
ninth edition
Prescott's
Microbiology
Willey
Sherwood
Woolverton

ninth edition

Prescott's Microbiology

Joanne M. Willey
HOFSTRA UNIVERSITY

Linda M. Sherwood
MONTANA STATE UNIVERSITY

Christopher J. Woolverton
KENT STATE UNIVERSITY

PRESCOTT'S MICROBIOLOGY, NINTH EDITION

This book is printed on acid-free paper.

1 2 3 4 5 6 7 8 9 0 DOW/DOW 1 0 9 8 7 6 5 4 3

ISBN 978–0–07–340240–6
MHID 0–07–340240–0

Senior Vice President, Products & Markets: *Kurt L. Strand*
Vice President, General Manager, Products & Markets: *Marty Lange*
Vice President, Content Production & Technology Services: *Kimberly Meriwether David*
Managing Director: *Michael S. Hackett*
Director, Biology: *Lynn M. Breithaupt*
Brand Manager: *Amy L. Reed*
Director of Development: *Rose Koos*
Development Editor: *Kathleen Timp/Angela FitzPatrick*
Director of Digital Content Development: *Barbekka Hurtt, Ph.D.*
Digital Product Manager: *Amber Bettcher*
Content Project Manager: *Sandy Wille*
Senior Buyer: *Sandy Ludovissy*
Senior Designer: *David W. Hash*
Cover/Interior Designer: *Christopher Reese*
Cover Image: *Sebastian Kaulitzki/Alamy*
Lead Content Licensing Specialist: *Carrie K. Burger*
Photo Research: *Mary Reeg*
Compositor: *Aptara®, Inc.*
Typeface: *10/12 Minion Pro*
Printer: *R. R. Donnelley*

Library of Congress Cataloging-in-Publication Data

Cataloging-in-Publication Data has been requested from the Library of Congress.

www.mhhe.com

About the Authors

Joanne M. Willey has been a professor at Hofstra University on Long Island, New York, since 1993, where she is Professor of Microbiology; she holds a joint appointment with the Hofstra University School of Medicine. Dr. Willey received her B.A. in Biology from the University of Pennsylvania, where her interest in microbiology began with work on cyanobacterial growth in eutrophic streams. She earned her Ph.D. in biological oceanography (specializing in marine microbiology) from the Massachusetts Institute of Technology–Woods Hole Oceanographic Institution Joint Program in 1987. She then went to Harvard University, where she spent her postdoctoral fellowship studying the filamentous soil bacterium *Streptomyces coelicolor*. Dr. Willey continues to investigate this fascinating microbe and has coauthored a number of publications that focus on its complex developmental cycle. She is an active member of the American Society for Microbiology (ASM), and served on the editorial board of the journal *Applied and Environmental Microbiology* for nine years and as Chair of the Division of General Microbiology. Dr. Willey regularly teaches microbiology to biology majors as well as medical students. She also teaches courses in cell biology, marine microbiology, and laboratory techniques in molecular genetics. Dr. Willey lives on the north shore of Long Island with her husband and two sons. She is an avid runner and enjoys skiing, hiking, sailing, and reading. She can be reached at joanne.m.willey@hofstra.edu.

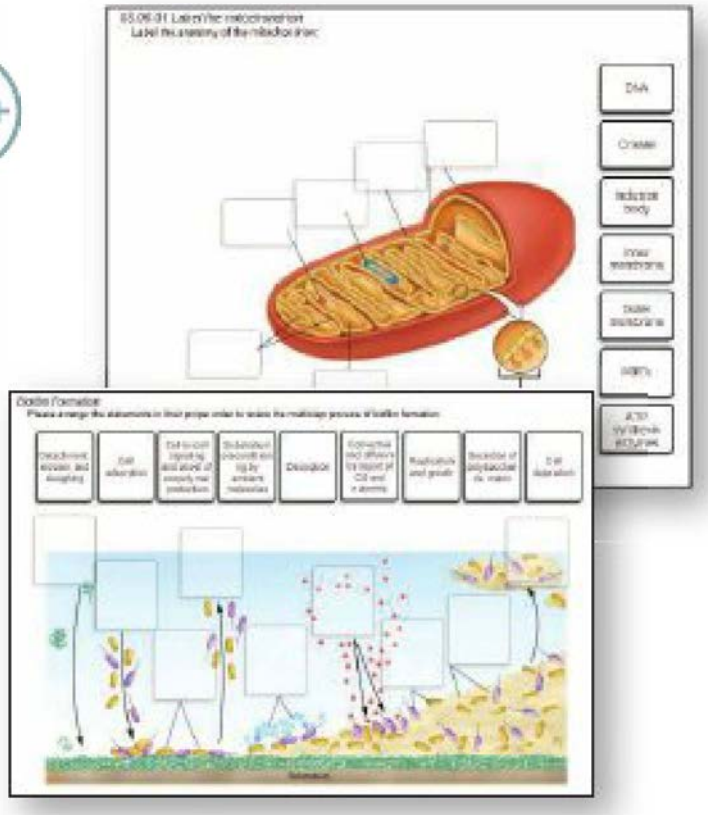
Linda M. Sherwood is a member of the Department of Microbiology at Montana State University. Her interest in microbiology was sparked by the last course she took to complete a B.S. degree in Psychology at Western Illinois University. She went on to complete an M.S. degree in Microbiology at the University of Alabama, where she studied histidine utilization by *Pseudomonas acidovorans*. She subsequently earned a Ph.D. in Genetics at Michigan State University, where she studied sporulation in *Saccharomyces cerevisiae*. She briefly left the microbial world to study the molecular biology of *dunce* fruit flies at Michigan State University before moving to Montana State University. Dr. Sherwood has always had a keen interest in teaching, and her psychology training has helped her to understand current models of cognition and learning and their implications for teaching. Over the years, she has taught courses in general microbiology, genetics, biology, microbial genetics, and microbial physiology. She has served as the editor for ASM's *Focus on Microbiology Education* and has participated in and contributed to numerous ASM Conferences for Undergraduate Educators (ASMCUE). She also has worked with K–12 teachers to develop a kit-based unit to introduce microbiology into the elementary school curriculum and has coauthored with Barbara Hudson a general microbiology laboratory manual, *Explorations in Microbiology: A Discovery Approach*, published by Prentice-Hall. Her association with McGraw-Hill began when she prepared the study guides for the fifth and sixth editions of *Microbiology*. Her non-academic interests focus primarily on her family. She also enjoys reading, hiking, gardening, and traveling. She can be reached at lsherwood@montana.edu.

Christopher J. Woolverton is founding professor of Environmental Health Science, College of Public Health at Kent State University (Kent, OH), and is the Director of the Kent State University (KSU) Center for Public Health Preparedness, overseeing its BSL-3 Training Facility. Dr. Woolverton serves on the KSU graduate faculty of the College of Public Health, the School of Biomedical Sciences, and the Department of Biological Sciences. He holds a joint appointment at Akron Children's Hospital (Akron, OH). He earned his B.S. in Biology from Wilkes College (PA), and his M.S. and Ph.D. in Medical Microbiology from West Virginia University, School of Medicine. He spent two years as a postdoctoral fellow at UNC-Chapel-Hill. Dr. Woolverton's current research is focused on real-time detection and identification of pathogens using a liquid crystal (LC) biosensor that he patented in 2001. Dr. Woolverton has published and lectured widely on the mechanisms by which LCs act as biosensors and on the LC characteristics of microbial proteins. Professor Woolverton teaches microbiology, communicable diseases, immunology, prevention and control of disease, and microbial physiology. He is on the faculty of the National Institutes of Health National Biosafety and Biocontainment Training Program, teaching laboratory safety, risk assessment, decontamination strategies, and bioterrorism readiness. An active member of the American Society for Microbiology, Woolverton serves on its Board of Education and as the editor-in-chief of its *Journal of Microbiology and Biology Education*. Woolverton and his wife, Nancy, have three daughters, a son-in-law, and a grandson. He enjoys time with his family, ultra-light hiking and camping, and is an avid cyclist. His e-mail address is cwoolver@kent.edu.

Digital Tools for Your Success

connect® plus+

LOG



Save time with auto-graded assessmen
Gather powerful performance data.

McGraw-Hill ConnectPlus® Microbiology provides online presentation, assignment, and assessment solutions, connecting your students with the tools and resources they'll need to achieve success.

Homework and Assessment

With **ConnectPlus Microbiology**, you can deliver auto-graded assignments, quizzes, and tests online. A robust set of interactive questions and activities using high-quality art from the textbook and animations is presented. Assignable content is available for every Learning Outcome in the book and is categorized according to the **ASM Curriculum Guidelines.** As an instructor, you can edit existing questions and author entirely new problems.

Detailed Reports

Track individual student performance—by question, assignment, or in relation to the class overall—with detailed grade reports. Integrate grade reports easily with Learning Management Systems (LMS), such as WebCT and Blackboard—and much more.

Lecture Capture

McGraw-Hill Tegrity® records and distributes your class lecture with just a click of a button. Students can view anytime, anywhere via computer or mobile device. Indexed as you record, students can use keywords to find exactly what they want to study.

Instructor Resources

Customize your lecture with tools such as PowerPoint® presentations, animations, and editable art from the textbook. An instructor's manual for the text and lab manual as well as answer keys to in-text questions save you time in developing your course.

eBook

ConnectPlus Microbiology provides students 24/7 online access to a media-rich version of the book, allowing seamless integration of text, media, and assessments.

Learn more at www.mcgrawhillconnect.com.

Digital Tools for Your Success

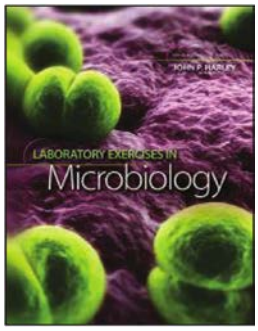
LearnSmart™

A diagnostic, adaptive learning system to increase preparedness.

Now Available for the Ninth Edition!

McGraw-Hill LearnSmart™ is an adaptive learning system designed to help students learn faster, study more efficiently, and retain more knowledge for greater success. LearnSmart assesses a student's knowledge of course content through a series of adaptive questions. It pinpoints concepts the student does not understand and maps out a **personalized study plan** for success. This innovative study tool also has features that allow instructors to see exactly what students have accomplished and a built-in assessment tool for graded assignments.

Visit www.mhlearnsmart.com for a demonstration.



Laboratory Exercises in Microbiology, Ninth Edition

John P. Harley has revised this laboratory manual to accompany the ninth edition of *Prescott's Microbiology.* The class-tested exercises are modular to allow instructors to easily incorporate them into their course. This balanced introduction to each area of microbiology now also has accompanying Connect content for additional homework and assessment opportunities. In addition, all artwork from the lab manual is now available through the Instructor Resources in Connect for incorporation into lectures.

create

With **McGraw-Hill Create™**, you can easily rearrange chapters, combine material from other content sources, and quickly upload content you have written, such as your course syllabus or teaching notes. Find the content you need in Create by searching through thousands of leading McGraw-Hill textbooks. Arrange your book to fit your teaching style. Create even allows you to personalize your book's appearance by selecting the cover and adding your name, school, and course information. Order a Create book and you'll receive a complimentary print review copy in three to five business days or a complimentary electronic review copy (eComp) via e-mail in minutes. Go to www.mcgrawhillcreate.com today and register to experience how McGraw-Hill Create empowers you to teach your students your way.

A Modern Approach to Microbiology

Evolution as a Framework

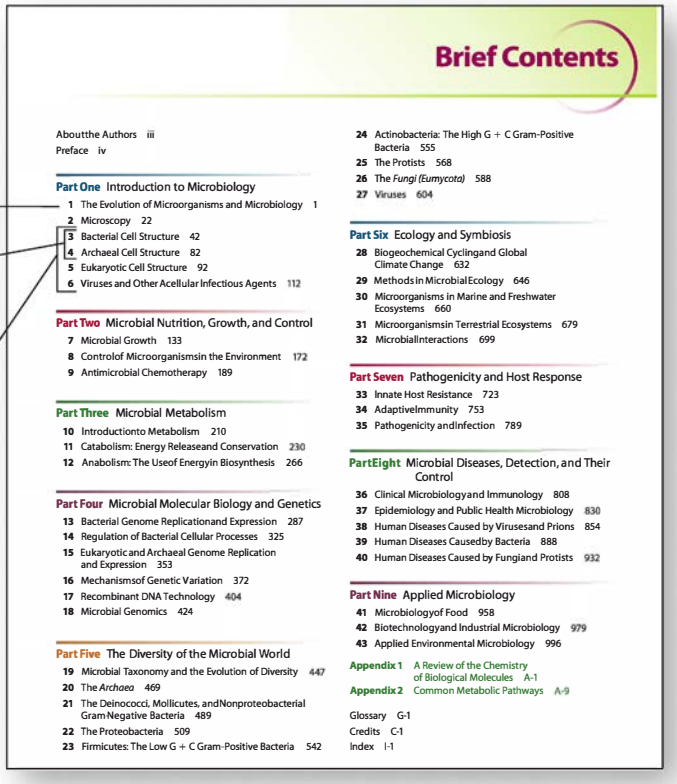
Introduced immediately in chapter 1 and used as an overarching theme throughout, evolution helps unite microbiological concepts and provides a framework upon which students can build their knowledge.

Separate Chapters on Bacteria and Archaea

In recognition of the importance and prevalence of archaea, the structure, genetics, and taxonomic and physiologic diversity of these microbes are now covered in chapters that are separate from those about bacteria.

An Introduction to the Entire Microbial World

Now covered in chapters 3–6, the separate chapters on the structure and function of bacteria and archaea are followed by the discussion of eukaryotic cells preceding viruses.



Brief Contents

About the Authors iii
Preface iv

Part One Introduction to Microbiology
1 The Evolution of Microorganisms and Microbiology 1
2 Microscopy 22
3 Bacterial Cell Structure 42
4 Archaeal Cell Structure 82
5 Eukaryotic Cell Structure 92
6 Viruses and Other Acellular Infectious Agents 112

Part Two Microbial Nutrition, Growth, and Control
7 Microbial Growth 133
8 Control of Microorganisms in the Environment 172
9 Antimicrobial Chemotherapy 189

Part Three Microbial Metabolism
10 Introduction to Metabolism 210
11 Catabolism: Energy Release and Conservation 230
12 Anabolism: The Use of Energy in Biosynthesis 266

Part Four Microbial Molecular Biology and Genetics
13 Bacterial Genome Replication and Expression 287
14 Regulation of Bacterial Cellular Processes 325
15 Eukaryotic and Archaeal Genome Replication and Expression 353
16 Mechanisms of Genetic Variation 372
17 Recombinant DNA Technology 404
18 Microbial Genomics 424

Part Five The Diversity of the Microbial World
19 Microbial Taxonomy and the Evolution of Diversity 447
20 The *Archaea* 469
21 The Deinococci, Mollicutes, and Nonproteobacterial Gram-Negative Bacteria 489
22 The Proteobacteria 509
23 Firmicutes: The Low G + C Gram-Positive Bacteria 542
24 Actinobacteria: The High G + C Gram-Positive Bacteria 555
25 The Protists 568
26 The *Fungi (Eumycota)* 588
27 Viruses 604

Part Six Ecology and Symbiosis
28 Biogeochemical Cycling and Global Climate Change 632
29 Methods In Microbial Ecology 646
30 Microorganisms in Marine and Freshwater Ecosystems 660
31 Microorganisms in Terrestrial Ecosystems 679
32 Microbial Interactions 699

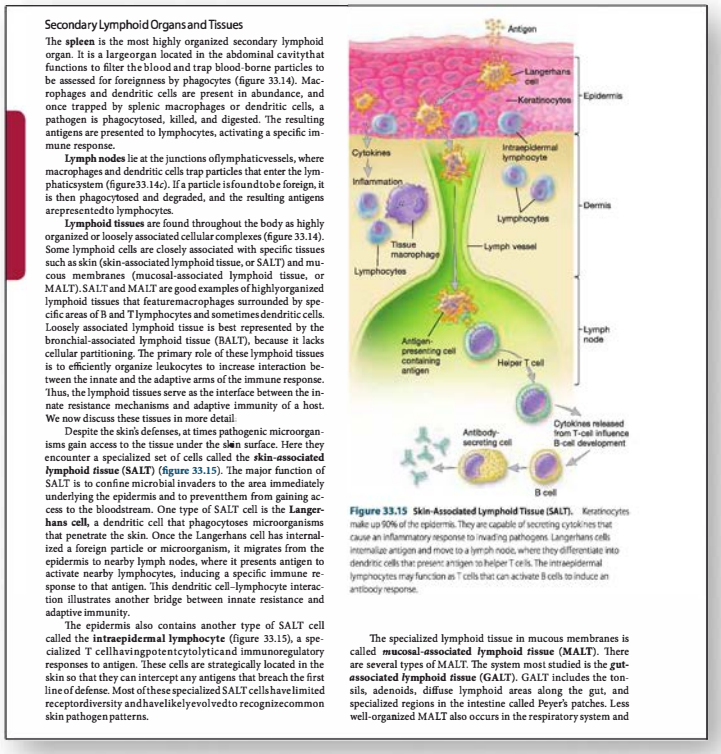
Part Seven Pathogenicity and Host Response
33 Innate Host Resistance 723
34 Adaptive Immunity 753
35 Pathogenicity and Infection 789

Part Eight Microbial Diseases, Detection, and Their Control
36 Clinical Microbiology and Immunology 808
37 Epidemiology and Public Health Microbiology 830
38 Human Diseases Caused by Viruses and Prions 854
39 Human Diseases Caused by Bacteria 888
40 Human Diseases Caused by Fungi and Protists 932

Part Nine Applied Microbiology
41 Microbiology of Food 958
42 Biotechnology and Industrial Microbiology 979
43 Applied Environmental Microbiology 996

Appendix 1 A Review of the Chemistry of Biological Molecules A-1
Appendix 2 Common Metabolic Pathways A-9

Glossary G-1
Credits C-1
Index I-1



Secondary Lymphoid Organs and Tissues

The **spleen** is the most highly organized secondary lymphoid organ. It is a large organ located in the abdominal cavity that functions to filter the blood and trap blood-borne particles to be assessed for foreignness by phagocytes (figure 33.14). Macrophages and dendritic cells are present in abundance, and once trapped by splenic macrophages or dendritic cells, a pathogen is phagocytosed, killed, and digested. The resulting antigens are presented to lymphocytes, activating a specific immune response.

Lymph nodes lie at the junctions of lymphatic vessels, where macrophages and dendritic cells trap particles that enter the lymphatic system (figure 33.14c). If a particle is found to be foreign, it is then phagocytosed and degraded, and the resulting antigens are presented to lymphocytes.

Lymphoid tissues are found throughout the body as highly organized or loosely associated cellular complexes (figure 33.14). Some lymphoid cells are closely associated with specific tissues such as skin (skin-associated lymphoid tissue, or SALT) and mucous membranes (mucosal-associated lymphoid tissue, or MALT). SALT and MALT are good examples of highly organized lymphoid tissues that feature macrophages surrounded by specific areas of B and T lymphocytes and sometimes dendritic cells. Loosely associated lymphoid tissue is best represented by the bronchial-associated lymphoid tissue (BALT), because it lacks cellular partitioning. The primary role of these lymphoid tissues is to efficiently organize leukocytes to increase interaction between the innate and the adaptive arms of the immune response. Thus, the lymphoid tissues serve as the interface between the innate resistance mechanisms and adaptive immunity of a host. We now discuss these tissues in more detail.

Despite the skin's defenses, at times pathogenic microorganisms gain access to the tissue under the skin surface. Here they encounter a specialized set of cells called the ***skin-associated lymphoid tissue* (SALT)** (figure 33.15). The major function of SALT is to confine microbial invaders to the area immediately underlying the epidermis and to prevent them from gaining access to the bloodstream. One type of SALT cell is the **Langerhans cell,** a dendritic cell that phagocytoses microorganisms that penetrate the skin. Once the Langerhans cell has internalized a foreign particle or microorganism, it migrates from the epidermis to nearby lymph nodes, where it presents antigen to activate nearby lymphocytes, inducing a specific immune response to that antigen. This dendritic cell–lymphocyte interaction illustrates another bridge between innate resistance and adaptive immunity.

The epidermis also contains another type of SALT cell called the **intraepidermal lymphocyte** (figure 33.15), a specialized T cell having potent cytolytic and immunoregulatory responses to antigen. These cells are strategically located in the skin so that they can intercept any antigens that breach the first line of defense. Most of these specialized SALT cells have limited receptor diversity and have likely evolved to recognize common skin pathogen patterns.

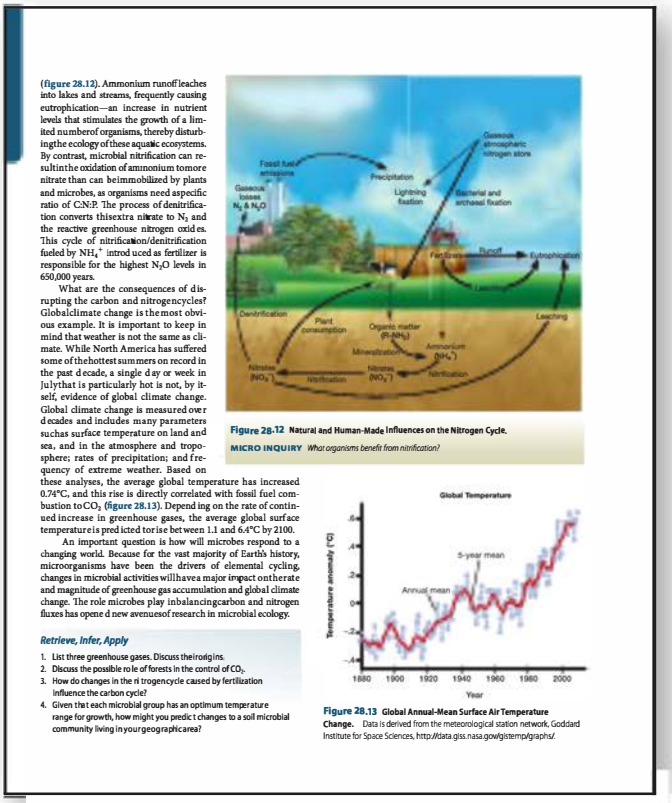
Figure 33.15 Skin-Associated Lymphoid Tissue (SALT). Keratinocytes make up 90% of the epidermis. They are capable of secreting cytokines that cause an inflammatory response to invading pathogens. Langerhans cells internalize antigen and move to a lymph node, where they differentiate into dendritic cells that present antigen to helper T cells. The intraepidermal lymphocytes may function as T cells that can activate B cells to induce an antibody response.

The specialized lymphoid tissue in mucous membranes is called ***mucosal-associated lymphoid tissue* (MALT)**. There are several types of MALT. The system most studied is the ***gut-associated lymphoid tissue* (GALT)**. GALT includes the tonsils, adenoids, diffuse lymphoid areas along the gut, and specialized regions in the intestine called Peyer's patches. Less well-organized MALT also occurs in the respiratory system and

Molecular Microbiology and Immunology

The ninth edition includes updates on genetics, biotechnology, genomics, and immunology. The discussion of eukaryotic and archaeal genetics has been expanded and makes up a separate chapter to reflect the relatedness of genetic information flow. A streamlined discussion of immunity with enhanced detail between innate and adaptive linkages helps students grasp the complexity and specificity of immune responses.

A Modern Approach to Microbiology



(figure 28.12). Ammonium runoff leaches into lakes and streams, frequently causing eutrophication—an increase in nutrient levels that stimulates the growth of a limited number of organisms, thereby disturbing the ecology of these aquatic ecosystems. By contrast, microbial nitrification can result in the oxidation of ammonium to more nitrate than can be immobilized by plants and microbes, as organisms need a specific ratio of C:N:P. The process of denitrification converts this extra nitrate to N_2 and the reactive greenhouse nitrogen oxides. This cycle of nitrification/denitrification fueled by NH_4^+ introduced as fertilizer is responsible for the highest N_2O levels in 650,000 years.

What are the consequences of disrupting the carbon and nitrogen cycles? Global climate change is the most obvious example. It is important to keep in mind that weather is not the same as climate. While North America has suffered some of the hottest summers on record in the past decade, a single day or week in July that is particularly hot is not, by itself, evidence of global climate change. Global climate change is measured over decades and includes many parameters such as surface temperature on land and sea, and in the atmosphere and troposphere; rates of precipitation; and frequency of extreme weather. Based on these analyses, the average global temperature has increased 0.74°C, and this rise is directly correlated with fossil fuel combustion to CO_2 (figure 28.13). Depending on the rate of continued increase in greenhouse gases, the average global surface temperature is predicted to rise between 1.1 and 6.4°C by 2100.

An important question is how will microbes respond to a changing world. Because for the vast majority of Earth's history, microorganisms have been the drivers of elemental cycling, changes in microbial activities will have a major impact on the rate and magnitude of greenhouse gas accumulation and global climate change. The role microbes play in balancing carbon and nitrogen fluxes has opened new avenues of research in microbial ecology.

Retrieve, Infer, Apply

1. List three greenhouse gases. Discuss their origins.
2. Discuss the possible role of forests in the control of CO_2.
3. How do changes in the nitrogen cycle caused by fertilization influence the carbon cycle?
4. Given that each microbial group has an optimum temperature range for growth, how might you predict changes to a soil microbial community living in your geographical area?

Figure 28.12 Natural and Human-Made Influences on the Nitrogen Cycle.
MICRO INQUIRY *What organisms benefit from nitrification?*

Figure 28.13 Global Annual-Mean Surface Air Temperature Change. Data is derived from the meteorological station network, Goddard Institute for Space Sciences, http://data.giss.nasa.gov/gistemp/graphs/.

21st-Century Microbiology

Prescott's Microbiology leads the way with updated text devoted to global climate change, biofuels, and microbial fuel cells. For more, see chapters 28, 30, 42, and 43.

Metagenomics and the Human Microbiome

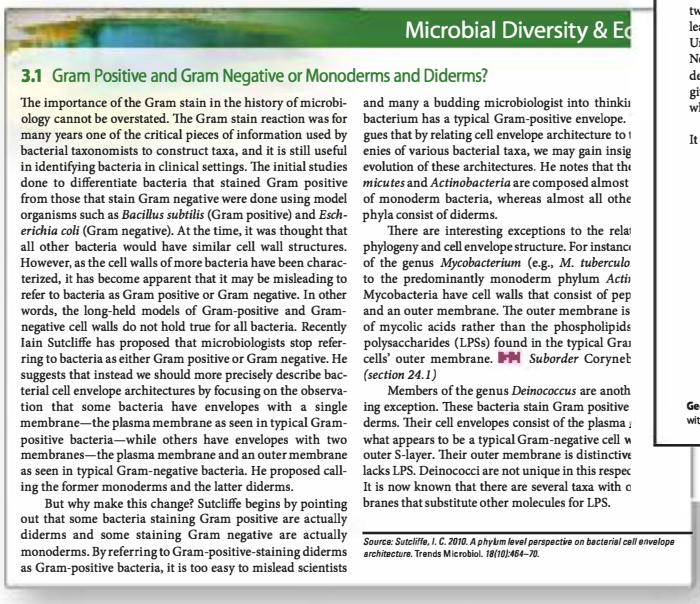
The updated genomics chapter covers the technical aspects of metagenomics, and the human microbiome is discussed in the context of microbial interactions in chapters 18 and 32.

Laboratory Safety

Reflecting forthcoming recommendations from the American Society for Microbiology, chapter 37 provides specific guidance for laboratory best practices to help instructors provide safe conditions during the teaching of laboratory exercises.

Special Interest Essays

Organized into four themes—Microbial Diversity & Ecology, Techniques & Applications, Historical Highlights, and Disease—these focused and interesting essays provide additional insight to relevant topics.



Microbial Diversity & Ec

3.1 Gram Positive and Gram Negative or Monoderms and Diderms?

The importance of the Gram stain in the history of microbiology cannot be overstated. The Gram stain reaction was for many years one of the critical pieces of information used by bacterial taxonomists to construct taxa, and it is still useful in identifying bacteria in clinical settings. The initial studies done to differentiate bacteria that stained Gram positive from those that stain Gram negative were done using model organisms such as *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative). At the time, it was thought that all other bacteria would have similar cell wall structures. However, as the cell walls of more bacteria have been characterized, it has become apparent that it may be misleading to refer to bacteria as Gram positive or Gram negative. In other words, the long-held models of Gram-positive and Gram-negative cell walls do not hold true for all bacteria. Recently Iain Sutcliffe has proposed that microbiologists stop referring to bacteria as either Gram positive or Gram negative. He suggests that instead we should more precisely describe bacterial cell envelope architectures by focusing on the observation that some bacteria have envelopes with a single membrane—the plasma membrane as seen in typical Gram-positive bacteria—while others have envelopes with two membranes—the plasma membrane and an outer membrane as seen in typical Gram-negative bacteria. He proposed calling the former monoderms and the latter diderms.

But why make this change? Sutcliffe begins by pointing out that some bacteria staining Gram positive are actually diderms and some staining Gram negative are actually monoderms. By referring to Gram-positive-staining diderms as Gram-positive bacteria, it is too easy to mislead scientists and many a budding microbiologist into thinki bacterium has a typical Gram-positive envelope. gues that by relating cell envelope architecture to enies of various bacterial taxa, we may gain insig evolution of these architectures. He notes that th *micutes* and *Actinobacteria* are composed almost of monoderm bacteria, whereas almost all othe phyla consist of diderms.

There are interesting exceptions to the rela phylogeny and cell envelope structure. For instanc of the genus *Mycobacterium* (e.g., *M. tuberculo* to the predominantly monoderm phylum *Acti* Mycobacteria have cell walls that consist of pep and an outer membrane. The outer membrane is of mycolic acids rather than the phospholipids polysaccharides (LPSs) found in the typical Gra cells' outer membrane. *Suborder* Corynet *(section 24.1)*

Members of the genus *Deinococcus* are anoth ing exception. These bacteria stain Gram positive derms. Their cell envelopes consist of the plasma what appears to be a typical Gram-negative cell w outer S-layer. Their outer membrane is distinctive lacks LPS. Deinococci are not unique in this respec It is now known that there are several taxa with c branes that substitute other molecules for LPS.

Source: Sutcliffe, I. C. 2010. A phylum level perspective on bacterial cell envelope architecture. Trends Microbiol. *18(10):464–70.*

Disease

26.1 White-Nose Syndrome Is Decimating North American Bat Populations

Bats evoke all kinds of images. Some people immediately think of vampire bats and are repulsed. Others think of the large fruit bats often called flying foxes. If you have spent a summer evening outdoors on the east coast of North America, mosquitoes and the small bats that eat them may come to mind. A new scene can now be added to these: bats with white fungal hyphae growing around their muzzles (box figure). This is the hallmark of white-nose syndrome (WNS), and if its rate of infection continues unchecked, it is projected to eliminate the most common bat species in eastern North America (*Myotis lucifugus*) by 2026.

WNS was first spotted in 2006 among bats hibernating in a cave near Albany, NY. Scientists quickly became alarmed for two reasons. First, it spreads rapidly—it's known to occur in at least six bat species and is now found from the mid-Atlantic United States, northward into Canada (Ontario, Quebec, and New Brunswick), and as far west as Oklahoma. Second, it is deadly. A population of bats declines from 30 to 99% in any given infected hibernacula (the place where bats hibernate, which unfortunately rhymes with Dracula).

WNS is caused by the ascomycete *Geomyces destructans*. It colonizes a bat's wings, muzzle, and ears where it first erodes the epidermis and then invades the underlying skin and connective tissue. Despite the name WNS, the primary site of infection (and the anatomical site harmed most) is the wing. Wings provide a large surface area for colonization, and once infected, the thin layer of skin is easily damaged, leading to adverse physiological changes during hibernation. These in turn result in premature awakening, loss of essential fat reserves, and strange behavior.

Where did this pathogen come from and why does it infect bats? The best hypothesis regarding its origin is that humans inadvertently brought it from Europe, where it causes mild infection in at least one hibernating bat species. This makes *G. destructans* an apparent case of pathogen pollution—the human introduction of invasive pathogens of wildlife and domestic animal populations that threaten biodiversity and ecosystem function.

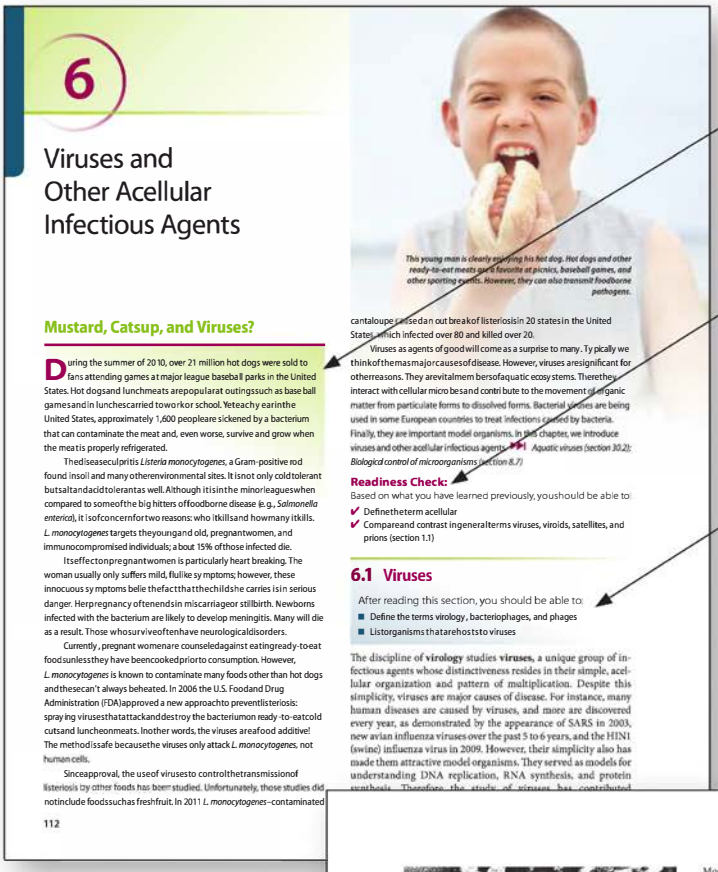
The capacity of *G. destructans* to sweep through bat populations results from a "perfect storm" of host- and pathogen-associated factors. *G. destructans* is psychrophilic, with a growth optimum around 12°C; it does not grow above 20°C. All infected bat species hibernate in cold and humid environments such as caves and mines. Because their metabolic rate is drastically reduced during hibernation, their body temperature reaches that of their surroundings, between 2 and 7°C. Thus WNS is only seen in hibernating bats or those that have just emerged from hibernation. When metabolically active, the bat's body temperature is too warm to support pathogen growth.

While it is too late to save the estimated 6 million bats that have already succumbed to WNS, microbiologists, conservationists, and government agencies are trying to limit the continued decline in bat populations. Caves have been closed to human traffic, and protocols for decontamination after visiting hibernacula have been developed to limit the spread from cave to cave. Although we cannot cure sick bats, it is our responsibility to stop the continued spread of this pathogen.

Geomyces destructans causes WNS. A little brown bat (*Myotis lucifugus*) with the white fungal hyphae (arrow) for which WNS is named.

Read more: Frick, W. F. et al., 2010. An emerging disease causes regional population collapse of a common North American bat species. Science 329:679–682.

Student-Friendly Organization



6

Viruses and Other Acellular Infectious Agents

This young man is clearly enjoying his hot dog. Hot dogs and other ready-to-eat meats are a favorite at picnics, baseball games, and other sporting events. However, they can also transmit foodborne pathogens.

Mustard, Catsup, and Viruses?

During the summer of 2010, over 21 million hot dogs were sold to fans attending games at major league baseball parks in the United States. Hot dogs and lunchmeats are popular at outings such as baseball games and in lunches carried to work or school. Yet each year in the United States, approximately 1,600 people are sickened by a bacterium that can contaminate the meat and, even worse, survive and grow when the meat is properly refrigerated.

The disease-causing bacteria *Listeria monocytogenes*, a Gram-positive rod found in soil and many other environmental sites. It is not only cold-tolerant but salt and acid tolerant as well. Although it is in the minor leagues when compared to some of the big hitters of foodborne disease (e.g., *Salmonella enterica*), it is of concern for two reasons: who it kills and how many it kills. *L. monocytogenes* targets the young and old, pregnant women, and immunocompromised individuals; about 15% of those infected die.

Its effect on pregnant women is particularly heart breaking. The woman usually only suffers mild, flu-like symptoms; however, these innocuous symptoms belie the fact that the child she carries is in serious danger. Her pregnancy often ends in miscarriage or stillbirth. Newborns infected with the bacterium are likely to develop meningitis. Many will die as a result. Those who survive often have neurological disorders.

Currently, pregnant women are counseled against eating ready-to-eat food unless they have been cooked prior to consumption. However, *L. monocytogenes* is known to contaminate many foods other than hot dogs and these aren't always reheated. In 2006 the U.S. Food and Drug Administration (FDA) approved a new approach to prevent listeriosis: spraying viruses that attack and destroy the bacterium on ready-to-eat cold cuts and luncheon meats. In other words, the viruses are a food additive! The method is safe because the viruses only attack *L. monocytogenes*, not human cells.

Since approval, the use of viruses to control the transmission of listeriosis by other foods has been studied. Unfortunately, those studies did not include foods such as fresh fruit. In 2011 *L. monocytogenes*–contaminated cantaloupes caused an outbreak of listeriosis in 20 states in the United States, which infected over 80 and killed over 20.

Viruses as agents of good will come as a surprise to many. Typically we think of them as major causes of disease. However, viruses are significant for other reasons. They are vital members of aquatic ecosystems. There they interact with cellular microbes and contribute to the movement of organic matter from particulate forms to dissolved forms. Bacterial viruses are being used in some European countries to treat infections caused by bacteria. Finally, they are important model organisms. In this chapter, we introduce viruses and other acellular infectious agents. ◀◀ *Aquatic viruses (section 30.2); Biological control of microorganisms (section 8.7)*

Readiness Check:

Based on what you have learned previously, you should be able to:

- ✔ Define the term acellular
- ✔ Compare and contrast in general terms viruses, viroids, satellites, and prions (section 1.1)

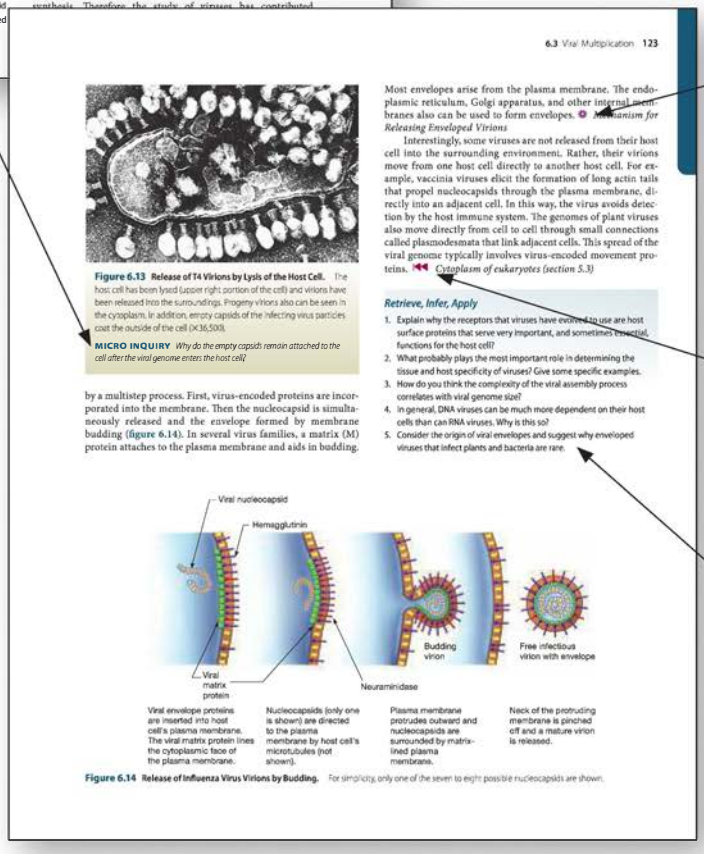
6.1 Viruses

After reading this section, you should be able to:

- Define the terms virology, bacteriophages, and phages
- List organisms that are hosts to viruses

The discipline of **virology** studies **viruses,** a unique group of infectious agents whose distinctiveness resides in their simple, acellular organization and pattern of multiplication. Despite this simplicity, viruses are major causes of disease. For instance, many human diseases are caused by viruses, and more are discovered every year, as demonstrated by the appearance of SARS in 2003, new avian influenza viruses over the past 5 to 6 years, and the H1N1 (swine) influenza virus in 2009. However, their simplicity also has made them attractive model organisms. They served as models for understanding DNA replication, RNA synthesis, and protein synthesis. Therefore the study of viruses has contributed

112



6.3 Viral Multiplication 123

Figure 6.13 **Release of T4 Virions by Lysis of the Host Cell.** The host cell has been lysed (upper right; portion of the cell) and virions have been released into the surroundings. Progeny virions also can be seen in the cytoplasm. In addition, empty capsids of the infecting virus particles coat the outside of the cell (×36,500).

MICRO INQUIRY *Why do the empty capsids remain attached to the cell after the viral genome enters the host cell?*

by a multistep process. First, virus-encoded proteins are incorporated into the membrane. Then the nucleocapsid is simultaneously released and the envelope formed by membrane budding (figure 6.14). In several virus families, a matrix (M) protein attaches to the plasma membrane and aids in budding.

Most envelopes arise from the plasma membrane. The endoplasmic reticulum, Golgi apparatus, and other internal membranes also can be used to form envelopes. ● *Mechanism for Releasing Enveloped Virions*

Interestingly, some viruses are not released from their host cell into the surrounding environment. Rather, their virions move from one host cell directly to another host cell. For example, vaccinia viruses elicit the formation of long actin tails that propel nucleocapsids through the plasma membrane, directly into an adjacent cell. In this way, the virus avoids detection by the host immune system. The genomes of plant viruses also move directly from cell to cell through small connections called plasmodesmata that link adjacent cells. This spread of the viral genome typically involves virus-encoded movement proteins. ◀◀ *Cytoplasm of eukaryotes (section 5.3)*

Retrieve, Infer, Apply

1. Explain why the receptors that viruses have evolved to use are host surface proteins that serve very important, and sometimes essential, functions for the host cell?
2. What probably plays the most important role in determining the tissue and host specificity of viruses? Give some specific examples.
3. How do you think the complexity of the viral assembly process correlates with viral genome size?
4. In general, DNA viruses can be much more dependent on their host cells than can RNA viruses. Why is this so?
5. Consider the origin of viral envelopes and suggest why enveloped viruses that infect plants and bacteria are rare.

Figure 6.14 **Release of Influenza Virus Virions by Budding.** For simplicity, only one of the seven to eight possible nucleocapsids are shown.

New! Newsworthy Stories—Each chapter begins with a real-life story illustrating the relevance of the content covered in the upcoming text.

New! Readiness Check—The introduction to each chapter includes a skills checklist that defines the prior knowledge a student needs to understand the material that follows.

New! Learning Outcomes—Every section in each chapter begins with a list of content-based activities students should be able to perform after reading.

Micro Inquiry—Select figures throughout every chapter contain probing questions, adding another assessment opportunity for the student.

Animation Icon—This symbol indicates material presented in the text is also accompanied by an animation on the text website at www.mhhe.com/willey9.

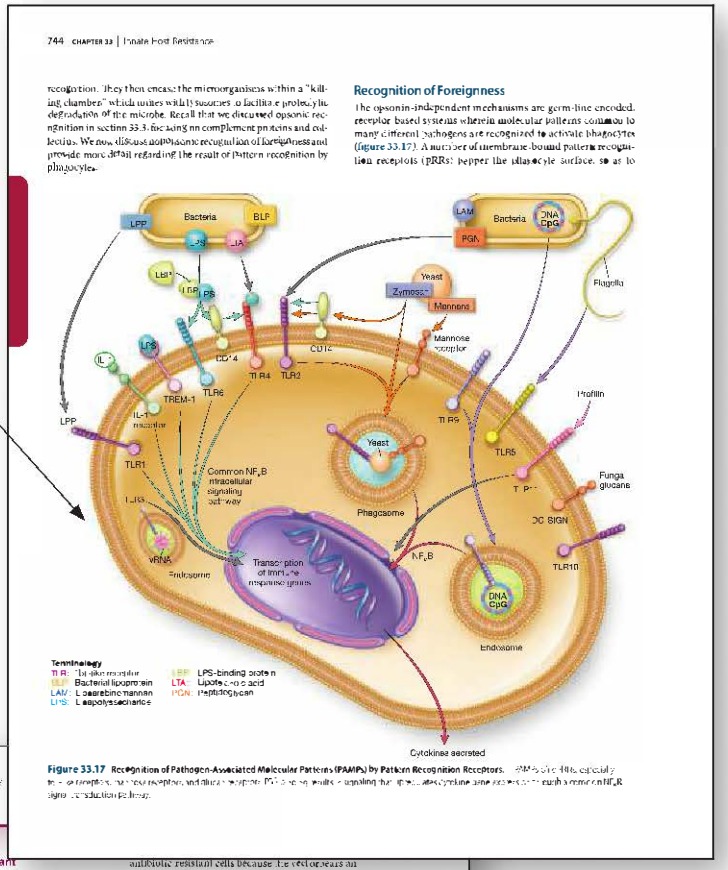
Cross-Referenced Notes—In-text references refer students to other parts of the book to review.

Retrieve, Infer, Apply—Questions within the narrative of each chapter assist students in mastering section concepts before moving on to other topics.

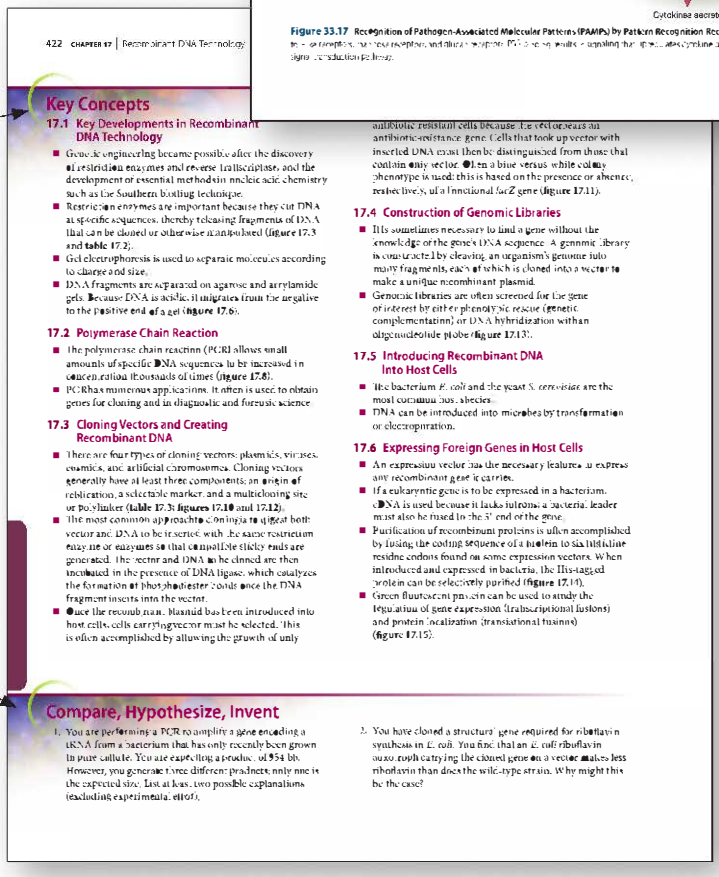
Student-Friendly Organization

Vivid Instructional Art Program—Three-dimensional renditions and bright, attractive colors enhance learning.

More Annotated Figures—All key metabolic pathways and molecular processes are now annotated, so that each step is clearly illustrated and explained.



Key Concepts—At the end of each chapter and organized by numbered headings, this feature distills the content to its essential components with completely cross-referenced figures and tables.



Compare, Hypothesize, Invent—Includes questions taken from current literature; designed to stimulate analytical problem-solving skills.

List of Content Changes

Each chapter has been thoroughly reviewed and many have undergone significant revision. All now feature pedagogical elements, including a Readiness Check for the chapter and Learning Outcomes for each section therein.

Part I

Chapter 1—Evolution is the driving force of all biological systems; this is made clear by introducing essential concepts of microbial evolution first.

Chapter 3—Coverage of bacterial cellular structure and function. The chapter now includes a discussion of nutrient uptake in the section on bacterial plasma membranes.

Chapter 4—Growing understanding of the distinctive characteristics of archaea has warranted the creation of a new chapter that focuses on their cell structure and function. Comparisons to bacteria are made throughout the chapter.

Chapter 5—An introduction to eukaryotic cell structure and function, with emphasis on eukaryotic microbes. More detailed information on protist and fungal cells is presented in chapters 25 (*The Protists*) and 26 (*The Fungi*), which also focus on the diversity of these microbes. Comparisons between bacteria, archaea, and eukaryotes are included throughout the chapter.

Chapter 6—This chapter, entitled *Viruses and Other Acellular Infectious Agents*, surveys the essential morphological, physiological, and genetic elements of viruses as well as viroids, satellites, and prions. This chapter completes our four-chapter introduction of microbial life.

Part II

Chapter 7—Reorganized to initially focus on the growth of microbes outside the laboratory (including growth in oligotrophic environments) and the environmental factors that influence microbial reproduction. Topics related to laboratory culture of microbes follow.

Chapter 8—Reorganized to reflect emphasis on interruption of normal growth and reproduction functions to control microorganisms.

Chapter 9—Content focuses on the mechanism of action of each antimicrobial agent and stresses usage to limit drug resistance.

Part III

Chapter 10—This introduction to metabolism includes a new section that outlines the nature of biochemical pathways and introduces the concept of metabolic flux through the interconnected biochemical pathways used by cells.

Chapter 11—The chapter now begins with an introduction to metabolic diversity and nutritional types.

Chapter 12—Updated coverage of CO_2-fixation pathways.

Part IV

Chapter 13—Now focuses on bacterial genetic information flow with improved coverage of bacterial promoters, sigma factors, termination of DNA replication, transcription cycle, and protein folding and secretion.

Chapter 14—Now focuses on the regulation of bacterial cellular processes. The coverage of regulation of complex cellular behaviors has been significantly updated and expanded, including new material on cyclic dimeric GMP.

Chapter 15—A new chapter that considers eukaryal and archaeal genome replication and expression together. In both cases, the discussion has been updated and expanded, and reflects the similarity of information flow as carried out by members of *Archaea* and *Eukarya*.

Chapter 16—Covers mutation, repair, and recombination in the context of processes that introduce genetic variation into populations. This is now related to the evolution of antibiotic-resistant bacteria.

Chapter 17—The use of recombinant DNA approaches to construct a synthetic genome is highlighted.

Chapter 18—New principles and applications of genomic techniques, including massively parallel genome sequencing and single cell genome sequencing, are now reviewed. The growing importance of metagenomics to environmental microbiology and its use in exploring the human microbiome are introduced here.

Part V

Chapter 19—Microbial evolution, introduced in chapter 1, is expanded with a complete discussion of the endosymbiotic theory, and the concept and definition of a microbial species.

Chapter 20—Expanded coverage of archaeal physiology includes new figures presenting archaeal-specific anabolic and catabolic pathways. The evolutionary advantage of each pathway is discussed in the context of archaeal ecology.

Chapter 21—Now includes mycoplasmas, in keeping with *Bergey's Manual;* new figures illustrating the life cycle of *Chlamydia* are included.

List of Content Changes

Chapter 22—Expanded coverage of proteobacterial physiology with content on C1 metabolism, including several figures.

Chapter 24—Increased coverage of streptomycetes, with new graphics illustrating their life cycle and their importance in antibiotic production.

Chapter 27—Updated discussion of virus taxonomy and phylogeny, including increased coverage of archaeal viruses and the CRISPR/CAS system.

Part VI

Chapter 28—The description of each nutrient cycle is accompanied by a new "student-friendly" figure that distinguishes between reductive and oxidative reactions. Expanded coverage of the interaction between nutrient cycles is also newly illustrated.

Chapter 29—This chapter continues to emphasize culture-based techniques as the "gold standard" and reviews some new, innovative approaches. The chapter also discusses a variety of culture-independent techniques used to assess populations and communities.

Chapter 30—Updated and expanded discussion of freshwater microbiology is complemented by discussion of carbon cycling in the open ocean and its implications for global climate change.

Chapter 31—New and updated coverage of mycorrhizae, with an emphasis on host-microbe communication and evolutionary similarities to rhizobia.

Chapter 32—Microbial relationships are presented along with human-microbe interactions, helping to convey the concept that the human body is an ecosystem. New and increased coverage of the human microbiome.

Part VII

Chapter 33—Reorganized and updated, this chapter on innate host resistance provides in-depth coverage of physical and chemical components of the nonspecific host response followed by an overview of cells, tissues, and organs of the immune system. This includes a step-by-step discussion of how microorganisms and damaged tissues are identified by the host using pattern recognition to remove them. Discussions of phagocytosis and inflammation are updated and reflect molecular mechanisms. The groundwork is laid for a full appreciation of the connections between the adaptive and innate arms of the immune system.

Chapter 34—Reorganized and updated to enhance linkages between innate and adaptive immune activities. Discussions integrate cell biology, physiology, and genetics concepts to present the immune system as a unified response having various components. Implications of dysfunctional immune actions are also discussed.

Chapter 35—This chapter has been re-titled *Pathogenicity and Infection,* reflecting its emphasis on microbial strategies for survival that can lead to human disease. The essential elements required for a pathogen to establish infection are introduced and virulence mechanisms highlighted. It follows the immunology chapters to stress that the host-parasite relationship is dynamic, with adaptations and responses offered by both host and parasite.

Part VIII

Chapter 36—This chapter has been updated to reflect the workflow and practice of a modern clinical laboratory. Emphasis is on modern diagnostic testing to identify infectious disease.

Chapter 37—Expanded focus on the important role of laboratory safety, especially in the teaching laboratory. Discussion emphasizes modern epidemiology as an investigative science and its role in preventative medicine. Disease prevention strategies are highlighted.

Chapter 38—Updated and expanded coverage includes viral pathogenesis and common viral infections.

Chapter 39—Expanded coverage of bacterial organisms and their common methods leading to human disease.

Chapter 40—Refocused to reflect disease transmission routes as well as expanded coverage of fungal and protozoal diseases.

Part IX

Chapter 41—Expanded discussion of probiotics in the context of the human microbiome.

Chapter 42—This chapter has been reorganized to illustrate the importance of industrial microbiology by presenting common microbial products—including biofuels—first. This is followed by an updated discussion of strain development, including in vivo and in vitro directed evolution.

Chapter 43—Updated discussion of water purification, wastewater treatment, and bioremediation. This includes the development and use of microbial fuel cells.

Acknowledgments

We would like to thank the Reviewers, who provided constructive reviews of every chapter. Their specialized knowledge helped us assimilate more reliable sources of information and find more effective ways of expressing an idea for the student reader.

Reviewers
Tamarah Adair, *Baylor University*
Richard Adler, *University of Michigan–Dearborn*
Fernando Agudelo-Silva, *College of Marin*
Shivanthi Anandan, *Drexel University*
Penny Antley, *University of Louisiana at Lafayette*
Suzanne Barth, *The University of Texas at Austin*
Larry Barton, *University of New Mexico*
Nancy Boury, *Iowa State University*
Ginger Brininstool, *Louisiana State University–Baton Rouge*
Linda Bruslind, *Oregon State University*
Alison Buchan, *University of Tennessee*
Jim Buritt, *University of Wisconsin–Stout*
Martha Smith Caldas, *Kansas State University*
Joseph Caruso, *Florida Atlantic University–Boca Raton*
Andrei Chistoserdov, *University of Louisiana at Lafayette*
Carlton Cooper, *University of Delaware*
Susan Deines, *Colorado State University*
John Dennehy, *Queens College*
James Dickson, *Iowa State University*
Ronald Dubreuil, *University of Illinois at Chicago*
Paul Dunlap, *University of Michigan–Ann Arbor*
Mary Farone, *Middle Tennessee State University*
Babu Fathepure, *Oklahoma State University–Stillwater*
Kathy Feldman, *University of Connecticut Storrs*
Bernard Frye, *University of Texas Arlington*
Sandra Gibbons, *University of Illinois at Chicago*
Elizabeth Good, *University of Illinois at Urbana-Champaign*
Melanie Griffin, *Kennesaw State University*
Janet Haynes, *Long Island University, Brooklyn*
Michael Ibba, *The Ohio State University*
David Jenkins, *The University of Alabama Birmingham*
Dennis Kitz, *Southern Illinois University Edwardsville*
James Koukl, *The University of Texas at Tyler*
Shashi Kumar, *Saint Mary Mercy Hospital*
Jeffrey Leblond, *Middle Tennessee State University*
Richard Long, *University of South Carolina*
Jean Lu, *Kennesaw State University*
Mark McBride, *University of Wisconsin–Milwaukee*
Vance McCracken, *Southern Illinois University Edwardsville*
Donald Mcgarey, *Kennesaw State University*
Robert McLean, *Texas State University*
Tamara Mcnealy, *Clemson University*
Rita Moyes, *Texas A&M University*
Karen Nakaoka, *Weber State University*
Comer Patterson, *Texas A&M University, College Station*
Ed Perry, *Faulkner State Community College*
Thomas Pistole, *University of New Hampshire*
Ronald Porter, *Penn State University–University Park*
Jackie Reynolds, *Richland College*
Margaret Richey, *Centre College*
Veronica Riha, *Madonna University*
Timberley Roane, *University of Colorado Denver*
Jerry Sanders, *University of Michigan–Flint*
Pratibha Saxena, *The University of Texas at Austin*
Mark Schneegurt, *Wichita State University*
Sasha A. Showsh, *University of Wisconsin–Eau Claire*
Khalifah Sidik, *University of Illinois College of Medicine at Rockford*
Deborah Siegele, *Texas A&M University*
Jack Steiert, *Missouri State University*
Raji Subramanian, *NOVA Community College Annandale*
Karen Sullivan, *Louisiana State University–Baton Rouge*
Cristina Takacs-Vesbach, *University of New Mexico*
Monica Tischler, *Benedictine University*
Virginia Young, *Mercer University*
Jianmin Zhong, *Humboldt State University*

The authors wish to extend their gratitude to our editors, Kathy Lowenberg, Kathleen Timp, Angela FitzPatrick, Sandy Wille, and Lynn Breithaupt. We would also like to thank our photo editor, Mary Reeg, and the tremendous talent and patience displayed by the artists. We are also very grateful to the many reviewers who provided helpful criticism and analysis. Finally, we thank our spouses and children who provided support and tolerated our absences (mental, if not physical) while we completed this demanding project.

Contents

About the Authors iii
Preface iv

Part One Introduction to Microbiology

1 The Evolution of Microorganisms and Microbiology 1
1.1 Members of the Microbial World 1
1.2 Microbial Evolution 4
1.3 Microbiology and Its Origins 11
1.4 Microbiology Today 17

2 Microscopy 22
2.1 Lenses and the Bending of Light 22
2.2 Light Microscopes 23
2.3 Preparation and Staining of Specimens 31
2.4 Electron Microscopy 34
2.5 Scanning Probe Microscopy 39

3 Bacterial Cell Structure 42
3.1 The "Prokaryote" Controversy 42
3.2 A Typical Bacterial Cell 43
3.3 Bacterial Plasma Membranes 47
3.4 Bacterial Cell Walls 53
Microbial Diversity & Ecology 3.1
Gram Positive and Gram Negative or Monoderms and Diderms? 54
3.5 Cell Envelope Layers Outside the Cell Wall 61
3.6 Bacterial Cytoplasm 62
3.7 External Structures 69
3.8 Bacterial Motility and Chemotaxis 72
3.9 Bacterial Endospores 76

4 Archaeal Cell Structure 82
4.1 A Typical Archaeal Cell 82
4.2 Archaeal Cell Envelopes 84
4.3 Archaeal Cytoplasm 87
4.4 External Structures 88
4.5 Comparison of *Bacteria* and *Archaea* 90

5 Eukaryotic Cell Structure 92
5.1 A Typical Eukaryotic Cell 92
5.2 Eukaryotic Cell Envelopes 94
5.3 Cytoplasm of Eukaryotes 96
5.4 Organelles of the Secretory and Endocytic Pathways 97
5.5 Organelles Involved in Genetic Control of the Cell 101
5.6 Organelles Involved in Energy Conservation 103
5.7 External Structures 104
Microbial Diversity & Ecology 5.1
There Was an Old Woman Who Swallowed a Fly 106
5.8 Comparison of Bacterial, Archaeal, and Eukaryotic Cells 108

6 Viruses and Other Acellular Infectious Agents 112
6.1 Viruses 112
6.2 Virion Structure 113
Microbial Diversity & Ecology 6.1
Host-Independent Growth of an Archaeal Virus 114
6.3 Viral Multiplication 119
6.4 Types of Viral Infections 124
6.5 Cultivation and Enumeration of Viruses 127
6.6 Viroids and Satellites 129
6.7 Prions 130

Part Two Microbial Nutrition, Growth, and Control

7 Microbial Growth 133
7.1 Reproductive Strategies 133
7.2 Bacterial Cell Cycle 134
Microbial Diversity & Ecology 7.1
Cytokinesis Without FtsZ 137
7.3 Influences of Environmental Factors on Growth 141
7.4 Microbial Growth in Natural Environments 149
7.5 Laboratory Culture of Cellular Microbes 154
7.6 Growth Curve: When One Becomes Two and Two Become Four . . . 160
7.7 Measurement of Microbial Population Size 164
7.8 Continuous Culture of Microorganisms 168

8 Control of Microorganisms in the Environment 172
8.1 Principles of Microbial Control 172
8.2 The Pattern of Microbial Death 174
8.3 Mechanical Removal Methods 175

Contents

8.4 Physical Control Methods 177
8.5 Chemical Control Agents 180
8.6 Evaluation of Antimicrobial Agent Effectiveness 184
8.7 Biological Control of Microorganisms 186

9 Antimicrobial Chemotherapy 189
9.1 The Development of Chemotherapy 189
9.2 General Characteristics of Antimicrobial Drugs 190
9.3 Determining the Level of Antimicrobial Activity 193
9.4 Antibacterial Drugs 195
9.5 Antifungal Drugs 201
9.6 Antiviral Drugs 203
9.7 Antiprotozoan Drugs 205
9.8 Factors Influencing Antimicrobial Drug Effectiveness 206

Part Three Microbial Metabolism

10 Introduction to Metabolism 210
10.1 Metabolism: Important Principles and Concepts 211
10.2 ATP: The Major Energy Currency of Cells 213
10.3 Redox Reactions: Reactions of Central Importance in Metabolism 215
10.4 Electron Transport Chains: Sets of Sequential Redox Reactions 216
10.5 Biochemical Pathways 219
10.6 Enzymes and Ribozymes 220
10.7 Regulation of Metabolism 224

11 Catabolism: Energy Release and Conservation 230
11.1 Metabolic Diversity and Nutritional Types 230
11.2 Chemoorganotrophic Fueling Processes 232
11.3 Aerobic Respiration 235
11.4 From Glucose to Pyruvate 235
11.5 Tricarboxylic Acid Cycle 239
11.6 Electron Transport and Oxidative Phosphorylation 239
11.7 Anaerobic Respiration 247
11.8 Fermentation 248
11.9 Catabolism of Organic Molecules Other Than Glucose 251
11.10 Chemolithotrophy 253
Microbial Diversity & Ecology 11.1
Acid Mine Drainage 255
11.11 Phototrophy 256

12 Anabolism: The Use of Energy in Biosynthesis 266
12.1 Principles Governing Biosynthesis 266
12.2 Precursor Metabolites 268
12.3 CO_2 Fixation 269
12.4 Synthesis of Carbohydrates 272
12.5 Synthesis of Amino Acids 274
12.6 Synthesis of Purines, Pyrimidines, and Nucleotides 281
12.7 Lipid Synthesis 283

Part Four Microbial Molecular Biology and Genetics

13 Bacterial Genome Replication and Expression 287
13.1 DNA as Genetic Material 288
13.2 Nucleic Acid and Protein Structure 288
13.3 DNA Replication in Bacteria 293
13.4 Bacterial Gene Structure 301
13.5 Transcription in Bacteria 304
13.6 The Genetic Code 309
13.7 Translation in Bacteria 311
13.8 Protein Maturation and Secretion 319

14 Regulation of Bacterial Cellular Processes 325
14.1 Levels of Regulation 326
14.2 Regulation of Transcription Initiation 326
14.3 Regulation of Transcription Elongation 333
14.4 Regulation of Translation 336
14.5 Regulating Complex Cellular Processes 338

Contents

15 Eukaryotic and Archaeal Genome Replication and Expression **353**

15.1 Why Consider Eukaryotic and Archaeal Genetics Together? 354
15.2 DNA Replication 354
15.3 Transcription 358
15.4 Translation and Protein Maturation and Localization 363
15.5 Regulation of Cellular Processes 367

16 Mechanisms of Genetic Variation **372**

16.1 Mutations 372
16.2 Detection and Isolation of Mutants 378
16.3 DNA Repair 380
16.4 Creating Additional Genetic Variability 383
16.5 Transposable Elements 385
16.6 Bacterial Conjugation 387
16.7 Bacterial Transformation 393
16.8 Transduction 396
16.9 Evolution in Action: The Development of Antibiotic Resistance in Bacteria 398

17 Recombinant DNA Technology **404**

17.1 Key Developments in Recombinant DNA Technology 405
Techniques & Applications 17.1
Streptavidin-Biotin Binding and Biotechnology 410
17.2 Polymerase Chain Reaction 411
17.3 Cloning Vectors and Creating Recombinant DNA 412
Techniques & Applications 17.2
How to Build a Microorganism 416
17.4 Construction of Genomic Libraries 417
17.5 Introducing Recombinant DNA into Host Cells 418
17.6 Expressing Foreign Genes in Host Cells 419

18 Microbial Genomics **424**

18.1 Determining DNA Sequences 424
18.2 Genome Sequencing 429
18.3 Bioinformatics 431
18.4 Functional Genomics 433
18.5 Proteomics 437
18.6 Systems Biology 440
18.7 Comparative Genomics 440
18.8 Metagenomics 443

Part Five The Diversity of the Microbial World

19 Microbial Taxonomy and the Evolution of Diversity **447**

19.1 Introduction to Microbial Taxonomy 448
19.2 Taxonomic Ranks 449
19.3 Exploring Microbial Taxonomy and Phylogeny 450
19.4 Phylogenetic Trees 456
19.5 Evolutionary Processes and the Concept of a Microbial Species 459
19.6 *Bergey's Manual of Systematic Bacteriology* 464
Microbial Diversity & Ecology 19.1
"Official" Nomenclature Lists—A Letter from *Bergey's* 465

20 The *Archaea* **469**

20.1 Overview of the *Archaea* 470
20.2 Phylum *Crenarchaeota* 476
20.3 Phylum *Euryarchaeota* 480

21 The Deinococci, Mollicutes, and Nonproteobacterial Gram-Negative Bacteria **489**

21.1 *Aquificae* and *Thermotogae* 490
21.2 *Deinococcus-Thermus* 490
21.3 Class *Mollicutes* (Phylum *Tenericutes*) 491
21.4 Photosynthetic Bacteria 494
21.5 Phylum *Planctomycetes* 501
21.6 Phylum *Chlamydiae* 501
21.7 Phylum *Spirochaetes* 504
21.8 Phylum *Bacteroidetes* 506
21.9 Phylum *Verrucomicrobia* 507

22 The Proteobacteria **509**

22.1 Class *Alphaproteobacteria* 510
22.2 Class *Betaproteobacteria* 518
22.3 Class *Gammaproteobacteria* 522
Microbial Diversity & Ecology 22.1
Bacterial Bioluminescence 530
22.4 Class *Deltaproteobacteria* 533
22.5 Class *Epsilonproteobacteria* 538

Contents

23 Firmicutes: The Low G + C Gram-Positive Bacteria **542**
23.1 Class *Clostridia* *543*
23.2 Class *Bacilli* 547

24 Actinobacteria: The High G + C Gram-Positive Bacteria **555**
24.1 Order *Actinomycetales* 557
24.2 Order *Bifidobacteriales* 566

25 The Protists **568**
25.1 Overview of Protists 569
25.2 Supergroup *Excavata* 571
25.3 Supergroup *Amoebozoa* 573
25.4 Supergroup *Rhizaria* 574
25.5 Supergroup *Chromalveolata* 577
25.6 Supergroup *Archaeplastida* 584

26 The *Fungi* (*Eumycota*) **588**
26.1 Overview of Fungal Biology 590
26.2 *Chytridiomycota* 593
26.3 *Zygomycota* 593
26.4 *Glomeromycota* 594
26.5 *Ascomycota* 595
26.6 *Basidiomycota* 598
Disease 26.1 White-Nose Syndrome Is Decimating North American Bat Populations 599
26.7 *Microsporidia* 601

27 Viruses **604**
27.1 Virus Taxonomy and Phylogeny 604
27.2 Double-Stranded DNA Viruses 606
Microbial Diversity & Ecology 27.1 What Is a Virus? 617
27.3 Single-Stranded DNA Viruses 617
27.4 RNA Viruses: Unity Amidst Diversity 619
27.5 Double-Stranded RNA Viruses 620
27.6 Plus-Strand RNA Viruses 622
27.7 Minus-Strand RNA Viruses 624
27.8 Retroviruses 626
27.9 Reverse Transcribing DNA Viruses 628

Part Six Ecology and Symbiosis

28 Biogeochemical Cycling and Global Climate Change **632**
28.1 Biogeochemical Cycling 633
28.2 Global Climate Change 642

29 Methods in Microbial Ecology **646**
29.1 Culturing Techniques 647
29.2 Assessing Microbial Diversity 651
29.3 Assessing Microbial Community Activity 655

30 Microorganisms in Marine and Freshwater Ecosystems **660**
30.1 Water as a Microbial Habitat 661
30.2 Microorganisms in Marine Ecosystems 662
30.3 Microorganisms in Freshwater Ecosystems 672

31 Microorganisms in Terrestrial Ecosystems **679**
31.1 Soils as a Microbial Habitat 680
31.2 Microorganisms in the Soil Environment 683
31.3 Microbe-Plant Interactions 684
31.4 The Subsurface Biosphere 696

32 Microbial Interactions **699**
32.1 Microbial Interactions 700
Microbial Diversity & Ecology 32.1 *Wolbachia pipientis:* The World's Most Infectious Microbe? 701
32.2 Human-Microbe Interactions 713
Microbial Diversity & Ecology 32.2 Do Bacteria Make People Fat? 714
32.3 Normal Microbiota of the Human Body 715

Part Seven Pathogenicity and Host Response

33 Innate Host Resistance **723**
33.1 Innate Resistance Overview 724
33.2 Physical and Mechanical Barrier Defenses of Innate Resistance 725
33.3 Chemical Mediators in Innate Resistance 728
33.4 Cells, Tissues, and Organs of the Immune System 735
33.5 Phagocytosis 743
33.6 Inflammation 748

Contents

34 Adaptive Immunity **753**
34.1 Overview of Adaptive Immunity 753
34.2 Antigens 755
34.3 Types of Adaptive Immunity 756
34.4 Recognition of Foreignness 757
34.5 T-Cell Biology 760
34.6 B-Cell Biology 764
34.7 Antibodies 767
Techniques & Applications 34.1
Monoclonal Antibody Therapy 776
34.8 Action of Antibodies 777
34.9 Acquired Immune Tolerance 778
34.10 Immune Disorders 779

35 Pathogenicity and Infection **789**
35.1 Pathogenicity and Infectious Disease 790
35.2 Virulence 793
35.3 Exposure and Transmission 802
Historical Highlights 35.1
The First Indications of Person-to-Person Spread of an Infectious Disease 803

Part Eight Microbial Diseases, Detection, and Their Control

36 Clinical Microbiology and Immunology **808**
36.1 Overview of the Clinical Microbiology Laboratory 808
36.2 Biosafety 809
36.3 Identification of Microorganisms from Specimens 812
36.4 Clinical Immunology 820

37 Epidemiology and Public Health Microbiology **830**
37.1 Epidemiology 830
Historical Highlights 37.1
The Birth of Public Health in the United States 831
Historical Highlights 37.2
John Snow, the First Epidemiologist 832
37.2 Epidemiological Methods 832
Historical Highlights 37.3
SARS: Evolution of a Virus 833
37.3 Measuring Infectious Disease Frequency 835
37.4 Patterns of Infectious Disease in a Population 836
Historical Highlights 37.4
"Typhoid Mary" 837
37.5 Emerging and Reemerging Infectious Diseases and Pathogens 839
37.6 Health-Care–Associated Infections 841
37.7 Prevention and Control of Epidemics 843
Historical Highlights 37.5
The First Immunizations 846
37.8 Bioterrorism Preparedness 848
Historical Highlights 37.6
1346—The First Recorded Biological Warfare Attack 849

38 Human Diseases Caused by Viruses and Prions **854**
38.1 Airborne Diseases 855
38.2 Arthropod-Borne Diseases 865
38.3 Direct Contact Diseases 865
38.4 Food-Borne and Waterborne Diseases 878
Historical Highlights 38.1
A Brief History of Polio 881
38.5 Zoonotic Diseases 881
Disease 38.2
Viral Hemorrhagic Fevers: A Microbial History Lesson 882
38.6 Prion Diseases 885

39 Human Diseases Caused by Bacteria **888**
39.1 Airborne Diseases 888
39.2 Arthropod-Borne Diseases 898
39.3 Direct Contact Diseases 901
Disease 39.1
A Brief History of Syphilis 909
Disease 39.2
Biofilms 910
39.4 Food-Borne and Waterborne Diseases 915
Techniques & Applications 39.3
Clostridial Toxins as Therapeutic Agents: Benefits of Nature's Most Toxic Proteins 919
39.5 Zoonotic Diseases 924
39.6 Opportunistic Diseases 926

Contents

40 Human Diseases Caused by Fungi and Protists 932
- 40.1 Pathogenic Fungi and Protists 932
- 40.2 Airborne Diseases 934
- 40.3 Arthropod-Borne Diseases 937
  - **Disease 40.1** A Brief History of Malaria 938
- 40.4 Direct Contact Diseases 944
- 40.5 Food-Borne and Waterborne Diseases 948
- 40.6 Opportunistic Diseases 952

Part Nine Applied Microbiology

41 Microbiology of Food 958
- 41.1 Microbial Growth and Food Spoilage 959
- 41.2 Controlling Food Spoilage 961
- 41.3 Food-Borne Disease Outbreaks 964
- 41.4 Detection of Food-Borne Pathogens 967
- 41.5 Microbiology of Fermented Foods 969
  - **Techniques & Applications 41.1** Chocolate: The Sweet Side of Fermentation 970
- 41.6 Probiotics 976

42 Biotechnology and Industrial Microbiology 979
- 42.1 Major Products of Industrial Microbiology 980
- 42.2 Biofuel Production 982
- 42.3 Growing Microbes in Industrial Settings 983
- 42.4 Microorganisms Used in Industrial Microbiology 985
- 42.5 Agricultural Biotechnology 990
- 42.6 Microbes as Products 992

43 Applied Environmental Microbiology 996
- 43.1 Water Purification and Sanitary Analysis 996
  - **Techniques & Applications 43.1** Waterborne Diseases, Water Supplies, and Slow Sand Filtration 999
- 43.2 Wastewater Treatment 1001
- 43.3 Microbial Fuel Cells 1008
- 43.4 Biodegradation and Bioremediation 1009

Appendix 1 A Review of the Chemistry of Biological Molecules A-1

Appendix 2 Common Metabolic Pathways A-9

Glossary G-1

Credits C-1

Index I-1

Brief Contents

About the Authors iii
Preface iv

Part One Introduction to Microbiology

1 The Evolution of Microorganisms and Microbiology 1
2 Microscopy 22
3 Bacterial Cell Structure 42
4 Archaeal Cell Structure 82
5 Eukaryotic Cell Structure 92
6 Viruses and Other Acellular Infectious Agents 112

Part Two Microbial Nutrition, Growth, and Control

7 Microbial Growth 133
8 Control of Microorganisms in the Environment 172
9 Antimicrobial Chemotherapy 189

Part Three Microbial Metabolism

10 Introduction to Metabolism 210
11 Catabolism: Energy Release and Conservation 230
12 Anabolism: The Use of Energy in Biosynthesis 266

Part Four Microbial Molecular Biology and Genetics

13 Bacterial Genome Replication and Expression 287
14 Regulation of Bacterial Cellular Processes 325
15 Eukaryotic and Archaeal Genome Replication and Expression 353
16 Mechanisms of Genetic Variation 372
17 Recombinant DNA Technology 404
18 Microbial Genomics 424

Part Five The Diversity of the Microbial World

19 Microbial Taxonomy and the Evolution of Diversity 447
20 The *Archaea* 469
21 The Deinococci, Mollicutes, and Nonproteobacterial Gram-Negative Bacteria 489
22 The Proteobacteria 509
23 Firmicutes: The Low G + C Gram-Positive Bacteria 542
24 Actinobacteria: The High G + C Gram-Positive Bacteria 555
25 The Protists 568
26 The *Fungi (Eumycota)* 588
27 Viruses 604

Part Six Ecology and Symbiosis

28 Biogeochemical Cycling and Global Climate Change 632
29 Methods in Microbial Ecology 646
30 Microorganisms in Marine and Freshwater Ecosystems 660
31 Microorganisms in Terrestrial Ecosystems 679
32 Microbial Interactions 699

Part Seven Pathogenicity and Host Response

33 Innate Host Resistance 723
34 Adaptive Immunity 753
35 Pathogenicity and Infection 789

Part Eight Microbial Diseases, Detection, and Their Control

36 Clinical Microbiology and Immunology 808
37 Epidemiology and Public Health Microbiology 830
38 Human Diseases Caused by Viruses and Prions 854
39 Human Diseases Caused by Bacteria 888
40 Human Diseases Caused by Fungi and Protists 932

Part Nine Applied Microbiology

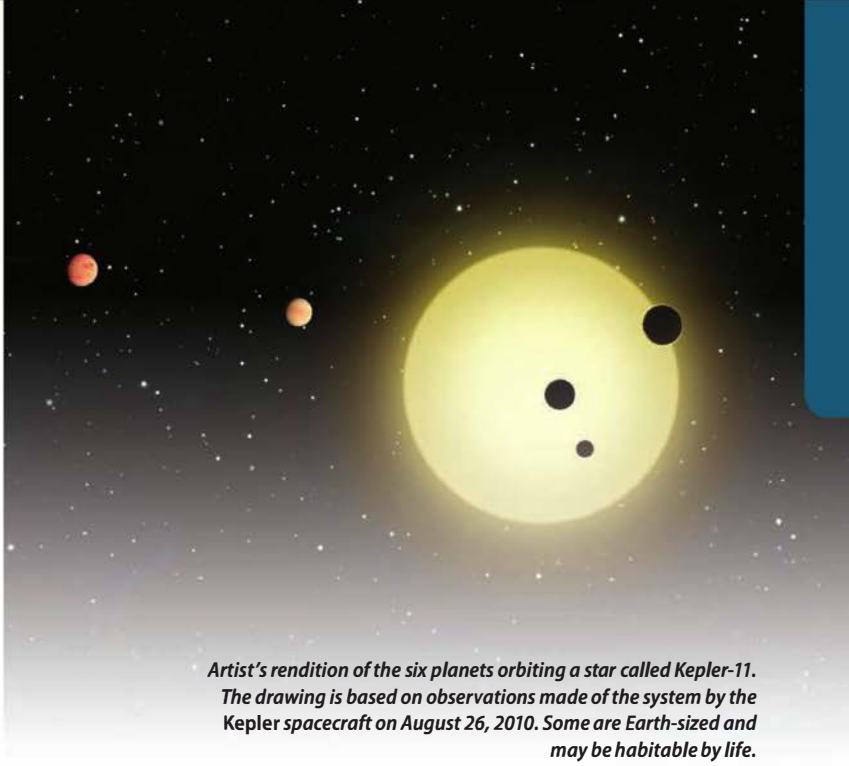
41 Microbiology of Food 958
42 Biotechnology and Industrial Microbiology 979
43 Applied Environmental Microbiology 996

Appendix 1 A Review of the Chemistry of Biological Molecules A-1
Appendix 2 Common Metabolic Pathways A-9

Glossary G-1
Credits C-1
Index I-1

1

The Evolution of Microorganisms and Microbiology



Artist's rendition of the six planets orbiting a star called Kepler-11. The drawing is based on observations made of the system by the **Kepler** *spacecraft on August 26, 2010. Some are Earth-sized and may be habitable by life.*

Over 2,000 Potential Planets Discovered

In February 2012, the National Aeronautics and Space Administration (NASA) reported that over 2,000 potential planets had been discovered by the 2009 *Kepler* mission. Using a telescope in space, the light emanating from stars as far as 3,000 light-years away had been monitored every half-hour. The *Kepler* telescope identified planets as they circulated their star and caused a brief decrease in emitted light; just as an object is detected as a blip by radar, a blip of "darkness" indicates a planet.

Unless you are a science fiction fan, you might wonder why NASA is interested in finding planets. By finding other planets, scientists can gather evidence to support or refute current models of planet formation. These models predict a process that is chaotic and violent. Planets are thought to begin as dust particles circling around newly formed stars. As these particles collide, they grow in size, forming larger chunks. Eventually a series of such collisions results in planet-sized bodies. Astrobiologists are interested in identifying characteristics of a planet that may allow it to support life. Using Earth as a model, they hypothesize that life-supporting planets will share many features with Earth. But how will life be recognized? Again, scientists look to life on Earth to answer this question, and increasingly they are turning to microbiologists for help.

Earth formed 4.5 billion years ago. Within the next billion years, the first cellular life forms—microbes—appeared. Since that time, microorganisms have evolved and diversified to occupy virtually every habitat on Earth: from oceanic geothermal vents to the coldest Arctic ice. The diversity of cellular microorganisms is best exemplified by their metabolic capabilities. Some carry out respiration, just as animals do. Others perform photosynthesis, rivaling plants in the amount of carbon dioxide they capture, forming organic matter and releasing oxygen into the atmosphere. Indeed, *Prochlorococcus*, a cyanobacterium (formerly called a blue-green alga), is thought to be the most abundant photosynthetic organism on Earth and thus a major contributor to the functioning of the biosphere. In addition to these familiar types of metabolism, other microbes are able to use inorganic molecules as sources of energy in both oxic (oxygen available) and anoxic (no oxygen) conditions. It is these microbes that are of particular interest to NASA scientists, as it is thought that the organisms on other planets may have similar unusual metabolisms.

Our goal in this chapter is to introduce you to this amazing group of organisms and to outline the history of their evolution and discovery. Microbiology is a biological science, and as such, much of what you will learn in this text is similar to what you have learned in high school and college biology classes that focus on large organisms. But microbes have unique properties, so microbiology has unique approaches to understanding them. These too will be introduced. But before you delve into this chapter, check to see if you have the background needed to get the most from it.

Readiness Check:

Based on what you have learned previously, you should be able to:

- ✔ List the features of eukaryotic cells that distinguish them from other cell types
- ✔ List the attributes that scientists use to determine if an object is alive

1.1 Members of the Microbial World

After reading this section, you should be able to:

- Differentiate the biological entities studied by microbiologists from those studied by other biologists
- Explain Carl Woese's contributions in establishing the three domain system for classifying cellular life
- Provide an example of the importance to humans of each of the major types of microbes
- Determine the type of microbe (e.g., bacterium, fungus, etc.) when given a description of a newly discovered microbe

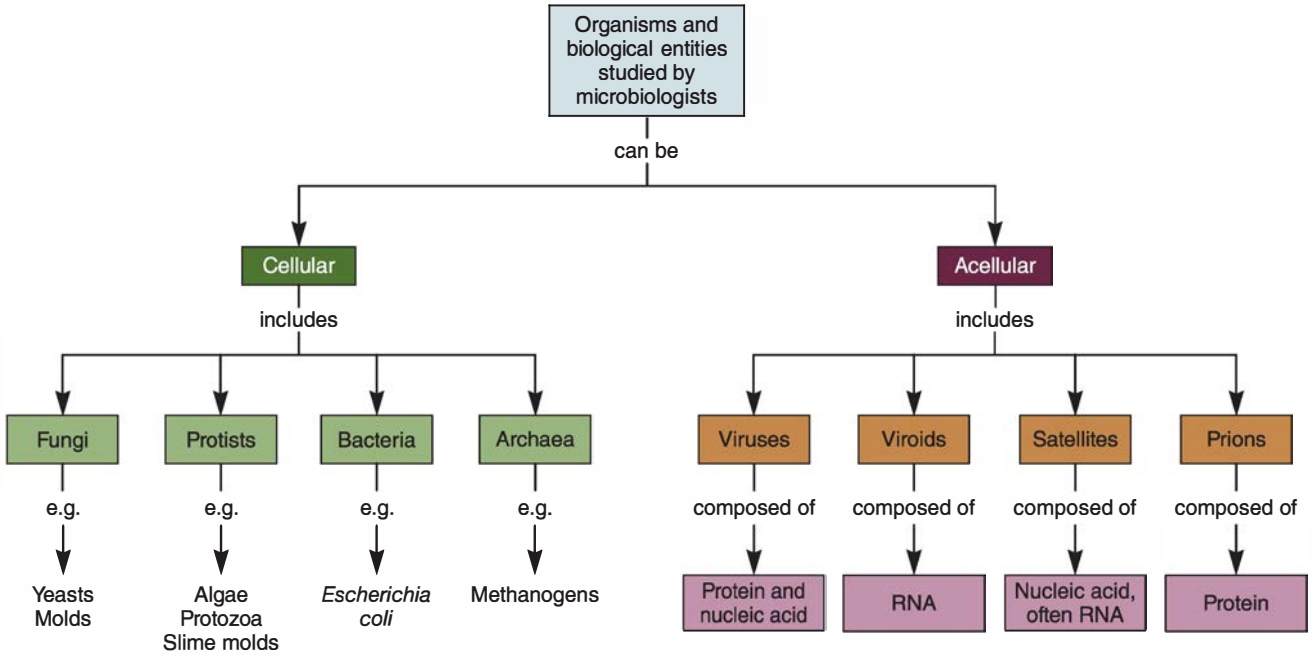


Figure 1.1 **Concept Map Showing the Types of Biological Entities Studied by Microbiologists.**

MICRO INQUIRY *How would you alter this concept map so that it also distinguishes the cellular organisms from each other?*

Microorganisms are defined as those organisms and acellular biological entities too small to be seen clearly by the unaided eye (**figure 1.1**). They are generally 1 millimeter or less in diameter. Although small size is an important characteristic of microbes, it alone is not sufficient to define them. Some cellular microbes, such as bread molds and filamentous photosynthetic microbes, are actually visible without microscopes. These macroscopic microbes are often colonial, consisting of small aggregations of cells. Some macroscopic microorganisms are multicellular. They are distinguished from other multicellular life forms such as plants and animals by their lack of highly differentiated tissues. Most unicellular microbes are microscopic. However, there are interesting exceptions, as we describe in chapter 3. In summary, cellular microbes are usually smaller than 1 millimeter in diameter, often unicellular and, if multicellular, lack differentiated tissues.

The diversity of microorganisms has always presented a challenge to microbial taxonomists. The early descriptions of cellular microbes as either plants or animals were too simple. For instance, some microbes are motile like animals but also have cell walls and are photosynthetic like plants. Such microbes cannot be placed easily into either kingdom. An important breakthrough in microbial taxonomy arose from studies of their cellular architecture, when it was discovered that cells exhibited one of two possible "floor plans." Cells that came to be called **prokaryotic cells** (Greek *pro,* before, and *karyon,* nut or kernel; organisms with a primordial nucleus) have an open floor plan. That is, their contents are not divided into compartments ("rooms") by membranes ("walls"). The most obvious characteristic of these cells is that they lack the membrane-delimited nucleus observed in **eukaryotic cells** (Greek *eu,* true, and *karyon,* nut or kernel). Eukaryotic cells not only have a nucleus but also many other membrane-bound organelles that separate some cellular materials and processes from others.

These observations eventually led to the development of a classification scheme that divided organisms into five kingdoms: *Monera, Protista, Fungi, Animalia,* and *Plantae.* Microorganisms (except for viruses and other acellular infectious agents, which have their own classification system) were placed in the first three kingdoms. In this scheme, all organisms with prokaryotic cell structure were placed in *Monera.* The five-kingdom system was an important development in microbial taxonomy, but it is no longer accepted by microbiologists. This is because not all "prokaryotes" are the same and therefore should not be grouped together in a single kingdom. Furthermore, it is currently argued that the term *prokaryote* is not meaningful and should be abandoned. As we describe next, this discovery required several advances in the tools used to study microbes. ⏭ *The "prokaryote" controversy (section 3.1)*

Great progress has been made in three areas that profoundly affect microbial classification. First, much has been learned about the detailed structure of microbial cells from the use of electron microscopy. Second, microbiologists have determined the biochemical and physiological characteristics of many different microorganisms. Third, the sequences of nucleic acids and

proteins from a wide variety of organisms have been compared. The comparison of ribosomal RNA (rRNA), begun by Carl Woese in the 1970s, was instrumental in demonstrating that there are two very different groups of organisms with prokaryotic cell architecture: *Bacteria* and *Archaea*. Later studies based on rRNA comparisons showed that *Protista* is not a cohesive taxonomic unit (i.e., taxon) and that it should be divided into three or more kingdoms. These studies and others have led many taxonomists to reject the five-kingdom system in favor of one that divides cellular organisms into three domains: *Bacteria* (sometimes referred to as true bacteria or eubacteria), *Archaea* (sometimes called archaeobacteria or archaebacteria), and *Eukarya* (all eukaryotic organisms) (**figure 1.2**). We use this system throughout the text. A brief description of the three domains and of the microorganisms placed in them follows. ▶▶| *Nucleic acids (appendix I); Proteins (appendix I)*

Members of domain ***Bacteria*** are usually single-celled organisms.[1] Most have cell walls that contain the structural molecule peptidoglycan. Although most bacteria exhibit typical prokaryotic cell structure (i.e., they lack a membrane-bound nucleus), a few members of the unusual phylum *Planctomycetes* have their genetic material surrounded by a membrane. This inconsistency is another argument made for abandoning the term "prokaryote." Bacteria are abundant in soil, water, and air, including sites that have extreme temperatures, pH, or salinity. Bacteria are also major inhabitants of our skin, mouth, and intestines. Indeed, more microbial cells are found in and on the human body than there are human cells. These microbes begin to colonize humans shortly after birth. As the microbes establish themselves, they contribute to the development of the body's immune system. Those microbes that inhabit the large intestine help the body digest food and produce vitamins. In these and other ways, microbes help maintain the health and well-being of their human hosts. ▶▶| *Phylum Planctomycetes (section 21.5)*

Unfortunately, some bacteria cause disease, and some of these diseases have had a huge impact on human history. In 1347 the plague (Black Death), an arthropod-borne disease, struck Europe with brutal force, killing one-third of the population (about 25 million people) within four years. Over the next 80 years, the disease struck repeatedly, eventually wiping out 75% of the European population. The plague's effect was so great that some historians believe it changed European culture and prepared the way for the Renaissance. Because of such plagues, it is easy for people to think that all bacteria are pathogens, but in fact, relatively few are. Most play beneficial roles, from global impact to maintaining human health. They break down dead plant and animal material and, in doing so, cycle elements in the biosphere. Furthermore, they are used extensively in industry to make bread, cheese, antibiotics, vitamins, enzymes, and other products.

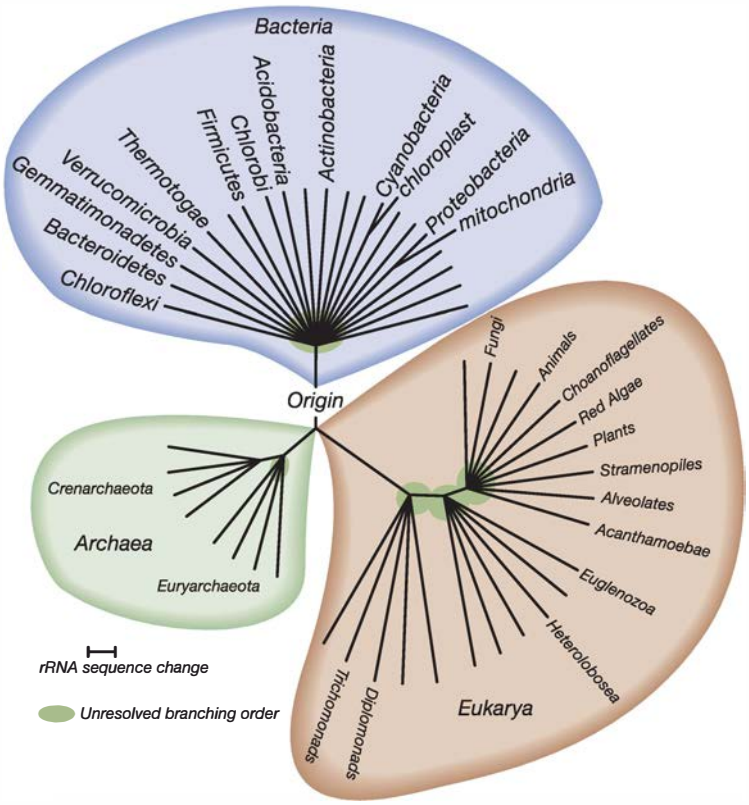


Figure 1.2 Universal Phylogenetic Tree. These evolutionary relationships are based on rRNA sequence comparisons. To save space, many lineages have not been identified.

MICRO INQUIRY *How many of the taxa listed in the figure include microbes?*

Members of domain ***Archaea*** are distinguished from bacteria by many features, most notably their distinctive rRNA sequences, lack of peptidoglycan in their cell walls, and unique membrane lipids. Some have unusual metabolic characteristics, such as the methanogens, which generate methane (natural) gas. Many archaea are found in extreme environments, including those with high temperatures (thermophiles) and high concentrations of salt (extreme halophiles). Although some archaea are members of a community of microbes involved in gum disease in humans, their role in causing disease has not been clearly established.

Domain ***Eukarya*** includes microorganisms classified as protists or fungi. Animals and plants are also placed in this domain. **Protists** are generally unicellular but larger than most bacteria and archaea. They have traditionally been divided into protozoa and algae. Despite their use, none of these terms has taxonomic value as protists, algae, and protozoa do

1 In this text, the term *bacteria* (s., *bacterium*) is used to refer to those microbes belonging to domain *Bacteria*, and the term *archaea* (s., *archaeon*) is used to refer to those that belong to domain *Archaea*. In some publications, the term *bacteria* is used to refer to all cells having prokaryotic cell structure. That is not the case in this text.

not form cohesive taxa. However, for convenience, we use them here.

The major types of protists are algae, protozoa, slime molds, and water molds. **Algae** are photosynthetic. They, together with cyanobacteria, produce about 75% of the planet's oxygen and are the foundation of aquatic food chains. **Protozoa** are unicellular, animal-like protists that are usually motile. Many free-living protozoa function as the principal hunters and grazers of the microbial world. They obtain nutrients by ingesting organic matter and other microbes. They can be found in many different environments, and some are normal inhabitants of the intestinal tracts of animals, where they aid in digestion of complex materials such as cellulose. A few cause disease in humans and other animals. **Slime molds** are protists that behave like protozoa in one stage of their life cycle but like fungi in another. In the protozoan phase, they hunt for and engulf food particles, consuming decaying vegetation and other microbes. **Water molds** are protists that grow on the surface of freshwater and moist soil. They feed on decaying vegetation such as logs and mulch. Some water molds have produced devastating plant infections, including the Great Potato Famine of 1846–1847 in Ireland. ▶▶| *The protists (chapter 25)*

Fungi are a diverse group of microorganisms that range from unicellular forms (yeasts) to molds and mushrooms. Molds and mushrooms are multicellular fungi that form thin, threadlike structures called hyphae. They absorb nutrients from their environment, including the organic molecules they use as sources of carbon and energy. Because of their metabolic capabilities, many fungi play beneficial roles, including making bread rise, producing antibiotics, and decomposing dead organisms. Some fungi associate with plant roots to form mycorrhizae. Mycorrhizal fungi transfer nutrients to the roots, improving growth of the plants, especially in poor soils. Other fungi cause plant diseases (e.g., rusts, powdery mildews, and smuts) and diseases in humans and other animals. ▶▶| *The Fungi (chapter 26)*

The microbial world also includes numerous acellular infectious agents. **Viruses** are acellular entities that must invade a host cell to multiply. The simplest viruses are composed only of proteins and a nucleic acid, and can be extremely small (the smallest is 10,000 times smaller than a typical bacterium). However, their small size belies their power: they cause many animal and plant diseases and have caused epidemics that have shaped human history. Viral diseases include smallpox, rabies, influenza, AIDS, the common cold, and some cancers. Viruses also play important roles in aquatic environments, and their role in shaping aquatic microbial communities is currently being explored. **Viroids** and **satellites** are infectious agents composed only of ribonucleic acid (RNA). Viroids cause numerous plant diseases, whereas satellites cause plant diseases and some important animal diseases such as hepatitis. Finally, **prions,** infectious agents composed only of protein, are responsible for causing a variety of spongiform encephalopathies such as scrapie and "mad cow disease." ▶▶| *Viruses and other acellular infectious agents (chapter 6)*

Retrieve, Infer, Apply

1. How did the methods used to classify microbes change, particularly in the last half of the twentieth century? What was the result of these technological advances?
2. Identify one characteristic for each of these types of microbes that distinguishes it from the other types: bacteria, archaea, protists, fungi, viruses, viroids, satellites, and prions.

1.2 Microbial Evolution

After reading this section, you should be able to:

- Propose a time line of the origin and history of microbial life and integrate supporting evidence into it
- Design a set of experiments that could be used to place a newly discovered cellular microbe on a phylogenetic tree based on small subunit (SSU) rRNA sequences
- Compare and contrast the definitions of plant and animal species, microbial species, and microbial strains

A review of figure 1.2 reminds us that in terms of the number of taxa, microbes are the dominant organisms on Earth. How has microbial life been able to radiate to such an astonishing level of diversity? To answer this question, we must consider microbial evolution. The field of microbial evolution, like any other scientific endeavor, is based on the formulation of hypotheses, the gathering and analysis of data, and the reformation of hypotheses based on newly acquired evidence. That is to say, the study of microbial evolution is based on the scientific method (**see www.mhhe.com/willey9**). To be sure, it is sometimes more difficult to amass evidence when considering events that occurred millions, and often billions, of years ago, but the advent of molecular methods has offered scientists a living record of life's ancient history. This section describes the outcome of this scientific research.

Evidence for the Origin of Life

Dating meteorites through the use of radioisotopes places our planet at an estimated 4.5 to 4.6 billion years old. However, conditions on Earth for the first 100 million years or so were far too harsh to sustain any type of life. Eventually bombardment by meteorites decreased, water appeared on the planet in liquid form, and gases were released by geological activity to form Earth's atmosphere. These conditions were amenable to the origin of the first life forms. But how did this occur, and what did these life forms look like?

Clearly, in order to find evidence of life and to develop hypotheses about its origin and subsequent evolution, scientists must be able to define life. Although even very young children can examine an object and correctly determine whether it is living or not, defining life succinctly has proven elusive for scientists. Thus most definitions of life consist of a set of attributes. The attributes of particular importance to paleobiologists are an orderly structure, the ability to obtain

and use energy (i.e., metabolism), and the ability to reproduce. Just as NASA scientists are using the characteristics of microbes on Earth today to search for life elsewhere (p. 1), so too are scientists examining **extant organisms,** those organisms present today, to explore the origin of life. Some extant organisms have structures and molecules that represent "relics" of ancient life forms. Furthermore, they can provide scientists with ideas about the type of evidence to seek when testing hypotheses.

The first direct evidence of primitive cellular life was the 1977 discovery of microbial fossils in the Swartkoppie chert. Chert is a type of granular sedimentary rock rich in silica. The Swartkoppie chert fossils as well as those from the Archaean Apex chert of Australia have been dated at about 3.5 billion years old (**figures 1.3** and **1.4**). Despite these findings, the microbial fossil record is understandably sparse. Thus to piece together the very early events that led to the origin of life, biologists must rely primarily on indirect evidence. Each piece of evidence must fit together as in a jigsaw puzzle for a coherent picture to emerge.

RNA World

The origin of life rests on a single question: How did early cells arise? At a minimum, modern cells consist of a plasma membrane enclosing water in which numerous chemicals are dissolved and subcellular structures float. It seems likely that the first self-replicating entity was much simpler than even the most primitive modern living cells. Before there was life, most evidence suggests that Earth was a very different place: hot and anoxic, with an atmosphere rich in water vapor, carbon dioxide, and nitrogen. In the oceans, hydrogen, methane, and carboxylic acids were formed by geological and chemical processes. Areas near hydrothermal vents or in shallow pools may have provided the conditions that allowed chemicals to react with one another, randomly "testing" the usefulness of the reaction and the stability of its products. Some reactions released energy and would eventually become the basis of modern cellular metabolism. Other reactions generated molecules that could function as catalysts, some aggregated with other molecules to form the predecessors of modern cell structures, and others were able to replicate and act as units of hereditary information.

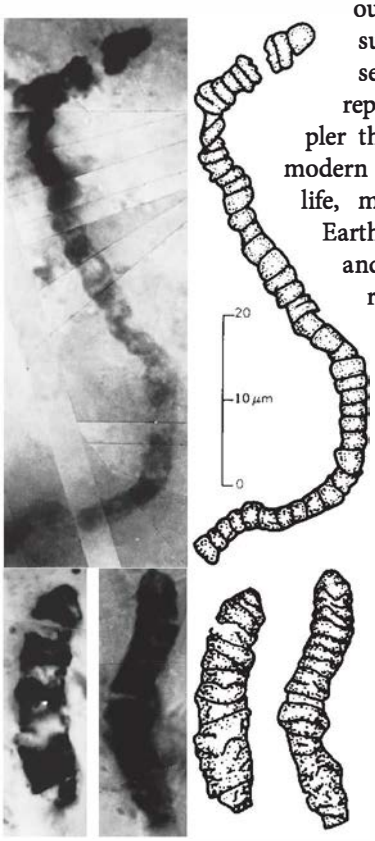


Figure 1.3 Microfossils of the Archaeon Apex Chert of Australia.
These microfossils are similar to modern filamentous cyanobacteria.

In modern cells, three different molecules fulfill the roles of catalysts, structural molecules, and hereditary molecules (**figure 1.5**). Proteins have two major roles in modern cells: structural and catalytic. Catalytic proteins are called **enzymes,** and they speed up the myriad of chemical reactions that occur in cells. DNA stores hereditary information and can be replicated to pass the information on to the next generation. RNA is involved in converting the information stored in DNA into protein. Any hypothesis about the origin of life must account for the evolution of these molecules, but the very nature of their relationships to each other in modern cells complicates attempts to imagine how they evolved. As demonstrated in figure 1.5, proteins can do cellular work, but their synthesis involves other proteins and RNA, and uses information stored in DNA. DNA can't do cellular work. It stores genetic information and serves as the template for its own replication, a process that requires proteins. RNA is synthesized using DNA as the template and proteins as the catalysts for the reaction.

Based on these considerations, it is hypothesized that at some time in the evolution of life, there must have been a single molecule that could do both cellular work and replicate itself. A possible molecule was suggested in 1981 when Thomas Cech discovered a catalytic RNA molecule in a protist (*Tetrahymena* sp.) that could cut out an internal section of itself and splice the remaining sections back together. Since then, other catalytic RNA molecules have been discovered, including an RNA found in ribosomes that is responsible for forming peptide bonds—the bonds that hold together amino acids, the building blocks of proteins. Catalytic RNA molecules are now called **ribozymes.**

The discovery of ribozymes suggested that RNA at some time had the ability to catalyze its own replication, using itself as the template. In 1986 Walter Gilbert coined the term **RNA world** to describe a precellular stage in the evolution of life in which RNA was capable of storing, copying, and expressing genetic information, as well as catalyzing other chemical reactions. However, for this precellular stage to proceed to the evolution of cellular life forms, a lipid membrane must have formed around the RNA (**figure 1.6**). This important evolutionary step is easier to imagine than other events in the origin of cellular life forms because lipids, major structural components of the membranes of modern organisms, spontaneously form liposomes—vesicles bounded by a lipid bilayer. A fascinating experiment performed by Marin Hanczyc, Shelly Fujikawa, and Jack Szostak in 2003 showed that clay triggers the formation of liposomes that actually grow and divide. Together with the data on ribozymes, these data suggest that early cells may have been liposomes containing RNA molecules (figure 1.6).

▶▶| *Lipids (appendix I)*

Apart from its ability to perform catalytic activities, the function of RNA suggests its ancient origin. Consider that much of the cellular pool of RNA in modern cells exists in the

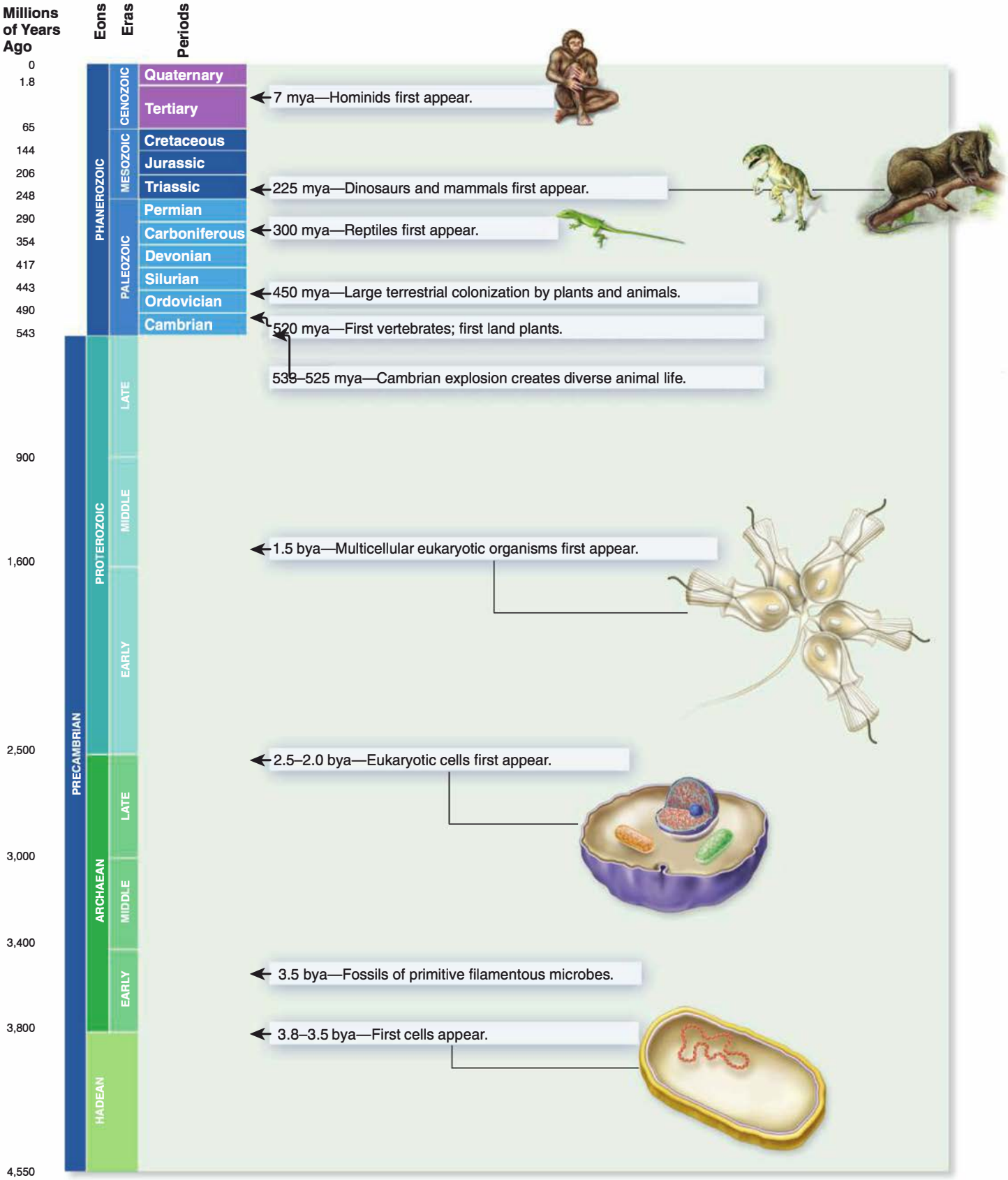


Figure 1.4 An Overview of the History of Life on Earth. mya = million years ago; bya = billion years ago.

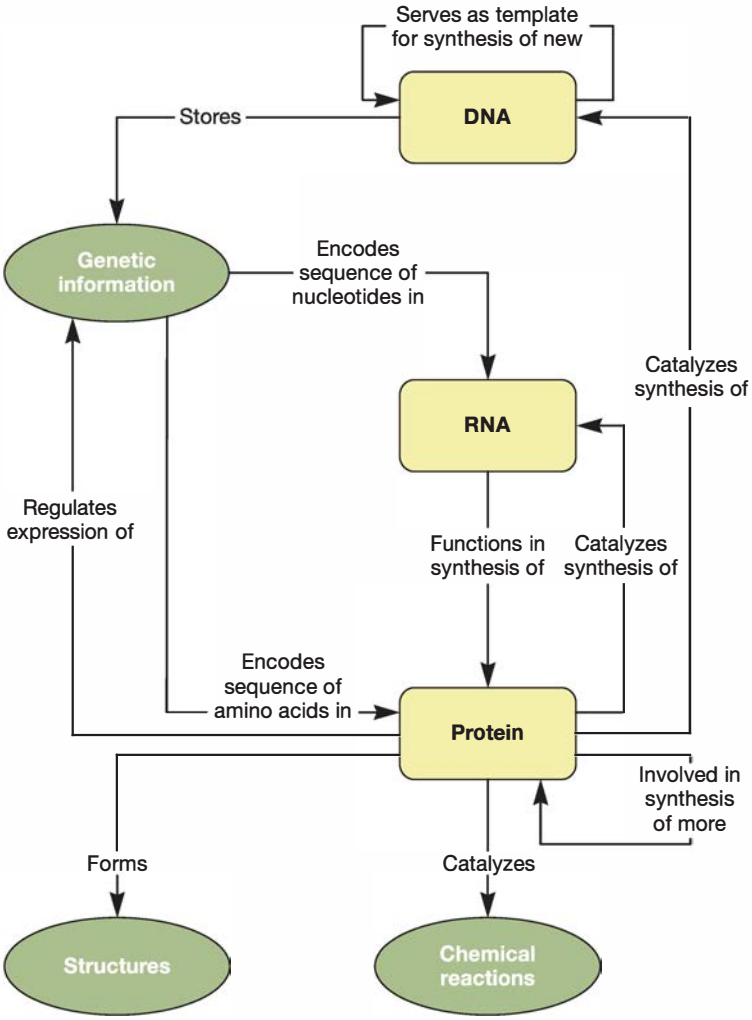


Figure 1.5 **Functions of DNA, RNA, and Protein, and Their Relationships to Each Other in Modern Cells.**

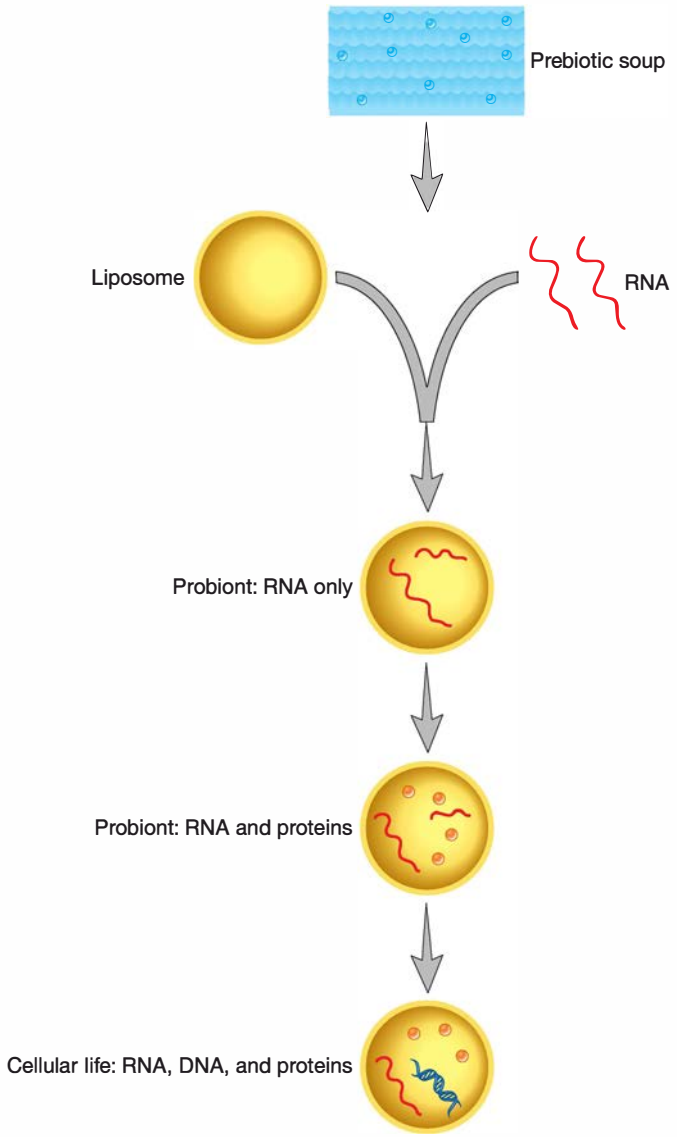
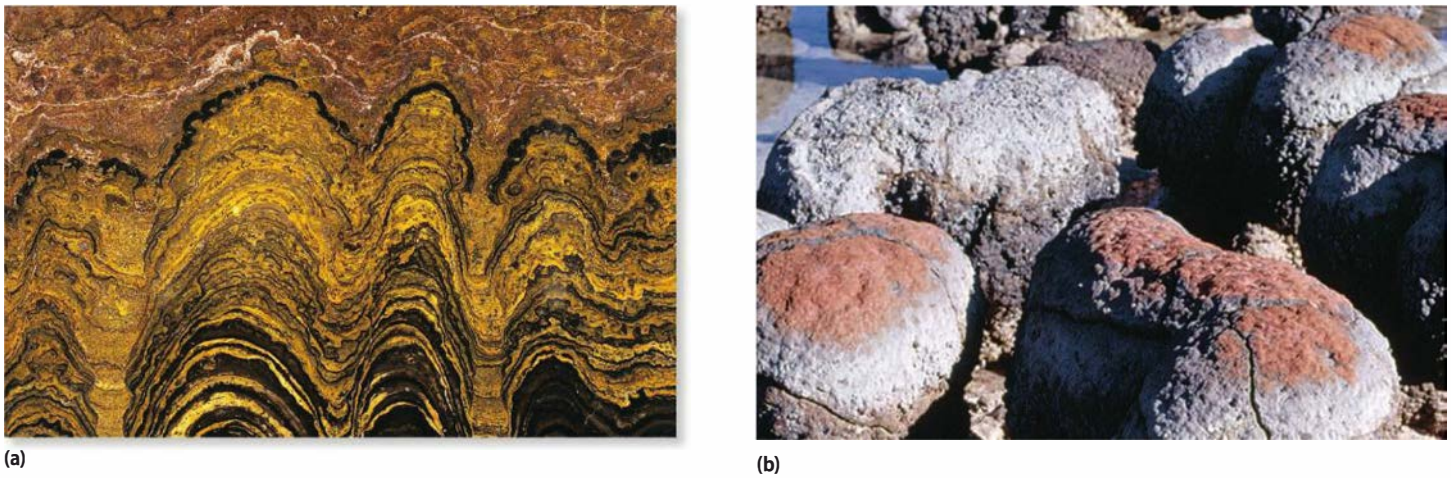


Figure 1.6 **The RNA World Hypothesis for the Origin of Life.**

MICRO INQUIRY *Why are the probionts pictured above not considered cellular life?*

ribosome, a structure that consists largely of rRNA and uses messenger RNA (mRNA) and transfer RNA (tRNA) to construct proteins. Also recall that rRNA itself catalyzes peptide bond formation during protein synthesis. Thus RNA seems to be well poised for its importance in the development of proteins. Because RNA and DNA are structurally similar, RNA could have given rise to double-stranded DNA. It is suggested that once DNA evolved, it became the storage facility for genetic information because it provided a more chemically stable structure. Two other pieces of evidence support the RNA world hypothesis: the fact that the energy currency of the cell, ATP, is a ribonucleotide and the more recent discovery that RNA can regulate gene expression. So it would seem that proteins, DNA, and cellular energy can be traced back to RNA. ▶▶| *ATP (section 10.2); Riboswitches (sections 14.3 and 14.4)*

Despite the evidence supporting the hypothesis of an RNA world, it is not without problems, and many argue against it. Another area of research is also fraught with considerable debate: the evolution of metabolism, in particular, the evolution of energy-conserving metabolic processes. Recall that early Earth was a hot environment that lacked oxygen. Thus the cells that arose there must have been able to use the available energy sources under these harsh conditions. Today there are heat-loving archaea capable of using inorganic molecules such as FeS as a source of energy. Some suggest that this interesting metabolic capability is a remnant of the first form of energy metabolism. Another metabolic strategy, oxygen-releasing photosynthesis, appears to have evolved perhaps as early as 2.5 billion years ago. Fossils of cyanobacteria-like cells found in rocks dating to that time support this hypothesis,



(a) (b)

Figure 1.7 Stromatolites. (a) Section of a fossilized stromatolite. Evolutionary biologists think the layers of material were formed when mats of cyanobacteria, layered one on top of each other, became mineralized. (b) Modern stromatolites from Western Australia. Each stromatolite is a rocklike structure, typically 1 m in diameter, containing layers of cyanobacteria.

as does the discovery of ancient stromatolites (**figure 1.7*a***). Stromatolites are layered rocks, often domed, that are formed by the incorporation of mineral sediments into layers of microorganisms growing as thick mats on surfaces (figure 1.7*b*). The appearance of cyanobacteria-like cells was an important step in the evolution of life on Earth. The oxygen they released is thought to have altered Earth's atmosphere to its current oxygen-rich state, allowing the evolution of additional energy-capturing strategies such as aerobic respiration, the oxygen-consuming metabolic process that is used by many microbes and animals.

Evolution of the Three Domains of Life

As noted in section 1.1, rRNA comparisons were an important breakthrough in the classification of microbes; this analysis also provides insights into the evolutionary history of all life. What began with the examination of rRNA from relatively few organisms has been expanded by the work of many others, including Norman Pace. Dr. Pace has developed a **universal phylogenetic tree** (figure 1.2) based on comparisons of small subunit rRNA molecules (SSU rRNA), the rRNA found in the small subunit of the ribosome. Here we examine how these comparisons are made and what the universal phylogenetic tree tells us. ▶▶| *Bacterial ribosomes (section 3.6); Exploring microbial taxonomy and phylogeny (section 19.3)*

Comparing SSU rRNA Molecules

The details of phylogenetic tree construction are discussed in chapter 19. However, the general concept is not difficult to understand. In one approach, the sequences of nucleotides in the genes that encode SSU rRNAs from diverse organisms are aligned, and pair-wise comparisons of the sequences are made. For each pair of SSU rRNA gene sequences, the number of differences in the nucleotide sequences is counted (**figure 1.8**). This value serves as a measure of the evolutionary distance between the organisms; the more differences counted, the greater the evolutionary distance. The evolutionary distances from many comparisons are used by sophisticated computer programs to construct the tree. Each branch in the tree represents one of the organisms used in the comparison. The distance from the tip of one branch to the tip of another is the evolutionary distance between the two organisms represented by the branches.

Two things should be kept in mind when examining phylogenetic trees developed in this way. The first is that they are molecular trees, not organismal trees. In other words, they represent, as accurately as possible, the evolutionary history of a molecule and the gene that encodes it. Second, the distance between branch tips is a measure of relatedness, not of time. If the distance along the lines is very long, then the two organisms are more evolutionarily diverged (i.e., less related). However, we do not know when they diverged from each other. This concept is analogous to a map that accurately shows the distance between two cities but because of many factors (traffic, road conditions, etc.) cannot show the time needed to travel that distance.

LUCA

What does the universal phylogenetic tree tell us about the evolution of life? At the center of the tree is a line labeled "Origin" (figure 1.2). This is where the data indicate the *l*ast *u*niversal *c*ommon *a*ncestor (LUCA) to all three domains should be placed. LUCA is on the bacterial branch, which means that *Archaea* and *Eukarya* evolved independently, separate from *Bacteria*. Thus the universal phylogenetic tree presents a picture in which all life, regardless of eventual domain, arose from a single common ancestor. One can envision the universal tree of life as a real tree that grows from a single seed.

The evolutionary relationship of *Archaea* and *Eukarya* is still the matter of considerable debate. According to the universal phylogenetic tree we show here, *Archaea* and *Eukarya* shared common ancestry but diverged and became separate domains. Other versions suggest that *Eukarya* evolved out of *Archaea*. The

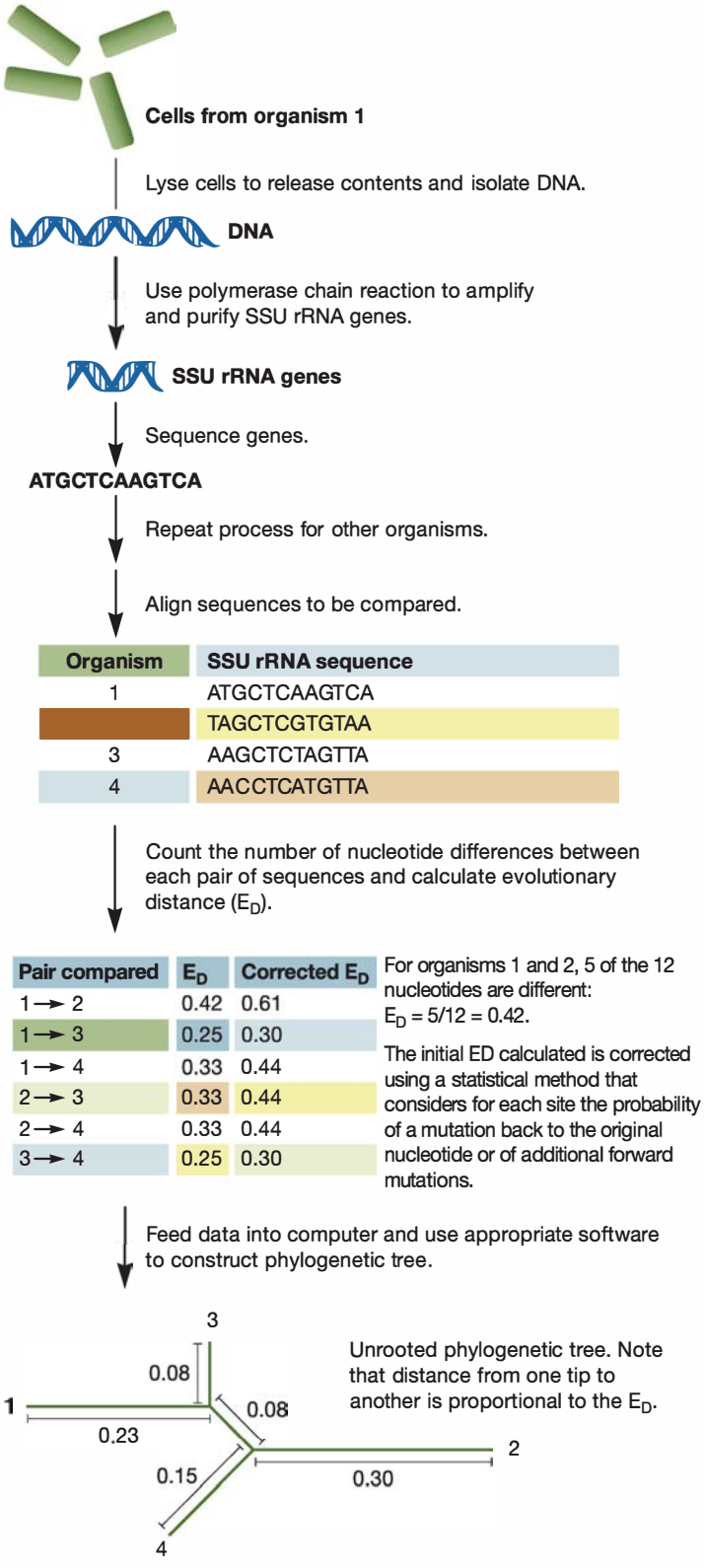


Figure 1.8 The Construction of Phylogenetic Trees Using a Distance Method.

MICRO INQUIRY *Why does the branch length indicate amount of evolutionary change but not the time it took for that change to occur?*

close evolutionary relationship of these two forms of life is still evident in the manner in which they process genetic information. For instance, certain protein subunits of archaeal and eukaryotic RNA polymerases, the enzymes that catalyze RNA synthesis, resemble each other to the exclusion of those of bacteria. However, archaea have other features that are most similar to their counterparts in bacteria (e.g., mechanisms for conserving energy). This has further complicated and fueled the debate. The evolution of the nucleus and endoplasmic reticulum is also at the center of many controversies. However, hypotheses regarding the evolution of other membrane-bound organelles are more widely accepted and are considered next.

Endosymbiotic Origin of Mitochondria, Chloroplasts, and Hydrogenosomes

The **endosymbiotic hypothesis** is generally accepted as the origin of three eukaryotic organelles: mitochondria, chloroplasts, and hydrogenosomes. Endosymbiosis is an interaction between two organisms in which one organism lives inside the other. The initial statement of the endosymbiotic hypothesis proposed that over time a bacterial endosymbiont of an ancestral cell in the eukaryotic lineage lost its ability to live independently, becoming either a mitochondrion, if the intracellular bacterium used aerobic respiration, or a chloroplast, if the endosymbiont was a photosynthetic bacterium (*see figure 19.11*).

Although the mechanism by which the endosymbiotic relationship was established is unknown, there is considerable evidence to support the hypothesis. Mitochondria and chloroplasts contain DNA and ribosomes; both are similar to bacterial DNA and ribosomes. Indeed, inspection of figure 1.2 shows that both organelles belong to the bacterial lineage based on SSU rRNA analysis. Further evidence for the origin of mitochondria comes from the genome sequence of the bacterium *Rickettsia prowazekii*, an obligate intracellular parasite and the cause of epidemic (lice-borne) typhus. Its genome is more similar to that of modern mitochondrial genomes than to any other bacterium. The chloroplasts of plants and green algae are thought to have descended from an ancestor of the cyanobacterial genus *Prochloron*, which contains species that live within marine invertebrates.

Recently the endosymbiotic hypothesis for mitochondria has been modified by the **hydrogen hypothesis.** This asserts that the endosymbiont was an anaerobic bacterium that produced H_2 and CO_2 as end products of its metabolism. Over time, the host became dependent on the H_2 produced by the endosymbiont. Ultimately the endosymbiont evolved into one of two organelles. If the endosymbiont developed the capacity to perform aerobic respiration, it evolved into a mitochondrion. However, if the endosymbiont did not develop this capacity, it evolved into a hydrogenosome—an organelle found in some extant protists that produce ATP by a process called fermentation (*see figure 5.16*).