing angle at the boundary between the materials. This increases the image's contrast between the specimen and the medium. As the light rays travel away from the specimen, they spread out and enter the objective lens, and the image is thereby magnified.

To achieve high magnification (1000×) with good resolution, the objective lens must be small. Although we want light traveling through the specimen and medium to refract differently, we do not want to lose light rays after they have passed through the stained specimen. To preserve the direction of light rays at the highest magnification, immersion oil is placed between the glass slide and the oil immersion objective lens (**Figure 3.3**). The immersion oil has the same refractive index as glass, so the oil becomes part of the optics of the glass of the microscope. Unless immersion oil is used, light rays are refracted as they enter the air from the slide, and the objective lens would have to be increased in diameter to capture most of them. The oil has the same effect as increasing the objective lens diameter; therefore, it improves the resolving power of the lenses. If oil is not used with an oil immersion objective lens, the image has poor resolution and becomes fuzzy.

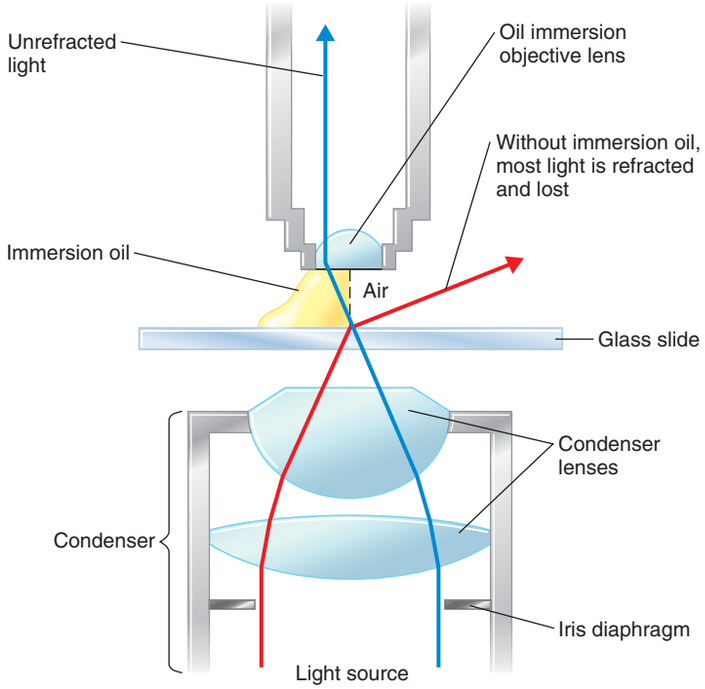


Figure 3.3 Refraction in the compound microscope using an oil immersion objective lens. Because the refractive indexes of the glass microscope slide and immersion oil are the same, the light rays do not refract when passing from one to the other when an oil immersion objective lens is used. Use of immersion oil is necessary at magnifications greater than 900×.

Q Why is immersion oil necessary at 1000× but not with the lower power objectives?

Under usual operating conditions, the field of vision in a compound light microscope is brightly illuminated. By focusing the light, the condenser produces a **brightfield illumination** (Figure 3.4a).

It is not always desirable to stain a specimen, but an unstained cell has little contrast with its surroundings and is therefore difficult to see. Unstained cells are more easily observed with the modified compound microscopes described in the next section.

CHECK YOUR UNDERSTANDING

- Through what lenses does light pass in a compound microscope? **3-2**
- What does it mean when a microscope has a resolution of 0.2 nm? **3-3**

Darkfield Microscopy

A **darkfield microscope** is used to examine live microorganisms that either are invisible in the ordinary light microscope, cannot be stained by standard methods, or are so distorted by staining that their characteristics are obscured. A darkfield microscope uses a darkfield condenser that contains an opaque disk. The disk blocks light that would enter the objective lens directly. Only light that is reflected off (turned away from) the specimen enters the objective lens. Because there is no direct background light, the specimen appears light against a black background—the dark field (Figure 3.4b). This technique is frequently used to examine unstained microorganisms suspended in liquid. One use for darkfield microscopy is the examination of very thin spirochetes, such as *Treponema pallidum* (trep-ō-NĒ-mah PAL-li-dum), the causative agent of syphilis.

Phase-Contrast Microscopy

Another way to observe microorganisms is with a **phase-contrast microscope.** Phase-contrast microscopy is especially useful because the internal structures of a cell become more sharply defined, permitting detailed examination of *living* microorganisms. In addition, it isn't necessary to fix (attach the microbes to the microscope slide) or stain the specimen—procedures that could distort or kill the microorganisms.

In a phase-contrast microscope, one set of light rays comes directly from the light source. The other set comes from light that is reflected or diffracted from a particular structure in the specimen. (*Diffraction* is the scattering of light rays as they "touch" a specimen's edge. The diffracted rays are bent away from the parallel light rays that pass farther from the specimen.) When the two sets of light rays—direct rays and reflected or diffracted rays—are brought together, they form an image of the specimen on the ocular lens, containing areas that are relatively light (in phase), through shades of gray, to black (out of phase; Figure 3.4c).

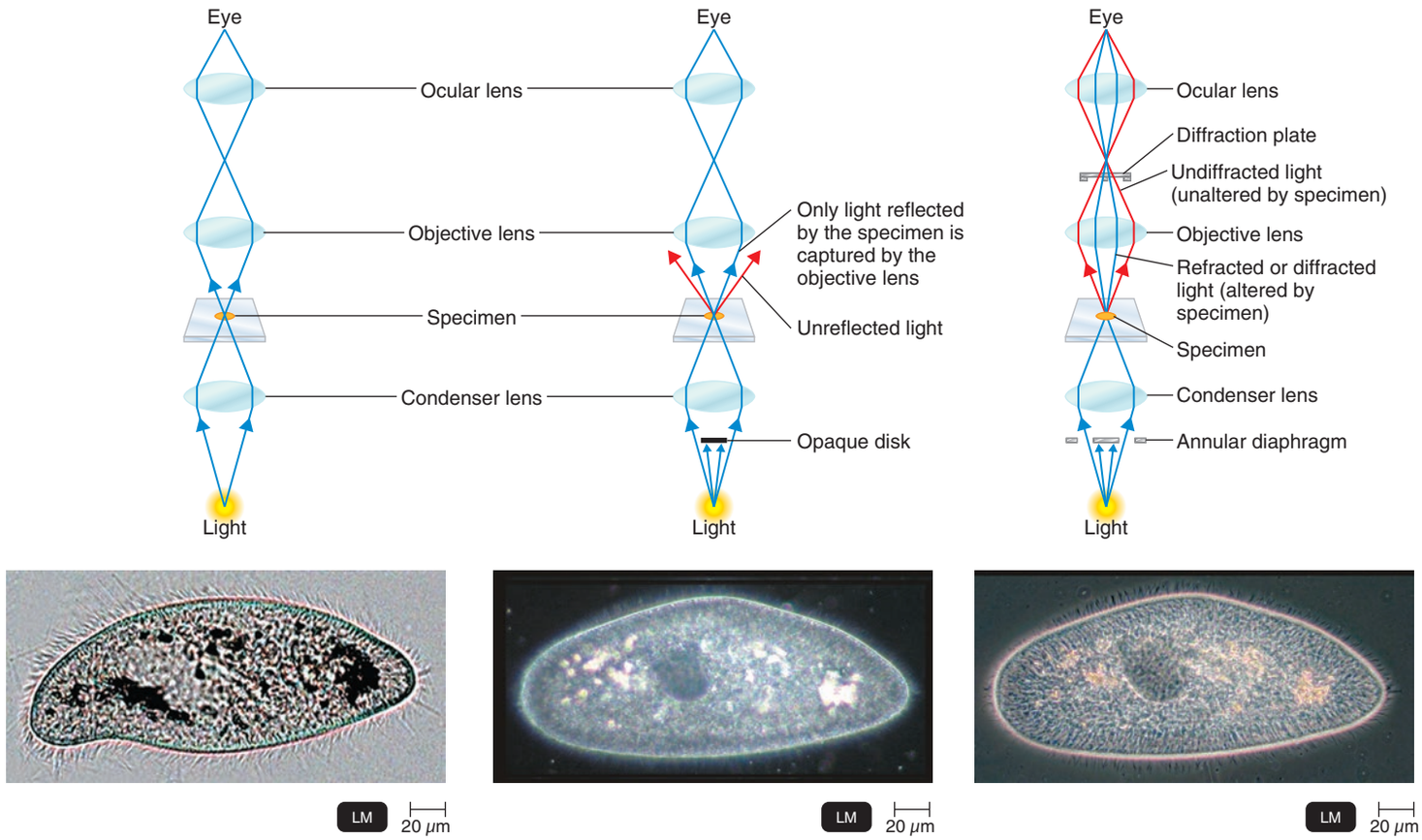
Differential Interference Contrast (DIC) Microscopy

Differential interference contrast (DIC) microscopy is similar to phase-contrast microscopy in that it uses differences in refractive indexes. However, a DIC microscope uses two beams of light instead of one. In addition, prisms split each light beam, adding contrasting colors to the specimen. Therefore, the resolution of a DIC microscope is higher than that of a standard phase-contrast microscope. Also, the image is brightly colored and appears nearly three-dimensional (Figure 3.5).

Fluorescence Microscopy

Fluorescence microscopy takes advantage of **fluorescence,** the ability of substances to absorb short wavelengths of light (ultraviolet) and give off light at a longer wavelength (visible). Some organisms fluoresce naturally under ultraviolet light; if the specimen to be viewed does not naturally fluoresce, it is stained with one of a group of fluorescent dyes called *fluorochromes.* When microorganisms stained with a fluorochrome are examined under a fluorescence microscope with an ultraviolet or near-ultraviolet light source, they appear as luminescent, bright objects against a dark background.

Fluorochromes have special attractions for different microorganisms. For example, the fluorochrome auramine O, which



(a) Brightfield. (Top) The path of light in brightfield microscopy, the type of illumination produced by regular compound light microscopes. (Bottom) Brightfield illumination shows internal structures and the outline of the transparent pellicle (external covering).

(b) Darkfield. (Top) The darkfield microscope uses a special condenser with an opaque disk that eliminates all light in the center of the beam. The only light that reaches the specimen comes in at an angle; thus, only light reflected by the specimen (blue lines) reaches the objective lens. (Bottom) Against the black background seen with darkfield microscopy, edges of the cell are bright, some internal structures seem to sparkle, and the pellicle is almost visible.

(c) Phase-contrast. (Top) In phase-contrast microscopy, the specimen is illuminated by light passing through an annular (ring-shaped) diaphragm. Direct light rays (unaltered by the specimen) travel a different path from light rays that are reflected or diffracted as they pass through the specimen. These two sets of rays are combined at the eye. Reflected or diffracted light rays are indicated in blue; direct rays are red. (Bottom) Phase-contrast microscopy shows greater differentiation of internal structures and clearly shows the pellicle.

Figure 3.4 Brightfield, darkfield, and phase-contrast microscopy. The photographs compare the protozoan *Paramecium* using these three different microscopy techniques.

Q What are the advantages of brightfield, darkfield, and phase-contrast microscopy?

glows yellow when exposed to ultraviolet light, is strongly absorbed by *Mycobacterium tuberculosis,* the bacterium that causes tuberculosis. When the dye is applied to a sample of material suspected of containing the bacterium, the bacterium can be detected by the appearance of bright yellow organisms against a dark background. *Bacillus anthracis,* the causative agent of anthrax, appears apple green when stained with another fluorochrome, fluorescein isothiocyanate (FITC).

The principal use of fluorescence microscopy is a diagnostic technique called the **fluorescent-antibody (FA) technique,** or **immunofluorescence. Antibodies** are natural defense molecules that are produced by humans and many animals in reaction to a foreign substance, or **antigen.** Fluorescent antibodies for a particular antigen are obtained as follows: an animal is injected with a specific antigen, such as a bacterium, and the animal then begins to produce antibodies against that antigen. After a sufficient time, the antibodies are removed from the serum of the animal. Next, as shown in **Figure 3.6a**, a fluorochrome is chemically combined with the antibodies. These fluorescent antibodies are then added to a microscope slide containing an unknown bacterium. If this unknown bacterium is the same bacterium that was injected into the animal,

Figure 3.5 Differential interference contrast (DIC) microscopy. Like phase-contrast, DIC uses differences in refractive indexes to produce an image, in this case a *Paramecium*. The colors in the image are produced by prisms that split the two light beams used in this process.

Q Why is a DIC micrograph brightly colored?

the fluorescent antibodies bind to antigens on the surface of the bacterium, causing it to fluoresce.

This technique can detect bacteria or other pathogenic microorganisms, even within cells, tissues, or other clinical specimens (Figure 3.6b). Of paramount importance, it can be used to identify a microbe in minutes. Immunofluorescence is especially useful in diagnosing syphilis and rabies. We will say more about antigen–antibody reactions and immunofluorescence in Chapter 18.

Confocal Microscopy

Confocal microscopy is a technique in light microscopy used to reconstruct three-dimensional images. Like fluorescent microscopy, specimens are stained with fluorochromes so they will emit, or return, light. But instead of illuminating the entire field, one plane of a small region of a specimen is illuminated with a short-wavelength (blue) light which passes the returned light through an aperture aligned with the illuminated region. Each plane corresponds to an image of a fine slice that has been physically cut from a specimen. Successive planes and regions are illuminated until the entire specimen has been scanned. Because confocal microscopy uses a pinhole aperture, it eliminates blurring that occurs with other microscopes. As a result, exceptionally clear two-dimensional images can be obtained, with improved resolution of up to 40% over that of other microscopes.

Most confocal microscopes are used in conjunction with computers to construct three-dimensional images. The scanned planes of a specimen, which resemble a stack of images, are converted to a digital form that can be used by a computer to construct a three-dimensional representation. The reconstructed images can be rotated and viewed in any orientation. This technique has been used to obtain three-dimensional images of entire cells and cellular components (**Figure 3.7**). In addition, confocal microscopy can be used to evaluate cellular physiology by monitoring the distributions

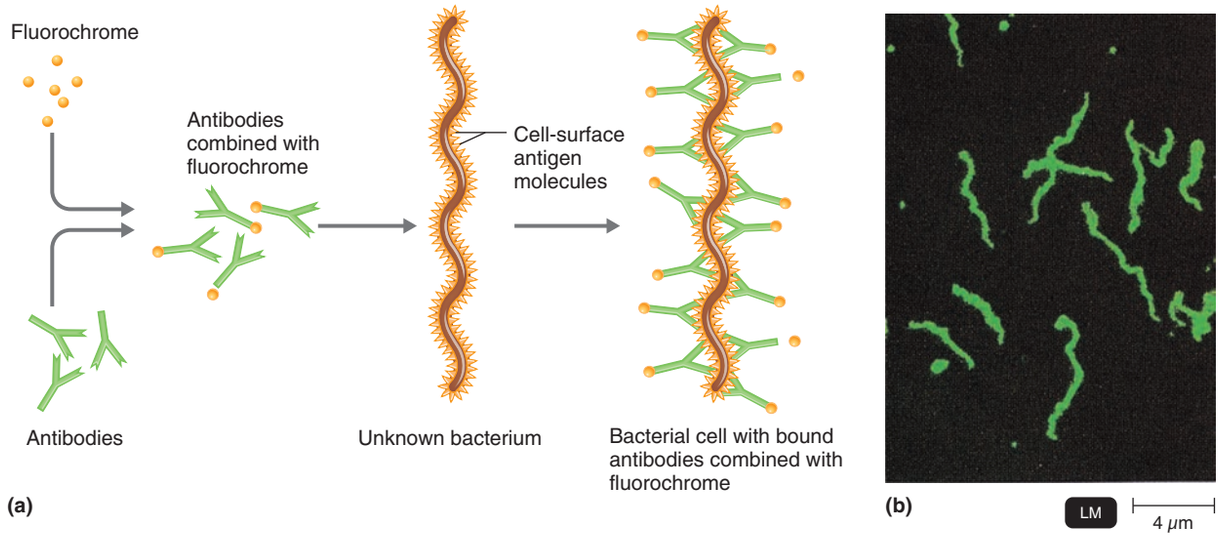


Figure 3.6 The principle of immunofluorescence. **(a)** A type of fluorochrome is combined with antibodies against a specific type of bacterium. When the preparation is added to bacterial cells on a microscope slide, the antibodies attach to the bacterial cells, and the cells fluoresce when illuminated with ultraviolet light. **(b)** In the fluorescent treponemal antibody absorption (FTA-ABS) test for syphilis shown here, *Treponema pallidum* shows up as green cells against a darker background.

Q Why won't other bacteria fluoresce in the FTA-ABS test?

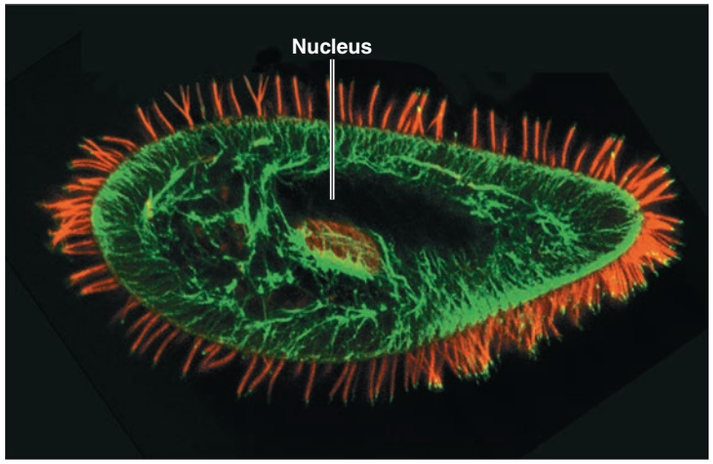


Figure 3.7 Confocal microscopy. Confocal microscopy produces three-dimensional images and can be used to look inside cells. Shown here is the nucleus in *Paramecium tetraurelia*.

Q What are the advantages of confocal microscopy?

and concentrations of substances such as ATP and calcium ions. MM **Animation** Light Microscopy

Two-Photon Microscopy

As in confocal microscopy, specimens are stained with a fluorochrome for **two-photon microscopy (TPM).** Two-photon microscopy uses long-wavelength (red) light, and therefore two photons, instead of one, are needed to excite the fluorochrome to emit light. The longer wavelength allows imaging of living cells in tissues up to 1 mm (1000 μm) deep (**Figure 3.8**). Confocal microscopy can image cells in detail only to a depth of less than 100 μm. Additionally, the longer wavelength is less likely to generate singlet oxygen, which damages cells (see page 155). Another advantage of TPM is that it can track the activity of cells in real time. For example, cells of the immune system have been observed responding to an antigen.

Scanning Acoustic Microscopy

Scanning acoustic microscopy (SAM) basically consists of interpreting the action of a sound wave sent through a specimen. A sound wave of a specific frequency travels through the specimen, and a portion of it is reflected back every time it hits an interface within the material. The resolution is about 1 μm. SAM is used to study living cells attached to another surface, such as cancer cells, artery plaque, and bacterial biofilms that foul equipment (**Figure 3.9**).

CHECK YOUR UNDERSTANDING

How are brightfield, darkfield, phase-contrast, and fluorescence microscopy similar? **3-4**

Electron Microscopy

Objects smaller than about 0.2 μm, such as viruses or the internal structures of cells, must be examined with an **electron microscope.** In electron microscopy, a beam of electrons is used instead of light. Like light, free electrons travel in waves. The resolving power of the electron microscope is far greater than that of the other microscopes described here so far. The better resolution of electron microscopes is due to the shorter wavelengths of electrons; the wavelengths of electrons are about 100,000 times smaller than the wavelengths of visible light. Thus, electron microscopes are used to examine structures too small to be resolved with light microscopes. Images produced by electron microscopes are always black and white, but they may be colored artificially to accentuate certain details.

Instead of using glass lenses, an electron microscope uses electromagnetic lenses to focus a beam of electrons onto a specimen. There are two types: the transmission electron microscope and the scanning electron microscope.

Transmission Electron Microscopy

In the **transmission electron microscope (TEM),** a finely focused beam of electrons from an electron gun passes through a specially prepared, ultrathin section of the specimen (**Figure 3.10a**). The beam is focused on a small area of the specimen by an electromagnetic condenser lens that performs roughly the same function as the condenser of a light microscope—directing the beam of electrons in a straight line to illuminate the specimen.

Instead of being placed on a glass slide, as in light microscopes, the specimen is usually placed on a copper mesh grid. The beam

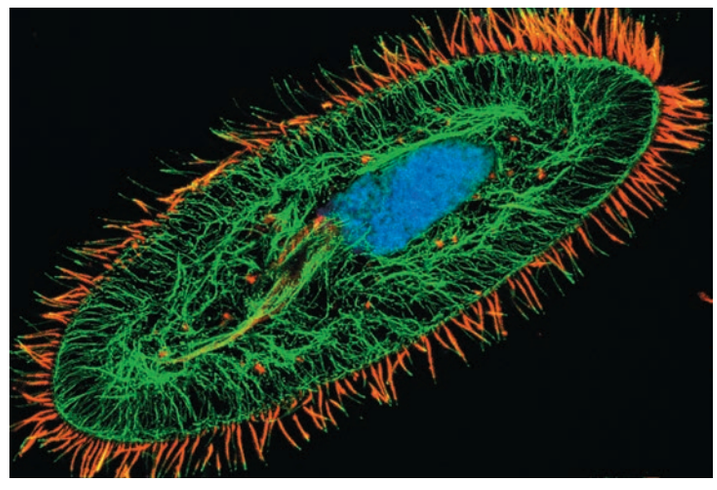


Figure 3.8 Two-photon microscopy (TPM). This procedure makes it possible to image cells up to 1 mm deep in detail. This image shows a *Paramecium*. Immunofluorescence is used to show microtubules and the nucleus.

Q What are the differences between TPM and confocal microscopy?

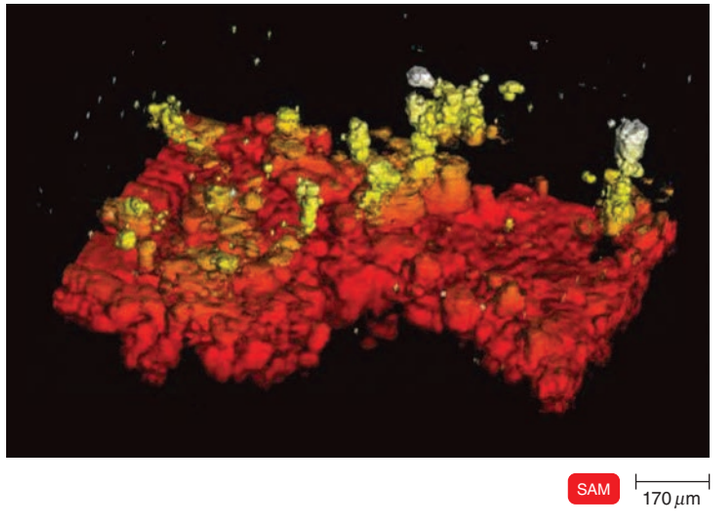


Figure 3.9 Scanning acoustic microscopy (SAM) of a bacterial biofilm on glass. Scanning acoustic microscopy essentially consists of interpreting the action of sound waves through a specimen. © 2006 IEEE.

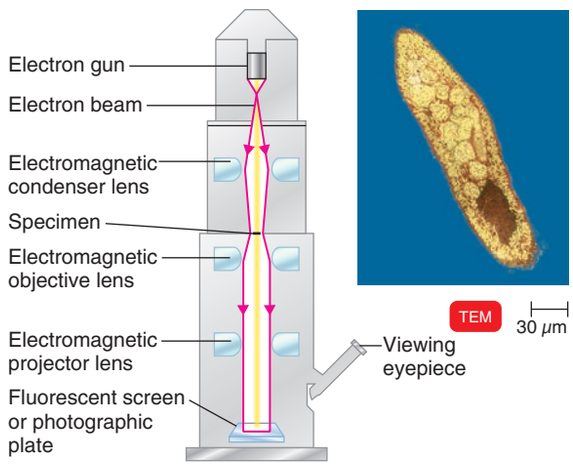
Q What is the principal use of SAM?

of electrons passes through the specimen and then through an electromagnetic objective lens, which magnifies the image. Finally, the electrons are focused by an electromagnetic projector lens (rather than by an ocular lens as in a light microscope) onto a fluorescent screen or photographic plate. The final image, called a *transmission electron micrograph,* appears as many light and dark areas, depending on the number of electrons absorbed by different areas of the specimen.

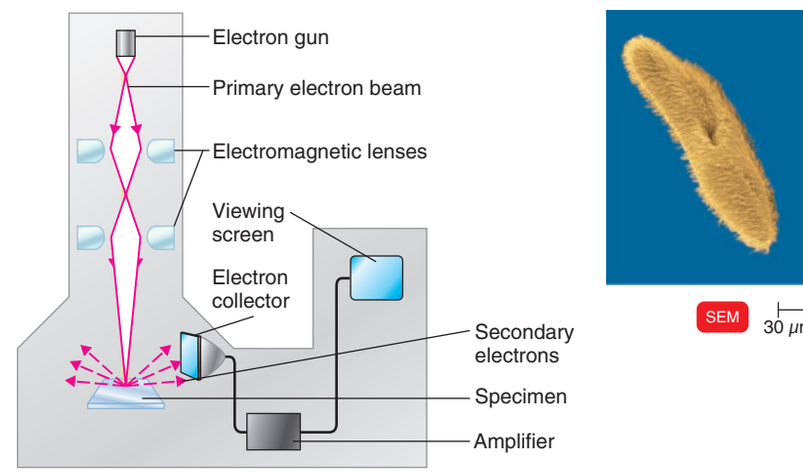
The transmission electron microscope can resolve objects as close together as 10 pm, and objects are generally magnified 10,000 to 100,000×. Because most microscopic specimens are so thin, the contrast between their ultrastructures and the background is weak. Contrast can be greatly enhanced by using a "dye" that absorbs electrons and produces a darker image in the stained region. Salts of various heavy metals, such as lead, osmium, tungsten, and uranium, are commonly used as stains. These metals can be fixed onto the specimen (*positive staining*) or used to increase the electron opacity of the surrounding field (*negative staining*). Negative staining is useful for the study of the very smallest specimens, such as virus particles, bacterial flagella, and protein molecules.

In addition to positive and negative staining, a microbe can be viewed by a technique called *shadow casting.* In this procedure, a heavy metal such as platinum or gold is sprayed at an angle of about 45° so that it strikes the microbe from only one side. The metal piles up on one side of the specimen, and the uncoated area on the opposite side of the specimen leaves a clear area behind it as a shadow. This gives a three-dimensional effect to the specimen and provides a general idea of the size and shape of the specimen (see the TEM in Figure 4.6, page 76).

Transmission electron microscopy has high resolution and is extremely valuable for examining different layers of specimens.



(a) Transmission. (Left) In a transmission electron microscope, electrons pass through the specimen and are scattered. Magnetic lenses focus the image onto a fluorescent screen or photographic plate. (Right) This colorized transmission electron micrograph (TEM) shows a thin slice of *Paramecium*. In this type of microscopy, the internal structures present in the slice can be seen.



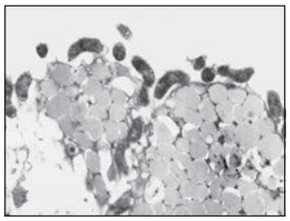
(b) Scanning. (Left) In a scanning electron microscope, primary electrons sweep across the specimen and knock electrons from its surface. These secondary electrons are picked up by a collector, amplified, and transmitted onto a viewing screen or photographic plate. (Right) In this colorized scanning electron micrograph (SEM), the surface structures of *Paramecium* can be seen. Note the three-dimensional appearance of this cell, in contrast to the two-dimensional appearance of the transmission electron micrograph in part (a).

Figure 3.10 Transmission and scanning electron microscopy. The illustrations show a *Paramecium* viewed with both of these types of microscopes. Although electron micrographs are normally black and white, these and other electron micrographs in this book have been artificially colorized for emphasis.

Q How do TEM and SEM images of the same organism differ?

Clinical Case

Helicobacter pylori is a spiral-shaped, gram-negative bacterium with multiple flagella. It is the most common cause of peptic ulcers in humans and can also cause stomach cancer. The first electron micrograph of *H. pylori* was viewed in the 1980s, when Australian physician Robin Warren used an electron microscope to see *H. pylori* in stomach tissue.



Why was an electron microscope necessary to see the *H. pylori* bacteria?

52 61 66 67

However, it does have certain disadvantages. Because electrons have limited penetrating power, only a very thin section of a specimen (about 100 nm) can be studied effectively. Thus, the specimen has no three-dimensional aspect. In addition, specimens must be fixed, dehydrated, and viewed under a high vacuum to prevent electron scattering. These treatments not only kill the specimen, but also cause shrinkage and distortion, sometimes to the extent that there may appear to be additional structures in a prepared cell. Structures that appear as a result of the preparation method are called *artifacts*.

Scanning Electron Microscopy

The **scanning electron microscope (SEM)** overcomes the sectioning problems associated with a transmission electron microscope. It provides striking three-dimensional views of specimens (Figure 3.10b). An electron gun produces a finely focused beam of electrons called the primary electron beam. These electrons pass through electromagnetic lenses and are directed over the surface of the specimen. The primary electron beam knocks electrons out of the surface of the specimen, and the secondary electrons thus produced are transmitted to an electron collector, amplified, and used to produce an image on a viewing screen or photographic plate. The image is called a *scanning electron micrograph*. This microscope is especially useful in studying the surface structures of intact cells and viruses. In practice, it can resolve objects as close together as 10 nm, and objects are generally magnified 1000 to 10,000×.

MM **Animation** Electron Microscopy

CHECK YOUR UNDERSTANDING

Why do electron microscopes have greater resolution than light microscopes? **3-5**

Scanned-Probe Microscopy

Since the early 1980s, several new types of microscopes, called **scanned-probe microscopes,** have been developed. They use various kinds of probes to examine the surface of a specimen using electric current, which does not modify the specimen or expose it to damaging, high-energy radiation. Such microscopes can be used to map atomic and molecular shapes, to characterize magnetic and chemical properties, and to determine temperature variations inside cells. Among the new scanned-probe microscopes are the scanning tunneling microscope and the atomic force microscope, discussed next.

Scanning Tunneling Microscopy

Scanning tunneling microscopy (STM) uses a thin tungsten probe that scans a specimen and produces an image that reveals the bumps and depressions of the atoms on the surface of the specimen (**Figure 3.11a**). The resolving power of an STM is much greater than that of an electron microscope; it can resolve features that are only about 1/100 the size of an atom. Moreover, special preparation of the specimen for observation is not needed. STMs are used to provide incredibly detailed views of molecules such as DNA.

Atomic Force Microscopy

In **atomic force microscopy (AFM),** a metal-and-diamond probe is gently forced down onto a specimen. As the probe moves along the surface of the specimen, its movements are recorded, and a three-dimensional image is produced (Figure 3.11b). As with STM, AFM does not require special specimen preparation. AFM

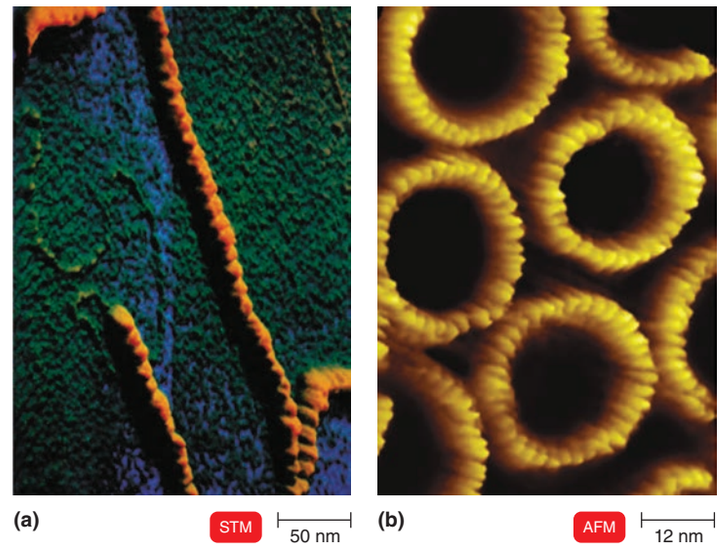


Figure 3.11 Scanned-probe microscopy. **(a)** Scanning tunneling microscopy (STM) image of RecA protein from *E. coli*. This protein is involved in repair of DNA. **(b)** Atomic force microscopy (AFM) image of perfringolysin O toxin from *Clostridium perfringens*. This protein makes holes in human plasma membranes.

Q What is the principle employed in scanned-probe microscopy?

is used to image both biological substances (in nearly atomic detail) (see also Figure 17.4c on page 473) and molecular processes (such as the assembly of fibrin, a component of a blood clot).

The various types of microscopy just described are summarized in Table 3.2.

CHECK YOUR UNDERSTANDING

For what is TEM used? SEM? Scanned-probe microscopy? **3-6**

Preparation of Specimens for Light Microscopy

LEARNING OBJECTIVES

3-7 Differentiate an acidic dye from a basic dye.

3-8 Explain the purpose of simple staining.

3-9 List Gram stain steps, and describe the appearance of gram-positive and gram-negative cells after each step.

3-10 Compare and contrast the Gram stain and the acid-fast stain.

3-11 Explain why each of the following is used: capsule stain, endospore stain, flagella stain.

Most microorganisms appear almost colorless when viewed through with brightfield microscopy, so we must prepare them for observation. One way is to stain (color) the specimen. Next we discuss several different staining procedures.

Preparing Smears for Staining

Most initial observations of microorganisms are made with stained preparations. **Staining** simply means coloring the microorganisms with a dye that emphasizes certain structures. Before the microorganisms can be stained, however, they must be **fixed** (attached) to the microscope slide. Fixing simultaneously kills the microorganisms and fixes them to the slide. It also preserves various parts of microbes in their natural state with only minimal distortion.

When a specimen is to be fixed, a thin film of material containing the microorganisms is spread over the surface of the slide. This film, called a **smear,** is allowed to air dry. In most staining procedures the slide is then fixed by passing it through the flame of a Bunsen burner several times, smear side up, or by covering the slide with methanol for 1 minute. Stain is applied and then washed off with water; then the slide is blotted with absorbent paper. Without fixing, the stain might wash the microbes off the slide. The stained microorganisms are now ready for microscopic examination.

Stains are salts composed of a positive and a negative ion, one of which is colored and is known as the *chromophore*. The color of so-called **basic dyes** is in the cation; in **acidic dyes,** it is in the anion. Bacteria are slightly negatively charged at pH 7. Thus, the colored cation in a basic dye is attracted to the negatively charged bacterial cell. Basic dyes, which include crystal

TABLE 3.2 A Summary of Various Types of Microscopes

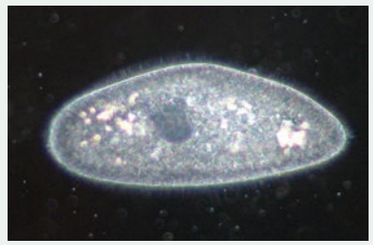
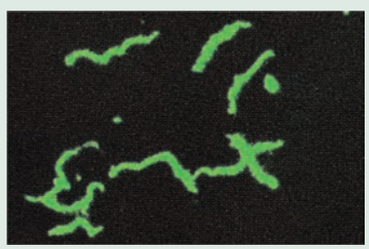
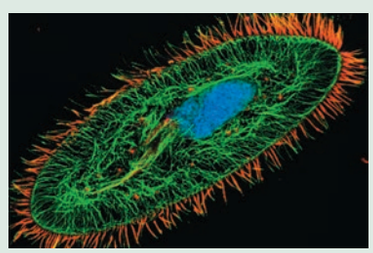
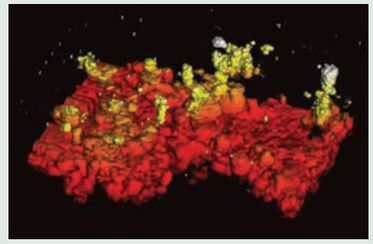
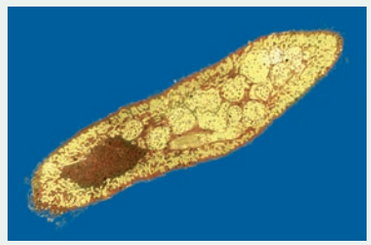
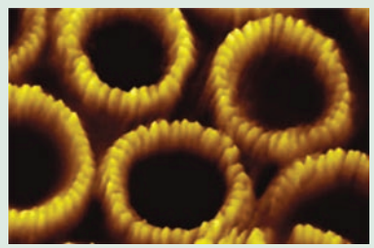
Microscope Type	Distinguishing Features	Typical Image	Principal Uses
Light			
Brightfield	Uses visible light as a source of illumination; cannot resolve structures smaller than about 0.2 µm; specimen appears against a bright background. Inexpensive and easy to use.	*Paramecium* LM 25 µm	To observe various stained specimens and to count microbes; does not resolve very small specimens, such as viruses.
Darkfield	Uses a special condenser with an opaque disk that blocks light from entering the objective lens directly; light reflected by specimen enters the objective lens, and the specimen appears light against a black background.	 *Paramecium* LM 25 µm	To examine living microorganisms that are invisible in brightfield microscopy, do not stain easily, or are distorted by staining; frequently used to detect *Treponema pallidum* in the diagnosis of syphilis.

TABLE 3.2 *(continued)*

Microscope Type	Distinguishing Features	Typical Image	Principal Uses
Phase-contrast	Uses a special condenser containing an annular (ring-shaped) diaphragm. The diaphragm allows direct light to pass through the condenser, focusing light on the specimen and a diffraction plate in the objective lens. Direct and reflected or diffracted light rays are brought together to produce the image. No staining required.	*Paramecium* LM 25 μm	To facilitate detailed examination of the internal structures of living specimens.
Differential interference contrast (DIC)	Like phase-contrast, uses differences in refractive indexes to produce images. Uses two beams of light separated by prisms; the specimen appears colored as a result of the prism effect. No staining required.	*Paramecium* LM 23 μm	To provide three-dimensional images.
Fluorescence	Uses an ultraviolet or near-ultraviolet source of illumination that causes fluorescent compounds in a specimen to emit light.	 *Treponema pallidum* LM 2 μm	For fluorescent-antibody techniques (immunofluorescence) to rapidly detect and identify microbes in tissues or clinical specimens.
Confocal	Uses a single photon to illuminate one plane of a specimen at a time.	*Paramecium* CF 25 μm	To obtain two- and three-dimensional images of cells for biomedical applications.
Two-Photon	Uses two photons to illuminate a specimen.	 *Paramecium* TPM 22 μm	To image living cells, up to depth of 1 mm, reduce phototoxicity, and observe cell activity in real time.

(continued)

TABLE 3.2 A Summary of Various Types of Microscopes *(continued)*

Microscope Type	Distinguishing Features	Typical Image	Principal Uses
Scanning Acoustic	Uses a sound wave of specific frequency that travels through the specimen with a portion being reflected when it hits an interface within the material.	 Biofilm SAM 180 μm	To examine living cells attached to another surface, such as cancer cells, artery plaque, and biofilms.
Electron			
Transmission	Uses a beam of electrons instead of light; electrons pass through the specimen; because of the shorter wavelength of electrons, structures smaller than 0.2 μm can be resolved. The image produced is two-dimensional.	 *Paramecium* TEM 25 μm	To examine viruses or the internal ultrastructure in thin sections of cells (usually magnified 10,000–100,000×).
Scanning	Uses a beam of electrons instead of light; electrons are reflected from the specimen; because of the shorter wavelength of electrons, structures smaller than 0.2 μm can be resolved. The image produced appears three-dimensional.	*Paramecium* SEM 25 μm	To study the surface features of cells and viruses (usually magnified 1000–10,000×).
Scanned-Probe			
Scanning tunneling	Uses a thin metal probe that scans a specimen and produces an image that reveals the bumps and depressions of the atoms on the surface of the specimen. Resolving power is much greater than that of an electron microscope. No special preparation required.	RecA protein from *E. coli* STM 45 nm	Provides very detailed views of molecules inside cells.
Atomic force	Uses a metal-and-diamond probe that is gently forced down along the surface of the specimen. Produces a three-dimensional image. No special preparation required.	 Perfringolysin O toxin from *Clostridium perfringens* AFM 9 nm	Provides three-dimensional images of biological specimens at high resolution in nearly atomic detail and can measure physical properties of biological specimens and molecular processes.

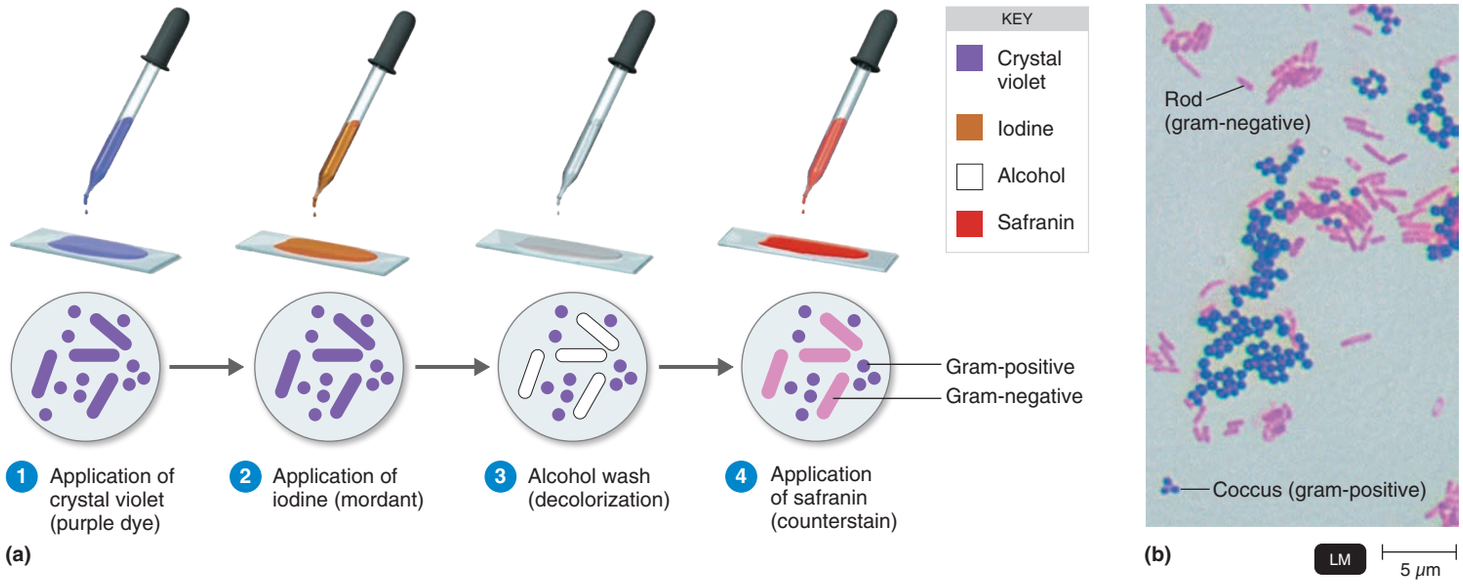


Figure 3.12 Gram staining. **(a)** Procedure. **(b)** Micrograph of Gram-stained bacteria. The cocci (purple) are gram-positive, and the rods (pink) are gram-negative.

Q How can the Gram reaction be useful in prescribing antibiotic treatment?

violet, methylene blue, malachite green, and safranin, are more commonly used than acidic dyes. Acidic dyes are not attracted to most types of bacteria because the dye's negative ions are repelled by the negatively charged bacterial surface, so the stain colors the background instead. Preparing colorless bacteria against a colored background is called **negative staining.** It is valuable for observing overall cell shapes, sizes, and capsules because the cells are made highly visible against a contrasting dark background (see Figure 3.14a on page 67). Distortions of cell size and shape are minimized because fixing isn't necessary and the cells don't pick up the stain. Examples of acidic dyes are eosin, acid fuchsin, and nigrosin.

To apply acidic or basic dyes, microbiologists use three kinds of staining techniques: simple, differential, and special.

Simple Stains

A **simple stain** is an aqueous or alcohol solution of a single basic dye. Although different dyes bind specifically to different parts of cells, the primary purpose of a simple stain is to highlight the entire microorganism so that cellular shapes and basic structures are visible. The stain is applied to the fixed smear for a certain length of time and then washed off. The slide is dried and examined. Occasionally, a chemical is added to the solution to intensify the stain; such an additive is called a **mordant.** One function of a mordant is to increase the affinity of a stain for a biological specimen; another is to coat a structure (such as a flagellum) to make it thicker and easier to see after it is stained with a dye. Some of the simple stains commonly used in the laboratory are methylene blue, carbolfuchsin, crystal violet, and safranin.

CHECK YOUR UNDERSTANDING

- Why doesn't a negative stain color a cell? **3-7**
- Why is fixing necessary for most staining procedures? **3-8**

Differential Stains

Unlike simple stains, **differential stains** react differently with different kinds of bacteria and thus can be used to distinguish them. The differential stains most frequently used for bacteria are the Gram stain and the acid-fast stain.

Gram Stain

The **Gram stain** was developed in 1884 by the Danish bacteriologist Hans Christian Gram. It is one of the most useful staining procedures because it classifies bacteria into two large groups: gram-positive and gram-negative.

In this procedure (**Figure 3.12a**),

1. A heat-fixed smear is covered with a basic purple dye, usually crystal violet. Because the purple stain imparts its color to all cells, it is referred to as a **primary stain.**
2. After a short time, the purple dye is washed off, and the smear is covered with iodine, a mordant. When the iodine is washed off, both gram-positive and gram-negative bacteria appear dark violet or purple.
3. Next, the slide is washed with alcohol or an alcohol-acetone solution. This solution is a **decolorizing agent,** which removes the purple from the cells of some species but not from others.

4 The alcohol is rinsed off, and the slide is then stained with safranin, a basic red dye. The smear is washed again, blotted dry, and examined microscopically.

The purple dye and the iodine combine in the cytoplasm of each bacterium and color it dark violet or purple. Bacteria that retain this color after the alcohol has attempted to decolorize them are classified as **gram-positive;** bacteria that lose the dark violet or purple color after decolorization are classified as **gram-negative** (Figure 3.12b). Because gram-negative bacteria are colorless after the alcohol wash, they are no longer visible. This is why the basic dye safranin is applied; it turns the gram-negative bacteria pink. Stains such as safranin that have a contrasting color to the primary stain are called **counterstains.** Because gram-positive bacteria retain the original purple stain, they are not affected by the safranin counterstain.

As you will see in Chapter 4, different kinds of bacteria react differently to the Gram stain because structural differences in their cell walls affect the retention or escape of a combination of crystal violet and iodine, called the crystal violet–iodine (CV–I) complex. Among other differences, gram-positive bacteria have a thicker peptidoglycan cell wall (disaccharides and amino acids) than gram-negative bacteria. In addition, gram-negative bacteria contain a layer of lipopolysaccharide (lipids and polysaccharides) as part of their cell wall (see Figure 4.13, page 82). When applied to both gram-positive and gram-negative cells, crystal violet and then iodine readily enter the cells. Inside the cells, the crystal violet and iodine combine to form CV–I. This complex is larger than the crystal violet molecule that entered the cells. Because of its size, it cannot be washed out of the intact peptidoglycan layer of gram-positive cells by alcohol. Consequently, gram-positive cells retain the color of the crystal violet dye. In gram-negative cells, however, the alcohol wash disrupts the outer lipopolysaccharide layer, and the CV–I complex is washed out through the thin layer of peptidoglycan. As a result, gram-negative cells are colorless until counterstained with safranin, after which they are pink.

In summary, gram-positive cells retain the dye and remain purple. Gram-negative cells do not retain the dye; they are colorless until counterstained with a red dye.

The Gram method is one of the most important staining techniques in medical microbiology. But Gram staining results are not universally applicable, because some bacterial cells stain poorly or not at all. The Gram reaction is most consistent when it is used on young, growing bacteria.

The Gram reaction of a bacterium can provide valuable information for the treatment of disease. Gram-positive bacteria tend to be killed easily by penicillins and cephalosporins. Gram-negative bacteria are generally more resistant because the antibiotics cannot penetrate the lipopolysaccharide layer. Some resistance to these antibiotics among both gram-positive and gram-negative bacteria is due to bacterial inactivation of the antibiotics.

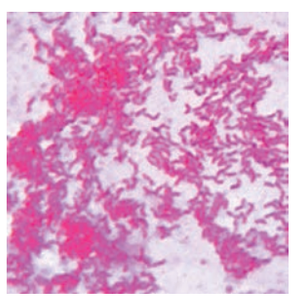
Acid-Fast Stain

Another important differential stain (one that differentiates bacteria into distinctive groups) is the **acid-fast stain,** which binds strongly only to bacteria that have a waxy material in their cell walls. Microbiologists use this stain to identify all bacteria in the genus *Mycobacterium* (mī′kō-bak-TI-rē-um), including the two important pathogens *Mycobacterium tuberculosis,* the causative agent of tuberculosis, and *Mycobacterium leprae* (LEP-rī), the causative agent of leprosy. This stain is also used to identify the pathogenic strains of the genus *Nocardia* (nō-KAR-dē-ah). Bacteria in the genera *Mycobacterium* and *Nocardia* are acid-fast.

In the acid-fast staining procedure, the red dye carbolfuchsin is applied to a fixed smear, and the slide is gently heated for several minutes. (Heating enhances penetration and retention of the dye.) Then the slide is cooled and washed with water. The smear is next treated with acid-alcohol, a decolorizer, which removes the red stain from bacteria that are not acid-fast. The acid-fast microorganisms retain the pink or red color because the carbolfuchsin is more soluble in the cell wall lipids than in the acid-alcohol (**Figure 3.13**). In non–acid-fast bacteria, whose cell walls lack the lipid components, the

Clinical Case

The resolving power of the electron microscope is much greater than that of a light microscope. The higher resolution provided unequivocal proof of the presence of spiral bacteria.



Although bismuth (the key ingredient in Pepto-Bismol) kills *H. pylori,* it is not a cure. Maryanne's physician prescribes the antibiotic clarithromycin. However, one week after finishing treatment, Maryanne's symptoms continue. To see whether *H. pylori* is still present, her physician orders a stomach biopsy to obtain a sample of the mucous lining of Maryanne's stomach. The lab uses light microscopy and a Gram stain to view the sample.

What does the Gram stain above show?

52 61 66 67

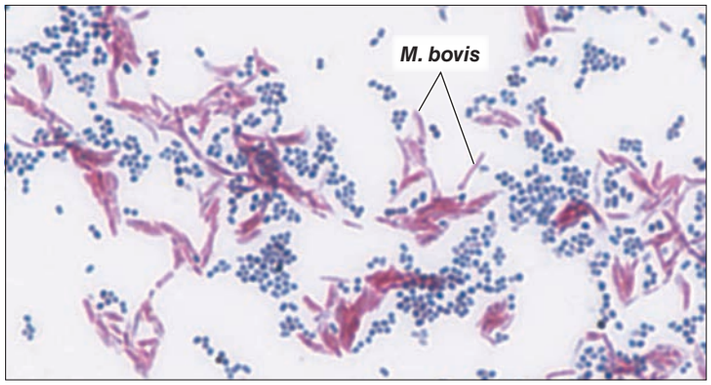


Figure 3.13 Acid-fast bacteria. The *Mycobacterium bovis* bacteria that have infected this tissue have been stained pink or red with an acid-fast stain. Non–acid-fast cells (*Staphylococcus*) are stained with the methylene blue counterstain.

Q Why is *Mycobacterium tuberculosis* easily identified by the acid-fast stain?

carbolfuchsin is rapidly removed during decolorization, leaving the cells colorless. The smear is then stained with a methylene blue counterstain. Non–acid-fast cells appear blue after the counterstain is applied.

CHECK YOUR UNDERSTANDING

- Why is the Gram stain so useful? **3-9**
- Which stain would be used to identify microbes in the genera *Mycobacterium* and *Nocardia*? **3-10**

Special Stains

Special stains are used to color parts of microorganisms, such as endospores, flagella, or capsules.

Clinical Case Resolved

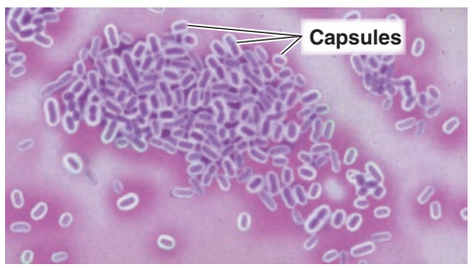
Because it's gram-negative, *H. pylori* turns pink from the counterstain. Lab results indicate the *H. pylori* is still present in Maryanne's stomach lining. Suspecting the bacteria are resistant to clarithromycin, Maryanne's physician now prescribes tetracycline and metronidazole. This time, Maryanne's symptoms do not return. Soon, she feels like her old self again and is back in the office full time.

52 61 66 **67**

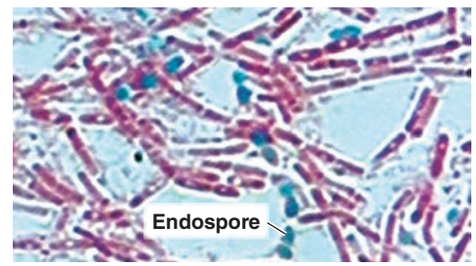
Negative Staining for Capsules

Many microorganisms contain a gelatinous covering called a **capsule.** (We will discuss capsules in our examination of the prokaryotic cell in Chapter 4.) In medical microbiology, demonstrating the presence of a capsule is a means of determining the organism's **virulence,** the degree to which a pathogen can cause disease.

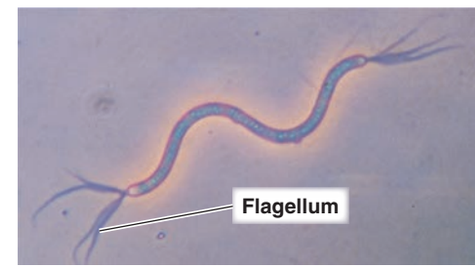
Capsule staining is more difficult than other types of staining procedures because capsular materials are water soluble and may be dislodged or removed during rigorous washing. To demonstrate the presence of capsules, a microbiologist can mix the bacteria in a solution containing a fine colloidal suspension of colored particles (usually India ink or nigrosin) to provide a contrasting background and then stain the bacteria with a simple stain, such as safranin (**Figure 3.14a**). Because of their chemical composition, capsules do not accept most biological dyes, such as safranin, and thus appear as halos surrounding each stained bacterial cell.



(a) Negative staining LM 5 μm



(b) Endospore staining LM 12 μm



(c) Flagella staining LM 7 μm

Figure 3.14 Special staining. **(a)** Capsule staining provides a contrasting background, so the capsules of these bacteria, *Klebsiella pneumoniae,* show up as light areas surrounding the stained cells. **(b)** Endospores are seen as green ovals in these rod-shaped cells of the bacterium *Bacillus cereus,* using the Schaeffer-Fulton endospore stain. **(c)** Flagella appear as wavy extensions from the ends of these cells of the bacterium *Spirillum volutans.* In relation to the body of the cell, the flagella are much thicker than normal because layers of the stain have accumulated from treatment of the specimen with a mordant.

Q Of what value are capsules, endospores, and flagella to bacteria?

TABLE 3.3 A Summary of Various Stains and Their Uses

Stain	Principal Uses
Simple (methylene blue, carbolfuchsin, crystal violet, safranin)	Used to highlight microorganisms to determine cellular shapes and arrangements. Aqueous or alcohol solution of a single basic dye stains cells. (Sometimes a mordant is added to intensify the stain.)
Differential	Used to distinguish different kinds of bacteria.
Gram	Classifies bacteria into two large groups: gram-positive and gram-negative. Gram-positive bacteria retain the crystal violet stain and appear purple. Gram-negative bacteria do not retain the crystal violet stain; they remain colorless until counterstained with safranin and then appear pink.
Acid-fast	Used to distinguish *Mycobacterium* species and some species of *Nocardia*. Acid-fast bacteria, once stained with carbolfuchsin and treated with acid-alcohol, remain pink or red because they retain the carbolfuchsin stain. Non–acid-fast bacteria, when stained and treated the same way and then stained with methylene blue, appear blue because they lose the carbolfuchsin stain and are then able to accept the methylene blue stain.
Special	Used to color and isolate various structures, such as capsules, endospores, and flagella; sometimes used as a diagnostic aid.
Negative	Used to demonstrate the presence of capsules. Because capsules do not accept most stains, the capsules appear as unstained halos around bacterial cells and stand out against a contrasting background.
Endospore	Used to detect the presence of endospores in bacteria. When malachite green is applied to a heat-fixed smear of bacterial cells, the stain penetrates the endospores and stains them green. When safranin (red) is then applied, it stains the remainder of the cells red or pink.
Flagella	Used to demonstrate the presence of flagella. A mordant is used to build up the diameters of flagella until they become visible microscopically when stained with carbolfuchsin.

Endospore (Spore) Staining

An **endospore** is a special resistant, dormant structure formed within a cell that protects a bacterium from adverse environmental conditions. Although endospores are relatively uncommon in bacterial cells, they can be formed by a few genera of bacteria. Endospores cannot be stained by ordinary methods, such as simple staining and Gram staining, because the dyes don't penetrate the endospore's wall.

The most commonly used endospore stain is the *Schaeffer-Fulton endospore stain* (Figure 3.14b). Malachite green, the primary stain, is applied to a heat-fixed smear and heated to steaming for about 5 minutes. The heat helps the stain penetrate the endospore wall. Then the preparation is washed for about 30 seconds with water to remove the malachite green from all of the cells' parts except the endospores. Next, safranin, a counterstain, is applied to the smear to stain portions of the cell other than endospores. In a properly prepared smear, the endospores appear green within red or pink cells. Because endospores are highly refractive, they can be detected under the light microscope when unstained, but without a special stain they cannot be differentiated from inclusions of stored material.

Flagella Staining

Bacterial **flagella** (singular: **flagellum**) are structures of locomotion too small to be seen with a light microscope without staining. A tedious and delicate staining procedure uses a mordant and the stain carbolfuchsin to build up the diameters of the flagella until they become visible under the light microscope (Figure 3.14c). Microbiologists use the number and arrangement of flagella as diagnostic aids. **Animation** Staining

A summary of stains is presented in **Table 3.3**. In the next chapters we will take a closer look at the structure of microbes and how they protect, nourish, and reproduce themselves.

CHECK YOUR UNDERSTANDING

How do unstained endospores appear? Stained endospores? **3-11**

Study Outline

MasteringMicrobiology®

Study microbiology where it matters: everywhere.
Check out the Study Area of MasteringMicrobiology to explore Interactive Microbiology. Study on-the-go with Dynamic Study Modules. Check your understanding with chapter quizzes and animations—and much more!

Units of Measurement (p. 52)

1. Microorganisms are measured in micrometers, μm (10^{-6} m), and in nanometers, nm (10^{-9} m).

Microscopy: The Instruments (pp. 52–62)

1. A simple microscope consists of one lens; a compound microscope has multiple lenses.

Light Microscopy (pp. 53–59)

2. The most common microscope used in microbiology is the compound light microscope (LM).
3. The total magnification of an object is calculated by multiplying the magnification of the objective lens by the magnification of the ocular lens.
4. The compound light microscope uses visible light.
5. The maximum resolution, or resolving power (the ability to distinguish two points) of a compound light microscope is 0.2 μm; maximum magnification is 2000×.
6. Specimens are stained to increase the difference between the refractive indexes of the specimen and the medium.
7. Immersion oil is used with the oil immersion lens to reduce light loss between the slide and the lens.
8. Brightfield illumination is used for stained smears.
9. Unstained cells are more productively observed using darkfield, phase-contrast, or DIC microscopy.
10. The darkfield microscope shows a light silhouette of an organism against a dark background. It is most useful for detecting the presence of extremely small organisms.
11. A phase-contrast microscope brings direct and reflected or diffracted light rays together (in phase) to form an image of the specimen on the ocular lens. It allows the detailed observation of living organisms.
12. The DIC microscope provides a colored, three-dimensional image of living cells.
13. In fluorescence microscopy, specimens are first stained with fluorochromes and then viewed through a compound microscope by using an ultraviolet light source.
14. The microorganisms appear as bright objects against a dark background.
15. Fluorescence microscopy is used primarily in a diagnostic procedure called fluorescent-antibody (FA) technique, or immunofluorescence.
16. In confocal microscopy, a specimen is stained with a fluorescent dye and illuminated with short-wavelength light.
17. Using a computer to process the images, two-dimensional and three-dimensional images of cells can be produced.

Two-Photon Microscopy (p. 59)

18. In TPM, a live specimen is stained with a fluorescent dye and illuminated with long-wavelength light.

Scanning Acoustic Microscopy (p. 59)

19. Scanning acoustic microscopy (SAM) is based on the interpretation of sound waves through a specimen.
20. It is used to study living cells attached to surfaces such as biofilms.

Electron Microscopy (pp. 59–61)

21. Instead of light, a beam of electrons is used with an electron microscope.
22. Instead of glass lenses, electromagnets control focus, illumination, and magnification.
23. Thin sections of organisms can be seen in an electron micrograph produced using a transmission electron microscope (TEM). Magnification: 10,000–100,000×. Resolving power: 10 pm.
24. Three-dimensional views of the surfaces of whole microorganisms can be obtained with a scanning electron microscope (SEM). Magnification: 1000–10,000×. Resolution: 10 nm.

Scanned-Probe Microscopy (pp. 61–62)

25. Scanning tunneling microscopy (STM) and atomic force microscopy (AFM) produce three-dimensional images of the surface of a molecule.

Preparation of Specimens for Light Microscopy (pp. 62–68)

Preparing Smears for Staining (pp. 62–65)

1. Staining means coloring a microorganism with a dye to make some structures more visible.
2. Fixing uses heat or alcohol to kill and attach microorganisms to a slide.
3. A smear is a thin film of material used for microscopic examination.
4. Bacteria are negatively charged, and the colored positive ion of a basic dye will stain bacterial cells.
5. The colored negative ion of an acidic dye will stain the background of a bacterial smear; a negative stain is produced.

Simple Stains (p. 65)

6. A simple stain is an aqueous or alcohol solution of a single basic dye.
7. A mordant may be used to improve bonding between the stain and the specimen.

Differential Stains (pp. 65–67)

8. Differential stains, such as the Gram stain and acid-fast stain, differentiate bacteria according to their reactions to the stains.
9. The Gram stain procedure uses a purple stain, iodine as a mordant, an alcohol decolorizer, and a red counterstain.
10. Gram-positive bacteria remain purple after decolorization; gram-negative bacteria do not, and appear pink from the counterstain.
11. Acid-fast microbes, such as members of the genera *Mycobacterium* and *Nocardia,* retain carbolfuchsin after acid-alcohol decolorization and appear red; non–acid-fast microbes take up the methylene blue counterstain and appear blue.

Special Stains (pp. 67–68)

12. Negative staining is used to make microbial capsules visible.
13. The endospore stain and flagella stain are special stains that are used to visualize specific structures in bacterial cells.

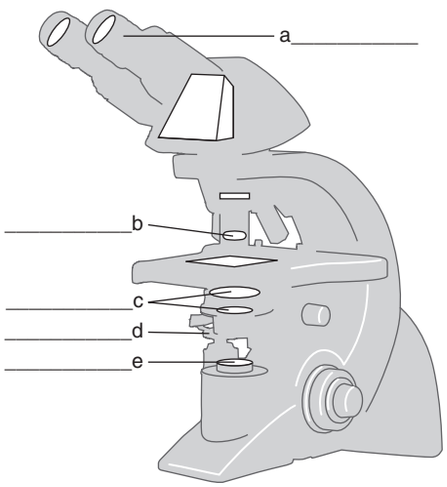
Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. Fill in the following blanks.
   a. 1 μm = _____ m
   b. 1 _____ = 10^{-9} m
   c. 1 μm = _____ nm
2. Which type of microscope would be best to use to observe each of the following?
   a. a stained bacterial smear
   b. unstained bacterial cells: the cells are small, and no detail is needed
   c. unstained live tissue when it is desirable to see some intracellular detail
   d. a sample that emits light when illuminated with ultraviolet light
   e. intracellular detail of a cell that is 1 μm long
   f. unstained live cells in which intracellular structures are shown in color
3. DRAW IT Label the parts of the compound light microscope in the figure below, and then draw the path of light from the illuminator to your eye.



4. Calculate the total magnification of the nucleus of a cell being observed through a compound light microscope with a 10× ocular lens and an oil immersion lens.
5. The maximum magnification of a compound microscope is (a) _____; that of an electron microscope, (b) _____. The maximum resolution of a compound microscope is (c) _____; that of an electron microscope, (d) _____. One advantage of a scanning electron microscope over a transmission electron microscope is (e) _____.
6. Why is a mordant used in the Gram stain? In the flagella stain?
7. What is the purpose of a counterstain in the acid-fast stain?
8. What is the purpose of a decolorizer in the Gram stain? In the acid-fast stain?
9. Fill in the following table regarding the Gram stain:

	Appearance After This Step	
Steps	**Gram-Positive Cells**	**Gram-Negative Cells**
Crystal violet	a. _____	e. _____
Iodine	b. _____	f. _____
Alcohol-acetone	c. _____	g. _____
Safranin	d. _____	h. _____

10. NAME IT A sputum sample from Calle, a 30-year-old Asian elephant, was smeared onto a slide and air dried. The smear was fixed, covered with carbolfuchsin, and heated for 5 minutes. After washing with water, acid-alcohol was placed on the smear for 30 seconds. Finally, the smear was stained with methylene blue for 30 seconds, washed with water, and dried. On examination at 1000×, the zoo veterinarian saw red rods on the slide. What microbe do these results suggest?

Multiple Choice

1. Assume you stain *Bacillus* by applying malachite green with heat and then counterstain with safranin. Through the microscope, the green structures are
   a. cell walls.
   b. capsules.
   c. endospores.
   d. flagella.
   e. impossible to identify.
2. Three-dimensional images of live cells can be produced with
   a. darkfield microscopy.
   b. fluorescence microscopy.
   c. transmission electron microscopy.
   d. confocal microscopy.
   e. phase-contrast microscopy.
3. Carbolfuchsin can be used as a simple stain and a negative stain. As a simple stain, the pH is
   a. 2.
   b. higher than the negative stain.
   c. lower than the negative stain.
   d. the same as the negative stain.
4. Looking at the cell of a photosynthetic microorganism, you observe the chloroplasts are green in brightfield microscopy and red in fluorescence microscopy. You conclude:
   a. chlorophyll is fluorescent.
   b. the magnification has distorted the image.
   c. you're not looking at the same structure in both microscopes.
   d. the stain masked the green color.
   e. none of the above
5. Which of the following is *not* a functionally analogous pair of stains?
   a. nigrosin and malachite green
   b. crystal violet and carbolfuchsin
   c. safranin and methylene blue
   d. ethanol-acetone and acid-alcohol
   e. All of the above pairs are functionally analogous.

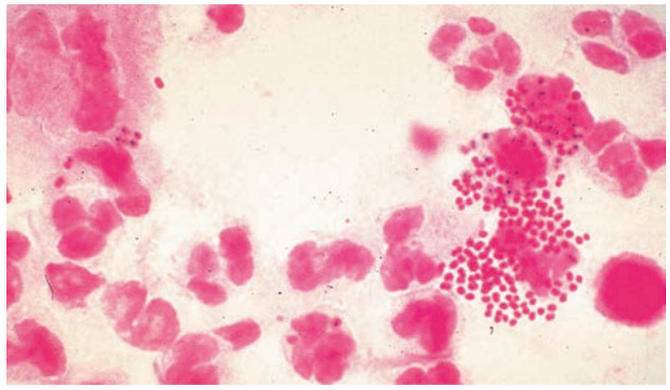
6. Which of the following pairs is *mismatched*?
   a. capsule—negative stain
   b. cell arrangement—simple stain
   c. cell size—negative stain
   d. Gram stain—bacterial identification
   e. none of the above
7. Assume you stain *Clostridium* by applying a basic stain, carbolfuchsin, with heat, decolorizing with acid-alcohol, and counterstaining with an acidic stain, nigrosin. Through the microscope, the endospores are __1__, and the cells are stained __2__.
   a. 1—red; 2—black
   b. 1—black; 2—colorless
   c. 1—colorless; 2—black
   d. 1—red; 2—colorless
   e. 1—black; 2—red
8. Assume that you are viewing a Gram-stained field of red cocci and blue rods through the microscope. You can safely conclude that you have
   a. made a mistake in staining.
   b. two different species.
   c. old bacterial cells.
   d. young bacterial cells.
   e. none of the above
9. In 1996, scientists described a new tapeworm parasite that had killed at least one person. The initial examination of the patient's abdominal mass was most likely made using
   a. brightfield microscopy.
   b. darkfield microscopy.
   c. electron microscopy.
   d. phase-contrast microscopy.
   e. fluorescence microscopy.
10. Which of the following is *not* a modification of a compound light microscope?
    a. brightfield microscopy
    b. darkfield microscopy
    c. electron microscopy
    d. phase-contrast microscopy
    e. fluorescence microscopy

Analysis

1. In a Gram stain, a step could be omitted and still allow differentiation between gram-positive and gram-negative cells. What is it?
2. Using a good compound light microscope with a resolving power of 0.3 μm, a 10× ocular lens, and a 100× oil immersion lens, would you be able to discern two objects separated by 3 μm? 0.3 μm? 300 nm?
3. Why isn't the Gram stain used on acid-fast bacteria? If you did Gram stain acid-fast bacteria, what would their Gram reaction be? What is the Gram reaction of non–acid-fast bacteria?
4. Endospores can be seen as refractile structures in unstained cells and as colorless areas in Gram-stained cells. Why is it necessary to do an endospore stain to verify the presence of endospores?

Clinical Applications and Evaluation

1. In 1882, German bacteriologist Paul Erhlich described a method for staining *Mycobacterium* and noted, "It may be that all disinfecting agents which are acidic will be without effect on this [tubercle] bacillus, and one will have to be limited to alkaline agents." How did he reach this conclusion without testing disinfectants?
2. Laboratory diagnosis of *Neisseria gonorrhoeae* infection is based on microscopic examination of Gram-stained pus. Locate the bacteria in this light micrograph. What is the disease?



3. Assume that you are viewing a Gram-stained sample of vaginal discharge. Large (10 μm) nucleated red cells are coated with small (0.5 μm wide by 1.5 μm long) blue cells on their surfaces. What is the most likely explanation for the red and blue cells?

In the Clinic

As a pediatric nurse, you see an 8-year-old patient, Sophia, who has just been diagnosed with a urinary tract infection (UTI). You explain to Sophia's mother that UTIs are quite common in children, especially girls. When you give the mother a prescription for streptomycin, she asks why Sophia can't just have penicillin again—that was the drug she received last winter to treat a chest infection. **How would you answer the mother's question?**

Hint: Read about the cell wall on pages 80–85.

Note: Answers to In the Clinic are found online at MasteringMicrobiology.

4 Functional Anatomy of Prokaryotic and Eukaryotic Cells

Despite their complexity and variety, all living cells can be classified into two groups, prokaryotes and eukaryotes, based on certain structural and functional characteristics. In general, prokaryotes are structurally simpler and smaller than eukaryotes. The DNA (genetic material) of prokaryotes is usually a single, circularly arranged chromosome and is not surrounded by a membrane; the DNA of eukaryotes is found in multiple chromosomes in a membrane-enclosed nucleus. Prokaryotes lack membrane-enclosed organelles, specialized structures that carry on various activities.

Plants and animals are entirely composed of eukaryotic cells. In the microbial world, bacteria and archaea are prokaryotes. Other cellular microbes—fungi (yeasts and molds), protozoa, and algae—are eukaryotes. Both eukaryotic and prokaryotic cells can have a sticky glycocalyx surrounding them. In nature, most bacteria are found sticking to solid surfaces, including other cells, rather than free-floating. The glycocalyx is the glue that holds the cells in place. The *Serratia* bacteria in the photograph are attached to plastic; the sticky glycocalyx dried into filaments during microscopic examination. An example of the problem posed by biofilms in hospital water supplies is described in the Clinical Case.

***Serratia* bacteria attached to plastic.**

Comparing Prokaryotic and Eukaryotic Cells: An Overview

LEARNING OBJECTIVE

4-1 Compare the cell structure of prokaryotes and eukaryotes.

Prokaryotes and eukaryotes both contain nucleic acids, proteins, lipids, and carbohydrates. They use the same kinds of chemical reactions to metabolize food, build proteins, and store energy. It is primarily the structure of cell walls and membranes, and the absence of *organelles* (specialized cellular structures that have specific functions), that distinguish prokaryotes from eukaryotes. The chief distinguishing characteristics of **prokaryotes** (from the Greek words meaning prenucleus) are as follows:

1. Typically their DNA is not enclosed within a membrane and is usually a singular, circularly arranged chromosome. *Gemma obscuriglobus* has a double membrane around its nucleus. (Some bacteria, such as *Vibrio cholerae,* have two chromosomes, and some bacteria have a linearly arranged chromosome.)
2. Their DNA is not associated with histones (special chromosomal proteins found in eukaryotes); other proteins are associated with the DNA.
3. They generally lack organelles. Advances in microscopy reveal a few membrane-enclosed organelles (for example, some inclusions). However, prokaryotes lack other membrane-enclosed organelles such as nuclei, mitochondria, and chloroplasts.
4. Their cell walls almost always contain the complex polysaccharide peptidoglycan.
5. They usually divide by **binary fission,** where DNA is copied, and the cell splits into two cells. This involves fewer structures and processes than eukaryotic cell division.

Eukaryotes (from the Greek words meaning true nucleus) have the following distinguishing characteristics:

1. Their DNA is found in the cell's nucleus, which is separated from the cytoplasm by a nuclear membrane, and the DNA is found in multiple chromosomes.
2. Their DNA is consistently associated with chromosomal proteins called histones and with nonhistones.
3. They have a number of membrane-enclosed organelles, including mitochondria, endoplasmic reticulum, Golgi complex, lysosomes, and sometimes chloroplasts.
4. Their cell walls, when present, are chemically simple.
5. Cell division usually involves mitosis, in which chromosomes replicate and an identical set is distributed into each of two nuclei. Division of the cytoplasm and other organelles follows so that the two cells produced are identical to each other.

CHECK YOUR UNDERSTANDING

What is the main feature that distinguishes prokaryotes from eukaryotes? **4-1**

Clinical Case: Infection Detection

Irene Matthews, an infection control nurse, is in a quandary. Three patients in her hospital have contracted postprocedure bacterial septicemia. All have a fever and dangerously low blood pressure. The patients are in different units, and had different procedures. The first patient, Joe, a 32-year-old construction worker, is recovering from rotator cuff surgery. He is in relatively good health, otherwise. The second patient, Jessie, a 16-year-old student in intensive care, is in critical condition following an automobile accident. She is on a ventilator and cannot breathe on her own. The third patient, Maureen, a 57-year-old grandmother, is recovering from coronary artery bypass surgery. As far as Irene can tell, the only thing these patients have in common is the infectious agent—*Klebsiella pneumoniae.*

How can three patients in different parts of a hospital contract *Klebsiella pneumoniae*? Read on to find out.

73 83 85 91 94

The Prokaryotic Cell

Prokaryotes make up a vast group of very small unicellular organisms that include bacteria and archaea. The majority are bacteria. Although bacteria and archaea look similar, their chemical composition is different. The thousands of species of bacteria are differentiated by many factors, including morphology (shape), chemical composition, nutritional requirements, biochemical activities, and sources of energy. It is estimated that 99% of the bacteria in nature exist in biofilms (see pages 54 and 156–157).

The Size, Shape, and Arrangement of Bacterial Cells

LEARNING OBJECTIVE

4-2 Identify the three basic shapes of bacteria.

Most bacteria range from 0.2 to 2.0 μm in diameter and from 2 to 8 μm in length. They may be spherical **coccus** (plural: **cocci,** meaning berries), rod-shaped **bacillus** (plural: **bacilli,** meaning little rods or walking sticks), and **spiral.**

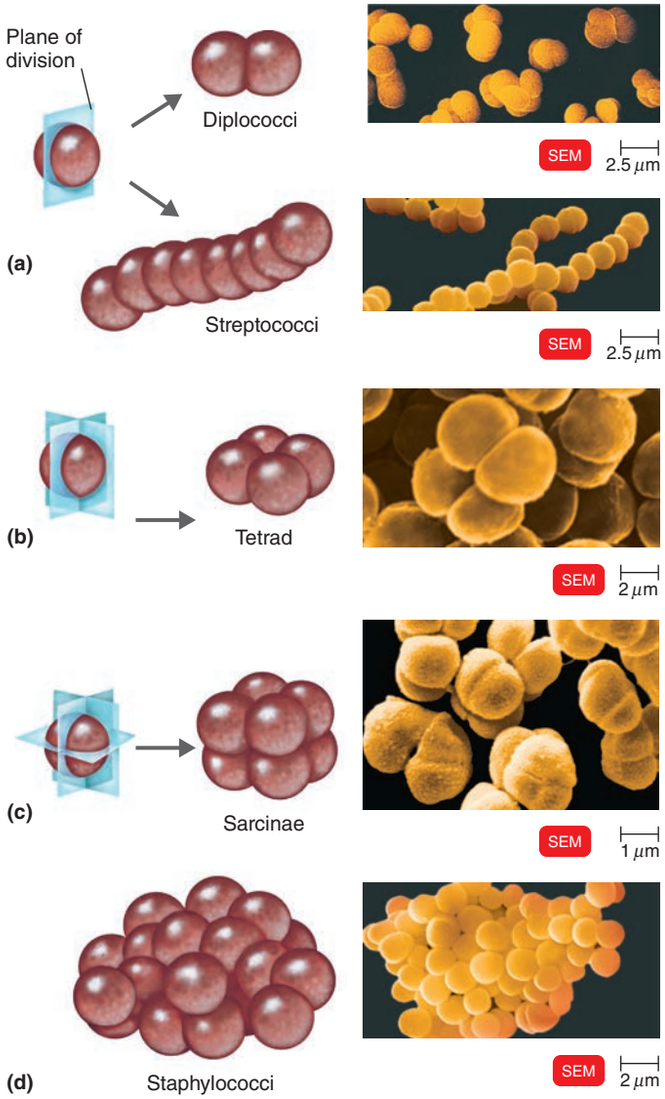


Figure 4.1 Arrangements of cocci. **(a)** Division in one plane produces diplococci and streptococci. **(b)** Division in two planes produces tetrads. **(c)** Division in three planes produces sarcinae, and **(d)** division in multiple planes produces staphylococci.

Q How do planes of division determine cell arrangement?

Cocci are usually round but can be oval, elongated, or flattened on one side. When cocci divide to reproduce, the cells can remain attached to one another. Cocci that remain in pairs after dividing are called **diplococci;** those that divide and remain attached in chainlike patterns are called **streptococci** (Figure 4.1a). Those that divide in two planes and remain in groups of four are known as **tetrads** (Figure 4.1b). Those that divide in three planes and remain attached in cubelike groups of eight are called **sarcinae** (Figure 4.1c). Those that divide in multiple planes and form grapelike clusters or broad sheets are called **staphylococci** (Figure 4.1d). These group characteristics are frequently helpful in identifying certain cocci.

Bacilli divide only across their short axis, so there are fewer groupings of bacilli than of cocci. Most bacilli appear as single rods, called **single bacilli.** (Figure 4.2a). **Diplobacilli** appear in pairs after division (Figure 4.2b), and **streptobacilli** occur in chains (Figure 4.2c). Some bacilli look like straws. Others have tapered ends, like cigars. Still others are oval and look so much like cocci that they are called **coccobacilli** (Figure 4.2d).

"Bacillus" has two meanings in microbiology. As we have just used it, bacillus refers to a bacterial shape. When capitalized and italicized, it refers to a specific genus. For example, the bacterium *Bacillus anthracis* is the causative agent of anthrax. Bacillus cells often form long, twisted chains of cells (Figure 4.3).

Spiral bacteria have one or more twists; they are never straight. Bacteria that look like curved rods are called **vibrios** (Figure 4.4a). Others, called **spirilla** (singular: **spirillum**), have a helical shape, like a corkscrew, and fairly rigid bodies (Figure 4.4b). Yet another group of spirals are helical and flexible; they are called **spirochetes** (Figure 4.4c). Unlike the spirilla, which use propeller-like external appendages called flagella to move,

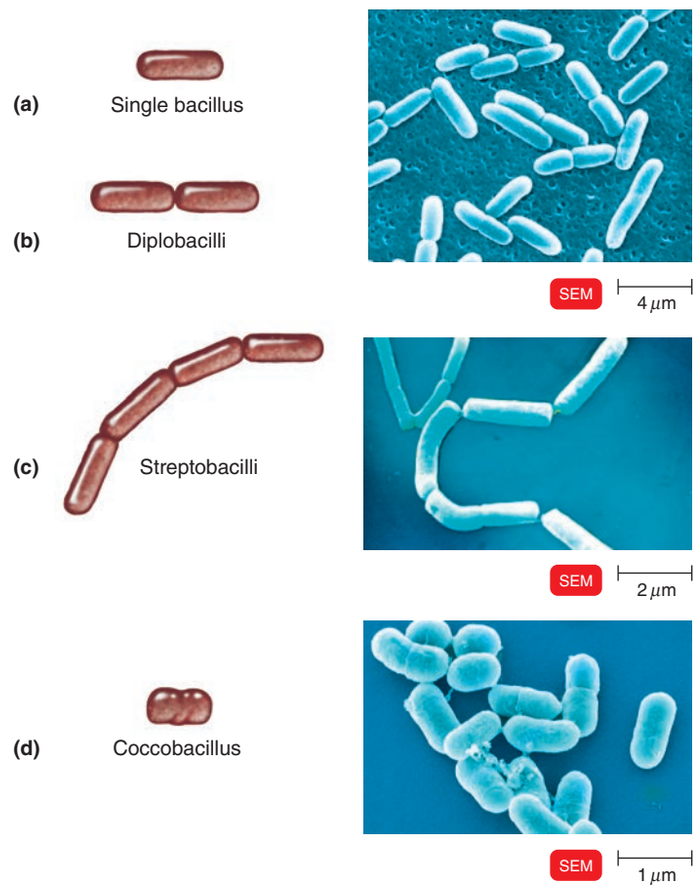


Figure 4.2 Bacilli. **(a)** Single bacilli. **(b)** Diplobacilli. In the top micrograph, a few joined pairs of bacilli could serve as examples of diplobacilli. **(c)** Streptobacilli. **(d)** Coccobacilli.

Q Why don't bacilli form tetrads or clusters?

Figure 4.3 Gram-stained *Bacillus anthracis*. As *Bacillus* cells age, their walls thin and their gram reaction is variable.

Q What is the difference between the term bacillus and *Bacillus*?

spirochetes move by means of axial filaments, which resemble flagella but are contained within a flexible external sheath. There are also star-shaped and rectangular prokaryotes (Figure 4.5).

The shape of a bacterium is determined by heredity. Genetically, most bacteria are **monomorphic;** that is, they maintain a single shape. However, a number of environmental conditions can alter that shape. If the shape is altered, identification becomes difficult. Moreover, some bacteria, such as *Rhizobium* (rī-ZŌ-bē-um) and *Corynebacterium* (kor′ī-nē-bak-TI-rē-um), are genetically **pleomorphic,** which means they can have many shapes, not just one.

The structure of a typical prokaryotic cell is shown in Figure 4.6. We will discuss structures external to the cell wall, the cell wall itself, and structures internal to the cell wall.

CHECK YOUR UNDERSTANDING

How can you identify streptococci with a microscope? **4-2**

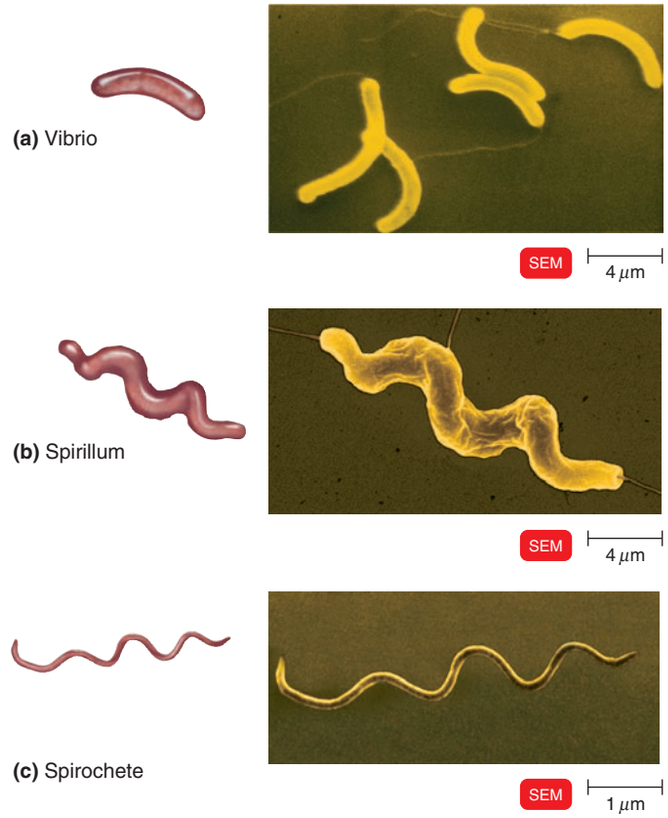


Figure 4.4 Spiral bacteria.

Q What is the distinguishing feature of spirochete bacteria?

Structures External to the Cell Wall

LEARNING OBJECTIVES

4-3 Describe the structure and function of the glycocalyx.

4-4 Differentiate flagella, axial filaments, fimbriae, and pili.

Among the possible structures external to the prokaryotic cell wall are the glycocalyx, flagella, axial filaments, fimbriae, and pili.

Glycocalyx

Many prokaryotes secrete on their surface a substance called glycocalyx. **Glycocalyx** (meaning sugar coat) is the general term used for substances that surround cells. The bacterial glycocalyx is a viscous (sticky), gelatinous polymer that is external to the cell wall and composed of polysaccharide, polypeptide, or both. Its chemical composition varies widely with the species. For the most part, it is made inside the cell and secreted to the cell surface. If the substance is organized and is firmly attached to the cell wall, the glycocalyx is described as a **capsule.** The presence of a capsule can be determined by using negative staining, described in Chapter 3 (see Figure 3.14a, page 67). If the

ASM: Bacteria have unique cell structures that are targets for antibiotics, immunity, and phage infection.

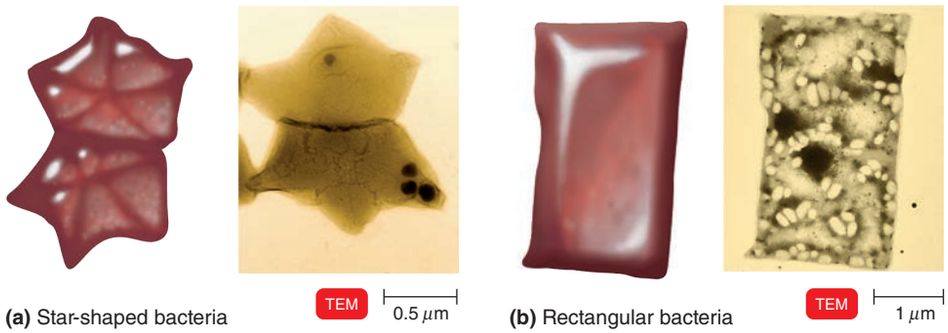


Figure 4.5 Star-shaped and rectangular prokaryotes. **(a)** *Stella* (star-shaped). **(b)** *Haloarcula,* a genus of halophilic archaea (rectangular cells).

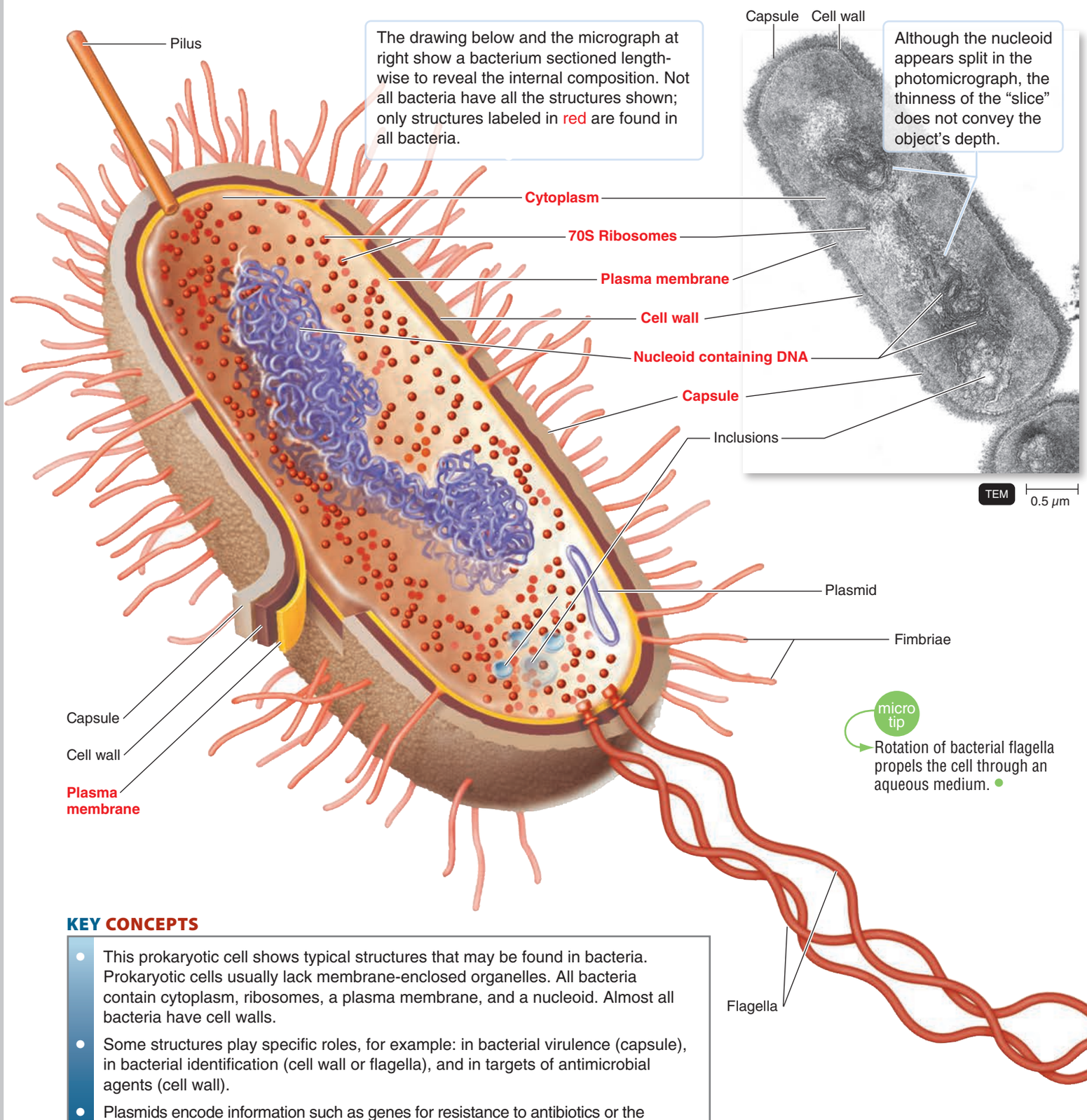
Q What are the common bacterial shapes?

FOUNDATION FIGURE 4.6

The Structure of a Prokaryotic Cell

The drawing below and the micrograph at right show a bacterium sectioned lengthwise to reveal the internal composition. Not all bacteria have all the structures shown; only structures labeled in red are found in all bacteria.

Although the nucleoid appears split in the photomicrograph, the thinness of the "slice" does not convey the object's depth.



micro tip

Rotation of bacterial flagella propels the cell through an aqueous medium.

KEY CONCEPTS

- This prokaryotic cell shows typical structures that may be found in bacteria. Prokaryotic cells usually lack membrane-enclosed organelles. All bacteria contain cytoplasm, ribosomes, a plasma membrane, and a nucleoid. Almost all bacteria have cell walls.
- Some structures play specific roles, for example: in bacterial virulence (capsule), in bacterial identification (cell wall or flagella), and in targets of antimicrobial agents (cell wall).
- Plasmids encode information such as genes for resistance to antibiotics or the production of toxins. Plasmids may be exchanged between bacteria.

(a) Peritrichous **(b)** Monotrichous and polar **(c)** Lophotrichous and polar **(d)** Amphitrichous and polar

Figure 4.7 Arrangements of bacterial flagella.
(a) Peritrichous. **(b)–(d)** Polar.

Q Not all prokaryotic cells have flagella. What are bacteria without flagella called?

substance is unorganized and only loosely attached to the cell wall, the glycocalyx is described as a **slime layer.**

In certain species, capsules are important in contributing to bacterial virulence (the degree to which a pathogen causes disease). Capsules often protect pathogenic bacteria from phagocytosis by the cells of the host. (As you will see later, phagocytosis is the ingestion and digestion of microorganisms and other solid particles.) For example, *Bacillus anthracis* produces a capsule of D-glutamic acid. (Recall from Chapter 2 that the D forms of amino acids are unusual.) Because only encapsulated *B. anthracis* causes anthrax, it is speculated that the capsule may prevent it from being destroyed by phagocytosis.

Another example involves *Streptococcus pneumoniae* (strep′tō-KOK-kus noo-MŌ-nē-ī), which causes pneumonia only when the cells are protected by a polysaccharide capsule. Unencapsulated *S. pneumoniae* cells cannot cause pneumonia and are readily phagocytized. The polysaccharide capsule of *Klebsiella* also prevents phagocytosis and allows the bacterium to adhere to and colonize the respiratory tract.

The glycocalyx is a very important component of biofilms (see pages 156–157). A glycocalyx that helps cells in a biofilm attach to their target environment and to each other is called an **extracellular polymeric substance (EPS).** The EPS protects the cells within it, facilitates communication among them, and enables the cells to survive by attaching to various surfaces in their natural environment.

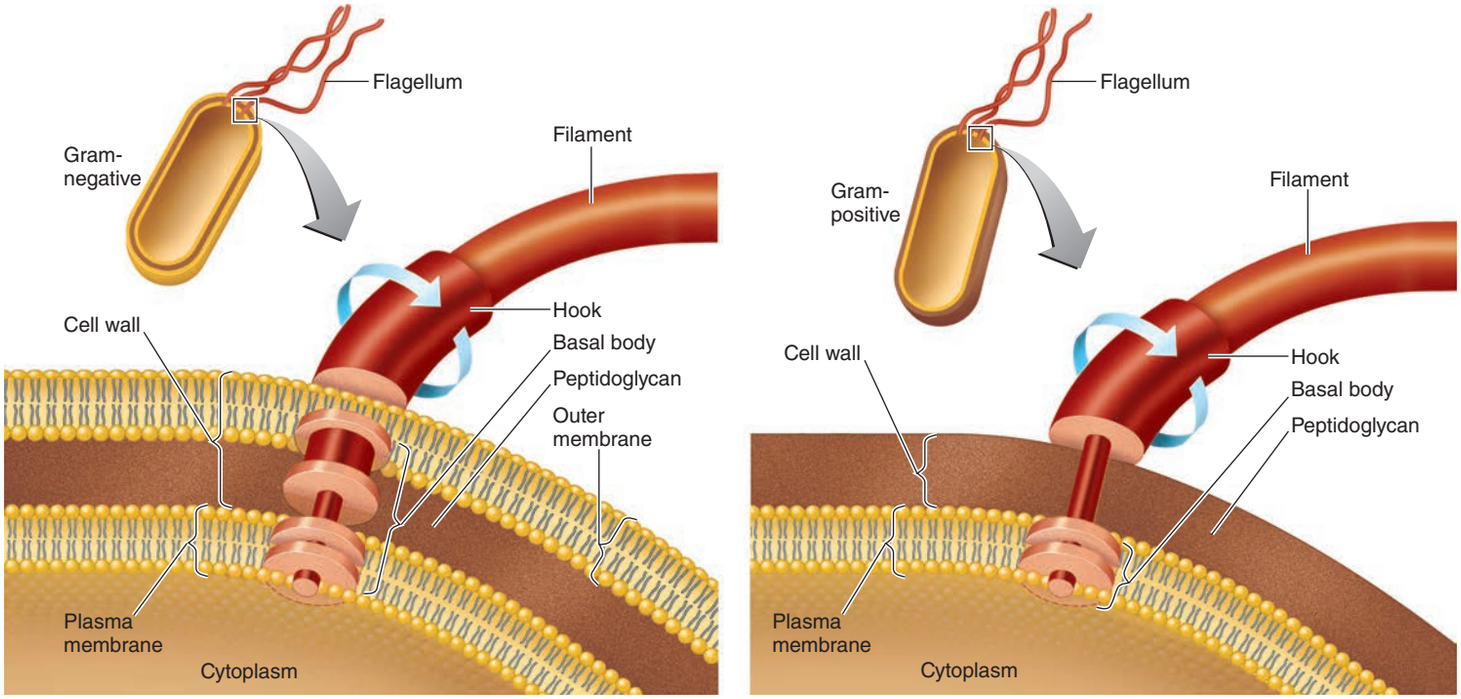
Through attachment, bacteria can grow on diverse surfaces such as rocks in fast-moving streams, plant roots, human teeth, medical implants, water pipes, and even other bacteria. *Streptococcus mutans* (MŪ-tanz), an important cause of dental caries, attaches itself to the surface of teeth by a glycocalyx. *S. mutans* may use its capsule as a source of nutrition by breaking it down and utilizing the sugars when energy stores are low. *Vibrio cholerae* (VIB-rē-ō KOL-er-ī), the cause of cholera, produces a glycocalyx that helps it attach to the cells of the small intestine. A glycocalyx also can protect a cell against dehydration, and its viscosity may inhibit the movement of nutrients out of the cell.

Flagella

Some prokaryotic cells have **flagella** (singular: **flagellum**), which are long filamentous appendages that propel bacteria. Bacteria that lack flagella are referred to as **atrichous** (without projections). Flagella may be **peritrichous** (distributed over the entire cell; Figure 4.7a) or **polar** (at one or both poles or ends of the cell). If polar, flagella may be **monotrichous** (a single flagellum at one pole; Figure 4.7b), **lophotrichous** (a tuft of flagella coming from one pole; Figure 4.7c), or **amphitrichous** (flagella at both poles of the cell; Figure 4.7d).

A flagellum has three basic parts (Figure 4.8). The long outermost region, the *filament,* is constant in diameter and contains the globular (roughly spherical) protein *flagellin* arranged in several chains that intertwine and form a helix around a hollow core. In most bacteria, filaments are not covered by a membrane or sheath, as in eukaryotic cells. The filament is attached to a slightly wider *hook,* consisting of a different protein. The third portion of a flagellum is the *basal body,* which anchors the flagellum to the cell wall and plasma membrane.

The basal body is composed of a small central rod inserted into a series of rings. Gram-negative bacteria contain two pairs of rings; the outer pair of rings is anchored to various portions of the cell wall, and the inner pair of rings is anchored to the plasma membrane. In gram-positive bacteria, only the inner pair is present. As you will see later, the flagella (and cilia) of eukaryotic cells are more complex than those of prokaryotic cells.



(a) Parts and attachment of a flagellum of a gram-negative bacterium

(b) Parts and attachment of a flagellum of a gram-positive bacterium

Figure 4.8 The structure of a prokaryotic flagellum. The parts and attachment of a flagellum of a gram-negative bacterium and gram-positive bacterium are shown in these highly schematic diagrams.

Q How do the basal bodies of gram-negative and gram-positive bacteria differ?

Each prokaryotic flagellum is a semirigid, helical structure that moves the cell by rotating from the basal body. The rotation of a flagellum is either clockwise or counterclockwise around its long axis. (Eukaryotic flagella, by contrast, undulate in a wavelike motion.) The movement of a prokaryotic flagellum results from rotation of its basal body and is similar to the movement of the shaft of an electric motor. As the flagella rotate, they form a bundle that pushes against the surrounding liquid and propels the bacterium. Flagellar rotation depends on the cell's continuous generation of energy.

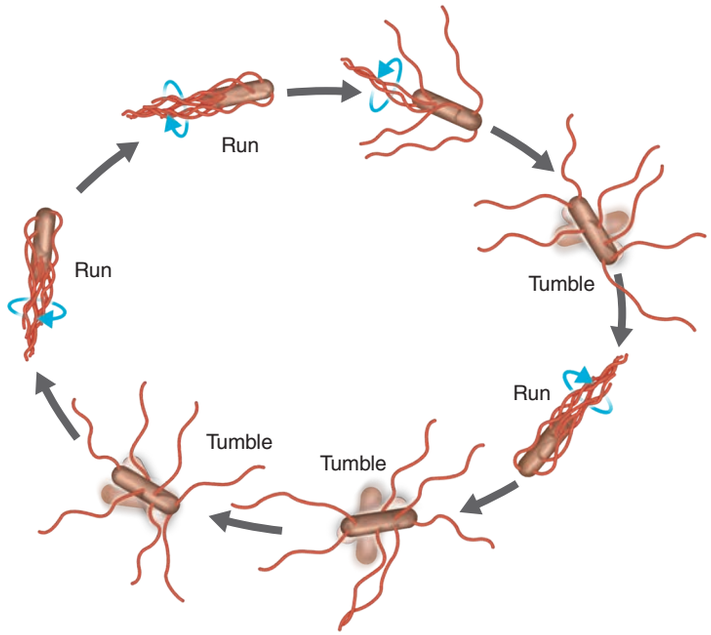
Bacterial cells can alter the speed and direction of rotation of flagella and thus are capable of various patterns of **motility,** the ability of an organism to move by itself. When a bacterium moves in one direction for a length of time, the movement is called a "run" or "swim." "Runs" are interrupted by periodic, abrupt, random changes in direction called "tumbles." Then, a "run" resumes. "Tumbles" are caused by a reversal of flagellar rotation (Figure 4.9a). Some species of bacteria endowed with many flagella—*Proteus* (PRŌ-tē-us), for example (Figure 4.9b)—can "swarm," or show rapid wavelike movement across a solid culture medium.

One advantage of motility is that it enables a bacterium to move toward a favorable environment or away from an adverse one. The movement of a bacterium toward or away from a particular stimulus is called **taxis.** Such stimuli include chemicals (**chemotaxis**) and light (**phototaxis**). Motile bacteria contain receptors in various locations, such as in or just under the cell wall. These receptors pick up chemical stimuli, such as oxygen, ribose, and galactose. In response to the stimuli, information is passed to the flagella. If the chemotactic signal is positive, called an *attractant,* the bacteria move toward the stimulus with many runs and few tumbles. If the chemotactic signal is negative, called a *repellent,* the frequency of tumbles increases as the bacteria move away from the stimulus.

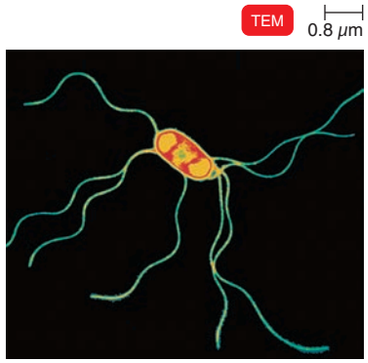
The flagellar protein called **H antigen** is useful for distinguishing among **serovars,** or variations within a species, of gram-negative bacteria (see page 299). For example, there are at least 50 different H antigens for *E. coli.* Those serovars identified as *E. coli* O157:H7 are associated with foodborne epidemics (see Chapter 1, pages 17–18). MM **Animations** Motility; Flagella: Structure, Movement, Arrangement

Axial Filaments

Spirochetes are a group of bacteria that have unique structure and motility. One of the best-known spirochetes is *Treponema pallidum* (trep-ō-NĒ-mah PAL-li-dum), the causative agent of syphilis. Another spirochete is *Borrelia burgdorferi* (bor-RĒ-lē-ah burg-DOR-fer-ē), the causative agent of Lyme disease. Spirochetes move by means of **axial filaments,** or **endoflagella,** bundles of fibrils that arise at the ends of the cell beneath an outer sheath and spiral around the cell (Figure 4.10).



(a) A bacterium running and tumbling. Notice that the direction of flagellar rotation (blue arrows) determines which of these movements occurs. Gray arrows indicate direction of movement of the microbe.



(b) A *Proteus* cell in the swarming stage may have more than 1000 peritrichous flagella.

Figure 4.9 Flagella and bacterial motility.

Do bacterial flagella push or pull a cell?

Axial filaments, which are anchored at one end of the spirochete, have a structure similar to that of flagella. The rotation of the filaments produces a movement of the outer sheath that propels the spirochetes in a spiral motion. This type of movement is similar to the way a corkscrew moves through a cork. This corkscrew motion probably enables a bacterium such as *T. pallidum* to move effectively through bodily fluids.

Animation Spirochetes

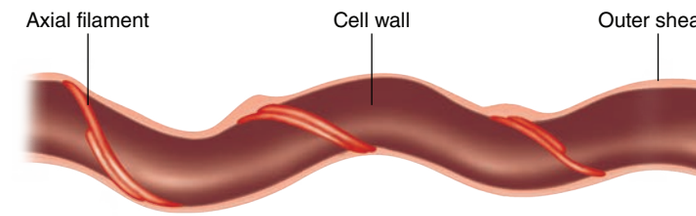
Fimbriae and Pili

Many gram-negative bacteria contain hairlike appendages that are shorter, straighter, and thinner than flagella. These structures, which consist of a protein called *pilin* arranged helically around a central core, are divided into two types, fimbriae and pili, having very different functions. (Some microbiologists use the two terms interchangeably to refer to all such structures, but we distinguish between them.)

Fimbriae (singular: **fimbria**) can occur at the poles of the bacterial cell or can be evenly distributed over the entire surface of the cell. They can number anywhere from a few to several hundred per cell (**Figure 4.11**). Fimbriae have a tendency to adhere to each other and to surfaces. As a result, they are involved in forming biofilms and other aggregations on the surfaces of liquids, glass, and rocks. Fimbriae can also help bacteria adhere to epithelial surfaces in the body. For example, fimbriae on the bacterium *Neisseria*

ASM: Bacteria and archaea have specialized structures (flagella, endorspores, and pili) that often confer critical capabilities.

(a) A photomicrograph of the spirochete *Leptospira,* showing an axial filament



(b) A diagram of axial filaments wrapping around part of a spirochete

Figure 4.10 Axial filaments.

How are endoflagella different from flagella?

Figure 4.11 Fimbriae. The fimbriae seem to bristle from this *E. coli* cell, which is beginning to divide.

Q Why are fimbriae necessary for colonization?

gonorrhoeae (nī-SE-rē-ah go-nōr-RĒ-ī), the causative agent of gonorrhea, help the microbe colonize mucous membranes. Once colonization occurs, the bacteria can cause disease. The fimbriae of *E. coli* O157 enable this bacterium to adhere to the lining of the small intestine, where it causes a severe watery diarrhea. When fimbriae are absent (because of genetic mutation), colonization cannot happen, and no disease ensues.

Pili (singular: **pilus**) are usually longer than fimbriae and number only one or two per cell. Pili are involved in motility and DNA transfer. In one type of motility, called **twitching motility,** a pilus extends by the addition of subunits of pilin, makes contact with a surface or another cell, and then retracts (powerstroke) as the pilin subunits are disassembled. This is called the *grappling hook model* of twitching motility and results in short, jerky, intermittent movements. Twitching motility has been observed in *Pseudomonas aeruginosa, Neisseria gonorrhoeae,* and some strains of *E. coli*. The other type of motility associated with pili is **gliding motility,** the smooth gliding movement of myxobacteria. Although the exact mechanism is unknown for most myxobacteria, some utilize pilus retraction. Gliding motility provides a means for microbes to travel in environments with a low water content, such as biofilms and soil.

Some pili are used to bring bacteria together allowing the transfer of DNA from one cell to another, a process called conjugation. Such pili are called **conjugation (sex) pili** (see pages 228–229 or Figure 8.27a, page 229). In this process, the conjugation pilus of one bacterium called an F^+ cell connects to receptors on the surface of another bacterium of its own species or a different species. The two cells make physical contact, and DNA from the F^+ cell is transferred to the other cell. The exchanged DNA can add a new function to the recipient cell, such as antibiotic resistance or the ability to digest its medium more efficiently.

CHECK YOUR UNDERSTANDING

- Why are bacterial capsules medically important? **4-3**
- How do bacteria move? **4-4**

The Cell Wall

LEARNING OBJECTIVES

4-5 Compare and contrast the cell walls of gram-positive bacteria, gram-negative bacteria, acid-fast bacteria, archaea, and mycoplasmas.

4-6 Compare and contrast archaea and mycoplasmas.

4-7 Differentiate *protoplast, spheroplast,* and *L form*.

The **cell wall** of the bacterial cell is a complex, semirigid structure responsible for the shape of the cell. Almost all prokaryotes have a cell wall that surrounds the underlying, fragile plasma (cytoplasmic) membrane and protects it and the interior of the cell from adverse changes in the outside environment (see Figure 4.6).

The major function of the cell wall is to prevent bacterial cells from rupturing when the water pressure inside the cell is greater than that outside the cell (see Figure 4.18d, page 89). It also helps maintain the shape of a bacterium and serves as a point of anchorage for flagella. As the volume of a bacterial cell increases, its plasma membrane and cell wall extend as needed. Clinically, the cell wall is important because it contributes to the ability of some species to cause disease and is the site of action of some antibiotics. In addition, the chemical composition of the cell wall is used to differentiate major types of bacteria.

Although the cells of some eukaryotes, including plants, algae, and fungi, have cell walls, their walls differ chemically from those of prokaryotes, are simpler in structure, and are less rigid.

Composition and Characteristics

The bacterial cell wall is composed of a macromolecular network called **peptidoglycan** (also known as *murein*), which is present either alone or in combination with other substances. Peptidoglycan consists of a repeating disaccharide connected by polypeptides to form a lattice that surrounds and protects the entire cell. The disaccharide portion is made up of monosaccharides called N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) (from *murus,* meaning wall), which are related to glucose. The structural formulas for NAG and NAM are shown in **Figure 4.12**.

The various components of peptidoglycan are assembled in the cell wall (**Figure 4.13a**). Alternating NAM and NAG molecules are linked in rows of 10 to 65 sugars to form a carbohydrate "backbone" (the glycan portion of peptidoglycan). Adjacent rows are linked by **polypeptides** (the peptide portion of peptidoglycan). Although the structure of the polypeptide link varies, it always includes *tetrapeptide side chains,* which

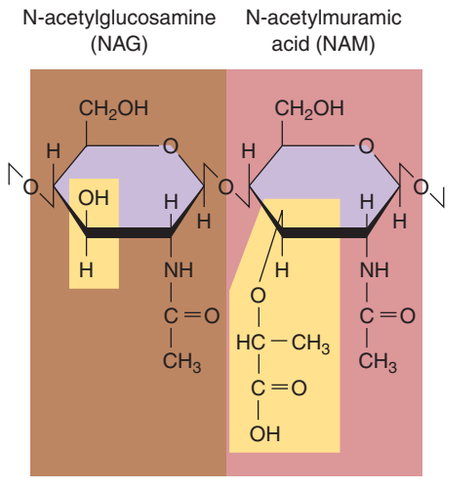


Figure 4.12 N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) joined as in a peptidoglycan. The gold areas show the differences between the two molecules. The linkage between them is called a β-1,4 linkage.

Q What kind of molecules are these: carbohydrates, lipids,or proteins?

consist of four amino acids attached to NAMs in the backbone. The amino acids occur in an alternating pattern of D and L forms (see Figure 2.13, page 40). This is unique because the amino acids found in other proteins are L forms. Parallel tetrapeptide side chains may be directly bonded to each other or linked by a *peptide cross-bridge,* consisting of a short chain of amino acids.

Penicillin interferes with the final linking of the peptidoglycan rows by peptide cross-bridges (see Figure 4.13a). As a result, the cell wall is greatly weakened and the cell undergoes **lysis,** destruction caused by rupture of the plasma membrane and the loss of cytoplasm.

Gram-Positive Cell Walls

In most gram-positive bacteria, the cell wall consists of many layers of peptidoglycan, forming a thick, rigid structure (Figure 4.13b). By contrast, gram-negative cell walls contain only a thin layer of peptidoglycan (Figure 4.13c). The space between the cell wall and plasma membrane of gram-positive bacteria is the plasmic space. It contains the granular layer, which is composed of lipoteichoic acid. In addition, the cell walls of gram-positive bacteria contain *teichoic acids,* which consist primarily of an alcohol (such as glycerol or ribitol) and phosphate. There are two classes of teichoic acids: *lipoteichoic acid,* which spans the peptidoglycan layer and is linked to the plasma membrane, and *wall teichoic acid,* which is linked to the peptidoglycan layer. Because of their negative charge (from the phosphate groups), teichoic acids may bind and regulate the movement of cations (positive ions) into and out of the cell. They may also assume a role in cell growth, preventing extensive wall breakdown and possible cell lysis. Finally, teichoic acids provide much of the wall's antigenic specificity and thus make it possible to identify gram-positive bacteria by certain laboratory tests (see Chapter 10). Similarly, the cell walls of gram-positive streptococci are covered with various polysaccharides that allow them to be grouped into medically significant types.

Gram-Negative Cell Walls

The cell walls of gram-negative bacteria consist of one or a very few layers of peptidoglycan and an outer membrane (see Figure 4.13c). The peptidoglycan is bonded to lipoproteins in the outer membrane and is in the *periplasm*, a gel-like fluid in the periplasmic space of gram negative bacteria, the region between the outer membrane and the plasma membrane. The periplasm contains a high concentration of degradative enzymes and transport proteins. Gram-negative cell walls do not contain teichoic acids. Because the cell walls of gram-negative bacteria contain only a small amount of peptidoglycan, they are more susceptible to mechanical breakage.

The *outer membrane* of the gram-negative cell consists of lipopolysaccharides (LPS), lipoproteins, and phospholipids (see Figure 4.13c). The outer membrane has several specialized functions. Its strong negative charge is an important factor in evading phagocytosis and the actions of complement (lyses cells and promotes phagocytosis), two components of the defenses of the host (discussed in detail in Chapter 16). The outer membrane also provides a barrier to detergents, heavy metals, bile salts, certain dyes, antibiotics (for example, penicillin), and digestive enzymes such as lysozyme.

However, the outer membrane doesn't block all environmental substances because nutrients must enter to sustain the metabolism of the cell. Part of the permeability of the outer membrane is due to proteins in the membrane, called **porins,** that form channels. Porins permit the passage of molecules such as nucleotides, disaccharides, peptides, amino acids, vitamin B_{12}, and iron.

The **lipopolysaccharide (LPS)** of the outer membrane is a large, complex molecule that contains lipids and carbohydrates and consists of three components: (1) lipid A, (2) a core polysaccharide, and (3) an O polysaccharide. **Lipid A** is the lipid portion of the LPS and is embedded in the top layer of the outer membrane. When gram-negative bacteria die, they release lipid A, which functions as an endotoxin (Chapter 15). Lipid A is responsible for the symptoms associated with infections by gram-negative bacteria, such as fever, dilation of blood vessels, shock, and blood clotting. The **core polysaccharide** is attached to lipid A and contains unusual sugars. Its role is structural—to provide stability. The **O polysaccharide** extends outward from the core polysaccharide and is composed of sugar molecules. The O polysaccharide functions as an antigen and is useful for distinguishing species of gram-negative bacteria. For example, the foodborne pathogen *E. coli* O157:H7 is distinguished from other serovars by certain laboratory tests that test for these specific antigens. This role is comparable to that of teichoic acids in gram-positive cells.

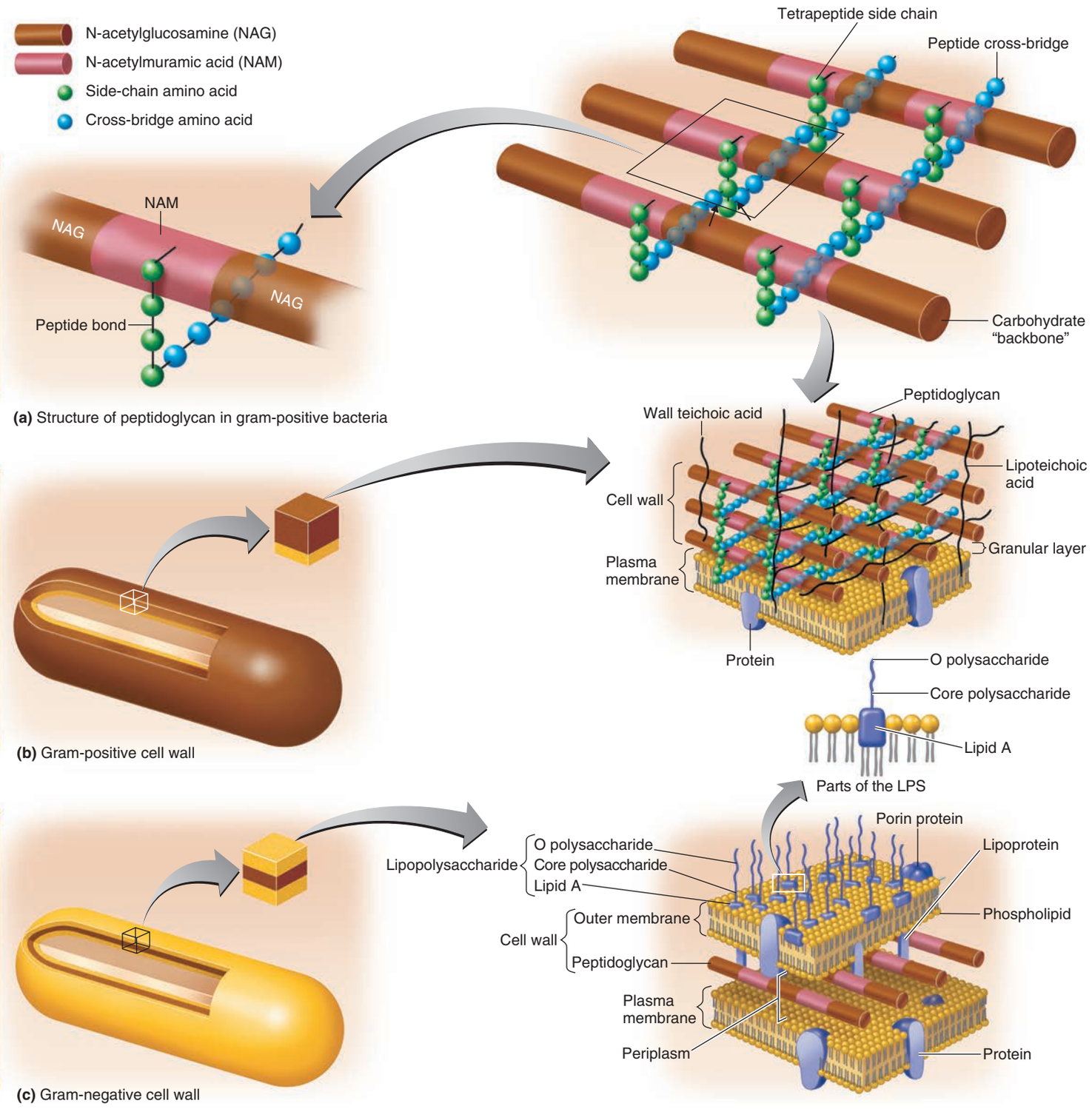


Figure 4.13 Bacterial cell walls **(a)** The structure of peptidoglycan in gram-positive bacteria. Together the carbohydrate backbone (glycan portion) and tetrapeptide side chains (peptide portion) make up peptidoglycan. The frequency of peptide cross-bridges and the number of amino acids in these bridges vary with species of bacteria. The small arrows indicate where penicillin interferes with the linkage of peptidoglycan rows by peptide cross-bridges. **(b)** A gram-positive cell wall. **(c)** A gram-negative cell wall.

Q What are the major structural differences between gram-positive and gram-negative cell walls?

Clinical Case

Irene reviews what she knows about gram-negative *K. pneumoniae* bacteria. Although this bacterium is part of normal intestinal microbiota, outside its typical environment it can cause serious infection. *K. pneumoniae* accounts for about 8% of all health care–associated infections. Irene surmises that the bacteria had to come from the hospital somewhere.

What is causing the patients' fever and low blood pressure?

73 83 85 91 94

Cell Walls and the Gram Stain Mechanism

Now that you've studied the Gram stain (in Chapter 3, page 65) and the chemistry of the bacterial cell wall (in the previous section), it's easier to understand how the Gram stain works. The mechanism of the Gram stain is based on differences in the cell wall structure of gram-positive and gram-negative bacteria and how each reacts to various reagents (substances used for producing a chemical reaction). Crystal violet, the primary stain, stains both gram-positive and gram-negative cells purple because the dye enters the cytoplasm of both types of cells. When iodine (the mordant) is applied, it forms large crystals with the dye that are too large to escape through the cell wall. The application of alcohol dehydrates the peptidoglycan of gram-positive cells to make it more impermeable to the crystal violet-iodine. The effect on gram-negative cells is quite different; alcohol dissolves the outer membrane of gram-negative cells and even leaves small holes in the thin peptidoglycan layer through which crystal violet-iodine diffuse. Because gram-negative bacteria are colorless after the alcohol wash, the addition of safranin (the counterstain) turns the cells pink or red. Safranin provides a contrasting color to the primary stain (crystal violet). Although gram-positive and gram-negative cells both absorb safranin, the pink or red color of safranin is masked by the darker purple dye previously absorbed by gram-positive cells.

In any population of cells, some gram-positive cells will give a gram-negative response. These cells are usually dead. However, there are a few gram-positive genera that show an increasing number of gram-negative cells as the culture ages. *Bacillus* and *Clostridium* are examples and are often described as *gram-variable* (see Figure 4.3).

Table 4.1 compares some of the characteristics of gram-positive and gram-negative bacteria.

Atypical Cell Walls

Among prokaryotes, certain types of cells have no walls or have very little wall material. These include members of the genus *Mycoplasma* (mī-kō-PLAZ-mah) and related organisms (see Figure 11.24, page 311). Mycoplasmas are the smallest known bacteria that can grow and reproduce outside living host cells. Because of their size and because they have no cell walls, they pass through most bacterial filters and were first mistaken for viruses. Their plasma membranes are unique among bacteria in having lipids called *sterols,* which are thought to help protect them from lysis (rupture).

Archaea may lack walls or may have unusual walls composed of polysaccharides and proteins but not peptidoglycan. These walls do, however, contain a substance similar to peptidoglycan called *pseudomurein*. Pseudomurein contains N-acetyltalosaminuronic acid instead of NAM and lacks the D-amino acids found in bacterial cell walls. Archaea generally cannot be Gram-stained but appear gram-negative because they do not contain peptidoglycan.

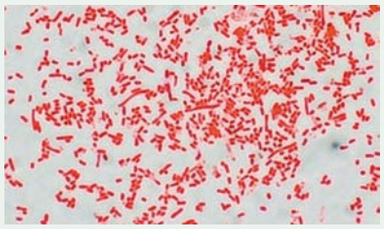
Acid-Fast Cell Walls

Recall from Chapter 3 that the acid-fast stain is used to identify all bacteria of the genus *Mycobacterium* and pathogenic species of *Nocardia*. These bacteria contain high concentrations (60%) of a hydrophobic waxy lipid (**mycolic acid**) in their cell wall that prevents the uptake of dyes, including those used in the Gram stain. The mycolic acid forms a layer outside of a thin layer of peptidoglycan. The mycolic acid and peptidoglycan are held together by a polysaccharide. The hydrophobic waxy cell wall causes both cultures of *Mycobacterium* to clump and stick to the walls of the flask. Acid-fast bacteria can be stained with carbolfuchsin, which penetrates bacteria more effectively when heated. The carbolfuchsin penetrates the cell wall, binds to the cytoplasm, and resists removal by washing with acid-alcohol. Acid-fast bacteria retain the red color of carbolfuchsin because it's more soluble in the cell wall's mycolic acid than in the acid-alcohol. If the mycolic acid layer is removed from the cell wall of acid-fast bacteria, they will stain gram-positive with the Gram stain.

Damage to the Cell Wall

Chemicals that damage bacterial cell walls, or interfere with their synthesis, often do not harm the cells of an animal host because the bacterial cell wall is made of chemicals unlike those in eukaryotic cells. Thus, cell wall synthesis is the target for some antimicrobial drugs. One way the cell wall can be damaged is by exposure to the digestive enzyme *lysozyme*. This enzyme occurs naturally in some eukaryotic cells and is a constituent of perspiration, tears, mucus, and saliva. Lysozyme is particularly active on the major cell wall components of most gram-positive bacteria, making them vulnerable to lysis. Lysozyme catalyzes hydrolysis of the bonds between the sugars in the repeating disaccharide "backbone" of peptidoglycan. This act is analogous to cutting the steel supports of a bridge with a cutting torch: the gram-positive cell wall is almost completely destroyed by lysozyme. The cellular contents that remain surrounded by the plasma membrane may remain intact if lysis does not occur;

TABLE 4.1 Some Comparative Characteristics of Gram-Positive and Gram-Negative Bacteria

Characteristic	Gram-Positive	Gram-Negative
	LM 12 μm	 LM 15 μm
Gram Reaction	Retain crystal violet dye and stain blue or purple	Can be decolorized to accept counterstain (safranin) and stain pink or red
Peptidoglycan Layer	Thick (multilayered)	Thin (single-layered)
Teichoic Acids	Present in many	Absent
Periplasmic Space	Granular layer	Periplasm
Outer Membrane	Absent	Present
Lipopolysaccharide (LPS) Content	Virtually none	High
Lipid and Lipoprotein Content	Low (acid-fast bacteria have lipids linked to peptidoglycan)	High (because of presence of outer membrane)
Flagellar Structure	2 rings in basal body	4 rings in basal body
Toxins Produced	Exotoxins	Endotoxin and exotoxins
Resistance to Physical Disruption	High	Low
Cell Wall Disruption by Lysozyme	High	Low (requires pretreatment to destabilize outer membrane)
Susceptibility to Penicillin and Sulfonamide	High	Low
Susceptibility to Streptomycin, Chloramphenicol, and Tetracycline	Low	High
Inhibition by Basic Dyes	High	Low
Susceptibility to Anionic Detergents	High	Low
Resistance to Sodium Azide	High	Low
Resistance to Drying	High	Low

this wall-less cell is termed a **protoplast.** Typically, a protoplast is spherical and is still capable of carrying on metabolism.

Some members of the genus *Proteus,* as well as other genera, can lose their cell walls and swell into irregularly shaped cells called **L forms,** named for the Lister Institute, where they were discovered. They may form spontaneously or develop in response to penicillin (which inhibits cell wall formation) or lysozyme (which removes the cell wall). L forms can live and divide repeatedly or return to the walled state.

When lysozyme is applied to gram-negative cells, usually the wall is not destroyed to the same extent as in gram-positive cells; some of the outer membrane also remains. In this case, the cellular contents, plasma membrane, and remaining outer wall layer are called a **spheroplast,** also a spherical structure. For lysozyme to exert its effect on gram-negative cells, the cells are first treated with EDTA (ethylenediaminetetraacetic acid). EDTA weakens ionic bonds in the outer membrane and thereby damages it, giving the lysozyme access to the peptidoglycan layer.

Protoplasts and spheroplasts burst in pure water or very dilute salt or sugar solutions because the water molecules from the surrounding fluid rapidly move into and enlarge the cell, which has

Clinical Case

The outer membrane of *K. pneumoniae*'s gram-negative cell wall contains the endotoxin, lipid A, which causes fever and capillary dilation.

Irene works with Joe's, Jessie's, and Maureen's physicians to combat this potentially deadly infection. Irene is particularly concerned about Jessie because of her already weakened respiratory condition. All three patients are treated with a β-lactam antibiotic, imipenem. *Klebsiella* bacteria are resistant to many antibiotics, but imipenem seems to be working for Joe and Maureen. Jessie, however, is getting worse.

Why are Jessie's symptoms worsening if the bacteria are being killed?

73 83 85 91 94

a much lower internal concentration of water. This rupturing, called **osmotic lysis,** will be discussed in detail shortly.

As noted earlier, certain antibiotics, such as penicillin, destroy bacteria by interfering with the formation of the peptide cross-bridges of peptidoglycan, thus preventing the formation of a functional cell wall. Most gram-negative bacteria are not as susceptible to penicillin as gram-positive bacteria are because the outer membrane of gram-negative bacteria forms a barrier that inhibits the entry of this and other substances, and gram-negative bacteria have fewer peptide cross-bridges. However, gram-negative bacteria are quite susceptible to some β-lactam antibiotics that penetrate the outer membrane better than penicillin. Antibiotics will be discussed in more detail in Chapter 20.

CHECK YOUR UNDERSTANDING

- Why are drugs that target cell wall synthesis useful? **4-5**
- Why are mycoplasmas resistant to antibiotics that interfere with cell wall synthesis? **4-6**
- How do protoplasts differ from L forms? **4-7**

Structures Internal to the Cell Wall

LEARNING OBJECTIVES

4-8 Describe the structure, chemistry, and functions of the prokaryotic plasma membrane.

4-9 Define *simple diffusion, facilitated diffusion, osmosis, active transport,* and *group translocation*.

4-10 Identify the functions of the nucleoid and ribosomes.

4-11 Identify the functions of four inclusions.

4-12 Describe the functions of endospores, sporulation, and endospore germination.

Thus far, we have discussed the prokaryotic cell wall and structures external to it. We will now look inside the prokaryotic cell and discuss the structures and functions of the plasma membrane and components within the cytoplasm of the cell.

The Plasma (Cytoplasmic) Membrane

The **plasma (cytoplasmic) membrane** (or *inner membrane*) is a thin structure lying inside the cell wall and enclosing the cytoplasm of the cell (see Figure 4.6). The plasma membrane of prokaryotes consists primarily of phospholipids (see Figure 2.10, page 38), which are the most abundant chemicals in the membrane, and proteins. Eukaryotic plasma membranes also contain carbohydrates and sterols, such as cholesterol. Because they lack sterols, prokaryotic plasma membranes are less rigid than eukaryotic membranes. One exception is the wall-less prokaryote *Mycoplasma,* which contains membrane sterols.

Structure

In electron micrographs, prokaryotic and eukaryotic plasma membranes (and the outer membranes of gram-negative bacteria) look like two-layered structures; there are two dark lines with a light space between the lines (**Figure 4.14a**). The phospholipid molecules are arranged in two parallel rows, called a *lipid bilayer* (Figure 4.14b). Each phospholipid molecule (see Chapter 2) contains a polar head, composed of a phosphate group and glycerol that is hydrophilic (water-loving) and soluble in water, and nonpolar tails, composed of fatty acids that are hydrophobic (water-fearing) and insoluble in water (Figure 4.14c). The polar heads are on the two surfaces of the lipid bilayer, and the nonpolar tails are in the interior of the bilayer.

The protein molecules in the membrane can be arranged in a variety of ways. Some, called *peripheral proteins,* are easily removed from the membrane by mild treatments and lie at the inner or outer surface of the membrane. They may function as enzymes that catalyze chemical reactions, as a "scaffold" for support, and as mediators of changes in membrane shape during movement. Other proteins, called *integral proteins,* can be removed from the membrane only after disrupting the lipid bilayer (by using detergents, for example). Most integral proteins penetrate the membrane completely and are called *transmembrane proteins*. Some integral proteins are channels that have a pore, or hole, through which substances enter and exit the cell.

Many of the proteins and some of the lipids on the outer surface of the plasma membrane have carbohydrates attached to them. Proteins attached to carbohydrates are called **glycoproteins,** and lipids attached to carbohydrates are called **glycolipids.** Both glycoproteins and glycolipids help protect and lubricate the cell and are involved in cell-to-cell interactions. For example, glycoproteins play a role in certain infectious diseases. The influenza virus and the toxins that cause cholera and botulism enter their target cells by first binding to glycoproteins on their plasma membranes.

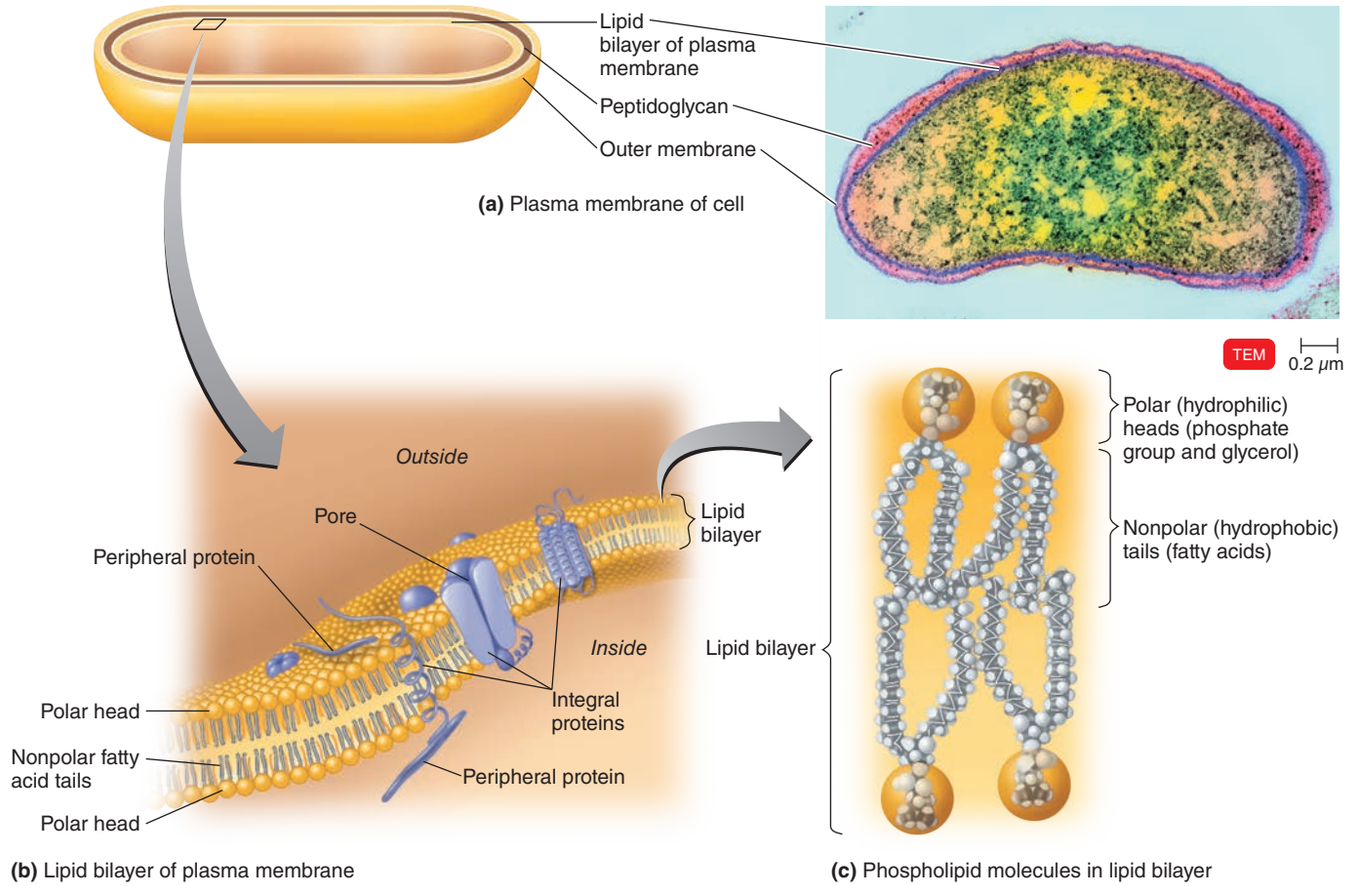


Figure 4.14 Plasma membrane. **(a)** A diagram and micrograph showing the lipid bilayer forming the inner plasma membrane of the gram-negative bacterium *Vibrio cholerae*. Layers of the cell wall, including the outer membrane, can be seen outside the inner membrane. **(b)** A portion of the inner membrane showing the lipid bilayer and proteins. The outer membrane of gram-negative bacteria is also a lipid bilayer. **(c)** Space-filling models of several phospholipid molecules as they are arranged in the lipid bilayer.

Q What is the difference between a peripheral and an integral protein?

Studies have demonstrated that the phospholipid and protein molecules in membranes are not static but move quite freely within the membrane surface. This movement is most probably associated with the many functions performed by the plasma membrane. Because the fatty acid tails cling together, phospholipids in the presence of water form a self-sealing bilayer; as a result, breaks and tears in the membrane heal themselves. The membrane must be about as viscous as olive oil, which allows membrane proteins to move freely enough to perform their functions without destroying the structure of the membrane. This dynamic arrangement of phospholipids and proteins is referred to as the **fluid mosaic model.**

Functions

The most important function of the plasma membrane is to serve as a selective barrier through which materials enter and exit the cell. In this function, plasma membranes have **selective permeability** (sometimes called *semipermeability*). This term indicates that certain molecules and ions are allowed to pass through the membrane but others are stopped. The permeability of the membrane depends on several factors. Large molecules (such as proteins) cannot pass through the plasma membrane, possibly because these molecules are larger than the pores in integral proteins that function as channels. But smaller molecules (such as water, oxygen, carbon dioxide, and some simple sugars) usually pass through easily. Ions penetrate the membrane very slowly. Substances that dissolve easily in lipids (such as oxygen, carbon dioxide, and nonpolar organic molecules) enter and exit more easily than other substances because the membrane consists mostly of phospholipids. The movement of materials across plasma membranes also depends on transporter molecules, which will be described shortly.

Plasma membranes are also important to the breakdown of nutrients and the production of energy. The plasma membranes of bacteria contain enzymes capable of catalyzing the chemical reactions that break down nutrients and produce ATP. In some bacteria, pigments and enzymes involved in photosynthesis are found in infoldings of the plasma membrane that extend

Figure 4.15 Chromatophores. In this micrograph of *Rhodospirillum rubrum,* a purple (nonsulfur) bacterium, the chromatophores are clearly visible.

Q What is the function of chromatophores?

into the cytoplasm. These membranous structures are called **chromatophores** (Figure 4.15).

When viewed with an electron microscope, bacterial plasma membranes often appear to contain one or more large, irregular folds called **mesosomes.** Many functions have been proposed for mesosomes. However, it is now known that they are artifacts, not true cell structures. Mesosomes are believed to be folds in the plasma membrane that develop by the process used for preparing specimens for electron microscopy. MM **Animations** Membrane Structure; Membrane Permeability

Destruction of the Plasma Membrane by Antimicrobial Agents

Because the plasma membrane is vital to the bacterial cell, it is not surprising that several antimicrobial agents exert their effects at this site. In addition to the chemicals that damage the cell wall and thereby indirectly expose the membrane to injury, many compounds specifically damage plasma membranes. These compounds include certain alcohols and quaternary ammonium compounds, which are used as disinfectants. By disrupting the membrane's phospholipids, a group of antibiotics known as the *polymyxins* cause leakage of intracellular contents and subsequent cell death. This mechanism will be discussed in Chapter 20.

The Movement of Materials across Membranes

Materials move across plasma membranes of both prokaryotic and eukaryotic cells by two kinds of processes: passive and active. In *passive processes,* substances cross the membrane from an area of high concentration to an area of low concentration (move with the concentration gradient, or difference), without any expenditure of energy by the cell. In *active processes,* the cell must use energy to move substances from areas of low concentration to areas of high concentration (against the concentration gradient).

Passive Processes

Passive processes include simple diffusion, facilitated diffusion, and osmosis.

Simple diffusion is the net (overall) movement of molecules or ions from an area of high concentration to an area of low concentration (Figure 4.16 and Figure 4.17a). The movement continues until the molecules or ions are evenly distributed. The point of even distribution is called *equilibrium*. Cells rely on simple diffusion to transport certain small molecules, such as oxygen and carbon dioxide, across their cell membranes.

In **facilitated diffusion,** integral membrane proteins function as channels or carriers that facilitate the movement of ions or large molecules across the plasma membrane. Such integral proteins are called *transporters* or *permeases*. Facilitated diffusion is similar to simple diffusion in that the cell *does not* expend energy, because the substance moves from a high to a low concentration. The process differs from simple diffusion in its use of transporters. Some transporters permit the passage of mostly small, inorganic ions that are too hydrophilic to penetrate the nonpolar interior of the lipid bilayer (Figure 4.17b). These transporters, which are common in prokaryotes, are nonspecific and allow a wide variety of ions or small molecules to pass through channels in integrated membrane proteins. Other transporters,

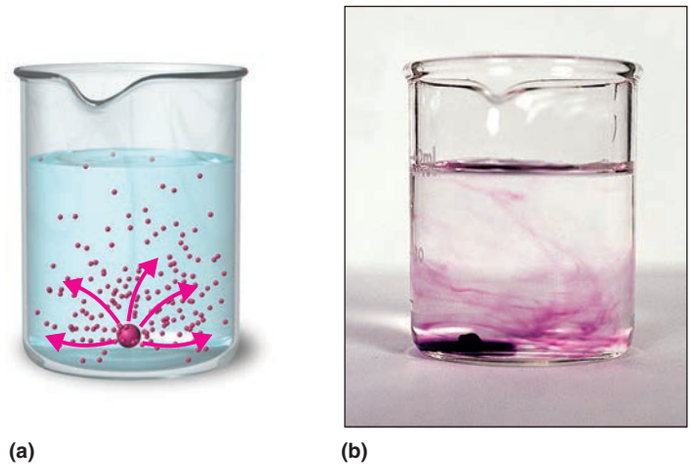


Figure 4.16 The principle of simple diffusion. **(a)** After a dye pellet is put into a beaker of water, the molecules of dye in the pellet diffuse into the water from an area of high dye concentration to areas of low dye concentration. **(b)** The dye potassium permanganate in the process of diffusing.

Q Why are passive processes important to a cell?

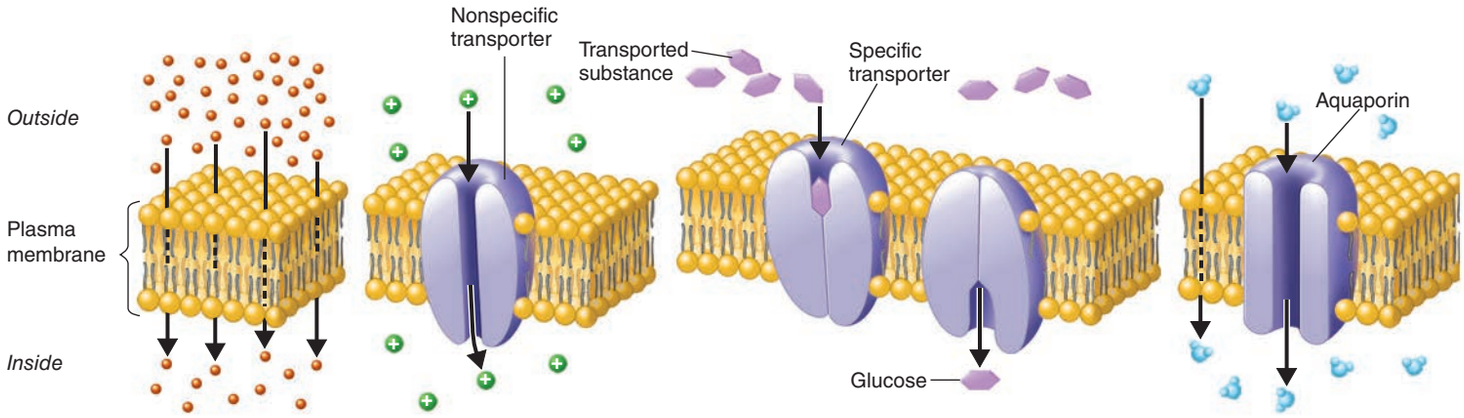


Figure 4.17 Passive processes.

Q How does simple diffusion differ from facilitated diffusion?

which are common in eukaryotes, are specific and transport only specific, usually larger, molecules, such as simple sugars (glucose, fructose, and galactose) and vitamins. In this process, the transported substance binds to a specific transporter (integrated membrane protein) on the outer surface of the plasma membrane, which undergoes a change of shape; then the transporter releases the substance on the other side of the membrane (Figure 4.17c).

In some cases, molecules that bacteria need are too large to be transported into the cells by these methods. Most bacteria, however, produce enzymes that can break down large molecules into simpler ones (such as proteins into amino acids, or polysaccharides into simple sugars). Such enzymes, which are released by the bacteria into the surrounding medium, are appropriately called *extracellular enzymes*. Once the enzymes degrade the large molecules, the subunits move into the cell with the help of transporters. For example, specific carriers retrieve DNA bases, such as the purine guanine, from extracellular media (substances outside the cell) and bring them into the cell's cytoplasm.

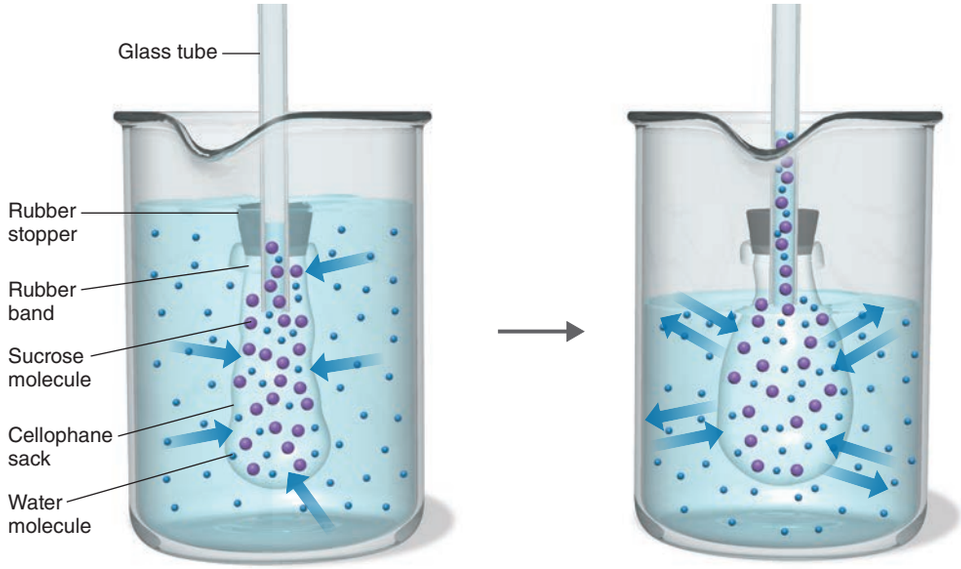
Osmosis is the net movement of water molecules across a selectively permeable membrane from an area with a high concentration of water molecules (low concentration of solute molecules) to an area of low concentration of water molecules (high concentration of solute molecules). Water molecules may pass through plasma membranes by moving through the lipid bilayer by simple diffusion or through integral membrane proteins, called *aquaporins,* that function as water channels (Figure 4.17d).

Osmosis may be demonstrated with the apparatus shown in **Figure 4.18a**. A sack constructed from cellophane, which is a selectively permeable membrane, is filled with a solution of 20% sucrose (table sugar). The cellophane sack is placed into a beaker containing distilled water. Initially, the concentrations of water on either side of the membrane are different. Because of the sucrose molecules, the concentration of water is lower inside the cellophane sack. Therefore, water moves from the beaker (where its concentration is higher) into the cellophane sack (where its concentration is lower).

Sugar does not move out of the cellophane sack into the beaker, however, because the cellophane is impermeable to molecules of sugar—the sugar molecules are too large to go through the pores of the membrane. As water moves into the cellophane sack, the sugar solution becomes increasingly dilute, and, because the cellophane sack has expanded to its limit as a result of an increased volume of water, water begins to move up the glass tube. In time, the water that has accumulated in the cellophane sack and the glass tube exerts a downward pressure that forces water molecules out of the cellophane sack and back into the beaker. This movement of water through a selectively permeable membrane produces osmotic pressure. **Osmotic pressure** is the pressure required to prevent the movement of pure water (water with no solutes) into a solution containing some solutes. In other words, osmotic pressure is the pressure needed to stop the flow of water across the selectively permeable membrane (cellophane). When water molecules leave and enter the cellophane sack at the same rate, equilibrium is reached (Figure 4.18b).

A bacterial cell may be subjected to any of three kinds of osmotic solutions: isotonic, hypotonic, or hypertonic. An **isotonic solution** is a medium in which the overall concentration of solutes equals that found inside a cell (*iso* means equal). Water leaves and enters the cell at the same rate (no net change); the cell's contents are in equilibrium with the solution outside the cytoplasmic membrane (Figure 4.18c).

Earlier we mentioned that lysozyme and certain antibiotics (such as penicillin) damage bacterial cell walls, causing the cells

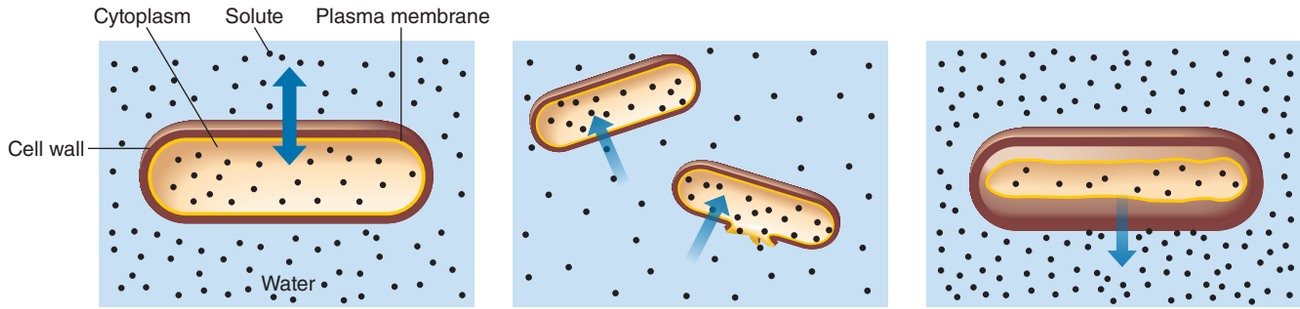


(a) At beginning of osmotic pressure experiment

(b) At equilibrium

Figure 4.18 The principle of osmosis. **(a)** Setup at the beginning of an osmotic pressure experiment. Water molecules start to move from the beaker into the sack along the concentration gradient. **(b)** Setup at equilibrium. The osmotic pressure exerted by the solution in the sack pushes water molecules from the sack back into the beaker to balance the rate of water entry into the sack. The height of the solution in the glass tube at equilibrium is a measure of the osmotic pressure. **(c)**–**(e)** The effects of various solutions on bacterial cells.

Q Why is osmosis important?



(c) Isotonic solution. No net movement of water occurs.

(d) Hypotonic solution. Water moves into the cell. If the cell wall is strong, it contains the swelling. If the cell wall is weak or damaged, the cell bursts (osmotic lysis).

(e) Hypertonic solution. Water moves out of the cell, causing its cytoplasm to shrink (plasmolysis).

to rupture, or lyse. Such rupturing occurs because bacterial cytoplasm usually contains such a high concentration of solutes that additional water enters the cell by osmosis when the wall is weakened or removed. The damaged (or removed) cell wall cannot constrain the swelling of the cytoplasmic membrane, and the membrane bursts. This is an example of osmotic lysis caused by immersion in a hypotonic solution. A **hypotonic solution** outside the cell is a medium whose concentration of solutes is lower than that inside the cell (*hypo* means under or less). Most bacteria live in hypotonic solutions, and the cell wall resists further osmosis and protects cells from lysis. Cells with weak cell walls, such as gram-negative bacteria, may burst or undergo osmotic lysis as a result of excessive water intake (Figure 4.18d).

A **hypertonic solution** is a medium having a higher concentration of solutes than that inside the cell (*hyper* means above or more). Most bacterial cells placed in a hypertonic solution shrink and collapse or *plasmolyze* because water leaves the cells by osmosis (Figure 4.18e). Keep in mind that the terms *isotonic, hypotonic,* and *hypertonic* describe the concentration of solutions outside the cell *relative to* the concentration inside the cell.

MM **Animations** Passive Transport: Principles of Diffusion, Special Types of Diffusion

Active Processes

Simple diffusion and facilitated diffusion are useful mechanisms for transporting substances into cells when the concentrations of the substances are greater outside the cell. However, when a bacterial cell is in an environment in which nutrients are in low concentration, the cell must use active processes, such as active transport and group translocation, to accumulate the needed substances.

In performing **active transport,** the cell *uses energy* in the form of ATP to move substances across the plasma membrane. Among the substances actively transported are ions (for example Na^+, K^+, H^+, Ca^{2+}, and Cl^-), amino acids, and simple sugars. Although these substances can also be moved into cells by passive processes, their movement by active processes can go against the concentration

gradient, allowing a cell to accumulate needed materials. The movement of a substance in active transport is usually from outside to inside, even though the concentration might be much higher inside the cell. Like facilitated diffusion, active transport depends on transporter proteins in the plasma membrane (see Figure 4.17b, c). There appears to be a different transporter for each substance or group of closely related substances. Active transport enables microbes to move substances across the plasma membrane at a constant rate, even if they are in short supply.

In active transport, the substance that crosses the plasma membrane is not altered by transport across the membrane. In **group translocation,** a special form of active transport that occurs exclusively in prokaryotes, the substance is chemically altered during transport across the membrane. Once the substance is altered and inside the cell, the plasma membrane is impermeable to it, so it remains inside the cell. This important mechanism enables a cell to accumulate various substances even though they may be in low concentrations outside the cell. Group translocation requires energy supplied by high-energy phosphate compounds, such as phosphoenolpyruvic acid (PEP).

One example of group translocation is the transport of the sugar glucose, which is often used in growth media for bacteria. While a specific carrier protein is transporting the glucose molecule across the membrane, a phosphate group is added to the sugar. This phosphorylated form of glucose, which cannot be transported out, can then be used in the cell's metabolic pathways.

Some eukaryotic cells (those without cell walls) can use two additional active transport processes called phagocytosis and pinocytosis. These processes, which do not occur in bacteria, are explained on page 97. MM **Animations** Active Transport: Types, Overview

CHECK YOUR UNDERSTANDING

- Which agents can cause injury to the bacterial plasma membrane? **4-8**
- How are simple diffusion and facilitated diffusion similar? How are they different? **4-9**

Cytoplasm

For a prokaryotic cell, the term **cytoplasm** refers to the substance of the cell inside the plasma membrane (see Figure 4.6). Cytoplasm is about 80% water and contains primarily proteins (enzymes), carbohydrates, lipids, inorganic ions, and many low-molecular-weight compounds. Inorganic ions are present in much higher concentrations in cytoplasm than in most media. Cytoplasm is thick, aqueous, semitransparent, and elastic. The major structures in the cytoplasm of prokaryotes are a nucleoid (containing DNA), particles called ribosomes, and reserve deposits called inclusions.

The term cytoskeleton is a collective term for a series of fibers (small roads and cylinders) in the cytoplasm. Not long ago, it was believed that the absence of a cytoskeleton was a distinguishing feature of prokaryotes. However, biologists recently discovered that prokaryotic cells have a cytoskeleton similar to eukaryotes. Components include MreB and ParM, cresetin, and FtsZ, which correspond to the microfilaments, intermediate filaments, and microtubules of the eukaryotic cytoskeleton, respectively. The prokaryotic cytoskeleton assumes roles in cell division, maintaining cell shape, growth, DNA movement, protein targeting, and alignment of organelles. The cytoplasm of prokaryotes is not capable of cytoplasmic streaming, which will be described later.

The Nucleoid

The **nucleoid** of a bacterial cell (see Figure 4.6) usually contains a single long, continuous, and frequently circularly arranged thread of double-stranded DNA called the **bacterial chromosome.** This is the cell's genetic information, which carries all the information required for the cell's structures and functions. Unlike the chromosomes of eukaryotic cells, bacterial chromosomes are not surrounded by a nuclear envelope (membrane) and do not include histones. The nucleoid can be spherical, elongated, or dumbbell-shaped. In actively growing bacteria, as much as 20% of the cell volume is occupied by DNA because such cells presynthesize DNA for future cells. The chromosome is attached to the plasma membrane. Proteins in the plasma membrane are believed to be responsible for replication of the DNA and segregation of the new chromosomes to daughter cells during cell division.

In addition to the bacterial chromosome, bacteria often contain small usually circular, double-stranded DNA molecules called **plasmids** (see the F factor in Figure 8.28a, page 230). These molecules are extrachromosomal genetic elements; that is, they are not connected to the main bacterial chromosome, and they replicate independently of chromosomal DNA. Research indicates that plasmids are associated with plasma membrane proteins. Plasmids usually contain from 5 to 100 genes that are generally not crucial for the survival of the bacterium under normal environmental conditions; plasmids may be gained or lost without harming the cell. Under certain conditions, however, plasmids are an advantage to cells. Plasmids may carry genes for such activities as antibiotic resistance, tolerance to toxic metals, the production of toxins, and the synthesis of enzymes. Plasmids can be transferred from one bacterium to another. In fact, plasmid DNA is used for gene manipulation in biotechnology.

Ribosomes

All eukaryotic and prokaryotic cells contain **ribosomes,** where protein synthesis takes place. Cells that have high rates of protein synthesis, such as those that are actively growing, have a large number of ribosomes. The cytoplasm of a prokaryotic cell contains tens of thousands of ribosomes, giving the cytoplasm a granular appearance (see Figure 4.6).

Ribosomes are composed of two subunits, each of which consists of protein and a type of RNA called *ribosomal RNA (rRNA).* Prokaryotic ribosomes differ from eukaryotic ribosomes

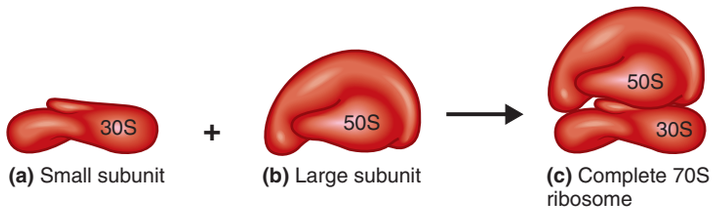


Figure 4.19 The prokaryotic ribosome. **(a)** A small 30S subunit and **(b)** a large 50S subunit make up **(c)** the complete 70S prokaryotic ribosome.

Q What is the importance of the differences between prokaryotic and eukaryotic ribosomes with regard to antibiotic therapy?

in the number of proteins and rRNA molecules they contain; they are also somewhat smaller and less dense than ribosomes of eukaryotic cells. Accordingly, prokaryotic ribosomes are called 70S ribosomes (Figure 4.19), and those of eukaryotic cells are known as 80S ribosomes. The letter S refers to Svedberg units, which indicate the relative rate of sedimentation during ultra-high-speed centrifugation. Sedimentation rate is a function of the size, weight, and shape of a particle. The subunits of a 70S ribosome are a small 30S subunit containing one molecule of rRNA and a larger 50S subunit containing two molecules of rRNA. (Note that the 70S "value" is not the sum of 30S plus 50S.) The apparent arithmetic error here has often puzzled students. However, you can think of a Svedberg unit as a unit of size rather than weight. Therefore, the combination here of 50S and 30S is not the same as combining 50 grams and 30 grams.

Several antibiotics work by inhibiting protein synthesis on prokaryotic ribosomes. Antibiotics such as streptomycin and gentamicin attach to the 30S subunit and interfere with protein synthesis. Other antibiotics, such as erythromycin and chloramphenicol, interfere with protein synthesis by attaching to the 50S subunit. Because of differences in prokaryotic and eukaryotic ribosomes, the microbial cell can be killed by the antibiotic while the eukaryotic host cell remains unaffected.

Inclusions

Within the cytoplasm of prokaryotic cells are several kinds of reserve deposits, known as **inclusions.** Cells may accumulate certain nutrients when they are plentiful and use them when the environment is deficient. Evidence suggests that macromolecules concentrated in inclusions avoid the increase in osmotic pressure that would result if the molecules were dispersed in the cytoplasm. Some inclusions are common to a wide variety of bacteria, whereas others are limited to a small number of species and therefore serve as a basis for identification. Some inclusions, such as magnetosomes, are membrane-enclosed organelles, while other inclusions, such as carboxysomes, are enclosed in protein complexes.

Metachromatic Granules

Metachromatic granules are large inclusions that take their name from the fact that they sometimes stain red with certain blue dyes such as methylene blue. Collectively they are known as **volutin.** Volutin represents a reserve of inorganic phosphate (polyphosphate) that can be used in the synthesis of ATP. It is generally formed by cells that grow in phosphate-rich environments. Metachromatic granules are found in algae, fungi, and protozoa, as well as in bacteria. These granules are characteristic of *Corynebacterium diphtheriae* (kor′ī-nē-bak-TI-rē-um dif-THI-rē-ī), the causative agent of diphtheria; thus, they have diagnostic significance.

Polysaccharide Granules

Inclusions known as **polysaccharide granules** typically consist of glycogen and starch, and their presence can be demonstrated when iodine is applied to the cells. In the presence of iodine, glycogen granules appear reddish brown and starch granules appear blue.

Lipid Inclusions

Lipid inclusions appear in various species of *Mycobacterium, Bacillus, Azotobacter* (ah-zō′tō-BAK-ter), *Spirillum* (spī-RIL-lum), and other genera. A common lipid-storage material, one unique to bacteria, is the polymer *poly-β-hydroxybutyric acid*. Lipid inclusions are revealed by staining cells with fat-soluble dyes, such as Sudan dyes.

Sulfur Granules

Certain bacteria—for example, the "sulfur bacteria" that belong to the genus *Acidithiobacillus*—derive energy by oxidizing sulfur

Clinical Case

The antibiotic killed the bacteria, but endotoxin is released when the cells die, causing Jessie's condition to worsen. Jessie's physician prescribes polymyxin, an antibiotic that does not cause release of endotoxin, and to which Jessie responds favorably.

As Irene sits with Jessie, she notices another patient being fed ice chips by a relative. On a hunch, Irene hurries back to her office to find out whether the ice machines had been swabbed. They have not. She immediately orders the machines to be swabbed and cultured. Her hunch turns out to be correct: the samples are positive for *K. pneumoniae*. Bacteria growing in the hospital's water pipes entered the ice machine with incoming water.

How can *K. pneumoniae* grow in water pipes?

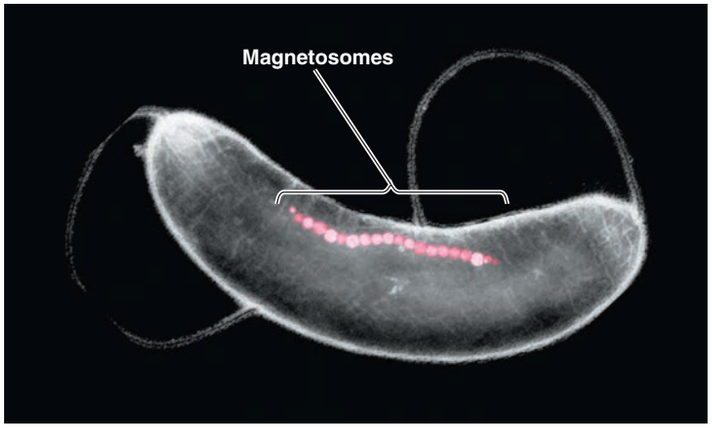


Figure 4.20 Magnetosomes. This micrograph of *Magnetospirillum magnetotacticum* shows a chain of magnetosomes. This bacterium is usually found in shallow freshwater mud.

Q How do magnetosomes behave like magnets?

and sulfur-containing compounds. These bacteria may deposit **sulfur granules** in the cell, where they serve as an energy reserve.

Carboxysomes

Carboxysomes are inclusions that contain the enzyme ribulose 1,5-diphosphate carboxylase. Photosynthetic bacteria use carbon dioxide as their sole source of carbon and require this enzyme for carbon dioxide fixation. Among the bacteria containing carboxysomes are nitrifying bacteria, cyanobacteria, and acidithiobacilli.

Gas Vacuoles

Hollow cavities found in many aquatic prokaryotes, including cyanobacteria, anoxygenic photosynthetic bacteria, and halobacteria are called **gas vacuoles.** Each vacuole consists of rows of several individual *gas vesicles,* which are hollow cylinders covered by protein. Gas vacuoles maintain buoyancy so that the cells can remain at the depth in the water appropriate for them to receive sufficient amounts of oxygen, light, and nutrients.

Magnetosomes

Magnetosomes are inclusions of iron oxide (Fe_3O_4) surrounded by invaginations of the plasma membrane. Magnetosomes are formed by several gram-negative bacteria such as *Magnetospirillum magnetotacticum* and act like magnets (Figure 4.20). Bacteria may use magnetosomes to move downward until they reach a suitable attachment site. In vitro, magnetosomes can decompose hydrogen peroxide, which forms in cells in the presence of oxygen. Researchers speculate that magnetosomes may protect the cell against hydrogen peroxide accumulation.

Endospores

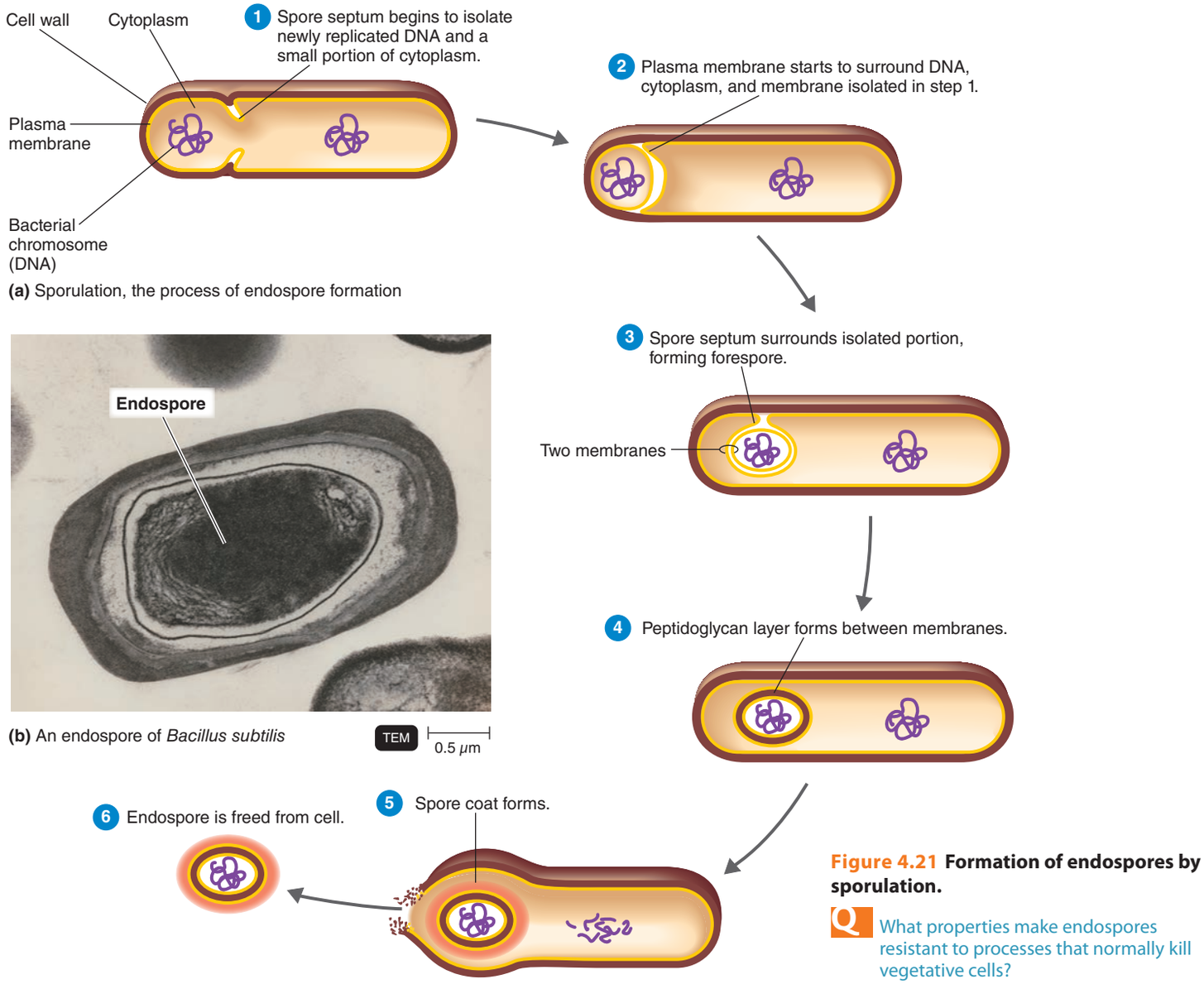
When essential nutrients are depleted, certain gram-positive bacteria, such as those of the genera *Clostridium* and *Bacillus,* form specialized "resting" cells called **endospores** (Figure 4.21). As you will see later, some members of the genus *Clostridium* cause diseases such as gangrene, tetanus, botulism, and food poisoning. Some members of the genus *Bacillus* cause anthrax and food poisoning. Unique to bacteria, endospores are highly durable dehydrated cells with thick walls and additional layers. They are formed internal to the bacterial cell membrane.

When released into the environment, they can survive extreme heat, lack of water, and exposure to many toxic chemicals and radiation. For example, 7500-year-old endospores of *Thermoactinomyces vulgaris* (ther´mō-ak-tin-ō-MĪ-sēz vul-GAR-is) from the freezing muds of Elk Lake in Minnesota have germinated when rewarmed and placed in a nutrient medium, and 25- to 40-million-year-old endospores found in the gut of a stingless bee entombed in amber (hardened tree resin) in the Dominican Republic are reported to have germinated when placed in nutrient media. Although true endospores are found in gram-positive bacteria, one gram-negative species, *Coxiella burnetii* (KOKS-ē-el-lah ber-NE-tē-ē), the cause of Q fever, usually a mild disease with flulike symptoms, forms endospore-like structures that resist heat and chemicals and can be stained with endospore stains (see Figure 24.13, page 692).

The process of endospore formation within a vegetative cell takes several hours and is known as **sporulation** or **sporogenesis** (Figure 4.21a). Vegetative cells of endospore-forming bacteria begin sporulation when a key nutrient, such as the carbon or nitrogen source, becomes scarce or unavailable. In the first observable stage of sporulation, a newly replicated bacterial chromosome and a small portion of cytoplasm are isolated by an ingrowth of the plasma membrane called a *spore septum*. The spore septum becomes a double-layered membrane that surrounds the chromosome and cytoplasm. This structure, entirely enclosed within the original cell, is called a *forespore*. Thick layers of peptidoglycan are laid down between the two membrane layers. Then a thick *spore coat* of protein forms around the outside membrane; this coat is responsible for the resistance of endospores to many harsh chemicals. The original cell is degraded, and the endospore is released.

The diameter of the endospore may be the same as, smaller than, or larger than the diameter of the vegetative cell. Depending on the species, the endospore might be located *terminally* (at one end), *subterminally* (near one end; Figure 4.21b), or *centrally* inside the vegetative cell. When the endospore matures, the vegetative cell wall ruptures (lyses), killing the cell, and the endospore is freed.

Most of the water present in the forespore cytoplasm is eliminated by the time sporulation is complete, and endospores do not carry out metabolic reactions. The endospore contains a large amount of an organic acid called *dipicolinic acid* (DPA),



(a) Sporulation, the process of endospore formation

(b) An endospore of *Bacillus subtilis*

Figure 4.21 Formation of endospores by sporulation.

Q What properties make endospores resistant to processes that normally kill vegetative cells?

which is accompanied by a large number of calcium ions. Evidence indicates that DPA protects the endospore DNA against damage. The highly dehydrated endospore core contains only DNA, small amounts of RNA, ribosomes, enzymes, and a few important small molecules. These cellular components are essential for resuming metabolism later.

Endospores can remain dormant for thousands of years. An endospore returns to its vegetative state by a process called **germination.** Germination is triggered by high heat, such as is used in canning, or small triggering molecules called *germinants*. Germinants identified to date are alanine and inosine (a nucleotide). The endospore's enzymes then break down the extra layers surrounding the endospore, water enters, and metabolism resumes. Because one vegetative cell forms a single endospore, which, after germination, remains one cell, sporulation in bacteria is *not* a means of reproduction. This process does not increase the number of cells. Bacterial endospores differ from spores formed by (prokaryotic) actinomycetes and the eukaryotic fungi and algae, which detach from the parent and develop into another organism and, therefore, represent reproduction.

Endospores are important from a clinical viewpoint and in the food industry because they're resistant to processes that normally kill vegetative cells. Such processes include heating, desiccation, use of chemicals, and radiation. Whereas most vegetative cells are killed by temperatures above 70°C, endospores can survive in boiling water for several hours or more. Endospores of thermophilic (heat-loving) bacteria can survive in boiling water for 19 hours. Endospore-forming bacteria

APPLICATIONS OF MICROBIOLOGY

Why Microbiologists Study Termites

Although termites are famous for their ability to eat wood, they are unable to digest it. To break down the cellulose, termites enlist the help of a variety of microorganisms.

Termites contain, within their digestive tracts, symbiotic microorganisms that digest the cellulose that the termites chew and swallow. In fact, these symbiotic microorganisms can survive only because of even smaller symbionts that live on and within them, without which they would not even be able to move. By studying how a single termite survives, microbiologists have begun to gain an entirely new understanding of symbiosis.

The termite's dependence on nitrogen-fixing bacteria to supply its nitrogen and on protozoans such as *Trichonympha sphaerica* to digest cellulose are examples of endosymbiosis, a symbiotic relationship with an organism that lives inside the body of the host organism (in this case, within the hindgut of the termite).

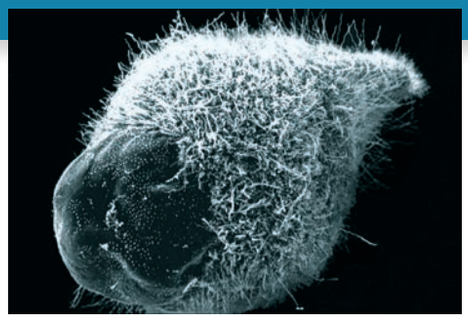
The picture is more complicated than this, however, for *T. sphaerica* is unable to digest cellulose without the aid of bacteria that live within its own body: in other words, the protozoan has its own body endosymbionts.

Certain hindgut flagellates such as *T. sphaerica* also demonstrate another form of symbiosis—ectosymbiosis, a symbiotic relationship with organisms that live outside its body. Advances in microscopy have shown that these flagellates are covered by precise rows consisting of thousands of bacteria, either rods or spirochetes. If these bacteria are killed, the protozoan is unable to move. Instead of using its own flagella, the protozoan relies on the rows of bacteria to row it about like oarsmen in a boat.

The protozoan *Mixotricha*, for example, has rows of spirochetes on its surface (see photo, right). The end of each spirochete abuts against a swelling known as a bracket. The spirochetes undulate in unison, creating waves of motion along *Mixotricha*'s surface.

Rod-shaped bacteria align in grooves that cover the surface of devescovinids, another group of termite-hindgut protozoans. Each rod has 12 flagella that overlap the flagella of the adjacent bacteria to form a continuous filament along the groove. The bacteria rotate their flagella, thus creating coordinated waves along all these rows of filaments, which propel the protozoan.

Sid Tamm and his colleagues at Boston University have found that the protozoa cannot control motility of the ectosymbiotics. *Mixotricha* uses its flagella to steer, and the bacteria push the protozoa forward—shoving and being shoved by its neighbors, much like bumper cars.



***Mixotricha,* a protozoan that lives in the termite gut.**

Clinical Case Resolved

It is the glycocalyx that enables bacteria in water to stick inside a pipe. The bacteria grow slowly in the nutrient-poor tap water but do not get washed away by the flowing water. A slimy layer of bacteria can accumulate in a pipe. Irene discovers that the disinfectant in the hospital's water supply was inadequate to prevent bacterial growth. Some bacteria can get dislodged by flowing water, and even normally harmless bacteria can infect a surgical incision or weakened host.

73 83 85 91 94

are a problem in the food industry because they are likely to survive underprocessing, and, if conditions for growth occur, some species produce toxins and disease. Special methods for controlling organisms that produce endospores are discussed in Chapter 7.

CHECK YOUR UNDERSTANDING

- Where is the DNA located in a prokaryotic cell? **4-10**
- What is the general function of inclusions? **4-11**
- Under what conditions do endospores form? **4-12**

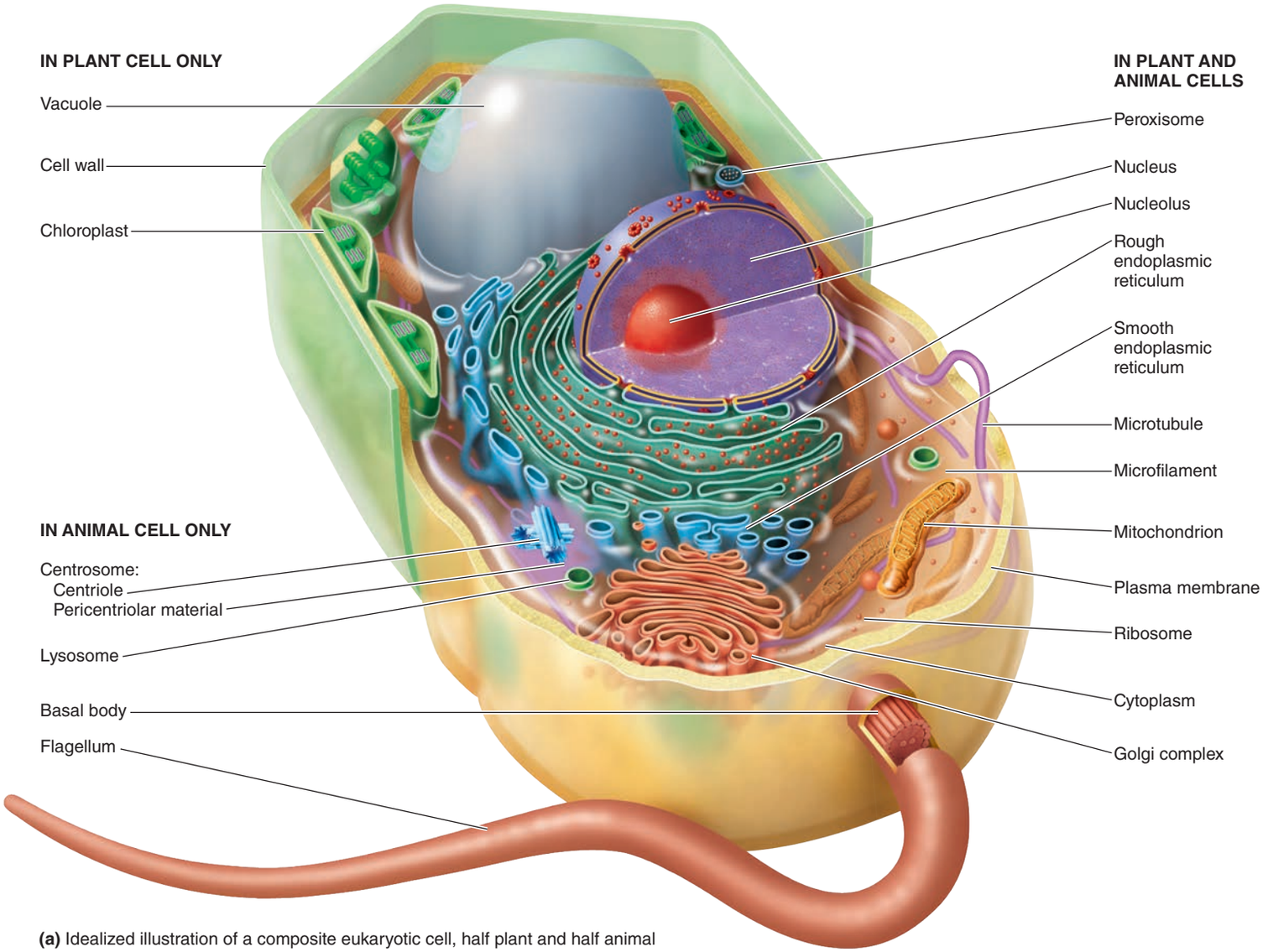
* * *

Having examined the functional anatomy of the prokaryotic cell, we will now look at the functional anatomy of the eukaryotic cell.

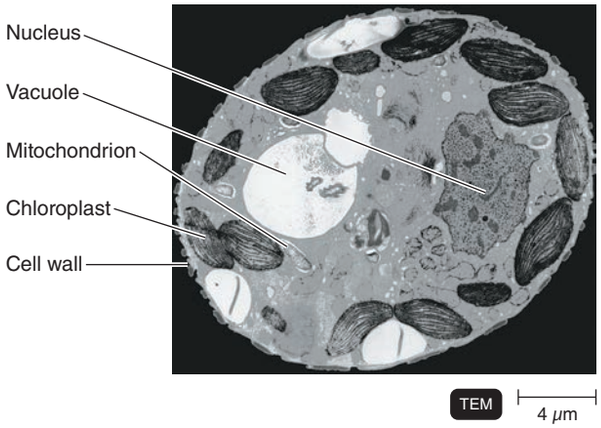
The Eukaryotic Cell

As mentioned earlier, eukaryotic organisms include algae, protozoa, fungi, plants, and animals. The eukaryotic cell is typically larger and structurally more complex than the prokaryotic cell (**Figure 4.22**). When the structure of the prokaryotic cell in Figure 4.6 is compared with that of the eukaryotic cell, the differences between the two types of cells become apparent. The principal differences between prokaryotic and eukaryotic cells are summarized in **Table 4.2** on page 96.

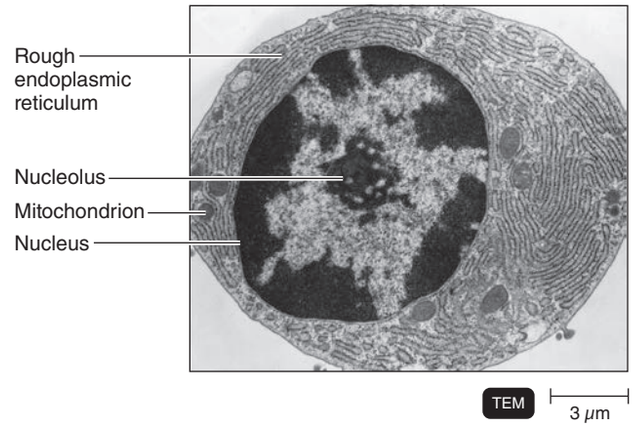
ASM: While microscopic eukaryotes (for example, fungi, protozoa, and algae) carry out some of the same processes as bacteria, many of the cellular properties are fundamentally different.



(a) Idealized illustration of a composite eukaryotic cell, half plant and half animal



(b) Transmission electron micrograph of plant cell



(c) Transmission electron micrograph of animal cell

Figure 4.22 Eukaryotic cells showing typical structures.

Q What kingdoms contain eukaryotic organisms?

TABLE 4.2 Principal Differences between Prokaryotic and Eukaryotic Cells

Characteristic	Prokaryotic	Eukaryotic
Size of Cell	Typically 0.2–2.0 µm in diameter	Typically 10–100 µm in diameter
Nucleus	Typically no nuclear membrane or nucleoli except *Gemmata* (see Figure 11.23)	True nucleus, consisting of nuclear membrane and nucleoli
Membrane-Enclosed Organelles	Relatively few	Present; examples include nuclei, lysosomes, Golgi complex, endoplasmic reticulum, mitochondria, and chloroplasts
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack a cell wall
Cell Wall	Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple (includes cellulose and chitin)
Plasma Membrane	Carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors
Cytoplasm	Cytoskeleton (MreB and ParM, cresetin, and FtsZ proteins); no cytoplasmic streaming	Cytoskeleton (microfilaments, intermediate filaments, and microtubules); cytoplasmic streaming
Ribosomes	Smaller size (70S)	Larger size (80S); smaller size (70S) in organelles
Chromosome (DNA)	Usually single circular chromosome; typically lacks histones	Multiple linear chromosomes with histones
Cell Division	Binary fission	Involves mitosis
Sexual Recombination	None; transfer of DNA only	Involves meiosis

The following discussion of eukaryotic cells will parallel our discussion of prokaryotic cells by starting with structures that extend to the outside of the cell.

Flagella and Cilia

LEARNING OBJECTIVE

4-13 Differentiate prokaryotic and eukaryotic flagella.

Many types of eukaryotic cells have projections that are used for cellular locomotion or for moving substances along the surface of the cell. These projections contain cytoplasm and are enclosed by the plasma membrane. If the projections are few and are long in relation to the size of the cell, they are called **flagella.** If the projections are numerous and short, they are called **cilia** (singular: **cilium**).

Algae of the genus *Euglena* (ū-GLĒ-nah) use a flagellum for locomotion, whereas protozoa, such as *Tetrahymena* (tet´rah-HĪ-me-nah), use cilia for locomotion (**Figure 4.23a** and Figure 4.23b). Both flagella and cilia are anchored to the plasma membrane by a basal body, and both consist of nine pairs of microtubules (doublets) arranged in a ring, plus another two microtubules in the center of the ring, an arrangement called a *9 + 2 array* (Figure 4.23c). **Microtubules** are long, hollow tubes made up of a protein called *tubulin*. A prokaryotic flagellum rotates, but a eukaryotic flagellum moves in a wavelike manner (Figure 4.23d). To help keep foreign material out of the lungs, ciliated cells of the human respiratory system move the material along the surface of the cells in the bronchial tubes and trachea toward the throat and mouth (see Figure 16.3, page 444).

The Cell Wall and Glycocalyx

LEARNING OBJECTIVE

4-14 Compare and contrast prokaryotic and eukaryotic cell walls and glycocalyxes.

Most eukaryotic cells have cell walls, although they are generally much simpler than those of prokaryotic cells. Many algae have cell walls consisting of the polysaccharide *cellulose* (as do all plants); other chemicals may be present as well. Cell walls of some fungi also contain cellulose, but in most fungi the principal structural component of the cell wall is the polysaccharide *chitin,* a polymer of N-acetylglucosamine (NAG) units. (Chitin is also the main structural component of the exoskeleton of crustaceans and insects.) The cell walls of yeasts contain the polysaccharides *glucan* and *mannan*. In eukaryotes that lack a cell wall, the plasma membrane may be the outer covering; however, cells that have direct contact with the environment may have coatings outside the plasma membrane. Protozoa do not have a typical cell wall; instead, they have a flexible outer protein covering called a *pellicle*.

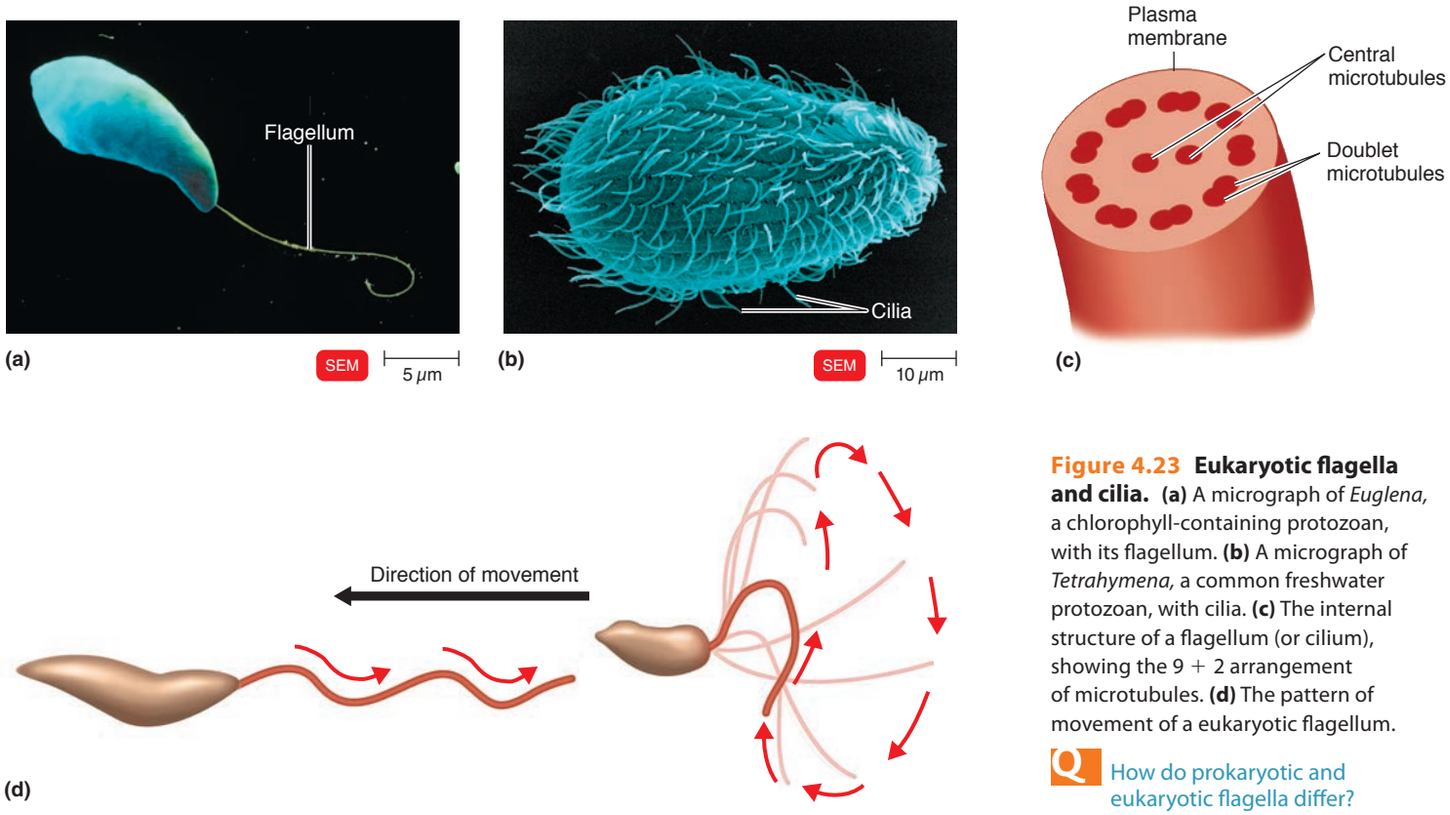


Figure 4.23 Eukaryotic flagella and cilia. **(a)** A micrograph of *Euglena,* a chlorophyll-containing protozoan, with its flagellum. **(b)** A micrograph of *Tetrahymena,* a common freshwater protozoan, with cilia. **(c)** The internal structure of a flagellum (or cilium), showing the 9 + 2 arrangement of microtubules. **(d)** The pattern of movement of a eukaryotic flagellum.

Q How do prokaryotic and eukaryotic flagella differ?

In other eukaryotic cells, including animal cells, the plasma membrane is covered by a **glycocalyx,** a layer of material containing substantial amounts of sticky carbohydrates. Some of these carbohydrates are covalently bonded to proteins and lipids in the plasma membrane, forming glycoproteins and glycolipids that anchor the glycocalyx to the cell. The glycocalyx strengthens the cell surface, helps attach cells together, and may contribute to cell–cell recognition.

Eukaryotic cells do not contain peptidoglycan, the framework of the prokaryotic cell wall. This is significant medically because antibiotics, such as penicillins and cephalosporins, act against peptidoglycan and therefore do not affect human eukaryotic cells.

The Plasma (Cytoplasmic) Membrane

LEARNING OBJECTIVE

4-15 Compare and contrast prokaryotic and eukaryotic plasma membranes.

The **plasma (cytoplasmic) membrane** of eukaryotic and prokaryotic cells is very similar in function and basic structure. There are, however, differences in the types of proteins found in the membranes. Eukaryotic membranes also contain carbohydrates, which serve as attachment sites for bacteria and as receptor sites that assume a role in such functions as cell–cell recognition. Eukaryotic plasma membranes also contain *sterols,* complex lipids not found in prokaryotic plasma membranes (with the exception of *Mycoplasma* cells). Sterols seem to be associated with the ability of the membranes to resist lysis resulting from increased osmotic pressure.

Substances can cross eukaryotic and prokaryotic plasma membranes by simple diffusion, facilitated diffusion, osmosis, or active transport. Group translocation does not occur in eukaryotic cells. However, eukaryotic cells can use a mechanism called **endocytosis.** This occurs when a segment of the plasma membrane surrounds a particle or large molecule, encloses it, and brings it into the cell.

The three types of endocytosis are phagocytosis, pinocytosis, and receptor-mediated endocytosis. During *phagocytosis,* cellular projections called pseudopods engulf particles and bring them into the cell. Phagocytosis is used by white blood cells to destroy bacteria and foreign substances (see Figure 16.8, page 451, and further discussion in Chapter 16). In *pinocytosis,* the plasma membrane folds inward, bringing extracellular fluid into the cell, along with whatever substances are dissolved in the fluid. In *receptor-mediated endocytosis,* substances (ligands) bind to receptors in the membrane. When binding occurs, the membrane folds inward. Receptor-mediated endocytosis is one of the ways viruses can enter animal cells (see Figure 13.14a, page 374).

Cytoplasm

LEARNING OBJECTIVE

4-16 Compare and contrast prokaryotic and eukaryotic cytoplasms.

The **cytoplasm** of eukaryotic cells encompasses the substance inside the plasma membrane and outside the nucleus (see Figure 4.22). The cytoplasm is the substance in which various cellular components are found. (The term **cytosol** refers to the fluid portion of cytoplasm.) The **cytoskeleton** of eukaryotes consists of small rods (*microfilaments* and *intermediate filaments*) and cylinders (*microtubules*). Recall these correspond to the MreB and ParM, crescetin, and FtsZ of the prokaryotic cytoskeleton, respectively. The cytoskeleton of eukaryotes provides support, shape, and assistance in transporting substances through the cell (and even in moving the entire cell, as in phagocytosis). The movement of eukaryotic cytoplasm from one part of the cell to another, which helps distribute nutrients and move the cell over a surface, is called **cytoplasmic streaming.** Another difference between prokaryotic and eukaryotic cytoplasm is that many of the important enzymes found in the cytoplasmic fluid of prokaryotes are sequestered in the organelles of eukaryotes.

Ribosomes

LEARNING OBJECTIVE

4-17 Compare the structure and function of eukaryotic and prokaryotic ribosomes.

Attached to the outer surface of the rough endoplasmic reticulum (discussed on page 99) are **ribosomes** (see Figure 4.25), which are also found free in the cytoplasm. As in prokaryotes, ribosomes are the sites of protein synthesis in the cell.

The ribosomes of cells are somewhat larger and denser than those of prokaryotic cells. These eukaryotic ribosomes are 80S ribosomes, each of which consists of a large 60S subunit containing three molecules of rRNA and a smaller 40S subunit with one molecule of rRNA. The subunits are made separately in the nucleolus and, once produced, exit the nucleus and join together in the cytosol. Chloroplasts and mitochondria contain 70S ribosomes, which indicates their evolution from prokaryotes. (This theory is discussed on page 101.) The role of ribosomes in protein synthesis will be discussed in more detail in Chapter 8.

Some ribosomes, called *free ribosomes,* are unattached to any structure in the cytoplasm. Primarily, free ribosomes synthesize proteins used *inside* the cell. Other ribosomes, called *membrane-bound ribosomes,* attach to the nuclear membrane and the endoplasmic reticulum. These ribosomes synthesize proteins destined for insertion in the plasma membrane or for export from the cell. Ribosomes located within mitochondria synthesize mitochondrial proteins. Ten to 20 ribosomes often join together in a stringlike arrangement called a *polyribosome*.

CHECK YOUR UNDERSTANDING

- Identify at least one significant difference between eukaryotic and prokaryotic flagella and cilia, cell walls, plasma membranes, and cytoplasm. **4-13–4-16**
- The antibiotic erythromycin binds with the 50S portion of a ribosome. What effect does this have on a prokaryotic cell? On a eukaryotic cell? **4-17**

Organelles

LEARNING OBJECTIVES

4-18 Define *organelle*.

4-19 Describe the functions of the nucleus, endoplasmic reticulum, Golgi complex, lysosomes, vacuoles, mitochondria, chloroplasts, peroxisomes, and centrosomes.

Organelles are structures with specific shapes and specialized functions and are characteristic of eukaryotic cells. They include the nucleus, endoplasmic reticulum, Golgi complex, lysosomes, vacuoles, mitochondria, chloroplasts, peroxisomes, and centrosomes. Not all of the organelles described are found in all cells, and certain cells have their own type and distribution of organelles based on specialization, age, and level of activity.

The Nucleus

The most characteristic eukaryotic organelle is the nucleus (see Figure 4.22). The **nucleus** (Figure 4.24) is usually spherical or oval, is frequently the largest structure in the cell, and contains almost all of the cell's hereditary information (DNA). Some DNA is also found in mitochondria and in the chloroplasts of photosynthetic organisms.

The nucleus is surrounded by a double membrane called the **nuclear envelope.** Both membranes resemble the plasma membrane in structure. Tiny channels in the membrane called **nuclear pores** allow the nucleus to communicate with the cytoplasm (Figure 4.24b). Nuclear pores control the movement of substances between the nucleus and cytoplasm. Within the nuclear envelope are one or more spherical bodies called **nucleoli** (singular: **nucleolus**). Nucleoli are actually condensed regions of chromosomes where ribosomal RNA is being synthesized. Ribosomal RNA is an essential component of ribosomes.

The nucleus also contains most of the cell's DNA, which is combined with several proteins, including some basic proteins called **histones** and nonhistones. The combination of about 165 base pairs of DNA and 9 molecules of histones is referred to as a *nucleosome*. When the cell is not reproducing, the DNA and its associated proteins appear as a threadlike mass called **chromatin.** During nuclear division, the chromatin coils into shorter and thicker rodlike bodies called **chromosomes.** Prokaryotic chromosomes do not undergo this process, do not have histones, and are not enclosed in a nuclear envelope.

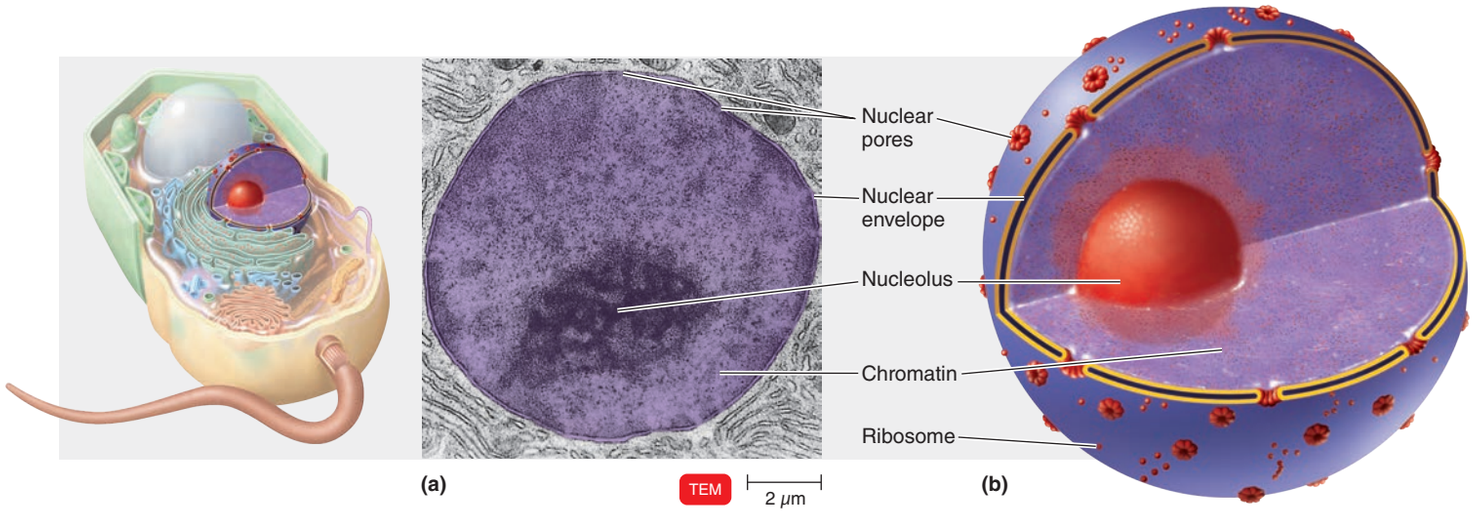


Figure 4.24 The eukaryotic nucleus. **(a)** A micrograph of a nucleus. **(b)** Drawing of details of a nucleus.

Q What keeps the nucleus suspended in the cell?

Eukaryotic cells require two elaborate mechanisms: mitosis and meiosis to segregate chromosomes prior to cell division. Neither process occurs in prokaryotic cells.

Endoplasmic Reticulum

Within the cytoplasm of eukaryotic cells is the **endoplasmic reticulum,** or **ER,** an extensive network of flattened membranous sacs or tubules called **cisternae** (Figure 4.25). The ER network is continuous with the nuclear envelope (see Figure 4.22a).

Most eukaryotic cells contain two distinct, but interrelated, forms of ER that differ in structure and function. The membrane of **rough ER** is continuous with the nuclear membrane and usually unfolds into a series of flattened sacs. The outer surface of rough ER is studded with ribosomes, the sites of protein synthesis. Proteins synthesized by ribosomes that are attached to rough ER enter cisternae within the ER for processing and sorting. In some cases, enzymes within the cisternae attach the proteins to carbohydrates to form glycoproteins.

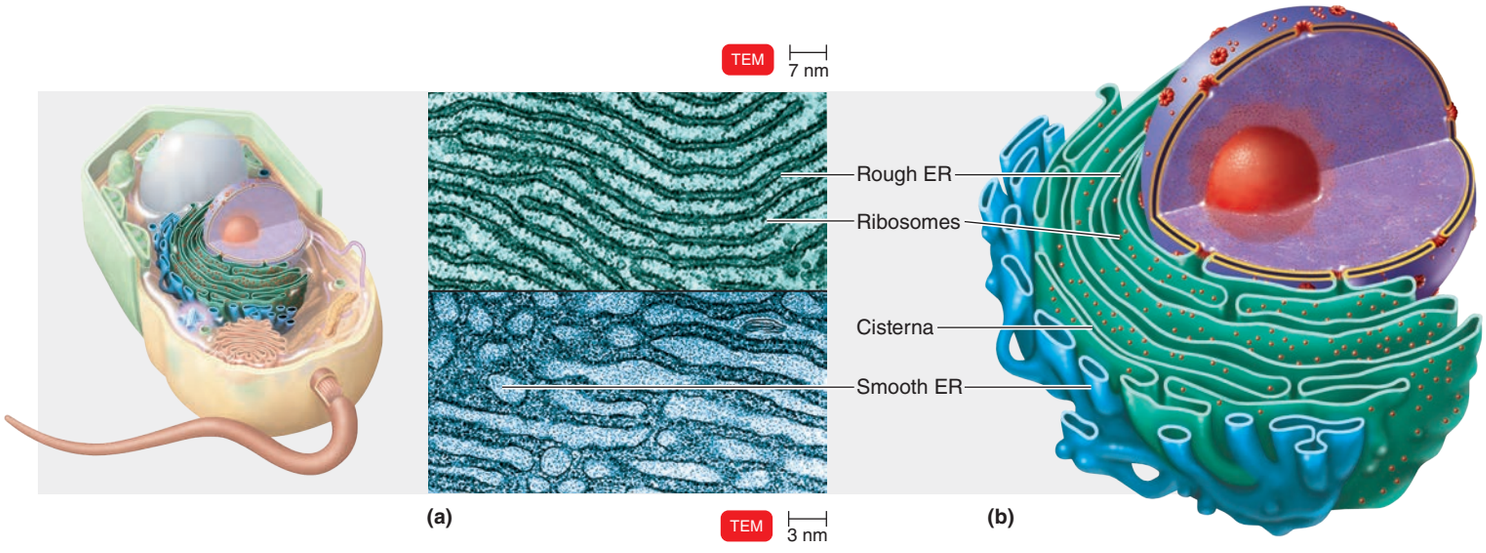


Figure 4.25 Rough endoplasmic reticulum and ribosomes. **(a)** A micrograph of the endoplasmic reticulum and ribosomes. **(b)** A drawing of details of the endoplasmic reticulum.

Q What functions of the smooth ER and rough ER are similar?

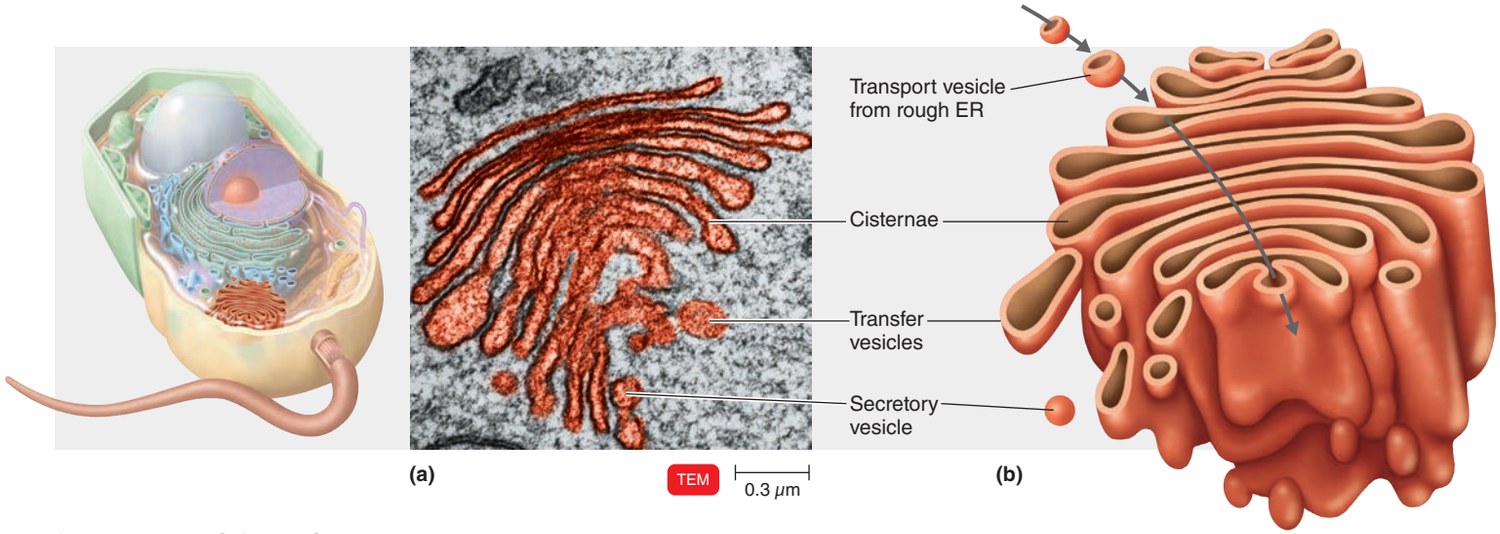


Figure 4.26 Golgi complex. **(a)** A micrograph of a Golgi complex. **(b)** A drawing of details of a Golgi complex.

What is the function of the Golgi complex?

In other cases, enzymes attach the proteins to phospholipids, also synthesized by rough ER. These molecules may be incorporated into organelle membranes or the plasma membrane. Thus, rough ER is a factory for synthesizing secretory proteins and membrane molecules.

Smooth ER extends from the rough ER to form a network of membrane tubules (see Figure 4.25). Unlike rough ER, smooth ER does not have ribosomes on the outer surface of its membrane. However, smooth ER contains unique enzymes that make it functionally more diverse than rough ER. Although it does not synthesize proteins, smooth ER does synthesize phospholipids, as does rough ER. Smooth ER also synthesizes fats and steroids, such as estrogens and testosterone. In liver cells, enzymes of the smooth ER help release glucose into the bloodstream and inactivate or detoxify drugs and other potentially harmful substances (for example, alcohol). In muscle cells, calcium ions released from the sarcoplasmic reticulum, a form of smooth ER, trigger the contraction process.

Golgi Complex

Most of the proteins synthesized by ribosomes attached to rough ER are ultimately transported to other regions of the cell. The first step in the transport pathway is through an organelle called the **Golgi complex.** It consists of 3 to 20 cisternae that resemble a stack of pita bread (Figure 4.26). The cisternae are often curved, giving the Golgi complex a cuplike shape.

Proteins synthesized by ribosomes on the rough ER are surrounded by a portion of the ER membrane, which eventually buds from the membrane surface to form a **transport vesicle.** The transport vesicle fuses with a cistern of the Golgi complex, releasing proteins into the cistern. The proteins are modified and move from one cistern to another via **transfer vesicles** that bud from the edges of the cisternae. Enzymes in the cisternae modify the proteins to form glycoproteins, glycolipids, and lipoproteins. Some of the processed proteins leave the cisternae in **secretory vesicles,** which detach from the cistern and deliver the proteins to the plasma membrane, where they are discharged by exocytosis. Other processed proteins leave the cisternae in vesicles that deliver their contents to the plasma membrane for incorporation into the membrane. Finally, some processed proteins leave the cisternae in vesicles that are called **storage vesicles.** The major storage vesicle is a lysosome, whose structure and functions are discussed next.

Lysosomes

Lysosomes are formed from Golgi complexes and look like membrane-enclosed spheres. Unlike mitochondria, lysosomes have only a single membrane and lack internal structure (see Figure 4.22). But they contain as many as 40 different kinds of powerful digestive enzymes capable of breaking down various molecules. Moreover, these enzymes can also digest bacteria that enter the cell. Human white blood cells, which use phagocytosis to ingest bacteria, contain large numbers of lysosomes.

Vacuoles

A **vacuole** (see Figure 4.22) is a space or cavity in the cytoplasm of a cell that is enclosed by a membrane called a *tonoplast*. In plant cells, vacuoles may occupy 5–90% of the cell volume, depending on the type of cell. Vacuoles are derived from the Golgi complex and have several diverse functions. Some vacuoles serve as temporary storage organelles for substances such as proteins, sugars,

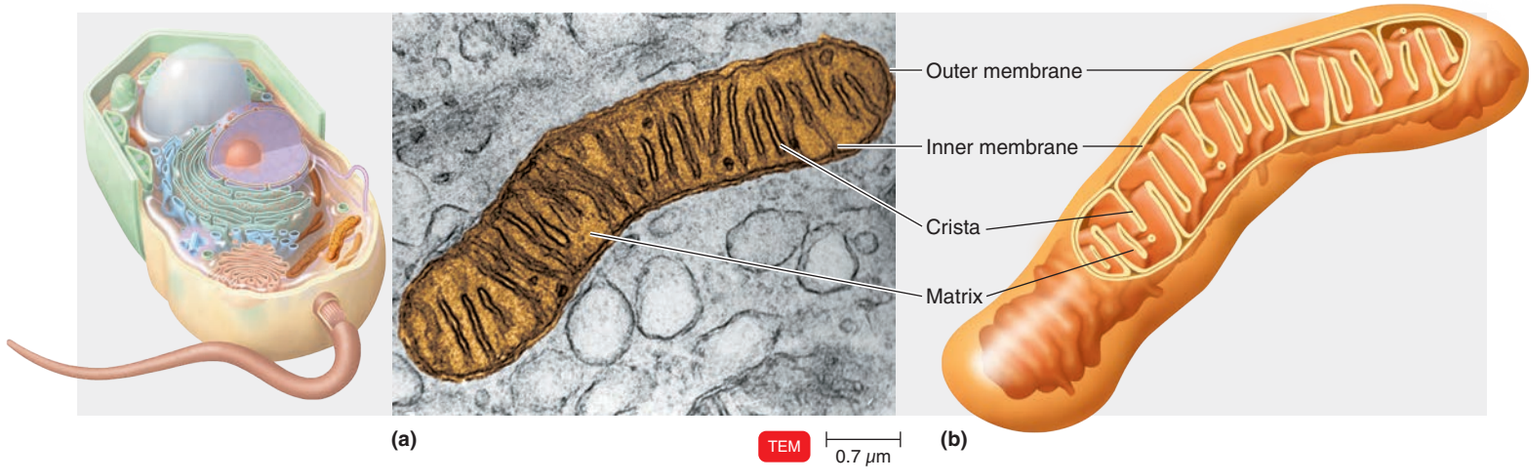


Figure 4.27 Mitochondria. **(a)** A micrograph of a mitochondrion from a rat pancreas cell. **(b)** A drawing of details of a mitochondrion.

Q How are mitochondria similar to prokaryotic cells?

organic acids, and inorganic ions. Other vacuoles form during endocytosis to help bring food into the cell. Many plant cells also store metabolic wastes and poisons that would otherwise be injurious if they accumulated in the cytoplasm. Finally, vacuoles may take up water, enabling plant cells to increase in size and also providing rigidity to leaves and stems.

Mitochondria

Elongated, irregularly shaped organelles called **mitochondria** (singular: **mitochondrion**) appear throughout the cytoplasm of most eukaryotic cells (see Figure 4.22). The number of mitochondria per cell varies greatly among different types of cells. For example, the protozoan *Giardia* has no mitochondria, whereas liver cells contain 1000 to 2000 per cell. A mitochondrion has two membranes similar in structure to the plasma membrane (Figure 4.27). The outer mitochondrial membrane is smooth, but the inner mitochondrial membrane is arranged in a series of folds called **cristae** (singular: **crista**). The center of the mitochondrion is a semifluid substance called the **matrix.** Because of the nature and arrangement of the cristae, the inner membrane provides an enormous surface area on which chemical reactions can occur. Some proteins that function in cellular respiration, including the enzyme that makes ATP, are located on the cristae of the inner mitochondrial membrane, and many of the metabolic steps involved in cellular respiration are concentrated in the matrix (see Chapter 5). Mitochondria are often called the "powerhouses of the cell" because of their central role in ATP production.

Mitochondria contain 70S ribosomes and some DNA of their own, as well as the machinery necessary to replicate, transcribe, and translate the information encoded by their DNA. In addition, mitochondria can reproduce more or less on their own by growing and dividing in two.

Chloroplasts

Algae and green plants contain a unique organelle called a **chloroplast** (Figure 4.28), a membrane-enclosed structure that contains both the pigment chlorophyll and the enzymes required for the light-gathering phases of photosynthesis (see Chapter 5). The chlorophyll is contained in flattened membrane sacs called **thylakoids;** stacks of thylakoids are called **grana** (singular: **granum**) (see Figure 4.28).

Like mitochondria, chloroplasts contain 70S ribosomes, DNA, and enzymes involved in protein synthesis. They are capable of multiplying on their own within the cell. The way both chloroplasts and mitochondria multiply—by increasing in size and then dividing in two—is strikingly reminiscent of bacterial multiplication.

Peroxisomes

Organelles similar in structure to lysosomes, but smaller, are called **peroxisomes** (see Figure 4.22). Although peroxisomes were once thought to form by budding off the ER, it is now generally agreed that they form by the division of preexisting peroxisomes.

Peroxisomes contain one or more enzymes that can oxidize various organic substances. For example, substances such as amino acids and fatty acids are oxidized in peroxisomes as part of normal metabolism. In addition, enzymes in peroxisomes oxidize toxic substances, such as alcohol. A by-product of the oxidation reactions is hydrogen peroxide (H_2O_2), a potentially toxic compound. However, peroxisomes also contain the enzyme *catalase,* which decomposes H_2O_2 (see Chapter 6, page 160). Because the generation and degradation of H_2O_2 occurs within the same organelle, peroxisomes protect other parts of the cell from the toxic effects of H_2O_2.

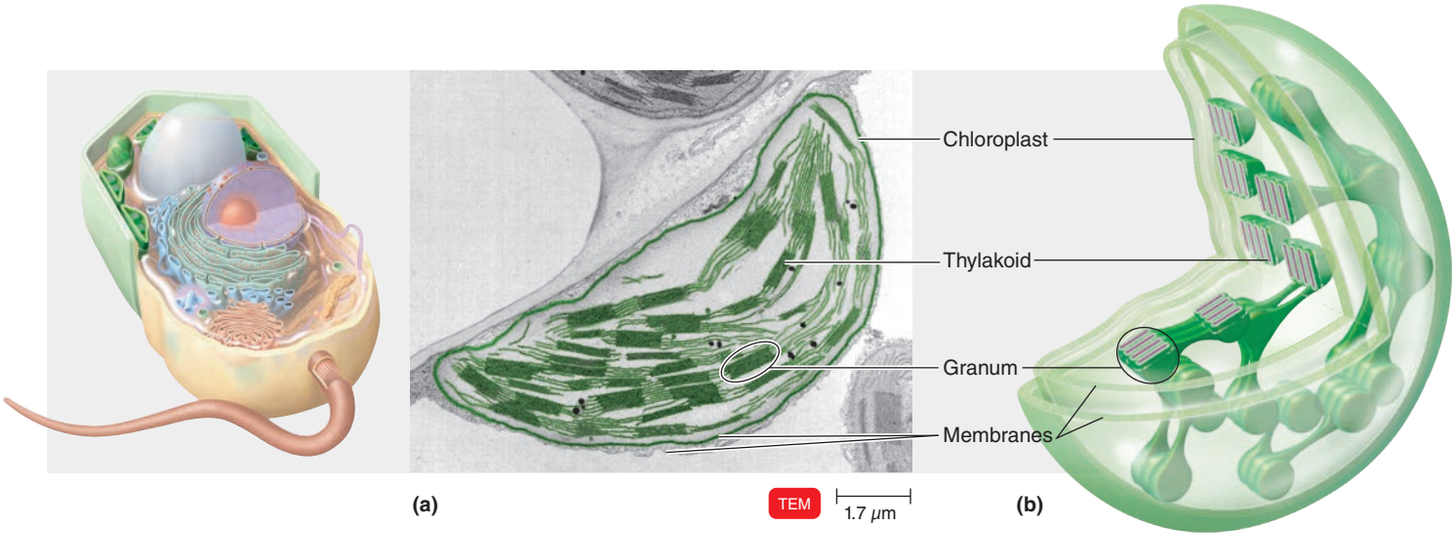


Figure 4.28 Chloroplasts. Photosynthesis occurs in chloroplasts; the light-trapping pigments are located on the thylakoids. **(a)** A micrograph of chloroplasts in a plant cell. **(b)** A drawing of details of a chloroplast, showing grana.

Q What are the similarities between chloroplasts and prokaryotic cells?

Centrosome

The **centrosome,** located near the nucleus, consists of two components: the pericentriolar area and centrioles (see Figure 4.22). The *pericentriolar material* is a region of the cytosol composed of a dense network of small protein fibers. This area is the organizing center for the mitotic spindle, which plays a critical role in cell division, and for microtubule formation in nondividing cells. Within the pericentriolar material is a pair of cylindrical structures called *centrioles,* each of which is composed of nine clusters of three microtubules (triplets) arranged in a circular pattern, an arrangement called a *9 + 0 array.* The 9 refers to the nine clusters of microtubules, and the 0 refers to the absence of microtubules in the center. The long axis of one centriole is at a right angle to the long axis of the other.

CHECK YOUR UNDERSTANDING

- Compare the structure of the nucleus of a eukaryote and the nucleoid of a prokaryote. **4-18**
- How do rough and smooth ER compare structurally and functionally? **4-19**

The Evolution of Eukaryotes

LEARNING OBJECTIVE

4-20 Discuss evidence that supports the endosymbiotic theory of eukaryotic evolution.

Biologists generally believe that life arose on Earth in the form of very simple organisms, similar to prokaryotic cells, about 3.5 to 4 billion years ago. About 2.5 billion years ago, the first eukaryotic cells evolved from prokaryotic cells. Recall that prokaryotes and eukaryotes differ mainly in that eukaryotes contain highly specialized organelles. The theory explaining the origin of eukaryotes from prokaryotes, pioneered by Lynn Margulis, is the **endosymbiotic theory.** According to this theory, larger bacterial cells lost their cell walls and engulfed smaller bacterial cells. This relationship, in which one organism lives within another, is called *endosymbiosis* (*symbiosis* = living together).

ASM: Cells, organelles (e.g., mitochondria and chloroplasts) and all major metabolic pathways evolved from early prokaryotic cells.

According to the endosymbiotic theory, the ancestral eukaryote developed a rudimentary nucleus when the plasma membrane folded around the chromosome (see Figure 10.2, page 268). This cell, called a nucleoplasm, may have ingested aerobic bacteria. Some ingested bacteria lived inside the host nucleoplasm. This arrangement evolved into a symbiotic relationship in which the host nucleoplasm supplied nutrients and the endosymbiotic bacterium produced energy that could be used by the nucleoplasm. Similarly, chloroplasts may be descendants of photosynthetic prokaryotes ingested by this early nucleoplasm. Eukaryotic flagella and cilia are believed to have originated from symbiotic associations between the plasma membrane of early eukaryotes and motile spiral bacteria called spirochetes. A living example that suggests how flagella developed is described in the box on page 94.

Studies comparing prokaryotic and eukaryotic cells provide evidence for the endosymbiotic theory. For example, both mitochondria and chloroplasts resemble bacteria in size and shape.

These organelles contain circular DNA, which is typical of prokaryotes, and the organelles can reproduce independently of their host cell. Moreover, mitochondrial and chloroplast ribosomes resemble those of prokaryotes, and their mechanism of protein synthesis is more similar to that found in bacteria than eukaryotes. Finally, the same antibiotics that inhibit protein synthesis on ribosomes in bacteria also inhibit protein synthesis on ribosomes in mitochondria and chloroplasts.

CHECK YOUR UNDERSTANDING

Which three organelles are not associated with the Golgi complex? What does this suggest about their origin? **4-20**

Study Outline

MasteringMicrobiology®
Study microbiology where it matters: every where.
Check out the Study Area of MasteringMicrobiology to explore Interactive Microbiology. Study on-the-go with Dynamic Study Modules. Check your understanding with chapter quizzes and animations—and much more!

Comparing Prokaryotic and Eukaryotic Cells: An Overview (p. 73)

1. Prokaryotic and eukaryotic cells are similar in their chemical composition and chemical reactions.
2. Prokaryotic cells typically lack membrane-enclosed organelles (including a nucleus).
3. Peptidoglycan is found in prokaryotic cell walls but not in eukaryotic cell walls.
4. Eukaryotic cells have a membrane-bound nucleus and other organelles.

The Prokaryotic Cell (pp. 73–94)

1. Bacteria are unicellular, and most of them multiply by binary fission.
2. Bacterial species are differentiated by morphology, chemical composition, nutritional requirements, biochemical activities, and source of energy.

The Size, Shape, and Arrangement of Bacterial Cells (pp. 73–75)

1. Most bacteria are 0.2 to 2.0 μm in diameter and 2 to 8 μm in length.
2. The three basic bacterial shapes are coccus (spherical), bacillus (rod-shaped), and spiral (twisted).
3. Pleomorphic bacteria can assume several shapes.

Structures External to the Cell Wall (pp. 75–80)

Glycocalyx (pp. 75–77)

1. The glycocalyx (capsule, slime layer, or extracellular polysaccharide) is a gelatinous polysaccharide and/or polypeptide covering.
2. Capsules may protect pathogens from phagocytosis.
3. Capsules enable adherence to surfaces, prevent desiccation, and may provide nutrients.

Flagella (pp. 77–78)

4. Flagella are relatively long filamentous appendages consisting of a filament, hook, and basal body.
5. Prokaryotic flagella rotate to push the cell.
6. Motile bacteria exhibit taxis; positive taxis is movement toward an attractant, and negative taxis is movement away from a repellent.
7. Flagellar (H) protein is an antigen.

Axial Filaments (pp. 78–79)

8. Spiral cells that move by means of an axial filament (endoflagellum) are called spirochetes.
9. Axial filaments are similar to flagella, except that they wrap around the cell.

Fimbriae and Pili (pp. 79–80)

10. Fimbriae help cells adhere to surfaces.
11. Pili are involved in twitching motility and DNA transfer.

The Cell Wall (pp. 80–85)

Composition and Characteristics (pp. 80–83)

1. The cell wall surrounds the plasma membrane and protects the cell from changes in water pressure.
2. The bacterial cell wall consists of peptidoglycan, a polymer consisting of NAG and NAM and short chains of amino acids.
3. Penicillin interferes with peptidoglycan synthesis.
4. Gram-positive cell walls consist of many layers of peptidoglycan and also contain teichoic acids.
5. Gram-negative bacteria have a lipopolysaccharide-lipoprotein-phospholipid outer membrane surrounding a thin peptidoglycan layer.
6. The outer membrane protects the cell from phagocytosis and from penicillin, lysozyme, and other chemicals.
7. Porins are proteins that permit small molecules to pass through the outer membrane; specific channel proteins allow other molecules to move through the outer membrane.
8. The lipopolysaccharide component of the outer membrane consists of sugars (O polysaccharides), which function as antigens, and lipid A, which is an endotoxin.

Cell Walls and the Gram Stain Mechanism (p. 83)

9. The crystal violet–iodine complex combines with peptidoglycan.
10. The decolorizer removes the lipid outer membrane of gram-negative bacteria and washes out the crystal violet.

Atypical Cell Walls (p. 83)

11. *Mycoplasma* is a bacterial genus that naturally lacks cell walls.
12. Archaea have pseudomurein; they lack peptidoglycan.
13. Acid-fast cell walls have a layer of mycolic acid outside a thin peptidoglycan layer.

Damage to the Cell Wall (pp. 83–85)

14. In the presence of lysozyme, gram-positive cell walls are destroyed, and the remaining cellular contents are referred to as a protoplast.
15. In the presence of lysozyme, gram-negative cell walls are not completely destroyed, and the remaining cellular contents are referred to as a spheroplast.
16. L forms are gram-positive or gram-negative bacteria that do not make a cell wall.
17. Antibiotics such as penicillin interfere with cell wall synthesis.

Structures Internal to the Cell Wall (pp. 85–94)

The Plasma (Cytoplasmic) Membrane (pp. 85–87)

1. The plasma membrane encloses the cytoplasm and is a lipid bilayer with peripheral and integral proteins (the fluid mosaic model).
2. The plasma membrane is selectively permeable.
3. Plasma membranes contain enzymes for metabolic reactions, such as nutrient breakdown, energy production, and photosynthesis.
4. Mesosomes, irregular infoldings of the plasma membrane, are artifacts, not true cell structures.
5. Plasma membranes can be destroyed by alcohols and polymyxins.

The Movement of Materials across Membranes (pp. 87–90)

6. Movement across the membrane may be by passive processes, in which materials move from areas of higher to lower concentration and no energy is expended by the cell.
7. In simple diffusion, molecules and ions move until equilibrium is reached.
8. In facilitated diffusion, substances are transported by transporter proteins across membranes from areas of high to low concentration.
9. Osmosis is the movement of water from areas of high to low concentration across a selectively permeable membrane until equilibrium is reached.
10. In active transport, materials move from areas of low to high concentration by transporter proteins, and the cell must expend energy.
11. In group translocation, energy is expended to modify chemicals and transport them across the membrane.

Cytoplasm (p. 90)

12. Cytoplasm is the fluid component inside the plasma membrane.
13. The cytoplasm is mostly water, with inorganic and organic molecules, DNA, ribosomes, inclusions, and cytoskeleton proteins.
14. A cytoskeleton is present, but cytoplasmic streaming does not occur.

The Nucleoid (p. 90)

15. The nucleoid contains the DNA of the bacterial chromosome.
16. Bacteria can also contain plasmids, which are circular, extrachromosomal DNA molecules.

Ribosomes (p. 91)

17. The cytoplasm of a prokaryote contains numerous 70S ribosomes; ribosomes consist of rRNA and protein.
18. Protein synthesis occurs at ribosomes; it can be inhibited by certain antibiotics.

Inclusions (pp. 91–92)

19. Inclusions are reserve deposits found in prokaryotic and eukaryotic cells.
20. Among the inclusions found in bacteria are metachromatic granules (inorganic phosphate), polysaccharide granules (usually glycogen or starch), lipid inclusions, sulfur granules, carboxysomes (ribulose 1,5-diphosphate carboxylase), magnetosomes (Fe_3O_4), and gas vacuoles.

Endospores (pp. 92–94)

21. Endospores are resting structures formed by some bacteria; they allow survival during adverse environmental conditions.

The Eukaryotic Cell (pp. 94–103)

Flagella and Cilia (p. 94)

1. Flagella are few and long in relation to cell size; cilia are numerous and short.
2. Flagella and cilia are used for motility, and cilia also move substances along the surface of the cells.
3. Both flagella and cilia consist of an arrangement of nine pairs and two single microtubules.

The Cell Wall and Glycocalyx (pp. 94–96)

1. The cell walls of many algae and some fungi contain cellulose.
2. The main material of fungal cell walls is chitin.
3. Yeast cell walls consist of glucan and mannan.
4. Animal cells are surrounded by a glycocalyx, which strengthens the cell and provides a means of attachment to other cells.

The Plasma (Cytoplasmic) Membrane (pp. 96–97)

1. Like the prokaryotic plasma membrane, the eukaryotic plasma membrane is a phospholipid bilayer containing proteins.
2. Eukaryotic plasma membranes contain carbohydrates attached to the proteins and sterols not found in prokaryotic cells (except *Mycoplasma* bacteria).
3. Eukaryotic cells can move materials across the plasma membrane by the passive processes used by prokaryotes and by active transport and endocytosis (phagocytosis, pinocytosis, and receptor-mediated endocytosis).

Cytoplasm (p. 97)

1. The cytoplasm of eukaryotic cells includes everything inside the plasma membrane and external to the nucleus.
2. The chemical characteristics of the cytoplasm of eukaryotic cells resemble those of the cytoplasm of prokaryotic cells.
3. Eukaryotic cytoplasm has a cytoskeleton and exhibits cytoplasmic streaming.

Ribosomes (pp. 97–98)

1. 80S ribosomes are found in the cytoplasm or attached to the rough endoplasmic reticulum.

Organelles (pp. 98–101)

1. Organelles are specialized membrane-enclosed structures in the cytoplasm of eukaryotic cells.
2. The nucleus, which contains DNA in the form of chromosomes, is the most characteristic eukaryotic organelle.
3. The nuclear envelope is connected to a system of membranes in the cytoplasm called the endoplasmic reticulum (ER).
4. The ER provides a surface for chemical reactions and serves as a transport network. Protein synthesis and transport occur on the rough ER; lipid synthesis occurs on the smooth ER.

5. The Golgi complex consists of flattened sacs called cisterns. It functions in membrane formation and protein secretion.
6. Lysosomes are formed from Golgi complexes. They store digestive enzymes.
7. Vacuoles are membrane-enclosed cavities derived from the Golgi complex or endocytosis. They are usually found in plant cells that store various substances and provide rigidity to leaves and stems.
8. Mitochondria are the primary sites of ATP production. They contain 70S ribosomes and DNA, and they multiply by binary fission.
9. Chloroplasts contain chlorophyll and enzymes for photosynthesis. Like mitochondria, they contain 70S ribosomes and DNA and multiply by binary fission.
10. A variety of organic compounds are oxidized in peroxisomes. Catalase in peroxisomes destroys H_2O_2.
11. The centrosome consists of the pericentriolar material and centrioles. Centrioles are 9 triplet microtubules involved in formation of the mitotic spindle and microtubules.

The Evolution of Eukaryotes (pp. 101–103)

1. According to the endosymbiotic theory, eukaryotic cells evolved from symbiotic prokaryotes living inside other prokaryotic cells.

Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

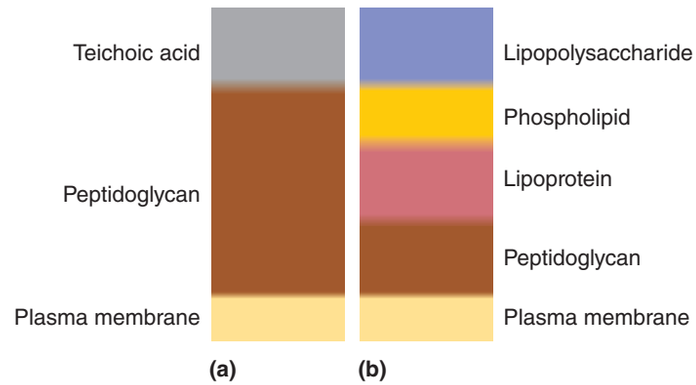
Knowledge and Comprehension

Review

1. DRAW IT Diagram each of the following flagellar arrangements:
   a. lophotrichous
   b. monotrichous
   c. peritrichous
   d. amphitrichous
   e. polar
2. Endospore formation is called (a) _____. It is initiated by (b) _____. Formation of a new cell from an endospore is called (c) _____. This process is triggered by (d) _____.
3. DRAW IT Draw the bacterial shapes listed in (a), (b), and (c). Then draw the shapes in (d), (e), and (f), showing how they are special conditions of a, b, and c, respectively.
   a. spiral
   b. bacillus
   c. coccus
   d. spirochetes
   e. streptobacilli
   f. staphylococci
4. Match the structures in column A to their functions in column B.

Column A	Column B
_____ **a**. Cell wall	**1**. Attachment to surfaces
_____ **b**. Endospore	**2**. Cell wall formation
_____ **c**. Fimbriae	**3**. Motility
_____ **d**. Flagella	**4**. Protection from osmotic lysis
_____ **e**. Glycocalyx	**5**. Protection from phagocytes
_____ **f**. Pili	**6**. Resting
_____ **g**. Plasma membrane	**7**. Protein synthesis
_____ **h**. Ribosomes	**8**. Selective permeability
	9. Transfer of genetic material

5. Why is an endospore called a resting structure? Of what advantage is an endospore to a bacterial cell?
6. Compare and contrast the following:
   a. simple diffusion and facilitated diffusion
   b. active transport and facilitated diffusion
   c. active transport and group translocation
7. Answer the following questions using the diagrams provided, which represent cross sections of bacterial cell walls.
   a. Which diagram represents a gram-positive bacterium? How can you tell?



   b. Explain how the Gram stain works to distinguish these two types of cell walls.
   c. Why does penicillin have no effect on most gram-negative cells?
   d. How do essential molecules enter cells through each wall?
   e. Which cell wall is toxic to humans?
8. Starch is readily metabolized by many cells, but a starch molecule is too large to cross the plasma membrane. How does a cell obtain the glucose molecules from a starch polymer? How does the cell transport these glucose molecules across the plasma membrane?
9. Match the characteristics of eukaryotic cells in column A with their functions in column B.

Column A	Column B
_____ **a**. Pericentriolar material	**1**. Digestive enzyme storage
_____ **b**. Chloroplasts	**2**. Oxidation of fatty acids
_____ **c**. Golgi complex	**3**. Microtubule formation
_____ **d**. Lysosomes	**4**. Photosynthesis
_____ **e**. Mitochondria	**5**. Protein synthesis
_____ **f**. Peroxisomes	**6**. Respiration
_____ **g**. Rough ER	**7**. Secretion

10. NAME IT What group of microbes is characterized by cells that form filaments, reproduce by spores, and have peptidoglycan in their cell walls?

Multiple Choice

1. Which of the following is *not* a distinguishing characteristic of prokaryotic cells?
   a. They usually have a single, circular chromosome.
   b. They lack membrane-enclosed organelles.
   c. They have cell walls containing peptidoglycan.
   d. Their DNA is not associated with histones.
   e. They lack a plasma membrane.

Use the following choices to answer questions 2–4.
   a. No change will result; the solution is isotonic.
   b. Water will move into the cell.
   c. Water will move out of the cell.
   d. The cell will undergo osmotic lysis.
   e. Sucrose will move into the cell from an area of higher concentration to one of lower concentration.

2. Which statement best describes what happens when a gram-positive bacterium is placed in distilled water and penicillin?
3. Which statement best describes what happens when a gram-negative bacterium is placed in distilled water and penicillin?
4. Which statement best describes what happens when a gram-positive bacterium is placed in an aqueous solution of lysozyme and 10% sucrose?
5. Which of the following statements best describes what happens to a cell exposed to polymyxins that destroy phospholipids?
   a. In an isotonic solution, nothing will happen.
   b. In a hypotonic solution, the cell will lyse.
   c. Water will move into the cell.
   d. Intracellular contents will leak from the cell.
   e. Any of the above might happen.
6. Which of the following is *false* about fimbriae?
   a. They are composed of protein.
   b. They may be used for attachment.
   c. They are found on gram-negative cells.
   d. They are composed of pilin.
   e. They may be used for motility.
7. Which of the following pairs is *mismatched*?
   a. glycocalyx—adherence
   b. pili—reproduction
   c. cell wall—toxin
   d. cell wall—protection
   e. plasma membrane—transport
8. Which of the following pairs is *mismatched*?
   a. metachromatic granules—stored phosphates
   b. polysaccharide granules—stored starch
   c. lipid inclusions—poly-β-hydroxybutyric acid
   d. sulfur granules—energy reserve
   e. ribosomes—protein storage
9. You have isolated a motile, gram-positive cell with no visible nucleus. You can assume this cell has
   a. ribosomes.
   b. mitochondria.
   c. an endoplasmic reticulum.
   d. a Golgi complex.
   e. all of the above
10. The antibiotic amphothericin B disrupts plasma membranes by combining with sterols; it will affect all of the following cells *except*
   a. animal cells.
   b. gram-negative bacterial cells.
   c. fungal cells.
   d. *Mycoplasma* cells.
   e. plant cells.

Analysis

1. How can prokaryotic cells be smaller than eukaryotic cells and still carry on all the functions of life?
2. The smallest eukaryotic cell is the motile alga *Micromonas*. What is the minimum number of organelles this alga must have?
3. Two types of prokaryotic cells have been distinguished: bacteria and archaea. How do these cells differ from each other? How are they similar?
4. In 1985, a 0.5-mm cell was discovered in surgeonfish and named *Epulopiscium fishelsoni* (see Figure 11.20, page 308). It was presumed to be a protozoan. In 1993, researchers determined that *Epulopiscium* is actually a gram-positive bacterium. Why do you suppose this organism was initially identified as a protozoan? What evidence would change the classification to bacterium?
5. When *E. coli* cells are exposed to a hypertonic solution, the bacteria produce a transporter protein that can move K^+ (potassium ions) into the cell. Of what value is the active transport of K^+, which requires ATP?

Clinical Applications and Evaluation

1. *Clostridium botulinum* is a strict anaerobe; that is, it is killed by the molecular oxygen (O_2) present in air. Humans can die of botulism from eating foods in which *C. botulinum* is growing. How does this bacterium survive on plants picked for human consumption? Why are home-canned foods most often the source of botulism?
2. A South San Francisco child enjoyed bath time at his home because of the colorful orange and red water. The water did not have this rusty color at its source, and the water department could not culture the *Acidithiobacillus* bacteria responsible for the rusty color from the source. How were the bacteria getting into the household water? What bacterial structures make this possible?
3. Live cultures of *Bacillus thuringiensis* (Dipel) and *B. subtilis* (Kodiak) are sold as pesticides. What bacterial structures make it possible to package and sell these bacteria? For what purpose is each product used? (*Hint*: Refer to Chapter 11.)

In the Clinic

As the nurse researcher in a large medical center, you are working with the gastroenterology physicians on a project to study the effect of diet on intestinal gas. People in the test group who developed the most gas ate broccoli and beans, which are high in raffinose and stachyose, and eggs, which are high in methionine and cysteine. Intestinal gas is composed of CO_2, CH_4, H_2S, and H_2. **How are these gases produced?**

Hint: Read about carbohydrates (pages 36–37), amino acids (pages 39–40), carbohydrate catabolism (pages 119–131), and protein catabolism (pages 131–133).

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

5

Microbial Metabolism

Now that you are familiar with the structure of prokaryotic cells, we can discuss the activities that enable these microbes to thrive. The life-support processes of even the most structurally simple organism involve a large number of complex biochemical reactions. Most, although not all, of the biochemical processes of bacteria also occur in eukaryotic microbes and in the cells of multicellular organisms, including humans. However, the reactions that are unique to bacteria are fascinating because they allow microorganisms to do things we cannot do. For example, some bacteria can live on cellulose, whereas others can live on petroleum. Through their metabolism, bacteria recycle elements after other organisms have used them. Still other bacteria can live on diets of inorganic substances such as carbon dioxide, iron, sulfur, hydrogen gas, and ammonia. Microbial metabolism allows some microorganisms to grow in or on the human body as shown in dental plaque in the photograph. An example of the bacterial metabolism that contributes to dental caries is discussed in the Clinical Case.

ASM: The interactions of microorganisms among themselves and with their environment are determined by their metabolic abilities.

This chapter examines some representative chemical reactions that either produce energy (the catabolic reactions) or use energy (the anabolic reactions) in microorganisms. We will also look at how these various reactions are integrated within the cell. The **Big Picture** feature on the next page highlights key principles of metabolism that are explained in greater detail throughout the chapter.

Dental plaque consists of bacteria (bubble-gum pink) embedded in a glycoprotein matrix (khaki).

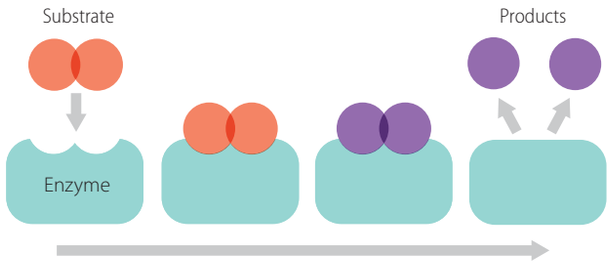
BIG PICTURE

Metabolism

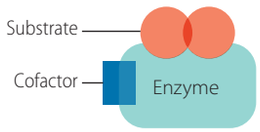
Metabolism is the buildup and breakdown of nutrients within a cell. These chemical reactions provide energy and create substances that sustain life.

Two key players in metabolism are **enzymes** and the molecule **adenosine triphosphate (ATP)**.

Enzymes catalyze reactions for specific molecules called **substrates**. During enzymatic reactions, substrates are transformed into new substances called **products**.

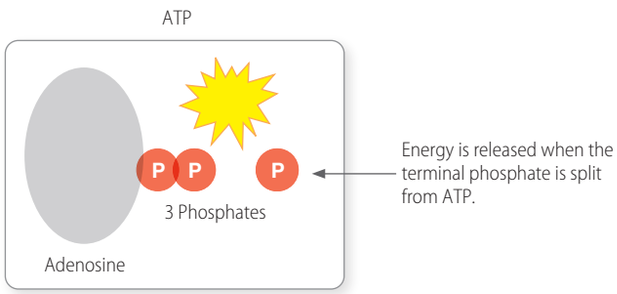


Enzymes, which are usually proteins, may need other nonprotein molecules called cofactors to work. Inorganic cofactors include metal ions. Organic cofactors, or coenzymes, include the electron carriers FAD, NAD^+ and $NADP^+$.



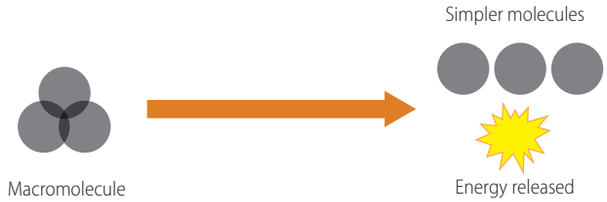
However, without energy, certain reactions will never occur, even if enzymes are present. **Adenosine triphosphate (ATP)** is a molecule that cells use to manage energy needs.

If a reaction results in excess energy, some can be captured in the form of ATP's bonds. A cell can then break those same bonds and use the released energy to fuel other reactions.



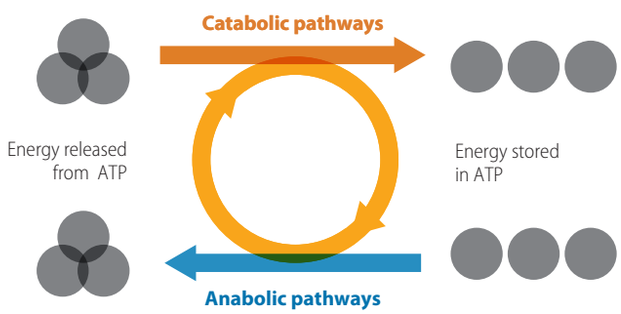
The chemistry of metabolism can seem overwhelming at first, with *pathways,* or sets of many coordinated reactions, working together toward common goals. But the basic rules of metabolism are actually quite simple. Pathways can be categorized into two general types—**catabolic** and **anabolic**.

Catabolic pathways break down macromolecules into simple component parts, releasing energy in the process.



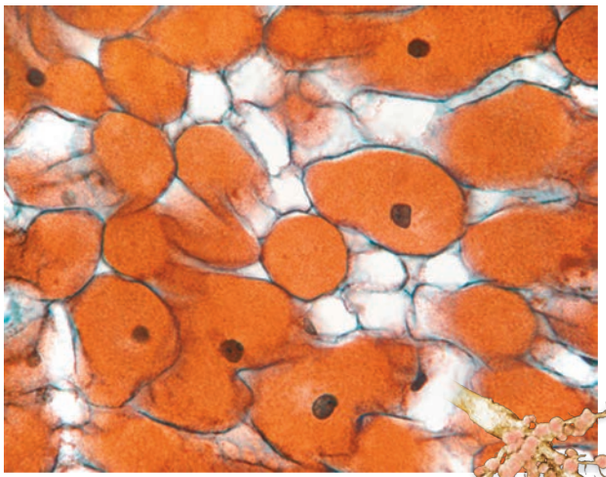
Anabolic pathways build up macromolecules by combining simpler molecules, using energy in the process.

In other words, catabolic and anabolic pathways are linked by **energy**. Catabolic reactions provide the energy needed for anabolic reactions.



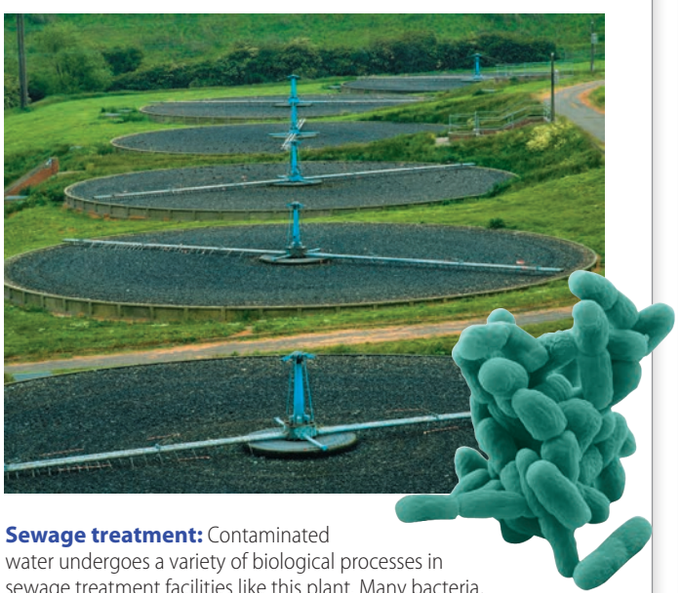
Scan this code to view MicroFlix: Metabolism

Although microbial metabolism can cause disease and food spoilage, many pathways are beneficial rather than pathogenic.



Nitrogen cycle: Nitrogen is a key component in proteins, DNA and RNA, and plant chlorophyll. But without microbes, there would be little nitrogen available for most life forms. Certain bacteria (such as these *Rhizobium*, above, within a soybean root nodule, shown at right) in the soil convert nitrogen from the atmosphere into forms that other life forms can use.

Beverages and food: Various bacteria and yeasts (such as *Saccharomyces cerevisiae,* shown at right) carry out catabolic reactions called fermentation. Beer, wine, and foods such as cheeses, yogurt, pickles, sauerkraut, and soy sauce tap microbial metabolism as a crucial part of production.



Sewage treatment: Contaminated water undergoes a variety of biological processes in sewage treatment facilities like this plant. Many bacteria, including some species of cyanobacteria (shown on the right) play a role in removing harmful organic matter.

Drugs: The pharmaceutical industry uses a variety of bacteria and fungi in the production of antibiotics, such as penicillin, (derived from the *Penicillium* fungus, shown on the right). Bacitracin, erythromycin, and other treatments such as vaccines, vitamins, and enzymes are also derived from microbial metabolism.

KEY CONCEPTS

- Enzymes facilitate metabolic reactions.
- ATP is used by microbes and other cells to manage energy needs.
- Catabolic reactions couple with ATP synthesis.
- Anabolic reactions couple with ATP breakdown.

Catabolic and Anabolic Reactions

LEARNING OBJECTIVES

5-1 Define *metabolism,* and describe the fundamental differences between anabolism and catabolism.

5-2 Identify the role of ATP as an intermediate between catabolism and anabolism.

We use the term **metabolism** to refer to the sum of all chemical reactions within a living organism. Because chemical reactions either release or require energy, metabolism can be viewed as an energy-balancing act. Accordingly, metabolism can be divided into two classes of chemical reactions: those that release energy and those that require energy.

In living cells, the enzyme-regulated chemical reactions that release energy are generally the ones involved in **catabolism,** the breakdown of complex organic compounds into simpler ones. These reactions are called *catabolic,* or *degradative,* reactions. Catabolic reactions are generally *hydrolytic* reactions (reactions which use water and in which chemical bonds are broken), and they are *exergonic* (produce more energy than they consume). An example of catabolism occurs when cells break down sugars into carbon dioxide and water.

The enzyme-regulated energy-requiring reactions are mostly involved in **anabolism,** the building of complex organic molecules from simpler ones. These reactions are called *anabolic,* or *biosynthetic,* reactions. Anabolic processes often involve *dehydration synthesis* reactions (reactions that release water), and they are *endergonic* (consume more energy than they produce). Examples of anabolic processes are the formation of proteins from amino acids, nucleic acids from nucleotides, and polysaccharides from simple sugars. These biosynthetic reactions generate the materials for cell growth.

Catabolic reactions provide building blocks for anabolic reactions and furnish the energy needed to drive anabolic reactions. This coupling of energy-requiring and energy-releasing reactions is made possible through the molecule adenosine triphosphate (ATP). (You can review its structure in Figure 2.18, page 46.) ATP stores energy derived from catabolic reactions and releases it later to drive anabolic reactions and perform other cellular work. Remember that a molecule of ATP consists of an adenine, a ribose, and three phosphate groups. When the terminal phosphate group is split from ATP, adenosine diphosphate (ADP) is formed, and energy is released to drive anabolic reactions. Using P to represent a phosphate group (P_i represents inorganic phosphate, which is not bound to any other molecule), we write this reaction as follows:

$$ATP \longrightarrow ADP + P_i + \text{energy}$$

Then, the energy from catabolic reactions is used to combine ADP and a P_i to resynthesize ATP:

$$ADP + P_i + \text{energy} \longrightarrow ATP$$

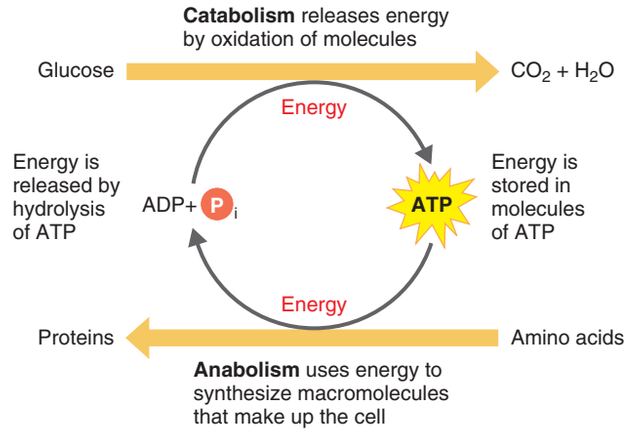


Figure 5.1 The role of ATP in coupling anabolic and catabolic reactions. When complex molecules are split apart (catabolism), some of the energy is transferred to and trapped in ATP, and the rest is given off as heat. When simple molecules are combined to form complex molecules (anabolism), ATP provides the energy for synthesis, and again some energy is given off as heat.

Q How does ATP provide the energy for synthesis?

Thus, anabolic reactions are coupled to ATP breakdown, and catabolic reactions are coupled to ATP synthesis. This concept of coupled reactions is very important; you will see why by the end of this chapter. For now, you should know that the chemical composition of a living cell is constantly changing: some molecules are broken down while others are being synthesized. This balanced flow of chemicals and energy maintains the life of a cell.

The role of ATP in coupling anabolic and catabolic reactions is shown in Figure 5.1. Only part of the energy released in catabolism is actually available for cellular functions because

Clinical Case: More Than a Sweet Tooth

Dr. Antonia Rivera is a pediatric dentist in St. Louis, Missouri. Her latest patient, 7-year-old Micah Thompson, has just left the office with strict instructions about brushing and flossing regularly. What most worries Dr. Rivera, however, is that Micah is her seventh patient this week to present with multiple dental caries, or cavities. Dr. Rivera is used to seeing some increase in tooth decay after Halloween and Easter, but why are all these children getting cavities in the middle of the summer? When possible, she has been speaking to each of the patient's parents or grandparents, but no one has noticed anything out of the ordinary in the children's diets.

Why do so many of Dr. Rivera's patients have multiple dental caries? Read on to find out.

110 128 133 135

part of the energy is lost to the environment as heat. Because the cell must use energy to maintain life, it has a continuous need for new external sources of energy.

Before we discuss how cells produce energy, let's first consider the principal properties of a group of proteins involved in almost all biologically important chemical reactions: enzymes. A cell's **metabolic pathways** (sequences of chemical reactions) are determined by its enzymes, which are in turn determined by the cell's genetic makeup. **Animation** Metabolism: Overview

CHECK YOUR UNDERSTANDING

- Distinguish catabolism from anabolism. **5-1**
- How is ATP an intermediate between catabolism and anabolism? **5-2**

Enzymes

LEARNING OBJECTIVES

5-3 Identify the components of an enzyme.
5-4 Describe the mechanism of enzymatic action.
5-5 List the factors that influence enzymatic activity.
5-6 Distinguish competitive and noncompetitive inhibition.
5-7 Define *ribozyme*.

Collision Theory

Chemical reactions occur when chemical bonds are formed or broken. For reactions to take place, atoms, ions, or molecules must collide. The **collision theory** explains how chemical reactions occur and how certain factors affect the rates of those reactions. The basis of the collision theory is that all atoms, ions, and molecules are continuously moving and colliding with one another. The energy transferred by the particles in the collision can disrupt their electron structures enough to break chemical bonds or form new bonds.

Several factors determine whether a collision will cause a chemical reaction: the velocities of the colliding particles, their energy, and their specific chemical configurations. Up to a point, the higher the particles' velocities, the more probable that their collision will cause a reaction. Also, each chemical reaction requires a specific level of energy. But even if colliding particles possess the minimum energy needed for reaction, no reaction will take place unless the particles are properly oriented toward each other.

Let's assume that molecules of substance AB (the reactant) are to be converted to molecules of substances A and B (the products). In a given population of molecules of substance AB, at a specific temperature, some molecules possess relatively little energy; the majority of the population possesses an average amount of energy; and a small portion of the population has high energy. If only the high-energy AB molecules are able to react and be converted to A and B molecules, then only relatively few molecules at any one time possess enough energy to react in a collision. The collision energy required for a chemical reaction is its **activation energy,** which is the amount of energy needed to disrupt the stable electronic configuration of any specific molecule so that the electrons can be rearranged.

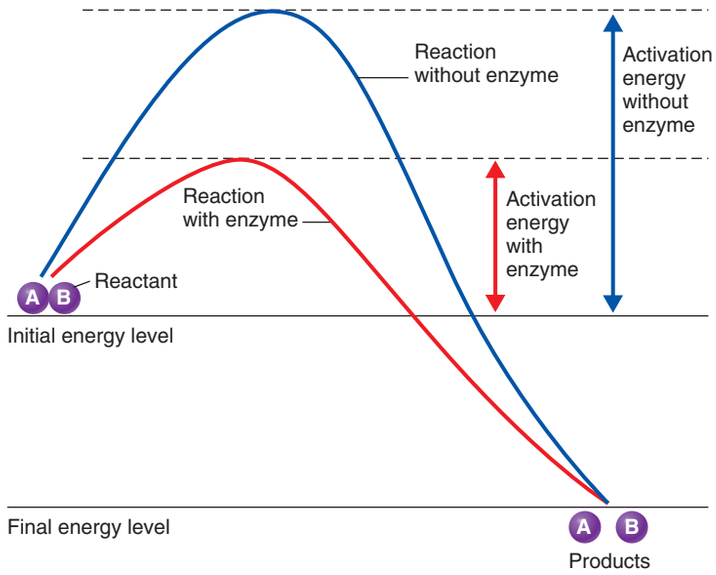


Figure 5.2 Energy requirements of a chemical reaction. This graph shows the progress of the reaction AB → A + B both without (blue line) and with (red line) an enzyme. The presence of an enzyme lowers the activation energy of the reaction (see arrows). Thus, more molecules of reactant AB are converted to products A and B because more molecules of reactant AB possess the activation energy needed for the reaction.

Q Why does a chemical reaction require increased activation energy without an enzyme as a biological catalyst?

The **reaction rate**—the frequency of collisions containing sufficient energy to bring about a reaction—depends on the number of reactant molecules at or above the activation energy level. One way to increase the reaction rate of a substance is to raise its temperature. By causing the molecules to move faster, heat increases both the frequency of collisions and the number of molecules that attain activation energy. The number of collisions also increases when pressure is increased or when the reactants are more concentrated (because the distance between molecules is thereby decreased). In living systems, enzymes increase the reaction rate without raising the temperature.

Enzymes and Chemical Reactions

Substances that can speed up a chemical reaction without being permanently altered themselves are called **catalysts.** In living cells, **enzymes** serve as biological catalysts. As catalysts, each enzyme acts on a specific substance, called the enzyme's **substrate** (or substrates, when there are two or more reactants), and each catalyzes only one reaction. For example, sucrose (table sugar) is the substrate of the enzyme sucrase, which catalyzes the hydrolysis of sucrose to glucose and fructose.

As catalysts, enzymes typically accelerate chemical reactions by lowering their activation energy (Figure 5.2). The enzyme

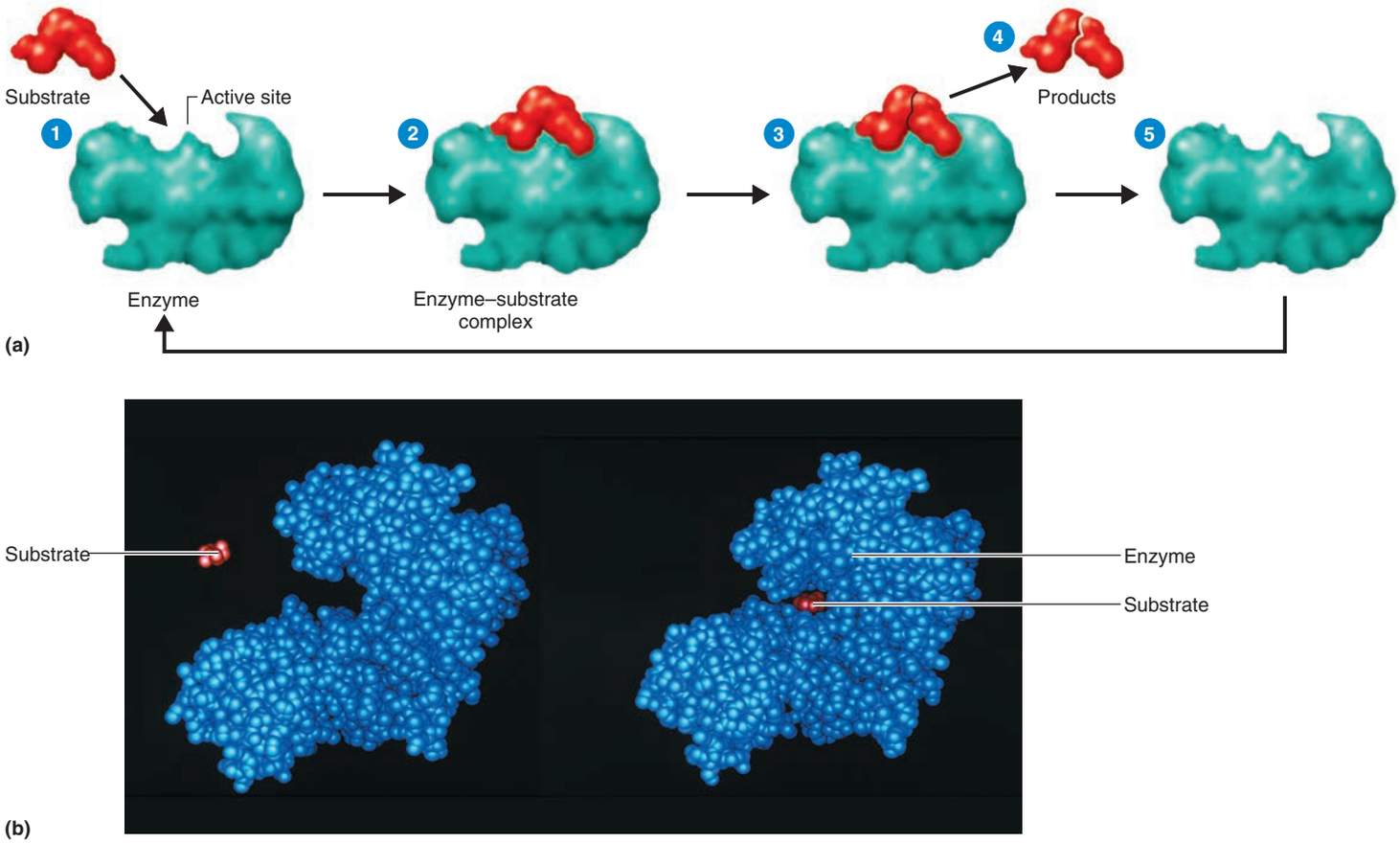


Figure 5.3 The mechanism of enzymatic action. **(a)** 1 The substrate contacts the active site on the enzyme to form 2 an enzyme–substrate complex. 3 The substrate is then transformed into products, 4 the products are released, and 5 the enzyme is recovered unchanged. In the example shown, the transformation into products involves a breakdown of the substrate into two products. Other transformations may occur, however. **(b)** Left: A molecular model of the enzyme in step 1 of part (a). The active site of the enzyme can be seen here as a groove on the surface of the protein. Right: As the enzyme and substrate meet in step 2 of part (a), the enzyme changes shape slightly to fit together more tightly with the substrate.

Q Give an example of enzymatic specificity.

therefore speeds up the reaction by increasing the number of AB molecules that attain sufficient activation energy to react. The general sequence of events in enzyme action is as follows (Figure 5.3a).

1. The surface of the substrate contacts a specific region of the surface of the enzyme molecule, called the *active site*.
2. A temporary intermediate compound forms, called an **enzyme–substrate complex.** The enzyme orients the substrate into a position that increases the probability of reaction, which enables the collisions to be more effective.
3. The substrate molecule is transformed by the rearrangement of existing atoms, the breakdown of the substrate molecule, or in combination with another substrate molecule.
4. The transformed substrate molecules—the products of the reaction—are released from the enzyme molecule because they no longer fit in the active site of the enzyme.
5. The unchanged enzyme is now free to react with other substrate molecules.

An enzyme's ability to accelerate a reaction without the need for an increase in temperature is crucial to living systems because a significant temperature increase would destroy cellular proteins. The crucial function of enzymes, therefore, is to speed up biochemical reactions at a temperature that is compatible with the normal functioning of the cell.

Enzyme Specificity and Efficiency

Enzymes have specificity for particular substrates. For example, a specific enzyme may be able to hydrolyze a peptide bond only between two specific amino acids. Other enzymes can hydrolyze starch but not cellulose; even though both starch and cellulose are polysaccharides composed of glucose subunits, the orientations of the subunits in the two polysaccharides differ. Each of the thousands of known enzymes have this specificity because the three-dimensional shape of the specific amino acids of the active site fits the substrate somewhat as a lock fits with its key (Figure 5.3b). The unique configuration of each enzyme enables it to "find" the

correct substrate from among the diverse molecules in a cell. However, the active site and substrate are flexible, and they change shape somewhat as they meet to fit together more tightly. The substrate is usually much smaller than the enzyme, and relatively few of the enzyme's amino acids make up the active site. A certain compound can be a substrate for several different enzymes that catalyze different reactions, so the fate of a compound depends on the enzyme that acts on it. At least four different enzymes can act on glucose 6-phosphate, a molecule important in cell metabolism, and each reaction will yield a different product.

Animations Enzymes: Overview, Steps in a Reaction

Enzymes are extremely efficient. Under optimum conditions, they can catalyze reactions at rates 10^8 to 10^{10} times (up to 10 billion times) higher than those of comparable reactions without enzymes. The **turnover number** (the maximum number of substrate molecules an enzyme molecule converts to product each second) is generally between 1 and 10,000 and can be as high as 500,000. For example, the enzyme DNA polymerase I, which participates in the synthesis of DNA, has a turnover number of 15, whereas the enzyme lactate dehydrogenase, which removes hydrogen atoms from lactic acid, has a turnover number of 1000.

Many enzymes exist in the cell in both active and inactive forms. The rate at which enzymes switch between these two forms is determined by the cellular environment.

Naming Enzymes

The names of enzymes usually end in *ase*. All enzymes can be grouped into six classes, according to the type of chemical reaction they catalyze (Table 5.1). Enzymes within each of the major classes are named according to the more specific types of reactions they assist. For example, the class called *oxidoreductases* is involved with oxidation-reduction reactions (described shortly). Enzymes in the oxidoreductase class that remove hydrogen (H) from a substrate are called *dehydrogenases;* those that add electrons to molecular oxygen (O_2) are called *oxidases*. As you will see later, dehydrogenase and oxidase enzymes have even more specific names, such as lactate dehydrogenase and cytochrome oxidase, depending on the specific substrates on which they act.

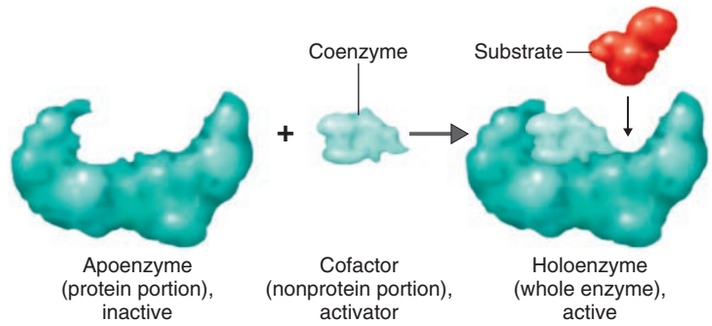


Figure 5.4 Components of a holoenzyme. Many enzymes require both an apoenzyme (protein portion) and a cofactor (nonprotein portion) to become active. The cofactor can be a metal ion, or if it is an organic molecule, it is called a coenzyme (as shown here). The apoenzyme and cofactor together make up the holoenzyme, or whole enzyme. The substrate is the reactant acted upon by the enzyme.

Q How does the enzyme–substrate complex lower the activation energy of the reaction?

Enzyme Components

Although some enzymes consist entirely of proteins, most consist of both a protein portion, called an **apoenzyme,** and a nonprotein component, called a **cofactor.** Ions of iron, zinc, magnesium, or calcium are examples of cofactors. If the cofactor is an organic molecule, it is called a **coenzyme.** Apoenzymes are inactive by themselves; they must be activated by cofactors. Together, the apoenzyme and cofactor form a **holoenzyme,** or whole, active enzyme (Figure 5.4). If the cofactor is removed, the apoenzyme will not function.

Cofactors may help catalyze a reaction by forming a bridge between an enzyme and its substrate. For example, magnesium (Mg^{2+}) is required by many phosphorylating enzymes (enzymes that transfer a phosphate group from ATP to another substrate). The Mg^{2+} can form a link between the enzyme and the ATP molecule. Most trace elements required by living cells are probably used in some such way to activate cellular enzymes.

TABLE 5.1 Enzyme Classification Based on Type of Chemical Reaction Catalyzed

Class	Type of Chemical Reaction Catalyzed	Examples
Oxidoreductase	Oxidation-reduction, in which oxygen and hydrogen are gained or lost	Cytochrome oxidase, lactate dehydrogenase
Transferase	Transfer of functional groups, such as an amino group, acetyl group, or phosphate group	Acetate kinase, alanine deaminase
Hydrolase	Hydrolysis (addition of water)	Lipase, sucrase
Lyase	Removal of groups of atoms without hydrolysis	Oxalate decarboxylase, isocitrate lyase
Isomerase	Rearrangement of atoms within a molecule	Glucose-phosphate isomerase, alanine racemase
Ligase	Joining of two molecules (using energy usually derived from the breakdown of ATP)	Acetyl-CoA synthetase, DNA ligase

TABLE 5.2 Selected Vitamins and Their Coenzymatic Functions

Vitamin	Function
Vitamin B_1 (Thiamine)	Part of coenzyme cocarboxylase; has many functions, including the metabolism of pyruvic acid
Vitamin B_2 (Riboflavin)	Coenzyme in flavoproteins; active in electron transfers
Niacin (Nicotinic Acid)	Part of NAD molecule*; active in electron transfers
Vitamin B_6 (Pyridoxine)	Coenzyme in amino acid metabolism
Vitamin B_{12} (Cyanocobalamin)	Coenzyme (methyl cyanocobalamide) involved in the transfer of methyl groups; active in amino acid metabolism
Pantothenic Acid	Part of coenzyme A molecule; involved in the metabolism of pyruvic acid and lipids
Biotin	Involved in carbon dioxide fixation reactions and fatty acid synthesis
Folic Acid	Coenzyme used in the synthesis of purines and pyrimidines
Vitamin E	Needed for cellular and macromolecular syntheses
Vitamin K	Coenzyme used in electron transport

*NAD = nicotinamide adenine dinucleotide

Coenzymes may assist the enzyme by accepting atoms removed from the substrate or by donating atoms required by the substrate. Some coenzymes act as electron carriers, removing electrons from the substrate and donating them to other molecules in subsequent reactions. Many coenzymes are derived from vitamins (Table 5.2). Two of the most important coenzymes in cellular metabolism are **nicotinamide adenine dinucleotide (NAD^+)** and **nicotinamide adenine dinucleotide phosphate ($NADP^+$).** Both compounds contain derivatives of the B vitamin niacin (nicotinic acid), and both function as electron carriers. Whereas NAD^+ is primarily involved in catabolic (energy-yielding) reactions, $NADP^+$ is primarily involved in anabolic (energy-requiring) reactions. The flavin coenzymes, such as **flavin mononucleotide (FMN)** and **flavin adenine dinucleotide (FAD),** contain derivatives of the B vitamin riboflavin and are also electron carriers. Another important coenzyme, **coenzyme A (CoA,)** contains a derivative of pantothenic acid, another B vitamin. This coenzyme plays an important role in the synthesis and breakdown of fats and in a series of oxidizing reactions called the Krebs cycle. We will come across all of these coenzymes in our discussion of metabolism later in the chapter.

Factors Influencing Enzymatic Activity

Enzymes are subject to various cellular controls. Two primary types are the control of enzyme *synthesis* (see Chapter 8) and the control of enzyme *activity* (how much enzyme is present versus how active it is).

Several factors influence the activity of an enzyme. Among the more important are temperature, pH, substrate concentration, and the presence or absence of inhibitors.

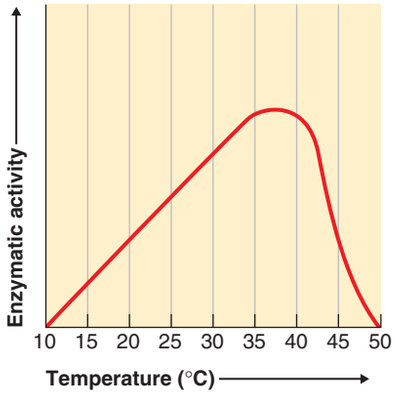
Temperature

The rate of most chemical reactions increases as the temperature increases. Molecules move more slowly at lower temperatures than at higher temperatures and so may not have enough energy to cause a chemical reaction. For enzymatic reactions, however, elevation beyond a certain temperature (the optimal temperature) drastically reduces the rate of reaction (Figure 5.5a). The optimal temperature for most disease-producing bacteria in the human body is between 35°C and 40°C. The rate of reaction declines beyond the optimal temperature because of the enzyme's **denaturation,** the loss of its characteristic three-dimensional structure (tertiary configuration) (Figure 5.6). Denaturation of a protein involves the breakage of hydrogen bonds and other noncovalent bonds; a common example is the transformation of uncooked egg white (a protein called albumin) to a hardened state by heat.

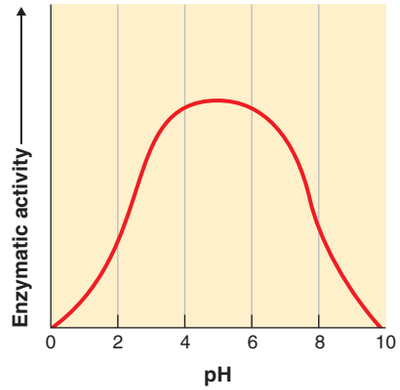
Denaturation of an enzyme changes the arrangement of the amino acids in the active site, altering its shape and causing the enzyme to lose its catalytic ability. In some cases, denaturation is partially or fully reversible. However, if denaturation continues until the enzyme has lost its solubility and coagulates, the enzyme cannot regain its original properties. Enzymes can also be denatured by concentrated acids, bases, heavy-metal ions (such as lead, arsenic, or mercury), alcohol, and ultraviolet radiation.

pH

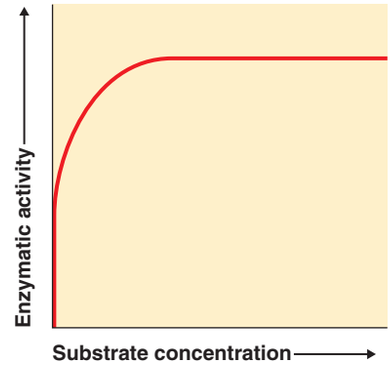
Typically, enzymes have an optimum pH at which they are most active. Above or below this pH value, enzyme activity, and therefore the reaction rate, decline (Figure 5.5b). When the H^+ concentration (pH) in the medium is changed drastically, the protein's three-dimensional structure is altered. Extreme changes in pH can cause denaturation. Acids (and bases) alter a protein's



(a) Temperature. The enzymatic activity (rate of reaction catalyzed by the enzyme) increases with increasing temperature until the enzyme, a protein, is denatured by heat and inactivated. At this point, the reaction rate falls steeply.



(b) pH. The enzyme illustrated is most active at about pH 5.0.



(c) Substrate concentration. With increasing concentration of substrate molecules, the rate of reaction increases until the active sites on all the enzyme molecules are filled, at which point the maximum rate of reaction is reached.

Figure 5.5 Factors that influence enzymatic activity, plotted for a hypothetical enzyme.

Q How will this enzyme act at 25°C? At 45°C? At pH 7?

three-dimensional structure because the H^+ (and OH^-) compete with hydrogen and ionic bonds in an enzyme, resulting in the enzyme's denaturation.

Substrate Concentration

Under conditions of high substrate concentration, an enzyme is said to be in **saturation;** that is, its active site is always occupied by substrate or product molecules, and it's catalyzing a specific reaction at its maximum rate. This maximum rate can be attained only when the concentration of substrate(s) is extremely high. In this condition, a further increase in substrate concentration will not affect the reaction rate because all active sites are already in use (Figure 5.5c). Under normal cellular conditions, enzymes are not saturated with substrate(s). At any given time, many of the enzyme molecules are inactive for lack of substrate; thus, the substrate concentration is likely to influence the rate of reaction.

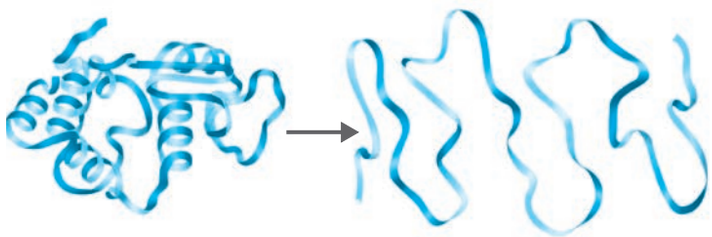


Figure 5.6 Denaturation of a protein. Breakage of the noncovalent bonds (such as hydrogen bonds) that hold the active protein in its three-dimensional shape renders the denatured protein nonfunctional.

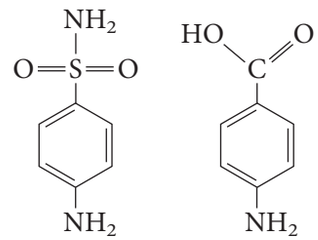
Q When is denaturation irreversible?

Inhibitors

An effective way to control the growth of bacteria is to control, or inhibit their enzymes. Certain poisons, such as cyanide, arsenic, and mercury, combine with enzymes and prevent the bacteria from functioning. As a result, the cells stop functioning and die.

Enzyme inhibitors are classified as either competitive or noncompetitive inhibitors (Figure 5.7). **Competitive inhibitors** fill the active site of an enzyme and compete with the normal substrate for the active site. A competitive inhibitor can do this because its shape and chemical structure are similar to those of the normal substrate (Figure 5.7b). However, unlike the substrate, it does not undergo any reaction to form products. Some competitive inhibitors bind irreversibly to amino acids in the active site, preventing any further interactions with the substrate. Others bind reversibly, alternately occupying and leaving the active site; these slow the enzyme's interaction with the substrate. Increasing the substrate concentration can overcome reversible competitive inhibition. As active sites become available, more substrate molecules than competitive inhibitor molecules are available to attach to the active sites of enzymes.

One good example of a competitive inhibitor is sulfanilamide (a potent antibacterial drug), which inhibits the enzyme whose normal substrate is *para*-aminobenzoic acid (PABA):



Sulfanilamide PABA

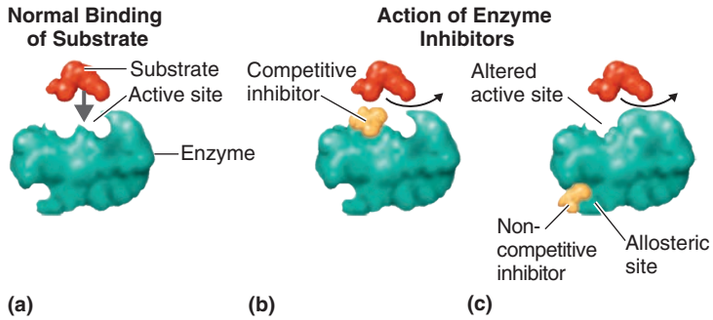


Figure 5.7 Enzyme inhibitors. **(a)** An uninhibited enzyme and its normal substrate. **(b)** A competitive inhibitor. **(c)** One type of noncompetitive inhibitor, causing allosteric inhibition.

Q How do competitive inhibitors operate in comparison to noncompetitive inhibitors?

PABA is an essential nutrient used by many bacteria in the synthesis of folic acid, a vitamin that functions as a coenzyme. When sulfanilamide is administered to bacteria, the enzyme that normally converts PABA to folic acid combines instead with the sulfanilamide. Folic acid is not synthesized, and the bacteria cannot grow. Because human cells do not use PABA to make their folic acid, sulfanilamide can kill bacteria but does not harm human cells.

Noncompetitive inhibitors do not compete with the substrate for the enzyme's active site; instead, they interact with another part of the enzyme (Figure 5.7c). In this process, called **allosteric** ("other space") **inhibition,** the inhibitor binds to a site on the enzyme other than the substrate's binding site, called the **allosteric site.** This binding causes the active site to change its shape, making it nonfunctional. As a result, the enzyme's activity is reduced. This effect can be either reversible or irreversible, depending on whether the active site can return to its original shape. In some cases, allosteric interactions can activate an enzyme rather than inhibit it. Another type of noncompetitive inhibition can operate on enzymes that require metal ions for their activity. Certain chemicals can bind or tie up the metal ion activators and thus prevent an enzymatic reaction. Cyanide can bind the iron in iron-containing enzymes, and fluoride can bind calcium or magnesium. Substances such as cyanide and fluoride are sometimes called *enzyme poisons* because they permanently inactivate enzymes. MM **Animations** Enzymes: Competitive Inhibition, Noncompetitive Inhibition

Feedback Inhibition

Noncompetitive, or allosteric, inhibitors play a role in a type of biochemical control called **feedback inhibition,** or **end-product inhibition.** This control mechanism stops the cell from making more of a substance than it needs and thereby wasting chemical resources. In some metabolic reactions, several steps are required for the synthesis of a particular chemical compound, called the **end-product.** The process is similar to an assembly line, with each step catalyzed by a separate enzyme (Figure 5.8). In many metabolic pathways, the end-product can allosterically inhibit the activity of one of the enzymes earlier in the pathway.

Feedback inhibition generally acts on the first enzyme in a metabolic pathway (similar to shutting down an assembly line by stopping the first worker). Because the enzyme is inhibited, the product of the first enzymatic reaction in the pathway is

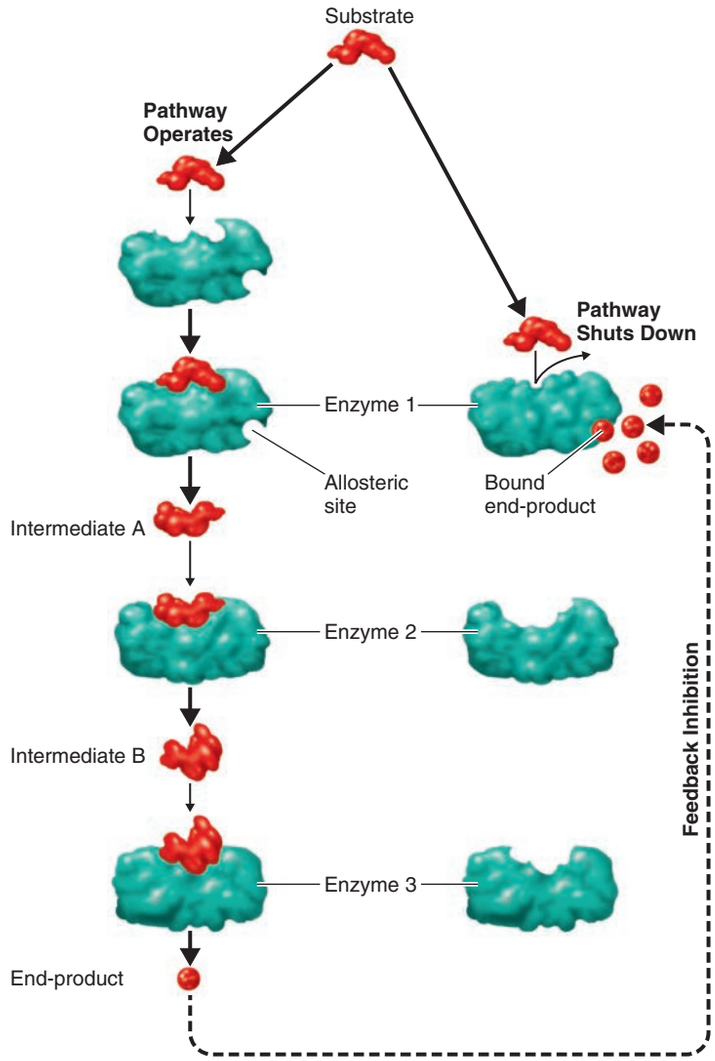


Figure 5.8 Feedback inhibition.

Q Explain the differences between competitive inhibition and feedback inhibition.

not synthesized. Because that unsynthesized product would normally be the substrate for the second enzyme in the pathway, the second reaction stops immediately as well. Thus, even though only the first enzyme in the pathway is inhibited, the entire pathway shuts down, and no new end-product is formed. By inhibiting the first enzyme in the pathway, the cell also keeps metabolic intermediates from accumulating. As the cell uses up the existing end-product, the first enzyme's allosteric site remains unbound more frequently, and the pathway resumes activity.

The bacterium *E. coli* can be used to demonstrate feedback inhibition in the synthesis of the amino acid isoleucine, which is required for the cell growth. In this metabolic pathway, the amino acid threonine is enzymatically converted to isoleucine in five steps. If isoleucine is added to the growth medium for *E. coli*, it inhibits the first enzyme in the pathway, and the bacteria stop synthesizing isoleucine. This condition is maintained until the supply of isoleucine is depleted. This type of feedback inhibition is also involved in regulating the cells' production of other amino acids, as well as vitamins, purines, and pyrimidines.

Ribozymes

Prior to 1982, it was believed that only protein molecules had enzymatic activity. Then researchers working on microbes discovered a unique type of RNA called a **ribozyme.** Like protein enzymes, ribozymes function as catalysts, have active sites that bind to substrates, and are not used up in a chemical reaction. Ribozymes cut and splice RNA and are involved in protein synthesis at ribosomes.

CHECK YOUR UNDERSTANDING

- What is a coenzyme? **5-3**
- Why is enzyme specificity important? **5-4**
- What happens to an enzyme below its optimal temperature? Above its optimal temperature? **5-5**
- Why is feedback inhibition noncompetitive inhibition? **5-6**
- What is a ribozyme? **5-7**

Energy Production

LEARNING OBJECTIVES

5-8 Explain the term *oxidation-reduction*.

5-9 List and provide examples of three types of phosphorylation reactions that generate ATP.

5-10 Explain the overall function of metabolic pathways.

Nutrient molecules, like all molecules, have energy associated with the electrons that form bonds between their atoms. When it's spread throughout the molecule, this energy is difficult for the cell to use. Various reactions in catabolic pathways, however, concentrate the energy into the bonds of ATP, which serves as a convenient energy carrier. ATP is generally referred to as having "high-energy" bonds. Actually, a better term is probably *unstable bonds*. Although the amount of energy in these bonds is not exceptionally large, it can be released quickly and easily. In a sense, ATP is similar to a highly flammable liquid such as kerosene. Although a large log might eventually burn to produce more heat than a cup of kerosene, the kerosene is easier to ignite and provides heat more quickly and conveniently. In a similar way, the "high-energy" unstable bonds of ATP provide the cell with readily available energy for anabolic reactions.

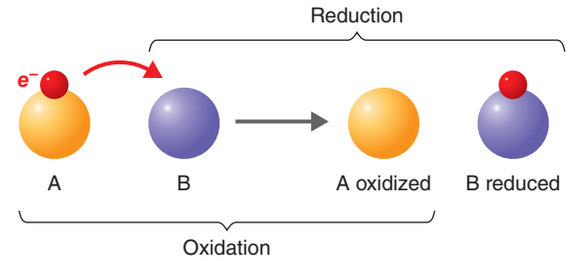


Figure 5.9 Oxidation-reduction. An electron is transferred from molecule A to molecule B. In the process, molecule A is oxidized, and molecule B is reduced.

Q How do oxidation and reduction differ?

Before discussing the catabolic pathways, we will consider two general aspects of energy production: the concept of oxidation-reduction and the mechanisms of ATP generation.

Oxidation-Reduction Reactions

Oxidation is the removal of electrons (*e*–) from an atom or molecule, a reaction that often produces energy. **Figure 5.9** shows an example of an oxidation in which molecule A loses an electron to molecule B. Molecule A has undergone oxidation (meaning that it has lost one or more electrons), whereas molecule B has undergone **reduction** (meaning that it has gained one or more electrons).* Oxidation and reduction reactions are always coupled: each time one substance is oxidized, another is simultaneously reduced. The pairing of these reactions is called **oxidation-reduction** or a **redox reaction.**

In many cellular oxidations, electrons and protons (hydrogen ions, H^+) are removed at the same time; this is equivalent to the removal of hydrogen atoms, because a hydrogen atom is made up of one proton and one electron (see Table 2.2, page 26). Because most biological oxidations involve the loss of hydrogen atoms,

*The terms do not seem logical until one considers the history of the discovery of these reactions. When mercury is heated, it gains weight as mercuric oxide is formed; this was called *oxidation*. Later it was determined that the mercury actually *lost* electrons, and the observed *gain* in oxygen was a direct result of this. Oxidation, therefore, is a *loss* of electrons, and reduction is a *gain* of electrons, but the gain and loss of electrons is not usually apparent as chemical-reaction equations are usually written. For example, in this chapter's equations for aerobic respiration on page 126, notice that each carbon in glucose had only one oxygen originally, and later, as carbon dioxide, each carbon now has two oxygens. However, the gain or loss of electrons in the equations is not apparent.

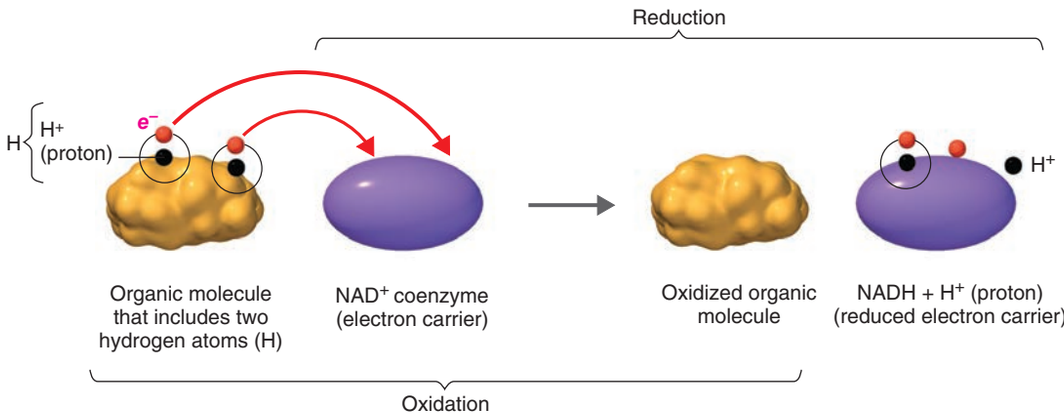


Figure 5.10 Representative biological oxidation. Two electrons and two protons (altogether equivalent to two hydrogen atoms) are transferred from an organic substrate molecule to a coenzyme, NAD^+. NAD^+ actually receives one hydrogen atom and two electrons, and one proton is released into the medium. NAD^+ is reduced to NADH, which is a more energy-rich molecule.

Q How do organisms use oxidation-reduction reactions?

they are also called **dehydrogenation** reactions. Figure 5.10 shows an example of a biological oxidation. An organic molecule is oxidized by the loss of two hydrogen atoms, and a molecule of NAD^+ is reduced. Remember that NAD^+ assists enzymes by accepting hydrogen atoms that have been removed from the substrate, in this case the organic molecule. As shown in Figure 5.10, NAD^+ accepts two electrons and one proton. One proton (H^+) is left over and is released into the surrounding medium. The reduced coenzyme, NADH, contains more energy than NAD^+. This energy can be used to generate ATP in later reactions.

It's important to remember that cells use biological oxidation-reduction reactions in catabolism to extract energy from nutrient molecules. Cells take nutrients, some of which serve as energy sources, and degrade them from highly reduced compounds (with many hydrogen atoms) to highly oxidized compounds. For example, when a cell oxidizes a molecule of glucose ($C_6H_{12}O_6$) to CO_2 and H_2O, the energy in the glucose molecule is removed in a stepwise manner and ultimately is trapped by ATP, which can then serve as an energy source for energy-requiring reactions. Compounds such as glucose that have many hydrogen atoms are highly reduced compounds, containing a large amount of potential energy. Thus, glucose is a valuable nutrient for organisms.

MM **Animation** Oxidation-Reduction Reactions

CHECK YOUR UNDERSTANDING

Why is glucose such an important molecule for organisms? **5-8**

The Generation of ATP

Much of the energy released during oxidation-reduction reactions is trapped within the cell by the formation of ATP. Specifically, an inorganic phosphate group, P_i, is added to ADP with the input of energy to form ATP:

$$\underbrace{\text{Adenosine}—P\sim P}_{\text{ADP}} + \text{Energy} + P_i \longrightarrow \underbrace{\text{Adenosine}—P\sim P\sim P}_{\text{ATP}}$$

The symbol ~ designates a "high-energy" bond that can readily be broken to release usable energy. The high-energy bond that attaches the third P contains the energy stored in this reaction, in a sense. When this P is removed, usable energy is released. The addition of P to a chemical compound is called **phosphorylation.** Organisms use three mechanisms of phosphorylation to generate ATP from ADP.

Substrate-Level Phosphorylation

In **substrate-level phosphorylation,** ATP is usually generated when a high-energy P is directly transferred from a phosphorylated compound (a substrate) to ADP. Generally, the P has acquired its energy during an earlier reaction in which the substrate itself was oxidized. The following example shows only the carbon skeleton and the P of a typical substrate:

$$C—C—C\sim P + ADP \rightarrow C—C—C + ATP$$

Oxidative Phosphorylation

In **oxidative phosphorylation,** electrons are transferred from organic compounds to one group of electron carriers (usually to NAD^+ and FAD). Then the electrons are passed through a series of different electron carriers to molecules of oxygen (O_2) or other oxidized inorganic and organic molecules. This process occurs in the plasma membrane of prokaryotes and in the inner mitochondrial membrane of eukaryotes. The sequence of electron carriers used in oxidative phosphorylation is called an **electron transport chain (system)** (see Figure 5.14). The transfer of electrons from one electron carrier to the next releases energy, some of which is used to generate ATP from ADP through a process called **chemiosmosis,** to be described on page 125.

Photophosphorylation

The third mechanism of phosphorylation, **photophosphorylation,** occurs only in photosynthetic cells, which contain light-trapping pigments such as chlorophylls. In photosynthesis, organic molecules, especially sugars, are synthesized with the energy of light from the energy-poor building blocks, carbon dioxide and water. Photophosphorylation starts this process by converting light

energy to the chemical energy of ATP and NADPH, which, in turn, are used to synthesize organic molecules. As in oxidative phosphorylation, an electron transport chain is involved.

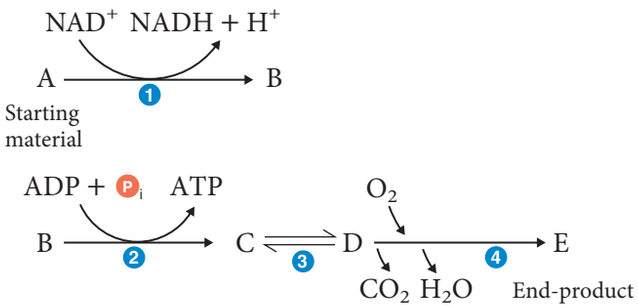
CHECK YOUR UNDERSTANDING

✓ Outline the three ways that ATP is generated. **5-9**

Metabolic Pathways of Energy Production

Organisms release and store energy from organic molecules by a series of controlled reactions rather than in a single burst. If the energy were released all at once as a large amount of heat, it could not be readily used to drive chemical reactions and would, in fact, damage the cell. To extract energy from organic compounds and store it in chemical form, organisms pass electrons from one compound to another through a series of oxidation-reduction reactions.

As noted earlier, a sequence of enzymatically catalyzed chemical reactions occurring in a cell is called a metabolic pathway. Below is a hypothetical metabolic pathway that converts starting material A to end-product E in a series of five steps.



1. Molecule A converts to molecule B. The curved arrow indicates that the reduction of coenzyme NAD^+ to NADH is coupled to that reaction; the electrons and protons come from molecule A.
2. Similarly, the arrow shows a coupling of two reactions. As B is converted to C, ADP is converted to ATP; the energy needed comes from B as it transforms into C.
3. The reaction converting C to D is readily reversible, as indicated by the double arrow.
4. The arrow leading from O_2 indicates that O_2 is a reactant. The arrows leading to CO_2 and H_2O indicate that these substances are secondary products produced in the reaction, in addition to E, the end-product that (presumably) interests us the most.

Secondary products, such as CO_2 and H_2O, are sometimes called *by-products* or *waste products*.

Keep in mind that almost every reaction in a metabolic pathway is catalyzed by a specific enzyme; sometimes the name of the enzyme is printed near the arrow.

CHECK YOUR UNDERSTANDING

✓ What is the purpose of metabolic pathways? **5-10**

Carbohydrate Catabolism

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LEARNING OBJECTIVES

- **5-11** Describe the chemical reactions of glycolysis.
- **5-12** Identify the functions of the pentose phosphate and Entner-Doudoroff pathways.
- **5-13** Explain the products of the Krebs cycle.
- **5-14** Describe the chemiosmotic model for ATP generation.
- **5-15** Compare and contrast aerobic and anaerobic respiration.
- **5-16** Describe the chemical reactions of, and list some products of, fermentation.

Most microorganisms oxidize carbohydrates as their primary source of cellular energy. **Carbohydrate catabolism,** the breakdown of carbohydrate molecules to produce energy, is therefore of great importance in cell metabolism. Glucose is the most common carbohydrate energy source used by cells. Microorganisms can also catabolize various lipids and proteins for energy production (page 131).

To produce energy from glucose, microorganisms use two general processes: *cellular respiration* and *fermentation*. (In discussing cellular respiration, we frequently refer to the process simply as respiration, but it should not be confused with breathing.) Both cellular respiration and fermentation usually start with the same first step, glycolysis, but follow different subsequent pathways (**Figure 5.11**). Before examining the details of glycolysis, respiration, and fermentation, we will first look at a general overview of the processes.

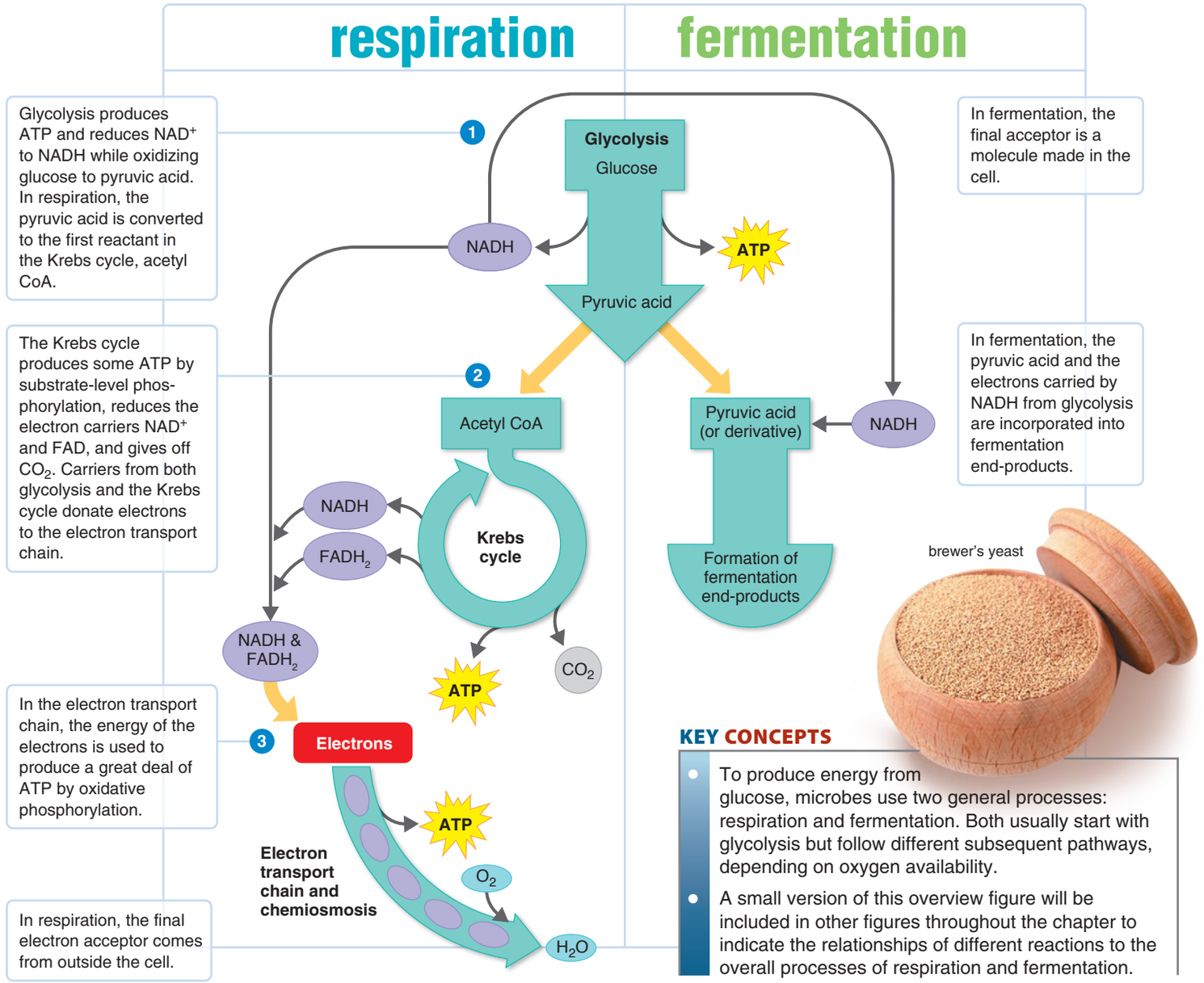
As shown in Figure 5.11, the respiration of glucose typically occurs in three principal stages: glycolysis, the Krebs cycle, and the electron transport chain (system).

1. Glycolysis is the oxidation of glucose to pyruvic acid with the production of some ATP and energy-containing NADH.
2. The Krebs cycle is the oxidation of acetyl CoA (a derivative of pyruvic acid) to carbon dioxide, with the production of some ATP, energy-containing NADH, and another reduced electron carrier, $FADH_2$ (the reduced form of flavin adenine dinucleotide).
3. In the electron transport chain (system), NADH and $FADH_2$ are oxidized, contributing the electrons they have carried from the substrates to a "cascade" of oxidation-reduction reactions involving a series of additional electron carriers. Energy from these reactions is used to generate a considerable amount of ATP. In respiration, most of the ATP is generated in the third step.

Because respiration involves a long series of oxidation-reduction reactions, the entire process can be thought of as involving a

FOUNDATION FIGURE 5.11

An Overview of Respiration and Fermentation



flow of electrons from the energy-rich glucose molecule to the relatively energy-poor CO_2 and H_2O molecules. The coupling of ATP production to this flow is somewhat analogous to producing electrical power by using energy from a flowing stream. Carrying the analogy further, you could imagine a stream flowing down a gentle slope during glycolysis and the Krebs cycle, supplying energy to turn two old-fashioned waterwheels. Then the stream rushes down a steep slope in the electron transport chain, supplying energy for a large modern power plant. In a similar way, glycolysis and the Krebs cycle generate a small amount of ATP and also supply the electrons that generate a great deal of ATP at the electron transport chain stage.

Typically, the initial stage of fermentation is also glycolysis (Figure 5.11). However, once glycolysis has taken place, the pyruvic acid is converted into one or more different products, depending on the type of cell. These products might include alcohol (ethanol) and lactic acid. Unlike respiration, there is no Krebs cycle or electron transport chain in fermentation. Accordingly, the ATP yield, which comes only from glycolysis, is much lower.

Glycolysis

Glycolysis, the oxidation of glucose to pyruvic acid, is usually the first stage in carbohydrate catabolism. Most microorganisms use this pathway; in fact, it occurs in most living cells.

Glycolysis is also called the *Embden-Meyerhof pathway*. The word *glycolysis* means splitting of sugar, and this is exactly what happens. The enzymes of glycolysis catalyze the splitting of glucose, a six-carbon sugar, into two three-carbon sugars. These sugars are then oxidized, releasing energy, and their atoms are rearranged to form two molecules of pyruvic acid. During glycolysis NAD^+ is reduced to NADH, and there is a net production of two ATP molecules by substrate-level phosphorylation. Glycolysis does not require oxygen; it can occur whether oxygen is present or not. This pathway is a series of ten chemical reactions, each catalyzed by a different enzyme. The steps are outlined in **Figure 5.12**; see also Figure A.2 in Appendix A for a more detailed representation of glycolysis.

To summarize the process, glycolysis consists of two basic stages, a preparatory stage and an energy-conserving stage:

1. First, in the preparatory stage (steps 1–4 in Figure 5.12), two molecules of ATP are used as a six-carbon glucose molecule is phosphorylated, restructured, and split into two three-carbon compounds: glyceraldehyde 3-phosphate (GP) and dihydroxyacetone phosphate (DHAP). 5 DHAP is readily converted to GP. (The reverse reaction may also occur.) The conversion of DHAP into GP means that from this point on in glycolysis, two molecules of GP are fed into the remaining chemical reactions.
2. In the energy-conserving stage (steps 6–10), the two three-carbon molecules are oxidized in several steps to two molecules of pyruvic acid. In these reactions, two molecules of NAD^+ are reduced to NADH, and four molecules of ATP are formed by substrate-level phosphorylation.

Because two molecules of ATP were needed to get glycolysis started and four molecules of ATP are generated by the process, *there is a net gain of two molecules of ATP for each molecule of glucose that is oxidized.* MM **Animations** Glycolysis: Overview, Steps

Additional Pathways to Glycolysis

Many bacteria have another pathway in addition to glycolysis for the oxidation of glucose. The most common alternative is the *pentose phosphate pathway;* another alternative is the *Entner-Doudoroff pathway*.

The Pentose Phosphate Pathway

The **pentose phosphate pathway** (or *hexose monophosphate shunt*) operates simultaneously with glycolysis and provides a means for the breakdown of five-carbon sugars (pentoses) as well as glucose (see Figure A.3 in Appendix A for a more detailed representation of the pentose phosphate pathway). A key feature of this pathway is that it produces important intermediate pentoses used in the synthesis of (1) nucleic acids, (2) glucose from carbon dioxide in photosynthesis, and (3) certain amino acids. The pathway is an important producer of the reduced coenzyme NADPH from $NADP^+$. The pentose phosphate pathway yields a net gain of only one molecule of ATP for each molecule of glucose oxidized. Bacteria that use the pentose phosphate pathway include *Bacillus subtilis* (SU-til-us), *E. coli, Leuconostoc mesenteroides* (loo-kō-NOS-tok mes′en-TER-oi-dēz), and *Enterococcus faecalis* (fē-KĀ-lis).

The Entner-Doudoroff Pathway

From each molecule of glucose, the **Entner-Doudoroff pathway** produces two molecules of NADPH and one molecule of ATP for use in cellular biosynthetic reactions (see Figure A.4 in Appendix A for a more detailed representation). Bacteria that have the enzymes for the Entner-Doudoroff pathway can metabolize glucose without either glycolysis or the pentose phosphate pathway. The Entner-Doudoroff pathway is found in some gram-negative bacteria, including *Rhizobium,* (rī-ZŌ-bē-um) *Pseudomonas* (soo′dō-MŌ-nas), and *Agrobacterium* (A-grō-bak-ti′rē-um); it is generally not found among gram-positive bacteria. Tests for the ability to oxidize glucose by this pathway are sometimes used to identify *Pseudomonas* in the clinical laboratory.

CHECK YOUR UNDERSTANDING

- What happens during the preparatory and energy-conserving stages of glycolysis? **5-11**
- What is the value of the pentose phosphate and Entner-Doudoroff pathways if they produce only one ATP molecule? **5-12**

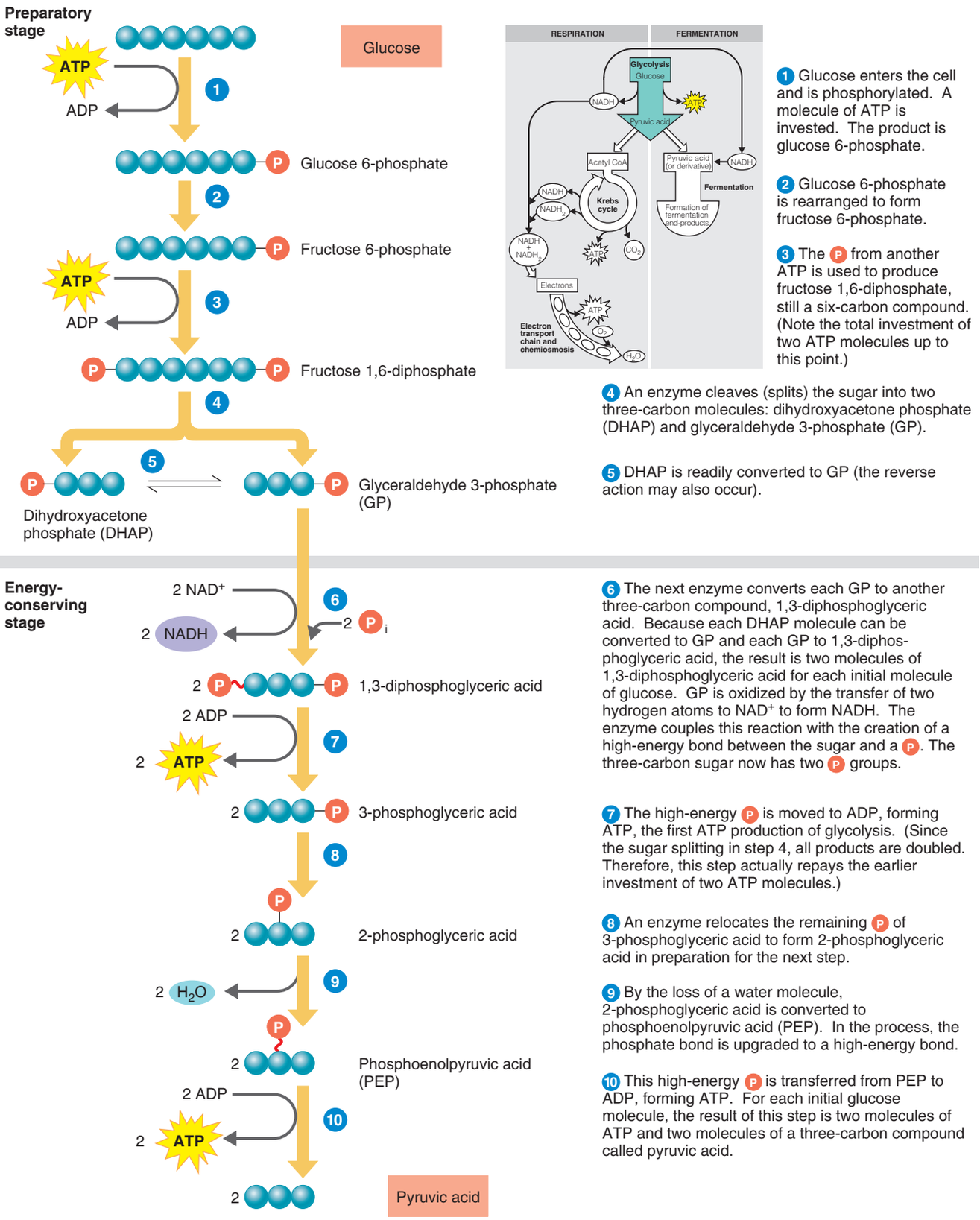
Cellular Respiration

After glucose has been broken down to pyruvic acid, the pyruvic acid can be channeled into the next step of either fermentation (page 127) or cellular respiration (see Figure 5.11). **Cellular respiration,** or simply **respiration,** is defined as an ATP-generating process in which molecules are oxidized and the final electron acceptor comes from outside the cell and is (almost always) an inorganic molecule. An essential feature of respiration is the operation of an electron transport chain.

There are two types of respiration, depending on whether an organism is an **aerobe**, which uses oxygen, or an **anaerobe,** which does not use oxygen and may even be killed by it. In **aerobic respiration,** the final electron acceptor is O_2; in **anaerobic respiration,** the final electron acceptor is an inorganic molecule other than O_2 or, rarely, an organic molecule. First we will describe respiration as it typically occurs in an aerobic cell.

Aerobic Respiration

The Krebs Cycle The **Krebs cycle,** also called the *tricarboxylic acid (TCA) cycle* or *citric acid cycle,* is a series of biochemical reactions in which the large amount of potential chemical energy stored in acetyl CoA is released step by step (see Figure 5.11).



1 Glucose enters the cell and is phosphorylated. A molecule of ATP is invested. The product is glucose 6-phosphate.

2 Glucose 6-phosphate is rearranged to form fructose 6-phosphate.

3 The P from another ATP is used to produce fructose 1,6-diphosphate, still a six-carbon compound. (Note the total investment of two ATP molecules up to this point.)

4 An enzyme cleaves (splits) the sugar into two three-carbon molecules: dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GP).

5 DHAP is readily converted to GP (the reverse action may also occur).

6 The next enzyme converts each GP to another three-carbon compound, 1,3-diphosphoglyceric acid. Because each DHAP molecule can be converted to GP and each GP to 1,3-diphosphoglyceric acid, the result is two molecules of 1,3-diphosphoglyceric acid for each initial molecule of glucose. GP is oxidized by the transfer of two hydrogen atoms to NAD^+ to form NADH. The enzyme couples this reaction with the creation of a high-energy bond between the sugar and a P. The three-carbon sugar now has two P groups.

7 The high-energy P is moved to ADP, forming ATP, the first ATP production of glycolysis. (Since the sugar splitting in step 4, all products are doubled. Therefore, this step actually repays the earlier investment of two ATP molecules.)

8 An enzyme relocates the remaining P of 3-phosphoglyceric acid to form 2-phosphoglyceric acid in preparation for the next step.

9 By the loss of a water molecule, 2-phosphoglyceric acid is converted to phosphoenolpyruvic acid (PEP). In the process, the phosphate bond is upgraded to a high-energy bond.

10 This high-energy P is transferred from PEP to ADP, forming ATP. For each initial glucose molecule, the result of this step is two molecules of ATP and two molecules of a three-carbon compound called pyruvic acid.

Figure 5.12 An outline of the reactions of glycolysis (Embden-Meyerhof pathway). The inset indicates the relationship of glycolysis to the overall processes of respiration and fermentation. A more detailed version of glycolysis is presented in Figure A.2 in Appendix A.

Q What is glycolysis?

In this cycle, a series of oxidations and reductions transfer that potential energy, in the form of electrons, to electron carrier coenzymes, chiefly NAD^+ and $FADH_2$. The pyruvic acid derivatives are oxidized; the coenzymes are reduced.

Pyruvic acid, the product of glycolysis, cannot enter the Krebs cycle directly. In a preparatory step, it must lose one molecule of CO_2 and become a two-carbon compound (**Figure 5.13**, at top). This process is called **decarboxylation.** The two-carbon compound, called an *acetyl group,* attaches to coenzyme A through a high-energy bond; the resulting complex is known as *acetyl coenzyme A (acetyl CoA)*. During this reaction, pyruvic acid is also oxidized, and NAD^+ is reduced to NADH.

Remember that the oxidation of one glucose molecule produces two molecules of pyruvic acid, so for each molecule of glucose, two molecules of CO_2 are released, two molecules of NADH are produced, and two molecules of acetyl CoA are formed. Once the pyruvic acid has undergone decarboxylation and its derivative (the acetyl group) has attached to CoA, the resulting acetyl CoA is ready to enter the Krebs cycle.

As acetyl CoA enters the Krebs cycle, CoA detaches from the acetyl group. The acetyl group combines with oxaloacetic acid to form citric acid. This synthesis reaction requires energy, which is provided by the cleavage of the high-energy bond between the acetyl group and CoA. The formation of citric acid is thus the first step in the Krebs cycle. The major chemical reactions of this cycle are outlined in Figure 5.13 (also see Figure A.5 in Appendix A for a more detailed representation of the Krebs cycle). Keep in mind that each reaction is catalyzed by a specific enzyme.

The chemical reactions of the Krebs cycle fall into several general categories; one of these is decarboxylation. For example, in step 3, isocitric acid is decarboxylated to a compound called α-ketoglutaric acid. Another decarboxylation takes place in step 4. All three carbon atoms in pyruvic acid are eventually released as CO_2 by the Krebs cycle. The conversion to CO_2 of all six carbon atoms contained in the original glucose molecule completed in two turns of the Krebs cycle.

Another general category of Krebs cycle chemical reactions is oxidation-reduction. For example, in step 3, isocitric acid is oxidized. Hydrogen atoms are also released in the Krebs cycle in steps 4, 6, and 8 and are picked up by the coenzymes NAD^+ and FAD. Because NAD^+ picks up two electrons but only one additional proton, its reduced form is represented as NADH; however, FAD picks up two complete hydrogen atoms and is reduced to $FADH_2$.

If we look at the Krebs cycle as a whole, we see that for every two molecules of acetyl CoA that enter the cycle, four molecules of CO_2 are liberated by decarboxylation, six molecules of NADH and two molecules of $FADH_2$ are produced by oxidation-reduction reactions, and two molecules of ATP are generated by substrate-level phosphorylation. A molecule of guanosine triphosphate (GTP), formed from guanosine diphosphate (GDP + P_i), is similar to ATP and serves as an intermediary at this point in the cycle. Many of the intermediates in the Krebs cycle also play a role in other pathways, especially in amino acid biosynthesis (page 141).

The CO_2 produced in the Krebs cycle is ultimately liberated into the atmosphere as a gaseous by-product of aerobic respiration. (Humans produce CO_2 from the Krebs cycle in most cells of the body and discharge it through the lungs during exhalation.) The reduced coenzymes NADH and $FADH_2$ are the most important products of the Krebs cycle because they contain most of the energy that was originally stored in glucose. During the next phase of respiration, a series of reductions indirectly transfers the energy stored in those coenzymes to ATP. These reactions are collectively called the electron transport chain. **Animations** Krebs Cycle: Overview, Steps

The Electron Transport Chain (System) An **electron transport chain (system)** consists of a sequence of carrier molecules that are capable of oxidation and reduction. As electrons are passed through the chain, there occurs a stepwise release of energy, which is used to drive the chemiosmotic generation of ATP, to be described shortly. The final oxidation is irreversible. In eukaryotic cells, the electron transport chain is contained in the inner membrane of mitochondria; in prokaryotic cells, it is found in the plasma membrane.

There are three classes of carrier molecules in electron transport chains:

1. **Flavoproteins** contain flavin, a coenzyme derived from riboflavin (vitamin B_2), and are capable of performing alternating oxidations and reductions. One important flavin coenzyme is flavin mononucleotide (FMN).
2. **Cytochromes** are proteins with an iron-containing group (heme) capable of existing alternately as a reduced form (Fe^{2+}) and an oxidized form (Fe^{3+}). The cytochromes involved in electron transport chains include cytochrome *b* (cyt *b*), cytochrome c_1 (cyt c_1), cytochrome *c* (cyt *c*), cytochrome *a* (cyt *a*), and cytochrome a_3 (cyt a_3).
3. **Ubiquinones**, or **coenzyme Q (Q),** are small nonprotein carriers.

The electron transport chains of bacteria are somewhat diverse, in that the particular carriers used by a bacterium and the order in which they function may differ from those of other bacteria and from those of eukaryotic mitochondrial systems. Even a single bacterium may have several types of electron transport chains. However, keep in mind that all electron transport chains achieve the same basic goal: to release energy while electrons are transferred from higher-energy compounds to lower-energy compounds. Much is known about the electron transport chain in the mitochondria of eukaryotic cells, so we'll describe its steps here.

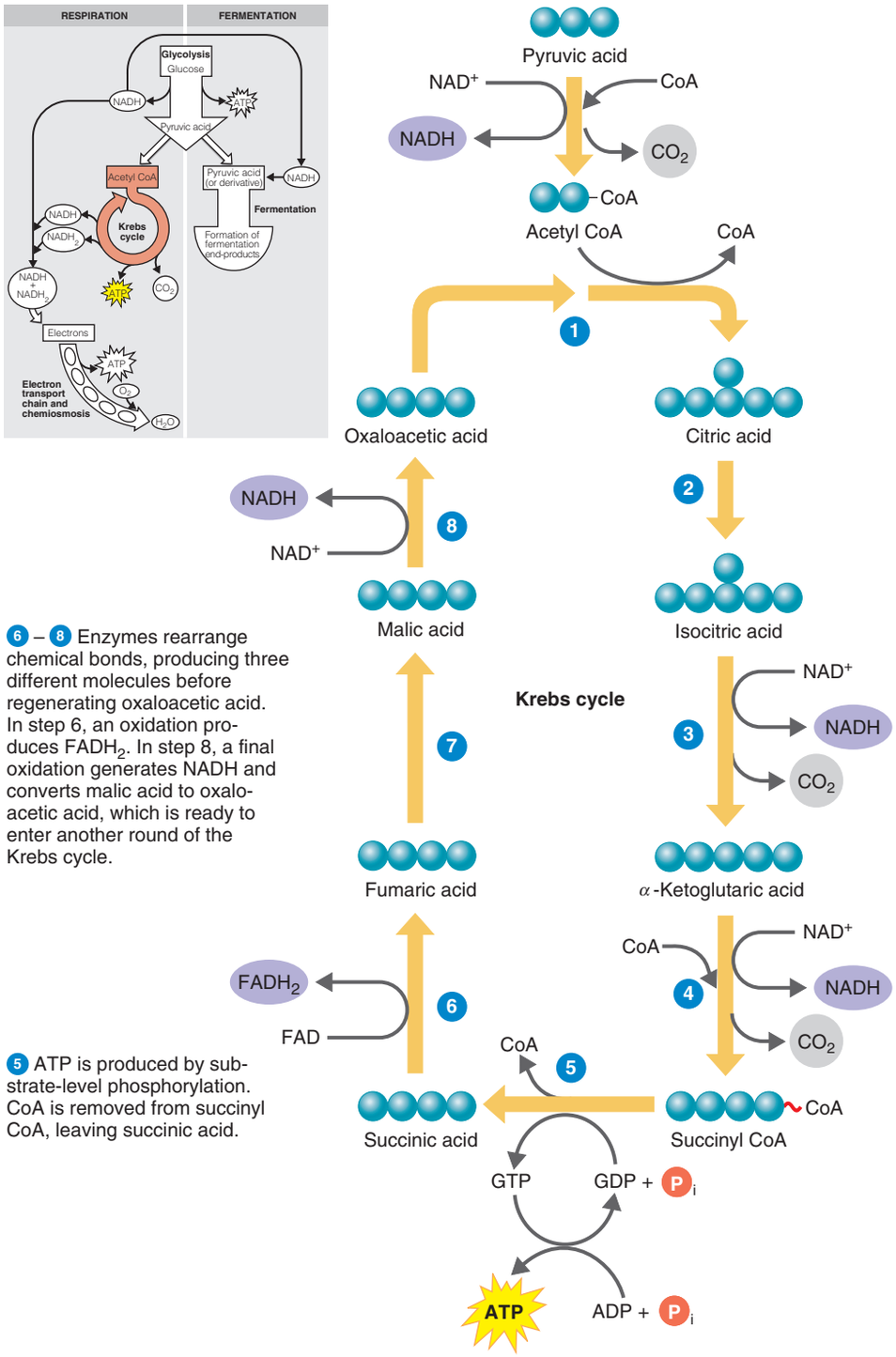


Figure 5.13 The Krebs cycle. The inset indicates the relationship of the Krebs cycle to the overall process of respiration. A more detailed version of the Krebs cycle is presented in Figure A.5 in Appendix A.

Q What are the products of the Krebs cycle?

1 High-energy electrons transfer from NADH to FMN, the first carrier in the chain (**Figure 5.14**). A hydrogen atom with two electrons passes to FMN, which picks up an additional H^+ from the surrounding aqueous medium. As a result NADH is oxidized to NAD^+, and FMN is reduced to $FMNH_2$.

2 $FMNH_2$ passes $2H^+$ to the other side of the mitochondrial membrane (see Figure 5.16) and passes two electrons to Q. As a result, $FMNH_2$ is oxidized to FMN. Q also picks up an additional $2H^+$ from the surrounding aqueous medium and releases it on the other side of the membrane.

3 Electrons are passed successively from Q to cyt *b*, cyt c_1, cyt *c*, cyt *a*, and cyt a_3. Each cytochrome in the chain is reduced as it picks up electrons and is oxidized as it gives up electrons. The last cytochrome, cyt a_3, passes its electrons to molecular oxygen (O_2), which becomes negatively charged and picks up protons from the surrounding medium to form H_2O.

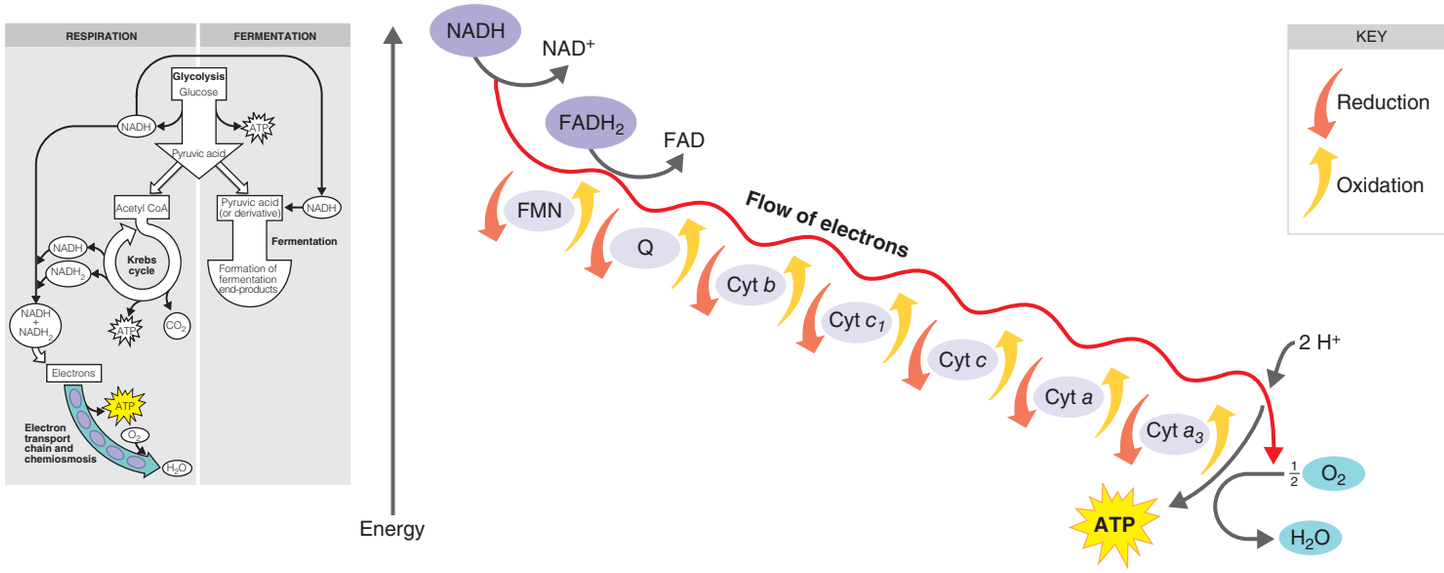


Figure 5.14 An electron transport chain (system). The inset indicates the relationship of the electron transport chain to the overall process of respiration. In the mitochondrial electron transport chain shown, the electrons pass along the chain in a gradual and stepwise fashion, so energy is released in manageable quantities (see Figure 5.16 to learn where ATP is formed).

Q What are the functions of the electron transport chain?

Notice that Figure 5.14 shows $FADH_2$, which is derived from the Krebs cycle, as another source of electrons. However, $FADH_2$ adds its electrons to the electron transport chain at a lower level than NADH. Because of this, the electron transport chain produces about one-third less energy for ATP generation when $FADH_2$ donates electrons than when NADH is involved.

An important feature of the electron transport chain is the presence of some carriers, such as FMN and Q, that accept and release protons and electrons, and other carriers, such as cytochromes, that transfer electrons only. Electron flow down the chain is accompanied at several points by the active transport (pumping) of protons from the matrix side of the inner mitochondrial membrane to the opposite side of the membrane. The result is a buildup of protons on one side of the membrane. Just as water behind a dam stores energy that can be used to generate electricity, this buildup of protons provides energy that the chemiosmotic mechanism uses to generate ADP.

The Chemiosmotic Mechanism of ATP Generation ATP synthesis using the electron transport chain is called **chemiosmosis,** and it involves **oxidative phosphorylation.** To understand chemiosmosis, we need to review several concepts related to the movement of materials across membranes. (See Chapter 4, page 87) Substances diffuse passively across membranes from areas of high concentration to areas of low concentration; this diffusion along a concentration gradient yields energy. The movement of substances *against* a concentration gradient *requires* energy that is usually provided by ATP. In chemiosmosis, the energy released when a substance moves along a gradient is used to *synthesize* ATP. The "substance" in this case refers to protons. In respiration, chemiosmosis is responsible for most of the ATP that is generated. The steps of chemiosmosis are as follows (Figure 5.15):

1. As energetic electrons from NADH (or chlorophyll) pass down the electron transport chain, some of the carriers in the chain pump—actively transport—protons across the membrane. Such carrier molecules are called *proton pumps*.
2. The phospholipid membrane is normally impermeable to protons, so this one-directional pumping establishes a proton gradient (a difference in the concentrations of protons on the two sides of the membrane). In addition to a concentration gradient, there is an electrical charge gradient. The excess H^+ on one side of the membrane makes that side positively charged compared with the other side. The resulting electrochemical gradient has potential energy, called the *proton motive force*.
3. The protons on the side of the membrane with the higher proton concentration can diffuse across the membrane only through special protein channels that contain an enzyme called *ATP synthase*. When this flow occurs, energy is released and is used by the enzyme to synthesize ATP from ADP and P_i.

The detailed steps that show how the electron transport chain operates in eukaryotes to drive the chemiosmotic mechanism are as follows (Figure 5.16):

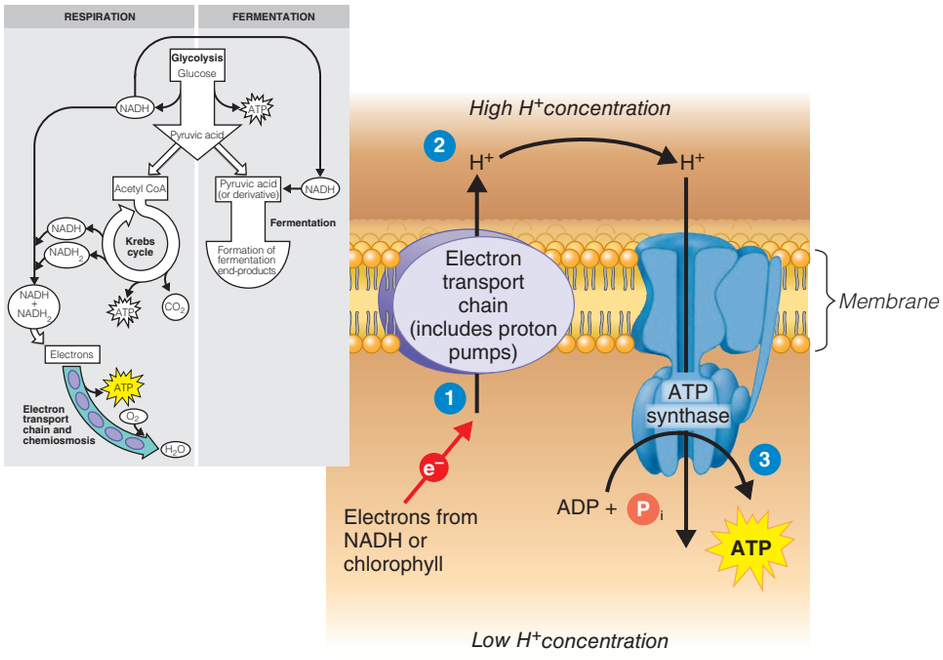


Figure 5.15 Chemiosmosis. An overview of the mechanism of chemiosmosis. The membrane shown could be a prokaryotic plasma membrane, a eukaryotic mitochondrial membrane, or a photosynthetic thylakoid. The numbered steps are described in the text.

Q What is the proton motive force?

1. Energetic electrons from NADH pass down the electron transport chains. Within the inner mitochondrial membrane, the carriers of the electron transport chain are organized into three complexes, with Q transporting electrons between the first and second complexes, and cyt *c* transporting them between the second and third complexes.
2. Three components of the system pump protons. At the end of the chain, electrons join with protons and oxygen (O_2) in the matrix fluid to form water (H_2O). Thus, O_2 is the final electron acceptor.
3. Both prokaryotic and eukaryotic cells use the chemiosmotic mechanism to generate energy for ATP production. However, in eukaryotic cells, the inner mitochondrial membrane contains the electron transport carriers and ATP synthase. In most prokaryotic cells, the plasma membrane does so. An electron transport chain also operates in photophosphorylation and is located in the thylakoid membrane of cyanobacteria and eukaryotic chloroplasts.

A Summary of Aerobic Respiration The electron transport chain regenerates NAD^+ and FAD, which can be used again in glycolysis and the Krebs cycle. The various electron transfers in the electron transport chain generate about 34 molecules of ATP from each molecule of glucose oxidized: approximately three from each of the ten molecules of NADH (a total of 30), and approximately two from each of the two molecules of $FADH_2$ (a total of four). In aerobic respiration among prokaryotes, each molecule of glucose generates 38 ATP molecules: 34 from chemiosmosis plus 4 generated by oxidation in glycolysis and the Krebs cycle. **Table 5.3** provides a detailed accounting of the ATP yield during prokaryotic aerobic respiration, and **Figure 5.17** presents a summary of the stages of aerobic respiration in prokaryotes.

Aerobic respiration among eukaryotes produces a total of only 36 molecules of ATP. There are fewer ATPs than in prokaryotes because some energy is lost when electrons are shuttled across the mitochondrial membranes that separate glycolysis (in the cytoplasm) from the electron transport chain. No such separation exists in prokaryotes. We can now summarize the overall reaction for aerobic respiration in prokaryotes as follows:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Oxygen}}{6\ O_2} + 38\ ADP + 38\ P_i \longrightarrow \underset{\text{Carbon dioxide}}{6\ CO_2} + \underset{\text{Water}}{6\ H_2O} + 38\ ATP$$

Anaerobic Respiration

In anaerobic respiration, the final electron acceptor is an inorganic substance other than oxygen (O_2). Some bacteria, such as *Pseudomonas* and *Bacillus,* can use a nitrate ion (NO_3^-) as a final electron acceptor; the nitrate ion is reduced to a nitrite ion (NO_2^-), nitrous oxide (N_2O), or nitrogen gas (N_2). Other bacteria, such as *Desulfovibrio* (dē-sul-fō-VIB-rē-ō), use sulfate (SO_4^{2-}) as the final electron acceptor to form hydrogen sulfide (H_2S). Still other bacteria use carbonate (CO_3^{2-}) to form methane (CH_4). Anaerobic respiration by bacteria using nitrate and sulfate as final acceptors is essential for the nitrogen and sulfur cycles that occur in nature. The amount of ATP generated in anaerobic respiration varies with the organism and the pathway. Because

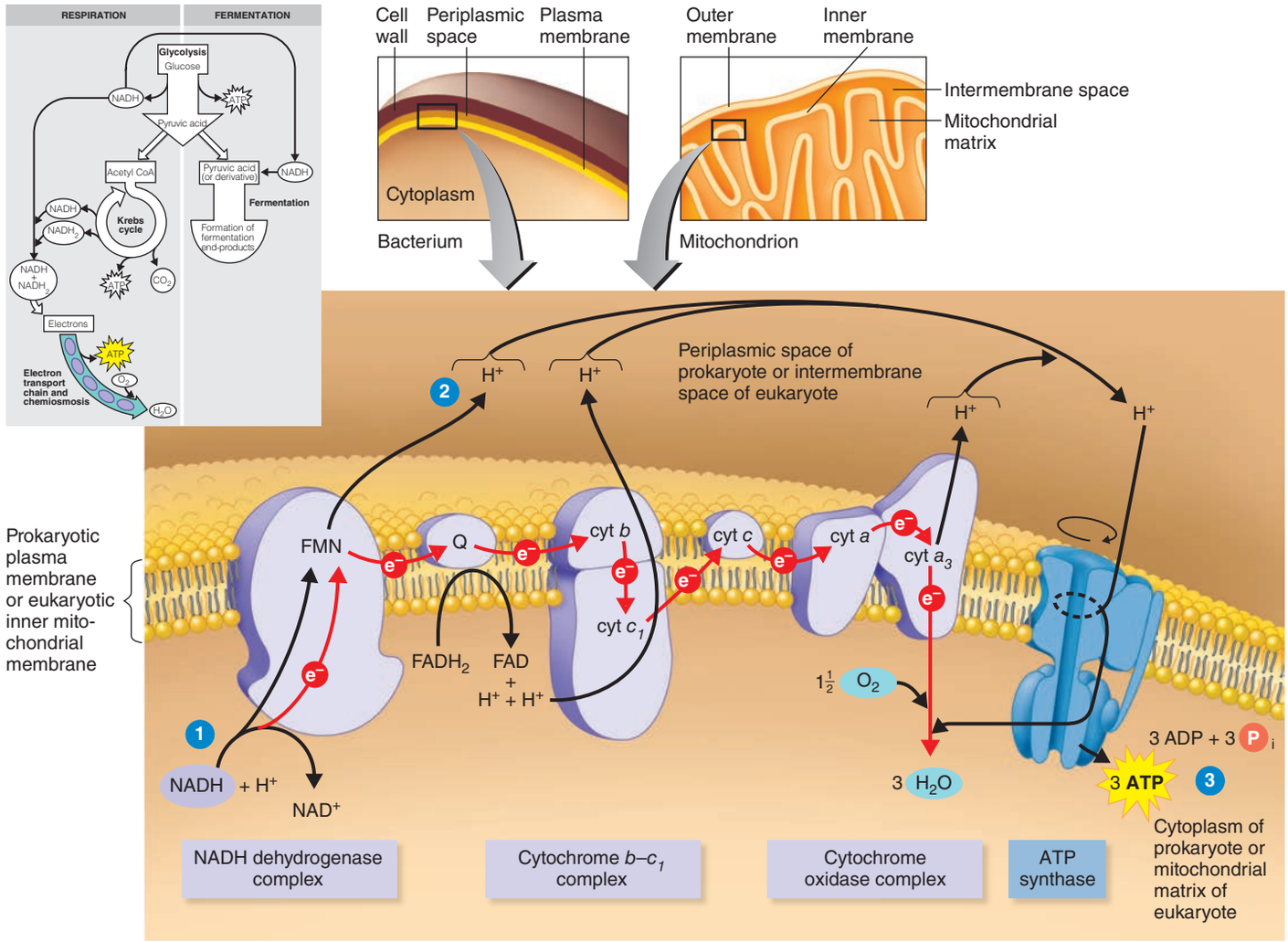


Figure 5.16 Electron transport and the chemiosmotic generation of ATP. Electron carriers are organized into three complexes, and protons (H^+) are pumped across the membrane at three points. In a prokaryotic cell, protons are pumped across the plasma membrane from the cytoplasmic side. In a eukaryotic cell, they are pumped from the matrix side of the mitochondrial membrane to the opposite side. The flow of electrons is indicated with red arrows.

Q Where does chemiosmosis occur in eukaryotes? In prokaryotes?

only part of the Krebs cycle operates under anaerobic conditions, and because only some of the carriers in the electron transport chain participate in anaerobic respiration, the ATP yield is never as high as in aerobic respiration. Accordingly, anaerobes tend to grow more slowly than aerobes. MM **Animations** Electron Transport Chain: Overview, The Process, Factors Affecting ATP Yield

CHECK YOUR UNDERSTANDING

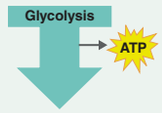
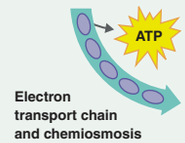
- What are the principal products of the Krebs cycle? **5-13**
- How do carrier molecules function in the electron transport chain? **5-14**
- Compare the energy yield (ATP) of aerobic and anaerobic respiration. **5-15**

Fermentation

After glucose has been broken down into pyruvic acid, the pyruvic acid can be completely broken down in respiration, as previously described, or it can be converted to an organic product in fermentation, whereupon NAD^+ and $NADP^+$ are regenerated and can enter another round of glycolysis (see Figure 5.11). **Fermentation** is defined as a process that

1. releases energy from sugars or other organic molecules, such as amino acids, organic acids, purines, and pyrimidines;
2. does not require oxygen (but can occur in its presence);
3. does not require the use of the Krebs cycle or an electron transport chain;

TABLE 5.3 ATP Yield during Prokaryotic Aerobic Respiration of One Glucose Molecule

Source		ATP Yield (Method)	
Glycolysis			
1. Oxidation of glucose to pyruvic acid		2 ATP (substrate-level phosphorylation)	
2. Production of 2 NADH		6 ATP (oxidative phosphorylation in electron transport chain)	
Preparatory Step			
1. Formation of acetyl CoA produces 2 NADH		6 ATP (oxidative phosphorylation in electron transport chain)	
Krebs Cycle			
1. Oxidation of succinyl CoA to succinic acid		2 GTP (equivalent of ATP; substrate-level phosphorylation)	
2. Production of 6 NADH		18 ATP (oxidative phosphorylation in electron transport chain)	
3. Production of 2 FADH		4 ATP (oxidative phosphorylation in electron transport chain)	
		Total: 38 ATP	

4. uses an organic molecule synthesized in the cell as the final electron acceptor;
5. produces only small amounts of ATP (only one or two ATP molecules for each molecule of starting material) because much of the original energy in glucose remains in the chemical bonds of the organic end-products, such as lactic acid or ethanol.

During fermentation, electrons are transferred (along with protons) from reduced coenzymes (NADH, NADPH) to pyruvic acid or its derivatives (**Figure 5.18a**). Those final electron acceptors are reduced to the end-products shown in Figure 5.18b. An essential function of the second stage of fermentation is to ensure a steady supply of NAD^+ and $NADP^+$ so that glycolysis can continue. In fermentation, ATP is generated only during glycolysis.

Microorganisms can ferment various substrates; the end-products depend on the particular microorganism, the substrate, and the enzymes that are present and active. Chemical analyses of these end-products are useful in identifying microorganisms. Two of the more important processes are lactic acid fermentation and alcohol fermentation.

Lactic Acid Fermentation

During glycolysis, which is the first phase of **lactic acid fermentation,** a molecule of glucose is oxidized to two molecules of pyruvic acid (**Figure 5.19**; see also Figure 5.10). This oxidation generates the energy that is used to form the two molecules of ATP. In the next step, the two molecules of pyruvic acid are reduced by two molecules of NADH to form two molecules of lactic acid (Figure 5.19a). Because lactic acid is the end-product of the reaction, it undergoes no further oxidation, and most of the energy produced by the reaction remains stored in the lactic acid. Thus, this fermentation yields only a small amount of energy.

Two important genera of lactic acid bacteria are *Streptococcus* and *Lactobacillus* (lak′tō-bah-SIL-lus). Because these microbes

Clinical Case

Feeling certain that there must be some connection between the increase in dental caries and the activities of her patients, Dr. Rivera starts to ask more questions about the children's activities. She finds out that they all attend a summer program at the same church in a nearby neighborhood. She also discovers that the culprit isn't candy, but bubblegum. The camp counselors have been giving out bubblegum as an incentive for attendance and good behavior. Although Dr. Rivera is pleased to hear that her patients have all been behaving themselves, she is concerned about the amount of bubblegum they have been chewing on a daily basis. The sucrose in gum causes a decrease in the pH of saliva, and the acid erodes the tooth enamel, thus exposing the tooth to bacterial decay.

If the pH of gum and sucrose is 7, what lowers the salivary pH?

110 128 133 135

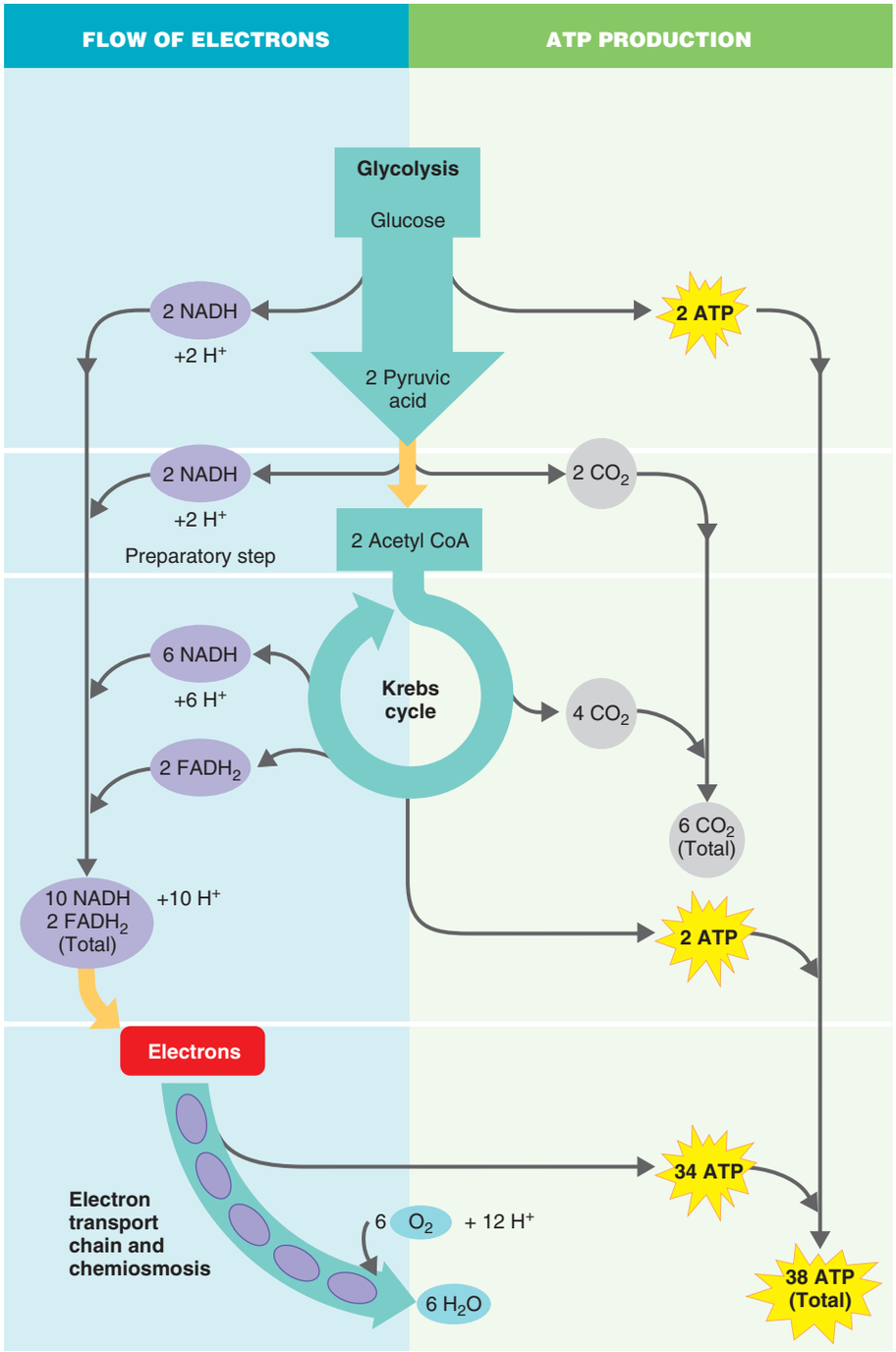


Figure 5.17 A summary of aerobic respiration in prokaryotes. Glucose is broken down completely to carbon dioxide and water, and ATP is generated. This process has three major phases: glycolysis, the Krebs cycle, and the electron transport chain. The preparatory step is between glycolysis and the Krebs cycle. The key event in aerobic respiration is that electrons are picked up from intermediates of glycolysis and the Krebs cycle by NAD^+ or FAD and are carried by NADH or $FADH_2$ to the electron transport chain. NADH is also produced during the conversion of pyruvic acid to acetyl CoA. Most of the ATP generated by aerobic respiration is made by the chemiosmotic mechanism during the electron transport chain phase; this is called oxidative phosphorylation.

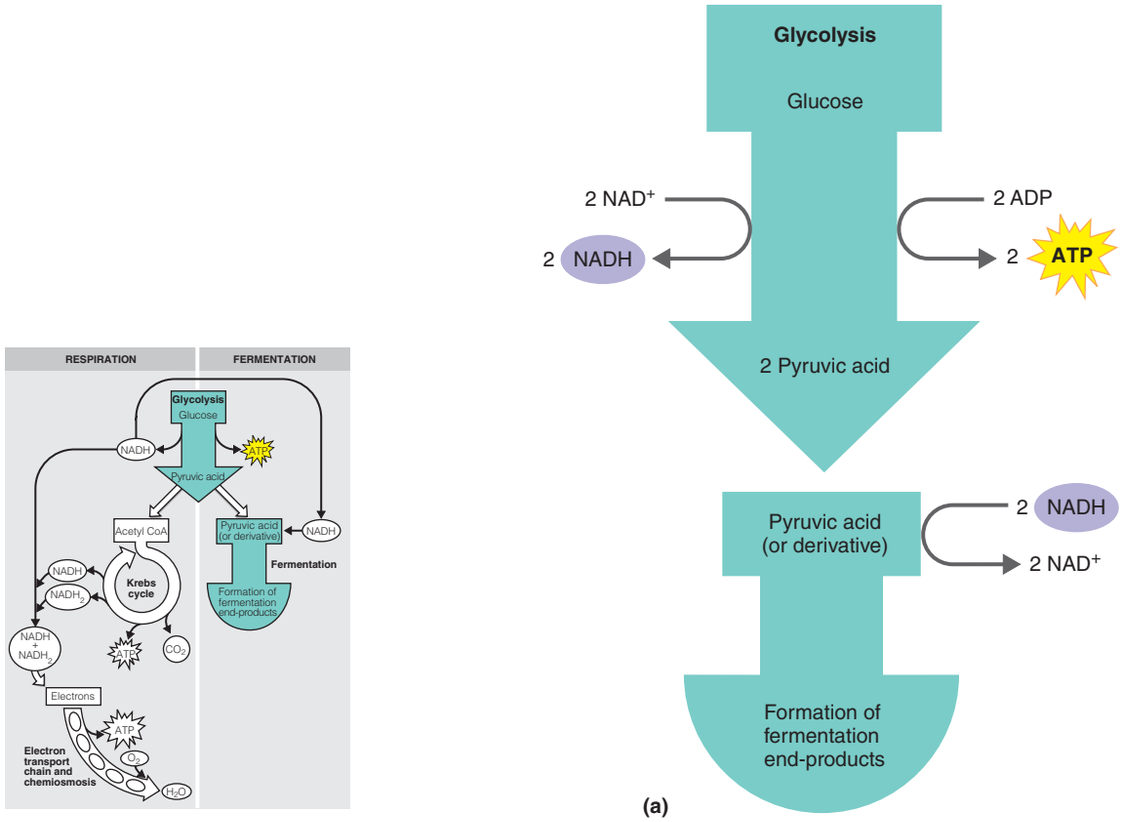
Q How do aerobic and anaerobic respiration differ?

produce only lactic acid, they are referred to as **homolactic** (or *homofermentative*). Lactic acid fermentation can result in food spoilage. However, the process can also produce yogurt from milk, sauerkraut from fresh cabbage, and pickles from cucumbers.

Alcohol Fermentation

Alcohol fermentation also begins with the glycolysis of a molecule of glucose to yield two molecules of pyruvic acid and two molecules of ATP. In the next reaction, the two molecules of pyruvic acid are converted to two molecules of acetaldehyde and two molecules of CO_2 (Figure 5.19b). The two molecules of acetaldehyde are reduced by two molecules of NADH to form two molecules of ethanol. Again, alcohol fermentation is a low-energy-yield process because most of the energy contained in the initial glucose molecule remains in the ethanol, the end-product.

Alcohol fermentation is carried out by a number of bacteria and yeasts. The ethanol and carbon dioxide produced by the yeast *Saccharomyces* (sak′kar-ō-MĪ-sēz) are waste products for yeast cells but are useful to humans. Ethanol made by yeasts is



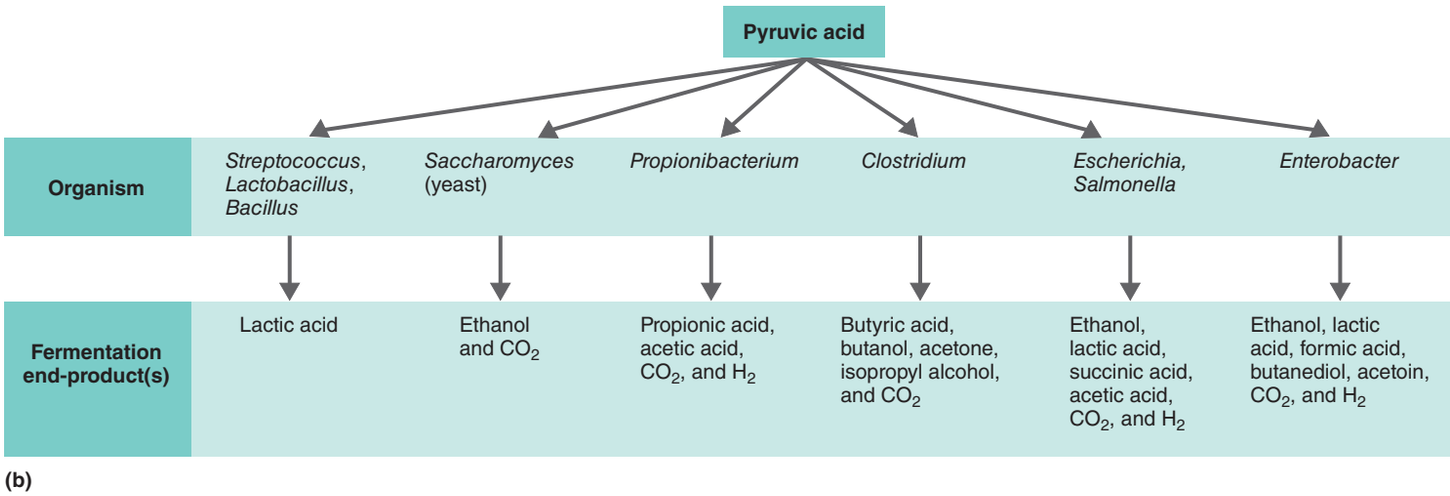


Figure 5.18 Fermentation. The inset indicates the relationship of fermentation to the overall energy-producing processes. **(a)** An overview of fermentation. The first step is glycolysis, the conversion of glucose to pyruvic acid. In the second step, the reduced coenzymes from glycolysis or its alternatives (NADH, NADPH) donate their electrons and hydrogen ions to pyruvic acid or a derivative to form a fermentation end-product. **(b)** End-products of various microbial fermentations.

Q During which phase of fermentation is ATP generated?

the alcohol in alcoholic beverages, and carbon dioxide made by yeasts causes bread dough to rise.

Organisms that produce lactic acid as well as other acids or alcohols are known as **heterolactic** (or *heterofermentative*) and often use the pentose phosphate pathway.

Table 5.4 lists some of the various microbial fermentations used by industry to convert inexpensive raw materials into useful end-products. **Table 5.5** provides a summary comparison of aerobic respiration, anaerobic respiration, and fermentation.

MM **Animation** Fermentation

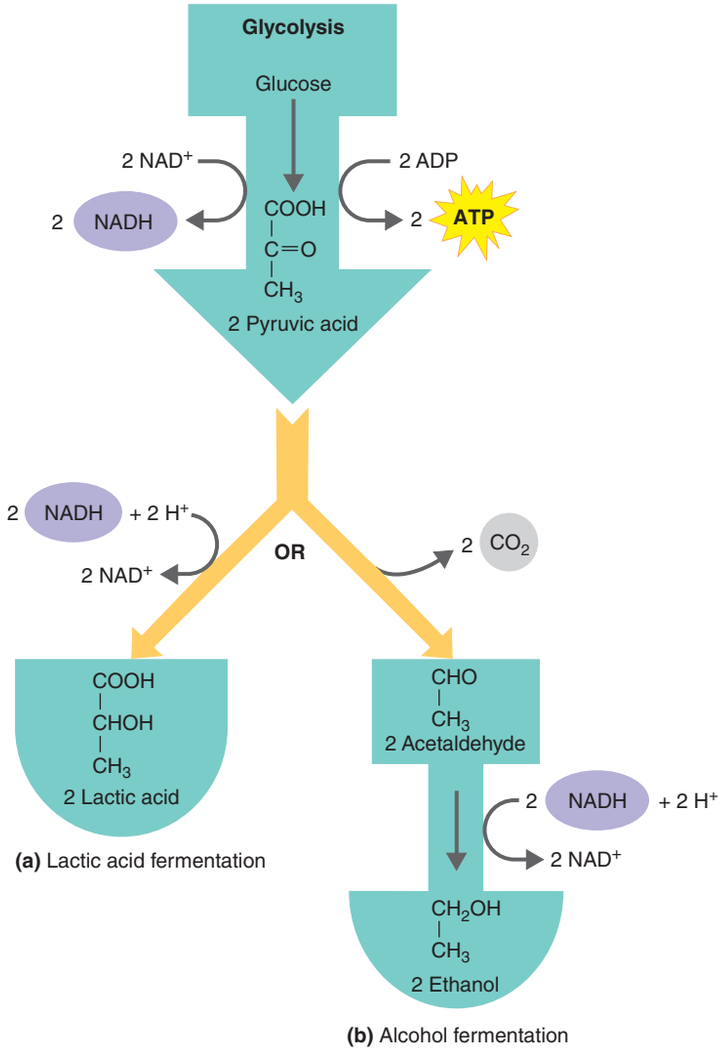


Figure 5.19 Types of fermentation.

Q What is the difference between homolactic and heterolactic fermentation?

CHECK YOUR UNDERSTANDING

✓ List four compounds that can be made from pyruvic acid by an organism that uses fermentation. **5-16**

Lipid and Protein Catabolism

LEARNING OBJECTIVE

5-17 Describe how lipids and proteins undergo catabolism.

Our discussion of energy production has emphasized the oxidation of glucose, the main energy-supplying carbohydrate. However, microbes also oxidize lipids and proteins, and the oxidations of all these nutrients are related.

Recall that fats are lipids consisting of fatty acids and glycerol. Microbes produce extracellular enzymes called *lipases* that break fats down into their fatty acid and glycerol components. Each component is then metabolized separately (Figure 5.20). The Krebs cycle functions in the oxidation of glycerol and fatty acids. Many bacteria that hydrolyze fatty acids can use the same enzymes to degrade petroleum products. Whereas beta-oxidation (fatty acid oxidation) of petroleum is a nuisance when these bacteria grow in a fuel storage tank, it is beneficial when they grow in oil spills. (See an illustration of beta-oxidation in the Applications of Microbiology box in Chapter 2, page 31.)

Proteins are too large to pass unaided through plasma membranes. Microbes produce extracellular *proteases* and *peptidases,* enzymes that break down proteins into their component amino acids, which can cross the membranes. However, before amino acids can be catabolized, they must be enzymatically converted to other substances that can enter the Krebs cycle. In one such conversion, called **deamination,** the amino group of an amino acid is removed and converted to an ammonium ion (NH_4^+), which can be excreted from the cell. The remaining organic acid can enter the Krebs cycle. Other conversions involve **decarboxylation** (the removal of —COOH) and **desulfurization** (removal of —SH).

A summary of the interrelationships of carbohydrate, lipid, and protein catabolism is shown in Figure 5.21.

CHECK YOUR UNDERSTANDING

✓ What are the end-products of lipid and protein catabolism? **5-17**

Biochemical Tests and Bacterial Identification

LEARNING OBJECTIVE

5-18 Provide two examples of the use of biochemical tests to identify bacteria in the laboratory.

Biochemical testing is frequently used to identify bacteria and yeasts because different species produce different enzymes. Such biochemical tests are designed to detect the presence of enzymes. One type of biochemical test detects amino acid catabolizing enzymes involved in decarboxylation and dehydrogenation (discussed on pages 123 and 118; Figure 5.22).

Another biochemical test is a **fermentation test** (Figure 5.23). The test medium contains protein, a single carbohydrate, a pH indicator, and an inverted Durham tube, which is used to capture gas. Bacteria inoculated into the tube can use the protein or carbohydrate as a carbon and energy source. If they catabolize the carbohydrate and produce acid, the pH indicator changes color. Some organisms produce gas as well as acid from carbohydrate catabolism. The presence of a bubble in the Durham tube indicates gas formation.

E. coli ferments the carbohydrate sorbitol. The pathogenic *E. coli* O157 strain, however, does not ferment sorbitol, a characteristic that differentiates it from nonpathogenic, commensal *E. coli*.

Another example of the use of biochemical tests is shown in Figure 10.8 on page 276.

TABLE 5.4 Some Industrial Uses for Different Types of Fermentations*

Fermentation End-Product(s)	Industrial or Commercial Use	Starting Material	Microorganism
Ethanol	Beer, wine	Starch, sugar	*Saccharomyces cerevisiae* (yeast, a fungus)
	Fuel	Agricultural wastes	*Saccharomyces cerevisiae* (yeast)
Acetic Acid	Vinegar	Ethanol	*Acetobacter*
Lactic Acid	Cheese, yogurt	Milk	*Lactobacillus, Streptococcus*
	Rye bread	Grain, sugar	*Lactobacillus delbrueckii*
	Sauerkraut	Cabbage	*Lactobacillus plantarum*
	Summer sausage	Meat	*Pediococcus*
Propionic Acid and Carbon Dioxide	Swiss cheese	Lactic acid	*Propionibacterium freudenreichii*
Acetone and Butanol	Pharmaceutical, industrial uses	Molasses	*Clostridium acetobutylicum*
Citric Acid	Flavoring	Molasses	*Aspergillus* (fungus)
Methane	Fuel	Acetic acid	*Methanosarcina* (archaeon)
Sorbose	Vitamin C (ascorbic acid)	Sorbitol	*Gluconobacter*

*Unless otherwise noted, the microorganisms listed are bacteria.

In some instances, the waste products of one microorganism can be used as a carbon and energy source by another species. *Acetobacter* (ah-sē′tō-BAK-ter) bacteria oxidize ethanol made by yeast. *Propionibacterium* (prō′-pē-on-ē-bak-TI-rē-um) can use lactic acid produced by other bacteria. Propionibacteria convert lactic acid to pyruvic acid in preparation for the Krebs cycle. During the Krebs cycle, propionic acid and CO_2 are made. The holes in Swiss cheese are formed by the accumulation of CO_2 gas.

Biochemical tests are used to identify bacteria that cause disease. All aerobic bacteria use the electron transport chain (ETC), but their ETCs are not all identical. Some bacteria have cytochrome *c*, but others do not. In the former, *cytochrome c oxidase* is the last enzyme, which transfers electrons to oxygen. The oxidase test is routinely used to quickly identify *Neisseria gonorrhoeae*. *Neisseria* is positive for cytochrome oxidase. The oxidase test can also be used to distinguish some gram-negative rods: *Pseudomonas* is oxidase-positive, and *Escherichia* is oxidase-negative.

Shigella causes dysentery and is differentiated from *E. coli* by biochemical tests. Unlike *E. coli*, *Shigella* does not produce gas from lactose.

Salmonella bacteria are readily distinguishable from *E. coli* by the production of hydrogen sulfide (H_2S). Hydrogen sulfide is released when the bacteria remove sulfur from amino acids (**Figure 5.24**).

TABLE 5.5 Aerobic Respiration, Anaerobic Respiration, and Fermentation

Energy-Producing Process	Growth Conditions	Final Hydrogen (Electron) Acceptor	Type of Phosphorylation Used to Generate ATP	ATP Molecules Produced per Glucose Molecule
Aerobic Respiration	Aerobic	Molecular oxygen (O_2)	Substrate-level and oxidative	36 (eukaryotes) 38 (prokaryotes)
Anaerobic Respiration	Anaerobic	Usually an inorganic substance (such as NO_3^-, SO_4^{2-}, or CO_3^{2-}) but not molecular oxygen (O_2)	Substrate-level and oxidative	Variable (fewer than 38 but more than 2)
Fermentation	Aerobic or anaerobic	An organic molecule	Substrate-level	2

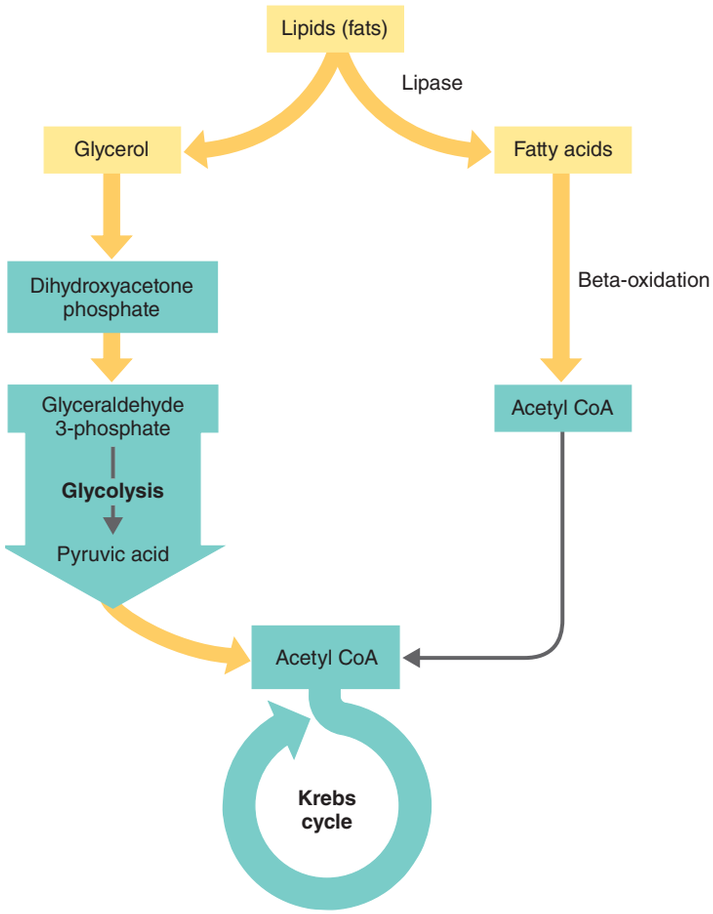


Figure 5.20 Lipid catabolism. Glycerol is converted into dihydroxyacetone phosphate (DHAP) and catabolized via glycolysis and the Krebs cycle. Fatty acids undergo beta-oxidation, in which carbon fragments are split off two at a time to form acetyl CoA, which is catabolized via the Krebs cycle.

Q What is the role of lipases?

Clinical Case

Dental caries are caused by oral streptococci, including *S. mutans, S. salivarius,* and *S. sobrinus,* that attach to tooth surfaces. Oral streptococci ferment sucrose and produce lactic acid, which lowers the salivary pH. Dr. Rivera decides to ask the camp counselors to substitute the bubblegum with a sugarless gum made with xylitol. A study has shown that chewing gum sweetened with xylitol, a naturally occuring sugar alcohol, can significantly lower the number of dental caries in children because it lowers the number of *S. mutans* in the mouth.

Why might xylitol reduce the number of *S. mutans*?

110 128 133 135

The Clinical Focus box on page 139 describes how biochemical tests were used to determine the cause of disease in a young child in Dallas, Texas.

CHECK YOUR UNDERSTANDING

On what biochemical basis are *Pseudomonas* and *Escherichia* differentiated? **5-18**

Photosynthesis

LEARNING OBJECTIVES

5-19 Compare and contrast cyclic and noncyclic photophosphorylation.

5-20 Compare and contrast the light-dependent and light-independent reactions of photosynthesis.

5-21 Compare and contrast oxidative phosphorylation and photophosphorylation.

In all of the metabolic pathways just discussed, organisms obtain energy for cellular work by oxidizing organic compounds. But where do organisms obtain these organic compounds? Some, including animals and many microbes, feed on matter produced by other organisms. For example, bacteria may catabolize compounds from dead plants and animals, or they may obtain nourishment from a living host.

Other organisms synthesize complex organic compounds from simple inorganic substances. The major mechanism for such synthesis is a process called **photosynthesis,** which is carried out by plants and many microbes. Essentially, photosynthesis is the conversion of light energy from the sun into chemical energy. The chemical energy is then used to convert CO_2 from the atmosphere to more reduced carbon compounds, primarily sugars. The word *photosynthesis* summarizes the process: *photo* means light, and *synthesis* refers to the assembly of organic compounds. This synthesis of sugars by using carbon atoms from CO_2 gas is also called **carbon fixation.** Continuation of life as we know it on Earth depends on the recycling of carbon in this way (see Figure 27.3 on page 775). Cyanobacteria, algae, and green plants all contribute to this vital recycling with photosynthesis.

Photosynthesis can be summarized with the following equations:

1. Plants, algae, and cyanobacteria use water as a hydrogen donor, releasing O_2.

$$6\,CO_2 + 12\,H_2O + \text{Light energy} \longrightarrow C_6H_{12}O_6 + 6\,H_2O + 6\,O_2$$

2. Purple sulfur and green sulfur bacteria use H_2S as a hydrogen donor, producing sulfur granules.

$$6\,CO_2 + 12\,H_2S + \text{Light energy} \longrightarrow C_6H_{12}O_6 + 6\,H_2O + 12\,S$$

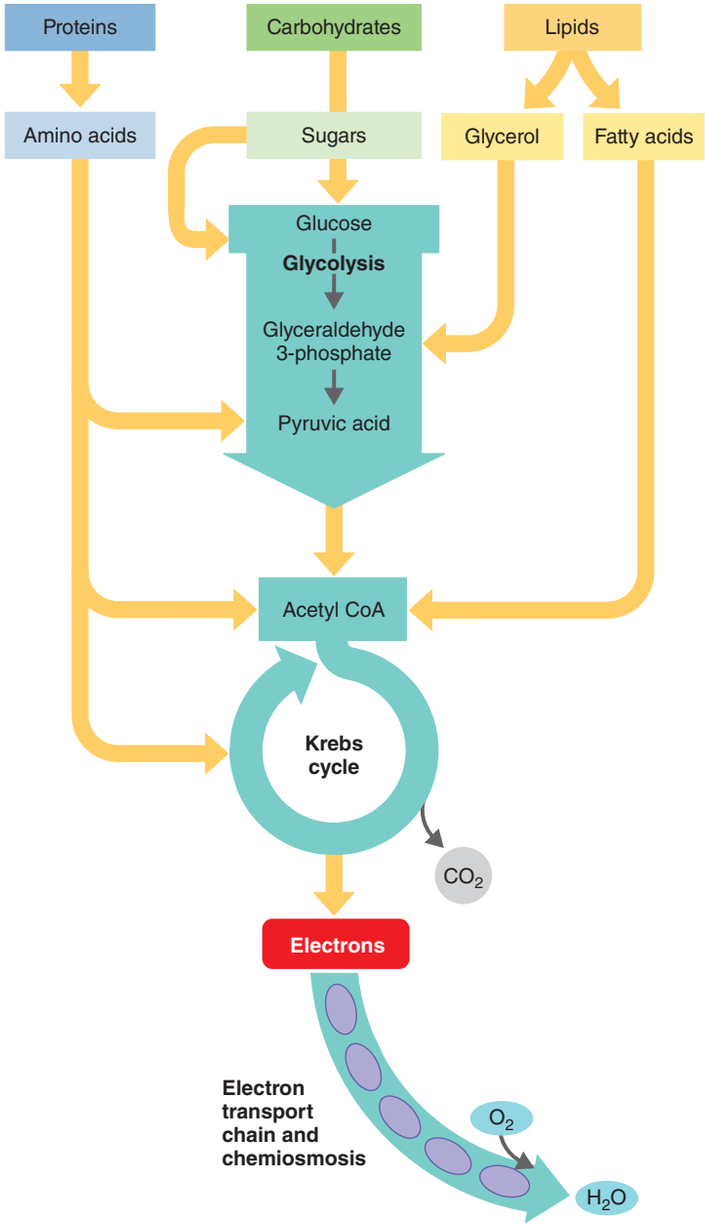


Figure 5.21 Catabolism of various organic food molecules. Proteins, carbohydrates, and lipids can all be sources of electrons and protons for respiration. These food molecules enter glycolysis or the Krebs cycle at various points.

Q What are the catabolic pathways through which high-energy electrons from all kinds of organic molecules flow on their energy-releasing pathways?

In the course of photosynthesis, electrons are taken from hydrogen atoms, an energy-poor molecule, and incorporated into sugar, an energy-rich molecule. The energy boost is supplied by light energy, although indirectly.

Photosynthesis takes place in two stages. In the first stage, called the **light-dependent (light) reactions,** light energy is used to convert ADP and P to ATP. In addition, in the predominant

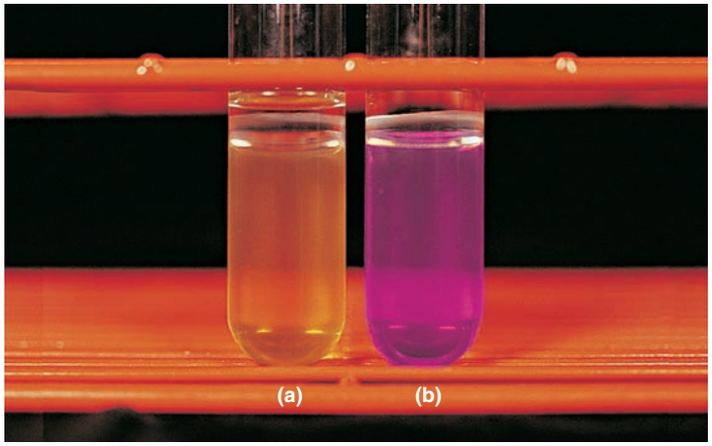


Figure 5.22 Detecting amino acid catabolizing enzymes in the lab. Bacteria are inoculated into tubes containing glucose, a pH indicator, and a specific amino acid. **(a)** The pH indicator turns to yellow when bacteria produce acid from glucose. **(b)** Alkaline products from decarboxylation turn the indicator to purple.

Q What is decarboxylation?

form of the light-dependent reactions, the electron carrier $NADP^+$ is reduced to NADPH. The coenzyme NADPH, like NADH, is an energy-rich carrier of electrons. In the second stage, the **light-independent (dark) reactions,** these electrons are used along with energy from ATP to reduce CO_2 to sugar.

MM **Animation** Photosynthesis: Overview

The Light-Dependent Reactions: Photophosphorylation

Photophosphorylation is one of the three ways ATP is formed, and it occurs only in photosynthetic cells. In this mechanism,

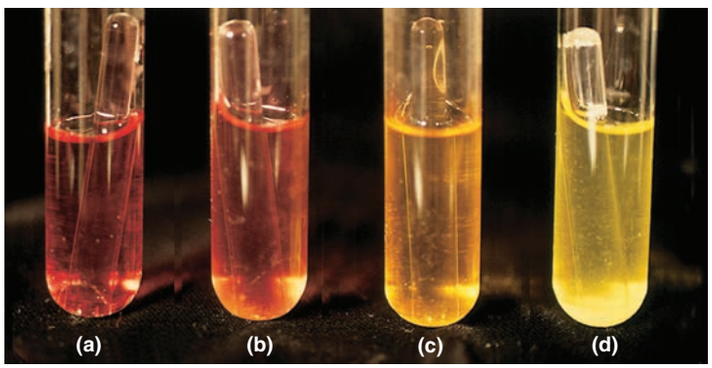


Figure 5.23 A fermentation test. **(a)** An uninoculated fermentation tube containing the carbohydrate mannitol. **(b)** *Staphylococcus epidermidis* grew on the protein but did not use the carbohydrate. This organism is described as mannitol −. **(c)** *Staphylococcus aureus* produced acid but not gas. This species is mannitol +. **(d)** *Escherichia coli* is also mannitol + and produced acid and gas from mannitol. The gas is trapped in the inverted Durham tube.

Q What is the *S. epidermidis* using as its energy source?

Figure 5.24 Use of peptone iron agar to detect the production of H_2S. H_2S produced in the tube precipitates with iron in the medium as ferrous sulfide.

Q What chemical reaction causes the release of H_2S?

light energy is absorbed by chlorophyll molecules in the photosynthetic cell, exciting some of the molecules' electrons. The chlorophyll principally used by green plants, algae, and cyanobacteria is *chlorophyll a*. It is located in the membranous thylakoids of chloroplasts in algae and green plants (see Figure 4.28, page 102) and in the thylakoids found in the photosynthetic structures of cyanobacteria. Other bacteria use *bacteriochlorophylls*.

The excited electrons jump from the chlorophyll to the first of a series of carrier molecules, an electron transport chain similar to that used in respiration. As electrons are passed along the series of carriers, protons are pumped across the membrane, and ADP is converted to ATP by chemiosmosis. Chlorophyll and other pigments are packed into thylakoids of chloroplasts (see Figure 4.28 on page 102) and are called **photosystems**. *Photosystem II* is so numbered because even though it was most likely the first photosystem to evolve, it was the second one discovered. It contains chlorophyll that is sensitive to wavelengths of light of 680 nm. *Photosystem I* contains chlorophyll that is sensitive to wavelengths of light of 700 nm. In **cyclic photophosphorylation,** the electrons released from chlorophyll in photosystem I eventually return to chlorophyll (**Figure 5.25a**). That is, the electrons in photosystem I remain in photosystem I. In **noncyclic photophosphorylation,** which is used in oxygenic organisms, both photosystems are required. The electrons released from the chlorophyll in photosystem II and photosystem I do not return to chlorophyll but become incorporated into NADPH (Figure 5.25b). The electrons lost from chlorophyll are replaced by electrons from H_2O. To summarize: the products of noncyclic photophosphorylation are ATP (formed by chemiosmosis using energy released in an electron transport chain), O_2 (from water molecules), and NADPH (carrying electrons from chlorophyll and protons derived ultimately from water). MM **Animations** Light Reaction: Cyclic Photophosphorylation; Light Reaction: Noncyclic Photophosphorylation

The Light-Independent Reactions: The Calvin-Benson Cycle

The light-independent reactions are so named because they don't require light directly. They include a complex cyclic pathway called the **Calvin-Benson cycle,** in which CO_2 is "fixed"—that is, it's used to synthesize sugars (**Figure 5.26**; see also Figure A.1 in Appendix A). MM **Animation** Light Independent Reactions

CHECK YOUR UNDERSTANDING

- How is photosynthesis important to catabolism? **5-19**
- What is made during the light-dependent reactions? **5-20**
- How are oxidative phosphorylation and photophosphorylation similar? **5-21**

Clinical Case Resolved

S. mutans cannot ferment xylitol; consequently, it doesn't grow and can't produce acid in the mouth. The camp counselors agree to switch to sugarless gum made with xylitol, and Dr. Rivera is pleased. She understands that there will be other sources of sucrose in the children's diets, but at least her patients are no longer going to be adversely affected by the camp's well-intentioned incentives. Researchers are still investigating ways that antimicrobials and vaccines can be used to reduce bacterial colonization. However, reducing consumption of sucrose-containing gum and candy may be an effective preventive measure.

110 128 133 135

A Summary of Energy Production Mechanisms

LEARNING OBJECTIVE

5-22 Write a sentence to summarize energy production in cells.

In the living world, energy passes from one organism to another in the potential energy contained in the bonds of chemical compounds. Organisms obtain the energy from oxidation reactions. To obtain energy in a usable form, a cell must have an electron (or hydrogen) donor, which serves as an initial energy source within the cell. Electron donors are diverse and can include photosynthetic pigments, glucose or other organic compounds, elemental sulfur, ammonia, or hydrogen gas (**Figure 5.27**). Next, electrons removed from the chemical energy sources are transferred to electron carriers, such as the coenzymes NAD^+, $NADP^+$, and FAD. This transfer is an oxidation-reduction reaction; the initial energy source is oxidized as this first electron carrier is reduced. During this phase, some ATP is produced. In the third stage, electrons are transferred from electron carriers to their

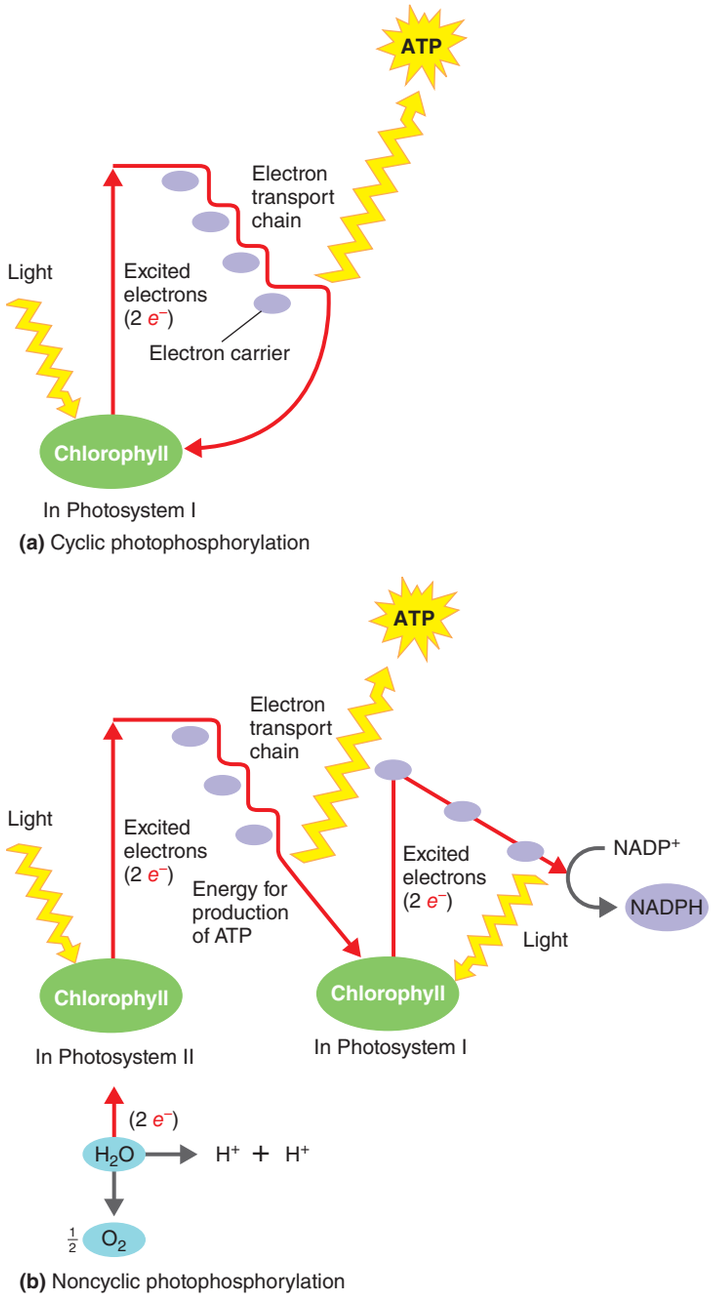


Figure 5.25 Photophosphorylation. **(a)** In cyclic photophosphorylation, electrons released from chlorophyll by light in photosystem I return to chlorophyll after passage along the electron transport chain. The energy from electron transfer is converted to ATP. **(b)** In noncyclic photophosphorylation, electrons released from chlorophyll in photosystem II are replaced by electrons from the hydrogen atoms in water. This process also releases hydrogen ions. Electrons from chlorophyll in photosystem I are passed along the electron transport chain to the electron acceptor $NADP^+$. $NADP^+$ combines with electrons and with hydrogen ions from water, forming NADPH.

Q How are oxidative phosphorylation and photophosphorylation similar?

final electron acceptors in further oxidation-reduction reactions, producing more ATP.

In aerobic respiration, oxygen (O_2) serves as the final electron acceptor. In anaerobic respiration, inorganic substances other than oxygen, such as nitrate ions (NO_3^-) or sulfate ions (SO_4^{2-}), serve as the final electron acceptors. In fermentation, organic compounds serve as the final electron acceptors. In aerobic and anaerobic respiration, a series of electron carriers called an electron transport chain releases energy that is used by the mechanism of chemiosmosis to synthesize ATP. Regardless of their energy sources, all organisms use similar oxidation-reduction reactions to transfer electrons and similar mechanisms to use the energy released to produce ATP.

CHECK YOUR UNDERSTANDING

Summarize how oxidation enables organisms to get energy from glucose, sulfur, or sunlight. **5-22**

Metabolic Diversity among Organisms

LEARNING OBJECTIVE

5-23 Categorize the various nutritional patterns among organisms according to carbon source and mechanisms of carbohydrate catabolism and ATP generation.

We have looked in detail at some of the energy-generating metabolic pathways that are used by animals and plants, as well as by many microbes. Some microbes can sustain themselves on inorganic substances by using pathways that are unavailable to either plants or animals. All organisms, including microbes, can be classified metabolically according to their *nutritional pattern*—their source of energy and their source of carbon.

ASM: Bacteria and Archaea exhibit extensive, and often unique, metabolic diversity.

First considering the energy source, we can generally classify organisms as phototrophs or chemotrophs. **Phototrophs** use light as their primary energy source, whereas **chemotrophs** depend on oxidation-reduction reactions of inorganic or organic compounds for energy. For their principal carbon source, **autotrophs** (self-feeders) use carbon dioxide, and **heterotrophs** (feeders on others) require an organic carbon source. Autotrophs are also referred to as *lithotrophs* (rock eating), and heterotrophs are also referred to as *organotrophs*.

If we combine the energy and carbon sources, we derive the following nutritional classifications for organisms: *photoautotrophs, photoheterotrophs, chemoautotrophs,* and *chemoheterotrophs* (Figure 5.28). Almost all of the medically important microorganisms discussed in this book are chemoheterotrophs. Typically, infectious organisms catabolize substances obtained from the host.

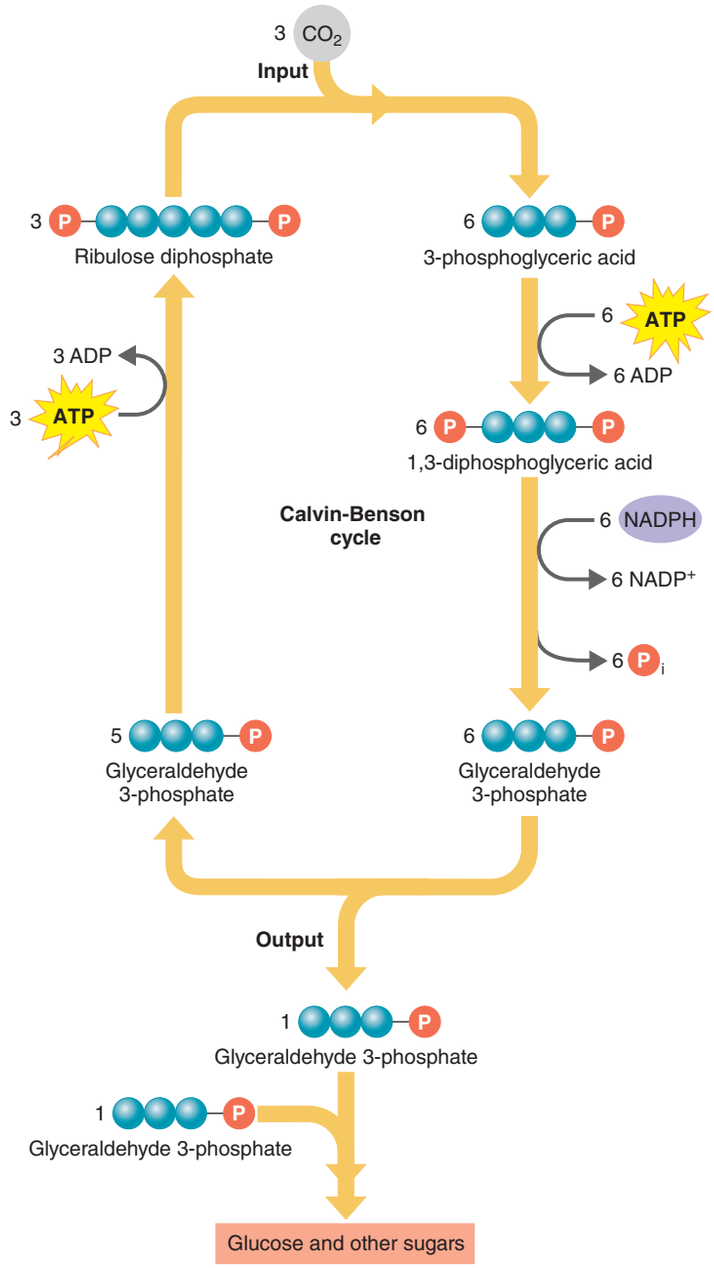


Figure 5.26 A simplified version of the Calvin-Benson cycle. This diagram shows three turns of the cycle, in which three molecules of CO_2 are fixed and one molecule of glyceraldehyde 3-phosphate is produced and leaves the cycle. Two molecules of glyceraldehyde 3-phosphate are needed to make one molecule of glucose. Therefore, the cycle must turn six times for each glucose molecule produced, requiring a total investment of 6 molecules of CO_2, 18 molecules of ATP, and 12 molecules of NADPH. A more detailed version of this cycle is presented in Figure A.1 in Appendix A.

Q In the Calvin-Benson cycle, which molecule is used to synthesize sugars?

Photoautotrophs

Photoautotrophs use light as a source of energy and carbon dioxide as their chief source of carbon. They include photosynthetic bacteria (green and purple bacteria and cyanobacteria), algae, and green plants. In the photosynthetic reactions of cyanobacteria, algae, and green plants, the hydrogen atoms of water are used to reduce carbon dioxide, and oxygen gas is given off. Because this photosynthetic process produces O_2 it is sometimes called **oxygenic.**

In addition to the cyanobacteria (see Figure 11.13, page 303), there are several other families of photosynthetic prokaryotes. Each is classified according to the way it reduces CO_2. These bacteria cannot use H_2O to reduce CO_2 and cannot carry on photosynthesis when oxygen is present (they must have an anaerobic environment). Consequently, their photosynthetic process does not produce O_2 and is called **anoxygenic.** The anoxygenic photoautotrophs are the green and purple bacteria. The **green bacteria,** such as *Chlorobium* (KLO-rō-bē-um), use sulfur (S), sulfur compounds (such as hydrogen sulfide, H_2S), or hydrogen gas (H_2) to reduce carbon dioxide and form organic compounds. Applying the energy from light and the appropriate enzymes, these bacteria oxidize sulfide (S^{2-}) or sulfur (S) to sulfate (SO_4^{2-}) or oxidize hydrogen gas to water (H_2O). The **purple bacteria,** such as *Chromatium* (krō-MĀ-shum), also use sulfur, sulfur compounds, or hydrogen gas to reduce carbon dioxide. They are distinguished from the green bacteria by their type of chlorophyll, location of stored sulfur, and ribosomal RNA.

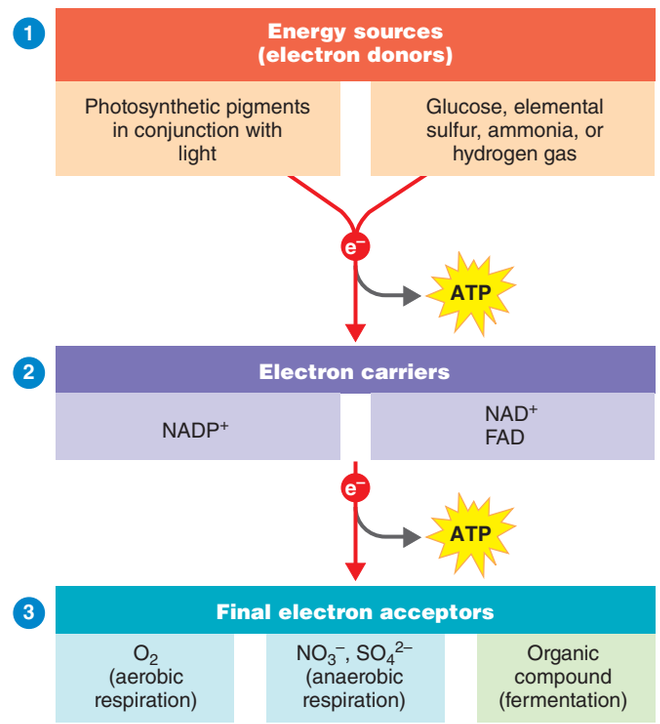


Figure 5.27 Requirements of ATP production. The production of ATP requires 1 an energy source (electron donor), 2 the transfer of electrons to an electron carrier during an oxidation-reduction reaction, and 3 the transfer of electrons to a final electron acceptor.

Q Are energy-generating reactions oxidations or reductions?

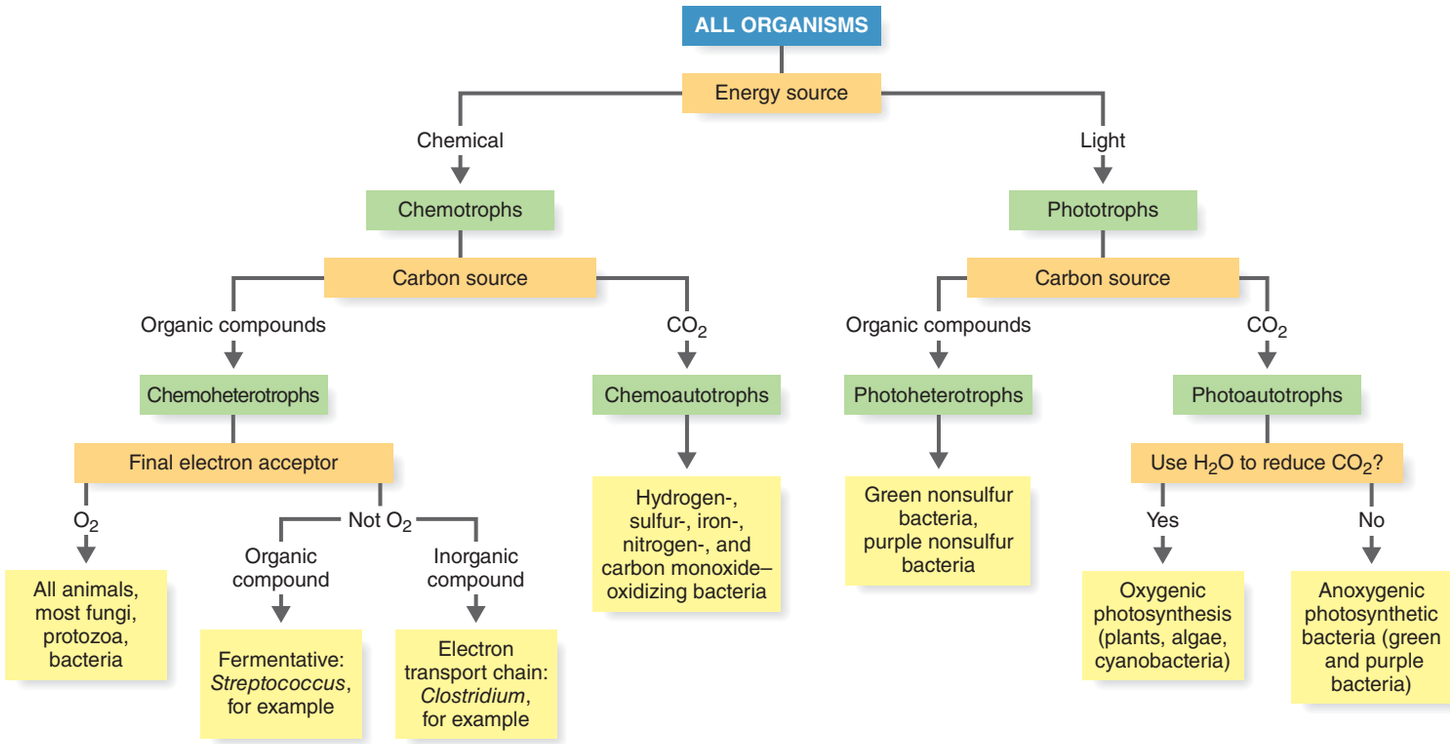


Figure 5.28 A nutritional classification of organisms.

What is the basic difference between chemotrophs and phototrophs?

The chlorophylls used by these photosynthetic bacteria are called *bacteriochlorophylls*, and they absorb light at longer wavelengths than that absorbed by chlorophyll *a*. Bacteriochlorophylls of green sulfur bacteria are found in vesicles called *chlorosomes* (or *chlorobium vesicles*) underlying and attached to the plasma membrane. In the purple sulfur bacteria, the bacteriochlorophylls are located in invaginations of the plasma membrane (*chromatophores*).

Table 5.6 summarizes several characteristics that distinguish eukaryotic photosynthesis from prokaryotic photosynthesis.

Animation Comparing Prokaryotes and Eukaryotes

Photoheterotrophs

Photoheterotrophs use light as a source of energy but cannot convert carbon dioxide to sugar; rather, they use organic compounds, such as alcohols, fatty acids, other organic acids, and carbohydrates, as sources of carbon. Photoheterotrophs are anoxygenic. The **green nonsulfur bacteria,** such as *Chloroflexus* (KLO-rō-flex-us), and **purple nonsulfur bacteria,** such as *Rhodopseudomonas* (rō′dō-soo′dō-MŌ-nas), are photoheterotrophs (see also page 304).

TABLE 5.6 Photosynthesis Compared in Selected Eukaryotes and Prokaryotes

Characteristic	Eukaryotes	Prokaryotes		
	Algae, Plants	Cyanobacteria	Green Bacteria	Purple Bacteria
Substance That Reduces CO_2	H atoms of H_2O	H atoms of H_2O	Sulfur, sulfur compounds, H_2 gas	Sulfur, sulfur compounds, H_2 gas
Oxygen Production	Oxygenic	Oxygenic (and anoxygenic)	Anoxygenic	Anoxygenic
Type of Chlorophyll	Chlorophyll *a*	Chlorophyll *a*	Bacteriochlorophyll *a*	Bacteriochlorophyll *a* or *b*
Site of Photosynthesis	Chloroplasts with thylakoids	Thylakoids	Chlorosomes	Chromatophores
Environment	Aerobic	Aerobic (and anaerobic)	Anaerobic	Anaerobic

CLINICAL FOCUS

Human Tuberculosis—Dallas, Texas

As you read through this box, you will encounter a series of questions that laboratory technicians ask themselves as they identify bacteria. Try to answer each question before going on to the next one.

1. Julia, a 12-month-old Latina, is brought by her parents to the emergency department of a Dallas, Texas, hospital. She has a fever of 39°C, a distended abdomen, some abdominal pain, and watery diarrhea. Julia is admitted to the pediatric wing of the hospital, pending results of laboratory and radiologic tests. Test results suggest peritoneal tuberculosis (TB). Caused by one of several closely related species in the *Mycobacterium tuberculosis* complex, TB is a reportable condition in the United States. Peritoneal TB is a disease of the intestines and abdominal cavity.
   What organ is usually associated with tuberculosis? How might someone get peritoneal TB?
2. Pulmonary TB is contracted by inhaling the bacteria; ingesting the bacteria can result in peritoneal TB. A laparoscopy reveals that nodules are present in Julia's abdominal cavity. A portion of a nodule is removed for biopsy so that it can be observed for the presence of acid-fast bacteria. Based on the presence of the abdominal nodules, Julia's physician begins conventional antituberculosis treatment. This long-term treatment can last up to 12 months.
   What is the next step?
3. The lab results confirm that acid-fast bacteria are indeed present in Julia's abdominal cavity. The laboratory now needs to identify the *Mycobacterium* species. Speciation of the *M. tuberculosis* complex is done by biochemical testing in reference laboratories (**Figure A**). The bacteria need to be grown in culture media. Slow-growing mycobacteria may take up to 6 weeks to form colonies.
   After colonies have been isolated, what is the next step?
4. Two weeks later, the laboratory results show that the bacteria are slow-growing. According to the identification scheme, the urease test (**Figure B**) should be performed.
   What is the result shown in Figure B?
5. Because the urease test is positive, the nitrate reduction test is performed. It shows that the bacteria do not produce the enzyme nitrate reductase. Julia's physician lets her parents know that they are very close to identifying the pathogen that is causing Julia's illness.
   What is the bacterium?

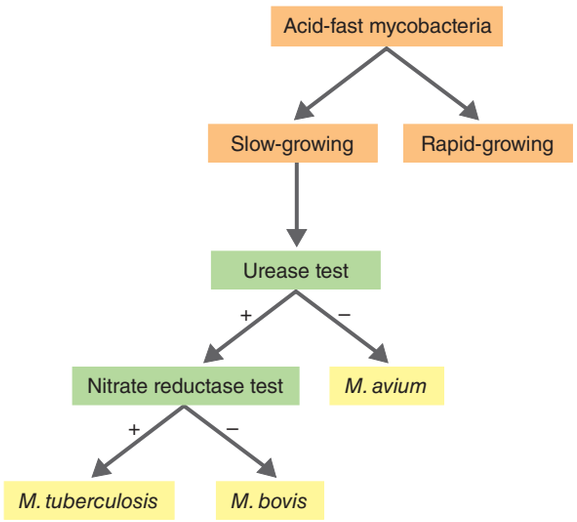


Figure 5.A An identification scheme for selected species of slow-growing mycobacteria.

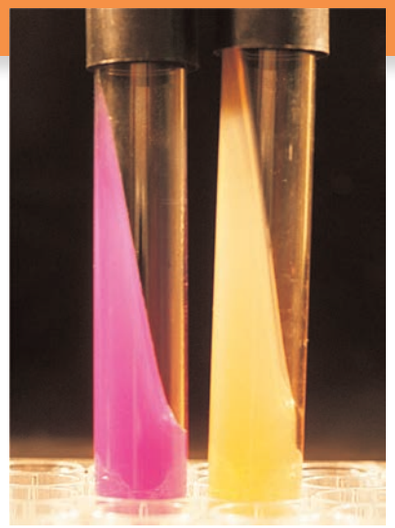


Figure 5.B The urease test. In a positive test, bacterial urease hydrolyzes urea, producing ammonia. The ammonia raises the pH, and the indicator in the medium turns to fuchsia.

6. *M. bovis* is a pathogen that primarily infects cattle. However, humans can become infected by consuming unpasteurized dairy products or inhaling infectious droplets from cattle. Human-to-human transmission occurs only rarely. The clinical and pathologic characteristics of *M. bovis* TB are indistinguishable from *M. tuberculosis* TB, but identification of the bacterium is important for prevention and treatment. Children may be at higher risk. In one study, almost half of the culture-positive pediatric TB cases were caused by *M. bovis*.

   Unfortunately, Julia does not recover from her illness. Her cadiovascular system collapses, and she dies. The official cause of death is peritoneal tuberculosis caused by *M. bovis*. Everyone should avoid consuming products from unpasteurized cow's milk, which carry the risk of transmitting *M. bovis* if imported from countries where the bacterium is common in cattle.

Source: Adapted from B. Müller et al., "Zoonotic Mycobacterium Bovis–Induced Tuberculosis in Humans," *Emerging Infectious Diseases*, 19(6), June 2013, http://dx.doi.org/10-3201/eid1906.120543.

Chemoautotrophs

Chemoautotrophs use the electrons from reduced inorganic compounds as a source of energy, and they use CO_2 as their principal source of carbon. They fix CO_2 in the Calvin-Benson Cycle (see Figure 5.26). Inorganic sources of energy for these organisms include hydrogen sulfide (H_2S) for *Beggiatoa* (BEJ-jē-ah-tō-ah); elemental sulfur (S) for *Acidithiobacillus thiooxidans;* ammonia (NH_3) for *Nitrosomonas* (NĪ-trō-sō-mō-nas); nitrite ions (NO_2^-) for *Nitrobacter* (ni'trō-BAK-ter); hydrogen gas (H_2) for *Cupriavidus* (KOO-prē-ah'vid-us); ferrous iron (Fe^{2+}) for *Acidithiobacillus ferrooxidans;* and carbon monoxide (CO) for *Pseudomonas carboxydohydrogena* (kar-box'id-ō-hī-DRO-je-nah). The energy derived

from the oxidation of these inorganic compounds is eventually stored in ATP, which is produced by oxidative phosphorylation.

Chemoheterotrophs

When we discuss photoautotrophs, photoheterotrophs, and chemoautotrophs, it's easy to categorize the energy and carbon sources because they occur as separate entities. However, in chemoheterotrophs, the distinction isn't as clear because the energy and carbon sources are usually the same organic compound—glucose, for example. **Chemoheterotrophs** specifically use the electrons from hydrogen atoms in organic compounds as their energy source.

Heterotrophs are further classified according to their source of organic molecules. **Saprophytes** live on dead organic matter, and **parasites** derive nutrients from a living host. Most bacteria, and all fungi, protozoa, and animals, are chemoheterotrophs.

Bacteria and fungi can use a wide variety of organic compounds for carbon and energy sources. This is why they can live in diverse environments. Understanding microbial diversity is scientifically interesting and economically important. In some situations microbial growth is undesirable, such as when rubber-degrading bacteria destroy a gasket or shoe sole. However, these same bacteria might be beneficial if they decomposed discarded rubber products, such as used tires. *Rhodococcus erythropolis* (rō-dō-KOK-kus er′i-THROP-ō-lis) is widely distributed in soil and can cause disease in humans and other animals. However, this species is able to replace sulfur atoms in petroleum with atoms of oxygen. Removing sulfur from crude oil is an important step in the oil-refining process. Sulfur corrodes equipment and pipelines, and contributes to acid rain, smog, and pollution-related respiratory problems in people. A Texas company is currently using *R. erythropolis* to produce desulfurized oil.

CHECK YOUR UNDERSTANDING

Almost all medically important microbes belong to which of the four aforementioned groups? **5-23**

* * *

We will next consider how cells use ATP pathways for the synthesis of organic compounds such as carbohydrates, lipids, proteins, and nucleic acids.

Metabolic Pathways of Energy Use

LEARNING OBJECTIVE

5-24 Describe the major types of anabolism and their relationship to catabolism.

Up to now we've been considering energy production. Through the oxidation of organic molecules, organisms produce energy by aerobic respiration, anaerobic respiration, and fermentation. Much of this energy is given off as heat. The complete metabolic oxidation of glucose to carbon dioxide and water is considered a very efficient process, but about 45% of the energy of glucose is lost as heat. Cells use the remaining energy, which is trapped in the bonds of ATP, in a variety of ways. Microbes use ATP to provide energy for the transport of substances across plasma membranes—the process called active transport. (See Chapter 4, page 90.) Microbes also use some of their energy for flagellar motion (also discussed in Chapter 4). Most of the ATP, however, is used in the production of new cellular components. This production is a continuous process in cells and, in general, is faster in prokaryotic cells than in eukaryotic cells.

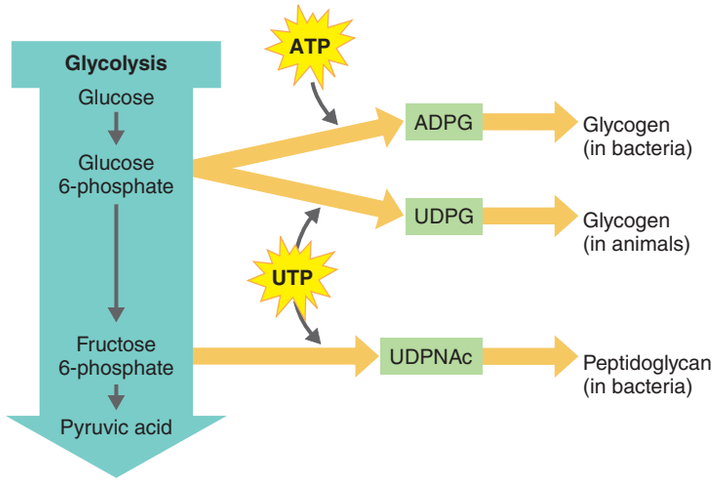


Figure 5.29 The biosynthesis of polysaccharides.

How are polysaccharides used in cells?

Autotrophs build their organic compounds by fixing carbon dioxide in the Calvin-Benson cycle (see Figure 5.26). This requires both energy (ATP) and electrons (from the oxidation of NADPH). Heterotrophs, by contrast, must have a ready source of organic compounds for biosynthesis—the production of needed cellular components, usually from simpler molecules. The cells use these compounds as both the carbon source and the energy source. We will next consider the biosynthesis of a few representative classes of biological molecules: carbohydrates, lipids, amino acids, purines, and pyrimidines. As we do so, keep in mind that synthesis reactions require a net input of energy.

Polysaccharide Biosynthesis

Microorganisms synthesize sugars and polysaccharides. The carbon atoms required to synthesize glucose are derived from the intermediates produced during processes such as glycolysis and the Krebs cycle, and from lipids or amino acids. After synthesizing glucose (or other simple sugars), bacteria may assemble it into more complex polysaccharides, such as glycogen. For bacteria to build glucose into glycogen, glucose units must be phosphorylated and linked. The product of glucose phosphorylation is glucose 6-phosphate. Such a process involves the expenditure of energy, usually in the form of ATP. In order for bacteria to synthesize glycogen, a molecule of ATP is added to glucose 6-phosphate to form *adenosine diphosphoglucose (ADPG)* (Figure 5.29). Once ADPG is synthesized, it is linked with similar units to form glycogen.

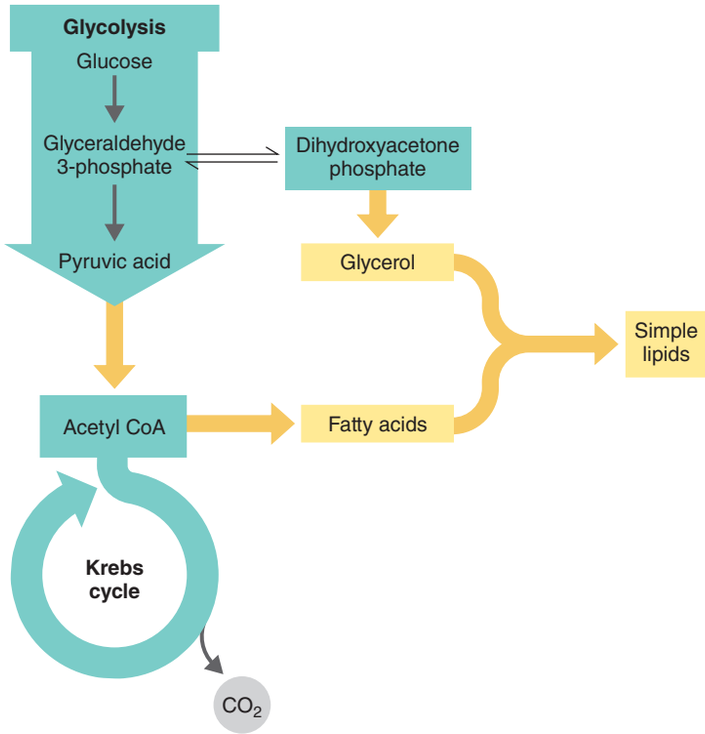


Figure 5.30 The biosynthesis of simple lipids.

What is the primary use of lipids in cells?

Using a nucleotide called uridine triphosphate (UTP) as a source of energy and glucose 6-phosphate, animals synthesize glycogen (and many other carbohydrates) from *uridine diphosphoglucose, UDPG* (see Figure 5.29). A compound related to UDPG, called *UDP-N-acetylglucosamine (UDPNAc),* is a key starting material in the biosynthesis of peptidoglycan, the substance that forms bacterial cell walls. UDPNAc is formed from fructose 6-phosphate, and the reaction also uses UTP.

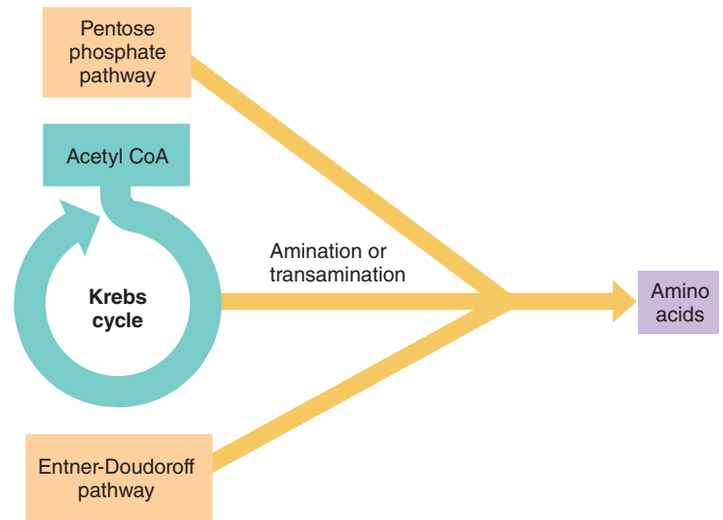
Lipid Biosynthesis

Because lipids vary considerably in chemical composition, they are synthesized by a variety of routes. Cells synthesize fats by joining glycerol and fatty acids. The glycerol portion of the fat is derived from dihydroxyacetone phosphate, an intermediate formed during glycolysis. Fatty acids, which are long-chain hydrocarbons (hydrogen linked to carbon), are built up when two-carbon fragments of acetyl CoA are successively added to each other (Figure 5.30). As with polysaccharide synthesis, the building units of fats and other lipids are linked via dehydration synthesis reactions that require energy, not always in the form of ATP.

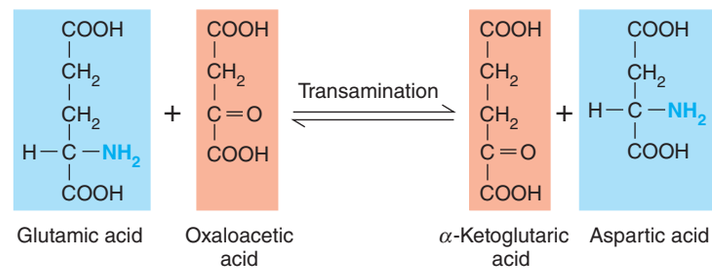
The most important role of lipids is to serve as structural components of biological membranes, and most membrane lipids are phospholipids. A lipid of a very different structure, cholesterol, is also found in plasma membranes of eukaryotic cells. Waxes are lipids that are important components of the cell wall of acid-fast bacteria. Other lipids, such as carotenoids, provide the red, orange, and yellow pigments of some microorganisms. Some lipids form portions of chlorophyll molecules. Lipids also function in energy storage. Recall that the breakdown products of lipids after biological oxidation feed into the Krebs cycle.

Amino Acid and Protein Biosynthesis

Amino acids are required for protein biosynthesis. Some microbes, such as *E. coli,* contain the enzymes necessary to use starting materials, such as glucose and inorganic salts, for the synthesis of all the amino acids they need. Organisms with the necessary enzymes can synthesize all amino acids directly or indirectly from intermediates of carbohydrate metabolism (Figure 5.31a). Other microbes require that the environment provide some preformed amino acids.



(a) Amino acid biosynthesis



(b) Process of transamination

Figure 5.31 The biosynthesis of amino acids. **(a)** Pathways of amino acid biosynthesis through amination or transamination of intermediates of carbohydrate metabolism from the Krebs cycle, pentose phosphate pathway, and Entner-Doudoroff pathway. **(b)** Transamination, a process by which new amino acids are made with the amine groups from old amino acids. Glutamic acid and aspartic acid are both amino acids; the other two compounds are intermediates in the Krebs cycle.

What is the function of amino acids in cells?

One important source of the *precursors* (intermediates) used in amino acid synthesis is the Krebs cycle. Adding an amine group to pyruvic acid or to an appropriate organic acid of the Krebs cycle converts the acid into an amino acid. This process is called **amination.** If the amine group comes from a preexisting amino acid, the process is called **transamination** (Figure 5.31b).

Most amino acids within cells are destined to be building blocks for protein synthesis. Proteins play major roles in the cell as enzymes, structural components, and toxins, to name just a few uses. The joining of amino acids to form proteins involves dehydration synthesis and requires energy in the form of ATP. The mechanism of protein synthesis involves genes and is discussed in Chapter 8.

Purine and Pyrimidine Biosynthesis

The informational molecules DNA and RNA consist of repeating units called *nucleotides,* each of which consists of a purine or pyrimidine, a pentose (five-carbon sugar), and a phosphate group. (See Chapter 2.) The five-carbon sugars of nucleotides are derived from either the pentose phosphate pathway or the Entner-Doudoroff pathway. Certain amino acids—aspartic acid, glycine, and glutamine—made from intermediates produced during glycolysis and in the Krebs cycle participate in the biosyntheses of purines and pyrimidines (**Figure 5.32**). The carbon and nitrogen atoms derived from these amino acids form the purine and pyrimidine rings, and the energy for synthesis is provided by ATP. DNA contains all the information necessary to determine the specific structures and functions of cells. Both RNA and DNA are required for protein synthesis. In addition, nucleotides such as ATP, NAD^+, and $NADP^+$ assume roles in stimulating and inhibiting the rate of cellular metabolism. The synthesis of DNA and RNA from nucleotides will be discussed in Chapter 8.

CHECK YOUR UNDERSTANDING

Where do amino acids required for protein synthesis come from? **5-24**

The Integration of Metabolism

LEARNING OBJECTIVE

5-25 Define *amphibolic pathways.*

We have seen thus far that the metabolic processes of microbes produce energy from light, inorganic compounds, and organic compounds. Reactions also occur in which energy is used for biosynthesis. With such a variety of activity, you might imagine that anabolic and catabolic reactions occur independently of each other in space and time. Actually, anabolic and catabolic reactions are joined through a group of common intermediates (identified as key intermediates in **Figure 5.33**). Both anabolic and catabolic reactions also share some metabolic pathways, such as the Krebs cycle. For example, reactions in the Krebs cycle not only participate in the oxidation of glucose but also produce intermediates that can be converted to amino acids. Metabolic pathways that function in both anabolism and catabolism are called **amphibolic pathways,** meaning that they are dual-purpose.

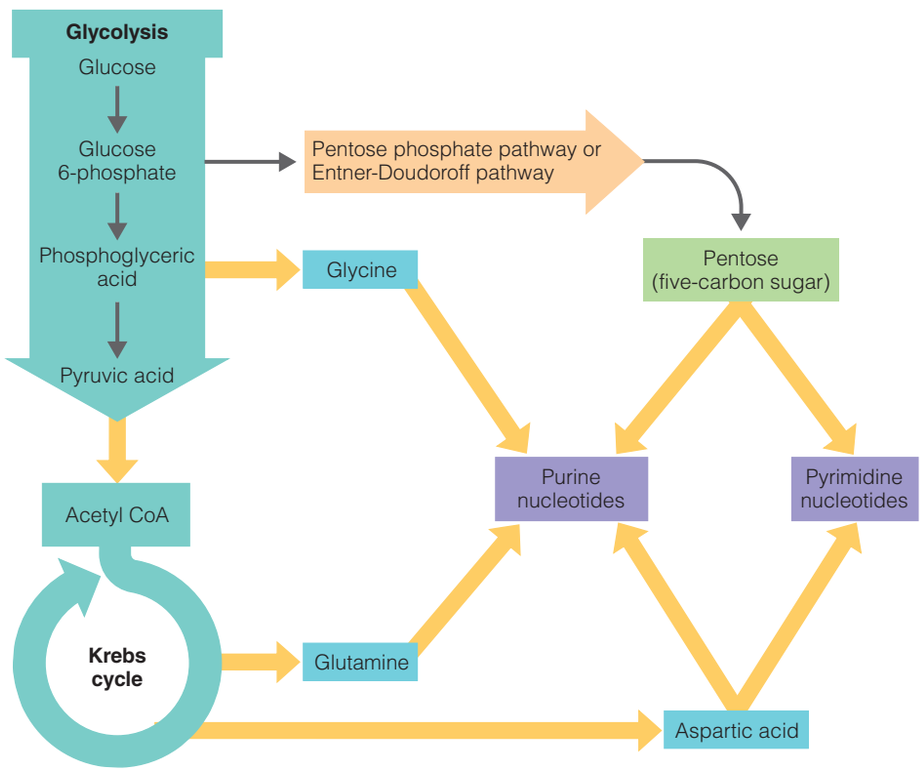


Figure 5.32 The biosynthesis of purine and pyrimidine nucleotides.

Q What are the functions of nucleotides in a cell?

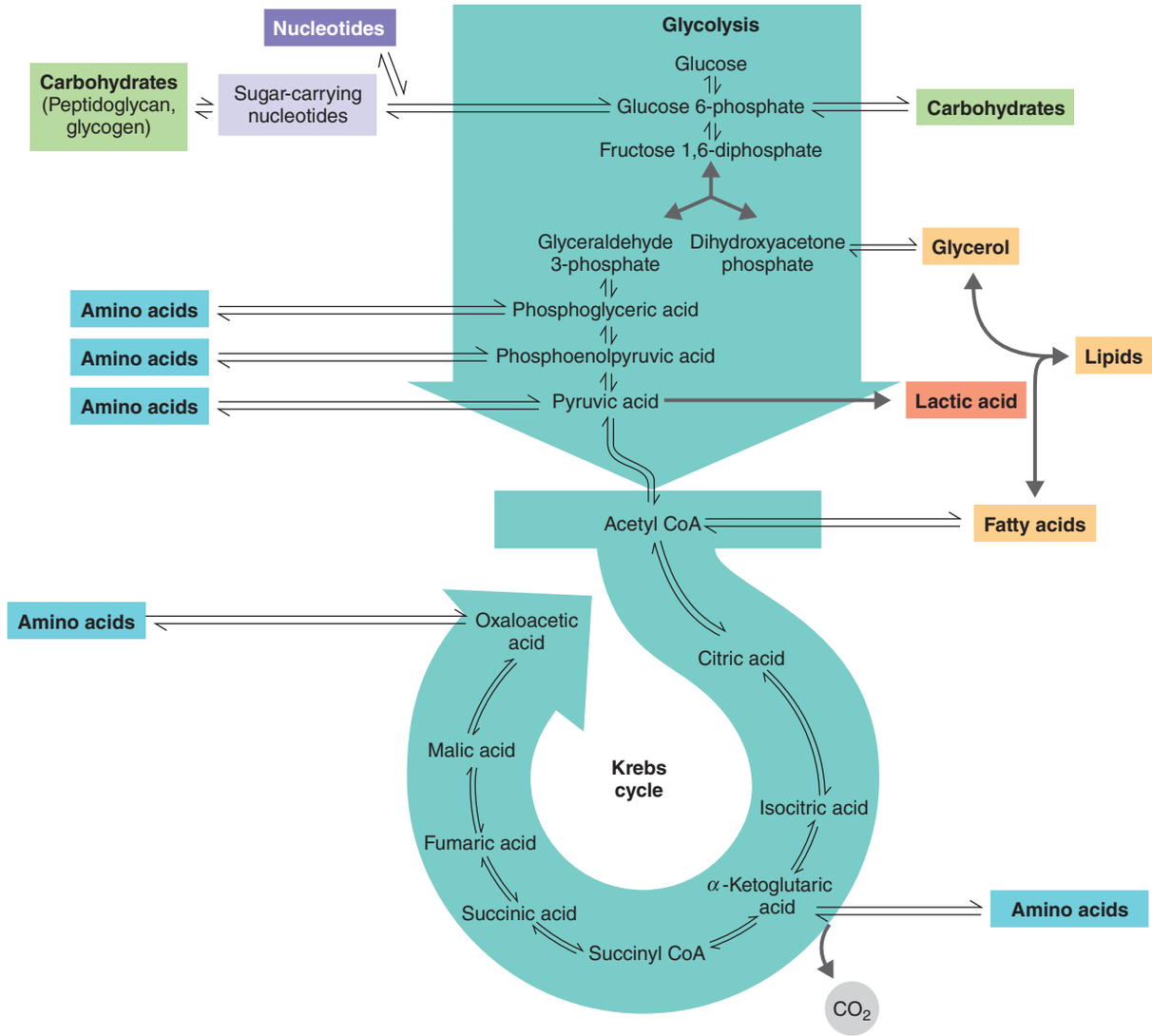


Figure 5.33 The integration of metabolism. Key intermediates are shown. Although not indicated in the figure, amino acids and ribose are used in the synthesis of purine and pyrimidine nucleotides (see Figure 5.32). The double arrows indicate amphibolic pathways.

Q What is the purpose of an amphibolic pathway?

Amphibolic pathways bridge the reactions that lead to the breakdown and synthesis of carbohydrates, lipids, proteins, and nucleotides. Such pathways enable simultaneous reactions to occur in which the breakdown product formed in one reaction is used in another reaction to synthesize a different compound, and vice versa. Because various intermediates are common to both anabolic and catabolic reactions, mechanisms exist that regulate synthesis and breakdown pathways and allow these reactions to occur simultaneously. One such mechanism involves the use of different coenzymes for opposite pathways. For example, NAD^+ is involved in catabolic reactions, whereas $NADP^+$ is involved in anabolic reactions. Enzymes can also coordinate anabolic and catabolic reactions by accelerating or inhibiting the rates of biochemical reactions.

The energy stores of a cell can also affect the rates of biochemical reactions. For example, if ATP begins to accumulate, feedback inhibition to an enzyme shuts down glycolysis; this control helps to synchronize the rates of glycolysis and the Krebs cycle. Thus, if citric acid consumption increases, either because of a demand for more ATP or because anabolic pathways are draining off intermediates of the Krebs cycle, glycolysis accelerates and meets the demand. MM **Animation** Metabolism: The Big picture

CHECK YOUR UNDERSTANDING

Summarize the integration of metabolic pathways using peptidoglycan synthesis as an example. **5-25**

Study Outline

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Catabolic and Anabolic Reactions (pp. 110–111)

1. The sum of all chemical reactions within a living organism is known as metabolism.
2. Catabolism refers to chemical reactions that result in the breakdown of more complex organic molecules into simpler substances. Catabolic reactions usually release energy.
3. Anabolism refers to chemical reactions in which simpler substances are combined to form more complex molecules. Anabolic reactions usually require energy.
4. The energy of catabolic reactions is used to drive anabolic reactions.
5. The energy for chemical reactions is stored in ATP.

Enzymes (pp. 111–117)

1. Enzymes are proteins, produced by living cells, that catalyze chemical reactions by lowering the activation energy.
2. Enzymes are generally globular proteins with characteristic three-dimensional shapes.
3. Enzymes are efficient, can operate at relatively low temperatures, and are subject to various cellular controls.
4. When an enzyme and substrate combine, the substrate is transformed, and the enzyme is recovered.
5. Enzymes are characterized by specificity, which is a function of their active sites.

Naming Enzymes (p. 113)

6. Enzyme names usually end in *-ase*.
7. The six classes of enzymes are defined on the basis of the types of reactions they catalyze.

Enzyme Components (pp. 113–114)

8. Most enzymes are holoenzymes, consisting of a protein portion (apoenzyme) and a nonprotein portion (cofactor).
9. The cofactor can be a metal ion (iron, copper, magnesium, manganese, zinc, calcium, or cobalt) or a complex organic molecule known as a coenzyme (NAD^+, $NADP^+$, FMN, FAD, or coenzyme A).

Factors Influencing Enzymatic Activity (pp. 114–116)

10. At high temperatures, enzymes undergo denaturation and lose their catalytic properties; at low temperatures, the reaction rate decreases.
11. The pH at which enzymatic activity is maximal is known as the optimum pH.
12. Enzymatic activity increases as substrate concentration increases until the enzymes are saturated.
13. Competitive inhibitors compete with the normal substrate for the active site of the enzyme. Noncompetitive inhibitors act on other parts of the apoenzyme or on the cofactor and decrease the enzyme's ability to combine with the normal substrate.

Feedback Inhibition (pp. 116–117)

14. Feedback inhibition occurs when the end-product of a metabolic pathway inhibits an enzyme's activity near the start of the pathway.

Ribozymes (p. 117)

15. Ribozymes are enzymatic RNA molecules involved in protein synthesis.

Energy Production (pp. 117–119)

Oxidation-Reduction Reactions (p. 117–118)

1. Oxidation is the removal of one or more electrons from a substrate. Protons (H^+) are often removed with the electrons.
2. Reduction of a substrate refers to its gain of one or more electrons.
3. Each time a substance is oxidized, another is simultaneously reduced.
4. NAD^+ is the oxidized form; NADH is the reduced form.
5. Glucose is a reduced molecule; energy is released during a cell's oxidation of glucose.

The Generation of ATP (pp. 118–119)

6. Energy released during certain metabolic reactions can be trapped to form ATP from ADP and P_i (phosphate). Addition of a P_i molecule is called phosphorylation.
7. During substrate-level phosphorylation, a high-energy P from an intermediate in catabolism is added to ADP.
8. During oxidative phosphorylation, energy is released as electrons are passed to a series of electron acceptors (an electron transport chain) and finally to O_2 or another inorganic compound.
9. During photophosphorylation, energy from light is trapped by chlorophyll, and electrons are passed through a series of electron acceptors. The electron transfer releases energy used for the synthesis of ATP.

Metabolic Pathways of Energy Production (p. 119)

10. A series of enzymatically catalyzed chemical reactions called metabolic pathways store energy in and release energy from organic molecules.

Carbohydrate Catabolism (p. 119–131) interactivemicrobiology

See how aerobic respiration affects a patient's health @MasteringMicrobiology®.

1. Most of a cell's energy is produced from the oxidation of carbohydrates.
2. The two major types of carbohydrate catabolism are respiration, in which a sugar is completely broken down, and fermentation, in which the sugar is partially broken down.

Glycolysis (p. 121)

3. The most common pathway for the oxidation of glucose is glycolysis. Pyruvic acid is the end-product.
4. Two ATP and two NADH molecules are produced from one glucose molecule.

Additional Pathways to Glycolysis (p. 121)

5. The pentose phosphate pathway is used to oxidize five-carbon sugars; one ATP and 12 NADPH molecules are produced from one glucose molecule.
6. The Entner-Doudoroff pathway yields one ATP and two NADPH molecules from oxidation of one glucose molecule.

Cellular Respiration (pp. 121–127)

7. During respiration, organic molecules are oxidized. Energy is generated from oxidation of the electron transport chain.
8. In aerobic respiration, O_2 functions as the final electron acceptor.
9. In anaerobic respiration, the final electron acceptor is not O_2; the electron acceptors in anaerobic respiration include NO_3^-, SO_4^{2-}, and CO_3^{2-}.
10. Decarboxylation of pyruvic acid produces one CO_2 molecule and one acetyl group.
11. Two-carbon acetyl groups are oxidized in the Krebs cycle. Electrons are picked up by NAD^+ and FAD for the electron transport chain.
12. From one molecule of glucose, oxidation produces six molecules of NADH, two molecules of $FADH_2$, and two molecules of ATP.
13. Decarboxylation produces six molecules of CO_2.
14. NADH and $FADH_2$ carry electrons to the electron transport chain by NADH.
15. The electron transport chain consists of carriers, including flavoproteins, cytochromes, and ubiquinones.
16. Protons being pumped across the membrane generate a proton motive force as electrons move through a series of acceptors or carriers.
17. Energy produced from movement of the protons back across the membrane is used by ATP synthase to make ATP from ADP and P_i.
18. In eukaryotes, electron carriers are located in the inner mitochondrial membrane; in prokaryotes, electron carriers are in the plasma membrane.
19. In aerobic prokaryotes, 38 ATP molecules can be produced from complete oxidation of a glucose molecule in glycolysis, the Krebs cycle, and the electron transport chain.
20. In eukaryotes, 36 ATP molecules are produced from complete oxidation of a glucose molecule.
21. The total ATP yield in anaerobic respiration is less than in aerobic respiration because only part of the Krebs cycle operates under anaerobic conditions.

Fermentation (pp. 127–131)

22. Fermentation releases energy from sugars or other organic molecules by oxidation.
23. O_2 is not required in fermentation.
24. Two ATP molecules are produced by substrate-level phosphorylation.
25. Electrons removed from the substrate reduce NAD^+.
26. The final electron acceptor is an organic molecule.
27. In lactic acid fermentation, pyruvic acid is reduced by NADH to lactic acid.
28. In alcohol fermentation, acetaldehyde is reduced by NADH to produce ethanol.
29. Heterolactic fermenters can use the pentose phosphate pathway to produce lactic acid and ethanol.

Lipid and Protein Catabolism (p. 131)

1. Lipases hydrolyze lipids into glycerol and fatty acids.
2. Fatty acids and other hydrocarbons are catabolized by beta-oxidation.
3. Catabolic products can be further broken down in glycolysis and the Krebs cycle.
4. Before amino acids can be catabolized, they must be converted to various substances that enter the Krebs cycle.
5. Transamination, decarboxylation, and desulfurization reactions convert the amino acids to be catabolized.

Biochemical Tests and Bacterial Identification (pp. 131–133)

1. Bacteria and yeast can be identified by detecting action of their enzymes.
2. Fermentation tests are used to determine whether an organism can ferment a carbohydrate to produce acid and gas.

Photosynthesis (pp. 133–135)

1. Photosynthesis is the conversion of light energy from the sun into chemical energy; the chemical energy is used for carbon fixation.

The Light-Dependent Reactions: Photophosphorylation (pp. 134–135)

2. Chlorophyll *a* is used by green plants, algae, and cyanobacteria.
3. Electrons from chlorophyll pass through an electron transport chain, from which ATP is produced by chemiosmosis.
4. Photosystems are made up of chlorophyll and other pigments packed into thylakoid membranes.
5. In cyclic photophosphorylation, the electrons return to the chlorophyll.
6. In noncyclic photophosphorylation, the electrons are used to reduce $NADP^+$. The electrons from H_2O or H_2S replace those lost from chlorophyll.
7. When H_2O is oxidized by green plants, algae, and cyanobacteria, O_2 is produced; when H_2S is oxidized by the sulfur bacteria, S^0 granules are produced.

The Light-Independent Reactions: The Calvin-Benson Cycle (p. 135)

8. CO_2 is used to synthesize sugars in the Calvin-Benson cycle.

A Summary of Energy Production Mechanisms (pp. 135–136)

1. Sunlight is converted to chemical energy in oxidation reactions carried on by phototrophs. Chemotrophs can use this chemical energy.
2. In oxidation delete reactions, energy is derived from the transfer of electrons.
3. To produce energy, a cell needs an electron donor (organic or inorganic), a system of electron carriers, and a final electron acceptor (organic or inorganic).

Metabolic Diversity among Organisms (pp. 136–140)

1. Photoautotrophs obtain energy by photophosphorylation and fix carbon from CO_2 via the Calvin-Benson cycle to synthesize organic compounds.
2. Cyanobacteria are oxygenic phototrophs. Green bacteria and purple bacteria are anoxygenic phototrophs.
3. Photoheterotrophs use light as an energy source and an organic compound for their carbon source and electron donor.

4. Chemoautotrophs use inorganic compounds as their energy source and carbon dioxide as their carbon source.
5. Chemoheterotrophs use complex organic molecules as their carbon and energy sources.

Metabolic Pathways of Energy Use (pp. 140–142)

Polysaccharide Biosynthesis (pp. 140–141)

1. Glycogen is formed from ADPG.
2. UDPNAc is the starting material for the biosynthesis of peptidoglycan.

Lipid Biosynthesis (p. 141)

3. Lipids are synthesized from fatty acids and glycerol.
4. Glycerol is derived from dihydroxyacetone phosphate, and fatty acids are built fro m acetyl CoA.

Amino Acid and Protein Biosynthesis (pp. 141–142)

5. Amino acids are required for protein biosynthesis.
6. All amino acids can be synthesized either directly or indirectly from intermediates of carbohydrate metabolism, particularly from the Krebs cycle.

Purine and Pyrimidine Biosynthesis (p. 142)

7. The sugars composing nucleotides are derived from either the pentose phosphate pathway or the Entner-Doudoroff pathway.
8. Carbon and nitrogen atoms from certain amino acids form the backbones of the purines and pyrimidines.

The Integration of Metabolism (pp. 142–143)

1. Anabolic and catabolic reactions are integrated through a group of common intermediates.
2. Such integrated metabolic pathways are referred to as amphibolic pathways.

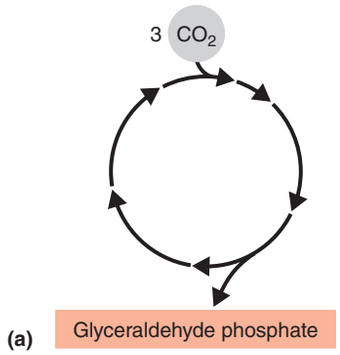
Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

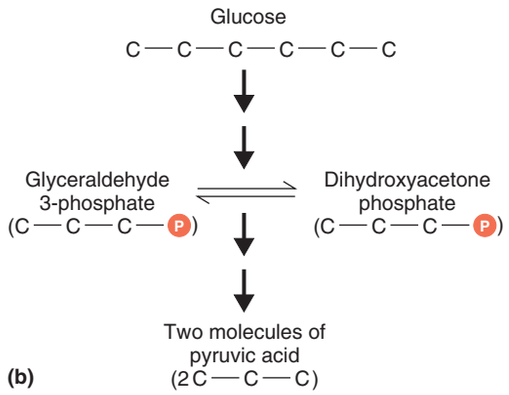
Knowledge and Comprehension

Review

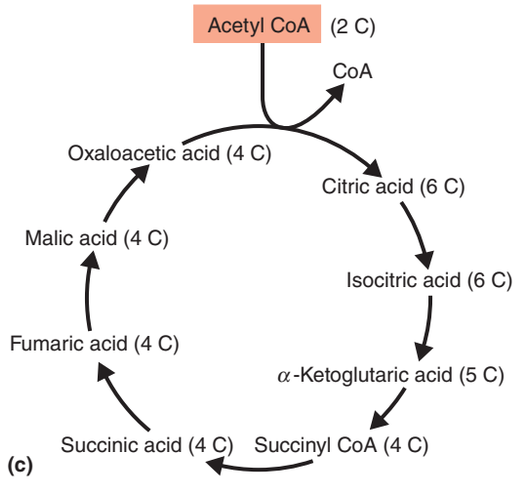
Use the following diagrams (a), (b), and (c) for question 1.



(a)

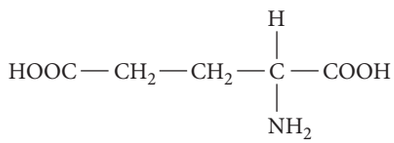


(b)



(c)

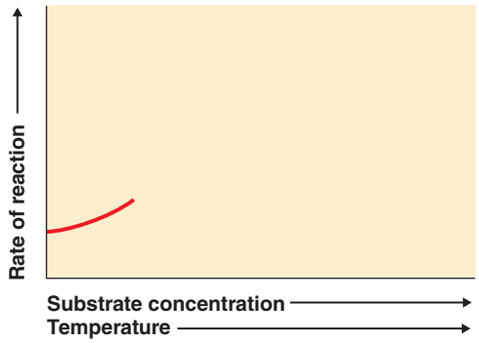
1. Name pathways diagrammed in parts (a), (b), and (c) of the figure.
   - **a.** Show where glycerol is catabolized and where fatty acids are catabolized.
   - **b.** Show where glutamic acid (an amino acid) is catabolized:



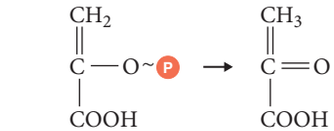
   - **c.** Show how these pathways are related.
   - **d.** Where is ATP required in pathways (a) and (b)?
   - **e.** Where is CO_2 released in pathways (b) and (c)?
   - **f.** Show where a long-chain hydrocarbon such as petroleum is catabolized.
   - **g.** Where is NADH (or $FADH_2$ or NADPH) used and produced in these pathways?
   - **h.** Identify four places where anabolic and catabolic pathways are integrated.

2. DRAW IT Using the diagrams below, show each of the following:
   a. where the substrate will bind
   b. where the competitive inhibitor will bind
   c. where the noncompetitive inhibitor will bind
   d. which of the four elements could be the inhibitor in feedback inhibition
   e. What effect will the reactions in (a), (b), and (c) have?

3. DRAW IT An enzyme and substrate are combined. The rate of reaction begins as shown in the following graph. To complete the graph, show the effect of increasing substrate concentration on a constant enzyme concentration. Show the effect of increasing temperature.



4. Define *oxidation-reduction,* and differentiate the following terms:
   a. aerobic and anaerobic respiration
   b. respiration and fermentation
   c. cyclic and noncyclic photophosphorylation
5. There are three mechanisms for the phosphorylation of ADP to produce ATP. Write the name of the mechanism that describes each of the reactions in the following table.

ATP Generated by	Reaction
a. _____	An electron, liberated from chlorophyll by light, is passed down an electron transport chain.
b. _____	Cytochrome *c* passes two electrons to cytochrome *a*.
c. _____	

Phosphoenolpyruvic acid → Pyruvic acid

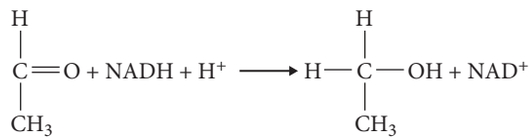
6. All of the energy-producing biochemical reactions that occur in cells, such as photophosphorylation and glycolysis, are _____ reactions.
7. Fill in the following table with the carbon source and energy source of each type of organism.

Organism	Carbon Source	Energy Source
Photoautotroph	a. _____	b. _____
Photoheterotroph	c. _____	d. _____
Chemoautotroph	e. _____	f. _____
Chemoheterotroph	g. _____	h. _____

8. Write your own definition of the chemiosmotic mechanism of ATP generation. On Figure 5.16, mark the following using the appropriate letter:
   a. the acidic side of the membrane
   b. the side with a positive electrical charge
   c. potential energy
   d. kinetic energy
9. Why must NADH be reoxidized? How does this happen in an organism that uses respiration? Fermentation?
10. NAME IT What nutritional type is a colorless microbe that uses the Calvin cycle, uses H_2 as the electron donor to its ETC, and uses elemental S as the final electron acceptor in the ETC?

Multiple Choice

1. Which substance in the following reaction is being reduced?



Acetaldehyde → Ethanol

   a. acetaldehyde
   b. NADH
   c. ethanol
   d. NAD^+
2. Which of the following reactions produces the most molecules of ATP during aerobic metabolism?
   a. glucose → glucose 6-phosphate
   b. phosphoenolpyruvic acid → pyruvic acid
   c. glucose → pyruvic acid
   d. acetyl CoA → $CO_2 + H_2O$
   e. succinic acid → fumaric acid
3. Which of the following processes does *not* generate ATP?
   a. photophosphorylation
   b. the Calvin-Benson cycle
   c. oxidative phosphorylation
   d. substrate-level phosphorylation
   e. All of the above generate ATP
4. Which of the following compounds has the greatest amount of energy for a cell?
   a. CO_2
   b. ATP
   c. glucose
   d. O_2
   e. lactic acid

5. Which of the following is the best definition of the Krebs cycle?
   a. the oxidation of pyruvic acid
   b. the way cells produce CO_2
   c. a series of chemical reactions in which NADH is produced from the oxidation of pyruvic acid
   d. a method of producing ATP by phosphorylating ADP
   e. a series of chemical reactions in which ATP is produced from the oxidation of pyruvic acid
6. Which of the following is the best definition of *respiration*?
   a. a sequence of carrier molecules with O_2 as the final electron acceptor
   b. a sequence of carrier molecules with an inorganic molecule as the final electron acceptor
   c. a method of generating ATP
   d. the complete oxidation of glucose to CO_2 and H_2O
   e. a series of reactions in which pyruvic acid is oxidized to CO_2 and H_2O

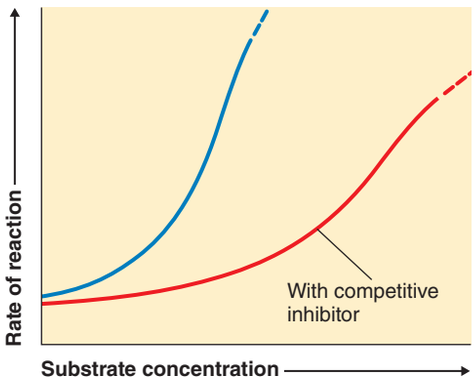
Use the following choices to answer questions 7–10.

   a. *E. coli* growing in glucose broth at 35°C with O_2 for 5 days
   b. *E. coli* growing in glucose broth at 35°C without O_2 for 5 days
   c. both a and b
   d. neither a nor b

7. Which culture produces the most lactic acid?
8. Which culture produces the most ATP?
9. Which culture uses NAD^+?
10. Which culture uses the most glucose?

Analysis

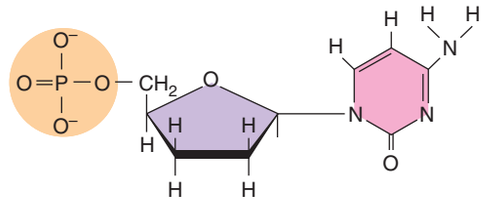
1. Explain why, even under ideal conditions, *Streptococcus* grows slowly.
2. The following graph shows the normal rate of reaction of an enzyme and its substrate (blue) and the rate when an excess of competitive inhibitor is present (red). Explain why the graph appears as it does.



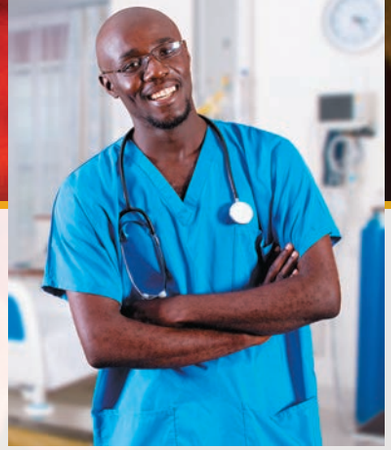
3. Compare and contrast carbohydrate catabolism and energy production in the following bacteria:
   a. *Pseudomonas*, an aerobic chemoheterotroph
   b. *Spirulina*, an oxygenic photoautotroph
   c. *Ectothiorhodospira*, an anoxygenic photoautotroph
4. How much ATP could be obtained from the complete oxidation of one molecule of glucose? From one molecule of butterfat containing one glycerol and three 12-carbon chains?
5. The chemoautotroph *Acidithiobacillus* can obtain energy from the oxidation of arsenic ($As^{3+} \rightarrow As^{5+}$). How does this reaction provide energy? How can humans put this bacterium to use?

Clinical Applications and Evaluation

1. *Haemophilus influenzae* requires hemin (X factor) to synthesize cytochromes and NAD^+ (V factor) from other cells. For what does it use these two growth factors? What diseases does *H. influenzae* cause?
2. The drug Hivid, also called ddC, inhibits DNA synthesis. It is used to treat HIV infection and AIDS. Compare the following illustration of ddC to the structure of DNA nucleotides in Figure 2.16 on page 44. How does this drug work?



3. The bacterial enzyme streptokinase is used to digest fibrin (blood clots) in patients with atherosclerosis. Why doesn't injection of streptokinase cause a streptococcal infection? How do we know the streptokinase will digest fibrin only and not good tissues?



In the Clinic

As a nurse in a plastic surgery clinic, you instruct patients on postsurgical care of their sutures. You tell patients to wash hands before removing bandages, to wash gently around the surgical site with soap and water, and to swab the wound with hydrogen peroxide. **One day a patient calls, alarmed that the hydrogen peroxide caused her wound to bubble.**

What would you tell the patient?

Hint: Read about catalase on page 155.

NOTE: Answers to In the Clinic questions are found online at MasteringMicrobiology

6 Microbial Growth

When we talk about microbial growth, we are really referring to the *number* of cells, not the *size* of the cells. Microbes that are "growing" are increasing in number, accumulating into *colonies* (groups of cells large enough to be seen without a microscope) of hundreds of thousands of cells or *populations* of billions of cells. Although individual cells approximately double in size during their lifetime, this change is not very significant compared with the size increases observed during the lifetime of plants and animals.

Many bacteria survive and grow slowly in nutrient-poor environments by forming biofilms. The *Serratia marcescens* bacteria in the photo may form biofilms on urinary catheters or on contact lenses. Biofilms are frequently sources of health care associated infections such as the one described in the Clinical Case.

Microbial populations can become incredibly large in a very short time. By understanding the conditions necessary for microbial growth, we can determine how to control the growth of microbes that cause diseases and food spoilage. We can also learn how to encourage the growth of helpful microbes and those we wish to study.

In this chapter we will examine the physical and chemical requirements for microbial growth, the various kinds of culture media, bacterial cell division, the phases of microbial growth, and the methods of measuring microbial growth.

***Serratia marcescens* on a cracker. This gram-negative rod produces the pigment prodigiosin, causing bright red colonies when the bacteria grow at room temperature.**

The Requirements for Growth

LEARNING OBJECTIVES

- **6-1** Classify microbes into five groups on the basis of preferred temperature range.
- **6-2** Identify how and why the pH of culture media is controlled.
- **6-3** Explain the importance of osmotic pressure to microbial growth.
- **6-4** Name a use for each of the four elements (carbon, nitrogen, sulfur, and phosphorus) needed in large amounts for microbial growth.
- **6-5** Explain how microbes are classified on the basis of oxygen requirements.
- **6-6** Identify ways in which aerobes avoid damage by toxic forms of oxygen.

ASM: The survival and growth of any microorganism in a given environment depends on its metabolic characteristics.

The requirements for microbial growth can be divided into two main categories: physical and chemical. Physical aspects include temperature, pH, and osmotic pressure. Chemical requirements include sources of carbon, nitrogen, sulfur, phosphorus, oxygen, trace elements, and organic growth factors.

Physical Requirements

Temperature

Most microorganisms grow well at the temperatures that humans favor. However, certain bacteria are capable of growing at extremes of temperature that would certainly hinder the survival of almost all eukaryotic organisms.

Microorganisms are classified into three primary groups on the basis of their preferred range of temperature: **psychrophiles** (cold-loving microbes), **mesophiles** (moderate-temperature–loving microbes), and **thermophiles** (heat-loving microbes).

Clinical Case: Glowing in the Dark

Reginald MacGruder, an investigator at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, has a mystery on his hands. Earlier this year, he was involved in the recall of an intravenous heparin solution that was blamed for causing *Pseudomonas fluorescens* bloodstream infections in patients in four different states. It seemed that everything was under control, but now, three months after the recall, 19 patients in two other states develop the same *P. fluorescens* bloodstream infections. It makes no sense to Dr. MacGruder; how could this infection be popping up again so soon after the recall? Could another heparin batch be tainted?

What is *P. fluorescens*? Read on to find out.

150 162 170 172

Most bacteria grow only within a limited range of temperatures, and their maximum and minimum growth temperatures are only about 30°C apart. They grow poorly at the high and low temperature extremes within their range.

Each bacterial species grows at particular minimum, optimum, and maximum temperatures. The **minimum growth temperature** is the lowest temperature at which the species will grow. The **optimum growth temperature** is the temperature at which the species grows best. The **maximum growth temperature** is the highest temperature at which growth is possible. By graphing the growth response over a temperature range, we can see that the optimum growth temperature is usually near the top of the range; above that temperature the rate of growth drops off rapidly (Figure 6.1). This happens presumably

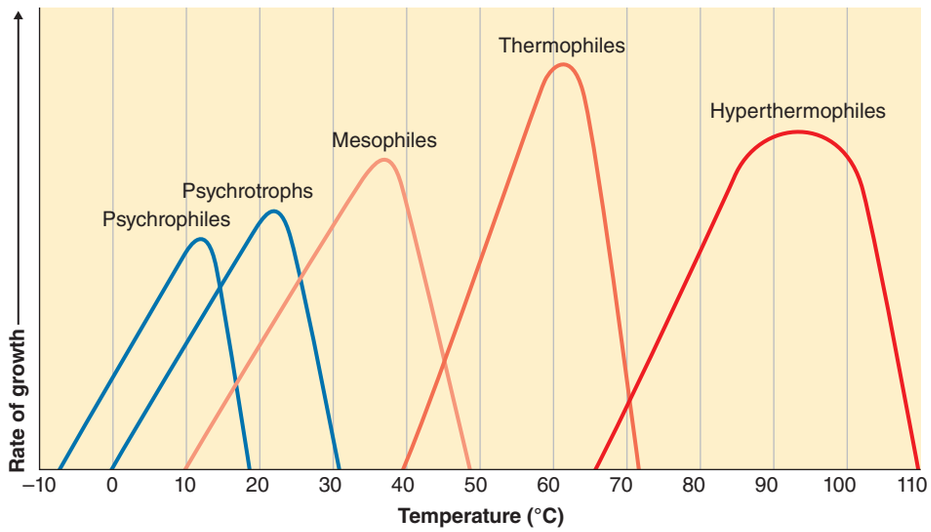


Figure 6.1 Typical growth rates of different types of microorganisms in response to temperature. The peak of the curve represents optimum growth (fastest reproduction). Notice that the reproductive rate drops off very quickly at temperatures only a little above the optimum. At either extreme of the temperature range, the reproductive rate is much lower than the rate at the optimum temperature.

Q Why is it difficult to define *psychrophile, mesophile,* and *thermophile*?

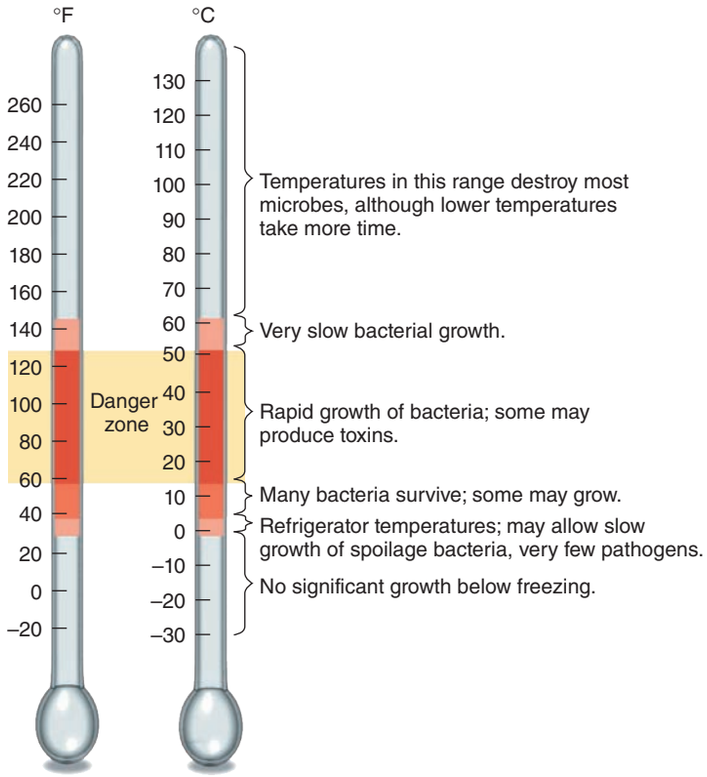


Figure 6.2 Food preservation temperatures. Low temperatures decrease microbial reproduction rates, which is the basic principle of refrigeration. There are always some exceptions to the temperature responses shown here; for example, certain bacteria grow well at high temperatures that would kill most bacteria, and a few bacteria can actually grow at temperatures well below freezing.

Q Which bacterium would theoretically be more likely to grow at refrigerator temperatures: a human intestinal pathogen or a soilborne plant pathogen?

because the high temperature has inactivated necessary enzymatic systems of the cell.

The ranges and maximum growth temperatures that define bacteria as psychrophiles, mesophiles, or thermophiles are not rigidly defined. Psychrophiles, for example, were originally considered simply to be organisms capable of growing at 0°C. However, there seem to be two fairly distinct groups capable of growth at that temperature. One group, composed of psychrophiles in the strictest sense, can grow at 0°C but has an optimum growth temperature of about 15°C. Most of these organisms are so sensitive to higher temperatures that they will not even grow in a reasonably warm room (25°C). Found mostly in the oceans' depths or in certain polar regions, such organisms seldom cause problems in food preservation. The other group that can grow at 0°C has higher optimum temperatures, usually 20–30°C and cannot grow above about 40°C. Organisms of this type are much more common than psychrophiles and are the most likely to be encountered in low-temperature food spoilage because they grow fairly well at refrigerator temperatures. We will use the term **psychrotrophs,** which food microbiologists favor, for this group of spoilage microorganisms.

Refrigeration is the most common method of preserving household food supplies. It is based on the principle that microbial reproductive rates decrease at low temperatures. Although microbes usually survive even subfreezing temperatures (they might become entirely dormant), they gradually decline in number. Some species decline faster than others. Psychrotrophs do not grow well at low temperatures, except in comparison with other organisms; given time, however, they are able to slowly degrade food. Such spoilage might take the form of mold mycelium, slime on food surfaces, or off-tastes or off-colors in foods. The temperature inside a properly set refrigerator will greatly slow the growth of most spoilage organisms and will entirely prevent the growth of all but a few pathogenic bacteria. **Figure 6.2** illustrates the importance of low temperatures for preventing the growth of spoilage and disease organisms. When large amounts of food must be refrigerated, it is important to remember that a large quantity of warm food cools at a relatively slow rate (**Figure 6.3**).

Mesophiles, with an optimum growth temperature of 25–40°C, are the most common type of microbe. Organisms that have adapted to live in the bodies of animals usually have an optimum temperature close to that of their hosts. The optimum

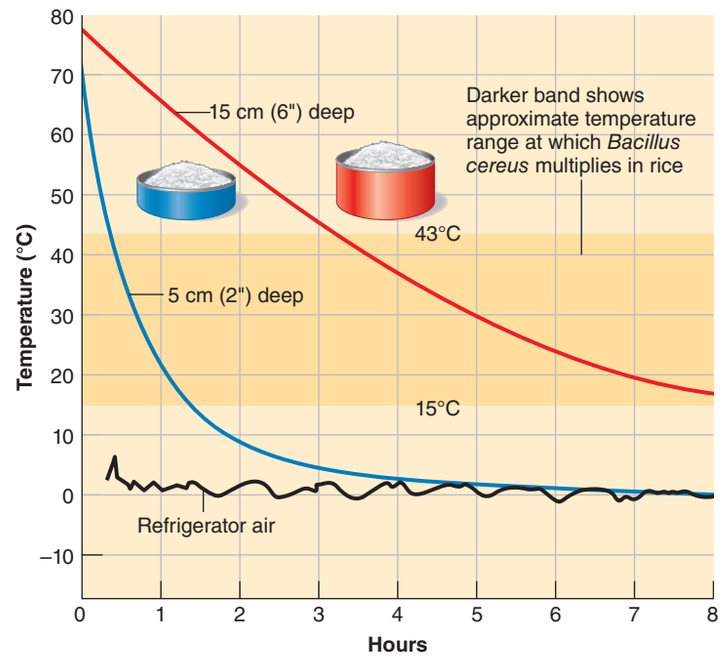
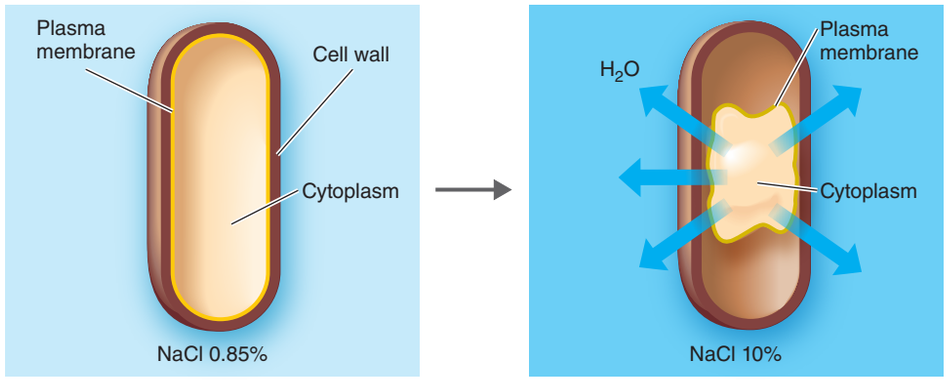


Figure 6.3 The effect of the amount of food on its cooling rate in a refrigerator and its chance of spoilage. Notice that in this example, the pan of rice with a depth of 5 cm (2 in) cooled through the incubation temperature range of the *Bacillus cereus* in about 1 hour, whereas the pan of rice with a depth of 15 cm (6 in) remained in this temperature range for about 5 hours.

Q Given a shallow pan and a deep pot with the same volume, which would cool faster? Why?



(a) Cell in isotonic solution. Under these conditions, the solute concentration in the cell is equivalent to a solute concentration of 0.85% sodium chloride (NaCl).

(b) Plasmolyzed cell in hypertonic solution. If the concentration of solutes such as NaCl is higher in the surrounding medium than in the cell (the environment is hypertonic), water tends to leave the cell. Growth of the cell is inhibited.

Figure 6.4 Plasmolysis.

Q Why is osmotic pressure an important factor in microbial growth?

temperature for many pathogenic bacteria is about 37°C, and incubators for clinical cultures are usually set at about this temperature. The mesophiles include most of the common spoilage and disease organisms.

Thermophiles are microorganisms capable of growth at high temperatures. Many of these organisms have an optimum growth temperature of 50–60°C, about the temperature of water from a hot water tap. Such temperatures can also be reached in sunlit soil and in thermal waters such as hot springs. Remarkably, many thermophiles cannot grow at temperatures below about 45°C. Endospores formed by thermophilic bacteria are unusually heat resistant and may survive the usual heat treatment given canned goods. Although elevated storage temperatures may cause surviving endospores to germinate and grow, thereby spoiling the food, these thermophilic bacteria are not considered a public health problem. Thermophiles are important in organic compost piles (see Figure 27.8 on page 779), in which the temperature can rise rapidly to 50–60°C.

Some microbes, members of the Archaea (page 4), have an optimum growth temperature of 80°C or higher. These organisms are called **hyperthermophiles,** or sometimes **extreme thermophiles.** Most of these organisms live in hot springs associated with volcanic activity, and sulfur is usually important in their metabolic activity. The known record for bacterial growth and replication at high temperatures is about 121°C near deep-sea hydrothermal vents. See the Applications of Microbiology box "Life in the Extreme." The immense pressure in the ocean depths prevents water from boiling even at temperatures well above 100°C.

pH

Recall from Chapter 2 (pages 33–34) that the pH refers to the acidity or alkalinity of a solution. Most bacteria grow best in a narrow pH range near neutrality, between pH 6.5 and 7.5. Very few bacteria grow at an acidic pH below about pH 4. This is why a number of foods, such as sauerkraut, pickles, and many cheeses, are preserved from spoilage by acids produced by bacterial fermentation. Nonetheless, some bacteria, called **acidophiles,** are remarkably tolerant of acidity. One type of chemoautotrophic bacteria, which is found in the drainage water from coal mines and oxidizes sulfur to form sulfuric acid, can survive at a pH 1. Molds and yeasts will grow over a greater pH range than bacteria will, but the optimum pH of molds and yeasts is generally below that of bacteria, usually about pH 5 to 6. Alkalinity also inhibits microbial growth but is rarely used to preserve foods.

When bacteria are cultured in the laboratory, they often produce acids that eventually interfere with their own growth. To neutralize the acids and maintain the proper pH, chemical buffers are included in the growth medium. The peptones and amino acids in some media act as buffers, and many media also contain phosphate salts. Phosphate salts have the advantage of exhibiting their buffering effect in the pH growth range of most bacteria. They are also nontoxic; in fact, they provide phosphorus, an essential nutrient.

Osmotic Pressure

Microorganisms obtain almost all their nutrients in solution from the surrounding water. Thus, they require water for growth, and their composition is 80–90% water. High osmotic pressures have the effect of removing necessary water from a cell. When a microbial cell is in a solution whose concentration of solutes is higher than in the cell (the environment is *hypertonic* to the cell), the cellular water passes out through the plasma membrane to the high solute concentration. (See the discussion of osmosis in Chapter 4, pages 88–89, and review Figure 4.18 for the three types of solution environments a cell may encounter.) This osmotic loss of water causes **plasmolysis,** or shrinkage of the cell's cytoplasm (Figure 6.4).

Life in the Extreme

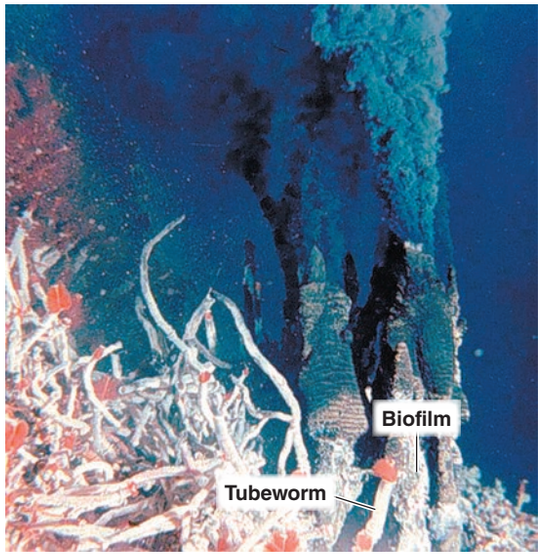
Until humans explored the deep-ocean floor, scientists believed that only a few forms of life could survive in that high-pressure, completely dark, oxygen-poor environment. Then, in 1977, *Alvin,* the deep-sea submersible, carried two scientists 2600 meters below the surface at the Galápagos Rift (about 350 km northeast of the Galápagos Islands). There, amid the vast expanse of barren basalt rocks, the scientists found unexpectedly rich oases of life. Superheated water from beneath the seafloor was rising through fractures in the Earth's crust called vents. They discovered that mats of bacteria were growing along the sides of the vents, where temperatures exceeded 100°C (see the figure).

Ecosystem of the Hydrothermal Vents

Life at the surface of the world's oceans depends on photosynthetic organisms, such as plants and algae, which harness the sun's energy to fix carbon dioxide (CO_2) to make carbohydrates. At the deep-ocean floor, where no light penetrates, photosynthesis is not possible. The scientists found that the primary producers at the ocean floor are chemoautotrophic bacteria. Using chemical energy from hydrogen sulfide (H_2S) as a source of energy to fix CO_2, the chemoautotrophs create an environment that supports higher life forms. Hydrothermal vents in the seafloor supply the H_2S and CO_2.

New Products from Hydrothermal Vents

Terrestrial fungi and bacteria have had a major impact on the development of biotechnology. Hydrothermal vents are the next frontier in the hunt for new products. In 2010 a peptide produced by *Thermovibrio ammonificans* was shown to induce apoptosis (cell death) and thus potential anticancer activity. Researchers are growing *Pyrococcus furiosus* because it produces alternative fuels, hydrogen gas and butanol. DNA polymerases (enzymes that synthesize DNA) isolated from two archaea living near deep-sea vents are being used in the polymerase chain reaction (PCR), a technique for making many copies of DNA. In PCR, single-stranded DNA is made by heating a chromosome fragment to 98°C and cooling it so that DNA polymerase can copy each strand. DNA polymerases from *Thermococcus litoralis,* called $Vent_R$, and from *Pyrococcus,* called Deep $Vent_R$, are not denatured at 98°C. These enzymes can be used in automatic thermalcyclers to repeat the heating and cooling cycles, allowing many copies of DNA to be made easily and quickly.



A white microbial biofilm is visible on this deep-sea hydrothermal vent. Giant tubeworms are visible. Water is being emitted through the ocean floor at temperatures above 100°C.

The growth of the cell is inhibited as the plasma membrane pulls away from the cell wall. Thus, the addition of salts (or other solutes) to a solution, and the resulting increase in osmotic pressure, can be used to preserve foods. Salted fish, honey, and sweetened condensed milk are preserved largely by this mechanism; the high salt or sugar concentrations draw water out of any microbial cells that are present and thus prevent their growth. These effects of osmotic pressure are roughly related to the *number* of dissolved molecules and ions in a volume of solution.

Some organisms, called **extreme halophiles,** have adapted so well to high salt concentrations that they actually require them for growth. In this case, they may be termed **obligate halophiles.** Organisms from such saline waters as the Dead Sea often require nearly 30% salt, and the inoculating loop (a device for handling bacteria in the laboratory) used to transfer them must first be dipped into a saturated salt solution. More common are **facultative halophiles,** which do not require high salt concentrations but are able to grow at salt concentrations up to 2%, a concentration that inhibits the growth of many other organisms. A few species of facultative halophiles can tolerate even 15% salt.

Most microorganisms, however, must be grown in a medium that is nearly all water. For example, the concentration of agar (a complex polysaccharide isolated from marine algae) used to solidify microbial growth media is usually about 1.5%. If markedly higher concentrations are used, the increased osmotic pressure can inhibit the growth of some bacteria.

If the osmotic pressure is unusually low (the environment is *hypotonic*)—such as in distilled water, for example—water tends to enter the cell rather than leave it. Some microbes that have a relatively weak cell wall may be lysed by such treatment.

CHECK YOUR UNDERSTANDING

- Why are hyperthermophiles that grow at temperatures above 100°C seemingly limited to oceanic depths? **6-1**
- Other than controlling acidity, what is an advantage of using phosphate salts as buffers in growth media? **6-2**
- Why might primitive civilizations have used food preservation techniques that rely on osmotic pressure? **6-3**

Chemical Requirements

Carbon

Besides water, one of the most important requirements for microbial growth is carbon. Carbon is the structural backbone of living matter; it is needed for all the organic compounds that make up a living cell. Half the dry weight of a typical bacterial cell is carbon. Chemoheterotrophs get most of their carbon from the source of their energy—organic materials such as proteins, carbohydrates, and lipids. Chemoautotrophs and photoautotrophs derive their carbon from carbon dioxide.

Nitrogen, Sulfur, and Phosphorus

In addition to carbon, microorganisms need other elements to synthesize cellular material. For example, protein synthesis requires considerable amounts of nitrogen as well as some sulfur. The syntheses of DNA and RNA also require nitrogen and some phosphorus, as does the synthesis of ATP, the molecule so important for the storage and transfer of chemical energy within the cell. Nitrogen makes up about 14% of the dry weight of a bacterial cell, and sulfur and phosphorus together constitute about another 4%.

Organisms use nitrogen primarily to form the amino group of the amino acids of proteins. Many bacteria meet this requirement by decomposing protein-containing material and reincorporating the amino acids into newly synthesized proteins and other nitrogen-containing compounds. Other bacteria use nitrogen from ammonium ions (NH_4^+), which are already in the reduced form and are usually found in organic cellular material. Still other bacteria are able to derive nitrogen from nitrates (compounds that dissociate to give the nitrate ion, NO_3^-, in solution).

Some important bacteria, including many of the photosynthesizing cyanobacteria (page 137), use gaseous nitrogen (N_2) directly from the atmosphere. This process is called **nitrogen fixation.** Some organisms that can use this method are free-living, mostly in the soil, but others live cooperatively in symbiosis with the roots of legumes such as clover, soybeans, alfalfa, beans, and peas. The nitrogen fixed in the symbiosis is used by both the plant and the bacterium (see Chapter 27).

Sulfur is used to synthesize sulfur-containing amino acids and vitamins such as thiamine and biotin. Important natural sources of sulfur include the sulfate ion (SO_4^{2-}), hydrogen sulfide, and the sulfur-containing amino acids.

Phosphorus is essential for the synthesis of nucleic acids and the phospholipids of cell membranes. Among other places, it is also found in the energy bonds of ATP. A source of phosphorus is the phosphate ion (PO_4^{3-}). Potassium, magnesium, and calcium are also elements that microorganisms require, often as cofactors for enzymes (see Chapter 5, pages 113–114).

Trace Elements

Microbes require very small amounts of other mineral elements, such as iron, copper, molybdenum, and zinc; these are referred to as **trace elements.** Most are essential for the functions of certain enzymes, usually as cofactors. Although these elements are sometimes added to a laboratory medium, they are usually assumed to be naturally present in tap water and other components of media. Even most distilled waters contain adequate amounts, but tap water is sometimes specified to ensure that these trace minerals will be present in culture media.

Oxygen

We are accustomed to thinking of molecular oxygen (O_2) as a necessity of life, but it is actually in a sense a poisonous gas. Very little molecular oxygen existed in the atmosphere during most of Earth's history—in fact, it is possible that life could not have arisen had oxygen been present. However, many current forms of life have metabolic systems that require oxygen for aerobic respiration. Hydrogen atoms that have been stripped from organic compounds combine with oxygen to form water, as shown in Figure 5.14 (page 125). This process yields a great deal of energy while neutralizing a potentially toxic gas—a very neat solution, all in all.

Microbes that use molecular oxygen (aerobes) extract more energy from nutrients than microbes that do not use oxygen (anaerobes). Organisms that require oxygen to live are called **obligate aerobes** (Table 6.1a).

Obligate aerobes are at a disadvantage because oxygen is poorly soluble in the water of their environment. Therefore, many of the aerobic bacteria have developed, or retained, the ability to continue growing in the absence of oxygen. Such organisms are called **facultative anaerobes** (Table 6.1b). In other words, facultative anaerobes can use oxygen when it is present but are able to continue growth by using fermentation or anaerobic respiration when oxygen is not available. However, their efficiency in producing energy decreases in the absence of oxygen. An example of facultative anaerobes is the familiar *Escherichia coli* that are found in the human intestinal tract. Many yeasts are also facultative anaerobes. Many microbes are able to substitute other electron acceptors, such as nitrate ions, for oxygen, which is something humans are unable to do. (See the discussion of anaerobic respiration in Chapter 5, page 126).

Obligate anaerobes (Table 6.1c) are bacteria that are unable to use molecular oxygen for energy-yielding reactions. In fact, most are harmed by it. The genus *Clostridium* (klos-TRID-ē-um), which contains the species that cause tetanus and botulism, is the most familiar example. These bacteria do use oxygen atoms present in cellular materials; the atoms are usually obtained from water.

TABLE 6.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; growth ceases in presence of oxygen.	Only anaerobic growth; but growth continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

Understanding how organisms can be harmed by oxygen requires a brief discussion of the toxic forms of oxygen:

1. **Singlet oxygen** ($^1O_2^-$) is normal molecular oxygen (O_2) that has been boosted into a higher-energy state and is extremely reactive.
2. **Superoxide radicals** (O_2^-), or **superoxide anions,** are formed in small amounts during the normal respiration of organisms that use oxygen as a final electron acceptor, forming water. In the presence of oxygen, obligate anaerobes also appear to form some superoxide radicals, which are so toxic to cellular components that all organisms attempting to grow in atmospheric oxygen must produce an enzyme, **superoxide dismutase (SOD),** to neutralize them. Their toxicity is caused by their great instability, which leads them to steal an electron from a neighboring molecule, which in turn becomes a radical and steals an electron, and so on. Aerobic bacteria, facultative anaerobes growing aerobically, and aerotolerant anaerobes (discussed shortly) produce SOD, with which they convert the superoxide radical into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2):

$$O_2^- + O_2^- + 2\,H^+ \longrightarrow H_2O_2 + O_2$$

3. The hydrogen peroxide produced in this reaction contains the **peroxide anion** O_2^{2-} and is also toxic. It is the active principle in the antimicrobial agents hydrogen peroxide and benzoyl peroxide. (See Chapter 7, page 194). Because the hydrogen peroxide produced during normal aerobic respiration is toxic, microbes have developed enzymes to neutralize it. The most familiar of these is **catalase,** which converts it into water and oxygen:

$$2\,H_2O_2 \longrightarrow 2H_2O + O_2$$

Catalase is easily detected by its action on hydrogen peroxide. When a drop of hydrogen peroxide is added to a colony of bacterial cells producing catalase, oxygen bubbles are released. Anyone who has put hydrogen peroxide on a wound will recognize that cells in human tissue also contain catalase. The other enzyme that breaks down hydrogen peroxide is **peroxidase,** which differs from catalase in that its reaction does not produce oxygen:

$$H_2O_2 + 2\,H^+ \longrightarrow 2\,H_2O$$

Another important form of reactive oxygen is **ozone (O_3)** (discussed on page 194).

4. The **hydroxyl radical** (OH·) is another intermediate form of oxygen and probably the most reactive. It is formed in

the cellular cytoplasm by ionizing radiation. Most aerobic respiration produces traces of hydroxyl radicals, but they are transient.

These toxic forms of oxygen are an essential component of one of the body's most important defenses against pathogens, phagocytosis (see page 450 and Figure 16.7). In the phagolysosome of the phagocytic cell, ingested pathogens are killed by exposure to singlet oxygen, superoxide radicals, peroxide anions of hydrogen peroxide, and hydroxyl radicals and other oxidative compounds.

Obligate anaerobes usually produce neither superoxide dismutase nor catalase. Because aerobic conditions probably lead to an accumulation of superoxide radicals in their cytoplasm, obligate anaerobes are extremely sensitive to oxygen.

Aerotolerant anaerobes (Table 6.1d) cannot use oxygen for growth, but they tolerate it fairly well. On the surface of a solid medium, they will grow without the use of special techniques (discussed later) required for obligate anaerobes. Many of the aerotolerant bacteria characteristically ferment carbohydrates to lactic acid. As lactic acid accumulates, it inhibits the growth of aerobic competitors and establishes a favorable ecological niche for lactic acid producers. Common examples of lactic acid–producing aerotolerant anaerobes are the lactobacilli used in the production of many acidic fermented foods, such as pickles and cheese. In the laboratory, they are handled and grown much like any other bacteria, but they make no use of the oxygen in the air. These bacteria can tolerate oxygen because they possess SOD or an equivalent system that neutralizes the toxic forms of oxygen previously discussed.

A few bacteria are **microaerophiles** (Table 6.1e). They are aerobic; they do require oxygen. However, they grow only in oxygen concentrations lower than those in air. In a test tube of solid nutrient medium, they grow only at a depth where small amounts of oxygen have diffused into the medium; they do not grow near the oxygen-rich surface or below the narrow zone of adequate oxygen. This limited tolerance is probably due to their sensitivity to superoxide radicals and peroxides, which they produce in lethal concentrations under oxygen-rich conditions.

Organic Growth Factors

Essential organic compounds an organism is unable to synthesize are known as **organic growth factors;** they must be directly obtained from the environment. One group of organic growth factors for humans is vitamins. Most vitamins function as coenzymes, the organic cofactors required by certain enzymes in order to function. Many bacteria can synthesize all their own vitamins and do not depend on outside sources. However, some bacteria lack the enzymes needed for the synthesis of certain vitamins, and for them those vitamins are organic growth factors. Other organic growth factors required by some bacteria are amino acids, purines, and pyrimidines.

CHECK YOUR UNDERSTANDING

- If bacterial cells were given a sulfur source containing radioactive sulfur (^{35}S) in their culture media, in what molecules would the ^{35}S be found in the cells? **6-4**
- How would one determine whether a microbe is a strict anaerobe? **6-5**
- Oxygen is so pervasive in the environment that it would be very difficult for a microbe to always avoid physical contact with it. What, therefore, is the most obvious way for a microbe to avoid damage? **6-6**

Biofilms

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LEARNING OBJECTIVE

6-7 Describe the formation of biofilms and their potential for causing infection.

In nature, microorganisms seldom live in the isolated single-species colonies that we see on laboratory plates. They more typically live in communities called **biofilms,** which are a thin, slimy layer encasing bacteria that adheres to a surface. This fact was not well appreciated until the development of confocal microscopy (see page 58) made the three-dimensional structure of biofilms more visible. A biofilm also can be considered a *hydrogel,* which is a complex polymer containing many times its dry weight in water. Cell-to-cell chemical communication, or *quorum sensing,* allows bacteria to coordinate their activity and group together into communities that provide benefits not unlike those of multicellular organisms (see the box in Chapter 3, page 54). Therefore, biofilms are not just bacterial slime layers but biological systems; the bacteria are organized into a coordinated, functional community. Biofilms are usually attached to a surface, such as a rock in a pond, a human tooth (plaque; see Figure 25.3 on page 710), or a mucous membrane. This community might be of a single species or of a diverse group of microorganisms. Biofilms also might take other, more varied forms. In fast-flowing streams, the biofilm might be in the form of filamentous streamers. Within a biofilm community, the bacteria are able to share nutrients and are sheltered from harmful factors in the environment, such as desiccation, antibiotics, and the body's immune system. The close proximity of microorganisms within a biofilm might also have the advantage of facilitating the transfer of genetic information by, for example, conjugation.

ASM: Most bacteria in nature live in biofilm communities.

A biofilm usually begins to form when a free-swimming (*planktonic*) bacterium attaches to a surface. (See Figure 1.8 on page 15.) If these bacteria grew in a uniformly thick monolayer, they would become overcrowded, nutrients would not be available in lower depths, and toxic wastes could accumulate. Microorganisms in biofilm communities sometimes avoid these problems by forming pillar-like structures (**Figure 6.5**) with

channels between them, through which water can carry incoming nutrients and outgoing wastes. This constitutes a primitive circulatory system. Individual microbes and clumps of slime occasionally leave the established biofilm and move to a new location where the biofilm becomes extended. Such a biofilm is generally composed of a surface layer about 10 μm thick, with pillars that extend up to 200 μm above it.

The microorganisms in biofilms can work cooperatively to carry out complex tasks. For example, the digestive systems of ruminant animals, such as cattle, require many different microbial species to break down cellulose. The microbes in a ruminant's digestive system are located mostly within biofilm communities. Biofilms are also essential elements in the proper functioning of sewage treatment systems, which we will discuss in Chapter 27. They can also, however, be a problem in pipes and tubing, where their accumulations impede circulation.

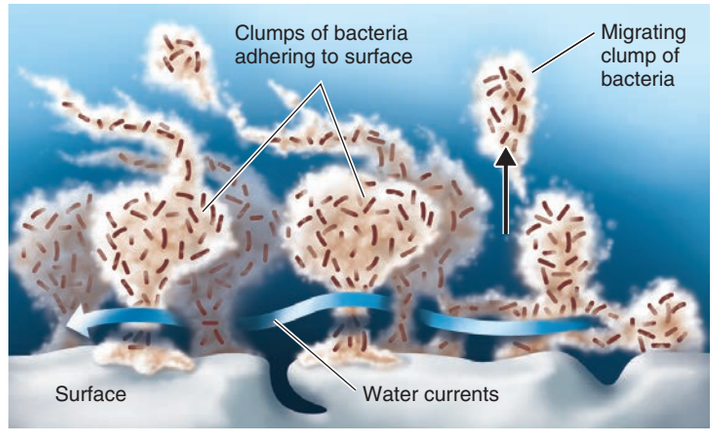
Biofilms are an important factor in human health. For example, microbes in biofilms are probably 1000 times more resistant to microbicides. Experts at the Centers for Disease Control and Prevention (CDC) estimate that 70% of human bacterial infections involve biofilms. Most health care associated infections are probably related to biofilms on medical catheters (see Figure 1.8 on page 15 and Figure 21.3 on page 582). In fact, biofilms form on almost all indwelling medical devices, including mechanical heart valves. Biofilms, which also can include those formed by fungi such as *Candida,* are encountered in many disease conditions, such as infections related to the use of contact lenses, dental caries (see page 709), and infections by pseudomonad bacteria (see page 296).

One approach to preventing biofilm formation is to incorporate antimicrobials into surfaces on which biofilms might form (see page 54). Because the chemical signals that allow quorum sensing are essential to biofilm formation, research is under way to determine the makeup of these chemical signals and perhaps block them. Another approach involves the discovery that lactoferrin (see page 461), which is abundant in many human secretions, can inhibit biofilm formation. Lactoferrin binds iron, especially among the pseudomonads that are responsible for cystic fibrosis biofilms, the cause of the pathology of this hereditary disease. The lack of iron inhibits the surface motility essential for the aggregation of the bacteria into biofilms.

Most laboratory methods in microbiology today use organisms being cultured in their planktonic mode. However, microbiologists now predict that there will be an increasing focus on how microorganisms actually live in relation to one another and that this will be considered in industrial and medical research.

CHECK YOUR UNDERSTANDING

Identify a way in which pathogens find it advantageous to form biofilms. **6-7**



Water currents move, as shown by the blue arrow, among pillars of slime formed by the growth of bacteria attached to solid surfaces. This allows efficient access to nutrients and removal of bacterial waste products. Individual slime-forming bacteria or bacteria in clumps of slime detach and move to new locations.

Figure 6.5 Biofilms.

Why is the prevention of biofilms important in a health care environment?

Culture Media

LEARNING OBJECTIVES

6-8 Distinguish chemically defined and complex media.

6-9 Justify the use of each of the following: anaerobic techniques, living host cells, candle jars, selective and differential media, enrichment medium.

6-10 Differentiate biosafety levels 1, 2, 3, and 4.

A nutrient material prepared for the growth of microorganisms in a laboratory is called a **culture medium.** Some bacteria can grow well on just about any culture medium; others require special media, and still others cannot grow on any nonliving medium yet developed. Microbes that are introduced into a culture medium to initiate growth are called an **inoculum.** The microbes that grow and multiply in or on a culture medium are referred to as a **culture.**

Suppose we want to grow a culture of a certain microorganism, perhaps the microbes from a particular clinical specimen. What criteria must the culture medium meet? First, it must contain the right nutrients for the specific microorganism we want to grow. It should also contain sufficient moisture, a properly adjusted pH, and a suitable level of oxygen, perhaps none at all. The medium must initially be **sterile**—that is, it must initially contain no living microorganisms—so that the culture will contain only the microbes (and their offspring) we add to the medium. Finally, the growing culture should be incubated at the proper temperature.

A wide variety of media are available for the growth of microorganisms in the laboratory. Most of these media, which

TABLE 6.2 A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

are available from commercial sources, have premixed components and require only the addition of water and then sterilization. Media are constantly being developed or revised for use in the isolation and identification of bacteria that are of interest to researchers in such fields as food, water, and clinical microbiology.

When it is desirable to grow bacteria on a solid medium, a solidifying agent such as agar is added to the medium. A complex polysaccharide derived from a marine alga, **agar** has long been used as a thickener in foods such as jellies and ice cream.

Agar has some very important properties that make it valuable to microbiology, and no satisfactory substitute has yet been found. Few microbes can degrade agar, so it remains solid. Also, agar liquefies at about 100°C (the boiling point of water) and at sea level remains liquid until the temperature drops to about 40°C. For laboratory use, agar is held in water baths at about 50°C. At this temperature, it does not injure most bacteria when it is poured over them (as shown in Figure 6.17a, page 168). Once the agar has solidified, it can be incubated at temperatures approaching 100°C before it again liquefies; this property is particularly useful when thermophilic bacteria are being grown.

Agar media are usually contained in test tubes or *Petri dishes*. The test tubes are called *slants* when their contents are allowed to solidify with the tube held at an angle so that a large surface area for growth is available. When the agar solidifies in a vertical tube, it is called a *deep*. Petri dishes, named for their inventor, are shallow dishes with a lid that nests over the bottom to prevent contamination; when filled, they are called *Petri* (or culture) *plates*.

Chemically Defined Media

To support microbial growth, a medium must provide an energy source, as well as sources of carbon, nitrogen, sulfur, phosphorus, and any organic growth factors the organism is unable to synthesize. A **chemically defined medium** is one whose exact chemical composition is known. For a chemoheterotroph, the chemically defined medium must contain organic growth factors that serve as a source of carbon and energy. For example, as shown in Table 6.2, glucose is included in the medium for growing the chemoheterotroph *E. coli*.

As Table 6.3 shows, many organic growth factors must be provided in the chemically defined medium used to cultivate a species of *Leuconostoc*. Organisms that require many growth factors are described as *fastidious*. Organisms of this type, such as *Lactobacillus* (page 310), are sometimes used in tests that determine the concentration of a particular vitamin in a substance. To perform such a *microbiological assay*, a growth medium is prepared that contains all the growth requirements of the bacterium except the vitamin being assayed. Then the medium, test substance, and bacterium are combined, and the growth of bacteria is measured. This bacterial growth, which is reflected by the amount of lactic acid produced, will be proportional to the amount of vitamin in the test substance. The more lactic acid, the more the *Lactobacillus* cells have been able to grow, so the more vitamin is present.

TABLE 6.3 Defined Culture Medium for *Leuconostoc mesenteroides*

Carbon and Energy
Glucose, 25 g
Salts
NH_4Cl, 3.0 g
K_2HPO_4*, 0.6 g
KH_2PO_4*, 0.6 g
$MgSO_4$, 0.1 g
Amino Acids, 100–200 μg each
Alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine
Purines and Pyrimidines, 10 mg of each
Adenine, guanine, uracil, xanthine
Vitamins, 0.01–1 mg each
Biotin, folate, nicotinic acid, pyridoxal, pyridoxamine, pyridoxine, riboflavin, thiamine, pantothenate, *p*-aminobenzoic acid
Trace Elements, 2–10 μg each
Fe, Co, Mn, Zn, Cu, Ni, Mo
Buffer, pH 7
Sodium acetate, 25 g
Distilled Water, 1000 ml
*Also serves as buffer.

TABLE 6.4 Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

Complex Media

Chemically defined media are usually reserved for laboratory experimental work or for the growth of autotrophic bacteria. Most heterotrophic bacteria and fungi, such as you would work with in an introductory lab course, are routinely grown on **complex media** made up of nutrients including extracts from yeasts, meat, or plants, or digests of proteins from these and other sources. The exact chemical composition varies slightly from batch to batch. Table 6.4 shows one widely used recipe.

In complex media, the energy, carbon, nitrogen, and sulfur requirements of the growing microorganisms are provided primarily by protein. Proteins are large, relatively insoluble molecules that only a minority of microorganisms can utilize directly. Partial digestion by acids or enzymes reduces proteins to shorter chains of amino acids called *peptones*. These small, soluble fragments can be digested by most bacteria.

Vitamins and other organic growth factors are provided by meat extracts or yeast extracts. The soluble vitamins and minerals from the meats or yeasts are dissolved in the extracting water, which is then evaporated, so these factors are concentrated. (These extracts also supplement the organic nitrogen and carbon compounds.) Yeast extracts are particularly rich in the B vitamins. If a complex medium is in liquid form, it is called **nutrient broth.** When agar is added, it is called **nutrient agar.** (This terminology can be confusing; just remember that agar itself is not a nutrient.)

Anaerobic Growth Media and Methods

The cultivation of anaerobic bacteria poses a special problem. Because anaerobes might be killed by exposure to oxygen, special media called **reducing media** must be used. These media contain ingredients, such as sodium thioglycolate, that chemically combine with dissolved oxygen and deplete the oxygen in the culture medium. To routinely grow and maintain pure cultures of obligate anaerobes, microbiologists use reducing media stored in ordinary, tightly capped test tubes. These media are heated shortly before use to drive off absorbed oxygen.

When the culture must be grown in Petri plates to observe individual colonies, several methods are available. Laboratories that work with relatively few culture plates at a time can use systems that can incubate the microorganisms in sealed boxes and jars in which the oxygen is chemically removed after the culture plates have been introduced and the container sealed as shown in Figure 6.6. The envelope of chemicals (the active ingredient is ascorbic acid) is simply opened to expose it to oxygen in the container's atmosphere. The atmosphere in such containers usually has less than 5% oxygen, about 18% CO_2, and no hydrogen. In a recently introduced system, each individual Petri plate (OxyPlate) becomes an anaerobic chamber. The medium in the plate contains an enzyme, oxyrase, which combines oxygen with hydrogen, removing oxygen as water is formed.

Laboratories that have a large volume of work with anaerobes often use an anaerobic chamber, such as that shown in Figure 6.7. The chamber is filled with inert gases (typically about 85% N_2, 10% H_2, and 5% CO_2) and is equipped with air locks to introduce cultures and materials.

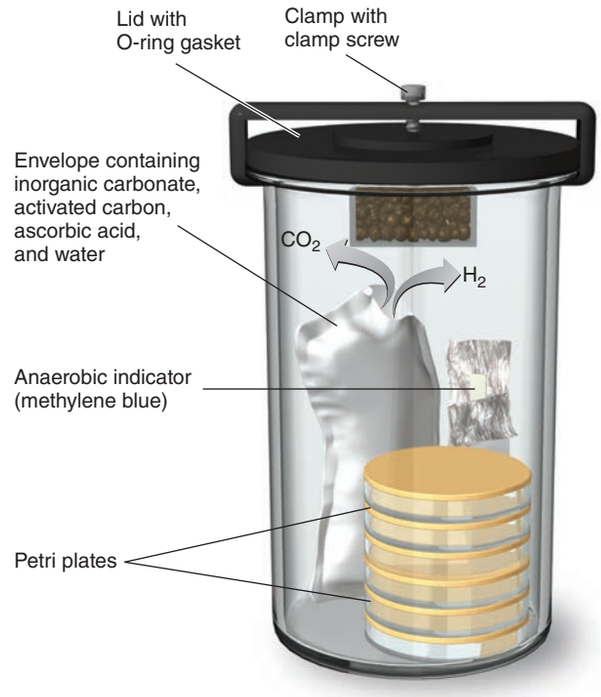


Figure 6.6 A jar for cultivating anaerobic bacteria on Petri plates. When water is mixed with the chemical packet containing sodium bicarbonate and sodium borohydride, hydrogen and carbon dioxide are generated. Reacting on the surface of a palladium catalyst in a screened reaction chamber, which may also be incorporated into the chemical packet, the hydrogen and atmospheric oxygen in the jar combine to form water. The oxygen is thus removed. Also in the jar is an anaerobic indicator containing methylene blue, which is blue when oxidized and turns colorless when the oxygen is removed (as shown here).

Q What is the technical name for bacteria that require a higher-than-atmospheric-concentration of CO_2 for growth?

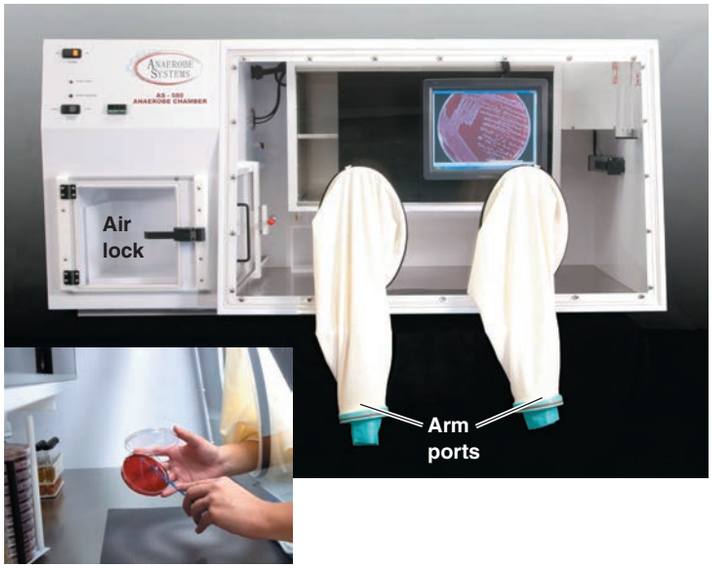


Figure 6.7 An anaerobic chamber. Materials are introduced through the small doors in the air-lock chamber at the left. The operator works through arm ports in airtight sleeves. The airtight sleeves extend into the cabinet when it is in use. This unit also features an internal camera and monitor.

Q In what way would an anaerobic chamber resemble the Space Laboratory orbiting in the vacuum of space?

Special Culture Techniques

Many bacteria have never been successfully grown on artificial laboratory media. *Mycobacterium leprae,* the leprosy bacillus, is now usually grown in armadillos, which have a relatively low body temperature that matches the requirements of the microbe. Another example is the syphilis spirochete, although certain nonpathogenic strains of this microbe have been grown on laboratory media. With few exceptions, the obligate intracellular bacteria, such as the rickettsias and the chlamydias, do not grow on artificial media. Like viruses, they can reproduce only in a living host cell. See the discussion of cell culture, page 367.

Many clinical laboratories have special *carbon dioxide incubators* in which to grow aerobic bacteria that require concentrations of CO_2 higher or lower than that found in the atmosphere. Desired CO_2 levels are maintained by electronic controls. High CO_2 levels are also obtained with simple *candle jars.* Cultures are placed in a large sealed jar containing a lighted candle, which consumes oxygen. The candle stops burning when the air in the jar has a lowered concentration of oxygen (at about 17% O_2, still adequate for the growth of aerobic bacteria). An elevated concentration of CO_2 (about 3%) is also present. Microbes that grow better at high CO_2 concentrations are called **capnophiles.** The low-oxygen, high-CO_2 conditions resemble those found in the intestinal tract, respiratory tract, and other body tissues where pathogenic bacteria grow.

Candle jars are still used occasionally, but more often commercially available chemical packets are used to generate carbon dioxide atmospheres in containers. When only one or two Petri plates of cultures are to be incubated, clinical laboratory investigators often use small plastic bags with self-contained chemical gas generators that are activated by crushing the packet or moistening it with a few milliliters of water. These packets are sometimes specially designed to provide precise concentrations of carbon dioxide (usually higher than can be obtained in candle jars) and oxygen for culturing organisms such as the microaerophilic *Campylobacter* bacteria (page 302).

Some microorganisms, such as the Ebola virus, are so dangerous that they can be handled only under extraordinary systems of containment called *biosafety level 4 (BSL-4).* Level 4 labs are popularly known as "the hot zone." Only a handful of such labs exists in the United States. The lab is a sealed environment within a larger building and has an atmosphere under negative pressure, so that aerosols containing pathogens will not escape. Both intake and exhaust air is filtered through high-efficiency particulate air filters (see HEPA filters, page 183); the exhaust air is filtered twice. All waste materials leaving the lab are rendered noninfectious. The personnel wear "space suits" that are connected to an air supply (**Figure 6.8**).

Less dangerous organisms are handled at lower levels of biosafety. For example, a basic microbiology teaching laboratory would be BSL-1. Organisms that present a moderate risk of infection can be handled at BSL-2 levels, that is, on open laboratory benchtops with appropriate gloves, lab coats, or possibly face and eye protection. BSL-3 labs are intended for highly infectious airborne pathogens such as the tuberculosis agent. Biological safety cabinets similar in appearance to the anaerobic chamber shown in Figure 6.7 are used. The laboratory itself should be negatively pressurized and equipped with air filters to prevent release of the pathogen from the laboratory.

Selective and Differential Media

In clinical and public health microbiology, it is frequently necessary to detect the presence of specific microorganisms associated with disease or poor sanitation. For this task, selective and differential media are used. **Selective media** are designed to suppress the growth of unwanted bacteria and encourage the growth of the desired microbes. For example, bismuth sulfite agar is one medium used to isolate the typhoid bacterium, the gram-negative *Salmonella typhi* (TĪ-fē), from feces. Bismuth sulfite inhibits gram-positive bacteria and most gram-negative intestinal bacteria (other than *S. typhi*), as well. Sabouraud's dextrose agar, which has a pH of 5.6, is used to isolate fungi that outgrow most bacteria at this pH.

Differential media make it easier to distinguish colonies of the desired organism from other colonies growing on the same plate. Similarly, pure cultures of microorganisms have

Figure 6.8 Technicians in a biosafety level 4 (BSL-4) laboratory. Personnel working in a BSL-4 facility wear a "space suit" that is connected to an outside air supply. Air pressure in the suit is higher than the atmosphere, preventing microbes from entering the suit.

Q If a technician were working with pathogenic prions, how would material leaving the lab be rendered noninfectious? (*Hint*: See Chapter 7.)

identifiable reactions with differential media in tubes or plates. Blood agar (which contains red blood cells) is a medium that microbiologists often use to identify bacterial species that destroy red blood cells. These species, such as *Streptococcus pyogenes* (pī-AH-jen-ēz), the bacterium that causes strep throat, show a clear ring around their colonies where they have lysed the surrounding blood cells (**Figure 6.9**).

Sometimes, selective and differential characteristics are combined in a single medium. Suppose we want to isolate the common bacterium *Staphylococcus aureus,* found in the nasal passages. This organism has a tolerance for high concentrations of sodium chloride; it can also ferment the carbohydrate mannitol to form acid. Mannitol salt agar contains 7.5% sodium chloride, which will discourage the growth of competing organisms and thus *select for* (favor the growth of) *S. aureus.* This salty medium also contains a pH indicator that changes color if the mannitol in the medium is fermented to acid; the mannitol-fermenting colonies of *S. aureus* are thus *differentiated from* colonies of bacteria that do not ferment mannitol. Bacteria that grow at the high salt concentration *and* ferment mannitol to acid can be readily identified by the color change (**Figure 6.10**). These are probably colonies of *S. aureus,* and their identification can be confirmed by additional tests. The use of differential media to identify toxin-producing *E. coli* is discussed in Chapter 5, page 136.

Enrichment Culture

Because bacteria present in small numbers can be missed, especially if other bacteria are present in much larger numbers, it is sometimes necessary to use an **enrichment culture.** This is often the case for soil or fecal samples. The medium (enrichment medium) for an enrichment culture is usually liquid and provides nutrients and environmental conditions that favor the growth of a particular microbe but not others. In this sense, it is also a selective medium, but it is designed to increase very small numbers of the desired type of organism to detectable levels.

Suppose we want to isolate from a soil sample a microbe that can grow on phenol and is present in much smaller numbers than other species. If the soil sample is placed in a liquid enrichment medium in which phenol is the only source of carbon and energy, microbes unable to metabolize phenol will not grow. The culture medium is allowed to incubate for a few days, and then a small amount of it is transferred into another flask of the same medium. After a series of such transfers, the surviving population will consist of bacteria capable of metabolizing phenol. The bacteria are given time to grow in the medium between transfers; this is the enrichment stage. (See the box in Chapter 28 page 801.) Any nutrients in the original inoculum are rapidly diluted out with the successive transfers. When the last dilution is streaked onto a solid medium of the same composition, only those colonies of organisms capable of using phenol should grow. A remarkable aspect of this particular technique is that phenol is normally lethal to most bacteria.

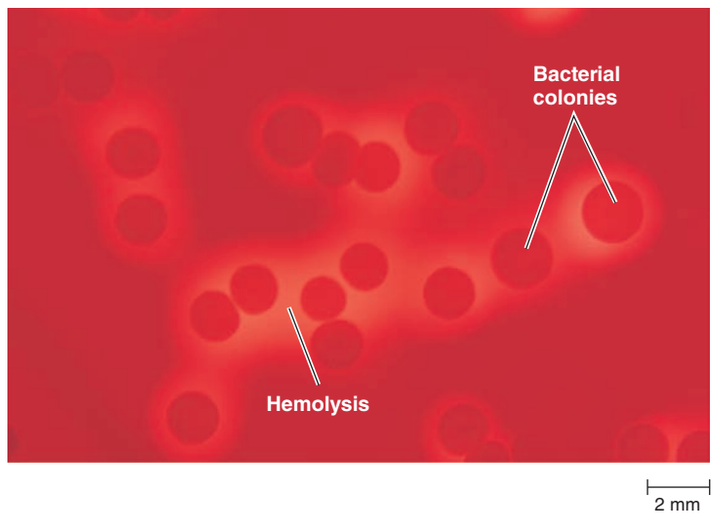


Figure 6.9 Blood agar, a differential medium containing red blood cells. The bacteria have lysed the red blood cells (beta-hemolysis), causing the clear areas around the colonies.

Q Of what value are hemolysins to pathogens?

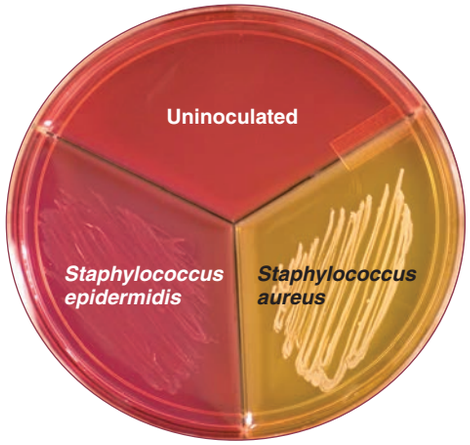


Figure 6.10 Differential medium. This medium is mannitol salt agar, and bacteria capable of fermenting the mannitol in the medium to acid (*Staphylococcus aureus*) cause the medium to change color to yellow. This **differentiates** between bacteria that can ferment mannitol and those that cannot. Actually, this medium is also **selective** because the high salt concentration prevents the growth of most bacteria but not *Staphylococcus* spp.

Q Are bacteria capable of growing at a high osmotic pressure likely to be capable of growing in the mucus found in nostrils?

Table 6.5 summarizes the purposes of the main types of culture media.

CHECK YOUR UNDERSTANDING

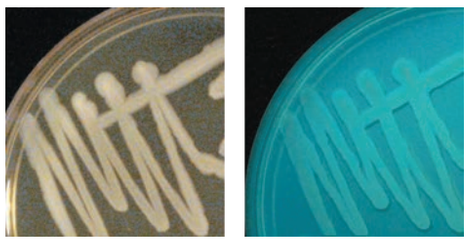
- Could humans exist on chemically defined media, at least under laboratory conditions? 6-8
- Could Louis Pasteur, in the 1800s, have grown rabies viruses in cell culture instead of in living animals? 6-9
- What BSL is your laboratory? 6-10

TABLE 6.5 Culture Media

Type	Purpose
Chemically Defined	Growth of chemoautotrophs and photoautotrophs; microbiological assays
Complex	Growth of most chemoheterotrophic organisms
Reducing	Growth of obligate anaerobes
Selective	Suppression of unwanted microbes; encouraging desired microbes
Differential	Differentiation of colonies of desired microbes from others
Enrichment	Similar to selective media but designed to increase numbers of desired microbes to detectable levels

Clinical Case

P. fluorescens is an aerobic, gram-negative rod that grows best between 25°C and 30°C and grows poorly at the standard hospital microbiology incubation temperatures (35°C to 37°C). The bacteria are so named because they produce a pigment that fluoresces under ultraviolet light. While reviewing the facts of the latest outbreak, Dr. MacGruder learns that the most recent patients were last exposed to the contaminated heparin 84 to 421 days before onset of their infections. On-site investigations confirmed that the patients' clinics are no longer using the recalled heparin and had, in fact, returned all unused inventory. Concluding that these patients did not develop infections during the previous outbreak, Dr. MacGruder must look for a new source of infection. The patients all have indwelling venous catheters: tubes that are inserted into a vein for long-term delivery of concentrated solutions, such as anticancer drugs. Dr. MacGruder orders cultures of the new heparin being used, but the results do not recover any organisms. He then orders blood and catheter cultures from each of the patients.



Illuminated with white light | Illuminated with ultraviolet light

The organism cultured from both the patients' blood and their catheters is shown in the figure. What organism is it?

150 162 170 172

Obtaining Pure Cultures

LEARNING OBJECTIVES

- 6-11 Define *colony.*
- 6-12 Describe how pure cultures can be isolated by using the streak plate method.

Most infectious materials, such as pus, sputum, and urine, contain several different kinds of bacteria; so do samples of soil, water, or food. If these materials are plated out onto the surface of a solid medium, colonies will form that are exact copies of the original organism. A visible **colony** theoretically arises from a single spore or vegetative cell or from a group of the same

microorganisms attached to one another in clumps or chains. Estimates are that only about 1% of bacteria in ecosystems produce colonies by conventional culture methods. Microbial colonies often have a distinctive appearance that distinguishes one microbe from another (see Figure 6.10). The bacteria must be distributed widely enough so that the colonies are visibly separated from each other.

Most bacteriological work requires pure cultures, or clones, of bacteria. The isolation method most commonly used to get pure cultures is the **streak plate method** (Figure 6.11). A sterile inoculating loop is dipped into a mixed culture that contains more than one type of microbe and is streaked in a pattern over the surface of the nutrient medium. As the pattern is traced, bacteria are rubbed off the loop onto the medium. The last cells to be rubbed off the loop are far enough apart to grow into isolated colonies. These colonies can be picked up with an inoculating loop and transferred to a test tube of nutrient medium to form a pure culture containing only one type of bacterium.

The streak plate method works well when the organism to be isolated is present in large numbers relative to the total population. However, when the microbe to be isolated is present only in very small numbers, its numbers must be greatly increased by selective enrichment before it can be isolated with the streak plate method.

CHECK YOUR UNDERSTANDING

- Can you think of any reason why a colony does not grow to an infinite size, or at least fill the confines of the Petri plate? **6-11**
- Could a pure culture of bacteria be obtained by the streak plate method if there were only one desired microbe in a bacterial suspension of billions? **6-12**

Preserving Bacterial Cultures

LEARNING OBJECTIVE

6-13 Explain how microorganisms are preserved by deep-freezing and lyophilization (freeze-drying).

Refrigeration can be used for the short-term storage of bacterial cultures. Two common methods of preserving microbial cultures for long periods are deep-freezing and lyophilization. **Deep-freezing** is a process in which a pure culture of microbes is placed in a suspending liquid and quick-frozen at temperatures ranging from −50°C to −95°C. The culture can usually be thawed and cultured even several years later. During **lyophilization (freeze-drying),** a suspension of microbes is quickly frozen at temperatures ranging from −54°C to −72°C, and the water is removed by a high vacuum (sublimation). While under vacuum, the container is sealed by melting the glass with a high-temperature torch. The remaining powderlike residue that contains the surviving microbes can be stored for years. The organisms can be revived at any time by hydration with a suitable liquid nutrient medium.

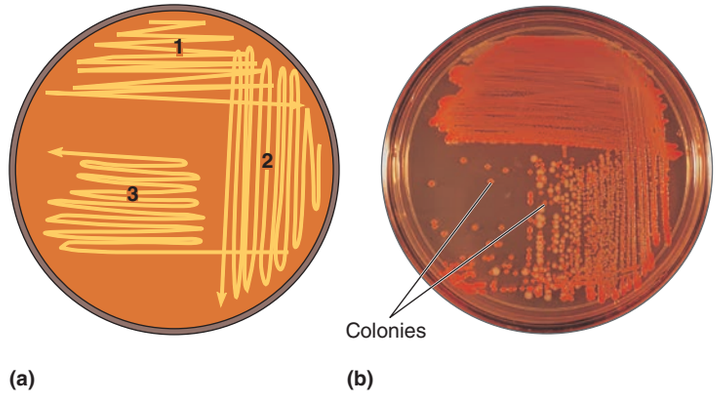


Figure 6.11 The streak plate method for isolating pure bacterial cultures. **(a)** Arrows indicate the direction of streaking. Streak series 1 is made from the original bacterial culture. The incoculating loop is sterilized following each streak series. In series 2 and 3, the loop picks up bacteria from the previous series, diluting the number of cells each time. There are numerous variants of such patterns. **(b)** In series 3 of this example, notice that well-isolated colonies of bacteria of two different types, red and yellow, have been obtained.

Q Is a colony formed as a result of streaking a plate always derived from a single bacterium? Why or why not?

CHECK YOUR UNDERSTANDING

- If the Space Station in Earth orbit suddenly ruptured, the humans on board would die instantly from cold and the vacuum of space. Would all the bacteria in the capsule also be killed? **6-13**

The Growth of Bacterial Cultures

LEARNING OBJECTIVES

6-14 Define *bacterial growth,* including *binary fission.*

6-15 Compare the phases of microbial growth, and describe their relation to generation time.

6-16 Explain four direct methods of measuring cell growth.

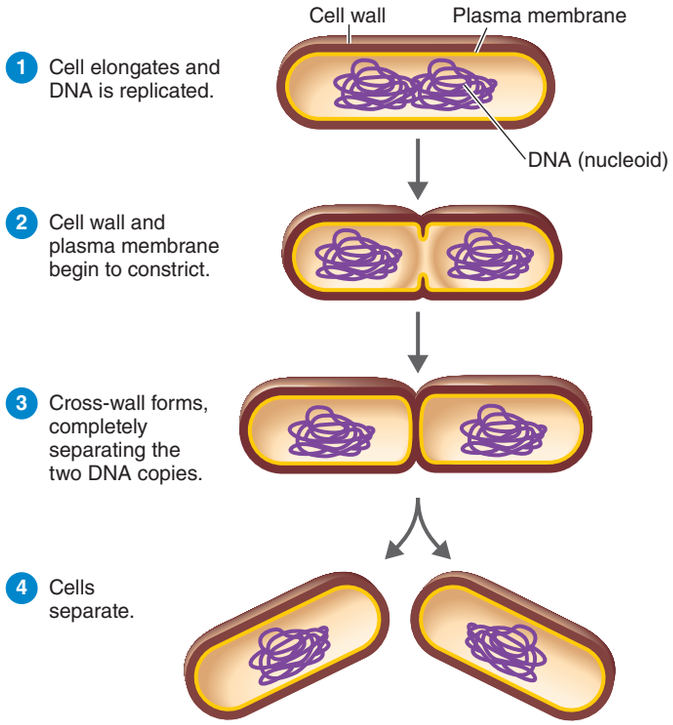
6-17 Differentiate direct and indirect methods of measuring cell growth.

6-18 Explain three indirect methods of measuring cell growth.

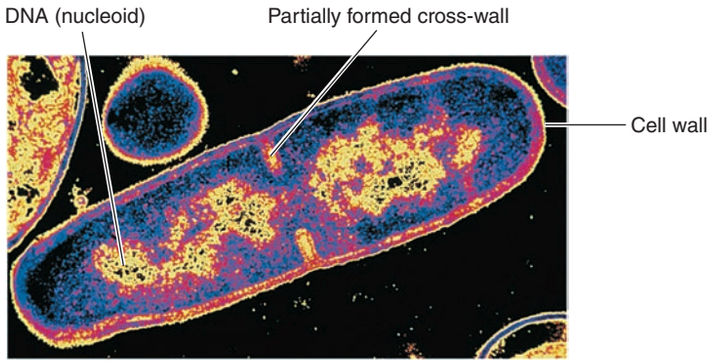
Being able to represent graphically the enormous populations resulting from the growth of bacterial cultures is an essential part of microbiology. It is also necessary to be able to determine microbial numbers, either directly, by counting, or indirectly, by measuring their metabolic activity.

Bacterial Division

As we mentioned at the beginning of the chapter, bacterial growth refers to an increase in bacterial numbers, not an increase in the size of the individual cells. Bacteria normally reproduce by **binary fission** (Figure 6.12).



(a) A diagram of the sequence of cell division



(b) A thin section of a cell of *Bacillus licheniformis* starting to divide

Figure 6.12 Binary fission in bacteria.

Q In what way is budding different from binary fission?

A few bacterial species reproduce by **budding;** they form a small initial outgrowth (a bud) that enlarges until its size approaches that of the parent cell, and then it separates. Some filamentous bacteria (certain actinomycetes) reproduce by producing chains of conidiospores (see Figure 11.XX) (an asexual spore) carried externally at the tips of the filaments. A few filamentous species simply fragment, and the fragments initiate the growth of new cells. MM **Animations** Binary Fission; Bacterial Growth: Overview

Generation Time

For purposes of calculating the generation time of bacteria, we will consider only reproduction by binary fission, which is by far the most common method. As you can see in Figure 6.13, one cell's division produces two cells, two cells' divisions produce four cells, and so on. When the number of cells in each generation is expressed as a power of 2, the exponent tells the number of doublings (generations) that have occurred.

The time required for a cell to divide (and its population to double) is called the **generation time.** It varies considerably among organisms and with environmental conditions, such as temperature. Most bacteria have a generation time of 1 to 3 hours; others require more than 24 hours per generation. (The math required to calculate generation times is presented in Appendix B.) If binary fission continues unchecked, an enormous number of cells will be produced. If a doubling occurred every 20 minutes—which is the case for *E. coli* under favorable conditions—after 20 generations a single initial cell would increase to over 1 million cells. This would require a little

Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

(a) Visual representation of increase in bacterial number over five generations. The number of bacteria doubles in each generation. The superscript indicates the generation; that is, 2^5 = 5 generations.

Generation Number	Number of Cells		Log_{10} of Number of Cells
0	2^0 =	1	0
5	2^5 =	32	1.51
10	2^{10} =	1,024	3.01
15	2^{15} =	32,768	4.52
16	2^{16} =	65,536	4.82
17	2^{17} =	131,072	5.12
18	2^{18} =	262,144	5.42
19	2^{19} =	524,288	5.72
20	2^{20} =	1,048,576	6.02

(b) Conversion of the number of cells in a population into the logarithmic expression of this number. To arrive at the numbers in the center column, use the y^x key on your calculator. Enter 2 on the calculator; press y^x; enter 5; then press the = sign. The calculator will show the number 32. Thus, the fifth-generation population of bacteria will total 32 cells. To arrive at the numbers in the right-hand column, use the log key on your calculator. Enter the number 32; then press the log key. The calculator will show, rounded off, that the log_{10} of 32 is 1.51.

Figure 6.13 Cell division.

Q If a single bacterium reproduced every 30 minutes, how many would there be in 2 hours?

less than 7 hours. In 30 generations, or 10 hours, the population would be 1 billion, and in 24 hours it would be a number trailed by 21 zeros. It is difficult to graph population changes of such enormous magnitude by using arithmetic numbers. This is why logarithmic scales are generally used to graph bacterial growth. Understanding logarithmic representations of bacterial populations requires some use of mathematics and is necessary for anyone studying microbiology. (See Appendix B.)

Logarithmic Representation of Bacterial Populations

To illustrate the difference between logarithmic and arithmetic graphing of bacterial populations, let's express 20 bacterial generations both logarithmically and arithmetically. In five generations (2^5), there would be 32 cells; in ten generations (2^{10}), there would be 1024 cells, and so on. (If your calculator has a y^x key and a log key, you can duplicate the numbers in the third column of Figure 6.13.)

In **Figure 6.14**, notice that the arithmetically plotted line (solid) does not clearly show the population changes in the early stages of the growth curve at this scale. In fact, the first ten generations do not even appear to leave the baseline, whereas the logarithmic plot point for the tenth generation (3.01) is halfway up the graph. Furthermore, another one or two arithmetic generations graphed to the same scale would greatly increase the height of the graph and take the line off the page.

The dashed line in Figure 6.14 shows how these plotting problems can be avoided by graphing the $\log_{10}$ of the population numbers. The $\log_{10}$ of the population is plotted at 5, 10, 15, and 20 generations. Notice that a straight line is formed and that a thousand times this population (1,000,000,000, or $\log_{10}$ 9.0) could be accommodated in relatively little extra space. However, this advantage is obtained at the cost of distorting our "common sense" perception of the actual situation. We are not accustomed to thinking in logarithmic relationships, but it is necessary for a proper understanding of graphs of microbial populations.

CHECK YOUR UNDERSTANDING

Can a complex organism, such as a beetle, divide by binary fission? **6-14**

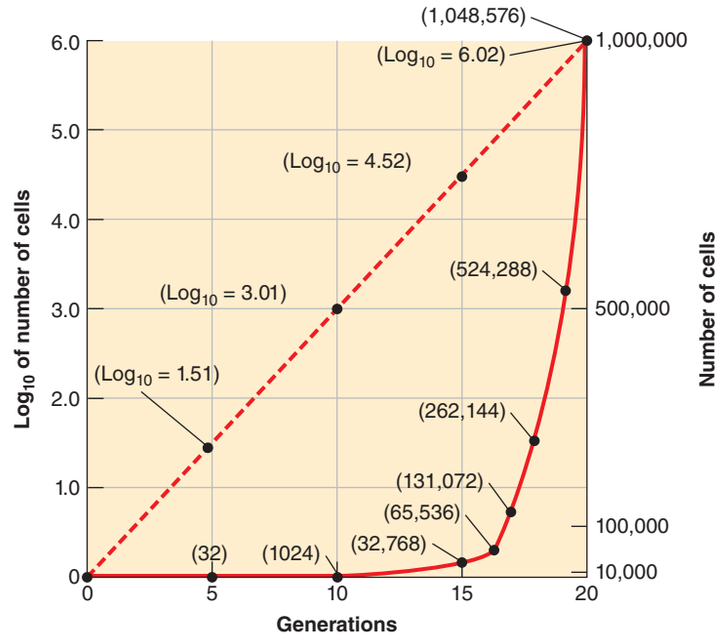


Figure 6.14 A growth curve for an exponentially increasing population, plotted logarithmically (dashed line) and arithmetically (solid line). For demonstration purposes, this graph has been drawn so that the arithmetic and logarithmic curves intersect at 1 million cells. This figure demonstrates why it is necessary to graph changes in the immense numbers of bacterial populations by logarithmic plots rather than by arithmetic numbers. For example, note that at ten generations the line representing arithmetic numbers has not even perceptibly left the baseline, whereas the logarithmic plot point for the tenth generation (3.01) is halfway up the graph.

Q If the arithmetic numbers (solid line) were plotted for two more generations, would the line still be on the page?

Phases of Growth

When a few bacteria are inoculated into a liquid growth medium and the population is counted at intervals, it is possible to plot a **bacterial growth curve** that shows the growth of cells over time (**Figure 6.15**). There are four basic phases of growth: the lag, log, stationary, and death phases. MM **Animation** Bacterial Growth Curve

The Lag Phase

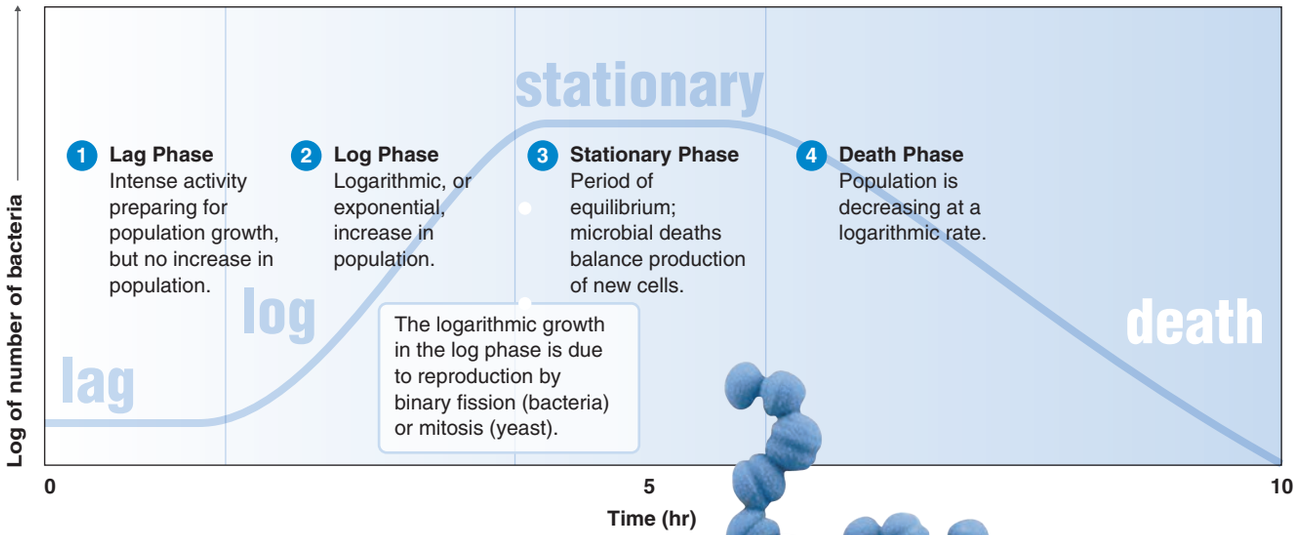
For a while, the number of cells changes very little because the cells do not immediately reproduce in a new medium. This period of little or no cell division is called the **lag phase,** and it can last for 1 hour or several days. During this time, however, the cells are not dormant. The microbial population is undergoing a period of intense metabolic activity involving, in particular, synthesis of enzymes and various molecules. (The situation is analogous to a factory being equipped to produce automobiles; there is considerable tooling-up activity but no immediate increase in the automobile population.)

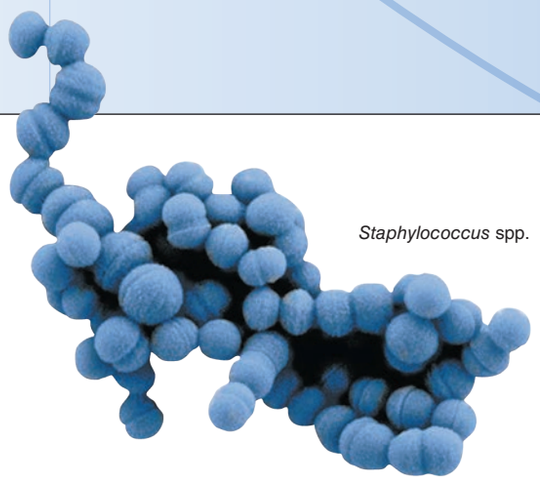
The Log Phase

Eventually, the cells begin to divide and enter a period of growth, or logarithmic increase, called the **log phase,** or **exponential growth phase.** Cellular reproduction is most active during this period, and generation time (intervals during which the population doubles) reaches a constant minimum. Because the generation time is constant, a logarithmic plot of growth during the log phase is a straight line. The log phase is the time when cells are most active metabolically and is preferred for industrial purposes where, for example, a product needs to be produced efficiently.

FOUNDATION FIGURE 6.15

Understanding the Bacterial Growth Curve





Staphylococcus spp.

KEY CONCEPTS

- Bacterial populations follow a sequential series of growth phases: the lag, log, stationary, and death phases.
- Knowledge of the bacterial growth curve is critical to understanding population dynamics and population control in the course of infectious diseases, in food preservation and spoilage, as well as in industrial microbiology processes, such as ethanol production.

The Stationary Phase

If exponential growth continued unchecked, startlingly large numbers of cells could arise. For example, a single bacterium (at a weight of 9.5×10^{-13} g per cell) dividing every 20 minutes for only 25.5 hours can theoretically produce a population equivalent in weight to that of an 80,000-ton aircraft carrier. In reality, this does not happen. Eventually, the growth rate slows, the number of microbial deaths balances the number of new cells, and the population stabilizes. This period of equilibrium is called the **stationary phase.**

What causes exponential growth to stop is not always clear. The exhaustion of nutrients, accumulation of waste products, and harmful changes in pH may all play a role.

The Death Phase

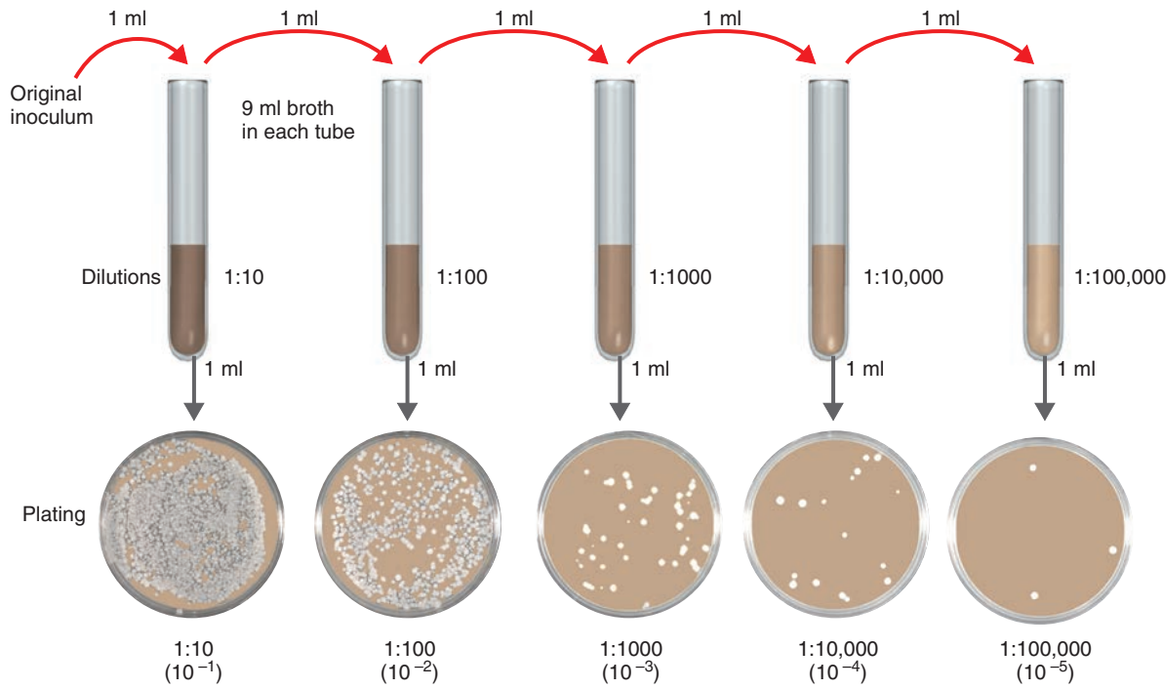
The number of deaths eventually exceeds the number of new cells formed, and the population enters the **death phase,** or **logarithmic decline phase.** This phase continues until the population is diminished to a tiny fraction of the number of cells in the previous phase or until the population dies out entirely. Some species pass through the entire series of phases in only a few days; others retain some surviving cells almost indefinitely. Microbial death will be discussed further in Chapter 7.

CHECK YOUR UNDERSTANDING

If two mice started a family within a fixed enclosure, with a fixed food supply, would the population curve be the same as a bacterial growth curve? **6-15**

Direct Measurement of Microbial Growth

The growth of microbial populations can be measured in a number of ways. Some methods measure cell numbers; other methods measure the population's total mass, which is often directly proportional to cell numbers. Population numbers are usually recorded as the number of cells in a milliliter of liquid or in a gram of solid material. Because bacterial populations are usually very large, most methods of counting them are based on direct or indirect counts of very small samples; calculations then determine the size of the total population. Assume, for example, that a millionth of a milliliter (10^{-6} ml) of sour milk is found to contain 70 bacterial cells. Then there must be 70 times 1 million, or 70 million, cells per milliliter.



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 54 colonies are on a plate of 1:1000 dilution, then the count is 54 × 1000 = 54,000 bacteria/ml in sample.)

Figure 6.16 Serial dilutions and plate counts. In serial dilutions, the original inoculum is diluted in a series of dilution tubes. In our example, each succeeding dilution tube will have only one-tenth the number of microbial cells as the preceding tube. Then, samples of the dilution are used to inoculate Petri plates, on which colonies grow and can be counted. This count is then used to estimate the number of bacteria in the original sample.

Q Why were the dilutions of 1:10,000 and 1:100,000 not counted? Theoretically, how many colonies should appear on the 1:100 plate?

However, it is not practical to measure out a millionth of a milliliter of liquid or a millionth of a gram of food. Therefore, the procedure is done indirectly, in a series of dilutions. For example, if we add 1 ml of milk to 99 ml of water, each milliliter of this dilution now has one-hundredth as many bacteria as each milliliter of the original sample had. By making a series of such dilutions, we can readily estimate the number of bacteria in our original sample. To count microbial populations in solid foods (such as hamburger), an homogenate of one part food to nine parts water is finely ground in a food blender. Samples of this initial one-tenth dilution can then be transferred with a pipette for further dilutions or cell counts.

Plate Counts

The most frequently used method of measuring bacterial populations is the **plate count.** An important advantage of this method is that it measures the number of viable cells. One disadvantage may be that it takes some time, usually 24 hours or more, for visible colonies to form. This can be a serious problem in some applications, such as quality control of milk, when it is not possible to hold a particular lot for this length of time.

Plate counts assume that each live bacterium grows and divides to produce a single colony. This is not always true, because bacteria frequently grow linked in chains or as clumps (see Figure 4.1, page 74). Therefore, a colony often results, not from a single bacterium, but from short segments of a chain or from a bacterial clump. To reflect this reality, plate counts are often reported as **colony-forming units (CFU).**

When a plate count is performed, it is important that only a limited number of colonies develop in the plate. When too many colonies are present, some cells are overcrowded and do not develop; these conditions cause inaccuracies in the count. The U.S. Food and Drug Administration convention is to count only plates with 25 to 250 colonies, but many microbiologists prefer plates with 30 to 300 colonies. To ensure that some colony counts will be within this range, the original inoculum is diluted several times in a process called **serial dilution** (Figure 6.16).

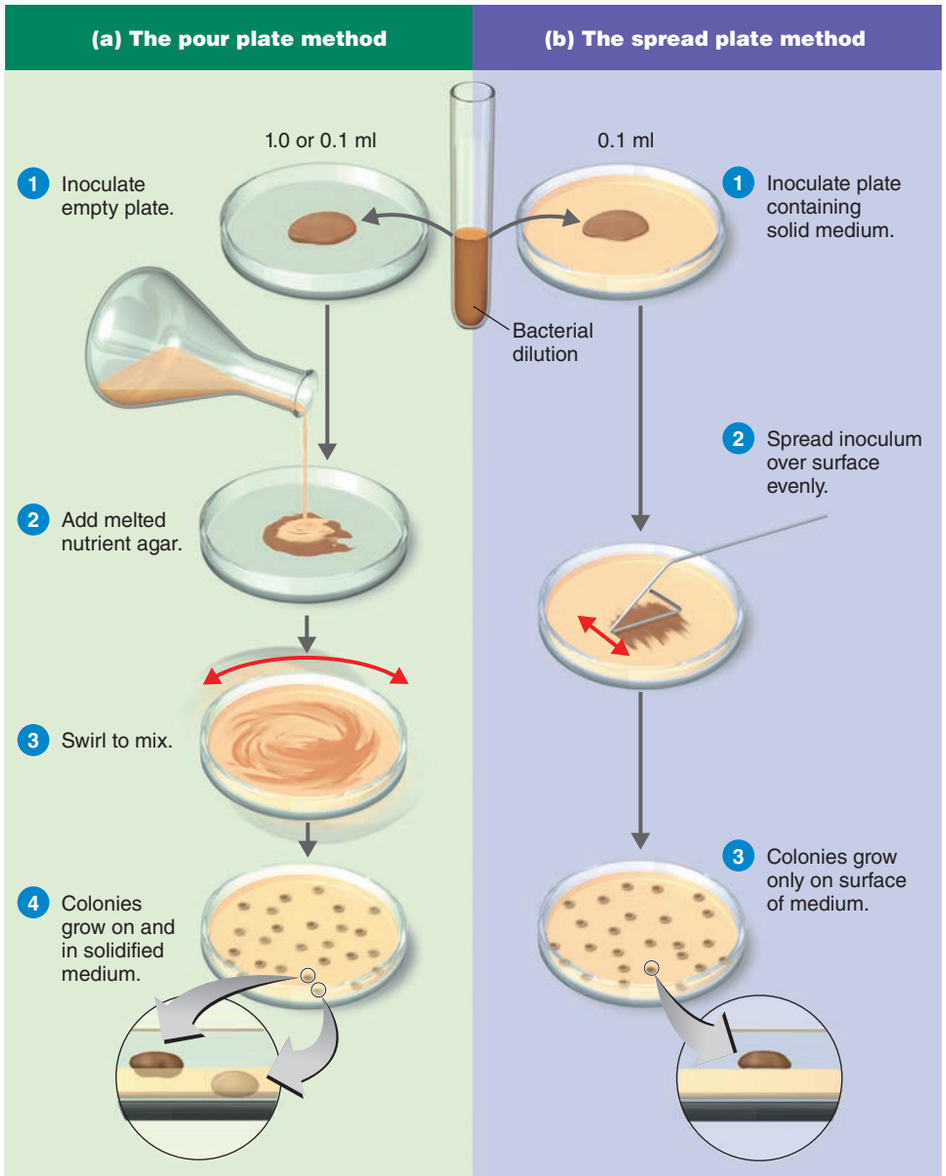


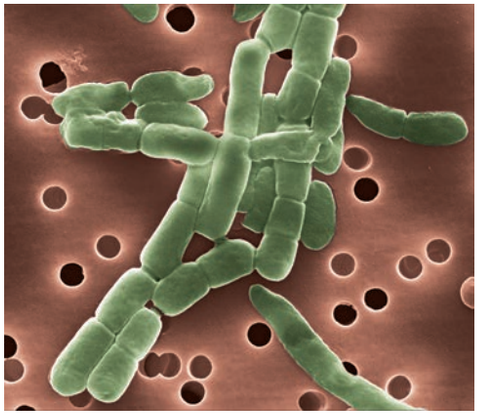
Figure 6.17 Methods of preparing plates for plate counts. **(a)** The pour plate method. **(b)** The spread plate method.

Q In what instances would the pour plate method be more appropriate than the spread plate method?

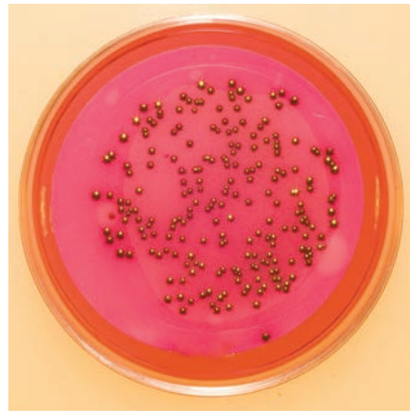
Serial Dilutions Let's say, for example, that a milk sample has 10,000 bacteria per milliliter. If 1 ml of this sample were plated out, there would theoretically be 10,000 colonies formed in the Petri plate of medium. Obviously, this would not produce a countable plate. If 1 ml of this sample were transferred to a tube containing 9 ml of sterile water, each milliliter of fluid in this tube would now contain 1000 bacteria. If 1 ml of this sample were inoculated into a Petri plate, there would still be too many potential colonies to count on a plate. Therefore, another serial dilution could be made. One milliliter containing 1000 bacteria would be transferred to a second tube of 9 ml of water. Each milliliter of this tube would now contain only 100 bacteria, and if 1 ml of the contents of this tube were plated out, potentially 100 colonies would be formed—an easily countable number.

Pour Plates and Spread Plates A plate count is done by either the pour plate method or the spread plate method. The **pour plate method** follows the procedure shown in Figure 6.17a. Either 1 ml or 0.1 ml of dilutions of the bacterial suspension is introduced into a Petri dish. The nutrient medium, in which the agar is kept liquid by holding it in a water bath at about 50°C, is poured over the sample, which is then mixed into the medium by gentle agitation of the plate. When the agar solidifies, the plate is incubated. With the pour plate technique, colonies will grow within the nutrient agar (from cells suspended in the nutrient medium as the agar solidifies) as well as on the surface of the agar plate.

This technique has some drawbacks because some relatively heat-sensitive microorganisms may be damaged by the melted agar and will therefore be unable to form colonies. Also, when



(a) The bacterial populations in bodies of water can be determined by passing a sample through a membrane filter. Here, the bacteria in a 100-ml water sample have been sieved out onto the surface of a membrane filter. These bacteria form visible colonies when placed on the surface of a suitable medium.



(b) A membrane filter with bacteria on its surface, as described in (a), has been placed on Endo agar. This medium is selective for gram-negative bacteria; lactose fermenters, such as the coliforms, form distinctive colonies. There are 214 colonies visible, so we would record 214 bacteria per 100 ml in the water sample.

Figure 6.18 Counting bacteria by filtration.

Q Could you make a pour plate in the usual Petri dish with a 10-ml inoculum? Why or why not?

certain differential media are used, the distinctive appearance of the colony on the surface is essential for diagnostic purposes. Colonies that form beneath the surface of a pour plate are not satisfactory for such tests. To avoid these problems, the **spread plate method** is frequently used instead (Figure 6.17b). A 0.1-ml inoculum is added to the surface of a prepoured, solidified agar medium. The inoculum is then spread uniformly over the surface of the medium with a specially shaped, sterilized glass or metal rod. This method positions all the colonies on the surface and avoids contact between the cells and melted agar.

Filtration

When the quantity of bacteria is very small, as in lakes or relatively pure streams, bacteria can be counted by **filtration** methods (**Figure 6.18**). In this technique, at least 100 ml of water are passed through a thin membrane filter whose pores are too small to allow bacteria to pass. Thus, the bacteria are filtered out and retained on the surface of the filter. This filter is then transferred to a Petri dish containing nutrient medium, where colonies arise from the bacteria on the filter's surface. This method is applied frequently to detection and enumeration of coliform bacteria, which are indicators of fecal contamination of food or water (see Chapter 27). The colonies formed by these bacteria are distinctive when a differential nutrient medium is used. (The colonies shown in Figure 6.18b are examples of coliforms.)

The Most Probable Number (MPN) Method

Another method for determining the number of bacteria in a sample is the **most probable number (MPN) method,** illustrated in **Figure 6.19**. This statistical estimating technique is based on the fact that the greater the number of bacteria in a sample, the more dilution is needed to reduce the density to the point at which no bacteria are left to grow in the tubes in a dilution series. The MPN method is most useful when the microbes being counted will not grow on solid media (such as the chemoautotrophic nitrifying bacteria). It is also useful when the growth of bacteria in a liquid differential medium is used to identify the microbes (such as coliform bacteria, which selectively ferment lactose to acid, in water testing). The MPN is only a statement that there is a 95% chance that the bacterial population falls within a certain range and that the MPN is statistically the most probable number.

Direct Microscopic Count

In the method known as the **direct microscopic count,** a measured volume of a bacterial suspension is placed within a defined area on a microscope slide. Because of time considerations, this method is often used to count the number of bacteria in milk. A 0.01-ml sample is spread over a marked square centimeter of slide, stain is added so that the bacteria can be seen, and the sample is viewed under the oil immersion objective lens. The area of the viewing field of this objective can be determined. Once the number of bacteria has been counted in several different fields, the average number of bacteria per viewing field can be calculated. From these data, the number of bacteria in the square centimeter over which the sample was spread can also be calculated. Because this area on the slide contained 0.01 ml of sample, the number of bacteria in each milliliter of the suspension is the number of bacteria in the sample times 100.

A specially designed slide called a *Petroff-Hausser cell counter* is used in direct microscopic counts (**Figure 6.20**).

Motile bacteria are difficult to count by this method, and, as happens with other microscopic methods, dead cells are about

Nutrient Medium Set (5 Tubes for Set)	Innoculum amount added	Number of Positive Tubes in Each Set
Set 1	10 ml	5
Set 2	1 ml	3
Set 3	0.1 ml	1

(a) Most probable number (MPN) dilution series.

Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	6.8	50
4-2-1	26	9.8	70
4-3-0	27	9.9	70
4-3-1	33	10	70
4-4-0	34	14	100
5-0-0	23		70
5-0-1	31	10	70
5-0-2	43	14	100
5-1-0	33	10	100
5-1-1	46	14	120
5-1-2	63	22	150
5-2-0	49	15	150
5-2-1	70	22	170
5-2-2	94	34	230
5-3-0	79	22	220
5-3-1	110	34	250
5-3-2	140	52	400

(b) MPN table. MPN tables enable us to calculate for a sample the microbial numbers that are statistically likely to lead to such a result. The number of positive (yellow) tubes is recorded for each set: in the shaded example, 5, 3, and 1. If we look up this combination in an MPN table, we find that the MPN index per 100 ml is 110. Statistically, this means that 95% of the water samples that give this result contain 34–250 bacteria, with 110 being the most probable number.

Figure 6.19 The most probable number (MPN) method.

Q Under what circumstances is the MPN method used to determine the number of bacteria in a sample?

as likely to be counted as live ones. In addition to these disadvantages, a rather high concentration of cells is required to be countable—about 10 million bacteria per milliliter. The chief advantage of microscopic counts is that no incubation time is required, and they are usually reserved for applications in which time is the primary consideration. This advantage also holds for *electronic cell counters*, sometimes known as *Coulter counters*, which automatically count the number of cells in a measured volume of liquid. These instruments are used in some research laboratories and hospitals.

CHECK YOUR UNDERSTANDING

Why is it difficult to measure realistically the growth of a filamentous mold isolate by the plate count method? 6-16

Estimating Bacterial Numbers by Indirect Methods

It is not always necessary to count microbial cells to estimate their numbers. In science and industry, microbial numbers and activity are determined by some of the following indirect means as well.

Turbidity

For some types of experimental work, estimating **turbidity** is a practical way of monitoring bacterial growth. As bacteria multiply in a liquid medium, the medium becomes turbid, or cloudy with cells.

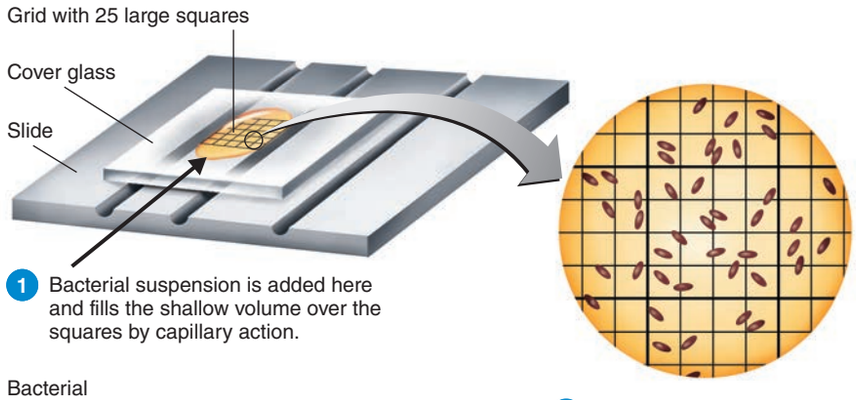
The instrument used to measure turbidity is a *spectrophotometer* (or colorimeter). In the spectrophotometer, a beam of light is transmitted through a bacterial suspension to a light-sensitive detector (Figure 6.21). As bacterial numbers increase, less light will reach the detector. This change of light will register on the instrument's scale as the *percentage of transmission* (*%T*). Also printed on the instrument's scale is a logarithmic expression called the *absorbance* (sometimes called *optical density*, or *OD*). The absorbance is used to plot bacterial growth. When the bacteria are in logarithmic growth or decline, a graph of absorbance versus time will form an approximately straight line. If absorbance readings are matched with plate counts of the same culture, this correlation can be used in future estimations of bacterial numbers obtained by measuring turbidity.

Clinical Case

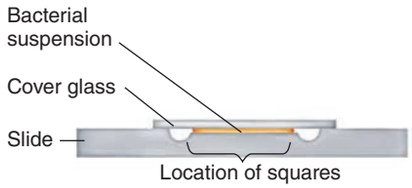
The bacteria in the blood and catheter cultures fluoresce under ultraviolet light. The results from the culture show that *P. fluorescens* is present in the blood of 15 patients, in 17 catheters, and in the blood and catheters of 4 patients. The bacteria survived even after the heparin recall. Dr. MacGruder would like to have some idea how many bacteria are colonizing a patient's catheter. Because the amount of nutrients in a patient's catheter is minimal, he concludes that the bacteria grow slowly. He does some calculations based on the assumption that five *Pseudomonas* cells, with a generation time of 35 hours, may have been originally introduced into the catheters.

Approximately how many cells would there be after a month?

150 162 170 172



1 Bacterial suspension is added here and fills the shallow volume over the squares by capillary action.



2 Cross section of a cell counter. The depth under the cover glass and the area of the squares are known, so the volume of the bacterial suspension over the squares can be calculated (depth × area).

3 Microscopic count: All cells in several large squares are counted, and the numbers are averaged. The large square shown here has 14 bacterial cells.

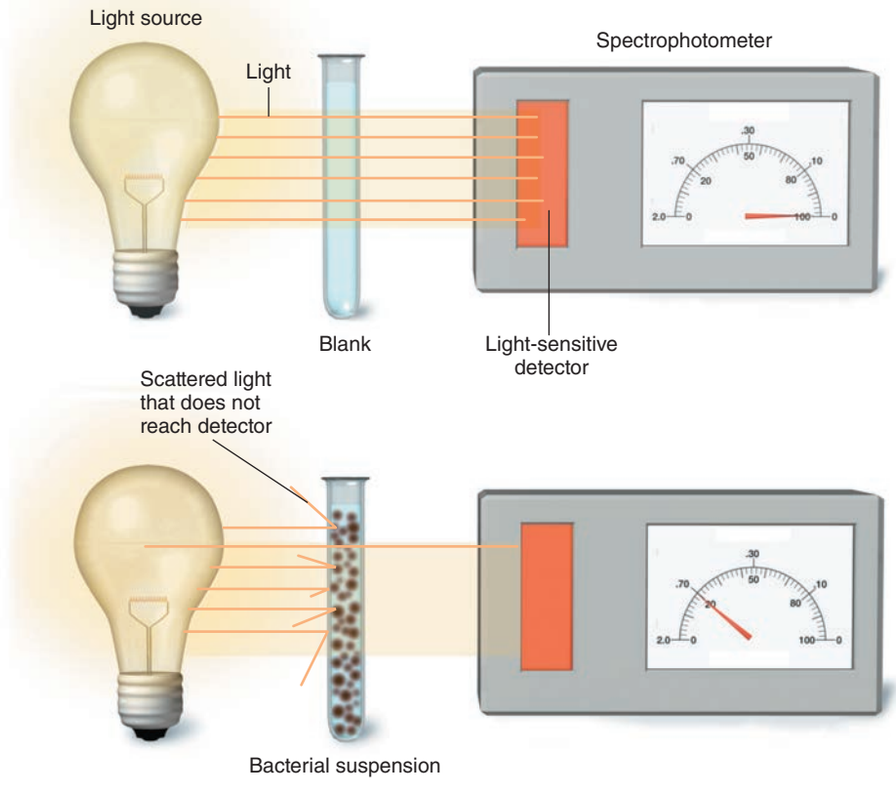
4 The volume of fluid over the large square is 1/1,250,000 of a milliliter. If it contains 14 cells, as shown here, then there are 14 × 1,250,000 = 17,500,000 cells in a milliliter.

Figure 6.20 Direct microscopic count of bacteria with a Petroff-Hausser cell counter. The average number of cells within a large square multiplied by a factor of 1,250,000 gives the number of bacteria per milliliter.

Q This type of counting, despite its obvious disadvantages, is often used in estimating the bacterial population in dairy products. Why?

Figure 6.21 Turbidity estimation of bacterial numbers. The amount of light striking the light-sensitive detector on the spectrophotometer is inversely proportional to the number of bacteria under standardized conditions. The less light transmitted, the more bacteria in the sample. The turbidity of the sample could be reported as either 20% transmittance or 0.7 absorbance. Readings in absorbance are a logarithmic function and are sometimes useful in plotting data.

Q Why is turbidity more useful in measuring contamination of liquids by large numbers, rather than small numbers, of bacteria?



More than a million cells per milliliter must be present for the first traces of turbidity to be visible. About 10 million to 100 million cells per milliliter are needed to make a suspension turbid enough to be read on a spectrophotometer. Therefore, turbidity is not a useful measure of contamination of liquids by relatively small numbers of bacteria.

Metabolic Activity

Another indirect way to estimate bacterial numbers is to measure a population's *metabolic activity*. This method assumes that the amount of a certain metabolic product, such as acid or CO_2, is in direct proportion to the number of bacteria present. An example of a practical application of a metabolic test is the microbiological assay (described on page 158) in which acid production is used to determine amounts of vitamins.

Dry Weight

For filamentous bacteria and molds, the usual measuring methods are less satisfactory. A plate count would not measure this increase in filamentous mass. In plate counts of actinomycetes (see Figure 11.26, page 313) and molds, it is mostly the number of asexual spores that is counted instead. This is not a good measure of growth. One of the better ways to measure the growth of filamentous organisms is by *dry weight*. In this procedure, the fungus is removed from the growth medium, filtered to remove extraneous material, and dried in a desiccator. It is then weighed. For bacteria, the same basic procedure is followed.

CHECK YOUR UNDERSTANDING

- Direct methods usually require an incubation time for a colony. Why is this not always feasible for analyzing foods? **6-17**
- If there is no good method for analyzing a product for its vitamin content, what is a feasible method of determining the vitamin content? **6-18**

* * *

You now have a basic understanding of the requirements for, and measurements of, microbial growth. In Chapter 7, we will look at how this growth is controlled in laboratories, hospitals, industry, and our homes.

Clinical Case Resolved

Biofilms are dense accumulations of cells. Five cells might go through 20 generations in a month, producing 7.79×10^6 cells. Now Dr. MacGruder knows that the *P. fluorescens* bacteria are present in the patients' indwelling catheters. He orders the catheters to be replaced and has the CDC examine the used catheters with scanning electron microscopy. They discover that the *P. fluorescens* colonized the inside of the catheters by forming biofilms. In his report to the CDC, Dr. MacGruder explains that the *P. fluorescens* bacteria may have entered the bloodstreams of these patients at the same time as the first outbreak, but not in sufficient quantities to cause symptoms at that time. Biofilm formation enabled the bacteria to persist in the patients' catheters. He notes that previous electron microscopy studies indicate that nearly all indwelling vascular catheters become colonized by microorganisms that are embedded in a biofilm layer and that heparin has been reported to stimulate biofilm formation. Dr. MacGruder concludes that the bacteria in the biofilm were dislodged by subsequent uncontaminated intravenous solutions and released into the bloodstream, finally causing infections months after initial colonization.

150 162 170 172

Study Outline

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The Requirements for Growth (pp. 150–156)

1. The growth of a population is an increase in the number of cells.
2. The requirements for microbial growth are both physical and chemical.

Physical Requirements (pp. 150–153)

3. On the basis of preferred temperature ranges, microbes are classified as psychrophiles (cold-loving), mesophiles (moderate-temperature–loving), and thermophiles (heat-loving).
4. The minimum growth temperature is the lowest temperature at which a species will grow, the optimum growth temperature is the temperature at which it grows best, and the maximum growth temperature is the highest temperature at which growth is possible.
5. Most bacteria grow best at a pH value between 6.5 and 7.5.
6. In a hypertonic solution, most microbes undergo plasmolysis; halophiles can tolerate high salt concentrations.

Chemical Requirements (pp. 154–156)

7. All organisms require a carbon source; chemoheterotrophs use an organic molecule, and autotrophs typically use carbon dioxide.
8. Nitrogen is needed for protein and nucleic acid synthesis. Nitrogen can be obtained from the decomposition of proteins or from NH_4^+ or NO_3^-; a few bacteria are capable of nitrogen (N_2) fixation.
9. On the basis of oxygen requirements, organisms are classified as obligate aerobes, facultative anaerobes, obligate anaerobes, aerotolerant anaerobes, and microaerophiles.

10. Aerobes, facultative anaerobes, and aerotolerant anaerobes must have the enzymes superoxide dismutase ($2\ O_2^- + 2\ H^+ \longrightarrow O_2 + H_2O_2$) and either catalase ($2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$) or peroxidase ($H_2O_2 + 2\ H^+ \longrightarrow 2\ H_2O$).
11. Other chemicals required for microbial growth include sulfur, phosphorus, trace elements, and, for some microorganisms, organic growth factors.

Biofilms (pp. 156–157) interactivemicrobiology

See how biofilms affect a patient's health @ MasteringMicrobiology®.

1. Microbes adhere to surfaces and accumulate as biofilms on solid surfaces in contact with water.
2. Most bacteria live in biofilms.
3. Microbes in biofilms are more resistant to antibiotics than are free-swimming microbes.

Culture Media (pp. 157–162)

1. A culture medium is any material prepared for the growth of bacteria in a laboratory.
2. Microbes that grow and multiply in or on a culture medium are known as a culture.
3. Agar is a common solidifying agent for a culture medium.

Chemically Defined Media (p. 158)

4. A chemically defined medium is one in which the exact chemical composition is known.

Complex Media (p. 159)

5. A complex medium is one in which the exact chemical composition varies slightly from batch to batch.

Anaerobic Growth Media and Methods (pp. 159–160)

6. Reducing media chemically remove molecular oxygen (O_2) that might interfere with the growth of anaerobes.
7. Petri plates can be incubated in an anaerobic jar, anaerobic chamber, or OxyPlate.

Special Culture Techniques (p. 160)

8. Some parasitic and fastidious bacteria must be cultured in living animals or in cell cultures.
9. CO_2 incubators or candle jars are used to grow bacteria that require an increased CO_2 concentration.
10. Procedures and equipment to minimize exposure to pathogenic microorganisms are designated as biosafety levels 1 through 4.

Selective and Differential Media (pp. 160–161)

11. By inhibiting unwanted organisms with salts, dyes, or other chemicals, selective media allow growth of only the desired microbes.
12. Differential media are used to distinguish different organisms.

Enrichment Culture (pp. 161–162)

13. An enrichment culture is used to encourage the growth of a particular microorganism in a mixed culture.

Obtaining Pure Cultures (pp. 162–163)

1. A colony is a visible mass of microbial cells that theoretically arose from one cell.
2. Pure cultures are usually obtained by the streak plate method.

Preserving Bacterial Cultures (p. 163)

1. Microbes can be preserved for long periods of time by deep-freezing or lyophilization (freeze-drying).

The Growth of Bacterial Cultures (p. 163)

Bacterial Division (pp. 163–164)

1. The normal reproductive method of bacteria is binary fission, in which a single cell divides into two identical cells.
2. Some bacteria reproduce by budding, aerial spore formation, or fragmentation.

Generation Time (pp. 164–165)

3. The time required for a cell to divide or a population to double is known as the generation time.

Logarithmic Representation of Bacterial Populations (p. 165)

4. Bacterial division occurs according to a logarithmic progression (two cells, four cells, eight cells, and so on).

Phases of Growth (pp. 165–166)

5. During the lag phase, there is little or no change in the number of cells, but metabolic activity is high.
6. During the log phase, the bacteria multiply at the fastest rate possible under the conditions provided.
7. During the stationary phase, there is an equilibrium between cell division and death.
8. During the death phase, the number of deaths exceeds the number of new cells formed.

Direct Measurement of Microbial Growth (pp. 166–170)

9. A heterotrophic plate count reflects the number of viable microbes and assumes that each bacterium grows into a single colony; plate counts are reported as number of colony-forming units (CFU).
10. A plate count may be done by either the pour plate method or the spread plate method.
11. In filtration, bacteria are retained on the surface of a membrane filter and then transferred to a culture medium to grow and subsequently be counted.
12. The most probable number (MPN) method can be used for microbes that will grow in a liquid medium; it is a statistical estimation.
13. In a direct microscopic count, the microbes in a measured volume of a bacterial suspension are counted with the use of a specially designed slide.

Estimating Bacterial Numbers by Indirect Methods (pp. 170–172)

14. A spectrophotometer is used to determine turbidity by measuring the amount of light that passes through a suspension of cells.
15. An indirect way of estimating bacterial numbers is measuring the metabolic activity of the population (for example, acid production or oxygen consumption).
16. For filamentous organisms such as fungi, measuring dry weight is a convenient method of growth measurement.

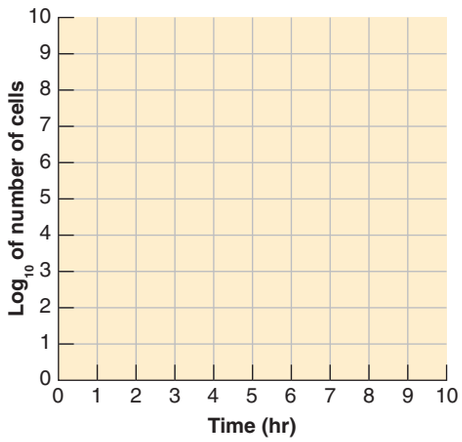
Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. Describe binary fission.
2. Macronutrients (needed in relatively large amounts) are often listed as CHONPS. What does each of these letters indicate, and why are they needed by the cell?
3. Define and explain the importance of each of the following:
   a. catalase
   b. hydrogen peroxide
   c. peroxidase
   d. superoxide radical
   e. superoxide dismutase
4. Seven methods of measuring microbial growth were explained in this chapter. Categorize each as either a direct or an indirect method.
5. By deep-freezing, bacteria can be stored without harm for extended periods. Why do refrigeration and freezing preserve foods?
6. A pastry chef accidentally inoculated a cream pie with six *S. aureus* cells. If *S. aureus* has a generation time of 60 minutes, how many cells would be in the cream pie after 7 hours?
7. Nitrogen and phosphorus added to beaches following an oil spill encourage the growth of natural oil-degrading bacteria. Explain why the bacteria do not grow if nitrogen and phosphorus are not added.
8. Differentiate complex and chemically defined media.
9. DRAW IT Draw the following growth curves for *E. coli,* starting with 100 cells with a generation time of 30 minutes at 35°C, 60 minutes at 20°C, and 3 hours at 5°C.
   a. The cells are incubated for 5 hours at 35°C.
   b. After 5 hours, the temperature is changed to 20°C for 2 hours.
   c. After 5 hours at 35°C, the temperature is changed to 5°C for 2 hours followed by 35°C for 5 hours.



10. NAME IT A prokaryotic cell hitched a ride to Earth on a space shuttle from some unknown planet. The organism is a psychrophile, an obligate halophile, and an obligate aerobe. Based on the characteristics of the microbe, describe the planet.

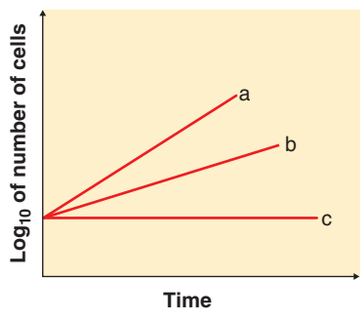
Multiple Choice

Use the following information to answer questions 1 and 2. Two culture media were inoculated with four different bacteria. After incubation, the following results were obtained:

Organism	Medium 1	Medium 2
Escherichia coli	Red colonies	No growth
Staphylococcus aureus	No growth	Growth
Staphylococcus epidermidis	No growth	Growth
Salmonella enterica	Colorless colonies	No growth

1. Medium 1 is
   a. selective.
   b. differential.
   c. both selective and differential.
2. Medium 2 is
   a. selective.
   b. differential.
   c. both selective and differential.

Use the following graph to answer questions 3 and 4.

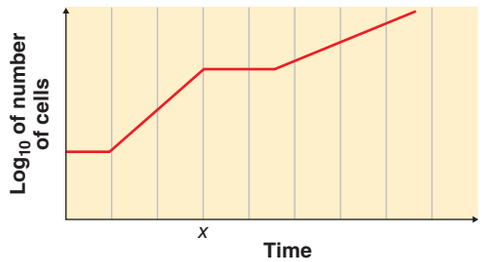


3. Which of the lines best depicts the log phase of a thermophile incubated at room temperature?
4. Which of the lines best depicts the log phase of *Listeria monocytogenes* growing in a human?
5. Assume you inoculated 100 facultatively anaerobic cells onto nutrient agar and incubated the plate aerobically. You then inoculated 100 cells of the same species onto nutrient agar and incubated the second plate anaerobically. After incubation for 24 hours, you should have
   a. more colonies on the aerobic plate.
   b. more colonies on the anaerobic plate.
   c. the same number of colonies on both plates.
6. The term *trace elements* refers to
   a. the elements CHONPS.
   b. vitamins.
   c. nitrogen, phosphorus, and sulfur.
   d. small mineral requirements.
   e. toxic substances.
7. Which one of the following temperatures would most likely kill a mesophile?
   a. −50°C
   b. 0°C
   c. 9°C
   d. 37°C
   e. 60°C

8. Which of the following is *not* a characteristic of biofilms?
   a. antibiotic resistance
   b. hydrogel
   c. iron deficiency
   d. quorum sensing
9. Which of the following types of media would *not* be used to culture aerobes?
   a. selective media
   b. reducing media
   c. enrichment media
   d. differential media
   e. complex media
10. An organism that has peroxidase and superoxide dismutase but lacks catalase is most likely an
    a. aerobe.
    b. aerotolerant anaerobe.
    c. obligate anaerobe.

Analysis

1. *E. coli* was incubated with aeration in a nutrient medium containing two carbon sources, and the following growth curve was made from this culture.
   a. Explain what happened at the time marked *x*.
   b. Which substrate provided "better" growth conditions for the bacteria? How can you tell?



2. *Clostridium* and *Streptococcus* are both catalase-negative. *Streptococcus* grows by fermentation. Why is *Clostridium* killed by oxygen, whereas *Streptococcus* is not?
3. Most laboratory media contain a fermentable carbohydrate and peptone because the majority of bacteria require carbon, nitrogen, and energy sources in these forms. How are these three needs met by glucose–minimal salts medium? (*Hint:* See Table 6.2.)
4. Flask A contains yeast cells in glucose–minimal salts broth incubated at 30°C with aeration. Flask B contains yeast cells in glucose–minimal salts broth incubated at 30°C in an anaerobic jar. The yeasts are facultative anaerobes.
   a. Which culture produced more ATP?
   b. Which culture produced more alcohol?
   c. Which culture had the shorter generation time?
   d. Which culture had the greater cell mass?
   e. Which culture had the higher absorbance?

Clinical Applications and Evaluation

1. Assume that after washing your hands, you leave 10 bacterial cells on a new bar of soap. You then decide to do a plate count of the soap after it was left in the soap dish for 24 hours. You dilute 1 g of the soap 1:10^6 and plate it on heterotrophic plate count agar. After 24 hours of incubation, there are 168 colonies. How many bacteria were on the soap? How did they get there?
2. Heat lamps are commonly used to maintain foods at about 50°C for as long as 12 hours in cafeteria serving lines. The following experiment was conducted to determine whether this practice poses a potential health hazard.

   Beef cubes were surface-inoculated with 500,000 bacterial cells and incubated at 43–53°C to establish temperature limits for bacterial growth. The following results were obtained from heterotrophic plate counts performed on beef cubes at 6 and 12 hours after inoculation:

		Bacteria per Gram of Beef After	
	Temp. (°C)	6 hr	12 hr
Staphylococcus aureus	43	140,000,000	740,000,000
	51	810,000	59,000
	53	650	300
Salmonella typhimurium	43	3,200,000	10,000,000
	51	950,000	83,000
	53	1,200	300
Clostridium perfringens	43	1,200,000	3,600,000
	51	120,000	3,800
	53	300	300

   Draw the growth curves for each organism. What holding temperature would you recommend? Assuming that cooking kills bacteria in foods, how could these bacteria contaminate the cooked foods? What disease does each organism cause? (*Hint:* See Chapter 25.)
3. The number of bacteria in saliva samples was determined by collecting the saliva, making serial dilutions, and inoculating nutrient agar by the pour plate method. The plates were incubated aerobically for 48 hours at 37°C.

	Bacteria per ml Saliva	
	Before Using Mouthwash	After Using Mouthwash
Mouthwash 1	13.1×10^6	10.9×10^6
Mouthwash 2	11.7×10^6	14.2×10^5
Mouthwash 3	9.3×10^5	7.7×10^5

   What can you conclude from these data? Did all the bacteria present in each saliva sample grow?

In the Clinic

As a hospital's infection control nurse, you become aware that 15 patients developed *Clostridium difficile* infections within a month. This rate of infection is 10 per 1000 patients—nearly 300% higher than the average of 2.7 cases per 1000 patients seen in previous months. You request that housekeeping clean rooms and equipment with a hypochlorite-based disinfectant rather than the standard hospital (quat) disinfectant normally used. The next month, the rate of infection is 3 cases per 1000. **Did your cleaning program work? How did you reach your conclusion?**

Hint: Read about chlorines (page 188) and quats (page 191); see Table 7.7.

Note: Answers to In the Clinic are found online at MasteringMicrobiology.

7 The Control of Microbial Growth

The scientific control of microbial growth began only about 100 years ago. Recall from Chapter 1 that Pasteur's work on microorganisms led scientists to believe that microbes were a possible cause of disease. In the mid-1800s, the Hungarian physician Ignaz Semmelweis and English physician Joseph Lister used this thinking to develop some of the first microbial control practices for medical procedures. These practices included washing hands with microbe-killing chloride of lime [$Ca(OCl)_2$] and using the techniques of **aseptic surgery** to prevent microbial contamination of surgical wounds. Until that time, hospital-acquired infections, or *nosocomial infections,* were the cause of death in at least 10% of surgical cases, and as high as 25% in delivering mothers. Ignorance of microbes was such that during the American Civil War, a surgeon might have cleaned his scalpel on his boot sole between incisions. We now know that handwashing is the best way to prevent transmission of pathogens such as the norovirus in the photo. Controlling noroviruses on environmental surfaces is the topic of the Clinical Case.

Over the last century, scientists have continued to develop a variety of physical methods and chemical agents to control microbial growth. In Chapter 20 we will discuss methods for controlling microbes once infection has occurred, mainly antibiotic chemotherapy.

Norovirus.

The Terminology of Microbial Control

LEARNING OBJECTIVE

7-1 Define the following key terms related to microbial control: *sterilization, disinfection, antisepsis, degerming, sanitization, biocide, germicide, bacteriostasis,* and *asepsis*.

A word frequently used, and misused, in discussing the control of microbial growth is *sterilization*. **Sterilization** is the removal or destruction of *all* living microorganisms. Heating is the most common method used for killing microbes, including the most resistant forms, such as endospores. A sterilizing agent is called a **sterilant.** Liquids or gases can be sterilized by filtration.

ASM: The growth of microorganisms can be controlled by physical, chemical, mechanical, or biological means.

One would think that canned food in the supermarket is completely sterile. In reality, the heat treatment required to ensure absolute sterility would unnecessarily degrade the quality of the food. Instead, food is subjected only to enough heat to destroy the endospores of *Clostridium botulinum* (BO-tū-lī′num), which can produce a deadly toxin. This limited heat treatment is termed **commercial sterilization.** The endospores of a number of thermophilic bacteria, capable of causing food spoilage but not human disease, are considerably more resistant to heat than *C. botulinum*. If present, they will survive, but their survival is usually of no practical consequence; they will not grow at normal food storage temperatures. If canned foods in a supermarket were incubated at temperatures in the growth range of these thermophiles (above about 45°C), significant food spoilage would occur.

Complete sterilization is often not required in other settings. For example, the body's normal defenses can cope with a few microbes entering a surgical wound. A drinking glass or a fork in a restaurant requires only enough microbial control to prevent the transmission of possibly pathogenic microbes from one person to another.

Control directed at destroying harmful microorganisms is called **disinfection.** It usually refers to the destruction of vegetative (non–endospore-forming) pathogens, which is not the same thing as complete sterility. Disinfection might make use of chemicals, ultraviolet radiation, boiling water, or steam. In practice, the term is most commonly applied to the use of a chemical (a *disinfectant*) to treat an inert surface or substance. When this treatment is directed at living tissue, it is called **antisepsis,** and the chemical is then called an *antiseptic*. Therefore, in practice the same chemical might be called a disinfectant for one use and an antiseptic for another. Of course, many chemicals suitable for wiping a tabletop would be too harsh to use on living tissue.

There are modifications of disinfection and antisepsis. For example, when someone is about to receive an injection, the skin is swabbed with alcohol—the process of **degerming** (or *degermation*), which mostly results in the mechanical removal, rather than the killing, of most of the microbes in a limited area. Restaurant glassware, china, and tableware are subjected to **sanitization,** which is intended to lower microbial counts to safe public health levels and minimize the chances of disease transmission from one user to another. This is usually accomplished by high-temperature washing or, in the case of glassware in a bar, washing in a sink followed by a dip in a chemical disinfectant.

Table 7.1 summarizes the terminology relating to the control of microbial growth.

Names of treatments that cause the outright death of microbes have the suffix *-cide,* meaning kill. A **biocide,** or **germicide,** kills microorganisms (usually with certain exceptions, such as endospores); a *fungicide* kills fungi; a *virucide* inactivates viruses; and so on. Other treatments only inhibit the growth and multiplication of bacteria; their names have the suffix *-stat* or *-stasis,* meaning to stop or to steady, as in **bacteriostasis.** Once a bacteriostatic agent is removed, growth might resume.

TABLE 7.1 Terminology Relating to the Control of Microbial Growth

	Definition	Comments
Sterilization	Destruction or removal of all forms of microbial life, including endospores but with the possible exception of prions.	Usually done by steam under pressure or a sterilizing gas, such as ethylene oxide.
Commercial Sterilization	Sufficient heat treatment to kill endospores of *Clostridium botulinum* in canned food.	More-resistant endospores of thermophilic bacteria may survive, but they will not germinate and grow under normal storage conditions.
Disinfection	Destruction of vegetative pathogens on inanimate objects.	May make use of physical or chemical methods.
Antisepsis	Destruction of vegetative pathogens on living tissue.	Treatment is almost always by chemical antimicrobials.
Degerming	Removal of microbes from a limited area, such as the skin around an injection site.	Mostly a mechanical removal by an alcohol-soaked swab.
Sanitization	Treatment is intended to lower microbial counts on eating and drinking utensils to safe public health levels.	May be done with high-temperature washing or by dipping into a chemical disinfectant.

TABLE 7.2 Microbial Exponential Death Rate: An Example

Time (min)	Deaths per Minute	Number of Survivors
0	0	1,000,000
1	900,000	100,000
2	90,000	10,000
3	9000	1000
4	900	100
5	90	10
6	9	1

Sepsis, from the Greek for decay or putrid, indicates bacterial contamination, as in septic tanks for sewage treatment. (The term is also used to describe a disease condition; see Chapter 23, page 639) *Aseptic* means that an object or area is free of pathogens. **Asepsis** is the absence of significant contamination (see Chapter 1). Aseptic techniques are important in surgery to minimize contamination from the instruments, operating personnel, and the patient.

CHECK YOUR UNDERSTANDING

The usual definition of *sterilization* is the removal or destruction of all forms of microbial life; how could there be practical exceptions to this simple definition? **7-1**

The Rate of Microbial Death

LEARNING OBJECTIVE

7-2 Describe the patterns of microbial death caused by treatments with microbial control agents.

When bacterial populations are heated or treated with antimicrobial chemicals, they usually die at a constant rate. For example, suppose a population of 1 million microbes has been treated for 1 minute, and 90% of the population has died. We are now left with 100,000 microbes. If the population is treated for another minute, 90% of *those* microbes die, and we are left with 10,000 survivors. In other words, for each minute the treatment is applied, 90% of the remaining population is killed (**Table 7.2**). If the death curve is plotted logarithmically, the death rate is constant, as shown by the straight line in **Figure 7.1a**.

Several factors influence the effectiveness of antimicrobial treatments:

- *The number of microbes.* The more microbes there are to begin with, the longer it takes to eliminate the entire population (Figure 7.1b).
- *Environmental influences.* Most disinfectants work somewhat better in warm solutions.

The presence of organic matter often inhibits the action of chemical antimicrobials. In hospitals, the presence of organic matter in blood, vomitus, or feces influences the selection of disinfectants. Microbes in surface biofilms, when they are encased in the mucoid matrix (see page 156), are difficult for biocides to reach effectively. Because their activity is due to temperature-dependent chemical reactions, disinfectants work somewhat better under warm conditions.

The nature of the suspending medium is also a factor in heat treatment. Fats and proteins are especially protective, and a medium rich in these substances protects microbes, which will then have a higher survival rate. Heat is also measurably more effective under acidic conditions.

- *Time of exposure.* Chemical antimicrobials often require extended exposure to affect more-resistant microbes or endospores. See the discussion of equivalent treatments on page 182.
- *Microbial characteristics.* The concluding section of this chapter discusses how microbial characteristics affect the choice of chemical and physical control methods.

CHECK YOUR UNDERSTANDING

How is it possible that a solution containing a million bacteria would take longer to sterilize than one containing a half-million bacteria? **7-2**

Actions of Microbial Control Agents

LEARNING OBJECTIVE

7-3 Describe the effects of microbial control agents on cellular structures.

In this section, we examine the ways various agents actually kill or inhibit microbes.

Clinical Case: A School Epidemic

It is 9:00 A.M. on a Wednesday morning, and Amy Garza, the school nurse at Westview Elementary School in Rockville, Maryland, has been on the phone since she came in to work at 7:00 A.M. So far this morning, she has received reports of students unable to attend school today because of some sort of gastrointestinal ailment. They all have the same symptoms: nausea and vomiting, diarrhea, and a low-grade fever. As Amy picks up the phone to call the principal to give her an update, she receives her eighth call of the day. Keith Jackson, a first-grade teacher who has been out sick since Monday, calls to tell Amy that his physician sent his stool sample to the laboratory for testing. The results came back positive for norovirus.

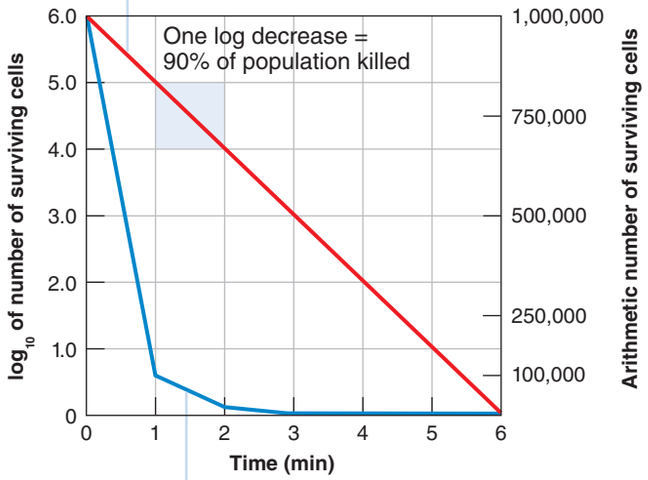
What is norovirus? Read on to find out.

178 192 194 195

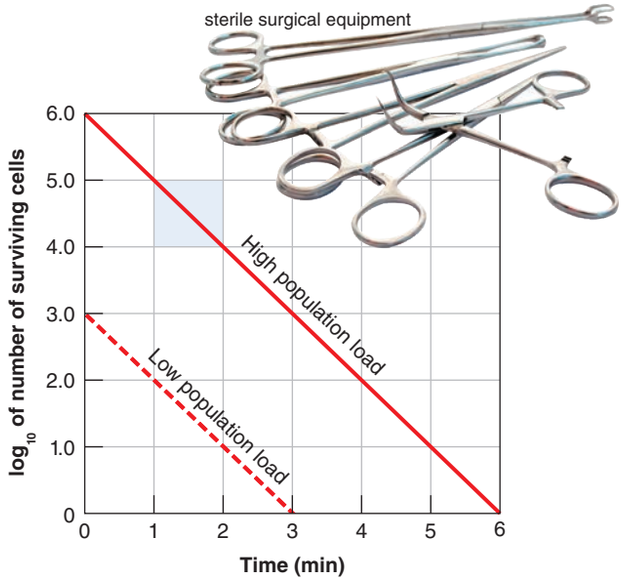
FOUNDATION FIGURE 7.1

Understanding the Microbial Death Curve

Plotting the typical microbial death curve **logarithmically** (**red line**) results in a straight line.



(a) Plotting the typical microbial death curve **arithmetically** (**blue line**) is impractical: at 3 minutes the population of 1000 cells would only be a hundredth of the graphed distance between 100,000 and the baseline.



(b) Logarithmic plotting (**red**) reveals that if the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one, whether using heat or chemical treatments.

KEY CONCEPTS

- Bacterial populations usually die at a constant rate when heated or when treated with antimicrobial chemicals.
- It is necessary to use logarithmic numbers to graph bacterial populations effectively.
- Understanding logarithmic death curves for microbial populations, including the elements of time and the size of the initial population, is especially useful in food preservation and in the sterilization of media or medical supplies.

Foods preserved by heat

Alteration of Membrane Permeability

A microorganism's plasma membrane (see Figure 4.14, page 86), located just inside the cell wall, is the target of many microbial control agents. This membrane actively regulates the passage of nutrients into the cell and the elimination of wastes from the cell. Damage to the lipids or proteins of the plasma membrane by antimicrobial agents causes cellular contents to leak into the surrounding medium and interferes with the growth of the cell.

Damage to Proteins and Nucleic Acids

Bacteria are sometimes thought of as "little bags of enzymes." Enzymes, which are primarily protein, are vital to all cellular activities. Recall that the functional properties of proteins are the result of their three-dimensional shape (see Figure 2.15, page 43). This shape is maintained by chemical bonds that link adjoining portions of the amino acid chain as it folds back and forth upon itself. Some of those bonds are hydrogen bonds, which are susceptible to breakage by heat or certain chemicals; breakage results in denaturation of the protein. Covalent bonds are stronger but are also subject to attack. For example, disulfide bridges, which play an important role in protein structure by joining amino acids with exposed sulfhydryl (—SH) groups, can be broken by certain chemicals or sufficient heat.

The nucleic acids DNA and RNA are the carriers of the cell's genetic information. Damage to these nucleic acids by heat, radiation, or chemicals is frequently lethal to the cell; the cell can no longer replicate, nor can it carry out normal metabolic functions such as the synthesis of enzymes.

CHECK YOUR UNDERSTANDING

- Would a chemical microbial control agent that affects plasma membranes affect humans? **7-3**

Physical Methods of Microbial Control

LEARNING OBJECTIVES

- **7-4** Compare the effectiveness of moist heat (boiling, autoclaving, pasteurization) and dry heat.
- **7-5** Describe how filtration, low temperatures, high pressure, desiccation, and osmotic pressure suppress microbial growth.
- **7-6** Explain how radiation kills cells.

As early as the Stone Age, humans likely were already using some physical methods of microbial control to preserve foods. Drying (desiccation) and salting (osmotic pressure) were probably among the earliest techniques.

When selecting methods of microbial control, one must consider what else, besides the microbes, a particular method will affect. For example, heat might inactivate certain vitamins or antibiotics in a solution. Repeated heating damages many laboratory and hospital materials, such as rubber and latex tubing. There are also economic considerations; for example, it may be less expensive to use presterilized, disposable plasticware than to repeatedly wash and resterilize glassware.

Heat

A visit to any supermarket will demonstrate that heat-preserved canned goods represent one of the most common methods of food preservation. Heat is also usually used to sterilize laboratory media and glassware and hospital instruments. Heat appears to kill microorganisms by denaturing their enzymes; the resultant changes to the three-dimensional shapes of these proteins inactivate them (see Figure 5.6, page 115).

Heat resistance varies among different microbes; these differences can be expressed through the concept of thermal death point. **Thermal death point (TDP)** is the lowest temperature at which all the microorganisms in a particular liquid suspension will be killed in 10 minutes.

Another factor to be considered in sterilization is the length of time required. This is expressed as **thermal death time (TDT),** the minimal length of time for all bacteria in a particular liquid culture to be killed at a given temperature. Both TDP and TDT are useful guidelines that indicate the severity of treatment required to kill a given population of bacteria.

Decimal reduction time (**DRT,** or *D value*) is a third concept related to bacterial heat resistance. DRT is the time, in minutes, in which 90% of a population of bacteria at a given temperature will be killed (in Table 7.2 and Figure 7.1a, DRT is 1 minute). In Chapter 28 you can find an important application of DRT in the canning industry.

Moist Heat Sterilization

Moist heat kills microorganisms primarily by coagulating proteins (denaturation), which is caused by breakage of the hydrogen bonds that hold the proteins in their three-dimensional structure. This coagulation process is familiar to anyone who has watched an egg white frying.

One type of moist heat "sterilization" is boiling, which kills vegetative forms of bacterial pathogens, almost all viruses, and fungi and their spores within about 10 minutes, usually much faster. Free-flowing (unpressurized) steam is essentially the same temperature as boiling water. Endospores and some viruses, however, are not destroyed this quickly. For example, some bacterial endospores can resist boiling for more than 20 hours. Boiling is therefore not always a reliable sterilization procedure. However, brief boiling, even at high altitudes, will kill most pathogens. The use of boiling to sanitize glass baby bottles is a familiar example.

Reliable sterilization with moist heat requires temperatures above that of boiling water. These high temperatures are most commonly achieved by steam under pressure in an **autoclave** (**Figure 7.2**). Autoclaving is the preferred method of sterilization in health care environments, unless the material to be sterilized can be damaged by heat or moisture. The higher the pressure in the autoclave, the higher the temperature. For example, when free-flowing steam at a temperature of 100°C is placed under a pressure of 1 atmosphere above sea level pressure—that is, about 15 pounds of pressure per square inch (psi)—the temperature rises to 121°C. Increasing the pressure to 20 psi raises the temperature to 126°C. The relationship between temperature and pressure is shown in **Table 7.3**.

Sterilization in an autoclave is most effective when the organisms either are contacted by the steam directly or are contained in a small volume of aqueous (primarily water) liquid. Under these conditions, steam at a pressure of about 15 psi (121°C) will kill *all* organisms (but not prions; see page 195) and their endospores in about 15 minutes. Sterilizing the surface of a solid requires that steam actually contact it. To sterilize dry glassware, bandages, and the like, care must be taken to ensure that steam contacts all surfaces. For example, aluminum foil is impervious to steam and should not be used to wrap dry materials that are to be sterilized; paper should be used instead. Care should also be taken to avoid trapping air in the bottom of a dry container: trapped air will not be replaced by steam, because steam is lighter than air. The trapped air is the equivalent of a small hot-air oven, which, as we will see shortly, requires a higher temperature and longer time to sterilize materials. Containers that can trap air should be placed in a tipped position so that the steam will force out the air. Products that do not permit penetration by moisture, such as mineral oil or petroleum jelly, are not sterilized by the same methods used to sterilize aqueous solutions. Large industrial autoclaves are called *retorts* (see Figure 7.2), but the same principle applies for the common household pressure cooker used in the home canning of foods.

Autoclaving is used to sterilize culture media, instruments, dressings, intravenous equipment, applicators, solutions, syringes, transfusion equipment, and numerous other items that can withstand high temperatures and pressures.

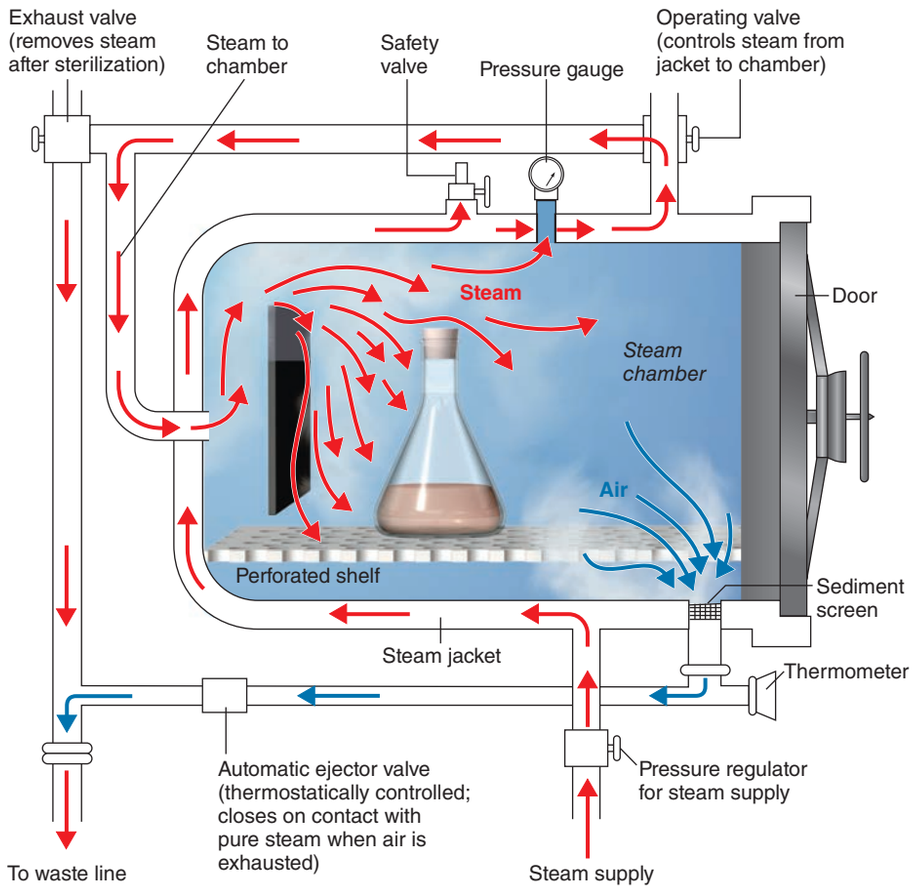


Figure 7.2 An autoclave. The entering steam forces the air out of the bottom (blue arrows). The automatic ejector valve remains open as long as an air-steam mixture is passing out of the waste line. When all the air has been ejected, the higher temperature of the pure steam closes the valve, and the pressure in the chamber increases.

Q How would an empty, uncapped flask be positioned for sterilization in an autoclave?

Heat requires extra time to reach the center of solid materials, such as canned meats, because such materials do not develop the efficient heat-distributing convection currents that occur in liquids. Heating large containers also requires extra time.

TABLE 7.3 The Relationship between the Pressure and Temperature of Steam at Sea Level*

Pressure (psi in Excess of Atmospheric Pressure)	Temperature (°C)
0	100
5	110
10	116
15	121
20	126
30	135

*At higher altitudes, the atmospheric pressure is less, a phenomenon that must be taken into account in operating an autoclave. For example, to reach sterilizing temperatures (121°C) in Denver, Colorado, whose altitude is 5280 feet (1600 meters), the pressure shown on the autoclave gauge would need to be higher than the 15 psi shown in the table.

Table 7.4 shows the different time requirements for sterilizing liquids in various container sizes.

Several commercially available methods can indicate whether heat treatment has achieved sterilization. Some of these are chemical reactions in which an indicator changes color when the proper times and temperatures have been reached (Figure 7.3). In some designs, the word *sterile* or *autoclaved* appears on wrappings or tapes. This test has a practical advantage; it does not require an incubation time for growth of a test organism, which is important if the sterilized product is intended for immediate consumption. A widely used test consists of preparations of specified species of bacterial endospores impregnated into paper strips. After the strips are autoclaved, they can then be aseptically inoculated into culture media. Growth in the culture media indicates survival of the endospores and therefore inadequate processing. Other designs use endospore suspensions that can be released, after heating, into a surrounding culture medium within the same vial.

Steam under pressure fails to sterilize when the air is not completely exhausted. This can happen with the premature closing of the autoclave's automatic ejector valve (see Figure 7.2). The principles of heat sterilization have a direct bearing on home canning. As anyone familiar with home canning knows, the steam must flow vigorously out of the valve in the lid for

Figure 7.3 Examples of sterilization indicators. The strips indicate whether the item has been properly sterilized. The word *NOT* appears if heating has been inadequate. In the illustration, the indicator that was wrapped with aluminum foil was not sterilized because steam couldn't penetrate the foil.

Q What should have been used to wrap the items instead of aluminum foil?

several minutes to carry with it all the air before the pressure cooker is sealed. If the air is not completely exhausted, the container will not reach the temperature expected for a given pressure. Because of the possibility of botulism, a kind of food poisoning resulting from improper canning methods (see Chapter 22, page 614), anyone doing home canning should obtain reliable directions and follow them exactly.

Pasteurization

In the early days of microbiology, Louis Pasteur found a practical method of preventing the spoilage of beer and wine (see Chapter 1). Pasteur used mild heating, which was sufficient to kill the organisms that caused the particular spoilage problem without seriously damaging the taste of the product. The same principle was later applied to milk to produce what we now call pasteurized milk. The intent of **pasteurization** of milk was to eliminate pathogenic microbes. It also lowers microbial numbers, which prolongs milk's good quality under refrigeration. Many relatively heat-resistant (**thermoduric**) bacteria survive pasteurization, but these are unlikely to cause disease or cause refrigerated milk to spoil.

Products other than milk, such as ice cream, yogurt, and beer, all have their own pasteurization times and temperatures, which often differ considerably. There are several reasons for these variations. For example, heating is less efficient in foods that are more viscous, and fats in food can have a protective effect on microorganisms. The dairy industry routinely uses a test to determine whether products have been pasteurized: the *phosphatase test* (phosphatase is an enzyme naturally present in milk). If the product has been pasteurized, phosphatase will have been inactivated.

Most milk pasteurization today uses temperatures of at least 72°C, but for only 15 seconds. This treatment, known as **high-temperature short-time (HTST) pasteurization,** is applied as the milk flows continuously past a heat exchanger. In addition to killing pathogens, HTST pasteurization lowers total bacterial counts, so the milk keeps well under refrigeration.

Sterilization

Milk can also be sterilized—something quite different from pasteurization—by **ultra-high-temperature (UHT) treatments.** It can then be stored for several months without refrigeration (also see *commercial sterilization,* page 795). UHT-treated milk is widely sold in Europe and is especially necessary in less developed parts of the world where refrigeration facilities are not always available. In the United States, UHT is sometimes used on the small containers of coffee creamers found in restaurants. To avoid giving the milk a cooked taste, the process avoids having the milk touch a surface hotter than the milk itself. Usually, the liquid milk (or juice) is sprayed through a nozzle into a chamber filled with high-temperature steam under pressure. A small volume of fluid sprayed into an atmosphere of high-temperature steam exposes a relatively large surface area on the fluid droplets to heating by the steam; sterilizing temperatures are reached almost instantaneously. After reaching a temperature of 140°C for 4 seconds, the fluid is rapidly cooled in a vacuum chamber. The milk or juice is then packaged in a presterilized, airtight container.

The heat treatments we have just discussed illustrate the concept of **equivalent treatments:** as the temperature is increased, much less time is needed to kill the same number of microbes. For example, suppose that the destruction of highly

TABLE 7.4 The Effect of Container Size on Autoclave Sterilization Times for Liquid Solutions*

Container Size	Liquid Volume	Sterilization Time (min)
Test tube: 18 × 150 mm	10 ml	15
Erlenmeyer flask: 125 ml	95 ml	15
Erlenmeyer flask: 2000 ml	1500 ml	30
Fermentation bottle: 9000 ml	6750 ml	70

*Sterilization times in the autoclave include the time for the contents of the containers to reach sterilization temperatures. For smaller containers, this is only 5 min or less, but for a 9000-ml bottle it might be as much as 70 min. Liquids in an autoclave boil vigorously, and so their containers are usually not filled past 75% of capacity.

resistant endospores might take 70 minutes at 115°C, whereas, in this hypothetical example, only 7 minutes might be needed at 125°C. Both treatments yield the same result.

Dry Heat Sterilization

Dry heat kills by oxidation effects. A simple analogy is the slow charring of paper in a heated oven, even when the temperature remains below the ignition point of paper. One of the simplest methods of dry heat sterilization is direct **flaming.** You will use this procedure many times in the microbiology laboratory when you sterilize inoculating loops. To effectively sterilize the inoculating loop, you heat the wire to a red glow. A similar principle is used in *incineration,* an effective way to sterilize and dispose of contaminated paper cups, bags, and dressings.

Another form of dry heat sterilization is **hot-air sterilization.** Items to be sterilized by this procedure are placed in an oven. Generally, a temperature of about 170°C maintained for nearly 2 hours ensures sterilization. The longer period and higher temperature (relative to moist heat) are required because the heat in water is more readily transferred to a cool body than is the heat in air. For example, imagine the different effects of immersing your hand in boiling water at 100°C (212°F) and of holding it in a hot-air oven at the same temperature for the same amount of time.

Filtration

Filtration is the passage of a liquid or gas through a screenlike material with pores small enough to retain microorganisms (often the same apparatus used for counting; see Figure 6.18, page 169). A vacuum is created in the receiving flask; air pressure then forces the liquid through the filter. Filtration is used to sterilize heat-sensitive materials, such as some culture media, enzymes, vaccines, and antibiotic solutions.

Some operating theaters and rooms occupied by burn patients receive filtered air to lower the numbers of airborne microbes. **High-efficiency particulate air (HEPA) filters** remove almost all microorganisms larger than about 0.3 µm in diameter.

In the early days of microbiology, hollow candle-shaped filters of unglazed porcelain were used to filter liquids. The long and indirect passageways through the walls of the filter adsorbed the bacteria. Unseen pathogens that passed through the filters (causing such diseases as rabies) were called *filterable viruses.* (See the discussion of filtration in modern water treatment on page 785.)

In recent years, **membrane filters,** composed of such substances as cellulose esters or plastic polymers, have become popular for industrial and laboratory use (**Figure 7.4**). These filters are only 0.1 mm thick. The pores of membrane filters, for example, 0.22-µm and 0.45-µm sizes, are intended for bacteria. Some very flexible bacteria, such as spirochetes, or the wall-less mycoplasma, will sometimes pass through such filters, however. Filters are available with pores as small as 0.01 µm, a size that will retain viruses and even some large protein molecules.

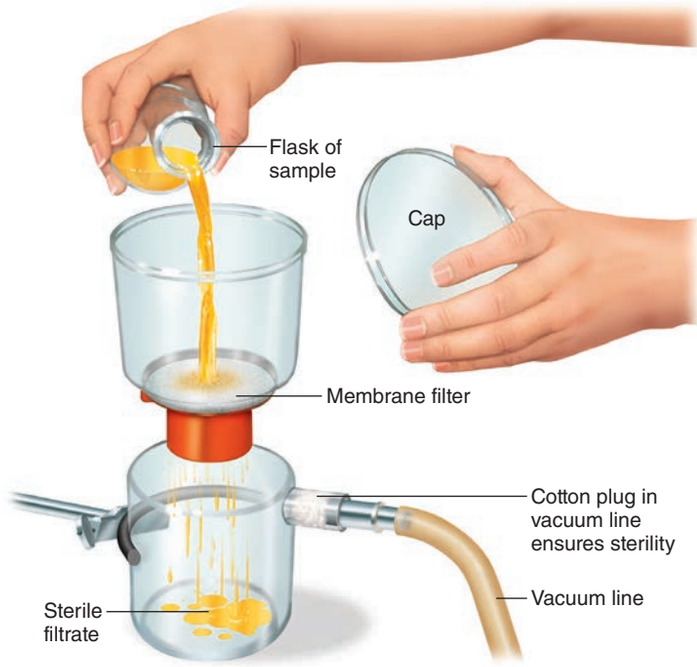


Figure 7.4 Filter sterilization with a disposable, presterilized plastic unit. The sample is placed into the upper chamber and forced through the membrane filter by a vacuum in the lower chamber. Pores in the membrane filter are smaller than the bacteria, so bacteria are retained on the filter. The sterilized sample can then be decanted from the lower chamber. Similar equipment with removable filter disks is used to count bacteria in samples (see Figure 6.18).

How is a plastic filtration apparatus presterilized? (Assume the plastic cannot be heat sterilized.)

Low Temperatures

The effect of low temperatures on microorganisms depends on the particular microbe and the intensity of the application. For example, at temperatures of ordinary refrigerators (0–7°C), the metabolic rate of most microbes is so reduced that they cannot reproduce or synthesize toxins. In other words, ordinary refrigeration has a bacteriostatic effect. Yet psychrotrophs do grow slowly at refrigerator temperatures and will alter the appearance and taste of foods over time. For example, a single microbe reproducing only three times a day would reach a population of more than 2 million within a week. Pathogenic bacteria generally will not grow at refrigerator temperatures, but *Listeria* is one important exception (see the discussion of listeriosis in Chapter 22, page 611).

Surprisingly, some bacteria can grow at temperatures several degrees below freezing. Most foods remain unfrozen until –2°C or lower. Rapidly attained subfreezing temperatures tend to render microbes dormant but do not necessarily kill them. Slow freezing is more harmful to bacteria; the ice crystals that form and grow disrupt the cellular and molecular structure of the bacteria. Thawing, being inherently slower, is actually the more

damaging part of a freeze-thaw cycle. Once frozen, one-third of the population of some vegetative bacteria might survive a year, whereas other species might have very few survivors after this time. Many eukaryotic parasites, such as the roundworms that cause human trichinellosis, are killed by several days of freezing temperatures. Some important temperatures associated with microorganisms and food spoilage are shown in Figure 6.2 (page 151).

High Pressure

High pressure applied to liquid suspensions is transferred instantly and evenly throughout the sample. If the pressure is high enough, it alters the molecular structures of proteins and carbohydrates, resulting in the rapid inactivation of vegetative bacterial cells. Endospores are relatively resistant to high pressure. Fruit juices preserved by high-pressure treatments are sold in Japan and the United States. An advantage is that these treatments preserve the flavors, colors, and nutrient values of the products.

Desiccation

In the absence of water, known as **desiccation,** microorganisms cannot grow or reproduce but can remain viable for years. Then, when water is made available to them, they can resume their growth and division. This is the principle that underlies lyophilization, or freeze-drying, a laboratory process for preserving microbes described in Chapter 6 (page 163). Certain foods are also freeze-dried (for example, coffee and some fruit additives for dry cereals).

The resistance of vegetative cells to desiccation varies with the species and the organism's environment. For example, the gonorrhea bacterium can withstand desiccation for only about an hour, but the tuberculosis bacterium can remain viable for months. Viruses are generally resistant to desiccation, but they are not as resistant as bacterial endospores, some of which have survived for centuries. This ability of certain dried microbes and endospores to remain viable is important in a hospital setting. Dust, clothing, bedding, and dressings might contain infectious microbes in dried mucus, urine, pus, and feces.

Osmotic Pressure

The use of high concentrations of salts and sugars to preserve food is based on the effects of *osmotic pressure*. High concentrations of these substances create a hypertonic environment that causes water to leave the microbial cell (see Figure 6.4, page 152). This process resembles preservation by desiccation, in that both methods deny the cell the moisture it needs for growth. The principle of osmotic pressure is used in the preservation of foods. For example, concentrated salt solutions are used to cure meats, and thick sugar solutions are used to preserve fruits.

As a general rule, molds and yeasts are much more capable than bacteria of growing in materials with low moisture or high osmotic pressures. This property of molds, sometimes combined with their ability to grow under acidic conditions, is the reason that molds, rather than bacteria, cause spoilage of fruits and grains. It is also part of the reason molds are able to form mildew on a damp wall or a shower curtain.

Radiation

Radiation has various effects on cells, depending on its wavelength, intensity, and duration. Radiation that kills microorganisms (sterilizing radiation) is of two types: ionizing and nonionizing.

Ionizing radiation—gamma rays, X rays, and high-energy electron beams—has a wavelength shorter than that of nonionizing radiation, less than about 1 nm. Therefore, it carries much more energy (**Figure 7.5**). *Gamma rays* are emitted by certain radioactive elements such as cobalt. Electron beams are produced by accelerating electrons to high energies in special machines, and *X rays,* which are produced by machines in a manner similar to the production of electron beams, are similar to gamma rays. Gamma rays penetrate deeply but may require hours to sterilize large masses; *high-energy electron beams* have much lower penetrating power but usually require only a few seconds of exposure. The principal effect of ionizing radiation is the ionization of water, which forms highly reactive hydroxyl radicals (see the discussion of toxic forms of oxygen in Chapter 6, pages 155–156). These radicals kill organisms by reacting with organic cellular components, especially DNA, and damaging them.

The so-called target theory of damage by radiation supposes that ionizing particles, or packets of energy, pass through or close to vital portions of the cell; these constitute "hits." One, or a few, hits may only cause nonlethal mutations, some of them conceivably useful. More hits are likely to cause sufficient mutations to kill the microbe.

The food industry is expanding the use of radiation for food preservation (discussed more fully in Chapter 28). Low-level ionizing radiation, used for years in many countries, has been approved in the United States for processing spices and certain meats and vegetables. Ionizing radiation, especially high-energy electron beams, is used to sterilize pharmaceuticals and disposable dental and medical supplies, such as plastic syringes, surgical gloves, suturing materials, and catheters. As a protection against bioterrorism, the postal service often uses electron beam radiation to sterilize certain classes of mail.

Nonionizing radiation has a wavelength longer than that of ionizing radiation, usually greater than about 1 nm. The best example of nonionizing radiation is ultraviolet (UV) light. UV light damages the DNA of exposed cells by causing bonds to form between adjacent pyrimidine bases, usually thymines, in DNA chains (see Figure 8.21, page 222). These *thymine dimers* inhibit

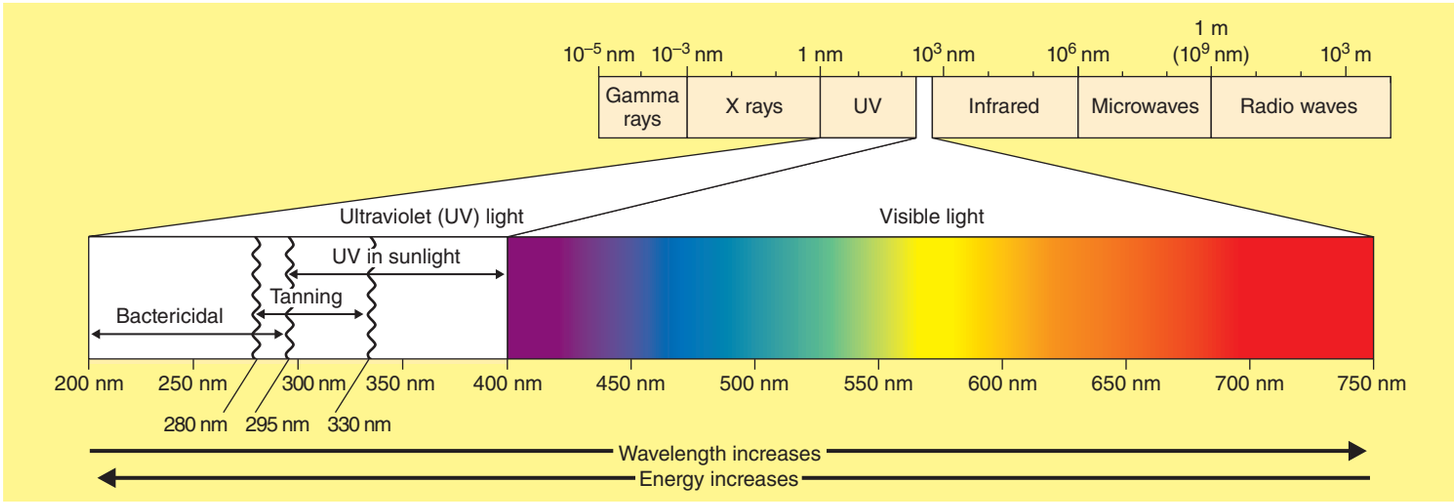


Figure 7.5 The radiant energy spectrum. Visible light and other forms of radiant energy radiate through space as waves of various lengths. Ionizing radiation, such as gamma rays and X rays, has a wavelength shorter than 1 nm. Nonionizing radiation, such as ultraviolet (UV) light, has a wavelength between 1 nm and about 380 nm, where the visible spectrum begins.

Q How might increased UV radiation (due to decrease in the ozone layer) affect the Earth's ecosystems?

correct replication of the DNA during cell reproduction. The UV wavelengths most effective for killing microorganisms are about 260 nm; these specific wavelengths are absorbed by cellular DNA. UV radiation is also used to control microbes in the air. A UV, or "germicidal," lamp is commonly found in hospital rooms, nurseries, operating rooms, and cafeterias. UV light is also used to disinfect vaccines and other medical products. A major disadvantage of UV light as a disinfectant is that the radiation is not very penetrating, so the organisms to be killed must be directly exposed to the rays. Organisms protected by solids and such coverings as paper, glass, and textiles are not affected. Another potential problem is that UV light can damage human eyes, and prolonged exposure can cause burns and skin cancer in humans.

Sunlight contains some UV radiation, but the shorter wavelengths—those most effective against bacteria—are screened out by the ozone layer of the atmosphere. The antimicrobial effect of sunlight is due almost entirely to the formation of singlet oxygen in the cytoplasm (see Chapter 6, page 155). Many pigments produced by bacteria provide protection from sunlight.

Microwaves do not have much direct effect on microorganisms, and bacteria can readily be isolated from the interior of recently operated microwave ovens. Moisture-containing foods are heated by microwave action, and the heat will kill most vegetative pathogens. Solid foods heat unevenly because of the uneven distribution of moisture. For this reason, pork cooked in a microwave oven has been responsible for outbreaks of trichinellosis.

Table 7.5 summarizes the physical methods of microbial control.

CHECK YOUR UNDERSTANDING

- How is microbial growth in canned foods prevented? **7-4**
- Why would a can of pork take longer to sterilize at a given temperature than a can of soup that also contained pieces of pork? **7-5**
- What is the connection between the killing effect of radiation and hydroxyl radical forms of oxygen? **7-6**

Chemical Methods of Microbial Control

LEARNING OBJECTIVE

7-7 List the factors related to effective disinfection.
7-8 Interpret the results of use-dilution tests and the disk-diffusion method.
7-9 Identify the methods of action and preferred uses of chemical disinfectants.
7-10 Differentiate halogens used as antiseptics from halogens used as disinfectants.
7-11 Identify the appropriate uses for surface-active agents.
7-12 List the advantages of glutaraldehyde over other chemical disinfectants.
7-13 Identify chemical sterilizers.

Chemical agents are used to control the growth of microbes on both living tissue and inanimate objects. Unfortunately, few chemical agents achieve sterility; most of them merely reduce microbial populations to safe levels or remove vegetative forms

TABLE 7.5 Physical Methods Used to Control Microbial Growth

Methods	Mechanism of Action	Comment	Preferred Use
Heat			
1. Moist heat			
a. Boiling or flowing steam	Protein denaturation	Kills vegetative bacterial and fungal pathogens and almost all viruses within 10 min; less effective on endospores.	Dishes, basins, pitchers, various equipment
b. Autoclaving	Protein denaturation	Very effective method of sterilization; at about 15 psi of pressure (121°C), all vegetative cells and their endospores are killed in about 15 min.	Microbiological media, solutions, linens, utensils, dressings, equipment, and other items that can withstand temperature and pressure
2. Pasteurization	Protein denaturation	Heat treatment for milk (72°C for about 15 sec) that kills all pathogens and most nonpathogens.	Milk, cream, and certain alcoholic beverages (beer and wine)
3. Dry heat			
a. Direct flaming	Burning contaminants to ashes	Very effective method of sterilization.	Inoculating loops
b. Incineration	Burning to ashes	Very effective method of sterilization.	Paper cups, contaminated dressings, animal carcasses, bags, and wipes
c. Hot-air sterilization	Oxidation	Very effective method of sterilization but requires temperature of 170°C for about 2 hr.	Empty glassware, instruments, needles, and glass syringes
Filtration	Separation of bacteria from suspending liquid	Removes microbes by passage of a liquid or gas through a screenlike material; most filters in use consist of cellulose acetate or nitrocellulose.	Useful for sterilizing liquids (e.g., enzymes, vaccines) that are destroyed by heat
Cold			
1. Refrigeration	Decreased chemical reactions and possible changes in proteins	Has a bacteriostatic effect.	Food, drug, and culture preservation
2. Deep-freezing (see Chapter 6, page 163)	Decreased chemical reactions and possible changes in proteins	An effective method for preserving microbial cultures, in which cultures are quick-frozen between – 50° and – 95°C.	Food, drug, and culture preservation
3. Lyophilization (see Chapter 6, page 163)	Decreased chemical reactions and possible changes in proteins	Most effective method for long-term preservation of microbial cultures; water removed by high vacuum at low temperature.	Food, drug, and culture preservation
High Pressure	Alteration of molecular structure of proteins and carbohydrates	Preserves colors, flavors, nutrient values.	Fruit juices
Desiccation	Disruption of metabolism	Involves removing water from microbes; primarily bacteriostatic.	Food preservation
Osmotic Pressure	Plasmolysis	Results in loss of water from microbial cells.	Food preservation
Radiation			
1. Ionizing	Destruction of DNA	Not widespread in routine sterilization.	Sterilizing pharmaceuticals and medical and dental supplies
2. Nonionizing	Damage to DNA	Radiation is not very penetrating.	Control of closed environment with UV (germicidal) lamp

of pathogens from objects. A common problem in disinfection is the selection of an agent. No single disinfectant is appropriate for all circumstances.

Principles of Effective Disinfection

By reading the label, we can learn a great deal about a disinfectant's properties. Usually the label indicates what groups of organisms the disinfectant is effective against. Remember that the concentration of a disinfectant affects its action, so it should always be diluted exactly as specified by the manufacturer.

Also consider the nature of the material being disinfected. For example, are organic materials present that might interfere with the action of the disinfectant? Similarly, the pH of the medium often has a great effect on a disinfectant's activity.

Another very important consideration is whether the disinfectant will easily make contact with the microbes. An area might need to be scrubbed and rinsed before the disinfectant is applied. In general, disinfection is a gradual process. Thus, to be effective, a disinfectant might need to be left on a surface for several hours.

Evaluating a Disinfectant

Use-Dilution Tests

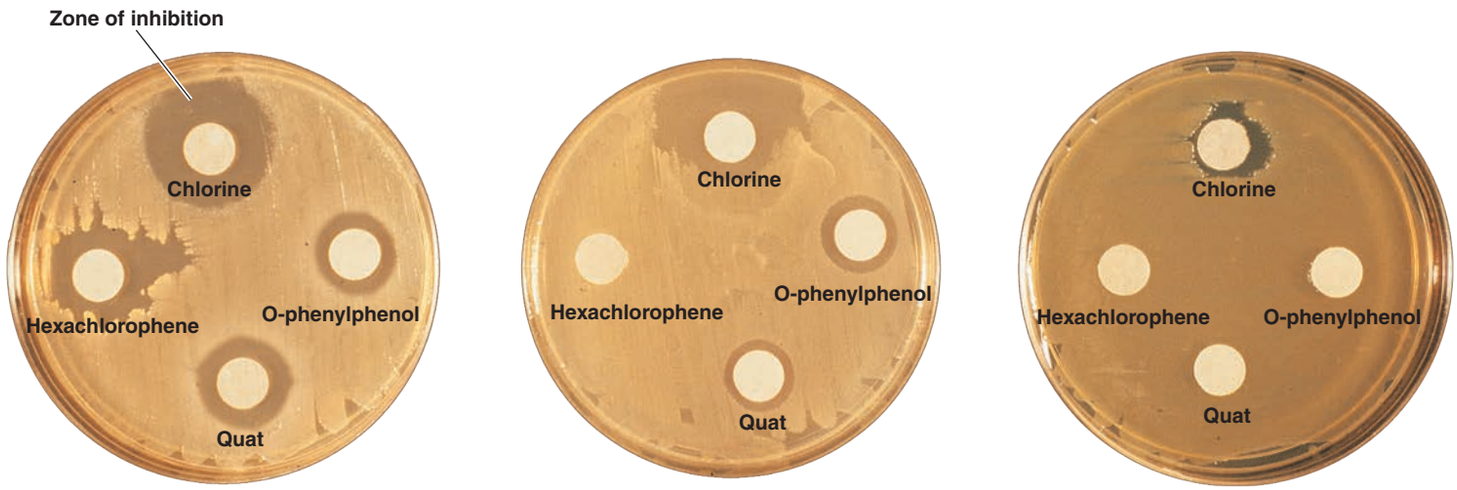
There is a need to evaluate the effectiveness of disinfectants and antiseptics. The current standard is the American Official Analytical Chemist's **use-dilution test.** Metal or glass cylinders (8 mm × 10 mm) are dipped into standardized cultures of the test bacteria grown in liquid media, removed, and dried at 37°C for a short time. The dried cultures are then placed into a solution of the disinfectant at the concentration recommended by the manufacturer and left there for 10 minutes at 20°C. Following this exposure, the cylinders are transferred to a medium that permits the growth of any surviving bacteria. The effectiveness of the disinfectant can then be determined by the number of cultures that grow.

Variations of this method are used for testing the effectiveness of antimicrobial agents against endospores, viruses, fungi, and mycobacteria that cause tuberculosis, because they are difficult to control with chemicals. Also, tests of antimicrobials intended for special purposes, such as dairy utensil disinfection, can substitute for other test bacteria.

The Disk-Diffusion Method

The **disk-diffusion method** is used in teaching laboratories to evaluate the efficacy of a chemical agent. A disk of filter paper is soaked with a chemical and placed on an agar plate that has been previously inoculated and incubated with the test organism. After incubation, if the chemical is effective, a clear zone representing inhibition of growth can be seen around the disk (Figure 7.6).

Disks containing antibiotics are commercially available and used to determine microbial susceptibility to antibiotics (see Figure 20.17, page 568).



Staphylococcus aureus **(gram-positive)**

Escherichia coli **(gram-negative)**

Pseudomonas aeruginosa **(gram-negative)**

Figure 7.6 Evaluation of disinfectants by the disk-diffusion method. In this experiment, paper disks are soaked in a solution of disinfectant and placed on the surface of a nutrient medium on which a culture of test bacteria has been spread to produce uniform growth.

At the top of each plate, the tests show that chlorine (as sodium hypochlorite) was effective against all the test bacteria but was more effective against gram-positive bacteria.

At the bottom row of each plate, the tests show that the quaternary ammonium compound ("quat") was also more effective against the gram-positive bacteria, but it did not affect the pseudomonads at all.

At the left side of each plate, the tests show that hexachlorophene was effective against gram-positive bacteria only.

At the right sides, O-phenylphenol was ineffective against pseudomonads but was almost equally effective against the gram-positive bacteria and the gram-negative bacteria.

All four chemicals worked against the gram-positive test bacteria, but only one of the four chemicals affected pseudomonads.

Q Why are the pseudomonads less affected by the four chemicals shown in the figure?

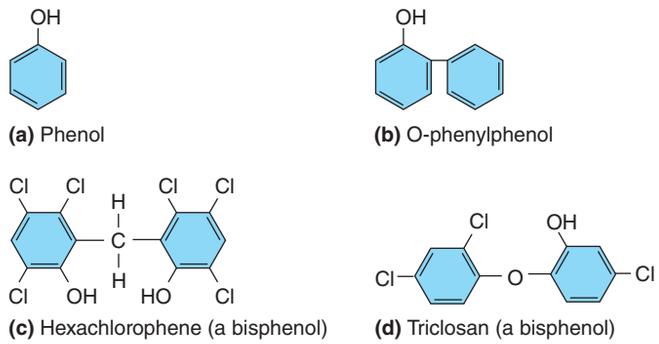


Figure 7.7 The structure of phenolics and bisphenols.

Some lozenges intended to alleviate the symptoms of a sore throat contain phenol. Why include this ingredient?

Types of Disinfectants

Phenol and Phenolics

Lister was the first to use **phenol** (carbolic acid) to control surgical infections in the operating room. Its use had been suggested by its effectiveness in controlling odor in sewage. It is now rarely used as an antiseptic or disinfectant because it irritates the skin and has a disagreeable odor. It is often used in throat lozenges for its local anesthetic effect but has little antimicrobial effect at the low concentrations used. At concentrations above 1% (such as in some throat sprays), however, phenol has a significant antibacterial effect. The structure of a phenol molecule is shown in Figure 7.7a.

Derivatives of phenol, called **phenolics,** contain a molecule of phenol that has been chemically altered to reduce its irritating qualities or increase its antibacterial activity in combination with a soap or detergent. Phenolics exert antimicrobial activity by injuring lipid-containing plasma membranes, which results in leakage of cellular contents. The cell wall of mycobacteria, the causes of tuberculosis and leprosy, are rich in lipids, which make them susceptible to phenol derivatives. A useful property of phenolics as disinfectants is that they remain active in the presence of organic compounds, are stable, and persist for long periods after application. For these reasons, phenolics are suitable agents for disinfecting pus, saliva, and feces.

One of the most frequently used phenolics is derived from coal tar, a group of chemicals called *cresols*. A very important cresol is *O-phenylphenol* (see Figure 7.6 and Figure 7.7b), the main ingredient in most formulations of Lysol. Cresols are very good surface disinfectants.

Bisphenols

Bisphenols are derivatives of phenol that contain two phenolic groups connected by a bridge (*bis* indicates *two*). One bisphenol, *hexachlorophene* (Figure 7.6 and Figure 7.7c), is an ingredient of a prescription lotion, pHisoHex, used for surgical and hospital microbial control procedures. Gram-positive staphylococci and streptococci, which can cause skin infections in newborns, are particularly susceptible to hexachlorophene, so it is often used to control such infections in nurseries.

Another widely used bisphenol is *triclosan* (Figure 7.7d), an ingredient in antibacterial soaps and at least one toothpaste. Triclosan has even been incorporated into kitchen cutting boards and the handles of knives and other plastic kitchenware. Its use is now so widespread that resistant bacteria have been reported, and concerns about its effect on microbes' resistance to certain antibiotics have been raised. Triclosan inhibits an enzyme needed for the biosynthesis of fatty acids (lipids), which mainly affects the integrity of the plasma membrane. It is especially effective against gram-positive bacteria but also works well against yeasts and gram-negative bacteria. There are certain exceptions, such as *Pseudomonas aeruginosa,* a gram-negative bacterium that is very resistant to triclosan, as well as to many other antibiotics and disinfectants (see the discussions on pages 296, 403, and 586).

Biguanides

Biguanides have a broad spectrum of activity, with a mode of action primarily affecting bacterial cell membranes. They are especially effective against gram-positive bacteria. Biguanides are also effective against gram-negative bacteria, with the significant exception of most pseudomonads. Biguanides are not sporicidal but have some activity against enveloped viruses. The best known biguanide is *chlorhexidine,* which is frequently used for microbial control on skin and mucous membranes. Combined with a detergent or alcohol, chlorhexidine is very often used for surgical hand scrubs and preoperative skin preparation in patients. *Alexidine* is a similar biguanide and is more rapid in its action than chlorhexidine. Eventually, alexidine is expected to replace Betadine in many applications (see below).

Halogens

The **halogens,** particularly iodine and chlorine, are effective antimicrobial agents, both alone and as constituents of inorganic or organic compounds. *Iodine* (I_2) is one of the oldest and most effective antiseptics. It is active against all kinds of bacteria, many endospores, various fungi, and some viruses. Iodine impairs protein synthesis and alters cell membranes, apparently by forming complexes with amino acids and unsaturated fatty acids.

Iodine is available as a **tincture**—that is, in solution in aqueous alcohol—and as an iodophor. An **iodophor** is a combination of iodine and an organic molecule, from which the iodine is released slowly. Iodophors have the antimicrobial activity of iodine, but they do not stain and are less irritating. The most common commercial preparation is Betadine, which is a *povidone-iodine.* Povidone is a surface-active iodophor that improves the wetting action and serves as a reservoir of free iodine. Iodines are used mainly for skin disinfection and wound treatment. Many campers are familiar with using iodine for water treatment.

Chlorine (Cl_2), as a gas or in combination with other chemicals, is another widely used disinfectant. Its germicidal action is caused by the hypochlorous acid (HOCl) that forms when chlorine is added to water:

(1)

$$\underset{\text{Chlorine}}{Cl_2} + \underset{\text{Water}}{H_2O} \rightleftharpoons \underset{\text{Hydrogen ion}}{H^+} + \underset{\text{Chloride ion}}{Cl^-} + \underset{\text{Hypochlorous acid}}{HOCl}$$

(2)

$$\underset{\text{Hypochlorous acid}}{HOCl} \rightleftharpoons \underset{\text{Hydrogen ion}}{H^+} + \underset{\text{Hypochlorite ion}}{OCl^-}$$

Hypochlorite is a strong oxidizing agent that prevents much of the cellular enzyme system from functioning. Hypochlorous acid is the most effective form of chlorine because it is neutral in electrical charge and diffuses as rapidly as water through the cell wall. Because of its negative charge, the hypochlorite ion (OCl^-) cannot enter the cell freely.

A liquid form of compressed chlorine gas is used extensively for disinfecting municipal drinking water, water in swimming pools, and sewage. Several compounds of chlorine are also effective disinfectants. For example, solutions of *calcium hypochlorite* [$Ca(OCl)_2$] are used to disinfect dairy equipment and restaurant eating utensils. This compound, once called chloride of lime, was used as early as 1825, long before the concept of a germ theory for disease, to soak hospital dressings in Paris hospitals. It was also the disinfectant used in the 1840s by Semmelweis to control hospital infections during childbirth, as mentioned in Chapter 1, page 9. Another chlorine compound, *sodium hypochlorite* (NaOCl; see Figure 7.6), is used as a household disinfectant and bleach (Clorox), and as a disinfectant in dairies, food-processing establishments, and hemodialysis systems. When the quality of drinking water is in question, household bleach can provide a rough equivalent of municipal chlorination. After two drops of bleach are added to a liter of water (four drops if the water is cloudy) and the mixture has sat for 30 minutes, the water is considered safe for drinking under emergency conditions.

The food-processing industry makes wide use of chlorine dioxide solution as a surface disinfectant because it does not leave residual tastes or odors. As a disinfectant, it has a broad spectrum of activity against bacteria and viruses and at high concentrations is even effective against cysts and endospores. At low concentrations, chlorine dioxide can be used as an antiseptic. (Also, see page 193 for the use of chlorine dioxide as a sterilant and disinfectant.)

An important group of chlorine compounds are the *chloramines,* combinations of chlorine and ammonia. Most municipal water-treatment systems mix ammonia with chlorine to form chloramines. (Chloramines are toxic to aquarium fish, but pet shops sell chemicals to neutralize them.) U.S. military forces in the field are issued tablets (Chlor-Floc) that contain *sodium dichloroisocyanurate,* a chloramine combined with an agent that flocculates (coagulates) suspended materials in a water sample, causing them to settle out, clarifying the water. Chloramines are also used to sanitize glassware and eating utensils and to treat dairy and food-manufacturing equipment. They are relatively stable compounds that release chlorine over long periods. Chloramines are relatively effective in organic matter but have the disadvantages of acting more slowly and being less effective than hypochlorite.

Alcohols

Alcohols effectively kill bacteria and fungi but not endospores and nonenveloped viruses. Alcohol usually denatures protein, but it can also disrupt membranes and dissolve many lipids, including the lipid component of enveloped viruses. Alcohols have the advantage of acting and then evaporating rapidly and leaving no residue. When the skin is swabbed (degermed) before an injection, most of the microbial control activity comes from simply wiping away dirt and microorganisms, along with skin oils. However, alcohols are unsatisfactory antiseptics when applied to wounds. They cause coagulation of a layer of protein under which bacteria continue to grow.

Two of the most commonly used alcohols are ethanol and isopropanol. The recommended optimum concentration of *ethanol* is 70%, but concentrations between 60% and 95% seem to kill as well (**Table 7.6**). Pure ethanol is less effective than aqueous solutions (ethanol mixed with water) because denaturation requires water. *Isopropanol,* often sold as rubbing alcohol, is slightly superior to ethanol as an antiseptic and disinfectant. Moreover, it is less volatile, less expensive, and more easily obtained than ethanol.

Alcohol-based (about 62% alcohol) hand sanitizers such as Purell and Germ-X are very popular for use when hands are not visibly soiled. Rub the product over the surfaces of the hands and fingers until they are dry. Claims that products will kill 99.9% of germs should be viewed with caution; such effectiveness is seldom reached under typical user's conditions. Also, certain pathogens, such as the spore-forming *Clostridium*

TABLE 7.6 Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution against *Streptococcus pyogenes*

Concentration of Ethanol (%)	Time of Exposure (sec)				
	10	20	30	40	50
100	G	G	G	G	G
95	NG	NG	NG	NG	NG
90	NG	NG	NG	NG	NG
80	NG	NG	NG	NG	NG
70	NG	NG	NG	NG	NG
60	NG	NG	NG	NG	NG
50	G	G	NG	NG	NG
40	G	G	G	G	G

Note:
G = growth
NG = no growth

Figure 7.8 Oligodynamic action of heavy metals. Clear zones where bacterial growth has been inhibited are seen around the sombrero charm (pushed aside), the dime, and the penny. The charm and the dime contain silver; the penny contains copper.

Q The coins used in this demonstration were minted many years ago; why were more contemporary coins not used?

difficile and viruses that lack a lipid envelope, are comparatively resistant to alcohol-based hand sanitizers.

Ethanol and isopropanol are often used to enhance the effectiveness of other chemical agents. For example, an aqueous solution of Zephiran (described on page 191) kills about 40% of the population of a test organism in 2 minutes, whereas a tincture of Zephiran kills about 85% in the same period. To compare the effectiveness of tinctures and aqueous solutions, see Figure 7.10.

Heavy Metals and Their Compounds

Several heavy metals can be biocidal or antiseptic, including silver, mercury, and copper. The ability of very small amounts of heavy metals, especially silver and copper, to exert antimicrobial activity is referred to as **oligodynamic action** (*oligo* means few). Centuries ago, Egyptians found that putting silver coins in water barrels served to keep the water clean of unwanted organic growths. This action can be seen when we place a coin or other clean piece of metal containing silver or copper on a culture on an inoculated Petri plate. Extremely small amounts of metal diffuse from the coin and inhibit the growth of bacteria for some distance around the coin (**Figure 7.8**). This effect is produced by the action of heavy metal ions on microbes. When the metal ions combine with the sulfhydryl groups on cellular proteins, denaturation results.

Silver is used as an antiseptic in a 1% *silver nitrate* solution. At one time, many states required that the eyes of newborns be treated with a few drops of silver nitrate to guard against an infection of the eyes called ophthalmia neonatorum, which the infants might have contracted as they passed through the birth canal. In recent years, antibiotics have replaced silver nitrate for this purpose.

Recently, there has been renewed interest in the use of silver as an antimicrobial agent. Silver-impregnated dressings that slowly release silver ions have proven especially useful against antibiotic-resistant bacteria. The enthusiasm for incorporating silver in all manner of consumer products is increasing. Among the newer products being sold are plastic food containers infused with silver nanoparticles, which are intended to keep food fresher, and silver-infused athletic shirts and socks, which are claimed to minimize odors.

A combination of silver and the drug sulfadiazine, *silver-sulfadiazine,* is the most common formulation. It is available as a topical cream for use on burns. Silver can also be incorporated into indwelling catheters, which are a common source of hospital infections, and in wound dressings. *Surfacine* is a relatively new antimicrobial for application to surfaces, either animate or inanimate. It contains water-insoluble silver iodide in a polymer carrier and is very persistent, lasting at least 13 days. When a bacterium contacts the surface, the cell's outer membrane is recognized, and a lethal amount of silver ions is released.

Inorganic mercury compounds, such as *mercuric chloride,* have a long history of use as disinfectants. They have a very broad spectrum of activity; their effect is primarily bacteriostatic. However, their use is now limited because of their toxicity, corrosiveness, and ineffectiveness in organic matter. At present, the primary use of mercurials is to control mildew in paints.

Copper in the form of *copper sulfate* or other copper-containing additives is used chiefly to destroy green algae (algicide) that grow in reservoirs, stock ponds, swimming pools, and fish tanks. If the water does not contain excessive organic matter, copper compounds are effective in concentrations of one part per million of water. To prevent mildew, copper compounds such as *copper 8-hydroxyquinoline* are sometimes included in paint. In the nineteenth century, the wine regions of Europe were plagued by fungal diseases that affected the grapevines. It was observed that vines near the road were less affected than those further afield. The reason was that these roadside vines had been sprayed with a mixture of copper sulfate and lime (both visible and bitter to the taste) to deter passers-by on the road from eating the grapes. Because of this chance observation, mixtures based on copper ions (known as Bordeaux mixture) have long been used to control fungal diseases of plants.

Long term use of alcohol-based hand sanitizers often causes problems with skin dryness. A relatively new hand sanitizer, Xgel, does not contain alcohol but uses copper contained in a skin lotion formulation. X-gel may be more effective as an antimicrobial than alcohol-based hand sanitizers.

Another metal used as an antimicrobial is zinc. The effect of trace amounts of zinc can be seen on weathered roofs of buildings down-slope from galvanized (zinc-coated) fittings. The roof is lighter-colored where biological growth, mostly algae, is impeded. Copper- and zinc-treated shingles are available. *Zinc chloride* is a common ingredient in mouthwashes, and *zinc pyrithione* is an ingredient in antidandruff shampoos.

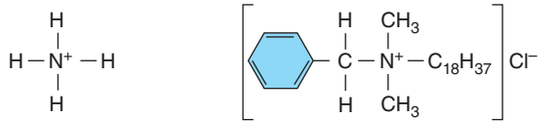


Figure 7.9 The ammonium ion and a quaternary ammonium compound, benzalkonium chloride (Zephiran). Notice how other groups replace the hydrogens of the ammonium ion.

Q Are quats most effective against gram-positive or gram-negative bacteria?

Surface-Active Agents

Surface-active agents, or **surfactants,** can decrease surface tension among molecules of a liquid. Such agents include soaps and detergents.

Soaps and Detergents Soap has little value as an antiseptic, but it does have an important function in the mechanical removal of microbes through scrubbing. The skin normally contains dead cells, dust, dried sweat, microbes, and oily secretions from oil glands. Soap breaks the oily film into tiny droplets, a process called *emulsification,* and the water and soap together lift up the emulsified oil and debris and float them away as the lather is washed off. In this sense, soaps are good degerming agents. Washing hands with soap and water is an effective sanitation method. Use soap and *warm* water (if possible), and rub hands together for 20 seconds (imagine singing "Happy Birthday" twice through). Then rinse, dry with a paper towel or air dryer, and try to use a paper towel to turn off the faucet.

Acid-Anionic Sanitizers *Acid-anionic* sanitizers are very important in cleaning food-processing facilities, especially dairy utensils and equipment. They are usually combinations of phosphoric acid with a surface-active agent. Their sanitizing ability is related to the negatively charged portion (anion) of the molecule, which reacts with the plasma membrane. These sanitizers, which act on a wide spectrum of microbes, including troublesome thermoduric bacteria, are odorless, nontoxic, noncorrosive, and fast acting.

Quaternary Ammonium Compounds (Quats) The most widely used surface-active agents are the cationic detergents, especially the **quaternary ammonium compounds (quats).** Their cleansing ability is related to the positively charged portion—the cation—of the molecule. Their name is derived from the fact that they are modifications of the four-valence ammonium ion, NH_4^+(Figure 7.9). Quaternary ammonium compounds are strongly bactericidal against gram-positive bacteria and less active against gram-negative bacteria (see Figure 7.6).

Quats are also fungicidal, amebicidal, and virucidal against enveloped viruses. They do not kill endospores or mycobacteria. (See the Clinical Focus box on page 193.) Their chemical mode of action is unknown, but they probably affect the plasma membrane. They change the cell's permeability and cause the loss of essential cytoplasmic constituents, such as potassium.

Two popular quats are Zephiran, a brand name of *benzalkonium chloride* (see Figure 7.9), and Cepacol, a brand name of *cetylpyridinium chloride.* They are strongly antimicrobial, colorless, odorless, tasteless, stable, easily diluted, and nontoxic, except at high concentrations. If your mouthwash bottle fills with foam when shaken, the mouthwash probably contains a quat. However, organic matter interferes with their activity, and they are rapidly neutralized by soaps and anionic detergents.

Anyone involved in medical applications of quats should remember that certain bacteria, such as some species of *Pseudomonas,* not only survive in quaternary ammonium compounds but actively grow in them. These microbes are resistant not only to the disinfectant solution but also to gauze and bandages moistened with it, because the fibers tend to neutralize the quats.

Before we move on to the next group of chemical agents, refer to Figure 7.10, which compares the effectiveness of some of the antiseptics we have discussed so far.

Chemical Food Preservatives

Chemical preservatives are frequently added to foods to retard spoilage. *Sulfur dioxide* (SO_2) has long been used as a disinfectant, especially in wine-making. Homer's *Odyssey,* written nearly 2800 years ago, mentions its use. Among the more common additives are sodium benzoate, sorbic acid, and calcium propionate. These chemicals are simple organic acids, or salts

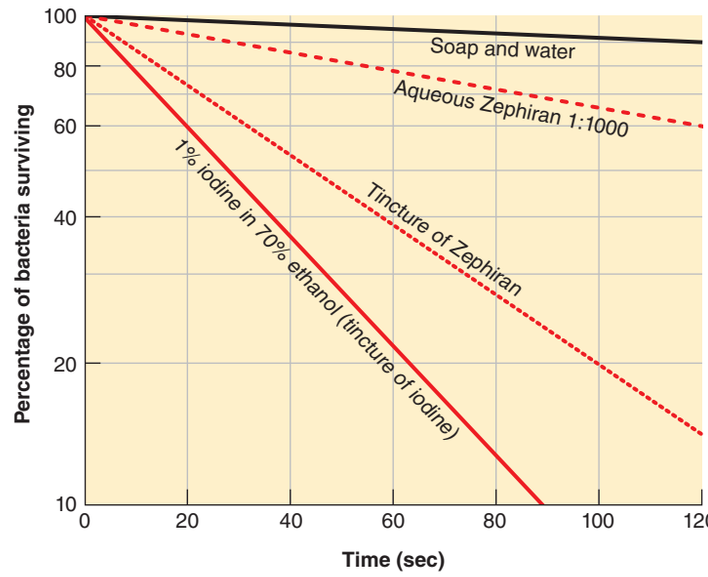


Figure 7.10 A comparison of the effectiveness of various antiseptics. The steeper the downward slope of the killing curve, the more effective the antiseptic is. A 1% iodine in 70% ethanol solution is the most effective; soap and water are the least effective. Notice that a tincture of Zephiran is more effective than an aqueous solution of the same antiseptic.

Q Why is the tincture of Zephiran more effective than the aqueous solution?

of organic acids, which the body readily metabolizes and which are generally judged to be safe in foods. *Sorbic acid,* or its more soluble salt *potassium sorbate,* and *sodium benzoate* prevent molds from growing in certain acidic foods, such as cheese and soft drinks. Such foods, usually with a pH of 5.5 or lower, are most susceptible to spoilage by molds. *Calcium propionate,* an effective fungistat used in bread, prevents the growth of surface molds and the *Bacillus* bacteria that produce the mucuslike secretion that cause ropy bread. These organic acids inhibit mold growth, not by affecting the pH but by interfering with the mold's metabolism or the integrity of the plasma membrane.

Sodium nitrate and *sodium nitrite* are added to many meat products, such as ham, bacon, hot dogs, and sausage. The active ingredient is sodium nitrite, which certain bacteria in the meats can also produce from sodium nitrate. These bacteria use nitrate as a substitute for oxygen under anaerobic conditions. The nitrite has two main functions: to preserve the pleasing red color of the meat by reacting with blood components in the meat, and to prevent the germination and growth of any botulism endospores that might be present. Nitrite selectively inhibits certain iron-containing enzymes of *Clostridium botulinum*. There has been some concern that the reaction of nitrites with amino acids can form certain carcinogenic products known as **nitrosamines,** and the amount of nitrites added to foods has generally been reduced in recent years for this reason. However, the use of nitrites continues because of their established value in preventing botulism. Because nitrosamines are formed in the body from other sources, the added risk posed by a limited use of nitrates and nitrites in meats is lower than was once thought.

Antibiotics

The antimicrobials discussed in this chapter are not useful for ingestion or injection to treat disease. Antibiotics are used for this purpose. At least two have considerable use in food preservation. Neither is of value for clinical purposes. *Nisin* is often added to cheese to inhibit the growth of certain endospore-forming spoilage bacteria. It is an example of a bacteriocin, a protein that is produced by one bacterium and inhibits another (see Chapter 8, page 231). Nisin is present naturally in small amounts in many dairy products. It is tasteless, readily digested, and nontoxic. *Natamycin* (pimaricin) is an antifungal antibiotic approved for use in foods, mostly cheese.

Aldehydes

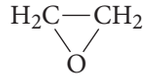
Aldehydes are among the most effective antimicrobials. Two examples are formaldehyde and glutaraldehyde. They inactivate proteins by forming covalent cross-links with several organic functional groups on proteins (—NH_2 —OH, —COOH, and —SH). *Formaldehyde gas* is an excellent disinfectant. However, it is more commonly available as *formalin,* a 37% aqueous solution of formaldehyde gas. Formalin was once used extensively to preserve biological specimens and inactivate bacteria and viruses in vaccines.

Glutaraldehyde is a chemical relative of formaldehyde that is less irritating and more effective than formaldehyde. Glutaraldehyde is used to disinfect hospital instruments, including endoscopes and respiratory therapy equipment, but they must be carefully cleaned first. When used in a 2% solution (Cidex), it is bactericidal, tuberculocidal, and virucidal in 10 minutes and sporicidal in 3 to 10 hours. Glutaraldehyde is one of the few liquid chemical disinfectants that can be considered a sterilizing agent. For practical purposes, 30 minutes is often considered the maximum time allowed for a sporicide to act, which is a criterion glutaraldehyde cannot meet. Both glutaraldehyde and formalin are used by morticians for embalming.

A possible replacement for glutaraldehyde for many uses is *ortho-phthalaldehyde* (OPA), which is more effective against many microbes and has fewer irritating properties.

Chemical Sterilization

Sterilization with liquid chemicals is possible, but even sporicidal chemicals such as glutaraldehyde are usually not considered to be practical sterilants. However, the gaseous chemosterilants are frequently used as substitutes for physical sterilization processes. Their application requires a closed chamber similar to a steam autoclave. Probably the most familiar example is *ethylene oxide:*



Its activity depends on *alkylation,* that is, replacing the proteins' labile hydrogen atoms in a chemical group (such as —SH, — COOH, or — CH_2CH_2OH) with a chemical radical. This leads

Clinical Case

Norovirus, a nonenveloped virus, is one cause of acute gastroenteritis. It can be spread by consuming fecally contaminated food or water, coming in direct contact with an infected person, or touching a contaminated surface. Amy is able to rule out foodborne transmission immediately. The small private school does not have a school lunch program; all students and staff bring their lunches from home. After meeting with the principal, Amy speaks to the custodial staff and directs them to use a quat to clean the school. She asks them to pay special attention to areas with high potential for fecal contamination, especially toilet seats, flush handles, toilet stall inner door handles, and restroom door inner handles. Amy is sure she has avoided a major outbreak, but by Friday, 42 students and six more staff members call in to report similar symptoms.

Why didn't the quat work to kill the virus?

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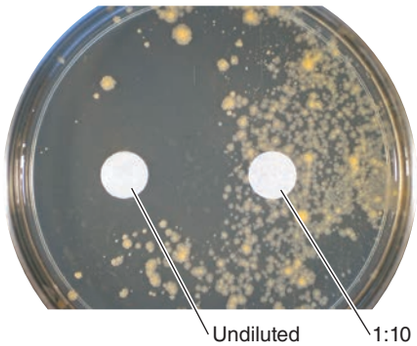
CLINICAL FOCUS

Infection Following Anesthesia Injection

As you read through this box, you will encounter a series of questions that infection control officers ask themselves as they track the source of infection. Try to answer each question before going on to the next one.

1. Dr. Priya Agarwal, an infectious disease physician, called the department of health to report that in the last 3 months, she had seen 12 patients with *Mycobacterium abscessus* soft-tissue infections. Slow-growing mycobacteria, including *M. tuberculosis* and *M. leprae,* are common human pathogens, but Dr. Agarwal was concerned because these infections were caused by rapidly growing mycobacteria (RGM).

   Where are these RGM normally found? (Hint: Read page 312.)



Disk-diffusion test of Zephiran against *M. abscessus*.

2. RGM are usually found in soil and water. In Dr. Agarwal's report, she noted that all 12 patients received injections for cosmetic procedures from the same physician. The injection procedure consisted of cleaning the skin with cotton balls soaked in diluted (1:10) Zephiran, painting the skin with commercially prepared iodine swabs, and anesthetizing the area with 0.5 ml of 1% lidocaine in a sterile 22-gauge needle and syringe.

   What did Dr. Agarwal need to do to determine the source of the infection?

3. Dr. Agarwal ordered cultures from the inside surface of an open metal container of forceps and the inside surface of a metal container of cotton balls. She also requested cultures of the iodine prep swabs, Zephiran-soaked cotton balls, lidocaine, diluted and undiluted Zephiran, and a sealed bottle of distilled water used to dilute the Zephiran.

   What type of disinfectant is Zephiran?

4. Zephiran is a quat. Results of cultures from the laboratory showed that only the cotton balls soaked in Zephiran had grown *M. abscessus*. Dr. Agarwal then performed a disk-diffusion assay (see the figure) of both diluted and undiluted Zephiran.

   What did the assay reveal? What should Dr. Agarwal tell the physician about preventing future infections?

5. Recent research has shown that soaking cotton balls in disinfectant selects for resistant bacteria. Additionally, the disinfecting ability of Zephiran and other quats is reduced by the presence of organic material, such as cotton balls or towels. Dr. Agarwal informed the physician that cotton balls should not be stored in the disinfectant; doing so can cause patients to be inoculated with bacteria.

Source: Adapted from *Journal of Antimicrobial Chemotherapy* 65(12): 2574–2581 (2010).

to cross-linking of nucleic acids and proteins and inhibits vital cellular functions. Ethylene oxide kills all microbes and endospores but requires a lengthy exposure period of several hours. It is toxic and explosive in its pure form, so it is usually mixed with a nonflammable gas, such as carbon dioxide. Among its advantages is that it carries out sterilization at ambient temperatures and it is highly penetrating. Larger hospitals often are able to sterilize even mattresses in special ethylene oxide sterilizers.

Chlorine dioxide is a short-lived gas that is usually manufactured at the place of use. Notably, it has been used to fumigate enclosed building areas contaminated with endospores of anthrax. It is much more stable in aqueous solution. Its most common use is in water treatment prior to chlorination, where its purpose is to remove, or reduce the formation of, certain carcinogenic compounds sometimes formed in the chlorination of water.

Plasmas

In addition to the traditional three states of matter—liquid, gas, and solid—a fourth state of matter exists, called plasma. **Plasma** is a state of matter in which a gas is excited, in this case by an electromagnetic field, to make a mixture of nuclei with assorted electrical charges and free electrons. Health care facilities are increasingly facing the challenge of sterilizing metal or plastic surgical instruments used for many newer procedures in arthroscopic or laparoscopic surgery. Such devices have long, hollow tubes, many with an interior diameter of only a few millimeters, and are difficult to sterilize. *Plasma sterilization* is a reliable method for this. The instruments are placed in a container in which a combination of a vacuum, electromagnetic field, and chemicals such as hydrogen peroxide (sometimes with peracetic acid, as well) form the plasma. Such plasmas have many free radicals that quickly destroy even endospore-forming microbes. The advantage of plasma sterilization, which has elements of both physical and chemical sterilization, is that it requires only low temperatures, but it is relatively expensive.

Supercritical Fluids

The use of supercritical fluids in sterilization combines chemical and physical methods. When carbon dioxide is compressed into a "supercritical" state, it has properties of both a liquid (with increased solubility) and a gas (with a lowered surface tension). Organisms exposed to *supercritical carbon dioxide* are inactivated, including most vegetative organisms that cause spoilage and foodborne pathogens. Even endospore inactivation requires a temperature of only about 45°C. Used for a number of years in treating certain foods, supercritical

carbon dioxide has more recently been used to decontaminate medical implants, such as bone, tendons, or ligaments taken from donor patients.

Peroxygens and Other Forms of Oxygen

Peroxygens are a group of oxidizing agents that includes hydrogen peroxide and peracetic acid.

Hydrogen peroxide is an antiseptic found in many household medicine cabinets and in hospital supply rooms. It is not a good antiseptic for open wounds. It is quickly broken down to water and gaseous oxygen by the action of the enzyme catalase, which is present in human cells (see Chapter 6, page 155). However, hydrogen peroxide does effectively disinfect inanimate objects; in such applications, it is even sporicidal at high concentrations. On a nonliving surface, the normally protective enzymes of aerobic bacteria and facultative anaerobes are overwhelmed by high concentrations of peroxide. Because of these factors, and its rapid degradation into harmless water and oxygen, the food industry is increasing its use of hydrogen peroxide for aseptic packaging (see page 787). The packaging material passes through a hot solution of the chemical before being assembled into a container. In addition, many wearers of contact lenses are familiar with hydrogen peroxide's use as a disinfectant. After the lens is disinfected, a platinum catalyst in the lens-disinfecting kit destroys residual hydrogen peroxide so that it does not persist on the lens, where it might cause eye irritation.

Heated, gaseous hydrogen peroxide can be used as a sterilant of atmosphere and surfaces. Hospital rooms, for example, can be decontaminated quickly and routinely with equipment available under the brand name Bioquell. The room is sealed with the generating apparatus inside and the controls on the outside. Once the sealed room has undergone a decontamination cycle, the hydrogen peroxide vapor is catalytically converted into water vapor and oxygen.

Peracetic acid (*peroxyactic acid,* or *PAA*) is one of the most effective liquid chemical sporicides available and can be used as a sterilant. Its mode of action is similar to that of hydrogen peroxide. It is generally effective on endospores and viruses within 30 minutes and kills vegetative bacteria and fungi in less than 5 minutes. PAA has many applications in the disinfection of food-processing and medical equipment, especially endoscopes, because it leaves no toxic residues (only water and small amounts of acetic acid) and is minimally affected by the presence of organic matter. The FDA has approved use of PAA for the washing of fruits and vegetables.

Other oxidizing agents include *benzoyl peroxide,* which is probably most familiar as the main ingredient in over-the-counter medications for acne. *Ozone* (O_3) is a highly reactive form of oxygen that is generated by passing oxygen through high-voltage electrical discharges. It is responsible for the air's rather fresh odor after a lightning storm, in the vicinity of electrical sparking, or around an ultraviolet light. Ozone is often used to supplement chlorine in the disinfection of water because it helps neutralize tastes and odors. Although ozone is a more effective killing agent than chlorine, its residual activity is difficult to maintain in water.

Clinical Case

Quats are virucidal against enveloped viruses. By Monday, a total of 103 out of 266 staff and students call in sick with vomiting and diarrhea. With almost half the school either out sick or returning to school after being sick, Amy decides to call the Maryland State Health Department. After going over her records with a health department statistician, she finds out that the most significant risk factors for infection are contact with an ill person or being in the first grade. All but five first-graders have reported sick with diarrheal illness. Because the school is so small, the first-grade classroom also houses the computer lab for the school. Both students and staff share these computers. The health department sends someone to swab the first-grade classroom, and norovirus is cultured from a computer mouse.

How did the virus get from a computer mouse in the first grade classroom to all of the other grades and staff?

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CHECK YOUR UNDERSTANDING

- If you wanted to disinfect a surface contaminated by vomit and a surface contaminated by a sneeze, why would your choice of disinfectant make a difference? **7-7**
- Which is more likely to be used in a medical clinic laboratory, a use-dilution test or a disk-diffusion test? **7-8**
- Why is alcohol effective against some viruses and not others? **7-9**
- Is Betadine an antiseptic or a disinfectant when it is used on skin? **7-10**
- What characteristics make surface-active agents attractive to the dairy industry? **7-11**
- What chemical disinfectants can be considered sporicides? **7-12**
- What chemicals are used to sterilize? **7-13**

Microbial Characteristics and Microbial Control

LEARNING OBJECTIVE

7-14 Explain how the type of microbe affects the control of microbial growth.

Many biocides tend to be more effective against gram-positive bacteria, as a group, than against gram-negative bacteria. A principal factor in this relative resistance to biocides is the

TABLE 7.7 Effectiveness of Chemical Antimicrobials against Endospores and Mycobacteria

Chemical Agent	Effect against Endospores	Effect against Mycobacteria
Glutaraldehyde	Fair	Good
Chlorines	Fair	Fair
Alcohols	Poor	Good
Iodine	Poor	Good
Phenolics	Poor	Good
Chlorhexidine	None	Fair
Bisphenols	None	None
Quats	None	None
Silver	None	None

external lipopolysaccharide layer of gram-negative bacteria. Within gram-negative bacteria, members of the genera *Pseudomonas* and *Burkholderia* are of special interest. These closely related bacteria are unusually resistant to biocides (see Figure 7.6) and will even grow actively in some disinfectants and antiseptics, most notably the quaternary ammonium compounds. These bacteria are also resistant to many antibiotics (see Chapter 20). This resistance to chemical antimicrobials is related mostly to the characteristics of their *porins* (structural openings in the wall of gram-negative bacteria; see Figure 4.13c, page 82). Porins are highly selective of molecules that they permit to enter the cell.

The mycobacteria are another group of non–endospore-forming bacteria that exhibit greater than normal resistance to chemical biocides. (See the Clinical Focus box on page 193.) This group includes *Mycobacterium tuberculosis,* the pathogen that causes tuberculosis. The cell wall of this organism and other members of this genus have a waxy, lipid-rich component. Instruction labels on disinfectants often state whether they are tuberculocidal, indicating that they are effective against mycobacteria. Special tuberculocidal tests have been developed to evaluate the effectiveness of biocides against this bacterial group.

Bacterial endospores are affected by relatively few biocides. (The activity of the major chemical antimicrobial groups against mycobacteria and endospores is summarized in **Table 7.7**.) The cysts and oocysts of protozoa are also relatively resistant to chemical disinfection.

The resistance of viruses to biocides largely depends on the presence or absence of an envelope. Antimicrobials that are lipid-soluble are more likely to be effective against enveloped viruses. The label of such an agent will indicate that it is effective against lipophilic viruses. Nonenveloped viruses, which have only a protein coat, are more resistant—fewer biocides are active against them.

A special problem, not yet completely solved, is the reliable killing of prions. Prions are infectious proteins that are the cause of neurological diseases known as spongiform encephalopathies, such as the popularly named mad cow disease (see Chapter 22, page 631). To destroy prions, infected animal carcasses are incinerated. A major problem is the disinfection of surgical instruments exposed to prion contamination. Normal autoclaving has proven to be inadequate. The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have recommended the combined use of a solution of sodium hydroxide and autoclaving at 134°C. Recent reports indicate that surgical instruments have been successfully treated to inactivate prions, which are proteins, by addition of protease enzymes to the cleaning solution. Surgeons sometimes resort to using disposable instruments.

In summary, it is important to remember that microbial control methods, especially biocides, are not uniformly effective against all microbes.

Table 7.8 summarizes chemical agents used to control microbial growth.

CHECK YOUR UNDERSTANDING

The presence or absence of endospores has an obvious effect on microbial control, but why are gram-negative bacteria more resistant to chemical biocides than gram-positive bacteria? **7-14**

The compounds discussed in this chapter are not generally useful in the treatment of diseases. Antibiotics and the pathogens against which they are active will be discussed in Chapter 20.

Clinical Case Resolved

Norovirus is an extremely contagious virus and can spread quickly from person to person. It is also nonenveloped, so it cannot be easily destroyed by a biocide. Amy asks the principal if, once the school is back to full capacity, she can hold an assembly to discuss the importance of handwashing with the students and staff. Proper washing with soap and water can eliminate the transmission of norovirus to other people or surfaces. Amy also meets again with the custodial staff to discuss the health department's recommendations. According to the health department, when cleaning environmental surfaces that are visibly soiled with feces or vomitus, the staff should wear masks and gloves, use a disposable towel that has been soaked in dilute detergent to wipe the surface for at least 10 seconds, and then apply a 1:10 household bleach solution for at least 1 minute. Although Amy knows this won't be the last time her school is affected by a virus, she is certain she has taken a positive step toward protecting her students and staff from this particular virus.

TABLE 7.8 Chemical Agents Used to Control Microbial Growth

Chemical Agent	Mechanism of Action	Preferred Use	Comment
Phenol and Phenolics			
Phenol	Disruption of plasma membrane, denaturation of enzymes.	Rarely used, except as a standard of comparison.	Seldom used as a disinfectant or antiseptic because of its irritating qualities and disagreeable odor.
Phenolics	Disruption of plasma membrane, denaturation of enzymes.	Environmental surfaces, instruments, skin surfaces, and mucous membranes.	Derivatives of phenol that are reactive even in the presence of organic material; O-phenylphenol is an example.
Bisphenols	Probably disruption of plasma membrane.	Disinfectant hand soaps and skin lotions.	Triclosan is an especially common example of a bisphenol. Broad spectrum, but most effective against gram-positives.
Biguanides (Chlorhexidine)	Disruption of plasma membrane.	Skin disinfection, especially for surgical hand scrubbing.	Bactericidal to gram-positives and gram-negatives; nontoxic, persistent.
Halogens	Iodine inhibits protein function and is a strong oxidizing agent; chlorine forms the strong oxidizing agent hypochlorous acid, which alters cellular components.	Iodine is an effective antiseptic available as a tincture and an iodophor; chlorine gas is used to disinfect water; chlorine compounds are used to disinfect dairy equipment, eating utensils, household items, and glassware.	Iodine and chlorine may act alone or as components of inorganic and organic compounds.
Alcohols	Protein denaturation and lipid dissolution.	Thermometers and other instruments. When the skin is swabbed with alcohol before an injection, most of the disinfecting action probably comes from a simple wiping away (degerming) of dirt and some microbes.	Bactericidal and fungicidal, but not effective against endospores or nonenveloped viruses; commonly used alcohols are ethanol and isopropanol.
Heavy Metals and Their Compounds	Denaturation of enzymes and other essential proteins.	Silver nitrate may be used to prevent ophthalmia neonatorum; silver-sulfadiazine is used as a topical cream on burns; copper sulfate is an algicide.	Heavy metals such as silver and mercury are biocidal.
Surface-Active Agents			
Soaps and detergents	Mechanical removal of microbes through scrubbing.	Skin degerming and removal of debris.	Many antibacterial soaps contain antimicrobials.
Acid-anionic sanitizers	Not certain; may involve enzyme inactivation or disruption.	Sanitizers in dairy and food-processing industries.	Wide spectrum of activity; nontoxic, noncorrosive, fast-acting.
Quaternary ammonium compounds (cationic detergents)	Enzyme inhibition, protein denaturation, and disruption of plasma membranes.	Antiseptic for skin, instruments, utensils, rubber goods.	Bactericidal, bacteriostatic, fungicidal, and virucidal against enveloped viruses. Examples of quats are Zephiran and Cepacol.
Chemical Food Preservatives			
Organic acids	Metabolic inhibition, mostly affecting molds; action not related to their acidity.	Sorbic acid and benzoic acid effective at low pH; parabens used in cosmetics, shampoos; calcium propionate used in bread.	Widely used to control mold and some bacteria in foods and cosmetics.
Nitrates/nitrites	Active ingredient is nitrite, which is produced by bacterial action on nitrate. Nitrite inhibits certain iron-containing enzymes of anaerobes.	Meat products such as ham, bacon, hot dogs, sausage.	Prevents growth of *Clostridium botulinum* in food; also imparts a red color.
Aldehydes	Protein denaturation.	Glutaraldehyde (Cidex) is less irritating than formaldehyde and is used for disinfecting medical equipment.	Very effective antimicrobials.
Chemical Sterilization			
Ethylene oxide and other gaseous sterilants	Inhibits vital cellular functions.	Mainly for sterilization of materials that would be damaged by heat.	Ethylene oxide is the most commonly used. Heated hydrogen peroxide and chlorine dioxide have special uses (below).

TABLE **7.8** (*continued*)

Chemical Agent	Mechanism of Action	Preferred Use	Comment
Plasma sterilization	Inhibits vital cellular functions.	Especially useful for tubular medical instruments.	Usually hydrogen peroxide excited in a vacuum by an electromagnetic field.
Supercritical fluids	Inhibits vital cellular functions.	Especially useful for sterilizing organic medical implants.	Carbon dioxide compressed to a supercritical state.
Peroxygens and Other Forms of Oxygen	Oxidation.	Contaminated surfaces; some deep wounds, in which they are very effective against oxygen-sensitive anaerobes.	Ozone is widely used as a supplement for chlorination; hydrogen peroxide is a poor antiseptic but a good disinfectant. Peracetic acid is especially effective.

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The Terminology of Microbial Control (pp. 177–178)

1. The control of microbial growth can prevent infections and food spoilage.
2. Sterilization is the process of removing or destroying all microbial life on an object.
3. Commercial sterilization is heat treatment of canned foods to destroy *C. botulinum* endospores.
4. Disinfection is the process of reducing or inhibiting microbial growth on a nonliving surface.
5. Antisepsis is the process of reducing or inhibiting microorganisms on living tissue.
6. The suffix *-cide* means to kill; the suffix *-stat* means to inhibit.
7. Sepsis is bacterial contamination.

The Rate of Microbial Death (p. 178)

1. Bacterial populations subjected to heat or antimicrobial chemicals usually die at a constant rate.
2. Such a death curve, when plotted logarithmically, shows this constant death rate as a straight line.
3. The time it takes to kill a microbial population is proportional to the number of microbes.
4. Microbial species and life cycle phases (e.g., endospores) have different susceptibilities to physical and chemical controls.
5. Organic matter may interfere with heat treatments and chemical control agents.
6. Longer exposure to lower heat can produce the same effect as shorter time at higher heat.

Actions of Microbial Control Agents (pp. 178–179)

Alteration of Membrane Permeability (p. 179)

1. The susceptibility of the plasma membrane is due to its lipid and protein components.
2. Certain chemical control agents damage the plasma membrane by altering its permeability.

Damage to Proteins and Nucleic Acids (p. 179)

3. Some microbial control agents damage cellular proteins by breaking hydrogen bonds and covalent bonds.
4. Other agents interfere with DNA and RNA and protein synthesis.

Physical Methods of Microbial Control (pp. 180–185)

Heat (pp. 180–183)

1. Heat is frequently used to kill microorganisms.
2. Moist heat kills microbes by denaturing enzymes.
3. Thermal death point (TDP) is the lowest temperature at which all the microbes in a liquid culture will be killed in 10 minutes.
4. Thermal death time (TDT) is the length of time required to kill all bacteria in a liquid culture at a given temperature.
5. Decimal reduction time (DRT) is the length of time in which 90% of a bacterial population will be killed at a given temperature.
6. Boiling (100°C) kills many vegetative cells and viruses within 10 minutes.
7. Autoclaving (steam under pressure) is the most effective method of moist heat sterilization. The steam must directly contact the material to be sterilized.
8. In HTST pasteurization, a high temperature is used for a short time (72°C for 15 seconds) to destroy pathogens without altering the flavor of the food. Ultra-high-temperature (UHT) treatment (140°C for 4 seconds) is used to sterilize dairy products.
9. Methods of dry heat sterilization include direct flaming, incineration, and hot-air sterilization. Dry heat kills by oxidation.
10. Different methods that produce the same effect (reduction in microbial growth) are called equivalent treatments.

Filtration (p. 183)

11. Filtration is the passage of a liquid or gas through a filter with pores small enough to retain microbes.
12. Microbes can be removed from air by high-efficiency particulate air (HEPA) filters.
13. Membrane filters composed of cellulose esters are commonly used to filter out bacteria, viruses, and even large proteins.

Low Temperatures (pp. 183–184)

14. The effectiveness of low temperatures depends on the particular microorganism and the intensity of the application.
15. Most microorganisms do not reproduce at ordinary refrigerator temperatures (0–7°C).
16. Many microbes survive (but do not grow) at the subzero temperatures used to store foods.

High Pressure (p. 184)

17. High pressure denatures proteins in vegetative cells.

Desiccation (p. 184)

18. In the absence of water, microorganisms cannot grow but can remain viable.
19. Viruses and endospores can resist desiccation.

Osmotic Pressure (p. 184)

20. Microorganisms in high concentrations of salts and sugars undergo plasmolysis.
21. Molds and yeasts are more capable than bacteria of growing in materials with low moisture or high osmotic pressure.

Radiation (pp. 184–185)

22. The effects of radiation depend on its wavelength, intensity, and duration.
23. Ionizing radiation (gamma rays, X rays, and high-energy electron beams) has a high degree of penetration and exerts its effect primarily by ionizing water and forming highly reactive hydroxyl radicals.
24. Ultraviolet (UV) radiation, a form of nonionizing radiation, has a low degree of penetration and causes cell damage by making thymine dimers in DNA that interfere with DNA replication; the most effective germicidal wavelength is 260 nm.
25. Microwaves can kill microbes indirectly as materials get hot.

Chemical Methods of Microbial Control (p. 187)

1. Chemical agents are used on living tissue (as antiseptics) and on inanimate objects (as disinfectants).
2. Few chemical agents achieve sterility.

Principles of Effective Disinfection (pp. 186–187)

3. Careful attention should be paid to the properties and concentration of the disinfectant to be used.
4. The presence of organic matter, degree of contact with microorganisms, and temperature should also be considered.

Evaluating a Disinfectant (p. 187)

5. In the use-dilution test, bacterial survival in the manufacturer's recommended dilution of a disinfectant is determined.
6. Viruses, endospore-forming bacteria, mycobacteria, and fungi can also be used in the use-dilution test.
7. In the disk-diffusion method, a disk of filter paper is soaked with a chemical and placed on an inoculated agar plate; a zone of inhibition indicates effectiveness.

Types of Disinfectants (pp. 188–194)

8. Phenolics exert their action by injuring plasma membranes.
9. Bisphenols such as triclosan (over the counter) and hexachlorophene (prescription) are widely used in household products.
10. Biguanides damage plasma membranes of vegetative cells.
11. Some halogens (iodine and chlorine) are used alone or as components of inorganic or organic solutions.
12. Iodine may combine with certain amino acids to inactivate enzymes and other cellular proteins.
13. Iodine is available as a tincture (in solution with alcohol) or as an iodophor (combined with an organic molecule).
14. The germicidal action of chlorine is based on the formation of hypochlorous acid when chlorine is added to water.
15. Alcohols exert their action by denaturing proteins and dissolving lipids.
16. In tinctures, alcohols enhance the effectiveness of other antimicrobial chemicals.
17. Aqueous ethanol (60–95%) and isopropanol are used as disinfectants.
18. Silver, mercury, copper, and zinc exert their antimicrobial action through oligodynamic action. When heavy metal ions combine with sulfhydryl (—SH) groups, proteins are denatured.
19. Soaps have limited germicidal action but assist in removing microorganisms.
20. Acid-anionic detergents are used to clean dairy equipment.
21. Quats are cationic detergents attached to NH_4^+.
22. By disrupting plasma membranes, quats allow cytoplasmic constituents to leak out of the cell.
23. Quats are most effective against gram-positive bacteria.
24. SO_2, sorbic acid, benzoic acid, and propionic acid inhibit fungal metabolism and are used as food preservatives.
25. Nitrate and nitrite salts prevent germination of *C. botulinum* endospores in meats.
26. Nisin and natamycin are antibiotics used to preserve foods, especially cheese.
27. Aldehydes such as formaldehyde and glutaraldehyde are among the most effective chemical disinfectants. They exert their antimicrobial effect by inactivating proteins.
28. Ethylene oxide is the gas most frequently used for sterilization. It penetrates most materials and kills all microorganisms by protein denaturation.
29. Free radicals in plasma gases are used to sterilize plastic instruments.
30. Supercritical fluids, which have properties of liquid and gas, can sterilize at low temperatures.
31. Hydrogen peroxide, peracetic acid, benzoyl peroxide, and ozone exert their antimicrobial effect by oxidizing molecules inside cells.

Microbial Characteristics and Microbial Control (pp. 194–197)

1. Gram-negative bacteria are generally more resistant than gram-positive bacteria to disinfectants and antiseptics.
2. Mycobacteria, endospores, and protozoan cysts and oocysts are very resistant to disinfectants and antiseptics.
3. Nonenveloped viruses are generally more resistant than enveloped viruses to disinfectants and antiseptics.
4. Prions are resistant to disinfection and autoclaving.

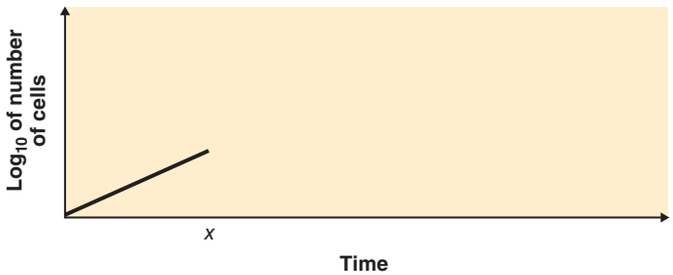
Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. The thermal death time for a suspension of *Bacillus subtilis* endospores is 30 minutes in dry heat and less than 10 minutes in an autoclave. Which type of heat is more effective? Why?
2. If pasteurization does not achieve sterilization, why is pasteurization used to treat food?
3. Thermal death point is not considered an accurate measure of the effectiveness of heat sterilization. List three factors that can alter thermal death point.
4. The antimicrobial effect of gamma radiation is due to (a) _____. The antimicrobial effect of ultraviolet radiation is due to (b) _____.
5. DRAW IT A bacterial culture was in log phase in the following figure. At time *x*, an antibacterial compound was added to the culture. Draw the lines indicating addition of a bactericidal compound and a bacteriostatic compound. Explain why the viable count does not immediately drop to zero at *x*.



6. How do autoclaving, hot air, and pasteurization illustrate the concept of equivalent treatments?
7. How do salts and sugars preserve foods? Why are these considered physical rather than chemical methods of microbial control? Name one food that is preserved with sugar and one preserved with salt. How do you account for the occasional growth of *Penicillium* mold in jelly, which is 50% sucrose?
8. The use-dilution values for two disinfectants tested under the same conditions are as follows: Disinfectant A—1:2; Disinfectant B—1:10,000. If both disinfectants are designed for the same purpose, which would you select?
9. A large hospital washes burn patients in a stainless steel tub. After each patient, the tub is cleaned with a quat. It was noticed that 14 of 20 burn patients acquired *Pseudomonas* infections after being bathed. Provide an explanation for this high rate of infection.
10. NAME IT What bacterium has porins, is resistant to triclosan, and survives and may grow in quats?

Multiple Choice

1. Which of the following does *not* kill endospores?
   a. autoclaving
   b. incineration
   c. hot-air sterilization
   d. pasteurization
   e. All of the above kill endospores.
2. Which of the following is most effective for sterilizing mattresses and plastic Petri dishes?
   a. chlorine
   b. ethylene oxide
   c. glutaraldehyde
   d. autoclaving
   e. nonionizing radiation
3. Which of these disinfectants does *not* act by disrupting the plasma membrane?
   a. phenolics
   b. phenol
   c. quats
   d. halogens
   e. biguanides
4. Which of the following *cannot* be used to sterilize a heat-labile solution stored in a plastic container?
   a. gamma radiation
   b. ethylene oxide
   c. supercritical fluids
   d. autoclaving
   e. short-wavelength radiation
5. Which of the following is used to control microbial growth in foods?
   a. organic acids
   b. alcohols
   c. aldehydes
   d. heavy metals
   e. all of the above

Use the following information to answer questions 6 and 7. The data were obtained from a use-dilution test comparing four disinfectants against *Salmonella choleraesuis*. *G* = growth, *NG* = no growth

	Bacterial Growth after Exposure to			
Dilution	Disinfectant A	Disinfectant B	Disinfectant C	Disinfectant D
1:2	NG	G	NG	NG
1:4	NG	G	NG	G
1:8	NG	G	G	G
1:16	G	G	G	G

6. Which disinfectant is the most effective?
7. Which disinfectant(s) is (are) bactericidal?
   a. A, B, C, and D
   b. A, C, and D
   c. A only
   d. B only
   e. none of the above
8. Which of the following is *not* a characteristic of quaternary ammonium compounds?
   a. bactericidal against gram-positive bacteria
   b. sporicidal
   c. amoebicidal
   d. fungicidal
   e. kills enveloped viruses
9. A classmate is trying to determine how a disinfectant might kill cells. You observed that when he spilled the disinfectant in your reduced litmus milk, the litmus turned blue again. You suggest to your classmate that
   a. the disinfectant might inhibit cell wall synthesis.
   b. the disinfectant might oxidize molecules.
   c. the disinfectant might inhibit protein synthesis.
   d. the disinfectant might denature proteins.
   e. he take his work away from yours.
10. Which of the following is most likely to be bactericidal?
    a. membrane filtration
    b. ionizing radiation
    c. lyophilization (freeze-drying)
    d. deep-freezing
    e. all of the above

Analysis

1. The disk-diffusion method was used to evaluate three disinfectants. The results were as follows:

Disinfectant	Zone of Inhibition
X	0 mm
Y	5 mm
Z	10 mm

   a. Which disinfectant was the most effective against the organism?
   b. Can you determine whether compound Y was bactericidal or bacteriostatic?

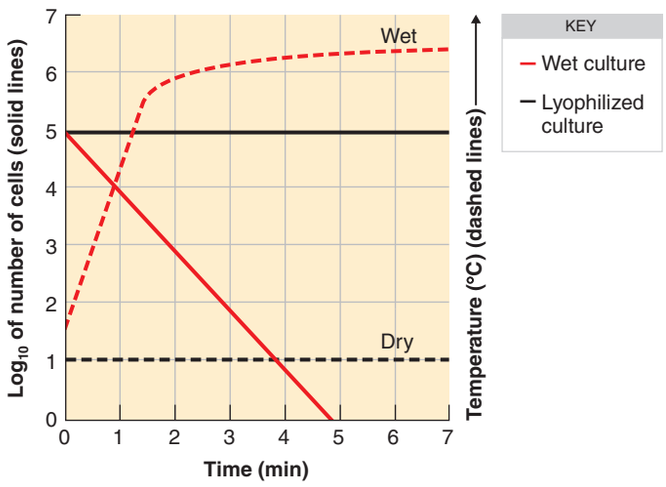
2. For each of the following bacteria, explain why it is often resistant to disinfectants.
   a. *Mycobacterium*
   b. *Pseudomonas*
   c. *Bacillus*

3. A use-dilution test was used to evaluate two disinfectants against *Salmonella choleraesuis*. The results were as follows:

	Bacterial Growth after Exposures		
Time of Exposure (min)	Disinfectant A	Disinfectant B Diluted with Distilled Water	Disinfectant B Diluted with Tap Water
10	G	NG	G
20	G	NG	NG
30	NG	NG	NG

   a. Which disinfectant was the most effective?
   b. Which disinfectant should be used against *Staphylococcus*?

4. To determine the lethal action of microwave radiation, two 10^5 suspensions of *E. coli* were prepared. One cell suspension was exposed to microwave radiation while wet, whereas the other was lyophilized (freeze-dried) and then exposed to radiation. The results are shown in the following figure. Dashed lines indicate the temperature of the samples. What is the most likely method of lethal action of microwave radiation? How do you suppose these data might differ for *Clostridium*?



Clinical Applications and Evaluation

1. *Entamoeba histolytica* and *Giardia intestinalis* were isolated from the stool sample of a 45-year-old man, and *Shigella sonnei* was isolated from the stool sample of an 18-year-old woman. Both patients experienced diarrhea and severe abdominal cramps, and prior to onset of digestive symptoms both had been treated by the same chiropractor. The chiropractor had administered colonic irrigations (enemas) to these patients. The device used for this treatment was a gravity-dependent apparatus using 12 liters of tap water. There were no check valves to prevent backflow, so all parts of the apparatus could have become contaminated with feces during each colonic treatment. The chiropractor provided colonic treatment to four or five patients per day. Between patients, the adaptor piece that is inserted into the rectum was placed in a "hot-water sterilizer."
   What two errors were made by the chiropractor?

2. Between March 9 and April 12, five chronic peritoneal dialysis patients at one hospital became infected with *Pseudomonas aeruginosa*. Four patients developed peritonitis (inflammation of the abdominal cavity), and one developed a skin infection at the catheter insertion site. All patients with peritonitis had low-grade fever, cloudy peritoneal fluid, and abdominal pain. All patients had permanent indwelling peritoneal catheters, which the nurse wiped with gauze that had been soaked with an iodophor solution each time the catheter was connected to or disconnected from the machine tubing. Aliquots of the iodophor were transferred from stock bottles to small in-use bottles. Cultures from the dialysate concentrate and the internal areas of the dialysis machines were negative; iodophor from a small in-use plastic container yielded a pure culture of *P. aeruginosa*.
   What improper technique led to this infection?

3. You are investigating a national outbreak of *Ralstonia mannitolilytica* associated with use of a contaminated oxygen-delivery device among pediatric patients. The device adds moisture to and warms the oxygen. Each hospital followed the manufacturer's recommendation to use a detergent to clean the reusable components of the device between patients. Tap water is permitted in the device because the device uses a reusable 0.01-µm filter as a biological barrier between the air and water compartments. *Ralstonia* is a gram-negative rod commonly found in water.
   Why did disinfection fail?
   What do you recommend for disinfecting? The device cannot be autoclaved.

In the Clinic

As a nurse at a U.S. military hospital, you treat service members injured in the recent Middle East conflicts. You notice that wounds infected by *Acinetobacter baumannii* are not responding to antibiotics. The Centers for Disease Control and Prevention reports that the antibiotic-resistance genes found in *A. baumannii* are the same as those in *Pseudomonas, Salmonella,* and *Escherichia*. Cephalosporin-resistance genes are on the chromosome, tetracycline resistance is encoded by a plasmid, and streptomycin resistance is associated with a transposon. **Can you suggest mechanisms by which *Acinetobacter* acquired this resistance?**

Hint: Read about genetic recombination on pages 225–230.

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

8 Microbial Genetics

Virtually all the microbial traits you have read about in earlier chapters are controlled or influenced by heredity. The inherited characteristics of microbes include shape, structural features, metabolism, ability to move, and interactions with other organisms. Individual organisms transmit these characteristics to their offspring through genes.

The development of antibiotic resistance in microorganisms is often carried on plasmids such as those in the photo, which are readily transferred between bacterial cells. They are responsible for the emergence of methicillin-resistant *Staphylococcus aureus* and the recent emergence of carbapenem-resistant *Klebsiella pneumoniae*. The emergence of vancomycin-resistant *S. aureus* (VRSA) poses a serious threat to patient care. In this chapter you will see how VRSA acquired this characteristic.

Emerging diseases provide another reason why it is important to understand genetics. New diseases are the results of genetic changes in some existing organism; for example, *E. coli* O157:H7 acquired the genes for Shiga toxin from *Shigella*.

Currently, microbiologists are using genetics to discover relatedness among organisms, to explore the origins of organisms such as HIV and H1N1 influenza virus, and to study how genes are expressed.

The **Big Picture** on the next page highlights key principles of genetics that are explained in greater detail throughout the chapter.

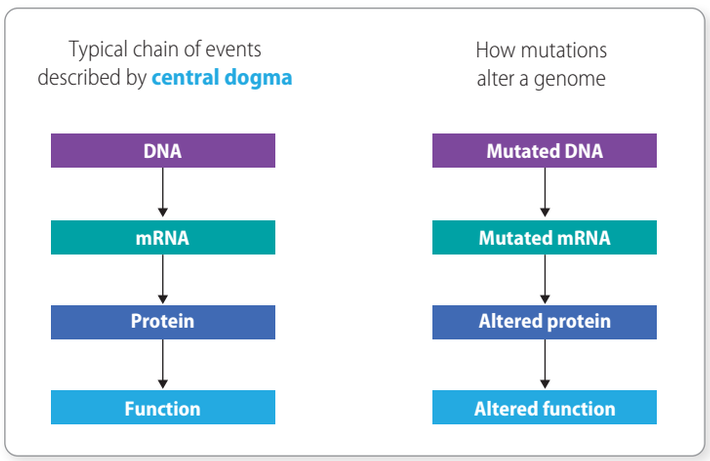
Plasmid DNA from *E. coli*.

BIG PICTURE

Genetics

Genetics is the science of heredity. It includes the study of genes: how they are replicated, expressed, and passed on from one generation to another.

The **central dogma** of molecular biology describes how, typically, DNA is transcribed to messenger RNA, which, in turn, is translated into proteins that carry out vital cellular functions. Mutations introduce change into this process—ultimately leading to new or lost functions.



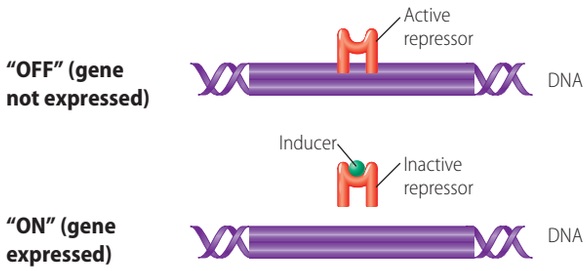
Mutations can be caused by **base substitutions** or **frameshift mutations**.

In **base substitution mutations**, a single DNA base pair is altered.

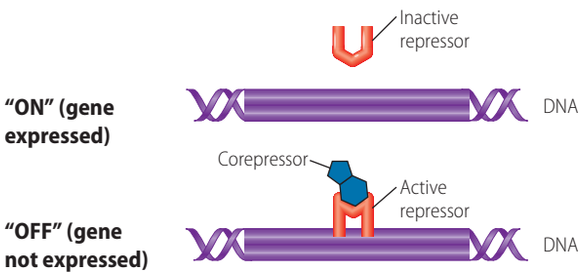
In **frameshift mutations**, DNA base pairs are added or removed from the sequence, causing a shift in the sequence reading.

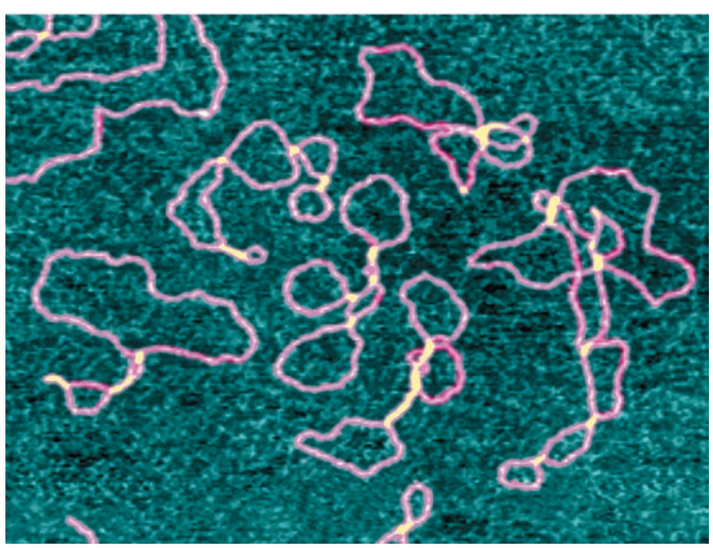
Bacterial gene expression can be regulated by operons, which can either be **inducible** or **repressible**.

An **inducible operon** includes genes that are in the "off" mode, with the repressor bound to the DNA, and is turned "on" by the environmental inducer.



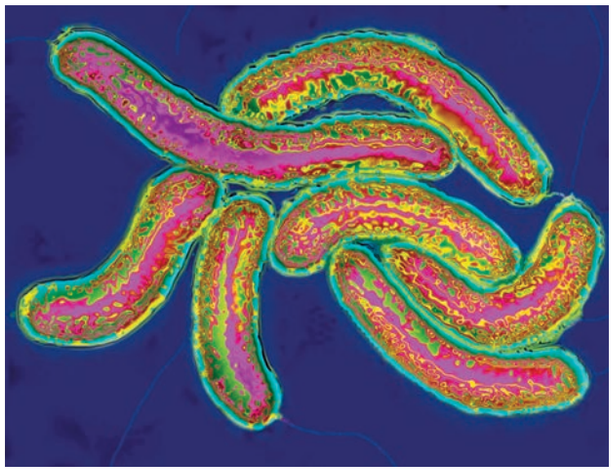
A **repressible operon** includes genes that are in the "on" mode, without the repressor bound to the DNA, and is turned "off" by the environmental corepressor and repressor.





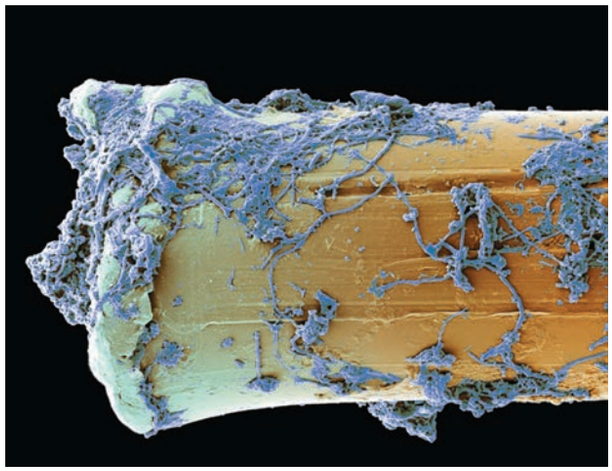
Atomic force micrograph showing DNA molecules AFM 7 nm

Alteration of bacterial genes and or gene expression may cause disease, prevent disease treatment, or be manipulated for human benefit.



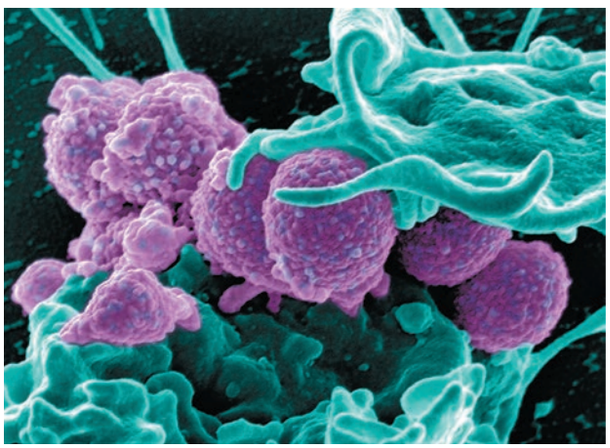
TEM 0.4 μm

Diseases: Many bacterial diseases are caused by the presence of toxic proteins that damage human tissue. These toxic proteins are coded for by bacterial genes. *Vibrio cholerae,* shown above, produces an enterotoxin that causes diarrhea and severe dehydration, which can be fatal if left untreated.



SEM 5 μm

Biofilms: Biofilms, such as the one seen here growing on a toothbrush bristle, are produced by altered bacterial gene expression when populations are large enough. Various *Streptococcus* species, including *S. mutans,* form biofilms on teeth and gums, contributing to the development of dental plaque and dental caries.



SEM 0.3 μm

Antibiotic Resistance: Mutations in the bacterial genome are one of the first steps toward the development of antibiotic resistance. This process has occurred with *Staphylococcus aureus,* which is currently resistant to beta-lactam antibiotics such as penicillin. Methicillin was introduced to treat penicillin-resistant *S. aureus*. Methicillin-resistant *S. aureus,* (MRSA) shown in purple above, is now a leading cause of healthcare-associated infections.

Biotechnology: Scientists can alter a microorganism's genome, adding genes that will produce human proteins used in treating disease. Insulin, used for treatment of diabetes, is produced in this manner.

KEY CONCEPTS

- DNA expression leads to cell function via the production of proteins.
- DNA expression can be controlled by operons.
- Mutations alter DNA sequences.
- DNA mutations can change bacterial function.

Structure and Function of the Genetic Material

LEARNING OBJECTIVES

- **8-1** Define *genetics, genome, chromosome, gene, genetic code, genotype, phenotype,* and *genomics*.
- **8-2** Describe how DNA serves as genetic information.
- **8-3** Describe the process of DNA replication.
- **8-4** Describe protein synthesis, including transcription, RNA processing, and translation.
- **8-5** Compare protein synthesis in prokaryotes and eukaryotes.

Genetics is the science of heredity. It includes the study of genes: how they carry information, how they replicate and pass to subsequent generations of cells or between organisms, and how the expression of their information within an organism determines its characteristics. The genetic information in a cell is called the **genome.** A cell's genome includes its chromosomes and plasmids. **Chromosomes** are structures containing DNA that physically carry hereditary information; the chromosomes contain the genes. **Genes** are segments of DNA (except in some viruses, in which they are made of RNA) that code for functional products. Usually these products are proteins, but they can also be RNAs (ribosomal RNA, transfer RNA, or microRNA).

We saw in Chapter 2 that DNA is a macromolecule composed of repeating units called *nucleotides*. Each nucleotide consists of a nucleobase (adenine, thymine, cytosine, or guanine), deoxyribose (a pentose sugar), and a phosphate group (see Figure 2.16, page 44). The DNA within a cell exists as long strands of nucleotides twisted together in pairs to form a double helix. Each strand has a string of alternating sugar and phosphate groups (its *sugar-phosphate backbone*), and a nitrogenous base is attached to each sugar in the backbone. The two strands are held together by hydrogen bonds between their nitrogenous bases. The **base pairs** always occur in a specific way: adenine always pairs with thymine, and cytosine always pairs with guanine. Because of this specific base pairing, the base sequence of one DNA strand determines the base sequence of the other strand. The two strands of DNA are thus *complementary*.

The structure of DNA helps explain two primary features of biological information storage. First, the linear sequence of bases provides the actual information. Genetic information is encoded by the sequence of bases along a strand of DNA, in much the same way as our written language uses a linear sequence of letters to form words and sentences. The genetic language, however, uses an alphabet with only four letters—the four kinds of nucleobases in DNA (or RNA). But 1000 of these four bases, the number contained in an average-sized gene, can be arranged in 4^{1000} different ways. This astronomically large number explains how genes can be varied enough to provide all the information a cell needs to grow and perform its functions.

The **genetic code,** the set of rules that determines how a nucleotide sequence is converted into the amino acid sequence of a protein, is discussed in more detail later in this chapter.

Second, the complementary structure allows for the precise duplication of DNA during cell division. Each offspring cell receives one of the original strands from the parent, thus ensuring one strand that functions correctly.

Much of cellular metabolism is concerned with translating the genetic message of genes into specific proteins. A gene usually codes for a messenger RNA (mRNA) molecule, which ultimately results in the formation of a protein. When the ultimate molecule for which a gene codes (a protein, for example) has been produced, we say that the gene has been *expressed*. The flow of genetic information can be shown as flowing from DNA to RNA to proteins, as follows:

This theory was called the **central dogma** by Francis Crick in 1956, when he first proposed that the sequence of nucleotides in DNA determines the sequence of amino acids in a protein.

ASM: Although the central dogma is universal in all cells, the processes differ in prokaryotes and eukaryotes, as we shall see in this chapter.

Genotype and Phenotype

The **genotype** of an organism is its genetic makeup—all its DNA—the information that codes for all the particular characteristics of the organism. The genotype represents *potential* properties, but not the properties themselves. **Phenotype** refers to *actual, expressed* properties, such as the organism's ability to perform a particular chemical reaction. Phenotype, then, is the manifestation of genotype.

In a sense, an organism's phenotype is its collection of proteins, because most of a cell's properties derive from the structures and functions of proteins. In microbes, most proteins are either *enzymatic* (catalyze particular reactions) or *structural* (participate in large functional complexes such as membranes or flagella). Even phenotypes that depend on structural macromolecules such as lipids or polysaccharides rely indirectly on proteins. For instance, the structure of a complex lipid or polysaccharide molecule results from catalytic activities of enzymes that synthesize, process, and degrade those molecules. Thus, saying that phenotypes are due to proteins is a useful simplification.

DNA and Chromosomes

Bacteria typically have a single circular chromosome consisting of a single circular molecule of DNA with associated proteins. The chromosome is looped and folded and attached at one or several points to the plasma membrane. The DNA of *E. coli* has

Figure 8.1 A prokaryotic chromosome.

Q How many times longer than the 2-μm cell is the chromosome?

about 4.6 million base pairs and is about 1 mm long—1000 times longer than the entire cell (Figure 8.1). However, the chromosome takes up only about 10% of the cell's volume because the DNA is twisted, or *supercoiled*.

The entire genome does not consist of back-to-back genes. Noncoding regions called **short tandem repeats (STRs)** occur in most genomes, including that of *E. coli*. STRs are repeating sequences of two to five-base sequences. These are used in DNA fingerprinting (discussed on page 254).

Now, the complete base sequences of chromosomes can be determined. Computers are used to search for *open reading frames*, that is, regions of DNA that are likely to encode a protein. As you will see later, these are base sequences between start and stop codons. The sequencing and molecular characterization of genomes is called **genomics.** The use of genomics to track West Nile virus is described in the Clinical Focus box on page 215.

The Flow of Genetic Information

DNA replication makes possible the flow of genetic information from one generation to the next. This is called **vertical gene transfer.** As shown in Figure 8.2, the DNA of a cell replicates before cell division so that each offspring cell receives a chromosome identical to the parent's. Within each metabolizing cell, the genetic information contained in DNA also flows in another way: it is transcribed into mRNA and then translated into protein. We describe the processes of transcription and translation later in this chapter.

CHECK YOUR UNDERSTANDING

- Give a clinical application of genomics. **8-1**
- Why is the base pairing in DNA important? **8-2**

DNA Replication

In DNA replication, one "parental" double-stranded DNA molecule is converted to two identical offspring molecules. The complementary structure of the nitrogenous base sequences in the DNA molecule is the key to understanding DNA replication. Because the bases along the two strands of double-helical DNA are complementary, one strand can act as a template for the production of the other strand (Figure 8.3a).

DNA replication requires the presence of several cellular proteins that direct a particular sequence of events. Enzymes involved in DNA replication and other processes are listed in Table 8.1. When replication begins, the supercoiling is relaxed by *topoisomerase* or *gyrase*. The two strands of parental DNA are unwound by *helicase* and separated from each other in one small DNA segment after another. Free nucleotides present in the cell cytoplasm are matched up to the exposed bases of the single-stranded parental DNA. Where thymine is present on the original strand, only adenine can fit into place on the new strand; where guanine is present on the original strand, only cytosine can fit into place, and so on. Any bases that are improperly base-paired are removed and replaced by replication enzymes. Once

Clinical Case: Where There's Smoke

Marcel DuBois, a 70-year-old grandfather of 12, quietly hangs up the phone. His doctor has just called him with the results of his stool DNA test that he undertook at the Mayo Clinic last week. Marcel's doctor suggested this new, noninvasive screening tool for colorectal cancer because Marcel is not comfortable with the colonoscopy procedure and usually tries to postpone getting one. The stool DNA test, however, uses stool samples, which contain cells that have been shed from the colon lining. The DNA from these cells is tested for DNA markers that may indicate the presence of precancerous polyps or cancerous tumors. Marcel makes an appointment to come in to see his doctor the next afternoon.

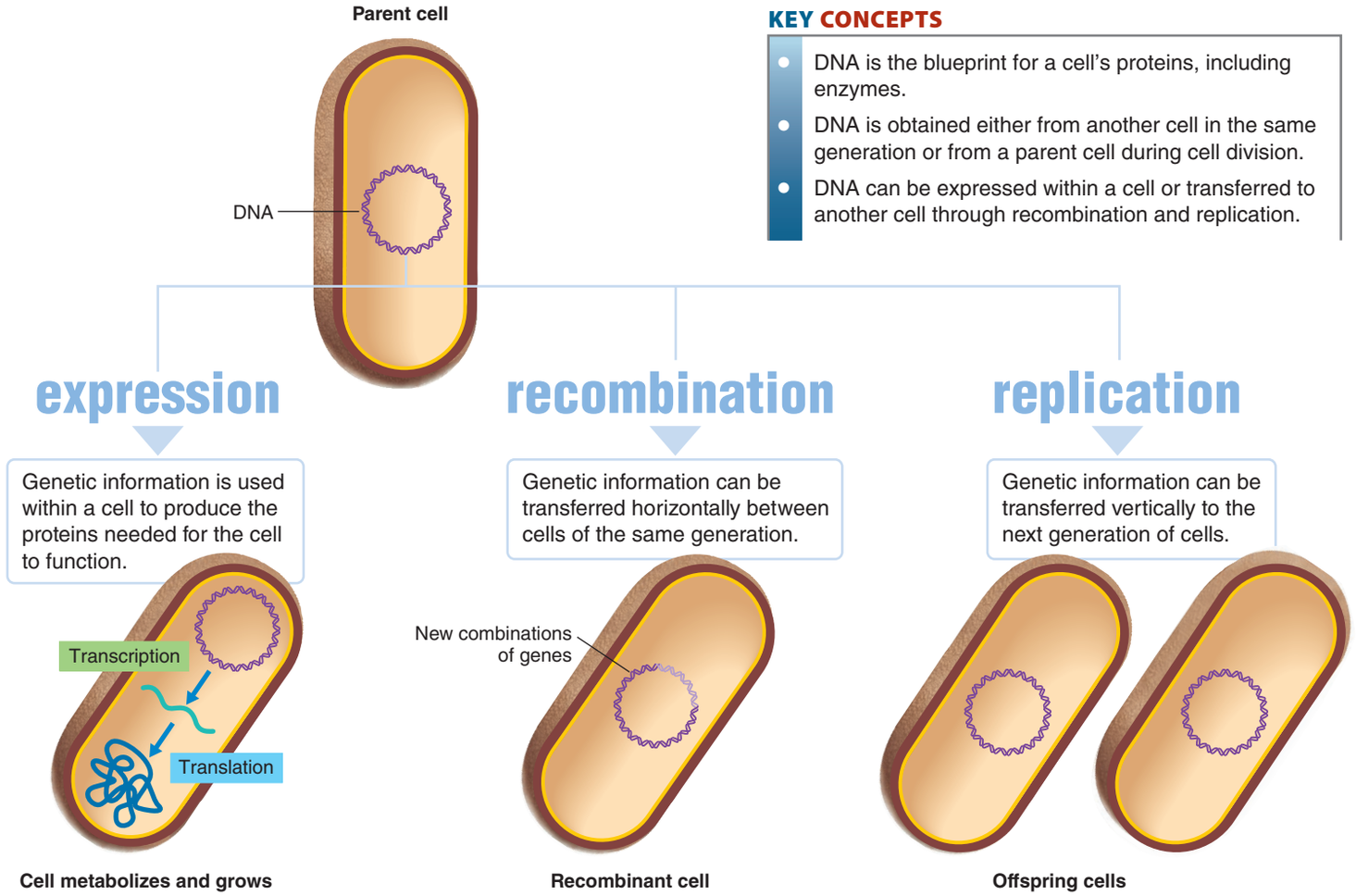
Once in the office, the doctor explains to Marcel and his wife, Janice, that the stool DNA test detected the presence of serrated colorectal polyps. This type of polyp is usually difficult to see with a colonoscopy because it is not raised and can be the same color as the colon wall.

How can DNA show whether a person has cancer? Read on to find out.

205 220 224 228

FOUNDATION FIGURE 8.2

The Flow of Genetic Information



aligned, the newly added nucleotide is joined to the growing DNA strand by an enzyme called **DNA polymerase.** Then the parental DNA is unwound a bit further to allow the addition of the next nucleotides. The point at which replication occurs is called the *replication fork*.

As the replication fork moves along the parental DNA, each of the unwound single strands combines with new nucleotides. The original strand and this newly synthesized daughter strand then rewind. Because each new double-stranded DNA molecule contains one original (conserved) strand and one new strand, the process of replication is referred to as **semiconservative replication.**

Before looking at DNA replication in more detail, let's discuss the structure of DNA (see Figure 2.16 on page 44 for an overview). It is important to understand that the paired DNA strands are oriented in opposite directions relative to each other. The carbon atoms of the sugar component of each nucleotide are numbered 1′ (pronounced "one prime") to 5′. For the paired bases to be next to each other, the sugar components in one strand are upside down relative to the other. The end with the hydroxyl attached to the 3′ carbon is called the 3′ end of the DNA strand; the end having a phosphate attached to the 5′ carbon is called the 5′ end. The way in which the two strands fit together dictates that the 5′ → 3′ direction of one strand runs counter to the 5′ → 3′ direction of the other strand (Figure 8.3b). This structure of DNA affects the replication process because DNA polymerases can add new nucleotides to the 3′ end only. Therefore, as the replication fork moves along the parental DNA, the two new strands must grow in different directions.

DNA replication requires a great deal of energy. The energy is supplied from the nucleotides, which are actually nucleoside triphosphates. You already know about ATP; the only difference between ATP and the adenine nucleotide in DNA is the sugar component. Deoxyribose is the sugar in the nucleosides used to synthesize DNA, and nucleoside triphosphates with ribose are used to synthesize RNA. Two phosphate groups are removed

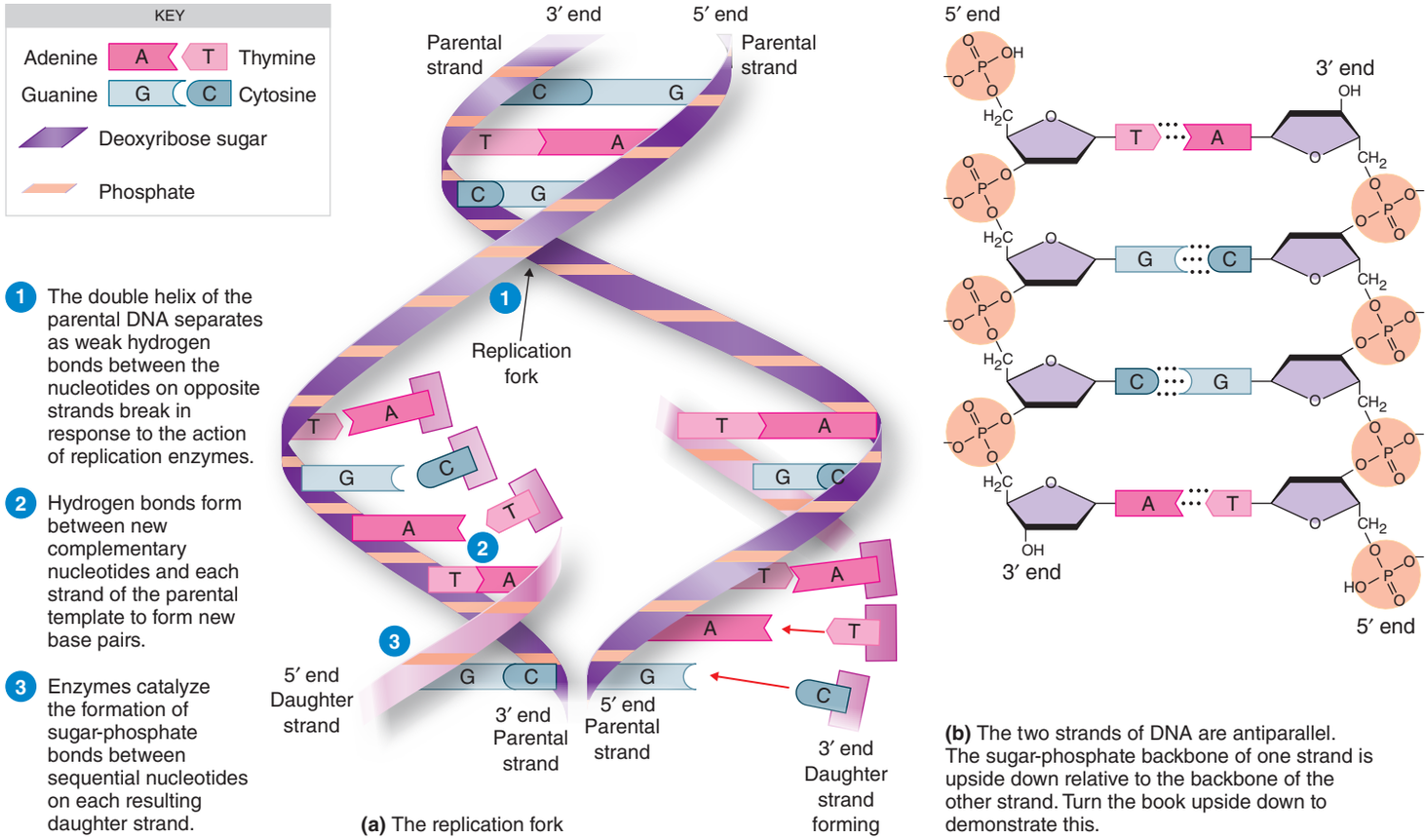


Figure 8.3 DNA replication.

Q What is the advantage of semiconservative replication?

TABLE **8.1** **Important Enzymes in DNA Replication, Expression, and Repair**

DNA Gyrase	Relaxes supercoiling ahead of the replication fork
DNA Ligase	Makes covalent bonds to join DNA strands; Okazaki fragments, and new segments in excision repair
DNA Polymerases	Synthesizes DNA; proofreads and repairs DNA
Endonucleases	Cut DNA backbone in a strand of DNA; facilitate repair and insertions
Exonucleases	Cut DNA from an exposed end of DNA; facilitate repair
Helicase	Unwinds double-stranded DNA
Methylase	Adds methyl group to selected bases in newly made DNA
Photolyase	Uses visible light energy to separate UV-induced pyrimidine dimers
Primase	An RNA polymerase that makes RNA primers from a DNA template
Ribozyme	RNA enzyme that removes introns and splices exons together
RNA Polymerase	Copies RNA from a DNA template
snRNP	RNA-protein complex that removes introns and splices exons together
Topoisomerase	Relaxes supercoiling ahead of the replication fork; separates DNA circles at the end of DNA replication
Transposase	Cuts DNA backbone, leaving single-stranded "sticky ends"

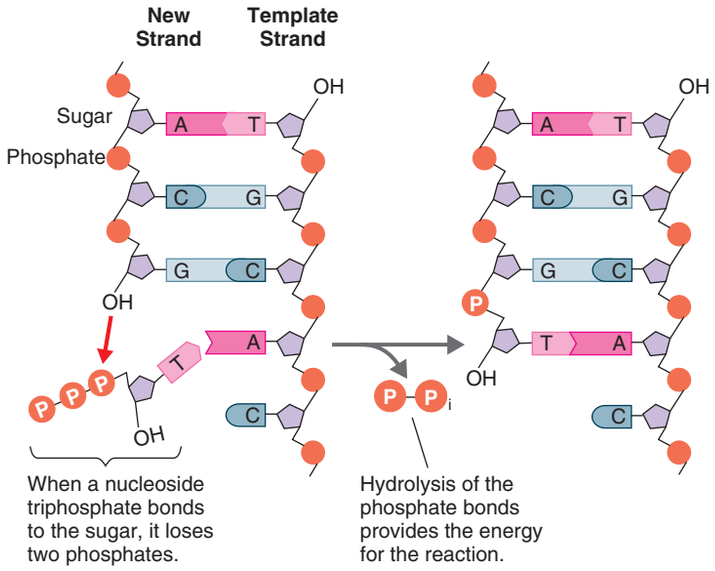


Figure 8.4 Adding a nucleotide to DNA.

Why is one strand "upside down" relative to the other strand? Why can't both strands "face" the same way?

to add the nucleotide to a growing strand of DNA; hydrolysis of the nucleoside is exergonic and provides energy to make the new bonds in the DNA strand (Figure 8.4).

Figure 8.5 provides more detail about the many steps that go into this complex process.

DNA replication by some bacteria, such as *E. coli,* goes *bidirectionally* around the chromosome (Figure 8.6). Two replication forks move in opposite directions away from the origin of replication. Because the bacterial chromosome is a closed loop, the replication forks eventually meet when replication is completed. The two loops must be separated by a topoisomerase. Much evidence shows an association between the bacterial plasma membrane and the origin of replication. After duplication, if each copy of the origin binds to the membrane at opposite poles, then each offspring cell receives one copy of the DNA molecule—that is, one complete chromosome.

DNA replication is an amazingly accurate process. Typically, mistakes are made at a rate of only 1 in every 10 billion bases incorporated. Such accuracy is largely due to the *proofreading* capability of DNA polymerase. As each new base is added, the enzyme evaluates whether it forms the proper complementary base-pairing structure. If not, the enzyme excises the improper base and replaces it with the correct one. In this way,

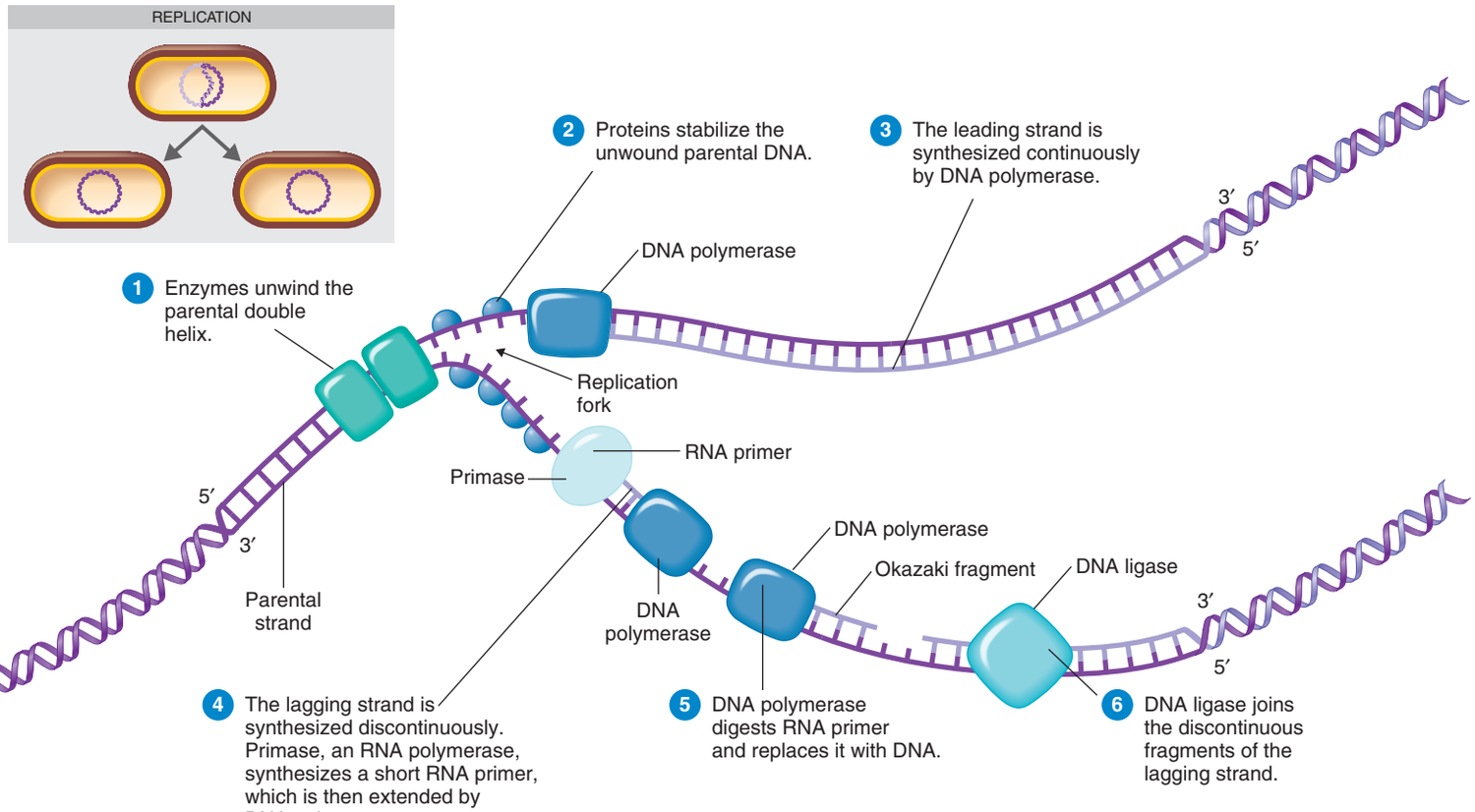
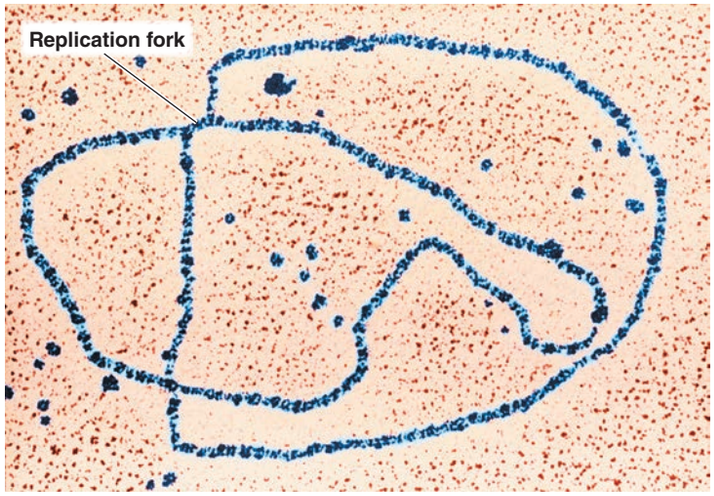
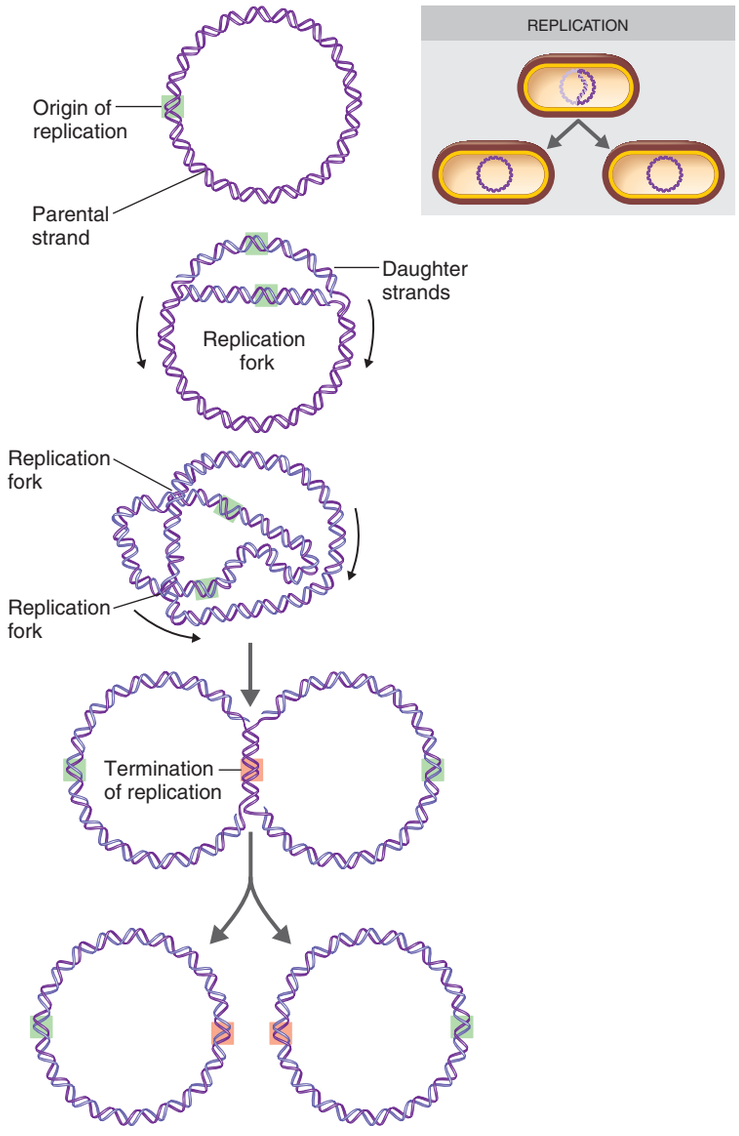


Figure 8.5 A summary of events at the DNA replication fork.

Why is one strand of DNA synthesized discontinuously?



(a) An *E. coli* chromosome in the process of replicating



(b) Bidirectional replication of a circular bacterial DNA molecule

Figure 8.6 Replication of bacterial DNA.

Q What is the origin of replication?

DNA can be replicated very accurately, allowing each daughter chromosome to be virtually identical to the parental DNA. MM **Animations** DNA Replication: Overview, Forming the Replication Fork, Replication Proteins, Synthesis

CHECK YOUR UNDERSTANDING

Describe DNA replication, including the functions of DNA gyrase, DNA ligase, and DNA polymerase. **8-3**

RNA and Protein Synthesis

How is the information in DNA used to make the proteins that control cell activities? In the process of *transcription*, genetic information in DNA is copied, or transcribed, into a complementary base sequence of RNA. The cell then uses the information encoded in this RNA to synthesize specific proteins through the process of *translation*. We now take a closer look at these two processes as they occur in a bacterial cell.

Transcription in Prokaryotes

Transcription is the synthesis of a complementary strand of RNA from a DNA template. We will discuss transcription in prokaryotic cells here. Transcription in eukaryotes is discussed on page 211.

Ribosomal RNA (rRNA) forms an integral part of ribosomes, the cellular machinery for protein synthesis. Transfer RNA is also involved in protein synthesis, as we will see. **Messenger RNA (mRNA)** carries the coded information for making specific proteins from DNA to ribosomes, where proteins are synthesized.

During transcription, a strand of mRNA is synthesized using a specific portion of the cell's DNA as a template. In other words, the genetic information stored in the sequence of nitrogenous bases of DNA is rewritten so that the same information appears in the base sequence of mRNA.

As in DNA replication, a guanine (G) in the DNA template dictates a cytosine (C) in the mRNA being made, and a C in the DNA template dictates a G in the mRNA. Likewise, a thymine (T) in the DNA template dictates an adenine (A) in the mRNA. However, an adenine in the DNA template dictates a uracil (U) in the mRNA, because RNA contains uracil instead of thymine. (Uracil has a chemical structure slightly different from thymine, but it base-pairs in the same way.) If, for example, the template portion of DNA has the base sequence 3′-ATGCAT, the newly synthesized mRNA strand will have the complementary base sequence 5′-UACGUA.

The process of transcription requires both an enzyme called *RNA polymerase* and a supply of RNA nucleotides (**Figure 8.7**).

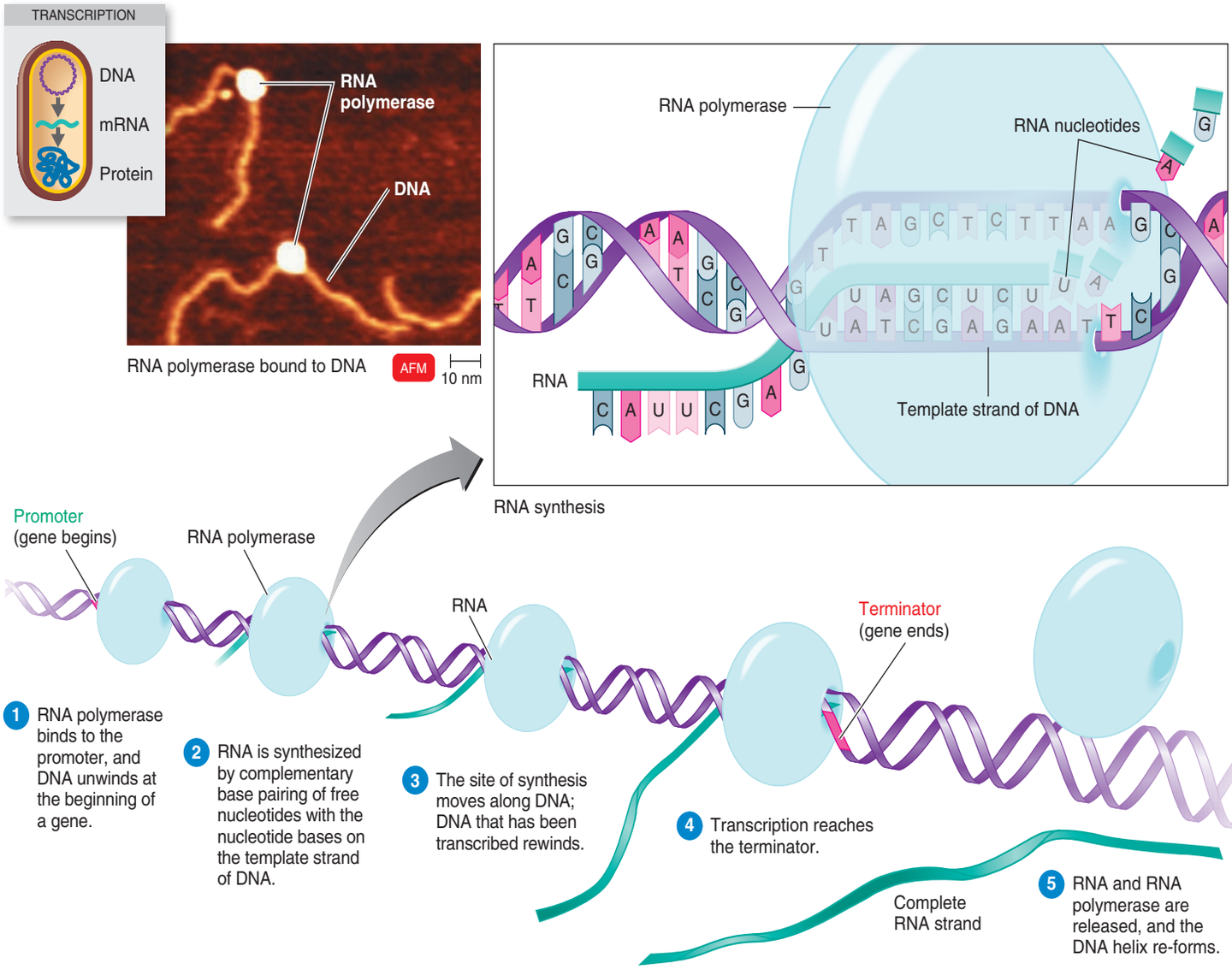


Figure 8.7 The process of transcription. The orienting diagram indicates the relationship of transcription to the overall flow of genetic information within a cell.

Q When does transcription stop?

Transcription begins when RNA polymerase binds to the DNA at a site called the **promoter.** Only one of the two DNA strands serves as the template for RNA synthesis for a given gene. Like DNA, RNA is synthesized in the 5′ → 3′ direction. RNA synthesis continues until RNA polymerase reaches a site on the DNA called the **terminator.**

Transcription allows the cell to produce short-term copies of genes that can be used as the direct source of information for protein synthesis. Messenger RNA acts as an intermediate between the permanent storage form, DNA, and the process that uses the information, translation. MM **Animations** Transcription: Overview, Process

Translation

We have seen how the genetic information in DNA transfers to mRNA during transcription. Now we will see how mRNA serves as the source of information for the synthesis of proteins. Protein synthesis is called **translation** because it involves decoding the "language" of nucleic acids and converting it into the "language" of proteins.

The language of mRNA is in the form of **codons,** groups of three nucleotides, such as AUG, GGC, or AAA. The sequence of codons on an mRNA molecule determines the sequence of amino acids that will be in the protein being synthesized. Each codon "codes" for a particular amino acid. This is the genetic code (Figure 8.8).

Codons are written in terms of their base sequence in mRNA. Notice in Figure 8.8 that there are 64 possible codons but only 20 amino acids. This means that most amino acids are signaled by several alternative codons, a situation referred to as the **degeneracy** of the code. For example, leucine has six codons, and alanine has four codons. Degeneracy allows for a certain amount of misreading of, or mutation in, the DNA without affecting the protein ultimately produced.

Of the 64 codons, 61 are sense codons, and 3 are nonsense codons. **Sense codons** code for amino acids, and **nonsense codons** (also called *stop codons*) do not. Rather, the nonsense codons—UAA, UAG, and UGA—signal the end of the protein molecule's synthesis. The start codon that initiates the synthesis of the protein molecule is AUG, which is also the codon for methionine. In bacteria, the start AUG codes for formylmethionine rather than the methionine found in other parts of the protein. The initiating methionine is often removed later, so not all proteins contain methionine.

During translation, codons of an mRNA are "read" sequentially; and, in response to each codon, the appropriate amino acid is assembled into a growing chain. The site of translation is the ribosome, and **transfer RNA (tRNA)** molecules both recognize the specific codons and transport the required amino acids.

Each tRNA molecule has an **anticodon,** a sequence of three bases that is complementary to a codon. In this way, a tRNA molecule can base-pair with its associated codon. Each tRNA can also carry on its other end the amino acid encoded by the codon that the tRNA recognizes. The functions of the ribosome are to direct the orderly binding of tRNAs to codons and to assemble the amino acids brought there into a chain, ultimately producing a protein.

Figure 8.9 shows the details of translation. The two ribosomal subunits, a tRNA with the anticodon UAC, and the mRNA molecule to be translated, along with several additional protein factors, all assemble. This sets up the start codon (AUG) in the proper position to allow translation to begin. After the ribosome joins the first two amino acids with a peptide bond, the first tRNA molecule leaves the ribosome. The ribosome then moves along the mRNA to the next codon. As the proper amino acids are brought into line one by one, peptide bonds are formed between them, and a polypeptide chain results. (Also see Figure 2.14, page 42.) Translation ends when one of the three nonsense codons in the mRNA is reached. The ribosome then comes apart into its two subunits, and the mRNA and newly synthesized polypeptide chain are released. The ribosome, the mRNA, and the tRNAs are then available to be used again.

The ribosome moves along the mRNA in the 5′ → 3′ direction. As a ribosome moves along the mRNA, it will soon allow the start codon to be exposed. Additional ribosomes can then assemble and begin synthesizing protein. In this way, there are usually a number of ribosomes attached to a single mRNA, all

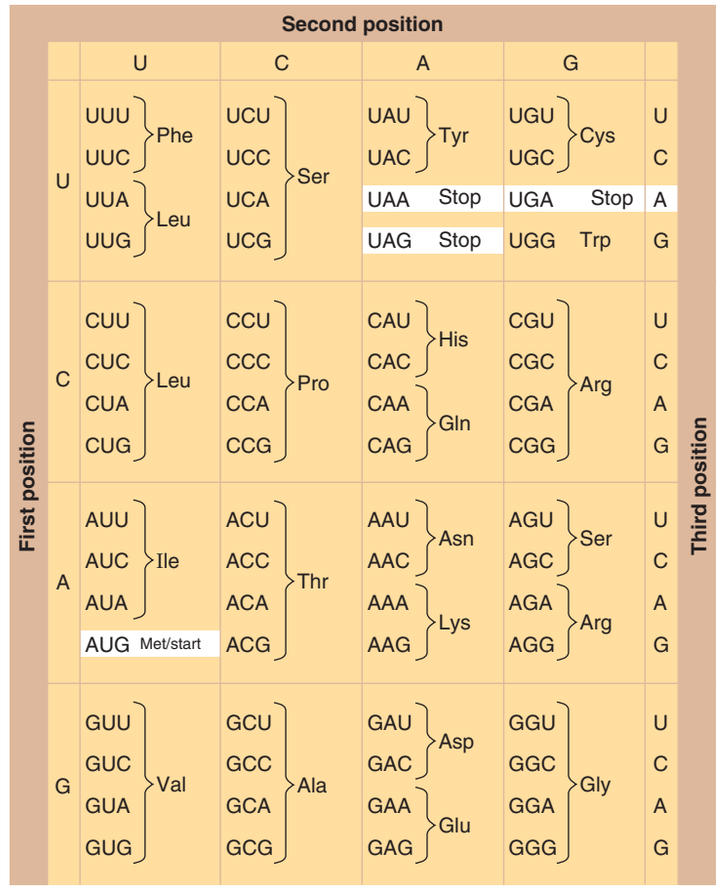


Figure 8.8 The genetic code. The three nucleotides in an mRNA codon are designated, respectively, as the first position, second position, and third position of the codon on the mRNA. Each set of three nucleotides specifies a particular amino acid, represented by a three-letter abbreviation (see Table 2.5, page 41). The codon AUG, which specifies the amino acid methionine, is also the start of protein synthesis. The word *Stop* identifies the nonsense codons that signal the termination of protein synthesis.

Q What is the advantage of the degeneracy of the genetic code?

at various stages of protein synthesis. In prokaryotic cells, the translation of mRNA into protein can begin even before transcription is complete (**Figure 8.10**). Because mRNA is produced in the cytoplasm in prokaryotes, the start codons of an mRNA being transcribed are available to ribosomes before the entire mRNA molecule is even made.

Transcription in Eukaryotes

In eukaryotic cells, transcription takes place in the nucleus. The mRNA must be completely synthesized and moved through the nuclear membrane to the cytoplasm before translation can begin. In addition, the RNA undergoes processing before it leaves the nucleus. In eukaryotic cells, the regions of genes that code for proteins are often interrupted by noncoding DNA. Thus, eukaryotic genes are composed of **exons,** the regions of DNA *expressed,* and **introns,** the *intervening* regions of DNA

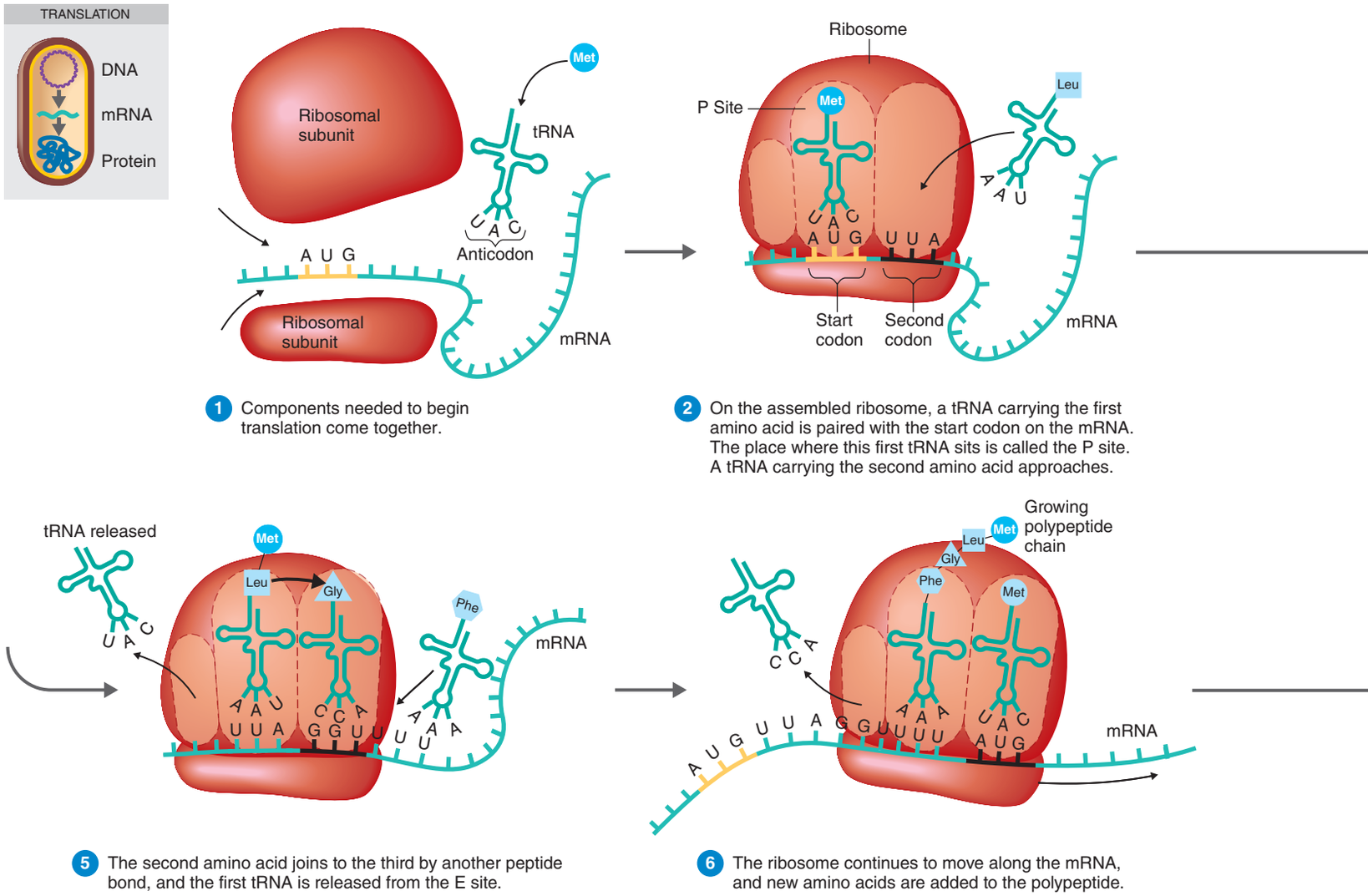


Figure 8.9 The process of translation. The overall goal of translation is to produce proteins using mRNAs as the source of biological information. The complex cycle of events illustrated here shows the primary role of tRNA and ribosomes in the decoding of this information. The ribosome acts as the site where the mRNA-encoded information is decoded, as well as the site where individual amino acids are connected into polypeptide chains. The tRNA molecules act as the actual "translators"—one end of each tRNA recognizes a specific mRNA codon, while the other end carries the amino acid encoded by that codon.

Q When does translation stop?

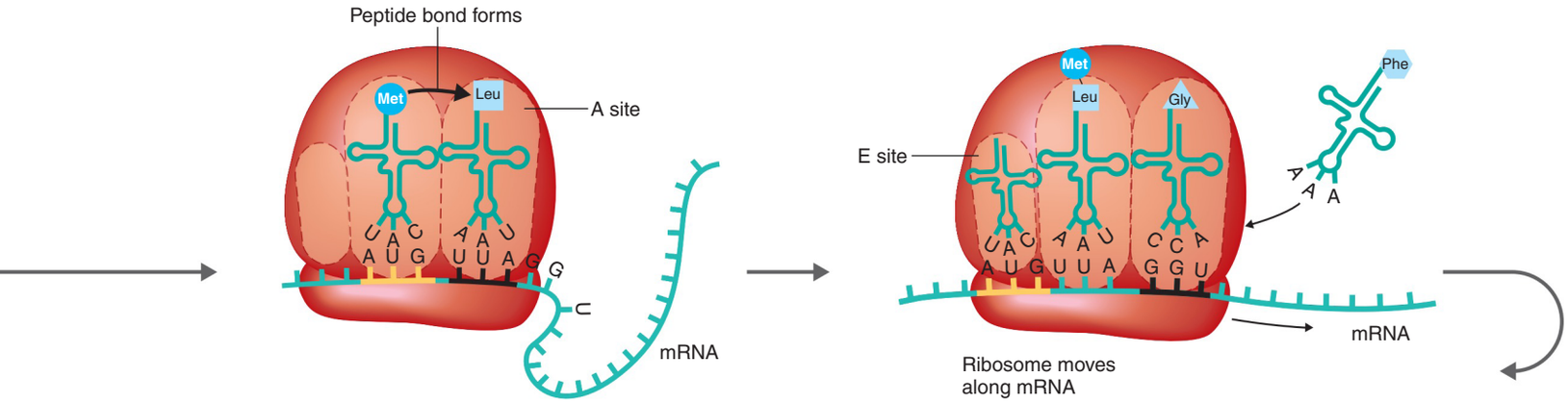
that do not encode protein. In the nucleus, RNA polymerase synthesizes a molecule called an RNA transcript that contains copies of the introns. Particles called **small nuclear ribonucleoproteins,** abbreviated **snRNPs** and pronounced "snurps," remove the introns and splice the exons together. In some organisms, the introns act as ribozymes to catalyze their own removal (Figure 8.11).

* * *

To summarize, genes are the units of biological information encoded by the sequence of nucleotide bases in DNA. A gene is expressed, or turned into a product within the cell, through the processes of transcription and translation. The genetic information carried in DNA is transferred to a temporary mRNA molecule by transcription. Then, during translation, the mRNA directs the assembly of amino acids into a polypeptide chain: a ribosome attaches to mRNA, tRNAs deliver the amino acids to the ribosome as directed by the mRNA codon sequence, and the ribosome assembles the amino acids into the chain that will be the newly synthesized protein. MM **Animations** Translation: Overview, Genetic Code, Process

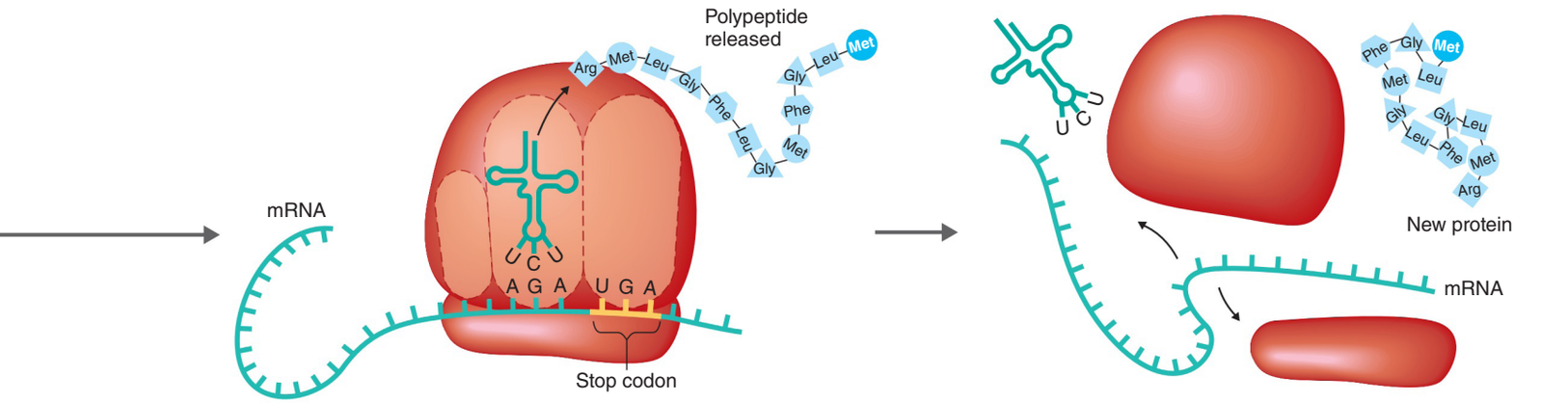
CHECK YOUR UNDERSTANDING

- What is the role of the promoter, terminator, and mRNA in transcription? **8-4**
- How does mRNA production in eukaryotes differ from the process in prokaryotes? **8-5**



3 The second codon of the mRNA pairs with a tRNA carrying the second amino acid at the A site. The first amino acid joins to the second by a peptide bond. This attaches the polypeptide to the tRNA in the P site.

4 The ribosome moves along the mRNA until the second tRNA is in the P site. The next codon to be translated is brought into the A site. The first tRNA now occupies the E site.



7 When the ribosome reaches a stop codon, the polypeptide is released.

8 Finally, the last tRNA is released, and the ribosome comes apart. The released polypeptide forms a new protein.

Figure 8.9 (*continued*)

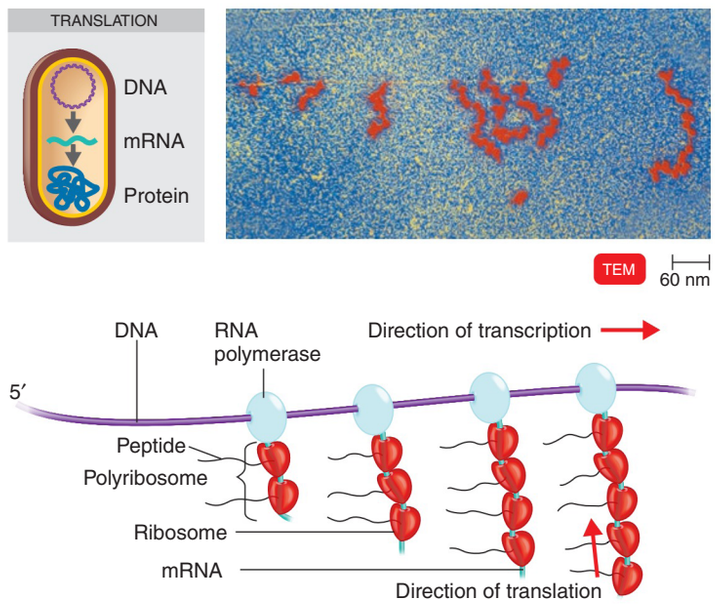


Figure 8.10 Simultaneous transcription and translation in bacteria. Many molecules of mRNA are being synthesized simultaneously. The longest mRNA molecules were the first to be transcribed at the promoter. Note the ribosomes attached to the newly forming mRNA. The micrograph shows a polyribosome (many ribosomes) in a single bacterial gene.

Q Why can translation begin before transcription is complete in prokaryotes but not in eukaryotes?

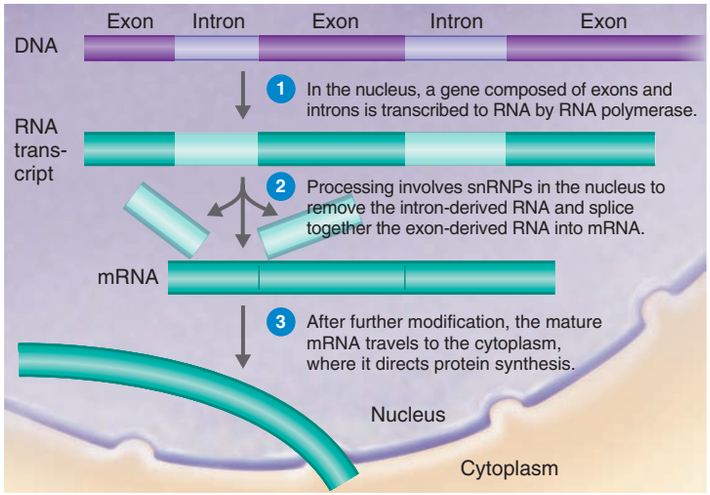


Figure 8.11 RNA processing in eukaryotic cells.

Q Why can't the RNA transcript be used for translation?

The Regulation of Bacterial Gene Expression

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LEARNING OBJECTIVES

- **8-6** Define *operon*.
- **8-7** Explain pre-transcriptional regulation of gene expression in bacteria.
- **8-8** Explain post-transcriptional regulation of gene expression.

A cell's genetic and metabolic machineries are integrated and interdependent. The bacterial cell carries out an enormous number of metabolic reactions (see Chapter 5). The common feature of all metabolic reactions is that they are catalyzed by enzymes that are proteins synthesized via transcription and translation. Feedback inhibition stops a cell from performing unneeded chemical reactions (Chapter 5, page 116) and stops enzymes that have already been synthesized. We will now look at mechanisms to prevent synthesis of enzymes that are not needed.

ASM: The regulation of gene expression is influenced by external and internal molecular cues and/or signals.

Because protein synthesis requires a huge amount of energy, cells save energy by making only those proteins needed at a particular time. The regulation of gene expression is influenced by internal and external molecular cues. Next we look at how chemical reactions are regulated by controlling gene expression.

Many genes, perhaps 60–80%, are not regulated but are instead *constitutive,* meaning that their products are constantly produced at a fixed rate. Usually these genes, which are effectively turned on all the time, code for enzymes that the cell needs in fairly large amounts for its major life processes. Glycolysis enzymes are examples. The production of other enzymes is regulated so that they are present only when needed. *Trypanosoma,* the protozoan parasite that causes African sleeping sickness, has hundreds of genes coding for surface glycoproteins. Each protozoan cell turns on only one glycoprotein gene at a time. As the host's immune system kills parasites with one type of surface molecule, parasites expressing a different surface glycoprotein can continue to grow.

Pre-transcriptional Control

Two genetic control mechanisms known as repression and induction regulate the transcription of mRNA, and consequently, the synthesis of enzymes from them. These mechanisms control the formation and amounts of enzymes in the cell, not the activities of the enzymes.

Repression

The regulatory mechanism that inhibits gene expression and decreases the synthesis of enzymes is called **repression.** Repression is usually a response to the overabundance of an end-product of a metabolic pathway; it causes a decrease in the rate of synthesis of the enzymes leading to the formation of that product. Repression is mediated by regulatory proteins called **repressors,** which block the ability of RNA polymerase to initiate transcription from the repressed genes. The default position of a repressible gene is *on*.

Induction

The process that turns on the transcription of a gene or genes is **induction.** A substance that initiates transcription of a gene is called an **inducer,** and enzymes that are synthesized in the presence of inducers are *inducible enzymes*. The genes required for lactose metabolism in *E. coli* are a well-known example of an inducible system. One of these genes codes for the enzyme β-galactosidase, which splits the substrate lactose into two simple sugars, glucose and galactose. (β refers to the type of linkage that joins the glucose and galactose.) If *E. coli* is placed into a medium in which no lactose is present, the organisms contain almost no β-galactosidase; however, when lactose is added to the medium, the bacterial cells produce a large quantity of the enzyme. Lactose is converted in the cell to the related compound allolactose, which is the inducer for these genes; the presence of lactose thus indirectly induces the cells to synthesize more enzyme. The default position of an inducible gene is *off*.

MM **Animations** Operons: Induction, Repression

The Operon Model of Gene Expression

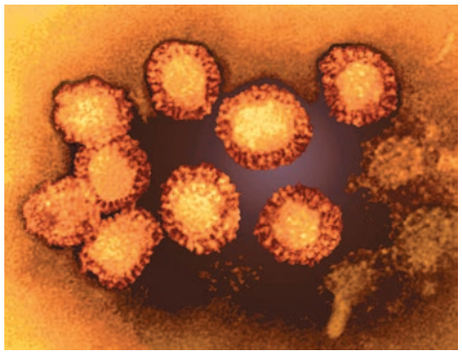
Details of the control of gene expression by induction and repression are described by the operon model formulated in the 1960s by François Jacob and Jacques Monod. The model shows induction of the enzymes of lactose catabolism in *E. coli*. In addition to β-galactosidase, these enzymes include lac permease, which is involved in the transport of lactose into the cell,

CLINICAL FOCUS

Tracking West Nile Virus

In the summer of 1999, the New York City Department of Health identified a cluster of six patients with encephalitis. At the same time, local health officials observed increased fatalities among New York City birds. No bacteria were cultured from the patients' blood or cerebrospinal fluid. Viruses transmitted by mosquitoes are a likely cause of aseptic encephalitis during the summer. Arboviruses, *ar*thropod-*bo*rne, are spread between susceptible vertebrate hosts by blood-feeding arthropods, such as mosquitoes.

Soon after, nucleic acid sequencing of isolates from birds was performed at the Centers for Disease Control and Prevention (CDC). Comparison of the nucleic acid sequences to databases indicated that the viruses were closely related to West Nile virus (WNV), which had never been isolated in the Western Hemisphere.



West Nile virus

By 2007, WNV had been found in birds in all states except Alaska and Hawaii. By 2009, the CDC considered West Nile virus endemic in the United States.

This Old Word flavivirus was first isolated in 1937 in the West Nile district of Uganda. In the early 1950s, scientists recognized WNV encephalitis outbreaks in humans in Egypt and Israel. Initially considered a minor arbovirus, WNV has emerged as a major public health and veterinary concern in southern Europe, the Mediterranean basin, and North America.

Researchers looked at the virus's genome for clues about its path around the world. The flavivirus genome consists of a positive, single-stranded RNA consisting of 10,948 base pairs. (Positive RNA can act as mRNA and be translated.) The virus has acquired several mutations, and researchers are looking for clues in these mutations to determine the virus's journey.

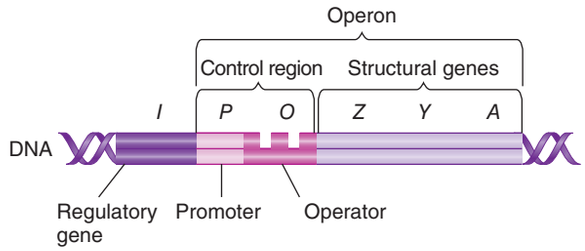
1. Using the portions of the genomes (shown below) that encode viral proteins, can you determine how similar these viruses are? Can you figure out its movement around the world?
   Determine the amino acids encoded, and group the viruses based on percentage of similarity to the Uganda strain.
2. Based on amino acids, there are two groups called clades.
   Can you identify the two groups?
3. The North American and Australian strains have accumulated more mutations, so these should be more recent.
   Calculate the percentage of difference between nucleotides to see how the viruses are related within their clade.
4. Although genetically related groups or clades can be seen, the actual journey of the virus remains elusive.

Source: Adapted from CDC data.

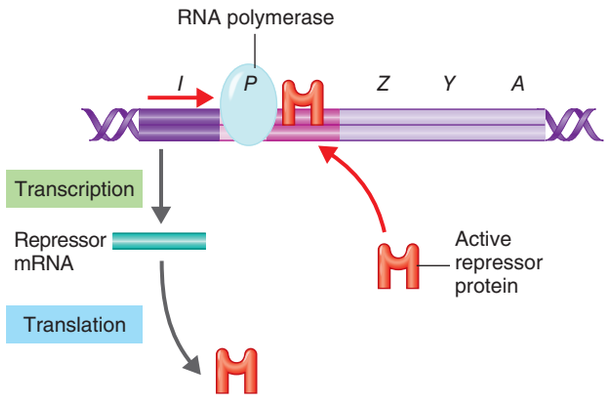
Australia	A	C	C	C	C	G	U	C	C	A	C	C	C	U	U	U	C	A	A	U	U
Egypt	A	A	U	C	G	A	U	C	A	U	C	U	U	C	G	U	C	G	A	U	C
France	A	A	U	C	G	A	U	C	A	U	C	G	U	C	G	U	C	G	A	U	C
Israel	A	U	C	C	A	U	U	C	A	U	C	C	U	C	A	U	C	G	A	U	U
Italy	A	U	C	C	A	C	U	C	A	U	C	C	U	C	G	U	C	G	A	U	U
Kenya	A	U	C	C	A	C	U	C	A	U	C	C	U	C	G	U	C	G	A	U	U
Mexico	A	A	C	C	C	U	U	C	C	U	C	C	C	C	U	U	C	G	A	U	U
United States	A	A	C	C	C	C	U	C	C	U	C	C	C	C	U	U	C	G	A	U	U
Uganda	A	U	A	C	G	A	U	C	A	U	G	C	U	C	G	U	C	C	A	U	C

and transacetylase, which metabolizes certain disaccharides other than lactose.

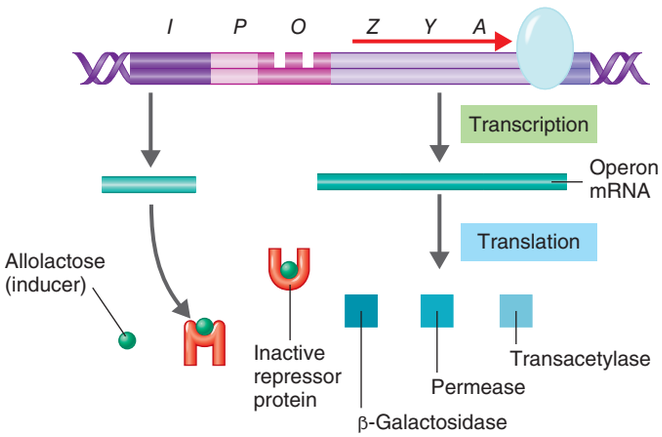
The genes for the three enzymes involved in lactose uptake and utilization are next to each other on the bacterial chromosome and are regulated together (**Figure 8.12**). These genes, which determine the structures of proteins, are called *structural genes* to distinguish them from an adjoining control region on the DNA. When lactose is introduced into the culture medium, the *lac* structural genes are all transcribed and translated rapidly and simultaneously. We will now see how this regulation occurs.



1 Structure of the operon. The operon consists of the promoter (*P*) and operator (*O*) sites and structural genes that code for the protein. The operon is regulated by the product of the regulatory gene (*I*).



2 Repressor active, operon off. The repressor protein binds with the operator, preventing transcription from the operon.



3 Repressor inactive, operon on. When the inducer allolactose binds to the repressor protein, the inactivated repressor can no longer block transcription. The structural genes are transcribed, ultimately resulting in the production of the enzymes needed for lactose catabolism.

Figure 8.12 An inducible operon. Lactose-digesting enzymes are produced in the presence of lactose. In *E. coli,* the genes for the three enzymes are in the *lac* operon. β-galactosidase is encoded by *lacZ*. The *lacY* gene encodes the lac permease, and *lacA* encodes transacetylase, whose function in lactose metabolism is still unclear.

Q What causes transcription of an inducible enzyme?

In the control region of the *lac* operon are two relatively short segments of DNA. One, the promoter, is the segment where RNA polymerase initiates transcription. The other is the **operator,** which is like a traffic light that acts as a go or stop signal for transcription of the structural genes. A set of operator and promoter sites and the structural genes they control define an **operon;** thus, the combination of the three *lac* structural genes and the adjoining control regions is called the *lac* operon.

A regulatory gene called the *I gene* encodes a repressor protein that switches inducible and repressible operons on or off. The *lac* operon is an **inducible operon** (see Figure 8.12). In the absence of lactose, the repressor binds to the operator site, thus preventing transcription. If lactose is present, the repressor binds to a metabolite of lactose instead of to the operator, and lactose-digesting enzymes are transcribed.

In repressible operons, the structural genes are transcribed until they are turned off (**Figure 8.13**). The genes for the enzymes involved in the synthesis of tryptophan are regulated in this manner. The structural genes are transcribed and translated, leading to tryptophan synthesis. When excess tryptophan is present, the tryptophan acts as a **corepressor** binding to the repressor protein. The repressor protein can now bind to the operator, stopping further tryptophan synthesis. **Animation** Operons: Overview

CHECK YOUR UNDERSTANDING

Use the following metabolic pathway to answer the questions that follow it. **8-6**

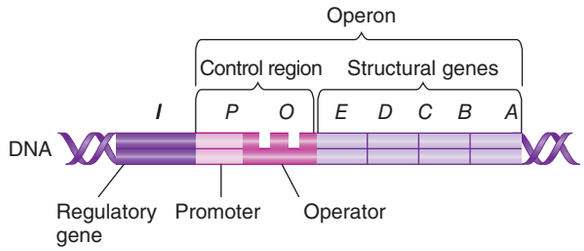
Substrate *A* —*enzyme a*→ Intermediate *B* —*enzyme b*→ End-product *C*

a. If enzyme *a* is inducible and is not being synthesized at present, a (1) _____ protein must be bound tightly to the (2) _____ site. When the inducer is present, it will bind to the (3) _____ so that (4) _____ can occur.

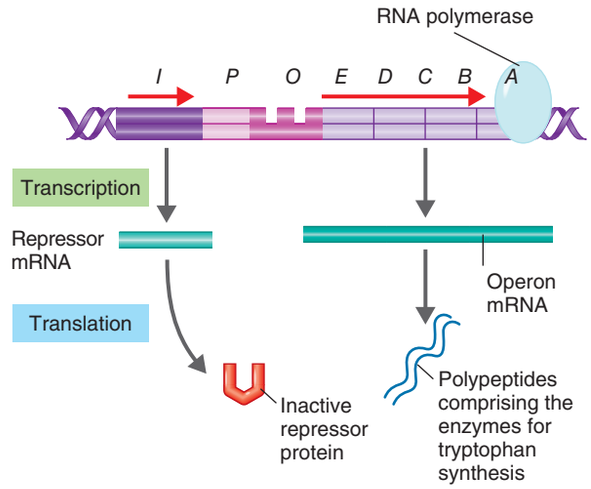
b. If enzyme *a* is repressible, end-product *C*, called a (1) _____, causes the (2) _____ to bind to the (3) _____. What causes derepression?

Positive Regulation

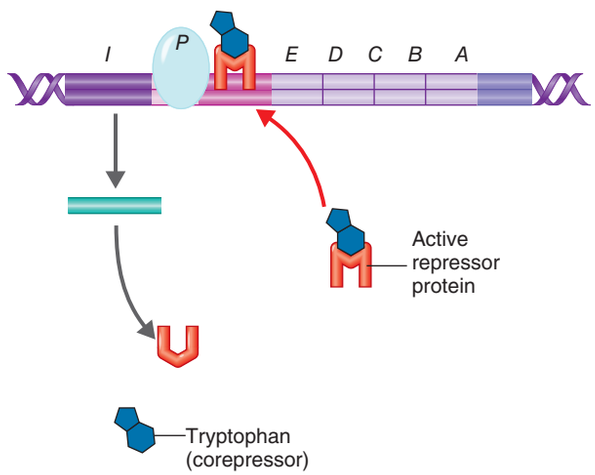
Regulation of the lactose operon also depends on the level of glucose in the medium, which in turn controls the intracellular level of the small molecule **cyclic AMP (cAMP),** a substance derived from ATP that serves as a cellular alarm signal. Enzymes that metabolize glucose are constitutive, and cells grow at their maximal rate with glucose as their carbon source because they can use it most efficiently (**Figure 8.14**). When glucose is no longer available, cAMP accumulates in the cell. The cAMP binds to the allosteric site of *catabolic activator protein (CAP)*. CAP then binds to the *lac* promoter, which initiates transcription by making it easier for RNA polymerase to bind to the promoter. Thus transcription of the *lac* operon requires both the presence of lactose and the absence of glucose (**Figure 8.15**).



1 Structure of the operon. The operon consists of the promoter (*P*) and operator (*O*) sites and structural genes that code for the protein. The operon is regulated by the product of the regulatory gene (*I*).



2 Repressor inactive, operon on. The repressor is inactive, and transcription and translation proceed, leading to the synthesis of tryptophan.



3 Repressor active, operon off. When the corepressor tryptophan binds to the repressor protein, the activated repressor binds with the operator, preventing transcription from the operon.

Figure 8.13 A repressible operon. Tryptophan, an amino acid, is produced by anabolic enzymes encoded by five structural genes. Accumulation of tryptophan represses transcription of these genes, preventing further synthesis of tryptophan. The *E. coli trp* operon is shown here.

Q What causes transcription of a repressible enzyme?

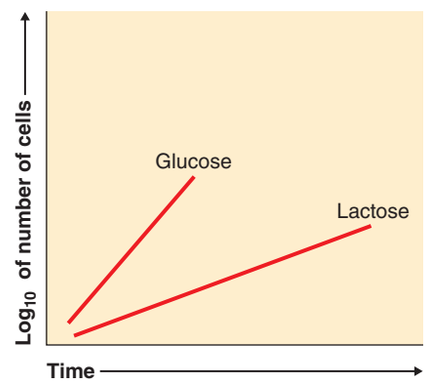
Cyclic AMP is an example of an *alarmone*, a chemical alarm signal that promotes a cell's response to environmental or nutritional stress. (In this case, the stress is the lack of glucose.) The same mechanism involving cAMP allows the cell to use other sugars. Inhibition of the metabolism of alternative carbon sources by glucose is termed **catabolite repression** (or the *glucose effect*). When glucose is available, the level of cAMP in the cell is low, and consequently CAP is not bound.

Epigenetic Control

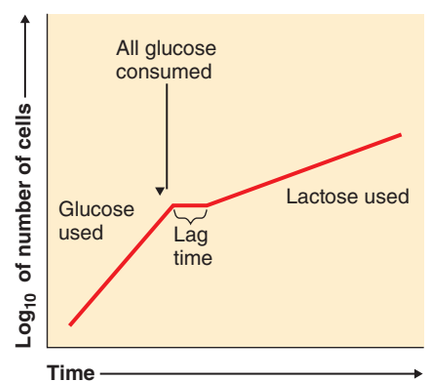
Eukaryotic and bacterial cells can turn genes off by methylating certain nucleotides—that is, by adding a methyl group ($-CH_3$). The methylated (off) genes are passed to offspring cells. Unlike mutations, this isn't permanent, and the genes can be turned on in a later generation. This is called *epigenetic inheritance* (*epigenetic* = on genes). Epigenetics may explain why bacteria behave differently in a biofilm (see the box on page 54).

Post-transcriptional Control

Some regulatory mechanisms stop protein synthesis after transcription has occurred. Single-stranded RNA molecules of approximately 22 nucleotides, called **microRNAs (miRNAs),** inhibit protein production in eukaryotic cells. In humans,



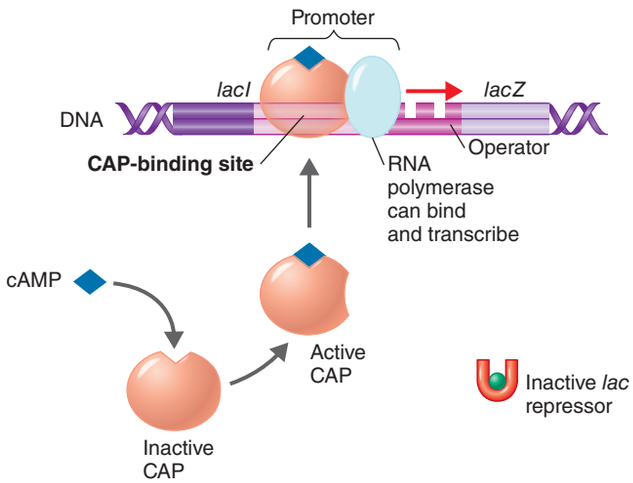
(a) Bacteria growing on glucose as the sole carbon source grow faster than on lactose.



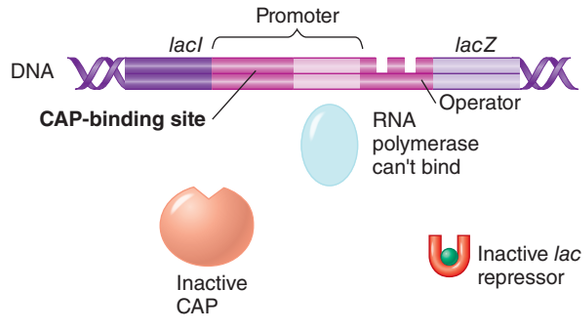
(b) Bacteria growing in a medium containing glucose and lactose first consume the glucose and then, after a short lag time, the lactose. During the lag time, intracellular cAMP increases, the *lac* operon is transcribed, more lactose is transported into the cell, and β-galactosidase is synthesized to break down lactose.

Figure 8.14 The growth rate of *E. coli* on glucose and lactose.

Q When both glucose and lactose are present, why will cells use glucose first?



(a) Lactose present, glucose scarce (cAMP level high). If glucose is scarce, the high level of cAMP activates CAP, and the *lac* operon produces large amounts of mRNA for lactose digestion.



(b) Lactose present, glucose present (cAMP level low). When glucose is present, cAMP is scarce, and CAP is unable to stimulate transcription.

Figure 8.15 Positive regulation of the *lac* operon.

Q Will transcription of the *lac* operon occur in the presence of lactose and glucose? In the presence of lactose and the absence of glucose? In the presence of glucose and the absence of lactose?

miRNAs produced during development allow different cells to produce different proteins. Heart cells and skin cells have the same genes, but the cells in each organ produce different proteins because of miRNAs produced in each cell type during development. Similar short RNAs in bacteria enable the cell to cope with environmental stresses, such as low temperature or oxidative damage. An miRNA base-pairs with a complementary mRNA, forming a double-stranded RNA. This double-stranded RNA is enzymatically destroyed so that the mRNA-encoded protein is not made (**Figure 8.16**). The action of another type of RNA, siRNA, is similar and is discussed on page 251.

CHECK YOUR UNDERSTANDING

- What is the role of cAMP in regulating gene expression? **8-7**
- How does miRNA stop protein synthesis? **8-8**

Changes in Genetic Material

LEARNING OBJECTIVES

- **8-9** Classify mutations by type.
- **8-10** Describe two ways mutations can be repaired.
- **8-11** Describe the effect of mutagens on the mutation rate.
- **8-12** Outline the methods of direct and indirect selection of mutants.
- **8-13** Identify the purpose of and outline the procedure for the Ames test.

A cell's DNA can be changed by mutations and horizontal gene transfer. Changes in DNA result in genetic variations that can impact microbial function (e.g., biofilm formation, pathogenicity, and antibiotic resistance). Survival and reproduction of the bacteria with a new genotype can be favored by natural and human-influenced environments and result in a huge diversity of microorganisms. The survival of new genotypes is called **natural selection.**

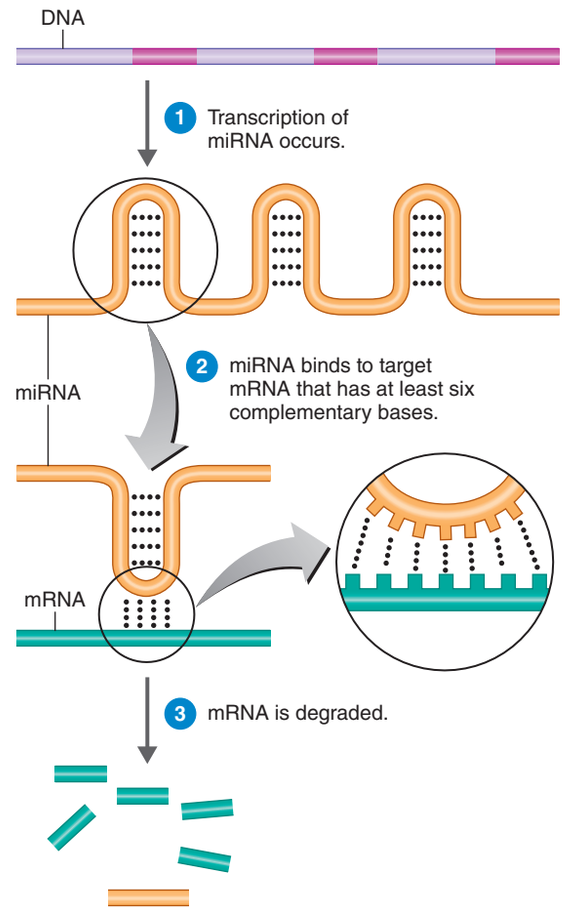


Figure 8.16 MicroRNAs control a wide range of activities in cells.

Q In mammals, some miRNAs hybridize with viral RNA. What would happen if a mutation occurred in the miRNA gene?

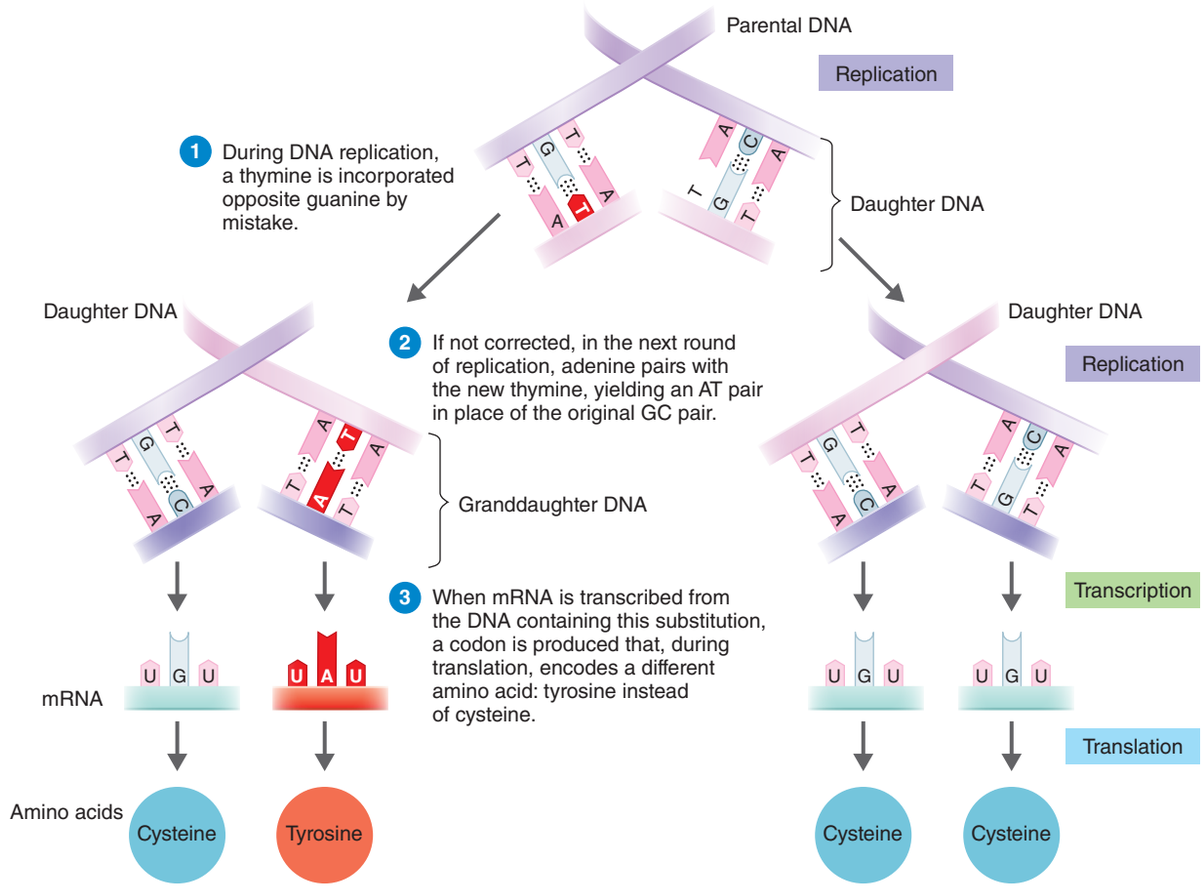


Figure 8.17 Base substitutions. This mutation leads to an altered protein in a grandchild cell.

Q Does a base substitution always result in a different amino acid?

Mutation

A **mutation** is a permanent change in the base sequence of DNA. Such a change will sometimes cause a change in the product encoded by that gene. For example, when the gene for an enzyme mutates, the enzyme encoded by the gene may become inactive or less active because its amino acid sequence has changed. Such a change in genotype may be disadvantageous, or even lethal, if the cell loses a phenotypic trait it needs. However, a mutation can be beneficial if, for instance, the altered enzyme encoded by the mutant gene has a new or enhanced activity that benefits the cell.

Types of Mutations

Many simple mutations are silent (neutral); the change in DNA base sequence causes no change in the activity of the product encoded by the gene. Silent mutations commonly occur when one nucleotide is substituted for another in the DNA, especially at a location corresponding to the third position of the mRNA codon. Because of the degeneracy of the genetic code, the resulting new codon might still code for the same amino acid. Even if the amino acid is changed, the function of the protein may not change if the amino acid is in a nonvital portion of the protein, or is chemically very similar to the original amino acid.

The most common type of mutation involving single base pairs is **base substitution** (or *point mutation*), in which a single base at one point in the DNA sequence is replaced with a different base. When the DNA replicates, the result is a substituted base pair (Figure 8.17). For example, AT might be substituted for GC, or CG for GC. If a base substitution occurs within a gene that codes for a protein, the mRNA transcribed from the gene will carry an incorrect base at that position. When the mRNA is translated into protein, the incorrect base may cause the insertion of an incorrect amino acid in the protein. If the base substitution results in an amino acid substitution in the synthesized protein, this change in the DNA is known as a **missense mutation** (Figure 8.18a and Figure 8.18b).

The effects of such mutations can be dramatic. For example, sickle cell disease is caused by a single change in the gene for globin, the protein component of hemoglobin. Hemoglobin is primarily responsible for transporting oxygen from the lungs to the tissues. A single change from an A to a T at a specific site results in the change from glutamic acid to valine in the protein. This causes the shape of the hemoglobin molecule to change under conditions of low oxygen, which, in turn, alters the shape of the red blood cells. Misshapen red blood cells don't move through capillaries well and can restrict blood flow, causing organ damage.

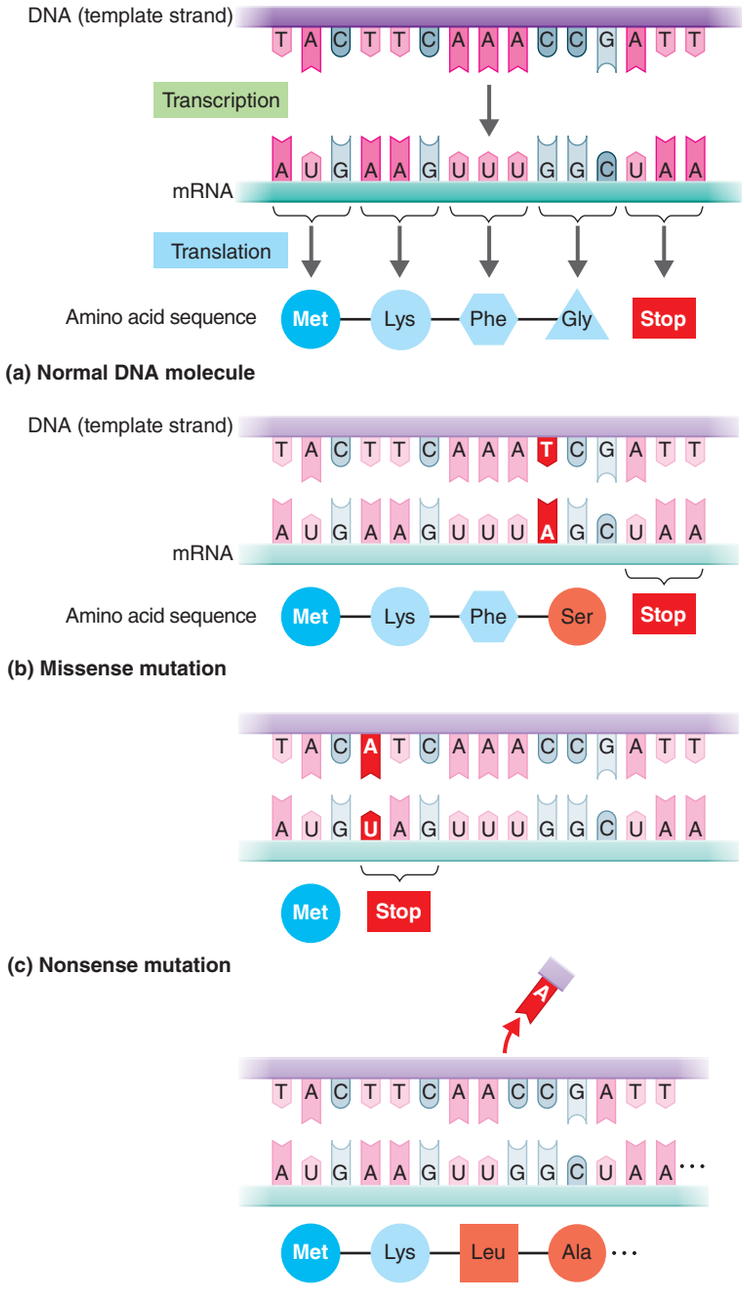


Figure 8.18 Types of mutations and their effects on the amino acid sequences of proteins.

Q What happens if base 9 in (a) is changed to a C?

By creating a nonsense (stop) codon in the middle of an mRNA molecule, some base substitutions effectively prevent the synthesis of a complete functional protein; only a fragment is synthesized. A base substitution resulting in a nonsense codon is thus called a **nonsense mutation** (Figure 8.18c).

Besides base-pair mutations, there are also changes in DNA called **frameshift mutations,** in which one or a few nucleotide pairs are deleted or inserted in the DNA (Figure 8.18d). This mutation can shift the "translational reading frame"—that is, the three-by-three grouping of nucleotides recognized as codons by the tRNAs during translation. For example, deleting one nucleotide pair in the middle of a gene causes changes in many amino acids downstream from the site of the original mutation. Frameshift mutations almost always result in a long stretch of altered amino acids and the production of an inactive protein from the mutated gene. In most cases, a nonsense codon will eventually be encountered and thereby terminate translation.

Occasionally, mutations occur where significant numbers of bases are added to (inserted into) a gene. Huntington's disease, for example, is a progressive neurological disorder caused by extra bases inserted into a particular gene.

Base substitutions and frameshift mutations may occur spontaneously because of occasional mistakes made during DNA replication. These **spontaneous mutations** apparently occur in the absence of any mutation-causing agents.

CHECK YOUR UNDERSTANDING

How can a mutation be beneficial? **8-9**

Mutagens

Chemical Mutagens

Agents in the environment, such as certain chemicals and radiation, that directly or indirectly bring about mutations are called **mutagens.** In the microbial world, certain mutations result in resistance to antibiotics (see the box in Chapter 26, page 756).

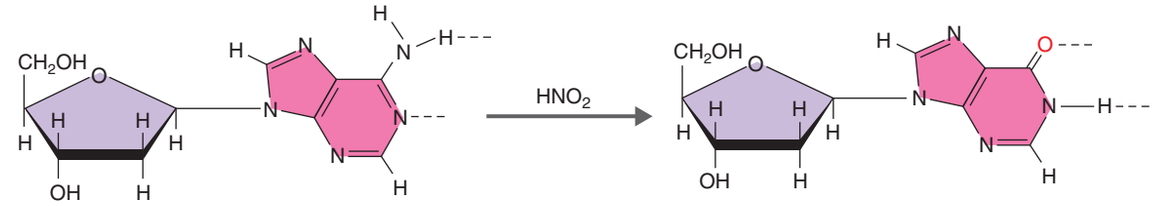
One of the many chemicals known to be a mutagen is nitrous acid. Figure 8.19 shows how exposing DNA to nitrous acid can convert the base adenine to a form that pairs with

Clinical Case

A person's DNA can undergo mutations. One improper nucleotide in DNA creates a mutation, which could alter the function of the gene. Cancer is abnormal cell growth caused by mutations. These mutations can be inherited.

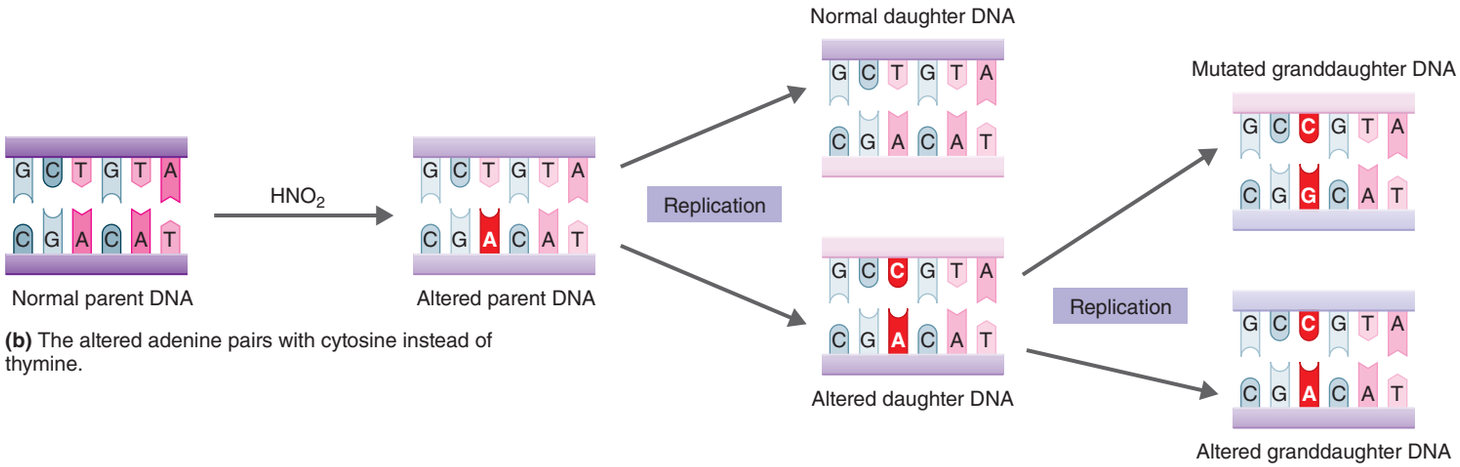
As Marcel and his wife, Janice, drive home from the doctor's office, they review Marcel's family history. Marcel's brother, Robert, passed away from colon cancer 10 years ago, but Marcel has always been the picture of health. Even at 70, he hasn't given a thought to retiring from his Memphis barbeque restaurant that he once co-owned with his brother until Robert's death.

What factors may have contributed to Marcel's colon cancer?



(a) Adenosine nucleoside normally base-pairs by hydrogen bonds with an oxygen and a hydrogen of a thymine or uracil nucleotide.

Altered adenine will hydrogen bond with a hydrogen and a nitrogen of a cytosine nucleotide.



(b) The altered adenine pairs with cytosine instead of thymine.

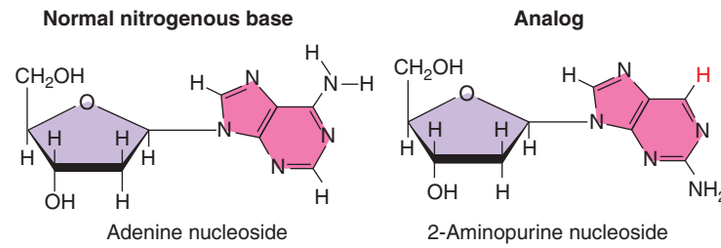
Figure 8.19 Oxidation of nucleotides makes a mutagen. The nitrous acid emitted into the air by burning fossil fuels oxidizes adenine.

Q What is a mutagen?

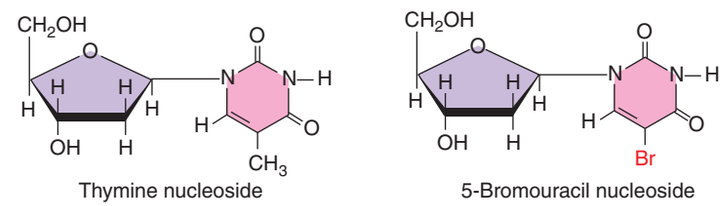
cytosine instead of the usual thymine. When DNA containing such modified adenines replicates, one daughter DNA molecule will have a base-pair sequence different from that of the parent DNA. Eventually, some AT base pairs of the parent will have been changed to GC base pairs in a granddaughter cell. Nitrous acid makes a specific base-pair change in DNA. Like all mutagens, it alters DNA at random locations.

Another type of chemical mutagen is the **nucleoside analog.** These molecules are structurally similar to normal nitrogenous bases, but they have slightly altered base-pairing properties. Examples, 2-aminopurine and 5-bromouracil, are shown in **Figure 8.20**. When nucleoside analogs are given to growing cells, the analogs are randomly incorporated into cellular DNA in place of the normal bases. Then, during DNA replication, the analogs cause mistakes in base pairing. The incorrectly paired bases will be copied during subsequent replication of the DNA, resulting in base-pair substitutions in the progeny cells. Some antiviral and antitumor drugs are nucleoside analogs, including AZT (azidothymidine), one of the primary drugs used to treat HIV infection.

Still other chemical mutagens cause small deletions or insertions, which can result in frameshifts. For instance, under certain conditions, benzopyrene, which is present in smoke and



(a) The 2-aminopurine is incorporated into DNA in place of adenine but can pair with cytosine, so an AT pair becomes a CG pair.



(b) The 5-bromouracil is used as an anticancer drug because it is mistaken for thymine by cellular enzymes but pairs with cytosine. In the next DNA replication, an AT pair becomes a GC pair.

Figure 8.20 Nucleoside analogs and the nitrogenous bases they replace. A nucleoside is phosphorylated, and the resulting nucleotide used to synthesize DNA.

Q Why do these drugs kill cells?

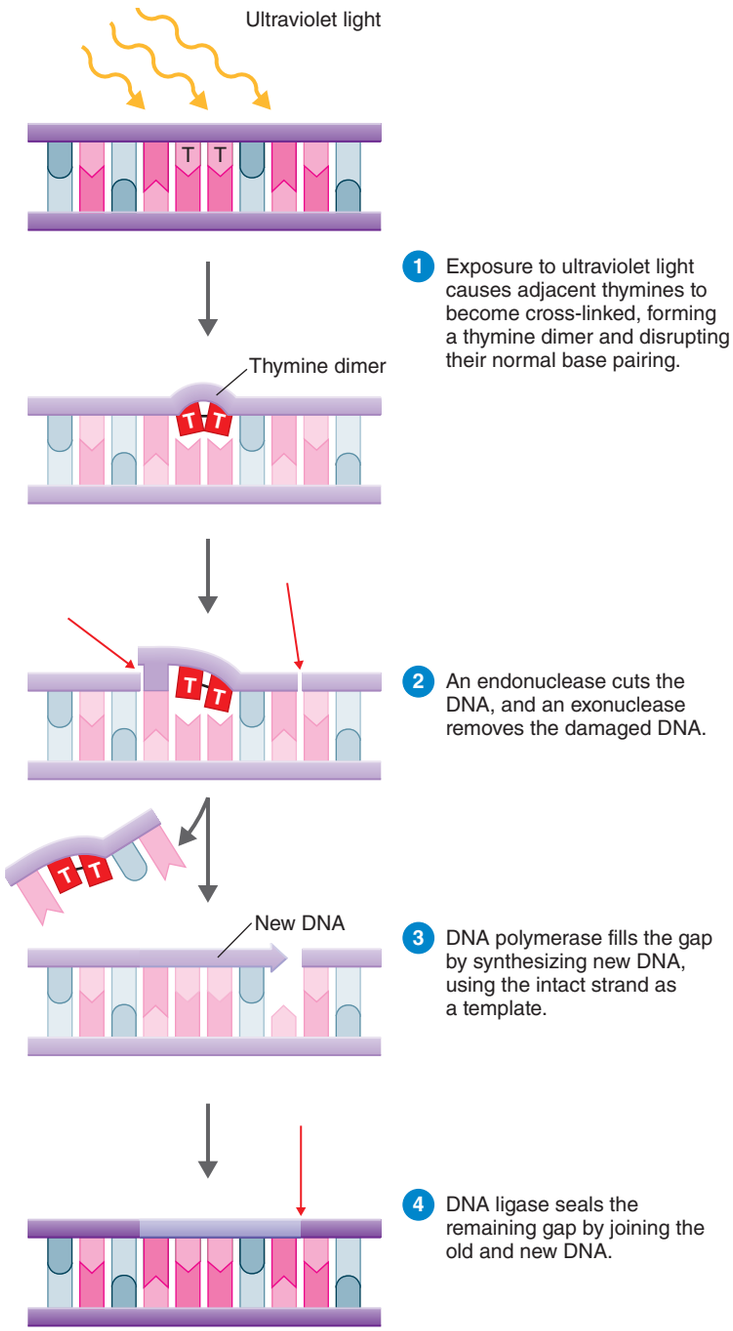


Figure 8.21 The creation and repair of a thymine dimer caused by ultraviolet light. After exposure to UV light, adjacent thymines can become cross-linked, forming a thymine dimer. In the absence of visible light, the nucleotide excision repair mechanism is used in a cell to repair the damage.

Q How do excision repair enzymes "know" which strand is incorrect?

soot, is an effective *frameshift mutagen*. Aflatoxin—produced by *Aspergillus flavus* (a-sper-JIL-lus FLĀ-vus), a mold that grows on peanuts and grain—is a frameshift mutagen, as are the acridine dyes used experimentally against herpesvirus infections. Frameshift mutagens usually have the right size and chemical properties to slip between the stacked base pairs of the DNA double helix. They may work by slightly offsetting the two strands of DNA, leaving a gap or bulge in one strand or the other. When the staggered DNA strands are copied during DNA synthesis, one or more base pairs can be inserted or deleted in the new double-stranded DNA. Interestingly, frameshift mutagens are often potent carcinogens.

Radiation

X rays and gamma rays are forms of radiation that are potent mutagens because of their ability to ionize atoms and molecules. The penetrating rays of ionizing radiation cause electrons to pop out of their usual shells (see Chapter 2). These electrons bombard other molecules and cause more damage, and many of the resulting ions and free radicals (molecular fragments with unpaired electrons) are very reactive. Some of these ions oxidize bases in DNA, resulting in errors in DNA replication and repair that produce mutations (see Figure 8.19). An even more serious outcome is the breakage of covalent bonds in the sugar-phosphate backbone of DNA, which causes physical breaks in chromosomes.

Another form of mutagenic radiation is ultraviolet (UV) light, a nonionizing component of ordinary sunlight. However, the most mutagenic component of UV light (wavelength 260 nm) is screened out by the ozone layer of the atmosphere. The most important effect of direct UV light on DNA is the formation of harmful covalent bonds between pyrimidine bases. Adjacent thymines in a DNA strand can cross-link to form thymine dimers. Such dimers, unless repaired, may cause serious damage or death to the cell because it cannot properly transcribe or replicate such DNA.

Bacteria and other organisms have enzymes that can repair UV-induced damage. **Photolyases,** also known as *light-repair enzymes,* use visible light energy to separate the dimer back to the original two thymines. **Nucleotide excision repair,** shown in **Figure 8.21**, is not restricted to UV-induced damage; it can repair mutations from other causes as well. Enzymes cut out the incorrect base and fill in the gap with newly synthesized DNA that is complementary to the correct strand. For many years biologists questioned how the incorrect base could be distinguished from the correct base if it was not physically distorted like a thymine dimer. In 1970, Hamilton Smith provided the answer with the discovery of **methylases.** These enzymes add a methyl group to selected bases soon after a DNA strand is made. A repair endonuclease then cuts the nonmethylated strand.

Exposure to UV light in humans, such as by excessive suntanning, causes a large number of thymine dimers in skin cells. Unrepaired dimers may result in skin cancers. Humans with xeroderma pigmentosum, an inherited condition that results in increased sensitivity to UV light, have a defect in nucleotide excision repair; consequently, they have an increased risk of skin cancer.

The Frequency of Mutation

The **mutation rate** is the probability that a gene will mutate when a cell divides. The rate is usually stated as a power of 10, and because mutations are very rare, the exponent is always a negative number. For example, if there is one chance in a million that a gene will mutate when the cell divides, the mutation rate is 1/1,000,000, which is expressed as 10^{-6}. Spontaneous mistakes in DNA replication occur at a very low rate, perhaps only once in 10^9 replicated base pairs (a mutation rate of one in a billion). Because the average gene has about 10^3 base pairs, the spontaneous rate of mutation is about one in 10^6 (a million) replicated genes.

Mutations usually occur more or less randomly along a chromosome. The occurrence of random mutations at low frequency is an essential aspect of the adaptation of species to their environment, for evolution requires that genetic diversity be generated randomly and at a low rate. For example, in a bacterial population of significant size—say, greater than 10^7 cells—a few new mutant cells will always be produced in every generation. Most mutations either are harmful and likely to be removed from the gene pool when the individual cell dies or are neutral. However, a few mutations may be beneficial. For example, a mutation that confers antibiotic resistance is beneficial to a population of bacteria that is regularly exposed to antibiotics. Once such a trait has appeared through mutation, cells carrying the mutated gene are more likely than other cells to survive and reproduce as long as the environment stays the same. Soon most of the cells in the population will have the gene; an evolutionary change will have occurred, although on a small scale.

A mutagen usually increases the spontaneous rate of mutation, which is about one in 10^6 replicated genes, by a factor of 10 to 1000 times. In other words, in the presence of a mutagen, the normal rate of 10^{-6} mutations per replicated gene becomes a rate of 10^{-5} to 10^{-3} per replicated gene. Mutagens are used experimentally to enhance the production of mutant cells for research on the genetic properties of microorganisms and for commercial purposes. MM **Animations** Mutations: Types, Repair

CHECK YOUR UNDERSTANDING

- How can mutations be repaired? **8-10**
- How do mutagens affect the mutation rate? **8-11**

Identifying Mutants

Mutants can be detected by selecting or testing for an altered phenotype. Whether or not a mutagen is used, mutant cells with specific mutations are always rare compared with other cells in the population. The problem is detecting such a rare event.

Experiments are usually performed with bacteria because they reproduce rapidly, so large numbers of organisms (more than 10^9 per milliliter of nutrient broth) can easily be used. Furthermore, because bacteria generally have only one copy of each gene per cell, the effects of a mutated gene are not masked by the presence of a normal version of the gene, as in many eukaryotic organisms.

Positive (direct) selection involves the detection of mutant cells by rejection of the unmutated parent cells. For example, suppose we were trying to find mutant bacteria that are resistant to penicillin. When the bacterial cells are plated on a medium containing penicillin, the mutant can be identified directly. The few cells in the population that are resistant (mutants) will grow and form colonies, whereas the normal, penicillin-sensitive parental cells cannot grow.

To identify mutations in other kinds of genes, **negative (indirect) selection** can be used. This process selects a cell that cannot perform a certain function, using the technique of **replica plating.** For example, suppose we wanted to use replica plating to identify a bacterial cell that has lost the ability to synthesize the amino acid histidine (**Figure 8.22**). First, about 100 bacterial cells are inoculated onto an agar plate. This plate, called the master plate, contains a medium with histidine on which all cells will grow. After 18 to 24 hours of incubation, each cell reproduces to form a colony. Then a pad of sterile material, such as latex, filter paper, or velvet, is pressed over the master plate, and some of the cells from each colony adhere to the velvet. Next, the velvet is pressed down onto two (or more) sterile plates. One plate contains a medium without histidine, and one contains a medium with histidine on which the original, nonmutant bacteria can grow. Any colony that grows on the medium with histidine on the master plate but that cannot synthesize its own histidine will not be able to grow on the medium without histidine. The mutant colony can then be identified on the master plate. Of course, because mutants are so rare (even those induced by mutagens), many plates must be screened with this technique to isolate a specific mutant.

Replica plating is a very effective means of isolating mutants that require one or more new growth factors. Any mutant microorganism having a nutritional requirement that is absent in the parent is known as an **auxotroph.** For example, an auxotroph may lack an enzyme needed to synthesize a particular amino acid and will therefore require that amino acid as a growth factor in its nutrient medium.

Identifying Chemical Carcinogens

Many known mutagens have been found to be **carcinogens,** substances that cause cancer in animals, including humans. In recent years, chemicals in the environment, the workplace, and the diet have been implicated as causes of cancer in humans. Animal testing procedures are time-consuming and expensive, so some faster and less expensive procedures for preliminary screening of potential carcinogens that do not use animals have been developed. One of these, called the **Ames test,** uses bacteria as carcinogen indicators.

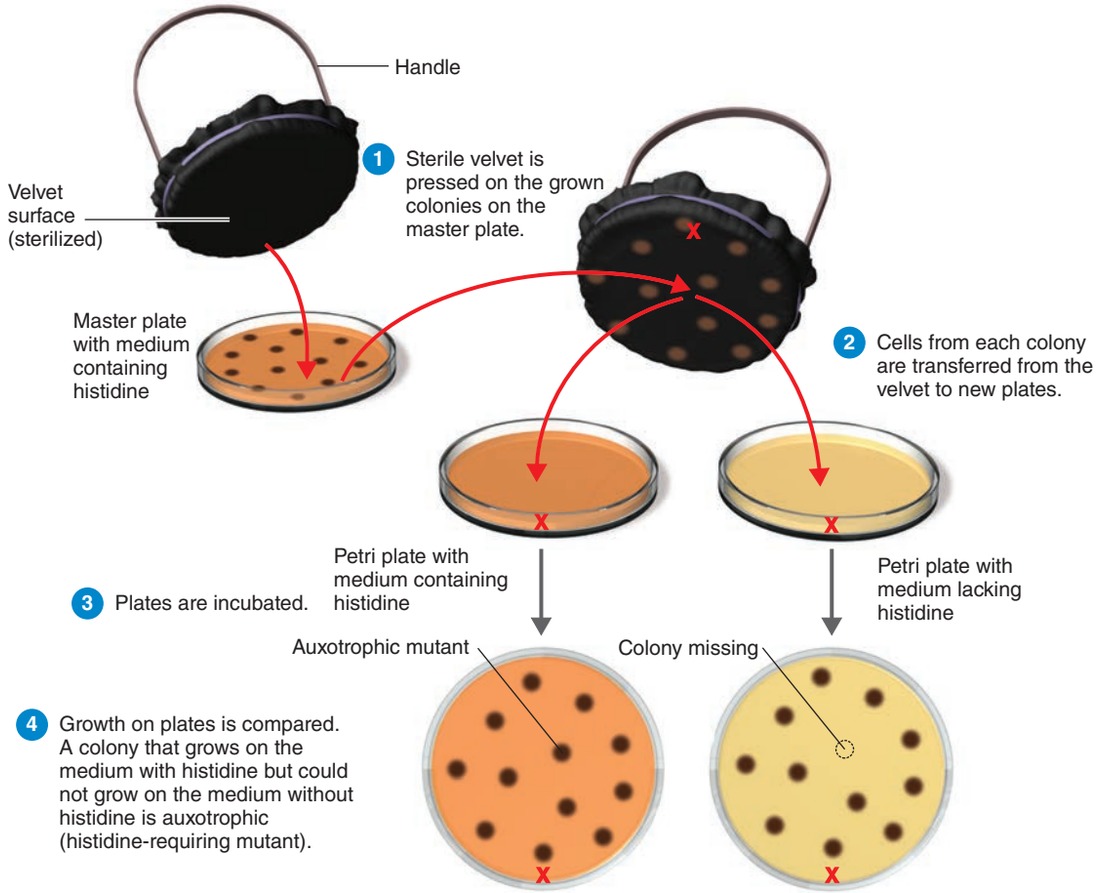


Figure 8.22 Replica plating. In this example, the auxotrophic mutant cannot synthesize histidine. The plates must be carefully marked (with an X here) to maintain orientation so that colony positions are known in relation to the original master plate.

Q What is an auxotroph?

The Ames test is based on the observation that exposure of mutant bacteria to mutagenic substances may cause new mutations that reverse the effect (the change in phenotype) of the original mutation. These are called *reversions*. Specifically, the test measures the reversion of histidine auxotrophs of *Salmonella* (so-called his^- cells, mutants that have lost the ability to synthesize histidine) to histidine-synthesizing cells (his^+) after treatment with a mutagen (**Figure 8.23**). Bacteria are incubated in both the presence and absence of the substance being tested. Because animal enzymes must activate many chemicals into forms that are chemically reactive for mutagenic or carcinogenic activity to appear, the chemical to be tested and the mutant bacteria are incubated together with rat liver extract, a rich source of activation enzymes. If the substance being tested is mutagenic, it will cause the reversion of his^- bacteria to his^+ bacteria at a rate higher than the spontaneous reversion rate. The number of observed revertants indicates the degree to which a substance is mutagenic and therefore possibly carcinogenic.

The test can be used in many ways. Several potential mutagens can be qualitatively tested by spotting the individual chemicals on small paper disks on a single plate inoculated with bacteria.

Clinical Case

Not all mutations are inherited; some are induced by genotoxins, that is, chemicals that damage a cell's genetic material. Marcel is not overweight, makes sure he spends time with his family, and has never smoked. Researchers have known since the 1970s that people who consume cooked meat and meat products are more likely to develop colon cancer. The suspect cancer-causing chemicals are aromatic amines that form during high-heat cooking.

Marcel has owned his Memphis barbeque restaurant for over 50 years. He is a hands-on type of employer and is always in the kitchen overseeing the cooking process. All of his barbequed meat is seared over high heat and then slow-cooked for hours. Marcel is considered the expert in this technique, but now it seems as if his profession could be a factor in his disease.

What test can be used to determine whether a chemical is genotoxic?

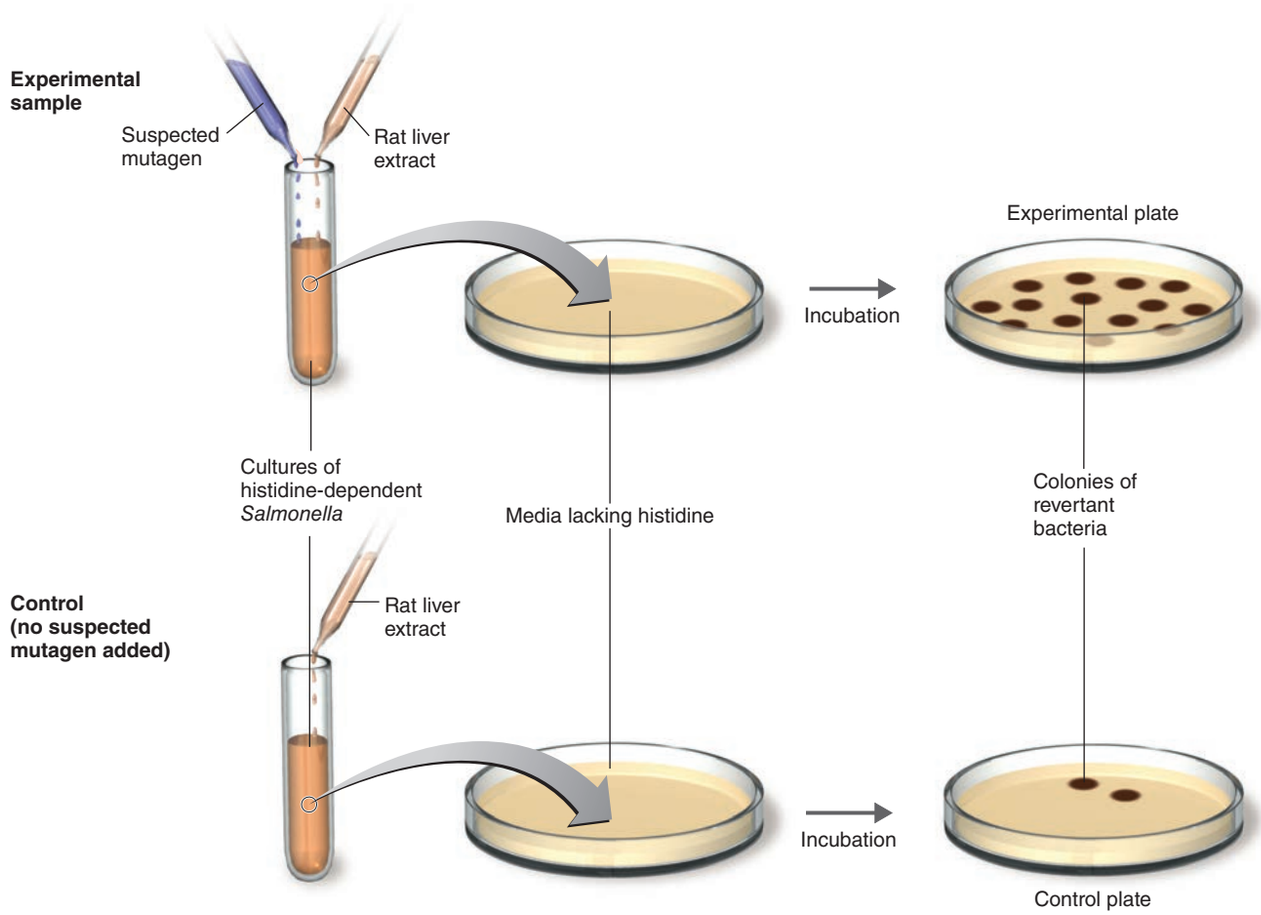


Figure 8.23 The Ames reverse gene mutation test.

Q Do all mutagens cause cancer?

The Ames test is routinely used to evaluate new chemicals and air and water pollutants.

About 90% of the substances found by the Ames test to be mutagenic have also been shown to be carcinogenic in animals. By the same token, the more mutagenic substances have generally been found to be more carcinogenic.

CHECK YOUR UNDERSTANDING

- How would you isolate an antibiotic-resistant bacterium? An antibiotic-sensitive bacterium? **8-12**
- What is the principle behind the Ames test? **8-13**

Genetic Transfer and Recombination

LEARNING OBJECTIVES

8-14 Differentiate horizontal and vertical gene transfer.

8-15 Compare the mechanisms of genetic recombination in bacteria.

8-16 Describe the functions of plasmids and transposons.

Genetic recombination refers to the exchange of genes between two DNA molecules to form new combinations of genes on a chromosome. **Figure 8.24** shows one mechanism for genetic recombination. If a cell picks up foreign DNA (called donor

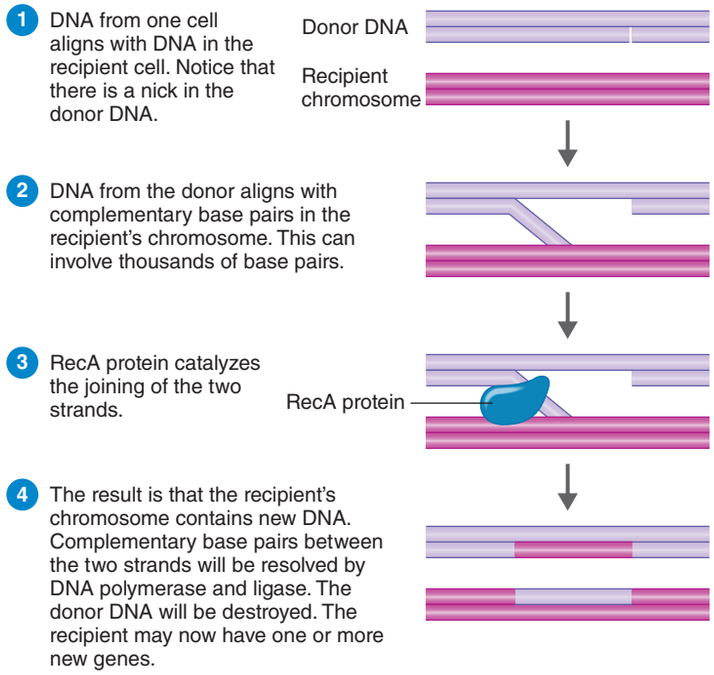


Figure 8.24 Genetic recombination by crossing over. Foreign DNA can be inserted into a chromosome by breaking and rejoining the chromosome. This can insert one or more new genes into the chromosome. A photograph of RecA protein is shown in Figure 3.11a, page 61.

What type of enzyme breaks the DNA?

DNA in the figure), some of it could insert into the cell's chromosome—a process called **crossing over**—and some of the genes carried by the chromosomes are shuffled. The DNA has recombined, so that the chromosome now carries a portion of the donor's DNA.

If A and B represent DNA from different individuals, how are they brought close enough together to recombine? In eukaryotes, genetic recombination is an ordered process that usually occurs as part of the sexual cycle of the organism. Crossing over generally takes place during the formation of reproductive cells, such that these cells contain recombinant DNA. In bacteria, genetic recombination can happen in a number of ways, which we will discuss in the following sections.

Like mutation, genetic recombination contributes to a population's genetic diversity, which is the source of variation in evolution. In highly evolved organisms such as present-day microbes, recombination is more likely to be beneficial than mutation because recombination will less likely destroy a gene's function and may bring together combinations of genes that enable the organism to carry out a valuable new function.

ASM: Genetic variations can impact microbial functions (e.g., in biofilm formation, pathogenicity, and drug resistance).

The major protein that constitutes the flagella of *Salmonella* is also one of the primary proteins that causes our immune systems to respond. However, these bacteria have the capability of producing two different flagellar proteins. As our immune system mounts a response against those cells containing one form of the flagellar protein, those organisms producing the second are not affected. Which flagellar protein is produced is determined by a recombination event that apparently occurs somewhat randomly within the chromosomal DNA. Thus, by altering the flagellar protein produced, *Salmonella* can better avoid the defenses of the host.

Vertical gene transfer occurs when genes are passed from an organism to its offspring. Plants and animals transmit their genes by vertical transmission. Bacteria can pass their genes not only to their offspring, but also laterally, to other microbes of the same generation. This is known as **horizontal gene transfer** (see Figure 8.2). Horizontal gene transfer between bacteria occurs in several ways. In all of the mechanisms, the transfer involves a **donor cell** that gives a portion of its total DNA to a **recipient cell.** Once transferred, part of the donor's DNA is usually incorporated into the recipient's DNA; the remainder is degraded by cellular enzymes. The recipient cell that incorporates donor DNA into its own DNA is called a *recombinant*. The transfer of genetic material between bacteria is by no means a frequent event; it may occur in only 1% or less of an entire population. Let's examine in detail the specific types of genetic transfer. **Animation** Horizontal Gene Transfer: Overview

Transformation in Bacteria

During the process of **transformation,** genes are transferred from one bacterium to another as "naked" DNA in solution. This process was first demonstrated over 70 years ago, although it was not understood at the time. Not only did transformation show that genetic material could be transferred from one bacterial cell to another, but study of this phenomenon eventually led to the conclusion that DNA is the genetic material. The initial experiment on transformation was performed by Frederick Griffith in England in 1928 while he was working with two strains of *Streptococcus pneumoniae*. One, a virulent strain, has a polysaccharide capsule that prevents phagocytosis. The bacteria grow and cause pneumonia. The other, an avirulent strain, lacks the capsule and does not cause disease.

Griffith was interested in determining whether injections of heat-killed bacteria of the encapsulated strain could be used to vaccinate mice against pneumonia. As he expected, injections of living encapsulated bacteria killed the mouse (**Figure 8.25a**); injections of live nonencapsulated bacteria (Figure 8.25b) or dead encapsulated bacteria (Figure 8.25c) did not kill the mouse. However, when the dead encapsulated bacteria were mixed with live nonencapsulated bacteria and injected into the mice, many of the mice died. In the blood of the dead mice, Griffith found living, encapsulated bacteria. Hereditary material (genes) from

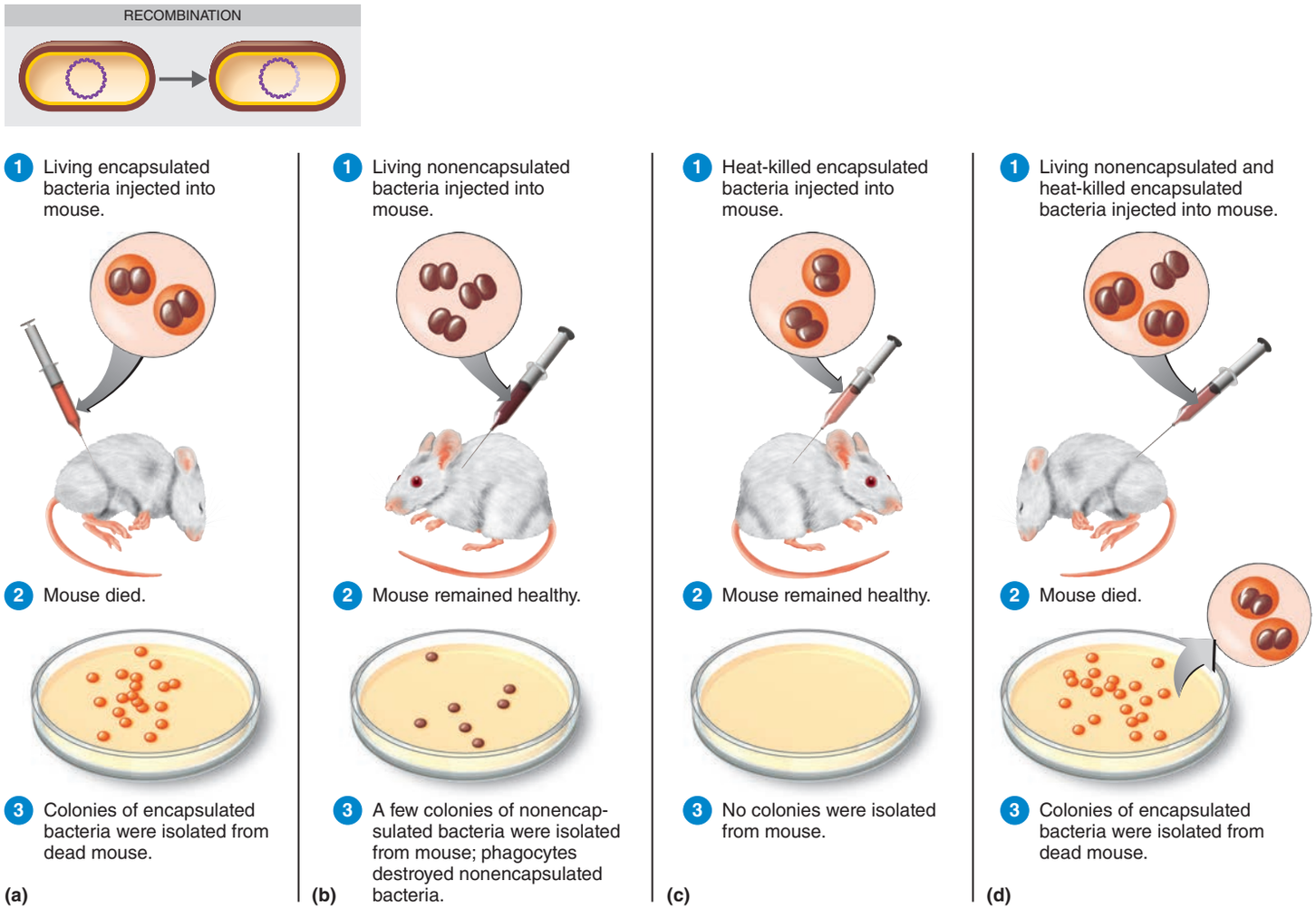


Figure 8.25 Griffith's experiment demonstrating genetic transformation. **(a)** Living encapsulated bacteria caused disease and death when injected into a mouse. **(b)** Living nonencapsulated bacteria are readily destroyed by the phagocytic defenses of the host, so the mouse remained healthy after injection. **(c)** After being killed by heat, encapsulated bacteria lost the ability to cause disease. **(d)** However, the combination of living nonencapsulated bacteria and heat-killed encapsulated bacteria (neither of which alone causes disease) did cause disease. Somehow, the live nonencapsulated bacteria were transformed by the dead encapsulated bacteria so that they acquired the ability to form capsules and therefore cause disease. Subsequent experiments proved the transforming factor to be DNA.

Q Why did encapsulated bacteria kill the mouse while nonencapsulated bacteria did not? What killed the mouse in (d)?

the dead bacteria had entered the live cells and changed them genetically so that their progeny were encapsulated and therefore virulent (Figure 8.25d).

Subsequent investigations based on Griffith's research revealed that bacterial transformation could be carried out without mice. A broth was inoculated with live nonencapsulated bacteria. Dead encapsulated bacteria were then added to the broth. After incubation, the culture was found to contain living bacteria that were encapsulated and virulent. The nonencapsulated bacteria had been transformed; they had acquired a new hereditary trait by incorporating genes from the killed encapsulated bacteria.

The next step was to extract various chemical components from the killed cells to determine which component caused the transformation. These crucial experiments were performed in the United States by Oswald T. Avery and his associates Colin M. MacLeod and Maclyn McCarty. After years of research, they announced in 1944 that the component responsible for transforming harmless *S. pneumoniae* into virulent strains was DNA. Their results provided one of the conclusive indications that DNA was indeed the carrier of genetic information.

Since the time of Griffith's experiment, considerable information has been gathered about transformation. In nature, some bacteria, perhaps after death and cell lysis, release their DNA into the environment. Other bacteria can then encounter the DNA and, depending on the particular species and growth conditions, take up fragments of DNA and integrate them into their own chromosomes by recombination. A protein called

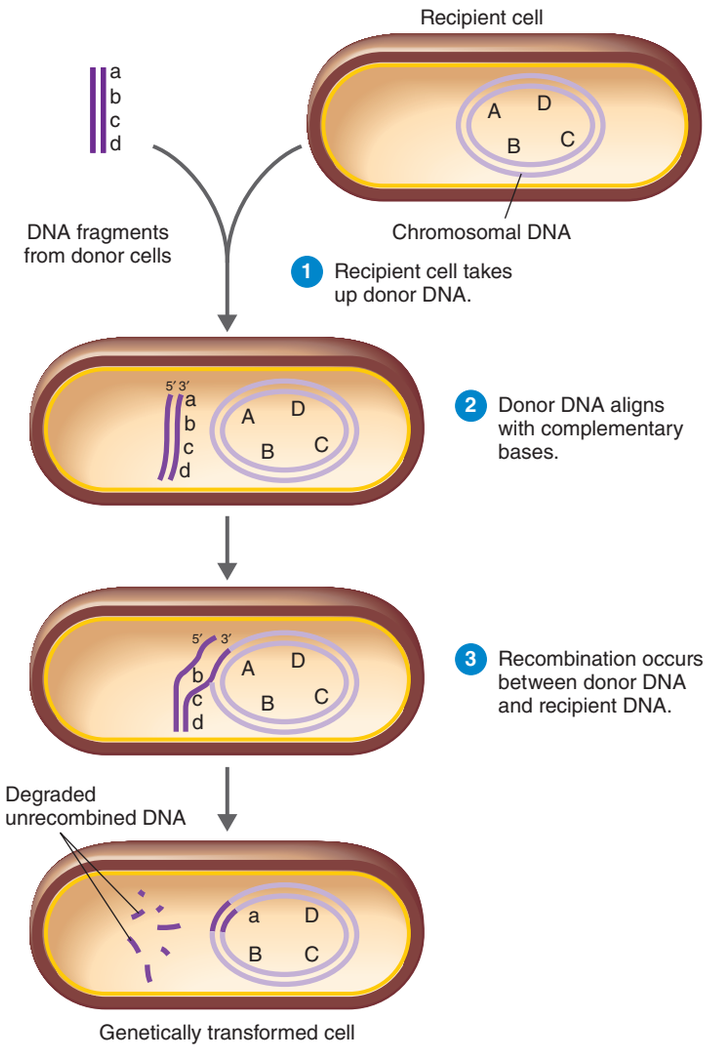


Figure 8.26 The mechanism of genetic transformation in bacteria. Some similarity is needed for the donor and recipient to align. Genes *a, b, c,* and *d* may be mutations of genes *A, B, C,* and *D*.

Q What type of enzyme cuts the donor DNA?

RecA (see Figure 3.11a, page 61) binds to the cell's DNA and then to donor DNA causing the exchange of strands. A recipient cell with this new combination of genes is a kind of hybrid, or recombinant cell (**Figure 8.26**). All the descendants of such a recombinant cell will be identical to it. Transformation occurs naturally among very few genera of bacteria, including *Bacillus, Haemophilus* (hē-MAH-fil-us), *Neisseria, Acinetobacter* (a-sin-ē-tō-bak′ter), and certain strains of the genera *Streptococcus* and *Staphylococcus*.

Even though only a small portion of a cell's DNA is transferred to the recipient, the molecule that must pass through the recipient cell wall and membrane is still very large. When a recipient cell is in a physiological state in which it can take up the donor DNA, it is said to be competent. **Competence** results from alterations in the cell wall that make it permeable to large DNA molecules. MM **Animation** Transformation

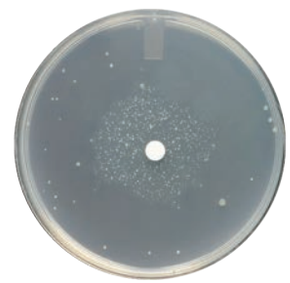
Conjugation in Bacteria

Another mechanism by which genetic material is transferred from one bacterium to another is known as **conjugation.** Conjugation is mediated by one kind of *plasmid,* a circular piece of DNA that replicates independently from the cell's chromosome (discussed on page 230). However, plasmids differ from bacterial chromosomes in that the genes they carry are usually not essential for the growth of the cell under normal conditions. The plasmids responsible for conjugation are transmissible between cells during conjugation.

Conjugation differs from transformation in two major ways. First, conjugation requires direct cell-to-cell contact. Second, the conjugating cells must generally be of opposite mating type; donor cells must carry the plasmid, and recipient cells usually do not. In gram-negative bacteria, the plasmid carries genes that code for the synthesis of *sex pili,* projections from the donor's cell surface that contact the recipient and help bring the two cells into direct contact (**Figure 8.27a**). Gram-positive bacterial cells produce sticky surface molecules that cause cells to come into direct contact with each other. In the process of conjugation, the plasmid is replicated during the transfer of a single-stranded copy

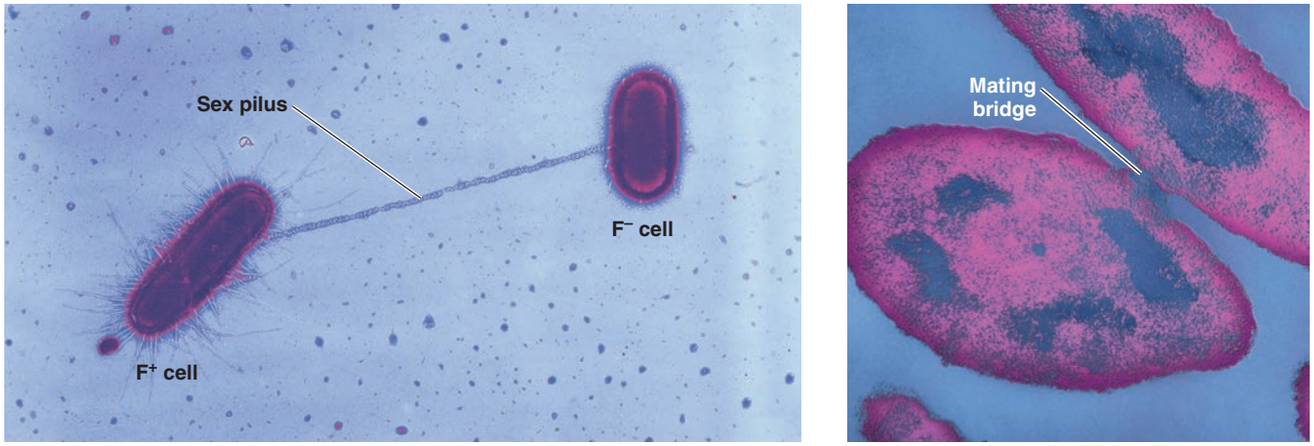
Clinical Case Resolved

The Ames test allows rapid screening of chemicals for genotoxicity. The his⁻ mutant *Salmonella* bacteria used in the Ames test are spread over glucose–minimal salts agar plates. A paper disk saturated with 2-aminofluorene (2-AF), an aromatic amine, is placed on the culture. The figure, for example, shows that reversion of the his⁻ mutation allowed the *Salmonella* to grow. This indicates that the chemical is mutagenic and is therefore potentially carcinogenic.



There are studies indicating that 2-AF activated by enzymes is more damaging than 2-AF alone, suggesting that the interaction between diet and intestinal microbiota is more likely to cause cancer than just diet. Variations in diet produce little change in the kinds of bacteria in the intestine, but they produce dramatic changes in the metabolic activity of the bacteria.

The detection of serrated colorectal polyps from Marcel's stool DNA test led to a diagnosis of an early, rather than late, stage of colorectal cancer. The offending polyps are found and removed, and Marcel undergoes chemotherapy to kill any missed cancer cells in his colon.



(a) Sex pilus **(b)** Mating bridge

Figure 8.27 Bacterial conjugation.

Q What is an F^+ cell?

of the plasmid DNA to the recipient, where the complementary strand is synthesized (Figure 8.27b).

Because most experimental work on conjugation has been done with *E. coli,* we will describe the process in this organism. In *E. coli,* the **F factor (fertility factor)** was the first plasmid observed to be transferred between cells during conjugation. Donors carrying F factors (F^+ cells) transfer the plasmid to recipients (F^- cells), which become F^+ cells as a result (**Figure 8.28a**). In some cells carrying F factors, the factor integrates into the chromosome, converting the F^+ cell to an **Hfr cell** (high frequency of recombination) (Figure 8.28b). When conjugation occurs between an Hfr cell and an F^- cell, the Hfr cell's chromosome (with its integrated F factor) replicates, and a parental strand of the chromosome is transferred to the recipient cell (Figure 8.28c). Replication of the Hfr chromosome begins in the middle of the integrated F factor, and a small piece of the F factor leads the chromosomal genes into the F^- cell. Usually, the chromosome breaks before it is completely transferred. Once within the recipient cell, donor DNA can recombine with the recipient's DNA. (Donor DNA that is not integrated is degraded.) Therefore, by conjugation with an Hfr cell, an F^- cell may acquire new versions of chromosomal genes (just as in transformation). However, it remains an F^- cell because it did not receive a complete F factor during conjugation.

Conjugation is used to map the location of genes on a bacterial chromosome (**Figure 8.29**). The genes for the synthesis of threonine (*thr*) and leucine (*leu*) are first, reading clockwise from 0. Their locations were determined by conjugation experiments. Assume that conjugation is allowed for only 1 minute between an Hfr strain that is his^+, pro^+, thr^+, and leu^+, and an F^- strain that is his^-, pro^-, thr^-, and leu^-. If the F^- acquired the ability to synthesize threonine, then the *thr* gene is located early in the chromosome, between 0 and 1 minute. If after 2 minutes the F^- cell now becomes thr^+ and leu^+, the order of these two genes on the chromosome must be *thr, leu.* MM **Animations** Conjugation: Overview, F Factor, Hfr Conjugation, Chromosome Mapping

Transduction in Bacteria

A third mechanism of genetic transfer between bacteria is **transduction.** In this process, bacterial DNA is transferred from a donor cell to a recipient cell inside a virus that infects bacteria, called a **bacteriophage,** or **phage.** (Phages will be discussed further in Chapter 13.)

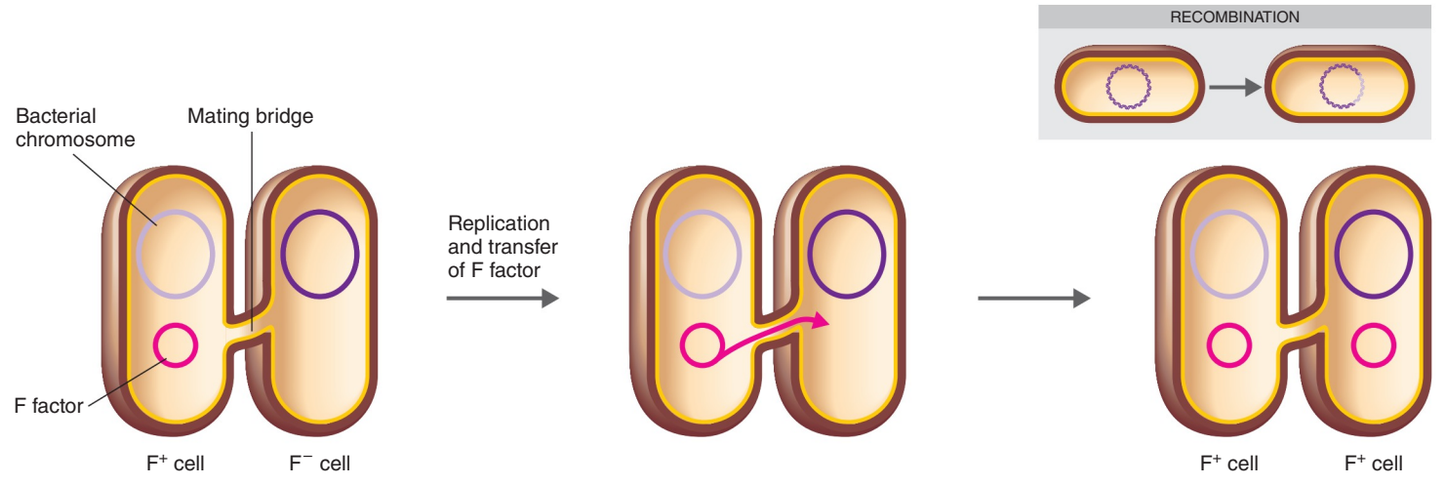
To understand how transduction works, we will consider the life cycle of one type of transducing phage of *E. coli;* this phage carries out **generalized transduction** (**Figure 8.30**).

During phage reproduction, phage DNA and proteins are synthesized by the host bacterial cell. The phage DNA should be packaged inside the phage protein coat. However, bacterial DNA, plasmid DNA, or even DNA of another virus may be packaged inside a phage protein coat.

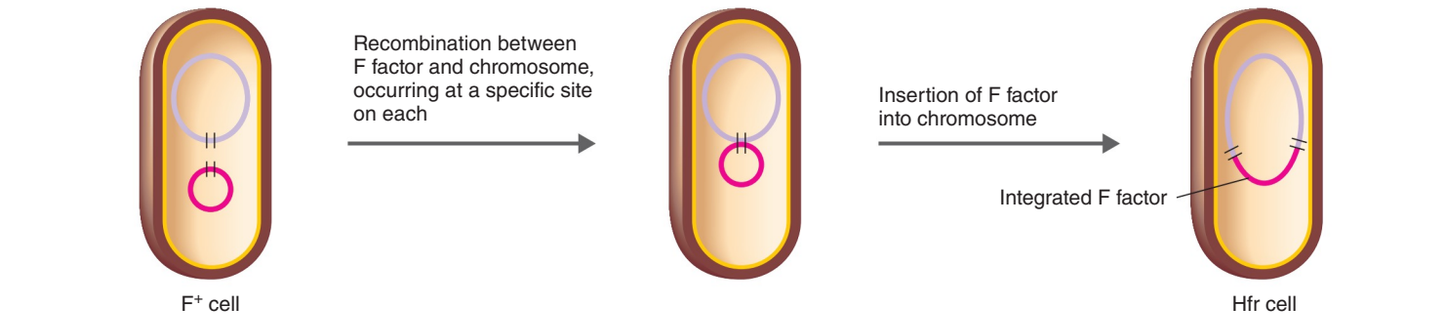
All genes contained within a bacterium infected by a generalized transducing phage are equally likely to be packaged in a phage coat and transferred. In another type of transduction, called **specialized transduction,** only certain bacterial genes are transferred (see page 372). In one type of specialized transduction, the phage codes for certain toxins produced by their bacterial hosts, such as diphtheria toxin for *Corynebacterium diphtheriae* (kor´ī-nē-bak-TI-rē-um dif-THI-rē-ī), erythrogenic toxin for *Streptococcus pyogenes,* and Shiga toxin for *E. coli* O157:H7. MM **Animation** Transduction: Generalized Transduction

CHECK YOUR UNDERSTANDING

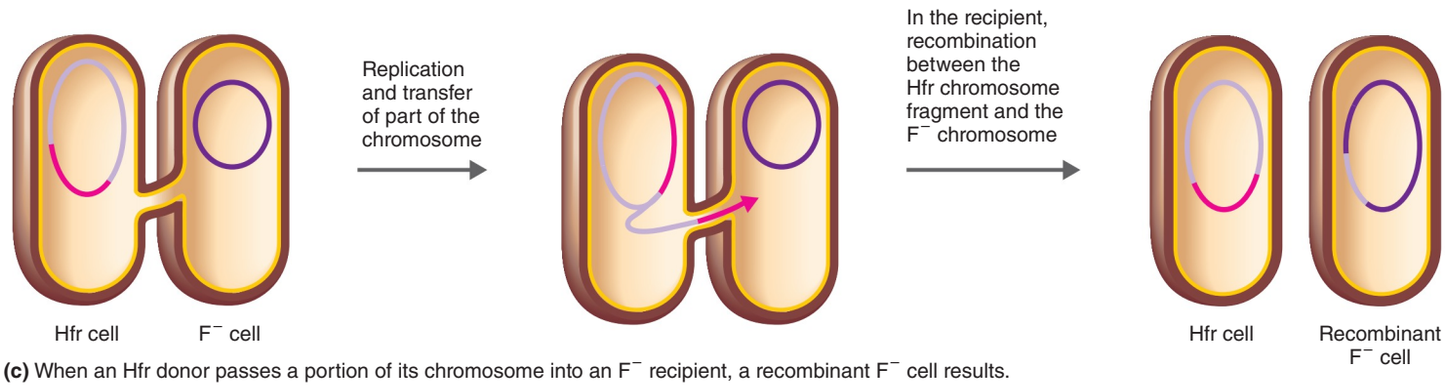
- Differentiate horizontal and vertical gene transfer. **8-14**
- Compare conjugation between the following pairs: $F^+ \times F^-$, Hfr $\times F^-$. **8-15**



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted to an F^+ cell.



(b) When an F factor becomes integrated into the chromosome of an F^+ cell, it makes the cell a high frequency of recombination (Hfr) cell.



(c) When an Hfr donor passes a portion of its chromosome into an F^- recipient, a recombinant F^- cell results.

Figure 8.28 Conjugation in *E. coli*.

Q Do bacteria reproduce during conjugation?

Plasmids and Transposons

Plasmids and transposons are genetic elements that provide additional mechanisms for genetic change. They occur in both prokaryotic and eukaryotic organisms, but this discussion focuses on their role in genetic change in prokaryotes.

Plasmids

Recall from Chapter 4 (page 90) that plasmids are self-replicating, gene-containing, circular pieces of DNA about 1–5% the size of the bacterial chromosome (Figure 8.31a). They are found mainly in bacteria but also in some eukaryotic microorganisms, such as *Saccharomyces cerevisiae*. The F factor is a **conjugative plasmid** that carries genes for sex pili and for the transfer of the plasmid to another cell. Although plasmids are usually dispensable, under certain conditions genes carried by plasmids can be crucial to the survival and growth of the cell. For example, **dissimilation plasmids** code for enzymes that trigger the catabolism of certain unusual sugars and hydrocarbons. Some species of *Pseudomonas*

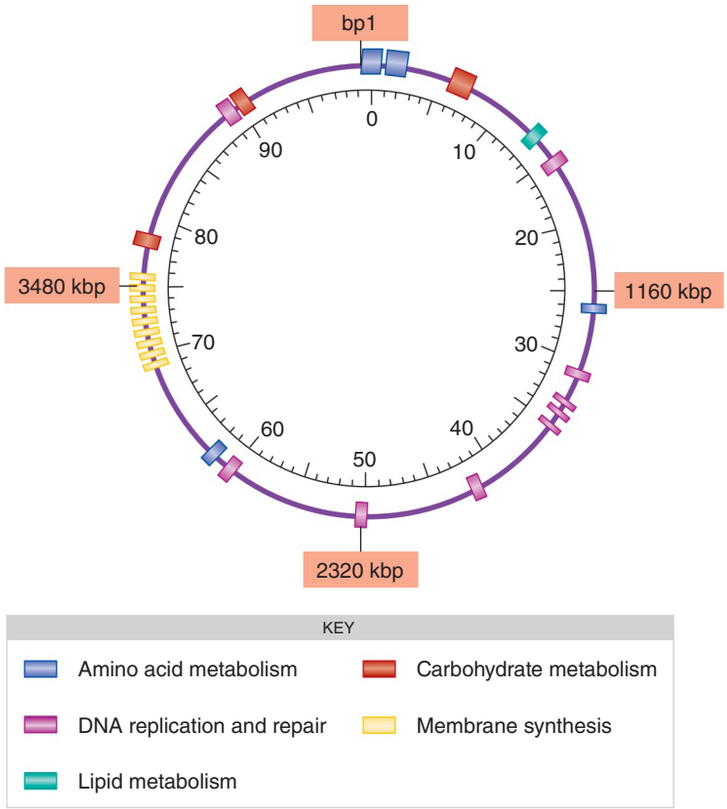


Figure 8.29 A genetic map of the chromosome of *E. coli*. This map is made by observing recombinant cells after conjugation. The numbers inside the circle indicate the number of minutes it takes to transfer the genes during mating between two cells; the numbers in colored boxes indicate the number of base pairs. 1 kbp = 1000 base pairs.

Q How many minutes of conjugation would be needed to transfer genes for membrane synthesis on this chromosome?

can actually use such exotic substances as toluene, camphor, and hydrocarbons of petroleum as primary carbon and energy sources because they have catabolic enzymes encoded by genes carried on plasmids. Such specialized capabilities permit the survival of those microorganisms in very diverse and challenging environments. Because of their ability to degrade and detoxify a variety of unusual compounds, many of them are being investigated for possible use in the cleanup of environmental wastes. (See the Applications box in Chapter 2, page 31.)

Other plasmids code for proteins that enhance the pathogenicity of a bacterium. The strain of *E. coli* that causes infant diarrhea and traveler's diarrhea carries plasmids that code for toxin production and for bacterial attachment to intestinal cells. Without these plasmids, *E. coli* is a harmless resident of the large intestine; with them, it is pathogenic. Other plasmid-encoded toxins include the exfoliative toxin of *Staphylococcus aureus, Clostridium tetani* neurotoxin, and toxins of *Bacillus anthracis*. Still other plasmids contain genes for the synthesis of **bacteriocins,** toxic proteins that kill other bacteria. These plasmids have been found in many bacterial genera, and they are useful markers for the identification of certain bacteria in clinical laboratories.

Resistance factors (R factors) are plasmids that have significant medical importance. They were first discovered in Japan in the late 1950s after several dysentery epidemics. In some of

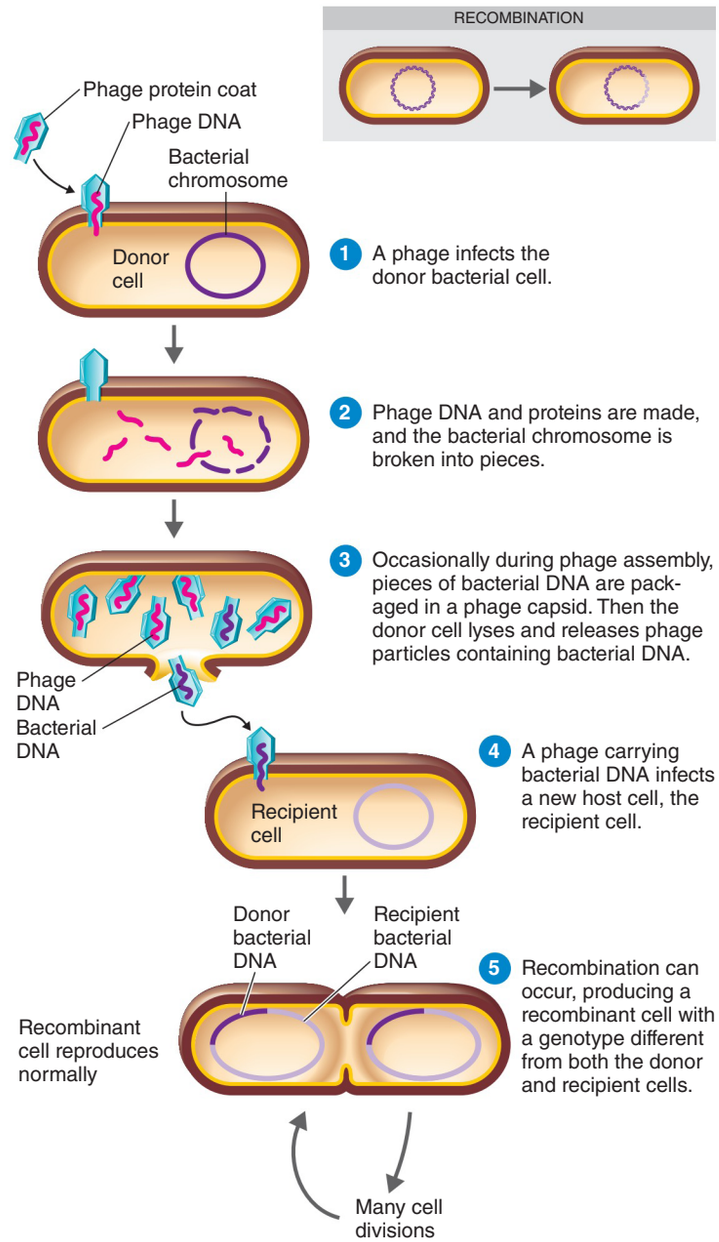
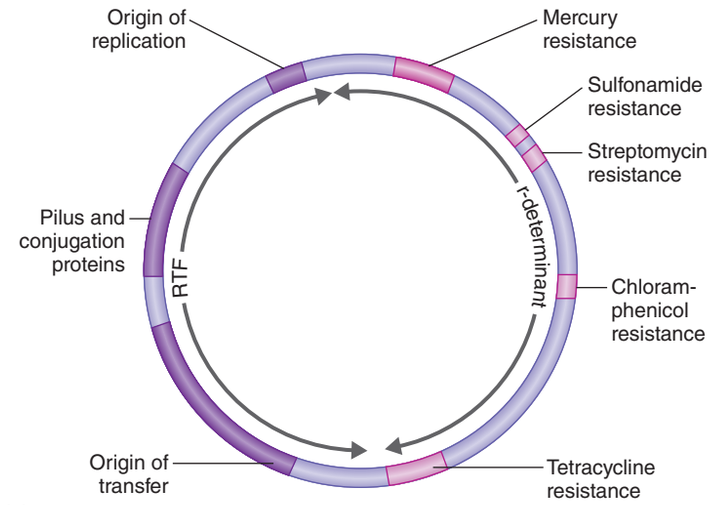
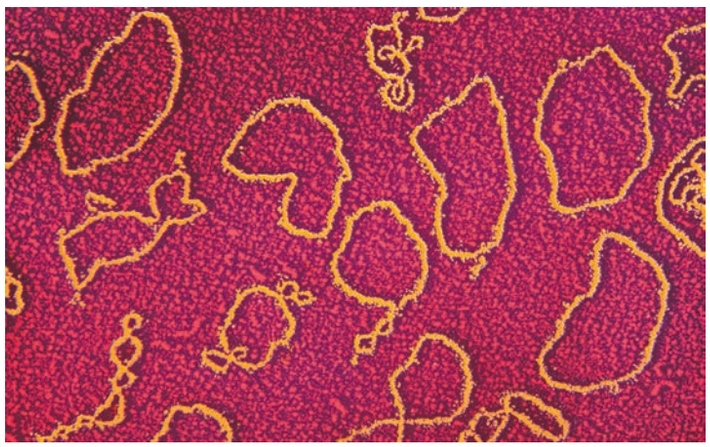


Figure 8.30 Transduction by a bacteriophage. Shown here is generalized transduction, in which any bacterial DNA can be transferred from one cell to another.

Q How could *E. coli* acquire the Shiga toxin gene?



(a)



(b)

Figure 8.31 R factor, a type of plasmid. **(a)** A diagram of an R factor, which has two parts: the RTF contains genes needed for plasmid replication and transfer of the plasmid by conjugation, and the r-determinant carries genes for resistance to four different antibiotics and mercury (*sul* = sulfonamide resistance, *str* = streptomycin resistance, *cml* = chloramphenicol resistance, *tet* = tetracycline resistance, *mer* = mercury resistance); numbers are base pairs × 1000. **(b)** Plasmids from *E. coli* bacteria.

Q Why are R factors important in the treatment of infectious diseases?

these epidemics, the infectious agent was resistant to the usual antibiotic. Following isolation, the pathogen was also found to be resistant to a number of different antibiotics. In addition, other normal bacteria from the patients (such as *E. coli*) proved to be resistant as well. Researchers soon discovered that these bacteria acquired resistance through the spread of genes from one organism to another. The plasmids that mediated this transfer are R factors.

R factors carry genes that confer upon their host cell resistance to antibiotics, heavy metals, or cellular toxins. Many R factors contain two groups of genes. One group is called the **resistance transfer factor (RTF)** and includes genes for plasmid replication and conjugation. The other group, the **r-determinant,** has the resistance genes; it codes for the production of enzymes that inactivate certain drugs or toxic substances (Figure 8.31a). Different R factors, when present in the same cell, can recombine to produce R factors with new combinations of genes in their r-determinants.

In some cases, the accumulation of resistance genes within a single plasmid is quite remarkable. For example, Figure 8.31a shows a genetic map of resistance plasmid R100. Carried on this plasmid are resistance genes for sulfonamides, streptomycin, chloramphenicol, and tetracycline, as well as genes for resistance to mercury. This particular plasmid can be transferred between a number of enteric genera, including *Escherichia, Klebsiella,* and *Salmonella.*

R factors present very serious problems for treating infectious diseases with antibiotics. The widespread use of antibiotics in medicine and agriculture (see the box in Chapter 20 on page 573) has led to the preferential survival (selection) of bacteria that have R factors, so populations of resistant bacteria grow larger and larger. The transfer of resistance between bacterial cells of a population, and even between bacteria of different genera, also contributes to the problem. The ability to reproduce sexually with members of its own species defines a eukaryotic species. However, a bacterial species can conjugate and transfer plasmids to other species. *Neisseria* may have acquired its penicillinase-producing plasmid from *Streptococcus,* and *Agrobacterium* can transfer plasmids to plant cells (see Figure 9.20, page 257). Nonconjugative plasmids may be transferred from one cell to another by inserting themselves into a conjugative plasmid or a chromosome or by transformation when released from a dead cell. Insertion is made possible by an insertion sequence, which will be discussed shortly.

Plasmids are an important tool for genetic engineering, discussed in Chapter 9 (pages 242–243).

Transposons

Transposons are small segments of DNA that can move (be "transposed") from one region of a DNA molecule to another. These pieces of DNA are 700 to 40,000 base pairs long.

In the 1950s, American geneticist Barbara McClintock discovered transposons in corn, but they occur in all organisms and have been studied most thoroughly in microorganisms. They may move from one site to another site on the same chromosome or to another chromosome or plasmid. As you might imagine, the frequent movement of transposons could wreak havoc inside a cell. For example, as transposons move about on chromosomes, they may insert themselves *within* genes, inactivating them. Fortunately, transposition occurs relatively rarely. The frequency of transposition is comparable to the spontaneous mutation rate that occurs in bacteria—that is, from 10^{-5} to 10^{-7} per generation.

All transposons contain the information for their own transposition. As shown in **Figure 8.32a**, the simplest transposons, also called **insertion sequences (IS),** contain only a gene that codes for an enzyme (*transposase,* which catalyzes the cutting and resealing of DNA that occurs in transposition) and recognition sites. *Recognition sites* are short inverted repeat sequences of DNA that the enzyme recognizes as recombination sites between the transposon and the chromosome.

Complex transposons also carry other genes not connected with the transposition process. For example, bacterial transposons may contain genes for enterotoxin or for antibiotic resistance (Figure 8.32b). Plasmids such as R factors are frequently made up of a collection of transposons (Figure 8.32c).

Transposons with antibiotic resistance genes are of practical interest, but there is no limitation on the kinds of genes that transposons can have. Thus, transposons provide a natural mechanism for the movement of genes from one chromosome to another. Furthermore, because they may be carried between cells on plasmids or viruses, they can also spread from one organism—or even species—to another. For example, vancomycin resistance was transferred from *Enterococcus faecalis* to *Staphylococcus aureus* via a transposon called Tn1546. Transposons are thus a potentially powerful mediator of evolution in organisms. MM **Animations** Transposons: Overview, Insertion Sequences, Complex Transposons

CHECK YOUR UNDERSTANDING

What types of genes do plasmids carry? **8-16**

Genes and Evolution

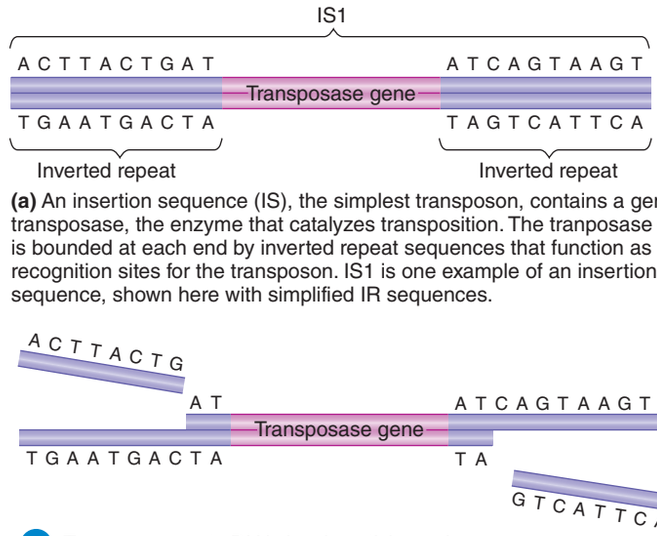
LEARNING OBJECTIVE

8-17 Discuss how genetic mutation and recombination provide material for natural selection to act upon.

We have now seen how gene activity can be controlled by the cell's internal regulatory mechanisms and how genes themselves can be altered or rearranged by mutation, transposition, and recombination. All these processes provide diversity in the descendants of cells. Diversity provides the raw material for evolution, and natural selection provides its driving force. Natural selection will act on diverse populations to ensure the survival of those fit for that particular environment. The different kinds of microorganisms that exist today are the result of a long history of evolution. Microorganisms have continually changed by alterations in their genetic properties and acquisition of adaptations to many different habitats. See the box on antibiotic resistance in Chapter 26, page 573, for an example of natural selection.

CHECK YOUR UNDERSTANDING

Natural selection means that the environment favors survival of some genotypes. From where does diversity in genotypes come? **8-17**



(a) An insertion sequence (IS), the simplest transposon, contains a gene for transposase, the enzyme that catalyzes transposition. The tranposase gene is bounded at each end by inverted repeat sequences that function as recognition sites for the transposon. IS1 is one example of an insertion sequence, shown here with simplified IR sequences.

(b) Complex transposons carry other genetic material in addition to transposase genes. The example shown here, Tn5, carries the gene for kanamycin resistance and has complete copies of the insertion sequence IS1 at each end.

(c) Insertion of the transposon Tn5 into R100 plasmid

Figure 8.32 Transposons and insertion.

Q Why are transposons sometimes referred to as "jumping genes"?

Study Outline

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Structure and Function of the Genetic Material (pp. 204–214)

1. Genetics is the study of what genes are, how they carry information, how their information is expressed, and how they are replicated and passed to subsequent generations or other organisms.
2. DNA in cells exists as a double-stranded helix; the two strands are held together by hydrogen bonds between specific nitrogenous base pairs: AT and CG.
3. A gene is a segment of DNA, a sequence of nucleotides, that encodes a functional product, usually a protein.
4. The DNA in a cell is duplicated before the cell divides, so each offspring cell receives the same genetic information.

Genotype and Phenotype (p. 204)

5. Genotype is the genetic composition of an organism, its entire complement of DNA.
6. Phenotype is the expression of the genes: the proteins of the cell and the properties they confer on the organism.

DNA and Chromosomes (pp. 204–205)

7. The DNA in a chromosome exists as one long double helix associated with various proteins that regulate genetic activity.
8. Genomics is the molecular characterization of genomes.

The Flow of Genetic Information (p. 205)

9. Following cell division, each offspring cell receives a chromosome that is virtually identical to the parent's.
10. Information contained in the DNA is transcribed into RNA and translated into proteins.

DNA Replication (pp. 205–209)

11. During DNA replication, the two strands of the double helix separate at the replication fork, and each strand is used as a template by DNA polymerases to synthesize two new strands of DNA according to the rules of complementary base pairing.
12. The result of DNA replication is two new strands of DNA, each having a base sequence complementary to one of the original strands.
13. Because each double-stranded DNA molecule contains one original and one new strand, the replication process is called semiconservative.
14. DNA is synthesized in one direction designated 5′ → 3′. At the replication fork, the leading strand is synthesized continuously and the lagging strand discontinuously.
15. DNA polymerase proofreads new molecules of DNA and removes mismatched bases before continuing DNA synthesis.

RNA and Protein Synthesis (pp. 209–214)

16. During transcription, the enzyme RNA polymerase synthesizes a strand of RNA from one strand of double-stranded DNA, which serves as a template.
17. RNA is synthesized from nucleotides containing the bases A, C, G, and U, which pair with the bases of the DNA strand being transcribed.
18. RNA polymerase binds the promoter; transcription begins at AUG; the region of DNA that is the end point of transcription is the terminator; RNA is synthesized in the 5′ → 3′ direction.
19. Translation is the process in which the information in the nucleotide base sequence of mRNA is used to dictate the amino acid sequence of a protein.
20. The mRNA associates with ribosomes, which consist of rRNA and protein.
21. Three-base segments of mRNA that specify amino acids are called codons.
22. The genetic code refers to the relationship among the nucleotide base sequence of DNA, the corresponding codons of mRNA, and the amino acids for which the codons code.
23. Specific amino acids are attached to molecules of tRNA. Another portion of the tRNA has a base triplet called an anticodon.
24. The base pairing of codon and anticodon at the ribosome results in specific amino acids being brought to the site of protein synthesis.
25. The ribosome moves along the mRNA strand as amino acids are joined to form a growing polypeptide; mRNA is read in the 5′ → 3′ direction.
26. Translation ends when the ribosome reaches a stop codon on the mRNA.

The Regulation of Bacterial Gene Expression (pp. 214–218)

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See how operons affect a patient's health @ MasteringMicrobiology®.

1. Regulating protein synthesis at the gene level is energy-efficient because proteins are synthesized only as they are needed.
2. Constitutive genes are expressed at a fixed rate. Examples are genes for the enzymes in glycolysis.

Pre-transcriptional Control (pp. 214–217)

3. When cells are exposed to a particular end-product, the synthesis of enzymes related to that product is repressed.
4. In the presence of certain chemicals (inducers), cells synthesize more enzymes. This process is called induction.
5. In bacteria, a group of coordinately regulated structural genes with related metabolic functions, plus the promoter and operator sites that control their transcription, is called an operon.
6. In the operon model for an inducible system, a regulatory gene codes for the repressor protein.
7. When the inducer is absent, the repressor binds to the operator, and no mRNA is synthesized.
8. When the inducer is present, it binds to the repressor so that it cannot bind to the operator; thus, mRNA is made, and enzyme synthesis is induced.

9. In repressible systems, the repressor requires a corepressor in order to bind to the operator site; thus, the corepressor controls enzyme synthesis.
10. Transcription of structural genes for catabolic enzymes (such as β-galactosidase) is induced by the absence of glucose. Cyclic AMP and CRP must bind to a promoter in the presence of an alternative carbohydrate.
11. Methylated nucleotides are not transcribed in epigenetic control.

Post-transcriptional Control (pp. 217–218)

12. MicroRNAs combine with mRNA; the resulting double-stranded RNA is destroyed.

Changes in Genetic Material (pp. 218–225)

1. Mutations and horizontal gene transfer can change a bacterium's genotype.

Mutation (p. 219)

2. A mutation is a change in the nitrogenous base sequence of DNA; that change causes a change in the product coded for by the mutated gene.
3. Many mutations are neutral, some are disadvantageous, and others are beneficial.

Types of Mutations (pp. 219–220)

4. A base substitution occurs when one base pair in DNA is replaced with a different base pair.
5. Alterations in DNA can result in missense mutations (which cause amino acid substitutions) or nonsense mutations (which create stop codons).
6. In a frameshift mutation, one or a few base pairs are deleted or added to DNA.
7. Spontaneous mutations occur without the presence of any mutagen.

Mutagens (pp. 220–223)

8. Mutagens are agents in the environment that cause permanent changes in DNA.
9. Chemical mutagens include base-pair mutagens, nucleoside analogs, and frameshift mutagens.
10. Ionizing radiation causes the formation of ions and free radicals that react with DNA; base substitutions or breakage of the sugar-phosphate backbone results.
11. Ultraviolet (UV) radiation is nonionizing; it causes bonding between adjacent thymines.

The Frequency of Mutation (p. 223)

12. Mutation rate is the probability that a gene will mutate when a cell divides; the rate is expressed as 10 to a negative power.
13. Mutations usually occur randomly along a chromosome.
14. A low rate of spontaneous mutations is beneficial in providing the genetic diversity needed for evolution.

Identifying Mutants (p. 223)

15. Mutants can be detected by selecting or testing for an altered phenotype.
16. Positive selection involves the selection of mutant cells and the rejection of nonmutated cells.
17. Replica plating is used for negative selection—to detect, for example, auxotrophs that have nutritional requirements not possessed by the parent (nonmutated) cell.

Identifying Chemical Carcinogens (pp. 223–225)

18. The Ames test is a relatively inexpensive and rapid test for identifying possible chemical carcinogens.
19. The test assumes that a mutant cell can revert to a normal cell in the presence of a mutagen and that many mutagens are carcinogens.

Genetic Transfer and Recombination (pp. 225–233)

1. Genetic recombination, the rearrangement of genes from separate groups of genes, usually involves DNA from different organisms; it contributes to genetic diversity.
2. In crossing over, genes from two chromosomes are recombined into one chromosome containing some genes from each original chromosome.
3. Vertical gene transfer occurs during reproduction when genes are passed from an organism to its offspring.
4. Horizontal gene transfer in bacteria involves a portion of the cell's DNA being transferred from donor to recipient.
5. When some of the donor's DNA has been integrated into the recipient's DNA, the resultant cell is called a recombinant.

Transformation in Bacteria (pp. 226–228)

6. During this process, genes are transferred from one bacterium to another as "naked" DNA in solution.

Conjugation in Bacteria (pp. 228–229)

7. This process requires contact between living cells.
8. One type of genetic donor cell is an F^+; recipient cells are F^-. F cells contain plasmids called F factors; these are transferred to the F^- cells during conjugation.

Transduction in Bacteria (pp. 229–230)

9. In this process, DNA is passed from one bacterium to another in a bacteriophage and is then incorporated into the recipient's DNA.
10. In generalized transduction, any bacterial genes can be transferred.

Plasmids and Transposons (pp. 230–233)

11. Plasmids are self-replicating circular molecules of DNA carrying genes that are not usually essential for the cell's survival.
12. There are several types of plasmids, including conjugative plasmids, dissimilation plasmids, plasmids carrying genes for toxins or bacteriocins, and resistance factors.
13. Transposons are small segments of DNA that can move from one region to another region of the same chromosome or to a different chromosome or a plasmid.
14. Complex transposons can carry any type of gene, including antibiotic-resistance genes, and are thus a natural mechanism for moving genes from one chromosome to another.

Genes and Evolution (p. 233)

1. Diversity is the precondition for evolution.
2. Genetic mutation and recombination provide diversity of organisms, and the process of natural selection allows the growth of those best adapted to a given environment.

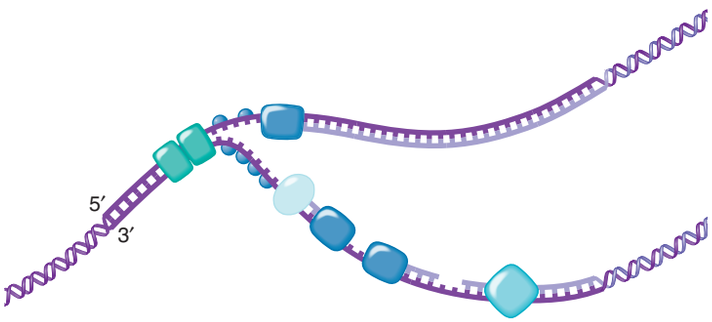
Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. Briefly describe the components of DNA, and explain its functional relationship to RNA and protein.
2. DRAW IT Identify and mark each of the following on the portion of DNA undergoing replication: replication fork, DNA polymerase, RNA primer, parent strands, leading strand, lagging strand, the direction of replication on each strand, and the 5′ end of each strand.



3. Match the following examples of mutagens.

Column A	Column B
_____ a. A mutagen that is incorporated into DNA in place of a normal base	1. Frameshift mutagen
_____ b. A mutagen that causes the formation of highly reactive ions	2. Nucleoside analog
_____ c. A mutagen that alters adenine so that it base-pairs with cytosine	3. Base-pair mutagen
_____ d. A mutagen that causes insertions	4. Ionizing radiation
_____ e. A mutagen that causes the formation of pyrimidine dimers	5. Nonionizing radiation

4. The following is a code for a strand of DNA.

DNA	3′	A	T	A	T	_	_	_	T	T	T	_	_	_	_	_	_	_	_
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
mRNA												C	G	U				U	G	A
tRNA														U	G	G				
Amino Acid						Met _____														

ATAT = Promoter sequence

   a. Using the genetic code provided in Figure 8.8, fill in the blanks to complete the segment of DNA shown.
   b. Fill in the blanks to complete the sequence of amino acids coded for by this strand of DNA.
   c. Write the code for the complementary strand of DNA completed in part (a).
   d. What would be the effect if C were substituted for T at base 10?
   e. What would be the effect if A were substituted for G at base 11?
   f. What would be the effect if G were substituted for T at base 14?
   g. What would be the effect if C were inserted between bases 9 and 10?
   h. How would UV radiation affect this strand of DNA?
   i. Identify a nonsense sequence in this strand of DNA.
5. When iron is not available, *E. coli* can stop synthesis of all proteins, such as superoxide dismutase and succinate dehydrogenase, that require iron. Describe a mechanism for this regulation.
6. Identify when (before transcription, after transcription but before translation, after translation) each of the following regulatory mechanisms functions.
   a. ATP combines with an enzyme, altering its shape.
   b. A short RNA is synthesized that is complementary to mRNA.
   c. Methylation of DNA occurs.
   d. An inducer combines with a repressor.
7. Which sequence is the best target for damage by UV radiation: AGGCAA, CTTTGA, or GUAAAU? Why aren't all bacteria killed when they are exposed to sunlight?
8. You are provided with cultures with the following characteristics:

   Culture 1: F^+, genotype $A^+ B^+ C^+$

   Culture 2: F^-, genotype $A^- B^- C^-$
   a. Indicate the possible genotypes of a recombinant cell resulting from the conjugation of cultures 1 and 2.
   b. Indicate the possible genotypes of a recombinant cell resulting from conjugation of the two cultures after the F^+ has become an Hfr cell.
9. Why are mutation and recombination important in the process of natural selection and the evolution of organisms?
10. NAME IT Normally a commensal in the human intestine, this bacterium became pathogenic after acquiring a toxin gene from a *Shigella* bacterium.

Multiple Choice

Match the following terms to the definitions in questions 1and 2.

   a. conjugation
   b. transcription
   c. transduction
   d. transformation
   e. translation

1. Transfer of DNA from a donor to a recipient cell by a bacteriophage.
2. Transfer of DNA from a donor to a recipient as naked DNA in solution.
3. Feedback inhibition differs from repression because feedback inhibition
   a. is less precise.
   b. is slower acting.
   c. stops the action of preexisting enzymes.
   d. stops the synthesis of new enzymes.
   e. all of the above
4. Bacteria can acquire antibiotic resistance by all of the following *except*
   a. mutation.
   b. insertion of transposons.
   c. conjugation.
   d. snRNPs.
   e. transformation.

5. Suppose you inoculate three flasks of minimal salts broth with *E. coli*. Flask A contains glucose. Flask B contains glucose and lactose. Flask C contains lactose. After a few hours of incubation, you test the flasks for the presence of β-galactosidase. Which flask(s) do you predict will have this enzyme?
   a. A
   b. B
   c. C
   d. A and B
   e. B and C
6. Plasmids differ from transposons in that plasmids
   a. become inserted into chromosomes.
   b. are self-replicated outside the chromosome.
   c. move from chromosome to chromosome.
   d. carry genes for antibiotic resistance.
   e. none of the above

Use the following choices to answer questions 7 and 8.

a. catabolite repression
b. DNA polymerase
c. induction
d. repression
e. translation

7. Mechanism by which the presence of glucose inhibits the *lac* operon.
8. The mechanism by which lactose controls the *lac* operon.
9. Two offspring cells are most likely to inherit which one of the following from the parent cell?
   a. a change in a nucleotide in mRNA
   b. a change in a nucleotide in tRNA
   c. a change in a nucleotide in rRNA
   d. a change in a nucleotide in DNA
   e. a change in a protein
10. Which of the following is *not* a method of horizontal gene transfer?
    a. binary fission
    b. conjugation
    c. integration of a transposon
    d. transduction
    e. transformation

Analysis

1. Nucleoside analogs and ionizing radiation are used in treating cancer. These mutagens can cause cancer, so why do you suppose they are used to treat the disease?
2. Replication of the *E. coli* chromosome takes 40 to 45 minutes, but the organism has a generation time of 26 minutes. How does the cell have time to make complete chromosomes for each offspring cell?
3. *Pseudomonas* has a plasmid containing the *mer* operon, which includes the gene for mercuric reductase. This enzyme catalyzes the reduction of the mercuric ion Hg^{2+} to the uncharged form of mercury, Hg^0. Hg^{2+} is quite toxic to cells; Hg^0 is not.
   a. What do you suppose is the inducer for this operon?
   b. The protein encoded by one of the *mer* genes binds Hg^{2+} in the periplasm and brings it into the cell. Why would a cell bring in a toxin?
   c. What is the value of the *mer* operon to *Pseudomonas*?

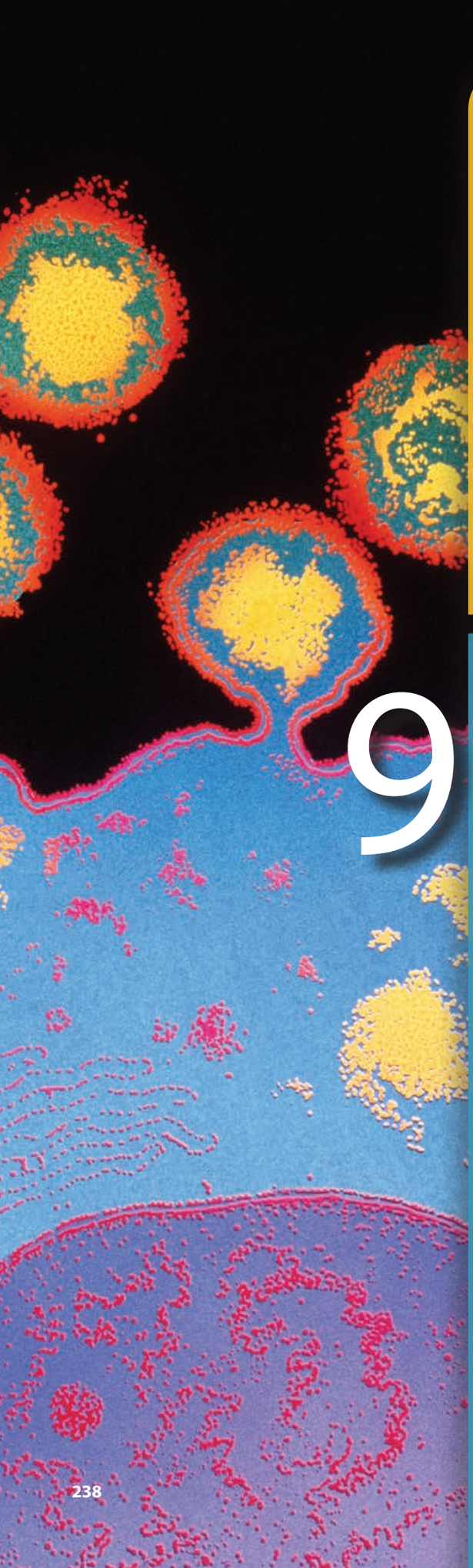
Clinical Applications and Evaluation

1. Ciprofloxacin, erythromycin, and acyclovir are used to treat microbial infections. Ciprofloxacin inhibits DNA gyrase. Erythromycin binds in front of the A site on the 50S subunit of a ribosome. Acyclovir is a guanine analog.
   a. What steps in protein synthesis are inhibited by each drug?
   b. Which drug is more effective against bacteria? Why?
   c. Which drugs will have effects on the host's cells? Why?
   d. Use the index to identify the disease for which acyclovir is primarily used. Why is it more effective than erythromycin for treating this disease?
2. HIV, the virus that causes AIDS, was isolated from three individuals, and the amino acid sequences for the viral coat were determined. Of the amino acid sequences shown below, which two of the viruses are most closely related? How can these amino acid sequences be used to identify the source of a virus?

Patient	Viral Amino Acid Sequence											
A	Asn	Gln	Thr	Ala	Ala	Ser	Lys	Asn	Ile	Asp	Ala	Leu
B	Asn	Leu	His	Ser	Asp	Lys	Ile	Asn	Ile	Ile	Leu	Leu
C	Asn	Gln	Thr	Ala	Asp	Ser	Ile	Val	Ile	Asp	Ala	Leu

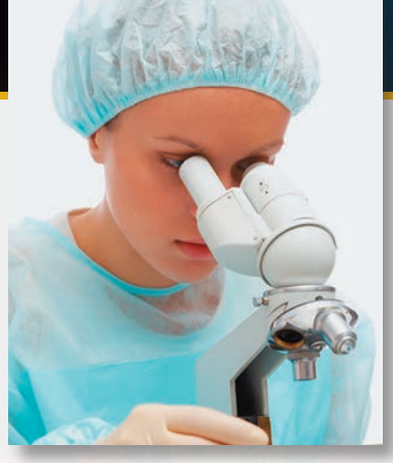
3. Human herpesvirus-8 (HHV-8) is common in parts of Africa, the Middle East, and the Mediterranean, but is rare elsewhere except in AIDS patients. Genetic analyses indicate that the African strain is not changing, whereas the Western strain is accumulating changes. Using the portions of the HHV-8 genomes (shown below) that encode one of the viral proteins, how similar are these two viruses? What mechanism can account for the changes? What disease does HHV-8 cause?

Western	3′-ATGGAGTTCTTCTGGACAAGA
African	3′-ATAAACTTTTTCTTGACAACG



9

Biotechnology and DNA Technology



In the Clinic

A crime suspect claims he is innocent. His clothing became bloodstained, he says, when he tried to resuscitate the victim. The pattern of blood on the suspect may have resulted from striking the victim, but it is also consistent with spatter from the victim's nose and mouth during CPR. As a forensic nurse for a police department, you collect a piece of bloodstained fabric from the suspect and another blood sample from the crime scene. You request PCR for *streptococci* on both samples. The test comes back positive for the fabric, but negative for the blood at the scene. **How can a PCR test detect evidence of *Streptococcus* bacteria in such small samples? Do these results help or hurt the suspect?**

Hint: Read about the polymerase chain reaction technique on page 243. As a nurse, you are aware that Streptococcus *bacteria are commonly found in the nose and mouth, but not in blood—and that the bacteria do not survive long on environmental surfaces.*

Note: Answers to In the clinic questions are found online at MasteringMicrobiology.

For thousands of years, people have been consuming foods produced by the action of microorganisms. Bread, chocolate, and soy sauce are some of the best-known examples. But it was only just over 100 years ago that scientists showed that microorganisms are responsible for these products. This knowledge opened the way for using microorganisms to produce other important products. Since World War I, microbes have been used to produce a variety of chemicals, such as ethanol, acetone, and citric acid. Since World War II, microorganisms have been grown on a large scale to produce antibiotics. More recently, microbes and their enzymes are replacing a variety of chemical processes involved in manufacturing such products as paper, textiles, and fructose. Using microbes or their enzymes instead of chemical syntheses offers several advantages: microbes may use inexpensive, abundant raw materials, such as starch; microbes work at normal temperatures and pressure, thereby avoiding the need for expensive and dangerous pressurized systems; and microbes don't produce toxic, hard-to-treat wastes. In the past 30 years, DNA technology has been added to the tools used to make products.

In this chapter you will learn the tools and techniques that are used to research and develop a product. You will also learn how DNA technology is used to track outbreaks of infectious disease and to provide evidence for courts of law in forensic microbiology. The Clinical Case illustrates the use of DNA technology to track HIV (see the photo).

Human immunodeficiency virus (HIV) budding from a host cell.

Introduction to Biotechnology

LEARNING OBJECTIVES

9-1 Compare and contrast biotechnology, genetic modification, and recombinant DNA technology.

9-2 Identify the roles of a clone and a vector in making recombinant DNA.

Biotechnology is the use of microorganisms, cells, or cell components to make a product. Microbes have been used in the commercial production of foods, vaccines, antibiotics, and vitamins for years. Bacteria are also used in mining to extract valuable elements from ore (see Figure 28.10, page 805). Additionally, animal cells have been used to produce viral vaccines since the 1950s. Until the 1980s, products made by living cells were all made by naturally occurring cells; the role of scientists was to find the appropriate cell and develop a method for their large-scale cultivation.

Now, microorganisms and plants are being used as "factories" to produce chemicals that the organisms don't naturally make. This is accomplished by inserting, deleting, or modifying genes with **recombinant DNA (rDNA) technology,** which is sometimes called *genetic engineering.* The development of rDNA technology is expanding the practical applications of biotechnology almost beyond imagination.

ASM: Cell genomes can be manipulated to alter cell function.

Recombinant DNA Technology

Recombination of DNA occurs naturally in microbes (see Chapter 8). In the 1970s and 1980s, scientists developed artificial techniques for making rDNA.

A gene from a vertebrate animal, including a human, can be inserted into the DNA of a bacterium, or a gene from a virus into a yeast may be used. In many cases, the recipient can then be made to express the gene, which may code for a commercially useful product. Thus, bacteria with genes for human insulin are now being used to produce insulin for treating diabetes, and a vaccine for hepatitis B is being made by yeast carrying a gene for part of the hepatitis virus (the yeast produces a viral coat protein). Scientists hope that such an approach may prove useful in producing vaccines against other infectious agents, thus eliminating the need to use whole organisms, as in conventional vaccines.

The rDNA techniques can also be used to make thousands of copies of the same DNA molecule—to *amplify* DNA—thus generating sufficient DNA for various kinds of experimentation and analysis. This technique has practical application for identifying microbes, such as viruses, that can't be cultured.

An Overview of Recombinant DNA Procedures

An overview of some of the procedures typically used for making rDNA, along with some promising applications, is shown in Figure 9.1. A **vector** is a DNA molecule that transports foreign DNA into a cell. (See more on vectors on page 242.) The gene of interest is inserted into the vector DNA in vitro. In Figure 9.1, the vector is a plasmid. The DNA molecule chosen as a vector must be self-replicating, such as a plasmid or a viral genome. This recombinant vector DNA is taken up by a cell such as a bacterium, where it can multiply. The cell containing the recombinant vector is then grown in culture to form a **clone** of many genetically identical cells, each of which carries copies of the vector, and therefore many copies of the gene of interest. This is why DNA vectors are often called *gene-cloning vectors,* or simply *cloning vectors.* (In addition to referring to a culture of identical cells, the word *clone* is also routinely used as a verb, to describe the entire process, as in "to clone a gene.")

The final step varies according to whether the gene itself or the product of the gene is of interest. From the cell clone, the researcher may isolate ("harvest") large quantities of the gene of interest, which may then be used for a variety of purposes. The gene may even be inserted into another vector for introduction into another kind of cell (such as a plant or animal cell). Alternatively, if the gene of interest is expressed (transcribed and translated) in the cell clone, its protein product can be harvested and used for a variety of purposes.

The advantages of using rDNA for obtaining such proteins is illustrated by one of its early successes, the production of human growth hormone (hGH) in *E. coli* bacteria. Some individuals don't produce adequate amounts of hGH, so their growth

Clinical Case: No Ordinary Checkup

Dr. B. is closing his dental practice after 20 years. Four years ago, he went to his family doctor because of debilitating exhaustion. He thought he had a flu virus that he could not shake, and he was also having night sweats. His doctor ordered a myriad of blood tests, but only one came back positive. Dr. B. had HIV. Although he immediately began an HIV treatment regimen, one year later he was diagnosed with AIDS. Now, two years later, Dr. B. is very ill and can no longer work.

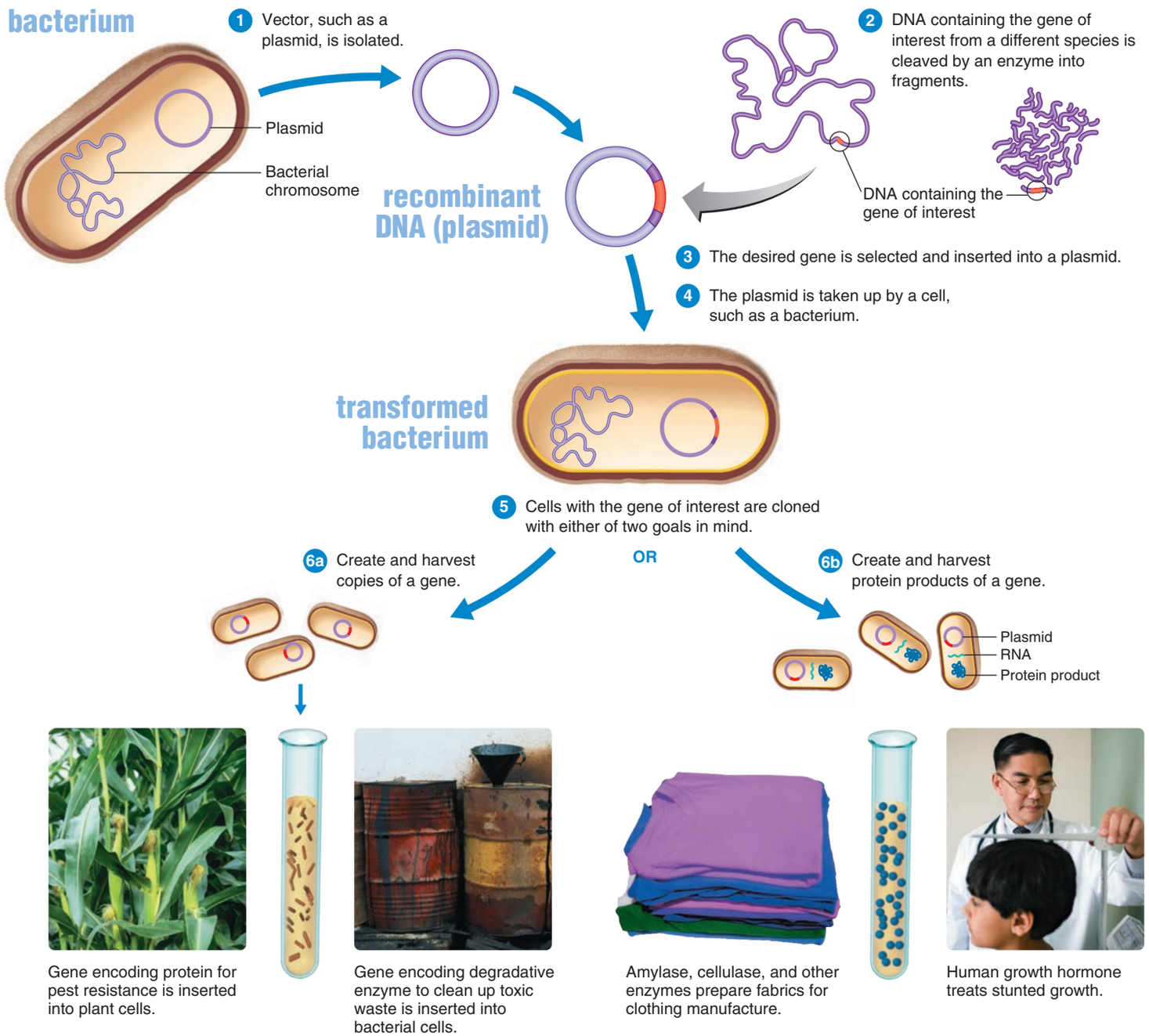
Dr. B. lets his employees know the situation and suggests that they all get tested for HIV. All of Dr. B.'s employees, including the hygienists, test negative. Dr. B. also writes an open letter to his patients informing them of his decision to close his practice and why he is doing so. This letter prompts 400 former patients to be tested for HIV, seven of whom test positive for antibodies against HIV.

What type of test can determine whether these patients contracted HIV from Dr. B.? Read on to find out.

239 245 248 250 253

FOUNDATION FIGURE 9.1

A Typical Genetic Modification Procedure



Gene encoding protein for pest resistance is inserted into plant cells.

Gene encoding degradative enzyme to clean up toxic waste is inserted into bacterial cells.

Amylase, cellulase, and other enzymes prepare fabrics for clothing manufacture.

Human growth hormone treats stunted growth.

KEY CONCEPTS

- Genes from one organism's cells can be inserted and expressed in another organism's cell.
- Genetically modified cells can be used to create a wide variety of useful products and applications.

is stunted. In the past, hGH had to be obtained from human pituitary glands at autopsy. (Growth hormone from other animals is not effective in humans.) This practice was not only expensive but also dangerous because on several occasions neurological diseases were transmitted with the hormone. Human growth hormone produced by genetically modified *E. coli* is a pure and cost-effective product. Recombinant DNA techniques also result in faster production of the hormone than traditional methods might allow.

CHECK YOUR UNDERSTANDING

- Differentiate biotechnology and rDNA technology. **9-1**
- In one sentence, describe how a vector and clone are used. **9-2**

Tools of Biotechnology

LEARNING OBJECTIVES

9-3 Compare selection and mutation.

9-4 Define *restriction enzymes,* and outline how they are used to make rDNA.

9-5 List the four properties of vectors.

9-6 Describe the use of plasmid and viral vectors.

9-7 Outline the steps in PCR, and provide an example of its use.

Research scientists and technicians isolate bacteria and fungi from natural environments such as soil and water to find, or *select,* the organisms that produce a desired product. The selected organism can be mutated to make more product or to make a better product.

Selection

In nature, organisms with characteristics that enhance survival are more likely to survive and reproduce than are variants that lack the desirable traits. This is called *natural selection.* Humans use **artificial selection** to select desirable breeds of animals or strains of plants to cultivate. As microbiologists learned how to isolate and grow microorganisms in pure culture, they were able to select the ones that could accomplish a desired objective, such as brewing beer more efficiently or producing a new antibiotic. Over 2000 strains of antibiotic-producing bacteria have been discovered by testing soil bacteria and selecting the strains that produce an antibiotic. The box in Chapter 28, page 801, describes how a bacterium was selected to convert a waste product into something useful.

Mutation

Mutations are responsible for much of the diversity of life (see Chapter 8). A bacterium with a mutation that confers resistance to an antibiotic will survive and reproduce in the presence of that antibiotic. Biologists working with antibiotic-producing microbes discovered that they could create new strains by exposing microbes to mutagens. After random mutations were created in penicillin-producing *Penicillium* by exposing fungal cultures to radiation, the highest-yielding variant among the survivors was selected for another exposure to a mutagen. Using mutations, biologists increased the amount of penicillin the fungus produced by over 1000 times.

Screening each mutant for penicillin production is a tedious process. **Site-directed mutagenesis** is more targeted and can be used to make a specific change in a gene. Suppose you determine that changing one amino acid will make a laundry enzyme work better in cold water. Using the genetic code (see Figure 8.8, page 211), you could, using the techniques described next, produce the sequence of DNA that encodes that amino acid and insert it into that enzyme's gene.

The science of molecular genetics has advanced to such a degree that many routine cloning procedures are performed using prepackaged materials and procedures that are very much like cookbook recipes. Scientists have a grab bag of methods from which to choose, depending on the ultimate application of their experiments. Next we describe some of the most important tools and techniques, and later we will consider some applications.

Restriction Enzymes

Recombinant DNA technology has its technical roots in the discovery of **restriction enzymes,** a special class of DNA-cutting enzymes that exist in many bacteria. First isolated in 1970, restriction enzymes in nature had actually been observed earlier, when certain bacteriophages were found to have a restricted host range. If these phages were used to infect bacteria other than their usual hosts, restriction enzymes in the new host destroyed almost all the phage DNA. Restriction enzymes protect a bacterial cell by hydrolyzing phage DNA. The bacterial DNA is protected from digestion because the cell **methylates** (adds methyl groups to) some of the cytosines in its DNA. The purified forms of these bacterial enzymes are used in today's laboratories.

What is important for rDNA techniques is that a restriction enzyme recognizes and cuts, or *digests,* only one particular sequence of nucleotide bases in DNA, and it cuts this sequence the same way each time. Typical restriction enzymes used in cloning experiments recognize four-, six-, or eight-base sequences. Hundreds of restriction enzymes are known, each producing DNA fragments with characteristic ends. A few restriction enzymes are listed in **Table 9.1**. You can see they are named for their bacterial source. Some of these enzymes (e.g., *Hae*III) cut both strands of DNA in the same place, producing **blunt ends,** and others make staggered cuts in the two strands—cuts that are not directly opposite each other (**Figure 9.2**). These staggered ends, or **sticky ends,** are most useful in rDNA because they can be used to join two different pieces of DNA that were cut by the same restriction

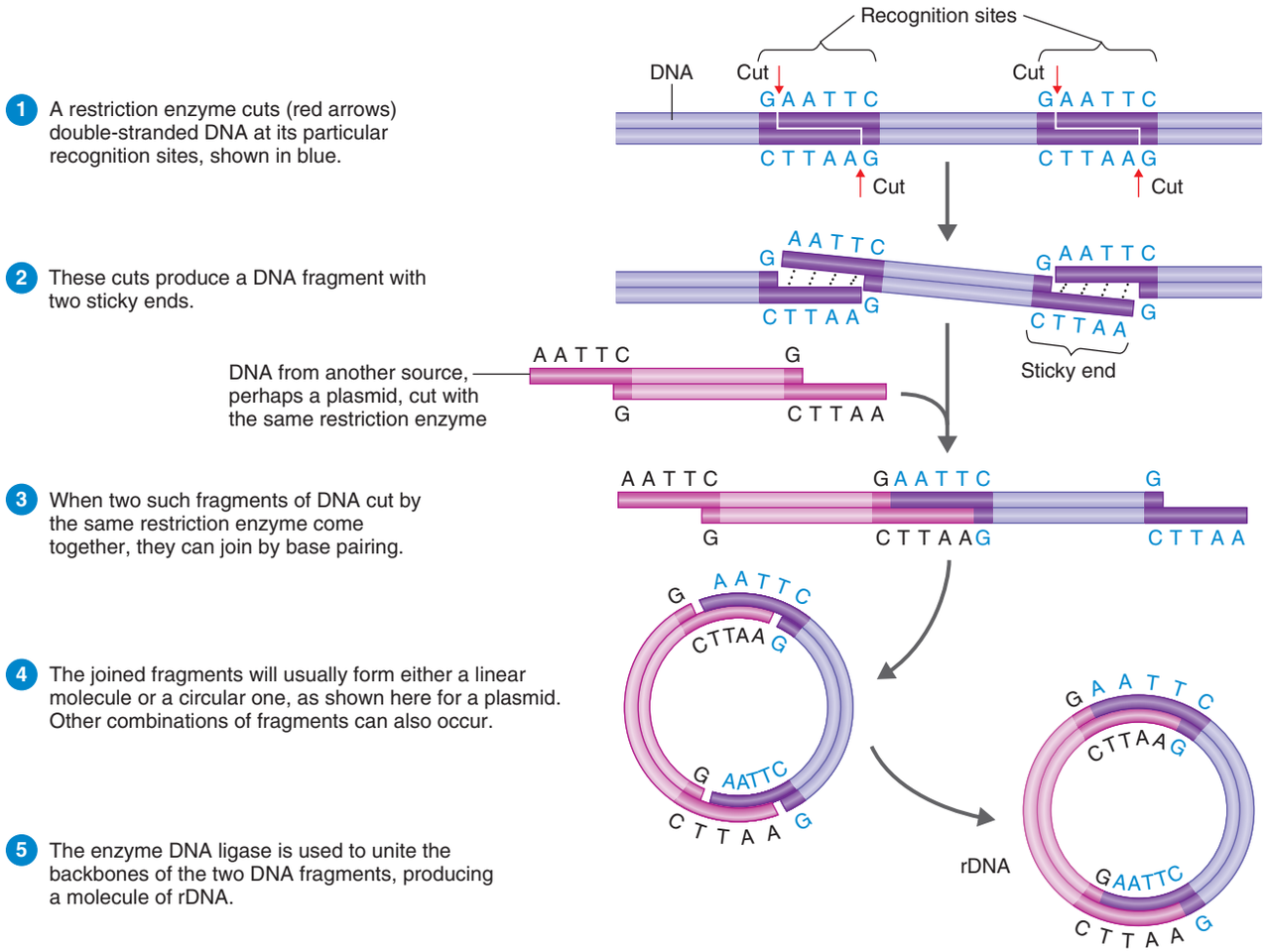


Figure 9.2 A restriction enzyme's role in making rDNA.

Q Why are restriction enzymes used to make rDNA?

TABLE 9.1 Selected Restriction Enzymes Used in rDNA Technology

Enzyme	Bacterial Source	Recognition Sequence
*Bam*HI	*Bacillus amyloliquefaciens*	G↓G A T C C G C T A G↑G
*Eco*RI	*Escherichia coli*	G↓A A T T C C T T A A↑G
*Hae*III	*Haemophilus aegyptius*	G G↓C C C C↑G G
*Hin*dIII	*Haemophilus influenzae*	A↓A G C T T T T C G A↑A

enzyme. The sticky ends "stick" to stretches of single-stranded DNA by complementary base pairing.

Notice in Figure 9.2 that the darker base sequences on the two strands are the same but run in opposite directions. Staggered cuts leave stretches of single-stranded DNA at the ends of the DNA fragments. If two fragments of DNA from different sources have been produced by the action of the same restriction enzyme, the two pieces will have identical sets of sticky ends and can be spliced (recombined) in vitro. The sticky ends join spontaneously by hydrogen bonding (base pairing). The enzyme DNA ligase is used to covalently link the backbones of the DNA pieces, producing an rDNA molecule.

MM **Animation** Recombinant DNA Technology

Vectors

Many different types of DNA molecules can serve as vectors, provided they have certain properties. The most important property is self-replication; once in a cell, a vector must be capable

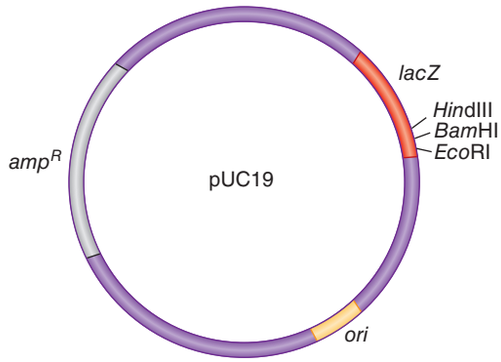


Figure 9.3 A plasmid used for cloning. A plasmid vector used for cloning in the bacterium *E. coli* is pUC19. An origin of replication (*ori*) allows the plasmid to be self-replicating. Two genes, one encoding resistance to the antibiotic ampicillin (amp^R) and one encoding the enzyme β-galactosidase (*lacZ*), serve as marker genes. Foreign DNA can be inserted at the restriction enzyme sites.

Q What is a vector in rDNA technology?

of replicating. Any DNA that is inserted in the vector will be replicated in the process. Thus, vectors serve as vehicles for the replication of desired DNA sequences.

Vectors also need to be large enough to be manipulated outside the cell during rDNA procedures. Smaller vectors are more easily manipulated than larger DNA molecules, which tend to be more fragile. Preservation is another important property of vectors. The DNA molecule's circular form protects the vector's DNA from being destroyed by its recipient. Notice in **Figure 9.3** that the DNA of a plasmid is circular. Another preservation mechanism occurs when a virus's DNA inserts itself quickly into the chromosome of the host (see Chapter 13, page 372).

When it is necessary to retrieve cells that contain the vector, a marker gene in the vector often helps make selection easy. Common selectable marker genes are for antibiotic resistance or for an enzyme that carries out an easily identified reaction.

Plasmids are one of the primary vectors in use, particularly variants of R factor plasmids. Plasmid DNA can be cut with the same restriction enzymes as the DNA that will be cloned, so that all pieces of the DNA will have the same sticky ends. When the pieces are mixed, the DNA to be cloned will be inserted into the plasmid (Figure 9.2). Note that other fragment combinations can occur as well, including the plasmid reforming a circle with no DNA inserted.

Some plasmids are capable of existing in several different species. They are called **shuttle vectors** and can be used to move cloned DNA sequences among organisms, such as among bacterial, yeast, and mammalian cells, or among bacterial, fungal, and plant cells. Shuttle vectors can be very useful in the process of genetically modifying multicellular organisms—for example, when herbicide resistance genes are inserted into plants.

A different kind of vector is viral DNA. This type of vector can usually accept much larger pieces of foreign DNA than plasmids can. After the DNA has been inserted into the viral vector, it can be cloned in the virus's host cells. The choice of a suitable vector depends on many factors, including the organism that will receive the new gene and the size of the DNA to be cloned. Retroviruses, adenoviruses, and herpesviruses are being used to insert corrective genes into human cells that have defective genes. Gene therapy is discussed on page 251.

CHECK YOUR UNDERSTANDING

- How are selection and mutation used in biotechnology? **9-3**
- What is the value of restriction enzymes in rDNA technology? **9-4**
- What criteria must a vector meet? **9-5**
- Why is a vector used in rDNA technology? **9-6**

Polymerase Chain Reaction

The **polymerase chain reaction (PCR)** is a technique by which small samples of DNA can be quickly amplified, that is, increased to quantities that are large enough for analysis.

Starting with just one gene-sized piece of DNA, PCR can be used to make billions of copies in only a few hours. The PCR process is shown in **Figure 9.4**.

Each strand of the target DNA will serve as a template for DNA synthesis. Added to this DNA are a supply of the four nucleotides (for assembly into new DNA) and the enzyme for catalyzing the synthesis, DNA polymerase (see Chapter 8, page 206). Short pieces of nucleic acid called primers are also added to help start the reaction. The primers are complementary to the ends of the target DNA and will hybridize to the fragments to be amplified. Then, the polymerase synthesizes new complementary strands. After each cycle of synthesis, the DNA is heated to convert all the new DNA into single strands. Each newly synthesized DNA strand serves in turn as a template for more new DNA.

As a result, the process proceeds exponentially. All of the necessary reagents are added to a tube, which is placed in a *thermal cycler.* The thermal cycler can be set for the desired temperatures, times, and number of cycles. Use of an automated thermal cycler is made possible by the use of DNA polymerase taken from a thermophilic bacterium such as *Thermus aquaticus;* the enzyme from such organisms can survive the heating phase without being destroyed. Thirty cycles, completed in just a few hours, will increase the amount of target DNA by more than a billion times.

The amplified DNA can be seen by gel electrophoresis. In *real-time PCR,* or *quantitative PCR* (*qPCR*), the newly made DNA is tagged with a fluorescent dye, so that the levels of fluorescence can be measured after every PCR cycle (that's the *real time* aspect). Another PCR procedure called *reverse-transcription PCR* uses viral RNA or a cell's mRNA as the template. The enzyme, reverse transcriptase, makes DNA from the RNA template, and the DNA is then amplified.

Note that PCR can only be used to amplify relatively small, specific sequences of DNA as determined by the choice of primers. It cannot be used to amplify an entire genome.

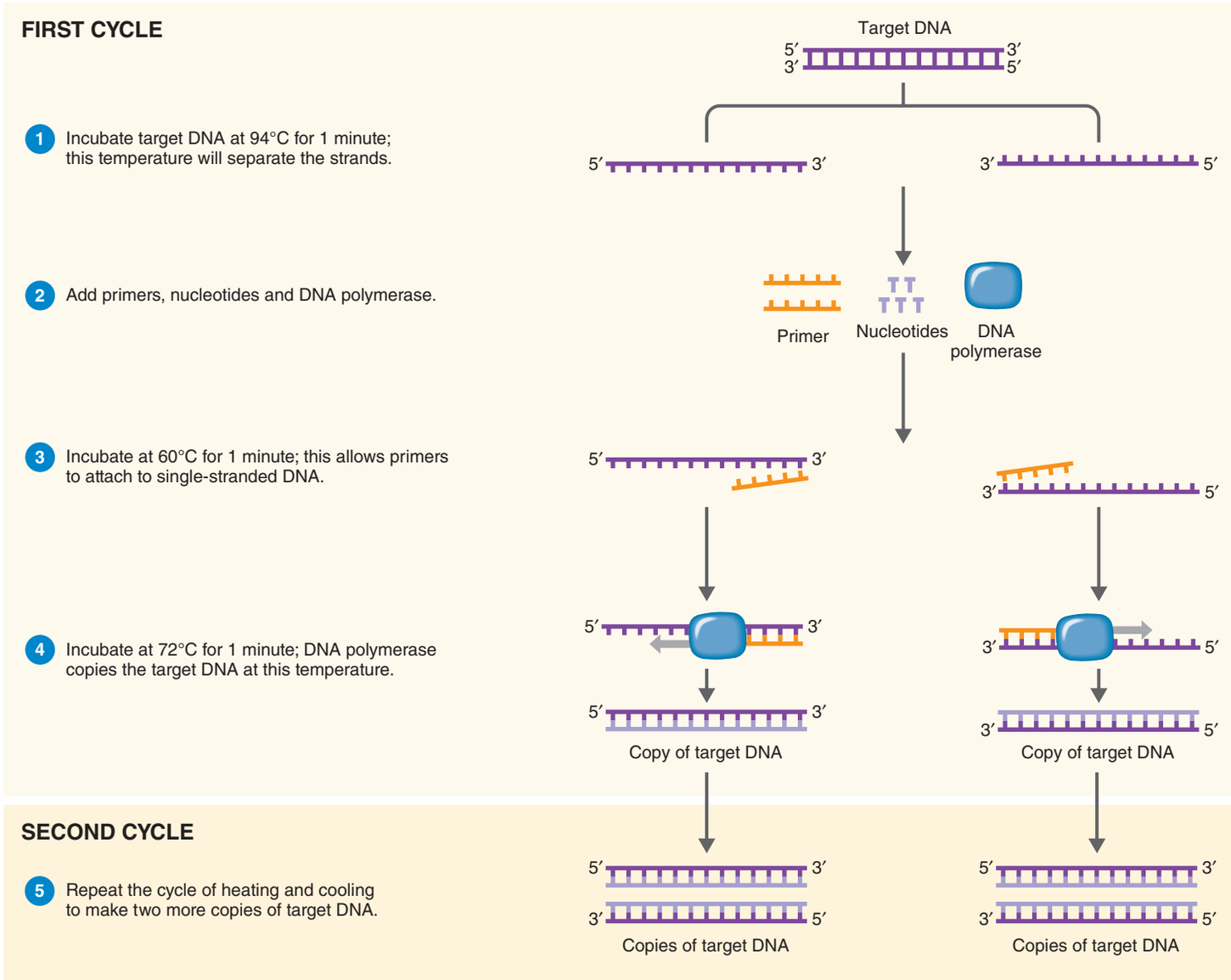


Figure 9.4 The polymerase chain reaction. Deoxynucleotides (dNTPs) base-pair with the target DNA: adenine pairs with thymine, and cytosine pairs with guanine.

Q How does *reverse-transcription PCR* differ from this figure?

PCR can be applied to any situation that requires the amplification of DNA. Especially noteworthy are diagnostic tests that use PCR to detect the presence of infectious agents in situations in which they would otherwise be undetectable. A qPCR test provides rapid identification of drug-resistant *Mycobacterium tuberculosis*. Otherwise, this bacterium can take up to 6 weeks to culture, leaving patients untreated for a significant period of time. MM **Animations** PCR: Overview, Components, Process

CHECK YOUR UNDERSTANDING

For what is each of the following used in PCR: primer, DNA polymerase, 94°C? **9-7**

Techniques of Genetic Modification

LEARNING OBJECTIVES

9-8 Describe five ways of getting DNA into a cell.

9-9 Describe how a genomic library is made.

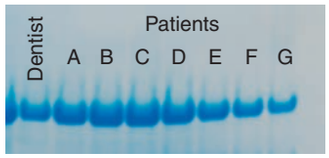
9-10 Differentiate cDNA from synthetic DNA.

9-11 Explain how each of the following is used to locate a clone: antibiotic-resistance genes, DNA probes, gene products.

9-12 List one advantage of modifying each of the following: *E. coli*, *Saccharomyces cerevisiae*, mammalian cells, plant cells.

Clinical Case

Reverse-transcription PCR using a primer for an HIV gene can be used to amplify DNA for analysis. The Centers for Disease Control and Prevention (CDC) interviews the seven former patients to determine whether their histories show any additional risk factors for contracting HIV. Five out of the seven have no identified risk factors for HIV other than having had invasive procedures performed on them by Dr. B. The CDC then performs reverse-transcription PCR on DNA from white blood cells in Dr. B.'s peripheral blood and the seven HIV-positive patients (see the figure).



What can be concluded from the PCR amplification in the figure?

239 245 248 250 253

Inserting Foreign DNA into Cells

Recombinant DNA procedures require that DNA molecules be manipulated outside the cell and then returned to living cells. There are several ways to introduce DNA into cells. The choice of method is usually determined by the type of vector and host cell being used.

In nature, plasmids are usually transferred between closely related microbes by cell-to-cell contact, such as in conjugation. To modify a cell, a plasmid must be inserted into a cell by **transformation,** a procedure during which cells can take up DNA from the surrounding environment (see Chapter 8, page 226). Many cell types, including *E. coli,* yeast, and mammalian cells, do not naturally transform; however, simple chemical treatments can make all of these cell types *competent,* or able to take up external DNA. For *E. coli,* the procedure for making cells competent is to soak them in a solution of calcium chloride for a brief period. Following this treatment, the now-competent cells are mixed with the cloned DNA and given a mild heat shock. Some of these cells will then take up the DNA.

There are other ways to transfer DNA to cells. A process called **electroporation** uses an electrical current to form microscopic pores in the membranes of cells; the DNA then enters the cells through the pores. Electroporation is generally applicable to all cells; those with cell walls often must be converted to protoplasts first. **Protoplasts** are produced by enzymatically removing the cell wall, thereby allowing more direct access to the plasma membrane.

The process of **protoplast fusion** also takes advantage of the properties of protoplasts. Protoplasts in solution fuse at a low but significant rate; the addition of polyethylene glycol increases the frequency of fusion (Figure 9.5). In the new hybrid cell, the DNA derived from the two "parent" cells may undergo natural recombination. This method is especially valuable in the genetic manipulation of plant and algal cells.

A remarkable way of introducing foreign DNA into plant cells is to literally shoot it directly through the thick cellulose walls using a gene gun (Figure 9.6). Microscopic particles of tungsten or gold are coated with DNA and propelled by a burst of helium through the plant cell walls. Some of the cells express the introduced DNA as though it were their own.

DNA can be introduced directly into an animal cell by **microinjection.** This technique requires the use of a glass micropipette with a diameter that is much smaller than the cell.

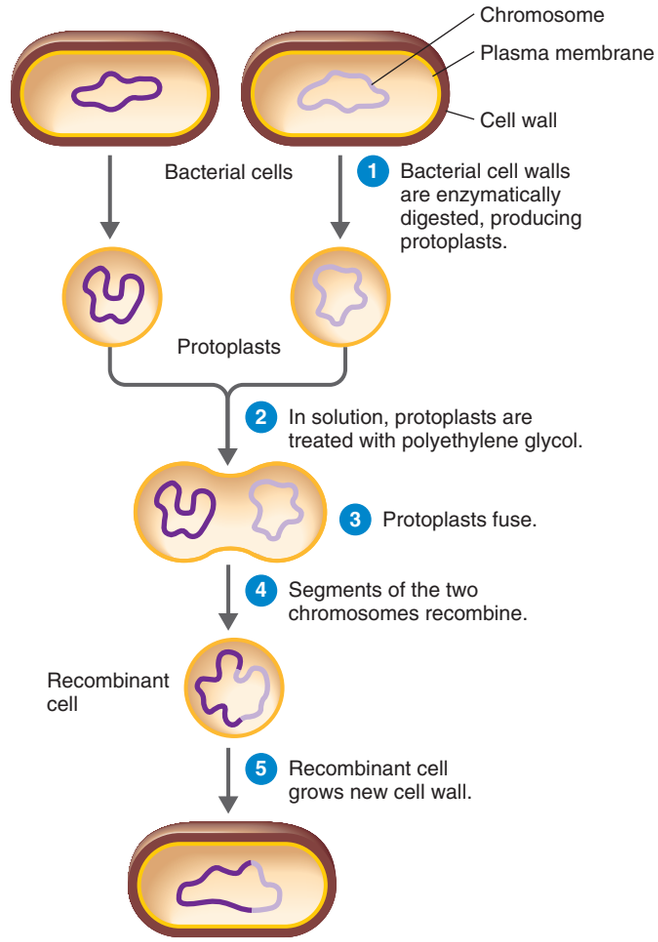


Figure 9.5 Protoplast fusion. Removal of the cell wall leaves only the delicate plasma membranes, which will fuse together, allowing the exchange of DNA.

Q What is a protoplast?

Figure 9.6 A gene gun, which can be used to insert DNA-coated "bullets" into a cell.

Q Name four other methods of inserting DNA into a cell.

The micropipette punctures the plasma membrane, and DNA can be injected through it (Figure 9.7).

Thus, there is a great variety of restriction enzymes, vectors, and methods of inserting DNA into cells. But foreign DNA will survive only if it's either present on a self-replicating vector or incorporated into one of the cell's chromosomes by recombination.

Figure 9.7 The microinjection of foreign DNA into an egg. The egg is first immobilized by applying mild suction to the large, blunt, holding pipette (right). Several hundred copies of the gene of interest are then injected into the nucleus of the cell through the tiny end of the micropipette (left).

Q Why is microinjection impractical for bacterial and fungal cells?

Obtaining DNA

We have seen how genes can be cloned into vectors by using restriction enzymes and how genes can be transformed or transferred into a variety of cell types. But how do biologists obtain the genes they are interested in? There are two main sources of genes: (1) genomic libraries containing either natural copies of genes or cDNA copies of genes made from mRNA, and (2) synthetic DNA.

Genomic Libraries

Isolating specific genes as individual pieces of DNA is seldom practical. Therefore, researchers interested in genes from a particular organism start by extracting the organism's DNA, which can be obtained from cells of any organism, whether plant, animal, or microbe, by lysing the cells and precipitating the DNA. This process results in a DNA mass that includes the organism's entire genome. After the DNA is digested by restriction enzymes, the restriction fragments are then spliced into plasmid or phage vectors, and the recombinant vectors are introduced into bacterial cells. The goal is to make a collection of clones large enough to ensure that at least one clone exists for every gene in the organism. This collection of clones containing different DNA fragments is called a **genomic library;** each "book" is a bacterial or phage strain that contains a fragment of the genome (Figure 9.8). Such libraries are essential for

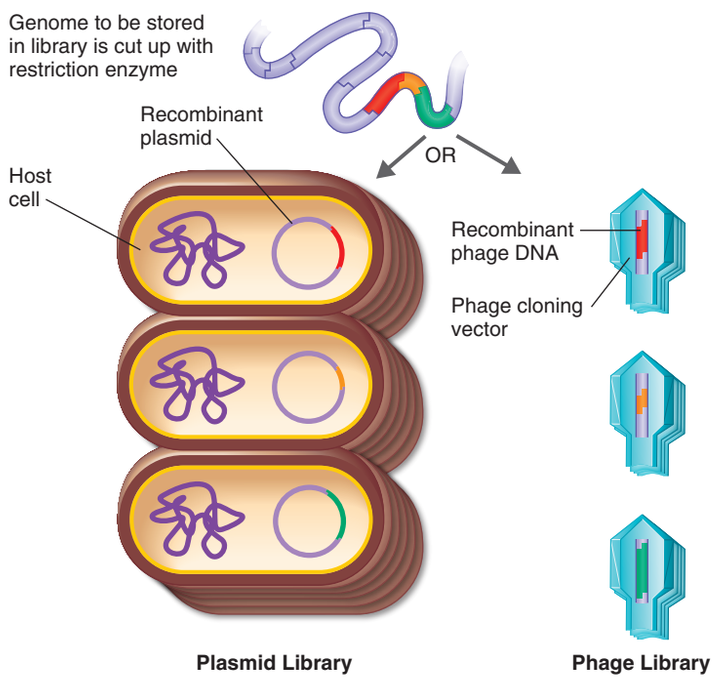


Figure 9.8 Genomic libraries. Each fragment of DNA, containing about one gene, is carried by a vector, either a plasmid within a bacterial cell or a phage.

Q Differentiate a restriction fragment from a gene.

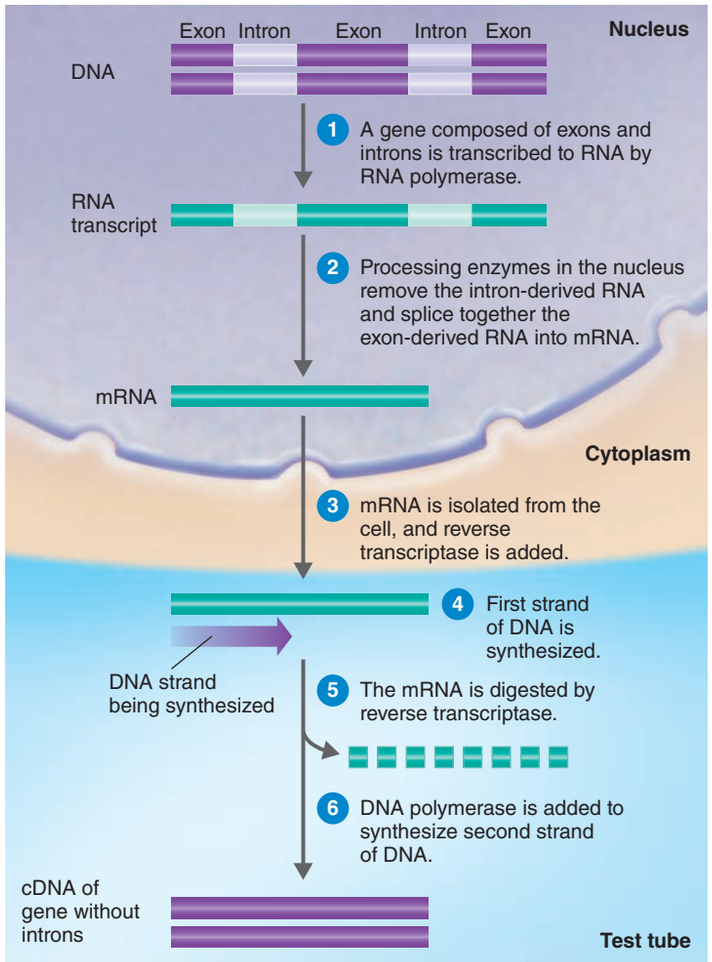


Figure 9.9 Making complementary DNA (cDNA) for a eukaryotic gene. Reverse transcriptase catalyzes the synthesis of double-stranded DNA from an RNA template.

Q How does reverse transcriptase differ from DNA polymerase?

maintaining and retrieving DNA clones; they can even be purchased commercially.

Cloning genes from eukaryotic organisms presents a specific problem. Genes of eukaryotic cells generally contain both **exons,** stretches of DNA that code for protein, and **introns,** intervening stretches of DNA that do not code for protein. When the RNA transcript of such a gene is converted to mRNA, the introns are removed (see Figure 8.11 on page 214). To clone genes from eukaryotic cells, it's desirable to use a version of the gene that lacks introns because a gene that includes introns may be too large to work with easily. In addition, if such a gene is put into a bacterial cell, the bacterium usually won't be able to remove the introns from the RNA transcript. Therefore, it won't be able to make the correct protein product. However, an artificial gene that contains only exons can be produced by using an enzyme called **reverse transcriptase** to synthesize **complementary DNA (cDNA)** from an mRNA template (Figure 9.9). This synthesis is the reverse of the normal DNA-to-RNA transcription process. A DNA copy of mRNA is produced by reverse transcriptase. Following this, the mRNA is enzymatically digested away. DNA polymerase then synthesizes a complementary strand of DNA, creating a double-stranded piece of DNA containing the information from the mRNA. Molecules of cDNA produced from a mixture of all the mRNAs from a tissue or cell type can then be cloned to form a cDNA library.

The cDNA method is the most common method of obtaining eukaryotic genes. A difficulty with this method is that long molecules of mRNA may not be completely reverse-transcribed into DNA; the reverse transcription often aborts, forming only parts of the desired gene.

Synthetic DNA

Under certain circumstances, genes can be made in vitro with the help of DNA synthesis machines (Figure 9.10). A keyboard on the machine is used to enter the desired sequence of nucleotides, much as letters are entered into a word processor to compose a sentence. A microprocessor controls the synthesis of the DNA from stored supplies of nucleotides and the other necessary reagents. A chain of about 200 nucleotides can be synthesized by this method. Unless the gene is very small, at least several chains must be synthesized separately and linked together to form an entire gene.

The difficulty of this approach, of course, is that the sequence of the gene must be known before it can be synthesized. If the gene hasn't already been isolated, then the only way to predict

Figure 9.10 A DNA synthesis machine. Short sequences of DNA can be synthesized by instruments such as this one.

Q What are some of the disadvantages of using a DNA synthesis machine?

Clinical Case

The primer amplifies all eight samples and confirms that Dr. B. and seven of his former patients are all infected with HIV. The CDC then sequences the amplified DNA and compares the sequencing to an HIV isolate from Cleveland (local control) and an isolate from Haiti (outlier). A portion of the coding (5′ to 3′) is shown below.

Patient A	GCTTG	GGCTG	GCGCT	GAAGT	GAGA
Patient B	GCTAT	TGCTG	GCGCT	GAATT	GCAC
Patient C	GCCAT	AGCTG	GCGCA	GAAGT	GCAC
Patient D	GCTAT	TGGCG	TGGCT	GACAG	AGAA
Patient E	GCACC	TGCTG	GCGCT	GAAGT	GAAA
Patient F	CAGAT	TGTGT	TGATT	GAACC	TCAC
Patient G	GCTAT	TGCTG	GCGCT	GAAGT	GAAA
Dentist	GCTAT	TGCTG	GCGCT	GAAGT	GCAC
Local control	CAGAC	TACTG	CTAGG	AAAAA	TATT
Outlier	GAAGA	CGAAA	GGACT	GCTAT	TCAG

What is the percent similarity among the viruses?

239 245 **248** 250 253

the DNA sequence is by knowing the amino acid sequence of the gene's protein product. If this amino acid sequence is known, in principle you can work backward through the genetic code to obtain the DNA sequence. Unfortunately, the degeneracy of the code prevents definitive determination; thus, if the protein contains a leucine, for example, which of the six codons for leucine is the one in the gene?

For these reasons, it's rare to clone a gene by synthesizing it directly, although some commercial products such as insulin, interferon, and somatostatin are produced from chemically synthesized genes. Desired restriction sites are added to the synthetic genes so the genes can be inserted into plasmid vectors and cloned in *E. coli*. Synthetic DNA plays a much more useful role in selection procedures, as we will see.

CHECK YOUR UNDERSTANDING

- Contrast the five ways of putting DNA into a cell. **9-8**
- What is the purpose of a genomic library? **9-9**
- Why isn't cDNA synthetic? **9-10**

Selecting a Clone

In cloning, it's necessary to select the particular cell that contains the specific gene of interest. This is difficult to do because out of millions of cells, only a very few might contain the desired gene. Here we'll examine a typical screening procedure known as *blue-white screening*, from the color of the bacterial colonies formed at the end of the screening process.

The plasmid vector used contains a gene (amp^R) encoding resistance to the antibiotic ampicillin. The host bacterium won't be able to grow on the test medium, which contains ampicillin, unless the vector has transferred the ampicillin-resistance gene. The plasmid vector also contains a second gene, this one for the enzyme β-galactosidase (*lacZ*). Notice in Figure 9.3 that there are several sites in *lacZ* that can be cut by restriction enzymes.

In the blue-white screening procedure shown in **Figure 9.11**, a library of bacteria is cultured in a medium called X-gal. X-gal

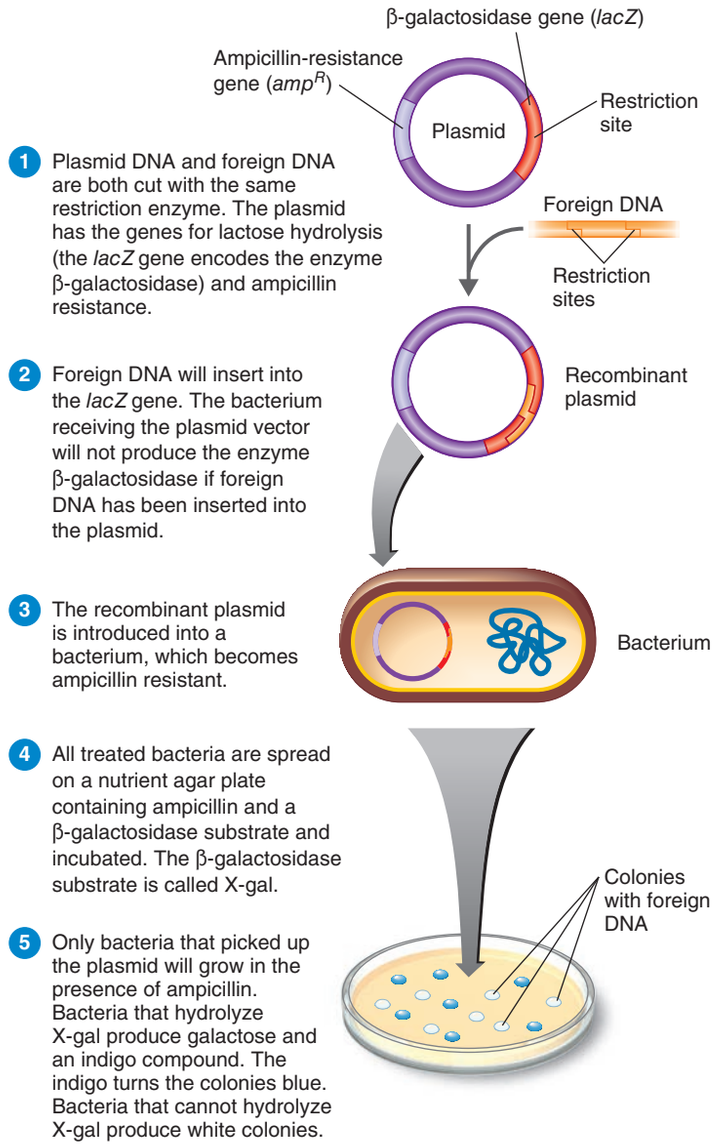


Figure 9.11 Blue-white screening, one method of selecting recombinant bacteria.

Q Why are some colonies blue and others white?

contains two essential components other than those necessary to support normal bacterial growth. One is the antibiotic ampicillin, which prevents the growth of any bacterium that has not successfully received the ampicillin-resistance gene from the plasmid. The other, called X-gal, is a substrate for β-galactosidase.

Only bacteria that picked up the plasmid will grow, because they are now ampicillin resistant. Bacteria that picked up the recombinant plasmid—in which the new gene was inserted into the *lacZ* gene—will not hydrolyze lactose and will produce white colonies. If a bacterium received the original plasmid containing the intact *lacZ* gene, the cells will hydrolyze X-gal to produce a blue-colored compound; the colony will be blue.

What remains to be done can still be difficult. The above procedure has isolated white colonies known to contain foreign DNA, but it is still not known whether it's the desired fragment of foreign DNA. A second procedure is needed to identify these bacteria. If the foreign DNA in the plasmid encodes the production of an identifiable product, the bacterial isolate only needs to be grown in culture and tested. However, in some cases the gene itself must be identified in the host bacterium.

Colony hybridization is a common method of identifying cells that carry a specific cloned gene. **DNA probes,** short segments of single-stranded DNA that are complementary to the desired gene, are synthesized. If the DNA probe finds a match, it will adhere to the target gene. The DNA probe is labeled with an enzyme or fluorescent dye so its presence can be detected. A typical colony hybridization experiment is shown in **Figure 9.12**. An array of DNA probes arranged in a DNA chip can be used to identify pathogens (see Figure 10.17, page 283).

Making a Gene Product

We have just seen how to identify cells carrying a particular gene. Gene products are frequently the reason for genetic modification. Most of the earliest work in genetic modification used *E. coli* to synthesize the gene products. *E. coli* is easily grown, and researchers are very familiar with the bacterium and its genetics. For example, some inducible promoters, such as that of the *lac* operon, have been cloned, and cloned genes can be attached to such promoters. The synthesis of great amounts of the cloned gene product can then be directed by the addition of an inducer. Such a method has been used to produce gamma interferon in *E. coli* (**Figure 9.13**). However, *E. coli* also has several disadvantages. Like other gram-negative bacteria, it produces endotoxins as part of the outer layer of its cell wall. Because endotoxins cause fever and shock in mammals, their accidental presence in products intended for human use would be a serious problem.

Another disadvantage of *E. coli* is it doesn't usually secrete protein products. To obtain a product, cells must usually be broken open and the product purified from the resulting "soup" of cell components. Recovering the product from such a mixture is

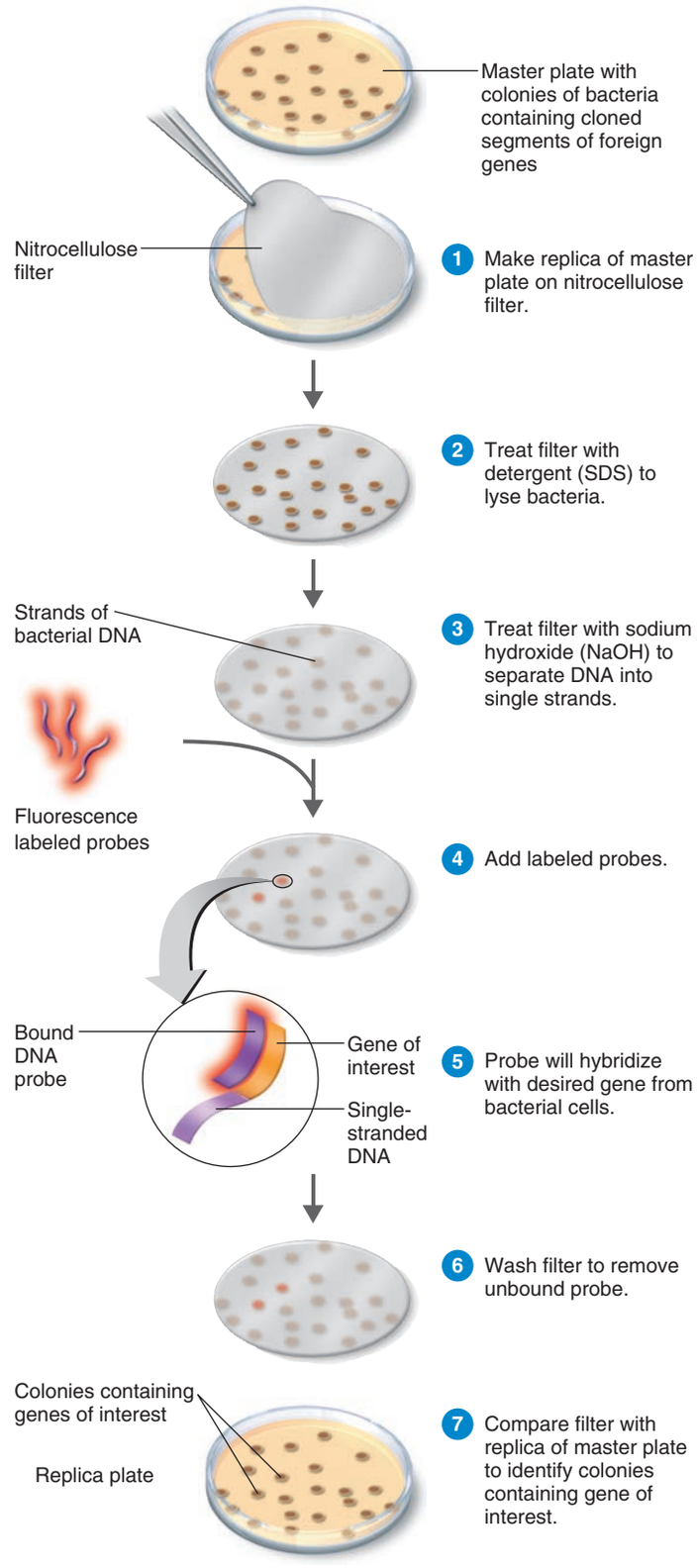


Figure 9.12 Colony hybridization: using a DNA probe to identify a cloned gene of interest.

Q What is a DNA probe?

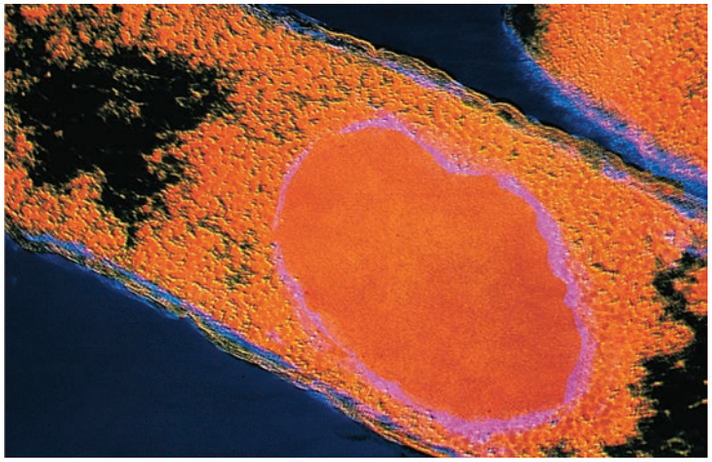


Figure 9.13 *E. coli* genetically modified to produce gamma interferon, a human protein that promotes an immune response. The product, visible here as an orange-colored substance, can be released by lysis of the cell.

Q What is one advantage of using *E. coli* for genetic engineering? One disadvantage?

expensive when done on an industrial scale. It's more economical to have an organism secrete the product so it can be recovered continuously from the growth medium. One approach has been to link the product to a natural *E. coli* protein that the bacterium does secrete. However, gram-positive bacteria, such as *Bacillus subtilis,* are more likely to secrete their products and are often preferred industrially for that reason.

Another microbe being used as a vehicle for expressing rDNA is baker's yeast, *Saccharomyces cerevisiae.* Its genome is only about four times larger than that of *E. coli* and is probably the best understood eukaryotic genome. Yeasts may carry plasmids, which are easily transferred into yeast cells whose cell walls have been removed. As eukaryotic cells, yeasts may be more successful in expressing foreign eukaryotic genes than bacteria. Furthermore, yeasts are likely to continuously secrete the product. Because of all these factors, yeasts have become the eukaryotic workhorse of biotechnology.

Mammalian cells in culture, even human cells, can be genetically modified much like bacteria to produce various products. Scientists have developed effective methods of growing certain mammalian cells in culture as hosts for growing viruses (see Chapter 13, page 367). Mammalian cells are often the best suited to making protein products for medical use because the cells secrete their products and there's a low risk of toxins or allergens. Using mammalian cells to make foreign gene products on an industrial scale often requires a preliminary step of cloning the gene in bacteria. Consider the example of colony-stimulating factor (CSF). A protein produced naturally in tiny amounts by white blood cells, CSF is valuable because it stimulates the growth of certain cells that protect against infection. To produce huge amounts of CSF industrially, the gene is first inserted into a plasmid. Bacteria are used to make multiple copies of the plasmid (see Figure 9.1), and the resulting recombinant plasmids are then inserted into mammalian cells that are grown in bottles.

Plant cells can also be grown in culture, altered by rDNA techniques, and then used to generate genetically modified plants. Such plants may prove useful as sources of valuable products, such as plant alkaloids (the painkiller codeine, for example), the isoprenoids that are the basis of synthetic rubber, and melanin (the animal skin pigment) for use in sunscreens. Genetically modified plants have many advantages for the production of human therapeutic agents, including vaccines and antibodies. The advantages include large-scale, low-cost agricultural production and a low risk of product contamination by mammalian pathogens or cancer-causing genes. Genetically modifying plants often requires use of a bacterium. We'll return to the topic of genetically modified plants later in the chapter (page 256).

CHECK YOUR UNDERSTANDING

- How are recombinant clones identified? **9-11**
- What types of cells are used for cloning rDNA? **9-12**

Applications of DNA Technology

LEARNING OBJECTIVES

9-13 List at least five applications of DNA technology.
9-14 Define RNAi.
9-15 Discuss the value of genome projects.
9-16 Define the following terms: *random shotgun sequencing, bioinformatics, proteomics.*
9-17 Diagram the Southern blotting procedure, and provide an example of its use.
9-18 Diagram DNA fingerprinting, and provide an example of its use.
9-19 Outline genetic engineering with *Agrobacterium.*

Clinical Case

The sequences from Dr. B. and patients A, B, C, E, and G share 87.5% of the nucleotide sequence, which is comparable to reported similarities for known linked infections.

Identify the amino acids encoded by the viral DNA. Did this change the percent similarity? (Hint: Refer to Figure 8.8 on page 211).

We have now described the entire sequence of events in cloning a gene. As indicated earlier, such cloned genes can be applied in a variety of ways. One is to produce useful substances more efficiently and less expensively (see the box in Chapter 1, page 3). Another is to obtain information from the cloned DNA that is useful for either basic research, medicine, or forensics. A third is to use cloned genes to alter the characteristics of cells or organisms. The box in Chapter 27, page 783, describes the use of recombinant cells to detect pollutants.

Therapeutic Applications

An extremely valuable pharmaceutical product is the hormone insulin, a small protein produced by the pancreas that controls the body's uptake of glucose from blood. For many years, people with insulin-dependent diabetes controlled their disease by injecting insulin obtained from the pancreases of slaughtered animals. Obtaining this insulin is an expensive process, and the insulin from animals is not as effective as human insulin.

Because of the value of human insulin and the protein's small size, producing human insulin by rDNA techniques was an early goal for the pharmaceutical industry. To produce the hormone, synthetic genes were first constructed for each of the two short polypeptide chains that make up the insulin molecule. The small size of these chains—only 21 and 30 amino acids long—made it possible to use synthetic genes. Following the procedure described earlier (page 245), each of the two synthetic genes was inserted into a plasmid vector and linked to the end of a gene coding for the bacterial enzyme β-galactosidase, so that the insulin polypeptide was coproduced with the enzyme. Two different *E. coli* bacterial cultures were used, one to produce each of the insulin polypeptide chains. The polypeptides were then recovered from the bacteria, separated from the β-galactosidase, and chemically joined to make human insulin. This accomplishment was one of the early commercial successes of DNA technology, and it illustrates a number of the principles and procedures discussed in this chapter.

Another human hormone that is now being produced commercially by genetic modification of *E. coli* is somatostatin. At one time 500,000 sheep brains were needed to produce 5 mg of animal somatostatin for experimental purposes. By contrast, only 8 liters of a genetically modified bacterial culture are now required to obtain the equivalent amount of the human hormone.

Subunit vaccines, consisting only of a protein portion of a pathogen, are being made by genetically modifying yeasts. Subunit vaccines have been produced for a number of diseases, notably hepatitis B. One of the advantages of a subunit vaccine is that there is no chance that the vaccine will cause an infection. The protein is harvested from genetically modified cells and purified for use as a vaccine. Animal viruses such as vaccinia virus can be genetically modified to carry a gene for another microbe's surface protein. When injected, the virus acts as a vaccine against the other microbe.

DNA vaccines are usually circular plasmids that include a gene encoding a viral protein that's under the transcriptional control of a promoter region active in human cells. The plasmids are then cloned in bacteria. Several vaccine trials against HIV, influenza, hepatitis, dengue, breast cancer, and malaria are being conducted. Vaccines are discussed in further detail in Chapter 18 (page 493). **Table 9.2** lists some other important rDNA products used in medical therapy.

The importance of rDNA technology to medical research cannot be emphasized enough. Artificial blood for use in transfusions can now be prepared with human hemoglobin produced in genetically modified pigs. Sheep have also been genetically modified to produce a number of drugs in their milk. This procedure has no apparent effect upon the sheep, and they provide a ready source of raw material for the product that does not require sacrificing animals.

Gene therapy may eventually provide cures for some genetic diseases. It is possible to imagine removing some cells from a person and transforming them with a normal gene to replace a defective or mutated gene. When these cells are returned to the person, they should function normally. For example, gene therapy has been used to treat hemophilia B and severe combined immunodeficiency. Adenoviruses and retroviruses are used most often to deliver genes; however, some researchers are working with plasmid vectors. An attenuated retrovirus was used as the vector when the first gene therapy to treat hemophilia in humans was performed in 1990. Several gene therapy trials are in progress that use genetically modified adenovirus carrying human genes to treat retinal diseases, to replace a heart protein, and to treat the degenerative brain disorder called Canavan disease. Antisense DNA (page 258) introduced into cells is also being explored to treat hepatitis, skin cancer, and high cholesterol.

Thus far, gene therapy results have not been impressive; there have even been a few deaths attributed to the viral vectors. A great deal of preliminary work remains to be done, but cures may not be possible for all genetic diseases.

Gene silencing is a natural process that occurs in a wide variety of eukaryotes and is apparently a defense against viruses and transposons. Gene silencing is similar to miRNA (page 217) in that a gene encoding a small piece of RNA is transcribed. Following transcription, RNAs called **small interfering RNAs (siRNAs)** are formed after processing by an enzyme called *Dicer*. The siRNA molecules bind to mRNA, which is then destroyed by proteins called the **RNA-induced silencing complex (RISC),** thus *silencing* the expression of a gene (**Figure 9.14**).

New technology called **RNA interference (RNAi)** holds promise for gene therapy for treating genetic diseases. A small DNA insert encoding siRNA against the gene of interest could be cloned into a plasmid. When transferred into a cell, the cell would produce the desired siRNA. Clinical trials are currently being conducted to test RNAi to treat macular degeneration and melanoma.

TABLE 9.2 Some Pharmaceutical Products of rDNA

Product	Comments
α-Glucosidase	Produced by genetically modified mammalian cells to treat Pompe disease
Cervical Cancer Vaccine	Consists of viral proteins; produced by *Saccharomyces cerevisiae* or by insect cells
Colony-Stimulating Factor	Counteracts effects of chemotherapy; improves resistance to infectious disease such as AIDS; used in treatment of leukemia; produced by *Escherichia coli* and *S. cerevisiae*
Epidermal Growth Factor (EGF)	Heals wounds, burns, ulcers; produced by *E. coli*
Erythropoietin (EPO)	Treatment of anemia; produced by mammalian cell culture
Interferon	
IFN–α	Therapy for leukemia, melanoma, and hepatitis; produced by *E. coli* and *S. cerevisiae* (yeast)
IFN–β	Treatment for multiple sclerosis; produced by mammalian cell culture
IFN–γ	Treatment of chronic granulomatous disease; produced by *E. coli*
Hepatitis B Vaccine	Produced by *S. cerevisiae* that carries hepatitis-virus gene on a plasmid
Human Growth Hormone (hGH)	Corrects growth deficiencies in children; produced by *E. coli*
Human Insulin	Therapy for diabetes; better tolerated than insulin extracted from animals; produced by *E. coli*
Influenza Vaccine	Vaccine made from *E. coli* or *S. cerevisiae* carrying virus genes
Interleukins	Regulate the immune system; possible treatment for cancer; produced by *E. coli*
Orthoclone OKT3 **Muromonab-CD3**	Monoclonal antibody used in transplant patients to help suppress the immune system, reducing the chance of tissue rejection; produced by mouse cells
Pulmozyme (rhDNase)	Enzyme used to break down mucous secretions in cystic fibrosis patients; produced by mammalian cell culture
Relaxin	Used to ease childbirth; produced by *E. coli*
Superoxide Dismutase (SOD)	Minimizes damage caused by oxygen free radicals when blood is resupplied to oxygen-deprived tissues; produced by *S. cerevisiae* and *Komagataella pastoris* (yeast)
Taxol	Plant product used for treatment for ovarian cancer; produced in *E. coli*
Tissue Plasminogen Activator	Dissolves the fibrin of blood clots; therapy for heart attacks; produced by mammalian cell culture
Tumor Necrosis Factor (TNF)	Causes disintegration of tumor cells; produced by *E. coli*
Veterinary Use	
Canine Distemper Vaccine	Canarypox virus carrying canine distemper virus genes
Feline Leukemia Vaccine	Canarypox virus carrying feline leukemia virus genes

CHECK YOUR UNDERSTANDING

- Explain how DNA technology can be used to treat disease and to prevent disease. **9-13**
- What is gene silencing? **9-14**

Genome Projects

The first genome to be sequenced was from a bacteriophage in 1977. In 1995, the genome of a free-living cell—*Haemophilus influenzae*—was sequenced. Since then, 1000 prokaryotic genomes and over 400 eukaryotic genomes have been sequenced.

In **shotgun sequencing,** small pieces of a genome of a free-living cell are sequenced, and the sequences are then assembled using a computer. Any gaps between the pieces then have to be found and sequenced (**Figure 9.15**). This technique can be used on environmental samples to study the genomes of microorganisms that haven't been cultured. The study of genetic material taken directly from environmental samples is called **metagenomics.**

The Human Genome Project was an international 13-year effort, formally begun in October 1990 and completed in 2003. The goal of the project was to sequence the entire human

Clinical Case Resolved

The amino acid sequence reflects the nucleotide sequence. Analysis of the amino acid signature pattern confirms that the viruses from the dentist and patients are closely related. HIV has a high mutation rate, so HIVs from different individuals are genetically distinct. Dr. B.'s HIV is different from the local control and from the outlier. Dr. B.'s amino acid sequences and those of patients A, B, C, E, and G are distinct from those in the control and in the outlier and from two dental patients with known behavioral risks for HIV infection.

PCR and RFLP analysis has made it possible to track transmission of disease between individuals, communities, and countries. This tracking works best with pathogens that have enough genetic variation to identify different strains.

* * *

Dr. B. died before the mode of transmission could be established. But in the era when he practiced dentistry, it was not always the norm to wear gloves when performing procedures. Patient interviews indicated that Dr. B. didn't like to wear gloves. It is likely that HIV was transmitted when a cut on the doctor's bare hands allowed the virus to enter patients' gums. Today the CDC and state health departments ask dental care providers to use universal precautions, including wearing gloves and masks and sterilizing equipment that is to be reused. Had Dr. B. used standard precautions, it is extremely unlikely he would have infected patients.

239 245 248 250 253

genome, approximately 3 billion nucleotide pairs, comprising 20,000 to 25,000 genes. Thousands of people in 18 countries participated in this project. Researchers collected blood (female) or sperm (male) samples from a large number of donors. Only a few samples were processed as DNA resources, and the source names are protected so that neither donors nor scientists knew whose samples were used. Development of shotgun sequencing greatly speeded the process, and 99% of the genome has been sequenced.

One surprising finding was that less than 2% of the genome encodes a functional product—the other 98% includes miRNA genes, viral remnants, repetitive sequences (called *short tandem repeats*), introns, the chromosome ends (called *telomeres*), and transposons (page 232). Currently, researchers are locating specific genes and determining their functions.

The next goal of researchers is the Human Proteome Project, which will map all the proteins expressed in human cells. Even before it is completed, however, it's yielding data that are of immense value to our understanding of biology. It will also eventually be of great medical benefit, especially for the diagnosis and treatment of genetic diseases.

Scientific Applications

Recombinant DNA technology can be used to make products, but this isn't its only important application. Because of its ability to produce many copies of DNA, it can serve as a sort of DNA "printing press." Once a large amount of a particular piece of DNA is available, various analytic techniques, discussed in this section, can be used to "read" the information contained in the DNA.

In 2010, researchers synthesized the smallest known cellular genome during the Minimal Genome Project. A copy of the *Mycoplasma mycoides* genome was synthesized and transplanted into an *M. capricolum* cell that had had its own DNA removed. The modified cell produced *M. mycoides* proteins. This experiment showed that large-scale changes to a genome can be made and that an existing cell will accept this DNA.

DNA sequencing has produced an enormous amount of information that has spawned the new field of **bioinformatics,** the science of understanding the function of genes through computer-assisted analysis. DNA sequences are stored in web-based databases referred to as GenBank. Genomic information can be

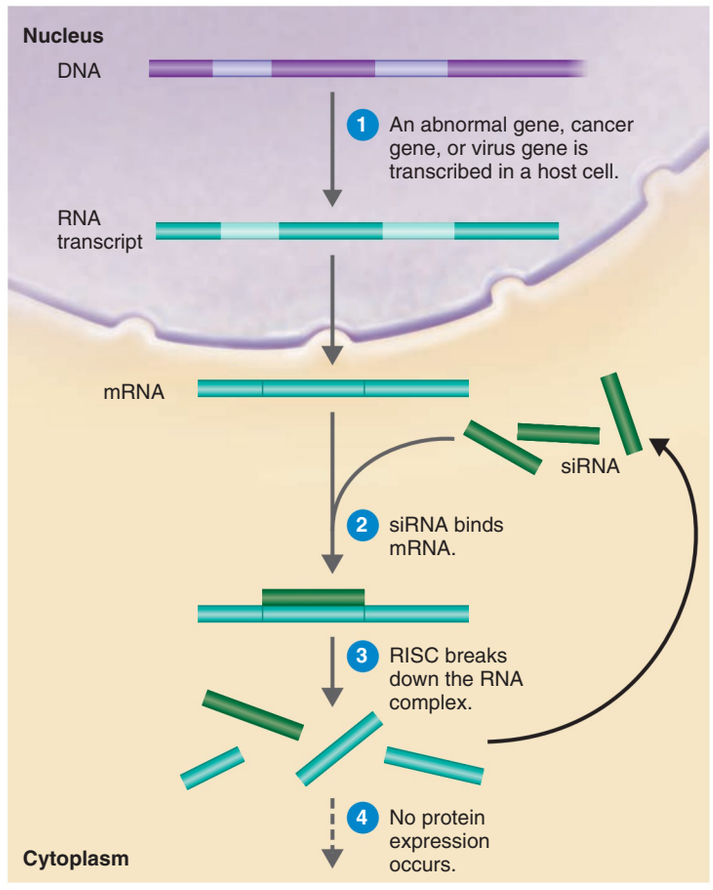


Figure 9.14 Gene silencing could provide treatments for a wide range of diseases.

Q Does RNAi act during or after transcription?

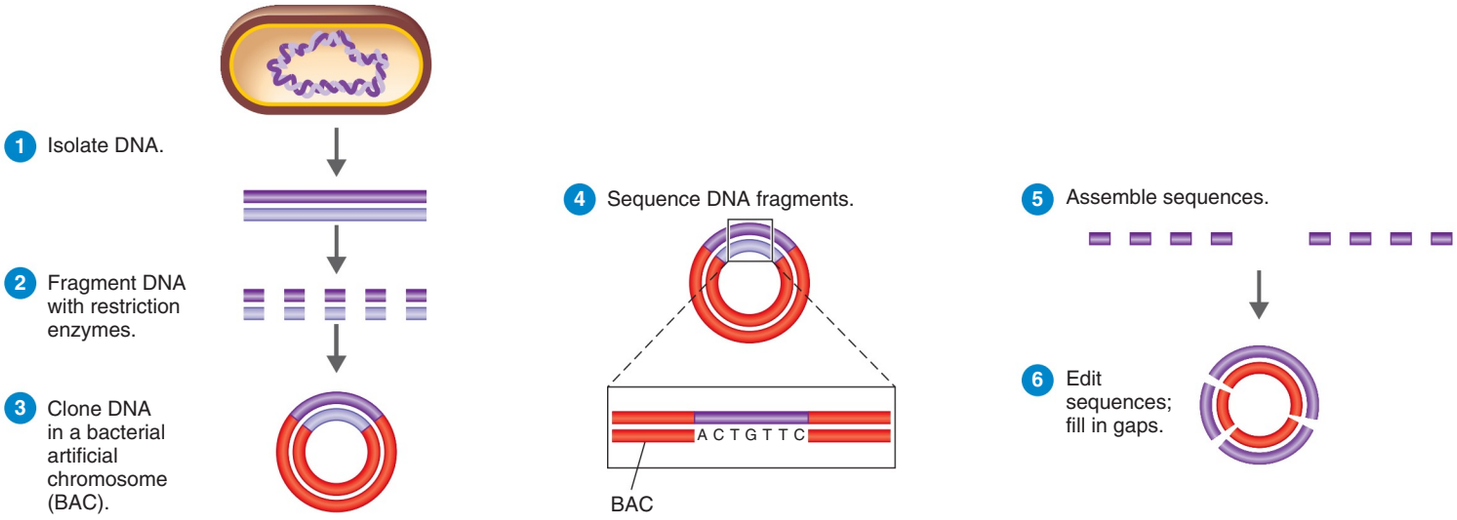


Figure 9.15 Shotgun sequencing. In this technique, a genome is cut into pieces, and each piece is sequenced. Then the pieces are fit together. There may be gaps if a specific DNA fragment was not sequenced.

Q Does this technique identify genes and their locations?

searched with computer programs to find specific sequences or to look for similar patterns in the genomes of different organisms. Microbial genes are now being searched to identify molecules that are the virulence factors of pathogens. By comparing genomes, researchers discovered that *Chlamydia trachomatis* (tra-KŌ-ma-tis) produces a toxin similar to that of *Clostridium difficile* (DIF-fi-sē-il).

The next goal is to identify the proteins encoded by these genes. **Proteomics** is the science of determining all of the proteins expressed in a cell.

Reverse genetics is an approach to discovering the function of a gene from a genetic sequence. Reverse genetics attempts to connect a given genetic sequence with specific effects on the organism. For example, if you mutate or block a gene (see the discussion of gene silencing on page 251), you can then look for a characteristic the organism lost.

An example of the use of human DNA sequencing is the identification and cloning of the mutant gene that causes cystic fibrosis (CF). CF is characterized by the oversecretion of mucus, leading to blocked respiratory passageways. The sequence of the mutated gene can be used as a diagnostic tool in a hybridization technique called **Southern blotting** (Figure 9.16), named for Ed Southern, who developed the technique in 1975.

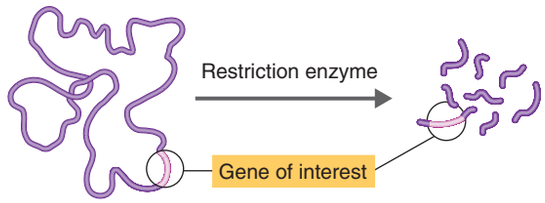
In this technique, subject DNA is digested with a restriction enzyme, yielding thousands of fragments of various sizes. The fragments are called **RFLPs** (pronounced "rif-lip"), for *restriction fragment length polymorphisms*. The different fragments are then separated by **gel electrophoresis**. The fragments are put in a well at one end of a layer of agarose gel. Then an electrical current is passed through the gel. While the charge is applied, the different-sized RFLPs migrate through the gel at different rates. The RFLPs are transferred onto a filter by blotting and are exposed to a labeled probe made from the cloned gene of interest, in this case the CF gene. The probe will hybridize to this mutant gene but not to the normal gene. Fragments to which the probe binds are identified by a colored dye. With this method, any person's DNA can be tested for the presence of the mutated gene.

Genetic testing can now be used to screen for several hundred genetic diseases. Such screening procedures can be performed on prospective parents and also on fetal tissue. Two of the more commonly screened genes are those associated with inherited forms of breast cancer and the gene responsible for Huntington's disease. Genetic testing can help a physician prescribe the correct medication for a patient. The drug herceptin, for example, is effective only in breast cancer patients with a specific nucleotide sequence in the HER2 gene.

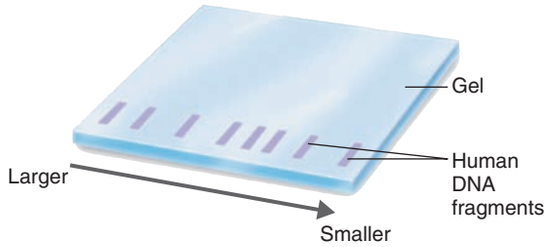
Forensic Microbiology

For several years, microbiologists have used RFLPs in a method of identification known as **DNA fingerprinting** to identify bacterial or viral pathogens (Figure 9.17).

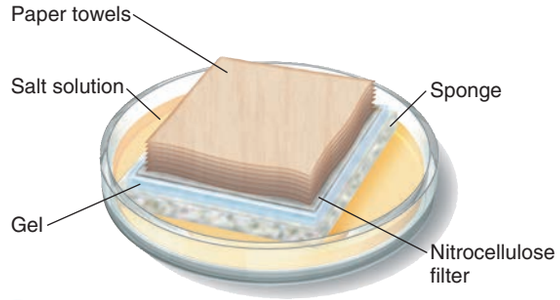
DNA chips (see Figure 10.17, page 283) or *PCR microarrays* that can screen a sample for multiple pathogens at once are now being used. In a DNA chip, up to 22 primers from different microorganisms can be used to initiate the PCR. A suspect microorganism is identified if DNA is copied from one of the primers. At the Centers for Disease Control and Prevention (CDC), PulseNet uses RFLPs to track outbreaks of foodborne disease. In some cases, PCR using specific primers can be used to track a bacterial strain to locate the source of an outbreak.



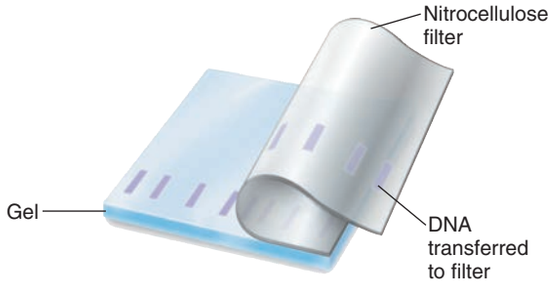
1 DNA containing the gene of interest is extracted from human cells and cut into fragments by restriction enzymes. Fragments are called restriction fragment length polymorphisms, or RFLPs (pronounced "rif-lips").



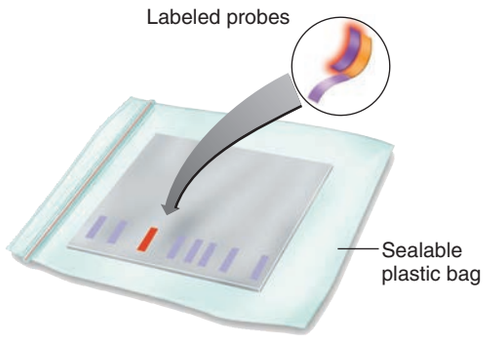
2 The fragments are separated according to size by gel electrophoresis. Each band contains many copies of a particular DNA fragment. The bands are invisible but can be made visible by staining.



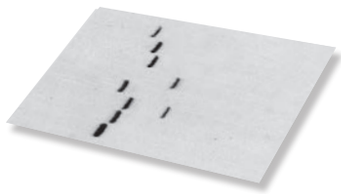
3 The DNA bands are transferred to a nitrocellulose filter by blotting. The solution passes through the gel and filter to the paper towels by capillary action.



4 This produces a nitrocellulose filter with DNA fragments positioned exactly as on the gel.



5 The filter is exposed to a labeled probe for a specific gene. The probe will base-pair (hybridize) with a short sequence present on the gene.



6 The fragment containing the gene of interest is identified by a band on the filter.

Figure 9.16 Southern blotting.

Q What is the purpose of Southern blotting?

The genomics of pathogens has become a mainstay of monitoring, preventing, and controlling infectious disease. The use of genomics to trace a disease outbreak is described in the Clinical Focus box on page 259. The new field of **forensic microbiology** developed because hospitals, food manufacturers, and individuals can be sued in courts of law and because microorganisms can be used as weapons. Microbial forensics has been used in court a few times. In the 1990s, DNA fingerprints of HIV were used for the first time to obtain a rape conviction. Since then, a physician was convicted of injecting his former lover with HIV from one of his patients, based on the DNA fingerprint of the HIV. In the 2001 anthrax attacks in the United States, DNA fingerprints of *Bacillus anthracis* were used to track the source and then the alleged attacker. Northern Arizona University researchers determined that the *B. anthracis* endospores used in a 1993 attack by a cult in Japan were actually a nonpathogenic vaccine strain. No one was hurt when those endospores were released. Currently, a DNA database is being developed for microorganisms that could be used in biological crimes.

The requirements to prove in a court of law the source of a microbe are stricter than for the medical community. For example, to prove intent to commit harm requires collecting evidence properly and establishing a chain of custody of that evidence. Microbial properties that are unimportant in public health may

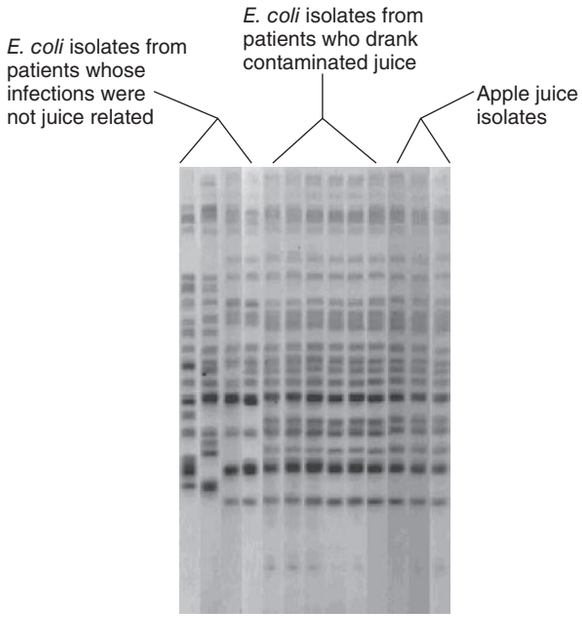


Figure 9.17 DNA fingerprints used to track an infectious disease. This figure shows the RFLP patterns of bacterial isolates from an outbreak of *Escherichia coli* O157:H7. The isolates from apple juice are identical to the patterns of isolates from patients who drank the contaminated juice but different from those from patients whose infections were not juice related.

Q What is forensic microbiology?

be important clues in forensic investigations. The American Academy of Microbiology recently proposed professional certification in forensic microbiology.

DNA can often be extracted from preserved and fossilized materials, including mummies and extinct plants and animals. Although such material is very rare, and usually partially degraded, PCR enables researchers to study environments and organisms that no longer exist in their natural form. The study of unusual organisms has also led to advances in basic taxonomy (discussed in Chapter 10).

Nanotechnology

Nanotechnology deals with the design and manufacture of extremely small electronic circuits and mechanical devices built at the molecular level of matter. Molecule-sized robots or computers can be used to detect contamination in food, diseases in plants, or biological weapons. However, the small machines require small (a nanometer is 10^{-9} meters; 1000 nm fit in 1 µm) wires and components. Bacteria may provide the needed small metals. Researchers at the U.S. Geological Survey have cultured several anaerobic bacteria that reduce toxic selenium, Se^{4+}, to nontoxic elemental Se^{0}, which forms into nanospheres (Figure 9.18). Nanotechnology research is growing, with researchers developing innovative ways of using bacteria to produce nanospheres for potential drug targeting and delivery. Researchers with the U.S. Department of Energy are using bacteria in nanoscale electrical circuits to make hydrogen gas. Swedish researchers are using *Acetobacter xylinum* to build cellulose nanofibers for artificial blood vessels.

CHECK YOUR UNDERSTANDING

- How are shotgun sequencing, bioinformatics, and proteomics related to genome projects? **9-15, 9-16**
- What is Southern blotting? **9-17**
- Why do RFLPs result in a DNA fingerprint? **9-18**

Agricultural Applications

The process of selecting for genetically desirable plants has always been time-consuming. Conventional plant cross-breeding is laborious and involves waiting for the planted seed to germinate and the resulting plant to mature in order to learn whether the plant has the desired traits. Plant breeding has been revolutionized by the use of plant cells grown in culture. Clones of plant cells, including cells that have been genetically altered by rDNA techniques, can be grown in large numbers. These cells can then be induced to regenerate whole plants, from which seeds can be harvested.

Recombinant DNA can be introduced into plant cells in several ways. Previously we mentioned protoplast fusion and the use of DNA-coated "bullets." The most elegant method, however, makes use of a plasmid called the **Ti plasmid** (*Ti* stands for tumor-inducing), which occurs naturally in the bacterium *Agrobacterium tumefaciens* (TOO-mah-fas′ē-enz). This bacterium infects certain plants, in which the Ti plasmid causes the formation of a tumorlike growth called a crown gall (Figure 9.19). A part of the Ti plasmid, called T-DNA, integrates into the genome of the infected plant. The T-DNA stimulates local cellular growth (the crown gall) and simultaneously causes the production of certain products used by the bacteria as a source of nutritional carbon and nitrogen.

Figure 9.18 *Bacillus* cells growing on selenium form chains of elemental selenium.

Q What might bacteria provide for nanotechnology?

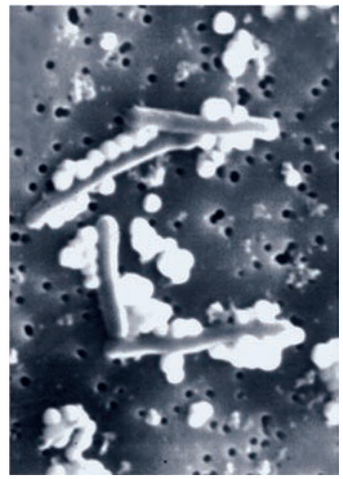


Figure 9.19 Crown gall disease on a rose plant. The tumorlike growth is stimulated by a gene on the Ti plasmid carried by the bacterium *Agrobacterium tumefaciens,* which has infected the plant.

Q What are some of the agricultural applications of rDNA technology?

For plant scientists, the attraction of the Ti plasmid is that it provides a vehicle for introducing rDNA into a plant (**Figure 9.20**). A scientist can insert foreign genes into the T-DNA, put the recombinant plasmid back into the *Agrobacterium* cell, and use the bacterium to insert the recombinant Ti plasmid into a plant cell. The plant cell with the foreign gene can then be used to generate a new plant. With luck, the new plant will express the foreign gene. Unfortunately, *Agrobacterium* does not naturally infect grasses, so it cannot be used to improve grains such as wheat, rice, or corn.

Noteworthy accomplishments of this approach are the introduction into plants of resistance to the herbicide glyphosate. Normally, the herbicide kills both weeds and useful plants by inhibiting an enzyme necessary for making certain essential amino acids. Some *Salmonella* bacteria happen to have this enzyme, but it is resistant to the herbicide. When the DNA for this enzyme is introduced into a crop plant, the crop becomes resistant to the herbicide, which then kills only the weeds. *Bacillus thuringiensis* bacteria are pathogenic to some insects because they produce a protein called Bt toxin that interferes with the insect digestive tract. The Bt gene has been inserted into a variety of crop plants, including cotton and potatoes, so insects that eat the plants will be killed. There is now a variety of plants in which different herbicide and pesticide resistances have been engineered. Resistance to drought, viral infection,

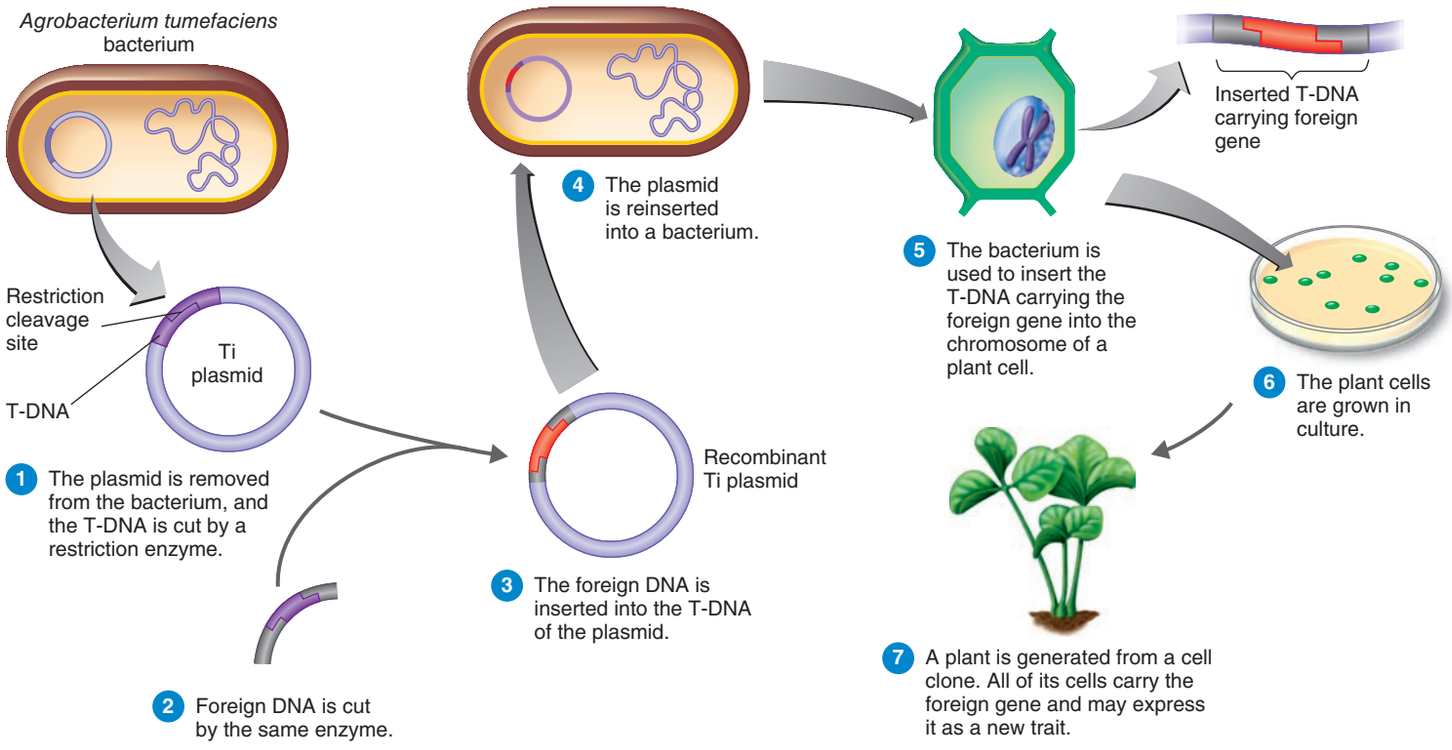


Figure 9.20 Using the Ti plasmid as a vector for genetic modification in plants.

Q Why is the Ti plasmid important to biotechnology?

TABLE 9.3 Some Agriculturally Important Products of rDNA Technology

Product	Comments
AGRICULTURAL PRODUCTS	
Bt cotton and Bt corn	Plants have toxin-producing gene from *Bacillus thuringiensis;* toxin kills insects that eat plants.
Genetically modified tomatoes, raspberries	Antisense gene blocks pectin degradation, so fruits have longer shelf life.
Pseudomonas fluorescens bacterium	Has toxin-producing gene from insect pathogen *B. thuringiensis;* toxin kills root-eating insects that ingest bacteria.
Pseudomonas syringae, ice-minus bacterium	Lacks normal protein product that initiates undesirable ice formation on plants.
Rhizobium meliloti bacterium	Modified for enhanced nitrogen fixation.
RoundUp (glyphosate)-resistant crops	Plants have bacterial gene; allows use of herbicide on weeds without damaging crops.
ANIMAL HUSBANDRY PRODUCTS	
Bovine growth hormone (bGH)	Improves weight gain and milk production in cattle; produced by *E. coli.*
Porcine growth hormone (pGH)	Improves weight gain in swine; produced by *E. coli.*
Transgenic animals	Genetic modification of animals to produce medically useful products in their milk.
OTHER FOOD PRODUCTION PRODUCTS	
Cellulase	Enzyme that degrades cellulose to make animal feedstocks; produced by *E. coli.*
Chymogen	Causes formation of milk curds in cheese-making; produced by *Aspergillus niger.*

and several other environmental stresses has also been engineered into crop plants.

Another example involves MacGregor tomatoes, which stay firm after harvest because the gene for polygalacturonase (PG), the enzyme that breaks down pectin, is suppressed. The suppression was accomplished by **antisense DNA** technology. First, a length of DNA complementary to the PG mRNA is synthesized. This antisense DNA is taken up by the cell and binds to the mRNA to inhibit translation. The DNA-RNA hybrid is broken down by the cell's enzymes, freeing the antisense DNA to disable another mRNA.

Perhaps the most exciting potential use of genetically modified plants concerns nitrogen fixation, the ability to convert the nitrogen gas in the air to compounds that living cells can use (see page 775). The availability of such nitrogen-containing nutrients is usually the main factor limiting crop growth. But in nature, only certain bacteria have genes for carrying out this process. Some plants, such as alfalfa, benefit from a symbiotic relationship with these microbes. Species of the symbiotic bacterium *Rhizobium* have already been genetically modified for enhanced nitrogen fixation. In the future, *Rhizobium* strains may be designed that can colonize such crop plants as corn and wheat, perhaps eliminating their requirement for nitrogen fertilizer. The ultimate goal would be to introduce functioning nitrogen-fixation genes directly into the plants. Although this goal cannot be achieved with our current knowledge, work toward it will continue because of its potential for dramatically increasing the world's food supply.

An example of a genetically modified bacterium now in agricultural use is *Pseudomonas fluorescens* that has been engineered to produce Bt toxin, normally produced by *Bacillus thuringiensis.* This toxin kills certain insects, such as the European corn borer. The genetically altered *Pseudomonas,* which produces much more toxin than *B. thuringiensis,* can be added to plant seeds and in time will enter the vascular system of the growing plant. Its toxin is ingested by the feeding borer larvae and kills them (but is harmless to humans and other warm-blooded animals).

Animal husbandry has also benefited from rDNA technology. We have seen how one of the early commercial products of rDNA was human growth hormone. By similar methods it is possible to manufacture bovine growth hormone (bGH). When bGH is injected into beef cattle, it increases their weight gain; in dairy cows, it also causes a 10% increase in milk production. Such procedures have met with resistance from consumers, primarily as a result of as-yet unsubstantiated fears that some of the bGH would be present in the milk or meat of these cattle and might be harmful to humans.

Table 9.3 lists these and several other rDNA products used in agriculture and animal husbandry.

CHECK YOUR UNDERSTANDING

✓ Of what value is the plant pathogen *Agrobacterium*? **9-19**

Safety Issues and the Ethics of Using DNA Technology

LEARNING OBJECTIVE

9-20 List the advantages of, and problems associated with, the use of genetic modification techniques.

Norovirus—Who Is Responsible for the Outbreak?

As you read through this box, you will encounter a series of questions that microbiologists ask themselves as they trace a disease outbreak. Whether the microbiologist is called as an expert witness in court will depend on whether a lawsuit is filed. Try to answer each question before going on to the next one.

1. On May 7, Nadia Koehler, a microbiologist at a county health department, is notified of a gastroenteritis outbreak among 115 people. The case is defined as vomiting and diarrhea and fever, cramps, or nausea.
   What information does Nadia need?
2. Nadia needs to find out where the ill people have been in the past 48 hours. After several interviews, Nadia finds out that the ill people include 23 school employees, 55 publishing company employees, 9 employees of a social service organization, and 28 other people (see **Figure A**).
   Now what does Nadia need to know?
3. Next, Nadia finds out what these 115 people have in common. In her investigation, Nadia discovers that on May 2, the school staff had been served a party-sized sandwich catered by a national franchise restaurant. On May 3, the publishing company and social service staff luncheons were catered by the same restaurant. The remaining 28 people ate sandwiches at the same restaurant, at varying times between these two days.
   What does Nadia do next?
4. Nadia analyzes exposures to 16 food items; the results show that eating lettuce is significantly associated with illness.
   What is Nadia's next step?
5. Nadia then requests a reverse-transcription PCR (RT-PCR) using a norovirus primer to be done on stool samples (**Figure B**).
   What did Nadia conclude?
6. RT-PCR confirmed norovirus infection. Nadia's next request is for a sequence analysis to be performed on 21 stool specimens. The results demonstrated 100% sequence homology for the 21 specimens.
   What should Nadia do next?
7. Nadia learns that a food handler employed by the restaurant had experienced vomiting and diarrhea on May 1. The food handler believes he had acquired the illness from his child. The child's illness was traced to an ill cousin who had been exposed to norovirus at a child-care center. The food handler's vomiting ended by the early morning of May 2, and he returned to work at the restaurant later that morning.
   What should Nadia look for now?
8. Now Nadia compares the virus strains from the food handler to the ones from the ill customers. She requests a sequence analysis on viruses from the food handler and eight ill customers. They are identical to the strains identified in step 6.
   Where does Nadia look next?
9. Nadia looks for any areas in the restaurant that still may be contaminated by the norovirus. She finds out that the lettuce was sliced each morning by the food handler who had been sick. Nadia's inspection reveals that the food preparation sink is also used for handwashing. The sink was not sanitized before and after the lettuce was washed. The health department closes the restaurant until it can be cleaned with the proper sanitizers.

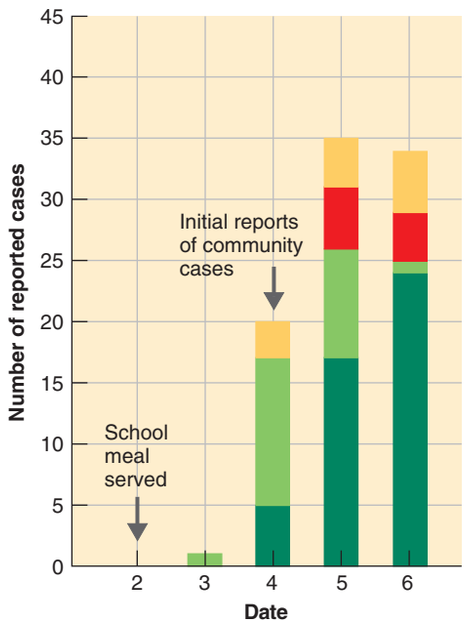


Figure A **Number of cases reported.**

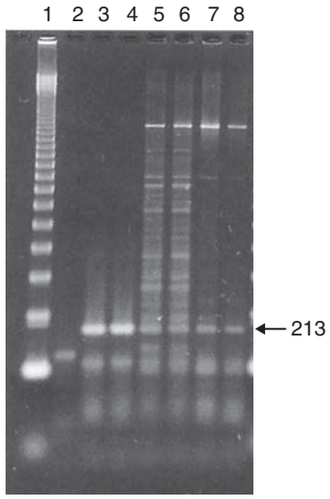


Figure B **Results of PCR of patient samples. Lane 1, 123-bp size ladders. Lane 2, negative RT-PCR control; Lanes 3–8, patient samples. Norovirus is identified by the 213-bp band of DNA.**

Noroviruses are the most common cause of outbreaks of acute gastroenteritis worldwide. During 2012, 287 norovirus outbreaks in the United States resulted in over 6000 infections.

Source: Adapted from CDC, Foodborne Outbreak Online Database (FOOD). http://www/cdc.gov.

There will always be concern about the safety of any new technology, and genetic modification and biotechnology are certainly no exceptions. One reason for this concern is it's nearly impossible to prove that something is entirely safe under all conceivable conditions. People worry that the same techniques that can alter a microbe or plant to make them useful to humans could also inadvertently make them pathogenic to humans or otherwise dangerous to living organisms or could create an ecological nightmare. Therefore, laboratories engaged in rDNA research must meet rigorous standards of control to avoid either accidental release of genetically modified organisms into the environment or exposure of humans to any risk of infection. To reduce risk further, microbiologists engaged in genetic modification often delete from the microbes' genomes certain genes that are essential for growth in environments outside the laboratory. Genetically modified organisms intended for use in the environment (in agriculture, for example) may be engineered to contain "suicide genes"—genes that eventually turn on to produce a toxin that kills the microbes, thus ensuring that they will not survive in the environment for very long after they have accomplished their task.

The safety issues in agricultural biotechnology are similar to those concerning chemical pesticides: toxicity to humans and to nonpest species. Although not shown to be harmful, genetically modified foods have not been popular with consumers. In 1999, researchers in Ohio noticed that humans may develop allergies to *Bacillus thuringiensis* (Bt) toxin after working in fields sprayed with the insecticide. And an Iowa study showed that the caterpillar stage of Monarch butterflies could be killed by ingesting windblown Bt-carrying pollen that landed on milkweed, the caterpillars' normal food. Crop plants can be genetically modified for herbicide resistance so that fields can be sprayed to eliminate weeds without killing the desired crop. However, if the modified plants pollinate related weed species, weeds could become resistant to herbicides, making it more difficult to control unwanted plants. An unanswered question is whether releasing genetically modified organisms will alter evolution as genes move to wild species.

These developing technologies also raise a variety of ethical issues. Genetic testing for diseases is becoming routine. Who should have access to this information? Should employers have the right to know the results of such tests? How can we be assured that such information will not be used to discriminate against certain groups? Should individuals be told they will get an incurable disease? If so, when?

Genetic counseling, which provides advice and counseling to prospective parents with family histories of genetic disease, is becoming more important in considerations about whether to have children.

There are probably just as many harmful applications of a new technology as there are helpful ones. It is particularly easy to imagine DNA technology being used to develop new and powerful biological weapons. In addition, because such research efforts are performed under top-secret conditions, it is virtually impossible for the general public to learn of them.

Perhaps more than most new technologies, molecular genetics holds the promise of affecting human life in previously unimaginable ways. It is important that society and individuals be given every opportunity to understand the potential impact of these new developments.

Like the invention of the microscope, the development of DNA techniques is causing profound changes in science, agriculture, and human health care. With this technology only slightly more than 30 years old, it is difficult to predict exactly what changes will occur. However, it is likely that within another 30 years, many of the treatments and diagnostic methods discussed in this book will have been replaced by far more powerful techniques based on the unprecedented ability to manipulate DNA precisely.

CHECK YOUR UNDERSTANDING

✓ Identify two advantages and two problems associated with genetically modified organisms. **9-20**

Study Outline

MasteringMicrobiology®

Study microbiology where it matters: everywhere.
Check out the Study Area of MasteringMicrobiology to explore Interactive Microbiology. Study on-the-go with Dynamic Study Modules. Check your understanding with chapter quizzes and animations—and much more!

Introduction to Biotechnology (pp. 239–241)

1. Biotechnology is the use of microorganisms, cells, or cell components to make a product.

Recombinant DNA Technology (p. 239)

2. Closely related organisms can exchange genes in natural recombination.
3. Genes can be transferred among unrelated species via laboratory manipulation, called rDNA technology.
4. Recombinant DNA is DNA that has been artificially manipulated to combine genes from two different sources.

An Overview of Recombinant DNA Procedures (pp. 239–241)

5. A desired gene is inserted into a DNA vector, such as a plasmid or a viral genome.

6. The vector inserts the DNA into a new cell, which is grown to form a clone.
7. Large quantities of the gene product can be harvested from the clone.

Tools of Biotechnology (pp. 241–244)

Selection (p. 241)

1. Microbes with desirable traits are selected for culturing by artificial selection.

Mutation (p. 241)

2. Mutagens are used to cause mutations that might result in a microbe with desirable traits.
3. Site-directed mutagenesis is used to change a specific codon in a gene.

Restriction Enzymes (pp. 241–242)

4. Prepackaged kits are available for rDNA techniques.
5. A restriction enzyme recognizes and cuts only one particular nucleotide sequence in DNA.
6. Some restriction enzymes produce sticky ends, short stretches of single-stranded DNA at the ends of the DNA fragments.
7. Fragments of DNA produced by the same restriction enzyme will spontaneously join by base pairing. DNA ligase can covalently link the DNA backbones.

Vectors (pp. 242–243)

8. Vectors are DNA used to transfer other DNA between cells.
9. A plasmid containing a new gene can be inserted into a cell by transformation.
10. A virus containing a new gene can insert the gene into a cell.

Polymerase Chain Reaction (pp. 243–244)

11. The polymerase chain reaction (PCR) is used to make multiple copies of a desired piece of DNA enzymatically.
12. PCR can be used to increase the amounts of DNA in samples to detectable levels. This may allow sequencing of genes, the diagnosis of genetic diseases, or the detection of viruses.

Techniques of Genetic Modification (pp. 244–250)

Inserting Foreign DNA into Cells (pp. 245–246)

1. Cells can take up naked DNA by transformation. Chemical treatments are used to make cells that are not naturally competent take up DNA.
2. Pores made in protoplasts and animal cells by electric current in the process of electroporation can provide entrance for new pieces of DNA.
3. Protoplast fusion is the joining of cells whose cell walls have been removed.
4. Foreign DNA can be introduced into plant cells by shooting DNA-coated particles into the cells.
5. Foreign DNA can be injected into animal cells by using a fine glass micropipette.

Obtaining DNA (pp. 246–248)

6. Genomic libraries can be made by cutting up an entire genome with restriction enzymes and inserting the fragments into bacterial plasmids or phages.
7. Complementary DNA (cDNA) made from mRNA by reverse transcription can be cloned in genomic libraries.
8. Synthetic DNA can be made in vitro by a DNA synthesis machine.

Selecting a Clone (pp. 248–249)

9. Antibiotic-resistance markers on plasmid vectors are used to identify cells containing the engineered vector by direct selection.
10. In blue-white screening, the vector contains the genes for amp^R and β-galactosidase.
11. The desired gene is inserted into the β-galactosidase gene site, destroying the gene.
12. Clones containing the recombinant vector will be resistant to ampicillin and unable to hydrolyze X-gal (white colonies). Clones containing the vector without the new gene will be blue. Clones lacking the vector will not grow.
13. Clones containing foreign DNA can be tested for the desired gene product.
14. A short piece of labeled DNA called a DNA probe can be used to identify clones carrying the desired gene.

Making a Gene Product (pp. 249–250)

15. *E. coli* is used to produce proteins using rDNA because *E. coli* is easily grown and its genomics are well understood.
16. Efforts must be made to ensure that *E. coli*'s endotoxin does not contaminate a product intended for human use.
17. To recover the product, *E. coli* must be lysed, or the gene must be linked to a gene that produces a naturally secreted protein.
18. Yeasts can be genetically modified and are likely to secrete a gene product continuously.
19. Genetically modified mammalian cells can be grown to produce proteins such as hormones for medical use.
20. Genetically modified plant cells can be grown and used to produce plants with new properties.

Applications of DNA Technology (pp. 250–258)

1. Cloned DNA is used to produce products, study the cloned DNA, and alter the phenotype of an organism.

Therapeutic Applications (pp. 251–252)

2. Synthetic genes linked to the β-galactosidase gene (*lacZ*) in a plasmid vector were inserted into *E. coli,* allowing *E. coli* to produce and secrete the two polypeptides used to make human insulin.
3. Cells and viruses can be modified to produce a pathogen's surface protein, which can be used as a vaccine.
4. DNA vaccines consist of rDNA cloned in bacteria.
5. Gene therapy can be used to cure genetic diseases by replacing the defective or missing gene.
6. RNAi may be useful to prevent expression of abnormal proteins.

Genome Projects (pp. 252–253)

7. Nucleotide sequences of genomes from more than 1000 organisms, including humans, have been completed.
8. This leads to determining the proteins produced in a cell.

Scientific Applications (pp. 253–256)

9. DNA can be used to increase understanding of DNA, for genetic fingerprinting, and for gene therapy.

10. DNA sequencing machines are used to determine the nucleotide base sequence of restriction fragments in shotgun sequencing.
11. Bioinformatics is the use of computer applications to study genetic data; proteomics is the study of a cell's proteins.
12. Southern blotting can be used to locate a gene in a cell.
13. DNA probes can be used to quickly identify a pathogen in body tissue or food.
14. Forensic microbiologists use DNA fingerprinting to identify the source of bacterial or viral pathogens.
15. Bacteria may be used to make nano-sized materials for nanotechnology machines.

Agricultural Applications (pp. 256–258)

16. Cells from plants with desirable characteristics can be cloned to produce many identical cells. These cells can then be used to produce whole plants from which seeds can be harvested.
17. Plant cells can be modified by using the Ti plasmid vector. The tumor-producing T genes are replaced with desired genes, and the rDNA is inserted into *Agrobacterium*. The bacterium naturally transforms its plant hosts.
18. Antisense DNA can prevent expression of unwanted proteins.

Safety Issues and the Ethics of Using DNA Technology (pp. 258–260)

1. Strict safety standards are used to avoid the accidental release of genetically modified microorganisms.
2. Some microbes used in rDNA cloning have been altered so that they cannot survive outside the laboratory.
3. Microorganisms intended for use in the environment may be modified to contain suicide genes so that the organisms do not persist in the environment.
4. Genetic testing raises a number of ethical questions: Should employers and insurance companies have access to a person's genetic records? Will some people be targeted for either breeding or sterilization? Will genetic counseling be available to everyone?
5. Genetically modified crops must be safe for consumption and for release in the environment.

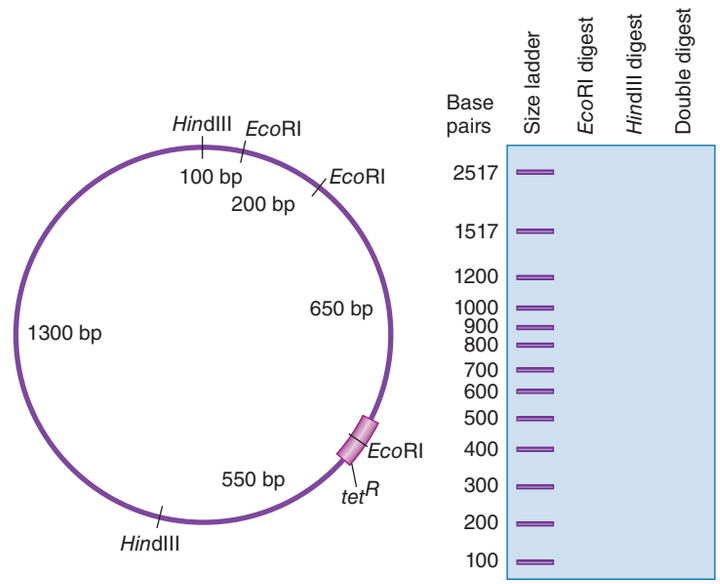
Study Questions

For answers to Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. Compare and contrast the following terms:
   a. *cDNA* and *gene*
   b. *RFLP* and *gene*
   c. *DNA probe* and *gene*
   d. *DNA polymerase* and *DNA ligase*
   e. *rDNA* and *cDNA*
   f. *genome* and *proteome*
2. Differentiate the following terms. Which one is "hit and miss"—that is, does *not* add a specific gene to a cell?
   a. protoplast fusion
   b. gene gun
   c. microinjection
   d. electroporation
3. Some commonly used restriction enzymes are listed in Table 9.1 on page 242.
   a. Indicate which enzymes produce sticky ends.
   b. Of what value are sticky ends in making rDNA?
4. Suppose you want multiple copies of a gene you have synthesized. How would you obtain the necessary copies by cloning? By PCR?
5. DRAW IT Using the following map of plasmid pMICRO, diagram the locations of the restriction fragments that result from digesting pMICRO with *Eco*RI, *Hin*dIII, and both enzymes together following electrophoresis. Which enzyme makes the smallest fragment containing the tetracycline resistance gene?



6. Describe an rDNA experiment in two or three sentences. Use the following terms: intron, exon, DNA, mRNA, cDNA, RNA polymerase, reverse transcriptase.
7. List at least two examples of the use of rDNA in medicine and in agriculture.
8. You are attempting to insert a gene for saltwater tolerance into a plant by using the Ti plasmid. In addition to the desired gene, you add a gene for tetracycline resistance (tet^R) to the plasmid. What is the purpose of the tet^R gene?
9. How does RNAi "silence" a gene?
10. NAME IT This virus family, normally associated with AIDS, may be useful for gene therapy.

Multiple Choice

1. Restriction enzymes were first discovered with the observation that
   a. DNA is restricted to the nucleus.
   b. phage DNA is destroyed in a host cell.
   c. foreign DNA is kept out of a cell.
   d. foreign DNA is restricted to the cytoplasm.
   e. all of the above
2. The DNA probe, 3′-GGCTTA, will hybridize with which of the following?
   a. 5′-CCGUUA
   b. 5′-CCGAAT
   c. 5′-GGCTTA
   d. 3′-CCGAAT
   e. 3′-GGCAAU
3. Which of the following is the fourth basic step to genetically modify a cell?
   a. transformation
   b. ligation
   c. plasmid cleavage
   d. restriction-enzyme digestion of gene
   e. isolation of gene
4. The following enzymes are used to make cDNA. What is the second enzyme used to make cDNA?
   a. reverse transcriptase
   b. ribozyme
   c. RNA polymerase
   d. DNA polymerase
5. If you put a gene in a virus, the next step in genetic modification would be
   a. insertion of a plasmid.
   b. transformation.
   c. transduction.
   d. PCR.
   e. Southern blotting.
6. You have a small gene that you want replicated by PCR. You add radioactively labeled nucleotides to the PCR thermal cycler. After three replication cycles, what percentage of the DNA single strands are radioactively labeled?
   a. 0%
   b. 12.5%
   c. 50%
   d. 87.5%
   e. 100%

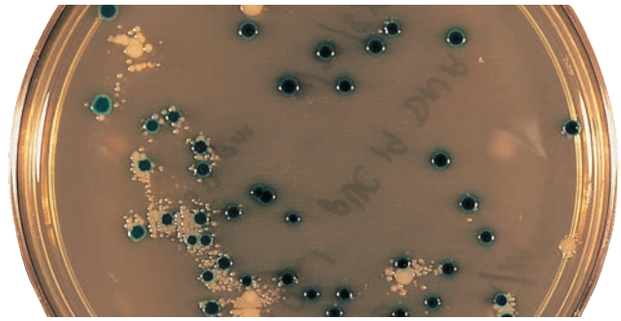
Match the following choices to the statements in questions 7 through 10.

a. antisense
b. clone
c. library
d. Southern blot
e. vector

7. Pieces of human DNA stored in yeast cells.
8. A population of cells carrying a desired plasmid.
9. Self-replicating DNA for transmitting a gene from one organism to another.
10. A gene that hybridizes with mRNA.

Analysis

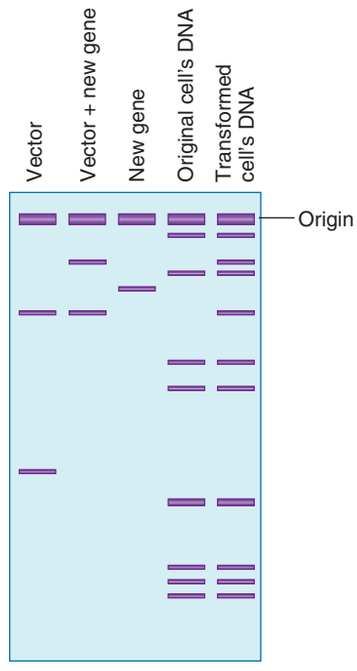
1. Design an experiment using vaccinia virus to make a vaccine against the AIDS virus (HIV).
2. Why did the use of DNA polymerase from the bacterium *Thermus aquaticus* allow researchers to add the necessary reagents to tubes in a preprogrammed heating block?
3. The following picture shows bacterial colonies growing on X-gal plus ampicillin in a blue-white screening test. Which colonies have the recombinant plasmid? The small satellite colonies do not have the plasmid. Why did they start growing on the medium 48 hours after the larger colonies?



Clinical Applications and Evaluation

1. PCR has been used to examine oysters for the presence of *Vibrio cholerae*. Oysters from different areas were homogenized, and DNA was extracted from the homogenates. The DNA was digested by the restriction enzyme *Hinc*II. A primer for the hemolysin gene of *V. cholerae* was used for the PCR reaction. After PCR, each sample was electrophoresed and stained with a probe for the hemolysin gene. Which of the oyster samples were (was) positive for *V. cholerae*? How can you tell? Why look for *V. cholerae* in oysters? What is the advantage of PCR over conventional biochemical tests to identify the bacteria?

2. Using the restriction enzyme *Eco*RI, the following gel electrophoresis patterns were obtained from digests of various DNA molecules from a transformation experiment. Can you conclude from these data that transformation occurred? Explain why or why not.



In the Clinic

As a hospice nurse, you are caring for a 75-year-old patient undergoing chemotherapy for cancer who recently developed pneumonia. The patient is homebound, and none of his visitors have been sick. While at the patient's house to collect a sputum sample for the laboratory, you notice the man's dog also has a cough. You end up swabbing the pet's nose and sending that sample in, too. **The cultures for both man and dog grow gram-negative, oxidase-positive, urease- and H_2S-positive bacteria. What is the causative agent of these infections?**

Hint: Look at the figure on page 274 to narrow the possibilities.

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

10 Classification of Microorganisms

The science of classification, especially the classification of living forms, is called *taxonomy* (from the Greek for orderly arrangement). The objective of taxonomy is to classify living organisms—that is, to establish the relationships between one group of organisms and another and to differentiate them. There may be as many as 100 million different living organisms, but fewer than 10% have been discovered, much less classified and identified.

Taxonomy also provides a common reference for identifying organisms already classified. For example, when a bacterium suspected of causing a specific disease is isolated from a patient, characteristics of that isolate are matched to lists of characteristics of previously classified bacteria to identify the isolate (see the Applications of Microbiology box on page 275). Finally, taxonomy is a basic and necessary tool for scientists, providing a universal language of communication.

Modern taxonomy is an exciting and dynamic field. The ability to rapidly sequence DNA, even entire genomes, has led to new insights into classification and evolution. In this chapter, you will learn the various classification systems, the different criteria used for classification, and tests that are used to identify microorganisms that have already been classified. The contribution of taxonomy in shedding new light on previously discovered organisms such as the *Pneumocystis jirovecii* shown in the photograph will be discussed in this chapter.

***Pneumocystis jirovecii* in human lung tissue.**

The Study of Phylogenetic Relationships

LEARNING OBJECTIVES

10-1 Define *taxonomy, taxon,* and *phylogeny*.

10-2 Discuss the limitations of a two-kingdom classification system.

10-3 Identify the contributions of Linnaeus, Whittaker, and Woese.

10-4 Discuss the advantages of the three-domain system.

10-5 List the characteristics of the Bacteria, Archaea, and Eukarya domains.

In 2001, an international project called the All Species Inventory was launched. The project's purpose is to identify and record every species of life on Earth in the next 25 years. These researchers have undertaken a challenging goal: whereas biologists have identified more than 1.7 million different organisms thus far, it is estimated that the number of living species ranges from 10 to 100 million.

Among these many and diverse organisms, however, are many similarities. For example, all organisms are composed of cells surrounded by a plasma membrane, use ATP for energy, and store their genetic information in DNA. These similarities are the result of evolution, or descent from a common ancestor. In 1859, the English naturalist Charles Darwin proposed that natural selection was responsible for the similarities as well as the differences among organisms. The differences can be attributed to the survival of organisms with traits best suited to a particular environment.

To facilitate research, scholarship, and communication, we use **taxonomy**—that is, we put organisms into categories, or **taxa** (singular: *taxon*), to show degrees of similarities among organisms. These similarities are due to relatedness—all organisms are related through evolution. **Systematics,** or **phylogeny,** is the study of the evolutionary history of organisms, and the hierarchy of taxa reflects their evolutionary, or *phylogenetic,* relationships.

The way we have classified organisms has changed greatly over the centuries. From the time of Aristotle, living organisms were categorized in just two ways, as either plants or animals. In 1735 Carolus Linnaeus introduced a formal system of classification with two kingdoms—Plantae and Animalia. As the biological sciences developed, a *natural classification* system—one that groups organisms based on ancestral relationships and allows us to see the order in life—was sought. In the 1800s, Carl von Nägeli proposed that bacteria and fungi be placed in the plant kingdom while Ernst Haeckel proposed the Kingdom Protista, to include bacteria, protozoa, algae, and fungi. For 100 years, biologists continued to follow von Nägeli's placement of bacteria and fungi in the plant kingdom—ironic, given that DNA sequencing shows that fungi are closer to animals than plants. Fungi were placed in their own kingdom in 1959.

The term *prokaryote* was introduced in 1937 to distinguish cells having no nucleus from the nucleated cells of plants and animals. In 1968, Robert G. E. Murray proposed the Kingdom Prokaryotae.

In 1969, Robert H. Whittaker founded the five-kingdom system in which prokaryotes were placed in the Kingdom Prokaryotae, or Monera, and eukaryotes comprised the other four kingdoms. The Kingdom Prokaryotae had been based on microscopic observations. Subsequent advances in molecular biology revealed that there are actually two types of prokaryotic cells and one type of eukaryotic cell.

CHECK YOUR UNDERSTANDING

- Of what value is taxonomy and systematics? **10-1**
- Why shouldn't bacteria be placed in the plant kingdom? **10-2, 10-3**

The Three Domains

The discovery of three cell types was based on the observations that ribosomes are not the same in all cells (see Chapter 4, page 91). Ribosomes are present in all cells. Comparing the sequences of nucleotides in ribosomal RNA (see page 283) from different kinds of cells shows that there are three distinctly different cell groups: the eukaryotes and two different types of prokaryotes—the bacteria and the archaea.

In 1978, Carl R. Woese proposed elevating the three cell types to a level above kingdom, called domain. Woese believed that the archaea and the bacteria, although similar in appearance, should form their own separate domains on the evolutionary tree (**Figure 10.1**). In addition to differences in rRNA, the three domains differ in membrane lipid structure, transfer RNA molecules, and sensitivity to antibiotics (**Table 10.1**).

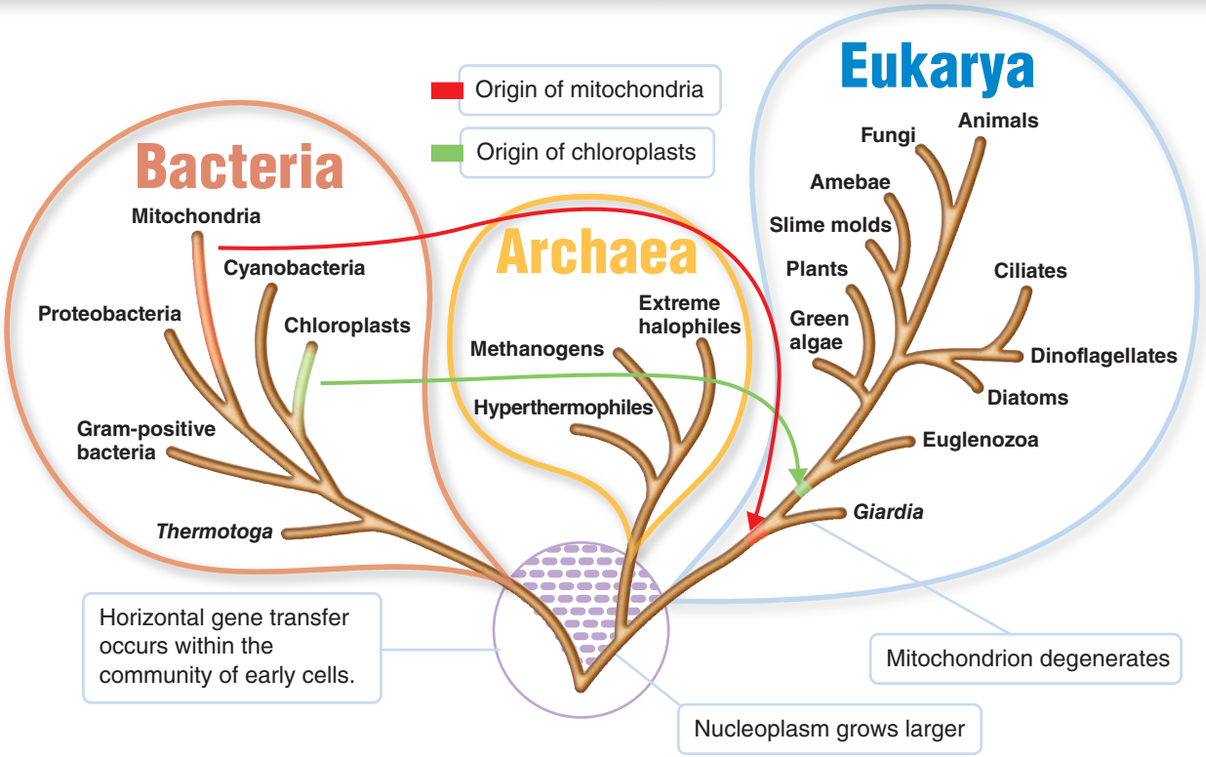
In this widely accepted scheme, animals, plants, and fungi are kingdoms in the Domain **Eukarya.** The Domain **Bacteria** includes all of the pathogenic prokaryotes as well as many of the nonpathogenic prokaryotes found in soil and water. The photoautotrophic prokaryotes are also in this domain. The Domain **Archaea** includes prokaryotes that do not have peptidoglycan in their cell walls. They often live in extreme environments and carry out unusual metabolic processes. Archaea include three major groups:

1. Methanogens, strict anaerobes that produce methane (CH_4) from carbon dioxide and hydrogen
2. Extreme halophiles, which require high concentrations of salt for survival
3. Hyperthermophiles, which normally grow in extremely hot environments

The evolutionary relationship of the three domains is the subject of current research by biologists. Based on rRNA analysis,

FOUNDATION FIGURE 10.1

Three-Domain System



KEY CONCEPTS

- All organisms evolved from cells that formed over 3 billion years ago.
- The DNA passed on from ancestors is described as *conserved*.
- The Domain Eukarya includes the Kingdoms Fungi, Plantae, and Animalia, as well as protists. The Domains Bacteria and Archaea are prokaryotes.

three cell lineages clearly emerged as cells were forming 3.5 billion years ago. That led to the Archaea, the Bacteria, and what eventually became the nucleoplasm of the eukaryotes. However, the three cell lines were not isolated from each other; horizontal gene transfer (page 226) appears to have occurred among them. Analysis of complete genomes shows that each domain shares genes with other domains. One-quarter of the genes of the bacterium *Thermotoga* were probably acquired from an archaeon. Gene transfer also has been seen between eukaryotic hosts and their prokaryote symbionts (see the box on page 297).

The oldest known fossils are the remains of prokaryotes that lived more than 3.5 billion years ago. Eukaryotic cells evolved more recently, about 2.5 billion years ago. According to endosymbiotic theory, eukaryotic cells evolved from prokaryotic cells living inside one another, as endosymbionts (see Chapter 4, page 102). In fact, the similarities between prokaryotic cells and eukaryotic organelles provide striking evidence for this endosymbiotic relationship (**Table 10.2**).

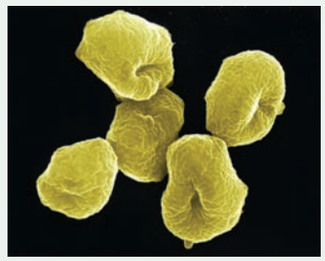
Clinical Case: Full-Flavor Outbreak

Monica Jackson, a 32-year-old production assistant at a Reno, Nevada, television station, has made an appointment with the nurse practitioner at her physician's office. Monica tells the nurse practitioner that she has had diarrhea, nausea, and abdominal cramping for almost 12 hours. She also feels tired and has a low-grade fever. Monica felt fine one minute, and the next she was violently ill. Monica informs the nurse practitioner that she and her good friend, who is also sick, had been at the same luncheon the day before. The nurse practitioner takes a stool sample and sends it to the hospital laboratory for analysis.

What will the laboratory do first to look for a bacterial pathogen? Read on to find out.

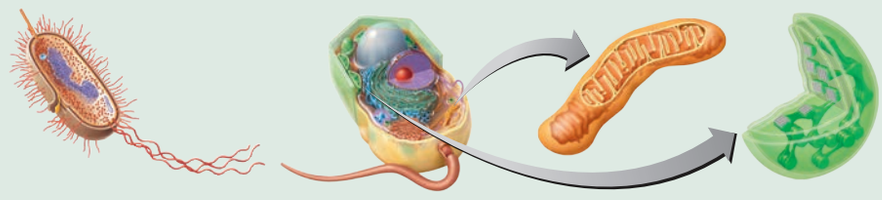
266 276 278 281 284 285

TABLE 10.1 Some Characteristics of Archaea, Bacteria, and Eukarya

	Archaea	Bacteria	Eukarya
	 *Sulfolobus* sp. SEM 1 μm	*E. coli* SEM 1 μm	*Amoeba* sp. SEM 5 μm
Cell Type	Prokaryotic	Prokaryotic	Eukaryotic
Cell Wall	Varies in composition; contains no peptidoglycan	Contains peptidoglycan	Varies in composition; contains carbohydrates
Membrane Lipids	Composed of branched carbon chains attached to glycerol by ether linkage	Composed of straight carbon chains attached to glycerol by ester linkage	Composed of straight carbon chains attached to glycerol by ester linkage
First Amino Acid in Protein Synthesis	Methionine	Formylmethionine	Methionine
Antibiotic Sensitivity	No	Yes	No
rRNA Loop*	Lacking	Present	Lacking
Common Arm of tRNA†	Lacking	Present	Present

*Binds to ribosomal protein; found in all bacteria.
†A sequence of bases in tRNA found in all eukaryotes and bacteria: guanine-thymine-pseudouridine-cytosine-guanine.

TABLE 10.2 Prokaryotic Cells and Eukaryotic Organelles Compared

	Prokaryotic Cell	Eukaryotic Cell	Eukaryotic Organelles (Mitochondria and Chloroplasts)
DNA	One circular; some two circular; some linear	Linear	Circular
Histones	In archaea	Yes	No
First Amino Acid in Protein Synthesis	Formylmethionine (bacteria) Methionine (archaea)	Methionine	Formylmethionine
Ribosomes	70S	80S	70S
Growth	Binary fission	Mitosis	Binary fission
			

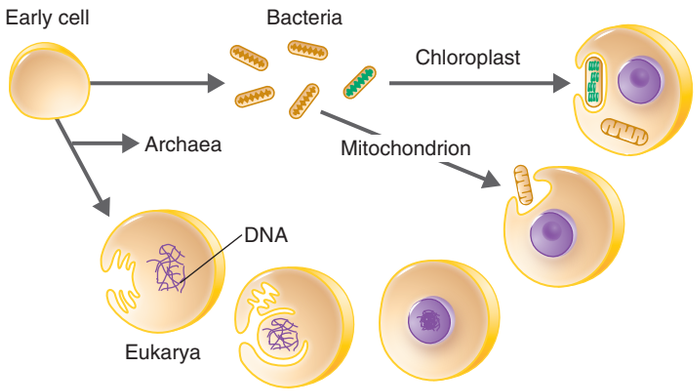


Figure 10.2 A model of the origin of eukaryotes. Invagination of the plasma membrane may have formed the nuclear envelope and endoplasmic reticulum. Similarities, including rRNA sequences, indicate that endosymbiotic prokaryotes gave rise to mitochondria and chloroplasts.

Q How many membranes make up the nuclear envelope of a eukaryotic cell?

The original nucleoplasmic cell was prokaryotic. However, infoldings in its plasma membrane may have surrounded the nuclear region to produce a true nucleus (Figure 10.2). Recently, French researchers provided support for this hypothesis with their observations of a true nucleus in *Gemmata* bacteria (see Figure 11.16 on page 306). Over time, the nucleoplasm's chromosome may have acquired pieces such as transposons (page 232). In some cells, this large chromosome may have fragmented into smaller linear chromosomes. Perhaps cells with linear chromosomes had an advantage in cell division over those with a large, unwieldy circular chromosome.

That nucleoplasmic cell provided the original host in which endosymbiotic bacteria developed into organelles (see page 103). An example of a modern prokaryote living in a eukaryotic cell is shown in Figure 10.3. The cyanobacterium-like cell and its eukaryotic host require each other for survival.

Taxonomy provides tools for clarifying the evolution of organisms, as well as their interrelationships. New organisms are being discovered every day, and taxonomists continue to search for a natural classification system that reflects phylogenetic relationships.

A Phylogenetic Tree

In a phylogenetic tree, grouping organisms according to common properties implies that a group of organisms evolved from a common ancestor; each species retains some of the characteristics of the ancestor. Some of the information used to classify and determine phylogenetic relationships in higher organisms comes from fossils. Bones, shells, or stems that contain mineral matter or have left imprints in rock that was once mud are examples of fossils.

ASM: The evolutionary relatedness of organisms is best reflected in phylogenetic trees.

The structures of most microorganisms are not readily fossilized. Some exceptions are the following:

- A marine protist whose fossilized colonies form the White Cliffs of Dover, England.
- Stromatolites, the fossilized remains of filamentous bacteria and sediments that flourished between 0.5 and 2 billion years ago (Figure 10.4a and Figure 10.4b).
- Cyanobacteria-like fossils found in rocks that are 3.0 to 3.5 billion years old. These are widely believed to be the oldest known fossils (Figure 10.4c).

Because fossil evidence isn't available for most prokaryotes, their phylogeny must be based on other evidence. In one notable exception, scientists may have isolated living bacteria and yeast that are 25 to 40 million years old. In 1995, the American microbiologist Raul Cano and his colleagues reported growing *Bacillus sphaericus* and other as yet unidentified microorganisms that had survived embedded in amber (fossilized plant resin) for millions of years. If confirmed, this discovery should provide more information about the evolution of microorganisms.

Similarities in genomes can be used to group organisms into taxa and to provide a timeline for the emergence of taxa. This is especially important for microorganisms that usually don't leave fossil evidence. This concept of a molecular clock based on the differences in amino acids in hemoglobin among different animals was first proposed in the 1960s. A **molecular clock** for evolution is based on nucleotide sequences in the genomes of organisms. Mutations accumulate in a genome at a constant rate. Some genes, such as those for rRNA, have few mutations—these

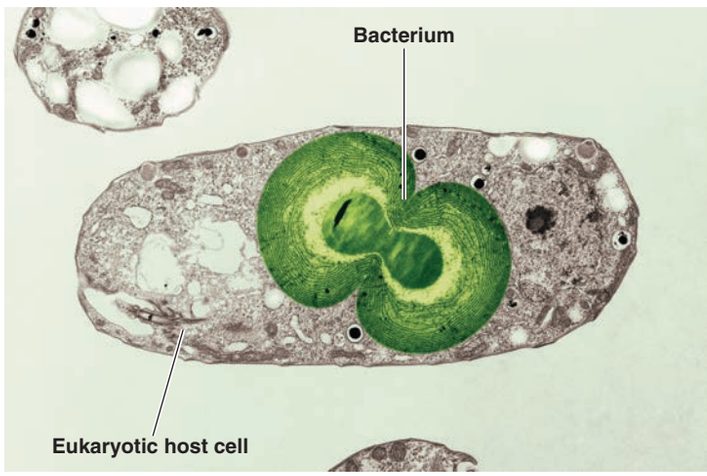
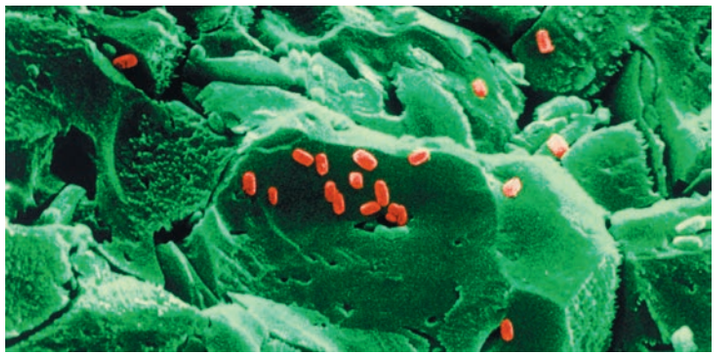


Figure 10.3 *Cyanophora paradoxa*. This organism, in which the eukaryotic host and the bacterium require each other for survival, provides a modern example of how eukaryotic cells might have evolved.

Q What features do chloroplasts, mitochondria, and bacteria have in common?

(a) Bacterial communities form rocklike pillars called stromatolites. These began growing about 3000 years ago. 30 cm

(b) Cut section through a fossilized stomatolite that flourished 2 billion years ago 2 cm



(c) Rod-shaped prokaryotes from the Early Precambrian (3.5 billion years ago) of South Africa SEM 5 μm

Figure 10.4 Fossilized prokaryotes.

Q What evidence is used to determine the phylogeny of prokaryotes?

are highly conserved genes. Other regions of a genome change with no apparent effect on the organism. Comparing the number of mutations between two organisms with the expected rate of change provides an estimate of when the two diverged from a common ancestor. This technique was used to track the path of West Nile virus to the United States. (See the box on page 215.)

Conclusions from rRNA sequencing and DNA hybridization studies (discussed on page 281) of selected orders and families of eukaryotes are in agreement with the fossil records. This has encouraged scientists to use DNA hybridization and rRNA sequencing to gain a better understanding of the evolutionary relationships among prokaryotic groups.

CHECK YOUR UNDERSTANDING

- What evidence supports classifying organisms into three domains? **10-4**
- Compare archaea and bacteria; bacteria and eukarya; and archaea and eukarya. **10-5**

Classification of Organisms

LEARNING OBJECTIVES

10-6 Explain why scientific names are used.
10-7 List the major taxa.
10-8 Differentiate *culture, clone,* and *strain*.
10-9 List the major characteristics used to differentiate the three kingdoms of multicellular Eukarya.
10-10 Define *protist*.
10-11 Differentiate eukaryotic, prokaryotic, and viral species.

Living organisms are grouped according to similar characteristics (classification), and each organism is assigned a unique scientific name. The rules for classifying and naming, which are used by biologists worldwide, are discussed next.

Scientific Nomenclature

In a world inhabited by millions of living organisms, biologists must be sure they know exactly which organism is being discussed. We cannot use common names, because the same name is often used for many different organisms in different locales. For example, there are two different organisms with the common name Spanish moss, and neither one is actually a moss. Plus, common names can be misleading and are in different languages. A system of scientific names was developed to solve this problem.

Every organism is assigned two names, or a binomial (Chapter 1, page 2). These names are the **genus** name and **specific epithet (species),** and both names are printed underlined or italicized. The genus name is always capitalized and is always a noun. The species name is lowercase and is usually an adjective. Because this system gives two names to each organism, the system is called **binomial nomenclature.**

Let's consider some examples. Our own genus and specific epithet are *Homo sapiens* (HŌ-mō SĀ-pē-enz). The genus means man; the specific epithet means wise. A mold that contaminates bread is called *Rhizopus stolonifer* (RĪ-zō-pus stō-LON-i-fer). *Rhizo-* (root) describes rootlike structures on the fungus; *stolo-* (a shoot) describes the long hyphae. Table 1.1 on page 4 contains more examples.

Binomials are used by scientists worldwide, regardless of their native language. This nomenclature enables them to share

knowledge efficiently and accurately. Several scientific entities are responsible for establishing rules governing the naming of organisms. Scientific names are taken from Latin (a genus name can be taken from Greek) or latinized by the addition of the appropriate suffix. Suffixes for order and family are *-ales* and *-aceae,* respectively.

As new laboratory techniques make more detailed characterizations of microbes possible, two genera may be reclassified as a single genus, or a genus may be divided into two or more genera. For example, rRNA analysis (see page 283) indicated that "Streptococcus faecalis" was only distantly related to the other streptococcal species; consequently, a new genus called *Enterococcus* was created, and this species was renamed *E. faecalis*. Making the transition to a new name can be confusing, so the old name is often written in parentheses. For example, a physician looking for information on the cause of a patient's pneumonia-like symptoms (melioidosis) would find the bacterial name *Burkholderia (Pseudomonas) pseudomallei* (berk-HŌL-der′ē-ah soo-dō-MAL-le-ē).

The Taxonomic Hierarchy

All organisms can be grouped into a series of subdivisions that make up the taxonomic hierarchy. Linnaeus developed this hierarchy for his classification of plants and animals. A **eukaryotic species** is a group of closely related organisms that breed among themselves. (Bacterial species will be discussed shortly.) A genus consists of species that differ from each other in certain ways but are related by descent. For example, *Quercus* (KWER-kus), the genus name for oak, consists of all types of oak trees (white oak, red oak, bur oak, velvet oak, and so on). Even though each species of oak differs from every other species, they are all related genetically. Just as a number of species make up a genus, related genera make up a **family.** A group of similar families constitutes an **order,** and a group of similar orders makes up a **class.** Related classes, in turn, make up a **phylum.** Thus, a particular organism (or species) has a genus name and specific epithet and belongs to a family, order, class, and phylum.

All phyla that are related to each other make up a **kingdom,** and related kingdoms are grouped into a **domain** (Figure 10.5).

CHECK YOUR UNDERSTANDING

- Using *Escherichia coli* and *Entamoeba coli* as examples, explain why the genus name must be written out on first use. Why is binomial nomenclature preferable to common names? **10-6**
- Find the gram-positive bacteria *Staphylococcus* in Appendix F. To which bacteria is this genus more closely related: *Bacillus* or *Streptococcus*? **10-7**

Classification of Prokaryotes

The taxonomic classification scheme for prokaryotes is found in *Bergey's Manual of Systematic Bacteriology,* 2nd edition (see Appendix F). In *Bergey's Manual,* prokaryotes are divided into two domains: Bacteria and Archaea. Each domain is divided into phyla. Remember, the classification is based on similarities in rRNA nucleotide sequences. Classes are divided into orders; orders, into families; families, into genera; and genera, into species.

A prokaryotic species is defined somewhat differently from a eukaryotic species, which is a group of closely related organisms that can interbreed. Unlike reproduction in eukaryotic organisms, cell division in bacteria is not directly tied to sexual conjugation, which is infrequent and does not always need to be species-specific. A **prokaryotic species,** therefore, is defined simply as a population of cells with similar characteristics. (The types of characteristics will be discussed later in this chapter.) The members of a bacterial species are nearly indistinguishable from each other but are distinguishable from members of other species, usually on the basis of several features. As you know, bacteria grown in media are called a culture. A pure culture is often a **clone,** a population of cells derived from a single parent cell. All cells in the clone should be identical, but in some cases, pure cultures of the same species are not identical in all ways. Each such group is called a **strain.** Strains are identified by numbers, letters, or names that follow the specific epithet.

ASM: The traditional concept of species is not readily applicable to microbes due to asexual reproduction and the frequent occurrence of horizontal gene transfer.

Bergey's Manual provides a reference for identifying bacteria in the laboratory, as well as a classification scheme for bacteria. One scheme for the evolutionary relationships of bacteria is shown in Figure 10.6. Characteristics used to classify and identify bacteria are discussed in Chapter 11.

Classification of Eukaryotes

Some kingdoms in the domain Eukarya are shown in Figure 10.1.

In 1969, simple eukaryotic organisms, mostly unicellular, were grouped as the Kingdom **Protista,** a catchall kingdom for a variety of organisms. Historically, eukaryotic organisms that didn't fit into other kingdoms were placed in the Protista. Approximately 200,000 species of protists have been identified thus far, and these organisms are nutritionally quite diverse—from photosynthetic to obligate intracellular parasite. Ribosomal RNA sequencing is making it possible to divide protists into groups based on their descent from common ancestors. Consequently, the organisms once classified as protists are being divided into **clades,** or genetically related groups. For convenience, we'll continue to use the term *protist* to refer to unicellular eukaryotes and their close relatives. These organisms will be discussed in Chapter 12.

Fungi, plants, and animals make up the three kingdoms of more complex eukaryotic organisms, most of which are multicellular.

The Kingdom **Fungi** includes the unicellular yeasts, multicellular molds, and macroscopic species such as mushrooms. A fungus absorbs dissolved organic matter through its plasma

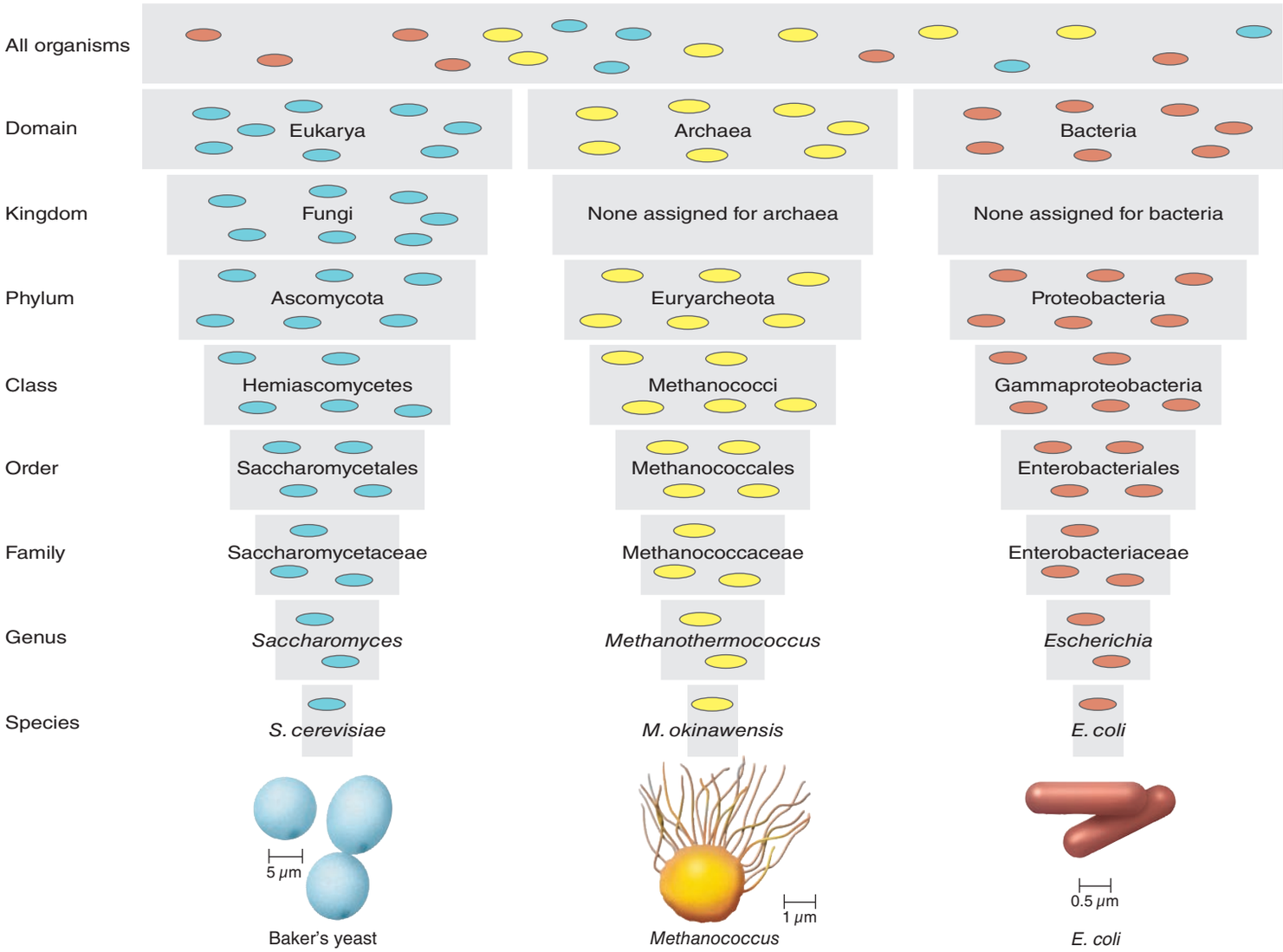


Figure 10.5 The taxonomic hierarchy. Organisms are grouped according to relatedness. Species that are closely related are grouped into a genus. For example, the baker's yeast belongs to the genus that includes sourdough yeast (*Saccharomyces exiguus*). Related genera, such as *Saccharomyces* and *Candida,* are placed in a family, and so on. Each group is more comprehensive. The domain Eukarya includes all organisms with eukaryotic cells.

Q What is the biological definition of *family*?

membrane to obtain raw materials for vital functions. The cells of a multicellular fungus are commonly joined to form thin tubes called *hyphae*. Fungi develop from spores or from fragments of hyphae. (See Figure 12.2, page 322.)

The Kingdom **Plantae** (plants) includes mosses, ferns, conifers, and flowering plants. All members of this kingdom are multicellular. To obtain energy, a plant uses photosynthesis, a process that converts carbon dioxide and water into organic molecules used by the cell.

The kingdom of multicellular organisms called **Animalia** (animals) includes sponges, various worms, insects, and animals with backbones (vertebrates). Animals obtain nutrients and energy by ingesting organic matter through a mouth of some kind.

Classification of Viruses

Viruses aren't classified as part of any of the three domains. They aren't composed of cells, and they use the anabolic machinery within living host cells to multiply. A viral genome can direct biosynthesis inside a host cell, and some viral genomes can be incorporated into the host genome. The ecological niche of a virus

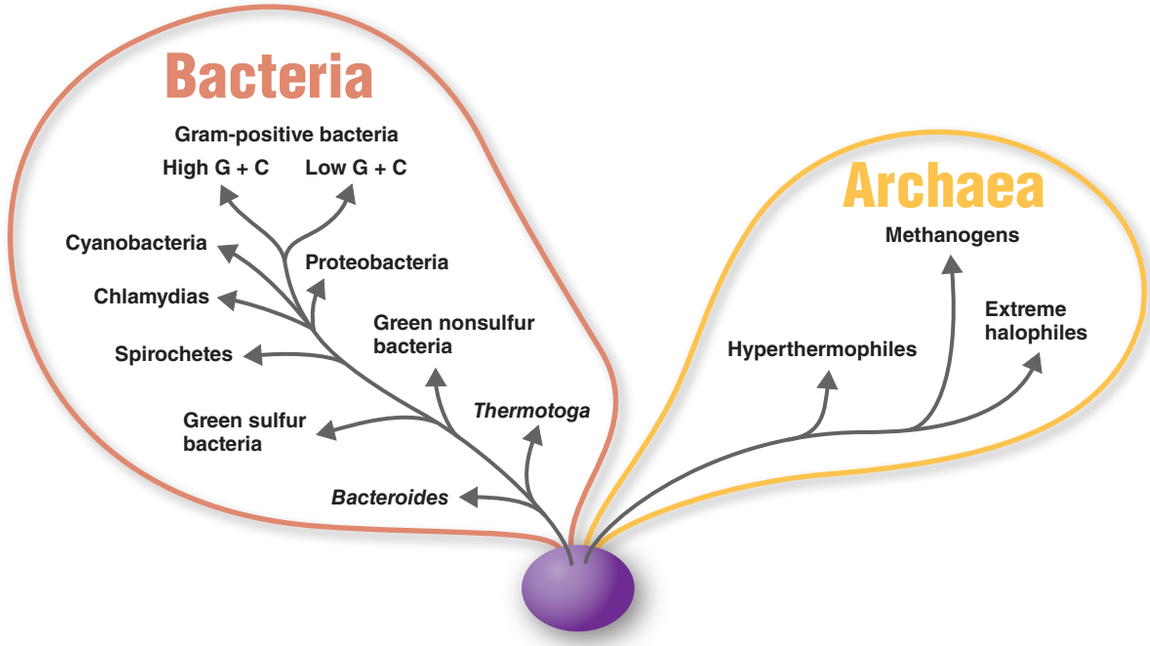


Figure 10.6 Phylogenetic relationships of prokaryotes. Arrows indicate major lines of descent of bacterial groups. Selected phyla are indicated.

Q Members of which phylum can be identified by Gram staining?

is its specific host cell, so viruses may be more closely related to their hosts than to other viruses. The International Committee on Taxonomy of Viruses defines a **viral species** as a population of viruses with similar characteristics (including morphology, genes, and enzymes) that occupies a particular ecological niche.

Viruses are obligatory intracellular parasites. Viral genes carried in the genomes of other organisms provide a record of viral evolution. Recent analysis shows that bornavirus genes integrated into mammals, including humans, at least 40 million years ago. There are three hypotheses on the origin of viruses: (1) They arose from independently replicating strands of nucleic acids (such as plasmids). (2) They developed from degenerative cells that, through many generations, gradually lost the ability to survive independently but could survive when associated with another cell. (3) They coevolved with host cells. For example, it has been hypothesized that the bacterial cell wall provided a selective advantage to avoid viral infections that could penetrate the bacterial cell wall. Viruses will be discussed in Chapter 13.

CHECK YOUR UNDERSTANDING

- Use the terms *species*, *culture*, *clone*, and *strain* in one sentence to describe growing methicillin-resistant *Staphylococcus aureus* (MRSA). **10-8**
- You discover a new multicellular, nucleated, heterotrophic organism with cell walls. To what kingdom does it belong? **10-9**
- Write your own definition of *protist*. **10-10**
- Why doesn't the definition viral species work for a bacteria? **10-11**

Methods of Classifying and Identifying Microorganisms

LEARNING OBJECTIVES

10-12 Compare and contrast classification and identification.
10-13 Explain the purpose of *Bergey's Manual*.
10-14 Describe how staining and biochemical tests are used to identify bacteria.
10-15 Differentiate Western blotting from Southern blotting.
10-16 Explain how serological tests and phage typing can be used to identify an unknown bacterium.
10-17 Describe how a newly discovered microbe can be classified by DNA base composition, DNA fingerprinting, and PCR.
10-18 Describe how microorganisms can be identified by nucleic acid hybridization, Southern blotting, DNA chips, ribotyping, and FISH.
10-19 Differentiate a dichotomous key from a cladogram.

A classification scheme provides a list of characteristics and a means for comparison to aid in the identification of an organism. Once an organism is identified, it can be placed into a previously devised classification scheme. Microorganisms are *identified* for practical purposes—for example, to determine an appropriate treatment for an infection. They are not necessarily identified by the same techniques by which they are

classified. Most identification procedures are easily performed in a laboratory and use as few procedures or tests as possible. Protozoa, parasitic worms, and fungi can usually be identified microscopically. Most prokaryotic organisms don't have distinguishing morphological features or even much variation in size and shape. Consequently, microbiologists have developed a variety of methods to test metabolic reactions and other characteristics to identify prokaryotes.

Bergey's Manual of Determinative Bacteriology has been a widely used reference since the first edition was published in 1923. *Bergey's Manual* does not classify bacteria according to evolutionary relatedness but instead provides identification (determinative) schemes based on such criteria as cell wall composition, morphology, differential staining, oxygen requirements, and biochemical testing.* The majority of Bacteria and Archaea haven't been cultured, and scientists estimate that only 1% of these microbes have been discovered.

Medical microbiology (the branch of microbiology dealing with human pathogens) has dominated the interest in microbes, and this interest is reflected in many identification schemes. However, to put the pathogenic properties of bacteria in perspective, of the more than 11,500 species listed in the *Approved Lists of Bacterial Names,* fewer than 5% are human pathogens.

We next discuss several criteria and methods for the classification and routine identification of microorganisms. In addition to properties of the organism itself, the source and habitat of a bacterial isolate are part of the identification processes. In clinical microbiology, a physician will swab a patient's pus or tissue surface. The swab is inserted into a tube of transport medium. **Transport media** are usually not nutritive and are designed to prolong viability of fastidious pathogens. The physician will note the type of specimen and test(s) requested on a lab requisition form (**Figure 10.7**). The lab technician's results will help the physician begin treatment (see the box in Chapter 5, page 139).

Morphological Characteristics

Morphological (structural) characteristics have helped taxonomists classify organisms for 200 years. Higher organisms are frequently classified according to observed anatomical detail. But many microorganisms look too similar to be classified by their structures alone. Organisms that might differ in metabolic or physiological properties may look alike under a microscope. Literally hundreds of bacterial species are small rods or small cocci.

Larger size and the presence of intracellular structures don't always mean easy classification, however. *Pneumocystis* (noo-mō-SIS-tis) pneumonia is the most common opportunistic infection in AIDS and other immunocompromised patients. Until the AIDS epidemic, the causative agent of this infection, *P. jirovecii* (ye-rō-VET-zē-ē) [formerly "P. carinii" (kar-I-nē-ē)] was rarely seen in humans. *Pneumocystis* lacks structures that can be easily used for identification (see Figure 24.19, page 700), and its taxonomic position has been uncertain since its discovery in 1909. It was originally classified as a protozoan; however, in 1988 rRNA sequencing showed that *Pneumocystis* is actually a member of the Kingdom Fungi. New treatments are being investigated as researchers take this organism's relatedness to fungi into account.

Cell morphology tells us little about phylogenetic relationships. However, morphological characteristics are still useful in identifying bacteria. For example, differences in such structures as endospores or flagella can be helpful.

Differential Staining

One of the first steps in identifying bacteria is differential staining (see Chapter 3). Most bacteria are either gram-positive or gram-negative. Other differential stains, such as the acid-fast stain, can be useful for a more limited group of microorganisms. Recall that these stains are based on the chemical composition of cell walls and therefore are not useful in identifying either the wall-less bacteria or the archaea with unusual walls. Microscopic examination of a Gram stain or an acid-fast stain is used to obtain information quickly in the clinical environment.

Biochemical Tests

Enzymatic activities are widely used to differentiate bacteria. Even closely related bacteria can usually be separated into distinct species by subjecting them to biochemical tests. For example, biochemical tests are used to identify bacteria in humans and in marine mammals (see the boxes on pages 139 and 275). Moreover, biochemical tests can provide insight into a species' niche in the ecosystem. For example, a bacterium that can fix nitrogen gas or oxidize elemental sulfur will provide important nutrients for plants and animals. This will be discussed in Chapter 27.

Enteric, gram-negative bacteria are a large heterogeneous group of microbes whose natural habitat is the intestinal tract of humans and other animals. This family contains several pathogens that cause diarrheal illness. A number of tests have been developed so technicians can quickly identify the pathogens, a clinician can then provide appropriate treatment, and epidemiologists can locate the source of an illness. All members of the family Enterobacteriaceae are oxidase-negative. Among the enteric bacteria are members of the genera *Escherichia, Enterobacter, Shigella, Citrobacter,* and *Salmonella*. *Escherichia, Enterobacter,* and *Citrobacter,* which ferment lactose to produce

*Both *Bergey's Manual of Systematic Bacteriology* (see page 270) and *Bergey's Manual of Determinative Bacteriology* are referred to simply as *Bergey's Manual;* the complete titles are used when the information under discussion is found in one but not the other, for example, an identification table.

MICROBIOLOGY REQUISITION

Lab:

Date, time received:

Date:

Time:

Slip prepared by:

Physician name:

Collected by:

Patient ID#:

DO NOT WRITE BELOW THIS LINE

USE SEPARATE SLIP FOR EACH REQUEST

GRAM STAIN REPORT

- ☐ GRAM POS. COCCI, GROUPS
- ☐ GRAM POS. COCCI, PAIRS/CHAIN
- ☐ GRAM POS. RODS
- ☒ GRAM NEG. COCCI
- ☐ GRAM NEG. RODS
- ☐ GRAM NEG. COCCOBACILLI
- ☐ YEAST
- ☐ OTHER

- ☐ NO GROWTH
- ☐ NO GROWTH IN ___DAYS
- ☐ MIXED MICROBIOTA
- ☐ SPECIMEN IMPROPERLY COLLECTED OR TRANSPORTED
- ☐ ___DIFFERENT TYPES OF ORGANISMS
- ☐ NEGATIVE FOR *SALMONELLA, SHIGELLA,* AND *CAMPYLOBACTER*
- ☐ NO OVA, CYSTS, OR PARASITES SEEN
- ☒ OXIDASE-POSITIVE GRAM-NEGATIVE DIPLOCOCCI
- ☐ PRESUMPTIVE BETA STREP GROUP A BY BACITRACIN

SOURCE OF SPECIMEN

- ☐ BLOOD
- ☐ CEREBROSPINAL FLUID
- ☐ FLUID (Specify Source) _____
- ☐ THROAT
- ☐ SPUTUM, expectorated
- ☐ OTHER Respiratory (Describe) _____
- ☐ URINE, Clean Catch Midstream
- ☐ URINE, Indwelling Catheter
- ☐ URINE, Straight Catheter
- ☐ URINE, Entire First Morning
- ☐ URINE, Other (Describe) _____
- ☐ STOOL
- ☑ GU (Specify Source) ___*vag.*
- ☐ ABSCESS (Specify Source) _____
- ☐ TISSUE (Specify Source) _____
- ☐ ULCER (Specify Source) _____
- ☐ WOUND (Specify Source) _____
- ☐ STERILIZER TEST

TEST(S) REQUESTED

Bacterial

- ☐ **Routine culture;** Gram stain, anaerobic culture, susceptibility testing. Throats done for Gp A Strep.
- ☐ *Legionella* culture
- ☐ *Bartonella*
- ☐ Blood Culture

Other Non-Routine Cultures

- ☐ *E. coli* O157:H7
- ☐ *Vibrio*
- ☐ *Yersinia*
- ☑ *H. ducreyi*
- ☐ *B. pertussis*
- ☐ Other _____

Screening Cultures

- ☑ Gonococci
- ☐ Group B Strep
- ☐ Group A Strep
- ☐ Other _____

☐ **ACID-FAST BACILLI**

☐ **FUNGAL**

VIRAL

- ☐ Routine culture
- ☐ Herpes simplex
- ☐ Direct FA for _____

PARASITOLOGY

- ☐ Exam for intestinal ova and parasites
- ☐ *Giardia* immunoassay
- ☐ *Cryptosporidium*
- ☐ Pinworm prep
- ☐ Blood parasites
- ☐ Filaria concentration
- ☐ *Trichomonas*
- ☐ Other _____

TOXIN ASSAY

- ☐ *Clostridium difficile*

DIRECT (Antigen Detection)

- ☐ Cryptococcal antigen-CSF only
- ☐ Bacterial antigens (Specify) _____

SPECIAL

- ☑ Antimicrobial tests (MIC)

Filled out by one person **Filled out by different person**

Figure 10.7 A clinical microbiology lab report form. In health care, morphology and differential staining are important in determining the proper treatment for microbial diseases. A clinician completes the form to identify the sample and specific tests. In this case, a genitourinary sample will be examined for sexually transmitted infections. The red notations are the lab technician's report of the Gram stain and culture results. (Minimal inhibitory concentration [MIC] of antibiotics will be discussed in Chapter 20, page 568.)

Q What diseases are suspected if the "acid-fast bacilli" box is checked?

acid and gas, can be distinguished from *Salmonella* and *Shigella*, which do not. Further biochemical testing, as represented in **Figure 10.8**, can differentiate among the genera.

The time needed to identify bacteria can be reduced considerably by the use of selective and differential media or by rapid identification methods. Selective media contain ingredients that suppress the growth of competing organisms and encourage the growth of desired ones, and differential media allow the desired organism to form a colony that is somehow distinctive (see Chapter 6, page 160).

Bergey's Manual doesn't evaluate the relative importance of each biochemical test or always describe strains. In diagnosing

Mass Deaths of Marine Mammals Spur Veterinary Microbiology

Over the past 20 years, thousands of marine mammals have died unexpectedly all over the world from a variety of infectious diseases. Notable outbreaks and problems include:

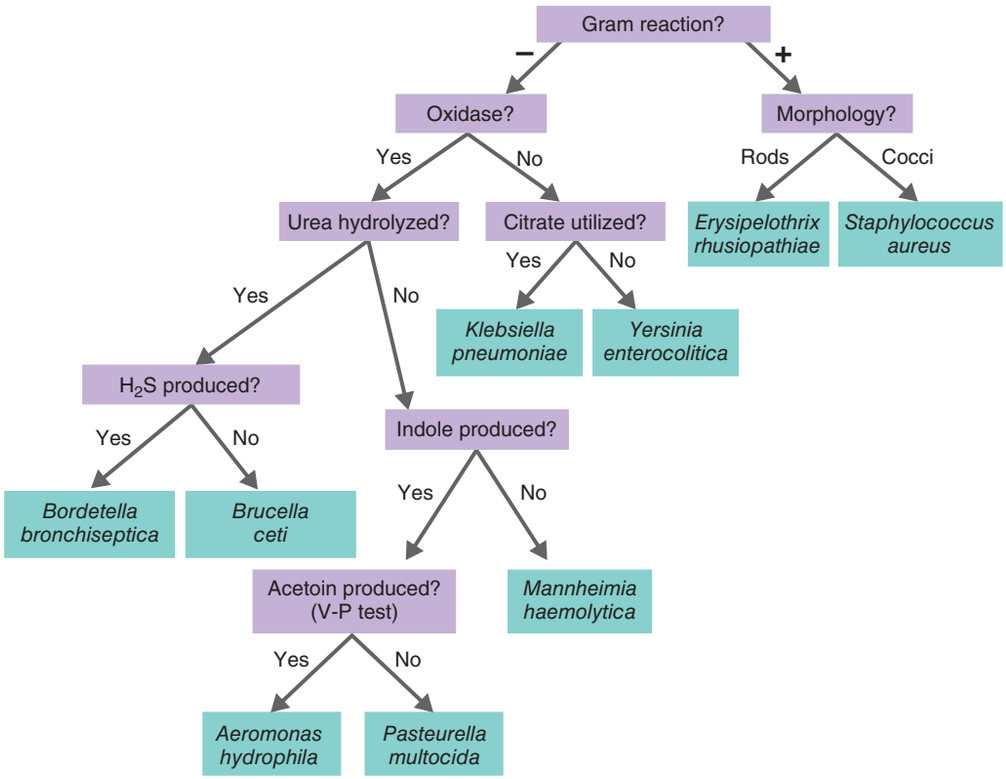
- The deaths of over 500 dolphins along the mid-Atlantic coast due to *Brucella* spp. during 2010–2013
- The decline of the California sea otter population in recent years, with toxoplasmosis and other bacterial species responsible for a 40% mortality rate
- The deaths of over 100 harbor seals along the New England coast in 2011, possibly due to influenza A H3N8
- The 2013 deaths of hundreds of bottlenose dolphins in the Atlantic Ocean from cetacean morbillivirus, likely transmitted to dolphins from pilot whales
- Large numbers of opportunistic pathogens, including 55 species of *Vibrio,* found in dolphins

Information Is Scarce

Such issues are the concern of veterinary microbiology, which until recently has been a neglected branch of medical microbiology. Although the diseases of cattle, chickens, and mink have been studied, partly because of their availability to researchers, the microbiology of wild animals, especially marine mammals, is a relatively new field. Gathering samples from animals that live in the open ocean and performing bacteriological analyses on them is very difficult. The animals being studied are those that have been stranded and those that come onto the shore to breed, such as the northern fur sea lion.

Microbiologists are identifying bacteria in marine mammals by using conventional test batteries (see figure) and genomic data of known species. The FISH technique is being used to find new species of bacteria in marine mammals (see page 283).

Veterinary microbiologists hope that studying the microbiology of wild animals, including marine mammals, will improve wildlife management and also provide models for studying human diseases.



Biochemical tests used to identify selected species of human pathogens isolated from marine mammals.

Q Assume you isolated a gram-negative rod that is oxidase-positive, is indole-negative, and does not produce urease or acetoin. What is the bacterium?

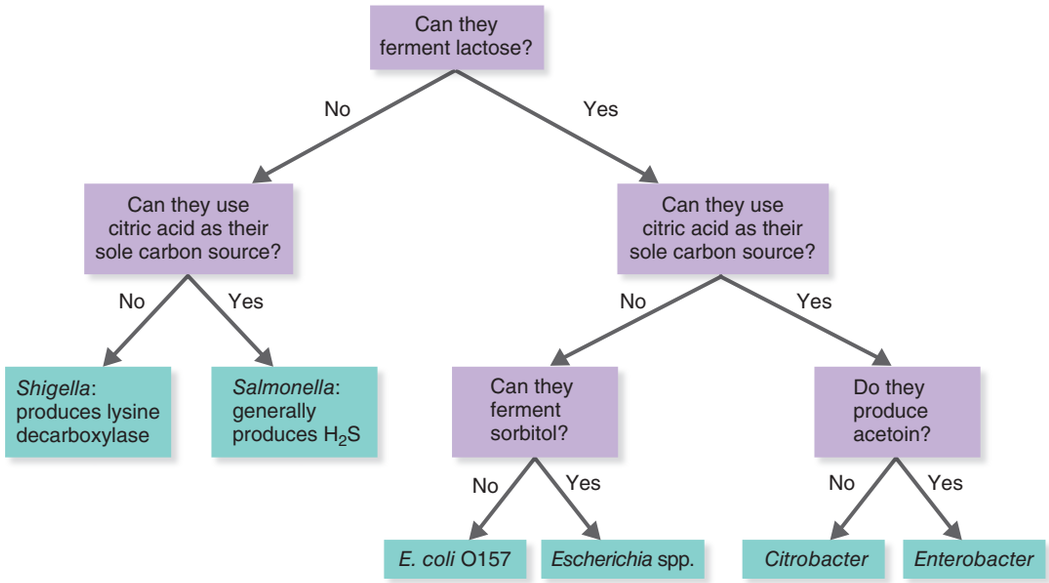


Figure 10.8 The use of metabolic characteristics to identify selected genera of enteric bacteria.

Q Assume you have a gram-negative bacterium that produces acid from lactose and cannot use citric acid as its sole carbon source. What is the bacterium?

an infection, clinicians must identify a particular species and even a particular strain to proceed with proper treatment. To this end, specific series of biochemical tests have been developed for fast identification in hospital laboratories. Rapid test systems have been developed for yeasts and other fungi, as well as bacteria.

Rapid identification methods are manufactured for groups of medically important bacteria, such as the enterics. Such tools are designed to perform several biochemical tests simultaneously and can identify bacteria within 4 to 24 hours. This is sometimes called **numerical identification** because the results of each test are assigned a number. In the simplest form, a positive test would be assigned a value of 1, and a negative is assigned a value of 0. In most commercial testing kits, test results are assigned numbers ranging from 1 to 4 that are based on the relative reliability and importance of each test, and the resulting total is compared to a database of known organisms.

In the example shown in Figure 10.9, an unknown enteric bacterium is inoculated into a tube designed to perform 15 biochemical tests. After incubation, results in each compartment are recorded. Notice that each test is assigned a value; a code number is derived from scoring all the tests. Fermentation of glucose is important, and a positive reaction is valued at 4, compared with the production of indole which has a value of 1.

A computerized interpretation of the simultaneous test results is essential and is provided by the manufacturer. A limitation of biochemical testing is that mutations and plasmid acquisition can result in strains with different characteristics. Unless a large number of tests is used, an organism could be incorrectly identified.

Clinical Case

The laboratory cannot just Gram stain a stool sample to look for a bacterial pathogen. The large number of gram-negative rods would be indistinguishable in a Gram stain made directly from feces. The stool sample should be cultured on selective and differential media to distinguish among bacteria in the stool. Monica's stool sample is cultured on bismuth sulfite agar. Black colonies are present on the agar after 24 hours.

Can gram-positive bacteria grow on this medium? Refer to Chapter 6 if you need a hint.

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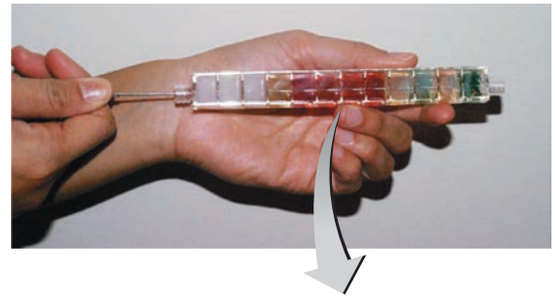
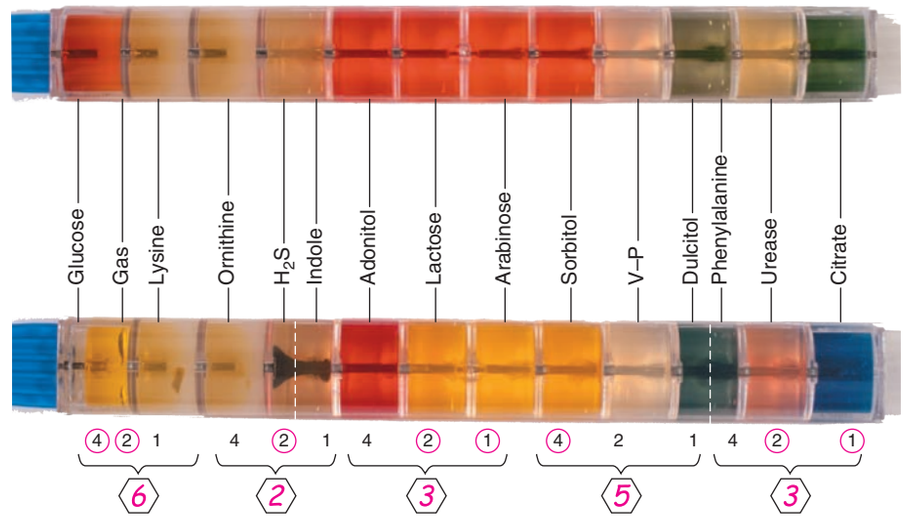
Serology

Serology is the science that studies serum and immune responses that are evident in serum (see Chapter 18). Microorganisms are antigenic; that is, microorganisms that enter an animal's body stimulate it to form antibodies. Antibodies are proteins that circulate in the blood and combine in a highly specific way with the bacteria that caused their production. For example, the immune system of a rabbit injected with killed typhoid bacteria (antigens) responds by producing antibodies against typhoid bacteria. Solutions of such antibodies used in the identification of many medically important microorganisms are commercially available; such a solution is called an **antiserum** (plural:

1 One tube containing media for 15 biochemical tests is inoculated with an unknown enteric bacterium.



2 After incubation, the tube is observed for results.



3 The value for each positive test is circled, and the numbers from each group of tests are added to give the code number.

4 Comparing the resultant code number with a computerized listing shows that the organism in the tube is *Citrobacter freundii*.

Code Number	Microorganism	Atypical Test Results
62352	*Citrobacter freundii*	Citrate
62353	*Citrobacter freundii*	None

Figure 10.9 One type of rapid identification method for bacteria: EnteroPluri test from BD Diagnostics. This example shows results for a typical strain of *C. freundii;* however, other strains may produce different test results, which are listed in the Atypical Test Results column.

Q How can one species have two different code numbers?

antisera). If an unknown bacterium is isolated from a patient, it can be tested against known antisera and often is identified quickly.

In a procedure called a **slide agglutination test,** samples of an unknown bacterium are placed in a drop of saline on each of several slides. Then a different known antiserum is added to each sample. The bacteria agglutinate (clump) when mixed with antibodies that were produced in response to that species or strain of bacterium; a positive test is indicated by the presence of agglutination. Positive and negative slide agglutination tests are shown in **Figure 10.10**.

Serological testing can differentiate not only among microbial species, but also among strains within species. Strains with different antigens are called **serotypes, serovars,** or **biovars.** See the discussion of *Escherichia* and *Salmonella* serovars on page 299. As mentioned in Chapter 1, Rebecca Lancefield was able to classify streptococcal serotypes by studying serological reactions. She found that the different antigens in the cell walls of various serotypes of streptococci stimulate the formation of different antibodies. In contrast, because closely related bacteria also produce some of the same antigens, serological testing can be used to screen bacterial isolates for possible similarities. If an antiserum reacts with proteins from different bacterial species or strains, these bacteria can be tested further for relatedness.

Serological testing was used to determine whether the increased number of necrotizing fasciitis cases in the United States and England since 1987 was due to a common source of the infections. No common source was located, but there has been an increase in two serotypes of *Streptococcus pyogenes* that have been dubbed the "flesh-eating" bacteria.

(a) Positive test **(b)** Negative test

Figure 10.10 A slide agglutination test. **(a)** In a positive test, the grainy appearance is due to the clumping (agglutination) of the bacteria. **(b)** In a negative test, the bacteria are still evenly distributed in the saline and antiserum.

Q Agglutination results when the bacteria are mixed with _____.

Clinical Case

Bismuth sulfite agar inhibits the growth of gram-positive bacteria; it is used to distinguish among gram-negative bacteria. The culture from Monica's stool sample reveals that she has been infected with *Salmonella* bacteria. There are only two species of *Salmonella: S. enterica* and *S. bongori.* Monica's infection is caused by *S. enterica*; however, there are over 2500 serovars of S. *enterica* that can infect people. Upon receiving the results from the lab, Monica's nurse practitioner calls the Nevada Department of Health to inform them of her patient's diagnosis and to let them know that Monica's friend has the same symptoms. It is important that the department of health identify the serovar to determine whether there is an outbreak from one source and to trace that source.

How will the department of health identify the correct *S. enterica* serovar?

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A test called the **enzyme-linked immunosorbent assay (ELISA)** is widely used because it is fast and can be read by a computer scanner (Figure 10.11; see also Figure 18.14, page 510). In a direct ELISA, known antibodies are placed in (and adhere to) the wells of a microplate, and an unknown type of bacterium is added to each well. A reaction between the known antibodies and the bacteria provides identification of the bacteria. An ELISA is used in AIDS testing to detect the presence of antibodies against human immunodeficiency virus (HIV), the virus that causes AIDS (see Figure 19.13, page 535).

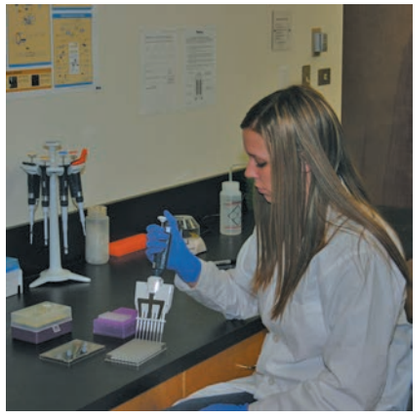
Another serological test, **Western blotting,** is also used to identify antibodies in a patient's serum (Figure 10.12). HIV infection is confirmed by Western blotting, and Lyme disease, caused by *Borrelia burgdorferi,* is often diagnosed by the Western blot.

Phage Typing

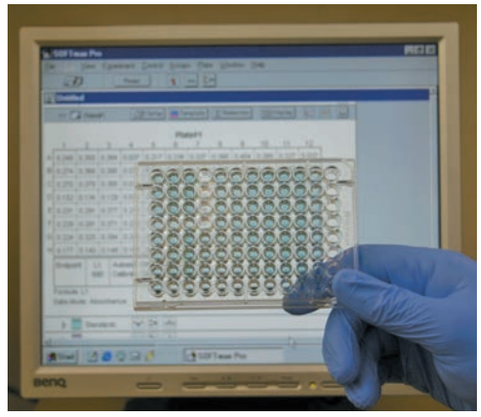
Like serological testing, phage typing looks for similarities among bacteria. Both techniques are useful in tracing the origin and course of a disease outbreak. **Phage typing** is a test for determining to which phages a bacterium is susceptible. Bacteriophages (phages) are bacterial viruses and that they usually cause lysis of the bacterial cells they infect (Chapter 13, page 369). They are highly specialized, in that they usually infect only members of a particular species, or even particular strains within a species. One bacterial strain might be susceptible to two different phages, whereas another strain of the same species might be susceptible to those two phages plus a third phage. Bacteriophages will be discussed further in Chapter 13.

Figure 10.11 An ELISA test.

Q What are the similarities between the slide agglutination test and the ELISA test?



(a) A technician uses a micropipette to add samples to a microplate for an ELISA.



(b) ELISA results are then read by the computer scanner.

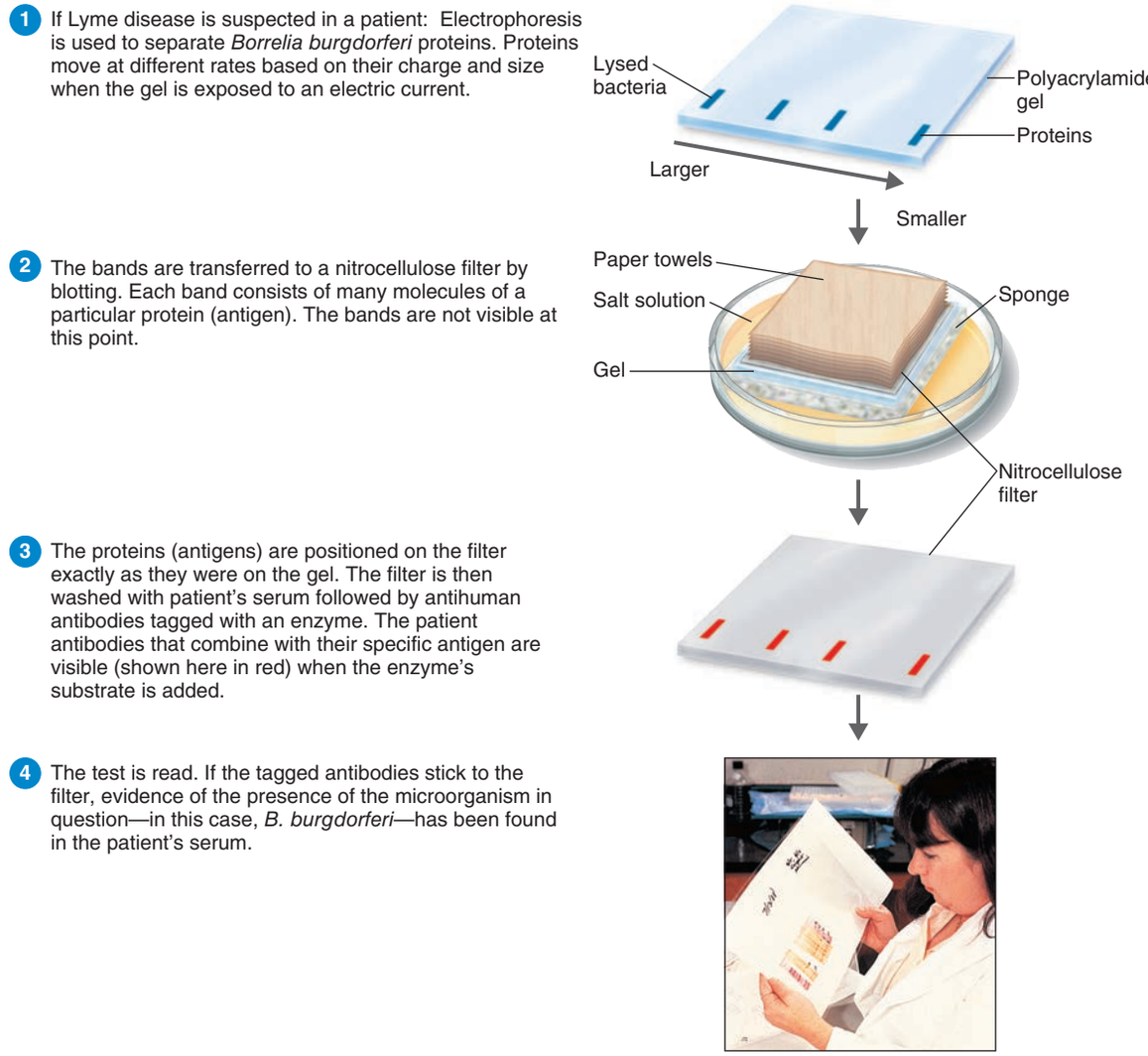


Figure 10.12 The Western blot. Proteins separated by electrophoresis can be detected by their reactions with antibodies.

Q Name two diseases that may be diagnosed by Western blotting.

The sources of food-associated infections can be traced by phage typing. One version of this procedure starts with a plate totally covered with bacteria growing on agar. A drop of each different phage type to be used in the test is then placed on the bacteria. Wherever the phages are able to infect and lyse the bacterial cells, clearings in the bacterial growth (called plaques) appear (**Figure 10.13**). Such a test might show, for instance, that bacteria isolated from a surgical wound have the same pattern of phage sensitivity as those isolated from the operating surgeon or surgical nurses. This result establishes that the surgeon or a nurse is the source of infection.

Fatty Acid Profiles

Bacteria synthesize a wide variety of fatty acids, and in general, these fatty acids are constant for a particular species. Commercial systems have been designed to separate cellular fatty acids to compare them to fatty acid profiles of known organisms. Fatty acid profiles, called **FAME** (*f*atty *a*cid *m*ethyl *e*ster), are widely used in clinical and public health laboratories.

Flow Cytometry

Flow cytometry can be used to identify bacteria in a sample without culturing the bacteria. In a *flow cytometer,* a moving fluid containing bacteria is forced through a small opening (see Figure 18.12, page 509). The simplest method detects the presence of bacteria by detecting the difference in electrical conductivity between cells and the surrounding medium. If the fluid passing through the opening is illuminated by a laser, the scattering of light provides information about the cell size, shape, density, and surface, which is analyzed by a computer. Fluorescence can be used to detect naturally fluorescent cells, such as *Pseudomonas,* or cells tagged with fluorescent dyes.

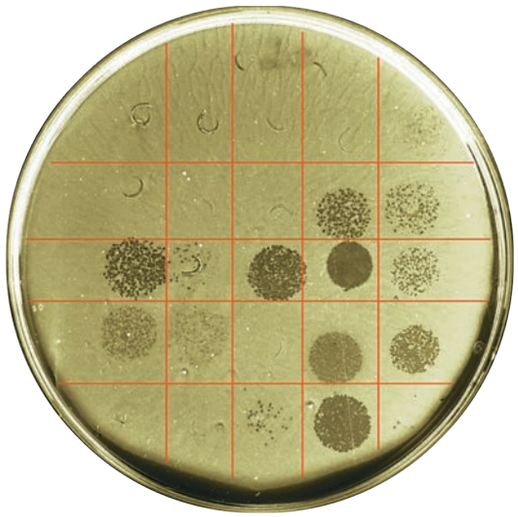


Figure 10.13 Phage typing of a strain of *Salmonella enterica*. The tested strain was grown over the entire plate. Plaques, or areas of lysis, were produced by bacteriophages, indicating that the strain was sensitive to infection by these phages. Phage typing is used to distinguish *S. enterica* serotypes and *Staphylococcus aureus* types.

Q What is being identified in phage typing?

A proposed test that uses flow cytometry to detect *Listeria* in milk could save time because the bacteria wouldn't need to be cultured for identification. Antibodies against *Listeria* can be labeled with a fluorescent dye and added to the milk to be tested. The milk is passed through the flow cytometer, which records the fluorescence of the antibody-labeled cells.

DNA Base Composition

Taxonomists can use an organism's **DNA base composition** to draw conclusions about relatedness. This base composition is usually expressed as the percentage of guanine plus cytosine (G + C). The base composition of a single species is theoretically a fixed property; thus, a comparison of the G + C content in different species can reveal the degree of species relatedness. Each guanine (G) in DNA has a complementary cytosine (C) (see Chapter 8). Similarly, each adenine (A) in the DNA has a complementary thymine (T). Therefore, the percentage of DNA bases that are GC pairs also tells us the percentage that are AT pairs (GC + AT = 100%). Two organisms that are closely related and have many identical or similar genes will have similar amounts of the various bases in their DNA. However, if there is a difference of more than 10% in their percentage of GC pairs (for example, if one bacterium's DNA contains 40% GC and another bacterium has 60% GC), then these two organisms probably aren't related. Of course, two organisms that have the same percentage of GC aren't necessarily closely related; other supporting data are needed to draw conclusions about their phylogenetic relationship.

During the last decade, comparison of DNA sequences has led to great strides in reclassifying known species and identifying new species. Genetic sequences of hundreds of organisms are compiled in databases that can be used online through the BioProject.

DNA Fingerprinting

Determining the entire sequence of bases in an organism's DNA is impractical for laboratory identification because it requires a great amount of time. However, the use of restriction enzymes enables researchers to compare the base sequences of different organisms. Restriction enzymes cut a molecule of DNA wherever a specific base sequence occurs, producing restriction fragments (as discussed in Chapter 9, page 254). For example, the enzyme *Eco*RI cuts DNA at the arrows in the sequence

$$\ldots G^{\downarrow}A\ A\ T\ T\ C \ldots$$
$$\ldots C\ T\ T\ AA_{\uparrow}G \ldots$$

In this technique, the DNA from two microorganisms is treated with the same restriction enzyme, and the restriction fragments (RFLPs) produced are separated by electrophoresis, producing a **DNA fingerprint** (see Figure 9.17, page 256). Comparing the number and sizes of restriction fragments that are produced from different organisms provides information about their genetic similarities and differences; the more similar the patterns, or *DNA fingerprints*, the more closely related the organisms are expected to be (**Figure 10.14**).

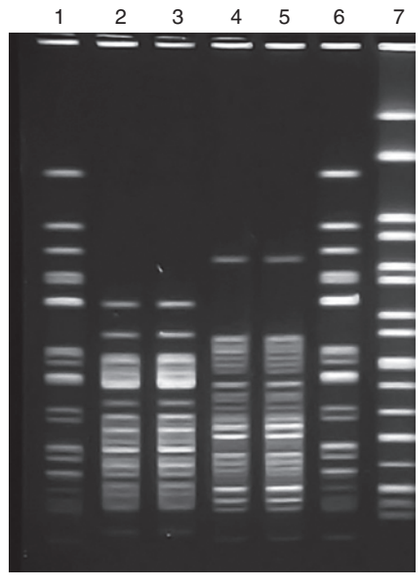


Figure 10.14 DNA fingerprints. DNA from seven different bacteria was digested with the same restriction enzyme. Each digest was put in a different well (origin) in the agarose gel. An electrical current was then applied to the gel to separate the fragments by size and electrical charge. The DNA was made visible by staining with a dye that fluoresces under ultraviolet light. Comparison of the lanes shows that DNA samples (and therefore the bacteria) in lanes 2 and 3; 4 and 5; and 1 and 6 are identical.

Q What is a RFLP?

DNA fingerprinting is used to determine the source of hospital-acquired infections. In one hospital, patients undergoing coronary bypass surgery developed infections caused by *Gordonia bronchialis* (gor-DŌ-nē-ah bron-kē-AL-is). The DNA fingerprints of the patients' bacteria and the bacteria of one nurse were identical. The hospital was thus able to break the infection's chain of transmission by encouraging the nurse to use aseptic technique.

This has led to interest in finding a few genes that are present in all species and that provide a large variation between species. Primers for these genes would be used for PCR to produce a *DNA bar code* for each species. This was first proposed in 2003 for eukaryotic species, but the necessary six to nine genes for bacterial identification have not been found yet.

Nucleic Acid Amplification Tests (NAATs)

When a microorganism cannot be cultured by conventional methods, the causative agent of an infectious disease might not be recognized. However, **nucleic acid amplification tests (NAATs)** can be used to increase the amount of microbial DNA to levels that can be tested by gel electrophoresis. NAATs use PCR, reverse-transcription PCR, and real-time PCR (see Chapter 9, page 243). If a primer for a specific microorganism is used, the presence of amplified DNA indicates that the microorganism is present.

In 1992, researchers used PCR to determine the causative agent of Whipple's disease, which was previously an unknown bacterium now named *Tropheryma whipplei* (trō-fer-Ē-mah WHIP-plē-ī). Whipple's disease was first described in 1907 by George Whipple as a gastrointestinal and nervous system disorder caused by an unknown bacillus. No one has been able to culture the bacterium to identify it; thus PCR provides the only reliable methods of diagnosing and treating the disease.

In recent years, PCR made possible several discoveries. For example, in 1992, Raul Cano used PCR to amplify DNA from *Bacillus* bacteria in amber that was 25 to 40 million years old. These primers were made from rRNA sequences in living *B. circulans* to amplify DNA coding for rRNA in the amber. These primers will cause amplification of DNA from other *Bacillus* species but do not cause amplification of DNA from other bacteria that might be present, such as *Escherichia* or *Pseudomonas*. The DNA was sequenced after amplification. This information was used to determine the relationships between the ancient bacteria and modern bacteria.

In 1993, microbiologists using PCR identified a *Hantavirus* as the cause of an outbreak of hemorrhagic fever in the American Southwest. The identification was made in record time—less than 2 weeks. PCR was used in 1994 to identify the causative agent of a new tickborne disease (human granulocytic ehrlichiosis) as the bacterium *Ehrlichia chaffeensis* (er-LIK-ē-ah chaf-FĒ-en-sis) (page 291). PCR is used to identify the source of rabies viruses, as well (see the box in Chapter 22, page 625).

In 2013, public health scientists used real-time PCR to identify a new strain of H7N9 influenza virus.

Clinical Case

Salmonella serovars are identified by serotyping with antisera against previously isolated serovars. The department of health identifies the serovar; Monica and her friend are infected with *Salmonella tennessee* bacteria. By now, the department of health has been inundated with calls; 27 additional cases of *Salmonella tennessee* infection have been identified and reported from all over the state of Nevada.

How can the department of health determine whether these 29 cases are related?

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Nucleic Acid Hybridization

If a double-stranded molecule of DNA is subjected to heat, the complementary strands will separate as the hydrogen bonds between the bases break. If the single strands are then cooled slowly, they will reunite to form a double-stranded molecule identical to the original double strand. (This reunion occurs because the single strands have complementary sequences.) When this technique is applied to separated DNA strands from two different organisms, it is possible to determine the extent of similarity between the base sequences of the two organisms. This method is known as **nucleic acid hybridization.** The procedure assumes that if two species are similar or related, a major portion of their nucleic acid sequences will also be similar. The procedure measures the ability of DNA strands from one organism to hybridize (bind through complementary base pairing) with the DNA strands of another organism (**Figure 10.15**). The greater the degree of hybridization, the greater the degree of relatedness.

Similar hybridization reactions can occur between any single-stranded nucleic acid chain: DNA-DNA, RNA-RNA, DNA-RNA. An RNA transcript will hybridize with the separated template DNA to form a DNA-RNA hybrid molecule. Nucleic acid hybridization reactions are the basis of several techniques (described below) that are used to detect the presence of microorganisms and to identify unknown organisms.

Southern Blotting

Nucleic acid hybridization can be used to identify unknown microorganisms by **Southern blotting** (see Figure 9.16, page 255). In addition, rapid identification methods using **DNA probes** are being developed. One method involves breaking DNA extracted from *Salmonella* into fragments with a restriction enzyme, then selecting a specific fragment as the probe for *Salmonella* (**Figure 10.16**). This fragment must be able to hybridize with the DNA of all *Salmonella* strains, but not with the DNA of closely related enteric bacteria.

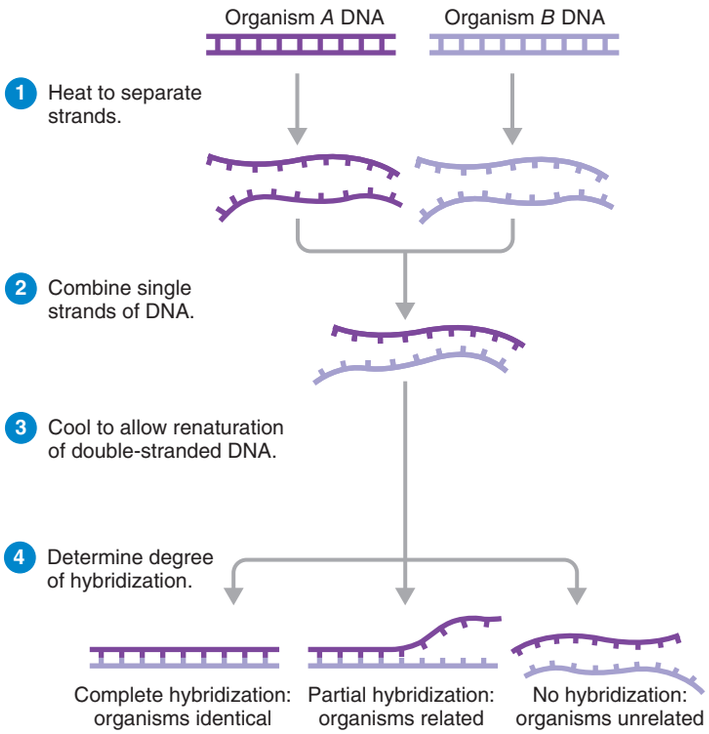
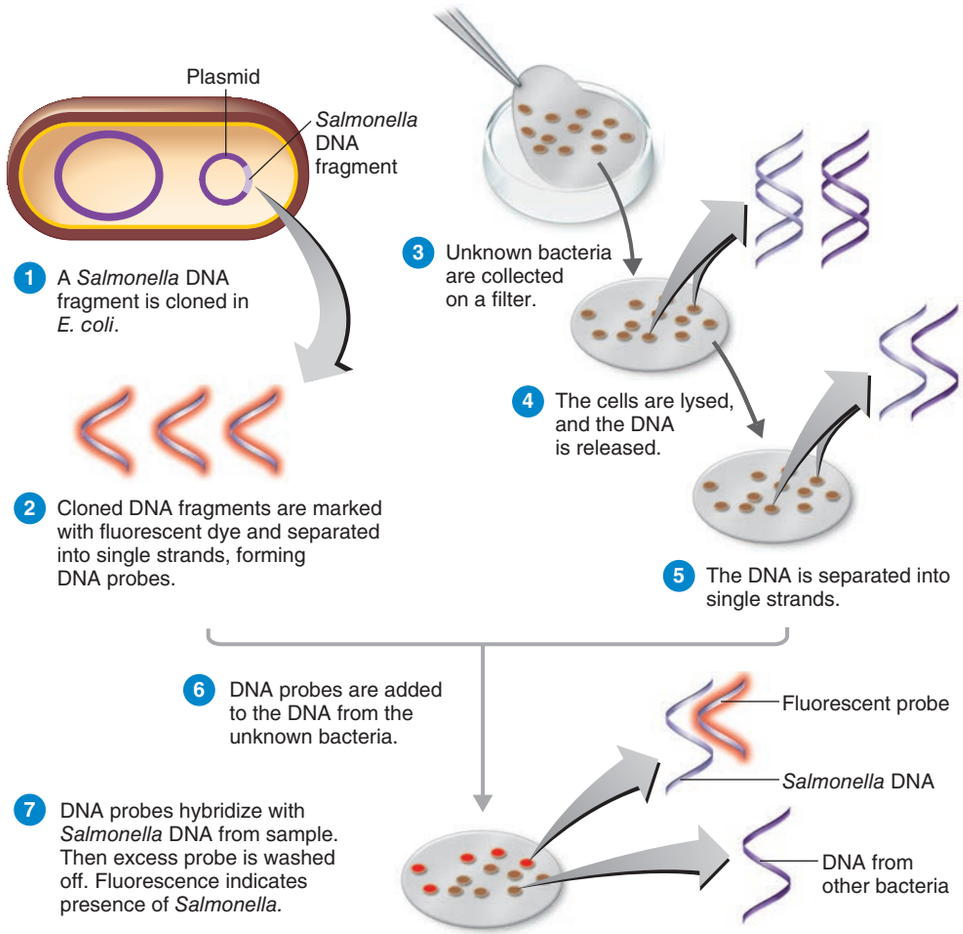


Figure 10.15 DNA-DNA hybridization. The greater the amount of pairing between DNA strands from different organisms (hybridization), the more closely the organisms are related.

Q What is the principle involved in DNA probes?

Figure 10.16 A DNA probe used to identify bacteria. Southern blotting is used to detect specific DNA. This modification of the Southern blot is used to detect *Salmonella*.

Q Why do the DNA probe and cellular DNA hybridize?



DNA Chips

An exciting new technology is the **DNA chip,** or **microarray,** which can quickly detect a pathogen in a host or the environment by identifying a gene that is unique to that pathogen (Figure 10.17). The DNA chip is composed of DNA probes. A sample containing DNA from an unknown organism is labeled with a fluorescent dye and added to the chip. Hybridization between the probe DNA and DNA in the sample is detected by fluorescence.

Ribotyping and Ribosomal RNA Sequencing

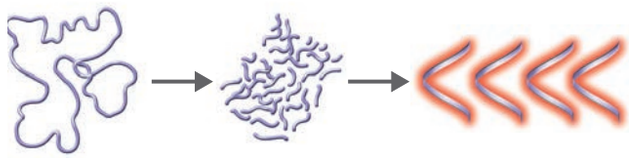
Ribotyping is currently being used to determine the phylogenetic relationships among organisms. There are several advantages to using rRNA. First, all cells contain ribosomes. Second, RNA genes have undergone few changes over time, so all members of a domain, phylum—and, in some cases, a genus—have the same "signature" sequences in their rRNA. The rRNA used most often is a component of the smaller portion of ribosomes. A third advantage of rRNA sequencing is that cells don't have to be cultured in the laboratory.

DNA can be amplified by PCR using an rRNA primer for specific signature sequences. The amplified fragments are subsequently cut with one or more restriction enzymes and separated by electrophoresis. The resulting band patterns can then be compared. The rRNA genes in the amplified fragments can be sequenced to determine evolutionary relationships between organisms. This technique is useful for classifying a newly discovered organism to domain or phylum or to determine the general types of organisms present in one environment. More specific probes (see page 249) are needed to identify individual species, however.

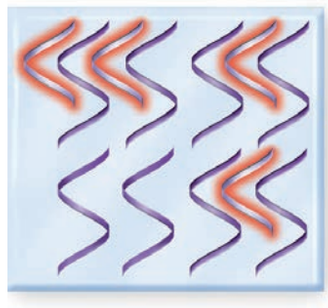
Fluorescent In Situ Hybridization (FISH)

Fluorescent dye–labeled RNA or DNA probes are used to stain microorganisms in place, or in situ. This technique is called **fluorescent in situ hybridization,** or **FISH.** Cells are treated so the probe enters the cells and reacts with target DNA in the cell (in situ). FISH is used to determine the identity, abundance, and relative activity of microorganisms in an environment and can be used to detect bacteria that have not yet been cultured. Using FISH, researchers discovered a tiny bacterium called *Pelagibacter* (pel-AJ-ē-bak-ter) in the ocean and determined that it's related to the rickettsia (page 293). As probes are developed, FISH can be used to detect bacteria in drinking water or bacteria in a patient without the normal 24-hour or longer wait required to culture the bacteria (Figure 10.18).

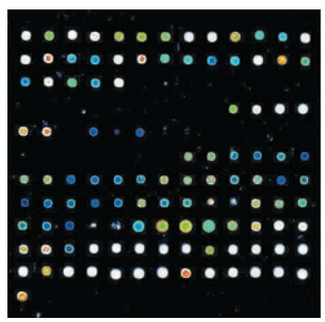
(a) A DNA chip can be manufactured to contain hundreds of thousands of synthetic single-stranded DNA sequences. Assume that each DNA sequence was unique to a different gene.



(b) Unknown DNA from a sample is separated into single strands, enzymatically cut, and labeled with a fluorescent dye.



(c) The unknown DNA is inserted into the chip and allowed to hybridize with the DNA on the chip.



(d) The tagged DNA will bind only to the complementary DNA on the chip. The bound DNA will be detected by its fluorescent dye and analyzed by a computer. In this *Salmonella* antimicrobial-resistance gene microarray, *S. typhimurium*–specific antibiotic-resistance gene probes are green, *S. typhi*–specific resistance gene probes are red, and antibiotic-resistance genes found in both serovars appear yellow/orange.

Figure 10.17 DNA chip. This DNA chip contains probes for antibiotic-resistance genes. It is used to detect antibiotic-resistant bacteria in samples collected from animals on a farm or in slaughter facilities.

Q What is on the chip to make it specific for a particular microorganism?

Putting Classification Methods Together

Morphological characteristics, differential staining, and biochemical testing were the only identification tools available just a few years ago. Technological advancements are making it possible to use nucleic acid analysis techniques, once reserved for classification, for routine identification. Information obtained about microbes is used to identify and classify the organisms. Two methods of using the information are described next.

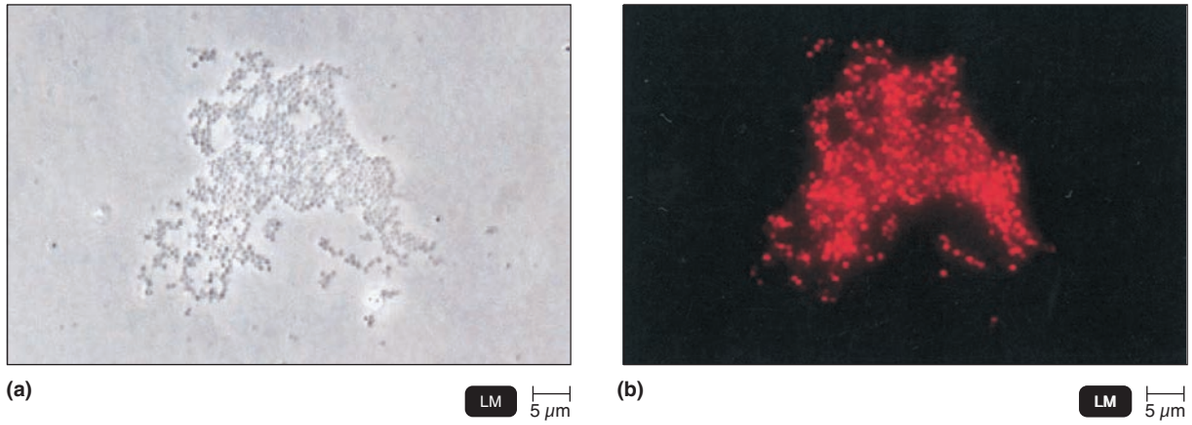
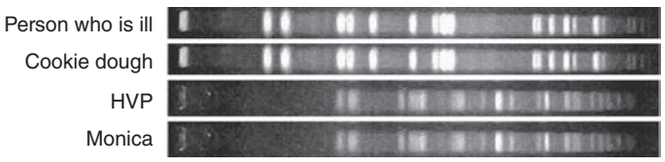


Figure 10.18 FISH, or fluorescent in situ hybridization. A DNA or RNA probe attached to fluorescent dyes is used to identify chromosomes. Bacteria seen with phase-contrast microscopy **(a)** are identified with a fluorescent-labeled probe that hybridizes with a specific sequence of DNA in *Staphylococcus aureus* **(b)**.

Q What is stained using the FISH technique?

Clinical Case

Salmonella isolates from each of the 29 infected people are sent to the state's public health laboratory for DNA fingerprinting. The DNA fingerprints are then sent to the Centers for Disease Control and Prevention (CDC). At the CDC, computer software compares each of the *Salmonella* DNA fingerprints to determine whether all 29 cases of *Salmonella tennessee* are identical. At this point, the CDC has received over 400 samples from 20 states, indicating a potential nationwide outbreak. Below is a figure of Monica's *Salmonella* DNA fingerprint along with other DNA fingerprint samples.



What can the CDC conclude about the outbreak based on these DNA fingerprints?

266 276 278 281 284 285

Dichotomous Keys

Dichotomous keys are widely used for identification. In a dichotomous key, identification is based on successive questions, and each question has two possible answers (*dichotomous* means cut in two). After answering one question, the investigator is directed to another question until an organism is identified. Although these keys often have little to do with phylogenetic relationships, they're invaluable for identification. For example, a dichotomous key for bacteria could begin with an easily determined characteristic, such as cell shape, and move on to the ability to ferment a sugar. Dichotomous keys are shown in Figure 10.8 and in the Applications of Microbiology boxes on pages 139 and 275. MM **Animations** Dichotomous Keys: Overview, Sample with Flowchart, Practice

Cladograms

Cladograms are maps that show evolutionary relationships among organisms (*clado-* means branch). Cladograms are shown in Figures 10.1 and 10.6. Each branch point on the cladogram is defined by a feature shared by various species on that branch. Historically, cladograms for vertebrates were made using fossil evidence; however, rRNA sequences are now being used to confirm fossil-based assumptions. As we said earlier, most microorganisms don't leave fossils; therefore, rRNA sequencing is primarily used to make cladograms for microorganisms. The small rRNA subunit used has 1500 bases, and computer programs do the calculations. The steps for constructing a cladogram are shown in **Figure 10.19**.

1. Two rRNA sequences are aligned.
2. The percentage of similarity between the sequences is calculated.
3. Then the horizontal branches are drawn in a length proportional to the calculated percent similarity. All species beyond a node (branch point) have similar rRNA sequences, suggesting that they arose from an ancestor at that node.

Figure 10.19 Building a cladogram.

Q Why do *L. brevis* and *L. acidophilus* branch from the same node?

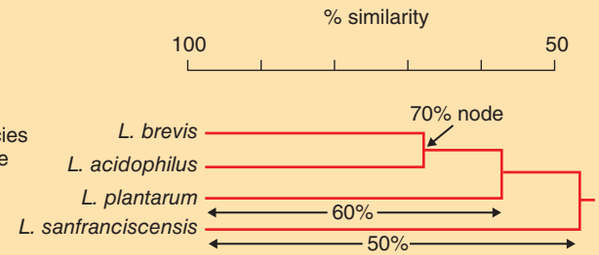
1 Determine the sequence of bases in an rRNA molecule for each organism. Only a short sequence of bases is shown for this example.

Lactobacillus brevis	AGUCCAGAGC
L. sanfranciscensis	GUAAAAGAGC
L. acidophilus	AGCGGAGAGC
L. plantarum	ACGUUAGAGC

2 Calculate the percentage of similarity in the nucleotide bases between pairs of species. For example, there is a 70% similarity between the sequences for *L. brevis* and *L. acidophilus*.

	Percent similarity
L. brevis → *L. sanfranciscensis*	50%
L. brevis → *L. acidophilus*	70%
L. brevis → *L. plantarum*	60%
L. sanfranciscensis → *L. acidophilus*	50%
L. sanfranciscensis → *L. plantarum*	50%
L. plantarum → *L. acidophilus*	60%

3 Construct a cladogram. The length of the horizontal lines corresponds to the percent similarity values. Each branch point, or node, in the cladogram represents an ancestor common to all species beyond that node. Each node is defined by a similarity in rRNA present in all species beyond that branch point.



Clinical Case Resolved

Early in this outbreak, there was a cluster of *Salmonella tennessee* illness due to consumption of raw eggs. Ill people and randomly chosen uninfected people completed questionnaires about foods they ate. The ill people were significantly more likely than well persons to report eating raw cookie dough, which contains uncooked eggs. However, the CDC soon determines that the cookie dough cluster involves a different strain of *Salmonella tennessee* from the strain involved in the current outbreak. This strain is associated with hydrolyzed vegetable protein (HVP), a flavor enhancer used in a variety of foods, including a vegetable dip that Monica and her friend ate the day before they became ill. In conjunction with the CDC and the U.S. Food and Drug Administration, the manufacturer recalls that batch of HVP. Monica and her friend fully recover.

Tracing *Salmonella* infections to their source is essential because *Salmonella* can be transmitted through a variety of foods. It causes an estimated 1.4 million illnesses and 400 deaths annually in the United States.

DNA fingerprinting is used in laboratories to distinguish among *Salmonella* strains because strain-specific primers aren't needed. RFLPs can also detect strains because they are made from the entire genome rather than from amplification of a few nucleotide sequences.

CHECK YOUR UNDERSTANDING

- What is in *Bergey's Manual*? **10-13**
- Design a rapid test for a *Staphylococcus aureus*. (*Hint:* See Figure 6.10, page 162.) **10-14**
- What is tested in Western blotting and Southern blotting? **10-15**
- What is identified by phage typing? **10-16**
- Why does PCR identify a microbe? **10-17**
- Which techniques involve nucleic acid hybridization? **10-18**
- Is a cladogram used for identification or classification? **10-12, 10-19**

Study Outline

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Introduction (p. 264)

1. Taxonomy is the science of the classification of organisms. Its goal is to show relationships among organisms.
2. Taxonomy also provides a means of identifying organisms.

The Study of Phylogenetic Relationships (pp. 265–268)

1. Phylogeny is the evolutionary history of a group of organisms.
2. The taxonomic hierarchy shows evolutionary, or phylogenetic, relationships among organisms.
3. Bacteria were separated into the Kingdom Prokaryotae in 1968.
4. Living organisms were divided into five kingdoms in 1969.

The Three Domains (pp. 265–268)

5. Living organisms are currently classified into three domains. A domain can be divided into kingdoms.
6. In this system, plants, animals, and fungi belong to the Domain Eukarya.
7. Bacteria (with peptidoglycan) form a second domain.
8. Archaea (with unusual cell walls) are placed in the Domain Archaea.

A Phylogenetic Tree (pp. 268–269)

9. Organisms are grouped into taxa according to phylogenetic relationships (from a common ancestor).
10. Some of the information for eukaryotic relationships is obtained from the fossil record.
11. Prokaryotic relationships are determined by rRNA sequencing.

Classification of Organisms (pp. 269–272)

Scientific Nomenclature (pp. 269–270)

1. According to scientific nomenclature, each organism is assigned two names, or a binomial: a genus and a specific epithet, or species.

The Taxonomic Hierarchy (p. 270)

2. A eukaryotic species is a group of organisms that interbreed with each other but do not breed with individuals of another species.
3. Similar species are grouped into a genus; similar genera are grouped into a family; families, into an order; orders, into a class; classes, into a phylum; phyla, into a kingdom; and kingdoms, into a domain.

Classification of Prokaryotes (p. 270)

4. *Bergey's Manual of Systematic Bacteriology* is the standard reference on bacterial classification.
5. A group of bacteria derived from a single cell is called a strain.
6. Closely related strains constitute a bacterial species.

Classification of Eukaryotes (pp. 270–271)

7. Eukaryotic organisms may be classified into the Kingdom Fungi, Plantae, or Animalia.
8. Protists are mostly unicellular organisms; these organisms are currently being assigned to kingdoms.
9. Fungi are absorptive chemoheterotrophs that develop from spores.
10. Multicellular photoautotrophs are placed in the Kingdom Plantae.
11. Multicellular ingestive heterotrophs are classified as Animalia.

Classification of Viruses (pp. 271–272)

12. Viruses are not placed in a kingdom. They are not composed of cells and cannot grow without a host cell.
13. A viral species is a population of viruses with similar characteristics that occupies a particular ecological niche.

Methods of Classifying and Identifying Microorganisms (pp. 272–285)

1. *Bergey's Manual of Determinative Bacteriology* is the standard reference for laboratory identification of bacteria.
2. Morphological characteristics are useful in identifying microorganisms, especially when aided by differential staining techniques.
3. The presence of various enzymes, as determined by biochemical tests, is used in identifying bacteria and yeasts.
4. Serological tests, involving the reactions of microorganisms with specific antibodies, are useful in determining the identity of strains and species, as well as relationships among organisms. ELISA and Western blotting are examples of serological tests.
5. Phage typing is the identification of bacterial species and strains by determining their susceptibility to various phages.
6. Fatty acid profiles can be used to identify some organisms.
7. Flow cytometry measures physical and chemical characteristics of cells.
8. The percentage of GC base pairs in the nucleic acid of cells can be used in the classification of organisms.
9. The number and sizes of DNA fragments, or DNA fingerprints, produced by restriction enzymes are used to determine genetic similarities.
10. NAATs can be used to amplify a small amount of microbial DNA in a sample. The presence or identification of an organism is indicated by amplified DNA.
11. Single strands of DNA, or of DNA and RNA, from related organisms will hydrogen-bond to form a double-stranded molecule; this bonding is called nucleic acid hybridization.
12. Southern blotting, DNA chips, and FISH are examples of nucleic acid hybridization techniques.
13. The sequence of bases in ribosomal RNA can be used in the classification of organisms.
14. Dichotomous keys are used for the identification of organisms. Cladograms show phylogenetic relationships among organisms.

Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. Which of the following organisms are most closely related? Are any two the same species? On what did you base your answer?

Characteristic	A	B	C	D
Morphology	Rod	Coccus	Rod	Rod
Gram Reaction	+	–	–	+
Glucose Utilization	Fermentative	Oxidative	Fermentative	Fermentative
Cytochrome Oxidase	Present	Present	Absent	Absent
GC Moles %	48–52	23–40	50–54	49–53

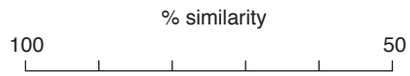
2. Here is some additional information on the organisms in question 1:

Organism	% DNA Hybridization
A and B	5–15
A and C	5–15
A and D	70–90
B and C	10–20
B and D	2–5

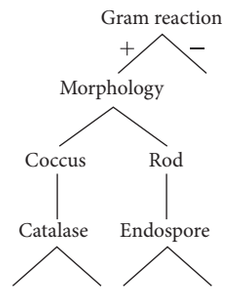
Which of these organisms are most closely related? Compare this answer with your response to review question 1.

3. DRAW IT Use the additional information below to construct a cladogram for some of the organisms used in question 4. What is the purpose of a cladogram? How does your cladogram differ from a dichotomous key for these organisms?

	Similarity in rRNA Bases
P. aeruginosa—M. pneumoniae	52%
P. aeruginosa—C. botulinum	52%
P. aeruginosa—E. coli	79%
M. pneumoniae—C. botulinum	65%
M. pneumoniae—E. coli	52%
E. coli—C. botulinum	52%



4. DRAW IT Use the information in the table below to complete the dichotomous key to these organisms. What is the purpose of a dichotomous key? Look up each genus in Chapter 11, and provide an example of why this organism is of interest to humans.



	Morphology	Gram Reaction	Acid from Glucose	Growth in Air (21% O_2)	Motile by Peritrichous Flagella	Presence of Cytochrome Oxidase	Produce Catalase
Staphylococcus aureus	Coccus	+	+	+	–	–	+
Streptococcus pyogenes	Coccus	+	+	+	–	–	–
Mycoplasma pneumoniae	Coccus	–	+	+ (Colonies < 1 mm)	–	–	+
Clostridium botulinum	Rod	+	+	–	+	–	–
Escherichia coli	Rod	–	+	+	+	–	+
Pseudomonas aeruginosa	Rod	–	+	+	–	+	+
Campylobacter fetus	Vibrio	–	–	–	–	+	+
Listeria monocytogenes	Rod	+	+	+	+	–	+

5. NAME IT Use the key in the Applications of Microbiology box on page 275 to identify the gram-negative rod causing pneumonia in a sea otter. It is H_2S-positive, indole-negative, and urease-positive.

Multiple Choice

1. *Bergey's Manual of Systematic Bacteriology* differs from *Bergey's Manual of Determinative Bacteriology* in that the former
   a. groups bacteria into species.
   b. groups bacteria according to phylogenetic relationships.
   c. groups bacteria according to pathogenic properties.
   d. groups bacteria into 19 species.
   e. all of the above
2. *Bacillus* and *Lactobacillus* are not in the same order. This indicates that which one of the following is *not* sufficient to assign an organism to a taxon?
   a. biochemical characteristics
   b. amino acid sequencing
   c. phage typing
   d. serology
   e. morphological characteristics
3. Which of the following is used to classify organisms into the Kingdom Fungi?
   a. ability to photosynthesize; possess a cell wall
   b. unicellular; possess cell wall; prokaryotic
   c. unicellular; lacking cell wall; eukaryotic
   d. absorptive; possess cell wall; eukaryotic
   e. ingestive; lacking cell wall; multicellular; prokaryotic
4. Which of the following is *false* about scientific nomenclature?
   a. Each name is specific.
   b. Names vary with geographical location.
   c. The names are standardized.
   d. Each name consists of a genus and specific epithet.
   e. It was first designed by Linnaeus.
5. You could identify an unknown bacterium by all of the following *except*
   a. hybridizing a DNA probe from a known bacterium with the unknown's DNA.
   b. making a fatty acid profile of the unknown.
   c. specific antiserum agglutinating the unknown.
   d. ribosomal RNA sequencing.
   e. percentage of guanine + cytosine.
6. The wall-less mycoplasmas are considered to be related to gram-positive bacteria. Which of the following would provide the most compelling evidence for this?
   a. They share common rRNA sequences.
   b. Some gram-positive bacteria and some mycoplasmas produce catalase.
   c. Both groups are prokaryotic.
   d. Some gram-positive bacteria and some mycoplasmas have coccus-shaped cells.
   e. Both groups contain human pathogens.

Use the following choices to answer questions 7 and 8.

   a. Animalia
   b. Fungi
   c. Plantae
   d. Firmicutes (gram-positive bacteria)
   e. Proteobacteria (gram-negative bacteria)

7. Into which group would you place a multicellular organism that has a mouth and lives inside the human liver?
8. Into which group would you place a photosynthetic organism that lacks a nucleus and has a thin peptidoglycan wall surrounded by an outer membrane?

Use the following choices to answer questions 9 and 10.

   1. 9 + 2 flagella
   2. 70S ribosome
   3. fimbria
   4. nucleus
   5. peptidoglycan
   6. plasma membrane

9. Which is (are) found in all three domains?
   a. 2, 6
   b. 5
   c. 2, 4, 6
   d. 1, 3, 5
   e. all six
10. Which is (are) found *only* in prokaryotes?
    a. 1, 4, 6
    b. 3, 5
    c. 1, 2
    d. 4
    e. 2, 4, 5

Analysis

1. The GC content of *Micrococcus* is 66–75 moles %, and of *Staphylococcus,* 30–40 moles %. According to this information, would you conclude that these two genera are closely related?
2. Describe the use of a DNA probe and PCR for:
   a. rapid identification of an unknown bacterium.
   b. determining which of a group of bacteria are most closely related.
3. SF medium is a selective medium, developed in the 1940s, to test for fecal contamination of milk and water. Only certain gram-positive cocci can grow in this medium. Why is it named SF? Using this medium, which genus will you culture? (*Hint:* Refer to page 270.)

Clinical Applications and Evaluation

1. A 55-year-old veterinarian was admitted to a hospital with a 2-day history of fever, chest pain, and cough. Gram-positive cocci were detected in his sputum, and he was treated for lobar pneumonia with penicillin. The next day, another Gram stain of his sputum revealed gram-negative rods, and he was switched to ampicillin and gentamicin. A sputum culture showed biochemically inactive gram-negative rods identified as *Pantoea (Enterobacter) agglomerans.* After fluorescent-antibody staining and phage typing, *Yersinia pestis* was identified in the patient's sputum and blood, and chloramphenicol and tetracycline were administered. The patient died 3 days after admission to the hospital. Tetracycline was given to his 220 contacts (hospital personnel, family, and co-workers). What disease did the patient have? Discuss what went wrong in the diagnosis and how his death might have been prevented. Why were the 220 other people treated? (*Hint:* Refer to Chapter 23.)
2. A 6-year-old girl was admitted to a hospital with endocarditis. Blood cultures showed a gram-positive, aerobic rod identified by the hospital laboratory as *Corynebacterium xerosis.* The girl died after 6 weeks of treatment with intravenous penicillin and chloramphenicol. The bacterium was tested by another laboratory and identified as *C. diphtheriae.* The following test results were obtained by each laboratory:

	Hospital Lab	Other Lab
Catalase	+	+
Nitrate reduction	+	+
Urea hydrolysis	–	–
Esculin hydrolysis	–	–
Glucose fermentation	+	+
Sucrose fermentation	–	+
Serological test for toxin production	Not done	+

Provide a possible explanation for the incorrect identification. What are the potential public health consequences of misidentifying *C. diphtheriae*? (*Hint:* Refer to Chapter 24.)

3. Using the following information, create a dichotomous key for distinguishing these unicellular organisms. Which cause human disease?

	Mitochondria?	Chlorophyll?	Nutritional Type?	Motile?
Euglena	+	+	Both	+
Giardia	–	–	Heterotroph	+
Nosema	–	–	Heterotroph	–
Pfiesteria	+	+	Autotroph	+
Trichomonas	–	–	Heterotroph	+
Trypanosoma	+	–	Heterotroph	+

Using the additional information shown below, create a dichotomous key for these organisms. Do your two keys differ? Explain why. Which key is more useful for laboratory identification? For classification?

	rRNA base #																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Euglena	C	C	A	G	G	U	U	G	U	U	C	C	A	G	U	U	U	U	A	A
Giardia	C	C	A	U	A	U	U	U	U	U	G	A	C	G	A	A	G	G	U	C
Nosema	C	C	A	U	A	U	U	U	U	U	A	A	C	G	A	A	G	G	C	C
Pfiesteria	C	C	A	A	C	U	U	A	U	U	C	C	A	G	U	U	U	C	A	G
Trichomonas	C	C	A	U	A	U	U	U	U	U	G	A	C	G	A	A	G	G	G	C
Trypanosoma	C	C	A	C	G	U	U	G	U	U	C	C	A	G	U	U	U	A	A	A

In the Clinic

As a perioperative clinical nurse specialist, you need to identify the source of infection in seven cardiovascular surgery patients. Nutrient-agar cultures from the patients grow red colonies consisting of gram-negative bacteria. You take cultures of selected hospital staff, and the same bacterium is cultured from a scrub nurse who wears artificial fingernails. **Removal of the fingernails ends the outbreak. What is the causative agent of the outbreak?**

Hint: The red pigment produced by this bacterium is distinctive. *(See page 300.)*

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

11 The Prokaryotes: Domains Bacteria and Archaea

When biologists first encountered microscopic bacteria, they were puzzled as to how to classify them. Bacteria were clearly not animals or rooted plants. Attempts to build a taxonomic system for bacteria based on the phylogenetic system developed for plants and animals failed (see page 265). In the earlier editions of *Bergey's Manual,* the main publication that focuses on classification, bacteria were grouped by morphology (rod, coccus), staining reactions, presence of endospores, and other obvious features. Although this system had its practical uses, it also had many limitations, somewhat like grouping bats and birds together on the basis of their having wings.

The knowledge of bacteria at the molecular level has now expanded to such a degree that it is possible to base the latest edition of *Bergey's Manual* on a phylogenetic system. For example, the genera *Rickettsia* and *Chlamydia* were once grouped together by their common requirement for intracellular growth. Members of the genus *Chlamydia* are now found in a phylum named Chlamydia, but the rickettsias are now grouped in a distant phylum, Proteobacteria, in the Alphaproteobacteria class. Some microbiologists find such changes upsetting, but they reflect important differences, primarily in the ribosomal RNA (rRNA) of the microbes, a genetic component that is slow to change (see page 283) and performs the same functions in all organisms.

Pathogenic bacteria isolated from patients, such as the *Streptococcus pyogenes* shown in the photo, must be identified quickly. Laboratory identification of bacterial species usually starts with Gram staining and morphology. Identification of this bacterium is discussed in the Clinical Case.

Streptococcus pyogenes **showing a typical chain arrangement.**

The Prokaryotic Groups

In the second (current) edition of *Bergey's Manual,* the prokaryotes are grouped into two **domains,** the **Archaea** and the **Bacteria.** Spelled without capitalization, archaea and bacteria denote organisms that fit into these domains. Each domain is divided into phyla, each phylum into classes, each class into orders, each order into families, each family into genera, and finally, each genus into species. Note that bacteria are also commonly distinguished by whether they are gram-negative or gram-positive.

ASM: Mutations and horizontal gene transfer, with the immense variety of microenvironments, have selected for a huge diversity of microorganisms.

The phyla discussed in this chapter are summarized in Table 11.1 (see also Appendix F).

Clinical Case: Mercy

Sheree Walker, a neonatologist at a local hospital, is checking on Mercy, a 48-hour-old infant girl. Mercy was delivered normally at 39 weeks and appeared to be a healthy baby. In the last 2 days, however, she has taken a bad turn and is admitted to the neonatal intensive care unit (NICU). Mercy is limp, has difficulty breathing, and has a body temperature of 35°C; but her lungs are clear, and her heart exam is normal. Dr. Walker speaks with Mercy's mother, who confirms that she received appropriate prenatal care and has no other medical problems. Dr. Walker orders a lumbar puncture for Mercy to check her cerebrospinal fluid (CSF) for possible infection. The report back from the lab shows blood in Mercy's CSF. Dr. Walker diagnoses Mercy with meningitis and then orders a venous blood culture to check for bacteria.

What bacteria could be the cause of Mercy's meningitis? Read on to find out.

291 310 312 313 314

TABLE 11.1 Classification of Selected Prokaryotes*

Domain	Phyla	Selected Classes	Notes
BACTERIA			
(Gram-Positive)	Firmicutes	• Bacilli • Clostridia	Firmicutes are low G + C gram-positive rods and cocci
	Actinobacteria	• Actinobacteria	Actinobacteria are high G + C gram-positive bacteria
BACTERIA			
(Gram-Negative)	Proteobacteria	• Alphaproteobacteria • Betaproteobacteria • Gammaproteobacteria • Deltaproteobacteria • Epsilonproteobacteria	Includes *Vibrio, Salmonella, Helicobacter,* and *Escherichia*
	Cyanobacteria	• Cyanobacteria	Oxygenic photosynthetic bacteria
	Chlorobi	• Chlorobia	Photosynthetic; anoxygenic; green sulfur bacteria
	Chloroflexi	• Chloroflexi	Include anoxygenic, photosynthetic, filamentous green nonsulfur bacteria
	Chlamydiae	• Chlamydiae	Grow only in eukaryotic host cells
	Plantomycetes	• Planctomycetacia	Aquatic bacteria; some are stalked
	Bacteroidetes	• Bacteroidetes • Flavobacteria • Sphingobacteria	Phylum members include opportunistic pathogens
	Fusobacteria	• Fusobacteria	Anaerobic; some cause tissue necrosis and septicemia in humans
	Spirochaetes	• Spirochaetes	Classes include pathogens that cause syphilis and Lyme disease
ARCHAEA			
	Crenarchaeota	• Thermoprotei	Members include mostly thermophiles and hyperthermophiles
	Euryarchaeota	• Methanobacteria • Halobacteria	Methanobacteria are important sources of methane

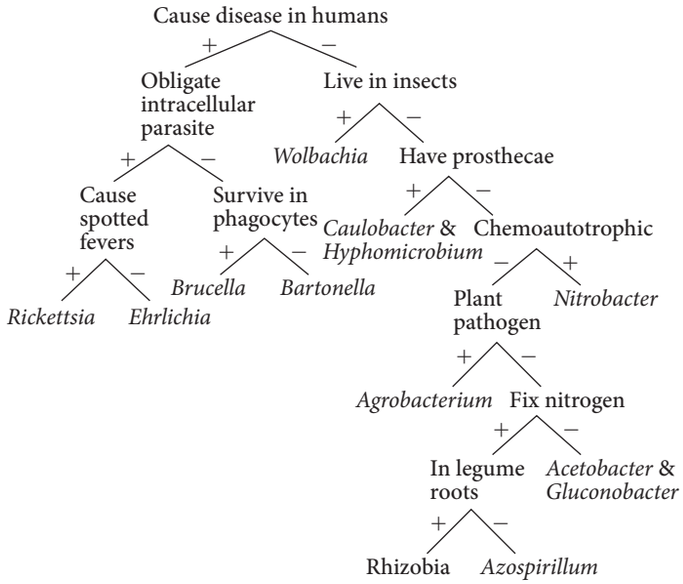
*For a complete list of genera discussed in this text, see Appendix F.

Domain Bacteria

Most of us think of bacteria as invisible, potentially harmful little creatures. Actually, relatively few species of bacteria cause disease in humans, animals, plants, or any other organisms. Once you have completed a course in microbiology, you'll realize that without bacteria, much of life as we know it would be impossible. In this chapter, you'll learn how bacterial groups are differentiated from each other and how important bacteria are in the world. Our discussion in this chapter emphasizes bacteria considered to be of practical importance, those that are important in medicine, or those that illustrate biologically unusual or interesting principles.

The Learning Objectives and Check Your Understanding questions throughout this chapter will help you to become familiar with these organisms and to look for similarities and differences between organisms. You'll also learn to draw dichotomous keys to differentiate the bacteria described in each group.

We've drawn the first of these dichotomous keys (for alphaproteobacteria) for you as an example (on the right).



Gram-Negative Bacteria

LEARNING OBJECTIVES

- **11-1** Differentiate the alphaproteobacteria described in this chapter by drawing a dichotomous key.
- **11-2** Differentiate the betaproteobacteria described in this chapter by drawing a dichotomous key.
- **11-3** Differentiate the gammaproteobacteria described in this chapter by drawing a dichotomous key.
- **11-4** Differentiate the deltaproteobacteria described in this chapter by drawing a dichotomous key.
- **11-5** Differentiate the epsilonproteobacteria described in this chapter by drawing a dichotomous key.

Proteobacteria

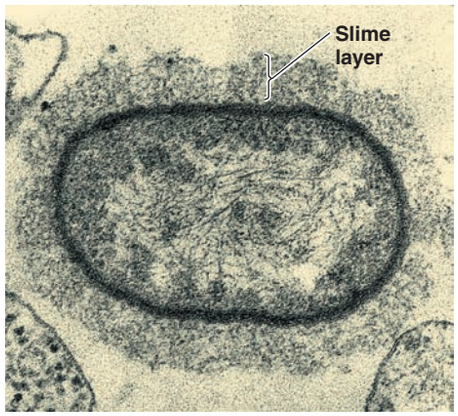
The **proteobacteria,** which include most of the gram-negative, chemoheterotrophic bacteria, are presumed to have arisen from a common photosynthetic ancestor. They are now the largest taxonomic group of bacteria. However, few are now photosynthetic; other metabolic and nutritional capacities have arisen to replace this characteristic. The phylogenetic relationship in these groups is based upon rRNA studies. The name *Proteobacteria* was taken from the mythological Greek god Proteus, who could assume many shapes. The proteobacteria are separated into five classes designated by Greek letters: alphaproteobacteria, betaproteobacteria, gammaproteobacteria, deltaproteobacteria, and epsilonproteobacteria.

The Alphaproteobacteria

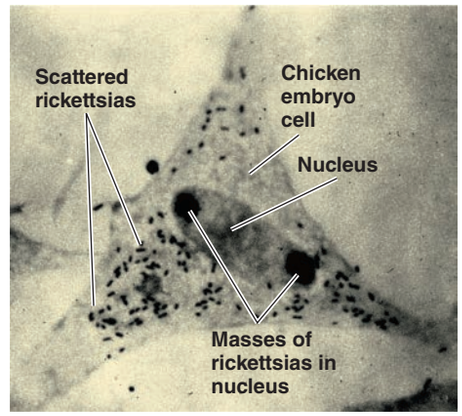
As a group, the alphaproteobacteria includes most of the proteobacteria that are capable of growth with very low levels of nutrients. Some have unusual morphology, including protrusions such as stalks or buds known as **prosthecae.** The alphaproteobacteria also include agriculturally important bacteria capable of nitrogen fixation in symbiosis with plants, and several plant and human pathogens.

Pelagibacter One of the most abundant microorganisms on Earth, certainly in the ocean environment, is *Pelagibacter ubique* (pel-AJ-ē-bak-ter OO-bēk). It is a member of a group of marine microbes named SAR 11 because of their original discovery in the Sargasso Sea. *P. ubique* is the first member of this group to be successfully cultivated. Its genome has been sequenced and found to have only 1354 genes. This number is very low for a free-living organism, although several mycoplasmas (see page 311) have even fewer genes. Bacteria in a symbiotic relationship have lower metabolic requirements and have the smallest genomes (see page 315). The bacterium is extremely small, a little over 0.3 µm diameter. Its small size and minimal genome probably give it a competitive advantage for survival in low-nutrient environments. In fact, it seems to be the most abundant living organism in the oceans on the basis of weight. (Part of its name, *ubique,* is derived from *ubiquitous*.) Its sheer numbers must give it an important role in the Earth's carbon cycle.

Azospirillum Agricultural microbiologists have been interested in members of the genus *Azospirillum* (ā-zō-spī-RIL-lum), a soil bacterium that grows in close association with the roots of many



(a) A rickettsial cell that has just been released from a host cell



(b) Rickettsias grow only within a host cell, such as the chicken embryo cell shown here. Note the scattered rickettsias within the cell and the compact masses of rickettsias in the cell nucleus.

Figure 11.1 Rickettsias.

How are rickettsias transmitted from one host to another?

plants, especially tropical grasses. It uses nutrients excreted by the plants and in return fixes nitrogen from the atmosphere. This form of nitrogen fixation is most significant in tropical grasses and in sugar cane, although the organism can be isolated from the root system of many temperate-climate plants, such as corn. The prefix *azo-* is frequently encountered in nitrogen-fixing genera of bacteria. It's derived from *a* (without) and *zo* (life), in reference to the early days of chemistry, when oxygen was removed, by a burning candle, from an experimental atmosphere. Presumably, mammalian life wasn't possible in this nitrogen-rich atmosphere. Hence, nitrogen came to be associated with absence of life.

Acetobacter* and *Gluconobacter *Acetobacter* (ah-SĒ-tō-bak-ter) and *Gluconobacter* (glū-kon'ō-BAK-ter) are industrially important aerobic organisms that convert ethanol into acetic acid (vinegar).

Rickettsia In the first edition of *Bergey's Manual,* the genera *Rickettsia, Coxiella,* and *Chlamydia* were grouped closely because they are all obligate intracellular parasites—that is, they reproduce only within a mammalian cell. In the second edition they are now widely separated. Rickettsias, chlamydias, and viruses are compared in Table 13.1, page 359.

The rickettsias are gram-negative rod-shaped bacteria, or coccobacilli (**Figure 11.1a**). One distinguishing feature of most rickettsias is that they are transmitted to humans by insect and tick bites, unlike the *Coxiella* (discussed later with gammaproteobacteria). Rickettsia enter their host cell by inducing phagocytosis. They quickly enter the cytoplasm of the cell and begin reproducing by binary fission (Figure 11.1b). They can usually be cultivated artificially in cell culture or chick embryos (Chapter 13, pages 363–368).

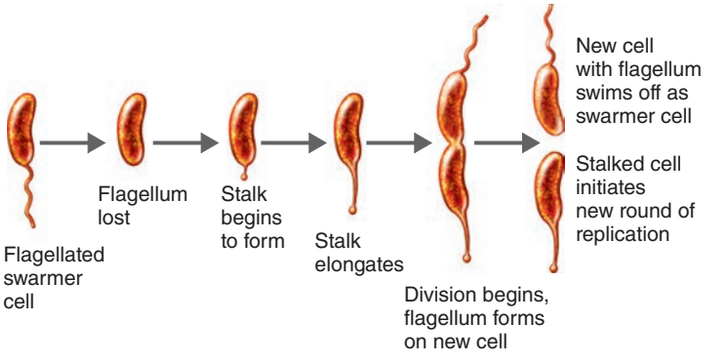
The rickettsias are responsible for a number of diseases known as the spotted fever group. These include epidemic typhus, caused by *Rickettsia prowazekii* (ri-KET-sē-ah prou-wah-ZE-kē-ē) and transmitted by lice (page 351); endemic murine typhus, caused by *R. typhi* (TĪ-fē) and transmitted by rat fleas; and Rocky Mountain spotted fever, caused by *R. rickettsii* (ri-KET-sē-ē) and transmitted by ticks (page 351). In humans, rickettsial infections damage the permeability of blood capillaries, which results in a characteristic spotted rash.

Ehrlichia Ehrlichiae are gram-negative, rickettsia-like bacteria that live obligately within white blood cells. *Ehrlichia* (er-LIK-ē-ah) species are transmitted by ticks to humans and cause ehrlichiosis, a sometimes fatal disease (page 653).

Caulobacter* and *Hyphomicrobium Members of the genus *Caulobacter* (kah-lō-BAK-ter) are found in low-nutrient aquatic environments, such as lakes. They feature stalks that anchor the organisms to surfaces (**Figure 11.2**). This arrangement increases their nutrient uptake because they are exposed to a continuously changing flow of water and because the stalk increases the surface-to-volume ratio of the cell. Also, if the surface to which they anchor is a living host, these bacteria can use the host's excretions as nutrients. When the nutrient concentration is exceptionally low, the size of the stalk increases, evidently to provide an even greater surface area for nutrient absorption.

Budding bacteria don't divide by binary fission into two nearly identical cells. The budding process resembles the asexual reproductive processes of many yeasts (see Figure 12.3, page 322). The parent cell retains its identity while the bud increases in size until it separates as a complete new cell. An example is the genus *Hyphomicrobium* (hī'fō-mĪ-KRŌ-bē-um), as shown in **Figure 11.3**. These bacteria, like the caulobacteria, are found in low-nutrient aquatic environments and have even been found growing in laboratory water baths. Both *Caulobacter* and *Hyphomicrobium* produce prominent prosthecae.

Rhizobium, Bradyrhizobium,* and *Agrobacterium The *Rhizobium* (rī-ZŌ-bē-um) and *Bradyrhizobium* (brā'dē-rī-ZŌ-bē-um) are



(a)

(b)

Figure 11.2 ***Caulobacter.***

Q What is the competitive advantage provided by attaching to a surface?

two of the more important genera of a group of agriculturally important bacteria that specifically infect the roots of leguminous plants, such as beans, peas, or clover. For simplicity these bacteria are known by the common name of **rhizobia.** The presence of rhizobia in the roots leads to formation of nodules in which the rhizobia and plant form a symbiotic relationship, resulting in the fixation of nitrogen from the air for use by the plant (see Figure 27.4 on page 777).

Like rhizobia, the genus *Agrobacterium* (ag-rō-bak-TI-rē-um) has the ability to invade plants. However, these bacteria do not induce root nodules or fix nitrogen. Of particular interest is *Agrobacterium tumefaciens*. This is a plant pathogen that causes a disease called crown gall; the crown is the area of the plant where the roots and stem merge. The tumorlike gall is induced when *A. tumefaciens* inserts a plasmid containing bacterial genetic information into the plant's chromosomal DNA (see Figure 9.19, page 257). For this reason, microbial geneticists are very interested in this organism. Plasmids are the most common vector that scientists use to carry new genes into a plant cell because the thick wall of plants is especially difficult to penetrate (see Figure 9.20, page 257).

Bartonella The genus *Bartonella* (BAR-tō-nel-lah) contains several members that are human pathogens. The best known is *Bartonella henselae,* a gram-negative bacillus that causes cat-scratch disease (page 647).

Brucella *Brucella* (broo-SEL-lah) bacteria are small nonmotile coccobacilli. All species of *Brucella* are obligate parasites of mammals and cause the disease brucellosis (page 643). Of medical interest is the ability of *Brucella* to survive phagocytosis, an important element of the body's defense against bacteria (see Chapter 16, page 450).

Nitrobacter* and *Nitrosomonas *Nitrobacter* (ni'trō-BAK-ter) and *Nitrosomonas* (NĪ-trō-sō-mō-nas) are genera of nitrifying bacteria that are of great importance to the environment and to agriculture. They are chemoautotrophs capable of using inorganic chemicals as energy sources and carbon dioxide as the only source of carbon, from which they synthesize all of their complex chemical makeup. The energy sources of the genera *Nitrobacter* and *Nitrosomonas* (the latter is a member of the betaproteobacteria) are reduced nitrogenous compounds. *Nitrosomonas* species oxidize ammonium (NH_4^+) to nitrite (NO_2^-), which is in turn oxidized by *Nitrobacter* species to nitrates (NO_3^-) in the process of *nitrification*. Nitrate is important to agriculture; it's a nitrogen form that's highly mobile in soil and therefore likely to be encountered and used by plants.

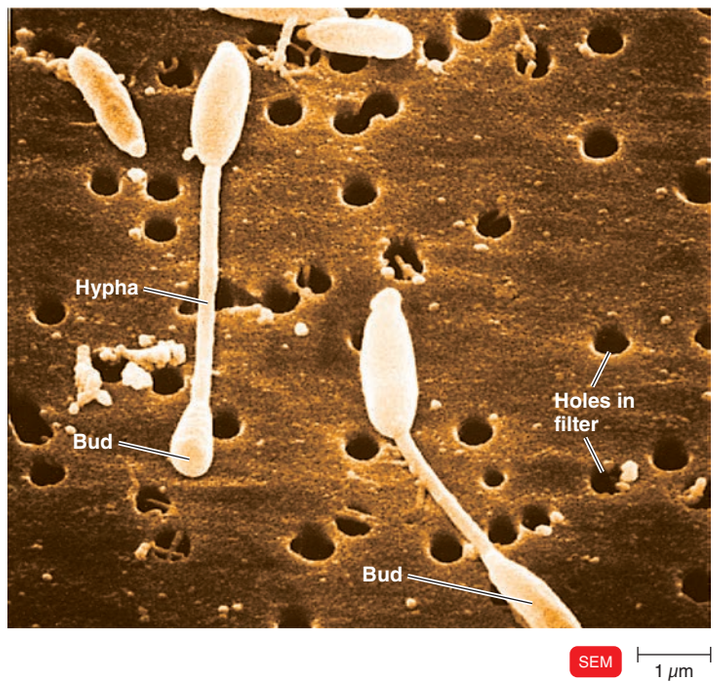


Figure 11.3 ***Hyphomicrobium,*** **a type of budding bacterium.**

Q Most bacteria do not reproduce by budding; what method do they use?

Figure 11.4 ***Spirillum volutans.*** These large helical bacteria are found in aquatic environments. Note the polar flagella.

Q Is this bacterium motile? How can you tell?

Wolbachia *Wolbachia* (wol-BAK-ē-ah) is probably the most common infectious bacterial genus in the world. Even so, little is known about these bacteria; they live only inside the cells of their hosts, usually insects (a relationship known as *endosymbiosis*). Therefore, *Wolbachia* escapes detection by the usual culture methods. This fascinating group of bacteria is described further in the Applications of Microbiology box on page 297.

CHECK YOUR UNDERSTANDING

Make a dichotomous key to distinguish the alphaproteobacteria described in this chapter. (*Hint:* See page 292 for a completed example.) **11-1**

The Betaproteobacteria

There is considerable overlap between the betaproteobacteria and the alphaproteobacteria, for example, among the nitrifying bacteria discussed earlier. The betaproteobacteria often use nutrient substances that diffuse away from areas of anaerobic decomposition of organic matter, such as hydrogen gas, ammonia, and methane. Several important pathogenic bacteria are found in this group.

Acidithiobacillus *Acidithiobacillus* (a′sid-ē-thi′ō-bah-SIL-lus) species and other sulfur-oxidizing bacteria are important in the sulfur cycle (see Figure 27.6, page 778). These chemoautotrophic bacteria are capable of obtaining energy by oxidizing the reduced forms of sulfur, such as hydrogen sulfide (H_2S), or elemental sulfur (S^0), into sulfates (SO_4^{2-}).

Spirillum The genus *Spirillum* (SPĪ-ril-lum) is found mainly in freshwater. *Spirillum* bacteria are motile by conventional polar flagella, an important morphological distinction from the helical spirochetes (page 307), which use axial filaments. The spirilla are relatively large, gram-negative, aerobic bacteria. *Spirillum volutans* (VOL-ū-tanz) is often used as a demonstration slide when microbiology students are first introduced to the operation of the microscope (**Figure 11.4**).

Sphaerotilus Sheathed bacteria, which include *Sphaerotilus natans* (sfer-O-ti-lus NĀ-tanz), are found in freshwater and in sewage. These gram-negative bacteria with polar flagella form a hollow, filamentous sheath in which to live (**Figure 11.5**). Sheaths are protective and also aid in nutrient accumulation. *Sphaerotilus* probably contributes to bulking, an important problem in sewage treatment (see Chapter 27).

Burkholderia The genus *Burkholderia* was formerly grouped with the genus *Pseudomonas,* which is now classified under the gammaproteobacteria. Like the pseudomonads, almost all *Burkholderia* species are motile by a single polar flagellum or tuft of flagella. The best known species is the aerobic, gram-negative rod *Burkholderia cepacia* (berk-HŌL-der′ē-ah se-PĀ-sē-ah). It has an extraordinary nutritional spectrum and is capable of degrading more than 100 different organic molecules. This capability is often a factor in the contamination of equipment and drugs in hospitals; these bacteria may actually grow in disinfectant solutions (see the Clinical Case in Chapter 15). This bacterium is also a problem for people with the genetic lung disease cystic fibrosis, in whom it metabolizes accumulated respiratory secretions.

Burkholderia pseudomallei (soo′dō-MAL-lē-ē) is a resident in moist soils and is the cause of a severe disease (melioidosis) endemic in southeast Asia and northern Australia (page 693).

Bordetella Of special importance is the nonmotile, aerobic, gram-negative rod *Bordetella pertussis* (bor′de-TEL-lah per-TUS-sis).

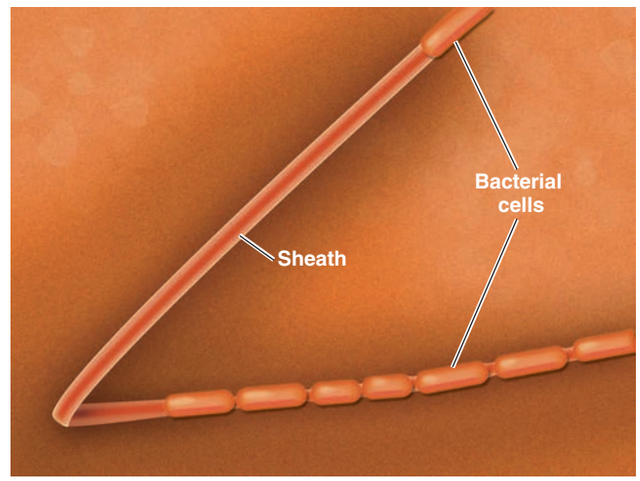


Figure 11.5 ***Sphaerotilus natans.*** These sheathed bacteria are found in dilute sewage and aquatic environments. They form elongated sheaths in which the bacteria live. The bacteria have flagella (not visible here) and can eventually swim free of the sheath.

Q How does the sheath help the cell?

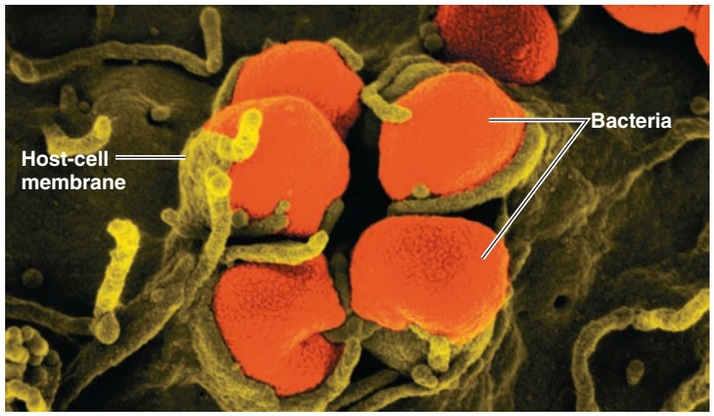


Figure 11.6 The gram-negative coccus *Neisseria gonorrhoeae*. This bacterium uses fimbriae and an outer membrane protein called Opa to attach to host cells. After attaching, the (green) host cell membrane surrounds the (red) bacteria.

Q How do fimbriae contribute to pathogenicity?

This serious pathogen is the cause of pertussis, or whooping cough (page 681).

Neisseria Bacteria of the genus *Neisseria* (nī-SE-rē-ah) are aerobic, gram-negative cocci that usually inhabit the mucous membranes of mammals. Pathogenic species include the gonococcus bacterium *Neisseria gonorrhoeae* (go-nōr-RĒ-ī), the causative agent of gonorrhoea (page 751, **Figure 11.6**, and the box in Chapter 26, page 756), and *N. meningitidis* (ME-nin-ji-ti-dis), the agent of meningococcal meningitis (page 610).

Zoogloea The genus *Zoogloea* (zō-ō-GLĒ-ah) is important in the context of aerobic sewage-treatment processes, such as the activated sludge system. As they grow, *Zoogloea* bacteria form fluffy, slimy masses that are essential to the proper operation of such systems.

CHECK YOUR UNDERSTANDING

✓ Make a dichotomous key to distinguish the betaproteobacteria described in this chapter. **11-2**

The Gammaproteobacteria

The gammaproteobacteria constitute the largest subgroup of the proteobacteria and include a great variety of physiological types. One species that is used in industrial microbiology is described in the box in Chapter 28 on page 801.

Thiotrichales Members of the order Thiotrichales include *Thiomargarita namibiensis*, which not only is the largest known bacterium but also exhibits several unusual characteristics. (See the discussion of microbial diversity, page 315). Other members of this order include the nutritionally distinctive genus *Beggiatoa* and *Francisella tularensis,* the pathogen causing tularemia.

Beggiatoa *Beggiatoa alba* (BEJ-jē-ah-tō-ah AL-bah), the only species of this unusual genus, grows in aquatic sediments at the interface between the aerobic and anaerobic layers. Morphologically, it resembles certain filamentous cyanobacteria (page 303), but it is not photosynthetic. Its motility is enabled by the production of slime, which attaches to the surface on which movement occurs and provides lubrication, allowing the organism to glide.

Nutritionally, *B. alba* uses hydrogen sulfide (H_2S) as an energy source and accumulates internal granules of sulfur. The ability of this organism to obtain energy from an inorganic compound was an important factor in the discovery of autotrophic metabolism.

Francisella *Francisella* (FRAN-sis-el-lah) is a genus of small, pleomorphic bacteria that grow only on complex media enriched with blood or tissue extracts. *Francisella tularensis* (TOO-lar-en-sis) causes the disease tularemia. (See the box in Chapter 23, page 642.)

Pseudomonadales Members of the order Pseudomonadales are gram-negative aerobic rods or cocci. The most important genus in this group is *Pseudomonas*. The order also includes *Azotobacter, Azomonas, Moraxella,* and *Acinetobacter.*

Pseudomonas A very important genus, *Pseudomonas* (soo′dō-MŌ-nas) consists of aerobic, gram-negative rods that are motile by polar flagella, either single or in tufts (**Figure 11.7**). Pseudomonads are very common in soil and other natural environments.

Figure 11.7 *Pseudomonas*. This photo of a pair of *Pseudomonas* bacteria shows polar flagella that are a characteristic of the genus. In some species only a single flagellum is present (see Figure 4.7b, page 77). Note that one cell (on the bottom) is beginning to divide.

Q How does the nutritional diversity of these bacteria make them a problem in hospitals?

Bacteria and Insect Sex

Wolbachia is quite possibly the most common infectious bacterial genus on Earth. Although these bacteria were first discovered in 1924, little had been known about them until the 1990s. They escape detection by the usual culture methods because they live as endosymbionts in the cells of insects and other invertebrates (**Figure A**).

Wolbachia infect over a million species of insects and other invertebrates. In all, as many as 75% of species of animals surveyed carry this bacterium. *Wolbachia* is essential to nematodes. If the bacterium is killed with antibiotics, the host worm dies.

In some insects, *Wolbachia* destroys males of its host species. *Wolbachia* can turn males into females by interfering with the male hormone. As shown in **Figure B**, if a male and female insect are uninfected with *Wolbachia,* they produce offspring normally. If only the male is infected, the insects fail to reproduce. If one or both insects of a mating pair are infected, only the infected females reproduce—and transmit *Wolbachia* in the cytoplasm of their eggs. Offspring produced without fertilization are female. The result is that the bacteria are transmitted to the next generation. This type of reproduction, called *parthenogenesis,* is seen in a variety of insects and in some amphibians and reptiles. Thus a question arises: Is *Wolbachia* always responsible?

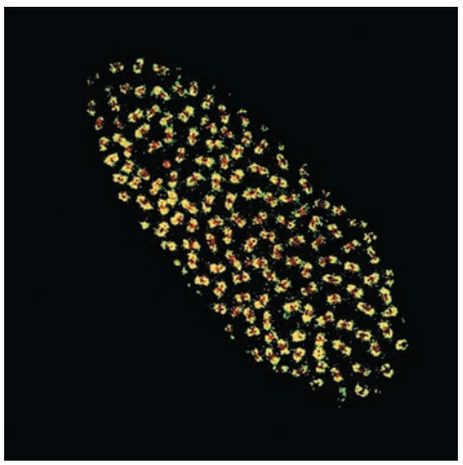


Figure A ***Wolbachia*** **are red inside the cells of this fruit fly embryo.**

Eukaryotic species are defined as organisms that reproduce only with members of their own species. This reproductive isolation prevents the production of hybrids and thus maintains the uniqueness of each species. In the laboratory, researchers have found that after antibiotic treatment, wasps of one species will produce hybrid offspring with another species. This raises the question about the influence *Wolbachia* has had on the evolution of insects. Did insects that were not infected reproduce successfully outside their species?

Wolbachia may be evolving into an organelle, much like ancient microbes evolved into mitochondria. Recently researchers discovered that *Wolbachia* can transfer its genes into the host cells and that the genes are expressed. This horizontal gene transfer could provide new traits in the host.

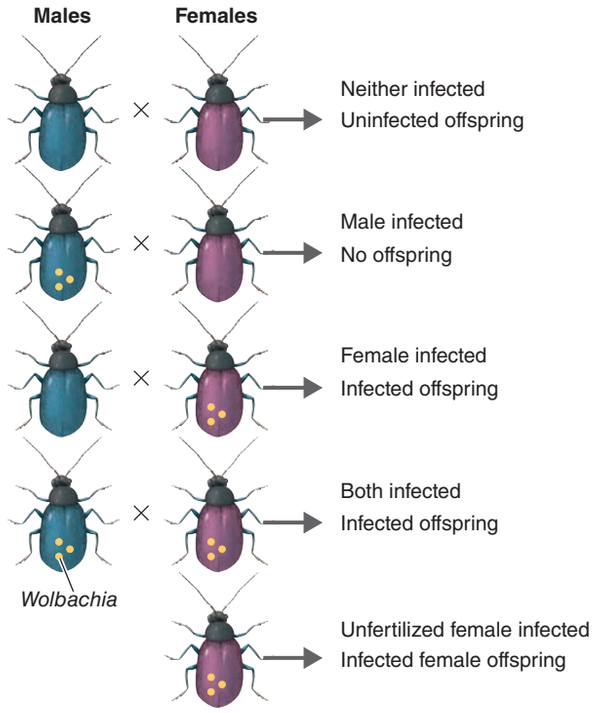


Figure B **In an infected pair, only female hosts can reproduce.**

A virulent strain of *Wolbachia* called "popcorn" causes host cells to lyse, or "pop," which eventually kills the host insect. On the one hand, the popcorn strain might be used to kill mosquitoes. On the other hand, eliminating *Wolbachia* from pest insects could result in a lower number of females, thus reducing population growth.

The unique biology of *Wolbachia* has attracted researchers interested in questions that range from the evolutionary implications of infection to commercial uses of *Wolbachia*.

Many species of pseudomonads excrete extracellular, water-soluble pigments that diffuse into their media. One species, *Pseudomonas aeruginosa* (Ā-roo-jĭ′nō-sah), produces a soluble, blue-green pigmentation. Under certain conditions, particularly in weakened hosts, this organism can infect the urinary tract, burns, and wounds, and can cause blood infections (sepsis; page 639), abscesses, and meningitis. Other pseudomonads produce soluble fluorescent pigments that glow when illuminated by ultraviolet light. One species, *P. syringae* (ser-IN-gī), is an occasional plant pathogen. (Some species of *Pseudomonas* have been transferred, based upon rRNA studies, to the genus *Burkholderia,* which was discussed previously with the betaproteobacteria.)

Pseudomonads have almost as much genetic capacity as the eukaryotic yeasts and almost half as much as a fruit fly. Although these bacteria are less efficient than some other heterotrophic bacteria in utilizing many of the more common nutrients, they compensate for this by making use of their genetic capabilities. For example, pseudomonads synthesize an unusually large

number of enzymes and can metabolize a wide variety of substrates. Therefore, they probably contribute significantly to the decomposition of uncommon chemicals, such as pesticides, that are added to soil.

In hospitals and other places where pharmaceutical agents are prepared, the ability of pseudomonads to grow on minute traces of unusual carbon sources, such as soap residues or capliner adhesives found in a solution, has been unexpectedly troublesome. Pseudomonads are even capable of growth in some antiseptics, such as quaternary ammonium compounds. Their resistance to most antibiotics has also been a source of medical concern. This resistance is probably related to the characteristics of the cell wall porins, which control the entrance of molecules through the cell wall (see Chapter 4, page 81). The large genome of pseudomonads also codes for several very efficient efflux pump systems (page 571) that eject antibiotics from the cell before they can function. Pseudomonads are responsible for about one in ten nosocomial infections (infections acquired in health care settings; see page 402), especially among infections in burn units. People with cystic fibrosis are also especially prone to infections by *Pseudomonas* and the closely related *Burkholderia*.

Although pseudomonads are classified as aerobic, some are capable of substituting nitrate for oxygen as a terminal electron acceptor. This process, anaerobic respiration, yields almost as much energy as aerobic respiration (see page 126). In this way, pseudomonads cause important losses of valuable nitrogen in fertilizer and soil. Nitrate (NO_3^-) is the form of nitrogen fertilizer most easily used by plants. Under anaerobic conditions, as in water-logged soil, pseudomonads eventually convert this valuable nitrate into nitrogen gas (N_2), which is lost to the atmosphere (see Figure 27.4, page 777).

Many pseudomonads can grow at refrigerator temperatures. This characteristic, combined with their ability to utilize proteins and lipids, makes them an important contributor to food spoilage.

Azotobacter and Azomonas Some nitrogen-fixing bacteria, such as *Azotobacter* (a-ZŌ-tō-bak-ter) and *Azomonas* (ā-zō-MŌ-nas), are free-living in soil. These large, ovoid, heavily capsulated bacteria are frequently used in laboratory demonstrations of nitrogen fixation. However, to fix agriculturally significant amounts of nitrogen, they would require energy sources, such as carbohydrates, that are in limited supply in soil.

Moraxella Members of the genus *Moraxella* (MOR-ax-el-lah) are strictly aerobic coccobacilli—that is, intermediate in shape between cocci and rods. *Moraxella lacunata* (LAH-koo-nah-tah) is implicated in conjunctivitis, an inflammation of the conjunctiva, the membrane that covers the eye and lines the eyelids (page 599).

Acinetobacter The genus *Acinetobacter* (ah-sin-Ē-tō-bak′ter) is aerobic and typically forms pairs. The bacteria occur naturally in soil and water. A member of this genus, *Acinetobacter baumanii* (BŌ-man-nē-ē), is an increasing concern to the medical community because of the rapidity with which it becomes resistant to antibiotics. Some strains are resistant to most available antibiotics. Not yet widespread in the United States, *A. baumanii* is an opportunistic pathogen primarily found in health care settings. The antibiotic resistance of the pathogen, combined with the weakened health of infected patients, has resulted in an unusually high mortality rate. *A. baumanii* is primarily a respiratory pathogen, but it also infects skin, soft tissues, and wounds and occasionally invades the bloodstream. It is more environmentally hardy than most gram-negative bacteria, and once established in a health care setting, it becomes difficult to eliminate.

Legionellales The genera *Legionella* and *Coxiella* are closely associated in the second edition of *Bergey's Manual,* where both are placed in the same order, Legionellales. Because the *Coxiella* share an intracellular lifestyle with the rickettsial bacteria, they were previously considered rickettsial in nature and grouped with them. *Legionella* bacteria grow readily on suitable artificial media.

Legionella *Legionella* (lē-JEN-el-lah) bacteria were originally isolated during a search for the cause of an outbreak of pneumonia now known as legionellosis (page 690). The search was difficult because these bacteria didn't grow on the usual laboratory isolation media then available. After intensive effort, special media were developed that enabled researchers to isolate and culture the first *Legionella*. Microbes of this genus are now known to be relatively common in streams, and they colonize such habitats as warm-water supply lines in hospitals and water in the cooling towers of air conditioning systems. (See the box in Chapter 24, page 694.) An ability to survive and reproduce within aquatic amebae often makes them difficult to eradicate in water systems.

Coxiella *Coxiella burnetii* (KOKS-ē-el-lah ber-NE-tē-ē), which causes Q fever (page 692), was formerly grouped with the rickettsias. Like them, *Coxiella* bacteria require a mammalian host cell to reproduce. Unlike rickettsias, *Coxiella* bacteria are not transmitted among humans by insect or tick bites. Although cattle ticks harbor the organism, it is most commonly transmitted by contaminated milk. A sporelike body is present in *C. burnetii* (see Figure 24.13, page 692). This might explain the bacterium's relatively high resistance to the stresses of airborne transmission and heat treatment.

Vibrionales Members of the order Vibrionales are facultatively anaerobic gram-negative rods. They are found mostly in aquatic habitats. *Vibrio* (VIB-rē-ō) species are rods that are often slightly curved (**Figure 11.8**). One important pathogen is *Vibrio cholerae* (KOL-er-ī), the causative agent of cholera (page 717). The disease is characterized by a profuse and watery diarrhea. *V. parahaemolyticus* (par′ah-hē-mō-LI-ti-kus) causes a less serious

form of gastroenteritis. Usually inhabiting coastal salt waters, it's transmitted to humans mostly by raw or undercooked shellfish.

Enterobacteriales The members of the order Enterobacteriales are facultatively anaerobic, gram-negative rods that are, if motile, peritrichously flagellated. Morphologically, the rods are straight. This is an important bacterial group, often commonly called **enterics.** This reflects the fact that they inhabit the intestinal tracts of humans and other animals. Most enterics are active fermenters of glucose and other carbohydrates.

Because of the clinical importance of enterics, there are many techniques to isolate and identify them. An identification method for some enterics is shown in Figure 10.9 (page 277), which incorporates a modern tool using 15 biochemical tests. Biochemical tests are especially important in clinical laboratory work and in food and water microbiology.

Enterics have fimbriae that help them adhere to surfaces or mucous membranes. Specialized sex pili aid in the exchange of genetic information between cells, which often includes antibiotic resistance (see Figures 8.26 and 8.27, pages 228 and 229).

Enterics, like many bacteria, produce proteins called bacteriocins that cause the lysis of closely related species of bacteria. Bacteriocins may help maintain the ecological balance of various enterics in the intestines. Important genera of the order include *Escherichia*, *Salmonella, Shigella, Klebsiella, Serratia, Proteus, Yersinia, Erwinia, Enterobacter,* and *Cronobacter*—all discussed next.

Escherichia The bacterial species *Escherichia coli* is one of the most common inhabitants of the human intestinal tract and is probably the most familiar organism in microbiology. A great deal is known about the biochemistry and genetics of *E. coli,* and it continues to be an important tool for basic biological research—many researchers consider it almost a laboratory pet. Its presence in water or food is an indication of fecal contamination (see Chapter 27, page 782). *E. coli* is not usually pathogenic. However, it can be a cause of urinary tract infections, and certain strains produce enterotoxins that cause traveler's diarrhea (page 722) and occasionally cause very serious foodborne disease (see *E. coli* O157:H7, page 719).

Salmonella Almost all members of the genus *Salmonella* (sal'mōn-EL-lah) are potentially pathogenic. Accordingly, there are extensive biochemical and serological tests to clinically isolate and identify salmonellae. Salmonellae are common inhabitants of the intestinal tracts of many animals, especially poultry and cattle. Under unsanitary conditions, they can contaminate food.

The nomenclature of the genus *Salmonella* is unusual. Instead of multiple species, members of the genus *Salmonella* that are infectious to warm-blooded animals can be considered for practical purposes to be a single species, *Salmonella enterica* (en-TER-i-kah). This species is divided into more than

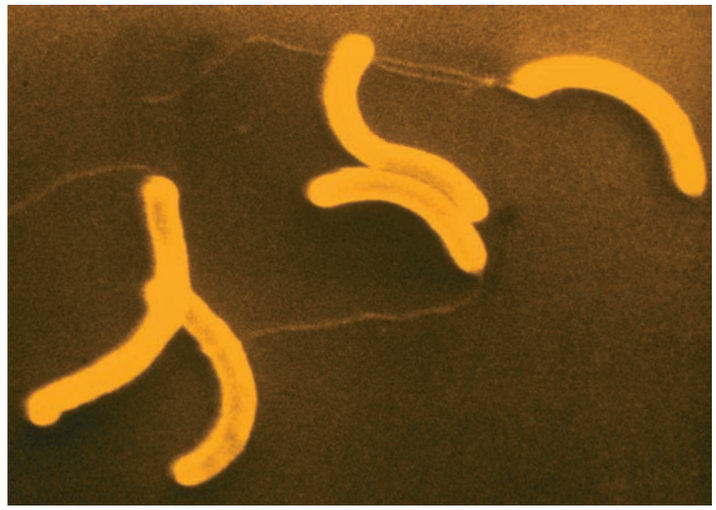


Figure 11.8 ***Vibrio cholerae.*** Notice the curvature of these rods, which is a characteristic of the genus.

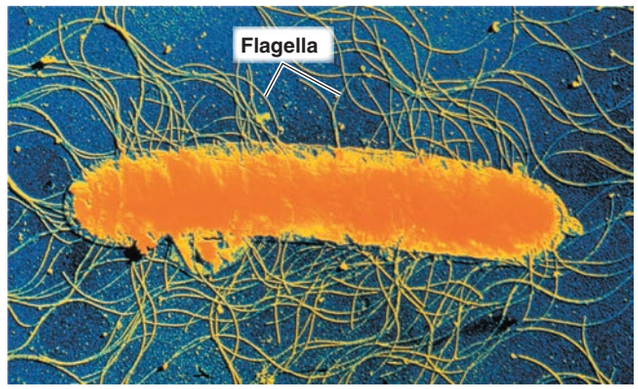
What disease does *Vibrio cholerae* cause?

2400 **serovars,** that is, *serological varieties*. The term **serotype** is often used to mean the same thing. By way of explanation of these terms, when salmonellae are injected into appropriate animals, their flagella, capsules, and cell walls serve as *antigens* that cause the animals to form *antibodies* in their blood that are specific for each of these structures. Thus, *serological* means are used to differentiate the microorganisms. Serology is discussed more fully in Chapter 18, but for now it's sufficient to state that it can be used to differentiate and identify bacteria.

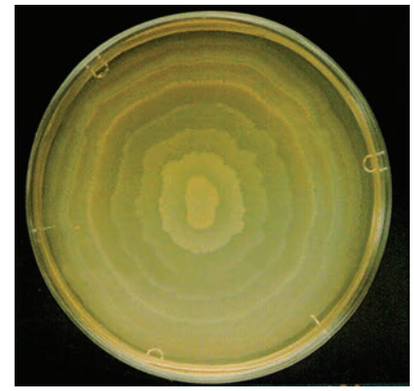
A serovar such as *Salmonella typhimurium* (tī-fi-MER-ē-um) is not a species and should be more properly written as "*Salmonella enterica* serovar Typhimurium." The convention now used by the Centers for Disease Control and Prevention (CDC) is to spell out the entire name at the first mention and then abbreviate it as, for example, *Salmonella* Typhimurium. For simplicity, we will identify serovars of salmonellae in this text as we would species, that is, *S. typhimurium,* etc.

Specific antibodies, which are available commercially, can be used to differentiate *Salmonella* serovars by a system known as the Kauffmann-White scheme. This scheme designates an organism by numbers and letters that correspond to specific antigens on the organism's capsule, cell wall, and flagella, which are identified by the letters K, O, and H, respectively. For example, the antigenic formula for the bacterium *S. typhimurium* is O1,4,[5],12:H,i,1,2*.

*The letters derive from the original German usage. Colonies that spread in a thin film over the agar surface were described by the German word for film, *Hauch*. The motility needed to form a film implied the presence of flagella, and the letter H came to be assigned to the antigens of flagella. Nonmotile bacteria were described as *ohne Hauch,* without film, and the O came to be assigned to the cell surface or body antigens. This terminology is also used in the naming of *E. coli* O157:H7, *Vibrio cholerae* O:1, and others.



(a) *Proteus mirabilis* with peritrichous flagella



(b) A swarming colony of *Proteus mirabilis*, showing concentric rings of growth

Figure 11.9 *Proteus mirabilis.* Chemical communication between bacterial cells causes changes from cells adapted to swimming in fluid (few flagella) to cells that are able to move on surfaces (numerous flagella). The concentric growth **(b)** results from periodic synchronized conversion to the highly flagellated form capable of movement on surfaces.

Q The photo of the *Proteus* cell is probably a swarmer cell. How would you know?

Many salmonellae are named only by their antigenic formulas. Serovars can be further differentiated by special biochemical or physiological properties into **biovars,** or **biotypes.**

A recent taxonomic arrangement based upon the latest molecular technology adds another species, *Salmonella bongori* (BON-gor-ē). This is a resident of "cold-blooded" animals—it was originally isolated from a lizard in the town of Bongor in the African desert nation of Chad—and is rarely found in humans.

Typhoid fever, caused by *Salmonella typhi* (TĪ-fē), is the most severe illness caused by any member of the genus *Salmonella* (page 716). A less severe gastrointestinal disease caused by other salmonellae is called salmonellosis (page 715). Salmonellosis is one of the most common forms of foodborne illness. (See the box in Chapter 25 on page 717.)

Shigella Species of *Shigella* (shi-GEL-lah) are responsible for a disease called bacillary dysentery, or shigellosis (page 714). Unlike salmonellae, they are found only in humans. Some strains of *Shigella* can cause life-threatening dysentery.

Klebsiella Members of the genus *Klebsiella* (kleb-SĒ-el-lah) are commonly found in soil or water. Many isolates are capable of fixing nitrogen from the atmosphere, which has been proposed as being a nutritional advantage in isolated populations with little protein nitrogen in their diet. The species *Klebsiella pneumoniae* (noo-MŌ-nē-ī) occasionally causes a serious form of pneumonia in humans.

Serratia *Serratia marcescens* (ser-RĀ-shah mar-SE-sens) is a bacterial species distinguished by its production of red pigment. In hospital situations, the organism can be found on catheters, in saline irrigation solutions, and in other supposedly sterile solutions. Such contamination is probably the cause of many urinary and respiratory tract infections in hospitals.

Proteus Colonies of *Proteus* (PRŌ-tē-us) bacteria growing on agar exhibit a swarming type of growth. Swarmer cells with many flagella (**Figure 11.9a**) move outward on the edges of the colony and then revert to normal cells with only a few flagella and reduced motility. Periodically, new generations of highly motile swarmer cells develop, and the process is repeated. As a result, a *Proteus* colony has the distinctive appearance of a series of concentric rings (Figure 11.9b). This genus of bacteria is implicated in many infections of the urinary tract and in wounds.

Yersinia *Yersinia pestis* (yer-SIN-ē-ah PES-tis) causes plague, the Black Death of medieval Europe (page 647). Urban rats in some parts of the world and ground squirrels in the American Southwest carry these bacteria. Fleas usually transmit the organisms among animals and to humans, although contact with respiratory droplets from infected animals and people can be involved in transmission.

Erwinia *Erwinia* (er-WI-nē-ah) species are primarily plant pathogens; some cause plant soft-rot diseases. These species produce enzymes that hydrolyze the pectin between individual plant cells. This causes the plant cells to separate from each other, a disease that plant pathologists term *soft rot*.

Enterobacter Two *Enterobacter* (en'ter-ō-BAK-ter) species, *E. cloacae* (KLŌ-ā-kī), and *E. aerogenes* (ar-O-jen-ēz), can cause urinary tract infections and hospital-acquired infections. They are widely distributed in humans and animals, as well as in water, sewage, and soil.

Cronobacter *Cronobacter* is a genus of gram-negative, rod-shaped bacteria of the family Enterobacteriaceae. This genus was introduced in 2007, and there are now seven named species. These bacteria are facultatively anaerobic and generally motile. The type species is *Cronobacter sakazakii,* which was previously known as *Enterobacter sakazakii*. This organism can cause meningitis and necrotizing enterocolitis in infants. It is widespread in a range of environments and foods. Most cases occur in adults, although the most publicized outbreaks have been associated with contaminated infant formulas.

Pasteurellales The bacteria in the order Pasteurellales are nonmotile; they are best known as human and animal pathogens.

Pasteurella The genus *Pasteurella* (PAS-tyer-el-lah) is primarily known as a pathogen of domestic animals. It causes sepsis in cattle, fowl cholera in chickens and other fowl, and pneumonia in several types of animals. The best-known species is *Pasteurella multocida* (MUL-tō-si-dah), which can be transmitted to humans by dog and cat bites.

Haemophilus *Haemophilus* (hē-MAH-fil-us) is a very important genus of pathogenic bacteria. These organisms inhabit the mucous membranes of the upper respiratory tract, mouth, vagina, and intestinal tract. The best-known species that affects humans is *Haemophilus influenzae* (IN-flū-en-zī), named long ago because of the erroneous belief that it was responsible for influenza.

The name *Haemophilus* is derived from the bacteria's requirement for blood in their culture medium (*hemo* = blood). They are unable to synthesize important parts of the cytochrome system needed for respiration, and they obtain these substances from the heme fraction, known as the **X factor,** of blood hemoglobin. The culture medium must also supply the cofactor nicotinamide adenine dinucleotide (from either NAD^+ or $NADP^+$), which is known as **V factor.** Clinical laboratories use tests for the requirement of X and V factors to identify isolates as *Haemophilus* species.

Haemophilus influenzae is responsible for several important diseases. It has been a common cause of meningitis in young children and is a frequent cause of earaches. Other clinical conditions caused by *H. influenzae* include epiglottitis (a life-threatening condition in which the epiglottis becomes infected and inflamed), septic arthritis in children, bronchitis, and pneumonia. *Haemophilus ducreyi* (doo-KRĀ-ē) is the cause of the sexually transmitted disease chancroid (page 761).

CHECK YOUR UNDERSTANDING

Make a dichotomous key to distinguish the orders of gamma-proteobacteria described in this chapter. **11-3**

The Deltaproteobacteria

The deltaproteobacteria are distinctive in that they include some bacteria that are predators on other bacteria. Bacteria in this group are also important contributors to the sulfur cycle.

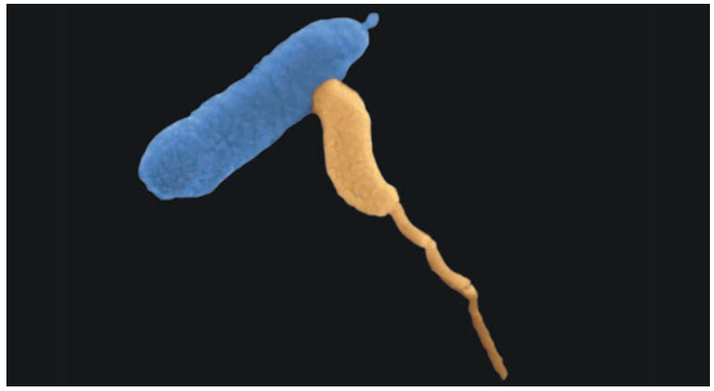


Figure 11.10 ***Bdellovibrio bacteriovorus.*** The yellow bacterium is *B. bacteriovorus*. It is attacking a bacterial cell shown in blue.

Q Would this bacterium attack *Staphylococcus aureus*?

Bdellovibrio *Bdellovibrio* (del-lō-VIB-rē-ō) is a particularly interesting genus. It attacks other gram-negative bacteria. It attaches tightly (*bdella* = leech; **Figure 11.10**), and after penetrating the outer layer of gram-negative bacteria, it reproduces within the periplasm. There, the cell elongates into a tight spiral, which then fragments almost simultaneously into several individual flagellated cells. The host cell then lyses, releasing the *Bdellovibrio* cells.

Desulfovibrionales Members of the order Desulfovibrionales are sulfur-reducing bacteria. They are obligately anaerobic bacteria that use oxidized forms of sulfur, such as sulfates (SO_4^{2-}) or elemental sulfur (S^0) rather than oxygen as electron acceptors. The product of this reduction is hydrogen sulfide (H_2S). (Because the H_2S is not assimilated as a nutrient, this type of metabolism is termed *dissimilatory.*) The activity of these bacteria releases millions of tons of H_2S into the atmosphere every year and plays a key part in the sulfur cycle (see Figure 27.6 on page 778). Sulfur-oxidizing bacteria such as *Beggiatoa* are able to use H_2S either as part of photosynthesis or as an autotrophic energy source.

Desulfovibrio (de′sul-fō-VIB-rē-ō), the best-studied sulfur-reducing genus, is found in anaerobic sediments and in the intestinal tracts of humans and animals. Sulfur-reducing and sulfate-reducing bacteria use organic compounds such as lactate, ethanol, or fatty acids as electron donors. This reduces sulfur or sulfate to H_2S. When H_2S reacts with iron it forms insoluble FeS, which is responsible for the black color of many sediments.

Myxococcales In earlier editions of *Bergey's Manual,* the Myxococcales were classified among the fruiting and gliding bacteria. They illustrate the most complex life cycle of all bacteria, part of which is predatory upon other bacteria.

Vegetative cells of the myxobacteria (*myxo* = mucus) move by gliding and leave behind a slime trail. *Myxococcus*

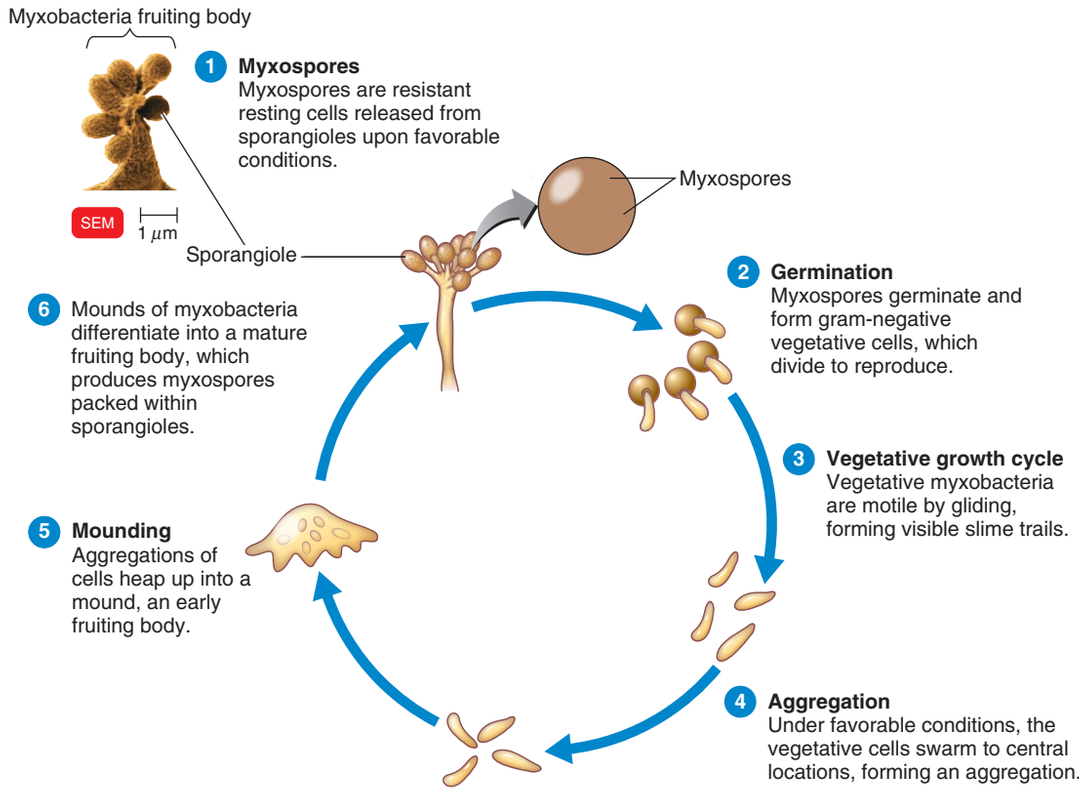


Figure 11.11 Myxococcales.

Q What is the feeding stage of this organism?

xanthus (mix′ō-KOK-us ZAN-thus) and *M. fulvus* (FUL-vus) are well-studied representatives of the genus *Myxococcus*. As they move, their source of nutrition is the bacteria they encounter, enzymatically lyse, and digest. Large numbers of these gram-negative microbes eventually aggregate (Figure 11.11). Where the moving cells aggregate, they differentiate and form a macroscopic stalked fruiting body that contains large numbers of resting cells called *myxospores*. Differentiation is usually triggered by low nutrients. Under proper conditions, usually a change in nutrients, the myxospores germinate and form new vegetative gliding cells. You might note the resemblance to the life cycle of the eukaryotic cellular slime molds in Figure 12.22 (page 344).

CHECK YOUR UNDERSTANDING

Make a dichotomous key to distinguish the deltaproteobacteria described in this chapter. **11-4**

The Epsilonproteobacteria

The epsilonproteobacteria are slender gram-negative rods that are helical or curved. We will discuss the two important genera, both of which are motile by means of flagella and are microaerophilic.

Campylobacter *Campylobacter* (KAM-pi-lō-bak-ter) bacteria are microaerophilic vibrios; each cell has one polar flagellum. One species, *C. fetus* (FĒ-tus), causes spontaneous abortion in domestic animals. Another species, *C. jejuni* (je-JU-nē), is a leading cause of outbreaks of foodborne intestinal disease.

Helicobacter *Helicobacter* bacteria are microaerophilic curved rods with multiple flagella. The species *Helicobacter pylori* (HĒ-lik-ō-bak′ter PĪ-lor-ē) has been identified as the most common cause of peptic ulcers in humans and a cause of stomach cancer (Figure 11.12; see also Figure 25.12 on page 723).

CHECK YOUR UNDERSTANDING

Make a dichotomous key to distinguish the epsilonproteobacteria described in this chapter. **11-5**

The Nonproteobacteria Gram-Negative Bacteria

LEARNING OBJECTIVES

11-6 Differentiate planctomycetes, chlamydias, Bacteroidetes, *Cytophaga,* and Fusobacteria by drawing a dichotomous key.

11-7 Compare and contrast purple and green photosynthetic bacteria with the cyanobacteria.

11-8 Describe the features of spirochetes and *Deinococcus*.

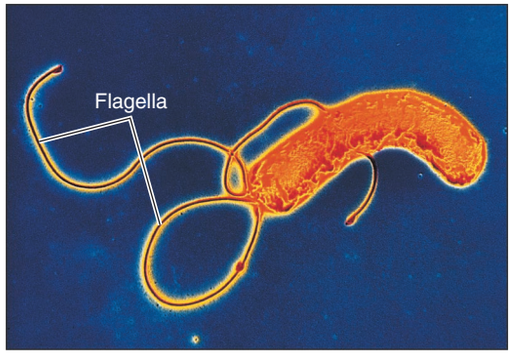


Figure 11.12 ***Helicobacter pylori.*** *H. pylori*, a curved rod, is an example of a helical bacterium that does not make a complete twist.

Q How do helical bacteria differ from spirochetes?

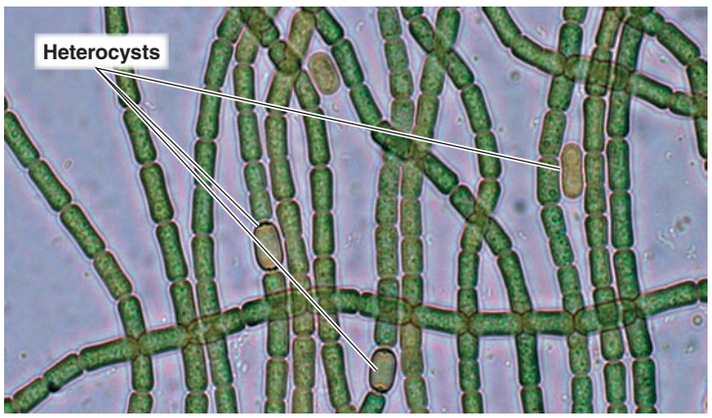
There are a number of important gram-negative bacteria that are not closely related to the gram-negative proteobacteria.

Cyanobacteria (The Oxygenic Photosynthetic Bacteria)

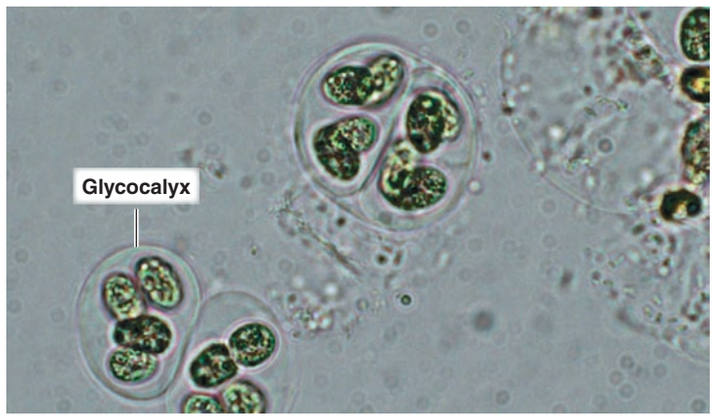
The cyanobacteria, named for their characteristic blue-green (*cyan*) pigmentation, were once called blue-green algae. Although they resemble the eukaryotic algae and often occupy the same environmental niches, this is a misnomer because they are bacteria, algae are not. However, cyanobacteria do carry out oxygenic photosynthesis, as do the eukaryotic plants and algae (see Chapter 12). Many of the cyanobacteria are capable of fixing nitrogen from the atmosphere. In most cases, this activity is located in specialized cells caleed **heterocysts,** which contain enzymes that fix nitrogen gas (N_2) into ammonium (NH_4^+) that can be used by the growing cell (Figure 11.13a). Species that grow in water usually have gas vacuoles that provide buoyancy, helping the cell float at a favorable environment. Cyanobacteria that move about on solid surfaces use gliding motility.

Cyanobacteria are morphologically varied. They range from unicellular forms that divide by simple binary fission (Figure 11.13b), to colonial forms that divide by multiple fission, to filamentous forms that reproduce by fragmentation of the filaments. The filamentous forms usually exhibit some differentiation of cells that are often bound together within an envelope or sheath.

Evidence indicates that oxygenic cyanobacteria played an important part in the development of life on Earth, which originally had very little free oxygen that would support life as we are familiar with it. Fossil evidence indicates that when cyanobacteria first appeared, the atmosphere contained only about 0.1% free oxygen. When oxygen-producing eukaryotic plants appeared millions of years later, the concentration of oxygen was more than 10%. The increase presumably was a result of photosynthetic activity by cyanobacteria. The atmosphere we breathe today contains about 20% oxygen. Cyanobacteria, especially those that fix nitrogen, are extremely important to the environment. They occupy environmental niches similar to those occupied by the eukaryotic algae (see Figure 12.10, page 328). Many of the cyanobacteria are able to fix nitrogen, making them even more adaptable than algae in nutritionally poor environments. The environmental role of cyanobacteria is presented more fully in Chapter 27, in the discussion of eutrophication (the nutritional overenrichment of bodies of water).



(a) Filamentous cyanobacterium showing heterocysts, in which nitrogen-fixing activity is located



(b) A unicellular, nonfilamentous cyanobacterium, *Gloeocapsa*. Groups of these cells, which divide by binary fission, are held together by the surrounding glycocalyx.

Figure 11.13 **Cyanobacteria.**

Q What does *anoxygenic* mean?

TABLE 11.2 Selected Characteristics of Photosynthesizing Bacteria

Common Name	Example	Phylum	Comments	Electron Donor for CO_2 Reduction	Oxygenic or Anoxygenic
Cyanobacteria	*Anabaena*	Cyanobacteria	Plantlike photosynthesis; some use bacterial photosynthesis under anaerobic conditions	Usually H_2O	Usually oxygenic
Green nonsulfur bacteria	*Chloroflexus*	Chloroflexi	Grow chemoheterotrophically in aerobic environments	Organic compounds	Anoxygenic
Green sulfur bacteria	*Chlorobium*	Chlorobi	Deposit sulfur granules inside cells	Usually H_2S	Anoxygenic
Purple nonsulfur bacteria	*Rhodospirillum*	Proteobacteria	Can grow chemoheterotrophically as well	Organic compounds	Anoxygenic
Purple sulfur bacteria	*Chromatium*	Proteobacteria	Deposit sulfur granules inside cells	Usually H_2S	Anoxygenic

The Phyla Chlorobi and Chloroflexi (The Anoxygenic Photosynthetic Bacteria)

The photosynthetic bacteria are taxonomically confusing, but they represent some interesting ecological niches. The phyla Cyanobacteria, Chlorobi, and Chloroflexi are gram-negative, but they are not included in the proteobacteria. Members of the photosynthetic phylum Chlorobi (representative genus: *Chlorobium*) are called **green sulfur bacteria.** Members of the phylum Chloroflexi (representative genus: *Chloroflexus*) are called **green nonsulfur bacteria.** Both these varieties of bacteria produce no oxygen during photosynthesis. The photosynthesizing bacteria are summarized in Table 11.2.

However, there are photosynthetic gram-negative bacteria that *are* genetically included in the proteobacteria. These are the **purple sulfur bacteria** and the **purple nonsulfur bacteria,** which are placed, respectively, in the alphaproteobacteria and gammaproteobacteria.

In these bacterial groups, the term *sulfur bacteria* indicates that the microbes can use H_2S as an electron donor (see the equations that follow). If classified as *nonsulfur bacteria,* the microbes have at least a limited capability of phototrophic growth, but without the production of oxygen. Cyanobacteria, as well as eukaryotic plants and algae, produce oxygen (O_2) from water (H_2O) as they carry out photosynthesis:

$$(1)\ 2H_2O + CO_2 \xrightarrow{\text{light}} (CH_2O) + H_2O + O_2$$

The *purple sulfur bacteria* and *green sulfur bacteria* use reduced sulfur compounds, such as hydrogen sulfide (H_2S), instead of water, and they produce granules of sulfur (S^0) rather than oxygen, as follows:

$$(2)\ 2H_2S + CO_2 \xrightarrow{\text{light}} (CH_2O) + H_2O + 2S^0$$

Chromatium (krō-MĀ-shum), shown in (Figure 11.14), is a representative genus. At one time, an important question in biology concerned the source of the oxygen produced by plant photosynthesis: was it from CO_2 or from H_2O? Until the introduction of radioisotope tracers, which traced the oxygen in water and carbon dioxide and settled the question, comparison of equations 1 and 2 was the best evidence that the oxygen source was H_2O. It is also important to compare these two equations to understand how reduced sulfur compounds, such as H_2S, can substitute for H_2O in photosynthesis. See "Life Without Sunshine" on page 776.

Other photoautotrophs, the *purple nonsulfur bacteria* and *green nonsulfur bacteria*, use organic compounds, such as acids and carbohydrates, for the photosynthetic reduction of carbon dioxide. Morphologically, the photosynthetic bacteria are very diverse, with spirals, rods, cocci, and even budding forms.

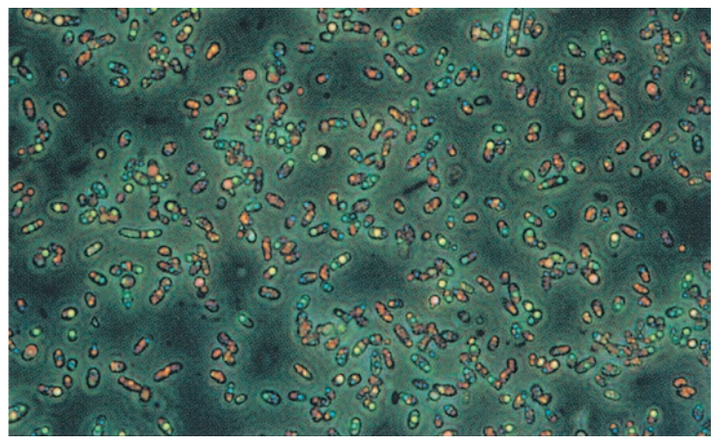
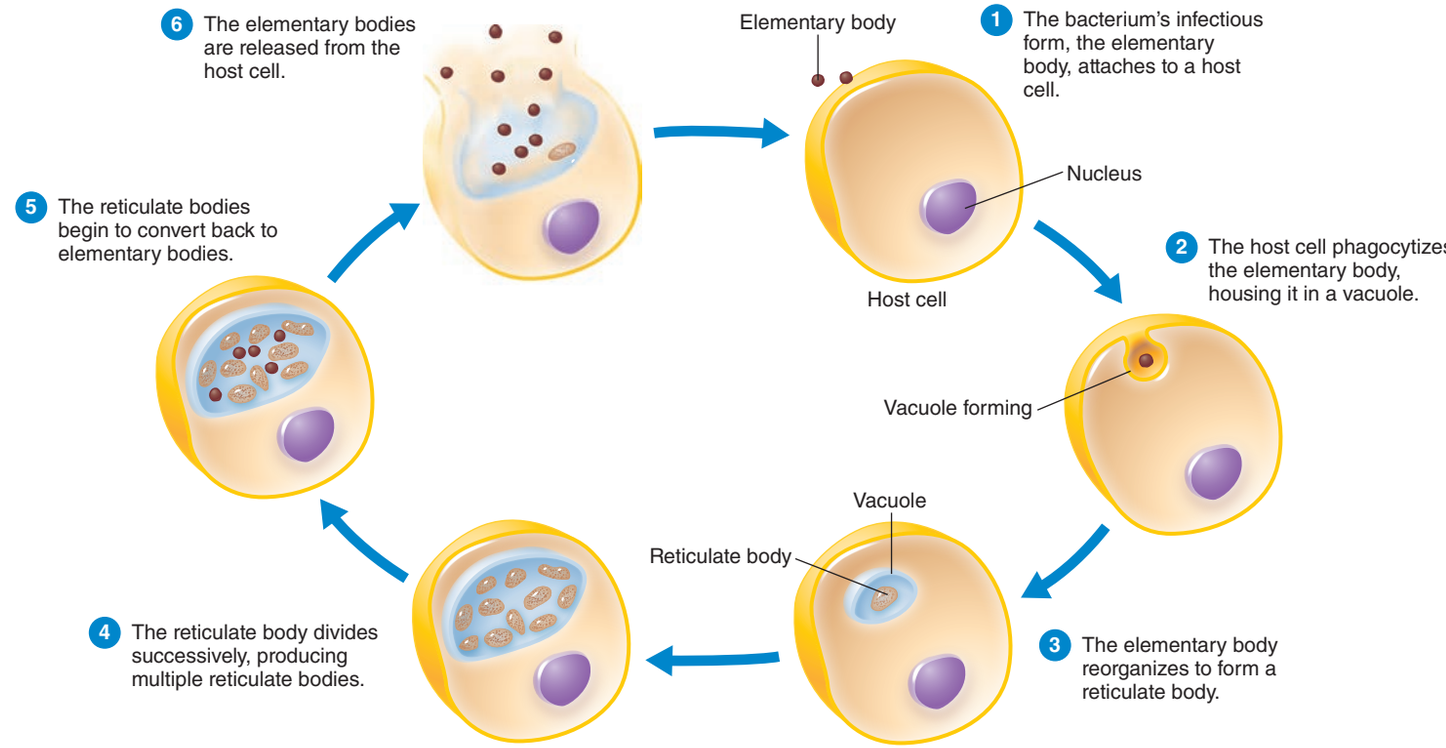
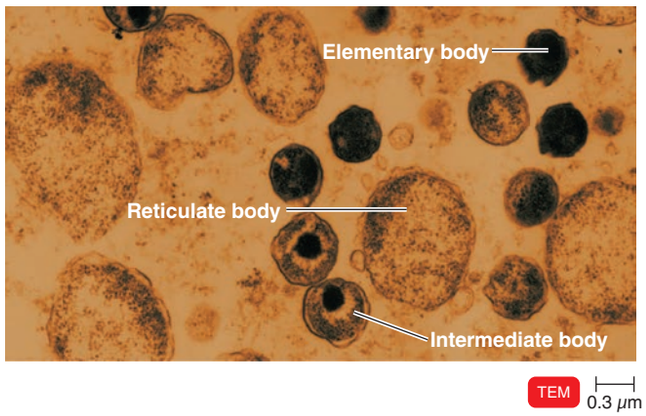


Figure 11.14 Purple sulfur bacteria. This photomicrograph of cells of the genus *Chromatium* shows the intracellular sulfur granules as multicolored refractile objects. The reason the sulfur accumulates can be surmised from inspection of equation 2 in the discussion.

Q How does the photosynthesis of cyanobacteria differ from that of the purple sulfur bacteria?



(a) Life cycle of the chlamydias, which takes about 48 hours to complete



(b) Micrograph of *Chlamydophila psittaci* in the cytoplasm of a host cell. The **elementary bodies** are the infectious stage; they are dense, dark, and relatively small. **Reticulate bodies,** the form in which chlamydias reproduce within the host cell, are larger with a speckled appearance. **Intermediate bodies,** a stage between the two, have a dark center.

Figure 11.15 Chlamydias.

Q Which stage of the life cycle is infectious to humans?

Chlamydiae

Members of the phylum Chlamydiae are grouped with other genetically similar bacteria that do not contain peptidoglycan in their cell walls. We will discuss only the genera *Chlamydia* and *Chlamydophila*. Earlier editions of *Bergey's Manual* grouped these bacteria with the rickettsial bacteria because they all grow intracellularly within host cells. The rickettsias are now classified according to their genetic content with the alphaproteobacteria.

Chlamydia* and *Chlamydophila *Chlamydia* and *Chlamydophila*, which we will call by the common name of the chlamydias, have a unique developmental cycle that is perhaps their most distinguishing characteristic (Figure 11.15a). They are gram-negative coccoid bacteria (Figure 11.15b). The **elementary body** shown in Figure 11.15 is the infective agent. Unlike the rickettsias, chlamydias do not require insects or ticks for transmission. They are transmitted to humans by interpersonal contact or by airborne respiratory routes. The chlamydias can be cultivated in laboratory animals, in cell cultures, or in the yolk sac of embryonated chicken eggs.

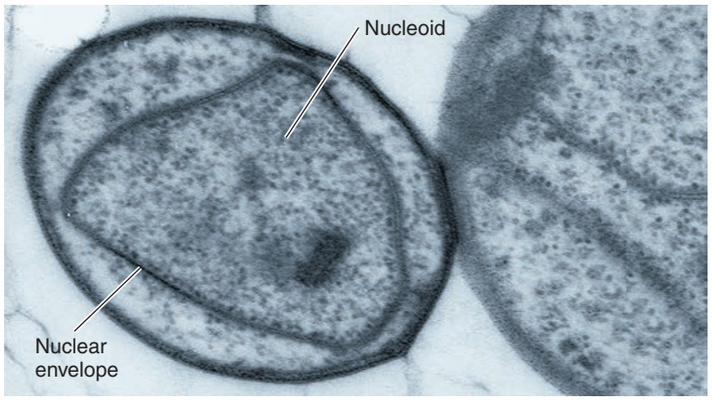


Figure 11.16 ***Gemmata obscuriglobus.*** This planctomycete exhibits a double membrane (nuclear envelope) surrounding its nucleoid (see Figure 4.6), which resembles a eukaryotic nucleus.

Q Can you see a resemblance between the double membrane around the nucleoid in this photo and the membrane around the nuclear envelope shown in Figure 4.24?

There are three species of the chlamydias that are significant pathogens for humans. *Chlamydia trachomatis* (kla-MI-dē-ah tra-KO-ma-tis) is the best known pathogen of the group and responsible for more than one major disease. These include trachoma, one of the most common causes of blindness in humans in the less-developed countries (page 602). It is also considered to be the primary causative agent of both nongonococcal urethritis, which may be the most common sexually transmitted disease in the United States, and lymphogranuloma venereum, another sexually transmitted disease (page 760).

Two members of the genus *Chlamydophila* (KLA-mi-dah'fil-ah) are well-known pathogens. *Chlamydophila psittaci* (SIT-tah-sē) is the causative agent of the respiratory disease psittacosis (ornithosis) (page 691). *Chlamydophila pneumoniae* (noo-MŌ-nē-ī) is the cause of a mild form of pneumonia that is especially prevalent in young adults.

Planctomycetes

The planctomycetes, a group of gram-negative, budding bacteria, are said to "blur the definition of what bacteria are." Although their DNA places them among the bacteria, they resemble the archaea in the makeup of their cell walls, and some even have organelles that resemble the nucleus of a eukaryotic cell. The members of the genus *Planctomyces* are aquatic bacteria that produce stalks resembling *Caulobacter* (page 293) and have cell walls similar to those of the archaea, that is, without peptidoglycan.

One species of planctomycetes, *Gemmata obscuriglobus* (JEM-mah-tah OB-scyer-i-glō'bus), has a double internal membrane around its DNA, resembling a eukaryotic nucleus (**Figure 11.16**). Biologists wonder whether this might make *Gemmata* a model for the origin of the eukaryotic nucleus.

Bacteroidetes

The phylum Bacteroidetes includes several genera of anaerobic bacteria. Included are the genus *Bacteroides,* a common inhabitant of the human intestinal tract, and the genus *Prevotella,* found in the human mouth. Also included in the phylum Bacteroidetes is the genus *Cytophaga,* important soil bacteria with gliding motility.

Bacteroides Bacteria of the genus *Bacteroides* (bak-ter-OI-dēz) live in the human intestinal tract in numbers approaching 1 billion per gram of feces. Some *Bacteroides* species also reside in anaerobic habitats such as the gingival crevice (see Figure 25.2 on page 709) and are also frequently recovered from deep-tissue infections. *Bacteroides* organisms are gram-negative, are nonmotile, and do not form endospores. Infections caused by *Bacteroides* often result from puncture wounds or surgery. *Bacteroides* are a frequent cause of peritonitis, an inflammation resulting from a perforated bowel.

Cytophaga Members of the genus *Cytophaga* (sī-TO-fa-gah) are important in the degradation of cellulose and chitin, which are both abundant in soil. Gliding motility places the microbe in close contact with these substrates, resulting in very efficient enzymatic action.

Fusobacteria

The fusiform bacteria comprise another phylum of anaerobes. These bacteria are often pleomorphic but, as their name suggests, may be spindle-shaped (*fuso* = spindle).

Fusobacterium Members of the genus *Fusobacterium* (fu′-so-bak-TI-rē-um) are long, slender, gram-negative rods with pointed

Figure 11.17 ***Fusobacterium.*** This is a common anaerobic rod found in the human intestine. Notice the characteristic pointed ends.

Q In what other place in the human body do you often find *Fusobacterium*?

rather than blunt ends (**Figure 11.17**). In humans, they are found most often in the gingival crevice of the gums and may be responsible for some dental abscesses.

Spirochaetes

The spirochetes have a coiled morphology, resembling a metal spring; some are more tightly coiled than others. The most distinctive characteristic of this order, however, is the cells' method of motility, which makes use of two or more **axial filaments** (or *endoflagella*) enclosed in the space between an outer sheath and the body of the cell. One end of each axial filament is attached near one of the cell's poles (see Figure 4.10, page 79, and **Figure 11.18a**). By rotating its axial filament, the cell rotates in the opposite direction, like a corkscrew, which is very efficient in moving the organism through liquids. For bacteria, this is more difficult than it might seem. At the scale of a bacterium, water is as viscous as molasses is to a human. However, a bacterium can typically move about 100 times its body length in a second (or about 50 μm/sec); by comparison, a large, fast fish, such as a tuna, can move only about 10 times its body length in this time.

Many spirochetes are found in the human oral cavity and are probably among the first microorganisms described by van Leeuwenhoek in the 1600s. An extraordinary location for spirochetes is on the surfaces of some of the cellulose-digesting protozoa found in termites, where they may function as substitutes for flagella. See the box on page 103.

Treponema The spirochetes include a number of important pathogenic bacteria. The best known is the genus *Treponema* (trep-ō-NĒ-mah), which includes *Treponema pallidum* (PAL-li-dum), the cause of syphilis (page 757) (Figure 11.18b).

Borrelia Members of the genus *Borrelia* (bor-RĒ-lē-ah) cause relapsing fever (page 651) and Lyme disease (page 651), serious diseases that are usually transmitted by ticks or lice.

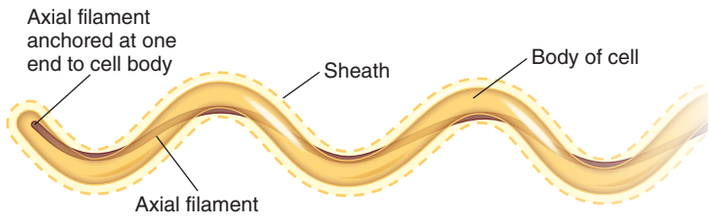
Leptospira Leptospirosis is a disease usually spread to humans by water contaminated by the *Leptospira* (LEP-tō-spī-rah) species (page 749). The bacteria are excreted in the urine of dogs, rats, and swine, so domestic dogs and cats are routinely immunized against leptospirosis. The tightly coiled cells of *Leptospira* are shown in Figure 26.4 on page 750.

Deinococci

The deinococci include two species of bacteria that have been widely studied because of their resistance to extremes in the environment. They stain gram-positive but have a cell wall that differs slightly in chemical structure from those of other gram-positives. *Deinococcus radiodurans* (dī-NŌ-kok-kus RĀ-dē-ō-der-anz) is exceptionally resistant to radiation, even more than are endospores. It can survive exposure to radiation doses as high as 15,000 Grays (see page 797), which is 1500 times the radiation dosage that would kill a human. The mechanism for this extraordinary resistance lies in a unique arrangement of its DNA that facilitates rapid repair of radiation damage. It is similarly resistant to many mutagenic chemicals.

Thermus aquaticus (THER-mus ah-KWA-ti-kus), another unique member of this group, is a bacterium that is unusually heat stable. It was isolated from a hot spring in Yellowstone National Park and is the source of the heat-resistant enzyme *Taq polymerase,* which is essential to the polymerase chain reaction (PCR). This is the method by which traces of DNA are amplified and used for identification (see page 243).

(a) This cross section of a spirochete shows numerous axial filaments between the dark cell and the outer sheath.



(b) The end of an axial filament (endoflagellum) is attached to the cell and extends through most of the length of the cell. Another axial filament is attached to the opposite end of the cell. These axial filaments do not extend away from the cell but remain between the body of the cell and the external sheath. Their contractions and relaxations cause the helical cell to rotate in a corkscrew fashion.

Figure 11.18 Spirochetes. Spirochetes are helical and have axial filaments under an outer sheath that enables them to move by a corkscrewlike rotation.

How does a spirochete's motility differ from that of *Spirillum* (see Figure 11.4)?

CHECK YOUR UNDERSTANDING

- Which gram-negative group has a life cycle that includes different stages? **11-6**
- Both the purple and green photosynthetic bacteria and the photosynthetic cyanobacteria use plantlike CO_2 fixation to make carbohydrates. In what way does the photosynthesis carried out by these two groups differ from plant photosynthesis? **11-7**
- The axial filament distinguishes what genera of bacteria? **11-8**

Figure 11.19 ***Clostridium difficile.*** The endospores of clostridia usually distend the cell wall, as shown here. The bacterial cell containing the endospore is dehydrated and flattened as a result of preparation for electron microscopy.

Q What physiological characteristic of *Clostridium* makes it a problem in contamination of deep wounds?

The Gram-Positive Bacteria

LEARNING OBJECTIVES

11-9 Differentiate the genera of firmicutes described in this chapter by drawing a dichotomous key.

11-10 Differentiate the actinobacteria described in this chapter by drawing a dichotomous key.

The gram-positive bacteria can be divided into two groups: those that have a high G + C ratio, and those that have a low G + C ratio (see "Nucleic Acids," page 45). To illustrate the variations in G + C ratio, the genus *Streptococcus* has a low G + C content of 33 to 44%; and the genus *Clostridium* has a low content of 21 to 54%. Included with the gram-positive, low G + C bacteria are the mycoplasmas, even though they lack a cell wall and therefore do not have a Gram reaction. Their G + C ratio is 23 to 40%.

By contrast, filamentous actinomycetes of the genus *Streptomyces* have a high G + C content of 69 to 73%. Gram-positive bacteria of a more conventional morphology, such as the genera *Corynebacterium* and *Mycobacterium,* have a G + C content of 51 to 63% and 62 to 70%, respectively.

These bacterial groups are placed into separate phyla, the **Firmicutes (low G + C ratios)** and **Actinobacteria (high G + C ratios).**

Firmicutes (Low G + C Gram-Positive Bacteria)

Low G + C gram-positive bacteria are assigned to the phylum Firmicutes. This group includes important endospore-forming bacteria such as the genera *Clostridium* and *Bacillus*. Also of extreme importance in medical microbiology are the genera *Staphylococcus, Enterococcus,* and *Streptococcus*. In industrial microbiology, the genus *Lactobacillus,* which produces lactic acid, is well known. The mycoplasma, which do not possess a cell wall, are also found in this phylum.

Clostridiales

Clostridium Members of the genus *Clostridium* (klos-TRID-ē-um) are obligate anaerobes. The rod-shaped cells contain endospores that usually distend the cell (**Figure 11.19**). The formation of endospores by bacteria is important to both medicine and the food industry because of the endospore's resistance to heat and many chemicals. Diseases associated with clostridia include tetanus (page 613), caused by *C. tetani* (TE-tan-ē); botulism (page 614), caused by *C. botulinum* (BO-tū-lī'num); and gas gangrene (page 646), caused by *C. perfringens* (per-FRIN-jenz) and other clostridia. *C. perfringens* is also the cause of a common form of foodborne diarrhea. *C. difficile* (DIF-fi-sē-il) is an inhabitant of the intestinal tract that may cause a serious diarrhea (page 724). This occurs only when antibiotic therapy alters the normal intestinal microbiota, allowing overgrowth by toxin-producing *C. difficile.*

Epulopiscium Biologists have long considered bacteria to be small by necessity because they lack the nutrient transport systems used by higher, eukaryotic organisms and because they depend on simple diffusion to obtain nutrients. These characteristics would seem to critically limit size. So, when a cigar-shaped organism living symbiotically in the gut of the Red Sea surgeonfish was first observed in 1985, it was considered to be a protozoan. Certainly, its size suggested this: the organism was as large as 80 μm × 600 μm —over half a millimeter in length—large enough to be seen with the unaided eye (**Figure 11.20**). Compared to the familiar bacterium *E. coli,* which is about 1 μm × 2 μm, this organism would be about a million times larger in volume.

Further investigation of the new organism showed that certain external structures thought to resemble the cilia of

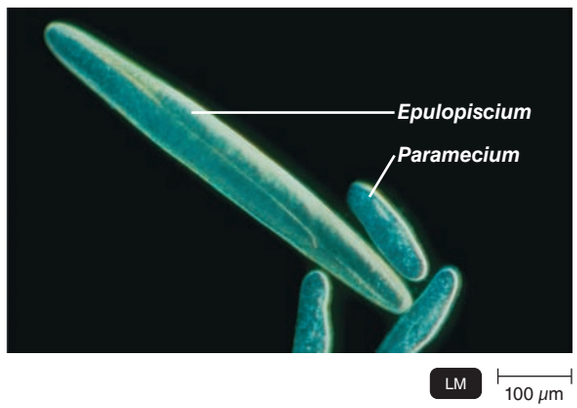
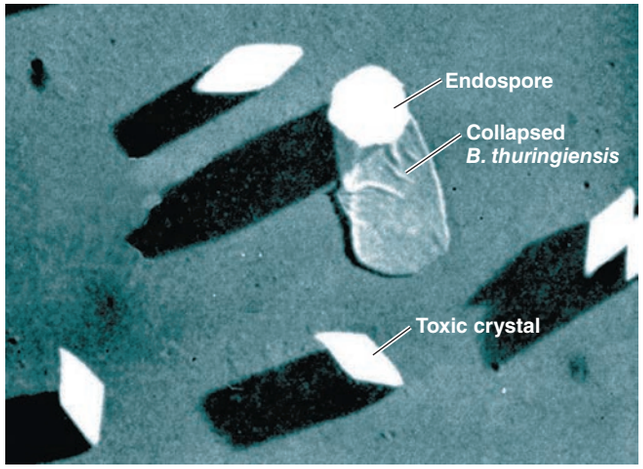


Figure 11.20 **A giant prokaryote, *Epulopiscium fishelsoni*.** *Paramecium* is a protozoan, a group whose members are normally larger than bacterial cells.

Q Why is *Epulopiscium* not in the same domain as *Paramecium*?



Bacillus thuringiensis. The diamond-shaped crystals shown next to the endospore are toxic to insects that ingest them. This electron micrograph was made using the technique of shadow casting.

Figure 11.21 ***Bacillus.***

Q What structure is made by both *Clostridium* and *Bacillus*?

protozoa were actually similar to bacterial flagella, and it didn't have a membrane-enclosed nucleus. Ribosomal RNA analysis conclusively placed *Epulopiscium* (ep′ū-lō-PIS-sē-um) with the prokaryotes. (The name means "guest at the banquet of a fish." It is literally bathed in semidigested food.) It most closely resembles gram-positive bacteria of the genus *Clostridium*. Strangely, the species *Epulopiscium fishelsoni* (fish-el-SŌ-ne) doesn't reproduce by binary fission. Daughter cells formed within the cell are released through a slit opening in the parent cell. This may be related to the evolutionary development of sporulation.

Recently it was discovered that this bacterium does not rely on diffusion to distribute nutrients. Instead, it makes use of its larger genetic capacity—it has 25 times as much DNA as a human cell and as many as 85,000 copies of at least one gene—to manufacture proteins at internal sites where they are needed. (We describe another, more recently discovered giant bacterium, *Thiomargarita,* on page 315.)

Bacillales

The order Bacillales includes several important genera of gram-positive rods and cocci.

Bacillus Bacteria of the genus *Bacillus* are typically rods that produce endospores. They are common in soil, and only a few are pathogenic to humans. Several species produce antibiotics.

Bacillus anthracis (bah-SIL-lus an-THRĀ-sis) causes anthrax, a disease of cattle, sheep, and horses that can be transmitted to humans (page 309). It's often mentioned as a possible agent of biological warfare. (See the box in Chapter 23 on page 648.) The anthrax bacillus is a nonmotile facultative anaerobe, often forming chains in culture. The centrally located endospore does not distend the walls. *Bacillus thuringiensis* (ther-IN-jē-en-sis) is probably the best-known microbial insect pathogen (**Figure 11.21**). It produces intracellular crystals when it sporulates. Commercial preparations containing endospores and crystalline toxin (Bt) of this bacterium are sold in gardening supply shops to be sprayed on plants. *Bacillus cereus* (SEER-ē-us) (Figure 11.21b) is a common bacterium in the environment and occasionally is identified as a cause of food poisoning, especially in starchy foods such as rice (page 724).

The three species of the genus *Bacillus* that we have just described are dramatically different in important ways, especially their disease-causing properties. However, they are so closely related that taxonomists consider them to be variants of a single species.

Staphylococcus Staphylococci typically occur in grapelike clusters (**Figure 11.22**). The most important staphylococcal species is *Staphylococcus aureus* (STAF-i-lō-kok′kus OR-ē-us), which is named for its yellow-pigmented colonies (*aureus* = golden). Members of this species are facultative anaerobes.

Some characteristics of the staphylococci account for their pathogenicity, which takes many forms. They grow comparatively well under conditions of high osmotic pressure and low moisture, which partially explains why they can grow and survive in nasal secretions (many of us carry the bacteria in our nostrils) and on the skin. This also explains how *S. aureus* can grow in some foods with high osmotic pressure (such as ham and other cured meats) or in low-moisture foods that tend to inhibit the growth of other organisms. The yellow pigment probably confers some protection from the antimicrobial effects of sunlight.

S. aureus produces many toxins that contribute to the bacterium's pathogenicity by increasing its ability to invade the body or damage tissue. The infection of surgical wounds by *S. aureus* is a common problem in hospitals. And its ability to develop resistance quickly to antibiotics such as penicillin makes it particularly dangerous to patients in hospital environments. (See the box in Chapter 14, page 411.) *S. aureus* produces the toxin responsible

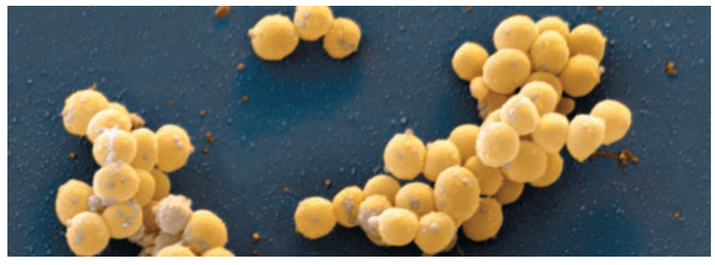


Figure 11.22 ***Staphylococcus aureus.*** Notice the grapelike clusters of these gram-positive cocci.

Q What is an environmental advantage of a pigment?

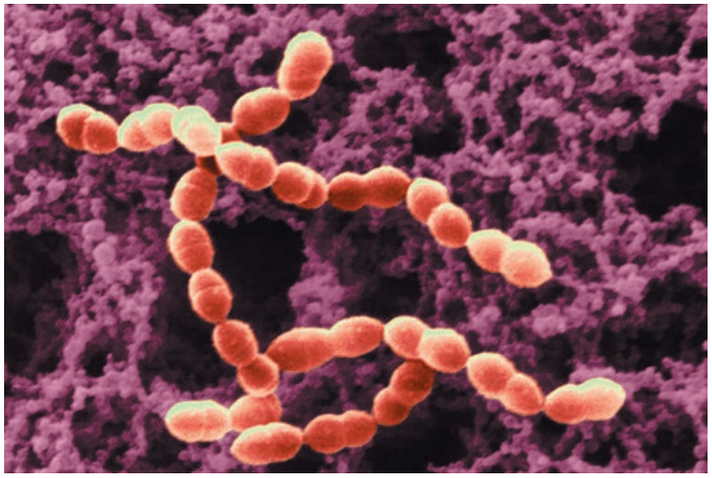


Figure 11.23 ***Streptococcus.*** Notice the chains of cells characteristic of most streptococci. Many of the spherical cells are dividing and are somewhat oval in appearance—especially when viewed with a light microscope, which has lower magnification than this electron micrograph.

Q How does the arrangement of *Streptococcus* differ from *Staphylococcus*?

for toxic shock syndrome, a severe infection characterized by high fever and vomiting, sometimes even death. *S. aureus* also produces an **enterotoxin** that causes vomiting and nausea when ingested; it is one of the most common causes of food poisoning (page 713).

Lactobacillales

Several important genera are found in the order Lactobacillales. The genus *Lactobacillus* is a representative of the industrially important lactic acid–producing bacteria. Most lack a cytochrome system and are unable to use oxygen as an electron acceptor. Unlike most obligate anaerobes, though, they are aerotolerant and capable of growth in the presence of oxygen. They grow poorly compared to oxygen-utilizing microbes. However, the production of lactic acid from simple carbohydrates inhibits the growth of competing organisms and allows them to grow competitively in spite of their inefficient metabolism. The genus *Streptococcus* shares the metabolic characteristics of the genus *Lactobacillus*. There are several industrially important species, but the streptococci are best known for their pathogenicity. The genera *Enterococcus* and *Listeria* are more conventional metabolically. Both are facultative anaerobes, and several species are important pathogens.

Lactobacillus In humans, bacteria of the genus *Lactobacillus* (lak′tō-bah-SIL-lus) are located in the vagina, intestinal tract, and oral cavity. Lactobacilli are used commercially in the production of sauerkraut, pickles, buttermilk, and yogurt. Typically, a succession of lactobacilli, each more acid tolerant than its predecessor, participates in these lactic acid fermentations.

Streptococcus Members of the genus *Streptococcus* (strep′tō-KOK-kus) are spherical, gram-positive bacteria that typically appear in chains (**Figure 11.23**). They are a taxonomically complex group, probably responsible for more illnesses and causing a greater variety of diseases than any other group of bacteria.

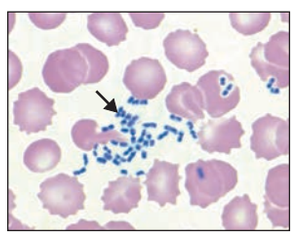
Pathogenic streptococci produce several extracellular substances that contribute to their pathogenicity. Among them are products that destroy phagocytic cells that ingest them. Enzymes produced by some streptococci spread infections by digesting the host's connective tissue, which may also result in extensive tissue destruction. (See the discussion of necrotizing fasciitis on page 585). Also, bacterial enzymes digest the fibrin (a threadlike protein) of blood clots, allowing infections to spread from the sites of injury.

A few nonpathogenic species of streptococci are important in the production of dairy products (see Chapter 28, page 799).

Beta-hemolytic streptococci A useful basis for classifying some streptococci is their colonial appearance when grown on blood agar. The *beta-hemolytic* species produce a hemolysin that forms a clear zone of hemolysis on blood agar (see Figure 6.9 on page 161). This group includes the principal pathogen of the streptococci, *Streptococcus pyogenes* (pī-AH-jen-ēz), also known as the beta-hemolytic group A streptococcus. Group A represents one of an antigenic group (A through G) within the hemolytic streptococci. Among the diseases caused by *S. pyogenes* (page 678) are scarlet fever, pharyngitis (sore throat), erysipelas, impetigo, and rheumatic fever. The most important virulence factor

Clinical Case

Two species of bacteria that can cause bacterial meningitis are *Neisseria meningitidis* and *Streptococcus pneumoniae*. Dr. Walker then requests that the lab perform a Gram stain of Mercy's CSF and venous blood. Below is the Gram stain of Mercy's venous blood.



What do you see that would cause you to modify your list of possible causes?

is the M protein on the bacterial surface (see Figure 21.6, page 585) by which the bacteria avoid phagocytosis. Another member of the beta-hemolytic streptococci is *Streptococcus agalactiae* (ā-gal-AK-tē-ī), in the beta-hemolytic group B. It is the only species with the group B antigen and is the cause of an important disease of the newborn, neonatal sepsis (page 640).

Non-beta-hemolytic streptococci Certain streptococci are not beta-hemolytic, but when grown on blood agar, their colonies are surrounded by a distinctive greening. These are the *alpha-hemolytic* streptococci. The greening represents a partial destruction of the red blood cells caused mostly by the action of bacteria-produced hydrogen peroxide, but it appears only when the bacteria grow in the presence of oxygen. The most important pathogen in this group is *Streptococcus pneumoniae,* the cause of pneumococcal pneumonia (page 689). Also included among the alpha-hemolytic streptococci are species of streptococci called *viridans streptococci*. However, not all species form the alpha-hemolytic greening (*virescent* = green), so this isn't really a satisfactory group name. Probably the most significant pathogen of the group is *Streptococcus mutans* (MŪ-tanz), the primary cause of dental caries (page 709).

Enterococcus The enterococci are adapted to areas of the body that are rich in nutrients but low in oxygen, such as the gastrointestinal tract, vagina, and oral cavity. They are also found in large numbers in human stool. Because they are relatively hardy microbes, they persist as contaminants in a hospital environment, on hands, bedding, and even as a fecal aerosol. In recent years they have become a leading cause of nosocomial infections, especially because of their high resistance to most antibiotics. Two species, *Enterococcus faecalis* (en′ter-ō-KOK-kus fē-KĀ-lis) and *Enterococcus faecium* (FĒ-sē-um), are responsible for much of the infections of surgical wounds and the urinary tract. In medical settings they frequently enter the bloodstream through invasive procedures, such as indwelling catheters.

Listeria The pathogenic species of the genus *Listeria, Listeria monocytogenes* (lis-TEER-ē-ah mon′ō-sī-TO-je-nēz), can contaminate food, especially dairy products. Important characteristics of *L. monocytogenes* are that it survives within phagocytic cells and is capable of growth at refrigeration temperatures. If it infects a pregnant woman, the organism poses the threat of stillbirth or serious damage to the fetus.

Mycoplasmatales

The mycoplasmas are highly pleomorphic because they lack a cell wall (**Figure 11.24**) and can produce filaments that resemble fungi, hence their name (*mykes* = fungus, and *plasma* = formed).

Cells of the genus *Mycoplasma* (mī-kō-PLAZ-mah) are very small, ranging in size from 0.1 to 0.25 μm, with a cell volume that is only about 5% of that of a typical bacillus. Because their size and plasticity allowed them to pass through filters that retained bacteria, they were originally considered to be viruses. Mycoplasmas may represent the smallest self-replicating organisms that are capable of a free-living existence. One species has only 517 genes; the minimum necessary is between 265 and 350. Studies of their DNA suggest that they are genetically related to the gram-positive bacterial group that includes the genera *Bacillus, Streptococcus,* and *Lactobacillus* but have gradually lost genetic material. The term *degenerative evolution* has been used to describe this process.

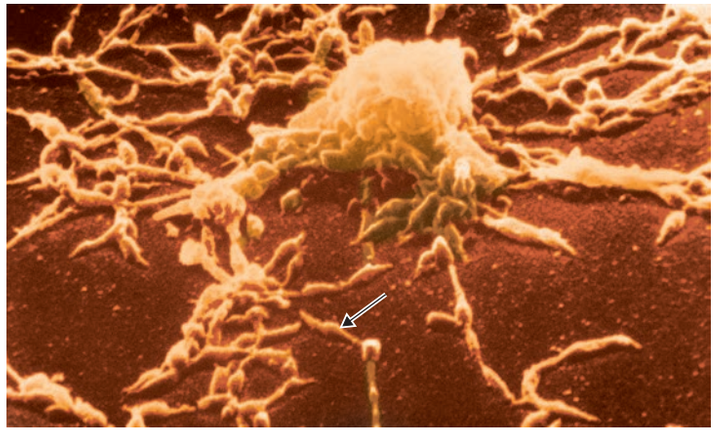


Figure 11.24 ***Mycoplasma pneumoniae.*** This micrograph shows the filamentous growth of *M. pneumoniae*. This bacterium does not have a cell wall; the cell membrane is the outermost layer. The cells are so small that they cannot be examined by light microscopy. Individual cells (arrow) have extensions at each end that probably aid in gliding motility and in attachment to the host cells. They depend on the host for survival and do not survive as free-living organisms.

Q How can the cell structure of mycoplasmas account for their pleomorphism?

The most significant human pathogen among the mycoplasmas is *M. pneumoniae* (noo-MŌ-nē-ī), which is the cause of a common form of mild pneumonia.

Mycoplasmas can be grown on artificial media that provide them with sterols (if necessary) and other special nutritional or physical requirements. Colonies are less than 1 mm in diameter and have a characteristic "fried egg" appearance when viewed under magnification (see Figure 24.13, page 692). For many purposes, cell culture methods are often more satisfactory. In fact, mycoplasmas grow so well by this method that they are a frequent contamination problem in cell culture laboratories.

CHECK YOUR UNDERSTANDING

To which genus is *Enterococcus* more closely related: *Staphylococcus* or *Lactobacillus*? **11-9**

Actinobacteria (High G + C Gram-Positive Bacteria)

High G + C gram-positive bacteria are in the phylum Actinobacteria. Many bacteria in this phylum are highly pleomorphic in their morphology; the genera *Corynebacterium* and *Gardnerella,* for example, and several genera such as *Streptomyces* grow only as extended, often-branching filaments. Several important pathogenic genera are found in the Actinobacteria, such as the *Mycobacterium* species causing tuberculosis and leprosy. The genera *Streptomyces, Frankia, Actinomyces,* and *Nocardia* are often informally called actinomycetes (from the Greek *actino* = ray) because they have a radiate, or starlike, form of growth by reason of their often-branching filaments. Superficially, their morphology resembles that of filamentous fungi; however, the actinomycetes are prokaryotic cells, and their filaments have a diameter much smaller than that of the eukaryotic molds. Some actinomycetes further resemble molds by their possession of externally carried asexual spores that are used for reproduction. Filamentous bacteria, like filamentous fungi, are very common inhabitants in soil, where a filamentous pattern of growth has advantages. The filamentous organism can bridge water-free gaps between soil particles to move to a new nutritional site. This morphology also gives the organism a much higher surface-to-volume ratio and improves its ability to absorb nutrients in the highly competitive soil environment.

Mycobacterium

The mycobacteria are aerobic, non–endospore-forming rods. The name *myco,* meaning funguslike, was derived from their occasional exhibition of filamentous growth (see Figure 24.7, page 684).

Clinical Case

The Gram stain of Mercy's venous blood shows gram-positive cocci among the red blood cells. Needing to identify the species of bacteria that is causing Mercy's meningitis, the lab then cultures the cocci on blood agar. See below for the results.

Based on this new information, what bacteria are responsible for Mercy's meningitis? (Hint: See Figure 6.9 on page 161.)

291 310 312 313 314

Many of the characteristics of mycobacteria, such as acid-fast staining, drug resistance, and pathogenicity, are related to their distinctive cell wall, which is structurally similar to gram-negative bacteria (see Figure 4.13c, page 82). However, the outermost lipopolysaccharide layer in mycobacteria is replaced by mycolic acids, which form a waxy, water-resistant layer. This makes the bacteria resistant to stresses such as drying. Also, few antimicrobial drugs are able to enter the cell. (See the box in Chapter 7 on page 193.) Nutrients enter the cell through this layer very slowly, which is a factor in the slow growth rate of mycobacteria; it sometimes takes weeks for visible colonies to appear. The mycobacteria include the important pathogens *Mycobacterium tuberculosis* (mī′kō-bak-TI-rē-um too′ber-kū-LŌ-sis), which causes tuberculosis (page 684), and *M. leprae* (LEP-rī), which causes leprosy (page 617).

The mycobacteria are generally separated into two groups: (1) the slow growers, such as *M. tuberculosis,* and (2) the fast, or rapid, growers, which form visible colonies on appropriate media within 7 days. The slow-growing mycobacteria are more likely to be pathogenic to humans. The rapidly growing group also contains a number of occasional, nontuberculous human pathogens, which most commonly infect wounds. However, these mycobacteria are more likely to be nonpathogenic soil and water microbes.

Corynebacterium

The corynebacteria (*coryne* = club-shaped) tend to be pleomorphic, and their morphology often varies with the age of the cells. The best-known species is *Corynebacterium diphtheriae* (kor′ī-nē-bak-TI-rē-um dif-THI-rē-ī), the causative agent of diphtheria (page 678).

Propionibacterium

The name of the genus *Propionibacterium* (pro-pē′on-ē-bak-TI-rē-um) is derived from the organism's ability to form propionic acid; some species are important in the fermentation of Swiss cheese. *Propionibacterium acnes* (AK-nēz) are bacteria that are commonly found on human skin and are implicated as the primary bacterial cause of acne.

Gardnerella

Gardnerella vaginalis (GARD-ner-el-lah VA-jin-al-is) is a bacterium that causes one of the most common forms of vaginitis (page 761). There has always been some difficulty in assigning a taxonomic position in this species, which is gram-variable and exhibits a highly pleomorphic morphology.

Frankia

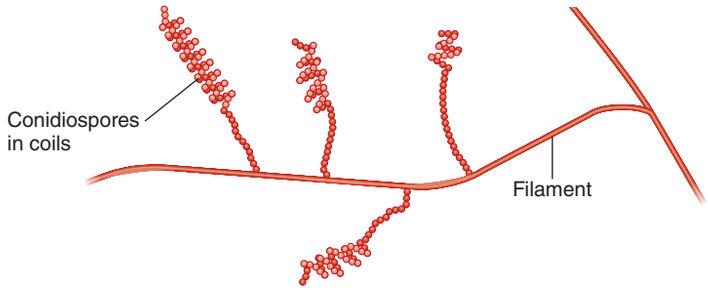
The genus *Frankia* (FRANK-ē-ah) causes nitrogen-fixing nodules to form in alder tree roots, much as rhizobia cause nodules on the roots of legumes (see Figure 27.4, page 777).

Streptomyces

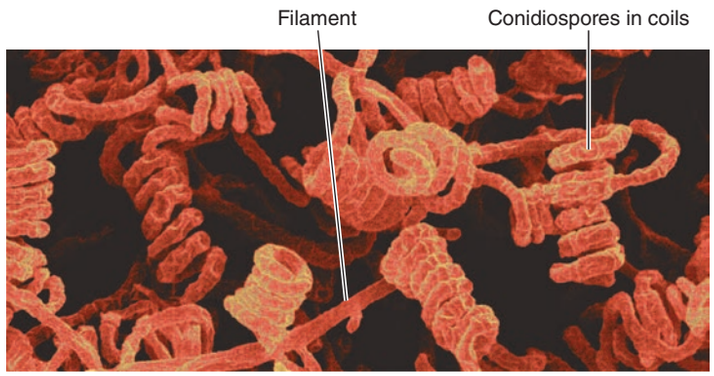
The genus *Streptomyces* (strep′tō-MĪ-sēs) is the best known of the actinomycetes and is one of the bacteria most commonly isolated from soil (**Figure 11.25**). The reproductive asexual spores of *Streptomyces* are formed at the ends of aerial filaments. If each spore lands on a suitable substrate, it is capable of germinating into a new colony. These organisms are strict aerobes. They often produce extracellular enzymes that enable them to utilize proteins, polysaccharides (such as starch and cellulose), and many other organic materials found in soil. *Streptomyces* characteristically produce a gaseous compound called *geosmin,* which gives fresh soil its typical musty odor. Species of *Streptomyces* are valuable because they produce most of our commercial antibiotics (see Table 20.1, page 550). This has led to intensive study of the genus—there are nearly 500 described species.

Actinomyces

The genus *Actinomyces* (ak-tin-ō-MĪ-sēs) consists of facultative anaerobes that are found in the mouth and throat of humans and animals. They occasionally form filaments that can fragment (**Figure 11.26**). One species, *Actinomyces israelii* (is-RĀ-lē-ē), causes actinomycosis, a tissue-destroying disease usually affecting the head, neck, or lungs.



(a) Drawing of a typical streptomycete showing filamentous, branching growth with asexual reproductive conidiospores at the filament tips



(b) Coils of conidiospores supported by filaments of the streptomycete

Figure 11.25 ***Streptomyces.***

Why is *Streptomyces* not classified with fungi?

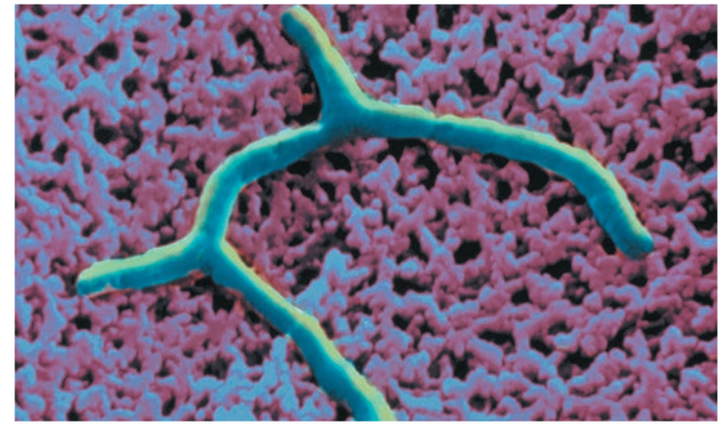


Figure 11.26 ***Actinomyces.*** Notice the branched filamentous morphology.

Why are these bacteria not classified as fungi?

Nocardia

The genus *Nocardia* (nō-KAR-dē-ah) morphologically resembles *Actinomyces;* however, these bacteria are aerobic. To reproduce, they form rudimentary filaments, which fragment into short rods. The structure of their cell wall resembles that of the mycobacteria; therefore, they are often acid-fast. *Nocardia* species are common in soil. Some species, such as *Nocardia asteroides* (AS-ter-oid-ēz), occasionally cause a chronic, difficult-to-treat pulmonary infection. *N. asteroides* is also one of the causative agents of mycetoma, a localized destructive infection of the feet or hands.

CHECK YOUR UNDERSTANDING

What group of bacteria makes most of the commercially important antibiotics? **11-10**

Clinical Case

The blood agar and Gram stain results show beta-hemolytic streptococci. Dr. Walker sends a request to the lab for Lancefield typing (see Chapter 1, page 12) of the blood culture to find out which *Streptococcus* species is causing Mercy's meningitis. The results confirm the presence of the group B Lancefield antigen, thus verifying a diagnosis of group B *Streptococcus* (GBS), or *S. agalactiae,* infection. Although Mercy's mother tested negative for GBS when she was pregnant, Dr. Walker asks that Mercy's mother be retested. This time the results come back positive.

What is GBS?

Domain Archaea

In the late 1970s, a distinctive type of prokaryotic cell was discovered. Most strikingly, the cell walls of these prokaryotes lacked the peptidoglycan common to most bacteria. It soon became clear that they also shared many rRNA sequences, and the sequences were different from both those of the Domain Bacteria and the eukaryotic organisms. These differences were so significant that these organisms now constitute a new taxonomic grouping, the Domain Archaea.

Diversity within the Archaea

LEARNING OBJECTIVE

11-11 Name a habitat for each group of archaea.

This exceptionally interesting group of prokaryotes is highly diverse. Most archaea are of conventional morphology, that is, rods, cocci, and helices, but some are of very unusual morphology, as illustrated in **Figure 11.27**. Some are gram-positive, others gram-negative; some may divide by binary fission, others by fragmentation or budding; a few lack cell walls. Cultivated members of the archaea (singular: *archaeon*) can be placed into five physiological or nutritional groups.

Physiologically, archaea are found under extreme environmental conditions. **Extremophiles,** as they are known, include halophiles, thermophiles, and acidophiles (see "Life in the Extreme" on page 153). There are no known pathogenic archaea.

Halophiles thrive in salt concentrations of more than 25%, such as found in the Great Salt Lake and solar evaporating ponds. Examples of these are found in the genus *Halobacterium* (hā′lo-bak-TI-rē-um), some of which may even require such salt concentrations in order to grow. The optimal growth temperatures of extremely thermophilic archaea is 80°C or higher. The present record growth temperature is 121°C, established by archaea growing near a hydrothermal vent at 2000 meters deep in the ocean. Acidophilic archaea can be found growing at pH values below zero, and frequently at elevated temperatures, as well. An example is *Sulfolobus* (SUL-fō-lō-bus), whose optimal pH is about 2 and optimal temperature is more than 70°C.

Nutritionally, the ocean contains numerous *nitrifying* archaea that oxidize ammonia for energy. Some might also be found in soils. Methanogens are strictly anaerobic archaea that produce methane as an end-product by combining hydrogen (H_2) with carbon dioxide (CO_2). There are no known bacterial methanogens. These archaea are of considerable economic importance when they are used in sewage treatment (see the discussion of sludge digestion in Chapter 27 on pages 787–788). Methanogens are also part of the microbiota of the human colon, vagina, and mouth.

CHECK YOUR UNDERSTANDING

What kind of archaea would populate solar evaporating ponds? **11-11**

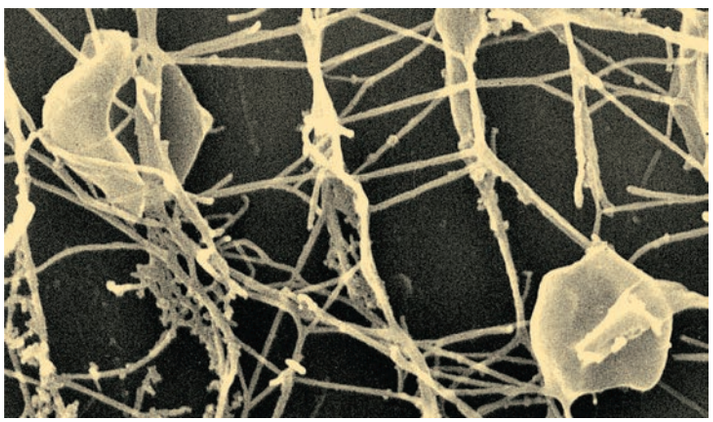


Figure 11.27 Archaea. *Pyrodictium abyssi*, an unusual member of the archaea found growing in deep-ocean sediment at a temperature of 110°C. The cells are disk-shaped with a network of tubules (cannulae). Most archaea are more conventional in their morphology.

Q Do the terms included in the name, *pyro* and *abyssi*, suggest a basis for the naming of this bacterium?

Clinical Case Resolved

GBS is often part of the normal intestinal or genitourinary microbiota, but it can cause disease in immunocompromised individuals. GBS emerged as a major cause of neonatal bacterial sepsis in the 1970s and is a leading infectious cause of neonatal morbidity in the United States. The bacterium, a common colonizer of the maternal genital tract, can infect the fetus during gestation, causing fetal death. GBS can also be acquired by the fetus during passage through the birth canal during delivery. Prevention includes screening all pregnant women for GBS at 35 to 37 weeks' gestation and administering antibiotics to carriers during labor. Although Mercy's mother tested negative during her pregnancy, her results were a rare false negative. Mercy is put on intravenous antibiotics and remains in the hospital for 10 days until the infection clears. She is sent home after 2 weeks and is now a healthy, happy 2-month-old girl.

Microbial Diversity

The Earth provides a seemingly infinite number of environmental niches, and novel life forms have evolved to fill them. Many of the microbes that exist in these niches cannot be cultivated by conventional methods on conventional growth media and have remained unknown. In recent years, however, isolation and identification methods have become much more sophisticated, and microbes that fill these niches are being identified—many without being cultivated. For example, see the *Pelagibacter* on page 292. Particularly interesting are bacteria that test the theoretical limits of size for prokaryotes.

ASM: Microorganisms are ubiquitous and live in diverse and dynamic ecosystems. Because the true diversity of microbial life is largely unknown, its effects and potential benefits have not been fully explored.

Discoveries Illustrating the Range of Diversity

LEARNING OBJECTIVE

11-12 List two factors that contribute to the limits of our knowledge of microbial diversity.

Earlier in this chapter, we described the giant bacterium *Epulopiscium*. In 1999, another, even larger, giant bacterium was discovered 100 meters deep in the sediments of the coastal waters off Namibia, on the southwestern coast of Africa. Named *Thiomargarita namibiensis* (THĪ-ō-mar-gar′ē-tah nah′mib-ē-EN-sis), meaning "sulfur pearl of Namibia," these spherical organisms, classified with the gammaproteobacteria, are as large as 750 µm in diameter (**Figure 11.28**). This is a bit larger than the size of a period at the end of this sentence.

As we have mentioned, a factor that limits the size of prokaryotic cells is that nutrients must enter the cytoplasm by simple diffusion. *T. namibiensis* minimizes this problem by resembling a fluid-filled balloon, the vacuole in the interior being surrounded by a relatively thin outer layer of cytoplasm. This cytoplasm is equal in volume to that of most other prokaryotes. Its energy source is essentially hydrogen sulfide, which is plentiful in the sediments in which it is normally found, and nitrate, which it must extract intermittently from nitrate-rich seawaters when storms stir the loose sediment. The cell's interior vacuole, which makes up about 98% of the bacterium's volume, serves as a storage space to hold the nitrate between recharging of its supply. The cell's energy is derived from the oxidation of hydrogen sulfide; the nitrate, although a source of nutritional nitrogen, primarily serves as an electron acceptor in the absence of oxygen.

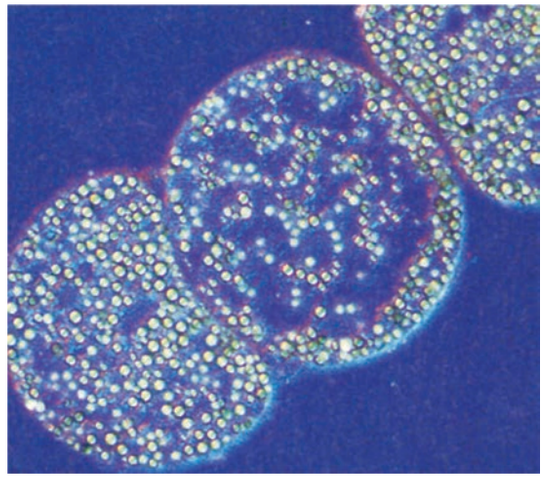


Figure 11.28 ***Thiomargarita namibiensis.*** *Thiomargarita namibiensis* gets its energy from reduced sulfur compounds such as hydrogen sulfide.

Q Is a bacterium of this size theoretically possible if the interior were cytoplasm rather than a fluid-filled vacuole?

The discovery of uniquely large bacteria has raised the question of how large a prokaryotic cell can be and still absorb nutrients. At the other extreme, is there a lower limit for the size of microorganisms—especially their genome? There are reports of bacteria as small as 0.02 to 0.08 µm (nanobacteria) found in deep rock formations and even meteorites. Most microbiologists have concluded that these are nonliving particles that have crystallized from minerals and suggest the name **nanons.** Theoretical considerations have been used to calculate that a cell with a significant metabolism would have to have a diameter of at least 0.1 µm. Certain bacteria have extraordinarily small genomes. For example, *Carsonella ruddii* (KAR-son-el-lah RUD-dē-ē) is a bacterium that lives in a *symbiotic relationship* with its insect host, a sap-eating psyllid (plant louse), and requires less genetic capability than would a free-living microbe. It has only 182 genes, which is close to the 151 genes that is the calculated theoretical minimum even for a microbe in such a symbiotic relationship. (Compare this with the minimal genetic requirements of the *free-living* mycoplasmas, on page 311). *C. ruddii* is not completely parasitic in its relationship with its host insect, but it supplies the host with some essential amino acids. It is therefore probably in the evolutionary process of becoming an organelle, like the mitochondria of mammalian cells (see page 268).

Until now, microbiologists have described only about 5000 bacterial species, of which about 3000 are listed in *Bergey's Manual*. The true number may be in the millions. Many bacteria in soil or water, or elsewhere in nature, cannot be cultivated with the media and conditions normally used for bacterial growth. Moreover, some bacteria are part of complex food chains and can grow only in the presence of other microbes that supply specific growth requirements. Recently, researchers have been using the polymerase chain reaction (PCR) to make millions of copies of genes found at random in a soil sample. By comparing the genes found in many repetitions of this process, researchers can estimate the different bacterial species in such a sample. One report indicates that a single gram of soil may contain 10,000 or so bacterial types—about twice as many as have ever been described.

CHECK YOUR UNDERSTANDING

How can you detect the presence of a bacterium that cannot be cultured? **11-12**

Study Outline

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Study microbiology where it matters: everywhere.
Check out the Study Area of MasteringMicrobiology to explore Interactive Microbiology. Study on-the-go with Dynamic Study Modules. Check your understanding with chapter quizzes and animations—and much more!

Introduction (p. 290)

1. *Bergey's Manual* categorizes bacteria into taxa based on rRNA sequences.
2. *Bergey's Manual* lists identifying characteristics such as Gram stain reaction, cellular morphology, oxygen requirements, and nutritional properties.

The Prokaryotic Groups (pp. 291–292)

1. Prokaryotic organisms are classified into two domains: Archaea and Bacteria.

Domain Bacteria (p. 292)

1. Bacteria are essential to life on Earth.

The Gram-Negative Bacteria (pp. 292–307)

The Proteobacteria (pp. 292–302)

1. Members of the phylum Proteobacteria are gram-negative.
2. Alphaproteobacteria include nitrogen-fixing bacteria, chemoautotrophs, and chemoheterotrophs.
3. Betaproteobacteria include chemoautotrophs and chemoheterotrophs.
4. Pseudomonadales, Legionellales, Vibrionales, Enterobacteriales, and Pasteurellales are classified as gammaproteobacteria.
5. *Bdellovibrio* and *Myxococcus* in the deltaproteobacteria prey on other bacteria.
6. Epsilonproteobacteria include *Campylobacter* and *Helicobacter*.

The Nonproteobacteria Gram-Negative Bacteria (pp. 302–307)

7. Cyanobacteria are photoautotrophs that use light energy and CO_2 and do produce O_2.
8. Purple and green photosynthetic bacteria are photoautotrophs that use light energy and CO_2 and do not produce O_2.
9. *Deinococcus* and *Thermus* are resistent to environmental extremes.
10. Planctomycetes, Chlamydiae, Spirochetes, Bacteroidetes, and Fusobacteria are phyla of gram-negative, chemoheterotrophic bacteria.

The Gram-Positive Bacteria (pp. 308–314)

1. In *Bergey's Manual,* gram-positive bacteria are divided into those that have low G + C ratio and those that have high G + C ratio.
2. Low G + C gram-positive bacteria include common soil bacteria, the lactic acid bacteria, and several human pathogens.
3. High G + C gram-positive bacteria include mycobacteria, corynebacteria, and actinomycetes.

Domain Archaea (p. 314)

1. Extreme halophiles, extreme thermophiles, and methanogens are included in the archaea.

Microbial Diversity (pp. 315–316)

1. Few of the total number of different prokaryotes have been isolated and identified.
2. PCR can be used to uncover the presence of bacteria that can't be cultured in the laboratory.

Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. The following outline can be used to identify important bacteria. Fill in a representative genus in the space provided.

	Representative Genus
I. Gram-positive	
A. Endospore-forming rod	
1. Obligate anaerobe	(a) _____
2. Not obligate anaerobe	(b) _____
B. Non–endospore-forming	
1. Cells are rods	
a. Produce conidiospores	(c) _____
b. Acid-fast	(d) _____
2. Cells are cocci	
a. Lack cytochrome system	(e) _____
b. Use aerobic respiration	(f) _____
II. Gram-negative	
A. Cells are helical or curved	
1. Axial filament	(g) _____
2. No axial filament	(h) _____
B. Cells are rods	
1. Aerobic, nonfermenting	(i) _____
2. Facultatively anaerobic	(j) _____
III. Lack cell walls	(k) _____
IV. Obligate intracellular parasites	
A. Transmitted by ticks	(l) _____
B. Reticulate bodies in host cells	(m) _____

2. Compare and contrast each of the following:
- **a.** Cyanobacteria and algae
- **b.** Actinomycetes and fungi
- **c.** *Bacillus* and *Lactobacillus*
- **d.** *Pseudomonas* and *Escherichia*
- **e.** *Leptospira* and *Spirillum*
- **f.** *Escherichia* and *Bacteroides*
- **g.** *Rickettsia* and *Chlamydia*
- **h.** *Mycobacterium* and *Mycoplasma*

3. DRAW IT Draw a key to differentiate the following bacteria: cyanobacteria, *Cytophaga, Desulfovibrio, Frankia, Hyphomicrobium,* methanogens, myxobacteria, *Nitrobacter,* purple bacteria, *Sphaerotilus,* and *Sulfolobus*.

4. NAME IT These organisms are important in sewage treatment and can produce a fuel used for home heating and for generating electricity.

Multiple Choice

1. If you Gram-stained the bacteria that live in the human intestine, you would expect to find mostly
- **a.** gram-positive cocci.
- **b.** gram-negative rods.
- **c.** gram-positive, endospore-forming rods.
- **d.** gram-negative, nitrogen-fixing bacteria.
- **e.** all of the above.

2. Which of the following does *not* belong with the others?
- **a.** Enterobacteriales
- **b.** Lactobacillales
- **c.** Legionellales
- **d.** Pasteurellales
- **e.** Vibrionales

3. Pathogenic bacteria can be
- **a.** motile.
- **b.** rods.
- **c.** cocci.
- **d.** anaerobic.
- **e.** all of the above

4. Which of the following is an intracellular parasite?
- **a.** *Rickettsia*
- **b.** *Mycobacterium*
- **c.** *Bacillus*
- **d.** *Staphylococcus*
- **e.** *Streptococcus*

5. Which of the following terms is the most specific?
- **a.** bacillus
- **b.** *Bacillus*
- **c.** gram-positive
- **d.** endospore-forming rods and cocci
- **e.** anaerobic

6. Which one of the following does *not* belong with the others?
- **a.** *Enterococcus*
- **b.** *Lactobacillus*
- **c.** *Staphylococcus*
- **d.** *Streptococcus*
- **e.** All are grouped together.

7. Which of the following pairs is *mismatched*?
- **a.** anaerobic endospore-forming gram-positive rods—*Clostridium*
- **b.** facultatively anaerobic gram-negative rods—*Escherichia*
- **c.** facultatively anaerobic gram-negative rods—*Shigella*
- **d.** pleomorphic gram-positive rods—*Corynebacterium*
- **e.** spirochete—*Helicobacter*

8. *Spirillum* is *not* classified as a spirochete because spirochetes
- **a.** do not cause disease.
- **b.** possess axial filaments.
- **c.** possess flagella.
- **d.** are prokaryotes.
- **e.** none of the above

9. When *Legionella* was newly discovered, why was it classified with the pseudomonads?
- **a.** It is a pathogen.
- **b.** It is an aerobic gram-negative rod.
- **c.** It is difficult to culture.
- **d.** It is found in water.
- **e.** none of the above

10. Unlike purple and green phototrophic bacteria, cyanobacteria
    a. produce oxygen during photosynthesis.
    b. do not require light.
    c. use H_2S as an electron donor.
    d. have a membrane-enclosed nucleus.
    e. all of the above

Analysis

1. Use of culture-independent techniques such as rRNA sequencing and FISH have increased our understanding of microbial diversity without cultivation. Do microbiologists still need to attempt to grow new species? Briefly explain.
2. To which of the following is the photosynthetic bacterium *Chromatium* most closely related? Briefly explain why.
    a. cyanobacteria
    b. *Chloroflexus*
    c. *Escherichia*
3. Bacteria are single-celled organisms that must absorb their nutrients by simple diffusion. The dimensions of *Thiomargarita namibiensis* are several hundred times larger than those of most bacteria, much too large for simple diffusion to operate. How does the bacterium solve this problem?

Clinical Applications and Evaluation

1. After contact with a patient's spinal fluid, a lab technician developed fever, nausea, and purple lesions on her neck and extremities. A throat culture grew gram-negative diplococci. What is the genus of the bacteria?
2. Between April 1 and May 15 of one year, 22 children in three states developed diarrhea, fever, and vomiting. The children had each received pet ducklings. Gram-negative, facultatively anaerobic bacteria were isolated from both the patients' and the ducks' feces; the bacteria were identified as serovar C2. What is the genus of these bacteria?
3. A woman complaining of lower abdominal pain with a temperature of 39°C gave birth soon after to a stillborn baby. Blood cultures from the infant revealed gram-positive rods. The woman had a history of eating unheated hot dogs during her pregnancy. Which organism is most likely involved?

In the Clinic

As a Peace Corps nurse in West Africa, you meet a 4-year-old girl with a particularly swollen stomach. The girl's mother shows you a large (10-cm) white worm that the girl coughed up. The worm is cylindrical with tapered ends. **What is the worm? How did she contract it?**

Hint: Read about helminths (pages 343–351).

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

12 The Eukaryotes: Fungi, Algae, Protozoa, and Helminths

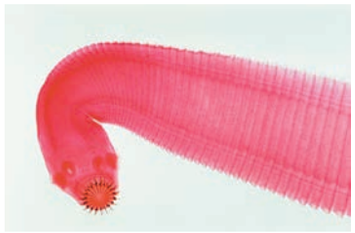
Over half of the world's population is infected with eukaryotic pathogens. The World Health Organization (WHO) ranks six parasitic diseases among the top 20 microbial causes of death in the world. Every year, over 5 million new cases of malaria, schistosomiasis, amebiasis, hookworm, African trypanosomiasis, and intestinal parasites are reported in developing countries. Emerging eukaryotic pathogens in developed countries include *Pneumocystis,* the cause of death in 10% of AIDS patients, and the protozoan *Cryptosporidium,* which causes 10,000 infections annually. Raccoon roundworm in humans has been identified as an emerging disease, and the CDC has announced that bloodborne parasites, *Plasmodium* spp., *Babesia* spp., *Trypanosoma cruzi,* and *Leishmania* spp., pose a public health threat in the United States. The emergence of the fungal pathogen *Cryptococcus gattii* (see the photograph) in North America is discussed in the Clinical Case.

In this chapter, we examine the eukaryotic microorganisms that affect humans: fungi, algae, protozoa, parasitic helminths, and the arthropods that transmit diseases. (For a comparison of their characteristics, see **Figure 12.1**.)

***Cryptococcus gattii,* an emerging fungal pathogen.**

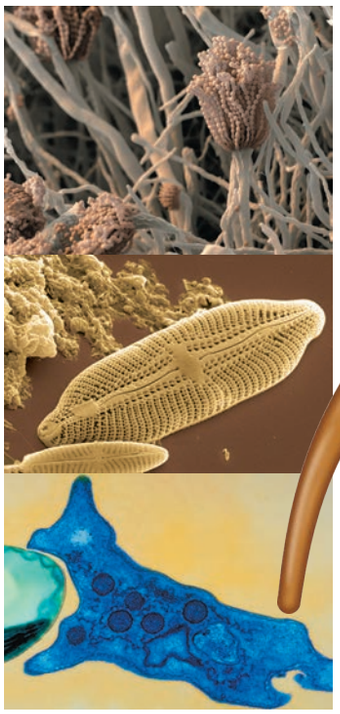
FOUNDATION FIGURE 12.1

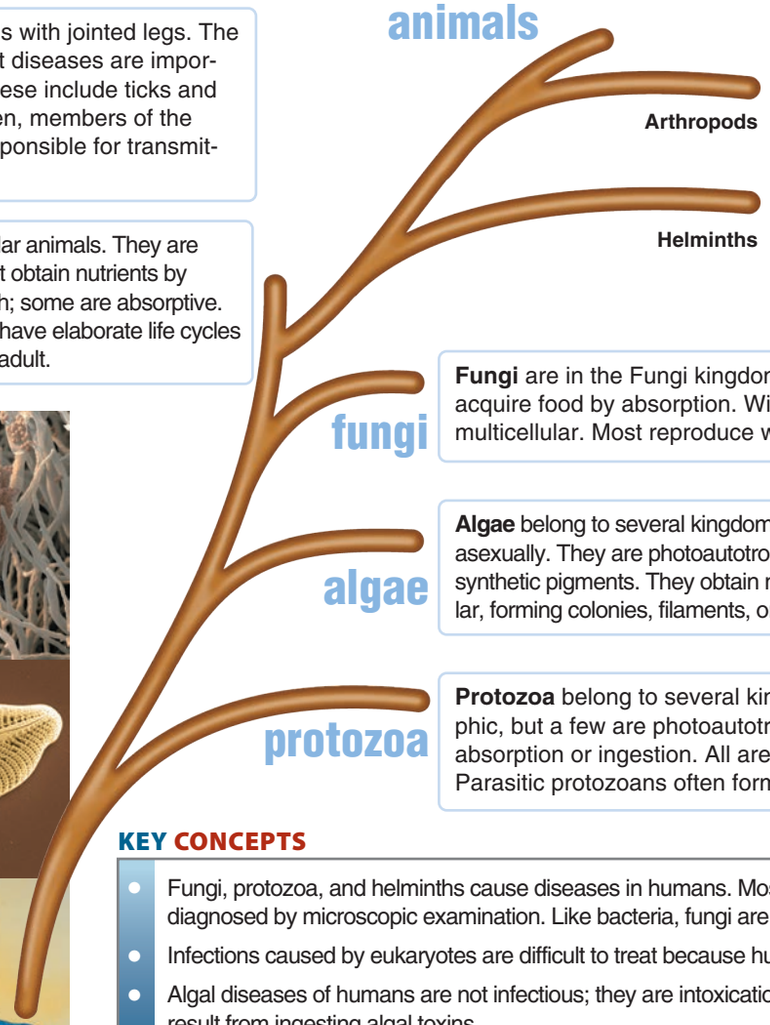
Exploring Pathogenic Eukaryotes



Arthropods are animals with jointed legs. The arthropods that transmit diseases are important in microbiology. These include ticks and some insects; most often, members of the mosquito family are responsible for transmitting disease.

Helminths are multicellular animals. They are chemoheterotrophs. Most obtain nutrients by ingestion through a mouth; some are absorptive. Parasitic helminths often have elaborate life cycles including egg, larva, and adult.





Fungi are in the Fungi kingdom. They are chemoheterotrophs and acquire food by absorption. With the exception of yeasts, fungi are multicellular. Most reproduce with sexual and asexual spores.

Algae belong to several kingdoms and can reproduce both sexually and asexually. They are photoautotrophs and produce several different photosynthetic pigments. They obtain nutrients by diffusion. Some are multicellular, forming colonies, filaments, or even tissues. A few produce toxins.

Protozoa belong to several kingdoms. Most are chemoheterotrophic, but a few are photoautotrophic. They obtain nutrients by absorption or ingestion. All are unicellular, and many are motile. Parasitic protozoans often form resistant cysts.

KEY CONCEPTS

- Fungi, protozoa, and helminths cause diseases in humans. Most of these diseases are diagnosed by microscopic examination. Like bacteria, fungi are cultured on laboratory media.
- Infections caused by eukaryotes are difficult to treat because humans have eukaryotic cells.
- Algal diseases of humans are not infectious; they are intoxications because the symptoms result from ingesting algal toxins.
- Arthropods that transmit infectious diseases are called vectors. Arthropod-borne diseases such as West Nile encephalitis are best controlled by limiting exposure to the arthropod.

Fungi

LEARNING OBJECTIVES

12-1 List the defining characteristics of fungi.

12-2 Differentiate asexual from sexual reproduction, and describe each of these processes in fungi.

12-3 List defining characteristics of the four phyla of fungi.

12-4 Identify two beneficial and two harmful effects of fungi.

Of the more than 100,000 species of fungi, only about 200 are pathogenic to humans and animals. However, over the last 10 years, the incidence of serious fungal infections has been increasing. These infections are occurring in health care settings and in people with compromised immune systems. In addition, thousands of fungal diseases afflict economically important plants, costing more than $1 billion annually.

Clinical Case: Man's Best Friend

Ethan, a 26-year-old computer programmer, is coaxing his dog, Waldo, into his truck. Waldo is very ill, and Ethan is taking him to the veterinary clinic in Bellingham, Washington, to get Waldo checked out. Waldo not only has nasal discharge, noisy breathing, coughing, and sneezing, but also is losing weight and having difficulty walking. Ethan already had to look all over the property to find Waldo, and by the time Ethan locates him in the barn, carries him to the driveway, and lifts the 60-pound Labrador into the bed of his truck, Ethan has to stop to rest. As a matter of fact, he thinks to himself, he doesn't look much better than Waldo these days! Ethan has been fighting what he thinks is some sort of virus as well.

The veterinarian examines Waldo and prescribes fluconazole, an antibiotic. Ethan, now very tired by this point, takes Waldo home. They both lie down to get some rest.

What type of infection could Waldo have? Read on to find out.

321 328 330 331

Fungi are also beneficial. They're important in the food chain because they decompose dead plant matter, thereby recycling vital elements. The hard parts of plants, which animals can't digest, are decomposed primarily by fungi through the use of extracellular enzymes, such as cellulases. Nearly all plants depend on symbiotic fungi, known as **mycorrhizae,** which help their roots absorb minerals and water from the soil (see Chapter 27). Fungi are also valuable to animals. Fungi-farming ants cultivate fungi that break down cellulose and lignin from plants, providing glucose that the ants can then digest. Humans use fungi for food (mushrooms) and to produce foods (bread and citric acid) and drugs (alcohol and penicillin).

The study of fungi is called **mycology.** A pathogen must be accurately identified to properly treat a disease and prevent its spread. We will first look at the structures that are the basis of fungal identification in a clinical laboratory. Then we will explore their life cycles and nutritional needs. All fungi are chemoheterotrophs, requiring organic compounds for energy and carbon. Fungi are aerobic or facultatively anaerobic; only a few anaerobic fungi are known.

Table 12.1 lists the basic differences between fungi and bacteria.

Characteristics of Fungi

Identifying yeasts and bacteria requires biochemical tests. However, multicellular fungi are identified on the basis of physical appearance, including colony characteristics and reproductive spores.

Vegetative Structures

Fungal colonies are described as **vegetative** structures because they're composed of the cells involved in catabolism and growth.

Molds and Fleshy Fungi The **thallus** (body) of a mold or fleshy fungus consists of long filaments of cells joined together; these filaments are called **hyphae** (singular: *hypha*). Hyphae can grow to immense proportions. Using DNA fingerprinting, scientists mapped the hyphae of a single fungus in Oregon (a mushroom) that extended over 4 square miles.

In most molds, the hyphae contain cross-walls called **septa** (singular: *septum*), which divide them into distinct, uninucleate (one-nucleus) cell-like units. These hyphae are called **septate hyphae** (**Figure 12.2a**). In a few classes of fungi, the hyphae contain no septa and appear as long, continuous cells with many nuclei. These are called **coenocytic hyphae** (Figure 12.2b). Even in fungi with septate hyphae, there are usually openings in the septa that make the cytoplasm of adjacent "cells" continuous; these fungi are actually coenocytic organisms, too.

Hyphae grow by elongating at the tips (Figure 12.2c). Each part of a hypha is capable of growth, and when a fragment breaks off, it can elongate to form a new hypha. In the

TABLE 12.1 Selected Features of Fungi and Bacteria Compared

	Fungi	Bacteria
Cell Type	Eukaryotic	Prokaryotic
Cell Membrane	Sterols present	Sterols absent, except in *Mycoplasma*
Cell Wall	Glucans; mannans; chitin (no peptidoglycan)	Peptidoglycan
Spores	Sexual and asexual reproductive spores	Endospores (not for reproduction); some asexual reproductive spores
Metabolism	Limited to heterotrophic; aerobic, facultatively anaerobic	Heterotrophic, autotrophic; aerobic, facultatively anaerobic, anaerobic

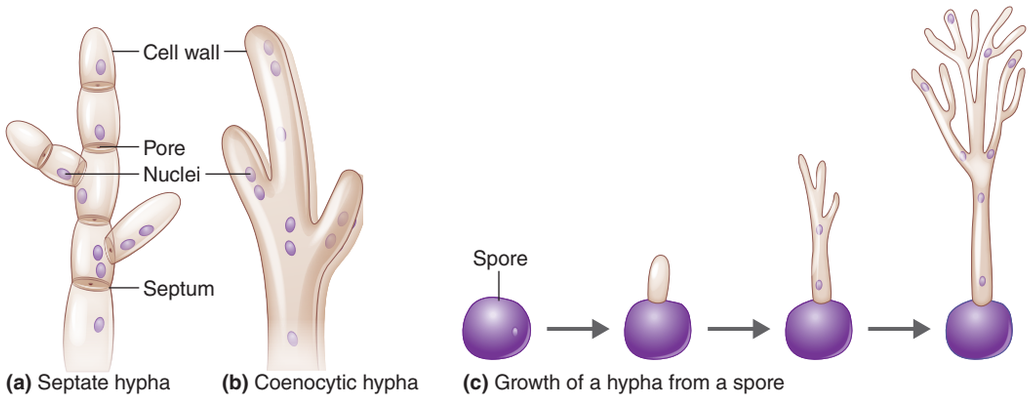


Figure 12.2 Characteristics of fungal hyphae. **(a)** Septate hyphae have cross-walls, or septa, dividing the hyphae into cell-like units. **(b)** Coenocytic hyphae lack septa. **(c)** Hyphae grow by elongating at the tips.

Q What is a hypha? A mycelium?

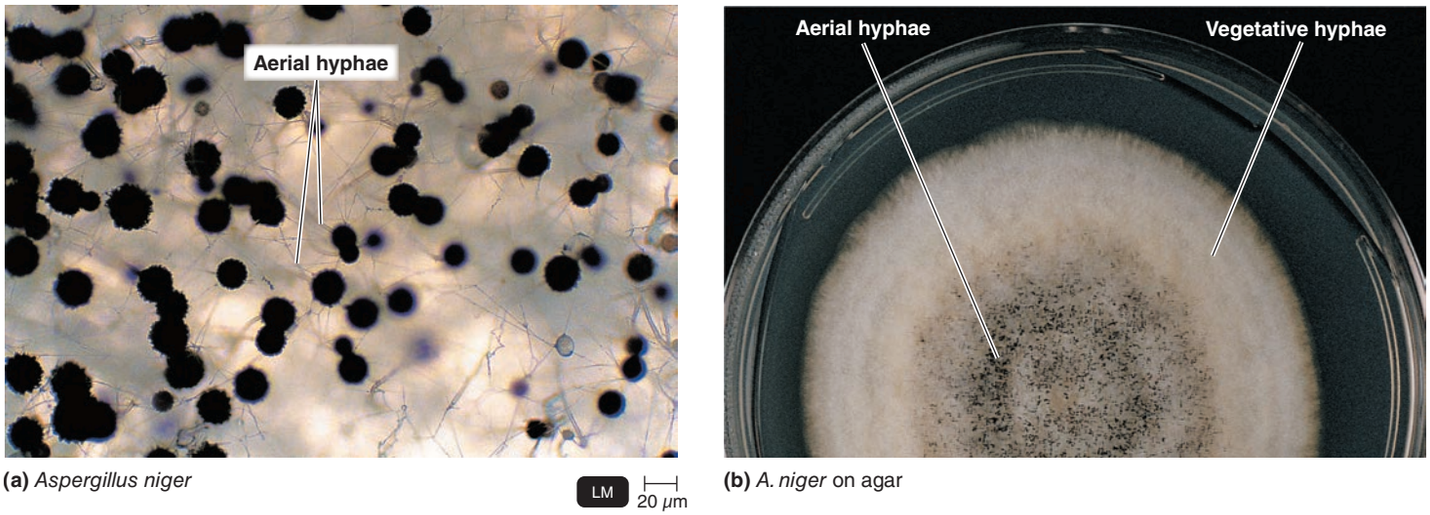
laboratory, fungi are usually grown from fragments obtained from a fungal thallus.

The portion of a hypha that obtains nutrients is called the *vegetative hypha;* the portion concerned with reproduction is the *reproductive* or *aerial hypha,* so named because it projects above the surface of the medium on which the fungus is growing. Aerial hyphae often bear reproductive spores (Figure 12.3a), discussed later. When environmental conditions are suitable, the hyphae grow to form a filamentous mass called a **mycelium,** which is visible to the unaided eye (Figure 12.3b).

Yeasts Yeasts are nonfilamentous, unicellular fungi that are typically spherical or oval. Like molds, yeasts are widely distributed in nature; they are frequently found as a white powdery coating on fruits and leaves. **Budding yeasts,** such as *Saccharomyces* (sak-kar-ō′MĪ′sēz), divide unevenly.

In budding (Figure 12.4), the parent cell forms a protuberance (bud) on its outer surface. As the bud elongates, the parent cell's nucleus divides, and one nucleus migrates into the bud. Cell wall material is then laid down between the bud and parent cell, and the bud eventually breaks away.

One yeast cell can in time produce up to 24 daughter cells by budding. Some yeasts produce buds that fail to detach themselves; these buds form a short chain of cells called a **pseudohypha.** *Candida albicans* (KAN-dē-dah AL-bi-kanz) attaches to human epithelial cells as a yeast but usually requires pseudohyphae to invade deeper tissues (see Figure 21.17a, page 597).



(a) *Aspergillus niger* **(b)** *A. niger* on agar

Figure 12.3 Aerial and vegetative hyphae. **(a)** A photomicrograph of aerial hyphae, showing reproductive spores. **(b)** A colony of *Aspergillus niger* grown on a glucose agar plate, showing both vegetative and aerial hyphae.

Q How do fungal colonies differ from bacterial colonies?

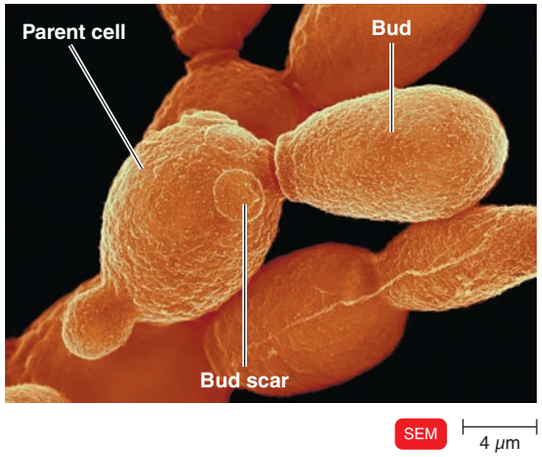


Figure 12.4 A budding yeast. A micrograph of *Saccharomyces cerevisiae* in various stages of budding.

Q How does a bud differ from a spore?

Fission yeasts, such as *Schizosaccharomyces* (SKI-zō-sak′kar-ō′mī-sēz), divide evenly to produce two new cells. During fission, the parent cell elongates, its nucleus divides, and two offspring cells are produced. Increases in the number of yeast cells on a solid medium produce a colony similar to a bacterial colony.

Yeasts are capable of facultative anaerobic growth, which allows these fungi to survive in various environments. If given access to oxygen, yeasts perform aerobic respiration to metabolize carbohydrates into carbon dioxide and water; denied oxygen, they ferment carbohydrates and produce ethanol and carbon dioxide. This fermentation is used in the brewing, wine-making, and baking industries. *Saccharomyces* species produce ethanol in brewed beverages and carbon dioxide for leavening bread dough.

Dimorphic Fungi Some fungi, most notably the pathogenic species, exhibit **dimorphism**—two forms of growth. Such fungi can grow either as a mold or as a yeast. The moldlike forms produce vegetative and aerial hyphae; the yeastlike forms reproduce by budding. Dimorphism in pathogenic fungi is temperature-dependent: at 37°C, the fungus is yeastlike, and at 25°C, it is moldlike. (See Figure 24.15, page 698.) However, the appearance of the dimorphic (in this instance, nonpathogenic) fungus shown in **Figure 12.5** changes with CO_2 concentration.

Life Cycle

Filamentous fungi can reproduce asexually by fragmentation of their hyphae. In addition, both sexual and asexual reproduction in fungi occurs by the formation of **spores.** In fact, fungi are usually identified by spore type.

Fungal spores, however, are quite different from bacterial endospores. Bacterial endospores allow a bacterial cell to survive adverse environmental conditions (see Chapter 4). A single vegetative bacterial cell forms one endospore, which eventually germinates to produce a single vegetative bacterial cell. This process isn't reproduction because it doesn't increase the total number of bacterial cells. But after a mold forms a spore, the spore detaches from the parent and germinates into a new mold (see Figure 12.2c). Unlike the bacterial endospore, this is a true reproductive spore; a second organism grows from the spore. Although fungal spores can survive for extended periods in dry or hot environments, most do not exhibit the extreme tolerance and longevity of bacterial endospores.

Spores are formed from aerial hyphae in a number of different ways, depending on the species. Fungal spores can be either asexual or sexual. **Asexual spores** are formed by the hyphae of one organism. When these spores germinate, they become organisms that are genetically identical to the parent. **Sexual spores** result from the fusion of nuclei from two opposite mating strains of the same species of fungus. Sexual spores require two different mating strains and so are made less frequently than asexual spores. Organisms that grow from sexual spores will have genetic characteristics of both parental strains. Because spores are of considerable importance in identifying fungi, next we'll look at some of the various types of asexual and sexual spores.

Asexual Spores Asexual spores are produced by an individual fungus through mitosis and subsequent cell division; there is no fusion of the nuclei of cells. Two types of asexual spores are produced by fungi. One type is a **conidiospore,** or **conidium** (plural: *conidia*), a unicellular or multicellular spore that is

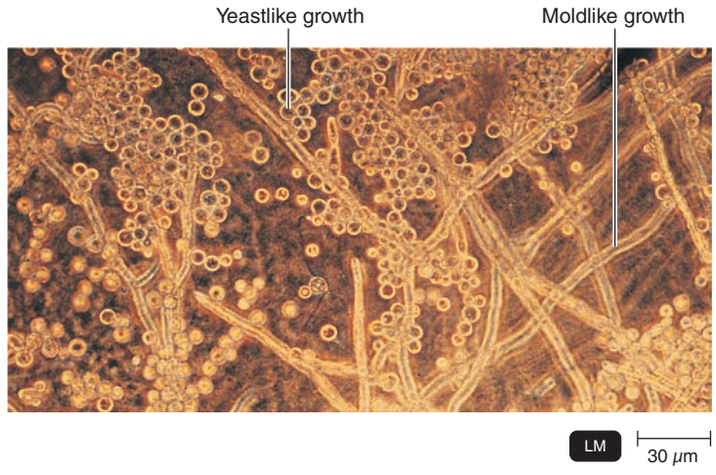
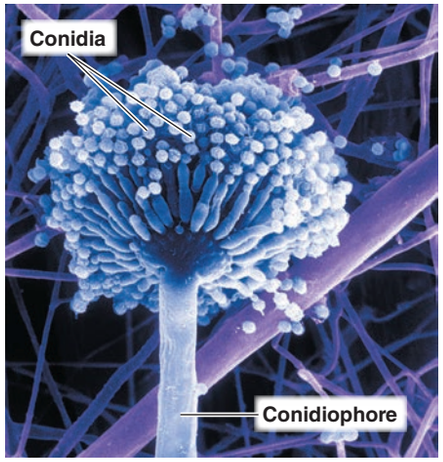
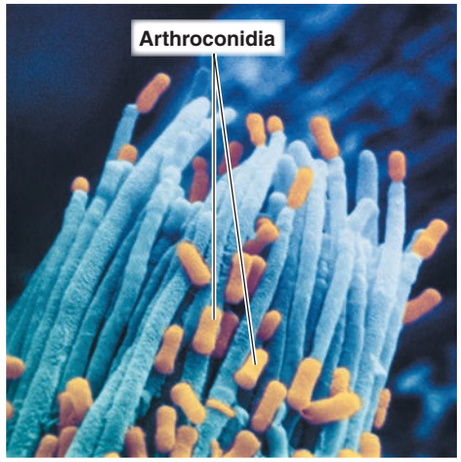


Figure 12.5 Fungal dimorphism. Dimorphism in the fungus *Mucor indicus* depends on CO_2 concentration. On the agar surface, *Mucor* exhibits yeastlike growth, but in the agar where CO_2 from metabolism has accumulated, it is moldlike.

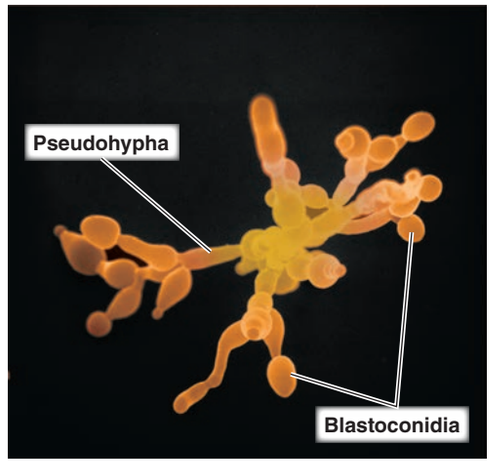
Q What is fungal dimorphism?



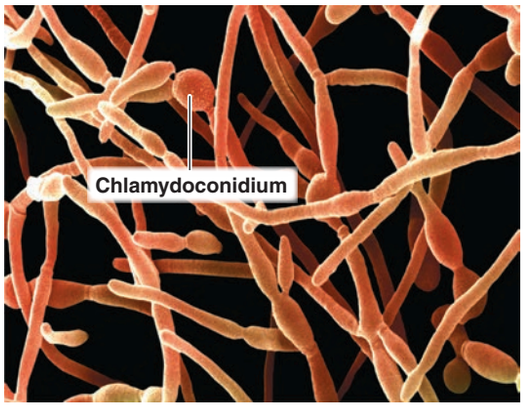
(a) Conidia are arranged in chains at the end of an *Aspergillus niger* conidiophore. SEM 12 μm



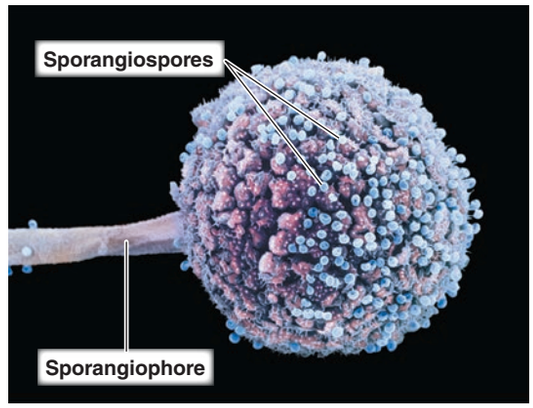
(b) Fragmentation of hyphae results in the formation of arthroconidia in *Ceratocystis ulmi*. SEM 2.5 μm



(c) Blastoconidia are formed from the buds of a parent cell of *Candida albicans*. SEM 13 μm



(d) Chlamydoconidia are thick-walled cells within hyphae of this *Candida albicans*. SEM 5 μm



(e) Sporangiospores are formed within a sporangium of *Rhizopus stolonifer*. SEM 5 μm

Figure 12.6 Representative asexual spores.

What are the green powdery structures on moldy food?

not enclosed in a sac (Figure 12.6a). Conidia are produced in a chain at the end of a **conidiophore.** Such spores are produced by *Penicillium* and *Aspergillus* (a-sper-JIL-lus). Conidia formed by the fragmentation of a septate hypha into single, slightly thickened cells are called **arthroconidia** (Figure 12.6b). One species that produces such spores is *Coccidioides immitis* (KOK-si-dē-oi-dēz IM-mi-tis) (see Figure 24.17, page 699). Another type of conidium, **blastoconidia,** are formed from the buds of its parent cell (Figure 12.6c). Such spores are found in some yeasts, such as *Candida albicans* and *Cryptococcus.* A **chlamydoconidium** is a thick-walled spore formed by rounding and enlargement within a hyphal segment (Figure 12.6d). A fungus that produces chlamydoconidia is the yeast *Candida albicans.*

The other type of asexual spore is a **sporangiospore,** formed within a **sporangium,** or sac, at the end of an aerial hypha called a **sporangiophore.** The sporangium can contain hundreds of sporangiospores (Figure 12.6e). Such spores are produced by *Rhizopus.*

Sexual Spores A fungal sexual spore results from sexual reproduction, which consists of three phases:

1. **Plasmogamy.** A haploid nucleus of a donor cell (+) penetrates the cytoplasm of a recipient cell (−).
2. **Karyogamy.** The (+) and (−) nuclei fuse to form a diploid zygote nucleus.
3. **Meiosis.** The diploid nucleus gives rise to haploid nuclei (sexual spores), some of which may be genetic recombinants.

The sexual spores produced by fungi characterize the phyla. Clinical identification is based on microscopic examination of

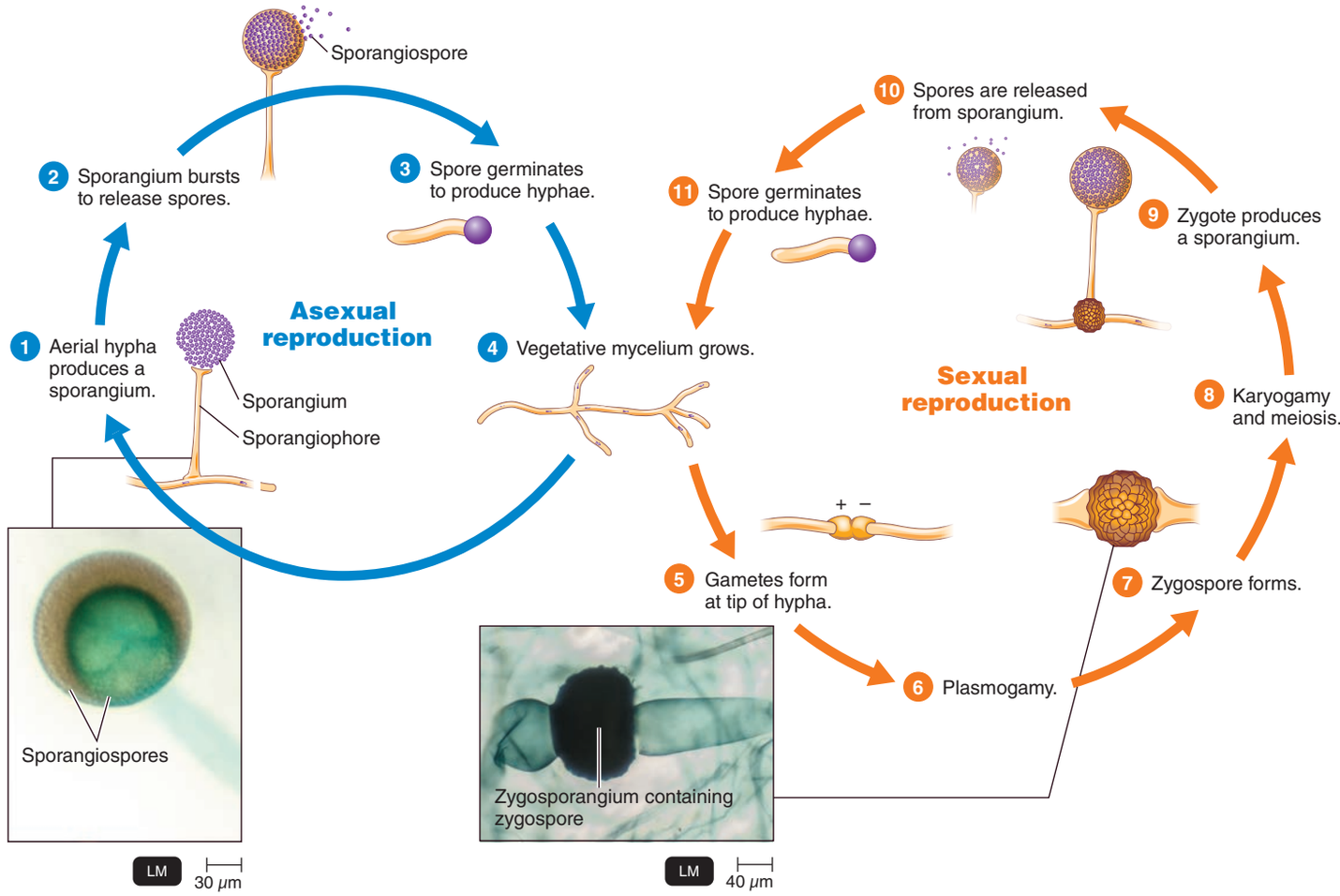


Figure 12.7 The life cycle of *Rhizopus*, a zygomycete. This fungus will reproduce asexually most of the time. Two opposite mating strains (designated + and −) are necessary for sexual reproduction.

Q What is an opportunistic mycosis?

asexual spores, because most fungi exhibit only asexual spores in laboratory settings.

Nutritional Adaptations

Fungi are generally adapted to environments that would be hostile to bacteria. Fungi are chemoheterotrophs, and, like bacteria, they absorb nutrients rather than ingesting them as animals do. However, fungi differ from bacteria in certain environmental requirements and in the following nutritional characteristics:

- Fungi usually grow better in an environment with a pH of about 5, which is too acidic for the growth of most common bacteria.
- Almost all molds are aerobic. Most yeasts are facultative anaerobes.
- Most fungi are more resistant to osmotic pressure than bacteria; most can therefore grow in relatively high sugar or salt concentrations.
- Fungi can grow on substances with a very low moisture content, generally too low to support the growth of bacteria.
- Fungi require somewhat less nitrogen than bacteria for an equivalent amount of growth.
- Fungi are often capable of metabolizing complex carbohydrates, such as lignin (a component of wood), that most bacteria can't use for nutrients.

These characteristics enable fungi to grow on such unlikely substrates as bathroom walls, shoe leather, and discarded newspapers.

CHECK YOUR UNDERSTANDING

- Assume you isolated a single-celled organism that has a cell wall. How would you determine that it is a fungus and not a bacterium? **12-1**
- Contrast the mechanism of conidiospore and ascospore formation. **12-2**

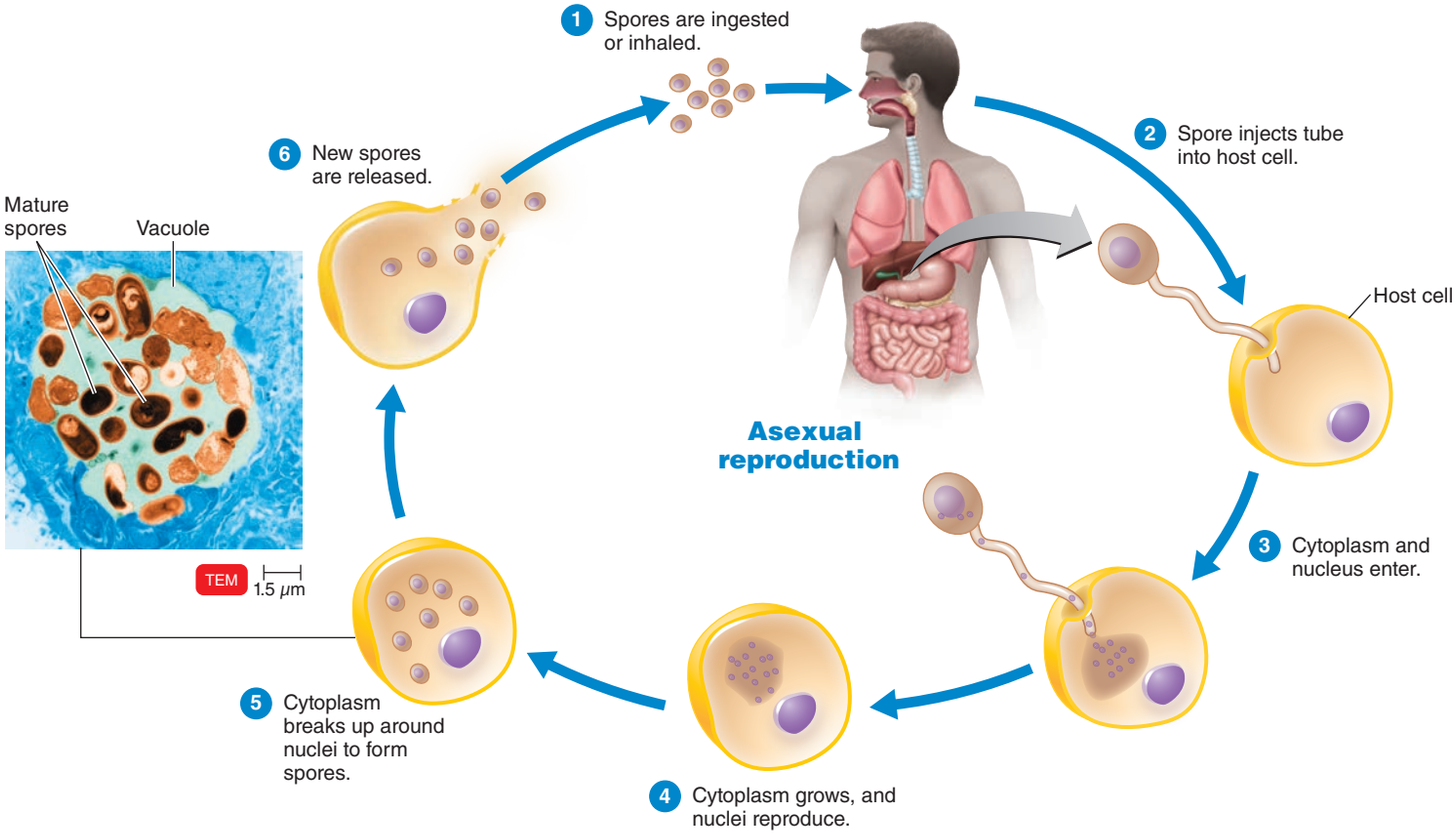


Figure 12.8 The life cycle of *Encephalitozoon,* a microsporidian. Microsporidiosis is an emerging opportunistic infection in immunocompromised patients and the elderly. *E. intestinalis* causes diarrhea. Sexual reproduction has not been observed.

Q Why were microsporidia so difficult to classify?

Medically Important Fungi

This section provides an overview of medically important phyla of fungi. The actual diseases they cause will be studied in Chapters 21 through 26. Note that only a relatively small number of fungi cause disease.

The genera named in the following phyla include many that are readily found as contaminants in foods and in laboratory bacterial cultures. Although these genera are not all of primary medical importance, they are typical examples of their respective groups.

Zygomycota

The Zygomycota, or conjugation fungi, are saprophytic molds that have coenocytic hyphae. An example is *Rhizopus stolonifer,* the common black bread mold. The asexual spores of *Rhizopus* are sporangiospores (Figure 12.7). The dark sporangiospores inside the sporangium give *Rhizopus* its descriptive common name. When the sporangium breaks open, the sporangiospores are dispersed. If they fall on a suitable medium, they will germinate into a new mold thallus.

The sexual spores are zygospores. A **zygospore** is a large spore enclosed in a thick wall (Figure 12.7, step 7). This type of spore forms when the nuclei of two cells that are morphologically similar to each other fuse.

Microsporidia

Microsporidia are unusual eukaryotes because they lack mitochondria. Microsporidia don't have microtubules (see Chapter 4, page 96), and they're obligate intracellular parasites. In 1857, when they were discovered, microsporidians were classified as fungi. They were reclassified as protists in 1983 because they lack mitochondria. Recent genome sequencing, however, reveals that the microsporidians are fungi. Sexual reproduction has not been observed but probably occurs within the host (Figure 12.8). Microsporidia have been reported to be the cause of a number of human diseases, including chronic diarrhea and keratoconjunctivitis (inflammation of the conjunctiva near the cornea), most notably in AIDS patients.

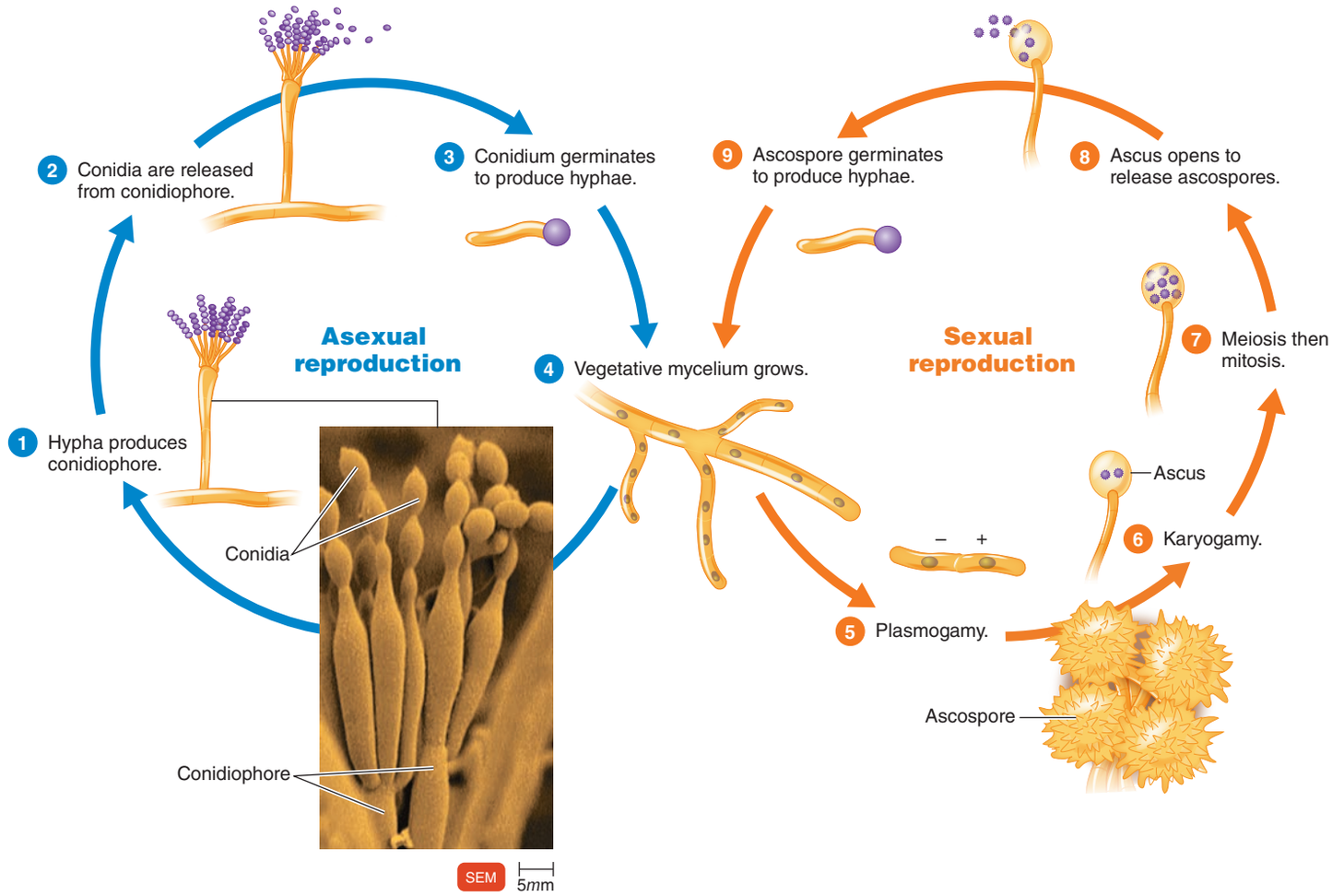


Figure 12.9 The life cycle of *Talaromyces,* an ascomycete. Occasionally, when two opposite mating cells from two different strains (+ and –) fuse, sexual reproduction occurs.

Q Name one ascomycete that can infect humans.

Ascomycota

The Ascomycota, or sac fungi, include molds with septate hyphae and some yeasts. Their asexual spores are usually conidia produced in long chains from the conidiophore. The term *conidia* means dust, and these spores freely detach from the chain at the slightest disturbance and float in the air like dust.

An **ascospore** forms when the nuclei of two cells that can be either morphologically similar or dissimilar fuse. These spores are produced in a saclike structure called an **ascus** (Figure 12.9, lower right). The members of this phylum are called sac fungi because of the ascus.

Basidiomycota

The Basidiomycota, or club fungi, also possess septate hyphae. This phylum includes fungi that produce mushrooms. **Basidiospores** are formed externally on a base pedestal called a **basidium** (Figure 12.10). (The common name of the fungus is derived from the club shape of the basidium.) There are usually four basidiospores per basidium. Some of the basidiomycota produce asexual conidiospores.

* * *

The fungi we have looked at thus far are **teleomorphs;** that is, they produce both sexual and asexual spores. Some ascomycetes have lost the ability to reproduce sexually. These asexual fungi are called **anamorphs.** *Penicillium* is an example of an anamorph that arose from a mutation in a teleomorph. Historically, fungi whose sexual cycle had not been observed were put in a "holding category" called *Deuteromycota.* Now, mycologists are using rRNA sequencing to classify these organisms. Most of these previously unclassified deuteromycetes are anamorphic phases of Ascomycota, and a few are basidiomycetes.

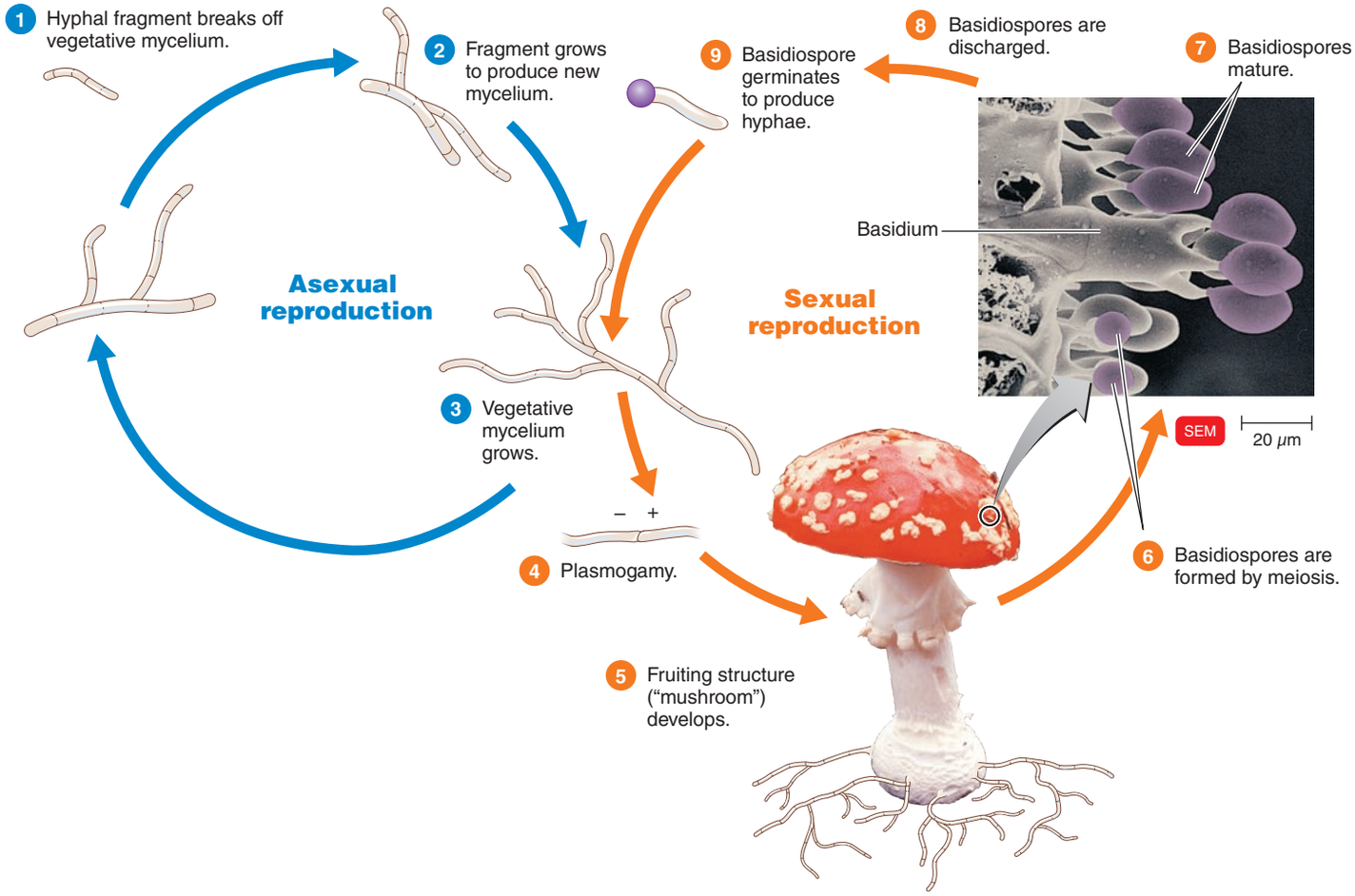


Figure 12.10 A generalized life cycle of a basidiomycete. Mushrooms appear after cells from two mating strains (+ and –) have fused.

Q On what basis are fungi classified into phyla?

Table 12.2 lists some fungi that cause human diseases. Both teleomorphic and anamorphic names are given for some of the fungi because some medically important fungi are known by their anamorph, or asexual, names.

Fungal Diseases

Any fungal infection is called a **mycosis.** Mycoses are generally chronic (long-lasting) infections because fungi grow slowly. Mycoses are classified into five groups according to the degree of tissue involvement and mode of entry into the host: systemic, subcutaneous, cutaneous, superficial, or opportunistic. Fungi are related to animals (as we saw in Chapter 10). Consequently, drugs that affect fungal cells may also affect animal cells, making fungal infections of humans and other animals often difficult to treat. Some fungi cause disease by producing toxins, and those are discussed in Chapter 15.

Systemic mycoses are fungal infections deep within the body. They are not restricted to any particular region of the

Clinical Case

Five days later, Ethan's doctor sends him to the hospital. For the past week Ethan has had shortness of breath, fever, chills, headache, night sweats, loss of appetite, nausea, and muscle pain. He has no other symptoms and is treated with amoxicillin for a presumed lower respiratory tract infection. Three days later, Ethan's condition deteriorates; his respiratory rate increases, but no other systemic symptoms are apparent. Based on Ethan's respiratory symptoms, his doctor orders a chest X-ray exam. The X-ray film shows that Ethan has a mass in his lungs.

Both Ethan and his dog seem to have acquired the same infection. Make a short list of possible pathogens based on this new information.

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TABLE 12.2 Characteristics of Some Pathogenic Fungi

Phylum	Growth Characteristics	Asexual Spore Types	Human Pathogens	Habitat	Type of Mycosis	Page
Zygomycota	Nonseptate hyphae	Sporangiospores	*Rhizopus*	Ubiquitous	Systemic	701
			Mucor	Ubiquitous	Systemic	701
Microsporidia	No hyphae	Nonmotile spores	*Encephalitozoon,*	Humans, other	Diarrhea,	326
			Nosema	animals	keratoconjunctivitis	330
Ascomycota		Conidia	*Aspergillus*	Ubiquitous	Systemic	701
			Claviceps purpurea	Grasses	Toxin ingestion	432
	Dimorphic		*Blastomyces** (or *Ajellomyces*†) *dermatitidis*	Unknown	Systemic	701
			*Histoplasma** (or *Ajellomyces*†) *capsulatum*	Soil	Systemic	698
	Septate hyphae, strong affinity for keratin	Conidia	*Microsporum*	Soil, animals	Cutaneous	596
		Arthroconidia Chlamydoconidia	*Trichophyton** (or *Arthroderma*†)	Soil, animals	Cutaneous	596
Anamorphs	Septate hyphae	Conidia	*Epidermophyton*	Soil, humans	Cutaneous	596
	Dimorphic		*Sporothrix schenckii, Stachybotrys*	Soil	Subcutaneous	596
		Arthroconidia	*Coccidioides immitis*	Soil	Systemic	699
	Yeastlike, pseudohyphae	Chlamydoconidia	*Candida albicans*	Human normal microbiota	Cutaneous, systemic, mucocutaneous	596
	Unicellular	None	*Pneumocystis*	Ubiquitous	Systemic	700
Basidiomycota	Septate hyphae; includes rusts and smuts, and plant pathogens; yeastlike encapsulated cells	Conidia	*Cryptococcus** (or *Filobasidiella*†)	Soil, bird feces	Systemic	621
			Malassezia	Human skin	Cutaneous	581
			Amanita spp.	Soil	Toxin ingestion	432

*Anamorph name.
†Teleomorph name.

body but can affect a number of tissues and organs. Systemic mycoses are usually caused by fungi that live in the soil. The spores are transmitted by inhalation; these infections typically begin in the lungs and then spread to other body tissues. They are not contagious from animal to human or from human to human. Two systemic mycoses, histoplasmosis and coccidioidomycosis, are discussed in Chapter 24.

Subcutaneous mycoses are fungal infections beneath the skin caused by saprophytic fungi that live in soil and on vegetation. Sporotrichosis is a subcutaneous infection acquired by gardeners and farmers (Chapter 21, page 596). Infection occurs by direct implantation of spores or mycelial fragments into a puncture wound in the skin.

Fungi that infect only the epidermis, hair, and nails are called **dermatophytes,** and their infections are called *dermatomycoses* or **cutaneous mycoses** (see Figure 21.16, page 596). Dermatophytes secrete keratinase, an enzyme that degrades **keratin,** a protein found in hair, skin, and nails. Infection is transmitted from human to human or from animal to human by direct contact or by contact with infected hairs and epidermal cells (as from barber shop clippers or shower room floors).

The fungi that cause **superficial mycoses** are localized along hair shafts and in superficial (surface) epidermal cells. These infections are prevalent in tropical climates.

An **opportunistic pathogen** is generally harmless in its normal habitat but can become pathogenic in a host who is seriously

debilitated or traumatized, who is under treatment with broad-spectrum antibiotics, whose immune system is suppressed by drugs or by an immune disorder, or who has a lung disease.

Pneumocystis is an opportunistic pathogen in individuals with compromised immune systems and is the most common life-threatening infection in AIDS patients (see Figure 24.19, page 700). It was first classified as a protozoan, but recent studies of its RNA reveal it's a unicellular anamorphic fungus. Another example of an opportunistic pathogen is the fungus *Stachybotrys* (STA-kē-bah-tris), which normally grows on cellulose found in dead plants but in recent years has been found growing on water-damaged walls of homes.

Mucormycosis is an opportunistic mycosis caused by *Rhizopus* and *Mucor* (MŪ-kor); the infection occurs mostly in patients who have diabetes mellitus, have leukemia, or are undergoing treatment with immunosuppressive drugs. Aspergillosis is also an opportunistic mycosis; it is caused by *Aspergillus* (see Figure 12.3). This disease occurs in people who have debilitating lung diseases or cancer and have inhaled *Aspergillus* spores. Opportunistic infections by *Cryptococcus* and *Penicillium* can cause fatal diseases in AIDS patients. These opportunistic fungi may be transmitted from one person to an uninfected person but do not usually infect immunocompetent people.

Clinical Case

Ethan's physician, suspecting that Ethan has a fungal infection, orders a biopsy of the lung mass. **Figure A** and **Figure B** show the microscopic examination and a culture from the biopsied tissue.

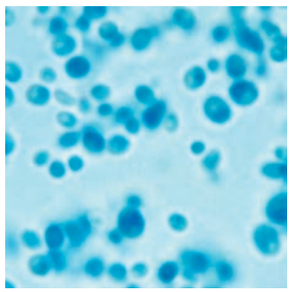

Figure A Microscopic examination from lung mass.

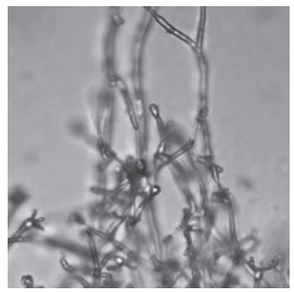

Figure B Microscopic appearance of culture.

Based on the figures, what is the most likely pathogen?

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Yeast infection, or candidiasis, is most frequently caused by *Candida albicans* and may occur as vulvovaginal candidiasis or thrush, a mucocutaneous candidiasis. Candidiasis frequently occurs in newborns, in people with AIDS, and in people being treated with broad-spectrum antibiotics (see Figure 21.17, page 597).

Some fungi cause disease by producing toxins. These toxins are discussed in Chapter 15.

Economic Effects of Fungi

Fungi have been used in biotechnology for many years. *Aspergillus niger* (NĪ-jer), for example, has been used to produce citric acid for foods and beverages since 1914. The yeast *Saccharomyces cerevisiae* is used to make bread and wine. It is also genetically modified to produce a variety of proteins, including hepatitis B vaccine. *Trichoderma* is used commercially to produce the enzyme cellulase, which is used to remove plant cell walls to produce a clear fruit juice. When the anticancer drug taxol, which is produced by yew trees, was discovered, there was concern that the yew forests of the U.S. Northwest coast would be decimated to harvest the drug. However, the fungus *Taxomyces* (TAKS-ō-mī-sēz) also produces taxol.

Fungi are used as biological controls of pests. In 1990, the fungus *Entomophaga* (en-tō-MŌ-fah-gah) unexpectedly proliferated and killed gypsy moths that were destroying trees in the eastern United States. Scientists are investigating the use of several fungi to kill pests:

- The fungus *Coniothyrium minitans* (kon′ē-ō-THER-ē-um MIN-i-tanz) feeds on fungi that destroy soybeans and other bean crops.
- A foam filled with *Paecilomyces fumosoroseus* is being used as a biological alternative to chemicals to kill termites hiding inside tree trunks and other hard-to-reach places.

In contrast to these beneficial effects, fungi can have undesirable effects for agriculture because of their nutritional adaptations. As most of us have observed, mold spoilage of fruits, grains, and vegetables is relatively common, but bacterial spoilage of such foods is not. There is little moisture on the unbroken surfaces of such foods, and the interiors of fruits are too acidic for many bacteria to grow there. Jams and jellies also tend to be acidic, and they have a high osmotic pressure from the sugars they contain. These factors all discourage bacterial growth but readily support the growth of molds. A paraffin layer on top of a jar of homemade jelly helps deter mold growth because molds are aerobic and the paraffin layer keeps out the oxygen. However, fresh meats and certain other foods are such good substrates for bacterial growth that bacteria not only outgrow molds but actively suppress mold growth by producing antifungal chemicals.

Clinical Case Resolved

Cryptococcus gattii, an emerging fungal infection in the United States, is a dimorphic fungus found in the soil. It grows as a yeast at 37°C and produces hyphae at 25°C. Based on the yeastlike appearance and presence of hyphae, the lab confirms the presence of *C. gattii* in Ethan's lung mass. Ethan regularly takes Waldo hiking in the Douglas fir forest of the Northwest, so it isn't possible to know exactly where or when they contracted their infections. Since the first reported case in 1999, over 200 cases have been reported in British Columbia alone. In the United States Pacific Northwest, 60 people and 50 companion animal cases have been confirmed since 2004.

Ethan is placed on intravenous therapy with the antifungal agents amphotericin B and flucytosine. After a 6-week hospital stay, Ethan and Waldo are back at home and almost ready to go hiking again.

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The spreading chestnut tree, of which Longfellow wrote, no longer grows in the United States except in a few widely isolated locations; a fungal blight killed virtually all of them. This blight was caused by the ascomycete *Cryphonectria parasitica* (kri-fō-NEK-trē-ah par-ah-SI-ti-kah), which was introduced from China around 1904. The fungus allows the tree roots to live and put forth shoots regularly, but then it kills the shoots just as regularly. *Cryphonectria*-resistant chestnuts are being developed. Another imported fungal plant disease is Dutch elm disease, caused by *Ceratocystis ulmi* (ser-AH-tō-sis-tis UL-mē). Carried from tree to tree by a bark beetle, the fungus blocks the afflicted tree's circulation. The disease has devastated the American elm population.

CHECK YOUR UNDERSTANDING

- List the asexual and sexual spores made by zygomycetes, ascomycetes, and basidiomycetes. **12-3**
- Why are microsporidia classified as fungi? **12-4**
- Are yeasts beneficial or harmful? **12-4**

Lichens

LEARNING OBJECTIVES

12-5 List the distinguishing characteristics of lichens, and describe their nutritional needs.

12-6 Describe the roles of the fungus and the alga in a lichen.

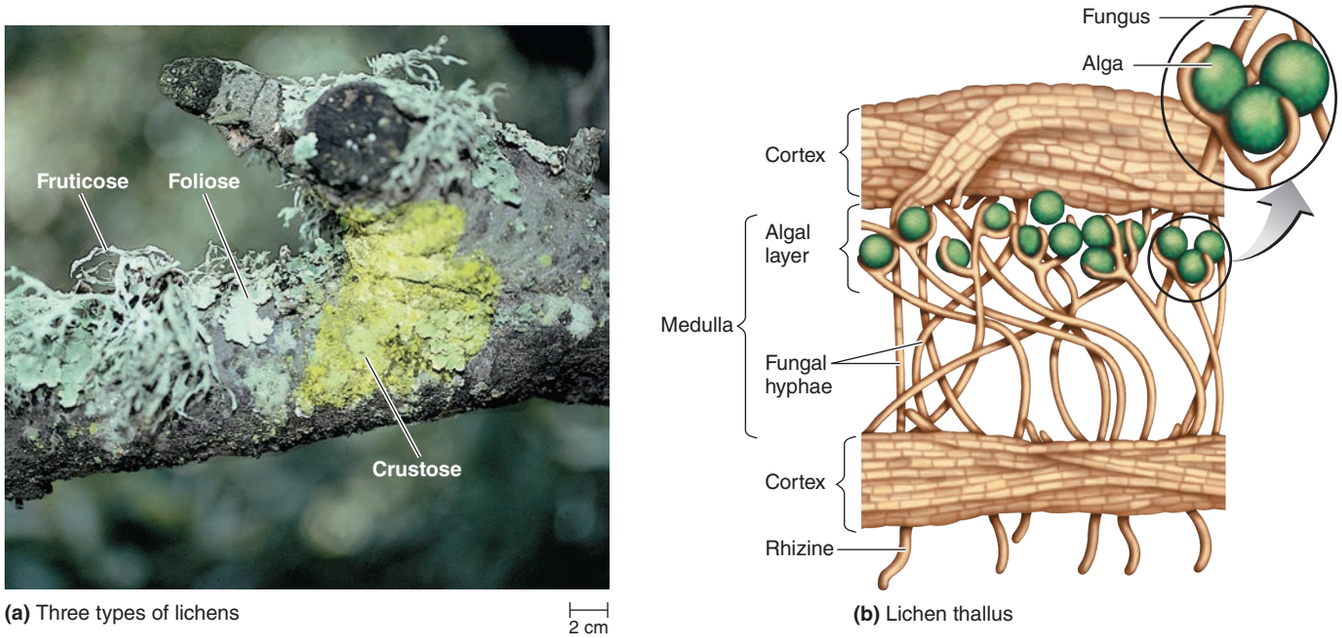
A **lichen** is a combination of a green alga (or a cyanobacterium) and a fungus. Lichens are placed in the Kingdom Fungi and are classified according to the fungal partner, most often an ascomycete. The two organisms exist in a *mutualistic* relationship, in which each partner benefits. The lichen is very different from either the alga or fungus growing alone, and if the partners are separated, the lichen no longer exists. Approximately 13,500 species of lichens occupy quite diverse habitats. Because they can inhabit areas in which neither fungi nor algae could survive alone, lichens are often the first life forms to colonize newly exposed soil or rock. Lichens secrete organic acids that chemically weather rock, and they accumulate nutrients needed for plant growth. Also found on trees, concrete structures, and rooftops, lichens are some of the slowest-growing organisms on Earth.

Lichens can be grouped into three morphologic categories (**Figure 12.11a**). *Crustose lichens* grow flush or encrusted onto the substratum, *foliose lichens* are more leaflike, and *fruticose lichens* have fingerlike projections. The lichen's thallus, or body, forms when fungal hyphae grow around algal cells to become the **medulla** (Figure 12.11b). Fungal hyphae project below the lichen body to form **rhizines,** or holdfasts. Fungal hyphae also form a **cortex,** or protective covering, over the algal layer and sometimes under it as well. After incorporation into a lichen thallus, the alga continues to grow, and the growing hyphae can incorporate new algal cells.

When the algal partner is cultured separately in vitro, about 1% of the carbohydrates produced during photosynthesis are released into the culture medium; however, when the alga is associated with a fungus, the algal plasma membrane is more permeable, and up to 60% of the products of photosynthesis are released to the fungus or are found as end-products of fungal metabolism. The fungus clearly benefits from this association. The alga, while giving up valuable nutrients, is in turn compensated; it receives from the fungus both attachment (rhizines) and protection from desiccation (cortex).

Lichens had considerable economic importance in ancient Greece and other parts of Europe as dyes for clothing. Usnic acid from *Usnea* is used as an antimicrobial agent in China. Erythrolitmin, the dye used in litmus paper to indicate changes in pH, is extracted from a variety of lichens. Some lichens or their acids can cause allergic contact dermatitis in humans.

Populations of lichens readily incorporate cations (positively charged ions) into their thalli. Therefore, the concentrations and types of cations in the atmosphere can be determined by chemical analyses of lichen thalli. In addition, the presence or absence of species that are quite sensitive to pollutants can be used to ascertain air quality. A 1985 study in the Cuyahoga Valley in Ohio revealed that 81% of the 172 lichen species that were present in 1917 were gone. Because this area is severely affected by air pollution, the inference is that air pollutants, primarily sulfur dioxide (the major contributor to acid precipitation), caused the death of sensitive species.



(a) Three types of lichens **(b)** Lichen thallus

Figure 12.11 Lichens. The lichen medulla is composed of fungal hyphae surrounding the algal layer. The protective cortex is a layer of fungal hyphae that covers the surface and sometimes the bottom of the lichen.

Q In what ways are lichens unique?

Lichens are the major food for tundra herbivores such as caribou and reindeer. After the 1986 Chernobyl nuclear disaster, 70,000 reindeer in Lapland that had been raised for food had to be destroyed because of high levels of radiation. The lichens on which the reindeer fed had absorbed radioactive cesium-137, which had spread in the air.

CHECK YOUR UNDERSTANDING

- What is the role of lichens in nature? **12-5**
- What is the role of the fungus in a lichen? **12-6**

Algae

LEARNING OBJECTIVES

12-7 List the defining characteristics of algae.

12-8 List the outstanding characteristics of the five phyla of algae discussed in this chapter.

12-9 Identify two beneficial and two harmful effects of algae.

Algae are familiar as the large brown kelp in coastal waters, the green scum in a puddle, and the green stains on soil or on rocks. A few algae are responsible for food poisonings. Some algae are unicellular; others form chains of cells (are filamentous); and a few have thalli.

"Algae" is not a taxonomic group; it is a way to describe photoautotrophs that lack the roots and stems of plants. Historically they were considered plants, but they lack the embryos of true plants. DNA analyses led to reclassification of algae into new kingdoms—see **Table 12.3**. Algae are mostly aquatic, although some are found in soil or on trees when sufficient moisture is available there. Unusual algal habitats include the hair of both the sedentary South American sloth and the polar bear. Water is necessary for physical support, reproduction, and the diffusion of nutrients. Generally, algae are found in cool temperate waters, although the large floating mats of the brown alga *Sargassum* (sar-GAS-sum) are found in the subtropical Sargasso Sea, and some species of brown algae grow in antarctic waters.

Characteristics of Algae

Algae are relatively simple eukaryotic photoautotrophs that lack the tissues (roots, stem, and leaves) of plants. The identification of unicellular and filamentous algae requires microscopic examination. Most algae are found in the ocean. Their locations depend on the availability of appropriate nutrients, wavelengths of light, and surfaces on which they can grow. Probable locations for representative algae are shown in **Figure 12.12a**.

Vegetative Structures

The body of a multicellular alga is called a thallus. Thalli of the larger multicellular algae, those commonly called seaweeds, consist of branched **holdfasts** (which anchor the alga to a rock), stemlike and often hollow **stipes,** and leaflike **blades** (Figure 12.12b).

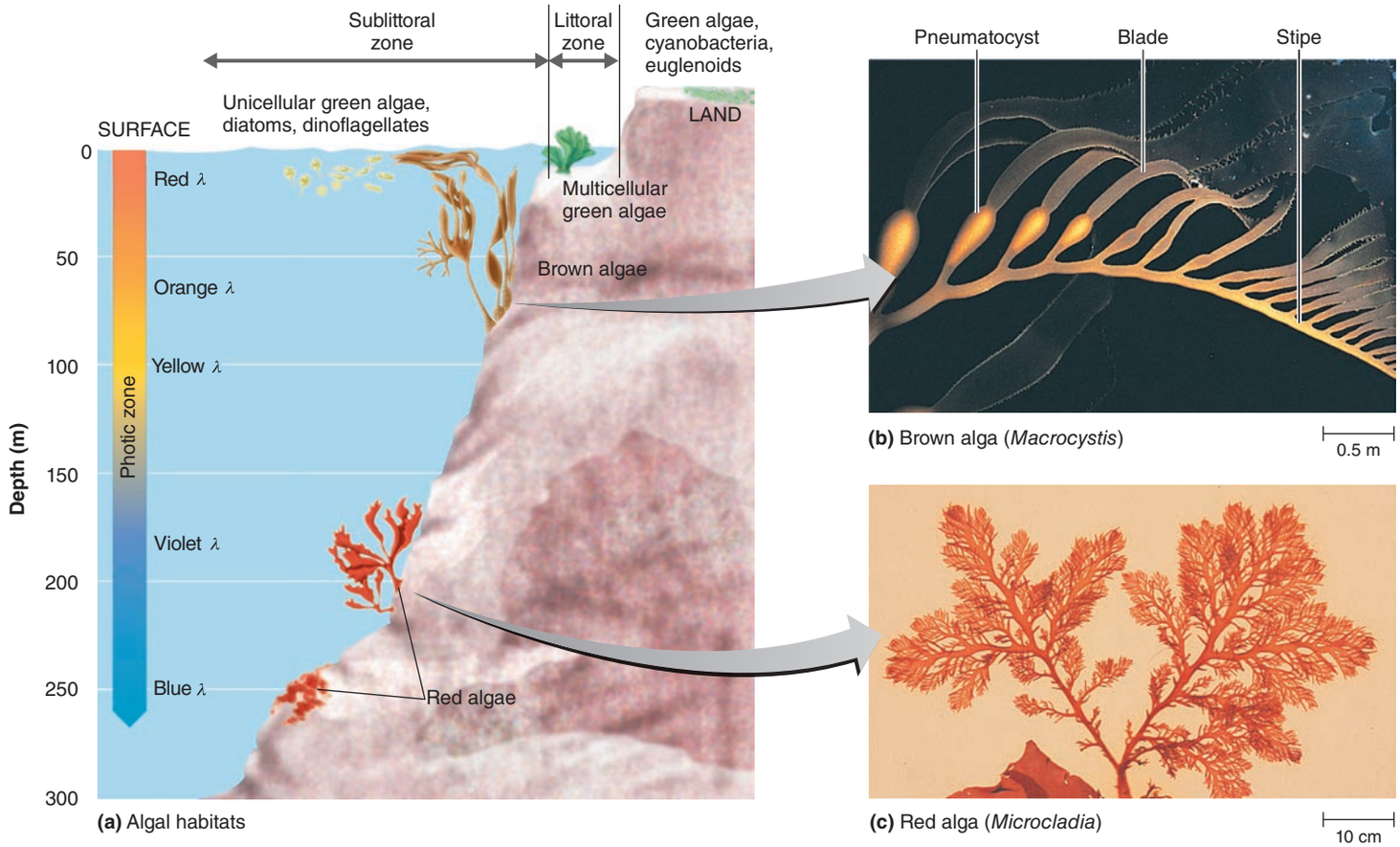


Figure 12.12 Algae and their habitats. **(a)** Although unicellular and filamentous algae can be found on land, they frequently exist in marine and freshwater environments as plankton. Multicellular green, brown, and red algae require a suitable attachment site, adequate water for support, and light of the appropriate wavelengths. **(b)** *Macrocystis porifera,* a brown alga. The hollow stipe and gas-filled pneumatocysts hold the thallus upright, ensuring that sufficient sunlight is received for growth. **(c)** *Microcladia,* a red alga. The delicately branched red algae get their color from phycobiliprotein accessory pigments.

Q What red alga is toxic for humans?

TABLE 12.3 Characteristics of Selected Algae

	Brown Algae	Diatoms	Dinoflagellates	Water Molds	Red Algae	Green Algae
Kingdom	Chromalveolata	Chromalveolata	Chromalveolata	Chromalveolata	Archaeplastida	Viridiplantae
Phylum	Phaeophyta	Bacillariophyta	Dinoflagellata	Oomycota	Rhodophyta	Chlorophyta
Color	Brownish	Brownish	Brownish	Colorless, white	Reddish	Green
Cell Wall	Cellulose and alginic acid	Pectin and silica	Cellulose in membrane	Cellulose	Cellulose	Cellulose
Cell Arrangement	Multicellular	Unicellular	Unicellular	Multicellular	Multicellular (most)	Unicellular and multicellular
Photosynthetic Pigments	Chlorophyll *a* and *c*, xanthophylls	Chlorophyll *a* and *c*, carotene, xanthophylls	Chlorophyll *a* and *c*, carotene, xanthins	None	Chlorophyll *a* and *d*, phycobiliproteins	Chlorophyll *a* and *b*
Sexual Reproduction	Yes	Yes	In a few	Yes (similar to Zygomycota)	Yes	Yes
Storage Material	Carbohydrate	Oil	Starch	None	Glucose polymer	Glucose polymer
Pathogenicity	None	Toxins	Toxins	Parasitic	A few produce toxins	None

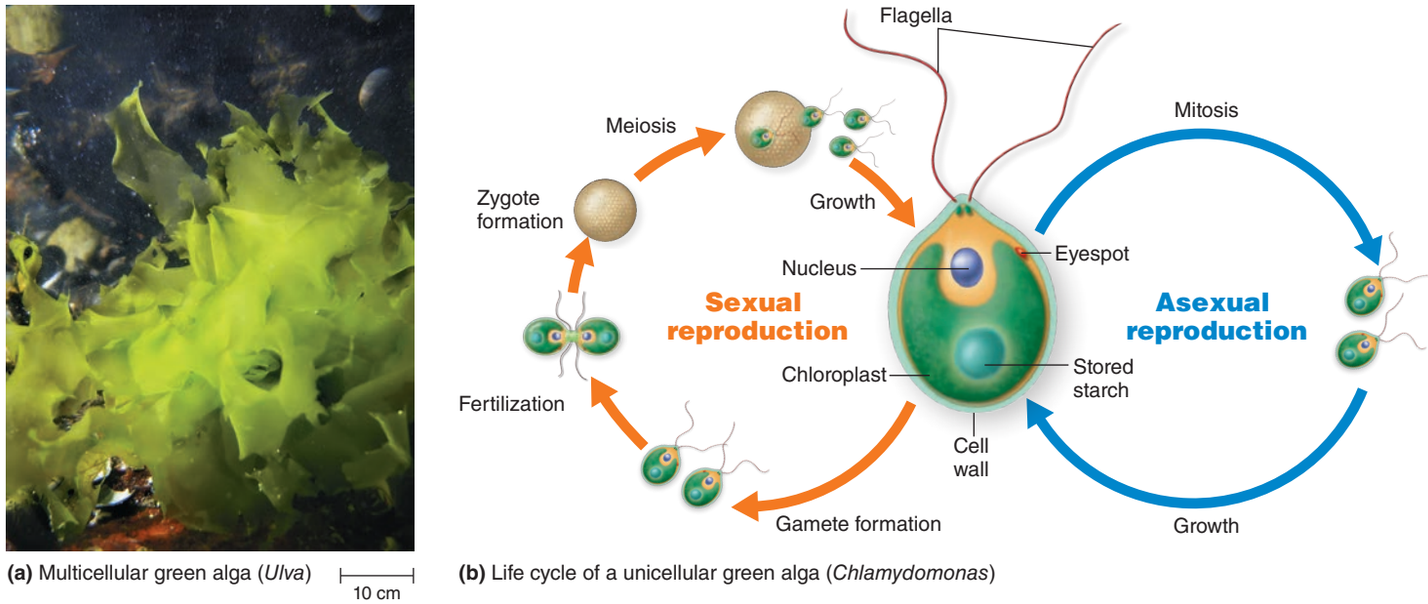


Figure 12.13 Green algae. **(a)** The multicellular green alga *Ulva*. **(b)** The life cycle of the unicellular green alga *Chlamydomonas*. Two whiplike flagella propel this cell.

Q What is the primary role of algae in the ecosystem?

The cells covering the thallus can carry out photosynthesis. The thallus lacks the conductive tissue (xylem and phloem) characteristic of vascular plants; algae absorb nutrients from the water over their entire surface. The stipe is not lignified or woody, so it does not offer the support of a plant's stem; instead, the surrounding water supports the algal thallus; some algae are also buoyed by a floating, gas-filled bladder called a *pneumatocyst.*

Life Cycle

All algae can reproduce asexually. Multicellular algae with thalli and filamentous forms can fragment; each piece is capable of forming a new thallus or filament. When a unicellular alga divides, its nucleus divides (mitosis), and the two nuclei move to opposite parts of the cell. The cell then divides into two complete cells (cytokinesis).

Algae can also reproduce sexually (Figure 12.13). In some species, asexual reproduction may occur for several generations and then, under different conditions, the same species reproduce sexually. Other species alternate generations so that the offspring resulting from sexual reproduction reproduce asexually, and the next generation then reproduces sexually.

Nutrition

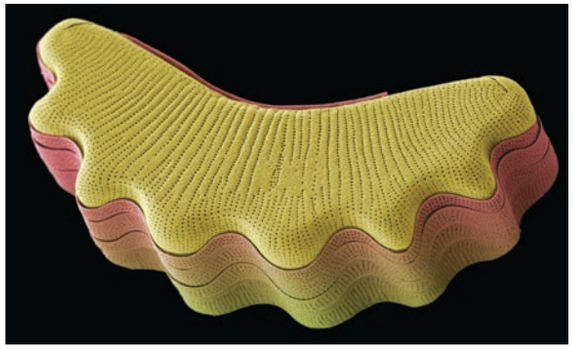
Algae is a common name that includes several phyla (Table 12.3). Most algae are photosynthetic; however, the oomycotes, or fungal-like algae, are chemoheterotrophs. Photosynthetic algae are found throughout the photic (light) zone of bodies of water. Chlorophyll *a* (a light-trapping pigment) and accessory pigments involved in photosynthesis are responsible for the distinctive colors of many algae.

Algae are classified according to their rRNA sequences, structures, pigments, and other qualities (see Table 12.3). Following are descriptions of some phyla of algae.

Selected Phyla of Algae

The *brown algae,* or kelp, are macroscopic; some reach lengths of 50 m (see Figure 12.12b). Most brown algae are found in coastal waters. Brown algae have a phenomenal growth rate. Some grow at rates exceeding 20 cm per day and therefore can be harvested regularly. **Algin,** a thickener used in many foods (such as ice cream and cake decorations), is extracted from their cell walls. Algin is also used in the production of a wide variety of nonfood goods, including rubber tires and hand lotion. The brown alga *Laminaria japonica* is used to induce vaginal dilation before surgical entry into the uterus through the vagina.

Most *red algae* have delicately branched thalli and can live at greater ocean depths than other algae (see Figure 12.12c). The thalli of a few red algae form crustlike coatings on rocks and shells. The red pigments enable red algae to absorb the blue light that penetrates deepest into the ocean. The agar used in microbiological media is extracted from many red algae. Another gelatinous material, carrageenan, comes from a species of red algae commonly called Irish moss. Carrageenan and agar can be a thickening ingredient in evaporated milk, ice cream, and pharmaceutical agents. *Gracilaria* species, which grow in the



(a) *Eunotia*, a freshwater diatom that grows in acidic water

(b) Asexual reproduction of a diatom

Figure 12.14 Diatoms. **(a)** In this micrograph of *Eunotia serra,* notice how the two parts of the cell wall fit together. **(b)** Asexual reproduction in a diatom. During mitosis, each daughter cell retains one-half of the cell wall from the parent (yellow) and must synthesize the remaining half (pink).

Q What human disease is caused by diatoms?

Pacific Ocean, are used by humans for food. However, some members of this genus can produce a lethal toxin.

Green algae have cellulose cell walls, contain chlorophyll *a* and *b,* and store starch, as plants do (see Figure 12.13a). Green algae are believed to have given rise to terrestrial plants. Most green algae are microscopic, although they may be either unicellular or multicellular. Some filamentous kinds form grass-green scum in ponds.

Diatoms, dinoflagellates, and water molds are grouped into the Kingdom Stramenopila. *Diatoms* (**Figure 12.14**) are unicellular or filamentous algae with complex cell walls that consist of pectin and a layer of silica. The two parts of the wall fit together like the halves of a Petri dish. The distinctive patterns of the walls are a useful tool in diatom identification. Diatoms store energy captured through photosynthesis in the form of oil.

The first reported outbreak of a neurological disease caused by diatoms was reported in 1987 in Canada. Affected people ate mussels that had been feeding on diatoms. The diatoms produced *domoic acid,* a toxin that was concentrated in the mussels. Symptoms occurred within 24 hours of eating and included diarrhea and memory loss. The fatality rate was less than 4%. Subsequent outbreaks showed that in some cases, the brain damage may be permanent. Since 1991, hundreds of marine birds and sea lions have died from the same **domoic acid toxicosis** in California.

Dinoflagellates are unicellular algae collectively called **plankton,** or free-floating organisms (**Figure 12.15**). Their rigid structure is due to cellulose embedded in the plasma membrane. Some dinoflagellates produce neurotoxins. In the last 20 years, a worldwide increase in toxic marine algae has killed millions of fish, hundreds of marine mammals, and even some humans. When fish swim through large numbers of the dinoflagellate *Karenia brevis* (kar-en-Ē-ah BREV-is), the algae trapped in the gills of the fish release a neurotoxin that stops the fish from breathing. Dinoflagellates in the genus *Alexandrium* (a′leks-AN-drē-um) produce neurotoxins (called **saxitoxins**) that cause **paralytic shellfish poisoning (PSP).** The toxin is concentrated when large numbers of dinoflagellates are eaten by mollusks, such as mussels or clams. Humans who eat these mollusks also develop PSP. Large concentrations of *Alexandrium* give the ocean a deep red color, from which the name **red tide** originates (Figure 27.10, page 782). Mollusks should not be harvested for consumption during a red tide. A disease called **ciguatera** occurs when the dinoflagellate *Gambierdiscus toxicus* (GAM-bē-er-dis-kus TOKS-i-kus) passes up the food chain and is concentrated in large fish. Ciguatera is endemic (constantly present) in the south Pacific Ocean and the Caribbean Sea. An emerging disease associated with heterotrophic *Pfiesteria* (fē-STEER-ē-ah) is responsible for periodic massive fish deaths along the Atlantic Coast.

Most *water molds,* or *Oomycota,* are decomposers. They form the cottony masses on dead algae and animals, usually in fresh water (**Figure 12.16**). Asexually, the oomycotes resemble the zygomycete fungi in that they produce spores in a sporangium (spore sac). However, oomycote spores, called **zoospores**

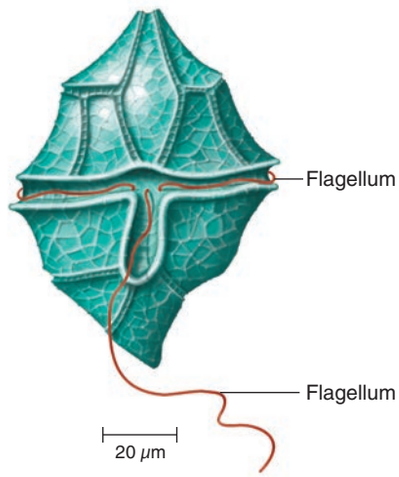


Figure 12.15 *Peridinium,* a dinoflagellate. Like all dinoflagellates, *Peridinium* has two flagella in perpendicular, opposing grooves. When the two flagella beat simultaneously, they cause the cell to spin.

Q What human diseases are caused by dinoflagellates?

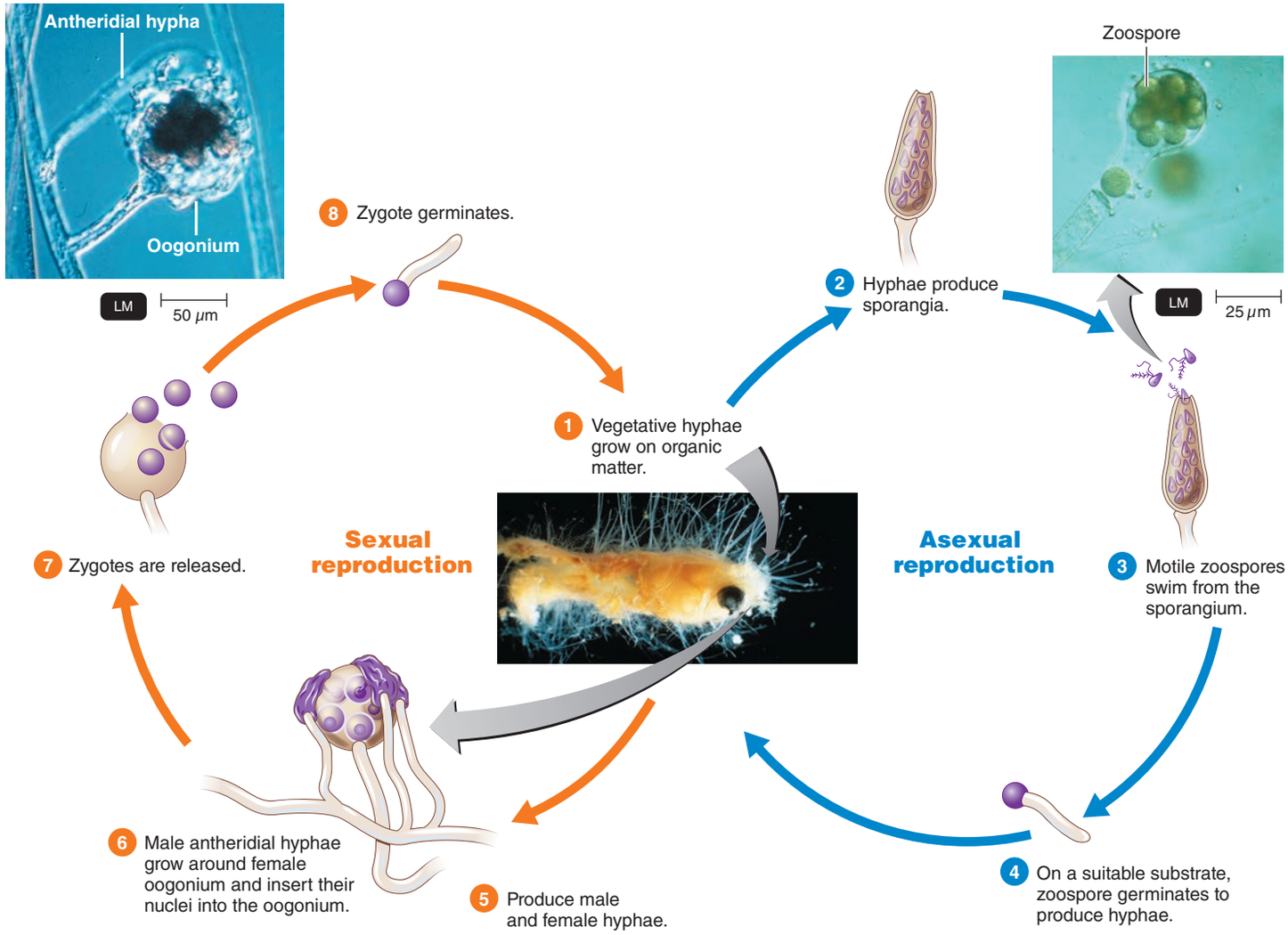


Figure 12.16 Oomycotes. These funguslike algae are common decomposers in fresh water. A few cause diseases of fish and terrestrial plants. Note the fuzzy mycelium of *Saprolegnia ferax* on the fish.

Q Is this oomycote more closely related to *Penicillium* or to diatoms?

(Figure 12.16, top right), have two flagella; fungi do not have flagella. Because of their superficial similarity to fungi, oomycotes were previously classified with the fungi. Their cellulose cell walls always raised the question about their relationship to algae, and recent DNA analyses have confirmed that oomycotes are more closely related to diatoms and dinoflagellates than to fungi. Many of the terrestrial oomycotes are plant parasites. The USDA inspects imported plants for white rust and other parasites. Often travelers, or even commercial plant importers, do not realize that one little blossom or seedling could carry a pest that is capable of causing millions of dollars' worth of damage to U.S. agriculture.

In Ireland during the mid-1800s, 1 million people died when the country's potato crop failed. The fungus that caused the great potato blight, *Phytophthora infestans* (fi-TOF-thor-ah in-FES-tans), was one of the first microorganisms to be associated with a disease. Today, *Phytophthora* infects soybeans, potatoes, and cocoa worldwide. Vegetative hyphae produce motile zoospores as well as specialized sex hyphae (see Figure 12.16). All of the U.S. strains were one mating type ("sex") called A1. In the 1990s, the other mating type, A2, was identified in the United States. When in close proximity, A1 and A2 will differentiate to produce haploid gametes that can mate to form a zygote. When the zygote germinates, the resulting alga will have genes from both parents.

In Australia, *P. cinnamoni* has infected about 20% of one species of *Eucalyptus* tree. *Phytophthora* was introduced into the United States in the 1990s and caused widespread damage to fruit and vegetable crops. When California oak trees suddenly started dying in 1995, University of California scientists identified the cause of this "sudden oak death" to be a new species, *P. ramorum*. *P. ramorum* also infects redwood trees.

Roles of Algae in Nature

Algae are an important part of any aquatic food chain because they fix carbon dioxide into organic molecules that can be consumed by chemoheterotrophs. Using the energy produced in photophosphorylation, algae convert carbon dioxide in the atmosphere into carbohydrates. Molecular oxygen (O_2) is a by-product of their photosynthesis. The top few meters of any body of water contain planktonic algae. As 75% of the Earth is covered with water, it is estimated that 80% of the Earth's O_2 is produced by planktonic algae.

Seasonal changes in nutrients, light, and temperature cause fluctuations in algal populations; periodic increases in numbers of planktonic algae are called **algal blooms.** Blooms of dinoflagellates are responsible for seasonal red tides. Blooms of a certain few species indicate that the water in which they grow is polluted because these algae thrive in high concentrations of organic materials that exist in sewage or industrial wastes. When algae die, the decomposition of the large numbers of cells associated with an algal bloom depletes the level of dissolved oxygen in the water. (This phenomenon is discussed in Chapter 27.)

Much of the world's petroleum was formed from diatoms and other planktonic organisms that lived several million years ago. When such organisms died and were buried by sediments, the organic molecules they contained did not decompose to be returned to the carbon cycle as CO_2. Heat and pressure resulting from the Earth's geologic movements altered the oil stored in the cells, as well as the cell membranes. Oxygen and other elements were eliminated, leaving a residue of hydrocarbons in the form of petroleum and natural gas deposits.

Many unicellular algae are symbionts in animals. The giant clam *Tridacna* (trī-DAK-nah) has evolved special organs that host dinoflagellates. As the clam sits in shallow water, the algae proliferate in these organs when they are exposed to the sun. The algae release glycerol into the clam's bloodstream, thus supplying the clam's carbohydrate requirement. In addition, evidence suggests that the clam gets essential proteins by phagocytizing old algae.

CHECK YOUR UNDERSTANDING

- How do algae differ from bacteria? From fungi? **12-7**
- List the cell wall composition and diseases caused by the following algae: diatoms, dinoflagellates, oomycotes. **12-8, 12-9**

Protozoa

LEARNING OBJECTIVES

12-10 List the defining characteristics of protozoa.

12-11 Describe the outstanding characteristics of the seven phyla of protozoa discussed in this chapter, and give an example of each.

12-12 Differentiate an intermediate host from a definitive host.

Protozoa are unicellular, eukaryotic organisms. Among the protozoa are many variations on this cell structure, as we shall see. Protozoa inhabit water and soil. The feeding and growing stage, or **trophozoite,** feeds upon bacteria and small particulate nutrients. Some protozoa are part of the normal microbiota of animals. Fire ants cause millions of dollars in agricultural damage each year and can cause painful stings. Researchers at the U.S. Department of Agriculture are studying an apicomplexan protozoan that reduces egg production by fire ants. Of the nearly 20,000 species of protozoa, relatively few cause human disease. Those few, however, have significant health and economic impact. Malaria is the fourth leading cause of death in children in Africa.

Characteristics of Protozoa

The term *protozoa* means "first animals," meant to describe animal-like nutrition. However, protozoa are quite different from animals—a few are photosynthetic, and many have complex life cycles that enable them to get from one host to the next. Protozoa are now classified into the same group of kingdoms as algae, based on DNA analyses (Table 12.4).

Life Cycle

Protozoa reproduce asexually by fission, budding, or schizogony. **Schizogony** is multiple fission; the nucleus undergoes multiple divisions before the cell divides. After many nuclei are formed, a small portion of cytoplasm concentrates around each nucleus, and then the single cell separates into daughter cells.

Sexual reproduction has been observed in some protozoa. The ciliates, such as *Paramecium,* reproduce sexually by **conjugation** (**Figure 12.17**), which is very different from the bacterial process of the same name (see Figure 8.27, page 229). During protozoan conjugation, two cells fuse, and a haploid nucleus (the micronucleus) from each cell migrates to the other cell. This haploid micronucleus fuses with the haploid micronucleus within the cell. The parent cells separate, each now a fertilized cell. When the cells later divide, they produce daughter cells with recombined DNA. Some protozoa produce **gametes (gametocytes),** which are haploid sex cells. During reproduction, two gametes fuse to form a diploid zygote.

Encystment Under certain adverse conditions, some protozoa produce a protective capsule called a **cyst.** A cyst permits the organism to survive when food, moisture, or oxygen are lacking, when temperatures are not suitable, or when toxic chemicals are present. A cyst also enables a parasitic species to survive outside a host. This is important because parasitic protozoa may have to be excreted from one host in order to get to a new host. The cyst formed in members of the phylum Apicomplexa is called an **oocyst.** It is a reproductive structure in which new cells are produced asexually.

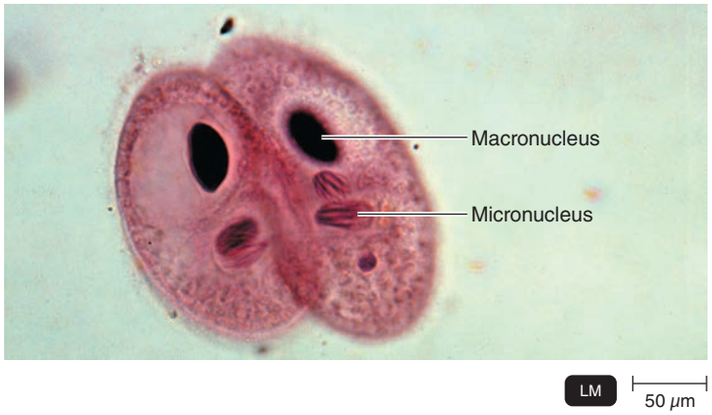


Figure 12.17 Conjugation in the ciliate protozoan *Paramecium*.
Sexual reproduction in ciliates is by conjugation. Each cell has two nuclei: a micronucleus and a macronucleus. The micronucleus is haploid and is specialized for conjugation. One micronucleus from each cell will migrate to the other cell during conjugation. Both cells will then go on to produce two daughter cells. Condensed chromosomes are visible in the micronuclei.

Q Does conjugation result in more cells?

Nutrition

Protozoa are mostly aerobic heterotrophs, although many intestinal protozoa are capable of anaerobic growth. Two chlorophyll-containing groups, dinoflagellates and euglenoids, are often studied with algae.

All protozoa live in areas with a large supply of water. Some protozoa transport food across the plasma membrane. However, some have a protective covering, or *pellicle,* and thus require specialized structures to take in food. Ciliates take in food by waving their cilia toward a mouthlike opening called a **cytostome.** Amebae engulf food by surrounding it with pseudopods and phagocytizing it. In all protozoa, digestion takes place in membrane-enclosed **vacuoles,** and waste may be eliminated through the plasma membrane or through a specialized **anal pore.**

Medically Important Protozoa

The biology of protozoa is discussed in this chapter. Diseases caused by protozoa are described in Part Four, Microorganisms and Human Disease.

Protozoa are a large and diverse group. Current schemes of classifying protozoan species into phyla are based on DNA data and morphology. As more information is obtained, some of the phyla discussed here may be grouped to form kingdoms.

Feeding Grooves

Single-celled eukaryotes with a feeding groove in the cytoskeleton have been placed in the Excavata. Most are spindle-shaped and possess flagella (Figure 12.18a). This superkingdom includes two phyla that lack mitochondria and the phylum Euglenozoa.

A parasite without mitochondria is *Giardia intestinalis* (JAR-dē-ah in′tes-tin-AL-is), sometimes called *G. lamblia* or *G. duodenalis.* The parasite (Figure 12.18b and Figure 25.16, page 733) is found in the small intestine of humans and other mammals. It is excreted in the feces as a cyst (Figure 12.18c) and survives in the environment before being ingested by the next host. Diagnosis of giardiasis, the disease caused by *G. intestinalis,* is often based on the identification of cysts in feces.

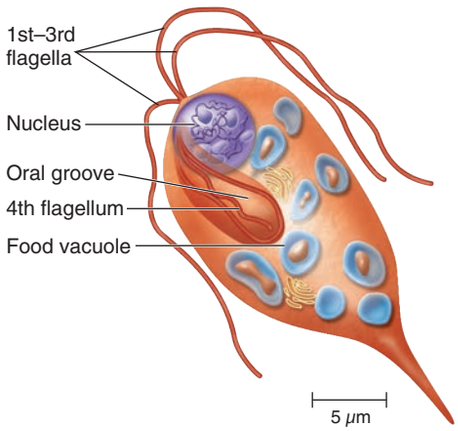
Another human parasite that lacks mitochondria is *Trichomonas vaginalis* (TRIK-ō-mō-nas VA-jin-al-is), shown in Figure 12.18d and Figure 26.16 on page 765. Like some other flagellates, *T. vaginalis* has an **undulating membrane,** which consists of a membrane bordered by a flagellum. *T. vaginalis* does not have a cyst stage and must be transferred from host to host quickly before desiccation occurs. *T. vaginalis* is found in the vagina and in the male urinary tract. It is usually transmitted by sexual intercourse but can also be transmitted by toilet facilities or towels.

Euglenozoa

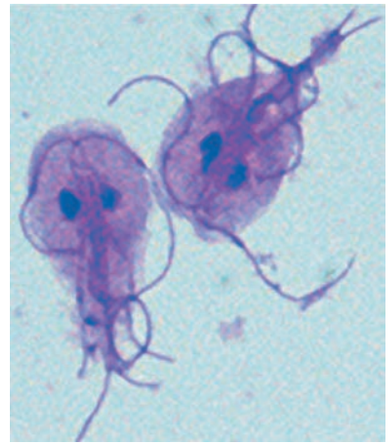
Two groups of flagellated cells are included in the **Euglenozoa** based on common rRNA sequences, disk-shaped mitochondria, and absence of sexual reproduction.

Euglenoids are photoautotrophs (Figure 12.18e). Euglenoids have a semirigid plasma membrane called a pellicle, and they move by means of a flagellum at the anterior end. Most euglenoids also have a red *eyespot* at the anterior end. This carotenoid-containing organelle senses light and directs the cell in the appropriate direction by using a *preemergent flagellum.* Some euglenoids are facultative chemoheterotrophs. In the dark, they ingest organic matter through a cytostome. Euglenoids are frequently studied with algae because they can photosynthesize.

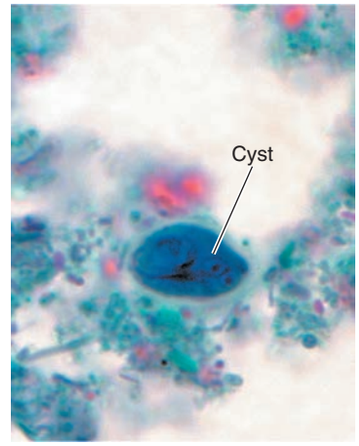
The **hemoflagellates** (blood parasites) are transmitted by the bites of blood-feeding insects and are found in the circulatory system of the bitten host. To survive in this viscous fluid, hemoflagellates usually have long, slender bodies and an undulating membrane. The genus *Trypanosoma* (TRI-pa-nō-sō-mah) includes the species that causes African sleeping sickness, *T. brucei* (BROOS-e-ē), which is transmitted by the tsetse fly. *T. cruzi* (KROOZ-ē; see Figure 23.22, page 662), the causative agent of Chagas' disease, is transmitted by the "kissing bug," so named because it bites on the face (see Figure 12.32d on page 352). After entering the insect, the trypanosome rapidly multiplies by schizogony. If the insect then defecates while biting a human, it can release trypanosomes that can contaminate the bite wound.



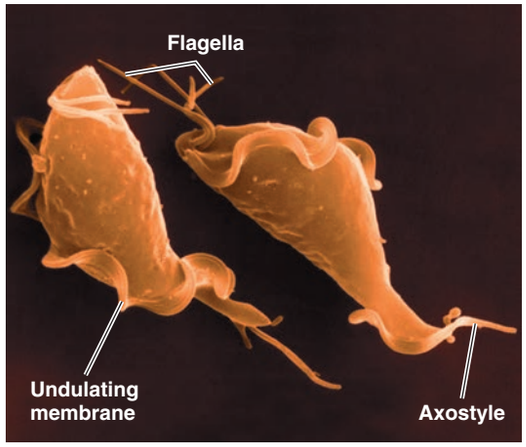
(a) *Chilomastix*. This flagellate, found in the human intestine, may be mildly pathogenic. The cysts can survive for months outside a human host. The fourth flagellum is used to move food into the oral groove, where food vacuoles are formed.



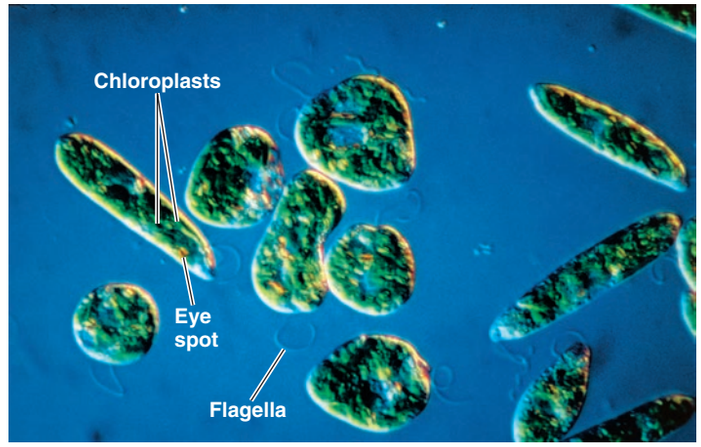
(b) *Giardia* trophozoites. The trophozoite of this intestinal parasite has eight flagella and two prominent nuclei, giving it a distinctive appearance. LM 7 μm



(c) *Giardia* cyst. The *Giardia* cyst provides protection from the environment before it is ingested by a new host. LM 10 μm



(d) *Trichomonas vaginalis*. This flagellate causes urinary and genital tract infections. Notice the small undulating membrane. This flagellate does not have a cyst stage. SEM 7.5 μm



(e) *Euglena*. Euglenoids are autotrophs. Semirigid rings supporting the pellicle allow *Euglena* to change shape. LM 20 μm

Figure 12.18 Members of the superkingdom Excavata.

How does *Giardia* obtain energy without mitochondria?

Amebae

The **amebae** move by extending blunt, lobelike projections of the cytoplasm called **pseudopods** (Figure 12.19a). Any number of pseudopods can flow from one side of the ameba, and the rest of the cell will flow toward the pseudopods.

Entamoeba histolytica (en-tah-MĒ-bah his-tō-LI-ti-kah) is the only pathogenic ameba found in the human intestine. As many as 10% of the human population may be colonized by this ameba. New techniques, including DNA analyses and lectin binding, have revealed that the ameba thought to be *E. histolytica* are actually two distinct species. The nonpathogenic species, *E. dispar* (DIS-par) is most common. The invasive *E. histolytica* (Figure 12.19b) causes amebic dysentery. In the human intestine, *E. histolytica* uses proteins called lectins to attach to the galactose of the plasma membrane and causes cell lysis. *E. dispar* does not have galactose-binding lectins. *Entamoeba* is transmitted between humans through ingestion of the cysts that are excreted in the feces of the infected person. *Acanthamoeba* (ah-kan-thah-MĒ-bah) growing in water, including tap water, can infect the cornea and cause blindness.

Since 1990, *Balamuthia* (bal-ah-MOO-thē-ah) has been reported as the cause of brain abscesses called granulomatous

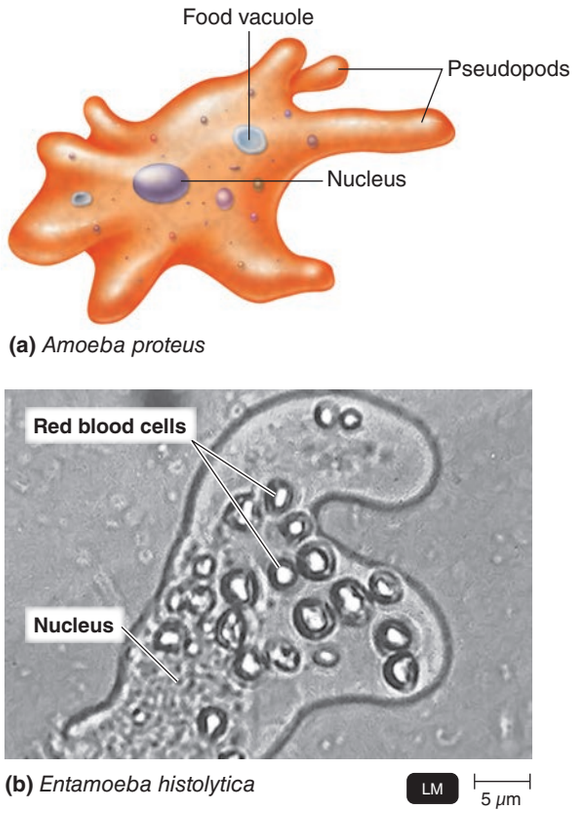


Figure 12.19 Amebae. **(a)** To move and to engulf food, amebae (such as this *Amoeba proteus*) extend cytoplasmic structures called pseudopods. Food vacuoles are created when pseudopods surround food and bring it into the cell. **(b)** *Entamoeba histolytica.* The presence of ingested red blood cells is diagnostic for *Entamoeba.*

Q How do amebic dysentery and bacillary dysentery differ?

amebic encephalitis in the United States and other countries. The ameba most often infects immunocompromised people. Like *Acanthamoeba, Balamuthia* is a free-living ameba found in water and is not transmitted from human to human.

Apicomplexa

The **Apicomplexa** are not motile in their mature forms and are obligate intracellular parasites. Apicomplexans are characterized by the presence of a complex of special organelles at the apexes (tips) of their cells (hence the phylum name). The organelles in these apical complexes contain enzymes that penetrate the host's tissues.

Apicomplexans have a complex life cycle that involves transmission between several hosts. An example of an apicomplexan is *Plasmodium* (plaz-MŌ-dē-um), the causative agent of malaria. Malaria affects 10% of the world's population, with 300 to 500 million new cases each year. The complex life cycle makes it difficult to develop a vaccine against malaria.

Plasmodium grows by sexual reproduction in the *Anopheles* (an-AH-fe-lēz) mosquito (**Figure 12.20**). When an *Anopheles* carrying the infective stage of *Plasmodium,* called a **sporozoite,** bites a human, sporozoites can be injected into the human. The sporozoites undergo schizogony in liver cells and produce thousands of progeny called **merozoites,** which infect red blood cells. The young trophozoite looks like a ring in which the nucleus and cytoplasm are visible. This is called a **ring stage** (see Figure 23.25b, page 665). The red blood cells eventually rupture and release more merozoites. Upon release of the merozoites, their waste products, which cause fever and chills, are also released. Most of the merozoites infect new red blood cells and perpetuate their cycle of asexual reproduction. However, some develop into male and female sexual forms (gametocytes). Even though the gametocytes themselves cause no further damage, they can be picked up by the bite of another *Anopheles* mosquito; they then enter the mosquito's intestine and begin their sexual cycle. Their progeny can then be injected into a new human host by the biting mosquito.

The mosquito is the **definitive host** because it harbors the sexually reproducing stage of *Plasmodium.* The host in which the parasite undergoes asexual reproduction (in this case, the human) is the **intermediate host.**

Malaria is diagnosed in the laboratory by microscopic observation of thick blood smears for the presence of *Plasmodium* (see Figure 23.25, page 665). A peculiar characteristic of malaria is that the interval between periods of fever caused by the release of merozoites is always the same for a given species of *Plasmodium* and is always a multiple of 24 hours. The reason and mechanism for such precision have intrigued scientists. After all, why should a parasite need a biological clock? *Plasmodium*'s development is regulated by the host's body temperature, which normally fluctuates over a 24-hour period. The parasite's careful timing ensures that gametocytes are mature at night, when *Anopheles* mosquitoes are feeding, and thereby facilitates transmission of the parasite to a new host.

Another apicomplexan parasite of red blood cells is *Babesia microti* (bah-BĒ-sē-ah mi-KRŌ-tē). *Babesia* causes fever and anemia in immunosuppressed individuals. In the United States, it is transmitted by the tick *Ixodes scapularis* (IKS-ō-des skap-ū-LAR-is).

Toxoplasma gondii (toks-ō-PLAZ-mah GON-dē-ē) is another apicomplexan intracellular parasite of humans. The life cycle of this parasite involves domestic cats. The trophozoites, called **tachyzoites,** reproduce sexually and asexually in an infected cat, and **oocysts,** each containing eight sporozoites, are excreted with feces. If the oocysts are ingested by humans or other animals, the sporozoites emerge as trophozoites, which can reproduce in the tissues of the new host (see Figure 23.23, page 663). *T. gondii* is dangerous to pregnant women because it can cause congenital infections in utero. Tissue examination and

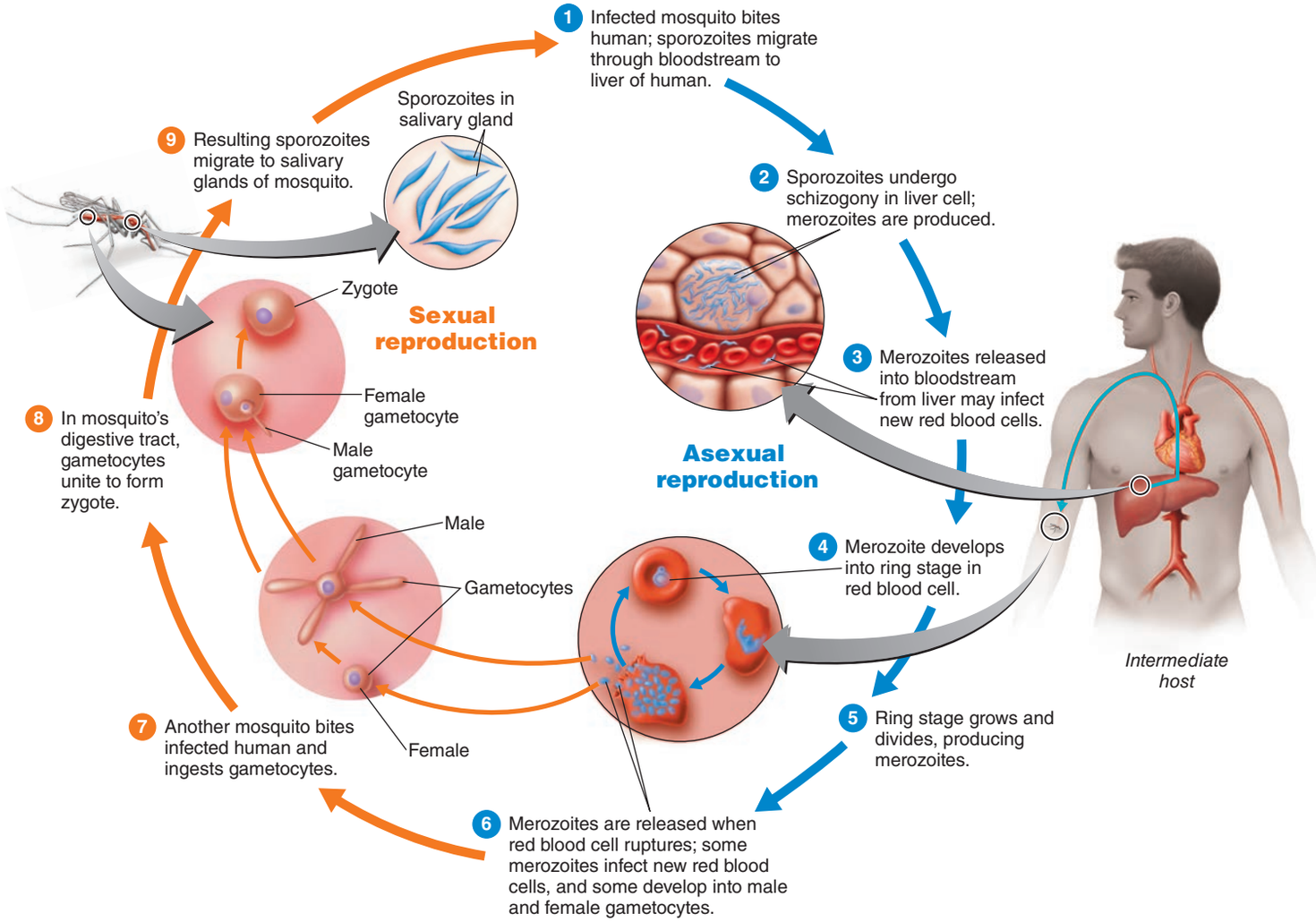


Figure 12.20 The life cycle of *Plasmodium vivax,* the apicomplexan that causes malaria. Asexual reproduction (schizogony) of the parasite takes place in the liver and in the red blood cells of a human host. Sexual reproduction occurs in the intestine of an *Anopheles* mosquito after the mosquito has ingested gametocytes.

Q What is the definitive host for *Plasmodium*?

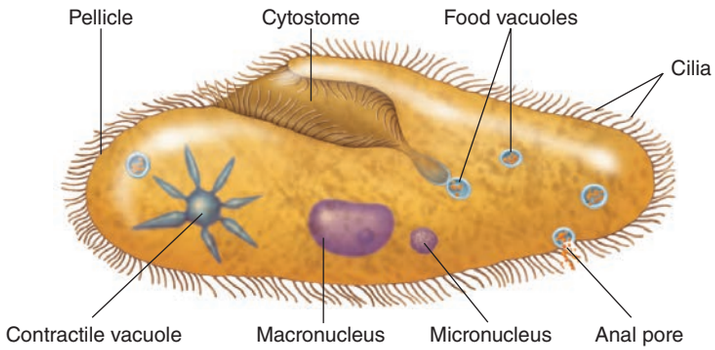
observation of *T. gondii* are used for diagnosis. Antibodies may be detected by ELISA and by indirect fluorescent-antibody tests (see Chapter 18).

Cryptosporidium (KRIP-tō-spor-i′dē-um) lives inside the cells lining the small intestine and can be transmitted to humans through the feces of cows, rodents, dogs, and cats. Inside the host cell, each *Cryptosporidium* organism forms four oocysts (see Figure 25.17, page 734), each containing four sporozoites. When the oocyst ruptures, sporozoites may infect new cells in the host or be released with the feces. See the Clinical Focus box on page 347.

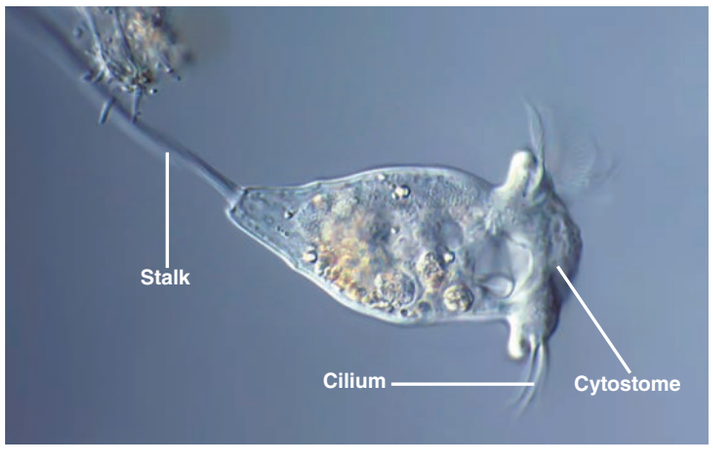
During the 1980s, epidemics of waterborne diarrhea were identified on every continent except Antarctica. The causative agent was misidentified as a cyanobacterium because the outbreaks occurred during warm months, and the disease agent looked like a prokaryotic cell. In 1993, the organism was identified as an apicomplexan similar to *Cryptosporidium.* In 2013, the new parasite, *Cyclospora cayetanensis* (sī-klō-SPOR-ah kī′ah-tan-EN-sis), was responsible for 600 cases of diarrhea associated with cilantro in the United States and Canada.

Ciliates

Ciliates have cilia that are similar to but shorter than flagella. The cilia are arranged in precise rows on the cell (**Figure 12.21**). They are moved in unison to propel the cell through its environment and to bring food particles to the mouth.



(a) *Paramecium*



(b) *Vorticella*

Figure 12.21 Ciliates. **(a)** *Paramecium* is covered with rows of cilia. It has specialized structures for ingestion (cytostome), elimination of wastes (anal pore), and the regulation of osmotic pressure (contractile vacuoles). The macronucleus is involved with protein synthesis and other ongoing cellular activities. The micronucleus functions in sexual reproduction. **(b)** *Vorticella* attaches to objects in water by the base of its stalk. The springlike stalk can expand, allowing *Vorticella* to feed in different areas. Cilia surround its cytostome.

Q What ciliate can cause disease in humans?

The only ciliate that is a human parasite is *Balantidium coli* (bal′an-TID-ē-um KŌ-lē), the causative agent of a severe, though rare, type of dysentery. When the host ingests cysts, they enter the large intestine, into which the trophozoites are released. The trophozoites produce proteases and other substances that destroy host cells. The trophozoite feeds on host cells and tissue fragments. Its cysts are excreted with feces.

Table 12.4 lists some typical parasitic protozoa and the diseases they cause.

CHECK YOUR UNDERSTANDING

- Identify three differences between protozoa and animals. **12-10**
- Do protozoa have mitochondria? **12-11**
- Where does *Plasmodium* undergo sexual reproduction? **12-12**

Slime Molds

LEARNING OBJECTIVE

12-13 Compare and contrast cellular slime molds and plasmodial slime molds.

Slime molds are closely related to amebae and are placed in the phylum Amoebozoa. There are two taxa of slime molds: cellular and plasmodial. **Cellular slime molds** are typical eukaryotic cells that resemble amebae. In the life cycle of cellular slime molds (Figure 12.22), the ameboid cells live and grow by ingesting fungi and bacteria by phagocytosis. Cellular slime molds are of interest to biologists who study cellular migration and aggregation, because when conditions are unfavorable, large numbers of ameboid cells aggregate to form a single structure. This aggregation occurs because some individual amebae produce the chemical cyclic AMP (cAMP), toward which the other amebae migrate. Some of the ameboid cells form a stalk; others swarm up the stalk to form a spore cap, and most of these differentiate into spores. When spores are released under favorable conditions, they germinate to form single amebae.

In 1973, a Dallas resident discovered a pulsating red blob in his backyard. The news media claimed that a "new life form" had been found. For some people, the "creature" evoked spine-chilling recollections of an old science fiction movie. Before imaginations got carried away too far, biologists calmed everyone's worst fears (or highest hopes). The amorphous mass was merely a plasmodial slime mold, they explained. But its unusually large size—46 cm in diameter—startled even scientists.

Plasmodial slime molds were first scientifically reported in 1729. A plasmodial slime mold exists as a mass of protoplasm with many nuclei (it is multinucleated). This mass of protoplasm is called a **plasmodium** (Figure 12.23). The entire plasmodium moves as a giant ameba; it engulfs organic debris and bacteria. Biologists have found that musclelike proteins forming microfilaments account for the movement of the plasmodium.

When plasmodial slime molds are grown in laboratories, a phenomenon called **cytoplasmic streaming** is observed, during which the protoplasm within the plasmodium moves and changes both its speed and direction so that the oxygen and nutrients are evenly distributed. The plasmodium continues to grow as long as there is enough food and moisture for it to thrive.

When either is in short supply, the plasmodium separates into many groups of protoplasm; each of these groups forms a stalked sporangium, in which haploid spores (a resistant, resting form of the slime mold) develop. When conditions improve, these spores germinate, fuse to form diploid cells, and develop into a multinucleated plasmodium.

TABLE 12.4 Some Representative Pathogenic Protozoa

Kingdom	Phylum	Human Pathogens	Distinguishing Features	Disease	Source of Human Infections	Page
Excavata	Diplomonads	*Giardia intestinalis*	Two nuclei, eight flagella	Giardial enteritis	Fecal contamination of drinking water	733
	Parabasalids	*Trichomonas vaginalis*	No encysting stage	Urethritis, vaginitis	Contact with vaginal-urethral discharge	765
	Euglenozoa	*Leishmania*	Flagellated form in sand fly; ovoid form in vertebrate host	Leishmaniasis	Sand fly bite (*Phlebotomus*)	667
		Naegleria fowleri	Flagellated and ameboid forms	Meningoencephalitis	Water (during swimming)	629
		Trypanosoma cruzi	Undulating membrane	Chagas' disease	*Triatoma* bite (kissing bug)	662
		T. brucei gambiense, T. b. rhodesiense		African trypanosomiasis	Tsetse fly bite	327
Not Ranked	Amoebozoa	*Acanthamoeba*	Pseudopods	Keratitis	Water	603
		Entamoeba histolytica, E. dispar		Amebic dysentery	Fecal contamination of drinking water	735
		Balamuthia		Encephalitis	Water	—
Chromalveolata	Apicomplexa	*Babesia microti*	Complex life cycles may require multiple hosts	Babesiosis	Domestic animals, ticks	668
		Cryptosporidium		Diarrhea	Water, humans, other animals	734
		Cyclospora		Diarrhea	Water	735
		Plasmodium		Malaria	*Anopheles* mosquito bite	664
		Toxoplasma gondii		Toxoplasmosis	Cats; beef; congenital	663
	Dinoflagellates	*Alexandrium, Pfiesteria*	Photosynthetic (most)	Paralytic shellfish poisoning; ciguatera	Ingestion of dinoflagellates in mollusks or fish	433
	Ciliates	*Balantidium coli*	Only parasitic ciliate of of humans	Balantidial dysentery	Fecal contamination of drinking water	—

CHECK YOUR UNDERSTANDING

Why are slime molds classified with amebae and not fungi? **12-13**

Helminths

LEARNING OBJECTIVES

12-14 List the distinguishing characteristics of parasitic helminths.

12-15 Provide a rationale for the elaborate life cycle of parasitic worms.

12-16 List the characteristics of the two classes of parasitic platyhelminths, and give an example of each.

12-17 Describe a parasitic infection in which humans serve as a definitive host, as an intermediate host, and as both.

12-18 List the characteristics of parasitic nematodes, and give an example of infective eggs and infective larvae.

12-19 Compare and contrast platyhelminths and nematodes.

A number of parasitic animals spend part or all of their lives in humans. Most of these animals belong to two phyla: Platyhelminthes (flatworms) and Nematoda (roundworms). These worms are commonly called **helminths.** There are also free-living species in these phyla, but we'll limit our discussion to the parasitic species. Diseases caused by parasitic worms are discussed in Part Four.

Characteristics of Helminths

Helminths are multicellular eukaryotic animals that generally possess digestive, circulatory, nervous, excretory, and reproductive systems. Parasitic helminths must be highly specialized to live inside their hosts. The following generalizations distinguish parasitic helminths from their free-living relatives:

1. *They may lack a digestive system.* They can absorb nutrients from the host's food, body fluids, and tissues.
2. *Their nervous system is reduced.* They do not need an extensive nervous system because they do not have to search for food or respond much to their environment. The environment within a host is fairly constant.

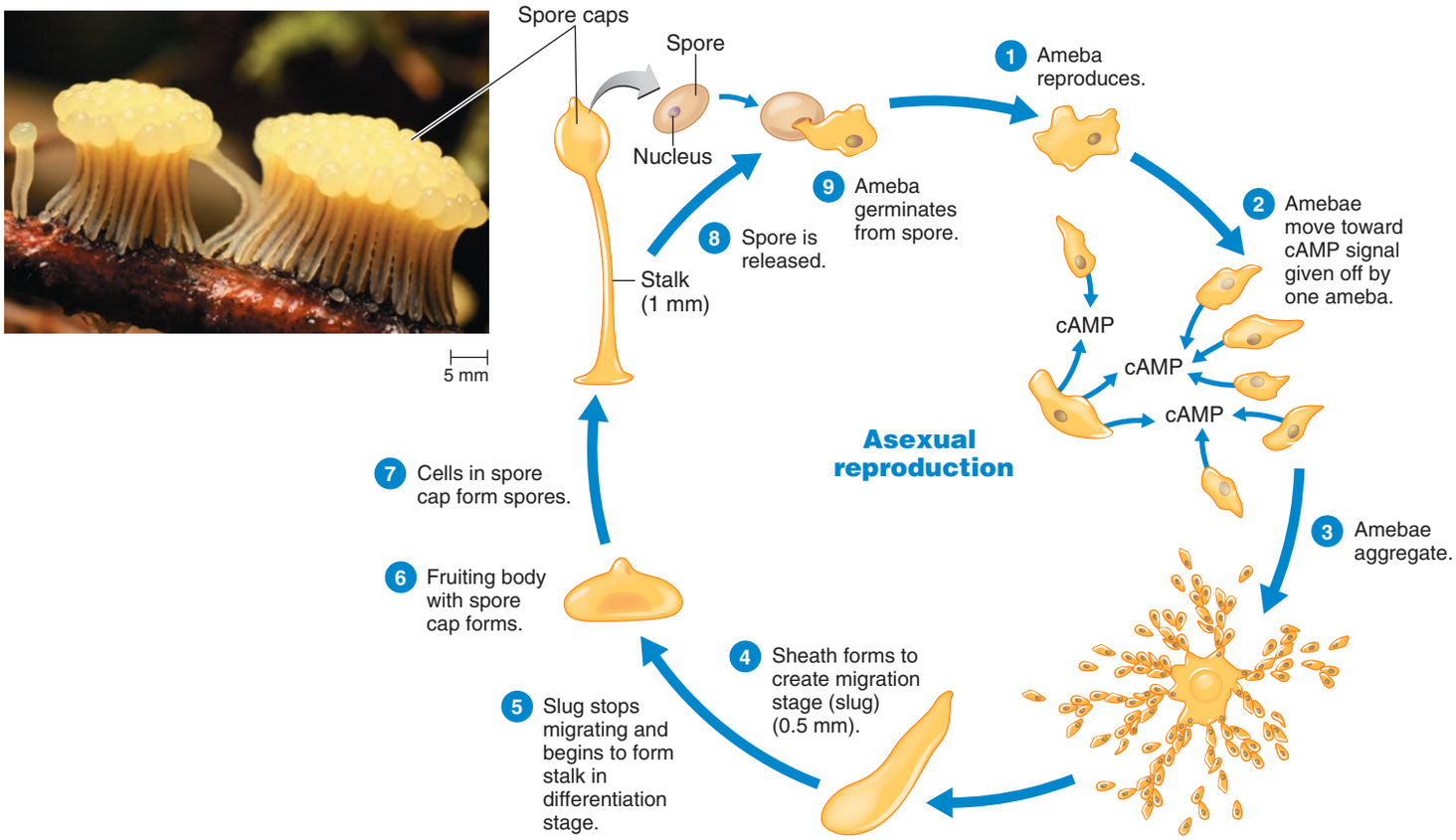


Figure 12.22 The generalized life cycle of a cellular slime mold. The micrograph shows a spore cap of *Dictyostelium*.

Q What characteristics do slime molds share with protozoa? With fungi?

3. *Their means of locomotion is occasionally reduced or completely lacking.* Because they are transferred from host to host, they don't need to search actively for a suitable habitat.
4. *The reproductive system is often complex.* Individuals produce large numbers of eggs, by which a suitable host is infected.

Life Cycle

The life cycle of parasitic helminths can be extremely complex, involving a succession of intermediate hosts for completion of each **larval** (developmental) stage of the parasite and a definitive host for the adult parasite.

Adult helminths may be **dioecious;** male reproductive organs are in one individual, and female reproductive organs are in another. In those species, reproduction occurs only when two adults of the opposite sex are in the same host.

Adult helminths may also be **monoecious,** or **hermaphroditic**—one animal has both male and female reproductive organs. Two hermaphrodites may copulate and simultaneously fertilize each other. A few types of hermaphrodites fertilize themselves.

CHECK YOUR UNDERSTANDING

- Why are drugs for parasitic helminths often toxic to the host? **12-14**
- Of what value is the complicated life cycle of parasitic helminths? **12-15**

Platyhelminths

Members of the phylum Platyhelminthes, the **flatworms,** are dorsoventrally flattened. The classes of parasitic flatworms include the trematodes and cestodes. These parasites cause disease or developmental disturbances in a wide variety of animals (**Figure 12.24**).

Trematodes

Trematodes, or **flukes,** often have flat, leaf-shaped bodies with a ventral sucker and an oral sucker (**Figure 12.25**). The suckers hold the organism in place. Flukes obtain food by absorbing it through their nonliving outer covering, called the **cuticle.**

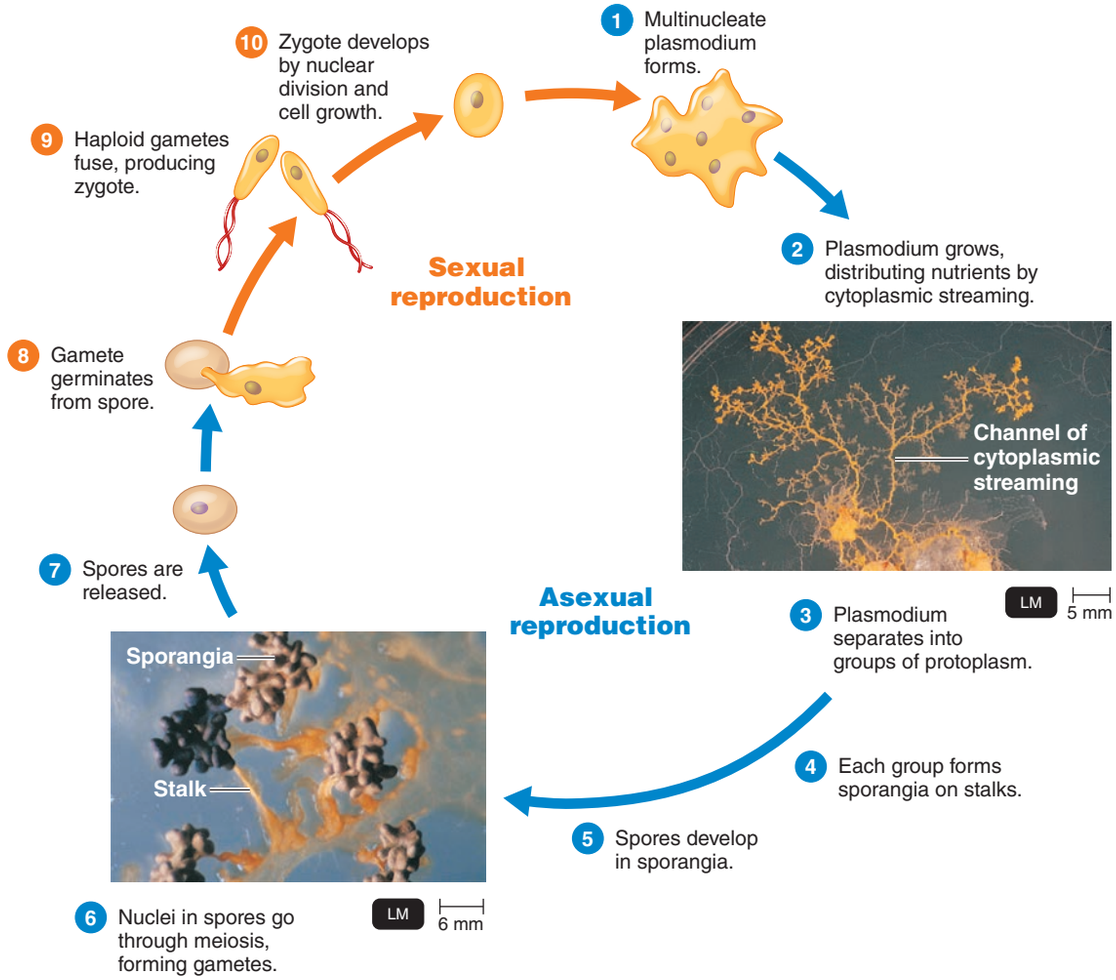


Figure 12.23 The life cycle of a plasmodial slime mold. *Physarum* is pictured in the photomicrographs.

Q How do cellular and acellular slime molds differ?

Flukes are given common names according to the tissue of the definitive host in which the adults live (for example, lung fluke, liver fluke, blood fluke). The Asian liver fluke *Clonorchis sinensis* (klon-OR-kis sin-EN-sis) is occasionally seen in immigrants in the United States, but it cannot be transmitted because its intermediate hosts are not in the United States.

To exemplify a fluke's life cycle, let's look at the lung fluke, *Paragonimus* spp. (par-ah-GŌ-nē-mus). *Paragonimus* species occur throughout the world. *P. kellicotti* (kel-li-KOT-tē), endemic in the United States, has been associated with eating raw crayfish on river raft trips. The adult lung fluke lives in the bronchioles of humans and other mammals and is approximately 6 mm wide and 12 mm long. The hermaphroditic adults liberate eggs into the bronchi. Because sputum that contains eggs is frequently swallowed, the eggs are usually excreted in feces of the definitive host. If the life cycle is to continue, the eggs must reach a body of water. A series of steps occurs that ensure adult flukes can mature in the lungs of a new host. The life cycle is shown in **Figure 12.26**.

In a laboratory diagnosis, sputum and feces are examined microscopically for fluke eggs. Infection results from eating

Figure 12.24 Infection by a parasitic platyhelminth. An increase in the trematode *Ribeiroia* in recent years has caused deformities in frogs. Frogs with multiple limbs have been found from Minnesota to California. Cercaria of the trematode infect tadpoles. The encysted metacercariae displace developing limb buds, causing abnormal limb development. The increase in the parasite may be due to fertilizer runoff that increases algae, which are food for the parasite's intermediate host snail.

Q What tailed stage of the parasite lives in a snail?

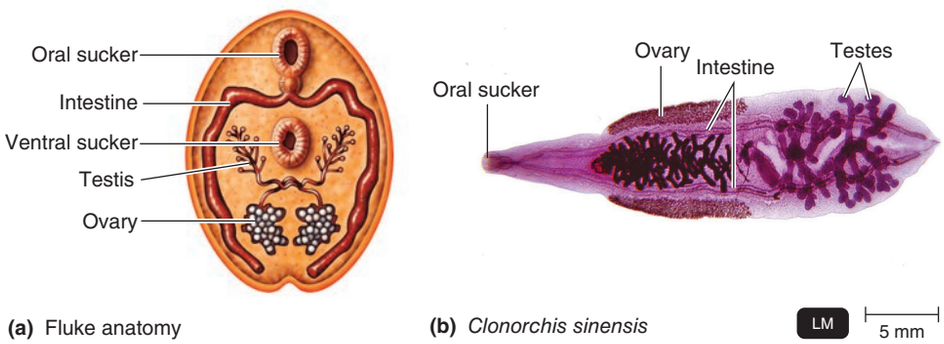


Figure 12.25 Flukes. **(a)** General anatomy of an adult fluke, shown in cross section. The oral and ventral suckers attach the fluke to the host. The mouth is located in the center of the oral sucker. Flukes are hermaphroditic; each animal contains both testes and ovaries. **(b)** The Asian liver fluke *Clonorchis sinensis*. Notice the incomplete digestive system. Heavy infestations may block bile ducts from the liver.

Q Why is the flatworm digestive system called "incomplete"?

undercooked freshwater crustaceans, and the disease can be prevented by thoroughly cooking crayfish and freshwater crabs.

The cercariae of the blood fluke *Schistosoma* (shis-tō-SŌ-mah) are not ingested. Instead, they burrow through the skin of the human host and enter the circulatory system. The adults are found in certain abdominal and pelvic veins. The disease schistosomiasis is a major world health problem; it will be discussed further in Chapter 23 (page 668).

Cestodes

Cestodes, or **tapeworms**, are intestinal parasites. Their structure is shown in Figure 12.27. The head, or **scolex** (plural: *scoleces*),

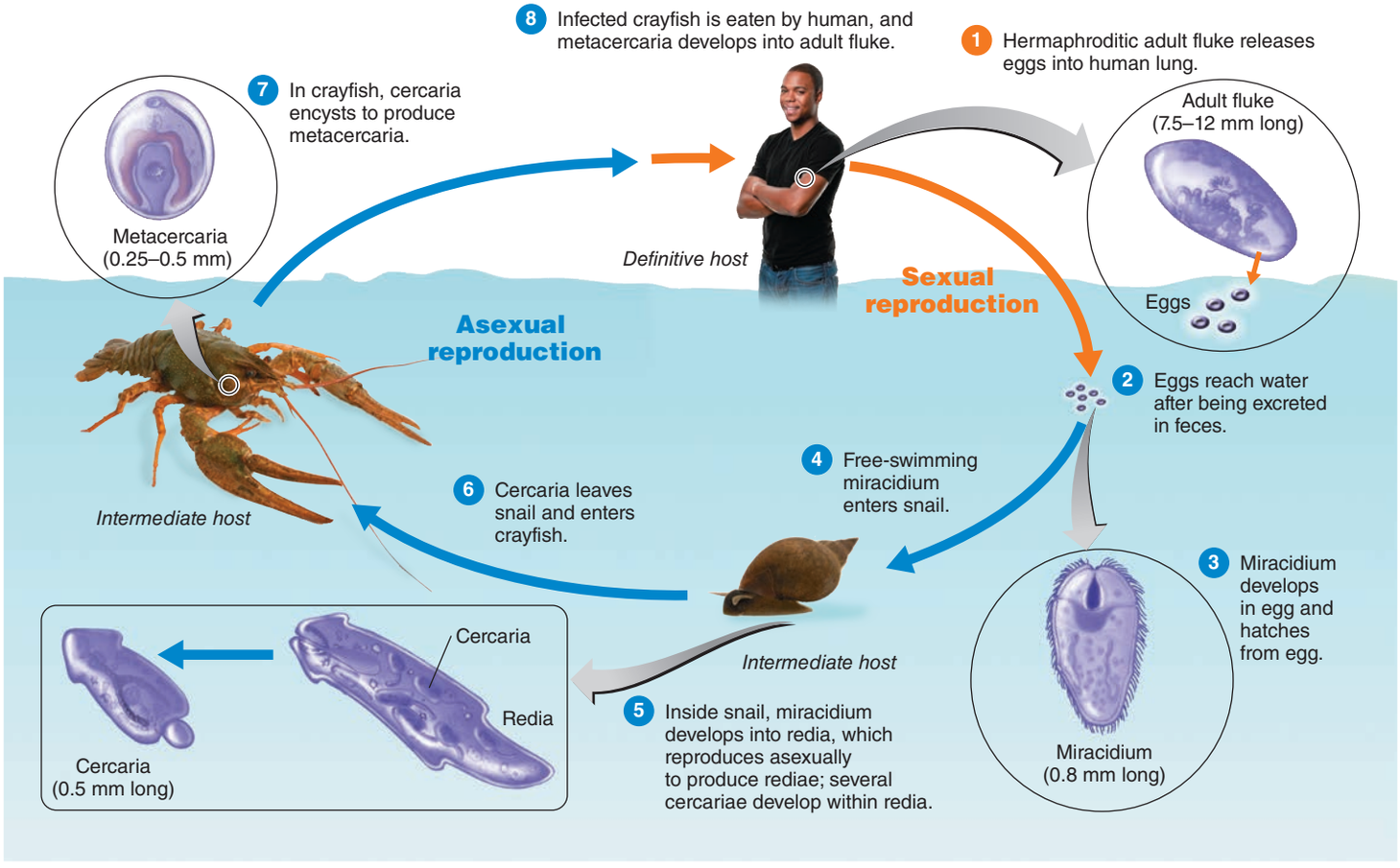


Figure 12.26 The life cycle of the lung fluke, *Paragonimus* spp. The trematode reproduces sexually in a human and asexually in a snail, its first intermediate host. Larvae encysted in the second intermediate host, freshwater crabs and crayfish, infect humans and other mammals when ingested. Also see the *Schistosoma* life cycle in Figure 23.27 (page 669).

Q Of what value is this complex life cycle to *Paragonimus*?

The Most Frequent Cause of Recreational Waterborne Diarrhea

As you read through this box, you'll encounter a series of questions that microbiologists ask themselves as they try to diagnose a disease. Try to answer each question before going on to the next one.

1. One week after her birthday party, 8-year-old Chloe had watery diarrhea, vomiting, and abdominal cramping. Her mother took her to the pediatrician because Chloe's symptoms weren't going away on their own.
   What diseases are possible? (*Hint:* See Diseases in Focus 25.2 on page 726.)
2. Possible diseases included giardiasis, cryptosporidiosis, *Cyclospora* diarrheal infection, and amebic dysentery. Chloe's pediatrician took a stool sample from Chloe and sent it to the lab for testing. The result of acid-fast staining of her stool is shown in **Figure A**.
   What is the disease?
3. The acid-fast staining stains the *Cryptosporidium* oocysts red, therefore making them easy to identify. In this case, the sporozoites are made visible inside the oocyst at the arrow. The oocysts are infectious immediately upon being excreted in feces.
   What else do you need to know?
4. Chloe's birthday party was held at a community water park. Chloe's mother immediately followed up with the parents of the other children who attended the party. She found out that the other 20 children also had watery diarrhea, vomiting, or abdominal cramps. All the children recovered from the infection 2 to 10 days after becoming ill.
   How is this disease transmitted?
5. *Cryptosporidium* infection is transmitted by the fecal–oral route. It results from ingesting *Cryptosporidium* oocysts through the consumption of fecally contaminated food or water or through direct person-to-person or animal-to-person contact. The infectious dose is low; feeding studies have demonstrated that ingesting as few as 10 to 30 oocysts can cause infection in healthy persons. Infected persons have been reported to shed 10^8 to 10^9 oocysts in a single bowel movement and to excrete oocysts for up to 50 days after cessation of diarrhea.

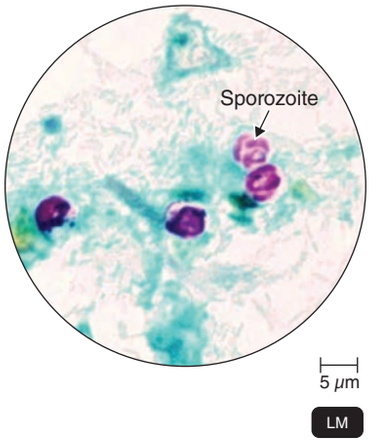


Figure A **Acid-fast stain of Chloe's feces.**

Cryptosporidium has emerged as the most frequently recognized cause of recreational water-associated outbreaks of gastroenteritis, even in venues with disinfected water. It became a reportable disease in 1994 (**Figure B**).

How Can *Cryptosporidium* Outbreaks Be Prevented?

Cryptosporidium species are known to be resistant to most chemical disinfectants, such as chlorine. Recommendations to reduce the risk of infection include the following:

- Do not swim during and for 2 weeks after diarrheal illness.
- Avoid swallowing pool water.
- Wash hands after using the restroom or changing diapers.

Source: Adapted from *MMWR* 61(19), 348–352, May 18, 2012.

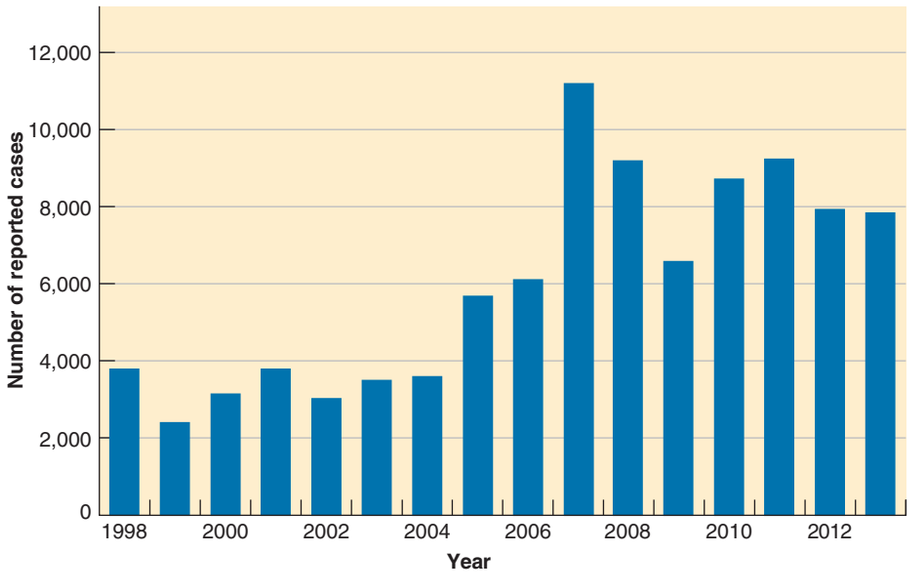


Figure B **Reported cases in the United States. CDC, 2014.**

has suckers for attaching to the intestinal mucosa of the definitive host; some species also have small hooks for attachment. Tapeworms do not ingest the tissues of their hosts; in fact, they completely lack a digestive system. To obtain nutrients from the small intestine, they absorb food through their cuticle. The body consists of segments called **proglottids.** Proglottids are continually produced by the neck region of the scolex, as long as the scolex is attached and alive. Each mature proglottid contains both male and female reproductive organs. The proglottids farthest away from the scolex are the mature ones containing eggs.

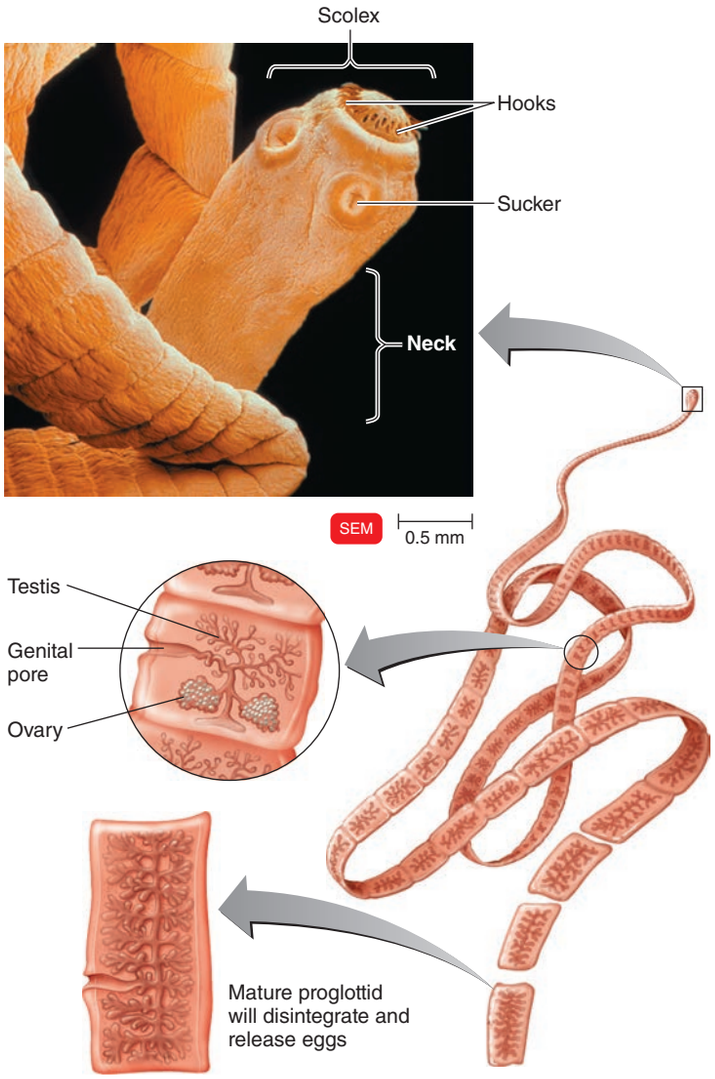


Figure 12.27 General anatomy of an adult tapeworm. The scolex, shown in the micrograph, consists of suckers and hooks that attach to the host's tissues. The body lengthens as new proglottids form at the neck. Each mature proglottid contains both testes and ovaries.

Q What are the similarities between tapeworms and flukes?

Mature proglottids are essentially bags of eggs, each of which is infective to the proper intermediate host.

Humans as Definitive Hosts The adults of *Taenia saginata* (TE-nē-ah sa-jin-AH-tah), the beef tapeworm, live in humans and can reach a length of 6 m. The scolex is about 2 mm long and is followed by a thousand or more proglottids. The feces of an infected human contain mature proglottids, each of which contains thousands of eggs. As the proglottids wriggle away from the fecal material, they increase their chances of being ingested by an animal that is grazing. Upon ingestion by cattle, the larvae hatch from the eggs and bore through the intestinal wall. The larvae migrate to muscle (meat), in which they encyst as **cysticerci.** When the cysticerci are ingested by humans, all but the scolex is digested. The scolex anchors itself in the small intestine and begins producing proglottids.

Diagnosis of tapeworm infection in humans is based on the presence of mature proglottids and eggs in feces. Cysticerci can be seen macroscopically in meat; their presence is referred to as "measly beef." Inspecting beef that is intended for human consumption for "measly" appearance is one way to prevent infections by beef tapeworm. Another method of prevention is to avoid the use of untreated human sewage as fertilizer in grazing pastures.

Humans are the only known definitive host of the pork tapeworm, *Taenia solium*. Adult worms living in the human intestine produce eggs, which are passed out in feces. When eggs are eaten by pigs, the larval helminth encysts in the pig's muscles; humans become infected when they eat undercooked pork. The human-pig-human cycle of *T. solium* is common in Latin America, Asia, and Africa. In the United States, however, *T. solium* is virtually nonexistent in pigs; the parasite is transmitted from human to human. Eggs shed by one person and ingested by another person hatch, and the larvae encyst in the brain and other parts of the body, causing cysticercosis (see Figure 25.20, page 736). The human hosting *T. solium*'s larvae is serving as an intermediate host. Approximately 7% of the few hundred cases reported in recent years were acquired by people who had never been outside the United States. They may have become infected through household contact with people who were born in or had traveled in other countries.

Humans as Intermediate Hosts Humans are the intermediate hosts for *Echinococcus granulosus* (ē-KĪ-nō-kok′kus gran-ū-LŌ-sus), shown in **Figure 12.28.** Dogs and coyotes are the definitive hosts for this minute (2–8 mm) tapeworm.

1. Eggs are excreted with feces.
2. Eggs are ingested by deer, sheep, or humans. Humans can also become infected by contaminating their hands with dog feces or saliva from a dog that has licked itself.
3. The eggs hatch in the human's small intestine, and the larvae migrate to the liver or lungs.
4. The larva develops into a **hydatid cyst.** The cyst contains "brood capsules," from which thousands of scoleces might be produced.
5. Humans are a dead-end for the parasite, but in the wild, the cysts might be in a deer that is eaten by a wolf.
6. The scoleces would be able to attach themselves in the wolf's intestine and produce proglottids.

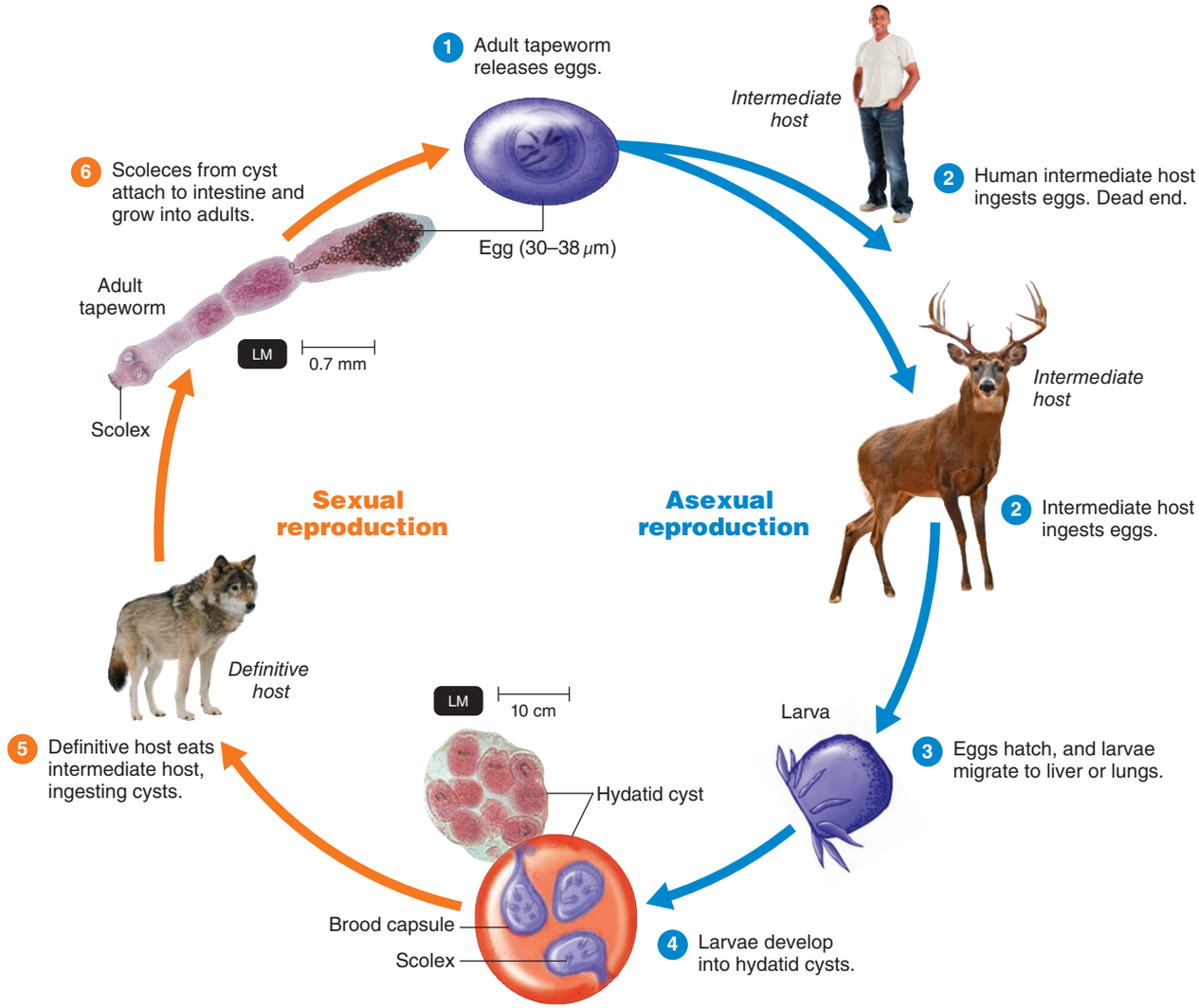


Figure 12.28 The life cycle of the tapeworm, *Echinococcus* spp. Dogs are the most common definitive host of *E. granulosus*. *E. multilocularis* infections in humans are rare. The parasite can complete its life cycle only if the cysts are ingested by a definitive host that eats the intermediate host.

Q Why isn't being in a human of benefit to *Echinococcus*?

Diagnosis of hydatid cysts is frequently made only on autopsy, although X rays can detect the cysts (see Figure 25.21, page 738).

CHECK YOUR UNDERSTANDING

Differentiate *Paragonimus* and *Taenia*. **12-16**

Nematodes

Members of the phylum Nematoda, the **roundworms,** are cylindrical and tapered at each end. Roundworms have a *complete* digestive system, consisting of a mouth, an intestine, and an anus. Most species are dioecious. Males are smaller than females and have one or two hardened **spicules** on their posterior ends. Spicules are used to guide sperm to the female's genital pore.

Some species of nematodes are free-living in soil and water, and others are parasites of plants and animals. Some nematodes pass their entire life cycle, from egg to mature adult, in a single host.

Intestinal roundworms are the most common causes of chronic infectious diseases. The most common are *Ascaris,* hookworms, and whipworms, infecting more than 2 billion people world wide. Nematode infections of humans can be divided into two categories: those in which the egg is infective, and those in which the larva is infective.

Eggs Infective for Humans

Ascaris lumbricoides (AS-kar-is lum-bri-KOY-dēz) is a large nematode (30 cm in length) that infects over 1 billion people worldwide

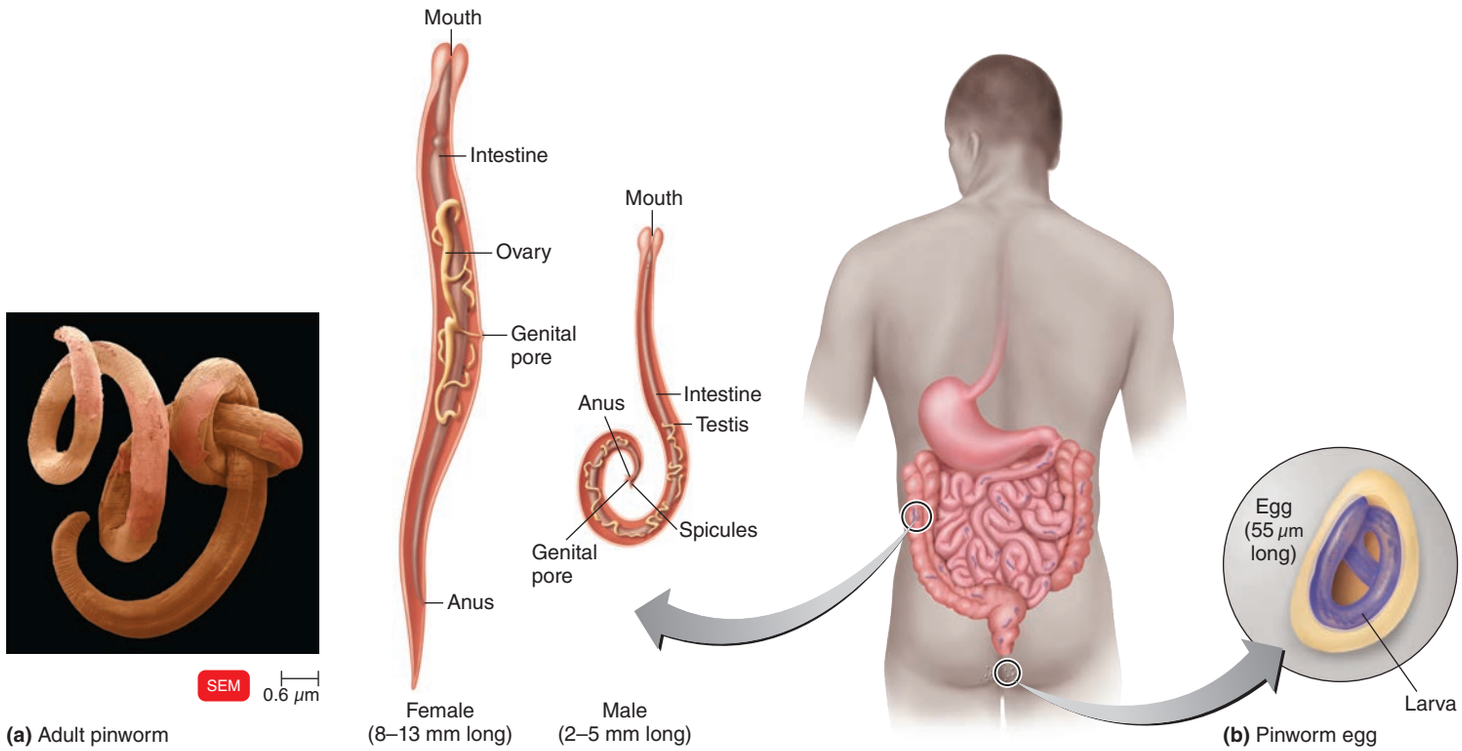


Figure 12.29 The pinworm, *Enterobius vermicularis*. **(a)** Adult pinworms live in the large intestine of humans. Most roundworms are dioecious, and the female (left and photomicrograph) is often distinctly larger than the male (right). **(b)** Pinworm eggs are deposited by the female on the perianal skin at night.

Q Are humans the definitive or intermediate host for pinworms?

(Figure 25.23, page 739). It is dioecious with **sexual dimorphism;** that is, the male and female worms look distinctly different, the male being smaller with a curled tail. The adult *Ascaris* lives in the small intestines of humans exclusively; it feeds primarily on semidigested food. Eggs, excreted with feces, can survive in the soil for long periods until accidentally ingested by another host. The eggs hatch in the small intestine of the host. The larvae then burrow out of the intestine and enter the blood. They are carried to the lungs, where they grow. The larvae will then be coughed up, swallowed, and returned to the small intestine, where they mature into adults.

Raccoon roundworm, *Baylisascaris procyonis,* is an emerging roundworm in North America. Raccoons are the definitive host, although the adult roundworm can also live in domestic dogs. Eggs are shed with feces and ingested by an intermediate host, usually a rabbit. The ingested eggs hatch in the intestines of rabbits and humans. The larvae migrate through a variety of tissues, causing a condition called *larva migrans*. Infection often results in severe neurological symptom or death. Larva migrans can also be caused by *Toxocara canis* (from dogs) and *T. cati* (from cats). These companion animals are the intermediate and definitive hosts, but humans can become infected by ingesting *Toxocara* eggs shed in the animals' feces. It is estimated that 14% of the U.S. population has been infected. Children are most likely to be infected probably because they play in soil and sandboxes, where animal feces can be found.

One billion people worldwide are infected with *Trichuris trichiura,* or whipworm. The worms are spread from person to person by fecal–oral transmission or through feces-contaminated food. The disease occurs most often in areas with tropical weather and poor sanitation practices and among children.

The pinworm *Enterobius vermicularis* (en-te-RŌ-bē-us ver-mi-kū-LAR-is) spends its entire life in a human host (**Figure 12.29**). Adult pinworms are found in the large intestine. From there, the female pinworm migrates to the anus to deposit her eggs on the perianal skin. The eggs can be ingested by the host or by another person exposed through contaminated clothing or bedding.

Larvae Infective for Humans

A few nematode larvae live in soil and can enter a human host directly through the skin. *Strongyloides* (stron-gel-OI-dēz) nematodes are endemic in the southeastern United States and Europe, and the CDC recently reported a reemergence of

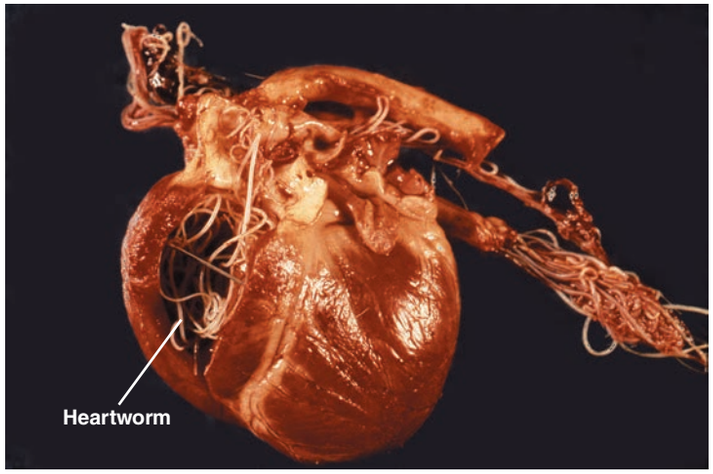


Figure 12.30 The heartworm, *Dirofilaria immitis*. Four adult *D. immitis* in the right ventricle of a dog's heart. Each worm is 12–30 cm long.

Q How do roundworms and flatworms differ?

infections. Most infections are limited to a rash where the nematode entered, but the larvae can migrate to the intestine, causing abdominal pain, or to the lungs, causing a cough.

Adult hookworms, *Necator americanus* (ne-KĀ-tor ah-mer-i-KĀ-nus) and *Ancylostoma duodenale* (an′sil-ō-STŌ-mah DOO-ah-den-al-ē), live in the small intestine of humans (Figure 25.22, page 738); the eggs are excreted in feces. The larvae hatch in the soil, where they feed on bacteria. A larva enters its host by penetrating the host's skin. It then enters a blood or lymph vessel, which carries it to the lungs. It is coughed up in sputum, swallowed, and finally carried to the small intestine.

Trichinellosis is caused by a nematode that the host acquires by eating encysted larvae in undercooked meat of infected animals (see page 740). The nematode, *Dirofilaria immitis* (dir-ō-fil-AIR-ē-ah IM-mi-tis), is spread from host to host through the bites of *Aedes* mosquitoes. It primarily affects dogs and cats, but it can infest human skin, conjunctiva, or lungs. Larvae injected by the mosquito migrate to various organs, where they mature into adults. The parasitic worm is called a **heartworm** because the adult stage is often in the animal host's heart, where it can kill its host through congestive heart failure (**Figure 12.30**). The disease occurs on every continent except Antarctica. *Wolbachia* bacteria appear to be essential to development of the worm embryos (see the box in Chapter 11 on page 297).

Four genera of roundworms called *anisakines,* or wriggly worms, can be transmitted to humans from infected fish and squid. Anisakine larvae are in the fish's intestinal mesenteries and migrate to the muscle when the fish dies. Freezing or thorough cooking will kill the larvae.

Table 12.5 on page 352 lists representative parasitic helminths of each phylum and class and the diseases they cause.

CHECK YOUR UNDERSTANDING

- What is the definitive host for *Enterobius*? **12-17**
- What stage of *Dirofilaria immitis* is infectious for dogs and cats? **12-18**
- You find a parasitic worm in a baby's diapers. How would you know whether it's a *Taenia* or a *Necator*? **12-19**

Arthropods as Vectors

LEARNING OBJECTIVES

12-20 Define *arthropod vector*.

12-21 Differentiate a tick from a mosquito, and name a disease transmitted by each.

Arthropods are animals characterized by segmented bodies, hard external skeletons, and jointed legs. With nearly 1 million species, this is the largest phylum in the animal kingdom. Although arthropods are not microbes themselves, we will briefly describe them here because a few suck the blood of humans and other animals and can transmit microbial diseases while doing so. Arthropods that carry pathogenic microorganisms are called **vectors.** Scabies and pediculosis are diseases that are caused by arthropods (see Chapter 21, pages 597–598).

Representative classes of arthropods include the following:

- Arachnida (eight legs): spiders, mites, ticks
- Crustacea (four antennae): crabs, crayfish
- Insecta (six legs): bees, flies, lice

Table 12.6 lists those arthropods that are important vectors, and **Figures 12.31** and **12.32** illustrate some of them. These insects and ticks reside on an animal only when they are feeding. An exception to this is the louse, which spends its entire life on its host and cannot survive for long away from a host.

(a) Mosquito

(b) Tick

Figure 12.31 Blood-sucking mosquitoes and ticks. Mosquitoes are the vector for several pathogens to humans, including yellow fever, malaria, and West Nile virus. Ticks are the vector for lyme disease.

Q When is a vector also a definitive host?

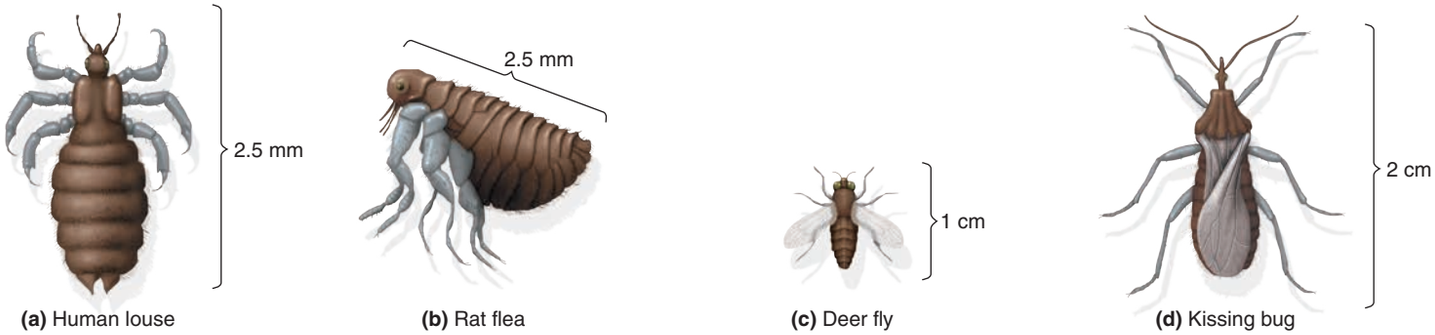


Figure 12.32 Arthropod vectors. **(a)** The human louse, *Pediculus*. **(b)** The rat flea, *Xenopsylla*. **(c)** The deer fly, *Chrysops*. **(d)** The kissing bug, *Triatoma*.

Q Name one pathogen carried by each of these vectors.

TABLE 12.5 Representative Parasitic Helminths

Phylum	Class	Human Parasites	Intermediate Host	Definitive Host Site	Stage Passed to Humans; Method	Disease	Figure Reference
Platyhelminthes	**Trematodes**	*Paragonimus*	Freshwater snails and crayfish	Humans; lungs	Metacercaria in crustaceans; ingested	Paragonimiasis (lung fluke)	12.26
		Schistosoma	Freshwater snails	Humans	Cercariae; through skin	Schistosomiasis	23.27 23.28
	Cestodes	*Echinococcus granulosus*	Humans	Dogs and other animals; intestines	Eggs from other animals; ingested	Hydatidosis	12.28 25.21
		Taenia saginata	Cattle	Humans; small intestine	Cysticerci in beef; ingested	Tapeworm	—
		Taenia solium	Humans; pigs	Humans	Eggs; ingested	Neurocysticercosis	25.20
Nematoda		*Ancylostoma duodenale*	—	Humans; small intestine	Larvae; through skin	Hookworm	25.22
		Anisakines	Marine fish and squid	Marine mammals	Larvae in fish; ingested	Anisakiasis (sashimi worms)	—
		Ascaris lumbricoides	—	Humans; small intestine	Eggs; ingested	Ascariasis	25.23
		Baylisascaris procyonis	Rabbits	Raccoons; large intestine	Eggs; ingested	Raccoon roundworm	—
		Enterobius vermicularis	—	Humans; large intestine	Eggs; ingested	Pinworm	12.29
		Necator americanus	—	Humans; small intestine	Larvae; through skin	Hookworm	—
		Strongyloides stercoralis	Soil	Humans	Larvae; through skin	Strongyloidiasis	—
		Trichinella spiralis	Humans and other mammals	Humans small intestine	Eggs; ingested	Trichinellosis	25.25
		Trichuris trichiura	—	Humans, pigs, and other mammals; small intestine	Larvae; ingested	Whipworm	25.24
		Toxocara canis, T. cati	Dogs, cats	Dogs, cats; small instestine	Eggs; ingested	Toxocariasis	—

TABLE 12.6 Important Arthropod Vectors of Human Diseases

Class	Order	Vector	Disease	Figure Reference
Arachnida	Mites and ticks	*Dermacentor* (tick)	Rocky Mountain spotted fever	—
		Ixodes (tick)	Lyme disease, babesiosis, ehrlichiosis	12.31
		Ornithodorus (tick)	Relapsing fever	—
Insecta	Sucking lice	*Pediculus* (human louse)	Epidemic typhus, relapsing fever	12.32a
	Fleas	*Xenopsylla* (rat flea)	Endemic murine typhus, plague	12.32b
	True flies	*Chrysops* (deer fly)	Tularemia	12.32c
		Aedes (mosquito)	Dengue fever, yellow fever, heartworm	12.31
		Anopheles (mosquito)	Malaria	—
		Culex (mosquito)	Arboviral encephalitis	—
		Glossina (tsetse fly)	African trypanosomiasis	—
	True bugs	*Triatoma* (kissing bug)	Chagas' disease	12.32d

Some vectors are just a mechanical means of transport for a pathogen. For example, houseflies lay their eggs on decaying organic matter, such as feces. While doing so, a housefly can pick up a pathogen on its feet or body and transport the pathogen to our food.

Some parasites multiply in their vectors. When this happens, the parasites can accumulate in the vector's feces or saliva. Large numbers of parasites can then be deposited on or in the host while the vector is feeding there. The spirochete that causes Lyme disease is transmitted by ticks in this manner (see Chapter 23, page 625), and the West Nile virus is transmitted in the same way by mosquitoes (see Chapter 22, page 625).

As discussed earlier, *Plasmodium* is an example of a parasite that requires that its vector also be the definitive host. *Plasmodium* can sexually reproduce only in the gut of an *Anopheles* mosquito. *Plasmodium* is introduced into a human host with the mosquito's saliva, which acts as an anticoagulant that keeps blood flowing.

To eliminate vectorborne diseases, health workers focus on eradicating the vectors.

CHECK YOUR UNDERSTANDING

- Vectors can be divided into three major types, according to the roles they play for the parasite. List the three types of vectors and a disease transmitted by each. **12-20**
- Assume you see an arthropod on your arm. How will you determine whether it is a tick or a flea? **12-21**

Study Outline

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Study microbiology where it matters: everywhere.
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Fungi (pp. 320–331)

1. Mycology is the study of fungi.
2. The number of serious fungal infections is increasing.
3. Fungi are aerobic or facultatively anaerobic chemoheterotrophs.
4. Most fungi are decomposers, and a few are parasites of plants and animals.

Characteristics of Fungi (pp. 321–325)

5. A fungal thallus consists of filaments of cells called hyphae; a mass of hyphae is called a mycelium.
6. Yeasts are unicellular fungi. To reproduce, fission yeasts divide symmetrically, whereas budding yeasts divide asymmetrically.
7. Buds that do not separate from the parent cell form pseudohyphae.
8. Pathogenic dimorphic fungi are yeastlike at 37°C and moldlike at 25°C.
9. Fungi are classified according to rRNA.
10. Sporangiospores and conidiospores are produced asexually.
11. Sexual spores are usually produced in response to special circumstances, often changes in the environment.
12. Fungi can grow in acidic, low-moisture, aerobic environments.
13. They are able to metabolize complex carbohydrates.

Medically Important Fungi (pp. 326–328)

14. The Zygomycota have coenocytic hyphae and produce sporangiospores and zygospores.
15. Microsporidia lack mitochondria and microtubules; they cause diarrhea in AIDS patients.

16. The Ascomycota have septate hyphae and produce ascospores and frequently conidiospores.
17. Basidiomycota have septate hyphae and produce basidiospores; some produce conidiospores.
18. Teleomorphic fungi produce sexual and asexual spores; anamorphic fungi produce asexual spores only.

Fungal Diseases (pp. 328–330)

19. Systemic mycoses are fungal infections deep within the body that affect many tissues and organs.
20. Subcutaneous mycoses are fungal infections beneath the skin.
21. Cutaneous mycoses affect keratin-containing tissues such as hair, nails, and skin.
22. Superficial mycoses are localized on hair shafts and superficial skin cells.
23. Opportunistic mycoses are caused by fungi that are not usually pathogenic.
24. Opportunistic mycoses can infect any tissues. However, they are usually systemic.

Economic Effects of Fungi (pp. 330–331)

25. *Saccharomyces* and *Trichoderma* are used in the production of foods.
26. Fungi are used for the biological control of pests.
27. Mold spoilage of fruits, grains, and vegetables is more common than bacterial spoilage of these products.
28. Many fungi cause diseases in plants.

Lichens (pp. 331–332)

1. A lichen is a mutualistic combination of an alga (or a cyanobacterium) and a fungus.
2. The alga photosynthesizes, providing carbohydrates for the lichen; the fungus provides a holdfast.
3. Lichens colonize habitats that are unsuitable for either the alga or the fungus alone.
4. Lichens may be classified on the basis of morphology as crustose, foliose, or fruticose.

Algae (pp. 332–337)

1. Algae are unicellular, filamentous, or multicellular (thallic).
2. Most algae live in aquatic environments.

Characteristics of Algae (pp. 332–334)

3. Algae are eukaryotic; most are photoautotrophs.
4. The thallus of multicellular algae usually consists of a stipe, a holdfast, and blades.
5. Algae reproduce asexually by cell division and fragmentation.
6. Many algae reproduce sexually.
7. Photoautotrophic algae produce oxygen.
8. Algae are classified according to their structures and pigments.

Selected Phyla of Algae (pp. 334–336)

9. Brown algae (kelp) may be harvested for algin.
10. Red algae grow deeper in the ocean than other algae.
11. Green algae have cellulose and chlorophyll *a* and *b* and store starch.
12. Diatoms are unicellular and have pectin and silica cell walls; some produce a neurotoxin.
13. Dinoflagellates produce neurotoxins that cause paralytic shellfish poisoning and ciguatera.
14. The oomycotes are heterotrophic; they include decomposers and pathogens.

Roles of Algae in Nature (p. 337)

15. Algae are the primary producers in aquatic food chains.
16. Planktonic algae produce most of the molecular oxygen in the Earth's atmosphere.
17. Petroleum is the fossil remains of planktonic algae.
18. Unicellular algae are symbionts in such animals as *Tridacna.*

Protozoa (pp. 337–342)

1. Protozoa are unicellular, eukaryotic chemoheterotrophs.
2. Protozoa are found in soil and water and as normal microbiota in animals.

Characteristics of Protozoa (pp. 337–338)

3. The vegetative form is called a trophozoite.
4. Asexual reproduction is by fission, budding, or schizogony.
5. Sexual reproduction is by conjugation.
6. During ciliate conjugation, two haploid nuclei fuse to produce a zygote.
7. Some protozoa can produce a cyst that provides protection during adverse environmental conditions.
8. Protozoa have complex cells with a pellicle, a cytostome, and an anal pore.

Medically Important Protozoa (pp. 338–342)

9. *Trichomonas* and *Giardia* lack mitochondria and have flagella.
10. Euglenozoa move by means of flagella and lack sexual reproduction; they include *Trypanosoma.*
11. Amebae include *Entamoeba* and *Acanthamoeba.*
12. Apicomplexa have apical organelles for penetrating host tissue; they include *Plasmodium* and *Cryptosporidium.*
13. Ciliates move by means of cilia; *Balantidium coli* is the human parasitic ciliate.

Slime Molds (pp. 342–343)

1. Cellular slime molds resemble amebae and ingest bacteria by phagocytosis.
2. Plasmodial slime molds consist of a multinucleated mass of protoplasm that engulfs organic debris and bacteria as it moves.

Helminths (pp. 343–351)

1. Parasitic flatworms belong to the phylum Platyhelminthes.
2. Parasitic roundworms belong to the phylum Nematoda.

Characteristics of Helminths (pp. 343–344)

3. Helminths are multicellular animals; a few are parasites of humans.
4. The anatomy and life cycle of parasitic helminths are modified for parasitism.

5. The adult stage of a parasitic helminth is found in the definitive host.
6. Each larval stage of a parasitic helminth requires an intermediate host.
7. Helminths can be monoecious or dioecious.

Platyhelminths (pp. 344–349)

8. Flatworms are dorsoventrally flattened animals; parasitic flatworms may lack a digestive system.
9. Adult trematodes, or flukes, have an oral and ventral sucker with which they attach to host tissue.
10. Eggs of trematodes hatch into free-swimming miracidia that enter the first intermediate host; two generations of rediae develop; the rediae become cercariae that bore out of the first intermediate host and penetrate the second intermediate host; cercariae encyst as metacercariae; the metacercariae develop into adults in the definitive host.
11. A cestode, or tapeworm, consists of a scolex (head) and proglottids.
12. Humans serve as the definitive host for the beef tapeworm, and cattle are the intermediate host.
13. Humans serve as the definitive host and can be an intermediate host for the pork tapeworm.
14. Humans serve as the intermediate host for *Echinococcus granulosus;* the definitive hosts are dogs, wolves, and foxes.

Nematodes (pp. 349–351)

15. Roundworms have a complete digestive system.
16. The nematodes that infect humans with their eggs include *Ascaris, Trichuris,* and *Enterobius.*
17. The nematodes that infect humans with their larvae include hookworms and *Trichinella*.

Arthropods as Vectors (pp. 351–353)

1. Jointed-legged animals, including ticks and insects, belong to the phylum Arthropoda.
2. Arthropods that carry diseases are called vectors.
3. Vectorborne diseases are most effectively eliminated by controlling or eradicating the vectors.

Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. Following is a list of fungi, their methods of entry into the body, and sites of infections they cause. Categorize each type of mycosis as cutaneous, opportunistic, subcutaneous, superficial, or systemic.

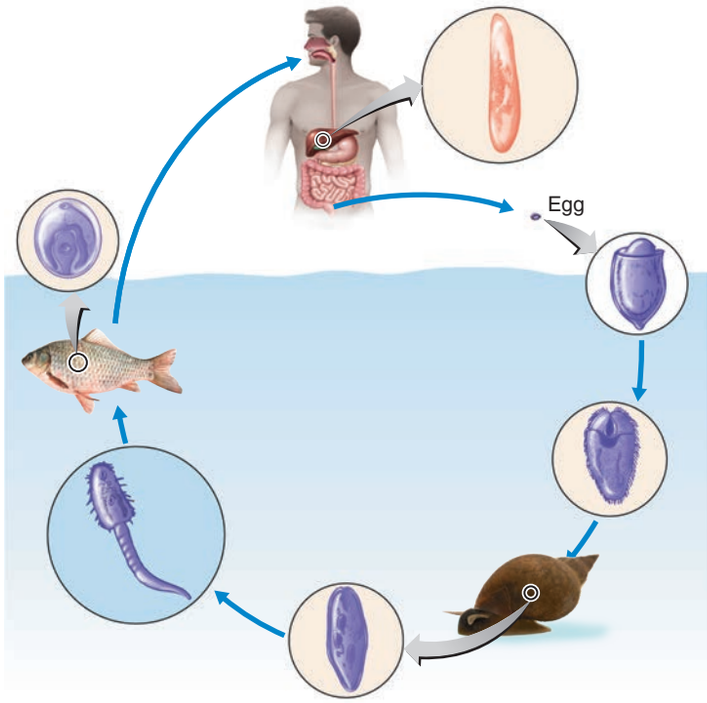
Genus	Method of Entry	Site of Infection	Mycosis
Blastomyces	Inhalation	Lungs	(a) _____
Sporothrix	Puncture	Ulcerative lesions	(b) _____
Microsporum	Contact	Fingernails	(c) _____
Trichosporon	Contact	Hair shafts	(d) _____
Aspergillus	Inhalation	Lungs	(e) _____

2. A mixed culture of *Escherichia coli* and *Penicillium chrysogenum* is inoculated onto the following culture media. On which medium would you expect each to grow? Why?
   a. 0.5% peptone in tap water
   b. 10% glucose in tap water
3. NAME IT Identify the structures of this eukaryote, which has an affinity for keratin.

4. Briefly discuss the importance of lichens in nature. Briefly discuss the importance of algae in nature.
5. Differentiate cellular and plasmodial slime molds. How does each survive adverse environmental conditions?
6. Complete the following table.

Phylum	Method of Motility	One Human Parasite
Diplomonads	(a) _____	(b) _____
Microsporidia	(c) _____	(d) _____
Amebae	(e) _____	(f) _____
Apicomplexa	(g) _____	(h) _____
Ciliates	(i) _____	(j) _____
Euglenozoa	(k) _____	(l) _____
Parabasalids	(m) _____	(n) _____

7. Why is it significant that *Trichomonas* does not have a cyst stage? Name a protozoan parasite that does have a cyst stage.
8. By what means are helminthic parasites transmitted to humans?
9. Most nematodes are dioecious. What does this term mean? To what phylum do nematodes belong?
10. DRAW IT A generalized life cycle of the liver fluke *Clonorchis sinensis* is shown below. Label the fluke's stages. Identify the intermediate host(s). Identify the definitive host(s). To what phylum and class does this animal belong?



Multiple Choice

1. How many phyla are represented in the following list of organisms: *Echinococcus*, *Cyclospora*, *Aspergillus*, *Taenia*, *Toxoplasma*, *Trichinella*?
   a. 1
   b. 2
   c. 3
   d. 4
   e. 5

Use the following choices to answer questions 2 and 3:
(1) metacercaria
(2) redia
(3) adult
(4) miracidium
(5) cercaria

2. Put the above stages in order of development, beginning with the egg.
   a. 5, 4, 1, 2, 3
   b. 4, 2, 5, 1, 3
   c. 2, 5, 4, 3, 1
   d. 3, 4, 5, 1, 2
   e. 2, 4, 5, 1, 3
3. If a snail is the first intermediate host of a parasite with these stages, which stage would be found in the snail?
   a. 1
   b. 2
   c. 3
   d. 4
   e. 5
4. Fleas are the intermediate host for *Dipylidium caninum* tapeworm, and dogs are the definitive host. Which stage of the parasite could be found in the flea?
   a. cysticerus larva
   b. proglottids
   c. scolex
   d. adult
5. Which of the following statements about yeasts are true?
   1. Yeasts are fungi.
   2. Yeasts can form pseudohyphae.
   3. Yeasts reproduce asexually by budding.
   4. Yeasts are facultatively anaerobic.
   5. All yeasts are pathogenic.
   6. All yeasts are dimorphic.
   a. 1, 2, 3, 4
   b. 3, 4, 5, 6
   c. 2, 3, 4, 5
   d. 1, 3, 5, 6
   e. 2, 3, 4
6. Which of the following events follows cell fusion in an ascomycete?
   a. conidiophore formation
   b. conidiospore germination
   c. ascus opening
   d. ascospore formation
   e. conidiospore release
7. The definitive host for *Plasmodium vivax* is
   a. human.
   b. *Anopheles.*
   c. a sporocyte.
   d. a gametocyte.

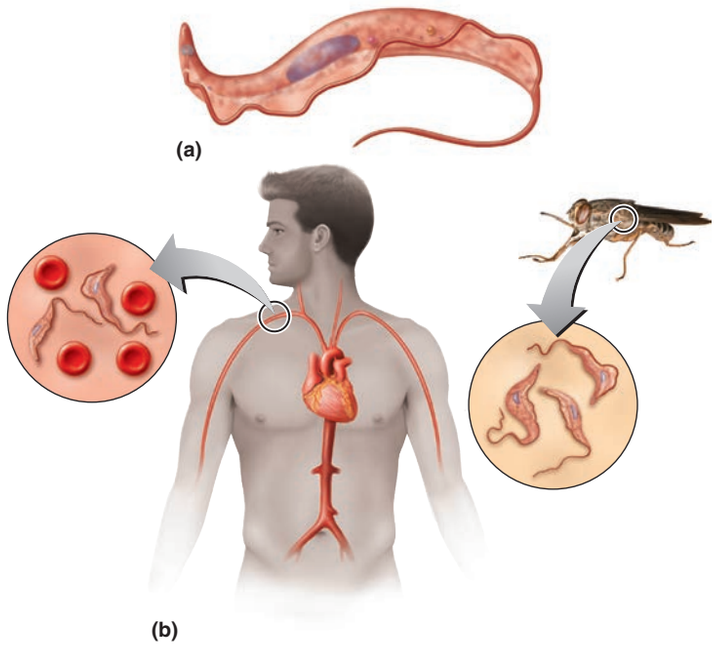
Use the following choices to answer questions 8–10:
a. Apicomplexa
b. ciliates
c. dinoflagellates
d. Microsporidia

8. These are obligate intracellular parasites that lack mitochondria.
9. These are nonmotile parasites with special organelles for penetrating host tissue.
10. These photosynthetic organisms can cause paralytic shellfish poisoning.

Analysis

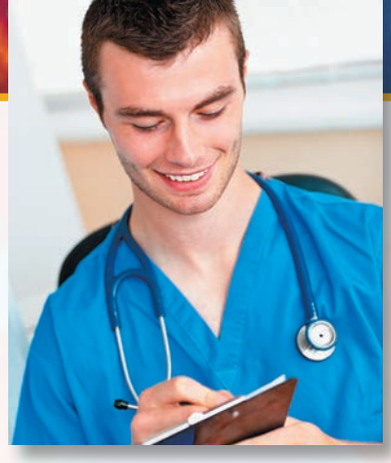
1. *Alexandrium* (red tide) has been called a plant, protist, protozoan, and alga in the past. Now it's in the Kingdom Chromalveolata, along with *Plasmodium* and *Paramecium*. Are all Chromalveolata photosynthetic? Are any? Explain why it has been so difficult to accurately classify *Alexandrium*.
2. The life cycle of the fish tapeworm *Diphyllobothrium* is similar to that of *Taenia saginata,* except that the intermediate host is fish. Describe the life cycle and method of transmission to humans. Why are freshwater fish more likely to be a source of tapeworm infection than marine fish?

3. *Trypanosoma brucei gambiense*—part (a) in the figure—is the causative agent of African sleeping sickness. To what phylum does it belong? Part (b) shows a simplified life cycle for *T. b. gambiense.* Identify the host and vector of this parasite.



Clinical Applications and Evaluation

1. A girl developed generalized seizures. A CT scan revealed a single brain lesion consistent with a tumor. Biopsy of the lesion showed a cysticercus. The patient lived in South Carolina and had never traveled outside the state. What parasite caused her disease? How is this disease transmitted? How might it be prevented?
2. A California farmer developed a low-grade fever, myalgia, and cough. A chest X-ray exam revealed an infiltrate in the lung. Microscopic examination of the sputum revealed round, budding cells. A sputum culture grew mycelia and arthroconidia. What organism is most likely the cause of the symptoms? How is this disease transmitted? How might it be prevented?
3. A teenaged male in California complained of remittent fever, chills, and headaches. A blood smear revealed ring-shaped cells in his red blood cells. He was successfully treated with primaquine and chloroquine. The patient lives near the San Luis Rey River and has no history of foreign travel, blood transfusion, or intravenous drug use. What is the disease? How was it acquired?



In the Clinic

A woman brings her 8-month-old daughter into the urgent care clinic where you work as a nurse practitioner. The baby has a runny nose and a fever of 39°C. The child's ears and lungs are free of infection. The woman is annoyed because she has already taken her daughter to see her pediatrician. She requested an antibiotic, but the doctor refused to prescribe it. She once again requests that the baby receive antibiotics. **Will you prescribe the medication the mother wants? Explain why or why not—and how you would discuss this with the mother.**

Hint: Read about virus structure on page 360 and multiplication of animal viruses on page 373.

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

13 Viruses, Viroids, and Prions

Viruses are too small to be seen with a light microscope and can't be cultured outside their hosts. Therefore, although viral diseases aren't new, the viruses themselves couldn't be studied until the twentieth century. In 1886, the Dutch chemist Adolf Mayer showed that tobacco mosaic disease (TMD) was transmissible from a diseased plant to a healthy plant. In 1892, in an attempt to isolate the cause of TMD, the Russian bacteriologist Dimitri Iwanowski filtered the sap of diseased plants through a porcelain filter that was designed to retain bacteria. He expected to find the microbe trapped in the filter; instead, he found that the infectious agent had passed through the minute pores of the filter. When he infected healthy plants with the filtered fluid, they contracted TMD. The first human disease associated with a filterable agent was yellow fever.

Advances in molecular biological techniques in the 1980s and 1990s led to the recognition of several new viruses, including human immunodeficiency virus (HIV) and SARS-associated coronavirus. Viral hepatitis is one of the most common infectious diseases in the world. Several different hepatitis viruses have been identified, including the bloodborne hepatitis B virus (shown in the photo) and hepatitis C virus, and the foodborne hepatitis A virus discussed in the Clinical Case. Human diseases caused by viruses will be discussed in Part Four. In this chapter, we'll study the biology of viruses.

Hepatitis B viruses.

General Characteristics of Viruses

LEARNING OBJECTIVE

13-1 Differentiate a virus from a bacterium.

One hundred years ago, researchers couldn't imagine submicroscopic particles, so they described the infectious agent as *contagium vivum fluidum*—a contagious fluid. By the 1930s, scientists had begun using the word *virus,* the Latin word for poison, to describe these filterable agents. The nature of viruses, however, remained elusive until 1935, when Wendell Stanley, an American chemist, isolated tobacco mosaic virus, making it possible for the first time to carry out chemical and structural studies on a purified virus. At about the same time, the invention of the electron microscope made it possible to see viruses.

The question of whether viruses are living organisms has an ambiguous answer. Life can be defined as a complex set of processes resulting from the actions of proteins specified by nucleic acids. The nucleic acids of living cells are in action all the time. Because viruses are inert outside living host cells, in this sense they aren't considered to be living organisms. However, once viruses enter a host cell, the viral nucleic acids become active, and viral multiplication results. In this sense, viruses are alive when they multiply in the host cells they infect. From a clinical point of view, viruses are alive because they cause infection and disease, just as pathogenic bacteria, fungi, and protozoa do. Depending on one's viewpoint, a virus may be regarded as an exceptionally complex aggregation of nonliving chemicals, or as an exceptionally simple living microorganism.

How, then, do we define *virus*? Viruses were originally distinguished from other infectious agents because they are especially small (filterable) and because they are **obligatory intracellular parasites**—that is, they absolutely require living host cells in order to multiply. However, both of these properties are shared by certain small bacteria, such as some rickettsias. Viruses and bacteria are compared in Table 13.1.

The truly distinctive features of viruses are now known to relate to their simple structural organization and their mechanism of multiplication. Accordingly, **viruses** are entities that

- Contain a single type of nucleic acid, either DNA or RNA.
- Contain a protein coat (sometimes itself enclosed by an envelope of lipids, proteins, and carbohydrates) that surrounds the nucleic acid.
- Multiply inside living cells by using the synthesizing machinery of the cell.
- Cause the synthesis of specialized structures that can transfer the viral nucleic acid to other cells.

Viruses have few or no enzymes of their own for metabolism; for example, they lack enzymes for protein synthesis and ATP generation. To multiply, viruses must take over the metabolic machinery of the host cell. This fact has considerable medical significance for the development of antiviral drugs, because most drugs that would interfere with viral multiplication would also interfere with the functioning of the host cell and therefore are too toxic for clinical use. (Antiviral drugs are discussed in Chapter 20.)

TABLE 13.1 Viruses and Bacteria Compared

	Bacteria		
	Typical Bacteria	Rickettsias/ Chlamydias	Viruses
Intracellular Parasite	No	Yes	Yes
Plasma Membrane	Yes	Yes	No
Binary Fission	Yes	Yes	No
Pass through Bacteriological Filters	No	No/Yes	Yes
Possess Both DNA and RNA	Yes	Yes	No
ATP-Generating Metabolism	Yes	Yes/No	No
Ribosomes	Yes	Yes	No
Sensitive to Antibiotics	Yes	Yes	No
Sensitive to Interferon	No	No	Yes

Host Range

The **host range** of a virus is the spectrum of host cells the virus can infect. There are viruses that infect invertebrates, vertebrates, plants, protists, fungi, and bacteria. However, most viruses are able to infect specific types of cells of only one host species. In rare cases, viruses cross the host-species barrier, thus expanding

Clinical Case: An Inconvenient Outbreak

Tina Markham, a 42-year-old pharmaceutical sales representative, has been home from work because of a very high, persistent fever (40°C). She is taking medications to reduce the fever, but they work only for a few hours. Tina makes an appointment to see her physician; he notices right away that Tina's skin is jaundiced. When he palpates her abdomen, she winces in pain; it is very tender. Sensing an issue with her liver, Tina's physician sends a blood sample to the local laboratory for a liver function test (LFT). The results show abnormal findings.

What disease could be causing Tina's symptoms? Read on to find out.

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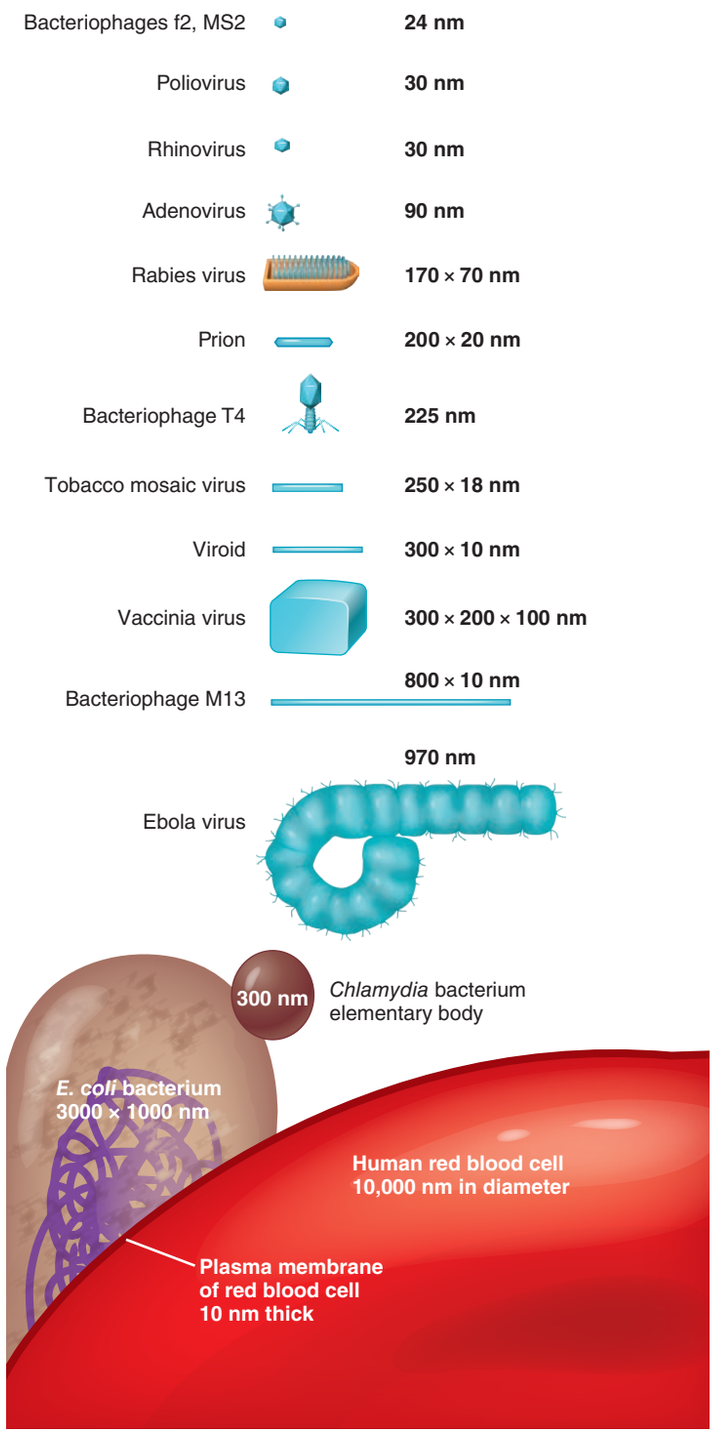


Figure 13.1 Virus sizes. The sizes of several viruses (teal blue) and bacteria (brown) are compared with a human red blood cell, shown below the microbes. Dimensions are given in nanometers (nm) and are either diameters or length by width.

Q How do viruses differ from bacteria?

their host range. An example is described in the Clinical Focus box on page 364. In this chapter, we're concerned mainly with viruses that infect either humans or bacteria. Viruses that infect bacteria are called **bacteriophages,** or **phages.**

The particular host range of a virus is determined by the virus's requirements for its specific attachment to the host cell and the availability within the potential host of cellular factors required for viral multiplication. For the virus to infect the host cell, the outer surface of the virus must chemically interact with specific receptor sites on the surface of the cell. The two complementary components are held together by weak bonds, such as hydrogen bonds. The combination of many attachment and receptor sites leads to a strong association between host cell and virus. For some bacteriophages, the receptor site is part of the cell wall of the host; in other cases, it is part of the fimbriae or flagella. For animal viruses, the receptor sites are on the plasma membranes of the host cells.

The potential to use viruses to treat diseases is intriguing because of their narrow host range and their ability to kill their host cells. The idea of *phage therapy*—using bacteriophages to treat bacterial infections—has been around for 100 years. Recent advances in our understanding of virus-host interactions have fueled new studies in the field of phage therapy.

Experimentally induced viral infections in cancer patients during the 1920s suggested that viruses might have antitumor activity. These tumor-destroying, or *oncolytic,* viruses may selectively infect and kill tumor cells or cause an immune response against tumor cells. Some viruses naturally infect tumor cells, and other viruses can be genetically modified to infect tumor cells. At present several studies are under way to determine the killing mechanism of oncolytic viruses and the safety of using viral therapy.

Viral Size

Viral sizes are determined with the aid of electron microscopy. Different viruses vary considerably in size. Although most are quite a bit smaller than bacteria, some of the larger viruses (such as the vaccinia virus) are about the same size as some very small bacteria (such as the mycoplasmas, rickettsias, and chlamydias). Viruses range from 20 to 1000 nm in length. The comparative sizes of several viruses and bacteria are shown in Figure 13.1.

CHECK YOUR UNDERSTANDING

How could the small size of viruses have helped researchers detect viruses before the invention of the electron microscope? 13-1

Viral Structure

LEARNING OBJECTIVE

13-2 Describe the chemical and physical structure of both an enveloped and a nonenveloped virus.

A **virion** is a complete, fully developed, infectious viral particle composed of nucleic acid and surrounded by a protein coat outside a host cell. Viruses are classified by their nucleic acid and by differences in the structures of their coats.

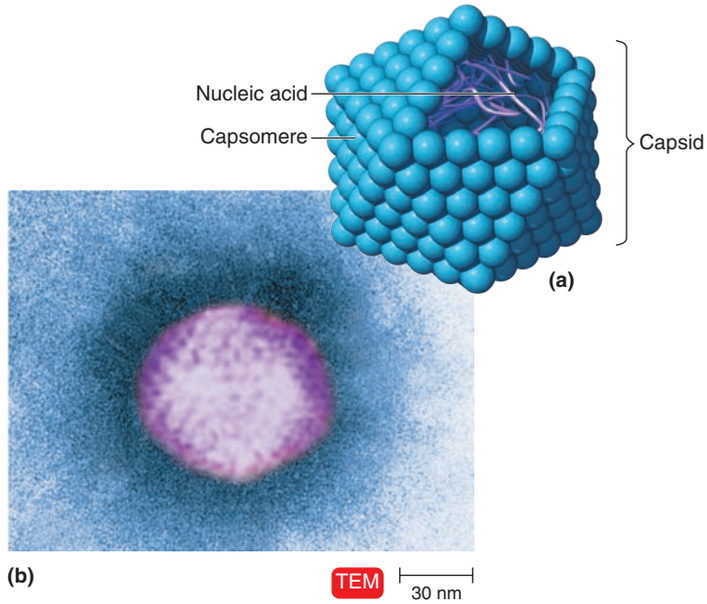


Figure 13.2 Morphology of a nonenveloped polyhedral virus. **(a)** A diagram of a polyhedral (icosahedral) virus. **(b)** A micrograph of the adenovirus *Mastadenovirus*. Individual capsomeres are visible.

Q What is the chemical composition of a capsid?

Nucleic Acid

In contrast to prokaryotic and eukaryotic cells, in which DNA is always the primary genetic material (and RNA plays an auxiliary role), a virus can have either DNA or RNA—but never both. The nucleic acid of a virus can be single-stranded or double-stranded. Thus, there are viruses with the familiar double-stranded DNA, with single-stranded DNA, with double-stranded RNA, and with single-stranded RNA. Depending on the virus, the nucleic acid can be linear or circular. In some viruses (such as the influenza virus), the nucleic acid is in several separate segments.

The percentage of nucleic acid in relation to protein is about 1% for the influenza virus and about 50% for certain bacteriophages. The total amount of nucleic acid varies from a few thousand nucleotides (or pairs) to as many as 250,000 nucleotides. (*E. coli*'s chromosome consists of approximately 4 million nucleotide pairs.)

Capsid and Envelope

The nucleic acid of a virus is protected by a protein coat called the **capsid** (Figure 13.2a). The structure of the capsid is ultimately determined by the viral nucleic acid and accounts for most of the mass of a virus, especially of small ones. Each capsid is composed of protein subunits called **capsomeres.** In some viruses, the proteins composing the capsomeres are of a single type; in other viruses, several types of protein may be present. Individual capsomeres are often visible in electron micrographs (see Figure 13.2b for an example). The arrangement of capsomeres is characteristic of a particular type of virus.

In some viruses, the capsid is covered by an **envelope** (Figure 13.3a), which usually consists of some combination of lipids, proteins, and carbohydrates. Some animal viruses are released from the host cell by an extrusion process that coats the virus with a layer of the host cell's plasma membrane; that layer becomes the viral envelope. In many cases, the envelope contains proteins determined by the viral nucleic acid and materials derived from normal host cell components.

Depending on the virus, envelopes may or may not be covered by **spikes,** which are carbohydrate-protein complexes that project from the surface of the envelope. Some viruses attach to host cells by means of spikes. Spikes are such a reliable characteristic of some viruses that they can be used as a means of identification. The ability of certain viruses, such as the influenza virus (Figure 13.3b), to clump red blood cells is associated with spikes. Such viruses bind to red blood cells and form bridges between them. The resulting clumping is called *hemagglutination* and is the basis for several useful laboratory tests. (See Figure 18.7, page 505.)

Viruses whose capsids aren't covered by an envelope are not known as **nonenveloped viruses** (see Figure 13.2). The capsid of a nonenveloped virus protects the nucleic acid from nuclease enzymes in biological fluids and promotes the virus's attachment to susceptible host cells.

When the host has been infected by a virus, the host's immune system is stimulated to produce antibodies (proteins

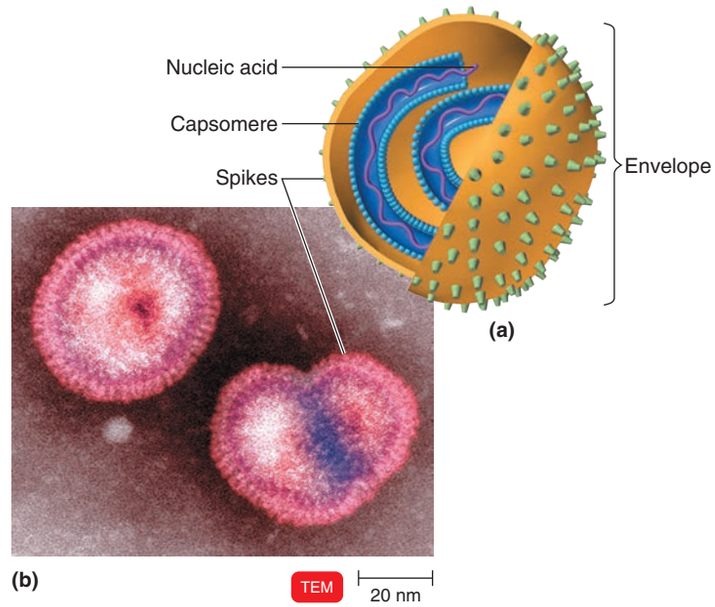


Figure 13.3 Morphology of an enveloped helical virus. **(a)** A diagram of an enveloped helical virus. **(b)** A micrograph of *Influenzavirus* A2. Notice the halo of spikes projecting from the outer surface of each envelope (see Chapter 24).

Q What is the nucleic acid in a virus?

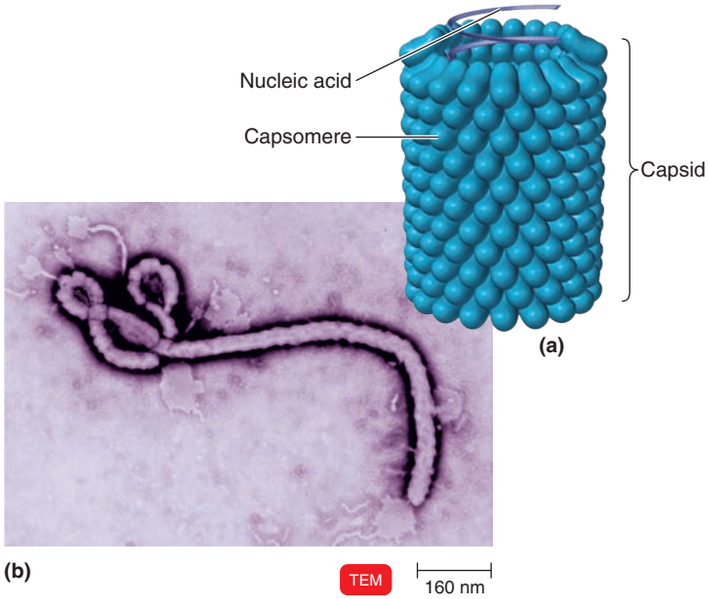


Figure 13.4 Morphology of a helical virus. **(a)** A diagram of a portion of a helical virus. A row of capsomeres has been removed to reveal the nucleic acid. **(b)** A micrograph of Ebola virus, a filovirus, showing a helical rod-like shape.

What is the chemical composition of a capsomere?

that react with the surface proteins of the virus). This interaction between host antibodies and virus proteins should inactivate the virus and stop the infection. However, some viruses can escape antibodies because regions of the genes that code for these viruses' surface proteins are susceptible to mutations. The progeny of mutant viruses have altered surface proteins, such that the antibodies aren't able to react with them. Influenza virus frequently undergoes such changes in its spikes. This is why you can get influenza more than once. Although you may have produced antibodies to one influenza virus, the virus can mutate and infect you again.

General Morphology

Viruses may be classified into several different morphological types on the basis of their capsid architecture. The structure of these capsids has been revealed by electron microscopy and a technique called X-ray crystallography.

Helical Viruses

Helical viruses resemble long rods that may be rigid or flexible. The viral nucleic acid is found within a hollow, cylindrical capsid that has a helical structure (Figure 13.4). The viruses that cause rabies and Ebola hemorrhagic fever are helical viruses.

Polyhedral Viruses

Many animal, plant, and bacterial viruses are polyhedral, or many-sided, viruses. The capsid of most polyhedral viruses is in the shape of an *icosahedron,* a regular polyhedron with 20 triangular faces and 12 corners (see Figure 13.2a). The capsomeres of each face form an equilateral triangle. An example of a polyhedral virus in the shape of an icosahedron is the adenovirus (shown in Figure 13.2b). Another icosahedral virus is the poliovirus.

Enveloped Viruses

As noted earlier, the capsid of some viruses is covered by an envelope. Enveloped viruses are roughly spherical. When helical or polyhedral viruses are enclosed by envelopes, they are called *enveloped helical* or *enveloped polyhedral viruses.* An example of an enveloped helical virus is the influenza virus (see Figure 13.3b). An example of an enveloped polyhedral (icosahedral) virus is the human herpes virus (see Figure 13.16b).

Complex Viruses

Some viruses, particularly bacterial viruses, have complicated structures and are called **complex viruses.** One example of a complex virus is a bacteriophage. Some bacteriophages have capsids to which additional structures are attached (Figure 13.5a). In this figure, notice that the capsid (head) is polyhedral and that the tail sheath is helical. The head contains the nucleic acid. Later in the chapter, we'll discuss the functions of the other structures, such as the tail sheath, tail fibers, baseplate, and pin. Another example of complex viruses are poxviruses, which don't contain clearly identifiable capsids but do have several coats around the nucleic acid (Figure 13.5b).

CHECK YOUR UNDERSTANDING

Diagram a nonenveloped polyhedral virus that has spikes. **13-2**

Taxonomy of Viruses

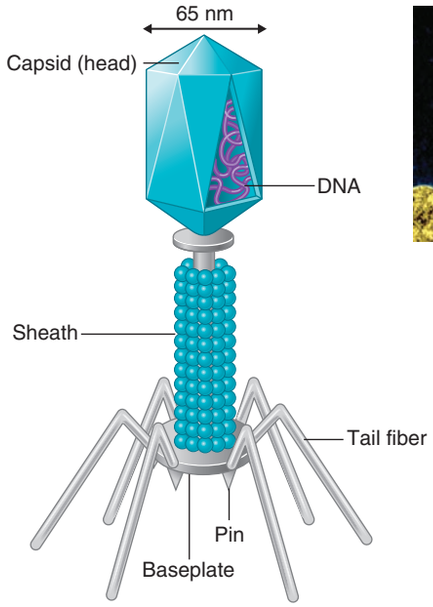
LEARNING OBJECTIVES

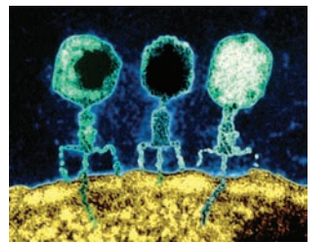
13-3 Define *viral species*.

13-4 Give an example of a family, genus, and common name for a virus.

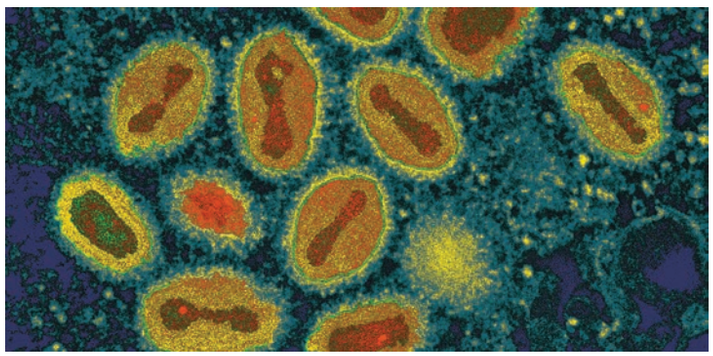
Just as we need taxonomic categories of plants, animals, and bacteria, we need viral taxonomy to help us organize and understand newly discovered organisms. The oldest classification of viruses is based on symptomatology, such as for diseases that affect the respiratory system. This system was convenient but not scientifically acceptable because the same virus may cause more than one disease, depending on the tissue affected. In addition, this system artificially grouped viruses that don't infect humans.

New, fast DNA sequencing allows the International Committee on Taxonomy of Viruses to group viruses into families based on genomics and structure. The suffix *-virus* is used for genus names; family names end in *-viridae;* and order names end in *-ales.* In formal usage, the family and genus names are





(a) A T-even bacteriophage



(b) *Orthopoxvirus*

Figure 13.5 Morphology of complex viruses. **(a)** A diagram and micrograph of a T-even bacteriophage. **(b)** A micrograph of variola virus, a species in the genus *Orthopoxvirus*, which causes smallpox.

Q What is the value of a capsid to a virus?

used in the following manner: Family Herpesviridae, genus *Simplexvirus,* human herpesvirus 2.

A **viral species** is a group of viruses sharing the same genetic information and ecological niche (host range). Specific epithets for viruses aren't used. Thus, viral species are designated by descriptive common names, such as human immunodeficiency virus (HIV), with subspecies (if any) designated by a number (HIV-1). **Table 13.2** presents a summary of the classification of viruses that infect humans.

CHECK YOUR UNDERSTANDING

- How does a virus species differ from a bacterial species? **13-3**
- Attach the proper endings to *Papilloma-* to show the family and genus that includes HPV, the cause of cervical cancer. **13-4**

Isolation, Cultivation, and Identification of Viruses

LEARNING OBJECTIVES

13-5 Describe how bacteriophages are cultured.
13-6 Describe how animal viruses are cultured.
13-7 List three techniques used to identify viruses.

The fact that viruses can't multiply outside a living host cell complicates their detection, enumeration, and identification. Viruses must be provided with living cells instead of a fairly simple chemical medium. Living plants and animals are difficult and expensive to maintain, and pathogenic viruses that grow only in higher primates and human hosts cause additional complications. However, viruses that use bacterial cells as a host (bacteriophages) are rather easily grown on bacterial cultures. This is one reason so much of our understanding of viral multiplication has come from bacteriophages.

Growing Bacteriophages in the Laboratory

Bacteriophages can be grown either in suspensions of bacteria in liquid media or in bacterial cultures on solid media. The use of solid media makes it possible to use the *plaque method* to detect and count viruses. A bacteriophage sample is mixed with host bacteria and melted agar. The agar containing the bacteriophages and host bacteria is then poured into a Petri plate containing a hardened layer of agar growth medium. The virus-bacteria mixture solidifies into a thin top layer that contains a layer of bacteria approximately one cell thick. Each virus infects a bacterium, multiplies, and releases several hundred new viruses. These newly produced viruses infect other bacteria in the immediate vicinity, and more new viruses are produced. Following several viral multiplication cycles, all the bacteria in the area surrounding the original virus are destroyed. This produces a number of clearings, or **plaques,** visible against a lawn of bacterial growth on the surface of the agar (**Figure 13.6**). While the plaques form, uninfected bacteria elsewhere in the Petri plate multiply rapidly and produce a turbid background.

Theoretically each plaque corresponds to a single virus in the initial suspension. Therefore, the concentrations of viral suspensions measured by the number of plaques are usually expressed in terms of **plaque-forming units (PFU).**

Growing Animal Viruses in the Laboratory

In the laboratory, three methods are commonly used for culturing animal viruses. These methods involve using living animals, embryonated eggs, or cell cultures.

In Living Animals

Some animal viruses can be cultured only in living animals, such as mice, rabbits, and guinea pigs. Most experiments to study the

CLINICAL FOCUS

Influenza: Crossing the Species Barrier

Influenza A viruses are found in many different animals, including birds, pigs, whales, horses, and seals. Sometimes influenza A viruses seen in one species can cross over and cause illness in another species. For example, up until 1998, only H1N1 viruses circulated widely in the U.S. pig population. In 1998, H3N2 viruses from humans were introduced into the pig population and caused widespread disease among pigs. The subtypes differ because of certain proteins on the surface of the virus (hemagglutinin [HA] and neuraminidase [NA] proteins). There are 16 different HA subtypes and 9 different NA subtypes of influenza A viruses.

How many different combinations of H and N proteins are possible?

Each combination is a different subtype. When we talk about "human flu viruses," we are referring to those subtypes that occur widely in humans. There are only three known subtypes of human influenza viruses (H1N1, H1N2, and H3N2).

What's different about bird flu?

H5 and H7 subtypes occur mainly in birds. Avian influenza (bird flu) viruses don't usually infect humans. All human cases of avian flu can be attributed to outbreaks in poultry, except one noteworthy probable transmission from a daughter to her mother. Avian influenza viruses may be transmitted to humans: (1) directly from birds or from avian-virus-contaminated environments or (2) through an intermediate host, such as a pig.

Why are pigs important?

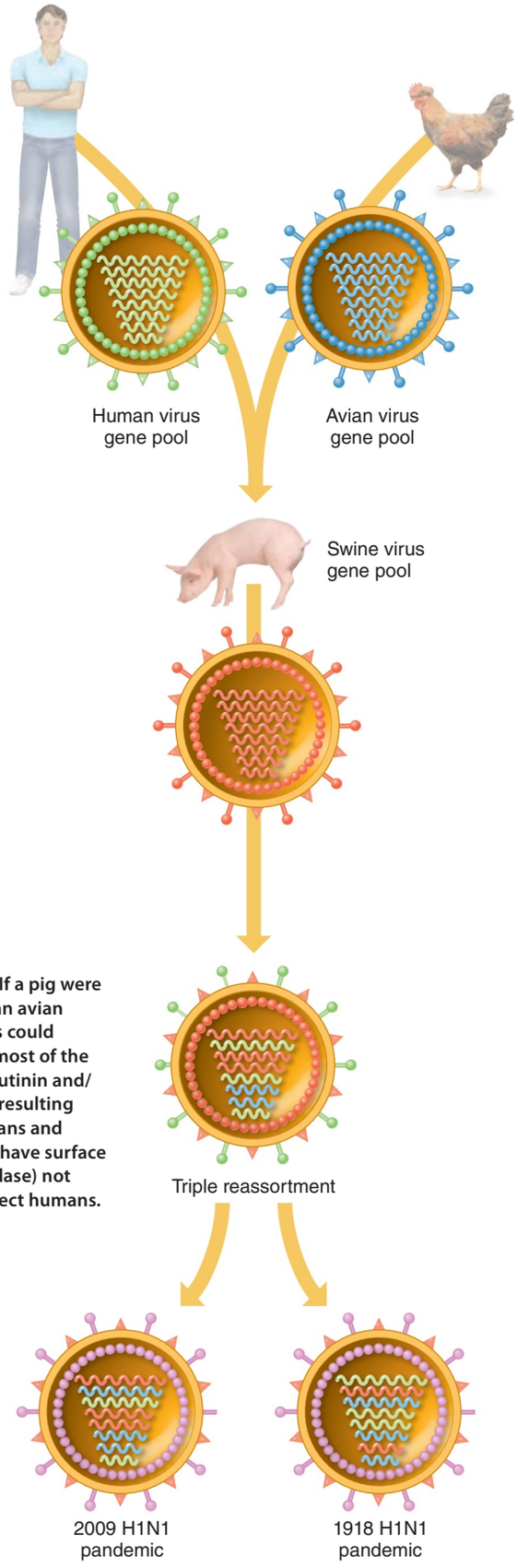
Pigs can be infected with both human and avian flu. The influenza virus genome is composed of eight separate segments. A segmented genome allows virus genes to mix and create a new influenza A virus if viruses from two different species infect the same person or animal (see the figure). This is known as *antigenic shift*.

The 2009 H1N1 virus was originally referred to as "swine flu" because laboratory testing showed that many of the genes in the virus were very similar to influenza viruses that normally occur in North American pigs. But further study has shown that the 2009 H1N1 is very different from that which normally circulates in North American pigs. It has two genes from flu viruses that normally circulate in pigs in Europe and Asia, avian influenza genes, and human genes. This is called a *quadruple reassortant* virus (see the figure).

Pandemics

During the last 100 years, the emergence of new influenza A virus subtypes caused three pandemics, all of which spread around the world within one year of being detected (see the table). Some genetic parts of all of these influenza A strains originally came from birds.

Source: Adapted from *MMWR* sources.

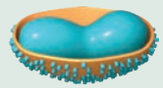


Model for antigenic shift in influenza virus. If a pig were infected with a human influenza virus and an avian influenza virus at the same time, the viruses could reassort and produce a new virus that had most of the genes from the human virus but a hemagglutinin and/or neuraminidase from the avian virus. The resulting new virus might then be able to infect humans and spread from person to person, but it would have surface proteins (hemagglutinin and/or neuraminidase) not previously seen in influenza viruses that infect humans.

Influenza A Pandemics During the Past 100 Years

1918–19	H1N1 caused up to 50 million deaths worldwide. Virus has avian flu–like genes.
1957–58	H2N2 caused about 70,000 deaths in the United States. First identified in China in late February 1957. Viruses contained a combination of genes from a human influenza virus and an avian influenza virus.
1968–69	H3N2 caused about 34,000 deaths in the United States. This virus contained genes from a human influenza virus and an avian influenza virus.
2009–10	H1N1 caused at least 14,000 deaths worldwide. A vaccine was available in developed and developing countries 3 months after the first cases.

TABLE 13.2 Families of Viruses That Affect Humans

Characteristics/ Dimensions	Viral Family	Important Genera	Clinical or Special Features
Single-Stranded DNA Nonenveloped			
18–25 nm	Parvoviridae	Human parvovirus B19	Fifth disease; anemia in immunocompromised patients. Refer to Chapter 21.
Double-Stranded DNA Nonenveloped			
70–90 nm	Adenoviridae	*Mastadenovirus*	Medium-sized viruses that cause various respiratory infections in humans; some cause tumors in animals.
40–57 nm	Papovaviridae	*Papillomavirus* (human wart virus) *Polyomavirus*	Small viruses that cause warts and cervical and anal cancer in humans belong to this family. Refer to Chapters 21 and 26.
Double-Stranded DNA Enveloped			
200–350 nm	Poxviridae 	*Orthopoxvirus* (vaccinia and smallpox viruses) *Molluscipoxvirus*	Very large, complex, brick-shaped viruses that cause smallpox (variola), molluscum contagiosum (wartlike skin lesion), and cowpox. Refer to Chapter 21.
150–200 nm	Herpesviridae	*Simplexvirus* (HHV-1 and -2) *Varicellovirus* (HHV-3) *Lymphocryptovirus* (HHV-4) *Cytomegalovirus* (HHV-5) *Roseolovirus* (HHV-6 and HHV-7) *Rhadinovirus* (HHV-8)	Medium-sized viruses that cause various human diseases: fever blisters, chickenpox, shingles, and infectious mononucleosis; cause a type of human cancer called Burkitt's lymphoma. Refer to Chapters 21, 23, and 26.
42 nm	Hepadnaviridae	*Hepadnavirus* (hepatitis B virus)	After protein synthesis, hepatitis B virus uses reverse transcriptase to produce its DNA from mRNA; causes hepatitis B and liver tumors. Refer to Chapter 25.
Single-Stranded RNA, + Strand Nonenveloped			
28–30 nm	Picornaviridae	*Enterovirus* *Rhinovirus* (common cold virus) Hepatitis A virus	Includes the polio-, coxsackie-, and echoviruses; hand-foot-mouth virus; more than 100 rhinoviruses exist and are the most common cause of colds. Refer to Chapters 22, 24, and 25.
35–40 nm	Caliciviridae	Hepatitis E virus *Norovirus*	Includes causes of gastroenteritis and one cause of human hepatitis. Refer to Chapter 25.
Single-Stranded RNA, + Strand Enveloped			
60–70 nm	Togaviridae	*Alphavirus* *Rubivirus* (rubella virus)	Includes many viruses transmitted by arthropods (*Alphavirus*); diseases include eastern equine encephalitis (EEE), western equine encephalitis (WEE), and chikungunya. Rubella virus is transmitted by the respiratory route. Refer to Chapters 21, 22, and 23.
40–50 nm	Flaviviridae	*Flavivirus* *Pestivirus* Hepatitis C virus	Can replicate in arthropods that transmit them; diseases include yellow fever, dengue and St. Louis and West Nile encephalitis. Refer to Chapters 22, 23, and 25.

(continued)

TABLE 13.2 Families of Viruses That Affect Humans *(continued)*

Characteristics/ Dimensions	Viral Family	Important Genera	Clinical or Special Features
80–160 nm	Coronaviridae	*Coronavirus*	Associated with upper respiratory tract infections and the common cold; SARS virus, MERS-CoV. Refer to Chapter 24.
− Strand, One Strand of RNA			
70–180 nm	Rhabdoviridae	*Vesiculovirus* (vesicular stomatatis virus) *Lyssavirus* (rabies virus)	Bullet-shaped viruses with a spiked envelope; cause rabies and numerous animal diseases. Refer to Chapter 22.
80–14,000 nm	Filoviridae	*Filovirus*	Enveloped, helical viruses; Ebola and Marburg viruses are filoviruses. Refer to Chapter 23.
150–300 nm	Paramyxoviridae	*Paramyxovirus* *Morbillivirus* (measles virus)	Paramyxoviruses cause parainfluenza, mumps, and Newcastle disease in chickens. Refer to Chapters 21, 24, and 25.
32 nm	Deltaviridae	Hepatitis D	Depend on coinfection with hepadnavirus. Refer to Chapter 25.
− Strand, Multiple Strands of RNA			
80–200 nm	Orthomyxoviridae	Influenza virus A, B, and C	Envelope spikes can agglutinate red blood cells. Refer to Chapter 24.
90–120 nm	Bunyaviridae	*Bunyavirus* (California encephalitis virus) *Hantavirus*	Hantaviruses cause hemorrhagic fevers such as Korean hemorrhagic fever and *Hantavirus* pulmonary syndrome; associated with rodents. Refer to Chapters 22 and 23.
110–130 nm	Arenaviridae	*Arenavirus*	Helical capsids contain RNA-containing granules; cause lymphocytic choriomeningitis, Venezuelan hemorrhagic fever, and Lassa fever. Refer to Chapter 23.
Single-Stranded RNA, Produce DNA			
100–120 nm	Retroviridae	Oncoviruses *Lentivirus* (HIV)	Includes all RNA tumor viruses. Oncoviruses cause leukemia and tumors in animals; *Lentivirus* causes AIDS. Refer to Chapter 19.
Double-Stranded RNA Nonenveloped			
60–80 nm	Reoviridae	*Reovirus* *Rotavirus*	Generally mild respiratory infections transmitted by arthropods; Colorado tick fever is the best-known. Refer to Chapter 25.

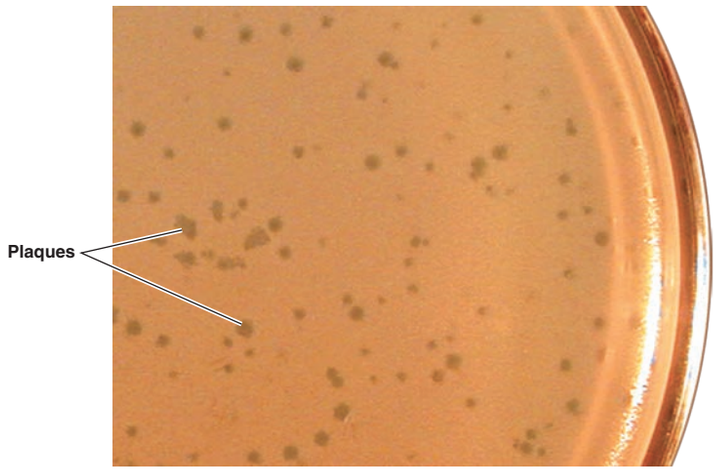


Figure 13.6 Viral plaques formed by bacteriophages. Clear viral plaques of various sizes have been formed by bacteriophage λ (lambda) on a lawn of *E. coli*.

Q What is a plaque-forming unit?

immune system's response to viral infections must also be performed in virally infected live animals. Animal inoculation may be used as a diagnostic procedure for identifying and isolating a virus from a clinical specimen. After the animal is inoculated with the specimen, the animal is observed for signs of disease or is killed so that infected tissues can be examined for the virus.

Some human viruses can't be grown in animals or can be grown but don't cause disease. The lack of natural animal models for AIDS has slowed our understanding of its disease process and prevented experimentation with drugs that inhibit growth of the virus in vivo. Chimpanzees can be infected with one subspecies of human immunodeficiency virus (HIV-1, genus *Lentivirus*), but because they don't show symptoms of the disease, they can't be used to study the effects of viral growth and disease treatments. AIDS vaccines are presently being tested in humans, but the disease progresses so slowly in humans that it can take years to determine the effectiveness of these vaccines. In 1986, simian AIDS (an immunodeficiency disease of green monkeys) was reported, followed in 1987 by feline AIDS (an immunodeficiency disease of domestic cats). These diseases are caused by lentiviruses, which are closely related to HIV, and the diseases develop within a few months, thus providing a model for studying viral growth in different tissues. In 1990, a way to infect mice with HIV was found when immunodeficient mice were grafted to produce human T cells and human gamma globulin. The mice provide a reliable model for studying viral replication, although they don't provide models for vaccine development.

In Embryonated Eggs

If the virus will grow in an *embryonated egg,* this can be a fairly convenient and inexpensive form of host for many animal viruses. A hole is drilled in the shell of the embryonated egg, and a viral suspension or suspected virus-containing tissue is injected into the egg's fluid. There are several membranes in an egg, and the virus is injected near the one most appropriate for its growth (Figure 13.7). Viral growth is signaled by the death of the embryo, by embryo cell damage, or by the formation of typical pocks or lesions on the egg membranes. This method was once the most widely used method of isolating and growing viruses, and it's still used to grow viruses for some vaccines. For this reason, you may be asked if you're allergic to eggs before receiving a vaccination, because egg proteins may be present in the viral vaccine preparations. (Allergic reactions will be discussed in Chapter 19.)

In Cell Cultures

Cell cultures have replaced embryonated eggs as the preferred type of growth medium for many viruses. Cell cultures consist of cells grown in culture media in the laboratory. Because these cultures are generally rather homogeneous collections of cells and can be propagated and handled much like bacterial cultures, they are more convenient to work with than whole animals or embryonated eggs.

Cell culture lines are started by treating a slice of animal tissue with enzymes that separate the individual cells (Figure 13.8). These cells are suspended in a solution that provides the osmotic pressure, nutrients, and growth factors needed for the cells to grow. Normal cells tend to adhere to the glass or plastic container and reproduce to form a monolayer. Viruses infecting

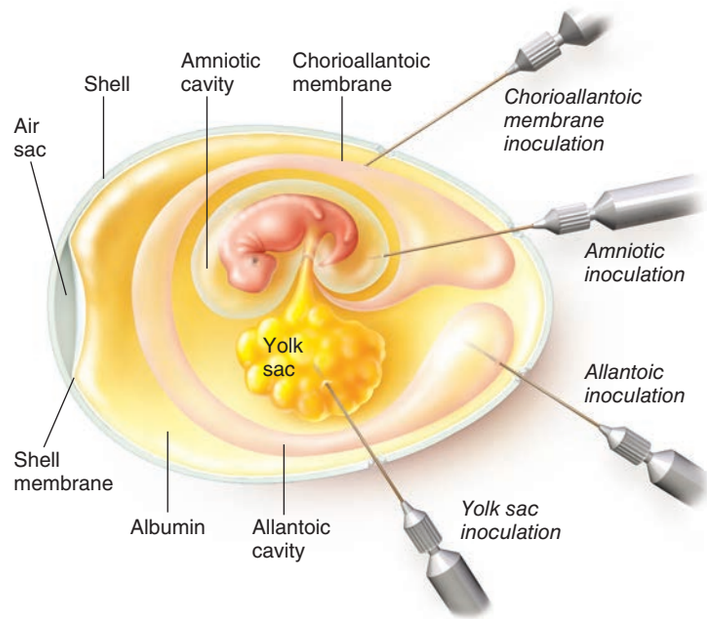


Figure 13.7 Inoculation of an embryonated egg. The viruses will grow on the membrane at the inoculation site.

Q Why are viruses grown in eggs and not in culture media?

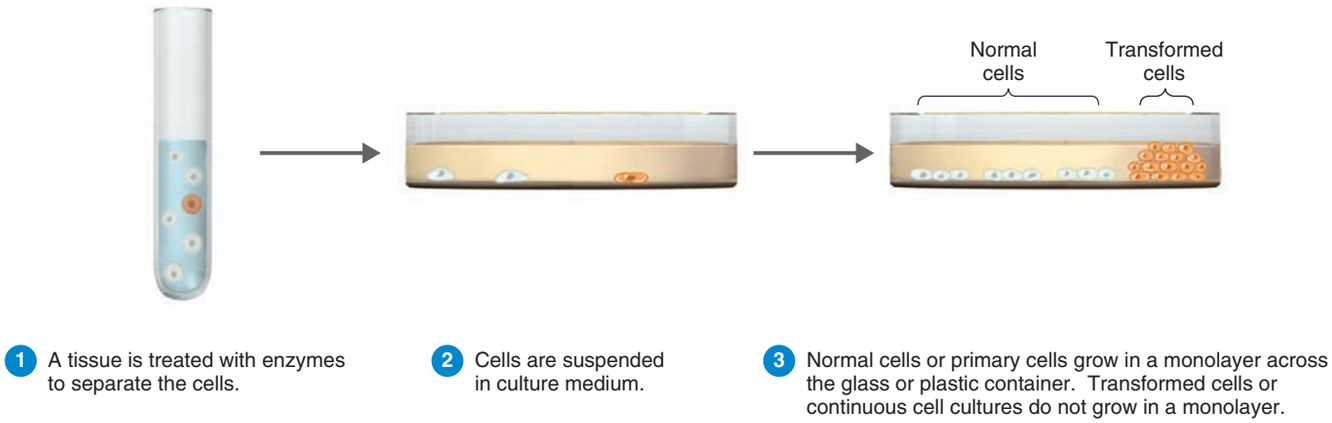


Figure 13.8 Cell cultures. Transformed cells can be grown indefinitely in laboratory culture.

Q Why are transformed cells referred to as "immortal"?

such a monolayer sometimes cause the cells of the monolayer to deteriorate as they multiply. This cell deterioration, called **cytopathic effect (CPE),** is illustrated in Figure 13.9. CPE can be detected and counted in much the same way as plaques caused by bacteriophages on a lawn of bacteria and reported as PFU/ml.

Viruses may be grown in primary or continuous cell lines. **Primary cell lines,** derived from tissue slices, tend to die out after only a few generations. Certain cell lines, called **diploid cell lines,** developed from human embryos can be maintained for about 100 generations and are widely used for culturing viruses that require a human host. Cell lines developed from embryonic human cells are used to culture rabies virus for a rabies vaccine called human diploid culture vaccine (see Chapter 22).

When viruses are routinely grown in a laboratory, **continuous cell lines** are used. These are transformed (cancerous) cells that can be maintained through an indefinite number of generations, and they're sometimes called immortal cell lines (see the discussion of transformation on page 381). One of these, the HeLa cell line, was isolated from the cancer of a woman (*Hen*rietta *Lac*ks) who died in 1951. After years of laboratory cultivation, many such cell lines have lost almost all the original characteristics of the cell, but these changes haven't interfered with the use of the cells for viral propagation. In spite of the success of cell culture in viral isolation and growth, there are still some viruses that have never been successfully cultivated in cell culture.

The idea of cell culture dates back to the end of the nineteenth century, but it wasn't a practical laboratory technique until the development of antibiotics in the years following World War II. A major problem with cell culture is that the cell lines must be kept free of microbial contamination. The maintenance of cell culture lines requires trained technicians with considerable experience working on a full-time basis. Because of these difficulties, most hospital laboratories and many state health laboratories don't isolate and identify viruses in clinical work. Instead, the tissue or serum samples are sent to central laboratories that specialize in such work.

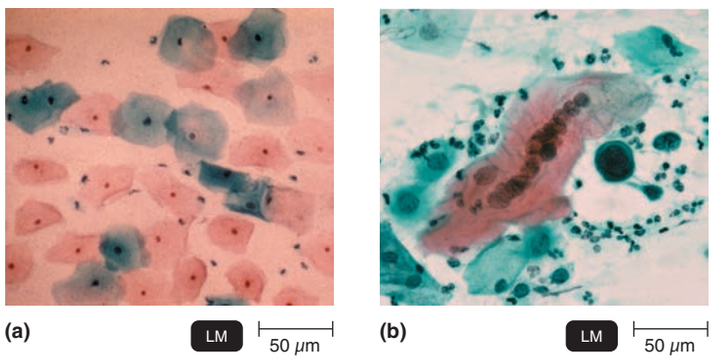


Figure 13.9 The cytopathic effect of viruses. **(a)** Uninfected human cervical cells; each has one nucleus. **(b)** After infection with HHV-2, the red cervical cell has many nuclei that are filled with viruses.

Q How did HHV-2 infection affect the cells?

Viral Identification

Identifying viral isolates isn't an easy task. For one thing, viruses can't be seen without the use of an electron microscope. Serological methods, such as Western blotting, are the most commonly used means of identification (see Figure 10.12, page 279). In these tests, the virus is detected and identified by its reaction with antibodies. We'll discuss antibodies in detail in Chapter 17 and a number of immunological tests for identifying viruses in Chapter 18. Observation of cytopathic effects, described in Chapter 15 (pages 430–432), is also useful for identifying a virus.

Virologists can identify and characterize viruses by using such modern molecular methods as use of restriction fragment

length polymorphisms (RFLPs) (Chapter 9, page 254) and the polymerase chain reaction (PCR) (Chapter 9, page 243). PCR was used to amplify viral RNA to identify the West Nile virus in 1999 in the United States and the SARS-associated coronavirus in China in 2002.

CHECK YOUR UNDERSTANDING

- What is the plaque method? **13-5**
- Why are continuous cell lines of more practical use than primary cell lines for culturing viruses? **13-6**
- What tests could you use to identify influenza virus in a patient? **13-7**

Viral Multiplication

LEARNING OBJECTIVES

13-8 Describe the lytic cycle of T-even bacteriophages.

13-9 Describe the lysogenic cycle of bacteriophage lambda.

13-10 Compare and contrast the multiplication cycle of DNA and RNA-containing animal viruses.

The nucleic acid in a virion contains only a few of the genes needed for the synthesis of new viruses. These include genes for the virion's structural components, such as the capsid proteins, and genes for a few of the enzymes used in the viral life cycle. These enzymes are synthesized and functional only when the virus is within the host cell. Viral enzymes are almost entirely concerned with replicating or processing viral nucleic acid. Enzymes needed for protein synthesis, ribosomes, tRNA, and energy production are supplied by the host cell and are used for synthesizing viral proteins, including viral enzymes. Although the smallest nonenveloped virions don't contain preformed enzymes, the larger virions may contain one or a few enzymes, which usually function in helping the virus penetrate the host cell or replicate its own nucleic acid.

ASM: The synthesis of viral genetic material and proteins is dependent on host cells.

Thus, for a virus to multiply, it must invade a host cell and take over the host's metabolic machinery. A single virion can give rise to several or even thousands of similar viruses in a single host cell. This process can drastically change the host cell and usually causes its death. In a few viral infections, cells survive and continue to produce viruses indefinitely.

The multiplication of viruses can be demonstrated with a **one-step growth curve** (Figure 13.10). The data are obtained by infecting every cell in a culture and then testing the culture medium and cells for virions and viral proteins and nucleic acids.

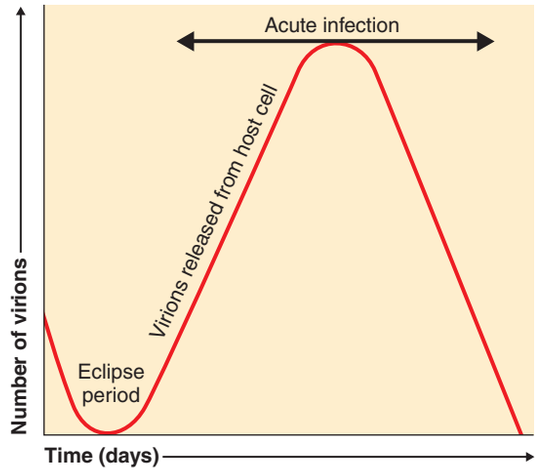


Figure 13.10 A viral one-step growth curve. No new infective virions are found in a culture until after biosynthesis and maturation have taken place. Most infected cells die as a result of infection; consequently, new virions won't be produced.

Q What can be found in the cell during biosynthesis and maturation?

Multiplication of Bacteriophages

Although the means by which a virus enters and exits a host cell may vary, the basic mechanism of viral multiplication is similar for all viruses. Bacteriophages can multiply by two alternative mechanisms: the lytic cycle or the lysogenic cycle. The **lytic cycle** ends with the lysis and death of the host cell, whereas the host cell remains alive in the **lysogenic cycle.** Because the *T-even bacteriophages* (T2, T4, and T6) have been studied most extensively, we'll describe the multiplication of T-even bacteriophages in their host, *E. coli,* as an example of the lytic cycle.

T-Even Bacteriophages: The Lytic Cycle

The virions of T-even bacteriophages are large, complex, and nonenveloped, with a characteristic head-and-tail structure shown in Figure 13.5a and Figure 13.11. The length of DNA contained in these bacteriophages is only about 6% of that contained in *E. coli,* yet the phage has enough DNA for over 100 genes. The multiplication cycle of these phages, like that of all viruses, occurs in five distinct stages: attachment, penetration, biosynthesis, maturation, and release.

Attachment 1 After a chance collision between phage particles and bacteria, *attachment,* or *adsorption,* occurs. During this process, an attachment site on the virus attaches to a complementary receptor site on the bacterial cell. This attachment is a chemical interaction in which weak bonds are formed between the attachment and receptor sites. T-even bacteriophages use fibers at the end of the tail as attachment sites. The complementary receptor sites are on the bacterial cell wall.

Penetration 2 After attachment, the T-even bacteriophage injects its DNA (nucleic acid) into the bacterium. To do this, the bacteriophage's tail releases an enzyme, **phage lysozyme,** which breaks down a portion of the bacterial cell wall. During the

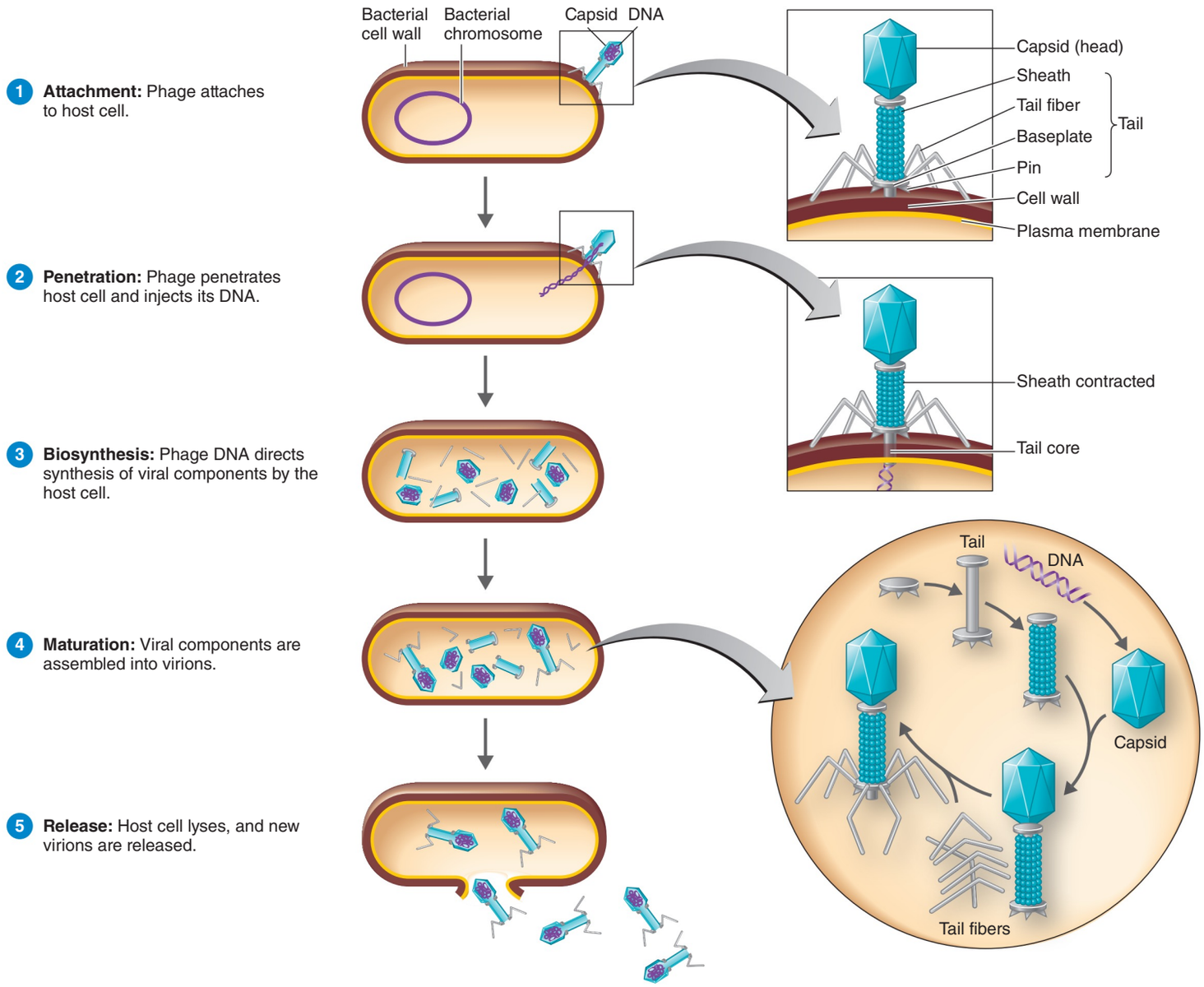


Figure 13.11 The lytic cycle of a T-even bacteriophage.

Q What is the result of the lytic cycle?

process of *penetration,* the tail sheath of the phage contracts, and the tail core is driven through the cell wall. When the tip of the core reaches the plasma membrane, the DNA from the bacteriophage's head passes through the tail core, through the plasma membrane, and enters the bacterial cell. The capsid remains outside the bacterial cell. Therefore, the phage particle functions like a hypodermic syringe to inject its DNA into the bacterial cell.

Biosynthesis 3 Once the bacteriophage DNA has reached the cytoplasm of the host cell, the biosynthesis of viral nucleic acid and protein occurs. Host protein synthesis is stopped by virus-induced degradation of the host DNA, viral proteins that interfere with transcription, or the repression of translation.

Initially, the phage uses the host cell's nucleotides and several of its enzymes to synthesize many copies of phage DNA. Soon after, the biosynthesis of viral proteins begins. Any RNA transcribed in the cell is mRNA transcribed from phage DNA for the biosynthesis of phage enzymes and capsid proteins. The host cell's ribosomes, enzymes, and amino acids are used for translation. Genetic controls regulate when different regions of phage DNA are transcribed into mRNA during the multiplication cycle. For example, early messages are translated into

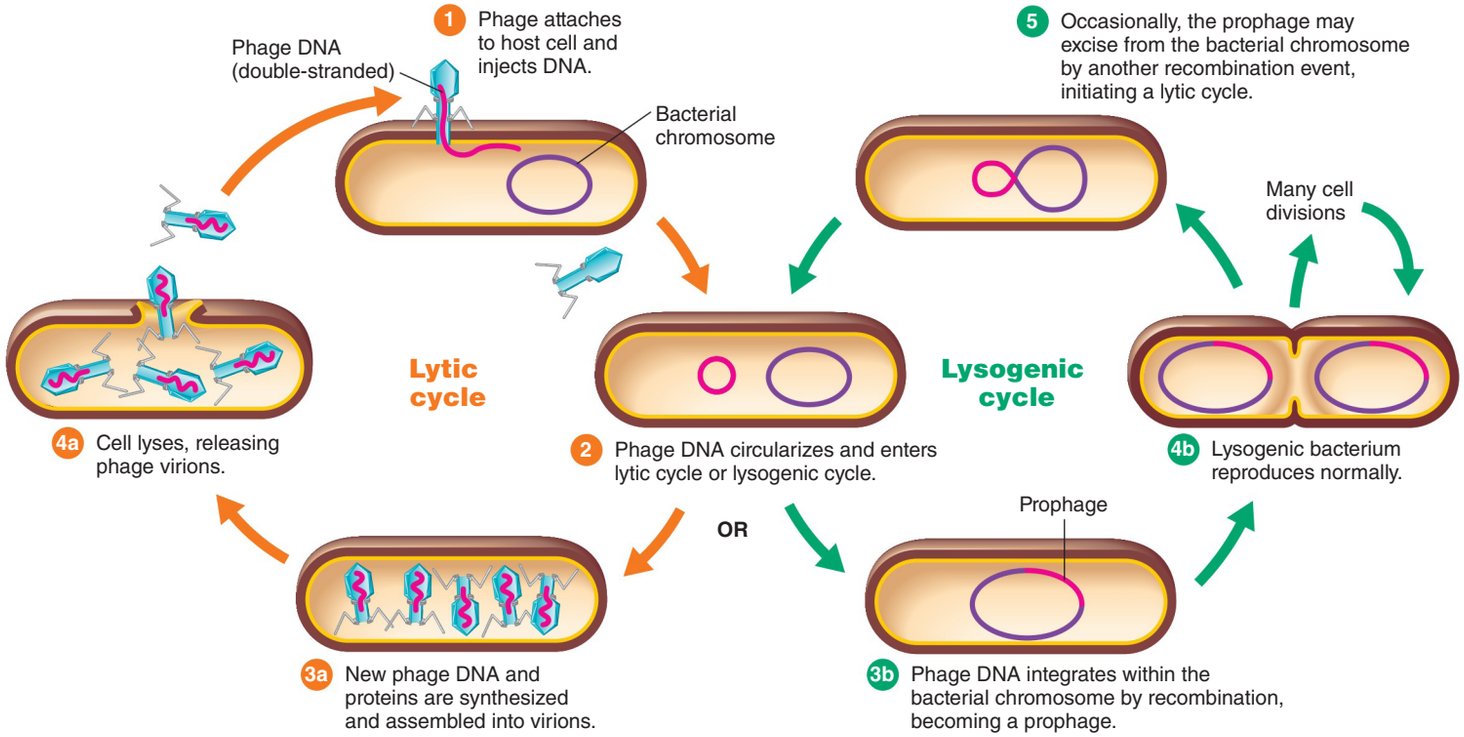


Figure 13.12 The lysogenic cycle of bacteriophage λ in *E. coli*.

Q How does lysogeny differ from the lytic cycle?

early phage proteins, the enzymes used in the synthesis of phage DNA. Also, late messages are translated into late phage proteins for the synthesis of capsid proteins.

For several minutes following infection, complete phages can't be found in the host cell. Only separate components—DNA and protein—can be detected. The period during viral multiplication when complete, infective virions aren't yet present is called the **eclipse period.**

Maturation 4 In the next sequence of events, *maturation* occurs. In this process, bacteriophage DNA and capsids are assembled into complete virions. The viral components essentially assemble into a viral particle spontaneously, eliminating the need for many nonstructural genes and gene products. The phage heads and tails are separately assembled from protein subunits, and the head is filled with phage DNA and attached to the tail.

Release 5 The final stage of viral multiplication is the *release* of virions from the host cell. The term **lysis** is generally used for this stage in the multiplication of T-even phages because in this case, the plasma membrane actually breaks open (lyses). Lysozyme, which is encoded by a phage gene, is synthesized within the cell. This enzyme causes the bacterial cell wall to break down, and the newly produced bacteriophages are released from the host cell. The released bacteriophages infect other susceptible cells in the vicinity, and the viral multiplication cycle is repeated within those cells.

Bacteriophage Lambda (λ): The Lysogenic Cycle

In contrast to T-even bacteriophages, some viruses don't cause lysis and death of the host cell when they multiply. These *lysogenic phages* (also called *temperate phages*) may indeed proceed through a lytic cycle, but they are also capable of incorporating their DNA into the host cell's DNA to begin a lysogenic cycle. In **lysogeny,** the phage remains latent (inactive). The participating bacterial host cells are known as *lysogenic cells.*

We will use the bacteriophage λ (lambda), a well-studied lysogenic phage, as an example of the lysogenic cycle (Figure 13.12).

1 Upon penetration into an *E. coli* cell,
1 the originally linear phage DNA forms a circle.
3a This circle can multiply and be transcribed,
4a leading to the production of new phage and to cell lysis (the lytic cycle).
3b Alternatively, the circle can recombine with and become part of the circular bacterial DNA (the lysogenic cycle). The inserted phage DNA is now called a **prophage.** Most of the prophage genes are repressed by two repressor proteins that are the products of phage genes. These repressors

stop transcription of all the other phage genes by binding to operators. Thus, the phage genes that would otherwise direct the synthesis and release of new virions are turned off, in much the same way that the genes of the *E. coli lac* operon are turned off by the *lac* repressor (Figure 8.12, page 216).

Every time the host cell's machinery replicates the bacterial chromosome,

4b it also replicates the prophage DNA. The prophage remains latent within the progeny cells.

5 However, a rare spontaneous event, or the action of UV light or certain chemicals, can lead to the excision (popping-out) of the phage DNA, and to initiation of the lytic cycle.

There are three important results of lysogeny. First, the lysogenic cells are immune to reinfection by the same phage. (However, the host cell isn't immune to infection by other phage types.) The second result of lysogeny is **phage conversion;** that is, the host cell may exhibit new properties. For example, the bacterium *Corynebacterium diphtheriae,* which causes diphtheria, is a pathogen whose disease-producing properties are related to the synthesis of a toxin. The organism can produce toxin only when it carries a lysogenic phage, because the prophage carries the gene coding for the toxin. As another example, only streptococci carrying a lysogenic phage are capable of causing toxic shock syndrome. The toxin produced by *Clostridium botulinum,* which causes botulism, is encoded by a prophage gene, as is the Shiga toxin produced by pathogenic strains of *E. coli.*

The third result of lysogeny is that it makes **specialized transduction** possible. Bacterial genes can be picked up in a phage coat and transferred to another bacterium in a process called generalized transduction (see Figure 8.30, page 231). Any bacterial genes can be transferred by generalized transduction because the host chromosome is broken down into fragments, any of which can be packaged into a phage coat. In specialized transduction, however, only certain bacterial genes can be transferred.

Specialized transduction is mediated by a lysogenic phage, which packages bacterial DNA *along with* its own DNA in the same capsid. When a prophage is excised from the host chromosome, adjacent genes from either side may remain attached to the phage DNA. In **Figure 13.13**, bacteriophage λ has picked up the *gal* gene for galactose fermentation from its galactose-positive host. The phage carries this gene to a galactose-negative cell, which then becomes galactose-positive.

Certain animal viruses can undergo processes very similar to lysogeny. Animal viruses that can remain latent in cells for long periods without multiplying or causing disease may become inserted into a host chromosome or remain separate from host DNA in a repressed state (as some lysogenic phages). Cancer-causing viruses may also be latent, as we'll discuss later in the chapter.

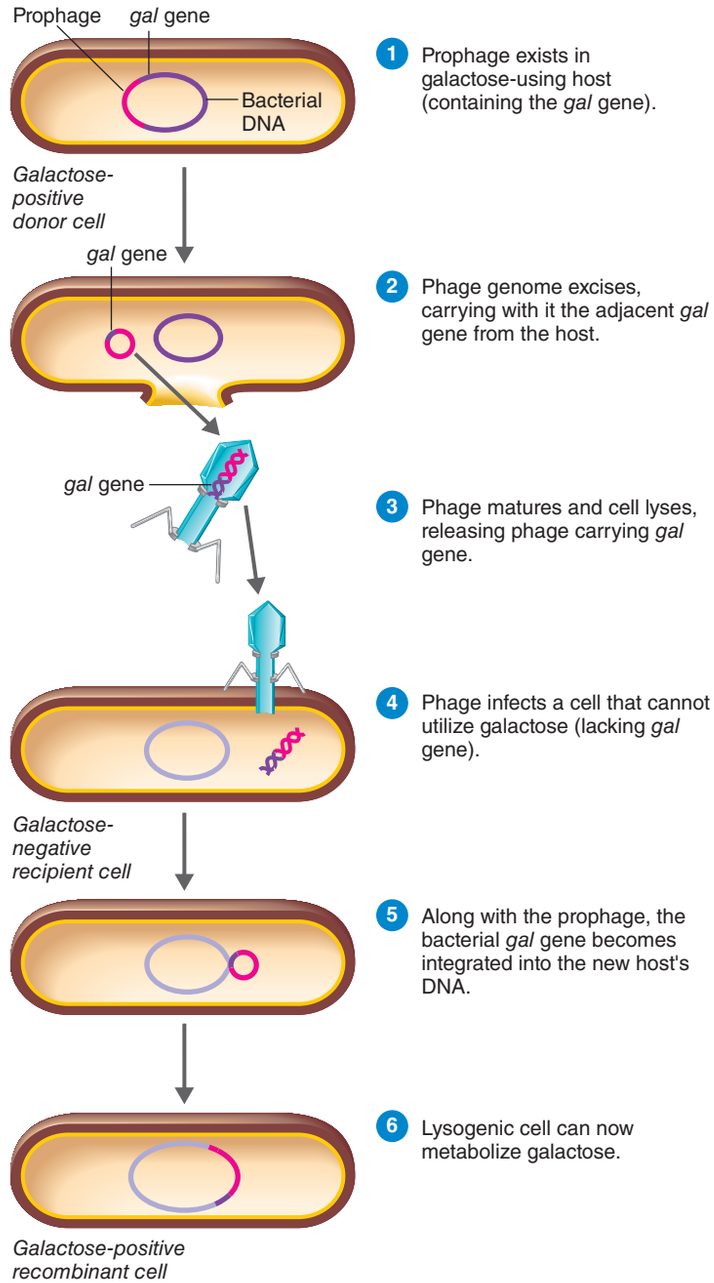


Figure 13.13 Specialized transduction. When a prophage is excised from its host chromosome, it can take with it a bit of the adjacent DNA from the bacterial chromosome.

Q How does specialized transduction differ from the lytic cycle?

MM **Animations** Viral Replication: Virulent Bacteriophages, Temperate Bacteriophages; Transduction: Specialized Transduction

CHECK YOUR UNDERSTANDING

- How do bacteriophages get nucleotides and amino acids if they don't have any metabolic enzymes? **13-8**
- *Vibrio cholerae* produces toxin and is capable of causing cholera only when it is lysogenic. What does this mean? **13-9**

TABLE 13.3 Bacteriophage and Animal Viral Multiplication Compared

Stage	Bacteriophages	Animal Viruses
Attachment	Tail fibers attach to cell wall proteins	Attachment sites are plasma membrane proteins and glycoproteins.
Entry	Viral DNA is injected into host cell	Capsid enters by receptor-mediated endocytosis or fusion.
Uncoating	Not required	Enzymatic removal of capsid proteins.
Biosynthesis	In cytoplasm	In nucleus (DNA viruses) or cytoplasm (RNA viruses).
Chronic infection	Lysogeny	Latency; slow viral infections; cancer.
Release	Host cell is lysed	Enveloped viruses bud out; nonenveloped viruses rupture plasma membrane.

Multiplication of Animal Viruses

The multiplication of animal viruses follows the basic pattern of bacteriophage multiplication but has several differences, summarized in Table 13.3. Animal viruses differ from phages in their mechanism of entering the host cell. Also, once the virus is inside, the synthesis and assembly of the new viral components are somewhat different, partly because of the differences between prokaryotic cells and eukaryotic cells. Animal viruses may have certain types of enzymes not found in phages. Finally, the mechanisms of maturation and release, and the effects on the host cell, differ in animal viruses and phages.

A virus needs live host cells but must stop synthesis of host proteins, so the viral genes are translated. Research currently indicates that viruses use several mechanisms to inhibit expression of host cell genes. Early proteins translated from the viral genome may block transcription, existing mRNA, or in-progress translation. In the following discussion of the multiplication of animal viruses, we'll consider the processes that are shared by both DNA- and RNA-containing animal viruses. These processes are attachment, entry, uncoating, and release. We'll also examine how DNA- and RNA-containing viruses differ with respect to their processes of biosynthesis.

Attachment

Like bacteriophages, animal viruses have attachment sites that attach to complementary receptor sites on the host cell's surface. However, the receptor sites of animal cells are proteins and glycoproteins of the plasma membrane. Moreover, animal viruses don't possess appendages like the tail fibers of some bacteriophages. The attachment sites of animal viruses are distributed over the surface of the virus, and the sites themselves vary from one group of viruses to another. In adenoviruses, which are icosahedral viruses, the attachment sites are small fibers at the corners of the icosahedron (see Figure 13.2b). In many of the enveloped viruses, such as influenza virus, the attachment sites are spikes located on the surface of the envelope (see Figure 13.3b). As soon as one spike attaches to a host receptor, additional receptor sites on the same cell migrate to the virus. Attachment is completed when many sites are bound.

Receptor sites are proteins of the host cell. The proteins have normal functions for the host and are hijacked by the virus. This could account for the individual differences in susceptibility to a particular virus. For example, people who lack the cellular receptor (called P antigen) for parvovirus B19 are naturally resistant to infection and don't get fifth disease (see page 595). Understanding the nature of attachment can lead to the development of drugs that prevent viral infections. Monoclonal antibodies (discussed in Chapter 18) that combine with a virus's attachment site or the cell's receptor site may soon be used to treat some viral infections.

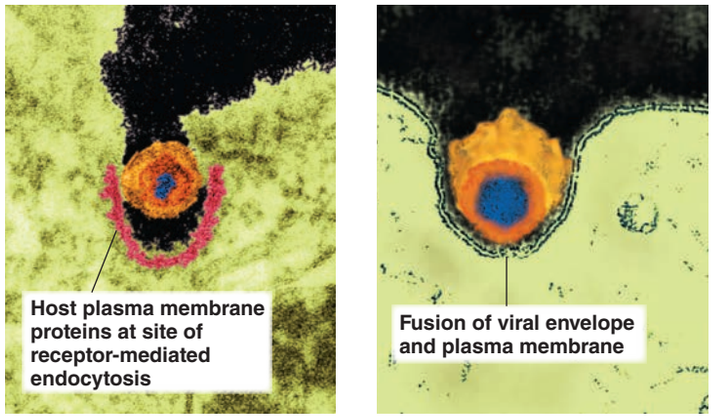
Entry

Following attachment, entry occurs. Many viruses enter into eukaryotic cells by **receptor-mediated endocytosis** (Chapter 4, page 97). A cell's plasma membrane continuously folds inward to form vesicles. These vesicles contain elements that originate outside the cell and are brought into the interior of the cell to be digested. If a virion attaches to the plasma membrane of a potential host cell, the host cell will enfold the virion and form a vesicle (Figure 13.14a).

Enveloped viruses can enter by an alternative method called **fusion,** in which the viral envelope fuses with the plasma membrane and releases the capsid into the cell's cytoplasm. (Figure 13.14b).

Uncoating

Viruses disappear during the eclipse period of an infection because they are taken apart inside the cell. **Uncoating** is the separation of the viral nucleic acid from its protein coat. This process varies with the type of virus. Some animal viruses accomplish uncoating by the action of lysosomal enzymes of the host cell. These enzymes degrade the proteins of the viral



(a) Entry of pig retrovirus by receptor-mediated endocytosis.

(b) Entry of herpesvirus by fusion.

Figure 13.14 The entry of viruses into host cells. After attachment, viruses enter host cells by **(a)** receptor-mediated endocytosis or **(b)** fusion of the viral envelope and cell membrane.

Q In which process is the cell actively taking in the virus?

capsid. The uncoating of poxviruses is completed by a specific enzyme encoded by the viral DNA and synthesized soon after infection. Uncoating of influenza virus occurs at the lower pH in a vesicle. Uncoating of togaviruses occurs at ribosomes in the host cytoplasm.

The biggest differences between viruses occur during biosynthesis of viral components. We'll discuss biosynthesis of DNA viruses and then biosynthesis of RNA viruses.

ASM: The replication cycles of viruses (lytic and lysogenic) differ among viruses and are determined by their unique structures and genomes.

The Biosynthesis of DNA Viruses

Generally, DNA-containing viruses replicate their DNA in the nucleus of the host cell by using viral enzymes, and they synthesize their capsid and other proteins in the cytoplasm by using host cell enzymes. Then the proteins migrate into the nucleus and are joined with the newly synthesized DNA to form virions. These virions are transported along the endoplasmic reticulum to the host cell's membrane for release. Herpesviruses, papovaviruses, adenoviruses, and hepadnaviruses all follow this pattern of biosynthesis (Table 13.4). Poxviruses are an exception because all of their components are synthesized in the cytoplasm.

As an example of the multiplication of a DNA virus, the sequence of events in papovavirus is shown in Figure 13.15.

1–2 Following attachment, entry, and uncoating, the viral DNA is released into the nucleus of the host cell.

3 Transcription of a portion of the viral DNA—the "early" genes—occurs next. Translation follows. The products of these genes are enzymes that are required for the multiplication of viral DNA. In most DNA viruses, early transcription is carried out with the host's transcriptase (RNA polymerase); poxviruses, however, contain their own transcriptase.

4 Sometime after the initiation of DNA replication, transcription and translation of the remaining "late" viral genes occur. Late proteins include capsid and other structural proteins.

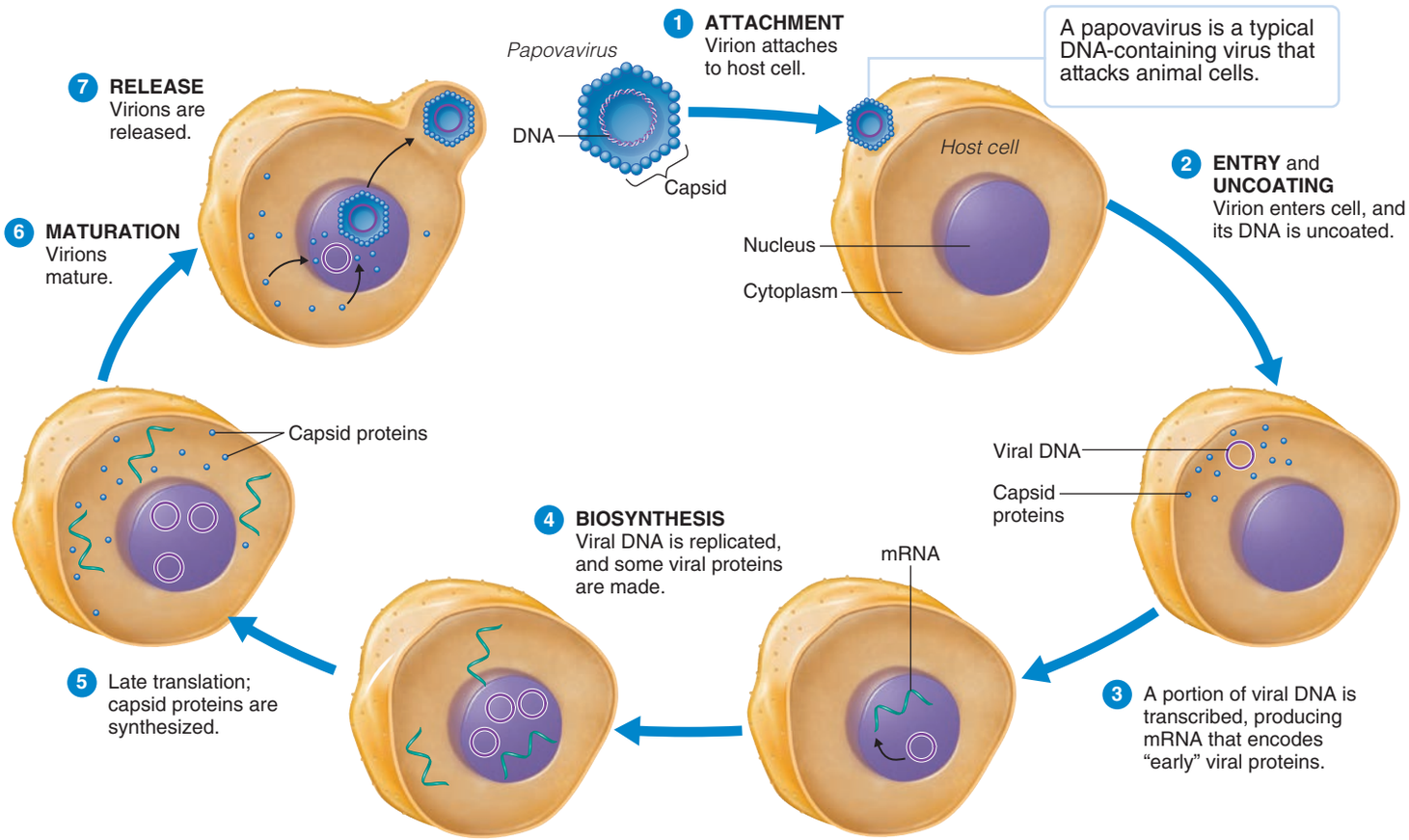
5 This leads to the synthesis of capsid proteins, which occurs in the cytoplasm of the host cell.

TABLE 13.4 The Biosynthesis of DNA and RNA Viruses Compared

Viral Nucleic Acid	Virus Family	Special Features of Biosynthesis
DNA, single-stranded	Parvoviridae	Cellular enzyme transcribes viral DNA in nucleus.
DNA, double-stranded	Herpesviridae	Cellular enzyme transcribes viral DNA in nucleus.
	Papovaviridae	
	Poxviridae	Viral enzyme transcribes viral DNA in cytoplasm.
DNA, reverse transcriptase	Hepadnaviridae	Cellular enzyme transcribes viral DNA in nucleus; reverse transcriptase copies mRNA to make viral DNA.
RNA, + strand	Picornaviridae Togaviridae	Viral RNA functions as a template for synthesis of RNA polymerase, which copies – strand RNA to make mRNA in cytoplasm.
RNA, – strand	Rhabdoviridae	Viral enzyme copies viral RNA to make mRNA in cytoplasm.
RNA, double-stranded	Reoviridae	Viral enzyme copies – strand RNA to make mRNA in virion, in cytoplasm.
RNA, reverse transcriptase	Retroviridae	Viral enzyme copies viral RNA to make DNA in cytoplasm; DNA moves to nucleus.

FOUNDATION FIGURE 13.15

Replication of a DNA-Containing Animal Virus



KEY CONCEPTS

- Viral replication in animals generally follows these steps: attachment, entry, uncoating, biosynthesis of nucleic acids and proteins, maturation, and release.
- Knowledge of viral replication phases is important for drug development strategies, and for understanding disease pathology.

6 After the capsid proteins migrate into the nucleus of the host cell, maturation occurs; the viral DNA and capsid proteins assemble to form complete viruses.

7 Complete viruses are then released from the host cell.

Some DNA viruses are described below.

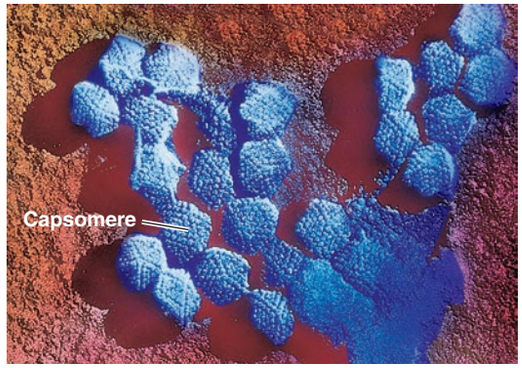
Adenoviridae Named after adenoids, from which they were first isolated, adenoviruses cause acute respiratory diseases—the common cold (**Figure 13.16a**).

Poxviridae All diseases caused by poxviruses, including smallpox and cowpox, include skin lesions (see Figure 21.10, page 591). *Pox* refers to pus-filled lesions. Viral multiplication is started by viral transcriptase; the viral components are synthesized and assembled in the cytoplasm of the host cell.

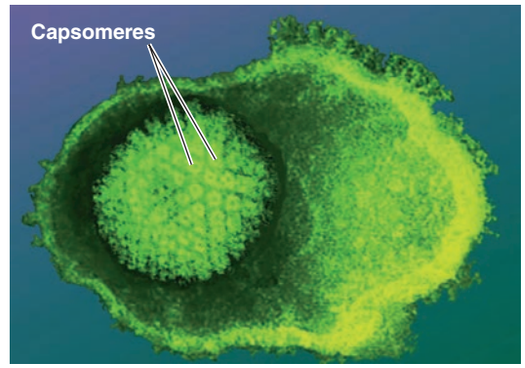
Herpesviridae Nearly 100 herpesviruses are known (Figure 13.16b). They are named after the spreading *(herpetic)* appearance of cold sores. Species of human herpesviruses (HHV) include HHV-1 and HHV-2, both in the genus *Simplexvirus,* which cause cold sores; HHV-3, genus *Varicellovirus,* which causes chickenpox; HHV-4, genus *Lymphocryptovirus,* which causes infectious mononucleosis; HHV-5, genus *Cytomegalovirus,* which causes CMV inclusion disease; HHV-6, genus *Roseolovirus,* which causes roseola; HHV-7, *Roseolovirus,* which infects most infants, causing measles-like rashes; and HHV-8, *Rhadinovirus,* which causes Kaposi's sarcoma, primarily in AIDS patients.

Figure 13.16 DNA-containing animal viruses. **(a)** Negatively stained adenoviruses that have been concentrated in a centrifuge. The individual capsomeres are clearly visible. **(b)** The envelope around this human herpes virus capsid has broken, giving a "fried egg" appearance.

Q What is the morphology of these viruses?



(a) *Mastadenovirus* SEM 100 nm



(b) *Simplexvirus* TEM 50 nm

Papovaviridae Papovaviruses are named for *pa*pillomas (warts), *po*lyomas (tumors), and *va*cuolation (cytoplasmic vacuoles produced by some of these viruses). Warts are caused by members of the genus *Papillomavirus*. Some *Papillomavirus* species are capable of transforming cells and causing cancer. Viral DNA is replicated in the host cell's nucleus along with host cell chromosomes. Host cells may proliferate, resulting in a tumor.

Hepadnaviridae Hepadnaviridae are so named because they cause *hepa*titis and contain *DNA* (Figure 25.14, page 727). The only genus in this family causes hepatitis B. (Hepatitis A, C, D, E, F, and G viruses, although not related to each other, are RNA viruses. Hepatitis is discussed in Chapter 25.) Hepadnaviruses differ from other DNA viruses because they synthesize DNA by copying RNA, using viral reverse transcriptase. This DNA is the template for viral mRNA and the virus's DNA genome. This enzyme is discussed later with the retroviruses, the only other family with reverse transcriptase.

The Biosynthesis of RNA Viruses

The multiplication of RNA viruses is essentially the same as that of DNA viruses, except RNA viruses multiply in the host cell's cytoplasm. Several different mRNA formation mechanisms occur among different groups of RNA viruses (see Table 13.4). Although the details of these mechanisms are beyond the scope of this text, for comparative purposes we'll trace the multiplication cycles of the four nucleic acid types of RNA viruses (three of which are shown in **Figure 13.17**). The major differences among the multiplication processes lie in how mRNA and viral RNA are produced. These viruses have **RNA-dependent RNA polymerase.** This enzyme isn't encoded in any cell's genome. Viral genes causes the enzyme to be made by a host cell. This enzyme catalyzes the synthesis of another strand of RNA, which is complementary in base sequence to the original infecting strand. Once viral RNA and viral proteins are synthesized, maturation occurs by similar means among all animal viruses, as we'll discuss shortly.

Picornaviridae Picornaviruses, such as enteroviruses and poliovirus (see Chapter 22, page 618), are single-stranded RNA viruses. They are the smallest viruses; and the prefix *pico-* (small) plus *RNA* gives these viruses their name. The RNA within the virion is called a **sense strand (+ strand),** because it can act as mRNA. After attachment, penetration, and uncoating are completed, the single-stranded viral RNA is translated into two principal proteins. One inhibits the host cell's synthesis of RNA, and the other is RNA-dependent RNA polymerase. This enzyme copies the virus sense strand and makes an **antisense strand (– strand),** which serves as a template to produce additional + strands. The + strands may serve as mRNA for the translation of capsid proteins, may become incorporated into capsid proteins to form a new virus, or may serve as a template for continued RNA multiplication. Once viral RNA and viral protein are synthesized, maturation occurs.

Clinical Case

Based on the abnormal LFT, Tina's physician diagnoses her with infectious hepatitis. This isn't the first case he's seen this month. As a matter of fact, the local health department has received reports of 31 other people with hepatitis. For a town of 4000 people, this is a large number. The health department needs to know what type of hepatitis they are dealing with because the term *hepatitis* describes any inflammation of the liver. Infectious hepatitis can be caused by a member of the Picornaviridae, Hepadnaviridae, or Flaviviridae.

The health department will need to differentiate among these viral families. List the method of transmission, morphology, nucleic acid, and type of replication for these three viral families. (*Hint*: See Diseases in Focus 25.3 on page 728 for a complete list of the hepatitis viruses.)

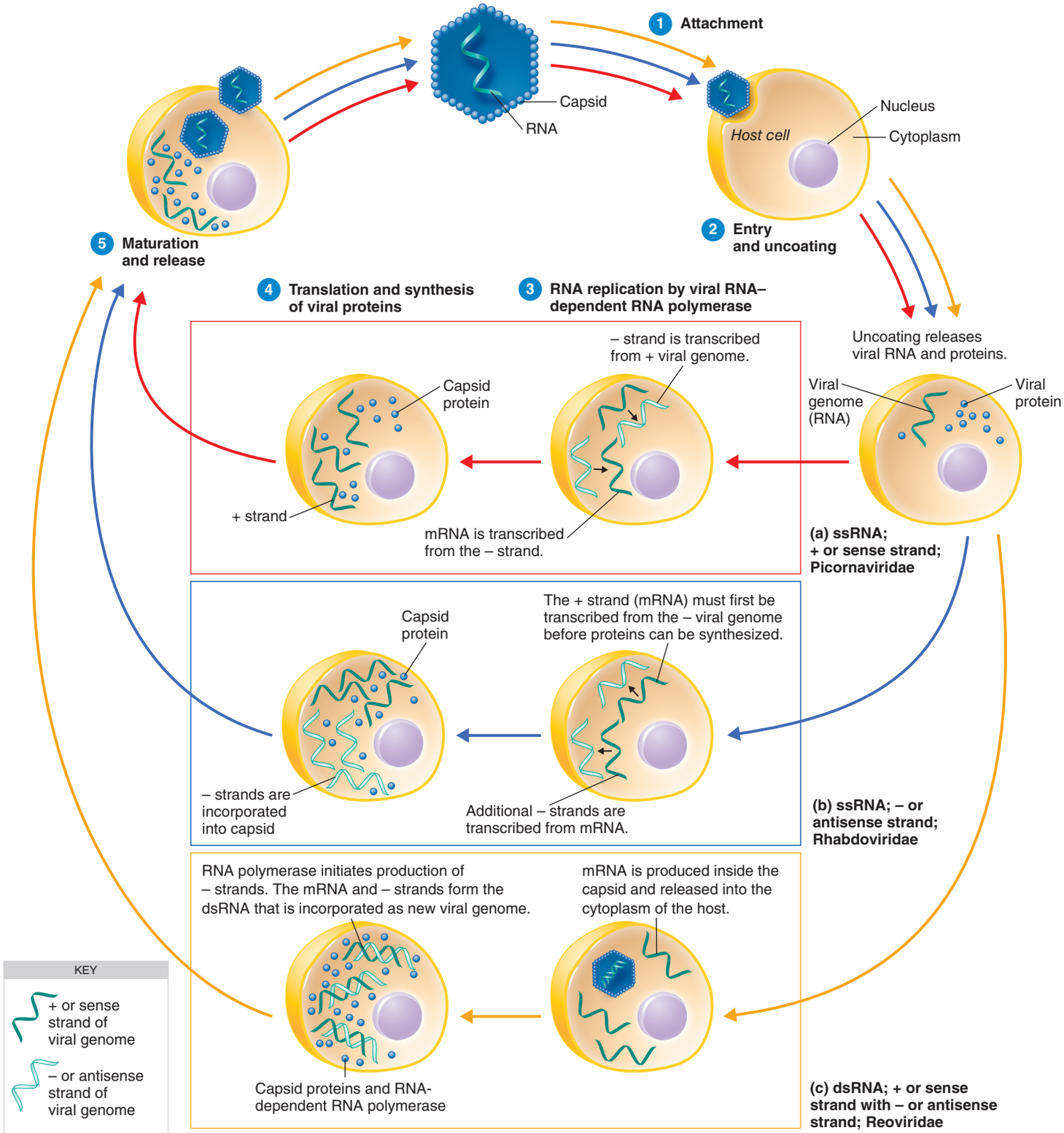
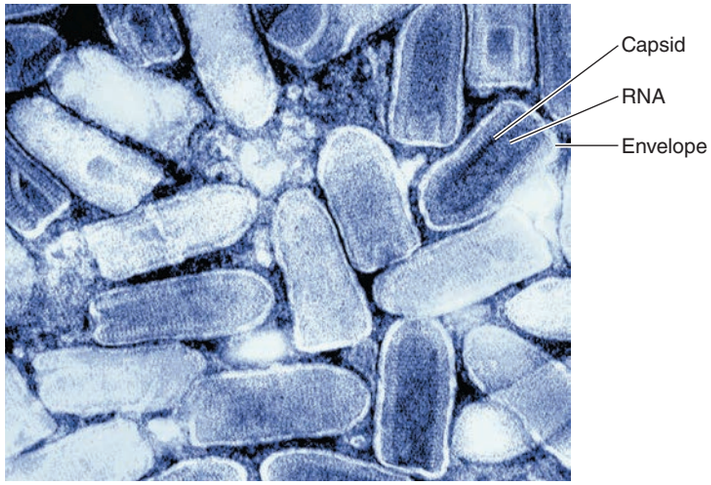


Figure 13.17 Pathways of multiplication used by various RNA-containing viruses. **(a)** After uncoating, single-stranded RNA (ssRNA) viruses with a + strand genome are able to synthesize proteins directly from their + strand. Using the + strand as a template, they transcribe – strands to produce additional + strands to serve as mRNA and be incorporated into capsid proteins as the viral genome. **(b)** The ssRNA viruses with a – strand genome must transcribe a + strand to serve as mRNA before they begin synthesizing proteins. The mRNA transcribes additional – strands for incorporation into capsid protein. Both ssRNA and **(c)** dsRNA viruses must use mRNA (+ strand) to code for proteins, including capsid proteins.

Q Why is – strand RNA made by picornaviruses and reoviruses? By rhabdoviruses?



(a) A rhabdovirus

(b) A filovirus

Figure 13.18 RNA-containing animal viruses. **(a)** Vesicular stomatitis viruses, a member of the family Rhabdoviridae. **(b)** Marburg virus, found in African cave bats, causes hemorrhagic fever in humans.

Q Why do viruses with a + strand of RNA make a – strand of RNA?

Togaviridae Togaviruses, which include *ar*thropod-*bo*rne *arbo*viruses in the genus *Alphavirus* (see Chapter 22, page 624), also contain a single + strand of RNA. Togaviruses are enveloped viruses; their name is from the Latin word for covering, *toga*. Keep in mind that these aren't the only enveloped viruses. After a – strand is made from the + strand, two types of mRNA are transcribed from the – strand. One type of mRNA is a short strand that codes for envelope proteins; the other, longer strand serves as mRNA for capsid proteins and can become incorporated into a capsid.

Rhabdoviridae Rhabdoviruses, such as rabies virus (genus *Lyssavirus;* see Chapter 22, page 620), are usually bullet-shaped (Figure 13.18a). *Rhabdo-* is from the Greek word for rod, which isn't really an accurate description of their morphology. They contain a single – strand of RNA. They also contain an RNA-dependent RNA polymerase that uses the – strand as a template from which to produce a + strand. The + strand serves as mRNA and as a template for synthesis of new viral RNA.

Reoviridae Reoviruses were named for their habitats: the respiratory and enteric (digestive) systems of humans. They weren't associated with any diseases when first discovered, so they were considered orphan viruses. Their name comes from the first letters of *r*espiratory, *e*nteric, and *o*rphan. Three serotypes are now known to cause respiratory tract and intestinal tract infections.

The capsid containing the double-stranded RNA is digested upon entering a host cell. Viral mRNA is produced in the cytoplasm, where it's used to synthesize more viral proteins. One of the newly synthesized viral proteins acts as RNA-dependent RNA polymerase to produce more – strands of RNA. The mRNA and – strand form the double-stranded RNA that is then surrounded by capsid proteins.

Biosynthesis of RNA Viruses That Use DNA

This group includes retroviruses and oncogenic RNA viruses.

Retroviridae Many retroviruses infect vertebrates (Figure 13.18b). One genus of retrovirus, *Lentivirus,* includes the subspecies HIV-1 and HIV-2, which cause AIDS (see Chapter 19, pages 535–544). The retroviruses that cause cancer will be discussed later in this chapter.

The formation of mRNA and RNA for new retrovirus virions is shown in Figure 13.19. These viruses carry **reverse transcriptase,** which uses the viral RNA as a template to produce complementary double-stranded DNA. This enzyme also degrades the original viral RNA. The name *retrovirus* is derived from the first letters of *re*verse *tr*anscriptase. The viral DNA is then integrated into a host cell chromosome as a **provirus.** Unlike a prophage, the provirus never comes out of the chromosome. As a provirus, HIV is protected from the host's immune system and antiviral drugs.

Sometimes the provirus simply remains in a latent state and replicates when the DNA of the host cell replicates. In other cases, the provirus is expressed and produces new viruses, which may infect adjacent cells. Mutagens such as gamma radiation can induce expression of a provirus. In oncogenic retroviruses, the provirus can also convert the host cell into a tumor cell; possible mechanisms for this phenomenon will be discussed later.

Maturation and Release

The first step in viral maturation is the assembly of the protein capsid; this assembly is usually a spontaneous process. The capsids of many animal viruses are enclosed by an envelope consisting of

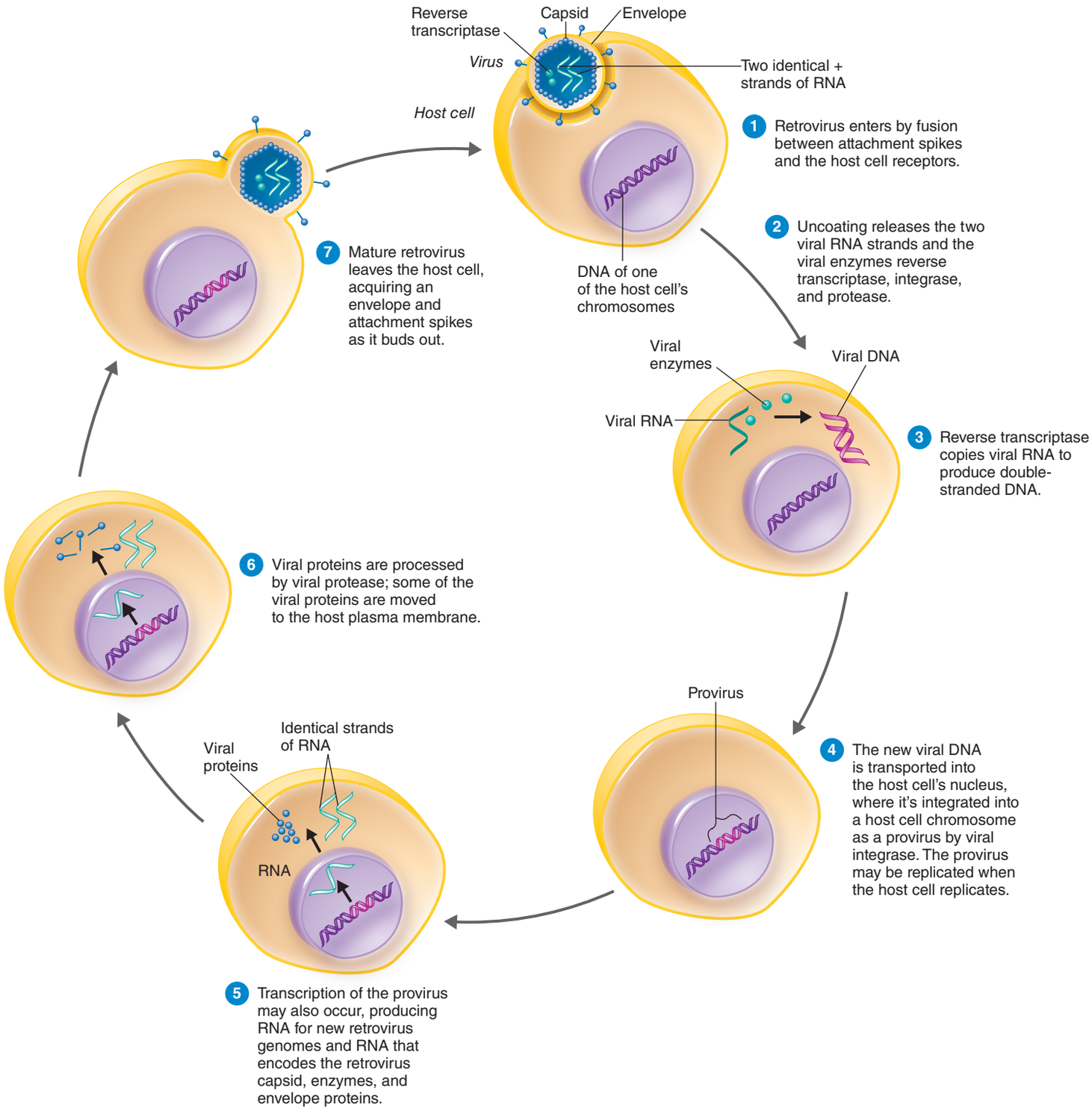
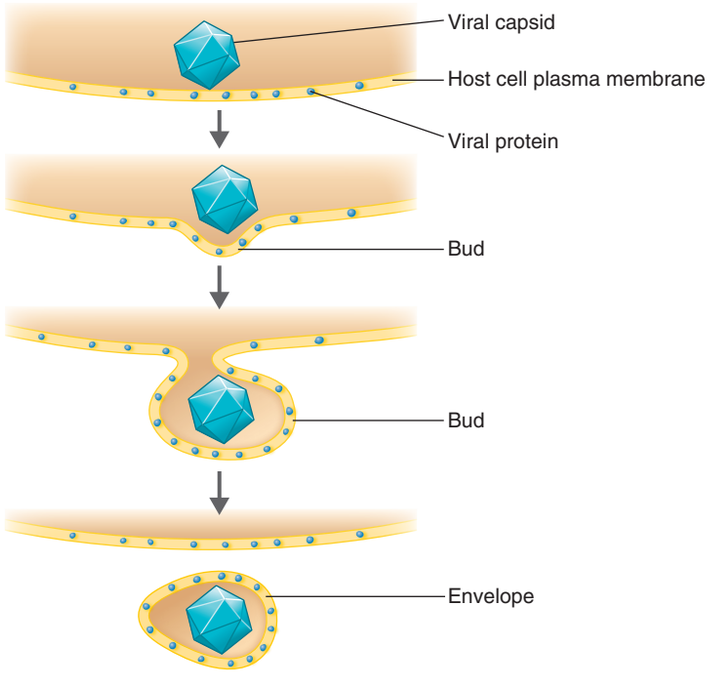
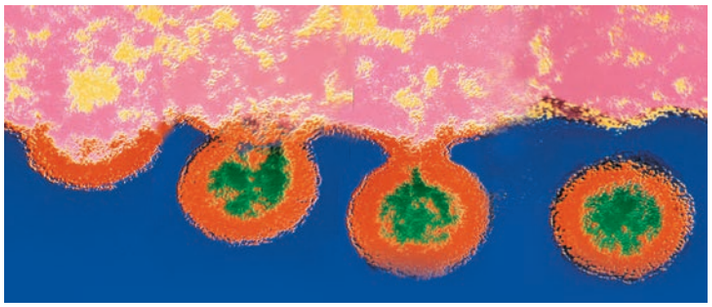


Figure 13.19 Multiplication and inheritance processes of the Retroviridae. A retrovirus may become a provirus that replicates in a latent state, and it may produce new retroviruses.

Q How does the biosynthesis of a retrovirus differ from that of other RNA viruses?



(a) Release by budding



(b) *Lentivirus*

TEM 50 nm

Figure 13.20 Budding of an enveloped virus. **(a)** A diagram of the budding process. **(b)** HIV budding from a T cell. Notice that the four budding viruses acquire their coats from the host plasma membrane.

Q Of what is a viral envelope composed?

protein, lipid, and carbohydrate, as noted earlier. Examples of such viruses include orthomyxoviruses and paramyxoviruses. The envelope protein is encoded by the viral genes and is incorporated into the plasma membrane of the host cell. The envelope lipid and carbohydrate are encoded by host cell genes and are present in the plasma membrane. The envelope actually develops around the capsid by a process called **budding** (Figure 13.20).

After the sequence of attachment, entry, uncoating, and biosynthesis of viral nucleic acid and protein, the assembled capsid containing nucleic acid pushes through the plasma membrane. As a result, a portion of the plasma membrane, now the envelope, adheres to the virus. This extrusion of a virus from a host cell is one method of release. Budding doesn't kill the host cell immediately, and in some cases the host cell survives.

Nonenveloped viruses are released through ruptures in the host cell plasma membrane. In contrast to budding, this type of release usually results in the death of the host cell. MM **Animations** Viral Replication: Overview; Animal Viruses

CHECK YOUR UNDERSTANDING

- Describe the principal events of attachment, entry, uncoating, biosynthesis, maturation, and release of an enveloped DNA-containing virus. **13-10**

Viruses and Cancer

LEARNING OBJECTIVES

13-11 Define *oncogene* and *transformed cell*.

13-12 Discuss the relationship between DNA- and RNA-containing viruses and cancer.

Several types of cancer are now known to be caused by viruses. Molecular biological research shows that the mechanisms of the diseases are similar, even when a virus doesn't cause the cancer.

The relationship between cancers and viruses was first demonstrated in 1908, when virologists Wilhelm Ellerman and Olaf Bang, working in Denmark, were trying to isolate the causative agent of chicken leukemia. They found that leukemia could be transferred to healthy chickens by cell-free filtrates that contained viruses. Three years later, F. Peyton Rous, working at the Rockefeller Institute in New York, found that a chicken **sarcoma** (cancer of connective tissue) can be similarly transmitted. Virus-induced **adenocarcinomas** (cancers of glandular epithelial tissue) in mice were discovered in 1936. At that time, it was clearly shown that mouse mammary gland tumors

Clinical Case

Hepatitis A virus, a nonenveloped + stranded RNA virus, is transmitted via the fecal–oral route. Hepatitis B virus is an enveloped double-stranded DNA virus. It has reverse transcriptase and is transmitted by the parenteral route (intravenous injection) or through sexual contact. Hepatitis C virus is also transmitted parenterally and is an enveloped + stranded RNA virus.

Based on this information, what conclusion can the health department make about which hepatitis virus is most likely infecting Tina and the other 31 people in this town?

are transmitted from mother to offspring through the mother's milk. The cancer-causing SE polyoma virus was discovered and isolated in 1953 by American scientists Bernice Eddy and Sarah Stewart.

The viral cause of cancer can often go unrecognized for several reasons. First, most of the particles of some viruses infect cells but don't induce cancer. Second, cancer might not develop until long after viral infection. Third, cancers, even those caused by viruses, don't seem to be contagious, as viral diseases usually are.

The Transformation of Normal Cells into Tumor Cells

Almost anything that can alter the genetic material of a eukaryotic cell has the potential to make a normal cell cancerous. These cancer-causing alterations to cellular DNA affect parts of the genome called **oncogenes.** Oncogenes were first identified in cancer-causing viruses and were thought to be a part of the normal viral genome. However, American microbiologists J. Michael Bishop and Harold E. Varmus received the 1989 Nobel Prize in Medicine for proving that the cancer-inducing genes carried by viruses are actually derived from animal cells. Bishop and Varmus showed that the cancer-causing *src* gene in avian sarcoma viruses is derived from a normal part of chicken genes.

Oncogenes can be activated to abnormal functioning by a variety of agents, including mutagenic chemicals, high-energy radiation, and viruses. Viruses capable of inducing tumors in animals are called **oncogenic viruses,** or *oncoviruses.* Approximately 10% of cancers are known to be virus-induced. A defining feature of all oncogenic viruses is that their genetic material integrates into the host cell's DNA and replicates along with the host cell's chromosome. This mechanism is similar to the phenomenon of lysogeny in bacteria, and it can alter the host cell's characteristics in the same way.

Tumor cells undergo **transformation;** that is, they acquire properties that are distinct from the properties of uninfected cells or from infected cells that don't form tumors. After being transformed by viruses, many tumor cells contain a virus-specific antigen on their cell surface, called **tumor-specific transplantation antigen (TSTA),** or an antigen in their nucleus, called the **T antigen.** Transformed cells tend to be irregularly shaped, compared to normal cells, and they tend to exhibit certain chromosomal abnormalities, such as unusual numbers of chromosomes and fragmented chromosomes.

Clinical Case

It is improbable that over 30 people of different ages and backgrounds are all IV drug users, so the most likely virus is hepatitis A. To investigate the source of the viral infection, the health department compares foods eaten by the 32 ill people with asymptomatic household members. All 32, including Tina, had eaten an ice-slush beverage purchased from a local convenience store. The health department determines that the convenience store clerk, unknowingly infected with hepatitis A virus, transferred the virus to the machine that makes the icy beverage. Over the next several months, Tina's symptoms subside, and her liver function returns to normal.

How can knowing the identity of the virus affect the health department's recommendation for treatment and for prevention of future outbreaks?

359 376 380 381 382

DNA Oncogenic Viruses

Oncogenic viruses are found within several families of DNA-containing viruses. These groups include the Adenoviridae, Herpesviridae, Poxviridae, Papovaviridae, and Hepadnaviridae. Among the papovaviruses, papillomaviruses cause uterine (cervical) cancer.

Virtually all cervical and anal cancers are caused by human papillomavirus (HPV). A vaccine against four HPVs is recommended for 11- to 12-year-old girls and boys.

Epstein-Barr (EB) virus was isolated from Burkitt's lymphoma cells in 1964 by Michael Epstein and Yvonne Barr. The proof that EB virus can cause cancer was accidentally demonstrated in 1985 when a 12-year-old boy known only as David received a bone marrow transplant. Several months after the transplant, he died of cancer. An autopsy revealed that the virus had been unwittingly introduced into the boy with the bone marrow transplant.

Another DNA virus that causes cancer is hepatitis B virus (HBV). Many animal studies have been performed that have clearly indicated the causal role of HBV in liver cancer. In one human study, virtually all people with liver cancer had previous HBV infections.

RNA Oncogenic Viruses

Among the RNA viruses, only the oncoviruses in the family Retroviridae cause cancer. The human T cell leukemia viruses (HTLV-1 and HTLV-2) are retroviruses that cause adult T cell leukemia and lymphoma in humans. (T cells are a type of white blood cell involved in the immune response.)

Sarcoma viruses of cats, chickens, and rodents, and the mammary tumor viruses of mice, are also retroviruses. Another retrovirus, feline leukemia virus (FeLV), causes leukemia in cats and is transmissible among cats. There is a test to detect the virus in cat serum.

Clinical Case Resolved

Drugs and vaccines work against specific viruses. There are no special treatments for hepatitis, but the preventive measures are different. For example, in this case, the health department recommends that everyone who ate at this convenience store in the previous 2 weeks receive hepatitis A vaccine and hepatitis A immunoglobulin.

Viruses were originally named for the symptoms they caused, hence the name "hepatitis virus" for a virus that affects the liver (from Latin *hepaticus*). This naming convention is imprecise but was the only method available until recently.

Molecular tools now allow viruses to be classified on the basis of genomes and morphology. Thus, related viruses, which may affect different tissues, are grouped into the same families. Differentiating viruses based on their genetic information provides valuable information for treatment and prevention.

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The ability of retroviruses to induce tumors is related to their production of a reverse transcriptase by the mechanism described earlier (see Figure 13.19). The provirus, which is the double-stranded DNA molecule synthesized from the viral RNA, becomes integrated into the host cell's DNA; new genetic material is thereby introduced into the host's genome and is the key reason retroviruses can contribute to cancer. Some retroviruses contain oncogenes; others contain promoters that turn on oncogenes or other cancer-causing factors.

Viruses to Treat Cancer

Oncolytic viruses infect and lyse cancer cells. Discovered in the early 1900s when physicians observed that tumors regressed in patients with concurrent viral infections, oncolytic viruses include adenovirus, vaccinia virus, and *Simplexvirus*. Clinical trials in humans and mice suggest that oncolytic viral therapy may provide treatment for some cancers. Several viruses are known to selectively infect cancer cells, and these are being genetically modified to remove virulence genes and add colony-stimulating factor genes to promote white blood cells.

CHECK YOUR UNDERSTANDING

- What is a provirus? **13-11**
- How can an RNA virus cause cancer if it doesn't have DNA to insert into a cell's genome? **13-12**

Latent Viral Infections

LEARNING OBJECTIVE

13-13 Provide an example of a latent viral infection.

A virus can remain in equilibrium with the host and not actually produce disease for a long period, often many years. The oncogenic viruses just discussed are examples of such latent infections. All of the human herpesviruses can remain in host cells throughout the life of an individual. When herpesviruses are reactivated by immunosuppression (for example, AIDS), the resulting infection may be fatal. The classic example of such a **latent infection** is the infection of the skin by *Simplexvirus*, which produces cold sores. This virus inhabits the host's nerve cells but causes no damage until it is activated by a stimulus such as fever or sunburn—hence the term *fever blister*.

In some individuals, viruses are produced, but symptoms never appear. Even though a large percentage of the human population carries *Simplexvirus*, only 10–15% of people carrying the virus exhibit the disease.

The chickenpox virus *(Varicellovirus)* can also exist in a latent state. Chickenpox (varicella) is a skin disease that is usually acquired in childhood. The virus gains access to the skin via the blood. From the blood, some viruses may enter nerves, where they remain latent. Later, changes in the immune (T cell) response can activate these latent viruses, causing shingles (zoster). The shingles rash appears on the skin along the nerve in which the virus was latent. Shingles occurs in 10–20% of people who have had chickenpox.

Persistent Viral Infections

LEARNING OBJECTIVE

13-14 Differentiate persistent viral infections from latent viral infections.

A **persistent viral infection** (or **chronic viral infection**) occurs gradually over a long period. Typically, persistent viral infections are fatal.

A number of persistent viral infections have in fact been shown to be caused by conventional viruses. For example, several years after causing measles, the measles virus can be responsible for a rare form of encephalitis called subacute sclerosing panencephalitis (SSPE). A persistent viral infection is apparently different from a latent viral infection in that, in most persistent viral infections, detectable infectious virus gradually builds up over a long period, rather than appearing suddenly (**Figure 13.21**).

Several examples of latent and persistent viral infections are listed in **Table 13.5**.

CHECK YOUR UNDERSTANDING

- Is shingles a persistent or latent infection? **13-13,13-14**

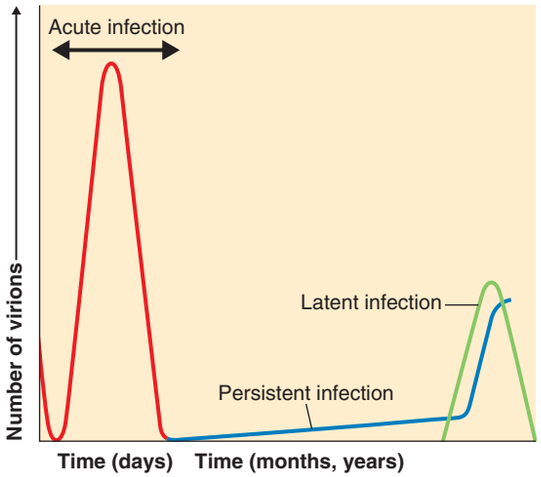


Figure 13.21 Latent and persistent viral infections.

Q How do latent and persistent infections differ?

Prions

LEARNING OBJECTIVE

13-15 Discuss how a protein can be infectious.

A few infectious diseases are caused by prions. In 1982, American neurobiologist Stanley Prusiner proposed that infectious proteins caused a neurological disease in sheep called scrapie. The infectivity of scrapie-infected brain tissue is reduced by treatment with proteases but not by treatment with radiation, suggesting that the infectious agent is pure protein. Prusiner coined the name **prion** for *pro*teinaceous *in*fectious particle.

Nine animal diseases now fall into this category, including the "mad cow disease" that emerged in cattle in Great Britain in 1987. All nine are neurological diseases called spongiform encephalopathies because large vacuoles develop in the brain (Figure 22.18b, page 630). The human diseases are kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia. (Neurological diseases are discussed in Chapter 22.) These diseases often run in families, which indicates a possible genetic cause. However, they aren't only inherited, because mad cow disease arose from feeding scrapie-infected sheep meat to cattle, and the new (bovine) variant was transmitted to humans who ate undercooked beef from infected cattle (see Chapter 1, page 17). Additionally, CJD has been transmitted with transplanted nerve tissue and contaminated surgical instruments.

These diseases are caused by the conversion of a normal host glycoprotein called PrP^C (for cellular prion protein) into an infectious form called PrP^{Sc} (for scrapie protein). The gene for PrP^C is located on chromosome 20 in humans. Recent evidence suggests that PrP^C is involved in regulating cell death. (See the discussion of apoptosis on page 483.) One hypothesis for how an infectious agent that lacks any nucleic acid can reproduce is shown in **Figure 13.22**.

The actual cause of cell damage isn't yet known. Fragments of PrP^{Sc} molecules accumulate in the brain, forming plaques; these plaques are used for postmortem diagnosis, but they don't appear to be the cause of cell damage. MM **Animations** Prion Reproduction: Overview, Characteristics, Diseases

Plant Viruses and Viroids

LEARNING OBJECTIVES

13-16 Differentiate virus, viroid, and prion.

13-17 Describe the lytic cycle for a plant virus.

TABLE 13.5 Examples of Latent and Persistent Viral Infections in Humans

Disease	Primary Effect	Causative Virus
Latent	**No symptoms during latency; viruses not usually released**	
Cold sores	Skin and mucous membrane lesions; genital lesions	Herpes simplex 1 and 2
Leukemia	Increased white blood cell growth	HTLV-1 and -2
Shingles	Skin lesions	*Varicellovirus* (Herpesvirus)
Persistent	**Viruses continuously released**	
Cervical cancer	Increased cell growth	Human papillomavirus
HIV/AIDS	Decreased $CD4^+$ T cells	HIV-1 and -2 (*Lentivirus*)
Liver cancer	Increased cell growth	Hepatitis B virus
Persistent enterovirus infection	Mental deterioration associated with AIDS	Echoviruses
Progressive encephalitis	Rapid mental deterioration	Rubella virus
Subacute sclerosing panencephalitis (SSPE)	Mental deterioration	Measles virus

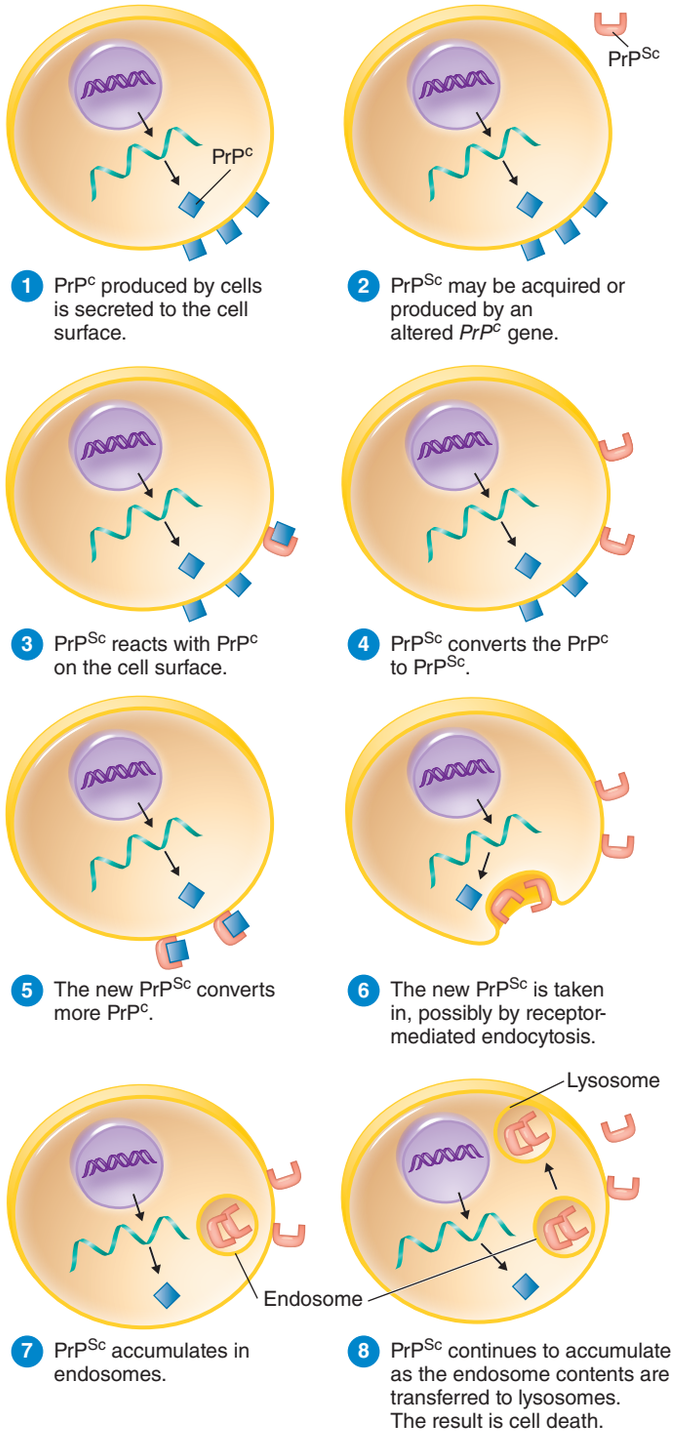


Figure 13.22 How a protein can be infectious. If an abnormal prion protein (PrP^{Sc}) enters a cell, it changes a normal prion protein to PrP^{Sc}, which now can change another normal PrP^{C}, resulting in an accumulation of the abnormal PrP^{Sc}.

Q How do prions differ from viruses?

Plant viruses resemble animal viruses in many respects: plant viruses are morphologically similar to animal viruses, and they have similar types of nucleic acid (Table 13.6). In fact, some plant viruses can multiply inside insect cells. Plant viruses cause many diseases of economically important crops, including beans (bean mosaic virus), corn and sugarcane (wound tumor virus), and potatoes (potato yellow dwarf virus). Viruses can cause color change, deformed growth, wilting, and stunted growth in their plant hosts. Some hosts, however, remain symptomless and only serve as reservoirs of infection.

Plant cells are generally protected from disease by an impermeable cell wall. Viruses must enter through wounds or be assisted by other plant parasites, including nematodes, fungi, and, most often, insects that suck the plant's sap. Once one plant is infected, it can spread infection to other plants in its pollen.

In laboratories, plant viruses are cultured in protoplasts (plant cells with the cell walls removed) and in insect cell cultures.

Some plant diseases are caused by **viroids,** short pieces of naked RNA, only 300 to 400 nucleotides long, with no protein coat. The nucleotides are often internally paired, so the molecule has a closed, folded, three-dimensional structure that presumably helps protect it from attack by cellular enzymes. The RNA doesn't code for any proteins. Thus far, viroids have been conclusively identified as pathogens only of plants. Annually, infections by viroids, such as potato spindle tuber viroid, result in losses of millions of dollars from crop damage (Figure 13.23).

Current research on viroids has revealed similarities between the base sequences of viroids and introns. Recall from Chapter 8 (page 212) that introns are sequences of genetic material that don't code for polypeptides. This observation has led to the hypothesis that viroids evolved from introns, leading to speculation that researchers may eventually discover animal viroids.

CHECK YOUR UNDERSTANDING

- Contrast viroids and prions, and for each name a disease it causes. **13-15, 13-16**
- How do plant viruses enter host cells? **13-17**

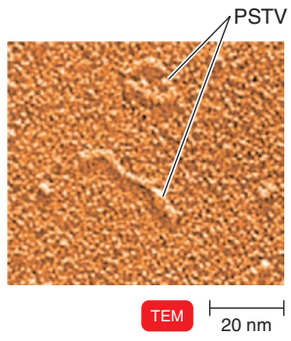
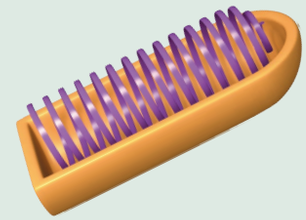


Figure 13.23 Linear and circular potato spindle tuber viroid (PSTV).

Q How do viroids differ from prions?

TABLE 13.6 Classification of Some Major Plant Viruses

Characteristic	Viral Family	Viral Genus or Unclassified Members	Morphology	Method of Transmission
Double-stranded DNA, nonenveloped	Caulimoviridae	Cauliflower mosaic virus		Aphids
Single-stranded RNA, + strand, nonenveloped	Bunyaviridae	Watermelon wilt		Whiteflies
	Virgaviridae	*Tobamovirus*		Wounds
Single-stranded RNA, – strand, enveloped	Rhabdoviridae	Potato yellow dwarf virus		Leafhoppers and aphids
Double-stranded RNA, nonenveloped	Reoviridae	Wound tumor virus		Leafhoppers

Study Outline

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General Characteristics of Viruses (pp. 359–360)

1. Depending on one's viewpoint, viruses may be regarded as exceptionally complex aggregations of nonliving chemicals or as exceptionally simple living microbes.
2. Viruses contain a single type of nucleic acid (DNA or RNA) and a protein coat, sometimes enclosed by an envelope composed of lipids, proteins, and carbohydrates.
3. Viruses are obligatory intracellular parasites. They multiply by using the host cell's synthesizing machinery to cause the synthesis of specialized elements that can transfer the viral nucleic acid to other cells.

Host Range (pp. 359–360)

4. *Host range* refers to the spectrum of host cells in which a virus can multiply.
5. Most viruses infect only specific types of cells in one host species.
6. Host range is determined by the specific attachment site on the host cell's surface and the availability of host cellular factors.

Viral Size (p. 360)

7. Viral size is ascertained by electron microscopy.
8. Viruses range from 20 to 1000 nm in length.

Viral Structure (pp. 360–362)

1. A virion is a complete, fully developed viral particle composed of nucleic acid surrounded by a coat.

Nucleic Acid (p. 361)

2. Viruses contain either DNA or RNA, never both, and the nucleic acid may be single- or double-stranded, linear or circular, or divided into several separate molecules.
3. The proportion of nucleic acid in relation to protein in viruses ranges from about 1% to about 50%.

Capsid and Envelope (pp. 361–362)

4. The protein coat surrounding the nucleic acid of a virus is called the capsid.
5. The capsid is composed of subunits, capsomeres, which can be a single type of protein or several types.
6. The capsid of some viruses is enclosed by an envelope consisting of lipids, proteins, and carbohydrates.
7. Some envelopes are covered with carbohydrate-protein complexes called spikes.

General Morphology (p. 362)

8. Helical viruses (for example, Ebola virus) resemble long rods, and their capsids are hollow cylinders surrounding the nucleic acid.
9. Polyhedral viruses (for example, adenovirus) are many-sided. Usually the capsid is an icosahedron.
10. Enveloped viruses are covered by an envelope and are roughly spherical but highly pleomorphic. There are also enveloped helical viruses (for example, influenza virus) and enveloped polyhedral viruses (for example, *Simplexvirus*).
11. Complex viruses have complex structures. For example, many bacteriophages have a polyhedral capsid with a helical tail attached.

Taxonomy of Viruses (pp. 362–363)

1. Classification of viruses is based on type of nucleic acid, strategy for replication, and morphology.
2. Virus family names end in *-viridae;* genus names end in *-virus.*
3. A viral species is a group of viruses sharing the same genetic information and ecological niche.

Isolation, Cultivation, and Identification of Viruses (pp. 363–369)

1. Viruses must be grown in living cells.
2. The easiest viruses to grow are bacteriophages.

Growing Bacteriophages in the Laboratory (p. 363)

3. The plaque method mixes bacteriophages with host bacteria and nutrient agar.
4. After several viral multiplication cycles, the bacteria in the area surrounding the original virus are destroyed; the area of lysis is called a plaque.
5. Each plaque originates with a single viral particle; the concentration of viruses is expressed as plaque-forming units.

Growing Animal Viruses in the Laboratory (pp. 363–368)

6. Cultivation of some animal viruses requires whole animals.
7. Simian AIDS and feline AIDS provide models for studying human AIDS.
8. Some animal viruses can be cultivated in embryonated eggs.
9. Cell cultures are animal or plant cells growing in culture media.
10. Primary cell lines and embryonic diploid cell lines grow for a short time in vitro.
11. Continuous cell lines can be maintained in vitro indefinitely.
12. Viral growth can cause cytopathic effects in the cell culture.

Viral Identification (pp. 368–369)

13. Serological tests are used most often to identify viruses.
14. Viruses may be identified by RFLPs and PCR.

Viral Multiplication (pp. 369–380)

1. Viruses don't contain enzymes for energy production or protein synthesis.
2. For a virus to multiply, it must invade a host cell and direct the host's metabolic machinery to produce viral enzymes and components.

Multiplication of Bacteriophages (pp. 369–372)

3. During the lytic cycle, a phage causes the lysis and death of a host cell.
4. Some viruses can either cause lysis or have their DNA incorporated as a prophage into the DNA of the host cell. The latter situation is called lysogeny.
5. During the attachment phase of the lytic cycle, sites on the phage's tail fibers attach to complementary receptor sites on the bacterial cell.
6. In penetration, phage lysozyme opens a portion of the bacterial cell wall, the tail sheath contracts to force the tail core through the cell wall, and phage DNA enters the bacterial cell. The capsid remains outside.
7. In biosynthesis, transcription of phage DNA produces mRNA coding for proteins necessary for phage multiplication. Phage DNA is replicated, and capsid proteins are produced. During the eclipse period, separate phage DNA and protein can be found.
8. During maturation, phage DNA and capsids are assembled into complete viruses.
9. During release, phage lysozyme breaks down the bacterial cell wall, and the new phages are released.
10. During the lysogenic cycle, prophage genes are regulated by a repressor coded for by the prophage. The prophage is replicated each time the cell divides.
11. Exposure to certain mutagens can lead to excision of the prophage and initiation of the lytic cycle.
12. Because of lysogeny, lysogenic cells become immune to reinfection with the same phage and may undergo phage conversion.
13. A lysogenic phage can transfer bacterial genes from one cell to another through transduction. Any genes can be transferred in generalized transduction, and specific genes can be transferred in specialized transduction.

Multiplication of Animal Viruses (pp. 373–380)

14. Animal viruses attach to the plasma membrane of the host cell.
15. Entry occurs by receptor-mediated endocytosis or fusion.
16. Animal viruses are uncoated by viral or host cell enzymes.
17. The DNA of most DNA viruses is released into the nucleus of the host cell. Transcription of viral DNA and translation produce viral DNA and, later, capsid proteins. Capsid proteins are synthesized in the cytoplasm of the host cell.
18. DNA viruses include Adenoviridae, Poxviridae, Herpesviridae, Papovaviridae, and Hepadnaviridae.
19. Multiplication of RNA viruses occurs in the cytoplasm of the host cell. RNA-dependent RNA polymerase synthesizes a double-stranded RNA.
20. Picornaviridae + strand RNA acts as mRNA and directs the synthesis of RNA-dependent RNA polymerase.
21. Togaviridae + strand RNA acts as a template for RNA-dependent RNA polymerase, and mRNA is transcribed from a new – RNA strand.
22. Rhabdoviridae – strand RNA is a template for viral RNA-dependent RNA polymerase, which transcribes mRNA.
23. Reoviridae are digested in host cell cytoplasm to release mRNA for viral biosynthesis.
24. Retroviridae reverse transcriptase (RNA-dependent DNA polymerase) transcribes DNA from RNA.
25. After assembly, viruses are released. One method of release (and envelope formation) is budding. Nonenveloped viruses are released through ruptures in the host cell membrane.

Viruses and Cancer (pp. 380–382)

1. The earliest relationship between cancer and viruses was demonstrated in the early 1900s, when chicken leukemia and chicken sarcoma were transferred to healthy animals by cell-free filtrates.

The Transformation of Normal Cells into Tumor Cells (p. 381)

2. When activated, oncogenes transform normal cells into cancerous cells.
3. Viruses capable of producing tumors are called oncogenic viruses.
4. Several DNA viruses and retroviruses are oncogenic.
5. The genetic material of oncogenic viruses becomes integrated into the host cell's DNA.
6. Transformed cells lose contact inhibition, contain virus-specific antigens (TSTA and T antigen), exhibit chromosome abnormalities, and can produce tumors when injected into susceptible animals.

DNA Oncogenic Viruses (p. 381)

7. Oncogenic viruses are found among the Adenoviridae, Herpesviridae, Poxviridae, Papovaviridae, and Hepadnaviridae.

RNA Oncogenic Viruses (pp. 381–382)

8. The virus's ability to produce tumors is related to the presence of reverse transcriptase. The DNA synthesized from the viral RNA becomes incorporated as a provirus into the host cell's DNA.
9. A provirus can remain latent, can produce viruses, or can transform the host cell.

Viruses to Treat Cancer (p. 382)

10. Oncolytic viruses infect and lyse cancer cells.

Latent Viral Infections (p. 382)

1. A latent viral infection is one in which the virus remains in the host cell for long periods without producing an infection.
2. Examples are cold sores and shingles.

Persistent Viral Infections (pp. 382–383)

1. Persistent viral infections are disease processes that occur over a long period and are generally fatal.
2. Persistent viral infections are caused by conventional viruses; viruses accumulate over a long period.

Prions (p. 383)

1. Prions are infectious proteins first discovered in the 1980s.
2. Prion diseases, such as CJD and mad cow disease, all involve the degeneration of brain tissue.
3. Prion diseases are the result of an altered protein; the cause can be a mutation in the normal gene for PrP^{C} or contact with an altered protein (PrP^{Sc}).

Plant Viruses and Viroids (pp. 383–385)

1. Plant viruses must enter plant hosts through wounds or with invasive parasites, such as insects.
2. Some plant viruses also multiply in insect (vector) cells.
3. Viroids are infectious pieces of RNA that cause some plant diseases, such as potato spindle tuber disease.

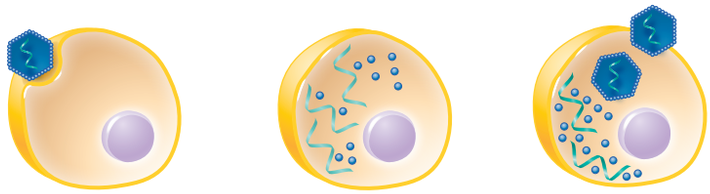
Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. Why do we classify viruses as obligatory intracellular parasites?
2. List the four properties that define a virus. What is a virion?
3. Describe the four morphological classes of viruses, then diagram and give an example of each.
4. DRAW IT Label the principal events of attachment, biosynthesis, entry, and maturation of a + stranded RNA virus. Draw in uncoating.



5. Compare biosynthesis of a + stranded RNA and a – stranded RNA virus.
6. Some antibiotics activate phage genes. MRSA releasing Panton-Valentine leukocidin causes a life-threatening disease. Why can this happen following antibiotic treatment?
7. Recall from Chapter 1 that Koch's postulates are used to determine the etiology of a disease. Why is it difficult to determine the etiology of
   a. a viral infection, such as influenza?
   b. cancer?
8. Persistent viral infections such as (a) _____ might be caused by (b) _____ that are (c) _____.
9. Plant viruses can't penetrate intact plant cells because (a) _____; therefore, they enter cells by (b) _____. Plant viruses can be cultured in (c) _____.
10. NAME IT Identify the viral family that infects skin, mucosa, and nerve cells; causes infections that can recur because of latency, and has polyhedral geometry.

Multiple Choice

1. Place the following in the most likely order for biosynthesis of a bacteriophage: (1) phage lysozyme; (2) mRNA; (3) DNA; (4) viral proteins; (5) DNA polymerase.
   a. 5, 4, 3, 2, 1
   b. 1, 2, 3, 4, 5
   c. 5, 3, 4, 2, 1
   d. 3, 5, 2, 4, 1
   e. 2, 5, 3, 4, 1

2. The molecule serving as mRNA can be incorporated in the newly synthesized virus capsids of all of the following *except*
   a. + strand RNA picornaviruses.
   b. + strand RNA togaviruses.
   c. − strand RNA rhabdoviruses.
   d. double-stranded RNA reoviruses.
   e. *Rotavirus.*
3. A virus with RNA-dependent RNA polymerase
   a. synthesizes DNA from an RNA template.
   b. synthesizes double-stranded RNA from an RNA template.
   c. synthesizes double-stranded RNA from a DNA template.
   d. transcribes mRNA from DNA.
   e. none of the above
4. Which of the following would be the first step in the biosynthesis of a virus with reverse transcriptase?
   a. A complementary strand of RNA must be synthesized.
   b. Double-stranded RNA must be synthesized.
   c. A complementary strand of DNA must be synthesized from an RNA template.
   d. A complementary strand of DNA must be synthesized from a DNA template.
   e. none of the above
5. An example of lysogeny in animals could be
   a. slow viral infections.
   b. latent viral infections.
   c. T-even bacteriophages.
   d. infections resulting in cell death.
   e. none of the above
6. The ability of a virus to infect an organism is regulated by
   a. the host species.
   b. the type of cells.
   c. the availability of an attachment site.
   d. cell factors necessary for viral replication.
   e. all of the above
7. Which of the following statements is *false?*
   a. Viruses contain DNA or RNA.
   b. The nucleic acid of a virus is surrounded by a protein coat.
   c. Viruses multiply inside living cells using viral mRNA, tRNA, and ribosomes.
   d. Viruses cause the synthesis of specialized infectious elements.
   e. Viruses multiply inside living cells.
8. Place the following in the order in which they are found in a host cell: (1) capsid proteins; (2) infective phage particles; (3) phage nucleic acid.
   a. 1, 2, 3
   b. 3, 2, 1
   c. 2, 1, 3
   d. 3, 1, 2
   e. 1, 3, 2
9. Which of the following does *not* initiate DNA synthesis?
   a. a double-stranded DNA virus (Poxviridae)
   b. a DNA virus with reverse transcriptase (Hepadnaviridae)
   c. an RNA virus with reverse transcriptase (Retroviridae)
   d. a single-stranded RNA virus (Togaviridae)
   e. none of the above
10. A viral species is *not* defined on the basis of the disease symptoms it causes. The best example of this is
    a. polio.
    b. rabies.
    c. hepatitis.
    d. chickenpox and shingles.
    e. measles.

Analysis

1. Discuss the arguments for and against the classification of viruses as living organisms.
2. In some viruses, capsomeres function as enzymes as well as structural supports. Of what advantage is this to the virus?
3. Why was the discovery of simian AIDS and feline AIDS important?
4. Prophages and proviruses have been described as being similar to bacterial plasmids. What similar properties do they exhibit? How are they different?

Clinical Applications and Evaluation

1. A 40-year-old man who was seropositive for HIV experienced abdominal pain, fatigue, and low-grade fever (38°C) for 2 weeks. A chest X-ray examination revealed lung infiltrates. Gram and acid-fast stains were negative. A viral culture revealed the cause of his symptoms: a large, enveloped polyhedral virus with double-stranded DNA. What is the disease? Which virus causes it? Why was a viral culture done after the Gram and acid-fast stain results were obtained?
2. A newborn female developed extensive vesicular and ulcerative lesions over her face and chest. What is the most likely cause of her symptoms? How would you determine the viral cause of this disease without doing a viral culture?
3. By May 14, two people living in the same household had died within 5 days of each other. Their illnesses were characterized by abrupt onset of fever, muscle pain, headache, and cough, followed by the rapid development of respiratory failure. By the end of the year, 36 cases of this disease, with a 50% mortality rate, had been confirmed. A member of the Orthomyxoviridae, Bunyaviridae, or Adenoviridae could cause this disease. Differentiate among these families by method of transmission, morphology, nucleic acid, and type of replication. The reservoir for this disease is mice. Name the disease. (*Hint*: Refer to Chapter 23.)

In the Clinic

As a county public health nurse, you follow up on reports of communicable diseases. The annual reported incidence of cryptosporidiosis in your state (population of 3.1 million) is 7.5 cases per 100,000 people. It is late December, and this year you have seen 65 cases of cryptosporidiosis in your county of 430,000 people. **Calculate the reported county incidence rate. How does this compare to the state rate? Assume that only one in ten people with diarrhea due to cryptosporidiosis seeks medical diagnosis and treatment. Under these circumstances, what is the true incidence in your county?**

Hint: Incidence per 100,000 allows us to compare occurrence of a disease in different areas because it is a rate that accounts for differences in population.

$$\text{Incidence} = \frac{\text{number of cases}}{\text{total population}} \times 100{,}000$$

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

14 Principles of Disease and Epidemiology

Now that you have a basic understanding of the structures and functions of microorganisms and some idea of the variety of microorganisms that exist, we can consider how the human body and various microorganisms interact in terms of health and disease.

We all have defenses to keep us healthy. In spite of these, however, we are still susceptible to **pathogens** (disease-causing microorganisms). A rather delicate balance exists between our defenses and the pathogenic mechanisms of microorganisms. When our defenses resist these pathogenic capabilities, we maintain our health—when the pathogen's capabilities overcome our defenses, disease results. After the disease has become established, an infected person may recover completely, suffer temporary or permanent damage, or die.

In the next section of the book we examine some of the principles of infection and disease, the mechanisms by which pathogens cause disease, the body's defenses against disease, and the ways that microbial diseases can be prevented by immunization and controlled by drugs. This chapter discusses the general principles of disease, starting with a discussion of the meaning and scope of pathology. In the last section of this chapter, "Epidemiology," you will learn how these principles are useful in studying and controlling disease. Understanding of these principles is vital to prevent disease transmission to patients in health care settings. Healthcare-associated infection by the *Clostridium difficile* bacteria shown in the photograph is discussed in the Clinical Case.

Clostrid

Pathology, Infection, and Disease

LEARNING OBJECTIVE

14-1 Define *pathology, etiology, infection,* and *disease*.

Pathology is the scientific study of disease (*pathos* = suffering; *logos* = science). Pathology is first concerned with the cause, or **etiology,** of disease. Second, it deals with **pathogenesis,** the manner in which a disease develops. Third, pathology is concerned with the *structural* and *functional changes* brought about by disease and their effects on the body.

Although the terms *infection* and *disease* are sometimes used interchangeably, they differ somewhat in meaning. **Infection** is the invasion or colonization of the body by pathogenic microorganisms; **disease** occurs when an infection results in any change from a state of health. Disease is an abnormal state in which part or all of the body is incapable of performing its normal functions. An infection may exist in the absence of detectable disease. For example, the body may be infected with the virus that causes AIDS but experience no symptoms of the disease.

The presence of a particular type of microorganism in a part of the body where it is not normally found is also called an infection—and may lead to disease. For example, although large numbers of *E. coli* are normally present in the healthy intestine, their infection of the urinary tract usually results in disease.

Few microorganisms are pathogenic. In fact, the presence of some microorganisms can even benefit the host. Therefore, before we discuss the role of microorganisms in causing disease, let's examine the relationship of microorganisms to the healthy human body.

CHECK YOUR UNDERSTANDING

What are the objectives of pathology? **14-1**

Normal Microbiota

LEARNING OBJECTIVES

14-2 Define *normal microbiota* and *transient microbiota*.

14-3 Compare commensalism, mutualism, and parasitism, and give an example of each.

14-4 Contrast normal microbiota and transient microbiota with opportunistic microorganisms.

Mammals, including humans, are generally free of microbes in utero. At birth, however, normal and characteristic microbial populations begin to establish themselves. Just before a woman gives birth, lactobacilli in her vagina multiply rapidly. The newborn's first contact with microorganisms is usually with these lactobacilli, and they become the predominant organisms in the newborn's intestine. More microorganisms are introduced to the newborn's body from the environment when breathing begins and feeding starts. After birth, *E. coli* and other bacteria acquired from foods begin to inhabit the large intestine. These microorganisms remain there throughout life and, in response to altered environmental conditions, may increase or decrease in number and contribute to disease.

Many other usually harmless microorganisms establish themselves inside other parts of the normal adult body and on its surface. A typical human body contains 1×10^{13} body cells, yet harbors an estimated 1×10^{14} bacterial cells (10 times more bacterial cells than human cells). This gives you an idea of the abundance of microorganisms that normally reside in the human body. The **Human Microbiome Project** (see the Big Picture, Chapter 19) began in 2007 to analyze microbial communities called *microbiomes* that live in and on the human body. Its goal is to determine the relationship between changes in the human microbiome and human health and disease. The human microbiome is more diverse than previously thought. Currently, researchers are comparing the microbiomes of healthy volunteers and volunteers with specific diseases. The microorganisms that establish more or less permanent residence (colonize) but that do not produce disease under normal conditions are members of the body's **normal microbiota,** or less commonly referred to as **normal flora*** (Figure 14.1). Others, called **transient microbiota,** may be present for several days, weeks, or months and then disappear. Microorganisms are not found throughout the entire human body but are localized in certain regions, as shown in Table 14.1 on page 392.

Many factors determine the distribution and composition of the normal microbiota. Among these are nutrients, physical and chemical factors, the host's defenses, and mechanical factors. Microbes vary with respect to the types of nutrients they can

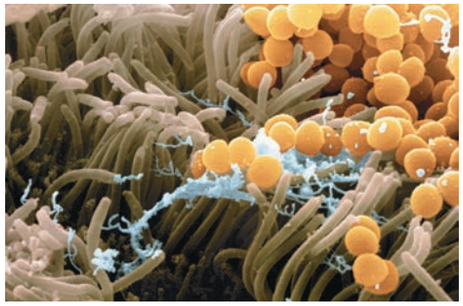
Clinical Case: Bathroom Break

Jamil Carter is in the bathroom, again. Ever since he was hospitalized for a urinary tract infection (UTI) 6 months ago, Jamil has been plagued with fever, chills, and severe diarrhea. He has lost 15 pounds since his hospitalization. Jamil is 75 years old, is retired, and lives with his wife and adult son. He does not smoke and rarely drinks alcohol. While in the hospital, Jamil was treated with the antibiotics ceftriaxone and ciprofloxacin for the UTI. He developed diarrhea 3 days after being discharged from the hospital and has had it ever since.

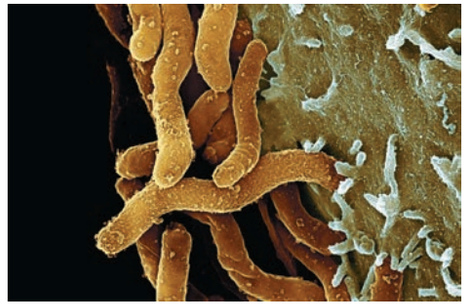
What could be causing Jamil's diarrhea and other symptoms? Read on to find out.

390 403 405 407 412

*Historically, bacteria once were classified as plants and thus bacteria on the human body were called normal flora.



(a) Bacteria (orange spheres) on the surface of the nasal epithelium



(b) Bacteria (brown) on the lining of the stomach

(c) Bacteria (orange) in the small intestine

Figure 14.1 Representative normal microbiota for different regions of the body.

Q Of what value are normal microbiota?

use as an energy source. Accordingly, microbes can colonize only those body sites that can supply the appropriate nutrients. These nutrients may be derived from dead cells, food in the gastrointestinal tract, secretory and excretory products of cells, and substances in body fluids.

A number of physical and chemical factors affect the growth of microbes and thus the growth and composition of the normal microbiota. Among these are temperature, pH, available oxygen and carbon dioxide, salinity, and sunlight.

You'll learn in Chapters 16 and 17 that the human body has certain defenses against microbes. These defenses include a variety of molecules and activated cells that kill microbes, inhibit their growth, prevent their adhesion to host cell surfaces, and neutralize toxins that microbes produce. Although these defenses are extremely important against pathogens, their role in determining and regulating the normal microbiota is unclear.

Certain regions of the body are subjected to mechanical forces that may affect colonization by the normal microbiota. For example, the chewing actions of the teeth and tongue movements can dislodge microbes attached to tooth and mucosal surfaces. In the gastrointestinal tract, the flow of saliva and digestive secretions and the various muscular movements of the throat, esophagus, stomach, and intestines can remove unattached microbes. The flushing action of urine also removes unattached microbes. In the respiratory system, mucus traps microbes, which cilia then propel toward the throat for elimination.

The conditions provided by the host at a particular body site vary from one person to another. Among the factors that also affect the normal microbiota are age, nutritional status, diet, health status, disability, hospitalization, stress, climate, geography, personal hygiene, living conditions, occupation, and lifestyle.

The principal normal microbiota in different regions of the body and some distinctive features of each region are listed in **Table 14.1.** Normal microbiota are also discussed more specifically in Part Four.

Animals with no microbiota whatsoever can be reared in the laboratory. Most germ-free mammals used in research are obtained by breeding them in a sterile environment. On the one hand, research with germ-free animals has shown that microbes aren't absolutely essential to animal life. On the other hand, this research has shown that germ-free animals have undeveloped immune systems and are unusually susceptible to infection and serious disease. Germ-free animals also require more calories and vitamins than do normal animals.

Relationships between the Normal Microbiota and the Host

Once established, the normal microbiota can benefit the host by preventing the overgrowth of harmful microorganisms. This phenomenon is called **microbial antagonism,** or **competitive exclusion.** Microbial antagonism involves competition among microbes. One consequence of this competition is that the normal microbiota protect the host against colonization by potentially pathogenic microbes by competing for nutrients, producing substances harmful to the invading microbes, and affecting conditions such as pH and available oxygen. When this balance between normal microbiota and pathogenic microbes is upset, disease can result. For example, the normal bacterial microbiota of the adult human vagina maintains a local pH of about 4. The presence of normal microbiota inhibits the overgrowth of the yeast *Candida albicans,* which can grow when the balance between normal microbiota and pathogens is upset and when pH is altered. If the bacterial population is eliminated by antibiotics, excessive douching, or deodorants, the pH of the vagina reverts to nearly neutral, and *C. albicans* can flourish and become the dominant microorganism there. This condition can lead to a form of vaginitis (vaginal infection).

Another example of microbial antagonism occurs in the large intestine. *E. coli* cells produce *bacteriocins,* proteins that inhibit the growth of other bacteria of the same or closely related species, such as pathogenic *Salmonella* and *Shigella.*

TABLE 14.1 Representative Normal Microbiota by Body Region

Region	Principal Components	Comments
Skin	*Propionibacterium, Staphylococcus, Corynebacterium, Micrococcus, Acinetobacter, Brevibacterium; Candida* (fungus), and *Malassezia* (fungus)	• Most of the microbes in direct contact with skin don't become residents because secretions from sweat and oil glands have antimicrobial properties. • Keratin is a resistant barrier, and the low pH of the skin inhibits many microbes. • The skin also has a relatively low moisture content.
Eyes (Conjunctiva)	*Staphylococcus epidermidis, S. aureus,* diphtheroids, *Propionibacterium, Corynebacterium*, streptococci, and *Micrococcus*	• The conjunctiva, a continuation of the skin or mucous membrane, contains basically the same microbiota found on the skin. • Tears and blinking also eliminate some microbes or inhibit others from colonizing.
Nose and Throat (Upper Respiratory System)	*Staphylococcus aureus, S. epidermidis,* and aerobic diphtheroids in the nose; *S. epidermidis, S. aureus,* diphtheroids, *Streptococcus pneumoniae, Haemophilus,* and *Neisseria* in the throat	• Although some normal microbiota are potential pathogens, their ability to cause disease is reduced by microbial antagonism. • Nasal secretions kill or inhibit many microbes, and mucus and ciliary action remove many microbes.
Mouth	*Streptococcus, Lactobacillus, Actinomyces, Bacteroides, Veillonella, Neisseria, Haemophilis, Fusobacterium, Treponema, Staphylococcus, Corynebacterium,* and *Candida* (fungus)	• Abundant moisture, warmth, and the constant presence of food make the mouth an ideal environment that supports very large and diverse microbial populations on the tongue, cheeks, teeth, and gums. • Biting, chewing, tongue movements, and salivary flow dislodge microbes. Saliva contains several antimicrobial substances.
Large Intestine	*Escherichia coli, Bacteroides, Fusobacterium, Lactobacillus, Enterococcus, Bifidobacterium, Enterobacter, Citrobacter, Proteus, Klebsiella,* and *Candida* (fungus)	• The large intestine contains the largest numbers of resident microbiota in the body because of its available moisture and nutrients. • Mucus and periodic shedding of the lining prevent many microbes from attaching to the lining of the gastrointestinal tract, and the mucosa produces several antimicrobiol chemicals. • Diarrhea also flushes out some of the normal microbiota.
Urinary and Reproductive Systems	*Staphylococcus, Micrococcus, Enterococcus, Lactobacillus, Bacteroides,* aerobic diphtheroids, *Pseudomonas, Klebsiella,* and *Proteus* in urethra; lactobacilli, *Streptococcus, Clostridium, Candida albicans* (fungus), and *Trichomonas vaginalis* (protozoan) in vagina	• The lower urethra in both sexes has a resident population; the vagina has its acid-tolerant population of microbes because of the nature of its secretions. • Mucus and periodic shedding of the lining prevent microbes from attaching to the lining; urine flow mechanically removes microbes, and the pH of urine and urea are antimicrobial. • Cilia and mucus expel microbes from the cervix of the uterus into the vagina, and the acidity of the vagina inhibits or kills microbes.

A bacterium that makes a particular bacteriocin isn't killed by that bacteriocin but may be killed by other ones. Bacteriocins are used in medical microbiology to help identify different strains of bacteria. Such identification helps determine whether several outbreaks of an infectious disease are caused by one or more strains of a bacterium.

A final example involves another bacterium, *Clostridium difficile,* also in the large intestine. The normal microbiota of the large intestine effectively inhibit *C. difficile,* possibly by making host receptors unavailable, competing for available nutrients, or producing bacteriocins. However, if the normal microbiota are eliminated (for example, by antibiotics), *C. difficile* can become a problem. This microbe is responsible for nearly all gastrointestinal infections that follow antibiotic therapy, from mild diarrhea to severe or even fatal colitis (inflammation of the colon). In 2013, a Canadian infectious disease specialist successfully treated *C. difficile* infections with pills containing normal intestinal microbiota. The normal microbiota were obtained from patients' relatives.

The relationship between the normal microbiota and the host is called **symbiosis,** a relationship between two organisms in which at least one organism is dependent on the other (Figure 14.2). In the symbiotic relationship called **commensalism,** one of the organisms benefits, and the other is unaffected. Many of the microorganisms that make up our normal microbiota are commensals; these include *Staphylococcus epidermidis* that inhabit the surface of the skin, the corynebacteria that inhabit the surface of the eye, and certain saprophytic mycobacteria that inhabit the ear and external genitals. These bacteria live on secretions and sloughed-off cells, and they bring no apparent benefit or harm to the host.

ASM: Microorganisms, cellular and viral, can interact with both human and nonhuman hosts in beneficial, neutral, or detrimental ways.

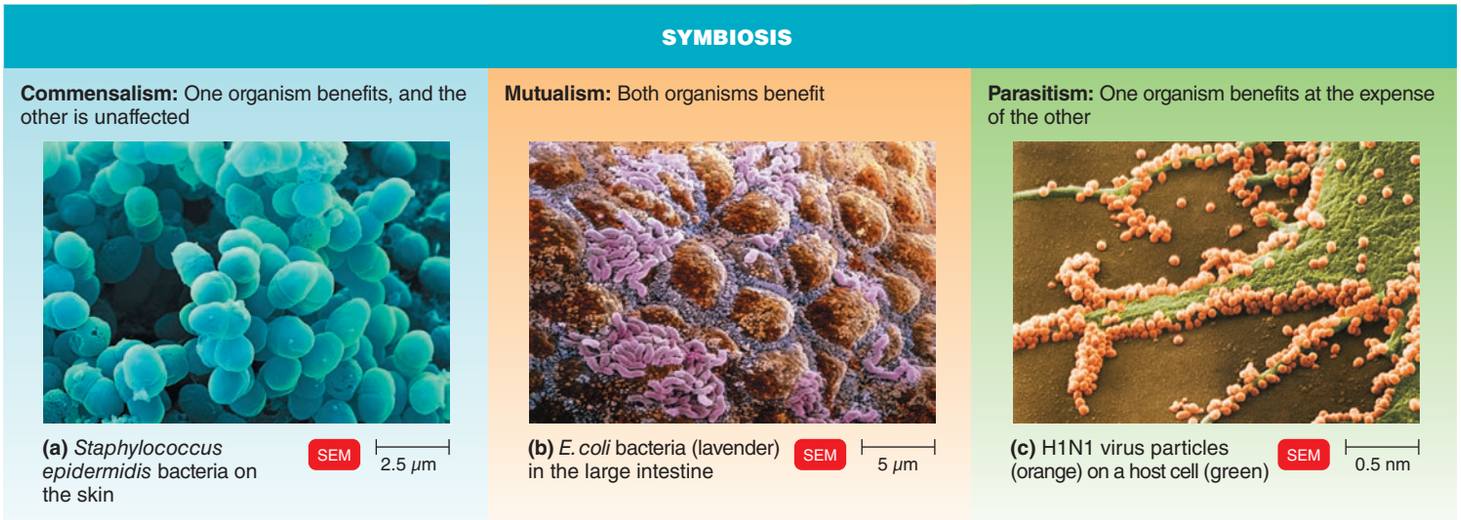


Figure 14.2 Symbiosis.

Q Which type of symbiosis is best represented by the relationship between humans and *E. coli*?

Mutualism is a type of symbiosis that benefits both organisms. For example, the large intestine contains bacteria, such as *E. coli,* that synthesize vitamin K and some B vitamins. These vitamins are absorbed into the bloodstream and distributed for use by body cells. In exchange, the large intestine provides nutrients used by the bacteria, allowing them to survive.

Recent genetics studies have found hundreds of antibiotic-resistance genes in the intestinal bacteria. It may seem desirable to have these bacteria survive while a person is taking antibiotics for an infectious disease; however, these beneficial bacteria may be able to transfer antibiotic-resistance genes to pathogens.

In still another kind of symbiosis, one organism benefits by deriving nutrients at the expense of the other; this relationship is called **parasitism.** Many disease-causing bacteria are parasites.

Opportunistic Microorganisms

Although categorizing symbiotic relationships by type is convenient, keep in mind that the relationship can change under certain conditions. For example, given the proper circumstances, a mutualistic organism, such as *E. coli,* can become harmful. *E. coli* is generally harmless as long as it remains in the large intestine; but if it gains access to other body sites, such as the urinary bladder, lungs, spinal cord, or wounds, it may cause urinary tract infections, pulmonary infections, meningitis, or abscesses, respectively. Microbes such as *E. coli* are called **opportunistic pathogens.** They don't cause disease in their normal habitat in a healthy person but may do so in a different environment. For example, microbes that gain access through broken skin or mucous membranes can cause opportunistic infections. Or, if the host is already weakened or compromised by infection, microbes that are usually harmless can cause disease. AIDS is often accompanied by a common opportunistic infection, *Pneumocystis* pneumonia, caused by the opportunistic organism *Pneumocystis jirovecii* (see Figure 24.19, page 700). This secondary infection can develop in AIDS patients because their immune systems are suppressed. Before the AIDS epidemic, this type of pneumonia was rare. Opportunistic pathogens possess other features that contribute to their ability to cause disease. For example, they're present in or on the body or in the external environment in relatively large numbers. Some opportunistic pathogens may be found in locations in or on the body that are somewhat protected from the body's defenses, and some are resistant to antibiotics.

In addition to the usual symbionts, many people carry other microorganisms that are generally regarded as pathogenic but that may not cause disease in those people. Among the pathogens that are frequently carried in healthy individuals are echoviruses (*echo* comes from *e*nteric *c*ytopathogenic *h*uman *o*rphan), which can cause intestinal diseases, and adenoviruses, which can cause respiratory diseases. *Neisseria meningitidis,* which often resides benignly in the respiratory tract, can cause meningitis, a disease that inflames the coverings of the brain and spinal cord. *Streptococcus pneumoniae,* a normal resident of the nose and throat, can cause a type of pneumonia.

Cooperation among Microorganisms

It isn't only competition among microbes that can cause disease; cooperation among microbes can also be a factor in causing disease. For example, pathogens that cause periodontal disease and gingivitis have been found to have receptors, not for the teeth, but for the oral streptococci that colonize the teeth.

CHECK YOUR UNDERSTANDING

- How do normal microbiota differ from transient microbiota? **14-2**
- Give several examples of microbial antagonism. **14-3**
- How can opportunistic pathogens cause infections? **14-4**

The Etiology of Infectious Diseases

LEARNING OBJECTIVE

14-5 List Koch's postulates.

Some diseases—such as polio, Lyme disease, and tuberculosis—have a well-known etiology. Some have an etiology that isn't completely understood, for example, the relationship between certain viruses and cancer. For still others, such as Alzheimer's disease, the etiology is unknown. Of course, not all diseases are caused by microorganisms. For example, the disease hemophilia is an *inherited (genetic) disease,* and osteoarthritis and cirrhosis are *degenerative diseases.* There are several other categories of disease, but here we'll discuss only *infectious diseases,* those caused by microorganisms. To see how microbiologists determine the etiology of an infectious disease, we'll discuss in greater detail the work of Robert Koch, which was introduced in Chapter 1 (page 9).

Koch's Postulates

In the historical overview of microbiology presented in Chapter 1, we briefly discussed Koch's famous postulates. Recall that Koch was a German physician who played a major role in establishing that microorganisms cause specific diseases. In 1877, he published some early papers on anthrax, a disease of cattle that can also occur in humans. Koch demonstrated that certain bacteria, today known as *Bacillus anthracis,* were always present in the blood of animals that had the disease and weren't present in healthy animals. He knew that the mere presence of the bacteria did not prove that they had caused the disease; the bacteria could have been there as a result of the disease. Thus, he experimented further.

He took a sample of blood from a sick animal and injected it into a healthy one. The second animal developed the same disease and died. He repeated this procedure many times, always with the same results. (A key criterion in the validity of any scientific proof is that experimental results be repeatable.) Koch also cultivated the microorganism in fluids outside the animal's body, and he demonstrated that the bacterium would cause anthrax even after many culture transfers.

Koch showed that a specific infectious disease (anthrax) is caused by a specific microorganism *(B. anthracis)* that can be isolated and cultured on artificial media. He later used the same methods to show that the bacterium *Mycobacterium tuberculosis* is the causative agent of tuberculosis.

Koch's research provides a framework for the study of the etiology of any infectious disease. Today, we refer to Koch's experimental requirements as **Koch's postulates** (Figure 14.3). They are summarized as follows:

1. The same pathogen must be present in every case of the disease.
2. The pathogen must be isolated from the diseased host and grown in pure culture.
3. The pathogen from the pure culture must cause the disease when it's inoculated into a healthy, susceptible laboratory animal.
4. The pathogen must be isolated from the inoculated animal and must be shown to be the original organism.

Exceptions to Koch's Postulates

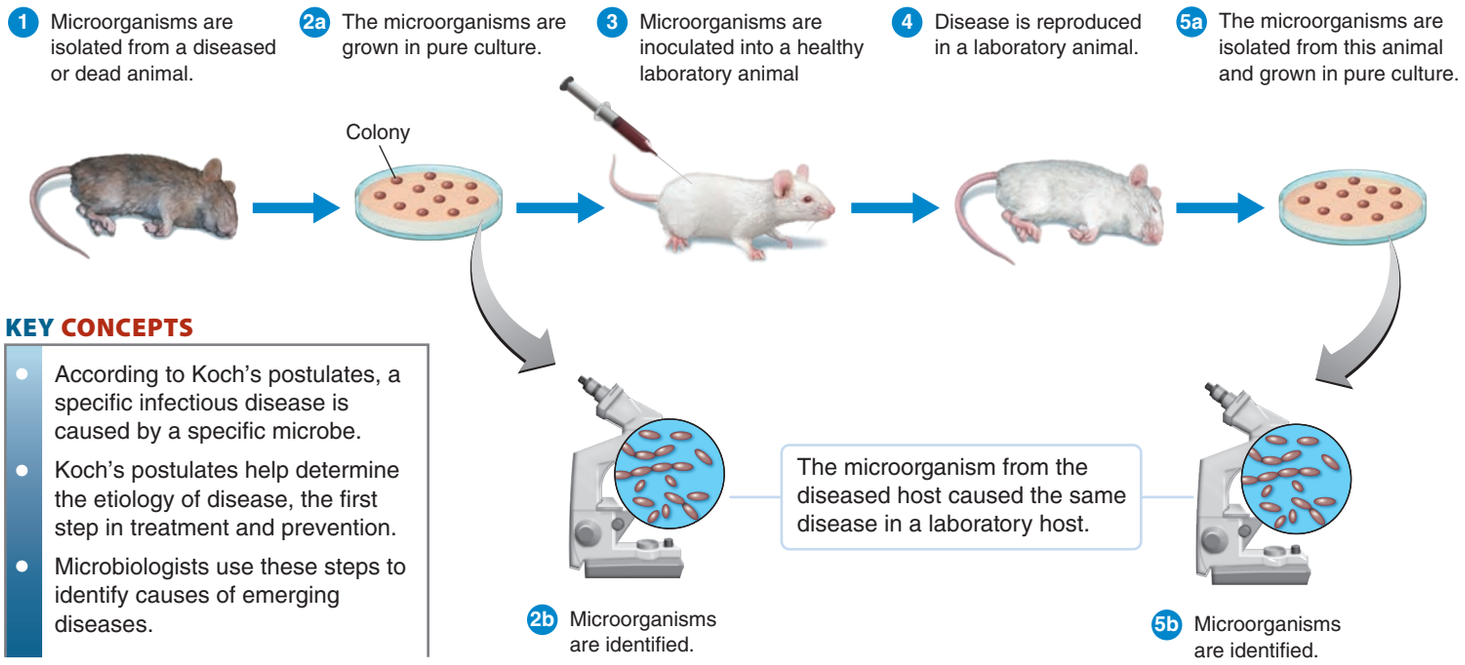
Although Koch's postulates are useful in determining the causative agent of most bacterial diseases, there are some exceptions. For example, some microbes have unique culture requirements. The bacterium *Treponema pallidum* is known to cause syphilis, but virulent strains have never been cultured on artificial media. The causative agent of leprosy, *Mycobacterium leprae,* has also never been grown on artificial media. Moreover, many rickettsial and viral pathogens cannot be cultured on artificial media because they multiply only within cells.

The discovery of microorganisms that can't grow on artificial media has necessitated some modifications of Koch's postulates and the use of alternative methods of culturing and detecting certain microbes. For example, when researchers looking for the microbial cause of legionellosis (Legionnaires' disease) were unable to isolate the microbe directly from a victim, they took the alternative step of inoculating a victim's lung tissue into guinea pigs. These guinea pigs developed the disease's pneumonia-like symptoms, whereas guinea pigs inoculated with tissue from an unafflicted person did not. Then tissue samples from the diseased guinea pigs were cultured in yolk sacs of chick embryos, a method (see Figure 13.7, page 367) that reveals the growth of extremely small microbes. After the embryos were incubated, electron microscopy revealed rod-shaped bacteria in the chick embryos. Finally, modern immunological techniques (discussed in Chapter 18) were used to show that the bacteria in the chick embryos were the same bacteria as those in the guinea pigs and in afflicted humans.

In a number of situations, a human host exhibits certain signs and symptoms that are associated only with a certain pathogen and its disease. For example, the pathogens responsible for diphtheria and tetanus cause distinguishing signs and symptoms that no other microbe can produce. They are unequivocally the only organisms that produce their respective diseases. But some infectious diseases are not as clear-cut and provide another exception to Koch's postulates. For example, nephritis (inflammation of the kidneys) can involve any of several different pathogens, all of which cause the same signs and

FOUNDATION FIGURE 14.3

Koch's Postulates: Understanding Disease



symptoms. Thus, it is often difficult to know which particular microorganism is causing a disease. Other infectious diseases that sometimes have poorly defined etiologies are pneumonia, meningitis, and peritonitis (inflammation of the peritoneum, the membrane that lines the abdomen and covers the organs within them).

Some pathogens can cause several disease conditions, resulting in still another exception to Koch's postulates. *Mycobacterium tuberculosis,* for example, is implicated in diseases of the lungs, skin, bones, and internal organs. *Streptococcus pyogenes* (strep′tō-KOK-kus pī-AH-jen-ēz) can cause sore throat, scarlet fever, skin infections (such as erysipelas), and osteomyelitis (inflammation of bone), among other diseases. When clinical signs and symptoms are used together with laboratory methods, these infections can usually be distinguished from infections of the same organs by other pathogens.

Ethical considerations may also impose an exception to Koch's postulates. For example, some agents that cause disease in humans have no other known host. An example is HIV, the cause of AIDS. This poses the ethical question of whether humans can be intentionally inoculated with infectious agents. In 1721, King George I told several condemned prisoners they could be inoculated with smallpox to test a smallpox vaccine (see Chapter 18). He promised their freedom if they lived. Human experiments with untreatable diseases are unacceptable today, although accidental inoculation does occur sometimes. For example, a contaminated red bone marrow transplant satisfied the third Koch's postulate to prove that a herpesvirus caused cancer (see page 381).

CHECK YOUR UNDERSTANDING

Explain some exceptions to Koch's postulates. **14-5**

Classifying Infectious Diseases

LEARNING OBJECTIVES

14-6 Differentiate a communicable from a noncommunicable disease.

14-7 Categorize diseases according to frequency of occurrence.

14-8 Categorize diseases according to severity.

14-9 Define *herd immunity.*

Every disease that affects the body alters body structures and functions in particular ways, and these alterations are usually indicated by several kinds of evidence. For example, the patient may experience certain **symptoms,** or changes in body function, such as pain and *malaise* (a vague feeling of body discomfort). These *subjective* changes aren't apparent to an observer. The patient can also exhibit **signs,** which are *objective* changes the physician can observe and measure. Frequently evaluated signs include lesions (changes produced in tissues by disease), swelling, fever, and paralysis. A specific group of symptoms or signs may always accompany a particular disease; such a group is called a **syndrome.** The diagnosis of a disease is made by

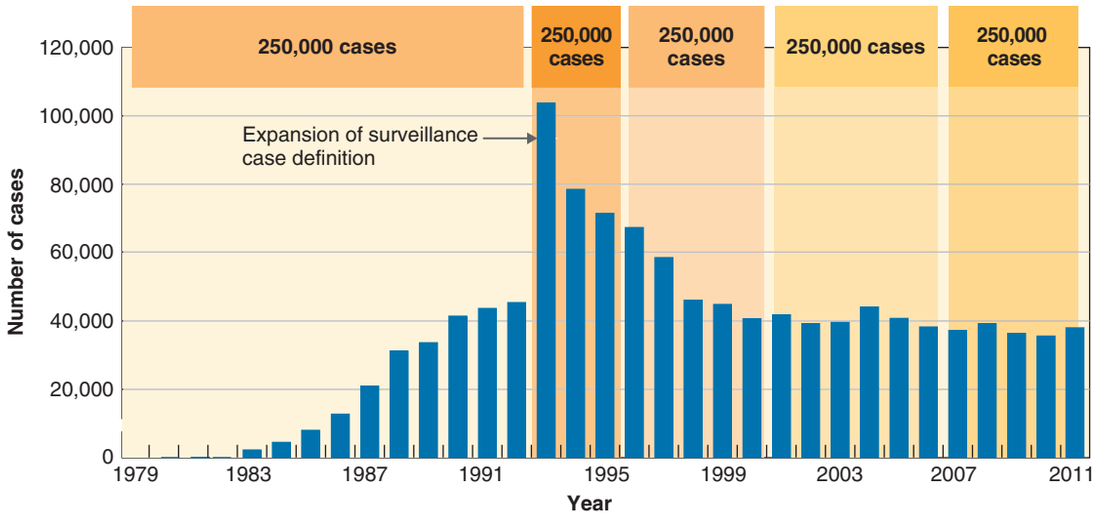


Figure 14.4 Reported AIDS cases in the United States. Notice that the first 250,000 cases occurred over a 12-year period, whereas the second through fifth 250,000 cases in this epidemic occurred in just 3 to 6 years. Much of the increase shown for 1993 is due to an expanded definition of AIDS cases adopted in that year. Likewise, in 2008 reported cases likely rose because of another definition change that expanded to include HIV infections in the number of AIDS cases.

Source: CDC.

Q What was the incidence of AIDS in 2010?

evaluation of the signs and symptoms, together with the results of laboratory tests.

Diseases are often classified in terms of how they behave within a host and within a given population. A **communicable disease** is a disease in which an infected person transmits an infectious agent, either directly or indirectly, to another person who in turn becomes infected. Chickenpox, measles, influenza, genital herpes, typhoid fever, and tuberculosis are examples. Chickenpox and measles are also examples of **contagious diseases,** that is, diseases that are very communicable and capable of spreading easily and rapidly from one person to another. A **noncommunicable disease** is not spread from one host to another. These diseases are caused by microorganisms that normally inhabit the body and only occasionally produce disease or by microorganisms that reside outside the body and produce disease only when introduced into the body. An example is tetanus: *Clostridium tetani* produces disease only when it is introduced into the body via abrasions or wounds.

Occurrence of a Disease

To understand the full scope of a disease, we should know something about its occurrence. The **incidence** of a disease is the number of people in a population who develop a disease during a particular time period. It's an indicator of the spread of the disease. The **prevalence** of a disease is the number of people in a population who develop a disease at a specified time, regardless of when it first appeared. Prevalence takes into account both old and new cases. It's an indicator of how seriously and how long a disease affects a population. For example, the incidence of AIDS in the United States in 2012 was 55,400 whereas the prevalence in that same year was estimated to be about 117,000. Knowing the incidence and the prevalence of a disease in different populations (for example, in populations representing different geographic regions or different ethnic groups) enables scientists to estimate the range of the disease's occurrence and its tendency to affect some groups of people more than others.

Frequency of occurrence is another criterion that is used in the classification of diseases. If a particular disease occurs only occasionally, it is called a **sporadic disease;** typhoid fever in the United States is such a disease. A disease constantly present in a population is called an **endemic disease;** an example of such a disease is the common cold. If many people in a given area acquire a certain disease in a relatively short period, it is called an **epidemic disease;** influenza is an example of a disease that often achieves epidemic status. **Figure 14.4** shows the epidemic incidence of AIDS in the United States. Some authorities consider gonorrhea and certain other sexually transmitted infections to be epidemic at this time as well (see Figure 26.5, page 754). An epidemic disease that occurs worldwide is called a **pandemic disease.** We experience pandemics of influenza from time to time. AIDS is another example of a pandemic disease.

Severity or Duration of a Disease

Another useful way of defining the scope of a disease is in terms of its severity or duration. An **acute disease** is one that develops rapidly but lasts only a short time; a good example is influenza. A **chronic disease** develops more slowly. The body's reactions may be less severe, but the disease is likely to continue or recur for long periods. Infectious mononucleosis, tuberculosis, and hepatitis B fall into this category. A disease that is intermediate between acute and chronic is described as a **subacute disease;** an example is subacute sclerosing panencephalitis, a rare brain disease characterized by diminished intellectual function and loss of nervous function. A **latent disease** is one in which the causative agent remains inactive for a time but then becomes active to produce symptoms of the disease; an example is shingles, one of the diseases caused by varicella virus.

The rate at which a disease or an epidemic spreads and the number of individuals involved are determined in part by the immunity of the population. Vaccination can provide long-lasting and sometimes lifelong protection of an individual against certain diseases. People who are immune to an infectious disease will not be carriers, thereby reducing the occurrence of the disease. Immune individuals act as a barrier to the spread of infectious agents. Even though a highly communicable disease may cause an epidemic, many nonimmune people will be protected because of the unlikelihood of their coming into contact with an infected person. A great advantage of vaccination is that enough individuals in a population will be protected from a disease to prevent its rapid spread to those in the population who aren't vaccinated. When many immune people are present in a community, **herd immunity** exists.

Extent of Host Involvement

Infections can also be classified according to the extent to which the host's body is affected. A **local infection** is one in which the invading microorganisms are limited to a relatively small area of the body. Some examples of local infections are boils and abscesses. In a **systemic (generalized) infection,** microorganisms or their products are spread throughout the body by the blood or lymph. Measles is an example of a systemic infection. Very often, agents of a local infection enter a blood or lymphatic vessel and spread to other specific parts of the body, where they are confined to specific areas of the body. This condition is called a **focal infection.** Focal infections can arise from infections in areas such as the teeth, tonsils, or sinuses.

Sepsis is a toxic inflammatory condition arising from the spread of microbes, especially bacteria or their toxins, from a focus of infection. **Septicemia,** also called blood poisoning, is a systemic infection arising from the multiplication of pathogens in the blood. Septicemia is a common example of sepsis. The presence of bacteria in the blood is known as **bacteremia. Toxemia** refers to the presence of toxins in the blood (as occurs in tetanus), and **viremia** refers to the presence of viruses in blood.

The state of host resistance also determines the extent of infections. A **primary infection** is an acute infection that causes the initial illness. A **secondary infection** is one caused by an opportunistic pathogen after the primary infection has weakened the body's defenses. Secondary infections of the skin and respiratory tract are common and are sometimes more dangerous than the primary infections. *Pneumocystis* pneumonia as a consequence of AIDS is an example of a secondary infection; streptococcal bronchopneumonia following influenza is an example of a secondary infection that is more serious than the primary infection. A **subclinical (inapparent) infection** is one that doesn't cause any noticeable illness. Poliovirus and hepatitis A virus, for example, can be carried by people who never develop the illness.

CHECK YOUR UNDERSTANDING

- Does *Clostridium perfringens* (page 723) cause a communicable disease? **14-6**
- Distinguish the incidence from the prevalence of a disease. **14-7**
- List two examples of acute and chronic diseases. **14-8**
- How does herd immunity develop? **14-9**

Patterns of Disease

LEARNING OBJECTIVES

14-10 Identify four predisposing factors for disease.

14-11 Put the following in proper sequence, according to the pattern of disease: period of decline, period of convalescence, period of illness, prodromal period, incubation period.

A definite sequence of events usually occurs during infection and disease. As you will learn shortly, for an infectious disease to occur, there must be a reservoir of infection as a source of pathogens. Next, the pathogen must be transmitted to a susceptible host by direct contact, by indirect contact, or by vectors. Transmission is followed by invasion, in which the microorganism enters the host and multiplies. Following invasion, the microorganism injures the host through a process called pathogenesis (discussed further in the next chapter). The extent of injury depends on the degree to which host cells are damaged, either directly or by toxins. Despite the effects of all these factors, the occurrence of disease ultimately depends on the resistance of the host to the activities of the pathogen.

Predisposing Factors

Certain predisposing factors also affect the occurrence of disease. A **predisposing factor** makes the body more susceptible to a disease and may alter the course of the disease. Gender is sometimes a predisposing factor; for example, females have a higher incidence of urinary tract infections than males, whereas males have higher rates of pneumonia and meningitis. Other aspects of genetic background may play a role as well. For example, sickle cell disease is a severe, life-threatening form of anemia that occurs when the genes for the disease are inherited from both parents. Individuals who carry only one sickle cell gene have a condition called sickle cell trait and appear normal unless specially tested. However, they are relatively resistant to the most serious form of malaria. The potential that individuals in a population might inherit a life-threatening disease is more than counterbalanced by protection from malaria among carriers of the gene for sickle cell trait. Of course, in countries where malaria isn't present, sickle cell trait is an entirely negative condition.

Climate and weather seem to have some effect on the incidence of infectious diseases. In temperate regions, the incidence of respiratory diseases increases during the winter. This increase may be related to the fact that when people stay indoors, the

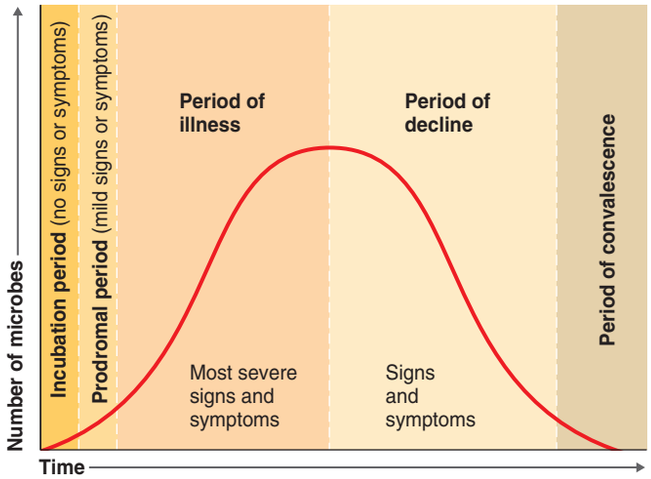


Figure 14.5 The stages of a disease.

Q During which periods can a disease be transmitted?

closer contact with one another facilitates the spread of respiratory pathogens.

Other predisposing factors include inadequate nutrition, fatigue, age, environment, habits, lifestyle, occupation, preexisting illness, and chemotherapy. It is often difficult to know the exact relative importance of the various predisposing factors.

Development of Disease

Once a microorganism overcomes the defenses of the host, development of the disease follows a certain sequence that tends to be similar whether the disease is acute or chronic (Figure 14.5).

Incubation Period

The **incubation period** is the interval between the initial infection and the first appearance of any signs or symptoms. In some diseases, the incubation period is always the same; in others, it is quite variable. The time of incubation depends on the specific microorganism involved, its virulence (degree of pathogenicity), the number of infecting microorganisms, and the resistance of the host. (See Table 15.1, page 419, for the incubation periods of a number of microbial diseases.)

Prodromal Period

The **prodromal period** is a relatively short period that follows the period of incubation in some diseases. The prodromal period is characterized by early, mild symptoms of disease, such as general aches and malaise.

Period of Illness

During the **period of illness,** the disease is most severe. The person exhibits overt signs and symptoms of disease, such as fever, chills, muscle pain (myalgia), sensitivity to light (photophobia), sore throat (pharyngitis), lymph node enlargement (lymphadenopathy), and gastrointestinal disturbances. During the period of illness, the number of white blood cells may increase or decrease. Generally, the patient's immune response and other defense mechanisms overcome the pathogen, and the period of illness ends. If the disease is not successfully overcome (or successfully treated), the patient dies during this period.

Period of Decline

During the **period of decline,** the signs and symptoms subside. The fever decreases, and the feeling of malaise diminishes. During this phase, which may take from less than 24 hours to several days, the patient is vulnerable to secondary infections.

Period of Convalescence

During the **period of convalescence,** the person regains strength and the body returns to its prediseased state. Recovery has occurred.

We all know that during the period of illness, people can serve as reservoirs of disease and can easily spread infections to other people. However, you should also know that people can spread infection during incubation and convalescence as well. This is especially true of diseases such as typhoid fever and cholera, in which the convalescing person carries the pathogenic microorganism for months or even years.

CHECK YOUR UNDERSTANDING

- What is a predisposing factor? **14-10**
- The incubation period for a cold is 3 days, and the period of disease is usually 5 days. If the person next to you has a cold, when will you know whether you contracted it? **14-11**

The Spread of Infection

LEARNING OBJECTIVES

14-12 Define *reservoir of infection.*

14-13 Contrast human, animal, and nonliving reservoirs, and give one example of each.

14-14 Explain three methods of disease transmission.

Now that you have an understanding of normal microbiota, the etiology of infectious diseases, and the types of infectious diseases, we'll examine the sources of pathogens and how diseases are transmitted.

Reservoirs of Infection

For a disease to perpetuate itself, there must be a continual source of the disease organisms. This source can be either a living organism or an inanimate object that provides a pathogen with adequate conditions for survival and multiplication and an opportunity for transmission. Such a source is called a **reservoir of infection.** These reservoirs may be human, animal, or nonliving.

Human Reservoirs

The principal living reservoir of human disease is the human body itself. Many people harbor pathogens and transmit them directly or indirectly to others. People with signs and symptoms of a disease may transmit the disease; in addition, some people can harbor pathogens and transmit them to others without exhibiting any signs of illness. These people, called **carriers,** are important living reservoirs of infection. Some carriers have inapparent infections for which no signs or symptoms are ever exhibited. Other people, such as those with latent diseases, carry a disease during its symptom-free stages—during the incubation period (before symptoms appear) or during the convalescent period (recovery). Typhoid Mary is an example of a carrier (see page 716). Human carriers play an important role in the spread of such diseases as AIDS, diphtheria, typhoid fever, hepatitis, gonorrhea, amebic dysentery, and streptococcal infections.

Animal Reservoirs

Both wild and domestic animals are living reservoirs of microorganisms that can cause human diseases. Diseases that occur primarily in wild and domestic animals and can be transmitted to humans are called **zoonoses** (ZŌ-ō-nō-sēz) (singular: *zoonosis*). Rabies (found in bats, skunks, foxes, dogs, and coyotes), and Lyme disease (found in field mice) are examples of zoonoses. Other representative zoonoses are presented in **Table 14.2**.

About 150 zoonoses are known. The transmission of zoonoses to humans can occur via one of many routes: by direct contact with infected animals; by direct contact with domestic pet waste (such as cleaning a litter box or bird cage); by contamination of food and water; by air from contaminated hides, fur, or feathers; by consuming infected animal products; or by insect vectors (insects that transmit pathogens).

Nonliving Reservoirs

The two major nonliving reservoirs of infectious disease are soil and water. Soil harbors such pathogens as fungi, which cause mycoses such as ringworm and systemic infections; *Clostridium botulinum,* the bacterium that causes botulism; and *C. tetani,* the bacterium that causes tetanus. Because both species of clostridia are part of the normal intestinal microbiota of horses and cattle, the bacteria are found especially in soil where animal feces are used as fertilizer.

Water that's been contaminated by the feces of humans and other animals is a reservoir for several pathogens, notably those responsible for gastrointestinal diseases. These include *Vibrio cholerae,* which causes cholera; *Cryptosporidium,* one cause of diarrhea; and *Salmonella typhi,* which causes typhoid fever. Other nonliving reservoirs include foods that are improperly prepared or stored. They may be sources of diseases such as trichinellosis and salmonellosis.

Transmission of Disease

The causative agents of disease can be transmitted from the reservoir of infection to a susceptible host by three principal routes: contact, vehicles, and vectors.

Contact Transmission

Contact transmission is the spread of a disease agent by direct contact, indirect contact, or droplet transmission. **Direct contact transmission,** also known as *person-to-person transmission,* is the direct transmission of an agent by physical contact between its source and a susceptible host; no intermediate object is involved (**Figure 14.6a**). The most common forms of direct contact transmission are touching, kissing, and sexual intercourse. Among the diseases that can be transmitted by direct contact are viral respiratory tract diseases (the common cold and influenza), staphylococcal infections, hepatitis A, measles, scarlet fever, and sexually transmitted infections (syphilis, gonorrhea, and genital herpes). Direct contact is also one way to spread AIDS and infectious mononucleosis. To guard against person-to-person transmission, health care workers use gloves and other protective measures (Figure 14.6b). Potential pathogens can also be transmitted by direct contact from animals (or animal products) to humans. Examples are the pathogens causing rabies (direct contact via bites) and anthrax.

Indirect contact transmission occurs when the agent of disease is transmitted from its reservoir to a susceptible host by means of a nonliving object. The general term for any nonliving object involved in the spread of an infection is a **fomite.** Examples of fomites are tissues, handkerchiefs, towels, bedding, diapers, drinking cups, eating utensils, toys, money, and thermometers (Figure 14.6c). Contaminated syringes serve as fomites in transmitting AIDS and hepatitis B. Other fomites may transmit diseases such as tetanus.

Droplet transmission is a third type of contact transmission in which microbes are spread in *droplet nuclei* (mucus droplets) that travel only short distances (Figure 14.6d). These droplets are discharged into the air by coughing, sneezing, laughing, or talking and travel less than 1 meter from the reservoir to the host. One sneeze may produce 20,000 droplets. Disease agents that travel such short distances are not considered airborne (airborne transmission is discussed shortly). Examples of diseases spread by droplet transmission are influenza, pneumonia, and pertussis (whooping cough).

Vehicle Transmission

Vehicle transmission is the transmission of disease agents by a medium, such as water, food, or air (**Figure 14.7**). Other media include blood and other body fluids, drugs, and intravenous fluids. An outbreak of *Salmonella* infections caused by vehicle transmission is described in the box in Chapter 25 (page 717). Here we'll discuss water, food, and air as transmission vehicles.

TABLE 14.2 Selected Zoonoses

Disease	Causative Agent	Reservoir	Transmission Due To	Chapter Reference
VIRAL				
Influenza (some types)	*Influenzavirus*	Swine, birds	Direct contact	24
Rabies	*Lyssavirus*	Bats, skunks, foxes, dogs, raccoons	Direct contact (bite)	22
West Nile encephalitis	*Flavivirus*	Horses, birds	*Aedes* and *Culex* mosquito bite	22
Hantavirus pulmonary syndrome	*Hantavirus*	Rodents (primarily deer mice)	Direct contact with rodent saliva, feces, or urine	23
BACTERIAL				
Anthrax	*Bacillus anthracis*	Domestic livestock	Direct contact with contaminated hides or animals; air; food	23
Brucellosis	*Brucella* spp.	Domestic livestock	Direct contact with contaminated milk, meat, or animals	23
Plague	*Yersinia pestis*	Rodents	Flea bites	23
Cat-scratch disease	*Bartonella henselae*	Domestic cats	Direct contact	23
Ehrlichiosis	*Ehrlichia* spp.	Deer, rodents	Tick bites	23
Leptospirosis	*Leptospira* spp.	Wild mammals, domestic dogs and cats	Direct contact with urine, soil, water	26
Lyme disease	*Borrelia burgdorferi*	Field mice	Tick bites	23
Psittacosis (ornithosis)	*Chlamydophila psittaci*	Birds, especially parrots	Direct contact	24
Rocky Mountain spotted fever	*Rickettsia rickettsii*	Rodents	Tick bites	23
Salmonellosis	*Salmonella enterica*	Poultry, reptiles	Ingestion of contaminated food and water and putting hands in mouth	25
Endemic typhus	*Rickettsia typhi*	Rodents	Flea bites	23
FUNGAL				
Ringworm	*Trichophyton* *Microsporum* *Epidermophyton*	Domestic mammals	Direct contact; fomites (nonliving objects)	21
PROTOZOAN				
Malaria	*Plasmodium* spp.	Monkeys	*Anopheles* mosquito bite	23
Toxoplasmosis	*Toxoplasma gondii*	Cats and other mammals	Ingestion of contaminated meat or by direct contact with infected tissues or fecal matter	23
HELMINTHIC				
Tapeworm (pork)	*Taenia solium*	Pigs	Ingestion of undercooked contaminated pork	25
Trichinellosis	*Trichinella spiralis*	Pigs, bears	Ingestion of undercooked contaminated meat	25

In **waterborne transmission,** pathogens are usually spread by water contaminated with untreated or poorly treated sewage. Diseases transmitted via this route include cholera, waterborne shigellosis, and leptospirosis. In **foodborne transmission,** pathogens are generally transmitted in foods that are incompletely cooked, poorly refrigerated, or prepared under unsanitary conditions. Foodborne pathogens cause diseases such as food poisoning and tapeworm infestation.

Airborne transmission refers to the spread of agents of infection by droplet nuclei in dust that travel more than 1 meter from the reservoir to the host. For example, microbes are spread by droplets, which may be discharged in a fine spray from the mouth and nose during coughing and sneezing (see Figure 14.6d). These droplets are small enough to remain airborne for prolonged periods. The virus that causes measles and the bacterium that causes tuberculosis can be transmitted via airborne droplets. Dust particles can harbor various pathogens. Staphylococci and streptococci can survive on dust and be transmitted by the airborne route. Spores produced by certain fungi are also transmitted by the airborne route and can cause such diseases

(a) Direct contact transmission

(b) Preventing direct contact transmission through the use of gloves, masks, and face shields

(c) Indirect contact transmission

(d) Droplet transmission

Figure 14.6 Contact transmission.

Q Name a disease transmitted by direct contact, a disease transmitted by indirect contact, and a disease transmitted by droplet transmission.

as histoplasmosis, coccidioidomycosis, and blastomycosis (see Chapter 24).

Vectors

Arthropods are the most important group of disease **vectors**—animals that carry pathogens from one host to another. (Insects and other arthropod vectors are discussed in Chapter 12, page 351.) Arthropod vectors transmit disease by two general methods. **Mechanical transmission** is the passive transport of the pathogens on the insect's feet or other body parts (Figure 14.8). If the insect makes contact with a host's food, pathogens can be transferred to the food and later swallowed by the host. Houseflies, for instance, can transfer the pathogens of typhoid fever and bacillary dysentery (shigellosis) from the feces of infected people to food.

Biological transmission is an active process and is more complex. The arthropod bites an infected person or animal and ingests some of the infected blood (see Figure 12.31, page 351). The pathogens then reproduce in the vector, and the increase in the number of pathogens increases the possibility that they will be transmitted to another host. Some parasites reproduce in the gut of the arthropod; these can be passed with feces. If the arthropod defecates or vomits while biting a potential host, the parasite can enter the wound. Other parasites reproduce in the vector's gut and migrate to the salivary gland; these are directly injected into a bite. Some protozoan and helminthic parasites use the vector as a host for a developmental stage in their life cycle.

Table 14.3 lists a few important arthropod vectors and the diseases they transmit.

CHECK YOUR UNDERSTANDING

- Why are carriers important reservoirs of infection? **14-12**
- How are zoonoses transmitted to humans? **14-13**
- Give an example of contact transmission, vehicle transmission, mechanical transmission, and biological transmission. **14-14**

(a) Water

(b) Food

(c) Air

Figure 14.7 Vehicle transmission.

Q How does vehicle transmission differ from contact transmission?

Figure 14.8 Mechanical transmission.

Q How do mechanical transmission and biological transmission by vectors differ?

Healthcare-Associated Infections (HAIs)

LEARNING OBJECTIVES

14-15 Define *healthcare-associated infections,* and explain their importance.

14-16 Define *compromised host.*

14-17 List several methods of disease transmission in hospitals.

14-18 Explain how healthcare-associated infections can be prevented.

Healthcare-associated infections (HAIs) are infections patients acquire while receiving treatment for other conditions at a health care facility, such as a nursing home, hospital, same-day surgery center, outpatient clinic, or in-home health care environment. Traditionally these were called **nosocomial infections** (nosocomial is Latin for hospital).

The Centers for Disease Control and Prevention (CDC) estimates that on any given day, about 1 in 25 hospital patients has at least one HAI. The work of pioneers in aseptic techniques such as Lister and Semmelweis (Chapter 1, page 9) decreased the rate of HAIs considerably. However, despite modern advances in sterilization techniques and disposable materials, the rate of HAIs has increased 36% during the last 20 years. In the United States, about 2 million people per year contract HAIs, and nearly 20,000 die as a result. HAIs represent the eighth leading cause of death in the United States (the top three are heart disease, cancer, and strokes).

HAIs result from the interaction of several factors: (1) microorganisms in the hospital environment, (2) the compromised (or weakened) status of the host, and (3) the chain of transmission in the hospital. Figure 14.9 illustrates that the presence of any one of these factors alone is generally not enough to cause infection; it is the interaction of all three factors that poses a significant risk of HAI.

Microorganisms in the Hospital

Although every effort is made to kill or check the growth of microorganisms in the hospital, the hospital environment is a major reservoir for a variety of pathogens. One reason is that

TABLE 14.3 Representative Arthropod Vectors and the Diseases They Transmit

Disease	Causative Agent	Arthropod Vector	Chapter Reference
Malaria	*Plasmodium* spp. (protozoan)	*Anopheles* (mosquito)	23
African trypanosomiasis	*Trypanosoma brucei gambiense* and *T. b. rhodesiense* (protozoan)	*Glossina* (tsetse fly)	22
Chagas' disease	*T. cruzi* (protozoan)	*Triatoma* (kissing bug)	23
Yellow fever	*Alphavirus* (yellow fever virus)	*Aedes* (mosquito)	23
Dengue	*Alphavirus* (dengue virus)	*A. aegypti* (mosquito)	23
Arthropod-borne encephalitis	*Alphavirus* (encephalitis virus)	*Culex* (mosquito)	22
Ehrlichiosis	*Ehrlichia* spp.	*Ixodes* spp. (tick)	23
Epidemic typhus	*Rickettsia prowazekii*	*Pediculus humanus* (louse)	23
Endemic murine typhus	*R. typhi*	*Xenopsylla cheopis* (rat flea)	23
Rocky Mountain spotted fever	*R. rickettsii*	*Dermacentor andersoni* and other species (tick)	23
Plague	*Yersinia pestis*	*X. cheopis* (rat flea)	23
Relapsing fever	*Borrelia* spp.	*Ornithodorus* spp. (soft tick)	23
Lyme disease	*B. burgdorferi*	*Ixodes* spp. (tick)	23

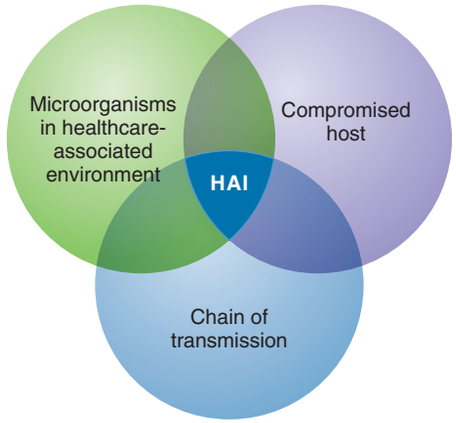


Figure 14.9 Healthcare-associated infections.

Q What are some settings were HAIs occur?

Clinical Case

Jamil calls his physician to discuss his symptoms and makes an appointment for that afternoon. His wife, Charlene, drives Jamil to the appointment; he is not sure he can make it that far without stopping. Initially, Jamil's physician thinks he has noroviral gastroenteritis, but the symptoms have gone on too long. She orders a stool sample to be sent to the local laboratory for culturing. The results return positive for *C. difficile*.

Where could Jamil have contracted *C. difficile*?

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certain normal microbiota of the human body are opportunistic and present a particularly strong danger to hospital patients. In fact, most of the microbes that cause HAIs don't cause disease in healthy people but are pathogenic only for individuals whose defenses have been weakened by illness or therapy (see the boxes on pages 193 and 411).

In the 1940s and 1950s, most HAIs were caused by gram-positive microbes. At one time, the gram-positive *Staphylococcus aureus* was the primary cause of HAIs. In the 1970s, gram-negative rods, such as *E. coli* and *Pseudomonas aeruginosa,* were the most common causes of HAIs. Then, during the 1980s, antibiotic-resistant gram-positive bacteria, *Staphylococcus aureus,* coagulase-negative staphylococci (see page 422), and *Enterococcus* spp., emerged as healthcare-associated pathogens. By the 1990s, these gram-positive bacteria accounted for 34% of nosocomial infections, and four gram-negative pathogens accounted for 32%. In the 2000s, antibiotic resistance in HAIs is a major concern. *Clostridium difficile* is now the leading cause of HAIs. In the past few years, improved prevention methods have led to a decrease in the total incidence of HAIs. The principal microorganisms involved in HAIs are summarized in **Table 14.4**.

In addition to being opportunistic, some microorganisms in the hospital become resistant to antimicrobial drugs, which are commonly used there. For example, *P. aeruginosa* and other such gram-negative bacteria tend to be difficult to control with

TABLE 14.4 Microorganisms Involved in Healthcare-Associated Infections

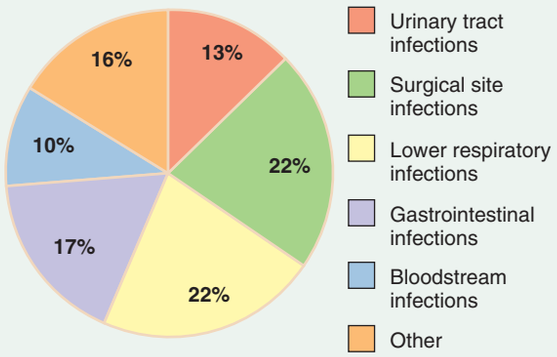
Microorganism	Most Common Infection Type	Percentage of Total Infections	Percentage Resistant to Antibiotics
Coagulase-negative staphylococci	Bloodstream	11%	Not reported
Staphylococcus aureus	Surgical wound	16%	55%
Clostridium difficile	Diarrhea after abdominal surgery	15%	Not reported
Enterococcus spp.	Bloodstream	14%	83%
Candida spp. (fungus)	Urinary tract infections	9%	Not reported
Escherichia coli	Urinary tract infections (most common cause)	12%	20%
Pseudomonas aeruginosa	Urinary tract and pneumonia	8%	10%
Klebsiella pneumoniae	All sites	8%	29%
Enterobacter spp.	All sites	5%	38%
Acinetobacter baumannii	All sites	2%	68%

Source: CDC, Healthcare-Associated Infections.

TABLE 14.5 Principal Sites of Healthcare-Associated Infections

Type of Infection	Comment
Urinary tract infections	Accounts for about 13% of all HAIs. Typically related to urinary catheterization.
Surgical site infections (cutaneous and subcutaneous)	Among the most common HAIs (22%).
Lower respiratory infections	Healthcare-associated pneumonias account for about 22% and have high mortality rates (13–55%). Most of these pneumonias are related to respiratory devices that aid breathing or administer medications.
Bacteremia, caused primarily by intravenous catheterizations	Bacteremias account for about 10% of HAIs. Intravenous catheterization is implicated in HAIs of the bloodstream, particularly infections caused by bacteria and fungi.
Gastrointestinal infections	Account for 17% of all HAIs. *Clostridium difficile* causes 12% of these.

Source: Data from CDC. Healthcare-associated infections. 2014.



antibiotics because of their R factors, which carry genes that determine resistance to antibiotics (see Chapter 8, page 231). As the R factors recombine, new and multiple resistance factors are produced. These strains become part of the microbiota of patients and hospital personnel and become progressively more resistant to antibiotic therapy. In this way, people become part of the reservoir (and chain of transmission) for antibiotic-resistant strains of bacteria. Usually, if the host's resistance is high, the new strains are not much of a problem. However, if disease, surgery, or trauma has weakened the host's defenses, secondary infections may be difficult to treat.

Compromised Host

A **compromised host** is one whose resistance to infection is impaired by disease, therapy, or burns. Two principal conditions can compromise the host: broken skin or mucous membranes, and a suppressed immune system.

As long as the skin and mucous membranes remain intact, they provide formidable physical barriers against most pathogens. Burns, surgical wounds, trauma (such as accidental wounds), injections, invasive diagnostic procedures, ventilators, intravenous therapy, and urinary catheters (used to drain urine) can all break the first line of defense and make a person more susceptible to disease in hospitals. Burn patients are especially susceptible to nosocomial infections because their skin is no longer an effective barrier to microorganisms.

The risk of infection is also related to other invasive procedures, such as administering anesthesia, which may alter breathing and contribute to pneumonia, and tracheotomy, in which an incision is made into the trachea to assist breathing. Patients who require invasive procedures usually have a serious underlying disease, which further increases susceptibility to infections. Invasive devices provide a pathway for microorganisms in the environment to enter the body; they also help transfer microbes from one part of the body to another. Pathogens can also proliferate on the devices themselves (see Figure 1.9 on page 16).

In healthy individuals, white blood cells called T cells (T lymphocytes) provide resistance to disease by killing pathogens directly, mobilizing phagocytes and other lymphocytes, and secreting chemicals that kill pathogens. White blood cells called B cells (B lymphocytes), which develop into antibody-producing cells, also protect against infection. Antibodies provide immunity by such actions as neutralizing toxins, inhibiting the attachment of a pathogen to host cells, and helping to lyse pathogens. Drugs, radiation therapy, steroid therapy, burns, diabetes, leukemia, kidney disease, stress, and malnutrition can all adversely affect the actions of T and B cells and compromise the host. In addition, the AIDS virus destroys certain T cells.

A summary of the principal sites of HAIs is presented in Table 14.5.

Chain of Transmission

Given the variety of pathogens (and potential pathogens) in health care settings and the compromised state of the host, routes of transmission are a constant concern. The principal routes of transmission of HAIs are (1) direct contact transmission from hospital staff to patient and from patient to patient and (2) indirect contact transmission through fomites and the hospital's ventilation system (airborne transmission).

Because health care personnel are in direct contact with patients, they can often transmit disease. For example, a physician or nurse may transmit microbes to a patient when changing a dressing, or a kitchen worker who carries *Salmonella* can contaminate a food supply.

Certain areas of health care facilities are reserved for specialized care; these include the burn, hemodialysis, recovery, intensive care, and oncology units. Unfortunately, these units

also group patients together and provide environments for the epidemic spread of nosocomial infections from patient to patient.

Many diagnostic and therapeutic hospital procedures provide a fomite route of transmission. The urinary catheter used to drain urine from the urinary bladder is a fomite in many HAIs. Intravenous catheters, which pass through the skin and into a vein to provide fluids, nutrients, or medication, can also transmit HAIs. Respiratory aids can introduce contaminated fluids into the lungs. Needles may introduce pathogens into muscle or blood, and surgical dressings can become contaminated and promote disease (see page 411).

Control of Healthcare-Associated Infections

Control measures aimed at preventing nosocomial infections vary from one institution to another, but certain procedures are generally implemented. It's important to reduce the number of pathogens to which patients are exposed by using aseptic techniques, handling contaminated materials carefully, insisting on frequent and thorough handwashing, educating staff members about basic infection control measures, and using isolation rooms and wards.

According to the CDC, handwashing is the single most important means of preventing the spread of infection. Nevertheless, the CDC reports that adherence of health care workers to recommended hand-washing procedures has been poor. On average, health care workers wash their hands before interacting with patients only 40% of the time.

In addition to handwashing, tubs used to bathe patients should be disinfected between uses so that bacteria from the previous patient won't contaminate the next one. Respirators and humidifiers provide both a suitable growth environment for some bacteria and a method of airborne transmission. These sources of HAIs must be kept scrupulously clean and disinfected, and materials used for bandages and intubation (insertion of tubes into organs, such as the trachea) should be single-use disposable or sterilized before use. Packaging used to maintain sterility should be removed aseptically. Physicians can help improve patients' resistance to infection by prescribing antibiotics only when necessary, avoiding invasive procedures if possible, and minimizing the use of immunosuppressive drugs.

Accredited hospitals should have an infection control committee. Most hospitals have at least an infection control nurse or epidemiologist (an individual who studies disease in populations). The role of these staff members is to identify problem sources, such as antibiotic-resistant strains of bacteria and improper sterilization techniques. The infection control officer should make periodic examinations of hospital equipment to determine the extent of microbial contamination. Samples should be taken from tubing, catheters, respirator reservoirs, and other equipment. MM **Animations** Healthcare-Associated Infections: Overview, Prevention

Clinical Case

C. difficile is a bacterium that is involved in 15–25% of all healthcare-associated infections (nosocomial infections) and almost half of all cases of diarrhea. It was first identified in 1935 as part of the normal intestinal microbiota. *C. difficile* was associated with diarrhea in 1977. Infection can range from asymptomatic colonization of patients to diarrhea or colitis. Mortality in older patients is 10–20%. After making sure Jamil is not taking any antibiotics, his physician prescribes the antibiotic metronidazole to treat the *C. difficile*.

Why does Jamil's physician make sure he is not taking antibiotics before she treats the *C. difficile* infection? (*Hint: See page* 391.)

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CHECK YOUR UNDERSTANDING

- What interacting factors result in nosocomial infections? **14-15**
- What is a compromised host? **14-16**
- How are nosocomial infections primarily transmitted, and how can they be prevented? **14-17, 14-18**

Emerging Infectious Diseases

LEARNING OBJECTIVE

14-19 List several probable reasons for emerging infectious diseases, and name one example for each reason.

Emerging infectious diseases (EIDs) are ones that are new or changing, showing an increase in incidence in the recent past, or a potential to increase in the near future (see Chapter 1). An emerging disease can be caused by a virus, a bacterium, a fungus, a protozoan, or a helminth. About 75% of emerging infectious diseases are zoonotic, mainly of viral origin, and are likely to be vector-borne. Several criteria are used for identifying an EID. For example, some diseases present symptoms that are clearly distinctive from all other diseases. Some are recognized because improved diagnostic techniques allow the identification of a new pathogen. Others are identified when a local disease becomes widespread, a rare disease becomes common, a mild disease becomes more severe, or an increase in life span permits a slow disease to develop. Examples

ASM: Human impact on the environment influences the evolution of microorganisms (e.g., emerging diseases and the selection of antibiotic resistance).

TABLE 14.6 Emerging Infectious Diseases

Microorganism	Year of Emergence	Disease Caused	Chapter Reference
BACTERIA			
Clostridium difficile	2004	Diarrhea, colitis, and hemorrhagic necrosis	25
Bordetella pertussis	2000	Whooping cough	24
Mycobacterium ulcerans	1998	Buruli ulcer	21
Methicillin-resistant *Staphylococcus aureus*	1997	Bacteremia, pneumonia	20
Vancomycin-resistant *Staphylococcus aureus*	1996	Bacteremia, pneumonia	20
Streptococcus pneumoniae	1995	Antibiotic-resistant pneumonia	24
Streptococcus pyogenes	1995	Streptococcal toxic shock syndrome	21
Corynebacterium diphtheriae	1994	Diphtheria epidemic, eastern Europe	24
Vibrio cholerae O139	1992	New serovar of cholera, Asia	25
Vancomycin-resistant enterococci	1988	Urinary tract infections, bacteremia, endocarditis	26, 23
Bartonella henselae	1983	Cat-scratch disease	23
Escherichia coli O157:H7	1982	Hemorrhagic diarrhea	25
FUNGI			
Coccidioides immitis	1993	Coccidioidomycosis	24
Pneumocystis jirovecii	1981	Pneumonia in immunocompromised individuals	24
PROTOZOA			
Trypanosoma cruzi	2007	Chagas' disease in United States	23
Cyclospora cayetanensis	1993	Severe diarrhea and wasting syndrome	25
HELMINTHS			
Baylisascaris procyonis	2001	Raccoon roundworm encephalitis, in humans	
VIRUSES			
Chikungunya virus	2013	Chikungunya disease, Americas	23
Middle East respiratory syndrome coronovirus (MERS-CoV)	2013	Middle East respiratory syndrome (MERS)	24
SARS-associated coronavirus	2002	Severe acute respiratory syndrome (SARS)	24
Ebola virus	2014, 2002, 1995, 1975	Ebola hemorrhagic fever	23
West Nile virus	1999	West Nile encephalitis	22
Nipah virus	1998	Encephalitis, Malaysia	22
Influenza A virus	1997, 2009	Avian influenza (H5N1), Swine flu (H1N1)	24
Hendra virus	1994	Encephalitis-like symptoms, Australia	24
Hantavirus	1993	*Hantavirus* pulmonary syndrome	23
Venezuelan hemorrhagic fever	1991	Hemorrhagic fever, South America	23
Hepatitis C virus	1989	Hepatitis	25
Monkeypox virus	1985	Chickenpox-like disease	21
Dengue virus	1984	Dengue	23
HIV	1983	AIDS	19
PRIONS			
Bovine spongiform encephalitis agent	1996	Mad cow disease, Great Britain	22

of emerging infectious diseases are listed in **Table 14.6** and described in the boxes in Chapters 8 and 13 (pages 215 and 364).

A variety of factors contribute to the emergence of new infectious diseases:

- New strains, such as *E. coli* O157:H7 and avian influenza (H5N1), may result from genetic recombination between organisms.
- A new serovar, such as *Vibrio cholerae* O139, may result from changes in or the evolution of existing microorganisms.
- The widespread, and sometimes unwarranted, use of antibiotics and pesticides encourages the growth of more resistant populations of microbes and the insects (mosquitoes and lice) and ticks that carry them.

- Global warming and changes in weather patterns may increase the distribution and survival of reservoirs and vectors, resulting in the introduction and dissemination of diseases such as malaria and *Hantavirus* pulmonary syndrome.
- Known diseases, such as chikungunya (or dengue) and West Nile virus, may spread to new geographic areas by modern transportation. This was less likely 100 years ago, when travel took so long that infected travelers either died or recovered during passage.
- Previously unrecognized infections may appear in individuals living or working in regions undergoing ecological changes brought about by natural disaster, construction, wars, and expanding human settlement. In California, the incidence of coccidioidomycosis increased tenfold following the Northridge earthquake of 1994. Workers clearing South American forests are now contracting Venezuelan hemorrhagic fever.
- Even animal control measures may affect the incidence of a disease. The increase in Lyme disease in recent years could be due to rising deer populations resulting from the killing of deer predators.
- Failures in public health measures may be a contributing factor to the emergence of previously controlled infections. For example, the failure of adults to get a diphtheria booster vaccination led to a diphtheria epidemic in the newly independent republics of the former Soviet Union in the 1990s.

The CDC, the National Institutes of Health (NIH), and the World Health Organization (WHO) have developed plans to address issues relating to EIDs. Their priorities include:

1. To detect, promptly investigate, and monitor emerging infectious pathogens, the diseases they cause, and factors that influence their emergence
2. To expand basic and applied research on ecological and environmental factors, microbial changes and adaptations, and host interactions that influence EIDs
3. To enhance the communication of public health information and the prompt implementation of prevention strategies regarding EIDs
4. To establish plans to monitor and control EIDs worldwide

Because of the importance of emerging infectious diseases to the scientific community, the CDC publishes a monthly journal called *Emerging Infectious Diseases.*

CHECK YOUR UNDERSTANDING

Give several examples of emerging infectious diseases. **14-19**

Clinical Case

Antibiotics can kill competing bacteria, thus allowing growth of *C. difficile.* When Jamil's physician learns of the cause of Jamil's diarrhea, she checks with the hospital to see whether any other patients have developed *C. difficile* diarrhea and colitis. It turns out that 20 other patients are also infected with *C. difficile.* The local health department completes an epidemiological study of the outbreak and releases the following information:

Rate of infection for patients	
Single room	7%
Double room	17%
Triple room	26%

Rate of environmental isolations of *C. difficile*	
Bed rail	10%
Commode	1%
Floor	18%
Call button	6%
Toilet	3%

C. difficile* on hands of hospital personnel after contact with patients who were culture-positive for *C. difficile	
Used gloves	0%
Did not use gloves	59%
Had *C. difficile* before patient contact	3%
Washed with nondisinfectant soap	40%
Washed with disinfectant soap	3%
Did not wash hands	20%

What is the most likely mode of transmission, and how can transmission be prevented?

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Epidemiology

LEARNING OBJECTIVES

14-20 Define *epidemiology,* and describe three types of epidemiologic investigations.

14-21 Identify the function of the CDC.

14-22 Define the following terms: *morbidity, mortality,* and *notifiable infectious diseases.*

In today's crowded, overpopulated world, in which frequent travel and the mass production and distribution of food and other goods are a way of life, diseases can spread rapidly. A contaminated food or water supply, for example, can affect many thousands of people very quickly. Identifying the causative agent of a disease is desirable so it can be effectively controlled and treated. It is also desirable to understand the mode of transmission and geographical distribution of the disease. The science that studies

when and where diseases occur and how they are transmitted in populations is called **epidemiology** (EP-i-dē-mē-ol-ō-jē).

Modern epidemiology began in the mid-1800s with three now-famous investigations. John Snow, a British physician, conducted a series of investigations related to outbreaks of cholera in London. As the cholera epidemic of 1848 to 1849 raged, Snow analyzed the death records attributed to cholera, gathered information about the victims, and interviewed survivors who lived in the neighborhood. Using the information he compiled, Snow made a map showing that most individuals who died of cholera drank or brought water from the Broad Street pump; those who used other pumps (or drank beer, like the workers at a nearby brewery) did not get cholera. He concluded that contaminated water from the Broad Street pump was the source of the epidemic. When the pump's handle was removed and people could no longer get water from this location, the number of cholera cases dropped significantly.

Between 1846 and 1848, Ignaz Semmelweis meticulously recorded the number of births and maternal deaths at Vienna General Hospital. The First Maternity Clinic had become a source of gossip throughout Vienna because the death rate due to puerperal sepsis ranged between 13% and 18%, four times that of the Second Maternity Clinic. Puerperal sepsis (childbirth fever) is a nosocomial infection that begins in the uterus as a result of childbirth or abortion. It is frequently caused by *Streptococcus pyogenes*. The infection progresses to the abdominal cavity (peritonitis) and in many cases to septicemia (proliferation of microbes in the blood). Wealthy women did not go to the clinic, and poor women had learned they had a better chance of surviving childbirth if they gave birth elsewhere before going to the hospital. Looking at his data, Semmelweis identified a common factor among the wealthy women and the poor women who had given birth prior to entering the clinic: they were not examined by the medical students, who had spent their mornings dissecting cadavers. In May 1847, he ordered all medical students to wash their hands with chloride of lime before entering the delivery room, and the mortality rate dropped to under 2%.

Florence Nightingale recorded statistics on epidemic typhus in the English civilian and military populations. In 1858, she published a thousand-page report using statistical comparisons to demonstrate that diseases, poor food, and unsanitary conditions were killing the soldiers. Her work resulted in reforms in the British Army and to her admission to the Statistical Society, their first female member.

These three careful analyses of where and when a disease occurred and how it was transmitted within a population constituted a new approach to medical research and demonstrated the importance of epidemiology. The works of Snow, Semmelweis, and Nightingale resulted in changes that lowered the incidence of diseases even though knowledge of the causes of infectious disease was limited. Most physicians believed that the symptoms they saw were the causes of the disease, not the result of disease. Koch's work on the germ theory of disease was still 30 years in the future.

An epidemiologist not only determines the etiology of a disease but also identifies other possibly important factors and patterns concerning the people affected. An important part of the epidemiologist's work is assembling and analyzing data such as age, sex, occupation, personal habits, socioeconomic status, history of immunization, presence of any other diseases, and the common history of affected individuals (such as eating the same food or visiting the same doctor's office). Also important for the prevention of future outbreaks is knowledge of the site at which a susceptible host came into contact with the agent of infection. In addition, the epidemiologist considers the period during which the disease occurs, either on a seasonal basis (to indicate whether the disease is prevalent during a particular season) or on a yearly basis (to indicate the effects of immunization or an emerging or reemerging disease).

An epidemiologist is also concerned with various methods for controlling a disease. The strategies for controlling diseases include the use of drugs (chemotherapy) and vaccines (immunization). Other methods include the control of human, animal, and nonliving reservoirs of infection, water treatment, proper sewage disposal (enteric diseases), cold storage, pasteurization, food inspection, adequate cooking (foodborne diseases), improved nutrition to bolster host defenses, changes in personal habits, and screening of transfused blood and transplanted organs.

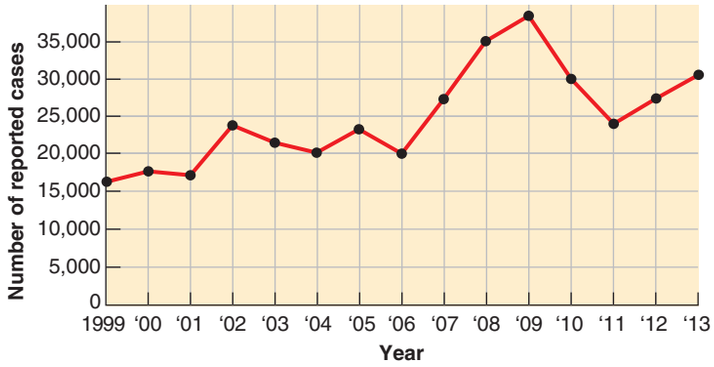
Figure 14.10 contains graphs indicating the incidence of selected diseases. Such graphs provide information about whether disease outbreaks are sporadic or epidemic and, if epidemic, how the disease might have spread. By establishing the frequency of a disease in a population and identifying the factors responsible for its transmission, an epidemiologist can provide physicians with information that is important in determining the prognosis and treatment of a disease. Epidemiologists also evaluate how effectively a disease is being controlled in a community—by a vaccination program, for example. Finally, epidemiologists can provide data to help in evaluating and planning overall health care for a community.

Epidemiologists use three basic types of investigations when analyzing the occurrence of a disease: descriptive, analytical, and experimental.

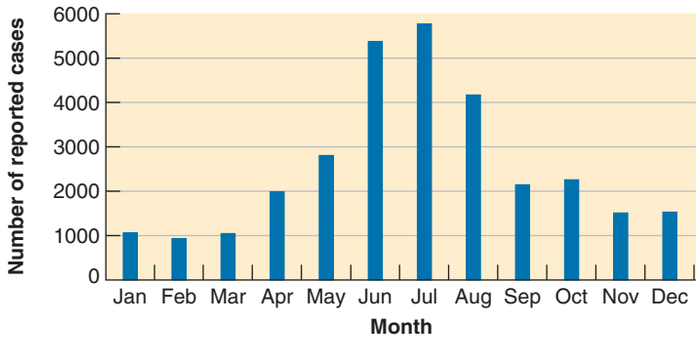
Descriptive Epidemiology

Descriptive epidemiology entails collecting all data that describe the occurrence of the disease under study. Relevant information usually includes information about the affected individuals and the place and period in which the disease occurred. Snow's search for the cause of the cholera outbreak in London is an example of descriptive epidemiology.

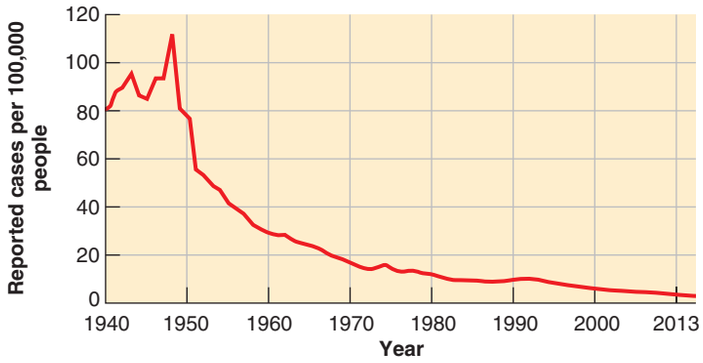
Such a study is generally *retrospective* (looking backward after the episode has ended). In other words, the epidemiologist backtracks to the cause and source of the disease (see the



(a) Lyme disease cases, 1999–2013



(b) Lyme disease by month, 2012



(c) Reported tuberculosis cases, 1940–2013

Figure 14.10 Epidemiological graphs. **(a)** Lyme disease cases, showing the annual occurrence of the disease during the covered period. **(b)** A different perspective of Lyme disease that enabled epidemiologists to draw some conclusions about the disease's epidemiology. This graph records the number of cases per 100,000 people, rather than the total number of cases. **(c)** A graph of the incidence of tuberculosis shows a rapid decrease in the rate of infection from 1948 to 1957.

Source: Data from CDC.

Q What does graph (b) indicate about transmission of Lyme disease? What can you conclude from graph (c)?

boxes in Chapters 21 through 26). The search for the cause of toxic shock syndrome is an example of a fairly recent retrospective study. In the initial phase of an epidemiological study, retrospective studies are more common than *prospective* (looking forward) studies, in which an epidemiologist chooses a group of people who are free of a particular disease to study. The group's subsequent disease experiences are then recorded for a given period. Prospective studies were used to test the Salk polio vaccine in 1954 and 1955.

Analytical Epidemiology

Analytical epidemiology analyzes a particular disease to determine its probable cause. This study can be done in two ways. With the *case control method,* the epidemiologist looks for factors that might have preceded the disease. A group of people who have the disease is compared with another group of people who are free of the disease. For example, one group with meningitis and one without the disease might be matched by age, sex, socioeconomic status, and location. These statistics are compared to determine which of all the possible factors—genetic, environmental, nutritional, and so forth—might be responsible for the meningitis. Nightingale's work was an example of analytical epidemiology, in which she compared disease in soldiers and civilians. With the *cohort method,* the epidemiologist studies two populations: one that has had contact with the agent causing a disease and another that has not (both groups are called *cohort groups*). For example, a comparison of one group composed of people who have received blood transfusions and one composed of people who have not could reveal an association between blood transfusions and the incidence of hepatitis B virus.

Experimental Epidemiology

Experimental epidemiology begins with a hypothesis about a particular disease; experiments to test the hypothesis are then conducted with a group of people. One such hypothesis could be the assumed effectiveness of a drug. A group of infected individuals is selected and divided randomly so that some receive the drug and others receive a *placebo,* a substance that has no effect. If all other factors are kept constant between the two groups, and if those people who received the drug recover more rapidly than those who received the placebo, it can be concluded that the drug was the experimental factor (variable) that made the difference.

Case Reporting

We noted earlier in this chapter that establishing the chain of transmission for a disease is extremely important. Once known, the chain can be interrupted to slow down or stop the spread of the disease.

An effective way to establish the chain of transmission is *case reporting,* a procedure that requires health care workers to report specified diseases to local, state, and national health officials. Examples of such diseases are AIDS, measles, gonorrhea, tetanus, and typhoid fever. Case reporting provides epidemiologists with an approximation of the incidence and prevalence of a disease. This information helps officials decide whether or not to investigate a given disease.

Nationally notifiable diseases, 2013		
Anthrax	Hemolytic uremic syndrome, post-diarrheal	Salmonellosis
Arboviral diseases: neuroinvasive, non-neuroinvasive	Hepatitis A, B, and C	Severe acute respiratory syndrome–associated coronavirus disease
Babesiosis	HIV Infection	Shiga toxin–producing *E. coli*
Botulism	Influenza-associated pediatric mortality	Shigellosis
Brucellosis	Invasive pneumococcal disease	Smallpox
Chancroid	Legionellosis	Spotted fever rickettsiosis
Chlamydia trachomatis infection	Listeriosis	Syphilis
Cholera	Lyme disease	Tetanus
Coccidioidomycosis	Malaria	Toxic shock syndrome (streptococcal and nonstreptococcal)
Cryptosporidiosis	Measles	Trichinellosis
Cyclosporiasis	Meningococcal disease	Tuberculosis
Dengue virus infections	Mumps	Tularemia
Diphtheria	Novel influenza A virus infections	Typhoid fever
Ehrlichiosis and anaplasmosis	Pertussis	Vancomycin-intermediate *Staphylococcus aureus* (VISA)
Giardiasis	Plague	Vancomycin-resistant *Staphylococcus aureus* (VRSA)
Gonorrhea	Poliomyelitis	Varicella
Haemophilus influenzae, invasive disease	Psittacosis	Vibriosis
Hansen's disease (leprosy)	Q fever	Viral hemorrhagic fever
Hantavirus pulmonary syndrome	Rabies, animal or human	Yellow fever
	Rubella	

Figure 14.11 **Nationally notifiable diseases, 2013.**

Q What is meant by a *notifiable infectious disease*?

Case reporting provided epidemiologists with valuable leads regarding the origin and spread of AIDS. In fact, one of the first clues about AIDS came from reports of young men with Kaposi's sarcoma, formerly a disease of older men. Using these reports, epidemiologists began various studies of the patients. If an epidemiological study shows that a large enough segment of the population is affected by a disease, an attempt is then made to isolate and identify its causative agent. Identification is accomplished by a number of different microbiological methods. Identifying the causative agent often provides valuable information regarding the reservoir for the disease.

Once the chain of transmission is discovered, it's possible to apply control measures to stop the disease from spreading. These might include elimination of the source of infection, isolation and segregation of infected people, the development of vaccines, and, as in the case of AIDS, education.

The Centers for Disease Control and Prevention (CDC)

Epidemiology is a major concern of state and federal public health departments. The **Centers for Disease Control and Prevention (CDC),** a branch of the U.S. Public Health Service located in Atlanta, Georgia, is a central source of epidemiological information in the United States.

The CDC issues a publication called the ***Morbidity and Mortality Weekly Report*** (www.cdc.gov). The ***MMWR,*** as it is called, is read by microbiologists, physicians, and other hospital and public health professionals. The *MMWR* contains data on **morbidity,** the incidence of specific notifiable diseases, and **mortality,** the number of deaths from these diseases. These data are usually organized by state. **Notifiable infectious diseases,** shown in Figure 14.11, are diseases for which physicians are

Healthcare-Associated Infections

In this box, you will encounter a series of questions the epidemiologist asks himself as he tries to trace an outbreak to its source. Try to answer each question before going to the next one.

1. Dwayne Jackson, the epidemiologist at a city hospital, would like to find out why during one year, 5287 patients developed bacteremia during their hospital stays. All patients had a fever (>38°C), chills, and low blood pressure; 14% had severe necrotizing fasciitis (see page 585). Dr. Jackson looks at the results of the blood cultures, which were grown on mannitol-salt agar; the bacteria are identified as coagulase-positive, gram-positive cocci (**Figure A**).
   What organisms are possible agents of infection?
2. The biochemical tests confirm that *Staphylococcus aureus* is the culprit. Antibiotic-sensitivity testing shows that all the isolates are methicillin-resistant. Six are vancomycin-intermediate resistant, and one is vancomycin-resistant. Methicillin-resistant *S. aureus* (MRSA) can cause a life-threatening, necrotizing illness due to a leukocidin toxin (see page 427).
   What else does Dr. Jackson need to know?
3. Polymerase chain reaction (PCR) is used to determine that strain USA100 caused 80% of the MRSA cases in Dr. Jackson's hospital. USA100 is the cause of 92% of healthcare-acquired strains. The majority (89%) of community-acquired MRSA infections are the USA300 strain. The incidence of MRSA in the community (not hospitalized) is 0.02–0.04%. Dr. Jackson compares the number of patients with MRSA to the procedures performed and cross-references that information with the antibiotic use of the patients (**Table A**).
   Based on the information in the table, which procedure increases the likelihood of infection most?
4. Each year, an estimated 250,000 cases of bloodstream infections occur in hospitals in the United States from inserting needles into veins to deliver intravenous (IV) solutions, and the estimated mortality for these infections is 12–25%. Dr. Jackson sees that people receiving hemodialysis are especially vulnerable to infections because they require access to veins for prolonged periods and undergo frequent punctures of the access site (**Figure B**).
   How does antimicrobial therapy contribute?
5. The first VRSA (vancomycin-resistant *S. aureus*) infection in the United States occurred in a dialysis patient in 2002. The patient had been treated with vancomycin for a MRSA infection. The VRSA isolate contained the *vanA* vancomycin-resistance gene from enterococci. VRSA are always methicillin resistant. Only ten VRSA cases have been reported in the United States; however, 82 VISA (vancomycin-intermediate *S. aureus*) cases were reported in 2011. Antimicrobial therapy for hemodialysis-associated infections increases the prevalence of antimicrobial resistance. Susceptible bacteria are killed, and bacteria with a mutation that confers resistance are able to grow without competition.

Figure A **The gram-positive cocci grown on mannitol-salt agar.**

Figure B **Hemodialysis procedure.**

Source: Adapted from *MMWR* 61(27): 501–504, July 13, 2012 and *MMWR* 60(53), July 5, 2013.

TABLE A

Procedure	MRSA-Infected Patients	Total Number of Patients Receiving Procedure
Hemodialysis	813	1807
Intravenous (IV) catheter	1057	16,516
Surgery	945	5659
Urinary bladder catheter	1750	7919
Ventilator (invasive airway)	722	7367
Antibiotic Use during the 6 Months Prior to Infection		
Vancomycin	21	41
Fluoroquinolone	49	113
Ceftriaxone	14	41

required by law to report cases to the U.S. Public Health Service. As of 2014, a total of 58 infectious diseases were reported at the national level. **Morbidity rate** is the number of people affected by a disease in a given period of time in relation to the total population. **Mortality rate** is the number of deaths resulting from a disease in a population in a given period of time in relation to the total population.

MMWR articles include reports of disease outbreaks, case histories of special interest, and summaries of the status of particular diseases during a recent period. These articles often include recommendations for procedures for diagnosis, immunization, and treatment. Several graphs and other data in this textbook are from *MMWR*, and the Clinical Focus boxes are adapted from reports from this publication. See the box on page 411, for example. **Animations** Epidemiology: Overview, Occurrence of Disease, Transmission of Disease

CHECK YOUR UNDERSTANDING

- After learning that 40 hospital employees developed nausea and vomiting, the hospital infection control officer determined that 39 ill people ate green beans in the hospital cafeteria, compared to 34 healthy people who ate in the cafeteria the same day but did not eat green beans in the hospital cafeteria. What type of epidemiology is this? **14-20**
- What is the CDC's function? **14-21**
- In 2012, the morbidity of West Nile encephalitis was 5674, and the mortality was 286. The morbidity of listeriosis was 121; the mortality was 13. Which disease is more likely to be fatal? **14-22**

* * *

In the next chapter, we consider the mechanisms of pathogenicity. We'll discuss in more detail the methods by which microorganisms enter the body and cause disease, the effects of disease on the body, and the means by which pathogens leave the body.

Clinical Case Resolved

Transmission of *C. difficile* can be prevented by wearing gloves for contact with all body substances, using disposable rectal thermometers, and stopping antibiotic overuse. *C. difficile* is acquired by ingesting the bacteria or its endospores from direct contact between people or indirect contact via fomites; it's the most common HAI and is considered an epidemic. Jamil responds well to treatment; he is gaining most of his weight back and no longer spends most of his time in the bathroom.

390 403 405 407 412

Study Outline

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Introduction (p. 389)

1. Disease-causing microorganisms are called pathogens.
2. Pathogenic microorganisms have special properties that allow them to invade the human body or produce toxins.
3. When a microorganism overcomes the body's defenses, a state of disease results.

Pathology, Infection, and Disease (p. 390)

1. Pathology is the scientific study of disease.
2. Pathology is concerned with the etiology (cause), pathogenesis (development), and effects of disease.
3. Infection is the invasion and growth of pathogens in the body.
4. A host is an organism that shelters and supports the growth of pathogens.
5. Disease is an abnormal state in which part or all of the body is not properly adjusted or is incapable of performing normal functions.

Normal Microbiota (pp. 390–394)

1. Animals, including humans, are usually germ-free in utero.
2. Microorganisms begin colonization in and on the surface of the body soon after birth.
3. Microorganisms that establish permanent colonies inside or on the body without producing disease make up the normal microbiota.
4. Transient microbiota are microbes that are present for various periods and then disappear.

Relationships between the Normal Microbiota and the Host (pp. 391–393)

5. The normal microbiota can prevent pathogens from causing an infection; this phenomenon is known as microbial antagonism.
6. Normal microbiota and the host exist in symbiosis (living together).
7. The three types of symbiosis are commensalism (one organism benefits, and the other is unaffected), mutualism (both organisms benefit), and parasitism (one organism benefits, and one is harmed).

Opportunistic Microorganisms (p. 393)

8. Opportunistic pathogens do not cause disease under normal conditions but cause disease under special conditions.

Cooperation among Microorganisms (pp. 393–394)

9. In some situations, one microorganism makes it possible for another to cause a disease or produce more severe symptoms.

The Etiology of Infectious Diseases (pp. 394–395)

Koch's Postulates (p. 394)

1. Koch's postulates are criteria for establishing that specific microbes cause specific diseases.
2. Koch's postulates have the following requirements: (1) the same pathogen must be present in every case of the disease; (2) the pathogen must be isolated in pure culture; (3) the pathogen isolated from pure culture must cause the same disease in a healthy, susceptible laboratory animal; and (4) the pathogen must be reisolated from the inoculated laboratory animal.

Exceptions to Koch's Postulates (pp. 394–395)

3. Koch's postulates are modified to establish etiologies of diseases caused by viruses and some bacteria, which cannot be grown on artificial media.
4. Some diseases, such as tetanus, have unequivocal signs and symptoms.
5. Some diseases, such as pneumonia and nephritis, may be caused by a variety of microbes.
6. Some pathogens, such as *S. pyogenes,* cause several different diseases.
7. Certain pathogens, such as HIV, cause disease in humans only.

Classifying Infectious Diseases (pp. 395–397)

1. A patient may exhibit symptoms (subjective changes in body functions) and signs (measurable changes), which a physician uses to make a diagnosis (identification of the disease).
2. A specific group of symptoms or signs that always accompanies a specific disease is called a syndrome.
3. Communicable diseases are transmitted directly or indirectly from one host to another.
4. A contagious disease is a very communicable disease that is capable of spreading easily and rapidly from one person to another.
5. Noncommunicable diseases are caused by microorganisms that normally grow outside the human body and are not transmitted from one host to another.

Occurrence of a Disease (p. 396)

6. Disease occurrence is reported by incidence (number of people contracting the disease) and prevalence (number of cases at a particular time).
7. Diseases are classified by frequency of occurrence: sporadic, endemic, epidemic, and pandemic.

Severity or Duration of a Disease (pp. 396–397)

8. The scope of a disease can be defined as acute, chronic, subacute, or latent.
9. Herd immunity is the presence of immunity to a disease in most of the population.

Extent of Host Involvement (p. 397)

10. A local infection affects a small area of the body; a systemic infection is spread throughout the body via the circulatory system.
11. A primary infection is an acute infection that causes the initial illness.
12. A secondary infection can occur after the host is weakened from a primary infection.
13. An inapparent, or subclinical, infection does not cause any signs of disease in the host.

Patterns of Disease (pp. 397–398)

Predisposing Factors (pp. 397–398)

1. A predisposing factor is one that makes the body more susceptible to disease or alters the course of a disease.
2. Examples include gender, climate, age, fatigue, and inadequate nutrition.

Development of Disease (p. 398)

3. The incubation period is the interval between the initial infection and the first appearance of signs and symptoms.
4. The prodromal period is characterized by the appearance of the first mild signs and symptoms.
5. During the period of illness, the disease is at its height, and all disease signs and symptoms are apparent.
6. During the period of decline, the signs and symptoms subside.
7. During the period of convalescence, the body returns to its prediseased state, and health is restored.

The Spread of Infection (pp. 398–402)

Reservoirs of Infection (pp. 398–399)

1. A continual source of infection is called a reservoir of infection.
2. People who have a disease or are carriers of pathogenic microorganisms are human reservoirs of infection.
3. Zoonoses are diseases that affect wild and domestic animals and can be transmitted to humans.
4. Some pathogenic microorganisms grow in nonliving reservoirs, such as soil and water.

Transmission of Disease (pp. 399–402)

5. Transmission by direct contact involves close physical contact between the source of the disease and a susceptible host.
6. Transmission by fomites (inanimate objects) constitutes indirect contact.
7. Transmission via saliva or mucus in coughing or sneezing is called droplet transmission.
8. Transmission by a medium such as water, food, or air is called vehicle transmission.
9. Airborne transmission refers to pathogens carried on water droplets or dust for a distance greater than 1 meter.
10. Arthropod vectors carry pathogens from one host to another by both mechanical and biological transmission.

Healthcare-Associated Infections (HAIs) (pp. 402–405)

1. Healthcare-associated infections (HAIs) include those acquired in settings such as hospitals, nursing homes, surgical centers, and health care clinics.
2. About 5–15% of patients acquire HAIs in the treatment environment.

Microorganisms in the Hospital (pp. 402–404)

3. Certain normal microbiota are often responsible for HAIs when they are introduced into the body through such medical procedures as surgery and catheterization.
4. Opportunistic bacteria are the most frequent causes of HAIs.

Compromised Host (p. 404)

5. Patients with burns, surgical wounds, and suppressed immune systems are the most susceptible to HAIs.

Chain of Transmission (pp. 404–405)

6. HAIs are transmitted by direct contact between staff members and patients and between patients.
7. Fomites such as catheters, syringes, and respiratory devices can transmit HAIs.

Control of Healthcare-Associated Infections (HAIs) (p. 405)

8. Aseptic techniques can prevent HAIs.
9. Hospital infection control staff members are responsible for overseeing the proper cleaning, storage, and handling of equipment and supplies.

Emerging Infectious Diseases (pp. 405–407)

1. New diseases and diseases with increasing incidences are called emerging infectious diseases (EIDs).
2. EIDs can result from the use of antibiotics and pesticides, climatic changes, travel, the lack of vaccinations, and improved case reporting.
3. The CDC, NIH, and WHO are responsible for surveillance and responses to emerging infectious diseases.

Epidemiology (pp. 407–412)

1. The science of epidemiology is the study of the transmission, incidence, and frequency of disease.
2. Modern epidemiology began in the mid-1800s with the works of Snow, Semmelweis, and Nightingale.
3. In descriptive epidemiology, data about infected people are collected and analyzed.
4. In analytical epidemiology, a group of infected people is compared with an uninfected group.
5. In experimental epidemiology, controlled experiments designed to test hypotheses are performed.
6. Case reporting provides data on incidence and prevalence to local, state, and national health officials.
7. The Centers for Disease Control and Prevention (CDC) is the main source of epidemiological information in the United States.
8. The CDC publishes the *Morbidity and Mortality Weekly Report* to provide information on morbidity (incidence) and mortality (deaths).

Study Questions

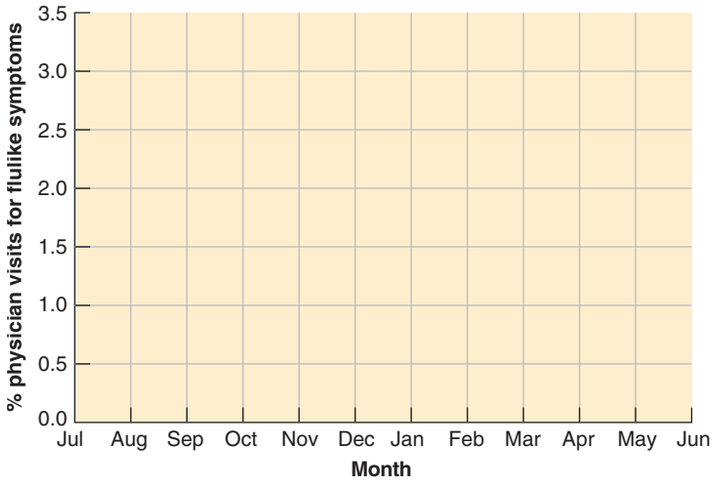
For answers to the Knowledge and Comprehension questions, turn to the Answers tab in the back of the textbook.

Knowledge and Comprehension

Review

1. Differentiate the terms in each of the following pairs:
   a. etiology and pathogenesis
   b. infection and disease
   c. communicable disease and noncommunicable disease
2. Define *symbiosis*. Differentiate commensalism, mutualism, and parasitism, and give an example of each.
3. Indicate whether each of the following conditions is typical of subacute, chronic, or acute infections.
   a. The patient experiences a rapid onset of malaise; symptoms last 5 days.
   b. The patient experiences cough and breathing difficulty for months.
   c. The patient has no apparent symptoms and is a known carrier.
4. Of all the hospital patients with infections, one-third do not enter the hospital with an infection. How do they acquire these infections? What is the method of transmission of these infections? What is the reservoir of infection?
5. Distinguish symptoms from signs as signals of disease.
6. How can a local infection become a systemic infection?
7. Why are some organisms that constitute the normal microbiota described as commensals, whereas others are described as mutualistic?
8. Put the following in the correct order to describe the pattern of disease: period of convalescence, prodromal period, period of decline, incubation period, period of illness.
9. NAME IT This microbe is acquired by humans as infants and is essential for good health. Acquiring a closely related strain causes severe stomach cramps, bloody diarrhea, and vomiting. What is the microbe?
10. DRAW IT Using the data below, draw a graph showing the incidence of influenza during a typical year. Indicate the endemic and epidemic levels.

Month	Percentage of Physician Visits for Influenza-like Symptoms
Jan	2.33
Feb	3.21
Mar	2.68
Apr	1.47
May	0.97
Jun	0.30
Jul	0.30
Aug	0.20
Sep	0.20
Oct	1.18
Nov	1.54
Dec	2.39



Multiple Choice

1. The emergence of new infectious diseases is probably due to all of the following *except*
   a. the need of bacteria to cause disease.
   b. the ability of humans to travel by air.
   c. changing environments (e.g., flood, drought, pollution).
   d. a pathogen crossing the species barrier.
   e. the increasing human population.
2. All members of a group of ornithologists studying barn owls in the wild have had salmonellosis (*Salmonella* gastroenteritis). One birder is experiencing her third infection. What is the most likely source of their infections?
   a. The ornithologists are eating the same food.
   b. They are contaminating their hands while handling the owls and nests.
   c. One of the workers is a *Salmonella* carrier.
   d. Their drinking water is contaminated.
3. Which of the following statements is *false*?
   a. *E. coli* never causes disease.
   b. *E. coli* provides vitamin K for its host.
   c. *E. coli* often exists in a mutualistic relationship with humans.
   d. *E. coli* gets nutrients from intestinal contents.
4. Which of the following is *not* one of Koch's postulates?
   a. The same pathogen must be present in every case of the disease.
   b. The pathogen must be isolated and grown in pure culture from the diseased host.
   c. The pathogen from pure culture must cause the disease when inoculated into a healthy, susceptible laboratory animal.
   d. The disease must be transmitted from a diseased animal to a healthy, susceptible animal by direct contact.
   e. The pathogen must be isolated in pure culture from an experimentally infected lab animal.
5. Which one of the following diseases is *not* correctly matched to its reservoir?
   a. influenza— animal
   b. rabies—animal
   c. botulism—nonliving
   d. anthrax—nonliving
   e. toxoplasmosis—cats

Use the following information to answer questions 6–7.

On September 6, a 6-year-old boy experienced fever, chills, and vomiting. On September 7, he was hospitalized with diarrhea and swollen lymph nodes under both arms. On September 3, the boy had been scratched and bitten by a cat. The cat was found dead on September 5, and *Yersinia pestis* was isolated from the cat. Chloramphenicol was administered to the boy from September 7, when *Y. pestis* was isolated from him. On September 17, the boy's temperature returned to normal; and on September 22, he was released from the hospital.

6. Identify the incubation period for this case of bubonic plague.
   a. September 3–5.
   b. September 3–6.
   c. September 6–7.
   d. September 6–17.
7. Identify the prodromal period for this disease.
   a. September 3–5.
   b. September 3–6.
   c. September 6–7.
   d. September 6–17.

Use the following information to answer questions 8–10.

A Maryland woman was hospitalized with dehydration; *Vibrio cholerae* and *Plesiomonas shigelloides* were isolated from the patient. She had neither traveled outside the United States nor eaten raw shellfish during the preceding month. She had attended a party 2 days before her hospitalization. Two other people at the party had acute diarrheal illness and elevated levels of serum antibodies against *Vibrio*. Everyone at the party ate crabs and rice pudding with coconut milk. Crabs left over from this party were served at a second party. One of the 20 people at the second party had onset of mild diarrhea; specimens from 14 of these people were negative for vibriocidal antibodies.

8. This is an example of
   a. vehicle transmission.
   b. airborne transmission.
   c. transmission by fomites.
   d. direct contact transmission.
   e. healthcare-associated transmission.
9. The etiologic agent of the disease is
   a. *Plesiomonas shigelloides.*
   b. crabs.
   c. *Vibrio cholerae.*
   d. coconut milk.
   e. rice.
10. The source of the disease was
    a. *Plesiomonas shigelloides.*
    b. crabs.
    c. *Vibrio cholerae.*
    d. coconut milk.
    e. rice.

Analysis

1. Ten years before Robert Koch published his work on anthrax, Anton De Bary showed that potato blight was caused by the alga *Phytophthora infestans*. Why do you suppose we use Koch's postulates instead of something called "De Bary's postulates"?
2. Florence Nightingale gathered the following data in 1855.

Population Sampled	Deaths from Contagious Diseases
Englishmen (in general population)	0.2%
English soldiers (in England)	18.7%
English soldiers (in Crimean War)	42.7%
English soldiers (in Crimean War) after Nightingale's sanitary reforms	2.2%

   Discuss how Nightingale used the three basic types of epidemiological investigation. The contagious diseases were primarily cholera and typhus; how are these diseases transmitted and prevented?
3. Name the method of transmission of each of the following diseases:
   a. malaria
   b. tuberculosis
   c. salmonellosis
   d. streptococcal pharyngitis
   e. mononucleosis
   f. measles
   g. hepatitis A
   h. tetanus
   i. hepatitis B
   j. chlamydial urethritis

4. The following graph shows the incidence of typhoid fever in the United States from 1954 through 2013. Mark the graph to show when this disease occurred sporadically and epidemically. What appears to be the endemic level? What would have to be shown to indicate a pandemic of this disease? How is typhoid fever transmitted?

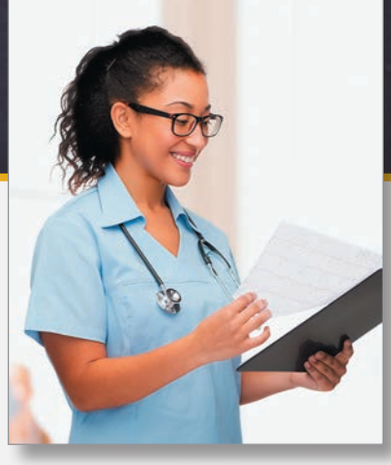
Clinical Applications and Evaluation

1. Three days before a nurse developed meningococcemia, she assisted with intubation of a patient with a *Neisseria meningitidis* infection. Of the 24 medical personnel involved, only this nurse became ill. The nurse recalled that she was exposed to nasopharyngeal secretions and did not receive antibiotic prophylaxis. What two mistakes did the nurse make? How is meningitis transmitted?
2. Three patients in a large hospital acquired infections of *Burkholderia cepacia* during their stay. All three patients received cryoprecipitate, which is prepared from blood that has been frozen in a standard plastic blood transfer pack. The transfer pack is then placed in a water bath to thaw. What is the probable origin of the infections? What characteristics of *Burkholderia* would allow it to be involved in this type of infection?
3. Following is a case history of a 49-year-old man. Identify each period in the pattern of disease that he experienced. On February 7, he handled a parakeet with a respiratory illness. On March 9, he experienced intense pain in his legs, followed by severe chills and headaches. On March 16, he had chest pains, cough, and diarrhea, and his temperature was 40°C. Appropriate antibiotics were administered on March 17, and his fever subsided within 12 hours. He continued taking antibiotics for 14 days. (*Note:* The disease is psittacosis. Can you find the etiology?)
4. *Mycobacterium avium-intracellulare* is prevalent in AIDS patients. In an effort to determine the source of this infection, hospital water systems were sampled. The water contained chlorine.

Percentage of Samples with *M. avium*

Hot Water		Cold Water	
February	88%	February	22%
June	50%	June	11%

What is the usual method of transmission for *Mycobacterium*? What is a probable source of infection in hospitals? How can such healthcare-associated infections be prevented?



In the Clinic

You are a transplant nurse taking care of a liver transplant patient. He tells you he is concerned that his physician stopped his iron supplements. The patient knows the supplements were used to treat his anemia. **What will you tell the patient?**

Hint: Read about siderophores on page 424.

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

15 Microbial Mechanisms of Pathogenicity

Now that you have a basic understanding of how microorganisms cause disease, we will take a look at some of the specific properties of microorganisms that contribute to pathogenicity, the ability to cause disease by overcoming the defenses of a host, and virulence, the degree or extent of pathogenicity. (As discussed throughout the chapter, the term *host* usually refers to humans.)

Microbes don't try to cause disease; the microbial cells are getting food and defending themselves. Sometimes the presence of microbial cells or cell parts can induce symptoms in a host. An example due to *Burkholderia* (in the photograph) is described in the Clinical Case.

To humans, it doesn't make sense for a parasite to kill its host. However, nature does not have a plan for evolution; the genetic variations that give rise to evolution are due to random mutations, not to logic. According to natural selection, organisms best adapted to their environments will reproduce. Coevolution between a parasite and its host seems to occur: the behavior of one influences that of the other. For example, the cholera pathogen, *Vibrio cholerae,* quickly induces diarrhea, threatening the host's life from a loss of fluids and salts but providing a way to transmit the pathogen to another person by contaminating the water supply.

Keep in mind that many of the properties contributing to microbial pathogenicity and virulence are unclear or unknown. We do know, however, that if the microbe overpowers the host defenses, disease results.

***Burkholderia* species like the ones shown here form biofilms that cause infections in hospitalized patients.**

How Microorganisms Enter a Host

LEARNING OBJECTIVES

15-1 Identify the principal portals of entry.

15-2 Define ID_{50} and LD_{50}.

15-3 Using examples, explain how microbes adhere to host cells.

As noted earlier, **pathogenicity** is the ability to cause disease by overcoming host defenses, whereas **virulence** is the degree of pathogenicity. To cause disease, most pathogens must gain access to the host, adhere to host tissues, penetrate or evade host defenses, and damage the host tissues. However, some microbes do not cause disease by directly damaging host tissue. Instead, disease is due to the accumulation of microbial waste products. Some microbes, such as those that cause dental caries and acne, can cause disease without penetrating the body. Pathogens can gain entrance to the human body and other hosts through several avenues, which are called **portals of entry.**

Portals of Entry

The portals of entry for pathogens are mucous membranes, skin, and direct deposition beneath the skin or membranes (the parenteral route).

Mucous Membranes

Many bacteria and viruses gain access to the body by penetrating mucous membranes lining the respiratory tract, gastrointestinal tract, genitourinary tract, and conjunctiva, a delicate membrane that covers the eyeballs and lines the eyelids. Most pathogens enter through the mucous membranes of the gastrointestinal and respiratory tracts.

The respiratory tract is the easiest and most frequently traveled portal of entry for infectious microorganisms. Microbes are inhaled into the nose or mouth in drops of moisture and dust particles. Diseases that are commonly contracted via the respiratory tract include the common cold, pneumonia, tuberculosis, influenza, and measles.

Microorganisms can gain access to the gastrointestinal tract in food and water and via contaminated fingers. Most microbes that enter the body in these ways are destroyed by hydrochloric acid and enzymes in the stomach or by bile and enzymes in the small intestine. Those that survive can cause disease. Microbes in the gastrointestinal tract can cause poliomyelitis, hepatitis A, typhoid fever, amebic dysentery, giardiasis, shigellosis (bacillary dysentery), and cholera. These pathogens are then eliminated with feces and can be transmitted to other hosts via contaminated water, food, or fingers.

The genitourinary tract is a portal of entry for pathogens that are contracted sexually. Some microbes that cause sexually transmitted infections (STIs) may penetrate an unbroken mucous membrane. Others require a cut or abrasion of some type. Examples of STIs are HIV infection, genital warts, chlamydia, herpes, syphilis, and gonorrhea.

Skin

The skin is the largest organ of the body, in terms of surface area and weight, and is an important defense against disease. Unbroken skin is impenetrable by most microorganisms. Some microbes gain access to the body through openings in the skin, such as hair follicles and sweat gland ducts. Larvae of the hookworm actually bore through intact skin, and some fungi grow on the keratin in skin or infect the skin itself.

The conjunctiva is a delicate mucous membrane that lines the eyelids and covers the white of the eyeballs. Although it is a relatively effective barrier against infection, certain diseases such as conjunctivitis, trachoma, and ophthalmia neonatorum are acquired through the conjunctiva.

The Parenteral Route

Other microorganisms gain access to the body when they are deposited directly into the tissues beneath the skin or into mucous membranes when these barriers are penetrated or injured. This route is called the **parenteral route.** Punctures, injections, bites, cuts, wounds, surgery, and splitting of the skin or mucous membrane due to swelling or drying can all establish parenteral routes. HIV, the hepatitis viruses, and bacteria that cause tetanus and gangrene can be transmitted parenterally.

Clinical Case: The Eyes Have It

Kerry Santos, a board-certified ophthalmologist for 20 years, has had a long day. She performed outpatient cataract surgery on ten patients (**Figure A**) today. As she checks over her patients in the recovery area, she notes that eight of the ten patients have an unusual degree of inflammation and that their pupils are fixed and do not respond to light.

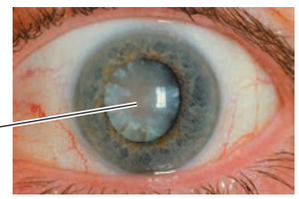


Figure A A cataract is a clouding of the eye lens. It distorts vision.

What could have caused this complication? Read on to find out.

TABLE 15.1 Portals of Entry for the Pathogens of Some Common Diseases

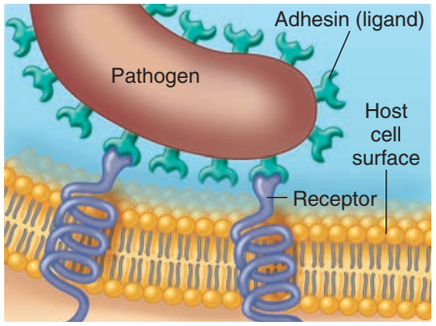
Portal of Entry	Pathogen*	Disease	Incubation Period
MUCOUS MEMBRANES			
Respiratory tract	*Streptococcus pneumoniae*	Pneumococcal pneumonia	1–3 days
	Mycobacterium tuberculosis†	Tuberculosis	2–12 weeks
	Bordetella pertussis	Whooping cough (pertussis)	12–20 days
	Influenza virus *(Influenzavirus)*	Influenza	18–36 hours
	Measles virus *(Morbillivirus)*	Measles (rubeola)	11–14 days
	Rubella virus *(Rubivirus)*	German measles (rubella)	2–3 weeks
	Epstein-Barr virus *(Lymphocryptovirus)*	Infectious mononucleosis	2–6 weeks
	Varicella-zoster virus *(Varicellovirus)*	Chickenpox (varicella) (primary infection)	14–16 days
	Histoplasma capsulatum (fungus)	Histoplasmosis	5–18 days
Gastrointestinal tract	*Shigella* spp.	Shigellosis (bacillary dysentery)	1–2 days
	Brucella spp.	Brucellosis (undulant fever)	6–14 days
	Vibrio cholerae	Cholera	1–3 days
	Salmonella enterica	Salmonellosis	7–22 hours
	Salmonella typhi	Typhoid fever	14 days
	Hepatitis A virus *(Hepatovirus)*	Hepatitis A	15–50 days
	Mumps virus *(Rubulavirus)*	Mumps	2–3 weeks
	Trichinella spiralis (helminth)	Trichinellosis	2–28 days
Genitourinary tract	*Neisseria gonorrhoeae*	Gonorrhea	3–8 days
	Treponema pallidum	Syphilis	9–90 days
	Chlamydia trachomatis	Nongonococcal urethritis	1–3 weeks
	Herpes simplex virus type 2	Herpes virus infections	4–10 days
	Human immunodeficiency virus (HIV)‡	AIDS	10 years
	Candida albicans (fungus)	Candidiasis	2–5 days
SKIN OR PARENTERAL ROUTE			
	Clostridium perfringens	Gas gangrene	1–5 days
	Clostridium tetani	Tetanus	3–21 days
	Rickettsia rickettsii	Rocky Mountain spotted fever	3–12 days
	Hepatitis B virus *(Hepadnavirus)*‡	Hepatitis B	6 weeks–6 months
	Rabiesvirus *(Lyssavirus)*	Rabies	10 days–1 year
	Plasmodium spp. (protozoan)	Malaria	2 weeks

*All pathogens are bacteria, unless indicated otherwise. For viruses, the viral species and/or genus name is given.
†These pathogens can also cause disease after entering the body via the gastrointestinal tract.
‡These pathogens can also cause disease after entering the body via the parenteral route. Hepatitis B virus and HIV can also cause disease after entering the body via the genitourinary tract.

Even after microorganisms have entered the body, they do not necessarily cause disease. The occurrence of disease depends on several factors, only one of which is the portal of entry.

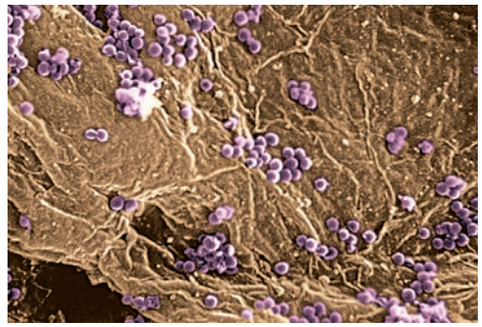
The Preferred Portal of Entry

Many pathogens have a preferred portal of entry that is a prerequisite to their being able to cause disease. If they gain access to the body by another portal, disease might not occur. For example, the bacteria of typhoid fever, *Salmonella typhi,* produce all the signs and symptoms of the disease when swallowed (preferred route), but if the same bacteria are rubbed on the skin, no reaction (or only a slight inflammation) occurs. Streptococci that are inhaled (preferred route) can cause pneumonia; those that are swallowed generally do not produce signs or symptoms. Some pathogens, such as *Yersinia pestis,* the microorganism that causes plague, and *Bacillus anthracis,* the causative agent of anthrax, can initiate disease from more than one portal of entry. The preferred portals of entry for some common pathogens are listed in **Table 15.1**.



(a) Surface molecules on a pathogen, called adhesins or ligands, bind specifically to complementary surface receptors on cells of certain host tissues.

(b) *E. coli* bacteria (yellow-green) on human urinary bladder cells SEM 1 μm



(c) Bacteria (purple) adhering to human skin SEM 9 μm

Figure 15.1 Adherence.

Q Of what chemicals are adhesins composed?

Numbers of Invading Microbes

If only a few microbes enter the body, they will probably be overcome by the host's defenses. However, if large numbers of microbes gain entry, the stage is probably set for disease. The likelihood of disease increases as the number of pathogens increases.

The virulence of a microbe is often expressed as the **ID_{50}** (infectious dose for 50% of a sample population). The 50 is not an absolute value; rather, it is used to compare relative virulence under experimental conditions. *Bacillus anthracis* can cause infection via three different portals of entry. The ID_{50} through the skin (cutaneous anthrax) is 10 to 50 endospores; the ID_{50} for inhalation anthrax is inhalation of 10,000 to 20,000 endospores; and the ID_{50} for gastrointestinal anthrax is ingestion of 250,000 to 1,000,000 endospores. These data show that cutaneous anthrax is significantly easier to acquire than either the inhalation or the gastrointestinal forms. A study of *Vibrio cholerae* showed that the ID_{50} is 10^8 cells; but if stomach acid is neutralized with bicarbonate, the number of cells required to cause an infection decreases significantly.

The potency of a toxin is often expressed as the **LD_{50}** (lethal dose for 50% of a sample population). For example, the LD_{50} for botulinum toxin in mice is 0.03 ng/kg;* for Shiga toxin, 250 ng/kg; and staphylococcal enterotoxin, 1350 ng/kg. In other words, compared to the other two toxins, a much smaller dose of botulinum toxin is needed to cause symptoms.

Adherence

Almost all pathogens have some means of attaching themselves to host tissues at their portal of entry. For most pathogens, this attachment, called **adherence** (or **adhesion**), is a necessary step in pathogenicity. (Of course, nonpathogens also have structures for attachment.) The attachment between pathogen and host is accomplished by means of surface molecules on the pathogen called **adhesins** or **ligands** that bind specifically to complementary surface **receptors** on the cells of certain host tissues (Figure 15.1). Adhesins may be located on a microbe's glycocalyx or on other microbial surface structures, such as pili, fimbriae, and flagella (see Chapter 4).

The majority of adhesins on the microorganisms studied so far are glycoproteins or lipoproteins. The receptors on host cells are typically sugars, such as mannose. Adhesins on different strains of the same species of pathogen can vary in structure. Different cells of the same host can also have different receptors that vary in structure. If adhesins, receptors, or both can be altered to interfere with adherence, infection can often be prevented (or at least controlled).

There is great diversity of adhesins. *Streptococcus mutans,* a bacterium that plays a key role in tooth decay, attaches to the surface of teeth by its glycocalyx. An enzyme produced by *S. mutans,* called glucosyltransferase, converts glucose into a sticky polysaccharide called dextran, which forms the glycocalyx. *Actinomyces* bacterial cells have fimbriae that adhere to the glycocalyx of *S. mutans.* The combination of *S. mutans, Actinomyces,* and dextran makes up dental plaque and contributes to dental caries (tooth decay; see Chapter 25, page 709).

Microbes have the ability to come together in masses, cling to surfaces, and take in and share available nutrients in communities called **biofilms** (discussed in more detail in Chapter 6, page 156). Examples of biofilms include the dental plaque on teeth, the algae on the walls of swimming pools, and the scum that accumulates on shower doors. A biofilm forms when microbes adhere to a particular surface that is typically moist and contains organic matter. The first microbes to attach are usually bacteria. Once they adhere to the surface, they multiply and secrete a glycocalyx that further attaches the bacteria to each other and to the surface (see Figure 6.5, page 157). In

*Nanogram (ng) equals one-billionth of a gram; kilogram (kg) equals 1000 grams.

some cases, biofilms can be several layers thick and may contain several types of microbes. Biofilms represent another method of adherence and are important because they resist disinfectants and antibiotics. This characteristic is significant, especially when biofilms colonize structures such as teeth, medical catheters, stents, heart valves, hip replacement components, and contact lenses. Dental plaque is actually a biofilm that mineralizes over time, creating what is commonly known as tartar. It is estimated that biofilms are involved in 65% of all human bacterial infections.

Enteropathogenic strains of *E. coli* (those responsible for gastrointestinal disease) have adhesins on fimbriae that adhere only to specific kinds of cells in certain regions of the small intestine. After adhering, *Shigella* and *E. coli* induce receptor-mediated endocytosis as a vehicle to enter host cells and then multiply within them (see Figure 25.7, page 714). *Treponema pallidum,* the causative agent of syphilis, uses its tapered end as a hook to attach to host cells. *Listeria monocytogenes,* which causes meningitis, spontaneous abortions, and stillbirths, produces an adhesin for a specific receptor on host cells. *Neisseria gonorrhoeae,* the causative agent of gonorrhea, also has fimbriae containing adhesins, which in this case permit attachment to cells with appropriate receptors in the genitourinary tract, eyes, and pharynx. *Staphylococcus aureus,* which can cause skin infections, binds to skin by a mechanism of adherence that resembles viral attachment (see Chapter 13).

CHECK YOUR UNDERSTANDING

- List three portals of entry, and describe how microorganisms gain access through each. **15-1**
- The LD_{50} of botulinum toxin is 0.03 ng/kg; the LD_{50} of *Salmonella* toxin is 12 mg/kg. Which is the more potent toxin? **15-2**
- How would a drug that binds mannose on human cells affect a pathogenic bacterium? **15-3**

How Bacterial Pathogens Penetrate Host Defenses

LEARNING OBJECTIVES

15-4 Explain how capsules and cell wall components contribute to pathogenicity.

15-5 Compare the effects of coagulases, kinases, hyaluronidase, and collagenase.

15-6 Define and give an example of *antigenic variation.*

15-7 Describe how bacteria use the host cell's cytoskeleton to enter the cell.

Although some pathogens can cause damage on the surface of tissues, most must penetrate tissues to cause disease. Here we will consider several factors that contribute to the ability of bacteria to invade a host.

Capsules

Recall from Chapter 4 that some bacteria make glycocalyx material that forms capsules around their cell walls; this property increases the virulence of the species. The capsule resists the host's defenses by impairing phagocytosis, the process by which certain cells of the body engulf and destroy microbes (see Chapter 16, page 452). The chemical nature of the capsule appears to prevent the phagocytic cell from adhering to the bacterium. However, the human body can produce antibodies against the capsule, and when these antibodies are present on the capsule surface, the encapsulated bacteria are easily destroyed by phagocytosis.

One bacterium that owes its virulence to the presence of a polysaccharide capsule is *Streptococcus pneumoniae,* the causative agent of pneumococcal pneumonia (see Figure 24.11, page 689). Strains of this bacterium with capsules are virulent, but strains without capsules are avirulent because they are susceptible to phagocytosis. Other bacteria that produce capsules related to virulence are *Klebsiella pneumoniae,* a causative agent of bacterial pneumonia; *Haemophilus influenzae,* a cause of pneumonia and meningitis in children; *Bacillus anthracis,* the cause of anthrax; and *Yersinia pestis,* the causative agent of plague. Keep in mind that capsules are not the only cause of virulence. Many nonpathogenic bacteria produce capsules, and the virulence of some pathogens is not related to the presence of a capsule.

Cell Wall Components

The cell walls of certain bacteria contain chemical substances that contribute to virulence. For example, *Streptococcus pyogenes* produces a heat-resistant and acid-resistant protein called **M protein** (see Figure 21.6, page 585). This protein is found on both the cell surface and fimbriae. It mediates attachment of the bacterium to epithelial cells of the host and helps the bacterium resist phagocytosis by white blood cells. The M protein thereby increases the virulence of the microorganism. Immunity to *S. pyogenes* depends on the body's production of an antibody specific to M protein. *Neisseria gonorrhoeae* grows inside human epithelial cells and leukocytes. These bacteria use **fimbriae** and an outer membrane protein called **Opa** to attach to host cells. Following attachment by both Opa and fimbriae, the host cells take in the bacteria. (Bacteria that produce Opa form *opa*que colonies on culture media.) The **waxy lipid** (mycolic acid) that makes up the cell wall of *Mycobacterium tuberculosis* also increases virulence by resisting digestion by phagocytes, and the bacteria can even multiply inside phagocytes.

Enzymes

Microbiologists think that the virulence of some bacteria is aided by the production of extracellular enzymes *(exoenzymes)* and related substances. These chemicals can digest materials between cells and form or digest blood clots, among other functions.

Streptococcus: Harmful or Helpful?

Louie, a 56-year-old man, wakes in the middle of the night with a burning pain in his chest. He is having a heart attack. Louie is rushed to the hospital, where his family is told that he has a blockage in one of his coronary arteries. The main cause of heart attack is blood clots that block the flow of blood in the coronary arteries (**Figure A**). Louie's physician injects him with the enzyme streptokinase to digest the blockage.

Ashley, a 4-month-old girl, has been having flulike symptoms, including tiredness and an intermittent, low-grade fever, for 4 days. Now her left leg has become red and swollen, and no puncture or scrape is visible. Her parents take her to the pediatrician, who orders that Ashley be hospitalized and given intravenous antibiotics. Despite the antibiotics treatment, after 2 days the area becomes dark, and fluid-filled vesicles appear (**Figure B**). Damaged tissue is blocking blood flow to her left leg. Ashley undergoes a fasciotomy (removal of the connective tissue over the muscles) of her left leg. Ashley has necrotizing fasciitis caused by *Streptococcus pyogenes*. Tissue destruction from *S. pyogenes* can occur as fast as 2 cm of tissue per hour—much faster than the bacteria are growing.

One of the enzymes responsible for the rapid spread is streptokinase. Strains that produce streptokinase digest the fibrin clot that the body uses to isolate an infection.

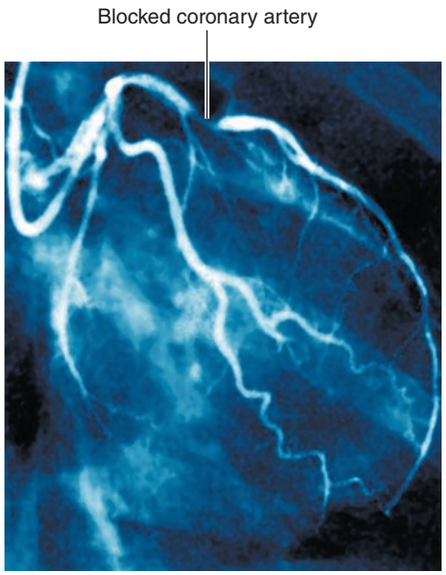


Figure A X-ray image of coronary arteries.

Figure B Necrotizing fasciitis.

What Do Louie and Ashley Have in Common?

Both Louie and Ashley have been affected by streptokinase. Louie has a positive experience:

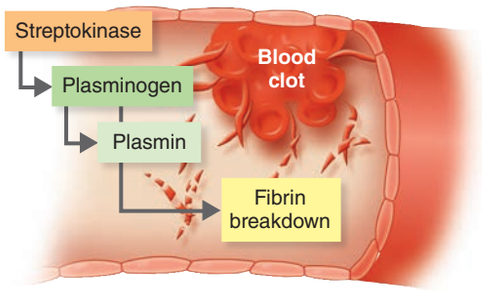


Figure C Mechanism of streptokinase.

the enzyme is used to hydrolize the blood clot that is blocking the artery to his heart. Ashley, however, is affected negatively: the streptokinase produced by *S. pyogenes* has destroyed tissue in her left leg. Normally the body produces plasmin to break down unneeded blood clots. Streptokinase breaks down the body's precursor, plasminogen, to produce plasmin (**Figure C**).

In the 1950s, physicians reported using streptokinase to successfully treat coronary artery blockage. Streptokinase became the mainstay for digesting blood clots when the U.S. Food and Drug Administration (FDA) approved its use in 1982.

Streptokinase is produced commercially from *Streptococcus equisimilis* H46A. The enzyme must be purified to ensure that toxins are not present. Isolation of the streptokinase gene has also allowed the enzyme to be produced by recombinant *E. coli*.

Coagulases are bacterial enzymes that coagulate (clot) the fibrinogen in blood. Fibrinogen, a plasma protein produced by the liver, is converted by coagulases into fibrin, the threads that form a blood clot. The fibrin clot may protect the bacterium from phagocytosis and isolate it from other defenses of the host. Coagulases are produced by some members of the genus *Staphylococcus;* they may be involved in the walling-off process in boils produced by staphylococci. However, some staphylococci that do not produce coagulases are still virulent. In these cases, capsules may be more important to their virulence.

Bacterial **kinases** are bacterial enzymes that break down fibrin and thus digest clots formed by the body to isolate the infection. One of the better-known kinases is *fibrinolysin (streptokinase),* which is produced by such streptococci as *Streptococcus pyogenes.* See the Applications of Microbiology box. Another kinase, *staphylokinase,* is produced by *Staphylococcus aureus.*

Hyaluronidase is another enzyme secreted by certain bacteria, such as streptococci. It hydrolyzes hyaluronic acid, a type of polysaccharide that holds together certain cells of the body, particularly cells in connective tissue. This digesting action is thought to be involved in the tissue blackening of infected wounds and to help the microorganism spread from its initial site of infection. Hyaluronidase is also produced by some clostridia that cause gas gangrene. For therapeutic use, hyaluronidase

may be mixed with a drug to promote the spread of the drug through a body tissue.

Another enzyme, **collagenase,** produced by several species of *Clostridium,* facilitates the spread of gas gangrene. Collagenase breaks down the protein collagen, which forms the connective tissue of muscles and other body organs and tissues.

As a defense against adherence of pathogens to mucosal surfaces, the body produces a class of antibodies called IgA antibodies. There are some pathogens with the ability to produce enzymes, called **IgA proteases,** that can destroy these antibodies. *N. gonorrhoeae* has this ability, as do *N. meningitidis*, the causative agent of meningococcal meningitis, and other microbes that infect the central nervous system.

Antigenic Variation

Adaptive immunity refers to a specific defensive response of the body to an infection or to antigens (see Chapter 17). In the presence of antigens, the body produces proteins called antibodies, which bind to the antigens and inactivate or destroy them. However, some pathogens can alter their surface antigens, by a process called **antigenic variation.** Thus, by the time the body mounts an immune response against a pathogen, the pathogen has already altered its antigens and is unaffected by the antibodies. Some microbes can activate alternative genes, resulting in antigenic changes. For example, *N. gonorrhoeae* has several copies of the Opa-encoding gene, resulting in cells with different antigens and in cells that express different antigens over time.

A wide range of microbes is capable of antigenic variation. Examples include *Influenzavirus,* the causative agent of influenza (flu); *Neisseria gonorrhoeae,* the causative agent of gonorrhea; and *Trypanosoma brucei gambiense* (tri-PA-nō-sō-mah BROOS-e-ē GAM-bē-ens), the causative agent of African trypanosomiasis (sleeping sickness). See Figure 22.16, page 629.

Penetration into the Host Cell Cytoskeleton

As previously noted, microbes attach to host cells by adhesins. The interaction triggers signals in the host cell that activate factors that can result in the entrance of some bacteria. The actual mechanism is provided by the host cell cytoskeleton. Eukaryotic cytoplasm has a complex internal structure (the cytoskeleton), consisting of protein filaments called microfilaments, intermediate filaments, and microtubules (see Chapter 4). A major component of the cytoskeleton is a protein called actin, which is used by some microbes to penetrate host cells and by others to move through and between host cells.

Salmonella strains and *E. coli* make contact with the host cell plasma membrane. This leads to dramatic changes in the membrane at the point of contact. The microbes produce surface proteins called **invasins** that rearrange nearby actin filaments of the cytoskeleton. For example, when *S. typhimurium* makes contact with a host cell, invasins of the microbe cause the appearance of the host cell plasma membrane to resemble the splash of a drop of a liquid hitting a solid surface. This effect, called *membrane ruffling,* is the result of disruption in the cytoskeleton of the host cell (**Figure 15.2**). The microbe sinks into the ruffle and is engulfed by the host cell.

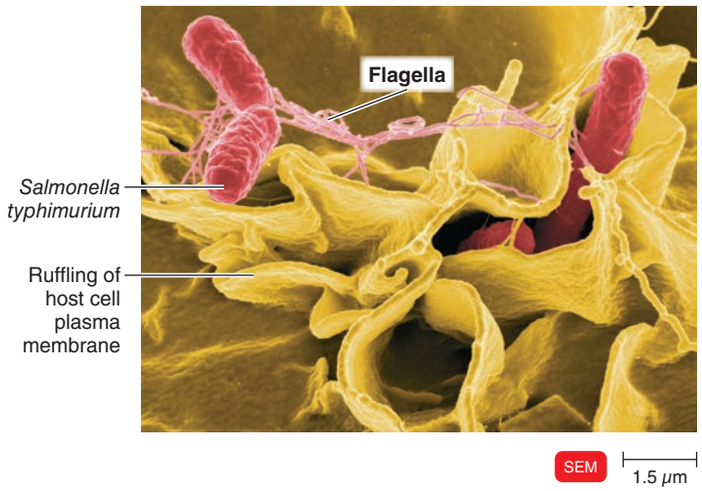


Figure 15.2 ***Salmonella*** **entering intestinal epithelial cells as a result of ruffling.**

What are invasins?

Once inside the host cell, certain bacteria such as *Shigella* species and *Listeria* species can actually use actin to propel themselves through the host cell cytoplasm and from one host cell to another. The condensation of actin on one end of the bacteria propels them through the cytoplasm. The bacteria also make contact with membrane junctions that form part of a transport network between host cells. The bacteria use a glycoprotein called *cadherin,* which bridges the junctions, to move from cell to cell.

The study of the numerous interactions between microbes and host cell cytoskeleton is a very intense area of investigation on virulence mechanisms.

CHECK YOUR UNDERSTANDING

- What function do capsules and M proteins have in common? **15-4**
- Would you expect a bacterium to make coagulase and kinase simultaneously? **15-5**
- Many vaccines provide years of protection against a disease. Why doesn't the influenza vaccine offer more than a few months of protection? **15-6**
- How does *E. coli* cause membrane ruffling? **15-7**

How Bacterial Pathogens Damage Host Cells

LEARNING OBJECTIVES

- **15-8** Describe the function of siderophores.
- **15-9** Provide an example of direct damage, and compare this to toxin production.
- **15-10** Contrast the nature and effects of exotoxins and endotoxins.
- **15-11** Outline the mechanisms of action of A-B toxins, membrane-disrupting toxins, superantigens, and genotoxins.
- **15-12** Identify the importance of the LAL assay.
- **15-13** Using examples, describe the roles of plasmids and lysogeny in pathogenicity.

When a microorganism invades a body tissue, it initially encounters phagocytes of the host. If the phagocytes are successful in destroying the invader, no further damage is done to the host. But if the pathogen overcomes the host's defense, then the microorganism can damage host cells in four basic ways:

1. By using the host's nutrients.
2. By causing direct damage in the immediate vicinity of the invasion.
3. By producing toxins, transported by blood and lymph, that damage sites far removed from the original site of invasion.
4. By inducing hypersensitivity reactions.

This fourth mechanism is considered in detail in Chapter 19. For now, we will discuss only the first three mechanisms.

Using the Host's Nutrients: Siderophores

Iron is required for the growth of most pathogenic bacteria. However, the concentration of free iron in the human body is fairly low because most of the iron is tightly bound to iron-transport proteins, such as lactoferrin, transferrin, and ferritin, as well as hemoglobin. These are discussed in more detail in Chapter 16. To obtain iron, some pathogens secrete proteins called **siderophores** (Figure 15.3). Siderophores are released into the medium, where they take the iron away from iron-transport proteins by binding the iron even more tightly. Once the iron-siderophore complex is formed, it is taken up by siderophore receptors on the bacterial surface. Then the iron is brought into the bacterium. In some cases, the iron is released from the complex to enter the bacterium; in other cases, the iron enters as part of the complex.

As an alternative to iron acquisition by siderophores, some pathogens have receptors that bind directly to iron-transport proteins and hemoglobin. Then these are taken into the bacterium directly along with the iron. Also, it is possible that some bacteria produce toxins (described shortly) when iron levels are low. The toxins kill host cells, releasing their iron and thereby making it available to the bacteria.

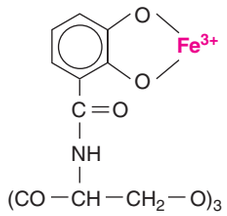


Figure 15.3 Structure of enterobactin, one type of bacterial siderophore. The iron (Fe^{3+}) is indicated in red.

Q Of what value are siderophores?

Direct Damage

Once pathogens attach to host cells, they can cause direct damage as the pathogens use the host cell for nutrients and produce waste products. As pathogens metabolize and multiply in cells, the cells usually rupture. Many viruses and some intracellular bacteria and protozoa that grow in host cells are released when the host cell ruptures. Following their release, pathogens that rupture cells can spread to other tissues in even greater numbers. Some bacteria, such as *E. coli, Shigella, Salmonella,* and *Neisseria gonorrhoeae,* can induce host epithelial cells to engulf them by a process that resembles phagocytosis. These pathogens can disrupt host cells as they pass through and can then be extruded from the host cells by a reverse phagocytosis process, enabling them to enter other host cells. Some bacteria can also penetrate host cells by excreting enzymes or by their own motility; such penetration can itself damage the host cell. Most damage by bacteria, however, is done by toxins. MM **Animations** Virulence Factors: Penetrating Host Tissues, Hiding from Host Defenses, Enteric Pathogens

Production of Toxins

Toxins are poisonous substances that are produced by certain microorganisms. They are often the primary factor contributing to the pathogenic properties of those microbes. The capacity of microorganisms to produce toxins is called **toxigenicity.** Toxins transported by the blood or lymph can cause serious, and sometimes fatal, effects. Some toxins produce fever, cardiovascular

Clinical Case

Dr. Santos suspects toxic anterior segment syndrome (TASS), which is a reaction to a toxin or other chemical. TASS is caused by (1) chemicals on surgical instruments, resulting from improper or insufficient cleaning; (2) products introduced into the eye during surgery, such as washing solutions or medications; or (3) other substances that enter the eye during or after surgery, such as topical ointments or talc from surgical gloves.

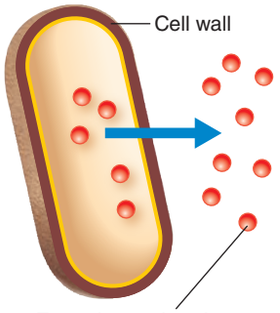
Why does Dr. Santos suspect an intoxication and not an infection?

FOUNDATION FIGURE 15.4

Mechanisms of Exotoxins and Endotoxins

exotoxins

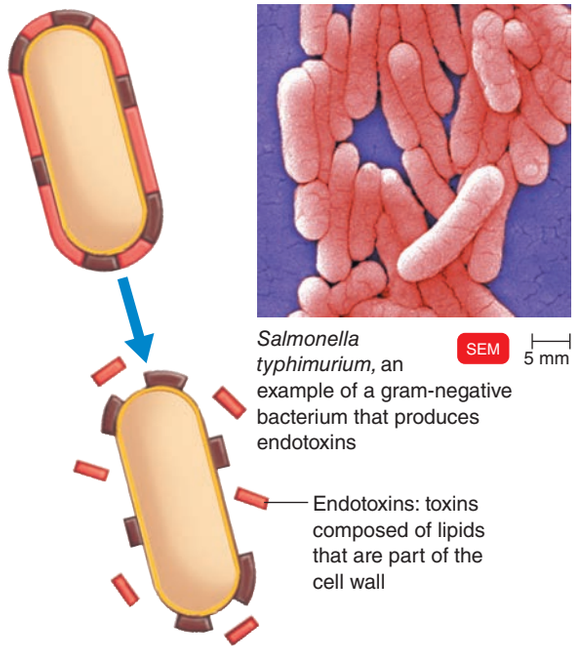
Proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted into the surrounding medium during log phase.



Clostridium botulinum, an example of a gram-positive bacterium that produces exotoxins

endotoxins

Lipid portions of lipopolysaccharides (LPS) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A). The endotoxins are liberated when the bacteria die and the cell wall lyses, or breaks apart.



Salmonella typhimurium, an example of a gram-negative bacterium that produces endotoxins

KEY CONCEPTS

- Toxins are of two general types: exotoxins and endotoxins.
- Bacterial toxins can cause damage to host cells.
- Toxins can elicit an inflammatory response in the host, as well as activate the complement system.
- Some gram-negative bacteria may release minute amounts of endotoxins, which may stimulate natural immunity.

disturbances, diarrhea, and shock. Toxins can also inhibit protein synthesis, destroy blood cells and blood vessels, and disrupt the nervous system by causing spasms. Of the 220 or so known bacterial toxins, nearly 40% cause disease by damaging eukaryotic cell membranes. The term **toxemia** refers to the presence of toxins in the blood. Toxins are of two general types, based on their position relative to the microbial cell: exotoxins and endotoxins. **Intoxications** are caused by the presence of a toxin; not by microbial growth.

Exotoxins

Exotoxins are produced inside some bacteria as part of their growth and metabolism and are secreted by the bacterium into the surrounding medium or released following lysis (Figure 15.4). *Exo-* refers to "outside," which in this context refers to the fact that exotoxins are secreted to the outside of the bacterial cells that produce them. Exotoxins are proteins, and many are enzymes that catalyze only certain biochemical reactions. Because of the enzymatic nature of most exotoxins, even small amounts are quite harmful because they can act over and over again. Bacteria that produce exotoxins may be gram-positive or gram-negative. The genes for most (perhaps all) exotoxins are carried on bacterial plasmids or phages. Because exotoxins are soluble in body fluids, they can easily diffuse into the blood and are rapidly transported throughout the body.

Exotoxins work by destroying particular parts of the host's cells or by inhibiting certain metabolic functions. They are highly specific in their effects on body tissues. Exotoxins are among the most lethal substances known. Only 1 milligram of the botulinum exotoxin is enough to kill 1 million guinea pigs. Fortunately, only a few bacterial species produce such potent exotoxins.

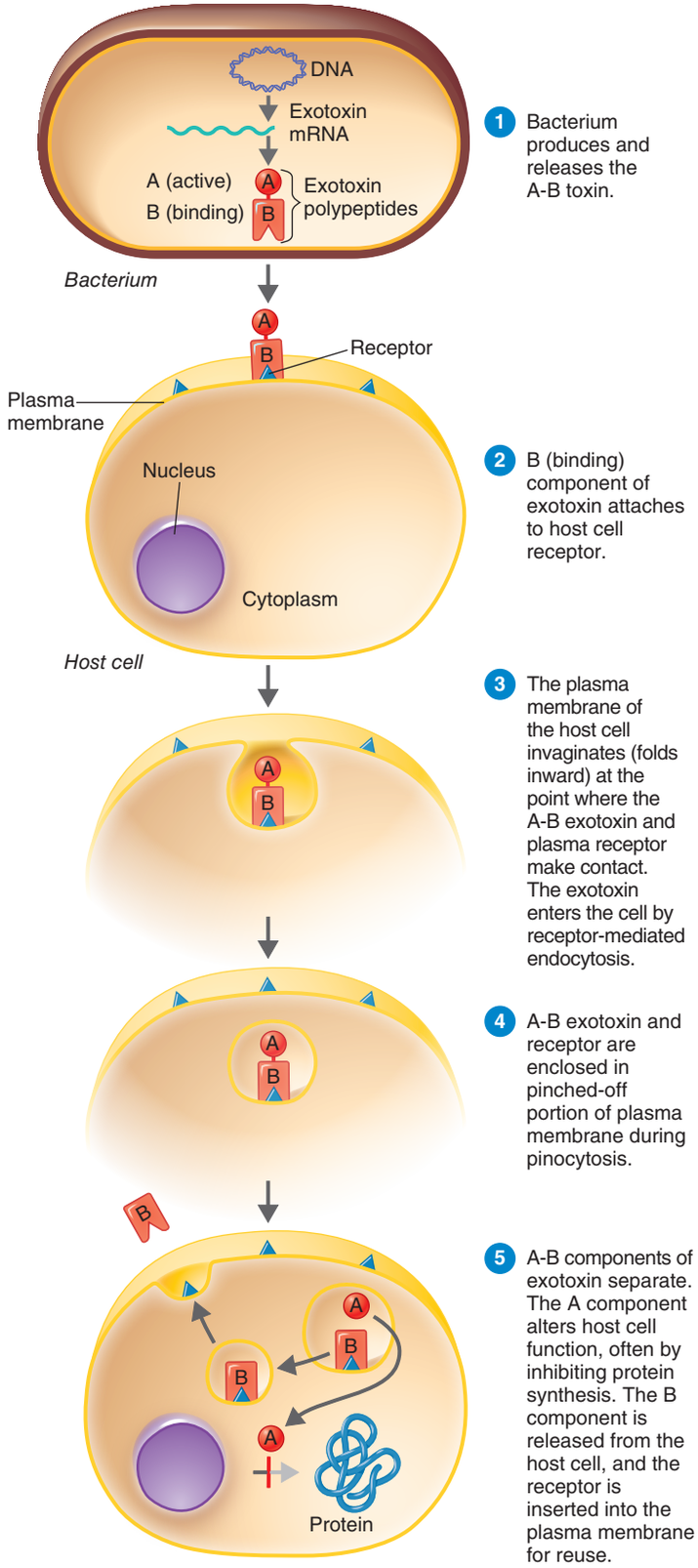


Figure 15.5 The action of an A-B exotoxin. A proposed model for the mechanism of action of diphtheria toxin.

Q Why is this called an A-B toxin?

Diseases caused by bacteria that produce exotoxins are often caused by minute amounts of exotoxins, not by the bacteria themselves. It is the exotoxins that produce the specific signs and symptoms of the disease. Thus, exotoxins are disease-specific. For example, botulism is usually due to ingestion of the exotoxin, not to a bacterial infection. Likewise, staphylococcal food poisoning is an *intoxication,* not an infection.

The body produces antibodies called **antitoxins** that provide immunity to exotoxins. When exotoxins are inactivated by heat or by formaldehyde, iodine, or other chemicals, they no longer cause the disease but can still stimulate the body to produce antitoxins. Such altered exotoxins are called **toxoids.** When toxoids are injected into the body as a vaccine, they stimulate antitoxin production so that immunity is produced. Diphtheria and tetanus can be prevented by toxoid vaccination.

Naming Exotoxins Exotoxins are named on the basis of several characteristics. One is the type of host cell that is attacked. For example, *neurotoxins* attack nerve cells, *cardiotoxins* attack heart cells, *hepatotoxins* attack liver cells, *leukotoxins* attack leukocytes, *enterotoxins* attack the lining of the gastrointestinal tract, and *cytotoxins* attack a wide variety of cells. Some exotoxins are named for the diseases with which they are associated. Examples include *diphtheria toxin* (cause of diphtheria) and *tetanus toxin* (cause of tetanus). Other exotoxins are named for the specific bacterium that produces them, for example, *botulinum toxin (Clostridium botulinum)* and *Vibrio enterotoxin (Vibrio cholerae).*

Types of Exotoxins Exotoxins are divided into three principal types on the basis of their structure and function: (1) A-B toxins, (2) membrane-disrupting toxins, and (3) superantigens.

A-B Toxins **A-B toxins** were the first toxins to be studied intensively and are so named because they consist of two parts designated A and B, both of which are polypeptides. Most exotoxins are A-B toxins. The A part is the active (enzyme) component, and the B part is the binding component. An example of an A-B toxin is the diphtheria toxin, which is illustrated in Figure 15.5.

Membrane-Disrupting Toxins **Membrane-disrupting toxins** cause lysis of host cells by disrupting their plasma membranes. Some do this by forming protein channels in the plasma membrane; others disrupt the phospholipid portion of the membrane. The cell-lysing exotoxin of *Staphylococcus aureus* is an example of an exotoxin that forms protein channels, whereas that of *Clostridium perfringens* is an example of an exotoxin that disrupts the phospholipids. Membrane-disrupting toxins contribute to virulence by killing host cells, especially phagocytes, and by aiding the escape of bacteria from sacs within phagocytes (phagosomes) into the host cell's cytoplasm.

TABLE 15.2 Diseases Caused by Exotoxins

Disease	Bacterium	Type of Exotoxin	Mechanism
Botulism	*Clostridium botulinum*	A-B	Neurotoxin prevents transmission of nerve impulses; flaccid paralysis results.
Tetanus	*Clostridium tetani*	A-B	Neurotoxin blocks nerve impulses to muscle relaxation pathway; results in uncontrollable muscle contractions.
Diphtheria	*Corynebacterium diphtheriae*	A-B	Cytotoxin inhibits protein synthesis, especially in nerve, heart, and kidney cells.
Scalded skin syndrome	*Staphylococcus aureus*	A-B	One exotoxin causes skin layers to separate and slough off.
Cholera	*Vibrio cholerae*	A-B	Enterotoxin causes secretion of large amounts of fluids and electrolytes that result in diarrhea.
Traveler's diarrhea	Enterotoxigenic *Escherichia coli* and *Shigella* spp.	A-B	Enterotoxin causes secretion of large amounts of fluids and electrolytes that result in diarrhea.
Anthrax	*Bacillus anthracis*	A-B	Two A components enter the cell via the same B. The A proteins cause shock and reduce the immune response.
Gas gangrene and food poisoning	*Clostridium perfringens* and other species of *Clostridium*	Membrane-disrupting	One exotoxin (cytotoxin) causes massive red blood cell destruction (hemolysis); another exotoxin (enterotoxin) is related to food poisoning and causes diarrhea.
Antibiotic-associated diarrhea	*Clostridium difficile*	Membrane-disrupting	Enterotoxin causes secretion of fluids and electrolytes that results in diarrhea; cytotoxin disrupts host cytoskeleton.
Food poisoning	*Staphylococcus aureus*	Superantigen	Enterotoxin causes secretion of fluids and electrolytes that results in diarrhea.
Toxic shock syndrome (TSS)	*Staphylococcus aureus*	Superantigen	Toxin causes secretion of fluids and electrolytes from capillaries that decreases blood volume and lowers blood pressure.
Stomach cancer	*Helicobacter* spp.	Genotoxin	Toxin causes breaks in eukaryotic DNA.

Membrane-disrupting toxins that kill phagocytic leukocytes (white blood cells) are called **leukocidins.** They act by forming protein channels. Leukocidins are also active against macrophages, the phagocytes present in tissues. Most leukocidins are produced by staphylococci and streptococci. The damage to phagocytes decreases host resistance. Membrane-disrupting toxins that destroy erythrocytes (red blood cells), also by forming protein channels, are called **hemolysins.** Important producers of hemolysins include staphylococci and streptococci. Hemolysins produced by streptococci are called **streptolysins.** One kind, called *streptolysin O (SLO),* is so named because it is inactivated by atmospheric oxygen. Another kind of streptolysin is called *streptolysin S (SLS)* because it is stable in an oxygen environment. Both streptolysins can cause lysis not only of red blood cells, but also of white blood cells (whose function is to kill the streptococci) and other body cells.

Superantigens **Superantigens** are antigens that provoke a very intense immune response. They are bacterial proteins. Through a series of interactions with various cells of the immune system, superantigens nonspecifically stimulate the proliferation of immune cells called T cells. These cells are types of white blood cells (lymphocytes) that act against foreign organisms and tissues (transplants) and regulate the activation and proliferation of other cells of the immune system. In response to superantigens, T cells are stimulated to release enormous amounts of chemicals called cytokines. *Cytokines* are small protein molecules produced by various body cells, especially T cells, that regulate immune responses and mediate cell-to-cell communication (see Chapter 17, page 470). The excessively high levels of cytokines released by T cells enter the bloodstream and give rise to a number of symptoms, including fever, nausea, vomiting, diarrhea, and sometimes shock and even death. Bacterial superantigens include the staphylococcal toxins that cause food poisoning and toxic shock syndrome. A summary of diseases produced by exotoxins is shown in **Table 15.2**.

Genotoxins Some gram-negative bacteria, including *Haemophilus ducreyi* and *Helicobacter* spp., make **genotoxins,** which damage DNA. This causes mutations, disrupts cell division, and may lead

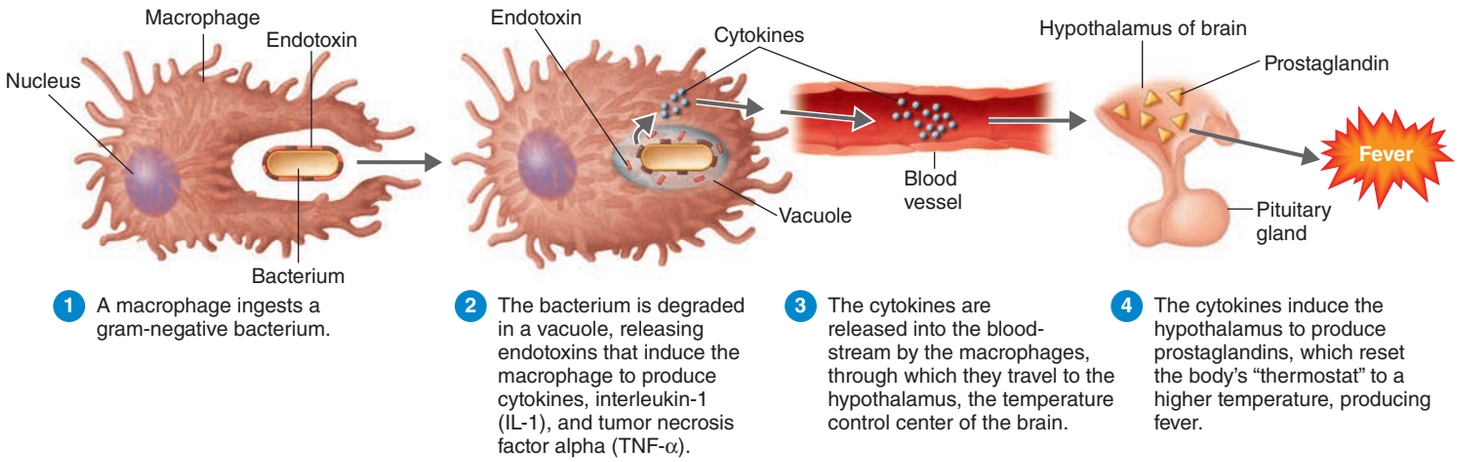


Figure 15.6 Endotoxins and the pyrogenic response. The proposed mechanism by which endotoxins cause fever.

Q What is an endotoxin?

to cancer. The first bacterial genotoxin to be discovered is cytolethal distending toxin. This toxin damages eukaryotic DNA.

Endotoxins

Endotoxins differ from exotoxins in several ways. *Endo-* means "within," in this context referring to the fact that the endotoxins are located within the bacterial cells. Endotoxins are part of the outer portion of the cell wall of gram-negative bacteria (Figure 15.4). Gram-negative bacteria have an outer membrane surrounding the peptidoglycan layer of the cell wall (see Chapter 4). This outer membrane consists of lipoproteins, phospholipids, and lipopolysaccharides (LPS) (see Figure 4.13c, page 82). The lipid portion of LPS, called **lipid A,** is the endotoxin. Thus, endotoxins are lipopolysaccharides, whereas exotoxins are proteins.

Endotoxins are released during bacterial multiplication and when gram-negative bacteria die and their cell walls undergo lysis. Antibiotics used to treat diseases caused by gram-negative bacteria can lyse the bacterial cells; this reaction releases endotoxin and may lead to an immediate worsening of the symptoms, but the condition usually improves as the endotoxin breaks down. Endotoxins exert their effects by stimulating macrophages to release cytokines in very high concentrations. At these levels, cytokines are toxic. All endotoxins produce the same signs and symptoms, regardless of the species of microorganism, although not to the same degree. These include chills, fever, weakness, generalized aches, and, in some cases, shock and even death. Endotoxins can also induce miscarriage.

Another consequence of endotoxins is the activation of blood-clotting proteins, causing the formation of small blood clots. These blood clots obstruct capillaries, and the resulting decreased blood supply induces the death of tissues. This condition is referred to as *disseminated intravascular coagulation (DIC).*

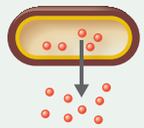
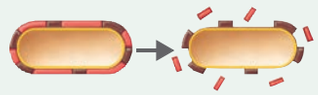
The fever (pyrogenic response) caused by endotoxins is believed to occur as depicted in Figure 15.6.

Bacterial cell death caused by lysis or antibiotics can also produce fever by this mechanism. Both aspirin and acetaminophen reduce fever by inhibiting the synthesis of prostaglandins. (The function of fever in the body is discussed in Chapter 16, page 455.) **Shock** refers to any life-threatening decrease in blood pressure. Shock caused by bacteria is called **septic shock.** Gram-negative bacteria cause *endotoxic shock.* Like fever, the shock produced by endotoxins is related to the secretion of a cytokine by macrophages. Phagocytosis of gram-negative bacteria causes the phagocytes to secrete tumor necrosis factor (TNF), sometimes called *cachectin.* TNF binds to many tissues in the body and alters their metabolism in a number of ways. One effect of TNF is damage to blood capillaries; their permeability is increased, and they lose large amounts of fluid. The result is a drop in blood pressure that results in shock. Low blood pressure has serious effects on the kidneys, lungs, and gastrointestinal tract. In addition, the presence of gram-negative bacteria such as *Haemophilus influenzae* type b in cerebrospinal fluid causes the release of IL-1 and TNF. These, in turn, cause a weakening of the blood–brain barrier that normally protects the central nervous system from infection. The weakened barrier lets phagocytes in, but this also lets more bacteria enter from the bloodstream. In the United States, about 3 out of every 1000 people develop septic shock each year. One-third of the patients die within a month, and nearly half die within 6 months.

Endotoxins do not promote the formation of effective antitoxins against the carbohydrate component of an endotoxin. Antibodies are produced, but they tend not to counter the effect of the toxin; sometimes, in fact, they actually enhance its effect.

Representative microorganisms that produce endotoxins are *Salmonella typhi* (the causative agent of typhoid fever), *Proteus*

TABLE 15.3 Exotoxins and Endotoxins

Property	Exotoxins	Endotoxins
		
Bacterial Source	Mostly from gram-positive bacteria	Gram-negative bacteria
Relation to Microorganism	Metabolic product of growing cell	Present in LPS of outer membrane of cell wall and released with destruction of cell or during cell division
Chemistry	Proteins, usually with two parts (A-B)	Lipid portion (lipid A) of LPS of outer membrane (lipopolysaccharide).
Pharmacology (Effect on Body)	Specific for a particular cell structure or function in the host (mainly affects cell functions, nerves, and gastrointestinal tract)	General, such as fever, weaknesses, aches, and shock; all produce the same effects
Heat Stability	Unstable; can usually be destroyed at 60–80°C (except staphylococcal enterotoxin)	Stable; can withstand autoclaving (121°C for 1 hour)
Toxicity (Ability to Cause Disease)	High	Low
Fever-Producing	No	Yes
Immunology (Relation to Antibodies)	Can be converted to toxoids to immunize against toxin; neutralized by antitoxin	Not easily neutralized by antitoxin; therefore, effective toxoids cannot be made to immunize against toxin
Lethal Dose	Small	Considerably larger
Representative Diseases	Gas gangrene, tetanus, botulism, diphtheria, scarlet fever	Typhoid fever, urinary tract infections, and meningococcal meningitis

spp. (frequently the causative agents of urinary tract infections), and *Neisseria meningitidis* (the causative agent of meningococcal meningitis).

It is important to have a sensitive test to identify the presence of endotoxins in drugs, medical devices, and body fluids. Materials that have been sterilized may contain endotoxins, even though no bacteria can be cultured from them. One such laboratory test is called the **Limulus amebocyte lysate (LAL) assay,** which can detect even minute amounts of endotoxin. The hemolymph (blood) of the Atlantic coast horseshoe crab, *Limulus polyphemus,* contains white blood cells called amebocytes, which have large amounts of a protein (lysate) that causes clotting. In the presence of endotoxin, amebocytes in the crab hemolymph lyse and liberate their clotting protein. The resulting gel-clot (precipitate) is a positive test for the presence of endotoxin. The degree of the reaction is measured using a spectrophotometer (see Figure 6.21, page 171).

Table 15.3 compares exotoxins and endotoxins. **Animations** Virulence Factors: Exotoxins, Endotoxins

Clinical Case

An infection could not have happened this quickly; infections usually take 3 to 4 days to show symptoms. Dr. Santos checks to make sure that the autoclave used to sterilize the ophthalmic equipment is functioning normally, and that single-use topical iodine antiseptic was used. A new sterile tip for the corneal extraction was used for each patient. Both the epinephrine used during surgery and the enzymatic solution for the ultrasonic bath used to clean surgical instruments are sterile, and medications with different lot numbers were used in each surgery. But Dr. Santos knows that the toxin had to have come from somewhere or something connected to the surgeries. Even though the enzymatic solution is sterile, Dr. Santos sends it to a laboratory for a LAL assay.

Why does Dr. Santos send the enzymatic solution for a LAL assay?

Plasmids, Lysogeny, and Pathogenicity

Plasmids are small, circular DNA molecules that are not connected to the main bacterial chromosome and are capable of independent replication. (See Chapter 4, page 90, and Chapter 8, page 230.) One group of plasmids, called R (resistance) factors, is responsible for the resistance of some microorganisms to antibiotics. In addition, a plasmid may carry the information that determines a microbe's pathogenicity. Examples of virulence factors that are encoded by plasmid genes are tetanus neurotoxin, heat-labile enterotoxin, and staphylococcal enterotoxin D. Other examples are dextransucrase, an enzyme produced by *Streptococcus mutans* that is involved in tooth decay; adhesins and coagulase produced by *Staphylococcus aureus;* and a type of fimbria specific to enteropathogenic strains of *E. coli.*

In Chapter 13, we noted that some bacteriophages (viruses that infect bacteria) can incorporate their DNA into the bacterial chromosome, becoming a prophage, and thus remain latent and do not cause lysis of the bacterium. Such a state is called *lysogeny,* and cells containing a prophage are said to be lysogenic. One outcome of lysogeny is that the host bacterial cell and its progeny may exhibit new properties encoded by the bacteriophage DNA. Such a change in the characteristics of a microbe due to a prophage is called **lysogenic conversion.** As a result of lysogenic conversion, the bacterial cell is immune to infection by the same type of phage. In addition, lysogenic cells are of medical importance because some bacterial pathogenesis is caused by the prophages they contain.

Among the bacteriophage genes that contribute to pathogenicity are the genes for diphtheria toxin, erythrogenic toxins, staphylococcal enterotoxin A and pyrogenic toxin, botulinum neurotoxin, and the capsule produced by *Streptococcus pneumoniae.* The gene for Shiga toxin in *E. coli* O157 is encoded by phage genes. Pathogenic strains of *Vibrio cholerae* carry lysogenic phages. These phages can transmit the cholera toxin gene to nonpathogenic *V. cholerae* strains, increasing the number of pathogenic bacteria.

CHECK YOUR UNDERSTANDING

- Of what value are siderophores? **15-8**
- How does toxigenicity differ from direct damage? **15-9**
- Differentiate an exotoxin from an endotoxin. **15-10**
- Food poisoning can be divided into two categories: food infection and food intoxication. On the basis of toxin production by bacteria, explain the difference between these two categories. **15-11**
- Washwater containing *Pseudomonas* was sterilized and used to wash cardiac catheters. Three patients developed fever, chills, and hypotension following cardiac catheterization. The water and catheters were sterile. Why did the patients show these reactions? How should the water have been tested? **15-12**
- How can lysogeny turn the normally harmless *E. coli* into a pathogen? **15-13**

Pathogenic Properties of Viruses

LEARNING OBJECTIVE

15-14 List nine cytopathic effects of viral infections.

The pathogenic properties of viruses depend on their gaining access to a host, evading the host's defenses, and then causing damage to or death of the host cell while reproducing themselves.

Viral Mechanisms for Evading Host Defenses

Viruses have a variety of mechanisms that enable them to evade destruction by the host's immune response (see Chapter 17, page 469). For example, viruses can penetrate and grow inside host cells, where components of the immune system cannot reach them. Viruses gain access to cells because they have attachment sites for receptors on their target cells. When such an attachment site is brought together with an appropriate receptor, the virus can bind to and penetrate the cell. Some viruses gain access to host cells because their attachment sites mimic substances useful to those cells. For example, the attachment sites of rabies virus can mimic the neurotransmitter acetylcholine. As a result, the virus can enter the host cell along with the neurotransmitter.

The AIDS virus (HIV) goes further by hiding its attachment sites from the immune response and by attacking components of the immune system directly. Like most viruses, HIV is cell-specific, in this case attacking only particular body cells that have a surface marker called the CD4 protein. Most of these cells are immune system T cells (T lymphocytes). Binding sites on HIV are complementary to the CD4 protein. The surface of the virus is folded to form ridges and valleys, and the HIV binding sites are located on the floors of the valleys. CD4 proteins are long enough and slender enough to reach these binding sites, whereas antibody molecules made against HIV are too large to make contact with the sites. As a result, it is difficult for these antibodies to destroy HIV.

Cytopathic Effects of Viruses

Infection of a host cell by an animal virus usually kills the host cell. Death can be caused by the accumulation of large numbers of multiplying viruses, by the effects of viral proteins on the permeability of the host cell's plasma membrane, or by inhibition of host DNA, RNA, or protein synthesis. The visible effects of viral infection are known as **cytopathic effects (CPE).** Those cytopathic effects that result in cell death are called *cytocidal effects;* those that result in cell damage but not cell death are called *noncytocidal effects.* CPEs are used to diagnose many viral infections.

Cytopathic effects vary by virus. One difference is the point in the viral infection cycle at which the effects occur. Some viral infections result in early changes in the host cell; in other infections, changes are not seen until a much later stage.

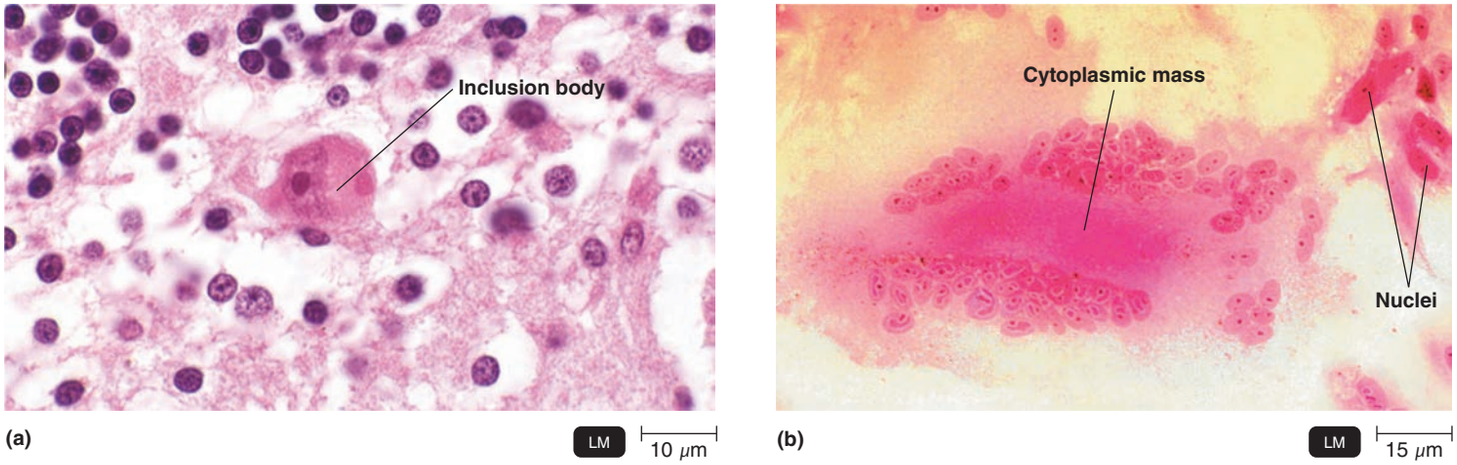


Figure 15.7 Some cytopathic effects of viruses. **(a)** Cytoplasmic inclusion body in brain tissue from a person who died of rabies. **(b)** Portion of a syncytium (giant cell) formed in a cell infected with measles virus. The cytoplasmic mass is probably the Golgi complexes of fused cells.

Q What are cytopathic effects?

A virus can produce one or more of the following cytopathic effects:

1. The macromolecular synthesis within the host cell stops. Some viruses, such as herpes simplex virus, irreversibly stop mitosis.
2. Host cell lysosomes are made to release their enzymes, resulting in destruction of intracellular contents and host cell death.
3. **Inclusion bodies** are found in the cytoplasm or nucleus of some infected cells (Figure 15.7a). These granules are sometimes viral parts—nucleic acids or proteins in the process of being assembled into virions. The granules vary in size, shape, and staining properties. They are characterized by their ability to stain with an acidic stain (acidophilic) or with a basic stain (basophilic). Other inclusion bodies arise at sites of earlier viral synthesis but do not contain assembled viruses or their components. Inclusion bodies are important because they can help identify the causative agent. For example, in most cases, rabies virus produces inclusion bodies (Negri bodies) in the cytoplasm of nerve cells, and their presence in animal brain tissue has been used as one diagnostic tool for rabies. Diagnostic inclusion bodies are also associated with measles virus, vaccinia virus, smallpox virus, herpesvirus, and adenoviruses.
4. At times, several adjacent infected cells fuse to form a very large multinucleate cell called a **syncytium** (Figure 15.7b). Such giant cells are produced from infections by viruses that cause measles, mumps, and the common cold.
5. Some viral infections result in changes in the host cell's functions with no visible changes in the infected cells. For example, when measles virus attaches to its receptor called CD46, the CD46 prompts the cell to reduce production of a cytokine called IL-12, reducing the host's ability to fight the infection. See the box in Chapter 17, page 471.
6. Many viral infections induce antigenic changes on the surface of the infected cells. These antigenic changes elicit a host antibody response against the infected cell, and thus they target the cell for destruction by the host's immune system.
7. Some viruses induce chromosomal changes in the host cell. For example, some viral infections result in chromosomal damage to the host cell, most often chromosomal breakage. Frequently, oncogenes (cancer-causing genes) may be contributed or activated by a virus.
8. Viruses capable of causing cancer *transform* host cells, as discussed in Chapter 13. Transformation results in an abnormal, spindle-shaped cell that does not recognize **contact inhibition,** meaning cells don't stop growing when they come in close contact with other cells. (Figure 15.8). The loss of contact inhibition results in unregulated cell growth.
9. Some virus-infected host cells produce substances called alpha and beta **interferons.** Viral infection induces cells to produce these interferons, but the host cell's DNA actually codes for them. Alpha and beta interferons protect neighboring uninfected cells from viral infection in two ways: (1) they inhibit synthesis of viral proteins and host cell proteins; and (2) they kill virus-infected host cells by apoptosis (programmed cell death). However, almost all viruses have mechanisms that evade interferons by partially blocking their synthesis.

Some representative viruses that cause cytopathic effects are presented in Table 15.4. In Part Four of the book we will discuss the pathological properties of viruses in more detail.

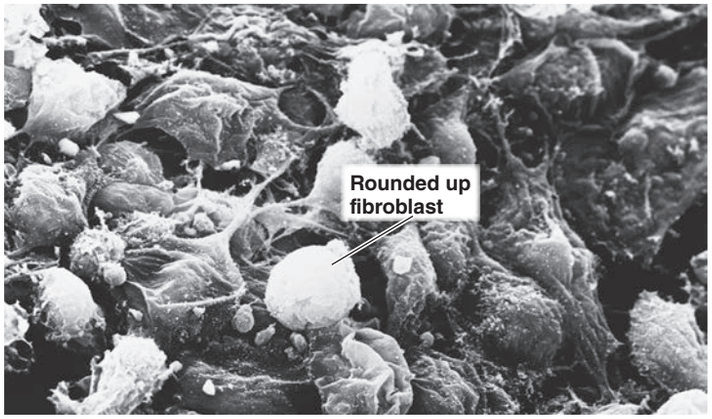


Figure 15.8 Human fibroblasts are transformed by Rous sarcoma virus. Normal fibroblasts grow as flat, spread-out cells.

Q What is contact inhibition?

CHECK YOUR UNDERSTANDING

Define *cytopathic effects,* and give five examples. **15-14**

Pathogenic Properties of Fungi, Protozoa, Helminths, and Algae

LEARNING OBJECTIVE

15-15 Discuss the causes of symptoms in fungal, protozoan, helminthic, and algal diseases.

This section describes some general pathological effects of fungi, protozoa, helminths, and algae that cause human disease. Specific diseases caused by fungi, protozoa, and helminths, along with the pathological properties of these organisms, are discussed in detail in Chapters 21 to 26.

Fungi

Although fungi cause disease, they do not have a well-defined set of virulence factors. Some fungi have metabolic products that are toxic to human hosts. In such cases, however, the toxin is only an indirect cause of disease, because the fungus is already growing in or on the host. Chronic fungal infections, such as athlete's foot, can also provoke an allergic response in the host.

Trichothecenes are fungal toxins that inhibit protein synthesis in eukaryotic cells. Ingestion of these toxins causes headaches, chills, severe nausea, vomiting, and visual disturbances. These toxins are produced by *Fusarium* (fū-SAR-ē-um) and *Stachybotrys* (STA-kē-bah-tris) growing on grains and wallboard in homes.

There is evidence that some fungi do have virulence factors. Two fungi that can cause skin infections, *Candida albicans* and *Trichophyton* (trik-ō-FĪ-ton), secrete proteases. These enzymes may modify host cell membranes to allow attachment of the fungi. *Cryptococcus neoformans* (KRIP-tō-kok-kus NĒ-ō-for-manz) is a fungus that causes a type of meningitis; it produces a capsule that helps it resist phagocytosis. Some fungi have become resistant to antifungal drugs by decreasing their synthesis of receptors for these drugs.

The disease called ergotism, common in Europe during the Middle Ages, is caused by a toxin produced by an ascomycete plant pathogen, *Claviceps purpurea* (KLA-vi-seps pur-pur-Ē-ah), that grows on grains. The toxin is contained in **sclerotia,** highly resistant portions of the mycelia of the fungus that can detach. The toxin itself, **ergot,** is an alkaloid that can cause hallucinations resembling those produced by LSD (lysergic acid diethylamide); in fact, ergot is a natural source of LSD. Ergot also constricts capillaries and can cause gangrene of the limbs by preventing proper blood circulation in the body. Although *C. purpurea* still occasionally occurs on grains, modern milling usually removes the sclerotia.

Several other toxins are produced by fungi that grow on grains or other plants. For example, peanut butter is occasionally recalled because of excessive amounts of **aflatoxin,** which has carcinogenic properties. Aflatoxin is produced by the growth of the mold *Aspergillus flavus.* When ingested, the toxin might be altered in a human body to a mutagenic compound.

A few mushrooms produce fungal toxins called **mycotoxins.** Examples are **phalloidin** and **amanitin,** produced by *Amanita phalloides* (A-man-ī-tah fal-LOI-dēz), commonly known as the deathcap. These neurotoxins are so potent that ingestion of the *Amanita* mushroom may result in death.

Protozoa

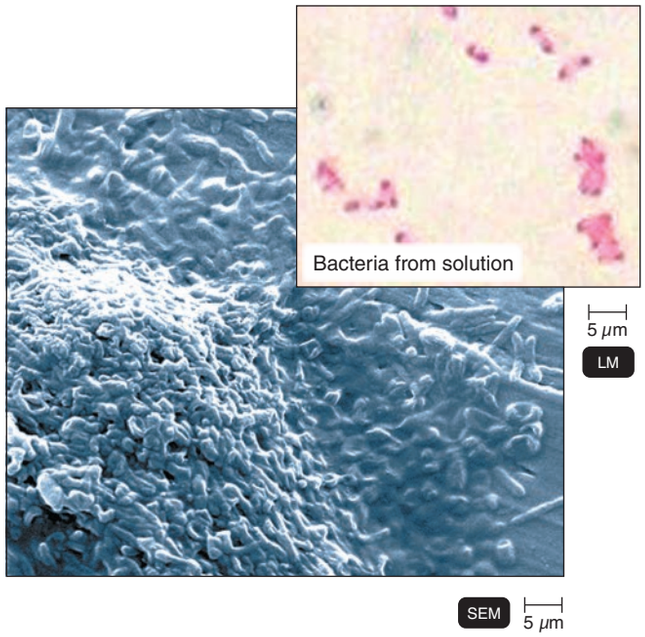
The presence of protozoa and their waste products often produces disease symptoms in the host (see Table 12.4, page 343).

TABLE 15.4 Cytopathic Effects of Selected Viruses

Virus (*Genus*)	Cytopathic Effect
Poliovirus (*Enterovirus*)	Cytocidal (cell death)
Genital warts virus (*Papillomavirus*)	Acidophilic inclusion bodies in nucleus
Adenovirus (*Mastadenovirus*)	Basophilic inclusion bodies in nucleus
Lyssavirus	Acidophilic inclusion bodies in cytoplasm
CMV (*Cytomegalovirus*)	Acidophilic inclusion bodies in nucleus and cytoplasm
Measles virus (*Morbillivirus*)	Cell fusion
Polyomavirus	Transformation
HIV (*Lentivirus*)	Destruction of T cells

Clinical Case

Although the sample was sterile, the lab test results show that the solution from the ultrasonic bath is positive for endotoxins. Gram-negative bacteria such as *Burkholderia* found in liquid reservoirs and moist environments can colonize water pipes (see the figure) and, in turn, the laboratory containers used to hold water. In this case, the bacteria from the biofilms were washed into the enzymatic solution.



How did endotoxins get in the sterile solutions?

418 424 429 433 435

Some protozoa, such as *Plasmodium,* the causative agent of malaria, invade host cells and reproduce within them, causing their rupture. *Toxoplasma* attaches to macrophages and gains entry by phagocytosis. The parasite prevents normal acidification and digestion; thus, it can grow in the phagocytic vacuole. Other protozoa, such as *Giardia intestinalis,* the causative agent of giardiasis, attach to host cells by a sucking disc (see Figure 25.26, page 733) and digest the cells and tissue fluids.

Some protozoa can evade host defenses and cause disease for very long periods of time. For example, *Giardia,* which causes diarrhea, and *Trypanosoma,* which causes African trypanosomiasis (sleeping sickness), both use antigenic variation (page 423) to stay one step ahead of the host's immune system. The immune system is alerted to recognize foreign substances called antigens; the presence of antigens causes the immune system to produce antibodies designed to destroy them (see Chapter 17). When *Trypanosoma* is introduced into the bloodstream by a tsetse fly, it produces and displays a specific antigen. In response, the body produces antibodies against that antigen. However, within 2 weeks, the microbe stops displaying the original antigen and instead produces and displays a different one (see Figure 22.16, page 629). Thus, the original antibodies are no longer effective. Because the microbe can make up to 1000 different antigens, such an infection can last for decades.

Helminths

The presence of helminths also often produces disease symptoms in a host (see Table 12.5, page 352). Some of these organisms actually use host tissues for their own growth or produce large parasitic masses; the resulting cellular damage evokes the symptoms. An example is the roundworm *Wuchereria bancrofti* (woo-kur-ER-ē-ah ban-KROF-tē), the causative agent of elephantiasis. This parasite blocks lymphatic circulation, leading to an accumulation of lymph and eventually causing grotesque swelling of the legs and other body parts. Waste products of the metabolism of these parasites can also contribute to the symptoms of a disease.

Algae

A few species of algae produce neurotoxins. For example, some genera of dinoflagellates, such as *Alexandrium,* are important medically because they produce a neurotoxin called **saxitoxin.** Although mollusks that feed on the dinoflagellates that produce saxitoxin show no symptoms of disease, people who eat the mollusks develop paralytic shellfish poisoning, with symptoms similar to botulism. Public health agencies frequently prohibit human consumption of mollusks during red tides (see Figure 27.10, page 782).

CHECK YOUR UNDERSTANDING

Identify one virulence factor that contributes to the pathogenicity of each of the following: fungi, protozoa, helminths, and algae. **15-15**

Portals of Exit

LEARNING OBJECTIVE

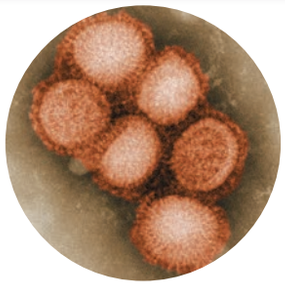
15-16 Differentiate portal of entry and portal of exit.

Just as microbes enter the body through a preferred route, they also leave the body via specific routes called **portals of exit** in secretions, excretions, discharges, or tissue that has been shed. In general, portals of exit relate to the infected part of the body, with microbes tending to use the same portal for entry and exit. Portals of exit let pathogens spread through a population by moving from one susceptible host to another. This type of information about the dissemination of a disease is very important to epidemiologists (see Chapter 14).

FOUNDATION FIGURE 15.9

Microbial Mechanisms of Pathogenicity

When the balance between host and microbe tips in favor of the microbe, an infection or disease results. Learning these mechanisms of microbial pathogenicity is fundamental to understanding how pathogens are able to overcome the host's defenses.



H1N1 flu virus

SEM 60 nm

portals of entry

Mucous membranes
- Respiratory tract
- Gastrointestinal tract
- Genitourinary tract
- Conjunctiva

Skin

Parenteral route

Number of invading microbes

Adherence

penetration or evasion of host defenses

Capsules
Cell wall components
Enzymes
Antigenic variation
Invasins
Intracellular growth

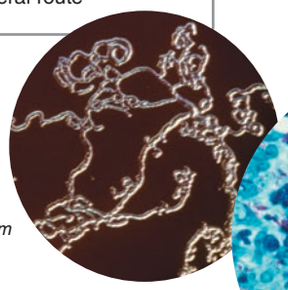
damage to host cells

Siderophores
Direct damage
Toxins
- Exotoxins
- Endotoxins

Lysogenic conversion
Cytopathic effects

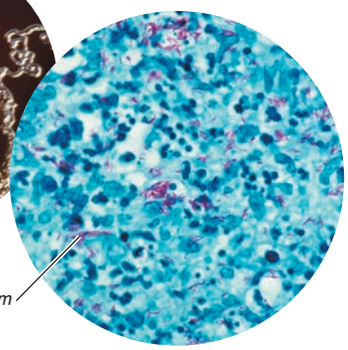
portals of exit

Generally the same as the portals of entry for a given microbe:
- Mucous membranes
- Skin
- Parenteral route



Clostridium tetani

SEM 5 μm



Mycobacterium intracellulare

SEM 5 μm

KEY CONCEPTS

- Several factors are required for a microbe to cause disease.
- After entering the host, most pathogens adhere to host tissue, penetrate or evade host defenses, and damage host tissues.
- Pathogens usually leave the body via specific portals of exit, which are generally the same sites where they entered initially.

The most common portals of exit are the respiratory and gastrointestinal tracts. Many pathogens living in the respiratory tract exit in discharges from the mouth and nose, expelled during coughing or sneezing. These microorganisms are found in droplets formed from mucus. Pathogens that cause tuberculosis, whooping cough, pneumonia, scarlet fever, meningococcal meningitis, chickenpox, measles, mumps, smallpox, and influenza are discharged through the respiratory route. Other pathogens exit via the gastrointestinal tract in feces or saliva. Feces may be contaminated with pathogens associated with salmonellosis, cholera, typhoid fever, shigellosis, amebic dysentery, and poliomyelitis. Saliva can also contain pathogens, such as those that cause rabies, mumps, and infectious mononucleosis.

Another important route of exit is the genitourinary tract. Microbes responsible for sexually transmitted infections are found in secretions from the penis and vagina. Urine can also contain the pathogens responsible for typhoid fever and brucellosis, which can exit via the urinary tract. Skin or wound infections are other portals of exit. Infections transmitted from the skin include yaws, impetigo, ringworm, herpes simplex, and warts. Drainage from wounds can spread infections to another person directly or by contact with a contaminated fomite. Infected blood can be removed and reinjected by biting insects and contaminated needles and syringes to spread infection within a population. Examples of diseases transmitted by biting insects are yellow fever, plague, tularemia, and malaria. AIDS and hepatitis B may be transmitted by contaminated needles and syringes.

CHECK YOUR UNDERSTANDING

Which are the most often used portals of exit? **15-16**

***

In the next chapter, we will examine a group of nonspecific defenses of the host against disease. But before proceeding, examine **Figure 15.9** carefully. It summarizes some key concepts of the microbial mechanisms of pathogenicity we have discussed in this chapter.

Clinical Case Resolved

Although the bacteria are killed by autoclaving, endotoxins can be released from the dead cells into solutions during autoclaving. Therefore, the solution was the disease source, even though it was sterile.

Dr. Santos treats her patients with prednisone, a topical anti-inflammatory drug, and they all fully recover from the reaction. (She did not prescribe antibiotics, because TASS is not an infection.) She holds a staff meeting to make sure the correct sterilizing procedures are being followed. Dr. Santos also stresses to her employees that preventing TASS depends primarily on using appropriate protocols for cleaning and sterilizing surgical equipment and paying careful attention to all solutions, medications, and ophthalmic devices used during surgery.

418 424 429 433 435

Study Outline

MasteringMicrobiology®

Study microbiology where it matters: every where.
Check out the study Area of MasteringMicrobiology to explore Interactive Microbiology. Study on-the-go with Dynamic Study Modules. Check your understanding with chapter guizzes and animations—and much more!

Introduction (p. 417)

1. Pathogenicity is the ability of a pathogen to produce a disease by overcoming the defenses of the host.
2. Virulence is the degree of pathogenicity.

How Microorganisms Enter a Host (pp. 418–421)

1. The specific route by which a particular pathogen gains access to the body is called its portal of entry.

Portals of Entry (pp. 418–419)

2. Many microorganisms can penetrate mucous membranes of the conjunctiva and the respiratory, gastrointestinal, and genitourinary tracts.
3. Most microorganisms cannot penetrate intact skin; they enter hair follicles and sweat ducts.
4. Some microorganisms can gain access to tissues by inoculation through the skin and mucous membranes in bites, injections, and other wounds. This route of penetration is called the parenteral route.

The Preferred Portal of Entry (p. 419)

5. Many microorganisms can cause infections only when they gain access through their specific portal of entry.

Numbers of Invading Microbes (p. 420)

6. Virulence can be expressed as LD_{50} (lethal dose for 50% of the inoculated hosts) or ID_{50} (infectious dose for 50% of the inoculated hosts).

Adherence (pp. 420–421)

7. Surface projections on a pathogen called adhesins (ligands) adhere to complementary receptors on the host cells.
8. Adhesins can be glycoproteins or lipoproteins and are frequently associated with fimbriae.
9. Mannose is the most common receptor.
10. Biofilms provide attachment and resistance to antimicrobial agents.

How Bacterial Pathogens Penetrate Host Defenses (pp. 421–423)

Capsules (p. 421)

1. Some pathogens have capsules that prevent them from being phagocytized.

Cell Wall Components (p. 421)

2. Proteins in the cell wall can facilitate adherence or prevent a pathogen from being phagocytized.

Enzymes (pp. 421–423)

3. Local infections can be protected in a fibrin clot caused by the bacterial enzyme coagulase.
4. Bacteria can spread from a focal infection by means of kinases (which destroy blood clots), hyaluronidase (which destroys a mucopolysaccharide that holds cells together), and collagenase (which hydrolyzes connective tissue collagen).
5. IgA proteases destroy IgA antibodies.

Antigenic Variation (p. 423)

6. Some microbes vary expression of antigens, thus avoiding the host's antibodies.

Penetration into the Host Cell Cytoskeleton (p. 423)

7. Bacteria may produce proteins that alter the actin of the host cell's cytoskeleton, allowing bacteria into the cell.

How Bacterial Pathogens Damage Host Cells (pp. 424–430)

Using the Host's Nutrients: Siderophores (p. 424)

1. Bacteria get iron from the host using siderophores.

Direct Damage (p. 424)

2. Host cells can be destroyed when pathogens metabolize and multiply inside the host cells.

The Production of Toxins (pp. 424–429)

3. Poisonous substances produced by microorganisms are called toxins; toxemia refers to the presence of toxins in the blood. The ability to produce toxins is called toxigenicity.
4. Exotoxins are produced by bacteria and released into the surrounding medium. Exotoxins, not the bacteria, produce the disease symptoms.
5. Antibodies produced against exotoxins are called antitoxins.
6. A-B toxins consist of an active component that inhibits a cellular process and a binding component that attaches the two portions to the target cell, e.g., diphtheria toxin.
7. Membrane-disrupting toxins cause cell lysis, e.g., hemolysins.
8. Superantigens cause release of cytokines, which cause fever, nausea, and other symptoms; e.g., toxic shock syndrome toxin.
9. Genotoxins alter the host's DNA.
10. Endotoxins are lipopolysaccharides (LPS), the lipid A component of the cell wall of gram-negative bacteria.
11. Bacterial cell death, antibiotics, and antibodies may cause the release of endotoxins.
12. Endotoxins cause fever (by inducing the release of interleukin-1) and shock (because of a TNF-induced decrease in blood pressure).
13. The Limulus amebocyte lysate (LAL) assay is used to detect endotoxins in drugs and on medical devices.

Plasmids, Lysogeny, and Pathogenicity (p. 430)

14. Plasmids may carry genes for antibiotic resistance, toxins, capsules, and fimbriae.
15. Lysogenic conversion can result in bacteria with virulence factors, such as toxins or capsules.

Pathogenic Properties of Viruses (pp. 430–432)

1. Viruses avoid the host's immune response by growing inside cells.
2. Viruses gain access to host cells because they have attachment sites for receptors on the host cell.
3. Visible signs of viral infections are called cytopathic effects (CPE).
4. Some viruses cause cytocidal effects (cell death), and others cause noncytocidal effects (damage but not death).
5. Cytopathic effects include stopping mitosis, lysis, formation of inclusion bodies, cell fusion, antigenic changes, chromosomal changes, and transformation.

Pathogenic Properties of Fungi, Protozoa, Helminths, and Algae (pp. 432–433)

1. Symptoms of fungal infections can be caused by capsules, toxins, and allergic responses.
2. Symptoms of protozoan and helminthic diseases can be caused by damage to host tissue or by the metabolic waste products of the parasite.
3. Some protozoa change their surface antigens while growing in a host, thus avoiding destruction by the host's antibodies.
4. Some algae produce neurotoxins that cause paralysis when ingested by humans.

Portals of Exit (pp. 433–435)

1. Pathogens leave a host by portals of exit.
2. Three common portals of exit are the respiratory tract via coughing or sneezing, the gastrointestinal tract via saliva or feces, and the genitourinary tract via secretions from the vagina or penis.
3. Arthropods and syringes provide a portal of exit for microbes in blood.

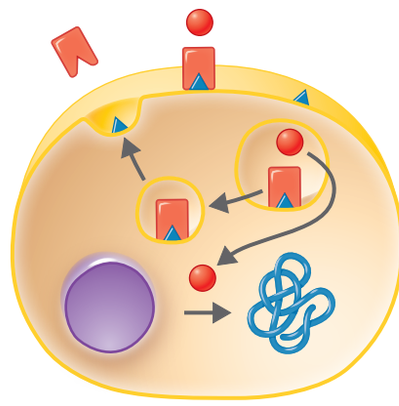
Study Questions

For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. Compare pathogenicity with virulence.
2. How are capsules and cell wall components related to pathogenicity? Give specific examples.
3. Describe how hemolysins, leukocidins, coagulase, kinases, hyaluronidase, siderophores, and IgA proteases might contribute to pathogenicity.
4. Explain how drugs that bind each of the following would affect pathogenicity:
   a. iron in the host's blood
   b. *Neisseria gonorrhoeae* fimbriae
   c. *Streptococcus pyogenes* M protein
5. Compare and contrast the following aspects of endotoxins and exotoxins: bacterial source, chemistry, toxicity, and pharmacology. Give an example of each toxin.
6. DRAW IT Label this diagram to show how the Shiga toxin enters and inhibits protein synthesis in a human cell.



7. Describe the factors contributing to the pathogenicity of fungi, protozoa, and helminths.
8. Which of the following genera is the most infectious?

Genus	ID_{50}	Genus	ID_{50}
Legionella	1 cell	*Shigella*	200 cells
Salmonella	10^5 cells	*Treponema*	52 cells

9. How can viruses and protozoa avoid being killed by the host's immune response?
10. NAME IT The *Opa* gene is used to identify this endotoxin-producing bacterium that grows well in the high-CO_2 conditions inside phagocytes.

Multiple Choice

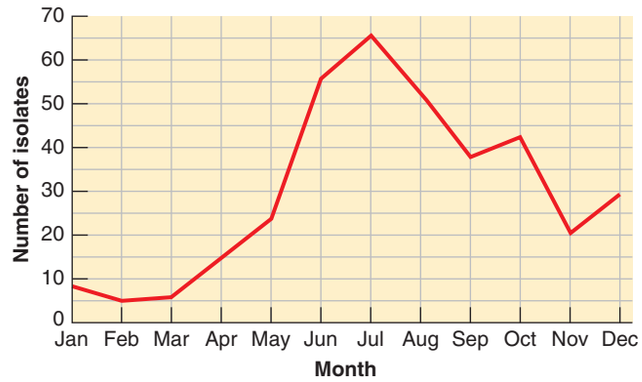
1. The removal of plasmids reduces virulence in which of the following organisms?
   a. *Clostridium tetani*
   b. *Escherichia coli*
   c. *Salmonella enterica*
   d. *Streptococcus mutans*
   e. *Clostridium botulinum*
2. What is the LD_{50} for the bacterial toxin tested in the example below?

Dilution (µg/kg)	No. of Animals Died	No. of Animals Survived
a. 6	0	6
b. 12.5	0	6
c. 25	3	3
d. 50	4	2
e. 100	6	0

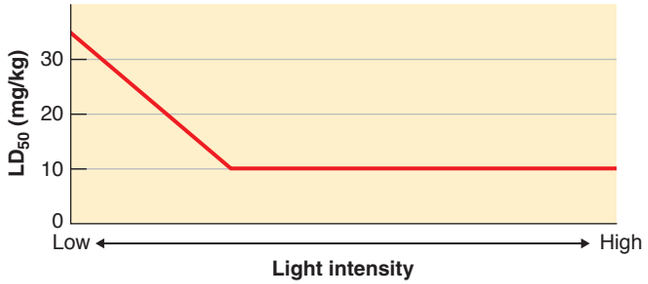
3. Which of the following is *not* a portal of entry for pathogens?
   a. mucous membranes of the respiratory tract
   b. mucous membranes of the gastrointestinal tract
   c. skin
   d. blood
   e. parenteral route
4. All of the following can occur during bacterial infection. Which would prevent all of the others?
   a. vaccination against fimbriae
   b. phagocytosis
   c. inhibition of phagocytic digestion
   d. destruction of adhesins
   e. alteration of cytoskeleton
5. The ID_{50} for *Campylobacter* sp. is 500 cells; the ID_{50} for *Cryptosporidium* sp. is 100 cells. Which of the following statements is *false*?
   a. Both microbes are pathogens.
   b. Both microbes produce infections in 50% of the inoculated hosts.
   c. *Cryptosporidium* is more virulent than *Campylobacter.*
   d. *Campylobacter* and *Cryptosporidium* are equally virulent; they cause infections in the same number of test animals.
   e. *Cryptosporidium* infections are acquired more easily than *Campylobacter* infections.
6. An encapsulated bacterium can be virulent because the capsule
   a. resists phagocytosis.
   b. is an endotoxin.
   c. destroys host tissues.
   d. kills host cells.
   e. has no effect; because many pathogens do not have capsules, capsules do not contribute to virulence.
7. A drug that binds to mannose on human cells would prevent
   a. the entrance of *Vibrio* enterotoxin.
   b. the attachment of pathogenic *E. coli.*
   c. the action of botulinum toxin.
   d. streptococcal pneumonia.
   e. the action of diphtheria toxin.
8. The earliest smallpox vaccines were infected tissue rubbed into the skin of a healthy person. The recipient of such a vaccine usually developed a mild case of smallpox, recovered, and was immune thereafter. What is the most likely reason this vaccine did not kill more people?
   a. Skin is the wrong portal of entry for smallpox.
   b. The vaccine consisted of a mild form of the virus.
   c. Smallpox is normally transmitted by skin-to-skin contact.
   d. Smallpox is a virus.
   e. The virus mutated.
9. Which of the following does *not* represent the same mechanism for avoiding host defenses as the others?
   a. Rabies virus attaches to the receptor for the neurotransmitter acetylcholine.
   b. *Salmonella* attaches to the receptor for epidermal growth factor.
   c. Epstein-Barr (EB) virus binds to the host receptor for complement.
   d. Surface protein genes in *Neisseria gonorrhoeae* mutate frequently.
   e. none of the above
10. Which of the following statements is true?
    a. The primary goal of a pathogen is to kill its host.
    b. Evolution selects for the most virulent pathogens.
    c. A successful pathogen doesn't kill its host before it is transmitted.
    d. A successful pathogen never kills its host.

Analysis

1. The graph below shows confirmed cases of enteropathogenic *E. coli.* Why is the incidence seasonal?



2. The cyanobacterium *Microcystis aeruginosa* produces a peptide that is toxic to humans. According to the graph below, when is this bacterium most toxic?



3. When injected into rats, the ID_{50} for *Salmonella typhimurium* is 10^6 cells. If sulfonamides are injected with the salmonellae, the ID_{50} is 35 cells. Explain the change in ID_{50} value.
4. How do each of the following strategies contribute to the virulence of the pathogen? What disease does each organism cause?

Strategy	Pathogen
Changes its cell wall after entry into host	*Yersinia pestis*
Uses urea to produce ammonia	*Helicobacter pylori*
Causes host to make more receptors	*Rhinovirus*

Clinical Applications and Evaluation

1. On July 8, a woman was given an antibiotic for presumptive sinusitis. However, her condition worsened, and she was unable to eat for 4 days because of severe pain and tightness of the jaw. On July 12, she was admitted to a hospital with severe facial spasms. She reported that on July 5 she had incurred a puncture wound at the base of her big toe; she cleaned the wound but did not seek medical attention. What caused her symptoms? Was her condition due to an infection or an intoxication? Can she transmit this condition to another person?
2. Explain whether each of the following examples is a food infection or intoxication. What is the probable etiological agent in each case?
   a. Eighty-two people in Louisiana developed diarrhea, nausea, headache, and fever from 4 hours to 2 days after eating shrimp.
   b. Two people in Vermont developed malaise, nausea, blurred vision, breathing difficulty, and numbness 3 to 6 hours after eating barracuda caught in Florida.
3. Cancer patients undergoing chemotherapy are normally *more* susceptible to infections. However, a patient receiving an antitumor drug that affects eukaryotic cytoskeletons was resistant to *Salmonella*. Provide a possible mechanism for the resistance.

In the Clinic

You are an emergency room nurse caring for Madge, a 30-year-old kidney transplant recipient. She is currently receiving treatment for septic shock—her third episode of this infection in her lifetime. Madge says that since childhood she has always been very susceptible to recurring infections. For this reason, she is especially thankful that so far, her transplanted kidney is functioning well and shows no signs of rejection or damage. You run tests that show leukocytosis, normal levels of antibodies, and a C6 deficiency. **What is the cause of Madge's frequent infections, and how does it relate to her transplant tolerance?**

Hint: Read about leukocyte response to infections on page 448, the complement system on pages 456–460, and testing for complement in the Applications of Microbiology box on page 462.

Note: Answers to In the Clinic questions are found online at MasteringMicrobiology.

16 Innate Immunity: Nonspecific Defenses of the Host

From our discussion to this point, you can see that pathogenic microorganisms are endowed with special properties that enable them to cause disease if given the right opportunity. If microorganisms never encountered resistance from the host, we would constantly be ill and would die of various diseases after a short life. In most cases, however, our body's defenses prevent this from happening. Some of these defenses are designed to keep out microorganisms altogether, other defenses remove the microorganisms if they do get in, and still others combat them if they remain inside.

In this chapter we discuss the first two lines of defense against pathogens, which we call the *innate immunity defenses*. The first line of defense is our skin and mucous membranes. The second line of defense consists of phagocytes, inflammation, fever, and antimicrobial substances produced by the body. The Clinical Case in this chapter describes one problem that can occur if phagocytes (green in the photograph) don't function properly.

See pages 440–441 for a **Big Picture** overview of the entire immune system.

A neutrophil (blue) phagocytizing *Aspergillus* spores (red).

BIG PICTURE

Immunity

Every day the human body wages war with microbial pathogens needing a place to live.

First-Line Defenses

First-line defenses keep pathogens on the outside or neutralize them before infection begins. The skin, mucous membranes, and certain antimicrobial substances are part of these defenses.

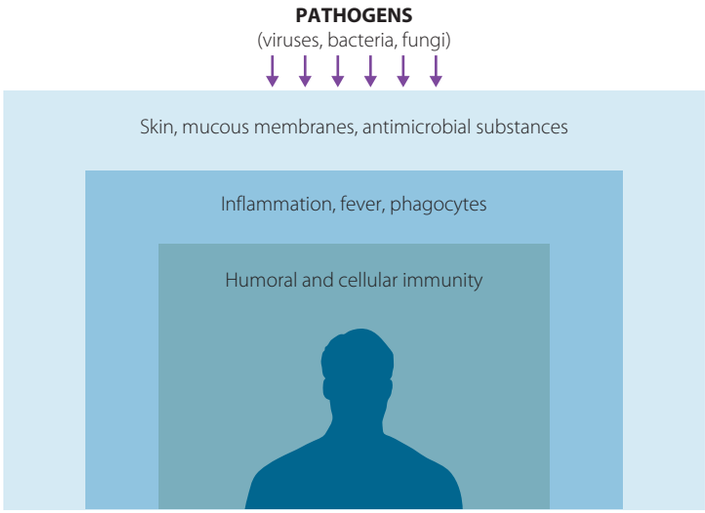
Second-Line Defenses

Second-line defenses slow or contain infections when first-line defenses fail. They include proteins that produce inflammation, fever that enhances cytokine activity, and phagocytes and NK cells, which attack and destroy cancer cells and virus-infected cells.

Third-Line Defenses

Third-line defenses include lymphocytes, shown in the table below, that target specific pathogens for destruction when the second-line defenses don't contain infections. It includes a memory component that allows the body to more effectively respond to that same pathogen in the future.

First- and second-line defenses are part of the **innate immune system**, whereas the third-line defenses are referred to as the **adaptive immune system.** Many leukocytes (white blood cells) coordinate efforts in controlling infections in the second and third lines of immune defense.

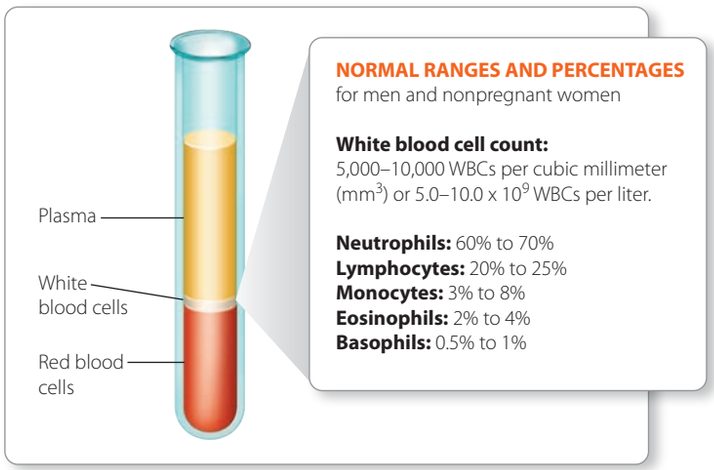


Innate or Adaptive	Cell Type	Description	Function
INNATE	Basophil	Granulocyte	Releases histamines that cause inflammation.
	Eosinophil	Granulocyte	Kills parasites with oxidative burst.
	Mast cell	Agranulocyte	Kills infected cells (often virus-infected) via cytolysis or apoptosis.
BOTH	Neutrophil	Granulocyte	Phagocytizes bacteria and fungi.
	Monocyte	Agranulocyte	Precursor to macrophages. Some macrophages are fixed in certain organs while others wander tissues, causing inflammation. All perform phagocytosis.
	Dendritic cell	Many surface projections	In skin and respiratory and intestinal mucosa, phagocytizes bacteria and presents antigens to T cells.
	Natural killer (NK) cell	Agranulocyte (lymphocyte)	Kills cancer cells and virus-infected cells.
ADAPTIVE	Plasma cell, B cell	Agranulocyte (lymphocyte)	Recognizes antigens and produces antibodies.
	T cells T helper (T_H) cell Cytotoxic T lymphocyte (CTL) T regulatory (T_{reg}) cell	Agranulocyte (lymphocyte)	T_H cells secrete cytokines. They are $CD4^+$ cells that bind MHC class II molecules on antigen-presenting cells (APCs). CTLs recognize and kill specific "nonself" cells. They are $CD8^+$ cells that bind to MHC class I molecules. T_{reg} cells are $CD4^+$ cells that destroy cells that do not correctly recognize "self" cells.

What Do Blood Cell Counts Tell Us About Patient Health?

White Blood Cell Counts

White blood cell (WBC) counts measure the number of leukocytes found in the blood. A related test, called *differential white blood cell count,* breaks down the white blood cell count further, identifying the percentage of eosinophils, basophils, neutrophils, monocytes, and lymphocytes. Abnormal blood cell counts give health care providers important clues for diagnosing infections and other conditions.



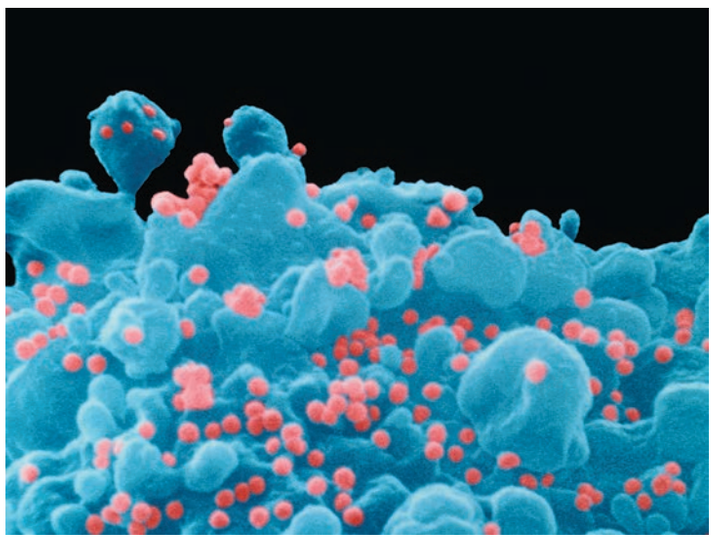
High White Blood Cell Counts

A high white blood cell count shows the patient is producing a higher than average number of leukocytes. This typically occurs when the patient battles a bacterial infection. High white blood cell counts may also stem from autoimmune disorders that result in too much inflammatory response, such as rheumatoid arthritis, and from leukemia, a cancer of the blood. Some drugs can cause high white blood cell counts as a side effect; these include certain asthma medications such as albuterol, epinephrine, corticosteroids, and the anticoagulant heparin.

◀ **Serial monitoring of the white blood cell count is used to monitor treatment for infants battling the bacterial infection pertussis. Rapidly rising high white blood cell counts have been shown to be associated with higher mortality among infants.**

Low White Blood Cell Counts

A low white blood cell count shows the patient has fewer leukocytes than expected. A low neutrophil count, in particular, is instructive. Even bacteria that generally live in the mouth and digestive track without being pathogenic may result in disease when the patient's neutrophil count drops below 500 neutrophils per mm^3 of blood. Low white blood cell counts may result from viral infections or pneumonia. They may also be caused from autoimmune diseases such as lupus; certain cancers, such as lymphoma; and radiation and other cancer treatments. White blood cell counts may also be low when a patient has an extremely severe bacterial infection, such as septicemia. Finally, numerous drugs may also cause low white blood cell counts, including a variety of antibiotics, diuretics, and anticancer medications.



▲ **T cell (blue) infected with HIV. Virus particles (pink) can be seen budding from the infected cell. HIV infects T_H lymphocytes.**

KEY CONCEPTS

- Innate immunity involves the first- and second-line defenses.
- Adaptive immunity involves third-line defenses.
- Innate immune actions are fast but nonspecific. Adaptive immune actions are slower but specific to pathogens and have a memory component.

The Concept of Immunity

LEARNING OBJECTIVES

16-1 Differentiate innate and adaptive immunity.

16-2 Define *Toll-like receptors.*

When microbes attack our bodies, we defend ourselves by utilizing our various mechanisms of immunity. **Immunity,** also called **resistance,** is the ability to ward off disease caused by microbes or their products and to protect against environmental agents such as pollen, chemicals, and animal dander. Lack of immunity is referred to as **susceptibility.** In general, there are two types of immunity: innate and adaptive. **Innate immunity** refers to defenses that are present at birth. They are always available to provide rapid responses to protect us against disease. Innate immunity does not involve recognition of a specific microbe. Further, innate immunity has no memory response, that is, a more rapid and stronger immune reaction to the same microbe at a later date. Innate immunity first-line defenses include skin and mucous membranes, and the second-line defenses include natural killer cells, phagocytes, inflammation, fever, and antimicrobial substances. Innate immune responses represent immunity's early-warning system and are designed to prevent microbes from gaining access into the body and to help eliminate those that do gain access.

Adaptive immunity is based on a specific response to a specific microbe once a microbe has breached the innate immunity defenses. It adjusts to handle a particular microbe. Unlike innate immunity, adaptive immunity is slower to respond, but it does have a memory component that allows the body to more effectively target the same pathogens in the future. Adaptive immunity involves lymphocytes (a type of white blood cell) called T cells (T lymphocytes) and B cells (B lymphocytes) and will be discussed in detail in Chapter 17. Here, we concentrate on innate immunity.

Responses of the innate system are activated by protein receptors in the plasma membranes of defensive cells. Among these activators are **Toll-like receptors (TLRs).** These TLRs attach to various components commonly found on pathogens that are called **pathogen-associated molecular patterns (PAMPs)** (see Figure 16.8). Examples include the lipopolysaccharide (LPS) of the outer membrane of gram-negative bacteria, the flagellin in the flagella of motile bacteria, the peptidoglycan in the cell wall of gram-positive bacteria, the DNA of bacteria, and the DNA and RNA of viruses. TLRs also attach to components of fungi and parasites.

You will learn later in this chapter that two of the defensive cells involved in innate immunity are called macrophages and dendritic cells and provide a link between innate immunity and adaptive immunity. When the TLRs on these cells encounter the PAMPs of microbes, such as the LPS of gram-negative bacteria, the TLRs induce the defensive cells to release chemicals called cytokines. **Cytokines** (*cyto-* = cell; *-kinesis* = motion) are proteins that regulate the intensity and duration of immune responses.

One role of cytokines is to recruit other macrophages and dendritic cells, as well as other defensive cells, to isolate and destroy the microbes as part of the inflammatory response. Cytokines can also activate the T cells and B cells involved in adaptive immunity. You will learn more about the different cytokines and their functions in Chapter 17. MM **Animation** Host Defenses: The Big Picture

CHECK YOUR UNDERSTANDING

- Which defense system, innate or adaptive immunity, prevents entry of microbes into the body? **16-1**
- What relationship do Toll-like receptors have to pathogen-associated molecular patterns? **16-2**

Clinical Case: Missing in Action

Two-year-old Jacob is back in the pediatrician's office with another high fever. Jacob has a history of recurrent skin infections, fever, and chronically enlarged lymph nodes.

Jacob's pediatrician notices that his lung sounds are not clear, so he sends Jacob for a chest X-ray exam. Results show a mass in Jacob's right lung. The mass is pneumonia, and Jacob's pediatrician treats him with antibiotics. A few weeks after he finishes his antibiotics, Jacob develops pneumonia again. This time Jacob's pediatrician orders a biopsy of the lung mass, and the culture reveals *Aspergillus* fungus.

Why isn't Jacob's innate immunity protecting him from infections? Read on to find out.

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First Line of Defense: Skin and Mucous Membranes

LEARNING OBJECTIVES

16-3 Describe the role of the skin and mucous membranes in innate immunity.

16-4 Differentiate physical from chemical factors, and list five examples of each.

16-5 Describe the role of normal microbiota in innate immunity.

The skin and mucous membranes are the body's first line of defense against environmental pathogens. This function results from both physical and chemical factors.

Physical Factors

Physical factors include barriers to entry and processes that remove microbes from the body's surface. The intact **skin** is the human body's largest organ in terms of surface area and weight and is an extremely important component of the first line of defense. It consists of the dermis and the epidermis (**Figure 16.1**). The **dermis,** the skin's inner, thicker portion, is composed of connective tissue. The **epidermis,** the outer, thinner portion, is

Figure 16.1 A section through human skin. The thin layers at the top of this photomicrograph contain keratin. These layers and the darker purple cells beneath them make up the epidermis. The region below the epidermis is the dermis.

Q What is the function of keratin in the epidermis?

in direct contact with the external environment. The epidermis consists of many layers of continuous sheets of tightly packed epithelial cells with little or no material between the cells. The top layer of epidermal cells is dead and contains a protective protein called **keratin.** The periodic shedding of the top layer helps remove microbes at the surface. In addition, the dryness of the skin is a major factor in inhibiting microbial growth on the skin. Although normal microbiota and other microbes are present on the entire skin, they are most numerous on moist areas. When the skin is frequently moist, as in hot, humid climates, skin infections are quite common, especially fungal infections such as athlete's foot. These fungi hydrolyze keratin when water is available.

If we consider the closely packed cells, continuous layering, the presence of keratin, and the dryness and shedding of the skin, we can see why the intact skin provides such a formidable barrier to the entrance of microorganisms. Microorganisms rarely, if ever, penetrate the intact surface of healthy epidermis. However, when the epithelial surface is broken as a result of burns, cuts, puncture wounds, or other conditions, a subcutaneous (below-the-skin) infection often develops. The bacteria most likely to cause infection are the staphylococci that normally inhabit the epidermis, hair follicles, and sweat and oil glands of the skin.

Epithelial cells called *endothelial cells* that line blood and lymphatic vessels are not closely packed like those of the epidermis. This arrangement permits defensive cells to move from blood into tissues during inflammation, but it also permits microbes to move into and out of blood and lymph.

Mucous membranes also consist of an epithelial layer and an underlying connective tissue layer. They are an important component of the first line of defense. Mucous membranes line the entire gastrointestinal, respiratory, and genitourinary tracts. The epithelial layer of a mucous membrane secretes a fluid called **mucus,** a slightly viscous (thick) glycoprotein produced by goblet cells of a mucous membrane. Among other functions, mucus prevents the tracts from drying out. Some pathogens that can thrive on the moist secretions are able to penetrate the membrane if the microorganism is present in sufficient numbers. *Treponema pallidum* is such a pathogen. This penetration may be facilitated by toxic substances produced by the microorganism, prior injury by viral infection, or mucosal irritation.

Besides the physical barrier presented by the skin and mucous membranes, several other physical factors help protect certain epithelial surfaces. One mechanism that protects eyes is the **lacrimal apparatus,** a group of structures that manufactures and drains tears (Figure 16.2). The lacrimal glands, located toward the upper, outermost portion of each eye socket, produce the tears and pass them under the upper eyelid. From here, tears pass toward the corner of the eye near the nose and into two small holes that lead through tubes (lacrimal canals) to the nose. The tears are spread over the surface of the eyeball by blinking. Normally, the tears evaporate or pass into the nose as fast as they are produced. This continual washing action helps keep microorganisms from settling on the surface of the eye. If an irritating substance or large numbers of microorganisms come in contact with the eye, the lacrimal glands start to secrete heavily, and the tears accumulate more rapidly than they can be carried away. This excessive

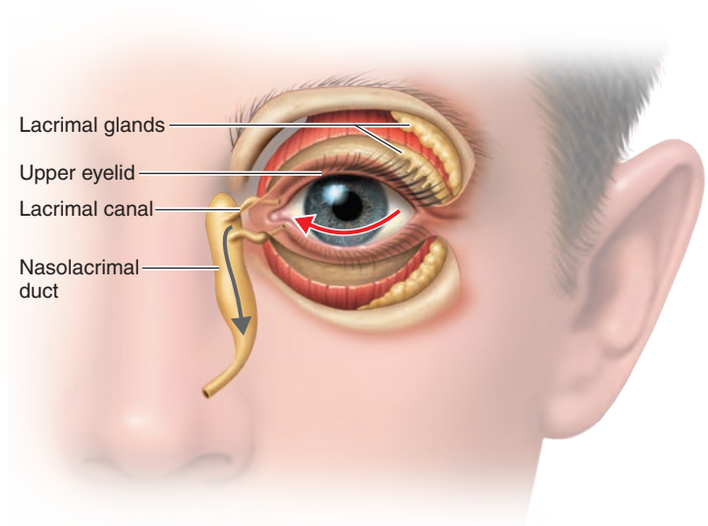


Figure 16.2 The lacrimal apparatus. The washing action of tears over the surface of the eyeball is shown by the red arrow. Tears produced by the lacrimal glands pass across the surface of the eyeball into two small holes that convey the tears into the lacrimal canals and the nasolacrimal duct. From here tears pass into the nose, as shown by the gray arrow.

Q How does the lacrimal apparatus protect the eyes against infections?

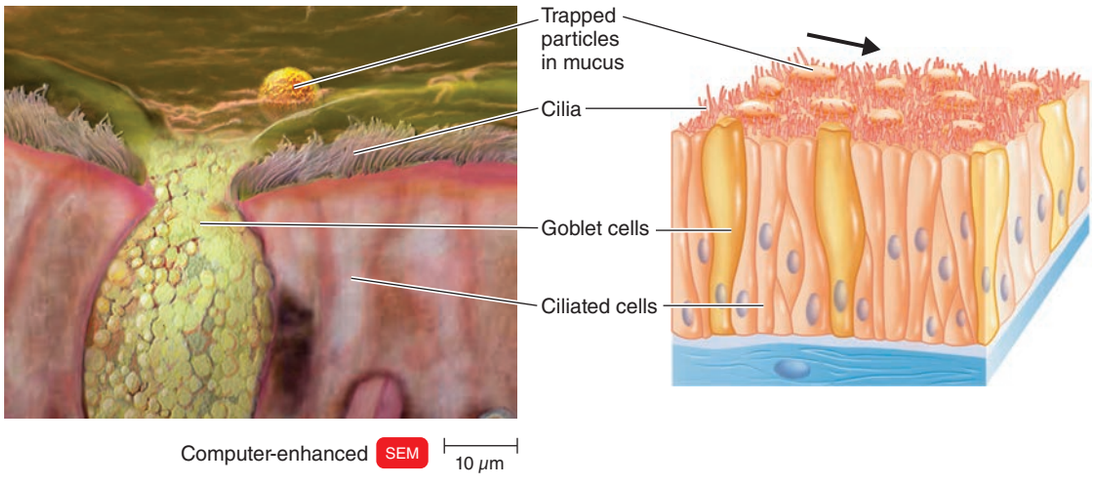


Figure 16.3 The ciliary escalator.

Q What can happen if the ciliary escalator is inhibited?

production is a protective mechanism because the excess tears dilute and wash away the irritating substance or microorganisms before infection can occur.

In a cleansing action very similar to that of tears, **saliva,** produced by the salivary glands, helps dilute the numbers of microorganisms and wash them from the surface of the teeth and the mucous membrane of the mouth. This helps prevent colonization by microbes.

The respiratory and gastrointestinal tracts have many physical forms of defense. **Mucus** traps many of the microorganisms that enter these tracts. The mucous membrane of the nose also has mucus-coated **hairs** that filter inhaled air and trap microorganisms, dust, and pollutants. The cells of the mucous membrane of the lower respiratory tract are covered with **cilia.** By moving synchronously, these cilia propel inhaled dust and microorganisms that have become trapped in mucus upward toward the throat. This so-called **ciliary escalator** (Figure 16.3) keeps the mucus blanket moving toward the throat at a rate of 1 to 3 cm per hour; coughing and sneezing speed up the escalator. Some substances in cigarette smoke are toxic to cilia and can seriously impair the functioning of the ciliary escalator by inhibiting or destroying the cilia. Mechanically ventilated patients are vulnerable to respiratory tract infections because the ciliary escalator mechanism is inhibited. Microorganisms are also prevented from entering the lower respiratory tract by a small lid of cartilage called the **epiglottis,** which covers the larynx (voicebox) during swallowing. The external ear canal contains hairs and **earwax** *(cerumen),* which help prevent microbes, dust, insects, and water from entering the ear.

The cleansing of the urethra by the flow of **urine** is another physical factor that prevents microbial colonization in the genitourinary tract. As you will see later, when urine flow is obstructed—by catheters, for example—urinary tract infections may develop. **Vaginal secretions** likewise move microorganisms out of the female body.

Peristalsis, defecation, vomiting, and **diarrhea** also expel microbes. Peristalsis is a series of coordinated contractions that propels food along the gastrointestinal tract. Mass peristalsis of large intestinal contents into the rectum results in defecation. In response to microbial toxins, the muscles of the gastrointestinal tract contract vigorously, resulting in vomiting and/or diarrhea, which may also rid the body of microbes.

Chemical Factors

Physical factors alone do not account for the high degree of resistance shown by skin and mucous membranes against microbial invasion. Certain chemical factors also play important roles.

Sebaceous (oil) glands of the skin produce an oily substance called **sebum** that prevents hair from drying and becoming brittle. Sebum also forms a protective film over the surface of the skin. One of the components of sebum is unsaturated fatty acids, which inhibit the growth of certain pathogenic bacteria and fungi. The low pH of the skin, between pH 3 and 5, is caused in part by the secretion of fatty acids and lactic acid. The skin's acidity probably discourages the growth of many other microorganisms.

Bacteria that live commensally on the skin decompose sloughed-off skin cells, and the resultant organic molecules and end-products of their metabolism produce body odor. Certain bacteria commonly found on the skin metabolize sebum, and this metabolism forms free fatty acids that cause the inflammatory response associated with acne (as we'll see in Chapter 21). Isotretinoin, a derivative of vitamin A that prevents sebum formation, is a treatment for a very severe type of acne called cystic acne.

The sweat glands of the skin produce **perspiration,** which helps maintain body temperature, eliminates certain wastes, and flushes microorganisms from the surface of the skin. Perspiration also contains **lysozyme,** an enzyme capable of breaking down cell walls of gram-positive bacteria and, to a lesser extent, gram-negative bacteria (see Figure 4.13, page 82). Specifically, lysozyme breaks chemical bonds on peptidoglycan,

which destroys the cell walls. Lysozyme is also found in tears, saliva, nasal secretions, tissue fluids, and urine, where it exhibits its antimicrobial activity. Alexander Fleming was studying lysozyme in 1929 when he accidentally discovered the antimicrobial effects of penicillin (see Figure 1.5, page 11).

Earwax, besides serving as a physical barrier, also functions as a chemical protectant. It is a mixture of secretions from glands producing earwax as well as from the sebaceous glands, which produce sebum. The secretions are rich in fatty acids, giving the ear canal a low pH, between 3 and 5, which inhibits the growth of many pathogenic microbes. Earwax also contains many dead cells from the lining of the ear canal.

Saliva contains not only the enzyme salivary amylase that digests starch, but also a number of substances that inhibit microbial growth. These include lysozyme, urea, and uric acid. The slightly acidic pH of saliva (6.55–6.85) also inhibits some microbes. Saliva additionally contains an antibody (lgA) that prevents attachment of microbes so that they cannot penetrate mucous membranes.

Gastric juice is produced by the glands of the stomach. It is a mixture of hydrochloric acid, enzymes, and mucus. The very high acidity of gastric juice (pH 1.2–3.0) is sufficient to destroy bacteria and most bacterial toxins, except those of *Clostridium botulinum* and *Staphylococcus aureus.* However, many enteric pathogens are protected by food particles and can enter the intestines via the gastrointestinal tract. In contrast, the bacterium *Helicobacter pylori* neutralizes stomach acid, thereby allowing the bacterium to grow in the stomach. Its growth initiates an immune response that results in gastritis and ulcers.

Vaginal secretions play a role in antibacterial activity in two ways. Glycogen produced by vaginal epithelial cells is broken down into lactic acid by *Lactobacillus acidophilus.* This creates an acidic pH (3–5) that inhibits microbes. Cervical mucus also has some antimicrobial activity.

Urine, in addition to containing lysozyme, has an acidic pH (average 6) that inhibits microbes.

Later in the chapter, we will discuss another group of chemicals, the antimicrobial peptides, which play a very important role in innate immunity.

Normal Microbiota and Innate Immunity

Technically speaking, the **normal microbiota** are not usually considered part of the first line of defense of the innate immune system, but they are discussed here because of the considerable protection they afford. Chapter 14 described several relationships between normal microbiota and host cells. Some of these relationships help prevent the overgrowth of pathogens and thus may be considered components of innate immunity. For example, in microbial antagonism, the normal microbiota prevent pathogens from colonizing the host by competing with them for nutrients (competitive exclusion), by producing substances that are harmful to the pathogens, and by altering conditions that affect the survival of the pathogens, such as pH and oxygen availability. The presence of normal microbiota in the vagina, for example, alters pH, thus preventing overpopulation by *Candida albicans,* a pathogenic yeast that causes vaginitis. In the large intestine, *E. coli* bacteria produce bacteriocins that inhibit the growth of *Salmonella* and *Shigella.*

In **commensalism,** one organism uses the body of a larger organism as its physical environment and may make use of the body to obtain nutrients. Thus in commensalism, one organism benefits while the other is unaffected. Most microbes that are part of the commensal microbiota are found on the skin and in the gastrointestinal tract. The majority of such microbes are bacteria that have highly specialized attachment mechanisms and precise environmental requirements for survival. Normally, such microbes are harmless, but they may cause disease if their environmental conditions change. These opportunistic pathogens include *E. coli, S. aureus, S. epidermidis, Enterococcus faecalis, Pseudomonas aeruginosa,* and oral streptococci.

Recent interest in the importance of bacteria to human health has led to the study of probiotics. **Probiotics** (*pro* = for, *bios* = life) are live microbial cultures applied to or ingested that are intended to exert a beneficial effect. Probiotics may be administered with *prebiotics,* which are chemicals that selectively promote the growth of beneficial bacteria. If these LAB colonize the large intestine, the lactic acid and bacteriocins they produce can inhibit the growth of certain pathogens. Several studies have shown that ingesting certain lactic acid bacteria (LAB) can alleviate diarrhea and prevent colonization by *Salmonella enterica* during antibiotic therapy. Researchers are also testing the use of LAB to prevent surgical wound infections caused by *S. aureus* and vaginal infections caused by *E. coli.* In a Stanford University study, HIV infection was reduced in women treated with a LAB that was genetically modified to produce CD4 protein that binds to HIV. Results of several studies suggest that giving probiotics with antibiotics reduces the risk of developing *Clostridium difficile*–associated diarrhea. However, probiotics may not always work. A recent French study found that although use of probiotics reduced incidence of pneumonia acquired in intensive care units (ICUs) and length of ICU stay, probiotics did not significantly reduce hospital mortality rates.

CHECK YOUR UNDERSTANDING

- Identify one physical factor and one chemical factor that prevent microbes from entering the body through skin and mucous membranes. **16-3**
- Identify one physical factor and one chemical factor that prevent microbes from entering or colonizing the body through the eyes, digestive tract, and respiratory tract. **16-4**
- Distinguish microbial antagonism from commensalism. **16-5**

Second Line of Defense

When microbes penetrate the first line of defense, they encounter a second line of defense that includes defensive cells, such as phagocytic cells; inflammation; fever; and antimicrobial substances.

Before we look at the phagocytic cells, it will be helpful to first have an understanding of the cellular components of blood.

Formed Elements in Blood

LEARNING OBJECTIVES

16-6 Classify leukocytes, and describe the roles of granulocytes and monocytes.

16-7 Describe the eight different types of white blood cells, and name a function for each type.

Blood consists of fluid, called **plasma,** and **formed elements**—that is, cells and cell fragments suspended in plasma. The formed elements include **erythrocytes,** or **red blood cells (RBCs); leukocytes,** or **white blood cells (WBCs);** and **platelets.** Formed elements are created in red bone marrow by stem cells in a process called **hematopoiesis** (hēm-a-tō-POY-ē-sis). It begins when a cell called a *pluripotent stem cell* develops into two other types of cells called *myeloid stem cells* and *lymphoid stem cells*. From these two stem cells, all of the formed elements develop. All of these blood cells are shown in **Figure 16.4**. More detailed descriptions of the formed elements that concern us most for innate immunity—the leukocytes—are found in **Table 16.1**.

Leukocytes are divided into two main categories based on their appearance under a light microscope: granulocytes and agranulocytes. **Granulocytes** owe their name to the presence of large granules in their cytoplasm that can be seen with a light microscope after staining. They are differentiated into three types of cells based on how the granules stain: neutrophils, basophils, and eosinophils. **Neutrophils** stain pale lilac with a mixture of acidic and basic dyes. Neutrophils are also commonly called *polymorphonuclear leukocytes (PMNs),* or *polymorphs.* (The term *polymorphonuclear* refers to the fact that the nuclei of neutrophils contain two to five lobes.) Neutrophils, which are highly phagocytic and motile, are active in the initial stages of an infection. They have the ability to leave the blood, enter an infected tissue, and destroy microbes and foreign particles.

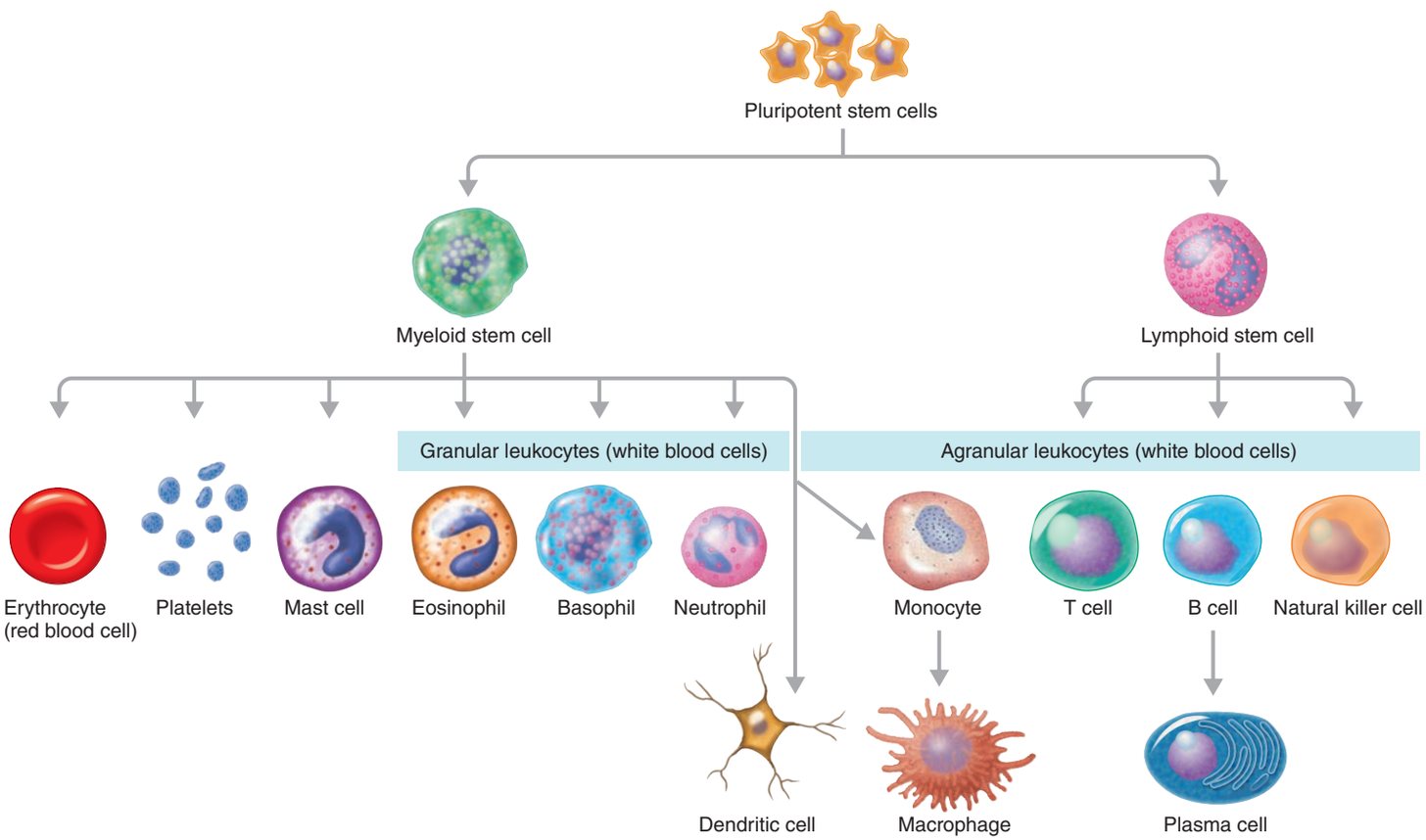
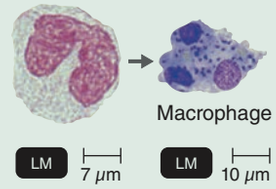
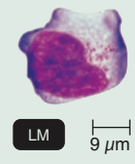


Figure 16.4 Hematopoiesis. The process begins in red bone marrow with a pluripotent stem cell.

Q What are the parent cells for granular leukocytes? For agranular leukocytes?

TABLE 16.1 Leukocytes (White Blood Cells)

Granulocytes		Agranulocytes	
Neutrophils (PMNs)(60–70% of leukocytes) Function: Phagocytosis		**Monocytes (3–8% total)** Function: Phagocytosis (when they mature into macrophages)	
Basophils (0.5–1%) Function: Production of histamine		**Dendritic cells** Functions: Phagocytosis and initiation of adaptive immune responses	
Eosinophils (2–4%) Functions: Production of toxic proteins against certain parasites; some phagocytosis		**Lymphocytes (20–25%)** • **Natural killer (NK) cells** Function: Destroy target cells by cytolysis and apoptosis	
		• **T cells** Function: Cell-mediated immunity	
		• **B cells** Function: Produce antibodies	

Basophils stain blue-purple with the basic dye methylene blue. Basophils release substances, such as histamine, that are important in inflammation and allergic responses.

Eosinophils stain red or orange with the acidic dye eosin. Eosinophils are somewhat phagocytic and also have the ability to leave the blood. Their major function is to produce toxic proteins against certain parasites, such as helminths. Although eosinophils are physically too small to ingest and destroy helminths, they can attach to the outer surface of the parasites and discharge peroxide ions that destroy them (see Figure 17.16, page 485). Their number increases significantly during certain parasitic worm infections and hypersensitivity (allergy) reactions.

Agranulocytes also have granules in their cytoplasm, but the granules are not visible under the light microscope after staining. There are three different types of agranulocytes: monocytes, dendritic cells, and lymphocytes. **Monocytes** are not actively phagocytic until they leave circulating blood, enter body tissues, and mature into **macrophages.** In fact, the proliferation of lymphocytes is one factor responsible for the swelling of lymph nodes during an infection. As blood and lymph that contain microorganisms pass through organs with macrophages, the microorganisms are removed by phagocytosis. Macrophages also dispose of worn out blood cells.

Dendritic cells are also believed to be derived from the same precursor cells as monocytes. They have long extensions that resemble the dendrites of nerve cells, thus their name. Dendritic cells are especially abundant in the epidermis of the skin, mucous membranes, the thymus, and lymph nodes. Dendritic cells destroy microbes by phagocytosis and initiate adaptive immunity responses (see Chapter 17, page 480).

Lymphocytes include natural killer cells, T cells, and B cells. **Natural killer (NK) cells** are found in blood and in the spleen, lymph nodes, and red bone marrow. NK cells have the ability to kill a wide variety of infected body cells and certain tumor cells.

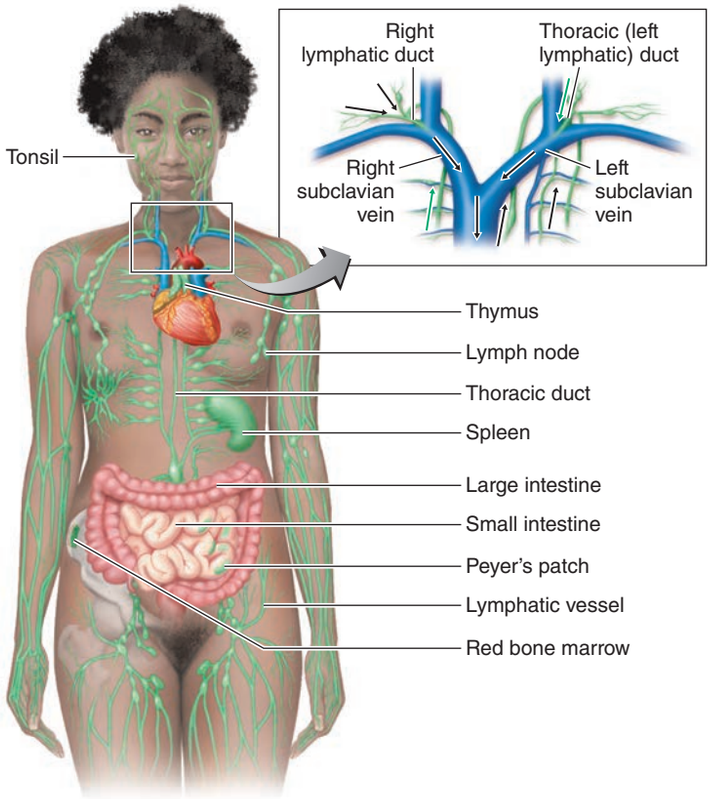


Figure 16.5 The lymphatic system. The arrows indicate the direction of lymph flow.

Q Why do lymph nodes swell during an infection?

NK cells attack any body cells that display abnormal or unusual plasma membrane proteins. The binding of NK cells to a target cell, such as an infected human cell, causes the release of vesicles containing toxic substances from NK cells. Some granules contain a protein called **perforin,** which inserts into the plasma membrane of the target cell and creates channels (perforations) in the membrane. As a result, extracellular fluid flows into the target cell and the cell bursts, a process called **cytolysis** (sī-TOL-i-sis; *cyto-* = cell; *-lysis* = loosening). Other granules of NK cells release **granzymes,** which are protein-digesting enzymes that induce the target cell to undergo apoptosis, or self-destruction. This type of attack kills infected cells but not the microbes inside the cells; the released microbes, which may or may not be intact, can be destroyed by phagocytes.

T cells and **B cells** are not usually phagocytic but play a key role in adaptive immunity (see Chapter 17). They occur in lymphoid tissues of the lymphatic system and also circulate in blood.

During many kinds of infections, especially bacterial infections, the total number of white blood cells increases as a protective response to combat the microbes; this increase is called *leukocytosis.* During the active stage of infection, the leukocyte count might double, triple, or quadruple, depending on the severity of the infection. Diseases that might cause such an elevation in the leukocyte count include meningitis, infectious mononucleosis, appendicitis, pneumococcal pneumonia, and gonorrhea. Other diseases, such as salmonellosis and brucellosis, and some viral and rickettsial infections may cause a *decrease* in the leukocyte count, called *leukopenia.* Leukopenia may be related to either impaired white blood cell production or the effect of increased sensitivity of white blood cell membranes to damage by complement, antimicrobial serum proteins discussed later in the chapter. Leukocyte increase or decrease can be detected by a **differential white blood cell count,** which is a calculation of the percentage of each kind of white cell in a sample of 100 white blood cells. The percentages in a normal differential white blood cell count are shown in parentheses in Table 16.1.

The Lymphatic System

LEARNING OBJECTIVE

16-8 Differentiate the lymphatic and blood circulatory systems.

The **lymphatic system** consists of a fluid called *lymph,* vessels called *lymphatic vessels,* a number of structures and organs containing *lymphoid tissue,* and *red bone marrow,* where stem cells develop into blood cells, including lymphocytes (Figure 16.5). Lymphoid tissue contains large numbers of lymphocytes, including T cells, B cells, and phagocytic cells that participate in immune responses. Lymph nodes are the sites of activation of T cells and B cells, which destroy microbes by immune responses (Chapter 17). Also within lymph nodes are reticular fibers, which trap microbes, and macrophages and dendritic cells, which destroy microbes by phagocytosis.

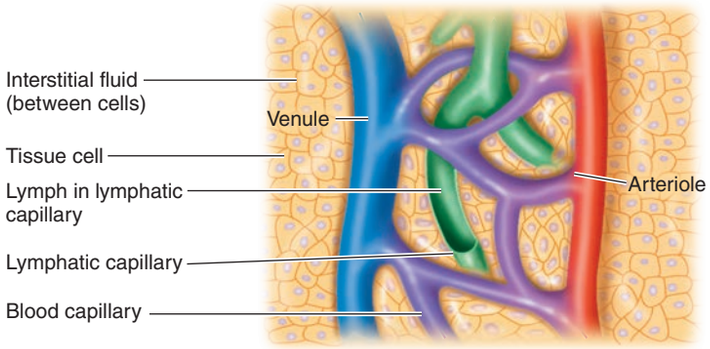
Lymphatic vessels begin as microscopic *lymphatic capillaries* located in spaces between cells (Figure 16.6). The lymphatic capillaries permit interstitial fluid derived from blood plasma to flow into them, but not out. Within the lymphatic capillaries, the fluid is called lymph. Lymphatic capillaries converge to form

Clinical Case

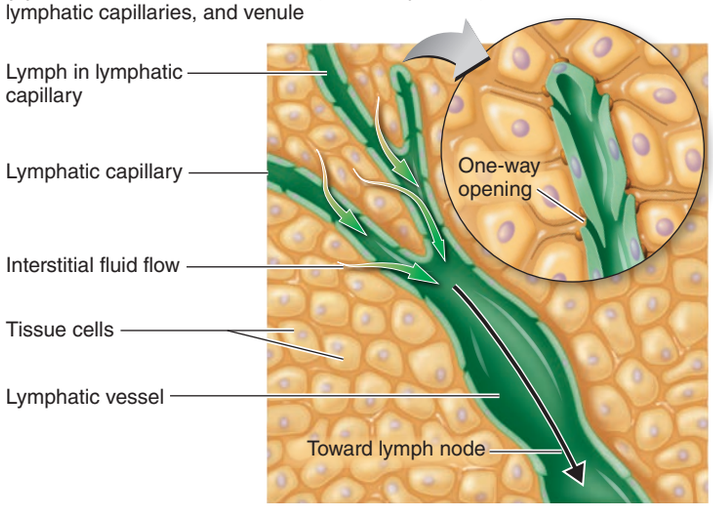
Jacob's pediatrician also sends Jacob's blood to the lab for a complete blood count (CBC). The results are shown below:

Red blood cells	4 million/μl
Neutrophils	9700/μl
Basophils	200/μl
Eosinophils	600/μl
Monocytes	1140/μl

Which cells should be protecting Jacob from infection? Based on the results of the CBC, how does Jacob's pediatrician know that something is wrong?



(a) Flow of fluid between arteriole, blood capillaries, lymphatic capillaries, and venule



(b) Lymphatic capillaries and lymphatic vein

Figure 16.6 Lymphatic capillaries. Fluid circulating between tissue cells (interstitial fluid) is picked up by lymphatic capillaries.

Q Where does lymph fluid go?

larger lymphatic vessels. These vessels, like veins, have one-way valves to keep lymph flowing in one direction only. At intervals along the lymphatic vessels, lymph flows through bean-shaped *lymph nodes* (Figure 16.5). All lymph eventually passes into the *thoracic (left lymphatic) duct* and *right lymphatic duct* and then into their respective subclavian veins, where the fluid is now called blood plasma. The blood plasma moves through the cardiovascular system and ultimately becomes interstitial fluid between tissue cells, and another cycle begins.

Lymphoid tissues and organs are scattered throughout the mucous membranes that line the gastrointestinal, respiratory, urinary, and reproductive tracts. They protect against microbes that are ingested or inhaled. Multiple large aggregations of lymphoid tissues are located in specific parts of the body. These include the *tonsils* in the throat and *Peyer's patches* in the small intestine. See Figure 17.9, page 479.

The *spleen* contains lymphocytes and macrophages that monitor the blood for microbes and secreted products such as toxins, much like lymph nodes monitor lymph. The *thymus* serves as a site for T cell maturation. It also contains dendritic cells and macrophages. MM **Animation** Host Defenses: Overview

CHECK YOUR UNDERSTANDING

- Compare the structures and function of monocytes and neutrophils. **16-6**
- Define *differential white blood cell count*. **16-7**
- What is the function of lymph nodes? **16-8**

Phagocytes

LEARNING OBJECTIVES

16-9 Define *phagocyte* and *phagocytosis.*

16-10 Describe the process of phagocytosis, and include the stages of adherence and ingestion.

16-11 Identify six mechanisms of avoiding destruction by phagocytosis.

Phagocytosis (from Greek words meaning eat and cell) is the ingestion of a microorganism or other substance by a cell. We have previously mentioned phagocytosis as the method of nutrition of certain protozoa. Phagocytosis is also involved in clearing away debris such as dead body cells and denatured proteins. In this chapter, phagocytosis is discussed as a means by which cells in the human body counter infection as part of the second line of defense.

Actions of Phagocytic Cells

Cells that perform phagocytosis are collectively called **phagocytes.** All are types of white blood cells or white blood cell derivatives. When an infection occurs, both granulocytes (especially neutrophils, but also eosinophils and dendritic cells) and monocytes migrate to the infected area. During this migration, monocytes enlarge and develop into actively phagocytic macrophages. These cells leave the blood and migrate into tissues where they enlarge and develop into macrophages. Some macrophages, called **fixed macrophages,** or *histiocytes* are resident in certain tissues and organs of the body. Fixed macrophages are found in the liver (Kupffer's cells), lungs (alveolar macrophages), nervous system (microglial cells), bronchial tubes, spleen (splenic macrophages), lymph nodes, red bone marrow, and the peritoneal cavity surrounding abdominal organs (peritoneal macrophages). Other macrophages are motile and are called **free (wandering) macrophages,** which roam the tissues and gather at sites of infection or inflammation. The various macrophages of the body constitute the **mononuclear phagocytic (reticuloendothelial) system.**

During the course of an infection, a shift occurs in the type of white blood cell that predominates in the bloodstream. Granulocytes, especially neutrophils, dominate during the initial phase of bacterial infection, at which time they are actively phagocytic; this dominance is indicated by their increased number in a differential white blood cell count. However, as the infection progresses, the macrophages dominate; they scavenge

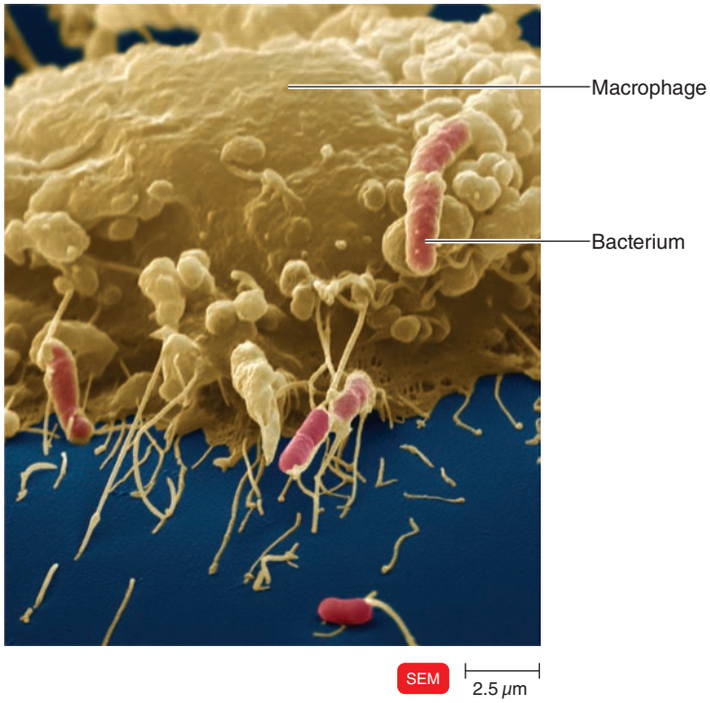


Figure 16.7 A macrophage engulfing rod-shaped bacteria. Macrophages in the mononuclear phagocytic system remove microorganisms after the initial phase of infection.

Q What are monocytes?

and phagocytize remaining living bacteria and dead or dying bacteria (Figure 16.7). The increased number of monocytes (which develop into macrophages) is also reflected in a differential white blood cell count.

The Mechanism of Phagocytosis

How does phagocytosis occur? For the convenience of study, we will divide phagocytosis into four main phases: chemotaxis, adherence, ingestion, and digestion (Figure 16.8).

Chemotaxis and Adherence

1 **Chemotaxis** is the chemical attraction of phagocytes to microorganisms. (The mechanism of chemotaxis is discussed in Chapter 4, page 78.) Among the chemotactic chemicals that attract phagocytes are microbial products, components of white blood cells and damaged tissue cells, cytokines released by other white blood cells, and, finally, peptides derived from complement—a system of host defense proteins discussed later in the chapter.

As it pertains to phagocytosis, **adherence** is the attachment of the phagocyte's plasma membrane to the surface of the microorganism or other foreign material. Adherence is facilitated by the attachment of pathogen-associated molecular patterns (PAMPs) of microbes to receptors, such as Toll-like receptors (TLRs), on the surface of phagocytes. The binding of PAMPs to TLRs not only initiates phagocytosis, but also induces the phagocyte to release specific cytokines that recruit additional phagocytes.

In some instances, adherence occurs easily, and the microorganism is readily phagocytized. Microorganisms can be more readily phagocytized if they are first coated with certain serum proteins that promote attachment of the microorganisms to the phagocyte. This coating process is called **opsonization.** The proteins that act as *opsonins* include some components of the complement system and antibody molecules (described later in this chapter and in Chapter 17).

Ingestion

2 Following adherence, **ingestion** occurs. The plasma membrane of the phagocyte extends projections called **pseudopods** that engulf the microorganism. (See also Figure 16.7.)

3 Once the microorganism is surrounded, the pseudopods meet and fuse, surrounding the microorganism with a sac called a **phagosome,** or *phagocytic vesicle.* The membrane of a phagosome has enzymes that pump protons (H^+) into the phagosome, reducing the pH to about 4. At this pH, hydrolytic enzymes activate.

Digestion

Next, the phagosome pinches off from the plasma membrane and enters the cytoplasm, where it contacts lysosomes that contain digestive enzymes and bactericidal substances (see Chapter 4, page 100).

4 On contact, the phagosome and lysosome membranes fuse to form a single, larger structure called a **phagolysosome.**

5 The contents of the phagolysosome brought in by ingestion are digested in the phagolysosome.

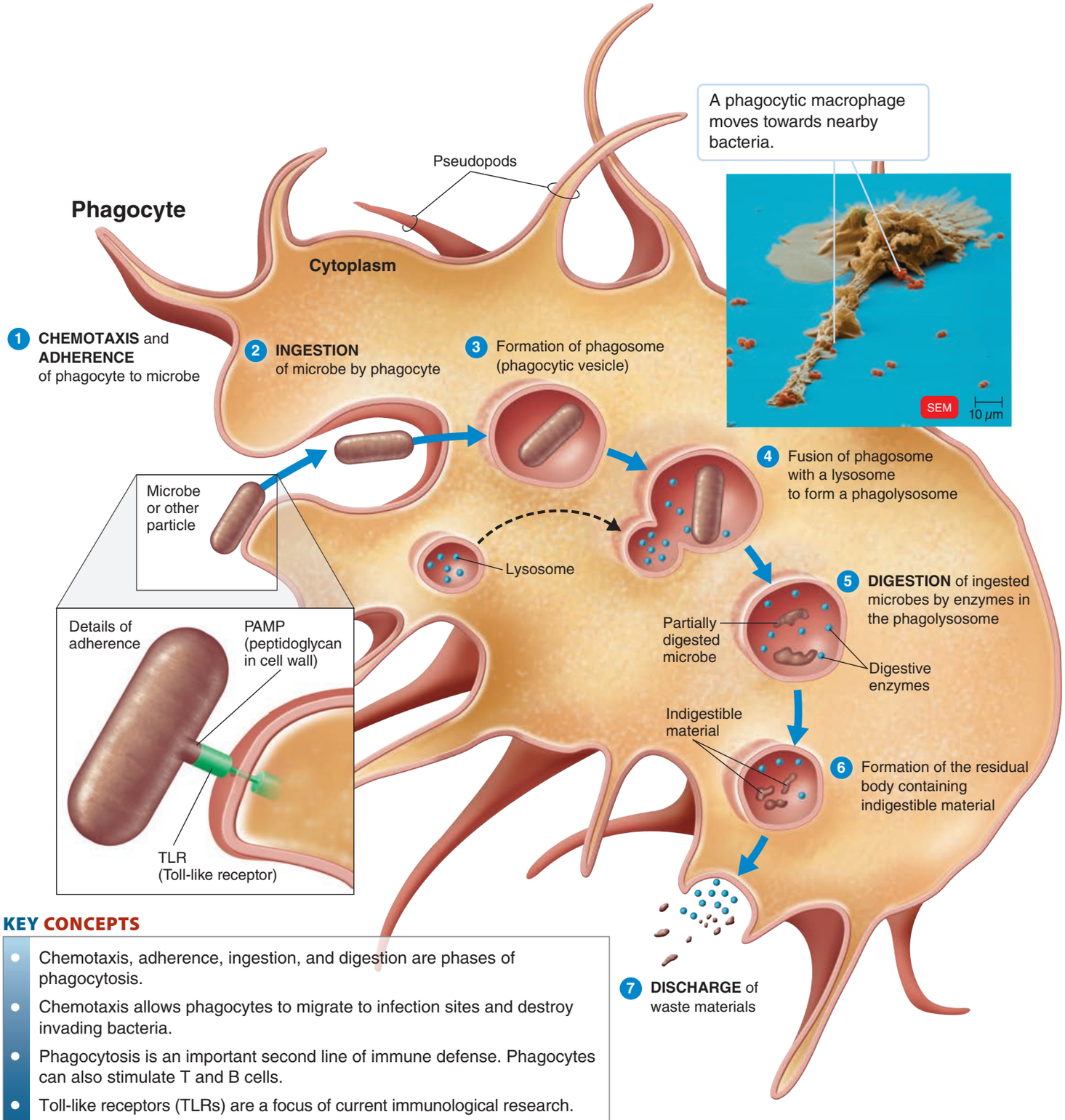
Lysosomal enzymes that attack microbial cells directly include lysozyme, which hydrolyzes peptidoglycan in bacterial cell walls. Lipases, proteases, ribonuclease, and deoxyribonuclease hydrolyze other macromolecular components of microorganisms. Lysosomes also contain enzymes that can produce toxic oxygen products such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), nitric oxide (NO), singlet oxygen ($^1O_2^-$), and hydroxyl radical (OH·) (see Chapter 6, pages 155–156). Toxic oxygen products are produced by an *oxidative burst.* Other enzymes can make use of these toxic oxygen products in killing ingested microorganisms. For example, the enzyme myeloperoxidase converts chloride (Cl^-) ions and hydrogen peroxide into highly toxic hypochlorous acid (HOCl). The acid contains hypochlorous ions, which are found in household bleach and account for its antimicrobial activity (see Chapter 7, page 000).

6 After enzymes have digested the contents of the phagolysosome brought into the cell by ingestion, the phagolysosome contains indigestible material and is called a *residual body.*

7 This residual body then moves toward the cell boundary and discharges its wastes outside the cell.

FOUNDATION FIGURE 16.8

The Phases of Phagocytosis



KEY CONCEPTS

- Chemotaxis, adherence, ingestion, and digestion are phases of phagocytosis.
- Chemotaxis allows phagocytes to migrate to infection sites and destroy invading bacteria.
- Phagocytosis is an important second line of immune defense. Phagocytes can also stimulate T and B cells.
- Toll-like receptors (TLRs) are a focus of current immunological research.

Microbial Evasion of Phagocytosis

The ability of a pathogen to cause disease is related to its ability to evade phagocytosis. Some bacteria have structures that inhibit adherence, such as M protein and capsules. The M protein of *Streptococcus pyogenes* inhibits the attachment of phagocytes and makes adherence more difficult (see Chapter 15, page 421). Organisms with large capsules include *Streptococcus pneumoniae* and *Haemophilus influenzae* type b. Heavily encapsulated microorganisms like these can be phagocytized only if the phagocyte traps the microorganism against a rough surface, such as a blood vessel, blood clot, or connective tissue fiber, from which the microbe cannot slide away.

Other microbes may be ingested but not killed. For example, *Staphylococcus aureus* produces leukocidins that may kill phagocytes by causing the release of the phagocyte's own lysosomal enzymes into its cytoplasm. Streptolysin released by streptococci has a similar mechanism.

A number of intracellular pathogens secrete pore-forming toxins that lyse phagocyte cell membranes once inside the phagocyte. For example, *Trypanosoma cruzi* (the causative agent of Chagas' disease) and *Listeria monocytogenes* (the causative agent of listeriosis), produce membrane attack complexes that lyse phagolysosome membranes and release the microbes into the cytoplasm of the phagocyte, where they propagate. Later the microbes secrete more membrane attack complexes that lyse the plasma membrane (see page 458), resulting in release of microbes from the phagocyte and infection of neighboring cells.

Still other microbes have the ability to survive inside phagocytes. *Coxiella burnetii,* the causative agent of Q fever, actually requires the low pH inside a phagolysosome to replicate. *L. monocytogenes, Shigella* (the causative agent of shigellosis), and *Rickettsia* species (the causative agent of Rocky Mountain spotted fever and typhus) have the ability to escape from a phagosome before it fuses with a lysosome. *Mycobacterium tuberculosis* (the causative agent of tuberculosis), HIV (the causative agent of AIDS), *Chlamydia* (the causative agent of trachoma, nongonococcal urethritis, and lymphogranuloma venereum), *Leishmania* (the causative agent of leishmaniasis), and *Plasmodium* (malarial parasites) can prevent both the fusion of a phagosome with a lysosome and the proper acidification of digestive enzymes. The microbes then multiply within the phagocyte, almost completely filling it. In most cases, the phagocyte dies and the microbes are released by autolysis to infect other cells. Still other microbes, such as the causative agents of tularemia and brucellosis, can remain dormant within phagocytes for months or years at a time.

Biofilms also play a role in evading phagocytes. Bacteria that are part of biofilms are much more resistant to phagocytosis because the phagocytes cannot detach bacteria from the biofilm prior to phagocytosis. Moreover, the neutrophil response against *Pseudomonas aeruginosa* in a biofilm is slower than against free-floating bacteria. In addition, although some bacteria in a biofilm, such as *P. aeruginosa,* can activate the oxidative burst response, it is weaker than in free-floating bacteria. **Animations** Virulence Factors: Hiding from Host Defenses, Inactivating Host Defenses; Phagocytosis: Overview, Mechanism, Microbes That Evade It

CHECK YOUR UNDERSTANDING

- What do fixed and wandering macrophages do? **16-9**
- What is the role of TLRs in phagocytosis? **16-10**
- How does each of these bacteria avoid destruction by phagocytes? *Streptococcus pneumoniae, Staphylococcus aureus, Listeria monocytogenes, Mycobacterium tuberculosis, Rickettsia* **16-11**

* * *

In addition to providing innate resistance for the host, phagocytosis plays a role in adaptive immunity. Macrophages help T and B cells perform vital adaptive immune functions—this will be discussed in Chapter 17.

In the next section, we will see how phagocytosis often occurs as part of another innate mechanism of resistance: inflammation.

Clinical Case

Leukocytes, including neutrophils and macrophages, are the cells listed in the test results that fight infections. According to the lab results, Jacob's white blood cell count is a little high. Leukocytosis, a white blood cell count above normal range, occurs during a fungal infection, but Jacob's pediatrician is concerned that the leukocytes are not doing their job. To check this, he then requests a nitroblue tetrazolium (NBT) test, which is performed on a blood smear on a microscope slide.

Normal neutrophils will reduce the yellow dye, NBT, to an insoluble blue precipitate. Jacob's neutrophils do not produce this result; his neutrophils are not working as they should. Normally, the adherence of a target cell, such as a bacterium, to the neutrophil's plasma membrane stimulates the neutrophil to produce NADPH. This is followed by a lethal oxidative burst of hydrogen peroxide.

What metabolic pathway produces NADPH for a cell?

442 448 452 456 461 464

Inflammation

LEARNING OBJECTIVES

16-12 List the stages of inflammation.

16-13 Describe the roles of vasodilation, kinins, prostaglandins, and leukotrienes in inflammation.

16-14 Describe phagocyte migration.

Damage to the body's tissues triggers a local defensive response called **inflammation,** another component of the second line of defense. The damage can be caused by microbial infection, physical agents (such as heat, radiant energy, electricity, or sharp

objects), or chemical agents (acids, bases, and gases). Inflammation is usually characterized by four signs and symptoms: *redness, pain, heat,* and *swelling.* Sometimes a fifth, *loss of function,* is present; its occurrence depends on the site and extent of damage.

Inflammation has the following functions: (1) to destroy the injurious agent, if possible, and to remove it and its by-products from the body; (2) if destruction is not possible, to limit the effects on the body by confining or walling off the injurious agent and its by-products; and (3) to repair or replace tissue damaged by the injurious agent or its by-products.

If the cause of an inflammation is removed in a relatively short period of time, the inflammatory response is intense and is referred to as an *acute inflammation.* An example is the response to a boil caused by *S. aureus.* If, instead, the cause of an inflammation is difficult or impossible to remove, the inflammatory response is longer lasting but less intense (although overall more destructive). This type of inflammation is referred to as a *chronic inflammation.* An example is the response to a chronic infection such as tuberculosis, caused by *M. tuberculosis.*

During the early stages of inflammation, microbial structures, such as flagellin, lipopolysaccharides (LPS), and bacterial DNA stimulate the Toll-like receptors of macrophages to produce cytokines, such as *tumor necrosis factor alpha* (*TNF-α*). In response to TNF-α in the blood, the liver synthesizes a group of proteins called **acute-phase proteins;** other acute-phase proteins are present in the blood in an inactive form and are converted to an active form during inflammation. Acute-phase proteins induce both local and systemic responses and include proteins such as C-reactive protein, mannose-binding lectin (page 457), and several specialized proteins such as fibrinogen for blood clotting and kinins for vasodilation.

All of the cells involved in inflammation have receptors for TNF-α and are activated by it to produce more of their own TNF-α. This amplifies the inflammatory response. Unfortunately, excessive production of TNF-α may lead to disorders such as rheumatoid arthritis and Crohn's disease. Monoclonal antibodies are used therapeutically to treat such inflammatory disorders (see Chapter 18, page 501).

For purposes of our discussion, we will divide the process of inflammation into three stages: vasodilation and increased permeability of blood vessels, phagocyte migration and phagocytosis, and tissue repair.

Vasodilation and Increased Permeability of Blood Vessels

Immediately following tissue damage (**Figure 16.9a**), blood vessels dilate (increase in diameter) in the area of damage, and their permeability increases (Figure 16.9b). Dilation of blood vessels, called **vasodilation,** is responsible for the redness (erythema) and heat associated with inflammation.

Increased permeability permits defensive substances normally retained in the blood to pass through the walls of the blood vessels and enter the injured area. The increase in permeability, which permits fluid to move from the blood into tissue spaces, is responsible for the **edema** (accumulation of fluid) of inflammation. The pain of inflammation can be caused by nerve damage, irritation by toxins, or the pressure of edema.

1. Vasodilation and the increase in permeability of blood vessels are caused by a number of chemicals released by damaged cells in response to injury. One such substance is **histamine,** a chemical present in many cells of the body, especially in mast cells in connective tissue, circulating basophils, and blood platelets. Histamine is released in direct response to the injury of cells that contain it; it is also released in response to stimulation by certain components of the complement system (discussed later). Phagocytic granulocytes attracted to the site of injury can also produce chemicals that cause the release of histamine.

Kinins are another group of substances that cause vasodilation and increased permeability of blood vessels. Kinins are present in blood plasma, and once activated, they play a role in chemotaxis by attracting phagocytic granulocytes, chiefly neutrophils, to the injured area.

Prostaglandins, substances released by damaged cells, intensify the effects of histamine and kinins and help phagocytes move through capillary walls. **Leukotrienes** are substances produced by mast cells (cells especially numerous in the connective tissue of the skin and respiratory system, and in blood vessels) and basophils. Leukotrienes cause increased permeability of blood vessels and help attach phagocytes to pathogens. Various components of the complement system stimulate the release of histamine, attract phagocytes, and promote phagocytosis.

Activated fixed macrophages also secrete **cytokines,** which bring about vasodilation and increased permeability. Vasodilation and the increased permeability of blood vessels also help deliver clotting elements of blood into the injured area.

2. The blood clots that form around the site of activity prevent the microbe (or its toxins) from spreading to other parts of the body.
3. As a result, there may be a localized collection of **pus,** a mixture of dead cells and body fluids, in a cavity formed by the breakdown of body tissues. This focus of infection is called an **abscess.** Common abscesses include pustules and boils.

Despite their positive role in the inflammatory process, prostaglandins are also associated with the pain related to inflammation. Drugs such as ibuprofen and aspirin are frequently used to alleviate pain because they inhibit prostaglandin production. Unfortunately, these drugs also interfere with the ability of the stomach to protect itself from acidic gastric juices. Thus, long-term use of these drugs can cause stomach upset, heartburn, and ulcers.

The next stage in inflammation involves the migration of phagocytes to the injured area.

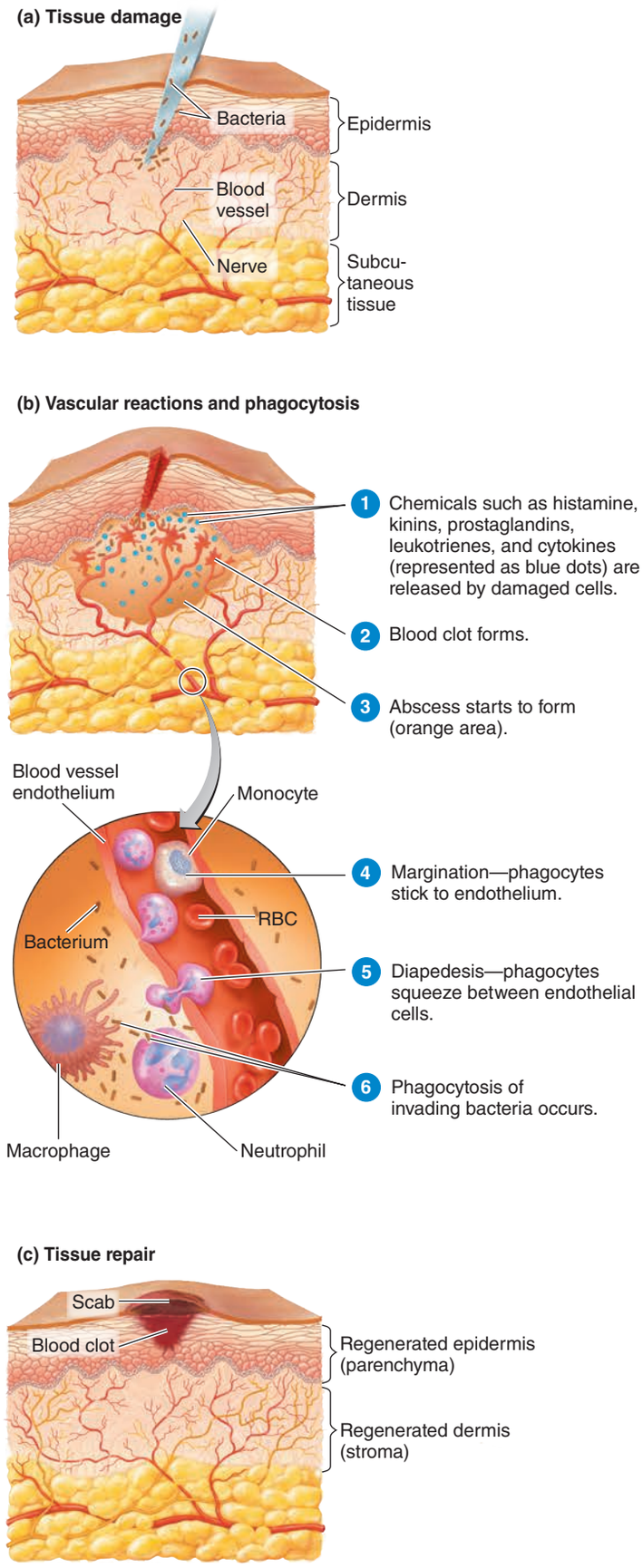


Figure 16.9 The process of inflammation. **(a)** Damage to otherwise healthy tissue—in this case, skin. **(b)** Vasodilation and increased permeability of blood vessels allows phagocyte migration. Phagocytosis by macrophages and neutrophils removes bacteria and cellular debris. Macrophages develop from monocytes. **(c)** The repair of damaged tissue.

Q What are the signs and symptoms of inflammation?

Phagocyte Migration and Phagocytosis

Generally, within an hour after the process of inflammation is initiated, phagocytes appear on the scene. 4 As the flow of blood gradually decreases, phagocytes (both neutrophils and monocytes) begin to stick to the inner surface of the endothelium (lining) of blood vessels. This sticking process in response to local cytokines is called **margination.** The cytokines alter cellular adhesion molecules on cells lining blood vessels, causing the phagocytes to stick at the site of inflammation. (Margination is also involved in red bone marrow, where cytokines can release phagocytes into circulation when they are needed.) 5 Then the collected phagocytes begin to squeeze between the endothelial cells of the blood vessel to reach the damaged area. This migration, which resembles ameboid movement, is called **diapedesis;** it can take as little as 2 minutes. 6 The phagocytes then begin to destroy invading microorganisms by phagocytosis.

As mentioned earlier, certain chemicals attract neutrophils to the site of injury (chemotaxis). These include chemicals produced by microorganisms and even other neutrophils; other chemicals are kinins, leukotrienes, chemokines, and components of the complement system. Chemokines are cytokines that are chemotactic for phagocytes and T cells and thus stimulate both the inflammatory response and an adaptive immune response. The availability of a steady stream of neutrophils is ensured by the production and release of additional granulocytes from red bone marrow.

As the inflammatory response continues, monocytes follow the granulocytes into the infected area. Once the monocytes are contained in the tissue, they undergo changes in biological properties and become free macrophages. The granulocytes predominate in the early stages of infection but tend to die off rapidly. Macrophages enter the picture during a later stage of the infection, once granulocytes have accomplished their function. They are several times more phagocytic than granulocytes and are large enough to phagocytize tissue that has been destroyed, granulocytes that have been destroyed, and invading microorganisms.

After granulocytes or macrophages engulf large numbers of microorganisms and damaged tissue, they themselves eventually die. As a result, pus forms, and its formation usually continues until the infection subsides. At times, the pus pushes to the surface of the body or into an internal cavity for dispersal. On other occasions the pus remains even after the infection is terminated. In this case, the pus is gradually destroyed over a period of days and is absorbed by the body.

As effective as phagocytosis is in contributing to innate resistance, there are times when the mechanism becomes less functional in response to certain conditions. For example, with age, there is a progressive decline in the efficiency of phagocytosis. Recipients of heart or kidney transplants have impaired innate defenses as a result of receiving drugs that prevent the rejection of the transplant. Radiation treatments can also depress innate immune responses by damaging red bone marrow. Even certain diseases such as AIDS and cancer can cause defective functioning of innate defenses. Finally, some individuals are born with an inability to produce phagocytes.

Tissue Repair

The final stage of inflammation is tissue repair, the process by which tissues replace dead or damaged cells (Figure 16.9c). Repair begins during the active phase of inflammation, but it cannot be completed until all harmful substances have been removed or neutralized at the site of injury. The ability to regenerate, or repair, depends on the type of tissue. For example, skin has a high capacity for regeneration, whereas cardiac muscle tissue has a low capacity to regenerate.

A tissue is repaired when its stroma or parenchyma produces new cells. The *stroma* is the supporting connective tissue, and the *parenchyma* is the functioning part of the tissue. For example, the capsule around the liver that encloses and protects it is part of the stroma because it is not involved in the functions of the liver; liver cells (hepatocytes) that perform the functions of the liver are part of the parenchyma. If only parenchymal cells are active in repair, a perfect or near-perfect reconstruction of the tissue occurs. A familiar example of perfect reconstruction is a minor skin cut, in which parenchymal cells are more active in repair. However, if repair cells of the stroma of the skin are more active, scar tissue is formed.

As noted earlier, some microbes have various mechanisms that enable them to evade phagocytosis. Such microbes often induce a chronic inflammatory response, which can result in significant damage to body tissues. The most significant feature of chronic inflammation is the accumulation and activation of macrophages in the infected area. Cytokines released by activated macrophages induce fibroblasts in the tissue stroma to synthesize collagen fibers. These fibers aggregate to form scar tissue, a process called *fibrosis.* Because scar tissue is not specialized to perform the functions of the previously healthy tissue, fibrosis can interfere with the normal function of the tissue.

Animation Inflammation: Overview, Steps

CHECK YOUR UNDERSTANDING

- What purposes does inflammation serve? **16-12**
- What causes the redness, swelling, and pain associated with inflammation? **16-13**
- What is margination? **16-14**

Fever

LEARNING OBJECTIVE

16-15 Describe the cause and effects of fever.

While inflammation is a local response of the body to injury, there are also systemic, or overall, responses. One of the most important is **fever,** an abnormally high body temperature, a third component of the second line of defense. The most frequent cause of fever is infection from bacteria (and their toxins) or viruses.

The brain's hypothalamus is sometimes called the body's thermostat, and it is normally set at 37°C (98.6°F). It is believed that certain substances affect the hypothalamus by setting it at a higher temperature. Recall from Chapter 15 that when phagocytes ingest gram-negative bacteria, the lipopolysaccharides (LPS) of the cell wall are released. Since LPS is an endotoxin, it causes the phagocytes to release the cytokines interleukin-1 along with TNF-α. These cytokines cause the hypothalamus to release prostaglandins that reset the hypothalamic thermostat at a higher temperature, thereby causing fever (see Figure 15.6, page 428).

Assume that the body is invaded by pathogens and that the thermostat setting is increased to 39°C (102.2°F). To adjust to the new thermostat setting, the body responds by constricting blood vessels, increasing the rate of metabolism, and **shivering,** all of which raise body temperature. Even though body temperature is climbing higher than normal, the skin remains cold. This condition, called a *chill,* is a definite sign that body temperature is rising. When body temperature reaches the setting of the thermostat, the chill disappears. The body will continue to maintain its temperature at 39°C until the cytokines are eliminated. The thermostat is then reset to 37°C. As the infection subsides, heat-losing mechanisms such as vasodilation and sweating go into operation. The skin becomes warm, and the person begins to sweat. This phase of the fever, called the **crisis,** indicates that body temperature is falling.

Up to a certain point, fever is considered a defense against disease. Interleukin-1 helps step up the production of T cells. High body temperature intensifies the effect of antiviral interferons (page 460) and increases production of transferrins that decrease the iron available to microbes (page 461). Also, because the high temperature speeds up the body's reactions, it may help body tissues repair themselves more quickly.

Among the complications of fever are tachycardia (rapid heart rate), which may compromise older persons with cardiopulmonary disease; increased metabolic rate, which may produce acidosis; dehydration; electrolyte imbalances; seizures in young children; and delirium and coma. As a rule, death results if body temperature rises above 44° to 46°C (112° to 114°F).

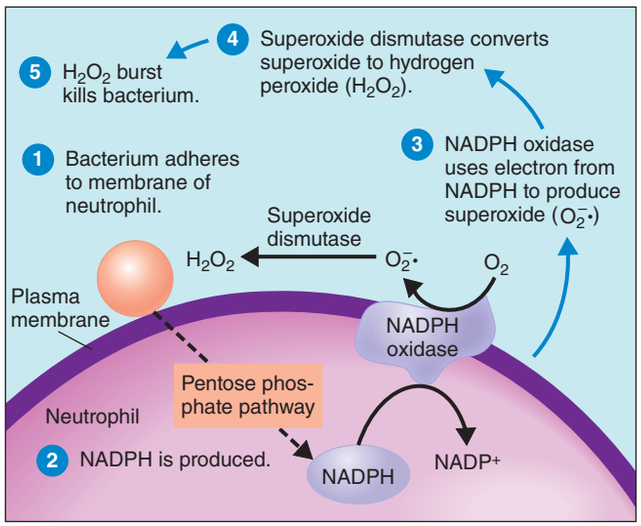
As with the pain associated with inflammation, ibuprofen and aspirin can also be used to reduce fever.

CHECK YOUR UNDERSTANDING

- Why does a chill indicate that a fever is about to occur? **16-15**

Clinical Case

The pentose pathway produces the NADPH. (See figure.) Jacob's pediatrician realizes that Jacob's neutrophils must be missing an oxidase and can't oxidize the NADPH. He diagnoses Jacob with chronic granulomatous disease (CGD), an inherited X-linked recessive disorder in which phagocytes do not function as they should. It is caused by a mutation in the gene for NADPH oxidase.



What is the function of NADPH oxidase? (Hint: See page 113.)

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Antimicrobial Substances

LEARNING OBJECTIVES

- **16-16** List the major components of the complement system.
- **16-17** Describe three pathways of activating complement.
- **16-18** Describe three consequences of complement activation.
- **16-19** Define *interferons.*
- **16-20** Compare and contrast the actions of IFN-α and IFN-β with IFN-γ.
- **16-21** Describe the role of iron-binding proteins in innate immunity.
- **16-22** Describe the role of antimicrobial peptides in innate immunity.

The body produces certain antimicrobial substances, a final component of the second line of defense, in addition to the chemical factors mentioned earlier. Among the most important of these are the proteins of the complement system, interferons, iron-binding proteins, and antimicrobial peptides.

The Complement System

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The **complement system** consists of over 30 proteins produced by the liver that circulate in blood serum and within tissues throughout the body. (See the box on page 462.) The system is so named because it "completes," or assists, cells of the immune system in destroying microbes. The complement system is not adaptable, never changing over a person's lifetime. Therefore it is considered part of the innate immune system. However, it can be recruited into action by the adaptive immune system. Together, proteins of the complement system destroy microbes by cytolysis, opsonization, and inflammation (see Figure 16.12), and they also prevent excessive damage to host tissues.

Complement proteins are inactive until split into fragments (products), which activates them. Activated fragments carry out the destructive actions. Complement proteins are usually designated by an uppercase letter C and are numbered C1 through C9, named for the order in which they were discovered. Activated fragments are indicated by lowercase letters *a* and *b*. For example, inactive complement protein C3 is split into activated fragments, C3a and C3b.

Complement proteins act in a *cascade,* where one reaction triggers another, which in turn triggers another. More product is formed with each succeeding reaction in the cascade, amplifying the effects. The cascade of complement proteins that occurs during an infection is called **complement activation.** It may occur in three pathways that end in the activation of C3.

The Classical Pathway

The **classical pathway** was the first discovered. It is initiated when antibodies bind to antigens, as shown in **Figure 16.10a**:

1. Antibodies attach to antigens (for example, proteins or large polysaccharides on the surface of a bacterium or other cell), forming antigen–antibody complexes. The antigen–antibody complexes bind to and activate C1.
2. Next, activated C1 activates C2 and C4 by splitting them. C2 splits into fragments C2a and C2b, and C4 is split into C4a and C4b.
3. C2a and C4b combine and together activate C3 by splitting it into C3a and C3b fragments. C3a participates in inflammation, and C3b functions in cytolysis and opsonization.

The Alternative Pathway

The **alternative pathway** is so named because it was discovered after the classical pathway. Unlike the classical pathway, it does not involve antibodies. The alternative pathway is activated by contact between certain complement proteins and a pathogen, as shown in Figure 16.10b:

1. C3, constantly present in the blood, combines with complement proteins called factor B, factor D, and factor P (properdin) on the microbe's surface. The complement proteins

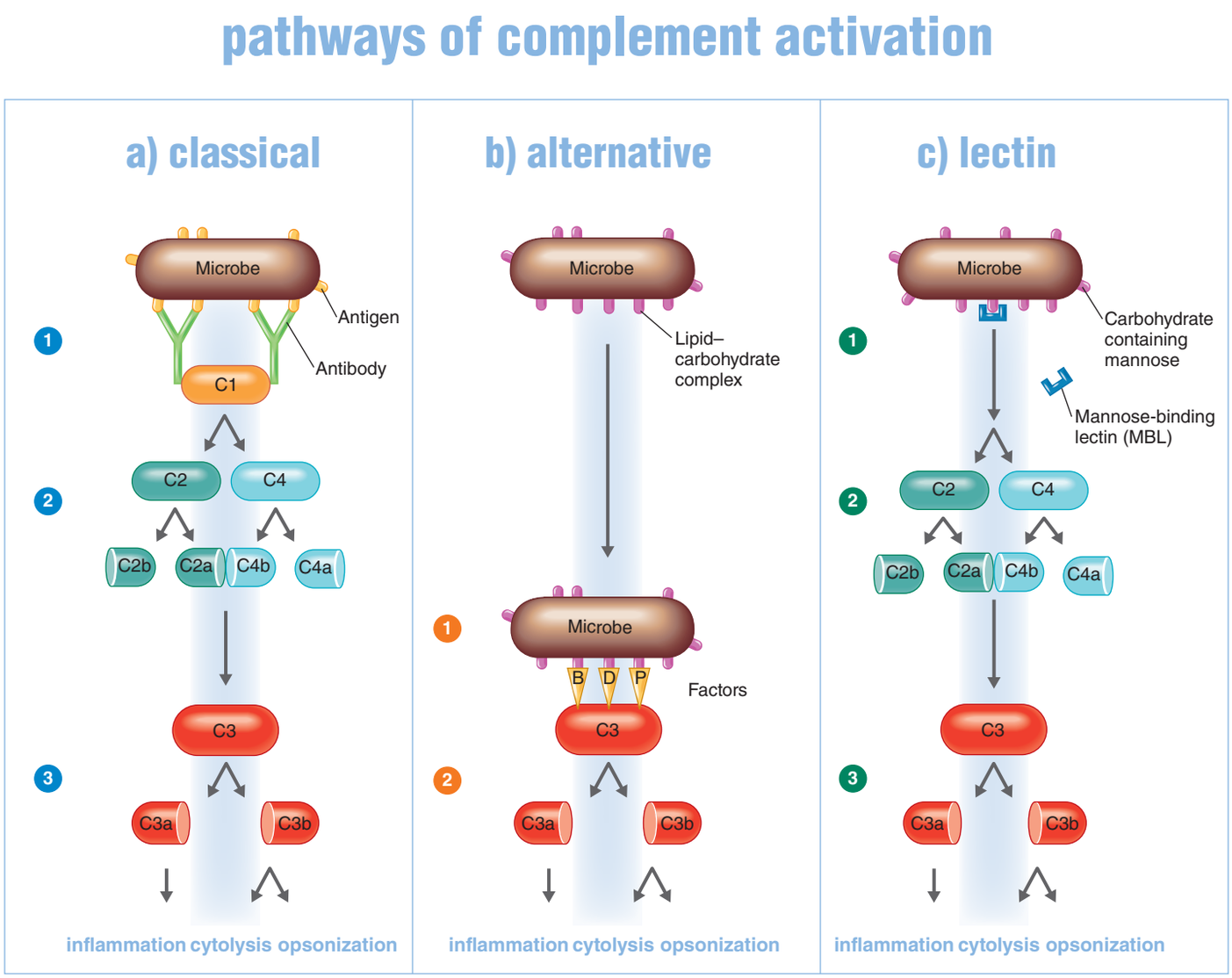


Figure 16.10 Pathways of complement activation. The classical pathway begins with an antigen–antibody reaction. The alternative pathway begins by contact between certain complement proteins and a pathogen; it doesn't involve antibodies. In the lectin pathway, lectin binds to mannose on the surface of a microbe.

Q How is the alternative pathway similar to the classical pathway, and how are the lectin and alternative pathways different from the classical pathway?

are attracted to microbial cell surface material (mostly lipid–carbohydrate complexes of certain bacteria and fungi).

2. Once the complement proteins combine and interact, C3 splits into fragments C3a and C3b. As in the classical pathway, C3a participates in inflammation, and C3b functions in cytolysis and opsonization.

The Lectin Pathway

The **lectin pathway** is the most recently discovered mechanism for complement activation. When macrophages ingest bacteria, viruses, and other foreign matter by phagocytosis, they release cytokines that stimulate the liver to produce **lectins,** proteins that bind to carbohydrates, as shown in Figure 16.10c:

1. **Mannose-binding lectin (MBL)** binds to the carbohydrate mannose. MBL binds to many pathogens because MBL molecules recognize a distinctive pattern of carbohydrates that includes mannose, which is found in bacterial cell walls and on some viruses.
2. As a result of binding, MBL functions as an opsonin to enhance phagocytosis and activates C2 and C4.
3. C2a and C4b activate C3. As with the other two mechanisms, C3 splits into fragments C3a, which participates in inflammation; and C3b, which functions in cytolysis and opsonization.

Outcomes of Complement Activation

As mentioned previously, the classical, alternative, and lectin pathways result in complement cascades that active C3. Activation of C3, in turn, can lead to cytolysis, opsonization, and inflammation.

Cytolysis *Cytolysis* of microbial cells involves the membrane attack complex (MAC), as shown in Figure 16.12a:

1. Activated C3 splits into C3a and C3b.
2. C3b splits C5 into C5a and C5b.
3. Fragments C5b, C6, C7, and C8 bind together sequentially and insert into the plasma membrane of the invading cell. C5b through C8 act as a receptor that attracts a C9 fragment. Additional C9 fragments are added to form a transmembrane channel.

Together, C5b through C8 and the multiple C9 fragments form the **membrane attack complex (MAC)** (Figure 16.11). The MAC creates a hole on a pathogen's cell membrane and makes transmembrane channels, allowing for flow of extracellular fluid into the pathogen. The fluid inflow bursts the microbial cell.

Plasma membranes of the host cell contain proteins that protect against lysis by preventing the MAC proteins from attaching to their surfaces. Also, the MAC forms the basis for the complement fixation test used to diagnose some diseases. This is explained in the Applications of Microbiology box on page 462 and in Chapter 18 (see Figure 18.10, page 507).

Gram-negative bacteria are more susceptible to cytolysis because they have only one or very few layers of peptidoglycan to protect the plasma membrane from the effects of complement. Gram-positive bacteria have many layers of peptidoglycan, which limit complement's access to the plasma membrane and thus interfere with cytolysis. Bacteria that are not killed by the MAC are said to be *MAC-resistant*.

Opsonization *Opsonization,* or immune adherence, promotes attachment of a phagocyte to a microbe. This enhances phagocytosis, as Figure 16.12b shows:

1. Activated C3 splits into activated C3a and C3b.
2. C3b binds to the surface of a microbe, and receptors on phagocytes attach to the C3b.

Inflammation This is outlined in Figure 16.12c:

1. Activated C3 splits into C3a and C3b.
2. C3a and C5a bind to mast cells and cause them to release histamine and other chemicals that increase blood vessel permeability during *inflammation*. C5a also functions as a very powerful chemotactic factor that attracts phagocytes to the site of an infection.

Figure 16.13 shows inflammation stimulated by complement in more detail than Figure 16.12c.

Figure 16.11 The MAC results in cytolysis. Micrograph of a rod-shaped bacterium before cytolysis (left) and after cytolysis (right).
Source: Reprinted from Schreiber, R.D., et al. "Bacterial Activity of the Alternative Complement Pathway Generated from 11 Isolated Plasma Proteins," *Journal of Experimental Medicine,* 149:870–882, 1979.

Q How does complement help fight infections?

Regulation of Complement

Once complement is activated, its destructive capabilities usually cease very quickly to minimize the destruction of host cells. This is accomplished by various regulatory proteins in the host's blood and on certain cells, such as blood cells. The regulatory proteins are present at higher concentrations than the complement proteins. The proteins bring about the breakdown or inhibition of activated complement. One example of a regulatory protein is *CD59,* which prevents the assembly of C9 molecules to form the MAC.

Complement and Disease

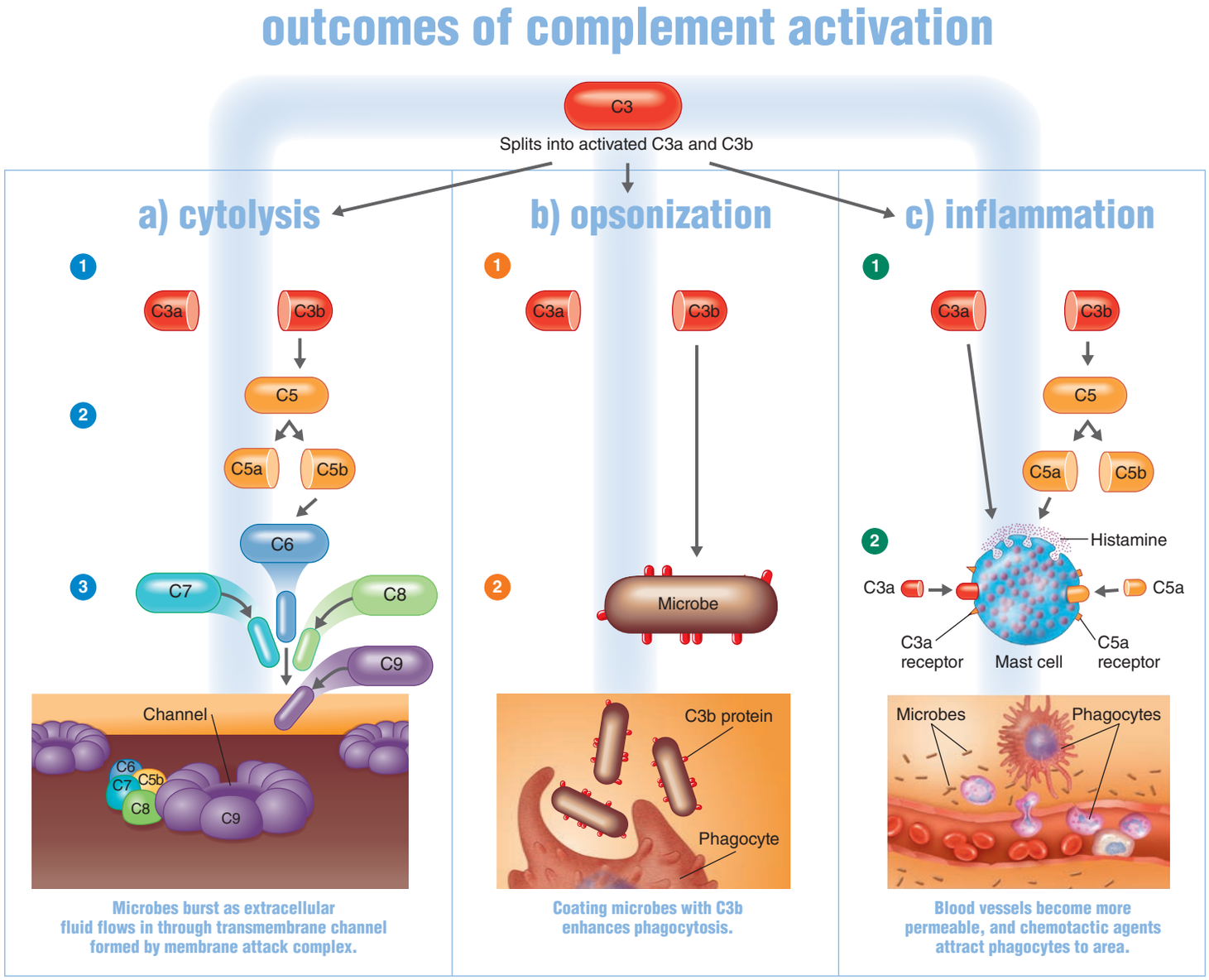
In addition to its importance in defense, the complement system assumes a role in causing disease as a result of inherited deficiencies. C1, C2, or C4 deficiencies cause collagen vascular disorders that result in hypersensitivity (anaphylaxis); deficiency of C3, though rare, results in increased susceptibility to recurrent infections with pyogenic (pus-producing) microbes; and C5 through C9 defects result in increased susceptibility to *Neisseria meningitidis* and *N. gonorrhoeae* infections. Complement may play a role in diseases with an immune component, such as systemic lupus erythematosus, asthma, various forms of arthritis, multiple sclerosis, and inflammatory bowel disease. Complement is also implicated in Alzheimer's disease and other neurodegenerative disorders.

Evading the Complement System

Some bacteria evade the complement system by means of their capsules, which prevent complement activation. For example, some capsules contain large amounts of a monosaccharide called

FOUNDATION FIGURE 16.12

Outcomes of Complement Activation



KEY CONCEPTS

- The complement system is another way the body fights infection and destroys pathogens. This component of innate immunity "complements" other immune reactions.
- Complement is a group of over 30 proteins circulating in serum that are activated in a cascade: one complement protein triggers the next.
- The cascade can be activated by a pathogen directly or by an antibody–antigen reaction.
- Together these proteins destroy microbes by (1) cytolysis, (2) enhanced phagocytosis, and (3) inflammation.

sialic acid, which discourages opsonization and MAC formation. Other capsules inhibit the formation of C3b and C4b and cover the C3b to prevent it from making contact with the receptor on phagocytes. Some gram-negative bacteria, such as *Salmonella,* can lengthen the O polysaccharide of their LPS (see page 81), which prevents MAC formation. Other gram-negative bacteria, such as *Neisseria gonorrhoeae, Bordetella pertussis,* and *influenzae type b,* attach their sialic acid to the sugars in the outer membrane,

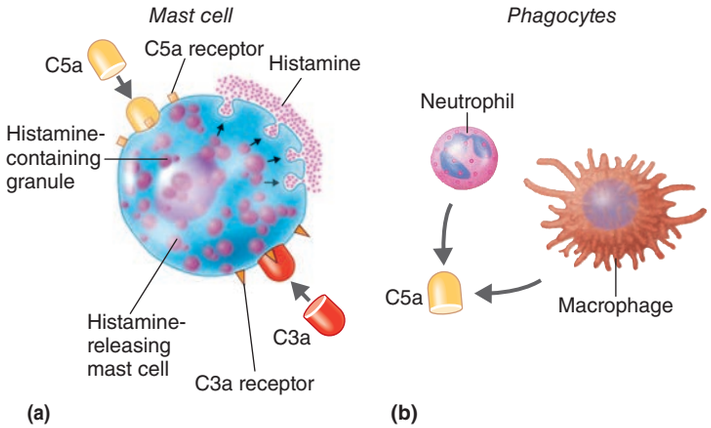


Figure 16.13 Inflammation stimulated by complement. **(a)** C3a and C5a bound to mast cells, basophils, and platelets trigger release of histamine, which increases blood vessel permeability. **(b)** C5a functions as a chemotactic factor and attracts phagocytes to the site of complement activation.

Q How is complement inactivated?

ultimately inhibiting MAC formation. Gram-positive cocci release an enzyme that breaks down C5a, the fragment that serves as a chemotactic factor that attracts phagocytes.

With respect to viruses, some viruses, such as the Epstein-Barr virus, attach to complement receptors on body cells to initiate their life cycle. MM **Animations** Complement System: Overview, Activation, Results

CHECK YOUR UNDERSTANDING

- What is complement? **16-16**
- List the steps of complement activation via the classical, alternative, and lectin pathways. **16-17**
- Summarize outcomes of complement activation. **16-18**

Interferons

Because viruses hijack host cells to carry out viral multiplication, it is difficult for the immune system to inhibit viral infections without affecting body cells too. One way an infected host cell counters viral infections is with a family of cytokines called **interferons (IFNs).** These are a class of proteins produced by certain animal cells, such as lymphocytes and macrophages. Just as different animal species produce different interferons, different types of cells within the same animal also produce different interferons. Interferons produced by people protect human cells, but they produce little antiviral activity for cells of other species, such as mice or chickens. However, the interferons of a species are active against a number of different viruses. They typically play a major role in infections that are acute, such as colds and influenza.

There are three main types of human interferons: **alpha interferon (IFN-α), beta interferon (IFN-β),** and **gamma interferon (IFN-γ).** The principal function of both IFN-α and IFN-β is to interfere with viral multiplication. There are also various subtypes of interferons within each of the principal groups. In humans, interferons are produced by fibroblasts in connective tissue and by lymphocytes and other leukocytes. Each type of interferon has a slightly different effect on the body. All interferons are small proteins, with molecular weights between 15,000 and 30,000. They are quite stable at low pH and are fairly resistant to heat.

Both IFN-α and IFN-β are produced by virus-infected host cells only in very small quantities that diffuse to uninfected neighboring cells (**Figure 16.14**). Both types are host-cell–specific but not virus-specific. They react with plasma or nuclear membrane receptors, inducing the uninfected neighboring cells to manufacture mRNA for the synthesis of **antiviral proteins (AVPs).** These proteins are enzymes that disrupt various stages of viral multiplication. For example, one AVP, called *oligoadenylate synthetase,* degrades viral mRNA. Another, called *protein kinase,* inhibits protein synthesis.

Gamma interferon is produced by lymphocytes and induces neutrophils and macrophages to kill bacteria. IFN-γ causes macrophages to produce nitric oxide that appears to kill bacteria as well as tumor cells by inhibiting ATP production. IFN-γ increases the expression of class I and class II molecules and increases antigen presentation.

Interferons would seem to be ideal antiviral substances, but certain problems do exist. They are stable for short periods of time in the body, so their effect is limited. When injected, interferons have side effects such as nausea, fatigue, headache, vomiting, weight loss, and fever. High concentrations of interferons are toxic to the heart, liver, kidneys, and red bone marrow. Another problem is that interferons have no effect on viral multiplication in cells already infected, and some viruses (such as adenoviruses) have resistance mechanisms that inhibit antiviral proteins. Further, some viruses, such as the hepatitis B virus, do not induce the production of sufficient amounts of interferon in host cells following viral stimulation.

The importance of interferons in protecting the body against viruses, as well as their potential as anticancer agents, has made their production in large quantities a top health priority. Several groups of scientists have successfully applied recombinant DNA technology in inducing certain species of bacteria to produce interferons. (This technique is described in Chapter 9.) The interferons produced with recombinant DNA techniques, called *recombinant interferons (rIFNs),* are important for two reasons: they are pure, and they are plentiful.

In clinical trials, IFNs have exhibited no effects against some types of tumors and only limited effects against others. Alpha interferon (Intron A) is approved in the United States for treating several virus-associated disorders. One is Kaposi's sarcoma, a cancer that often occurs in patients infected with HIV. Other approved uses for IFN-α include treating hepatitis B and C, malignant melanoma, and hairy cell leukemia. A form of IFN-β (Betaferon) slows the progression of multiple sclerosis (MS) and

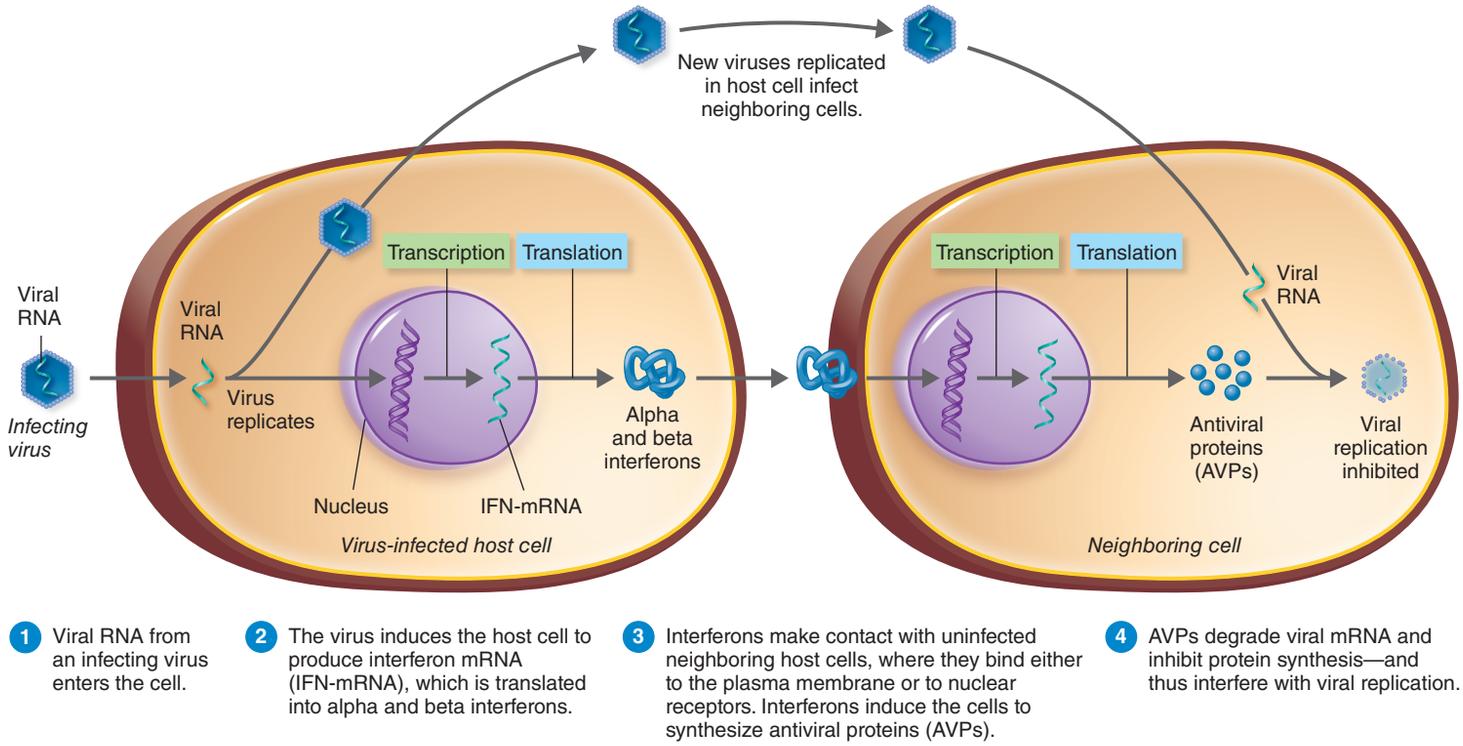


Figure 16.14 Antiviral action of alpha and beta interferons (IFNs). Interferons are host-cell–specific but not virus-specific.

How does interferon stop viruses?

lessens the frequency and severity of MS attacks. Another form of IFN-β (Actimmune) is being used to treat osteoporosis.

Iron-Binding Proteins

Most pathogenic bacteria require iron for their vegetative growth and reproduction (see Chapter 15). Humans use it as a component of cytochromes in the electron transport chain, a cofactor for enzyme systems, and as a part of hemoglobin, which transports oxygen in the body. Many pathogens also require iron to survive. So an infection creates a situation where pathogens and humans compete for available iron.

The concentration of free iron in the human body is low because most of it is bound to **iron-binding proteins**—molecules such as transferrin, lactoferrin, ferritin, and hemoglobin—whose function is to transport and store iron. **Transferrin** is found in blood and tissue fluids. **Lactoferrin** is found in milk, saliva, and mucus. **Ferritin** is located in the liver, spleen, and red bone marrow, and **hemoglobin** is located within red blood cells. The iron-binding proteins not only transport and store iron but also, by doing so, deprive most pathogens of the available iron.

To survive in the human body, many pathogenic bacteria obtain iron by secreting proteins called **siderophores** (see Figure 15.3 on page 424). Recall that siderophores compete to take away iron from iron-binding proteins by binding it more tightly. Once the iron–siderophore complex is formed, it is taken up by siderophore receptors on the bacterial surface and brought into the bacterium; then the iron is split from the siderophore and utilized. (In some cases, the iron enters the bacterium while the siderophore remains outside.)

A few pathogens do not use the siderophore mechanism to obtain iron. For example, *Neisseria meningitidis*, the causative agent

Clinical Case

In neutrophils, NADPH is reoxidized to $NADP^+$ by a membrane complex called NADP oxidase, which uses the electron to produce a superoxide radical ($O_2^{\cdot-}$) from O_2. The superoxide will be converted to hydrogen peroxide, and the resulting burst of H_2O_2 kills the pathogen. Because Jacob's neutrophils cannot function properly, his pediatrician needs to find a way to stimulate his immune system to kill the fungi that have invaded Jacob's bloodstream.

What enzyme converts superoxide radicals to hydrogen peroxide? (See the Clinical Case figure on page 456.) What treatment do you think Jacob's pediatrician should suggest for Jacob's CGD?

Serum Collection

It is common to draw more than one blood sample for laboratory tests. The blood is collected in tubes with caps of different colors (**Figure A**). Whole blood may be needed to culture microbes or to type the blood. Serum may be needed to test for enzymes or other chemicals in the blood. Serum is the straw-colored liquid remaining after blood is allow to clot. Blood plasma is the liquid remaining after formed elements are removed from unclotted blood by, for example, centrifugation.

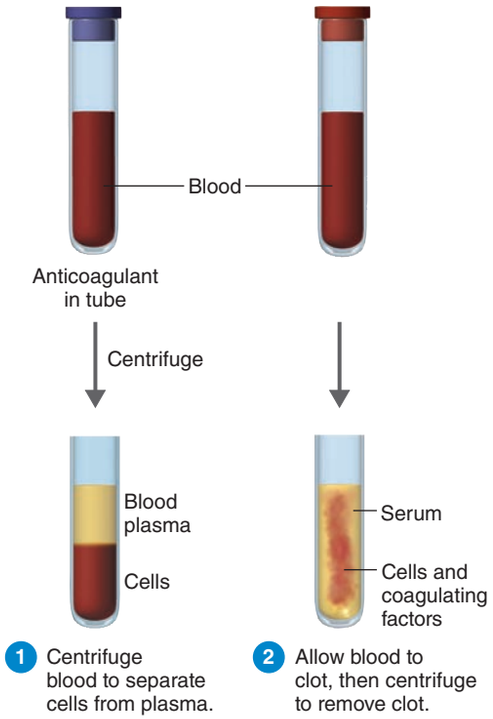


Figure A Collecting blood cells and serum.

Why test for complement?

Complement activity is measured because complement deficiency may be associated with recurrent bacterial infections. Moreover, complement is a key component in immune complex diseases. A decrease in serum complement, which occurs as complement is used in immune complexes, can be used to monitor progress and treatment of immune complex diseases such as systemic lupus erythematosus and rheumatoid arthritis.

Figure B shows how total complement activity is measured. Dilutions of the patient's serum are mixed with sheep red blood cells (RBCs) and antibodies against sheep RBCs (anti-RBCs). Following incubation at 37°C for 20 minutes, the degree of hemolysis, bursting of red blood cells, is determined.

What is the purpose of the RBCs and anti-RBCs?

The antibodies will react with the antigen (RBCs). This will activate complement in the patient's serum. The degree of lysis is relative to the amount of complement present and is expressed as a percentage of the hemolysis.

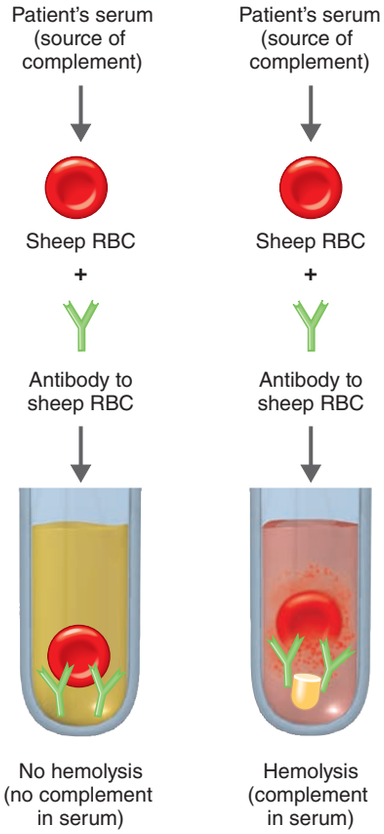


Figure B Testing for complement.

of meningitis, produces receptors on its surface that bind directly to human iron-binding proteins. Then the iron-binding protein, along with its iron, is taken into the bacterial cell. Some pathogens, such as *Streptococcus pyogenes,* release hemolysin, a protein that causes the lysis (destruction) of red blood cells. The hemoglobin is then degraded by other bacterial proteins to capture the iron.

Antimicrobial Peptides

Although fairly recently discovered, **antimicrobial peptides (AMPs)** may be one of the most important components of innate immunity (see also Chapter 20, page 575). Antimicrobial peptides are short peptides that consist of a chain of about 12 to 50 amino acids synthesized on ribosomes. They were first discovered in the skin of frogs, the lymph of insects, and human neutrophils; to date, over 600 AMPs have been discovered in nearly all plants and animals. AMPs have a broad spectrum of antimicrobial activities, including activity against bacteria, viruses, fungi, and eukaryotic parasites. Synthesis of AMPs is triggered by protein and sugar molecules on the surface of microbes. Cells produce AMPs when chemicals in microbes attach to Toll-like receptors (see page 442).

The modes of action of AMPs include inhibiting cell wall synthesis; forming pores in the plasma membrane, resulting in lysis; and destroying DNA and RNA. Among the AMPs produced by humans are *dermcidin,* produced by sweat glands; *defensins* and *cathelicidins,* produced by neutrophils, macrophages, and epithelium; and *thrombocidin,* produced by platelets.

Scientists are especially interested in AMPs for a number of reasons. Besides their broad spectrum of activity, AMPs have shown synergy (working together) with other antimicrobial agents, so that the effect of them working together is greater than that of either working separately. AMPs are also very stable over a wide range of pH. What is particularly significant is that

TABLE 16.2 Summary of Innate Immunity Defenses

Component	Functions
FIRST LINE OF DEFENSE: SKIN AND MUCOUS MEMBRANES	
PHYSICAL FACTORS	
Epidermis of skin	Intact skin forms a physical barrier to the entrance of microbes; shedding helps remove microbes.
Mucous membranes	Inhibit the entrance of many microbes, but not as effectively as intact skin.
Mucus	Traps microbes in respiratory and gastrointestinal tracts.
Lacrimal apparatus	Provides tears that wash away microbes; tears contain lysozyme, which destroys cell walls, especially of gram-positive bacteria.
Saliva	Dilutes and washes microbes from mouth.
Hairs	Filter and trap microbes and dust in nose.
Cilia	Together with mucus form a ciliary escalator, which traps and removes microbes from upper respiratory tract.
Epiglottis	Prevents microbes from entering lower respiratory tract.
Earwax	Prevents microbes from entering ear.
Urine	Washes microbes from urethra preventing colonization in genitourinary tract.
Vaginal secretions	Move microbes out of body.
Peristalsis, defecation, vomiting, and diarrhea	Expel microbes from body.
CHEMICAL FACTORS	
Sebum	Forms a protective acidic film over the skin surface that inhibits microbial growth.
Earwax	Fatty acids in earwax inhibit the growth of bacteria and fungi.
Perspiration	Flushes microbes from skin and contains lysozyme; lysozyme is also present in tears, saliva, nasal secretions, urine, and tissue fluids.
Saliva	Contains lysozyme, urea, and uric acid, which inhibit microbes; and immunoglobin A, which prevents attachment of microbes to mucous membranes. Slight acidity discourages microbial growth.
Gastric juice	High acidity destroys bacteria and most toxins in stomach.
Vaginal secretions	Glycogen breakdown into lactic acid provides slight acidity, which discourages bacterial and fungal growth.
Urine	Contains lysozyme. Slight acidity discourages microbial growth.
SECOND LINE OF DEFENSE	
DEFENSIVE CELLS	
Phagocytes	Phagocytosis by cells such as neutrophils, eosinophils, dendritic cells, and macrophages.
Natural killer (NK) cells	Kill infected cells by releasing granules of perforin and granzymes. Phagocytes then kill the cells.
INFLAMMATION	Confines and destroys microbes and initiates tissue repair.
FEVER	Intensifies the effects of interferons, inhibits growth of some microbes, and speeds up body reactions that aid repair.
ANTIMICROBIAL SUBSTANCES	
Complement system	Causes cytolysis of microbes, promotes phagocytosis, and contributes to inflammation.
Interferons	Protect uninfected host cells from viral infection. IFN-γ increases phagocytosis.
Iron-binding proteins	Inhibit growth of certain bacteria by reducing the amount of available iron.
Antimicrobial peptides (AMPs)	Inhibit cell wall synthesis, form pores in the plasma membrane that cause lysis; and destroy DNA and RNA.

microbes do not appear to develop resistance even though the microbes are exposed to them for long periods of time.

In addition to the killing effect of AMPs, they also participate in a number of other immune functions. For example, AMPs can sequester the LPS shed from gram-negative bacteria. Recall that the lipid A component of the LPS functions as an endotoxin and is responsible for the symptoms associated with infection by gram-negative bacteria (septic shock). AMPs have been found to vigorously attract dendritic cells, which destroy microbes by phagocytosis and initiate an adaptive immune response. AMPs have also been shown to recruit mast cells, which increase blood vessel permeability and vasodilation. This brings about inflammation, which destroys microbes, limits the extent of damage, and initiates tissue repair.

Table 16.2 contains a summary of innate immunity defenses.

Clinical Case Resolved

Superoxide dismutase (SOD) is an enzyme used to convert superoxide radicals into molecular oxygen and hydrogen peroxide. Jacob's pediatrician suggests treating Jacob with recombinant gamma interferon (IFN-γ), which will incite Jacob's neutrophils and macrophages to kill bacteria. The method of action isn't known, and IFN-γ isn't a cure; Jacob must take IFN-γ for the rest of his life or until he can receive a bone marrow transplant.

442 448 452 456 461 464

CHECK YOUR UNDERSTANDING

- What is interferon? **16-19**
- Why do IFN-α and IFN-β share the same receptor on target cells, yet IFN-γ has a different receptor? **16-20**
- What is the role of siderophores in infection? **16-21**
- Why are scientists interested in AMPs? **16-22**

Study Outline

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Introduction (p. 439)

1. The ability to ward off disease through body defenses is called immunity.
2. Lack of immunity is called susceptibility.

The Concept of Immunity (p. 442)

1. Innate immunity refers to all body defenses that protect the body against any kind of pathogen.
2. Adaptive immunity refers to defenses (antibodies) against specific microorganisms.
3. Toll-like receptors in plasma membranes of macrophages and dendritic cells bind to invading microbes.

First Line of Defense: Skin and Mucous Membranes (pp. 442–445)

1. The body's first line of defense against infections is a physical barrier and the nonspecific chemicals of the skin and mucous membranes.

Physical Factors (pp. 442–444)

1. The structure of intact skin and the waterproof protein keratin provide resistance to microbial invasion.
2. Some pathogens can penetrate mucous membranes.
3. The lacrimal apparatus protects the eyes from irritating substances and microorganisms.
4. Saliva washes microorganisms from teeth and gums.
5. Mucus traps many microorganisms that enter the respiratory and gastrointestinal tracts; in the lower respiratory tract, the ciliary escalator moves mucus up and out.
6. The flow of urine moves microorganisms out of the urinary tract, and vaginal secretions move microorganisms out of the vagina.

Chemical Factors (pp. 444–445)

1. Fatty acids in sebum and earwax inhibit the growth of pathogenic bacteria.
2. Perspiration washes microorganisms off the skin.
3. Lysozyme is found in tears, saliva, nasal secretions, and perspiration.
4. The high acidity (pH 1.2–3.0) of gastric juice prevents microbial growth in the stomach.

Normal Microbiota and Innate Immunity (p. 445)

1. Normal microbiota change the environment, a process that can prevent the growth of pathogens.

Second Line of Defense (pp. 446–464)

1. A microbe's penetration of the first line of defense encourages production of phagocytes, inflammation, fever, and antimicrobial substances.

Formed Elements in Blood (pp. 446–448)

1. Blood consists of plasma (fluid) and formed elements (cells and platelets).
2. Leukocytes (white blood cells) are divided into granulocytes (neutrophils, basophils, eosinophils) and agranulocytes.
3. During many infections, the number of leukocytes increases (leukocytosis); some infections are characterized by leukopenia (decrease in leukocytes).

The Lymphatic System (pp. 448–449)

1. The lymphatic system consists of lymph vessels, lymph nodes, and lymphoid tissue.
2. Interstitial fluid is returned to blood plasma via lymph vessels.

Phagocytes (pp. 449–452)

1. Phagocytosis is the ingestion of microorganisms or particulate matter by a cell.
2. Phagocytosis is performed by phagocytes, certain types of white blood cells or their derivatives.

Actions of Phagocytic Cells (pp. 449–450)

3. Enlarged monocytes become wandering macrophages and fixed macrophages.
4. Fixed macrophages are located in selected tissues and are part of the mononuclear phagocytic system.
5. Granulocytes, especially neutrophils, predominate during the early stages of infection, whereas monocytes predominate as the infection subsides.

The Mechanism of Phagocytosis (pp. 450–451)

6. Chemotaxis is the process by which phagocytes are attracted to microorganisms.
7. Toll-like receptors on a phagocyte adhere to the microbial cells. Adherence may be facilitated by opsonization—coating the microbe with serum proteins.
8. Pseudopods of phagocytes engulf the microorganism and enclose it in a phagosome to complete ingestion.
9. Many phagocytized microorganisms are killed by lysosomal enzymes and oxidizing agents.

Microbial Evasion of Phagocytosis (p. 452)

10. Some microbes are not killed by phagocytes and can even reproduce in phagocytes.
11. Evasion mechanisms include M protein, capsules, leukocidins, membrane attack complexes, and prevention of phagolysosome formation.

Inflammation (pp. 452–455)

1. Inflammation is a bodily response to cell damage; it is characterized by redness, pain, heat, swelling, and sometimes the loss of function.
2. TNF-α stimulates production of acute-phase proteins.

Vasodilation and Increased Permeability of Blood Vessels (pp. 453–454)

3. The release of histamine, kinins, and prostaglandins causes vasodilation and increased permeability of blood vessels.
4. Blood clots can form around an abscess to prevent dissemination of the infection.

Phagocyte Migration and Phagocytosis (pp. 454–455)

5. Phagocytes have the ability to stick to the lining of the blood vessels (margination) and also have the ability to squeeze through blood vessels (diapedesis).
6. Pus is the accumulation of damaged tissue and dead microbes, granulocytes, and macrophages.

Tissue Repair (p. 455)

7. A tissue is repaired when the stroma (supporting tissue) or parenchyma (functioning tissue) produces new cells.
8. Stromal repair by fibroblasts produces scar tissue.

Fever (pp. 455–456)

1. Fever is an abnormally high body temperature produced in response to a bacterial or viral infection.
2. Bacterial endotoxins, interleukin-1, and TNF-α can induce fever.
3. A chill indicates a rising body temperature; crisis (sweating) indicates that the body's temperature is falling.

Antimicrobial Substances (pp. 456–464)

The Complement System (pp. 456–460)

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1. The complement system consists of a group of serum proteins that activate one another to destroy invading microorganisms.
2. Complement proteins are activated in a cascade.
3. C3 activation can result in cell lysis, inflammation, and opsonization.
4. Complement is activated via the classical pathway, the alternative pathway, and the lectin pathway.
5. Complement is deactivated by host-regulatory proteins.
6. Complement deficiencies can result in an increased susceptibility to disease.
7. Some bacteria evade destruction by complement by means of capsules, surface lipid–carbohydrate complexes, and enzymatic destruction of C5a.

Interferons (pp. 460–461)

8. IFN-α and IFN-β are produced in response to viral infection.
9. IFN-α and IFN-β induce uninfected cells to produce antiviral proteins (AVPs) that prevent viral replication.
10. IFN-α and IFN-β are host-cell–specific but not virus-specific.
11. IFN-γ activates neutrophils and macrophages to kill bacteria.

Iron-Binding Proteins (pp. 461–462)

12. Iron-binding proteins transport and store iron, depriving most pathogens of the available iron.

Antimicrobial Peptides (pp. 462–464)

13. Antimicrobial peptides (AMPs) inhibit cell wall synthesis, form pores in plasma membranes, and destroy DNA and RNA.
14. Antimicrobial peptides are produced by nearly all plants and animals, and bacterial resistance to AMPs has not yet been seen.

Study Questions

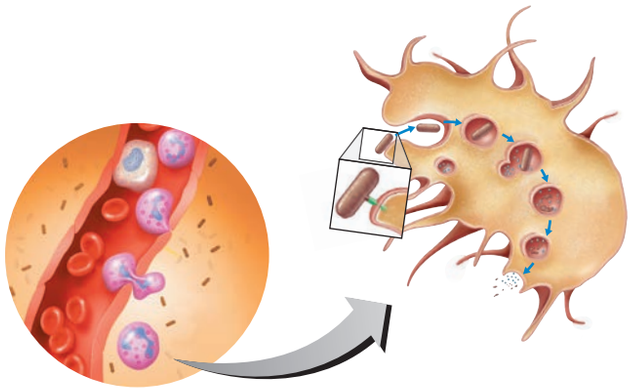
For answers to the Knowledge and Comprehension questions, turn to the Answers tab at the back of the textbook.

Knowledge and Comprehension

Review

1. Identify at least one physical factor and one chemical factor that prevent microbes from entering the body through each of the following:
   a. urinary tract
   b. reproductive tract
2. Define *inflammation,* and list its characteristics.
3. What are interferons? Discuss their roles in innate immunity.
4. How can the complement system cause endotoxic shock?
5. Patients with X-linked chronic granulomatous disease are susceptible to infections because their neutrophils don't generate an oxidative burst. What is the relation of the oxidative burst to infection?

6. Why does hemolysis of red blood cells occur when a person receives a transfusion of the wrong type of blood?
7. Give several examples of how microbes evade the complement system.
8. DRAW IT Label the following processes that result in phagocytosis: margination, diapedesis, adherence, and phagolysosome formation.



9. Are the following involved in innate or in adaptive immunity? Identify the role of each in immunity:
   a. TLRs
   b. transferrins
   c. antimicrobial peptides
10. NAME IT These agranulocytes are not phagocytic until they wander out of the blood.

Multiple Choice

1. *Legionella* uses C3b receptors to enter monocytes. This
   a. prevents phagocytosis.
   b. degrades complement.
   c. inactivates complement.
   d. prevents inflammation.
   e. prevents cytolysis.
2. *Chlamydia* can prevent the formation of phagolysosomes and therefore can
   a. avoid being phagocytized.
   b. avoid destruction by complement.
   c. prevent adherence.
   d. avoid being digested.
   e. none of the above
3. If the following are placed in the order of occurrence, which would be the *third* step?
   a. diapedesis
   b. digestion
   c. formation of a phagosome
   d. formation of a phagolysosome
   e. margination
4. If the following are placed in the order of occurrence, which would be the *third* step?
   a. activation of C5 through C9
   b. cell lysis
   c. antigen–antibody reaction
   d. activation of C3
   e. activation of C2 through C4
5. A human host can prevent a pathogen from getting enough iron by all of the following *except*
   a. reducing dietary intake of iron.
   b. binding iron with transferrin.
   c. binding iron with hemoglobin.
   d. binding iron with ferritin.
   e. binding iron with siderophores.
6. A decrease in the production of C3 would result in
   a. increased susceptibility to infection.
   b. increased numbers of white blood cells.
   c. increased phagocytosis.
   d. activation of C5 through C9.
   e. none of the above
7. In 1884, Elie Metchnikoff observed blood cells collected around a splinter inserted in a sea star embryo. This was the discovery of
   a. blood cells.
   b. sea stars.
   c. phagocytosis.
   d. immunity.
   e. none of the above
8. *Helicobacter pylori* uses the enzyme urease to counteract a chemical defense in the human organ in which it lives. This chemical defense is
   a. lysozyme.
   b. hydrochloric acid.
   c. superoxide radicals.
   d. sebum.
   e. complement.
9. Which of the following statements about IFN-α is *false*?
   a. It interferes with viral replication.
   b. It is host-cell–specific.
   c. It is released by fibroblasts.
   d. It is virus-specific.
   e. It is released by lymphocytes.
10. Which of the following does *not* stimulate phagocytes?
    a. cytokines
    b. IFN-γ
    c. C3b
    d. lipid A
    e. histamine

Analysis

1. What role does transferrin play in fighting an infection?
2. A variety of drugs with the ability to reduce inflammation are available. Comment on the danger of misuse of these anti-inflammatory drugs.
3. To be a successful parasite, a microbe must avoid destruction by complement. The following list provides examples of complement-evading techniques. For each microbe, identify the disease it causes, and describe how its strategy enables it to avoid destruction by complement.

Pathogen	Strategy
Group A streptococci	C3 does not bind to M protein
Haemophilus influenzae type b	Has a capsule
Pseudomonas aeruginosa	Sheds cell wall polysaccharides
Trypanosoma cruzi	Degrades C1

4. The list below identifies a virulence factor for a selected microorganism. Describe the effect of each factor listed. Name a disease caused by each organism.

Microorganism	Virulence Factor
Influenzavirus	Causes release of lysosomal enzymes
Mycobacterium tuberculosis	Inhibits lysosome fusion
Toxoplasma gondii	Prevents phagosome acidification
Trichophyton	Secretes keratinase
Trypanosoma cruzi	Lyses phagosomal membrane

Clinical Applications and Evaluation

1. People with *Rhinovirus* infections of the nose and throat have an 80-fold increase in kinins and no increase in histamine. What do you expect for rhinoviral symptoms? What disease is caused by rhinoviruses?
2. A hematologist often performs a differential white blood cell count on a blood sample. Such a count determines the relative numbers of white blood cells. Why are these numbers important? What do you think a hematologist would find in a differential white blood cell count of a patient with mononucleosis? With neutropenia? With eosinophilia?
3. Leukocyte adherence deficiency (LAD) is an inherited disease resulting in the inability of neutrophils to recognize C3b-bound microorganisms. What are the most likely consequences of LAD?
4. The neutrophils of individuals with Chédiak-Higashi syndrome (CHS) have fewer than normal chemotactic receptors and lysosomes that spontaneously rupture. What are the consequences of CHS?
5. Consider the following.
   a. In laboratory experiments, plant lectins can bind to mannose in human plasma membranes. Why doesn't human mannose-binding lectin bind to human cells?
   b. About 4% of the human population have a mannose-binding lectin deficiency. How might this deficiency affect a person?