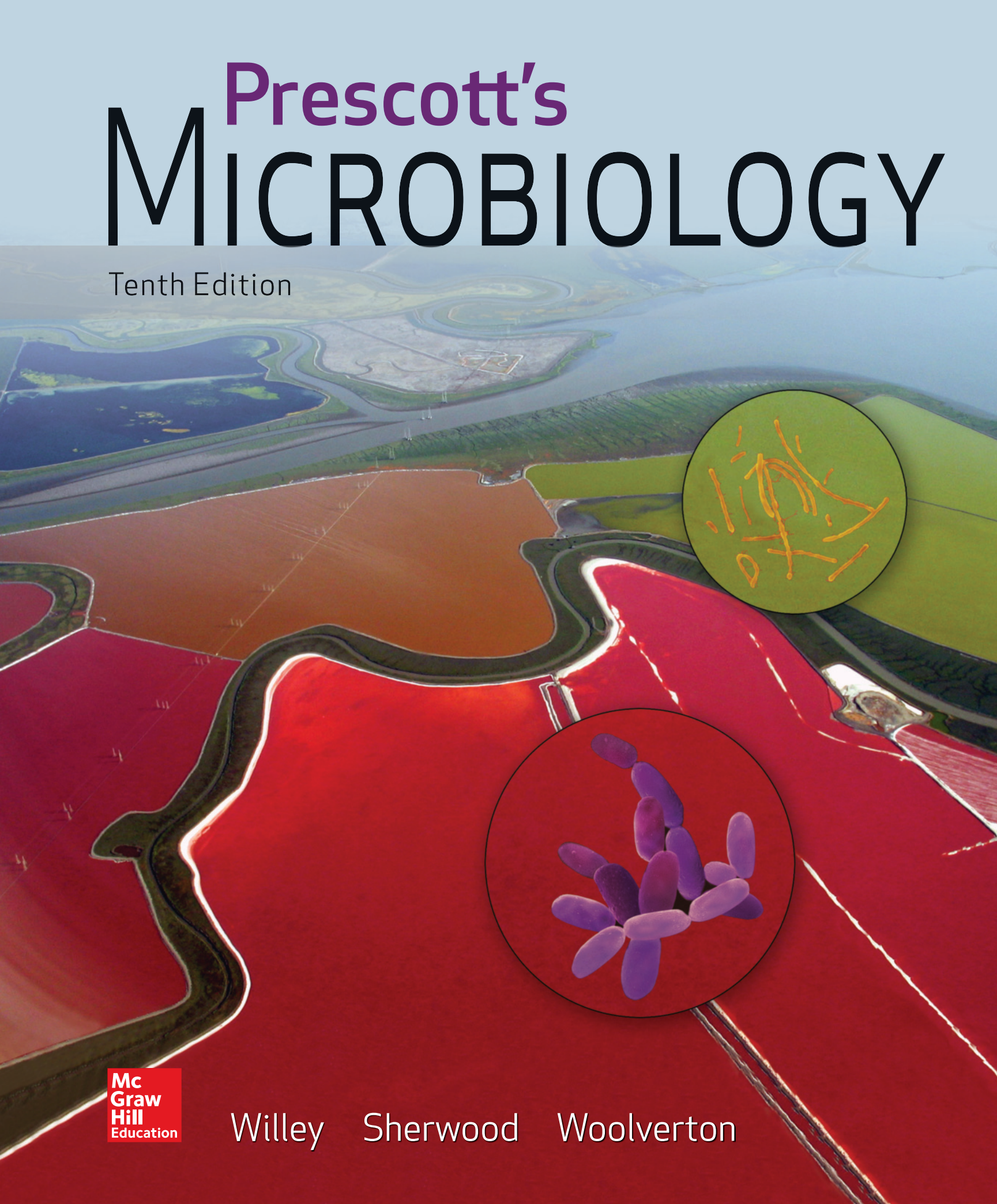
Prescott's MICROBIOLOGY

Tenth Edition



Willey Sherwood Woolverton

Mc Graw Hill Education

tenth edition

Prescott's Microbiology

Joanne M. Willey
HOFSTRA UNIVERSITY

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MONTANA STATE UNIVERSITY

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Mc Graw Hill Education

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Brief Contents

About the Authors

Joanne M. Willey has been a professor at Hofstra University on Long Island, New York, since 1993, where she is Professor and Chair of the Department of Science Education at the Hofstra North Shore-Long Island Jewish 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 taught microbiology to biology majors for 20 years and now teaches microbiology and infectious disease to medical students. She has taught 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; she has two grown 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 recently retired from the Department of Microbiology at Montana State University after over 20 years of service to the department. 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 helped her to understand current models of cognition and learning and their implications for teaching. She taught courses in general microbiology, genetics, biology, microbial genetics, and microbial physiology. She served as the editor for ASM's *Focus on Microbiology Education* and participated in and contributed to numerous ASM Conferences for Undergraduate Educators (ASMCUE). She also worked with K–12 teachers to develop a kit-based unit to introduce microbiology into the elementary school curriculum and coauthored with Barbara Hudson a general microbiology laboratory manual, *Explorations in Microbiology: A Discovery Approach*. 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 Sciences, College of Public Health at Kent State University (KSU), and is the Director of the KSU Center for Public Health Preparedness, overseeing its BSL-3 Training Facility. 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 research is focused on the detection and control of pathogens. Dr. Woolverton has published and lectured widely on the mechanisms by which liquid crystals (LCs) act as microbial biosensors and on the LC characteristics of microbial proteins. Professor Woolverton has taught zombie preparedness, general microbiology, communicable diseases, immunology, prevention and control of disease, and microbial physiology. On faculty of the National Institutes of Health National Biosafety and Biocontainment Training Program, he teaches laboratory safety, risk assessment, decontamination strategies, and bioterrorism readiness. An active member of the American Society for Microbiology, Woolverton has served on its Board of Education, its distinguished lecturer program, and as a Conference for Undergraduate Educators co-chair, and is the immediate past editor-in-chief of its *Journal of Microbiology and Biology Education*. Woolverton and his wife, Nancy, have three grown daughters, two sons (in-law), and two grandchildren. He enjoys family time, hiking, camping, and cycling. His e-mail address is cwoolver@kent.edu.

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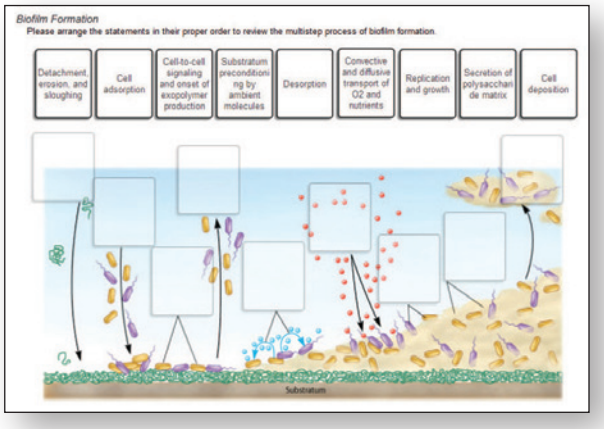

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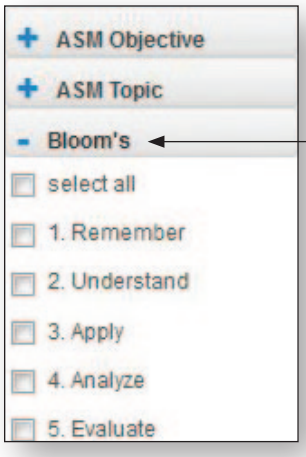

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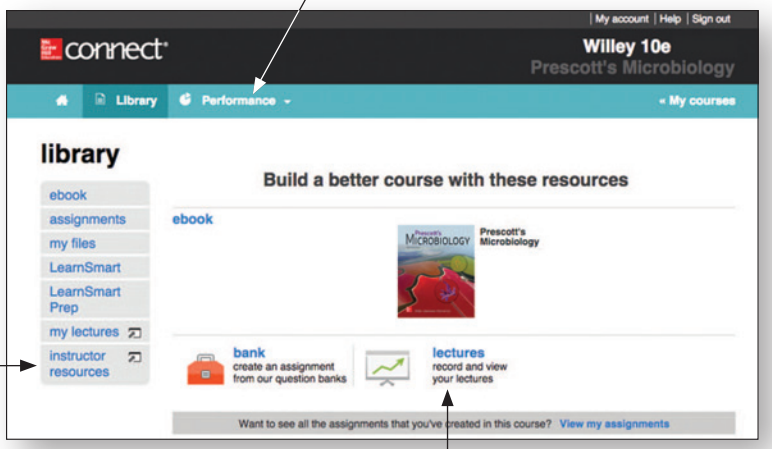

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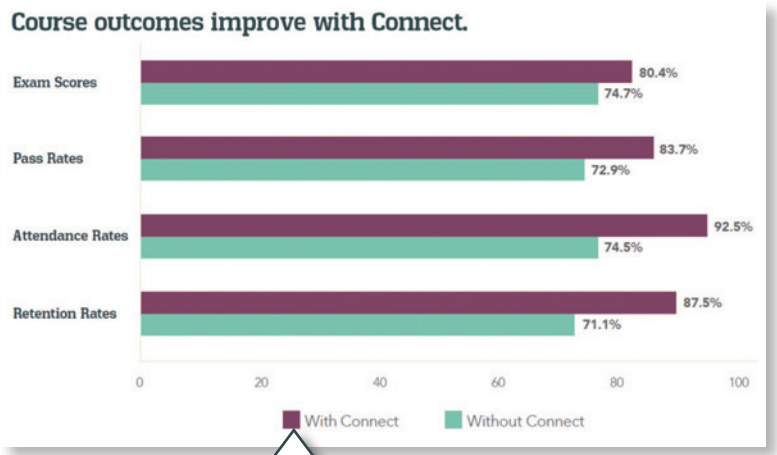

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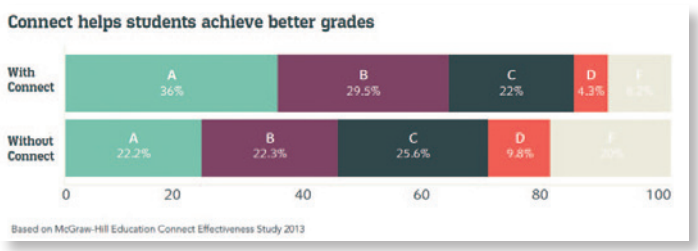

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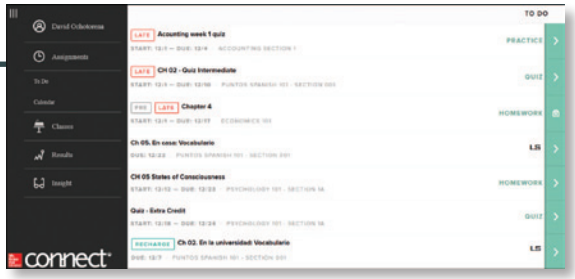

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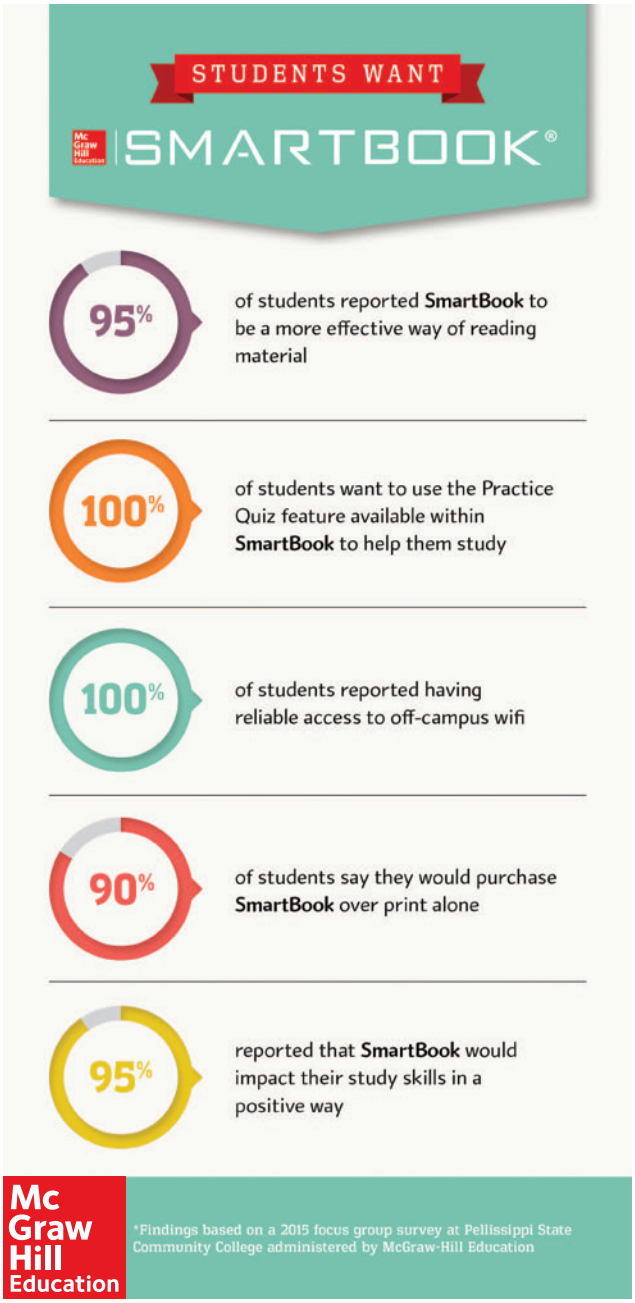


A Modern Approach to Microbiology

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.

An Introduction to the Entire Microbial World

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.

Broad Coverage of Microbial Ecology

The importance and multidisciplinary nature of microbial ecology is demonstrated by content that ranges from global climate change to the human microbiome.

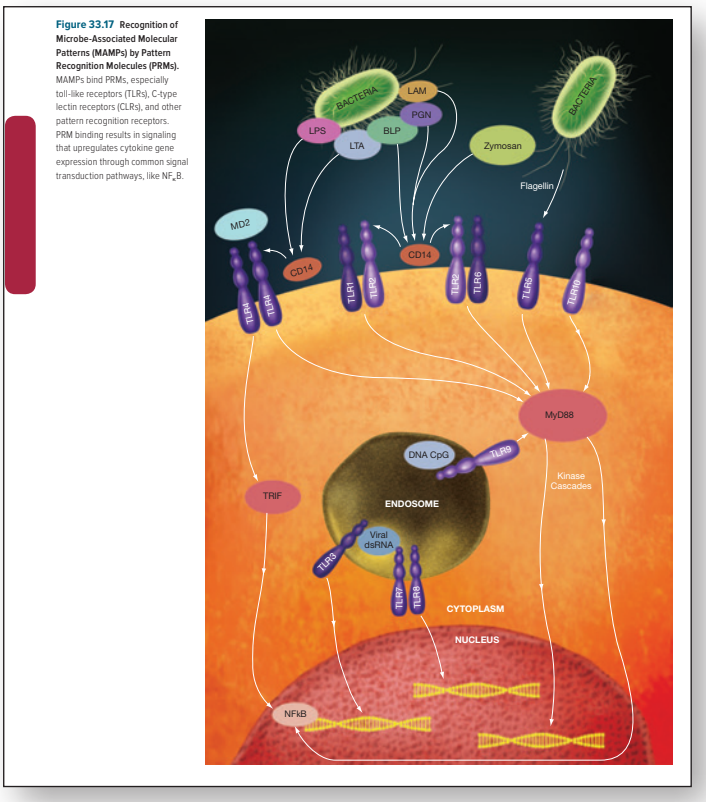


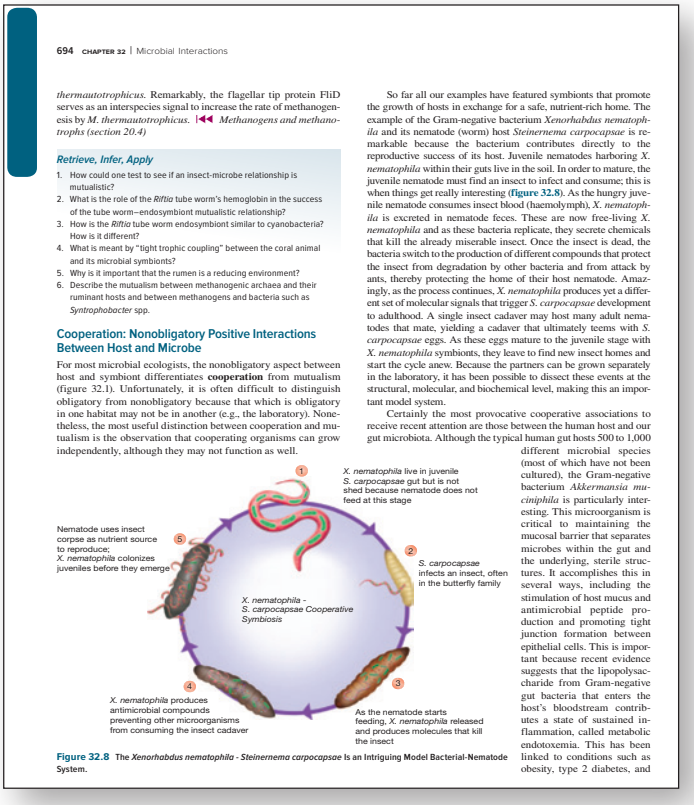
Figure 33.17 Recognition of Microbe-Associated Molecular Patterns (MAMPs) by Pattern Recognition Molecules (PRMs). MAMPs bind PRMs, especially toll-like receptors (TLRs), C-type lectin receptors (CLRs), and other pattern recognition receptors. PRM binding results in signaling that upregulates cytokine gene expression through common signal transduction pathways, like $NF_{\kappa}B$.

Brief Contents

Molecular Microbiology and Immunology

The tenth edition includes updates on genetics, biotechnology, genomics and metagenomics, and immunology. The discussion of eukaryotic and archaeal genetics has been expanded, strengthening our understanding of the relatedness of genetic information flow observed in these organisms. 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



694 CHAPTER 32 | Microbial Interactions

thermautotrophicus. Remarkably, the flagellar tip protein FliD serves as an interspecies signal to increase the rate of methanogenesis by *M. thermautotrophicus.* |◀◀ *Methanogens and methanotrophs (section 20.4)*

Retrieve, Infer, Apply

1. How could one test to see if an insect-microbe relationship is mutualistic?
2. What is the role of the *Riftia* tube worm's hemoglobin in the success of the tube worm–endosymbiont mutualistic relationship?
3. How is the *Riftia* tube worm endosymbiont similar to cyanobacteria? How is it different?
4. What is meant by "tight trophic coupling" between the coral animal and its microbial symbionts?
5. Why is it important that the rumen is a reducing environment?
6. Describe the mutualism between methanogenic archaea and their ruminant hosts and between methanogens and bacteria such as *Syntrophobacter* spp.

Cooperation: Nonobligatory Positive Interactions Between Host and Microbe

For most microbial ecologists, the nonobligatory aspect between host and symbiont differentiates **cooperation** from mutualism (figure 32.1). Unfortunately, it is often difficult to distinguish obligatory from nonobligatory because that which is obligatory in one habitat may not be in another (e.g., the laboratory). Nonetheless, the most useful distinction between cooperation and mutualism is the observation that cooperating organisms can grow independently, although they may not function as well.

So far all our examples have featured symbionts that promote the growth of hosts in exchange for a safe, nutrient-rich home. The example of the Gram-negative bacterium *Xenorhabdus nematophila* and its nematode (worm) host *Steinernema carpocapsae* is remarkable because the bacterium contributes directly to the reproductive success of its host. Juvenile nematodes harboring *X. nematophila* within their guts live in the soil. In order to mature, the juvenile nematode must find an insect to infect and consume; this is when things get really interesting (**figure 32.8**). As the hungry juvenile nematode consumes insect blood (haemolymph), *X. nematophila* is excreted in nematode feces. These are now free-living *X. nematophila* and as these bacteria replicate, they secrete chemicals that kill the already miserable insect. Once the insect is dead, the bacteria switch to the production of different compounds that protect the insect from degradation by other bacteria and from attack by ants, thereby protecting the home of their host nematode. Amazingly, as the process continues, *X. nematophila* produces yet a different set of molecular signals that trigger *S. carpocapsae* development to adulthood. A single insect cadaver may host many adult nematodes that mate, yielding a cadaver that ultimately teems with *S. carpocapsae* eggs. As these eggs mature to the juvenile stage with *X. nematophila* symbionts, they leave to find new insect homes and start the cycle anew. Because the partners can be grown separately in the laboratory, it has been possible to dissect these events at the structural, molecular, and biochemical level, making this an important model system.

Certainly the most provocative cooperative associations to receive recent attention are those between the human host and our gut microbiota. Although the typical human gut hosts 500 to 1,000 different microbial species (most of which have not been cultured), the Gram-negative bacterium *Akkermansia muciniphila* is particularly interesting. This microorganism is critical to maintaining the mucosal barrier that separates microbes within the gut and the underlying, sterile structures. It accomplishes this in several ways, including the stimulation of host mucus and antimicrobial peptide production and promoting tight junction formation between epithelial cells. This is important because recent evidence suggests that the lipopolysaccharide from Gram-negative gut bacteria that enters the host's bloodstream contributes a state of sustained inflammation, called metabolic endotoxemia. This has been linked to conditions such as obesity, type 2 diabetes, and

Figure 32.8 The *Xenorhabdus nematophila - Steinernema carpocapsae* Is an Intriguing Model Bacterial-Nematode System.

21st-Century Microbiology

Prescott's Microbiology leads the way with 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

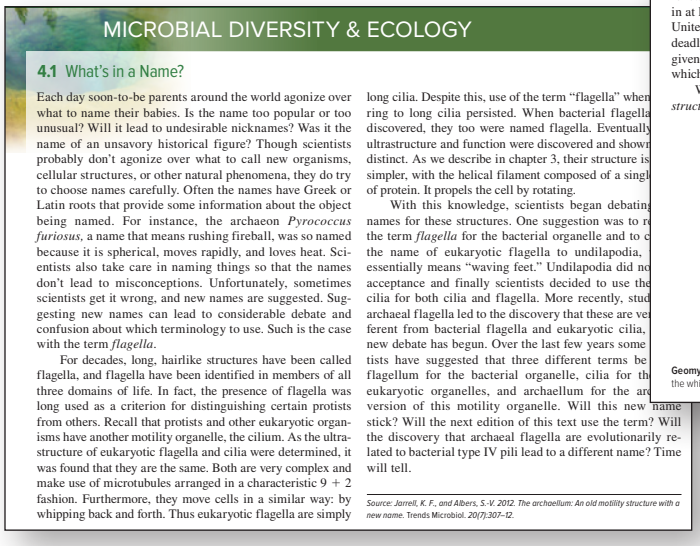
The importance of metagenomics in understanding the role of microbes in all environments and in exploring symbionts of invertebrates and humans is threaded throughout the text. Special emphasis on the power of metagenomics is found in chapters 1, 18, 28, 32, and 42.

Laboratory Safety

Reflecting recommendations from the Centers for Disease Control and Prevention, along with 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 & ECOLOGY

4.1 What's in a Name?

Each day soon-to-be parents around the world agonize over what to name their babies. Is the name too popular or too unusual? Will it lead to undesirable nicknames? Was it the name of an unsavory historical figure? Though scientists probably don't agonize over what to call new organisms, cellular structures, or other natural phenomena, they do try to choose names carefully. Often the names have Greek or Latin roots that provide some information about the object being named. For instance, the archaeon *Pyrococcus furiosus*, a name that means rushing fireball, was so named because it is spherical, moves rapidly, and loves heat. Scientists also take care in naming things so that the names don't lead to misconceptions. Unfortunately, sometimes scientists get it wrong, and new names are suggested. Suggesting new names can lead to considerable debate and confusion about which terminology to use. Such is the case with the term *flagella*.

For decades, long, hairlike structures have been called flagella, and flagella have been identified in members of all three domains of life. In fact, the presence of flagella was long used as a criterion for distinguishing certain protists from others. Recall that protists and other eukaryotic organisms have another motility organelle, the cilium. As the ultrastructure of eukaryotic flagella and cilia were determined, it was found that they are the same. Both are very complex and make use of microtubules arranged in a characteristic 9 + 2 fashion. Furthermore, they move cells in a similar way: by whipping back and forth. Thus eukaryotic flagella are simply long cilia. Despite this, use of the term "flagella" when ring to long cilia persisted. When bacterial flagella discovered, they too were named flagella. Eventually ultrastructure and function were discovered and shown distinct. As we describe in chapter 3, their structure is simpler, with the helical filament composed of a singl of protein. It propels the cell by rotating.

With this knowledge, scientists began debating names for these structures. One suggestion was to r the term *flagella* for the bacterial organelle and to c the name of eukaryotic flagella to undilapodia, essentially means "waving feet." Undilapodia did no acceptance and finally scientists decided to use the cilia for both cilia and flagella. More recently, stud archaeal flagella led to the discovery that these are ve ferent from bacterial flagella and eukaryotic cilia, new debate has begun. Over the last few years some tists have suggested that three different terms be flagellum for the bacterial organelle, cilia for th eukaryotic organelles, and archaellum for the ar version of this motility organelle. Will this new name stick? Will the next edition of this text use the term? Will the discovery that archaeal flagella are evolutionarily related to bacterial type IV pili lead to a different name? Time will tell.

Source: Jarrell, K. F., and Albers, S.-V. 2012. The archaellum: An old motility structure with a new name. Trends Microbiol. *20(7):307–12.*

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 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 11 bat species and is now found in 25 states in the United States and three Canadian provinces. Second, it is deadly. The 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 *Pseudogymnoascus destructans* (formerly *Geomyces destructans*). It colonizes a bat's wings, muzzle, and ears where it erodes the epidermis before invading 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 *P. 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.

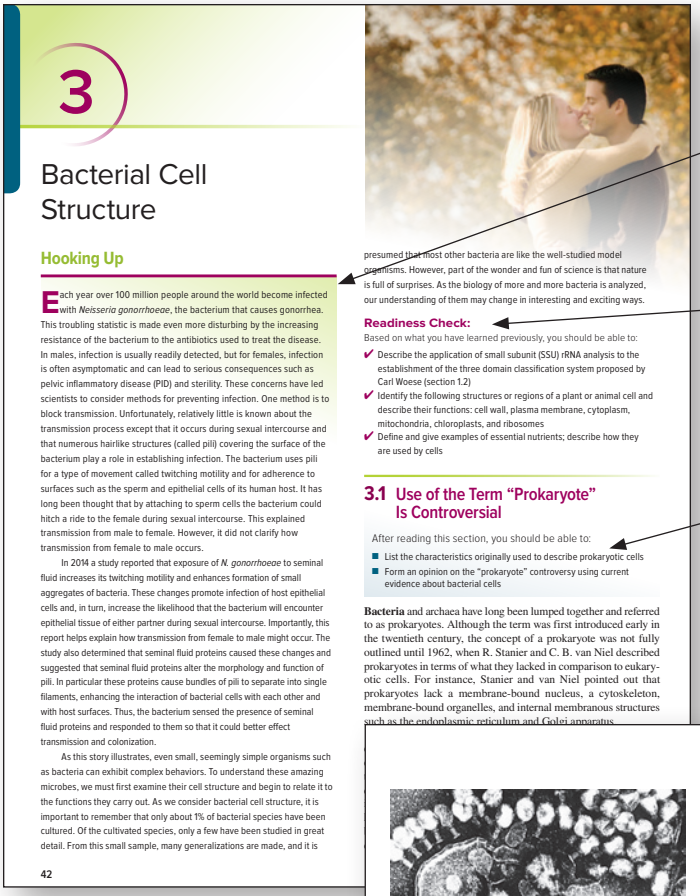
The capacity of *P. destructans* to sweep through bat populations results from a "perfect storm" of host- and pathogen-associated factors. *P. destructans* is psychrophilic, with a growth optimum around 12°C; it does not grow above 20°C. All infected bat species hibernate in cold and humid environments 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: Langwig, K.E., et al. 2014. Invasion dynamics of white-nose syndrome fungus, midwestern United States, 2012–2014. Emerging Infectious Diseases. *21: 1023–1026.*

Student-Friendly Organization



3

Bacterial Cell Structure

Hooking Up

Each year over 100 million people around the world become infected with *Neisseria gonorrhoeae*, the bacterium that causes gonorrhea. This troubling statistic is made even more disturbing by the increasing resistance of the bacterium to the antibiotics used to treat the disease. In males, infection is usually readily detected, but for females, infection is often asymptomatic and can lead to serious consequences such as pelvic inflammatory disease (PID) and sterility. These concerns have led scientists to consider methods for preventing infection. One method is to block transmission. Unfortunately, relatively little is known about the transmission process except that it occurs during sexual intercourse and that numerous hairlike structures (called pili) covering the surface of the bacterium play a role in establishing infection. The bacterium uses pili for a type of movement called twitching motility and for adherence to surfaces such as the sperm and epithelial cells of its human host. It has long been thought that by attaching to sperm cells the bacterium could hitch a ride to the female during sexual intercourse. This explained transmission from male to female. However, it did not clarify how transmission from female to male occurs.

In 2014 a study reported that exposure of *N. gonorrhoeae* to seminal fluid increases its twitching motility and enhances formation of small aggregates of bacteria. These changes promote infection of host epithelial cells and, in turn, increase the likelihood that the bacterium will encounter epithelial tissue of either partner during sexual intercourse. Importantly, this report helps explain how transmission from female to male might occur. The study also determined that seminal fluid proteins caused these changes and suggested that seminal fluid proteins alter the morphology and function of pili. In particular these proteins cause bundles of pili to separate into single filaments, enhancing the interaction of bacterial cells with each other and with host surfaces. Thus, the bacterium sensed the presence of seminal fluid proteins and responded to them so that it could better effect transmission and colonization.

As this story illustrates, even small, seemingly simple organisms such as bacteria can exhibit complex behaviors. To understand these amazing microbes, we must first examine their cell structure and begin to relate it to the functions they carry out. As we consider bacterial cell structure, it is important to remember that only about 1% of bacterial species have been cultured. Of the cultivated species, only a few have been studied in great detail. From this small sample, many generalizations are made, and it is presumed that most other bacteria are like the well-studied model organisms. However, part of the wonder and fun of science is that nature is full of surprises. As the biology of more and more bacteria is analyzed, our understanding of them may change in interesting and exciting ways.

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Readiness Check:

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

- ✔ Describe the application of small subunit (SSU) rRNA analysis to the establishment of the three domain classification system proposed by Carl Woese (section 1.2)
- ✔ Identify the following structures or regions of a plant or animal cell and describe their functions: cell wall, plasma membrane, cytoplasm, mitochondria, chloroplasts, and ribosomes
- ✔ Define and give examples of essential nutrients; describe how they are used by cells

3.1 Use of the Term "Prokaryote" Is Controversial

After reading this section, you should be able to:

- List the characteristics originally used to describe prokaryotic cells
- Form an opinion on the "prokaryote" controversy using current evidence about bacterial cells

Bacteria and archaea have long been lumped together and referred to as prokaryotes. Although the term was first introduced early in the twentieth century, the concept of a prokaryote was not fully outlined until 1962, when R. Stanier and C. B. van Niel described prokaryotes in terms of what they lacked in comparison to eukaryotic cells. For instance, Stanier and van Niel pointed out that prokaryotes lack a membrane-bound nucleus, a cytoskeleton, membrane-bound organelles, and internal membranous structures such as the endoplasmic reticulum and Golgi apparatus.

Micro Focus—Each chapter begins with a real-life story illustrating the relevance of the content covered in the upcoming text.

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.

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

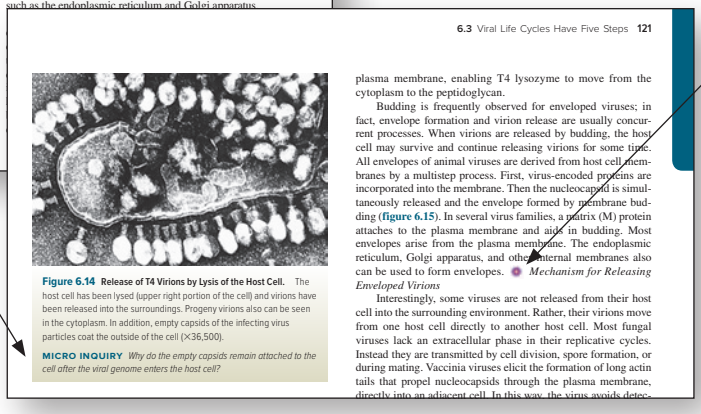


Figure 6.14 Release of T4 Virions by Lysis of the Host Cell. The host cell has been lysed (upper right portion of the cell) and virions have been released into the surroundings. Progeny virions also can be seen in the cytoplasm. In addition, empty capsids of the infecting virus particles coat the outside 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?*

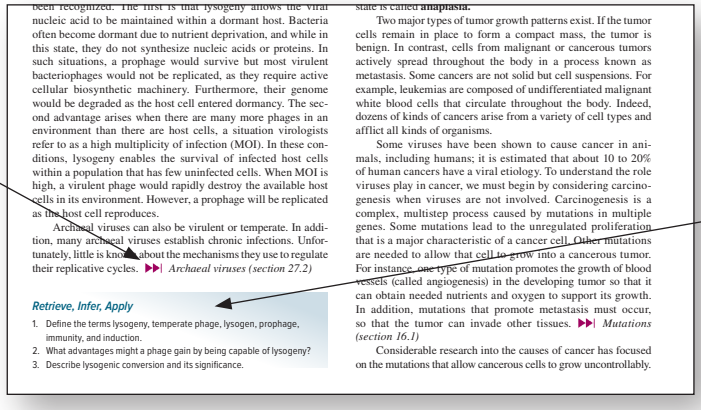
plasma membrane, enabling T4 lysozyme to move from the cytoplasm to the peptidoglycan.

Budding is frequently observed for enveloped viruses; in fact, envelope formation and virion release are usually concurrent processes. When virions are released by budding, the host cell may survive and continue releasing virions for some time. All envelopes of animal viruses are derived from host cell membranes 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 6.15**). 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. Most fungal viruses lack an extracellular phase in their replicative cycles. Instead they are transmitted by cell division, spore formation, or during mating. 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 detec-

Animation Icon—This symbol indicates that material presented in the text is accompanied by an animation within Instructor Resources in Connect. Create a file attachment assignment in Connect to have your students view the animation, or post it to your Learning Management System for students.

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



been recognized. The first is that lysogeny allows the viral nucleic acid to be maintained within a dormant host. Bacteria often become dormant due to nutrient deprivation, and while in this state, they do not synthesize nucleic acids or proteins. In such situations, a prophage would survive but most virulent bacteriophages would not be replicated, as they require active cellular biosynthetic machinery. Furthermore, their genome would be degraded as the host cell entered dormancy. The second advantage arises when there are many more phages in an environment than there are host cells, a situation virologists refer to as a high multiplicity of infection (MOI). In these conditions, lysogeny enables the survival of infected host cells within a population that has few uninfected cells. When MOI is high, a virulent phage would rapidly destroy the available host cells in its environment. However, a prophage will be replicated as the host cell reproduces.

Archaeal viruses can also be virulent or temperate. In addition, many archaeal viruses establish chronic infections. Unfortunately, little is known about the mechanisms they use to regulate their replicative cycles. ▶▶| *Archaeal viruses (section 27.2)*

Retrieve, Infer, Apply

1. Define the terms lysogeny, temperate phage, lysogen, prophage, immunity, and induction.
2. What advantages might a phage gain by being capable of lysogeny?
3. Describe lysogenic conversion and its significance.

state is called **anaplasia.**

Two major types of tumor growth patterns exist. If the tumor cells remain in place to form a compact mass, the tumor is benign. In contrast, cells from malignant or cancerous tumors actively spread throughout the body in a process known as metastasis. Some cancers are not solid but cell suspensions. For example, leukemias are composed of undifferentiated malignant white blood cells that circulate throughout the body. Indeed, dozens of kinds of cancers arise from a variety of cell types and afflict all kinds of organisms.

Some viruses have been shown to cause cancer in animals, including humans; it is estimated that about 10 to 20% of human cancers have a viral etiology. To understand the role viruses play in cancer, we must begin by considering carcinogenesis when viruses are not involved. Carcinogenesis is a complex, multistep process caused by mutations in multiple genes. Some mutations lead to the unregulated proliferation that is a major characteristic of a cancer cell. Other mutations are needed to allow that cell to grow into a cancerous tumor. For instance, one type of mutation promotes the growth of blood vessels (called angiogenesis) in the developing tumor so that it can obtain needed nutrients and oxygen to support its growth. In addition, mutations that promote metastasis must occur, so that the tumor can invade other tissues. ▶▶| *Mutations (section 16.1)*

Considerable research into the causes of cancer has focused on the mutations that allow cancerous cells to grow uncontrollably.

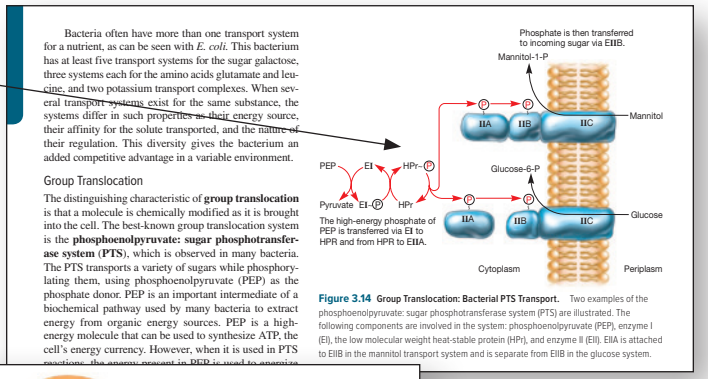
Cross-Referenced Notes—In-text references refer students to other parts of the book to review.

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

Student-Friendly Organization

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

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



Bacteria often have more than one transport system for a nutrient, as can be seen with *E. coli*. This bacterium has at least five transport systems for the sugar galactose, three systems each for the amino acids glutamate and leucine, and two potassium transport complexes. When several transport systems exist for the same substance, the systems differ in such properties as their energy source, their affinity for the solute transported, and the nature of their regulation. This diversity gives the bacterium an added competitive advantage in a variable environment.

Group Translocation

The distinguishing characteristic of **group translocation** is that a molecule is chemically modified as it is brought into the cell. The best-known group translocation system is the **phosphoenolpyruvate: sugar phosphotransferase system** (**PTS**), which is observed in many bacteria. The PTS transports a variety of sugars while phosphorylating them, using phosphoenolpyruvate (PEP) as the phosphate donor. PEP is an important intermediate of a biochemical pathway used by many bacteria to extract energy from organic energy sources. PEP is a high-energy molecule that can be used to synthesize ATP, the cell's energy currency. However, when it is used in PTS

Figure 3.14 **Group Translocation: Bacterial PTS Transport.** Two examples of the phosphoenolpyruvate: sugar phosphotransferase system (PTS) are illustrated. The following components are involved in the system: phosphoenolpyruvate (PEP), enzyme I (EI), the low molecular weight heat-stable protein (HPr), and enzyme II (EII). EIIA is attached to EIIB in the mannitol transport system and is separate from EIIB in the glucose system.

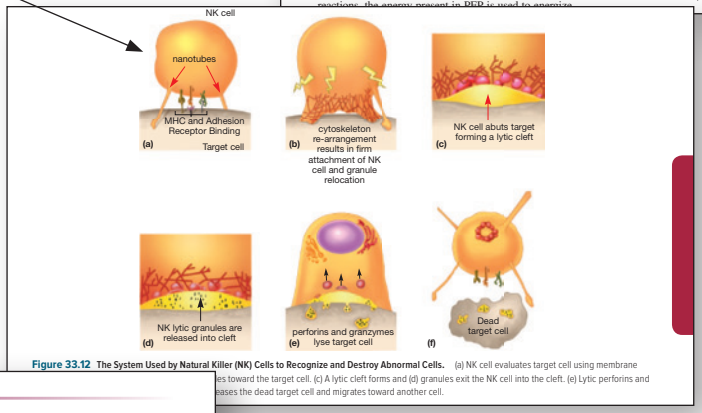


Figure 33.12 **The System Used by Natural Killer (NK) Cells to Recognize and Destroy Abnormal Cells.** (a) NK cell evaluates target cell using membrane ...es toward the target cell. (c) A lytic cleft forms and (d) granules exit the NK cell into the cleft. (e) Lytic perforins and ...eases the dead target cell and migrates toward another cell.

Key Concepts

17.1 Key Discoveries Led to the Development of Recombinant DNA Technology

- Genetic engineering became possible after the discovery of restriction enzymes and reverse transcriptase, and the development of essential methods in nucleic acid chemistry such as the Southern blotting technique.
- Restriction enzymes are important because they cut DNA at specific sequences, thereby releasing fragments of DNA that can be cloned or otherwise manipulated (**figure 17.3** and **table 17.1**).
- Gel electrophoresis is used to separate molecules according to charge and size.
- DNA fragments are separated on agarose and acrylamide gels. Because DNA is acidic, it migrates from the negative to the positive end of a gel (**figure 17.6**).

17.2 Polymerase Chain Reaction Amplifies Targeted DNA

- The polymerase chain reaction (PCR) allows small amounts of specific DNA sequences to be amplified, or increased in concentration thousands of times (**figure 17.8**).
- PCR consists of multiple cycles of 3 steps each: DNA denaturation, primer annealing, and DNA synthesis.
- PCR has numerous applications. It often is used to obtain genes for cloning and in diagnostic and forensic science.

17.3 Cloning Vectors Are Needed to Create Recombinant DNA

- There are four types of cloning vectors: plasmids, viruses, cosmids, and artificial chromosomes. Cloning vectors generally have at least three components: an origin of replication, a selectable marker, and a multicloning site or polylinker (**table 17.2; figures 17.10** and **17.12**).
- The most common approach to cloning is to digest both vector and DNA to be inserted with the same restriction enzyme or enzymes so that compatible sticky ends are generated. The vector and DNA to be cloned are then incubated in the presence of DNA ligase, which catalyzes the formation of phosphodiester bonds once the DNA fragment inserts into the vector.
- Once the recombinant plasmid has been introduced into host cells, cells carrying vector must be selected. This is often accomplished by allowing the growth of only antibiotic-resistant cells because the vector bears an antibiotic-resistance gene. Cells that took up vector with inserted DNA must then be distinguished from those that contain only vector. Often a blue-versus-white colony phenotype is used; this is based on the presence or absence, respectively, of a functional *lacZ* gene (**figure 17.11**).

17.4 Introducing Recombinant DNA into Host Cells

- The bacterium *E. coli* and the yeast *S. cerevisiae* are the most common host species.
- DNA can be introduced into microbes by transformation or electroporation.

17.5 Genomic Libraries: Cloning Genomes in Pieces

- It is sometimes necessary to find a gene without the knowledge of the gene's DNA sequence. A genomic library is constructed by cleaving an organism's genome into many fragments, each of which is cloned into a vector to make a unique recombinant plasmid.
- Genomic libraries are often screened for the gene of interest by either phenotypic rescue (genetic complementation) or DNA hybridization with an oligonucleotide probe (**figure 17.13**).

17.6 Expressing Foreign Genes in Host Cells

- An expression vector has the necessary features to express in high levels any recombinant gene it carries.
- If a eukaryotic gene is to be expressed in a bacterium, cDNA is used because it lacks introns; a bacterial leader must also be added to the 5′ end of the gene.
- Purification of recombinant proteins is often accomplished by fusing the coding sequence of a protein to six histidine residue codons found on some expression vectors. When introduced and expressed in bacteria, the His-tagged protein can be selectively purified (**figure 17.14**).
- Green fluorescent protein can be used to study the regulation of gene expression (transcriptional fusions) and protein localization (translational fusions) (**figure 17.15**).

Compare, Hypothesize, Invent

1. You are performing a PCR to amplify a gene encoding a tRNA from a bacterium that has only recently been grown in pure culture. You are expecting a product of 954 bp. However, you generate three different products; only one is the expected size. List at least two possible explanations (excluding experimental error).
2. You have cloned a structural gene required for riboflavin synthesis in *E. coli*. You find that an *E. coli* riboflavin auxotroph carrying the cloned gene on a vector makes less riboflavin than does the wild-type strain. Why might this be the case?

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

Part One

Chapter 1—Evolution is the driving force of all biological systems; this is made clear by introducing essential concepts of microbial evolution first. Advances in the discipline of microbiology and the increasing contributions of genomics and metagenomics are discussed.

Chapter 2—Microscopy was and is critical to the study of microorganisms and this chapter considers the most commonly used methods, including expanded coverage of phase-contrast microscopy.

Chapter 3—Coverage of bacterial cellular structure and function. A new chapter-opening story clearly establishes the importance of the material covered in this chapter.

Chapter 4—Growing understanding of the distinctive characteristics of archaea has warranted the creation of a new Microbial Diversity & Ecology box on the nature of motility organelles in the three domains of life. 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 (*Protists*) and 26 (*Fungi*), which also focus on the diversity of these microbes. The current thought on the evolution of mitochondria and mitochondria-like organelles is considered. Comparisons between bacteria, archaea, and eukaryotes are included throughout the chapter.

Chapter 6—This chapter surveys the essential morphological, physiological, and genetic elements of viruses as well as viroids, satellites, and prions. Updated discussion of the role of viruses in causing cancer. This chapter completes our four-chapter introduction to microbial life.

Part Two

Chapter 7—Discussion of the growth of microbes outside the laboratory, including expanded and updated coverage of the "persister cell" phenomenon, is followed by topics related to laboratory culture of microbes.

Chapter 8—Updated 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 Three

Chapter 10—This introduction to metabolism includes a section outlining the nature of biochemical pathways. The concept of metabolic flux is presented by discussing the interconnected biochemical pathways used by cells.

Chapter 11—An introduction to metabolic diversity and nutritional types is followed by an exploration of the energy-conserving process of each nutritional type. The coverage of oxygenic photosynthesis is expanded and updated.

Chapter 12—New coverage of pathways used to synthesize porphyrins, lipopolysaccharides, sterols, and isoprenoid lipids.

Part Four

Chapter 13—Updated coverage of protein splicing, folding, and secretion.

Chapter 14—The regulation of bacterial cellular processes, with updated coverage of regulation by small RNAs.

Chapter 15—Eukaryal and archaeal genome replication and expression are considered 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. A new chapter-opening story introduces the complexity of understanding the growing problem of antibiotic resistance.

Chapter 17—Students are guided through the steps of cloning a microbial gene—from DNA purification through protein purification.

Chapter 18—Next-generation nucleotide sequencing and single-cell genome sequencing are covered in the context of metagenomics as it relates to the microbial ecology of natural systems, including the human microbiome.

Part Five

Chapter 19—This overview of microbial evolution includes discussion of the concepts of ecotype, microbial species, and superphylum.

Chapter 20—Expanded coverage of archaeal physiology includes archaeal-specific catabolic and anabolic pathways with particular attention CO_2 fixation. The evolutionary advantage of each pathway is discussed in the context of archaeal ecology.

List of Content Changes

Chapter 21—In addition to the ecology and physiology of photosynthetic bacteria, the recently described *Planctomycetes, Verrucomicrobia, Chlamydia* (PVC) superphylum is introduced with an updated review of each of these genera.

Chapter 22—This chapter's coverage includes newly recognized genera, an expanded discussion of sulfur metabolism, and an updated discussion of gliding motility that reflects recent advances.

Chapter 24—This overview of actinobacteria incorporates new figures illustrating the mycobacterial cell wall and a new photo program.

Chapter 25—This chapter introduces protist morphology and diversity, with an emphasis on physiological adaptation and ecology.

Chapter 26—Fungal diversity is presented within a phylogenetic framework. Morphology, ecology, and reproductive strategies are stressed.

Chapter 27—Updated discussion of virus taxonomy and phylogeny.

Part Six

Chapter 28—The description of each nutrient cycle is accompanied by a "student-friendly" figure that distinguishes between reductive and oxidative reactions. Updated coverage of the role of biogeochemical cycling in global climate change.

Chapter 29—This chapter continues to emphasize culture-based techniques as the "gold standard" and reviews some new, innovative approaches such as mass spectrometry in the identification of microbial taxa as well as metatranscriptomics and metaproteomics in the study of community activity.

Chapter 30—Updated and expanded discussion of the role of marine microbes in the global carbon budget as well as an update on subsurface microbes.

Chapter 31—New and updated coverage of the microbial ecology of the phyllosphere, rhizoplane, and rhizosphere. Expanded discussion of fungal plant pathogens.

Chapter 32—Important model systems for the exploration of microbial symbiosis are presented, along with increased coverage of the human microbiome.

Part Seven

Chapter 33—Updated to reflect the increasing overlap with the acquired immune functions, 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. Uniting these components are the ever-expanding methods for recognition of microorganisms by immune cells. Barriers, chemical mediators, and immune cells define the molecular mechanisms that drive phagocytosis and inflammation.

Chapter 34—Updated to enhance linkages between innate and adaptive immune activities. Discussions integrate concepts of cell biology, physiology, and genetics 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 reorganized to reflect the host-microorganism interaction that can lead to human disease. The essential elements required for a pathogen to establish infection are introduced, and virulence mechanisms are highlighted. This chapter is placed after the immunology chapters to stress that the host-parasite relationship is dynamic, with adaptations and responses offered by both host and parasite.

Part Eight

Chapter 36—This chapter has been updated to reflect the technological advances 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, common viral infections, and prion-mediated diseases.

Chapter 39—Updated coverage of bacterial organisms and the ways in which they commonly lead to human disease.

Chapter 40—Updated and expanded coverage of fungal and protozoal diseases.

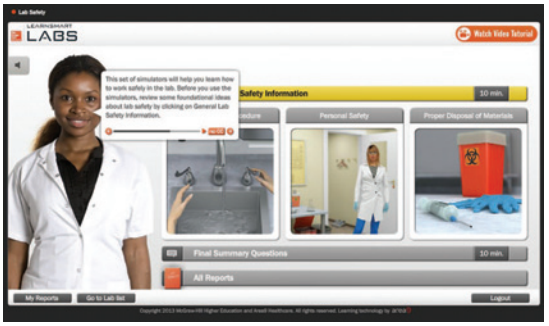
Part Nine

Chapter 41—Discussion of milk fermentation processes, including an updated description of cheese making.

Chapter 42—Includes updated coverage of biofuel production (first introduced in chapter 21) and an introduction to synthetic biology.

Chapter 43—This chapter complements our 21st-century approach to microbiology by emphasizing the importance of clean water and the power of microbial environmental remediation.

Lab Tools for Your Success

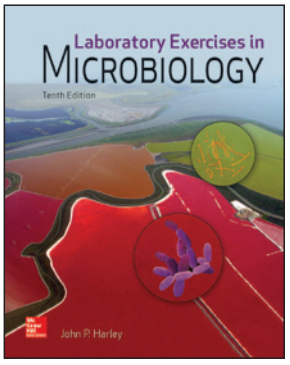


LearnSmart Labs® is an adaptive simulated lab experience that brings meaningful scientific exploration to students. Through a series of adaptive questions, LearnSmart Labs identifies a student's knowledge gaps and provides resources to quickly and efficiently close those gaps. Once students have mastered the necessary basic skills and concepts, they engage in a highly realistic simulated lab experience that allows for mistakes and the execution of the scientific method.

LearnSmart® Prep is an adaptive learning tool that prepares students for college-level work in Microbiology. LearnSmart Prep individually identifies concepts the student does not fully understand and provides learning resources to teach essential concepts so he or she enters the classroom prepared. Data-driven reports highlight areas where students are struggling, helping to accurately identify weak areas.

Laboratory Exercises in Microbiology, Tenth Edition

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



Acknowledgments

In the preparation of each edition, we have been guided by the collective wisdom of reviewers who are expert microbiologists and excellent teachers. They represent experience in community colleges, liberal arts colleges, comprehensive institutions, and research universities. We have followed their recommendations, while remaining true to our overriding goal of writing readable, student-centered content. Each feature incorporated into this edition has been carefully considered in terms of how it may be used to support student learning in both the traditional and the flipped learning environment.

Also in this edition, we are very excited to incorporate real student data points and input, derived from thousands of our LearnSmart users, to help guide our revision. With this information, we were able to hone both book and digital content.

The authors wish to extend their gratitude to our team at McGraw-Hill, including Marija Magner, Darlene Schueller, Kristine Rellihan, Jayne Klein, Tara McDermott, Christina Nelson, Lorraine Buczek, Carrie Burger, and David Tietz. Finally, we thank our spouses and children, who provided support and tolerated our absences (mental, if not physical) while we completed this demanding project.

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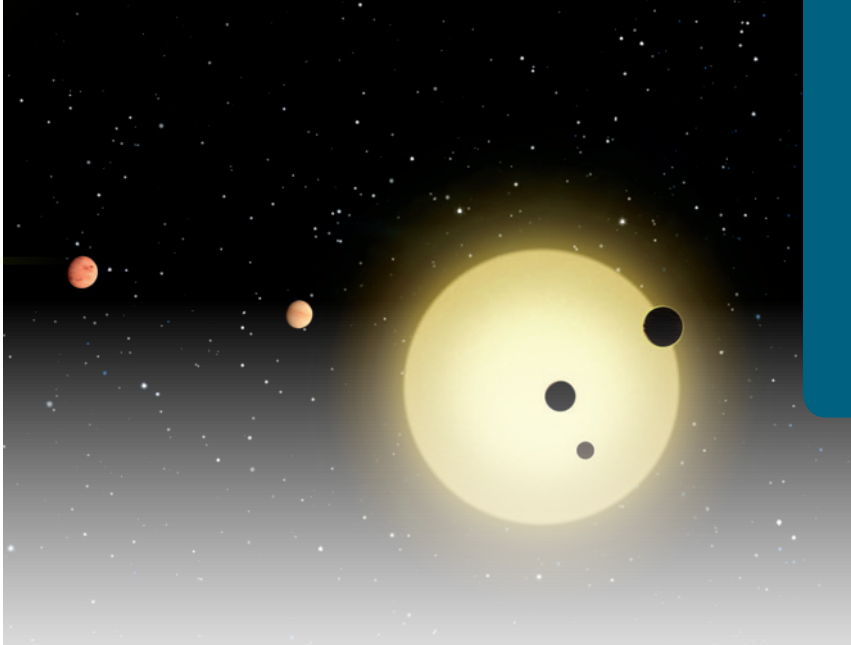
The Evolution of Microorganisms and Microbiology



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 4,000 Potential Planets Discovered

As of July 2015, the National Aeronautics and Space Administration (NASA) reported that over 4,000 potential planets and almost 1,000 confirmed 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 possible 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. NASA 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? 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. Still other microbes are able to use inorganic molecules as sources of energy in both oxic (oxygen available) and anoxic (no oxygen) conditions. Microbes also are diverse in terms of environmental conditions. Some withstand extremes of temperature, pressure, and pH. Indeed, studies have shown that some Earth microbes tolerate conditions that simulate those on Mars. These microbes are important for understanding what life might be like on other worlds.

Our goal in this chapter is to introduce you to the amazing world of microorganisms 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

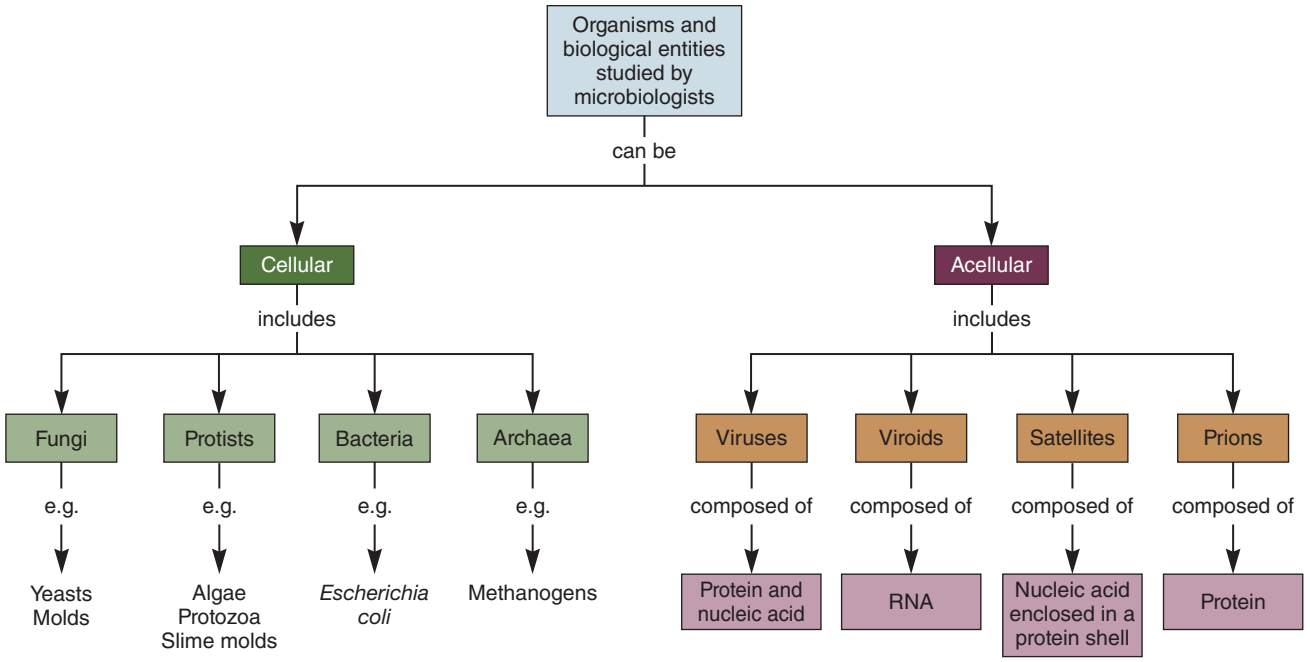


Figure 1.1 **Concept Map Showing the Types of Biological Entities Studied by Microbiologists.**

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

Microorganisms are defined as those organisms 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 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.

In addition to microorganisms, microbiologists study a variety of acellular biological entities (figure 1.1). These include viruses and subviral agents. Although the term "microorganism" is often applied only to cellular microbes, some texts use both "microorganism" and "microbe" when referring to these acellular agents.

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. 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. ▶▶| *Use of the term "prokaryote" is controversial (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 (1928–2012) 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*, *Archaea*, and *Eukarya* (all eukaryotic organisms) (**figure 1.2**).

▶▶| *Nucleic acids (appendix I); Proteins (appendix I)*

Although the three-domain tree is widely accepted, other trees are also possible. Perhaps the leading alternate tree is a two-domain tree consisting of *Archaea* and *Bacteria*. In this tree eukaryotes are simply a lineage within the archaeal domain. Both trees have proponents who continue to debate which tree best represents the evolutionary history of life on Earth. Until the debate is settled, we will use the three-domain system throughout this text. A brief description of the three domains and the microorganisms in them follows.

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 bodies, forming the human **microbiome.** 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, the human microbiome helps maintain our health and well-being.

▶▶| *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 conclude that all bacteria are pathogens, but in fact, relatively few are. Most play beneficial

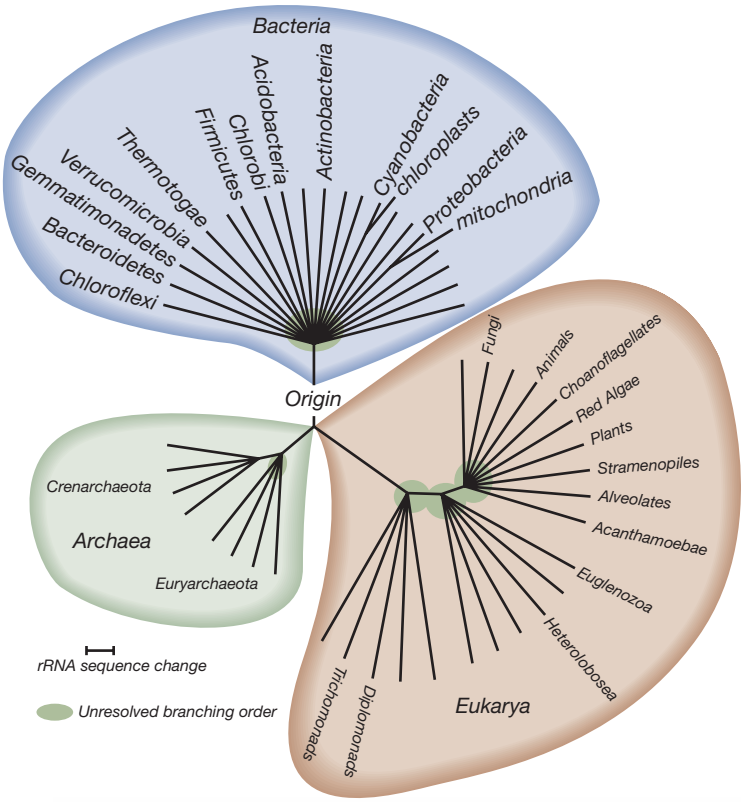


Figure 1.2 Universal Phylogenetic Tree. These evolutionary relationships are based on rRNA sequence comparisons. To save space, many lineages have not been identified.

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

roles. In addition to maintaining human health by forming our microbiomes, 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.

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

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.

protozoa and algae. Despite their use, none of these terms has taxonomic value as protists, algae, and protozoa do 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, which led to the mass exodus of Irish to the United States and other countries. ▶▶| *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 dough 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. ▶▶| 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 virus particles (also called virions) 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** are infectious agents composed only of ribonucleic acid (RNA). They cause numerous plant diseases. **Satellites** are composed of a nucleic acid enclosed in a protein shell. They 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 Microbes Have Evolved and Diversified for Billions of Years

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. 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.

Theories of the Origin of Life Depend Primarily on Indirect Evidence

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?

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 best direct evidence for the nature of primitive life would be a fossil record. There have been reports of microbial fossil discoveries since 1977 (**figure 1.3**). These have always met with skepticism because finding them involves preparing thin slices of ancient rocks and examining the slices for objects that look like cells. Unfortunately, some things that look like cells can be formed by geological forces that occurred as the rock was formed. The result is that the fossil record for microbes is sparse and always open to reinterpretation. Despite these problems, most scientists agree that life was present on Earth about 3.5 billion years ago (**figure 1.4**). To reach this conclusion, biologists have had to rely primarily on indirect evidence. Among the indirect evidence used are molecular fossils. These are chemicals found in rock or sediment that are chemically related to molecules found in cells. For instance, the presence in a rock of molecules called hopanes is an indication that when the rock was formed, bacteria were present. This conclusion is reached because hopanes are formed from hopanoids, which are found in the plasma membranes of bacteria. As you can see, no single piece of evidence can stand alone. Instead many pieces of evidence are put together in an attempt to get a coherent picture to emerge, much as for a jigsaw puzzle.

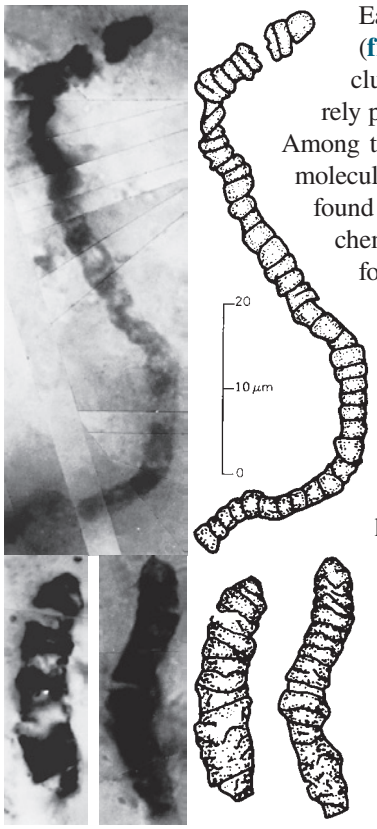


Figure 1.3 Possible Microfossils Found in the Archaeon Apex Chert of Australia. Chert is a type of granular sedimentary rock rich in silica. These structures were discovered in 1977. Because of their similarity to filamentous cyanobacteria they were proposed to be microfossils. In 2011 scientists reported that similar structures from the same chert were not biological in origin. They used spectrometry and microscopy techniques not available in 1977 to show that the structures were fractures in the rock filled with quartz and hematite. Scientists are still debating whether or not these truly are microfossils.

Early Life Was Probably RNA Based

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.

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. This idea was supported 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.

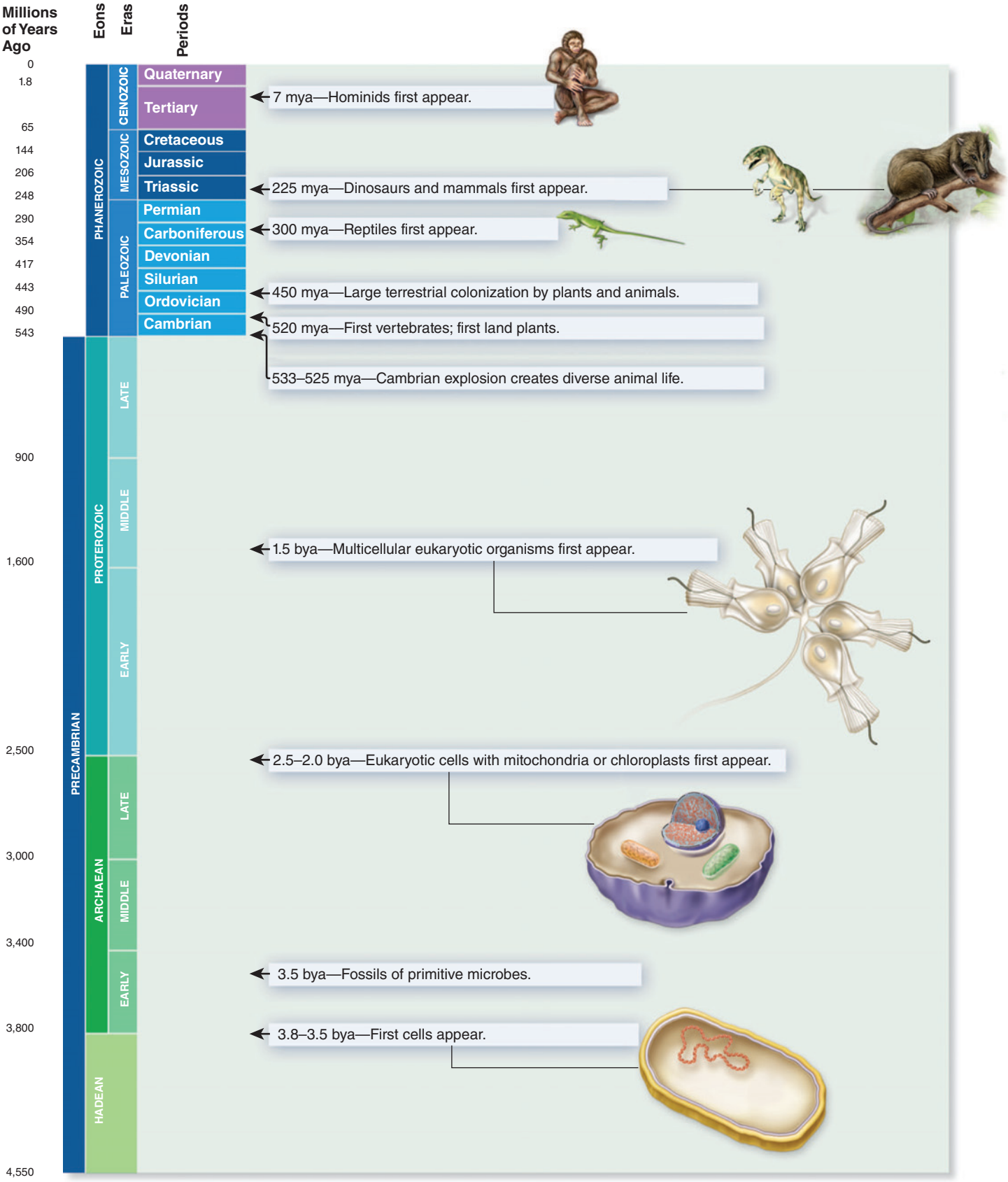


Figure 1.4 **An Overview of the History of Life on Earth.** mya = million years ago; bya = billion years ago.

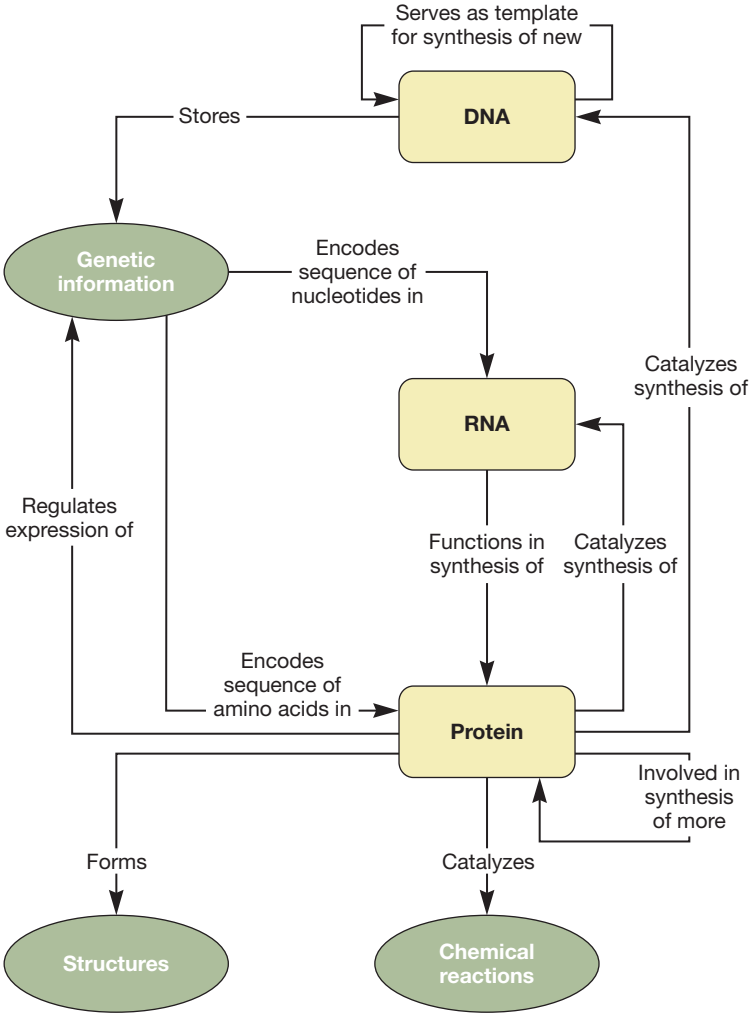


Figure 1.5 Functions of DNA, RNA, and Protein, and Their Relationships to Each Other in Modern Cells.

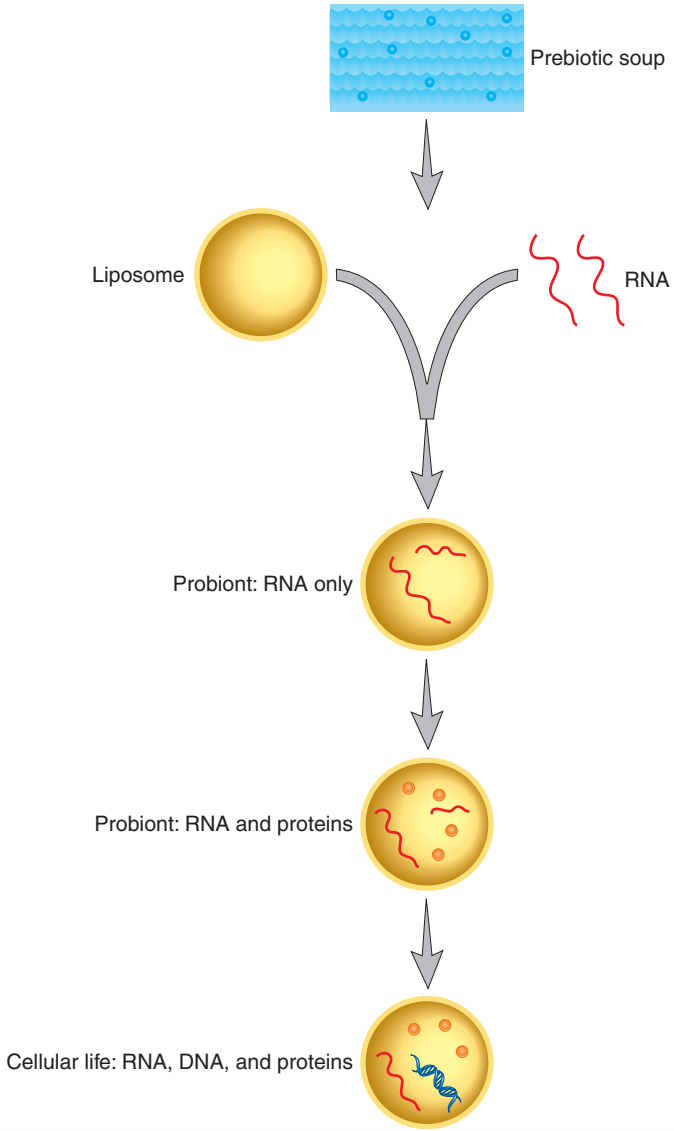


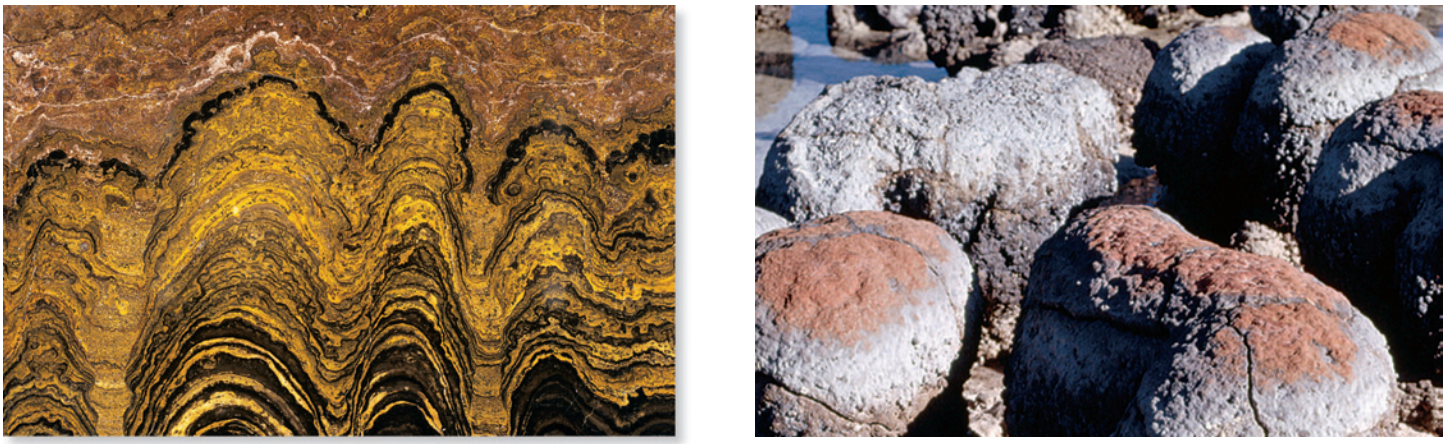
Figure 1.6 The RNA World Hypothesis for the Origin of Life.

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

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. ▶▶| *Lipids (appendix I)*

Jack Szostak is a leader in exploring how RNA-containing cells, so-called protocells, may have formed. His group has created liposomes using simpler fatty acids than those found in membranes today. These simpler fatty acids formed leaky liposomes that allowed single RNA nucleotides to move into the liposome, but prevented large RNA chains from moving out. Furthermore, they could prod the liposomes into growing and dividing. Szostak's group has also been able to create conditions in which an RNA molecule could serve as a template for synthesis of a complementary RNA strand. Thus, their experiments have recapitulated what may have happened in the early steps of the evolution of cells. As seen in figure 1.6, several other processes would need to occur to reach the level of complexity found in extant cells.

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 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



(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 the other, became mineralized. (b) Modern stromatolites from Western Australia. Each stromatolite is a rocklike structure, typically 1 m in diameter, containing layers of cyanobacteria.

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 cells, 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 (section 14.3); Translational riboswitches (section 14.4)*

Despite the evidence supporting the RNA world hypothesis, it is not without problems, and many argue against it. Another area of research also fraught with considerable debate is the evolution of metabolism, in particular the evolution of energy-conserving metabolic processes. The 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 (oxygenic photosynthesis), appears to have evolved perhaps as early as 2.7 billion years ago. Fossils of cyanobacteria-like cells found in rocks dating to that time support this hypothesis, 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*). Furthermore, chemical evidence, such as the presence of certain isotopes and oxidized minerals in rocks of this age, also support the antiquity of oxygenic photosynthesis. 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); Microbial taxonomy and phylogeny are largely based on molecular characterization (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. The tip of 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.

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

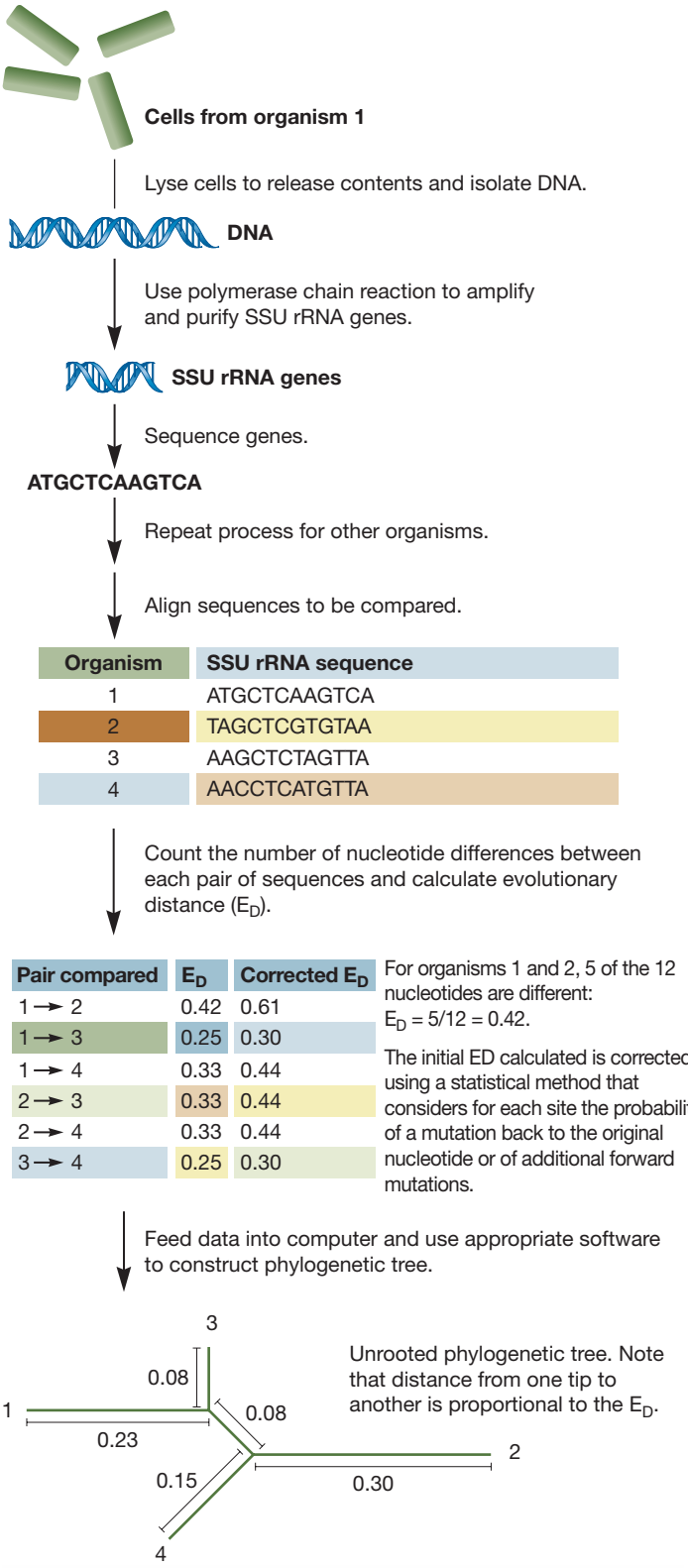


Figure 1.8 **The Construction of Phylogenetic Trees Using a Distance Method. The polymerase chain reaction is described in chapter 17.**

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

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 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 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.

Mitochondria, Mitochondria-Like Organelles, and Chloroplasts Evolved from Endosymbionts

The **endosymbiotic hypothesis** is generally accepted as the origin of several eukaryotic organelles, including 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 19.10*).

Although the mechanism by which the endosymbiotic relationship was established is unknown, there is considerable evidence to support the hypothesis. Mitochondria and chloroplasts