4

Structure of Bacterial and Archaeal Cells

Our planet has always been in the "Age of Bacteria," ever since the first fossils—bacteria of course—were entombed in rocks more than 3 billion years ago. On any possible, reasonable criterion, bacteria are—and always have been—the dominant forms of life on Earth.
—Paleontologist Stephen J. Gould (1941–2002)

"*Double, double toil and trouble*; *Fire burn, and cauldron bubble*" is the refrain repeated several times by the chanting witches in Shakespeare's *Macbeth* (Act IV, Scene 1). This image of a hot, boiling cauldron actually describes the environment in which many bacterial, and especially archaeal, species happily grow! For example, some species can be isolated from hot springs or the hot, acidic mud pits of volcanic vents (FIGURE 4.1).

When the eminent evolutionary biologist and geologist Stephen J. Gould wrote the opening quote of this chapter, he, as well as most microbiologists at the time, had no idea that embedded in these "bacteria" was another whole domain of organisms. Thanks to the pioneering studies of Carl Woese and his colleagues, it now is quite evident there are two distinctly different groups of "prokaryotes"—the Bacteria and the Archaea. Many of the organisms Woese and others studied are organisms that would live a happy life in a witch's cauldron because they can grow at high temperatures, produce methane gas, or survive in extremely acidic and hot environments—a real cauldron! Termed **extremophiles**, these members of the domains Bacteria and Archaea have a unique genetic makeup and have adapted to extreme environmental conditions.
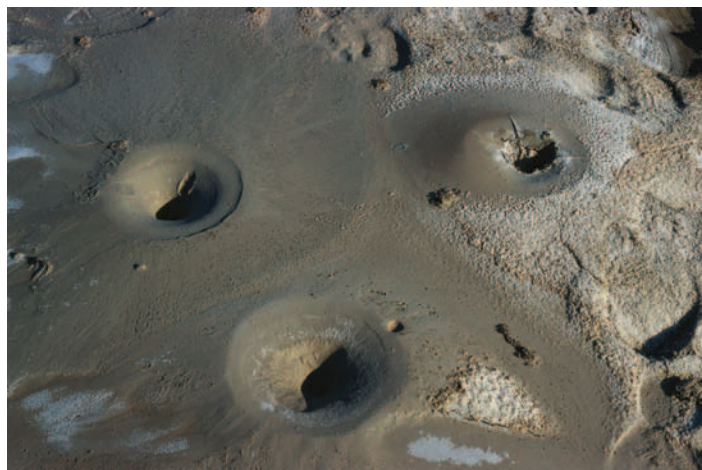
In fact, Gould's "first fossils" may have been archaeal species. Many microbiologists believe the ancestors of today's archaeal species might represent a type of organism that first inhabited planet Earth when it was a young, hot place. These unique characteristics led Woese to propose

Image courtesy of Dr. Fred Murphy/CDC.

(A)

(B)

FIGURE 4.1 **Life at the Edge.** Bacterial and archaeal extremophiles have been isolated from the edges of natural cauldrons, including (**A**) the Grand Prismatic Spring in Yellowstone National Park, Wyoming, where the water of the hot spring is over 70°C, or (**B**) the mud pools surrounding sulfurous steam vents of the Solfatara Crater in Pozzuoli, Italy, where the mud has a very low pH and a temperature above 90°C. (**A**) © Aleix Ventayol Farrés/ShutterStock, Inc. (**B**) © Corbis/age fotostock. »» How do extremophiles survive under these extreme conditions?

these organisms be lumped together and called the Archaebacteria (*archae* = "ancient").

Since then, the domain name has been changed to Archaea because (1) not all members are extremophiles or related to these possible ancient ancestors and (2) they are not Bacteria—they are Archaea. Some might also debate using the term prokaryotes when referring to both domains, as organisms in the two domains are as different from each other as they are from the Eukarya.

In this chapter, we examine briefly some of the organisms in the domains Bacteria and Archaea. However, because so many pathogens of humans are in the domain Bacteria, we emphasize structure within this domain. As we see in this chapter, a study of the structural features of bacterial cells provides a window to their activities and illustrates how the Bacteria relate to other living organisms.
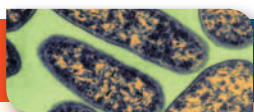
Chapter Challenge

As microbes have been studied in more detail using special microscopy optics and have had their genes sequenced, it is clear that there are unique as well as shared characteristics between species in the domains Bacteria, Archaea, and Eukarya. Once you finish studying this chapter on the structure of cells in the Bacteria and Archaea domains, you will be better prepared to wisely respond to this chapter challenge: "Studying the diversity of life only accentuates life's unity." Let's search for the evidence!

KEY CONCEPT 4.1   **There Is Tremendous Diversity Among the Bacteria and Archaea**

Currently, there are some 7,000 known bacterial and archaeal species and a suspected 10 million species. In this section, we will highlight a few phyla and groups using the phylogenetic tree in **FIGURE 4.2**.

The Domain Bacteria Contains Some of the Most Studied Microbial Organisms

There are about 25 assigned phyla of Bacteria that have been identified from culturing or nucleotide sequencing. It should come as no shock to you by now to read that the vast majority of these phyla play a positive role in nature (MicroFocus 4.1). Of course, we know from personal experience that some bacterial organisms are harmful—many human pathogens are members of the domain Bacteria. Certain species multiply within the human body, where they disrupt tissues or produce toxins that result in disease.
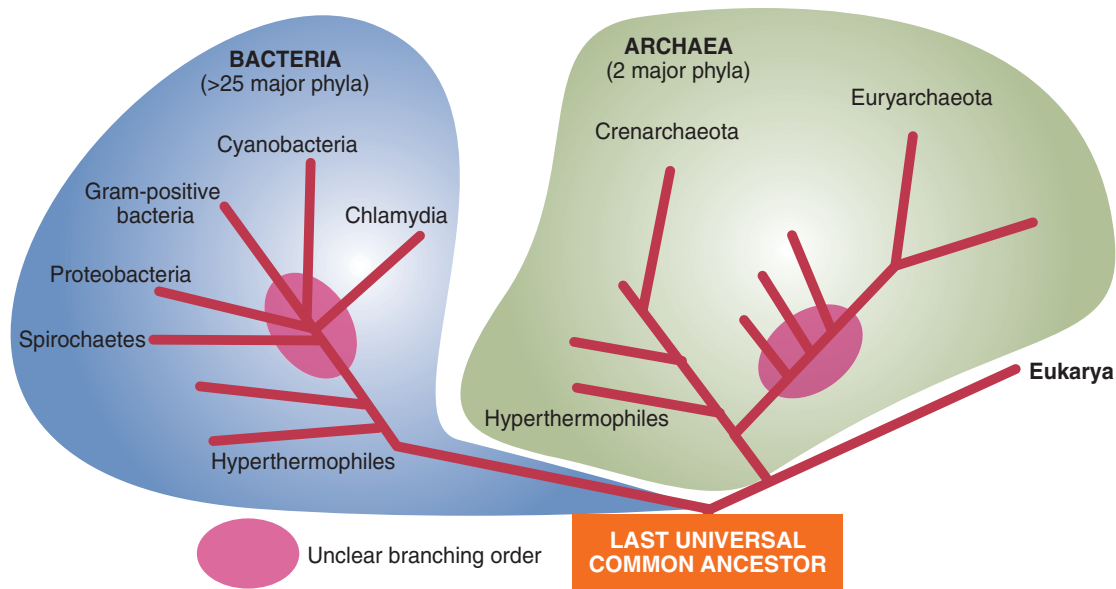
FIGURE 4.2 **The Phylogenetic Tree of Bacteria and Archaea.** The tree shows several of the bacterial and archaeal lineages discussed in this chapter. »» What is common to the branch base of both the Bacteria and Archaea?

The Bacteria have adapted to the diverse environments on Earth, inhabiting the air, soil, and water, and they exist in enormous numbers on the surfaces of virtually all plants and animals. They can be isolated from Arctic ice, thermal hot springs, the fringes of space, and the tissues of animals. Bacterial species, along with their archaeal relatives, have so completely colonized every part of the Earth that their mass is estimated to outweigh the mass of all plants and animals combined. Let's look briefly at some of the major phyla and other groups.

Proteobacteria. The **Proteobacteria** (*proteo* = "first") contains the largest and most diverse group of species. The phylum, spread among five classes, includes many familiar gram-negative genera, such as *Escherichia* (FIGURE 4.3A) and some of the most recognized human pathogens, including species of *Shigella, Salmonella, Neisseria* (responsible for gonorrhea), *Yersinia* (responsible for plague), and *Vibrio* (responsible for cholera). It is likely that the mitochondria of the Eukarya evolved through **endosymbiosis** from a free-living ancestor of the Proteobacteria.

The group also includes the **rickettsiae** (sing., rickettsia), which are called **obligate, intracellular parasites** because they can only reproduce once inside a host cell. These tiny bacterial cells are transmitted among humans primarily by arthropods, and are cultivated only in living tissues such as chick embryos. Different species cause a number of important diseases, including Rocky Mountain spotted fever and typhus fever.

The gram-positive bacteria rival the Proteobacteria in diversity and are divided into two phyla.

Firmicutes. The **Firmicutes** (*firm* = "strong"; *cuti* = "skin") consists of many species that are gram-positive. As we will see in this chapter, they share a similar thick "skin," which refers to their cell wall structure. Genera include *Bacillus* and *Clostridium,* specific species of which are responsible for anthrax and botulism, respectively. Species within the genera *Staphylococcus* (FIGURE 4.3B) and *Streptococcus* are responsible for several mild to life-threatening human illnesses.

Also within the Firmicutes is the genus *Mycoplasma,* which lacks a cell wall but is otherwise phylogenetically related to the gram-positive bacterial species (FIGURE 4.3C). Among the smallest free-living bacterial organisms, one species causes a form of pneumonia while another mycoplasmal illness represents a sexually-transmitted disease.

Actinobacteria. Another phylum of gram-positive species is the **Actinobacteria**. Often called the "actinomycetes," these soil organisms form a system of branched filaments that somewhat resemble the growth form of fungi (FIGURE 4.3D). The genus *Streptomyces* is the source for important antibiotics. Another medically important genus is *Mycobacterium,* one species of which is responsible for tuberculosis.

■ Host:
An organism on or in which a pathogen infects.

■ Arthropod:
An animal having jointed appendages and segmented body (e.g., ticks, lice, fleas, mosquitoes).

MICROFOCUS 4.1:
Bacteria in Eight Easy Lessons[1]

Mélanie Hamon, an assistante de recherché at the Institut Pasteur in Paris, says that when she introduces herself as a bacteriologist, she often is asked, "Just what does that mean?" To help explain her discipline, she gives us, in eight letters, what she calls "some demystifying facts about bacteria."

Basic principles: Their average size is 1/25,000th of an inch. In other words, hundreds of thousands of bacteria fit into the period at the end of this sentence. In comparison, human cells are 10 to 100 times larger with a more complex inner structure. While human cells have copious amounts of membrane-contained subcompartments, bacteria more closely resemble pocketless sacs. Despite their simplicity, they are self-contained living beings, unlike viruses, which depend on a host cell to carry out their life cycle.

Astonishing: Bacteria are the root of the evolutionary tree of life, the source of all living organisms. Quite successful evolutionarily speaking, they are ubiquitously distributed in soil, water, and extreme environments such as ice, acidic hot springs or radioactive waste. In the human body, bacteria account for 10% of dry weight, populating mucosal surfaces of the oral cavity, gastrointestinal tract, urogenital tract and surface of the skin. In fact, bacteria are so numerous on earth that scientists estimate their biomass to far surpass that of the rest of all life combined.

Crucial: It is a little known fact that most bacteria in our bodies are harmless and even essential for our survival. Inoffensive skin settlers form a protective barrier against any troublesome invader while approximately 1,000 species of gut colonizers work for our benefit, synthesizing vitamins, breaking down complex nutrients and contributing to gut immunity. Unfortunately for babies (and parents!), we are born with a sterile gut and "colic" our way through bacterial colonization.

Tools: Besides the profitable relationship they maintain with us, bacteria have many other practical and exploitable properties, most notably, perhaps, in the production of cream, yogurt and cheese. Less widely known are their industrial applications as antibiotic factories, insecticides, sewage processors, oil spill degraders, and so forth.

Evil: Unfortunately, not all bacteria are "good," and those that cause disease give them all an often undeserved and unpleasant reputation. If we consider the multitude of mechanisms these "bad" bacteria—pathogens—use to assail their host, it is no wonder that they get a lot of bad press. Indeed, millions of years of coevolution have shaped bacteria into organisms that "know" and "predict" their hosts' responses. Therefore, not only do bacterial toxins know their target, which is never missed, but bacteria can predict their host's immune response and often avoid it.

Resistant: Even more worrisome than their effectiveness at targeting their host is their faculty to withstand antibiotic therapy. For close to 50 years, antibiotics have revolutionized public health in their ability to treat bacterial infections. Unfortunately, overuse and misuse of antibiotics have led to the alarming fact of resistance, which promises to be disastrous for the treatment of such diseases.

Ingenious: The appearance of antibiotic-resistant bacteria is a reflection of how adaptable they are. Thanks to their large populations they are able to mutate their genetic makeup, or even exchange it, to find the appropriate combination that will provide them with resistance. Additionally, bacteria are able to form "biofilms," which are cellular aggregates covered in slime that allow them to tolerate antimicrobial applications that normally eradicate free-floating individual cells.

A long tradition: Although "little animalcules" were first observed in the 17th century, it was not until the 1850s that Louis Pasteur fathered modern microbiology. From this point forward, research on bacteria has developed into the flourishing field it is today. For many years to come, researchers will continue to delve into this intricate world, trying to understand how the good ones can help and how to protect ourselves from the bad ones. It is a great honor to be part of this tradition, working in the very place where it was born.

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[1]Republished with permission of the author, the Institut Pasteur, and the Pasteur Foundation. The original article appeared in *Pasteur Perspectives* Issue 20 (Spring 2007), the newsletter of the Pasteur Foundation, which may be found at www.pasteurfoundation.org © Pasteur Foundation.
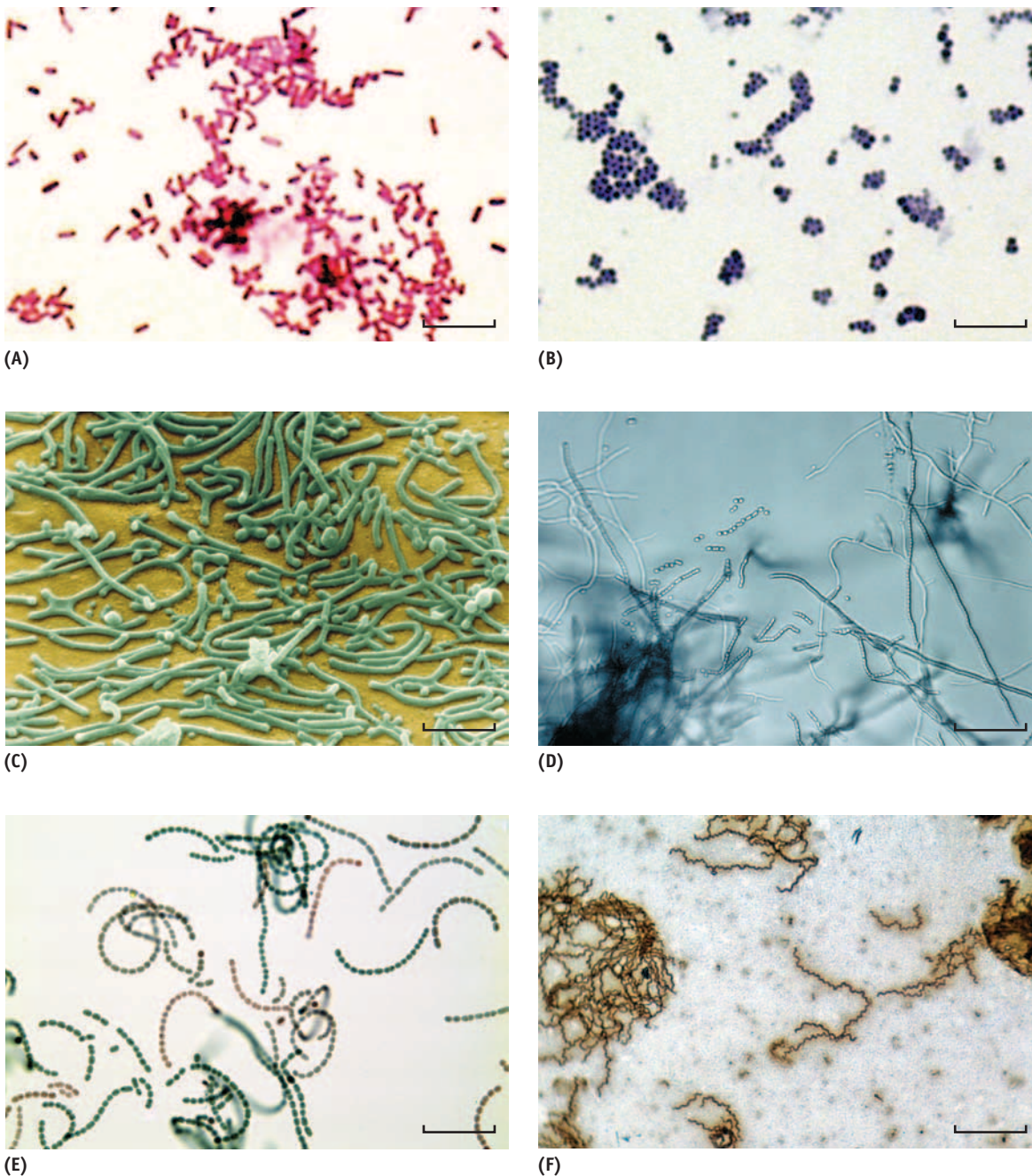
FIGURE 4.3 **Members of the Domain Bacteria.** (**A**) *Escherichia coli* (Bar = 10 μm.), (**B**) *Staphylococcus aureus* (Bar = 10 μm.), (**C**) *Mycoplasma* species (Bar = 2 μm.), (**D**) *Streptomyces* species (Bar = 20 μm.), (**E**) *Anabaena* species (Bar = 100 μm.), and (**F**) *Treponema pallidum* (Bar = 10 μm.). All images are light micrographs except (**C**), a false-color scanning electron micrograph. (**A, B, E, F**) Courtesy of Jeffrey Pommerville. (**C**) © Don W. Fawcett/Photo Researchers, Inc. (**D**) Courtesy of Dr. David Berd/CDC. »» What is the Gram staining result for *E. coli* and *S. aureus*?

Cyanobacteria. The members of cyano-bacteria are phylogenetically related to the gram-positive species and can exist as unicellular, filamentous, or colonial forms (FIGURE 4.3E). Once known as blue-green algae because of their pigmentation, pigments also may be black, yellow, green, or red. The periodic redness of the Red Sea, for example, is due to blooms of those cyanobacterial species that contain large amounts of red pigment.

The phylum **Cyanobacteria** is unique among bacterial groups because its members carry out photosynthesis similar to unicellular algae using the light-trapping pigment chlorophyll. Their

■ **Bloom:**
A sudden increase in the number of cells of an organism in an environment.

evolution on Earth was responsible for the "oxygen revolution" that transformed life on the young planet some 2 billion years ago. In addition, chloroplasts probably evolved through endosymbiosis from a free-living cyanobacterial ancestor.

Chlamydiae. Roughly half the size of the rickettsiae, members of the phylum **Chlamydiae** also are obligate, intracellular parasites and are cultivated only within living cells. Most species in the phylum are pathogens and one species causes the gonorrhea-like sexually transmitted disease (STD) chlamydia.

Spirochaetes. The phylum **Spirochaetes** contains more than 340 gram-negative species that possess a unique cell body that coils into a long helix and moves in a corkscrew pattern. The ecological niches for the spirochetes is diverse: from free-living species found in mud and sediments, to symbiotic species present in the digestive tracts of insects, to the pathogens found in the urogenital tracts of vertebrates. Many spirochetes are found in the human oral cavity; in fact, some of the first animalcules seen by Leeuwenhoek were probably spirochetes from his teeth scrapings. Among the human pathogens are *Treponema pallidum,* the causative agent of syphilis and one of the most common STDs (FIGURE 4.3F) and specific species of *Borrelia,* which are transmitted by ticks or lice and are responsible for Lyme disease and relapsing fever.

Other Phyla. There are many other phyla within the domain Bacteria. Several lineages branch off near the root of the domain. The common link between these organisms is that they are **hyperthermophiles**; they grow at high temperatures (70°C–85°C). Examples include *Aquifex* and *Thermotoga,* which typically are found in earthly cauldrons such as hot springs and other hydrothermal sites.

The Domain Archaea Contains Organisms with Diverse Physiologies

Classification within the domain Archaea has been more difficult than within the domain Bacteria, in large part because they have not been studied as long as their bacterial counterparts.

Archaeal organisms are found throughout the biosphere. Many genera are extremophiles, growing best at environmental extremes, such as very high temperatures, high salt concentrations, or extremes of pH. However, many more species exist in very cold environments. There also are archaeal genera that thrive under more modest conditions but there are no known species that cause disease in any plants or animals.

The archaeal genera can be placed into one of two phyla.

Euryarchaeota. The **Euryarchaeota** contain organisms with varying physiologies, many being extremophiles. Some groups, such as the **methanogens** (*methano* = "methane"; *gen* = "produce") are killed by oxygen gas and therefore are found in marine and freshwater environments (and animal gastrointestinal tracts) devoid of oxygen gas. The production of methane (natural) gas is important in their energy metabolism (FIGURE 4.4A). In fact, these archaeal species release more than 2 billion tons of methane gas into the atmosphere every year. About a third comes from the archaeal species living in the stomach (rumen) of cows.

Another group is the **extreme halophiles** (*halo* = "salt"; *phil* = "loving"). They are distinct from the methanogens in that they require oxygen gas for energy metabolism and need high concentrations of salt (up to 30% NaCl) to grow and reproduce. The fact that they often contain pink pigments makes their identification easy (FIGURE 4.4B). In addition, some extreme halophiles have been found in lakes where the pH is greater than 11.

A third group is the **hyperthermophiles** that grow optimally at temperatures above 80°C. They are typically found in volcanic terrestrial environments and deep-sea hydrothermal vents. Most also grow at very low pHs.

Crenarchaeota. The second phylum, the **Crenarchaeota**, are mostly hyperthermophiles, typically growing in hot springs and marine hydrothermal vents (FIGURE 4.4C). Other species are dispersed in open oceans, often inhabiting the cold ocean waters (−3°C) of the deep sea environments and polar seas.

(TABLE 4.1) summarizes some of the characteristics that are shared or are unique among the three domains.
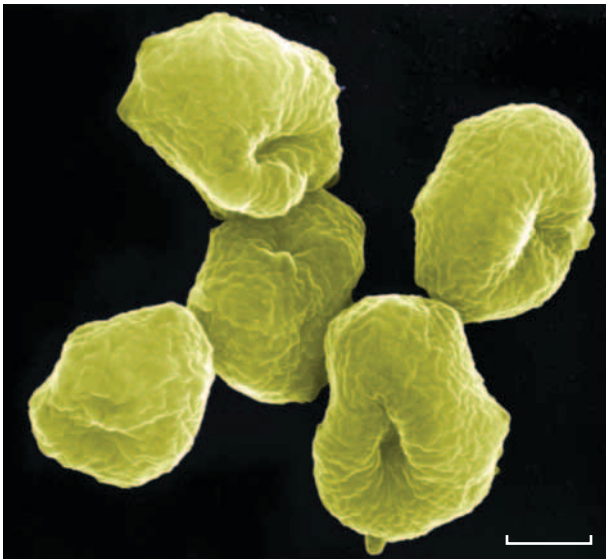
CONCEPT AND REASONING CHECKS 1

a. What three unique events occurred within the Proteobacteria and Cyanobacteria that contributed to the evolution of the Eukarya and the oxygen-rich atmosphere of Earth?

b. Compared to the more moderate environments in which some archaeal species grow, why have others adapted to such extreme environments?
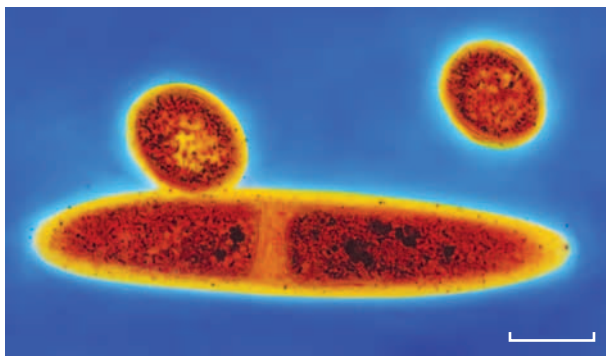
(A)

(B)

(C)

FIGURE 4.4 **Members of the Domain Archaea.** (**A**) A false-color transmission electron micrograph of the methanogen *Methanospirillum hungatei*. (Bar = 0.5 μm.) © Dr. Kari Lounatmaa/Photo Researchers, Inc. (**B**) An aerial view above Redwood City, California, of the salt ponds whose color is due to high concentrations of extreme halophiles. © Aerial Archives/Alamy Images. (**C**) A false-color scanning electron micrograph of *Sulfolobus,* a hyperthermophile that grows in waters as hot as 90°C. (Bar = 0.5 μm.) © Eye of Science/Photo Researchers, Inc. »» What advantage is afforded these species that grow in such extreme environments?

TABLE

4.1 **Some Major Differences Between Bacteria, Archaea, and Eukarya**

Characteristic	Bacteria	Archaea	Eukarya
Cell nucleus	No	No	Yes
Chromosome form	Single, circular	Single, circular	Multiple, linear
Histone proteins present	No	Yes	Yes
Peptidoglycan cell wall	Yes	No	No
Membrane lipids	Ester-linked	Ether-linked	Ester-linked
Ribosome sedimentation value	70S	70S	80S
Ribosome sensitivity to diphtheria toxin	No	Yes	Yes
First amino acid in a protein	Formylmethionine	Methionine	Methionine
Chlorophyll-based photosynthesis	Yes (cyanobacteria)	No	Yes (algae)
Growth above 80°C	Yes	Yes	No
Growth above 100°C	No	Yes	No
Pathogens	Yes	No	Yes

KEY CONCEPT 4.2	Prokaryotes Can Be Distinguished by Their Cell Shape and Arrangements

Bacterial and archaeal cells come in a variety of shapes that, in almost all members of the two domains, is determined by the cell wall and underlying cytoskeletal proteins. However, when viewing stained cells with the light microscope most, including the clinically significant ones, appear in one of three basic shapes: the rod, the sphere, or the spiral.

Variations in Cell Shape and Cell Arrangement Exist

A prokaryotic cell with a rod shape is called a **bacillus** (pl., bacilli) and depending on the species may be as long as 20 μm or as short as 0.5 μm. Certain bacilli are slender, such as those of *Salmonella typhi* that cause typhoid fever. Others, such as the agent of anthrax (*Bacillus anthracis*), are rectangular with squared ends; still others, such as the diphtheria bacilli (*Corynebacterium diphtheriae*), are club shaped. Most rods occur singly, in pairs called **diplobacillus**, or arranged into a long chain called **streptobacillus** (*strepto* = "chains") (FIGURE 4.5A). Realize there are two ways to use the word "bacillus": to denote a rod-shaped bacterial cell, and as a genus name (*Bacillus*).

A spherically shaped bacterial cell is known as a **coccus** (pl., cocci; *kokkos* = "berry") and tends to be quite small, being only 0.5 μm to 1.0 μm in diameter. Although they are usually round, they also may be oval, elongated, or indented on one side.

Many bacterial species that are cocci stay together after division and take on cellular arrangements characteristic of the species (FIGURE 4.5B). Cocci remaining in a pair after reproducing represent a **diplococcus**. The organism that causes gonorrhea, *Neisseria gonorrhoeae,* and one type of bacterial meningitis (*N. meningitidis*) are diplococci. Cocci that remain in a chain are called **streptococcus**. Certain species of streptococci are involved in strep throat (*Streptococcus pyogenes*) and tooth decay (*S. mutans*). Another arrangement of cocci is the **tetrad**, consisting of four spheres forming a square. A cube-like packet of eight cocci is called a **sarcina** (*sarcina* = "bundle"). *Micrococcus*

luteus, a common inhabitant of the skin, is one example. Other cocci may divide randomly and form an irregular grape-like cluster of cells called a **staphylococcus** (*staphylo* = "cluster"). A well-known example, *Staphylococcus aureus,* is often a cause of food poisoning, toxic shock syndrome, and several skin infections. The latter are known in the modern vernacular as "staph" infections. Notice again that the words "streptococcus" and "staphylococcus" can be used to describe cell shape and arrangement, or a bacterial genus (*Streptococcus* and *Staphylococcus*).

The third common morphology of bacterial cells is the **spiral**, which can take one of three forms (FIGURE 4.5C). The **vibrio** is a curved rod that resembles a comma. The cholera-causing organism *Vibrio cholerae* is typical. Another spiral form called **spirillum** (pl., spirilla) has a helical shape with a thick, rigid cell wall. The spiral-shaped form known as **spirochete** has a thin, flexible cell wall. The organism causing syphilis, *Treponema pallidum,* typifies a spirochete. Spiral-shaped bacterial cells can be from 1 μm to 100 μm in length.

In addition to the bacillus, coccus, and spiral shapes, other morphologies exist. Some bacterial species have appendaged bacterial cells while others consist of branching filaments; and some archaeal species have square and star shapes.

CONCEPT AND REASONING CHECKS 2

a. What morphology does a bacterial cell have that is said to be coccobacillus?
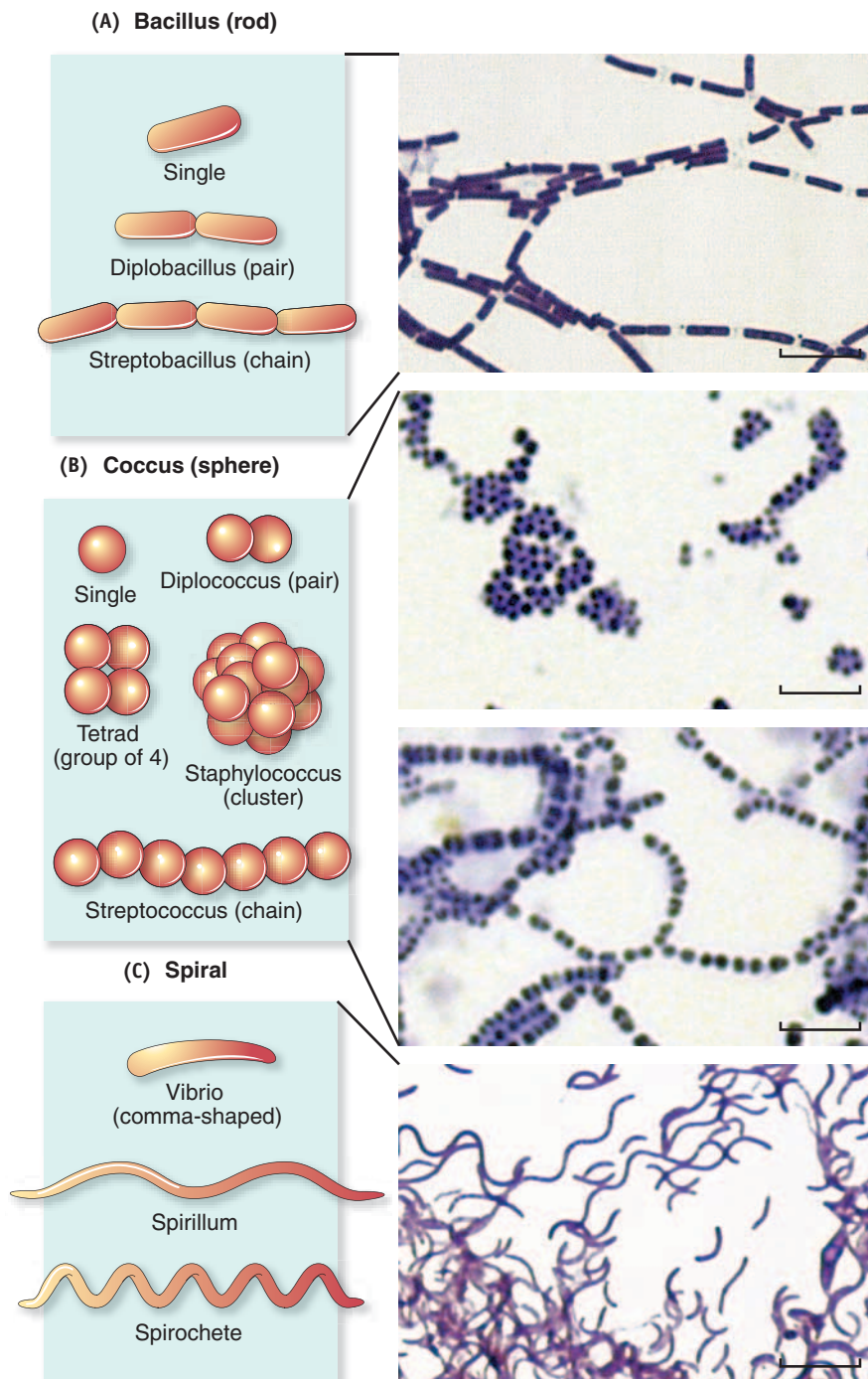
Chapter Challenge   A

So far you have learned more about the diverse groups in the domains Bacteria and Archaea, and have become familiar with their dissimilar cell shapes.

QUESTION A:
Based on what you have read up to this point, how has this diversity of life highlighted its unity?

Answers can be found on the Student Companion Website in **Appendix D**.

FIGURE 4.5 **Variation in Shape and Cell Arrangements.** Many bacterial and archaeal cells have a bacillus (**A**) or coccus (**B**) shape. Most spiral shaped-cells (**C**) are not organized into a specific arrangement (All bars = 10 µm.). Courtesy of Jeffrey Pommerville.
»» In Panel C, identify the vibrio and the spirillum forms in the photograph.

KEY CONCEPT 4.3   An Overview to Bacterial and Archaeal Cell Structure

In another chapter we discovered that bacterial and archaeal cells appear to have little visible structure when observed with a light microscope. This, along with their small size, gave the impression they are homogeneous, static structures with an organization very different from eukaryotic cells.

However, bacterial and archaeal species still have all the complex processes typical of eukaryotic cells. It is simply a matter that, in most cases, the structure and sometimes pattern to accomplish these processes is different from the membranous organelles typical of eukaryotic species.

### Cell Structure Organizes Cell Function

Recent advances in understanding bacterial and archaeal cell biology indicate these organisms exhibit a highly ordered intracellular organization. This organization is centered on three specific processes that need to be carried out (FIGURE 4.6). These are:

- **Sensing and responding to the surrounding environment.** Because most bacterial and archaeal cells are surrounded by a cell wall, some pattern of "external structures" is necessary to sense their en-

vironment and respond to it or other cells such as for quorum sensing and motility.

- **Compartmentation of metabolism.** Cell metabolism must be segregated from the exterior environment and yet be able to transport materials to and from that environment. In addition, protection from osmotic pressure due to water movement into cells must be in place. The "cell envelope" fulfills those roles.
- **Growth and reproduction.** Cell survival demands a complex metabolism that occurs within the aqueous "cytoplasm." These processes and reproduction exist as internal structures or subcompartments localized to specific areas within the cytoplasm.

Our understanding of bacterial and archaeal cell biology is still an emerging field of study. However, there is more to cell structure than previously thought—smallness does not equate with simplicity. For example, specific cellular proteins can be localized to precise regions of the cell. In *Streptococcus pyogenes*, many of the proteins that confer its pathogenic nature in causing diseases
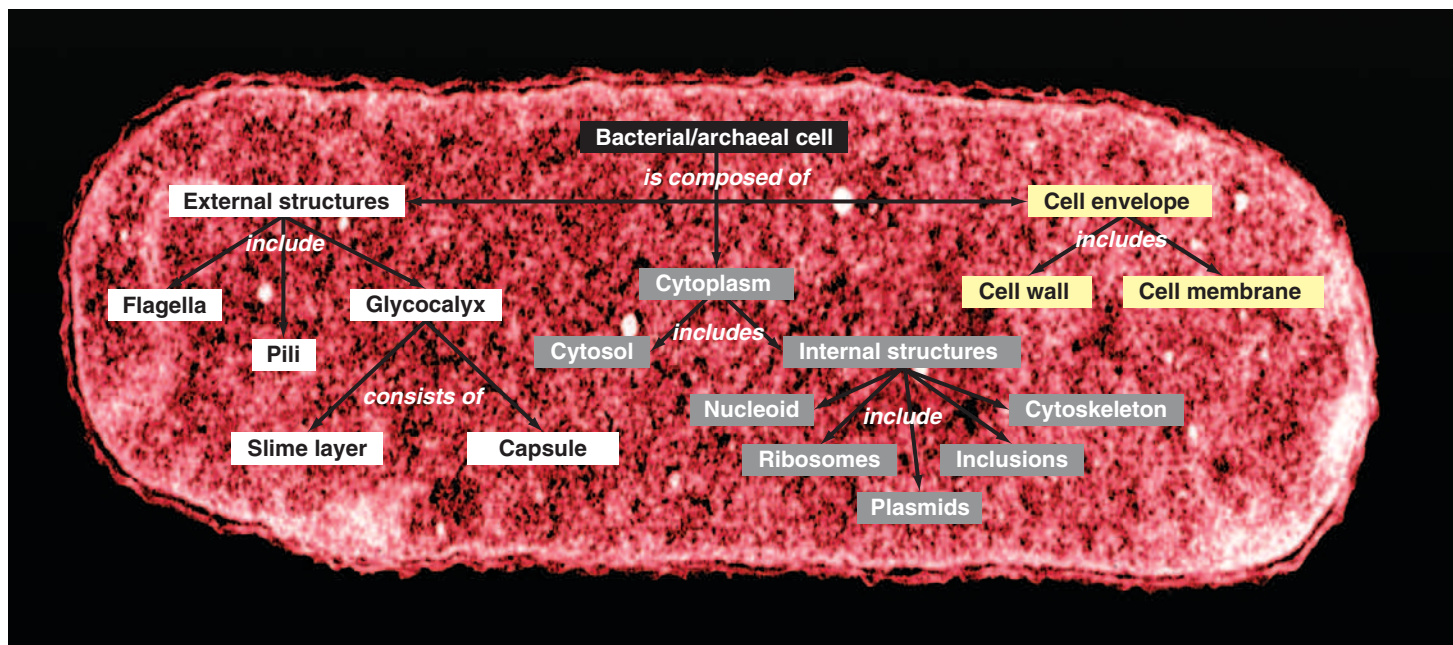
FIGURE 4.6   A Concept Map for Studying Bacterial and Archaeal Cell Structure. Not all cells have all the structures shown here. © George Chapman/ Visuals Unlimited. »» Why can't we see in the TEM image of a bacterial cell all the structures outlined in the concept map?

like strep throat are secreted from a specific area of the surface. *Yersinia pestis,* which is the agent responsible for plague, contains a specialized secretion apparatus through which toxic proteins are released. This apparatus only exists on the bacterial surface that is in contact with the target host cells.

The cell biology studies also may have important significance to clinical microbiology and the fight against infectious disease. As more is discovered about these cells and how they truly differ from eukaryotic cells, the better equipped we will be to develop new antimicrobial agents that will target the subcellular organization of pathogens. In an era when we have fewer effective antibiotics to fight infections, understanding of cell structure and function may be very important.

On the following pages, we examine some of the common structures found in an idealized bacterial cell, as no single species always contains all the structures (FIGURE 4.7). Our journey starts by examining the structures on or protruding from the surface of the cell. Then, we examine the cell envelope before plunging into the cell cytoplasm. Note: images of cells give the impression of a static, motionless structure. Realize such images are but a "snapshot" of a highly active living cell.

CONCEPT AND REASONING CHECKS 3

a. What is gained by bacterial and archaeal cells being organized into three general sets of structures—external, envelope, and cytoplasmic?
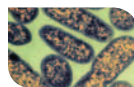
Chapter Challenge **B**

Prokaryotic cell structure is organized to efficiently carry out those functions required by the organism; that is, sensing and responding to the surrounding environment, compartmentalizing metabolism, and carrying out growth and reproduction.

QUESTION B:
Do you believe these three major functions are different from those in the eukaryotic microbial cell? Explain, keeping in mind the challenge of diversity emphasizing unity.

Answers can be found on the Student Companion Website in **Appendix D.**
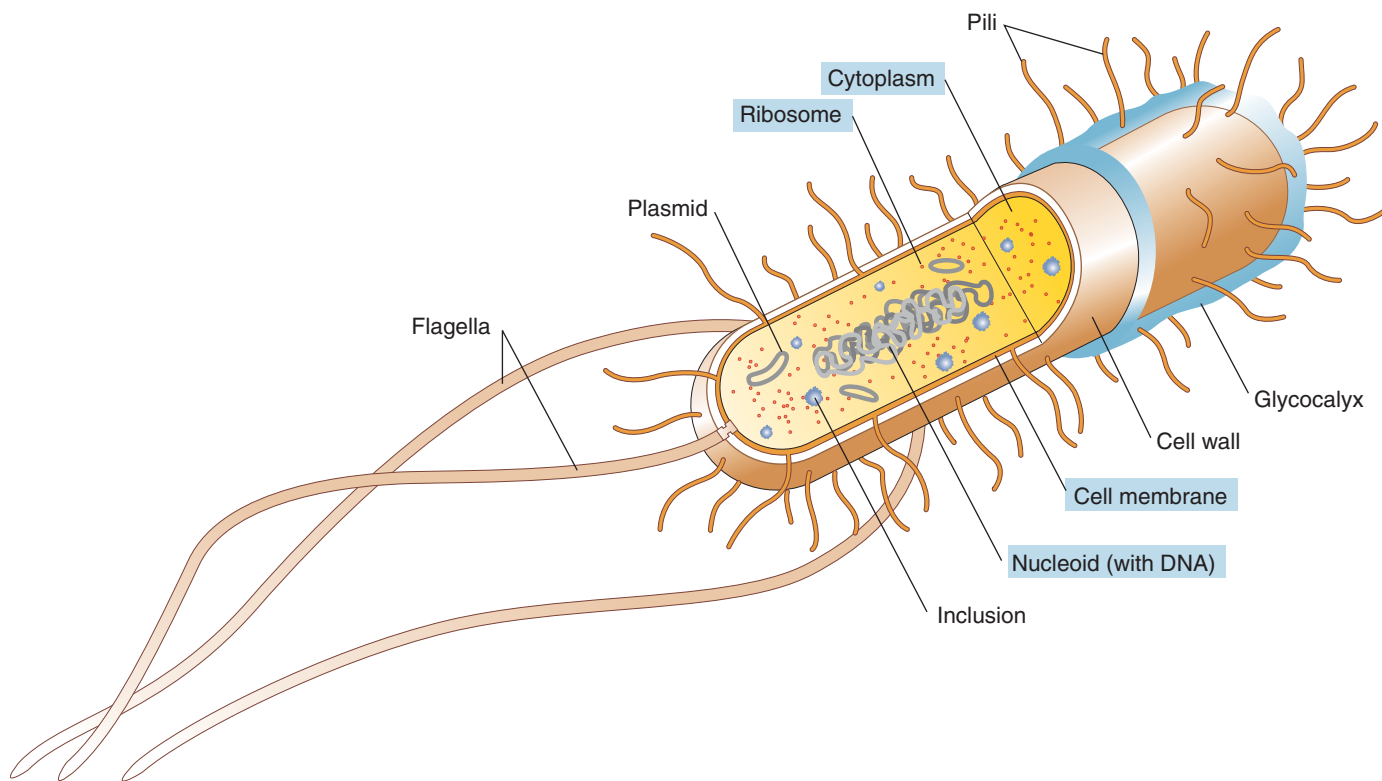
FIGURE 4.7 **Bacterial Cell Structure.** The structural features of a composite, "idealized" bacterial cell. Structures highlighted in blue are found in all bacterial and archaeal species. »» Which structures represent (a) external structures, (b) the cell envelope, and (c) cytoplasmic structures?

<div style="background:#e11; color:#fff">KEY CONCEPT 4.4</div> **External Cell Structures Interact with the Environment**

Bacterial and archaeal cells need to respond to and monitor their external environment. This is made difficult by having a cell wall that "blindfolds" the cell. Many cells have solved this sensing problem by possessing structures that extend from the cell surface into the environment.

Pili Are Primarily Used for Attachment

Numerous short, thin fibers, called **pili** (sing., pilus; *pilus* = "hair"), protrude from the surface of most gram-negative bacteria (FIGURE 4.8). It should be noted that microbiologists often use the term "pili" interchangeably with "fimbriae" (sing., fimbria; *fimbria* = "fiber"). The rigid fibers, composed of a protein called pilin, act as a scaffolding onto which specific adhesive molecules, called **adhesins**, are attached at the tip. Therefore, the primary function of most pili is to attach the cells to environmental surfaces or, in the case of human pathogens, to appropriate host cells and tissues. This requires that the pili on different bacterial species have specialized adhesins to recognize the appropriate cell. For example, the pili adhesins on *Neisseria gonorrhoeae* cells specifically anchor the cells to the mucosal surface of the urogenital tract whereas the adhesins on *Bordetella pertussis* (causative agent of whooping cough) adhere to cells of the mucosal surface of the upper respiratory tract. In this way, the pili act as a virulence factor by

■ Mucosal:
Referring to the mucous membranes lining many body cavities exposed to the environment.

■ Virulence factor:
A pathogen-produced molecule or structure that allows the cell to invade or evade the immune system and possibly cause disease.
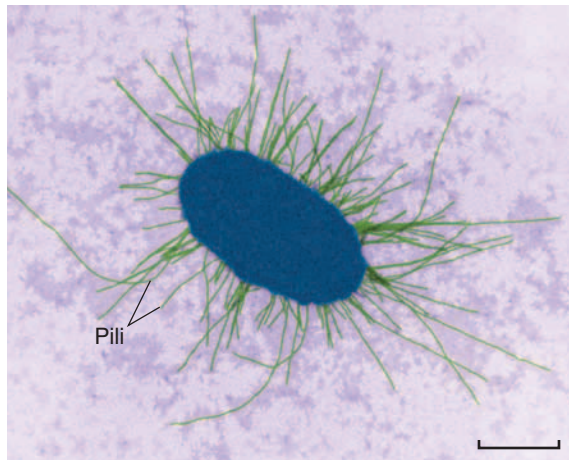
facilitating colonization and biofilm formation, and possibly leading to disease development. Without the chemical mooring line lashing the bacterial cells to host cells, it is less likely the cells could infect host tissue. Investigating the Microbial World 4 looks at pili as a virulence factor.

Until recently, attachment pili were thought to be specific to only certain species of gram-negative bacteria. However, extremely thin pili are present on at least some gram-positive bacteria, such as *Streptococcus*. These pili play a very similar role to the pili on gram-negative cells.

Besides these attachment pili, termed "type I pili," some bacterial species produce flexible **conjugation pili** that establish contact between appropriate cells, facilitating the transfer of genetic material from donor to recipient through a process called "conjugation." Conjugation pili are longer than attachment pili and only one or a few are produced on a cell. Pili in some cases function in cell movement. Several bacterial species possess "type IV pili" which, by pilus extension, attachment, and retraction, pull the bacterial cell toward the site of attachment. This movement, called either "twitching motility" or "gliding motility" depending on the species involved, helps cells move through drier environments, over cell tissues, or within biofilms.

Flagella Provide Motility

Many bacterial and archaeal cells are motile by using remarkable "nanomachines" called **flagella** (sing., flagellum). Depending on the species, one or more flagella may be attached to one or both ends of the cell, or at positions distributed over the cell surface (FIGURE 4.9A). In the domain Archaea, flagellar protein composition and structure differs significantly from that of the Bacteria; motility appears similar though.

Flagella range in length from 10 μm to 20 μm and are many times longer than the diameter of the cell. Because they are only about 20 nm thick, they cannot be seen with the light microscope unless stained. However, their existence can be inferred by using dark-field microscopy to watch the live cells dart about.

In the domain Bacteria, each flagellum is composed of a helical filament, hook, and basal body

Pili

FIGURE 4.8   Bacterial Pili. False-color transmission electron micrograph of an *Escherichia coli* cell (blue) with many pili (green). (Bar = 0.5 μm.) © Dennis Kunkel Microscopy, Inc./Visuals Unlimited/Corbis. »» What function do pili play?

Investigating the Microbial World 4

The Role of Pili

The cell surface of *Escherichia coli* (and many other bacterial species) is covered with tiny hair-like appendages called pili that function to attach the cells to human host cells—in this case, epithelial cells of the gut.

■ **OBSERVATION:** There are *E. coli* strains that normally exist in our gut but cause us no ill as long as they stay there. However, there are other strains that, if ingested from a contaminated food source, can cause illness and disease (such as diarrhea). So, what a group of researchers at Stanford University wanted to investigate is whether the pili on an enteropathogenic (intestinal disease-causing) strain of *E. coli* are necessary to initiate the disease process.

■ **QUESTION:** *Is* **E. coli** *attachment by pili to the intestinal epithelium required for the onset of gastrointestinal disease (diarrhea)?*

■ **HYPOTHESIS:** Pili-mediated adhesion of *E. coli* cells is necessary for the infection process leading to disease. If correct, then mutants of *E. coli* that lack pili should not cause diarrhea while mutants with an excessive number of pili should cause a more intense illness.

■ **EXPERIMENTAL DESIGN:** First, what was needed was a marker for infection. With the strain of *E. coli* used, this was easy—a good case of diarrhea would develop (as measured by the volume of liquid stools produced). Next, the Stanford researchers needed experimental subjects who would not complain if they developed a case of diarrhea. So, a tolerant group of healthy Stanford student volunteers were enrolled in the study—and paid $300 for their "contribution" to science! The volunteers were randomized into separate groups, each group receiving a slightly different form or dose of *E. coli*. Neither the volunteers nor the Stanford researchers knew who was drinking which liquid mixture; it was a so-called double-blind study. A number of nurses and doctors were on hand to help the volunteers through their ordeal.

■ **EXPERIMENT 1:** Each of three groups drank three doses of a fruit-flavored cocktail containing a mutant strain (muT) from the wild type having few pili, another mutant strain (muA) from the wild type also having few pili, or the diarrhea-causing strain (wild-type; wt) with normal numbers of pili. Each group was further divided into three subgroups based on the dose they would receive (5×10^8, 2.5×10^9, or 2×10^{10} *E. coli* cells). Over 48 hours, the cumulative volume of liquid stool collected from each volunteer was recorded.

■ **EXPERIMENT 2:** Two additional groups drank three doses of a fruit-flavored cocktail containing a mutant strain (muF) from the wild type having excessive pili that causes the cells to aggregate together in culture. One group received doses of 2.5×10^9 cells while the other group received doses of 2×10^{10} cells. Again, the volume of liquid stool collected from each volunteer over a 48 hour period was recorded.

■ **RESULTS:** See figure. In the figure, each vertical bar represents one volunteer and the cumulative volume of liquid stool. The short bars below the horizontal X axis represent volunteers who produced no liquid stools in the 48 hour period of the experiments.

■ **CONCLUSIONS:**

QUESTION *1: Was the hypothesis validated? Explain using the figure and identify the control in experiment 1.*

QUESTION *2: In experiment 1, explain why the volunteers drinking the cocktail with muT and muA did not suffer severe diarrhea? Were there any anomalies within these groups?*
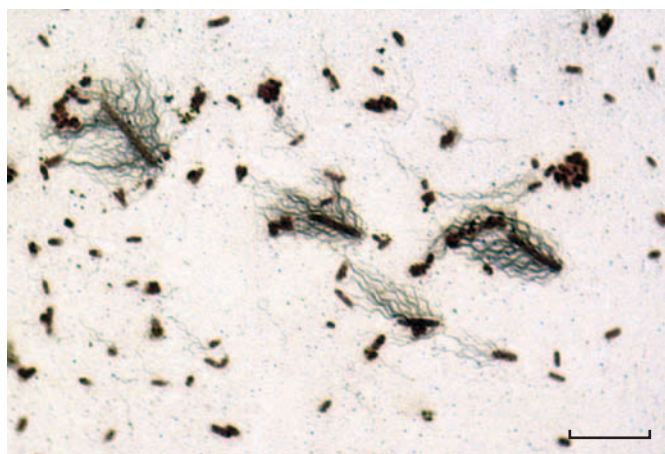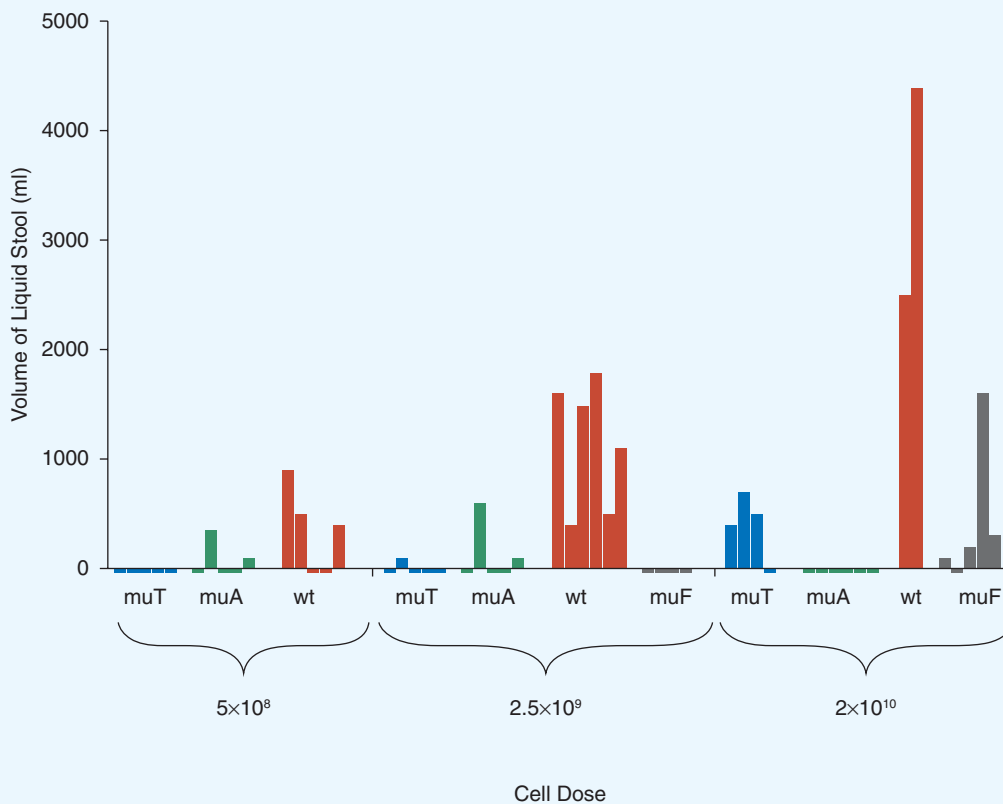
QUESTION *3: From experiment 2, propose a reason why the volunteers drinking the cocktail with muF did not suffer severe diarrhea? Were there any anomalies within these groups?*

Answers can be found on the Student Companion Website in **Appendix D**.

Investigating the Microbial World 4 (continued)

(A)

(B)

FIGURE 4.9   Bacterial Flagella.   (A) A light micrograph of stained *Proteus vulgaris* showing numerous flagella extending from the cell surface. (Bar = 10 μm.) Note that the length of a flagellum is many times the width of the cell. Courtesy of Jeffrey Pommerville. **(B)** The flagellum on a gram-negative bacterial cell is attached to the cell wall and membrane by two pairs of protein rings in the basal body. »» Why is the flagellum referred to as a "nanomachine"?
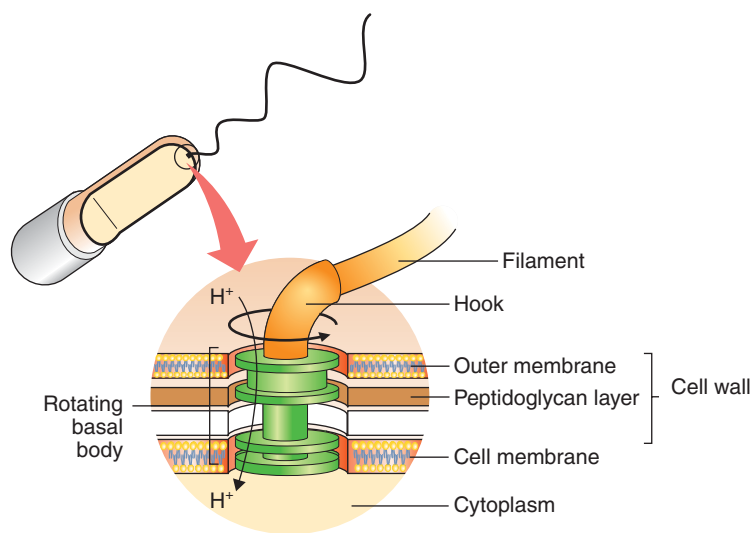
(FIGURE 4.9B). The hollow filament is composed of long, rigid strands of protein while the hook attaches the filament to a basal body (motor) anchored in the cell envelope.

The basal body is an assembly of more than 20 different proteins that form a central rod and set of enclosing rings. Gram-positive cells have a pair of rings embedded in the cell membrane and one ring in the cell wall, while gram-negative cells have a pair of rings embedded in the cell membrane and another pair in the cell wall.

The basal body represents a powerful biological motor or rotary engine that generates a propeller-type rotation of the rigid filament. The energy for rotation comes from the diffusion of protons (hydrogen ions; H^+) into the cell through proteins associated with the basal body. This energy is sufficient to produce up to 1,500 rpm by the filament, driving the cell forward.

What advantage is gained by cells having flagella? In nature, there are many chemical nutrients in the environment that cells need to survive. Cells will search out such **attractants** by using their flagella to move up the concentration gradient; that is, toward the attractant. The process is called **chemotaxis**.

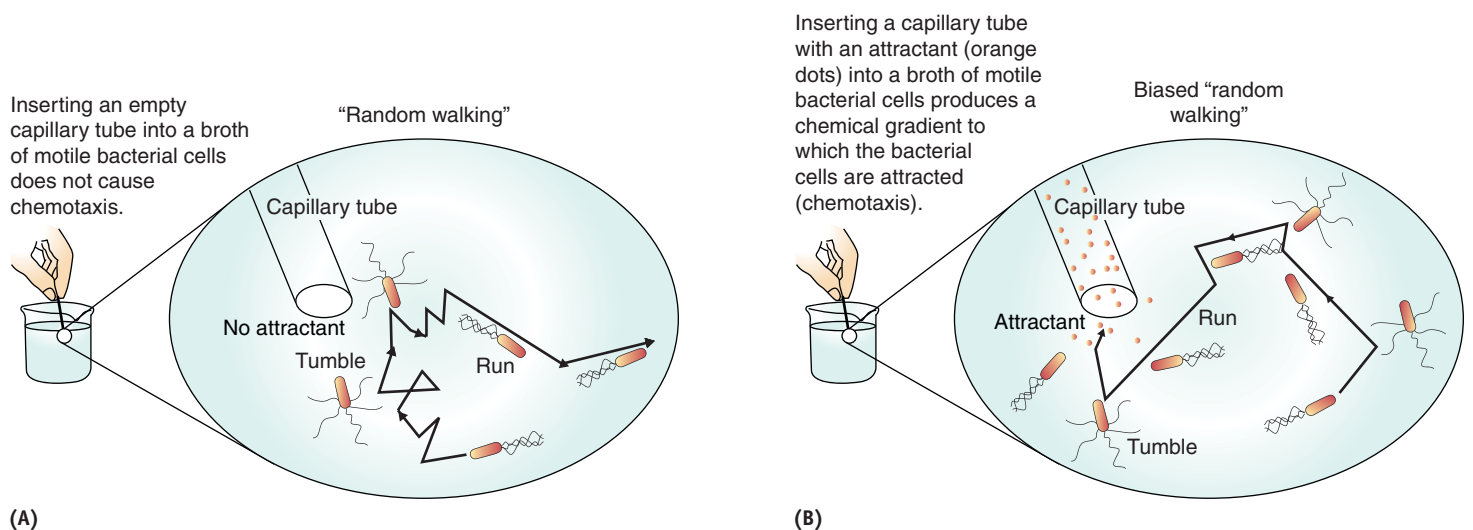Being so small, the cells sense their chemical surroundings using a temporal sensing system. In the absence of a gradient, the flagella all rotate as a bundle counterclockwise and the cell moves straight ahead in short bursts called "runs" (FIGURE 4.10A). These runs can last a few seconds and the cells can move up to 10 body lengths per second (the fastest human can run about 5–6 body lengths per second). A reversal of flagellar rotation (clockwise rotation) causes the cell to "tumble" randomly for a second as the flagella become unbundled and uncoordinated. Then, the motor again reverses direction and another run occurs in a new direction.

If an attractant gradient is present, cell behavior changes and the cells moving up the gradient now undergo longer periods when the motor turns counterclockwise (lengthened runs) and shorter periods when it turns clockwise (shortened tumbles) (FIGURE 4.10B). The combined result is a net movement toward the attractant; that is, up the concentration gradient.

Similar types of motile behavior are seen in photosynthetic organisms moving toward light (phototaxis) or other cells moving toward oxygen gas (aerotaxis). MicroFocus 4.2 investigates how flagella may have evolved.

One additional type of flagellar organization is found in the spirochetes, the group of gram-negative, coiled bacterial species described earlier in this chapter. The cells are motile by flagella that extend from one or both poles of the cell

■ Temporal sensing: One that compares the chemical environment and concentration from one moment to the next.

Inserting an empty capillary tube into a broth of motile bacterial cells does not cause chemotaxis.

"Random walking"

Capillary tube

No attractant

Tumble

Run

(A)

Inserting a capillary tube with an attractant (orange dots) into a broth of motile bacterial cells produces a chemical gradient to which the bacterial cells are attracted (chemotaxis).

Biased "random walking"

Capillary tube

Attractant

Run

Tumble

(B)

FIGURE 4.10 **Chemotaxis.** Chemotaxis represents a behavioral response to chemicals. (**A**) In a "random walk," rotation of the flagellum counterclockwise causes the bacterial cell to "run," while rotation of the flagellum clockwise causes the bacterial cell to "tumble," as shown. (**B**) During chemotaxis (biased "random walk") to an attractant, such as sugar, cellular behavior leads to longer runs and fewer tumbles, which will result in biased movement toward the attractant. »» Predict the behavior of a bacterial cell if it sensed a repellant; that is a potential harmful or lethal chemical.

MICROFOCUS 4.2: Evolution
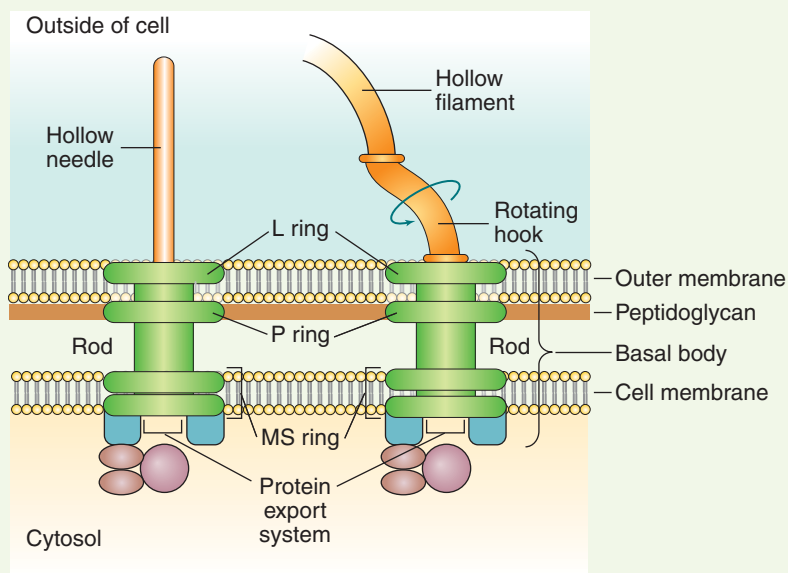### The Origin of the Bacterial Flagellum

Flagella are an assembly of protein parts forming a rotary engine that, like an outboard motor, propel the cell forward through its moist environment. Recent work has shown how such a nanomachine may have evolved.

Several bacterial species, including *Yersinia pestis,* the agent of bubonic plague, contain structures to inject toxins into an appropriate eukaryotic host cell. These bacterial cells have a hollow tube or needle to accomplish this process, just as the bacterial flagellum and filament are hollow (see diagram below). In addition, many of the flagellar proteins are similar to some of the injection proteins. In 2004, investigations discovered that *Y. pestis* cells actually contain all the genes needed for a flagellum—but the cells have lost the ability to use these genes for that purpose. *Y. pestis* is nonmotile and it appears that the cells use a subset of the flagellar proteins to build the injection device.

One scenario then is that an ancient cell evolved a structure that was the progenitor of the injection and flagellar systems. In fact, many of the proteins in the basal body of flagellar and injection systems are similar to proteins involved in proton (hydrogen ion; H^+) transport. Therefore, a proton transport system may have evolved into the injection device and, through diversification events, evolved into the motility structure present on many bacterial cells today.

The fascinating result of these investigations and proposals is it demonstrates that structures can evolve from other structures with a different function. It is not necessary that evolution "design" a structure from scratch but rather it can modify existing structures for other functions.

Individuals have proposed that the complexity of structures like the bacterial flagellum are just too complex to arise gradually through a step-by-step process. However, the investigations being conducted illustrate that a step-by-step evolution of a specific structure is not required. Rather, there can be cooperation, where one structure is modified to have other functions. The bacterial flagellum almost certainly falls into that category.

A bacterial injection device (left) compared to a bacterial flagellum (right). Both have a protein export system in the base of the basal body.

(A)   **(B)**
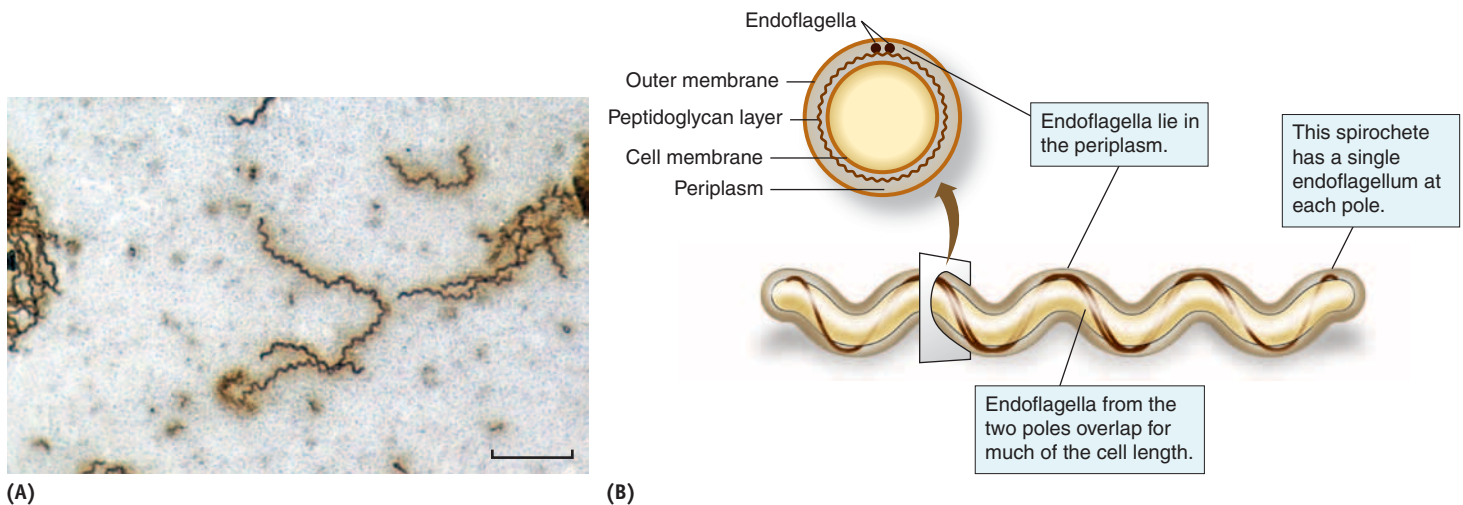
FIGURE 4.11   **The Spirochete Endoflagella.** (**A**) A light micrograph of *Treponema pallidum* shows the corkscrew-shaped spirochete cell. (Bar = 10 µm.) Courtesy of Jeffrey Pommerville. (**B**) Diagram showing the positioning of endoflagella in a spirochete. »» How are endoflagella different from true bacterial flagella?

but fold back along the cell body (FIGURE 4.11). Such **endoflagella** lie in an area called the "periplasm" (see below). Motility results from the torsion generated on the cell by the normal rotation of the flagella. The resulting motility is less regular and more jerky than with flagellar motility.

The Glycocalyx Serves Several Functions

Many bacterial species secrete an adhering layer of polysaccharides, or polysaccharides and small proteins, called the **glycocalyx** (*glyco* = "sweet"; *calyx* = "coat"). The layer can be thick and covalently bound to the cell, in which case it is known as a **capsule**. A thinner, loosely attached layer is referred to as a **slime layer**. Colonies containing cells with a glycocalyx appear moist and glistening. The actual capsule can be seen by light microscopy when observing cells in a negative stain preparation or by transmission electron microscopy (FIGURE 4.12).

The glycocalyx serves as a buffer between the cell and the external environment. Because of its high water content, the glycocalyx can protect cells from desiccation. Another major role of the glycocalyx is to allow the cells to stick to surfaces. The glycocalyx of *V. cholerae,* for example, permits the cells to attach to the intestinal wall of the host.

Website Animation: Bacterial Cell Motility and Chemotaxis
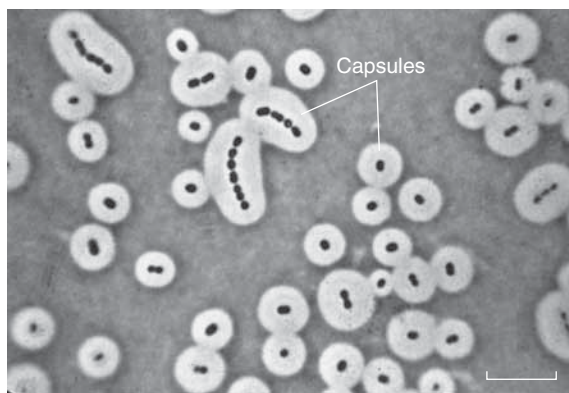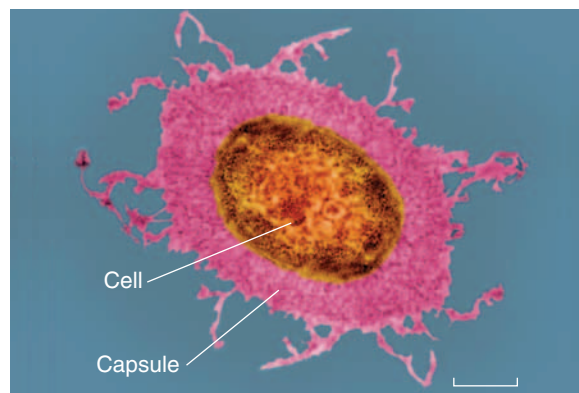
(A)   **(B)**

FIGURE 4.12   **The Bacterial Glycocalyx.** (**A**) Demonstration of the presence of a capsule in an *Acinetobacter* species by negative staining and observed by phase-contrast microscopy. (Bar = 10 µm.) Courtesy of Elliot Juni, Department of Microbiology and Immunology, The University of Michigan. (**B**) A false-color transmission electron micrograph of *Escherichia coli*. The cell is surrounded by a thick capsule (pink). (Bar = 0.5 µm.) © George Musil/Visuals Unlimited. »» How does the capsule provide protection for the bacterial cell?

The glycocalyx of pathogens therefore represents another virulence factor.

Other encapsulated pathogens, such as *Streptococcus pneumoniae* (a principal cause of bacterial pneumonia) and *Bacillus anthracis,* evade the immune system because they cannot be easily engulfed by white blood cells during phagocytosis. Scientists believe the repulsion between bacterial cell and phagocyte is due to strong negative charges on the capsule and phagocyte surface.

A slime layer usually contains a mass of tangled fibers of a polysaccharide called **dextran**. The fibers attach the bacterial cell to tissue surfaces. A case in point is *Streptococcus mutans,* an important cause of tooth decay. This species forms dental plaque, which represents a type of biofilm on the tooth surface. TEXTBOOK CASE 4 details a medical consequence of a biofilm.

■ **Encapsulated:**
Referring to a cell having a capsule.

■ **Phagocytosis:**
A process whereby certain white blood cells (phagocytes) engulf foreign matter and often destroy microorganisms.

CONCEPT AND REASONING CHECKS 4

a. What is the primary function for pili? What other roles can they have?
b. Explain how flagella move a cell during a "run."
c. Under what circumstances might it be advantageous to a bacterial cell to have a capsule rather than a slime layer?

KEY CONCEPT 4.5   **Most Bacterial and Archaeal Cells Have a Cell Envelope**

The **cell envelope** is a complex structure that forms the two "wrappers"—the **cell wall** and the **cell membrane**—surrounding the cell cytoplasm. The cell wall is relatively porous to the movement of substances whereas the cell membrane regulates transport of nutrients and metabolic products.

The Bacterial Cell Wall Is a Tough and Protective External Shell

The fact that most bacterial and archaeal cells have a semi-rigid cell wall suggests the critical role this structure must play. By covering the entire cell surface, the cell wall acts as an exoskeleton to protect the cell from injury and damage. It helps, along with the cytoskeleton, to maintain the shape of the cell and reinforce the cell envelope against the high intracellular water (osmotic) pressure pushing against the cell membrane. Most microbes live in an environment where there are more dissolved materials inside the cell than outside. This hypertonic condition in the cell means water diffuses inward, accounting for the increased osmotic pressure. Without a cell wall, the cell would rupture or undergo **osmotic lysis** (FIGURE 4.13). It is similar to blowing so much air into a balloon that the air pressure bursts the balloon.

The bacterial cell wall differs markedly from the walls of archaeal cells and cells of eukaryotic microorganisms (algae and fungi) in containing **peptidoglycan**, which is a network of disaccharide chains (glycan strands) cross-linked by short, elastic peptides (FIGURE 4.14A). Each disaccharide unit in this very large molecule is composed of two alternating monosaccharides, *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM). The carbohydrate backbone can occur in multiple layers connected by side chains of four amino acids and peptide cross-bridges.

There is more to a bacterial cell wall than just peptidoglycan, so several forms of cell wall architecture exist.

Gram-Positive Walls. Most gram-positive bacterial cells have a very thick, rigid peptidoglycan cell wall (FIGURE 4.14B). The abundance and thickness (25 nm) of this material may be one reason why they retain the crystal violet in the Gram stain procedure. The multiple layers of glycan strands are cross-linked to one another both in the same layer as well as between layers forming a three-dimensional mesh.

The gram-positive cell wall also contains a sugar-alcohol and phosphate polymer called **teichoic acid**. Teichoic acids, which are bound to the glycan chains or to the cell membrane, are essential for cell viability—if the genes for teichoic acid synthesis are deleted, cell death occurs. Still, the function of the teichoic acids remains unclear. They may protect the cell envelope by establishing a surface charge on the cell wall, controlling the activity of autolytic enzymes acting on the peptidoglycan, and/or acting as a virulence factor that triggers fever and inflammation.

The actinomycetes are phylogenetically related to the gram-positive bacteria. However, these bacterial cells have evolved another type of wall architecture to protect the cell membrane from rupture. In organisms like *Mycobacterium,* the cell wall is composed of a waxy lipid called

■ **Hypertonic:**
A solution with more dissolved material (solutes) than the surrounding solution.

■ **Autolytic enzyme:**
An enzyme that breaks bonds in the peptidoglycan, thereby causing lysis of the cell (e.g., lysozyme).

Textbook CASE 4

An Outbreak of *Enterobacter cloacae* Associated with a Biofilm

Hemodialysis is a treatment for people with severe chronic kidney disease (kidney failure). The treatment filters the patient's blood to remove wastes and excess water. Before a patient begins hemodialysis, an access site is created on the lower part of one arm. Similar to an intravenous (IV) site, a tiny tube runs from the arm to the dialysis machine. The patient's blood is pumped through the dialysis machine, passed through a filter or artificial kidney called a dialyzer, and the cleaned blood returned to the patient's body at the access site. The complete process can take 3 to 4 hours.

1 During September 1995, a patient at an ambulatory hemodialysis center in Montreal, Canada received treatment on a hemodialysis machine to help relieve the effects of kidney disease. The treatment was performed without incident.

2 The next day, a second patient received treatment on the same hemodialysis machine. His treatment also went normally, and he returned to his usual activities after the session was completed.

3 In the following days, both patients experienced bloodstream infections (BSIs). They had high fever, muscular aches and pains, sore throat, and impaired blood circulation. Because the symptoms were severe, the patients were hospitalized. The clinical microbiology lab reported that both patients had infections of *Enterobacter cloacae,* a gram-negative rod.

4 In the following months, an epidemiological investigation reviewed other hemodialysis patients at that center. In all, seven additional adult patients were identified who had used the same hemodialysis machine. They discovered all seven had similar BSIs.

5 Inspection of the hemodialysis machine used by these nine patients indicated the presence of biofilms containing *Enterobacter cloacae,* which was identical to those samples taken from the patients' bloodstreams (see figure).

6 Further study indicated that the dialysis machine was contaminated with *E. cloacae,* specifically where fluid flows.

7 It was discovered that hospital personnel were disinfecting the machines correctly. The problem was that the valves in the drain line were malfunctioning, allowing a backflow of contaminated material.

8 Health officials began a hospital education program to ensure that further outbreaks of infection would be minimized.
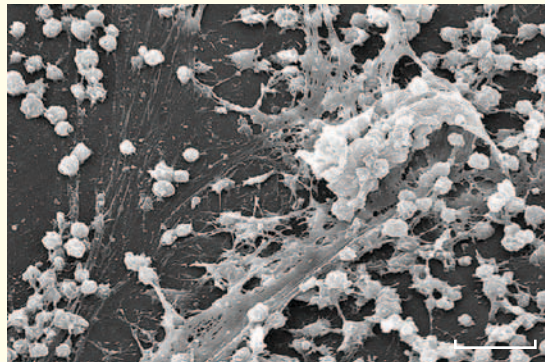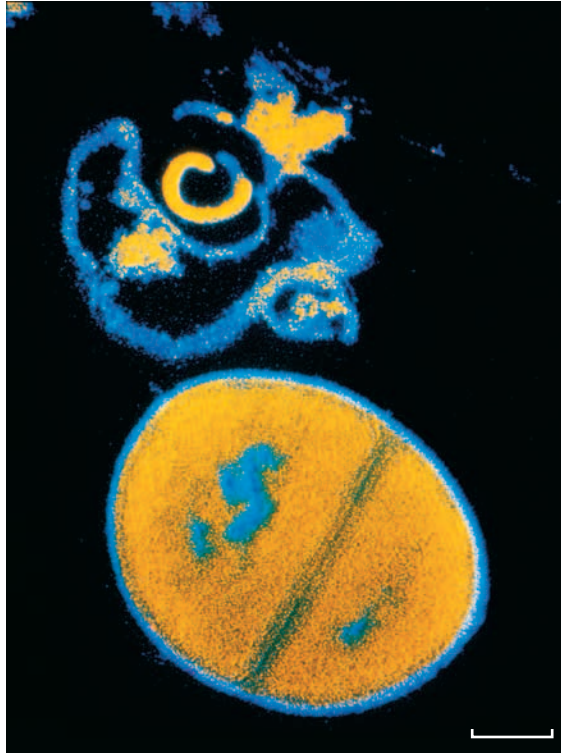

Similar to the description in this textbook case, biofilms consisting of *Staphylococcus* cells can contaminate hemodialysis machines. (Bar = 5 µm.) Courtesy of Dr. Rodney M. Donlan and Janice Carr/CDC.

Questions:

(Answers can be found on the Student Companion Website in **Appendix D**.)

A. Suggest how the hemodialysis machine originally became contaminated.

B. Why weren't the other five cases of BSI correlated with the hemodialysis machine until the epidemiological investigation was begun?

C. How could future outbreaks of infection be prevented?

For additional information see www.cdc.gov/mmwr/preview/mmwrhtml/00051244.htm.

Endotoxin:
A poison that can activate inflammatory responses, leading to high fever, shock, and organ failure.

Hydrophilic:
Pertaining to molecules or parts of molecules that are soluble in water.

Hydrophobic:
Pertaining to molecules or parts of molecules that are not soluble in water.

FIGURE 4.13 Cell Rupture (Lysis). A false-color electron micrograph showing the lysis of a *Staphylococcus aureus* cell. The addition of the antibiotic penicillin interferes with the construction of the peptidoglycan in new cells, and they quickly burst (top cell). (Bar = 0.25 µm.) © CNRI/Photo Researchers, Inc. »» Where is the concentration of dissolved substances (solutes) higher, inside the cell or outside? Explain how this leads to cell lysis.

mycolic acid that is arranged in two layers that are covalently attached to the underlying peptidoglycan. Such a hydrophobic layer is impervious to the Gram stains, so stain identification of *M. tuberculosis* is carried out using the acid-fast stain procedure.

Gram-Negative Walls. The cell wall of gram-negative bacterial cells is structurally quite different from that of the gram-positive wall (**FIGURE 4.14C**). The peptidoglycan strands compose just a single layer or two making the cell more susceptible to lysis. This is one reason why it loses the crystal violet dye during the Gram stain procedure. Also, there is no teichoic acid present.

The unique feature of the gram-negative cell wall is the presence of an **outer membrane**, which is separated by a gap, called the **periplasm**, from the cell membrane. This gel-like compartment contains digestive enzymes and transport proteins to speed entry of nutrients into the cell. The pep-

tidoglycan layer is located in the periplasm and attached to lipoproteins in the outer membrane.

The inner half of the outer membrane contains phospholipids similar to the cell membrane. However, the outer half is composed primarily of **lipopolysaccharide (LPS)**, which consists of polysaccharide attached to a unique anchoring lipid molecule known as **lipid A**. The so-called O polysaccharide is used to identify variants of a species (e.g., strain O157:H7 of *E. coli*). On cell death, lipid A is released and represents an endotoxin that can be toxic if ingested.

The outer membrane also contains unique proteins called **porins**. These proteins form pores in the outer membrane through which small, hydrophilic molecules (sugars, amino acids, some ions) diffuse passively into the periplasm. The outer membrane also contains substrate-specific active transporters for some needed compounds, such as vitamins. However, other larger, hydrophobic molecules cannot pass, partly accounting for the resistance of gram-negative cells to many antimicrobial agents, dyes, disinfectants, and lysozyme.

Before leaving the bacterial cell walls, a brief mention should be made of bacterial species that lack a cell wall. The mycoplasmas are a wall-less genus that is again phylogenetically related to the gram-positive bacteria. Taxonomists believe that the mycoplasmas once had a cell wall but lost it because of their parasitic relationship with their host. To help protect the cell membrane from rupture, the mycoplasmas are unusual in containing sterols in the cell membrane.

TABLE 4.2 summarizes the major differences between the three major types of bacterial cell walls.

The Archaeal Cell Wall Also Provides Mechanical Strength

Archaeal species vary in the type of wall they possess. None have the peptidoglycan typical of the Bacteria. Some species have a **pseudopeptidoglycan** where the NAM is replaced by *N*-acetyltalosamine uronic acid (NAT). Other archaeal cells have walls made of polysaccharide, protein, or both.

A common component of the cell wall among most archaeal species is a surface layer called the **S-layer**, which is also quite common in many

(A) Structure of peptidoglycan

(B) Gram-positive cell wall

(C) Gram-negative cell wall
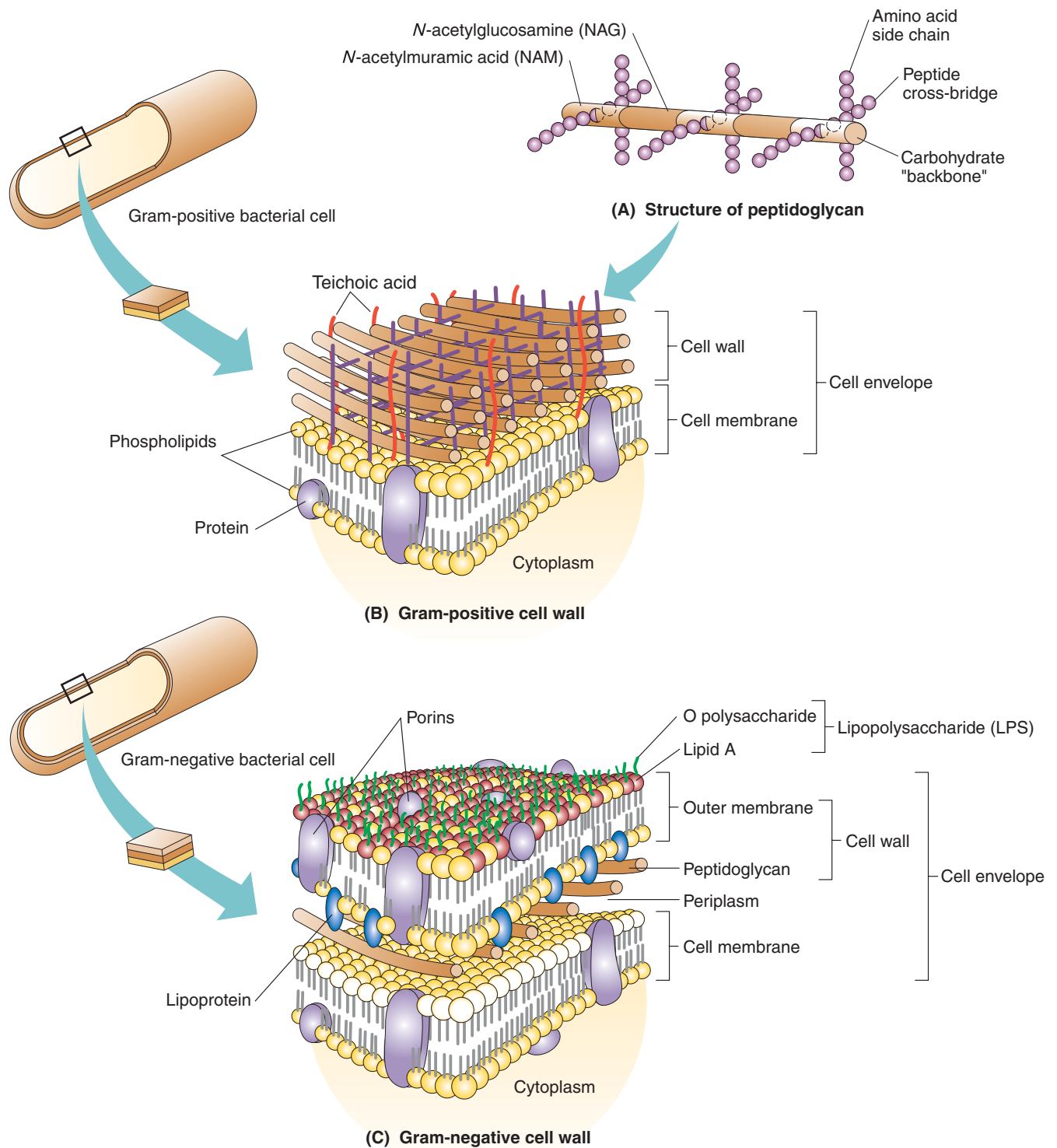
FIGURE 4.14 **A Comparison of the Cell Walls of Gram-Positive and Gram-Negative Bacterial Cells.** (**A**) The structure of peptidoglycan is shown as units of NAG and NAM joined laterally by amino acid cross-bridges and vertically by side chains of four amino acids. (**B**) The cell wall of a gram-positive bacterial cell is composed of multiple peptidoglycan layers combined with teichoic acid molecules. (**C**) In the gram-negative cell wall, the peptidoglycan layer is much thinner, and there is no teichoic acid. Moreover, an outer membrane overlies the peptidoglycan layer such that both comprise the cell wall. Note the structure of the outer membrane in this figure. It contains porin proteins and the outer half is unique in containing lipopolysaccharide.
»» Simply based on cell wall structure, assess the potential of gram-positive and gram-negative cells as pathogens.

TABLE 4.2 A Comparison of Gram-Positive and Gram-Negative Cell Walls			
Characteristic	Gram Positive	Gram Positive Acid-fast	Gram Negative
Peptidoglycan	Yes, thick layer	Yes	Yes, thin layer
Teichoic acids	Yes	No[1]	No
Outer membrane	No	Yes	Yes
Mycolic acid	No	Yes	No
Lipopolysaccharides (LPS)	No	No	Yes
Porin proteins	No	Yes	Yes
Periplasm	No	Yes	Yes

[1]Have a different type of glycopolymer.

groups of the Bacteria. It consists of hexagonal patterns of protein or glycoprotein that self-assemble into a crystalline lattice 5 nm to 25 nm thick.

Although the walls may be structurally different and the molecules form a different structural pattern, the function is the same as in bacterial species—to provide mechanical support and prevent osmotic lysis.

The Cell Membrane Represents a Selectively Permeable Barrier

A **cell** (or **plasma**) **membrane** is a universal structure that separates external from internal (cytoplasmic) environments, preventing soluble materials from simply diffusing into and out of the cell. One exception is water, which due to its small size and overall lack of charge can diffuse slowly across the membrane.

The bacterial cell membrane, which is about 7 nm thick, is 25% phospholipid (by weight) and 75% protein. In illustrations, the cell membrane appears very rigid (FIGURE 4.15). In reality, it is quite fluid, having the consistency of olive oil. This means the mosaic of phospholipids and proteins are not cemented in place, but rather they can move laterally in the membrane. This dynamic model of membrane structure therefore is called the **fluid mosaic model**.

The phospholipid molecules, typical of most biological membranes, are arranged in two parallel layers (a bilayer) and represent the barrier function of the membrane. The phospholipids contain a charged phosphate head group attached to two hydrophobic fatty acid chains. The fatty acid "tails" are the portion that forms the permeability barrier. In contrast, the hydrophilic head groups are exposed to the aqueous external or cytoplasmic environments.

Several antimicrobial substances act on the membrane bilayer. The antibiotic polymyxin pokes holes in the bilayer, while some detergents and alcohols dissolve the bilayer. Such action allows the cytoplasmic contents to leak out of bacterial cells, resulting in death through cell lysis.

A diverse population of membrane proteins populates the phospholipid bilayer. These mem-
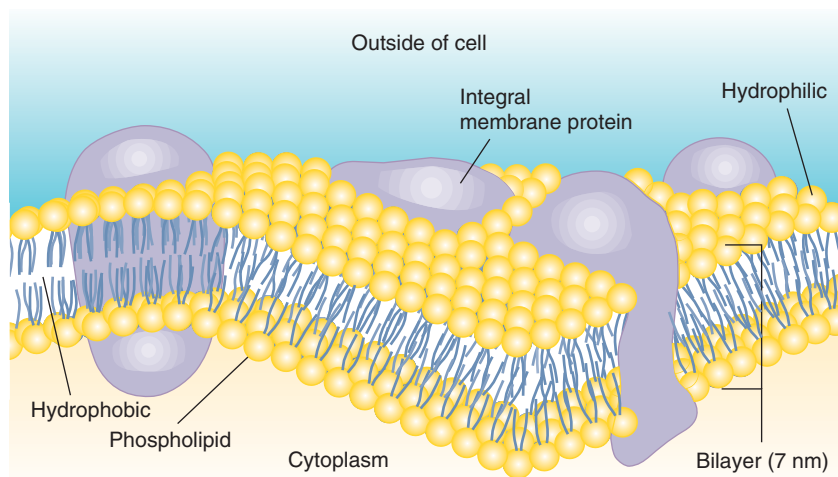
FIGURE 4.15 **The Structure of the Bacterial Cell Membrane.** The cell membrane of a bacterial cell consists of a phospholipid bilayer in which are embedded integral membrane proteins. Other proteins and ions may be associated with the integral proteins or the phospholipid heads. »» Why is the cell membrane referred to as a fluid mosaic structure?

brane proteins often have stretches of hydrophobic amino acids that interact with the hydrophobic fatty acid chains in the membrane. These proteins span the width of the bilayer and are referred to as "integral membrane proteins." Other proteins, called "peripheral membrane proteins," are associated with the polar heads of the bilayer or exposed parts of integral membrane proteins.

Bacterial and archaeal cell membrane proteins carry out numerous important functions, some of which are absent from the eukaryotic plasma membrane. This includes enzymes needed for cell wall synthesis and energy metabolism. As mentioned, bacterial and archaeal cells lack mitochondria and part of that organelle's function is carried out by the cell membrane. Other membrane proteins help anchor the DNA to the membrane during replication or act as receptors of chemical information, sensing changes in environment conditions and triggering appropriate behavioral responses as demonstrated through quorum sensing.

Perhaps the largest group of integral membrane proteins is involved as transporters of charged solutes, such as amino acids, simple sugars, and ions across the lipid bilayer. The transport proteins are highly specific though, only transporting a single molecular type or a very similar class of molecules. Therefore, there are many different transport proteins to regulate the diverse molecular traffic that must flow into or out of a cell.

The transport process can be passive or active. In **facilitated diffusion**, integral membrane proteins facilitate the movement of materials down their concentration gradient; that is, from an area of higher concentration to one of lower concentration (FIGURE 4.16). By acting as a conduit for diffusion or as a transporter through the hydrophobic bilayer, hydrophilic solutes can enter or leave without the need for cellular energy.

Unlike facilitated diffusion, **active transport** allows different concentrations of solutes to be established outside or inside of the cell against the concentration gradient. These membrane proteins act as "pumps" and, as such, demand an energy input from the cell. Cellular processes such as cell energy production and flagella rotation also depend on active transport.
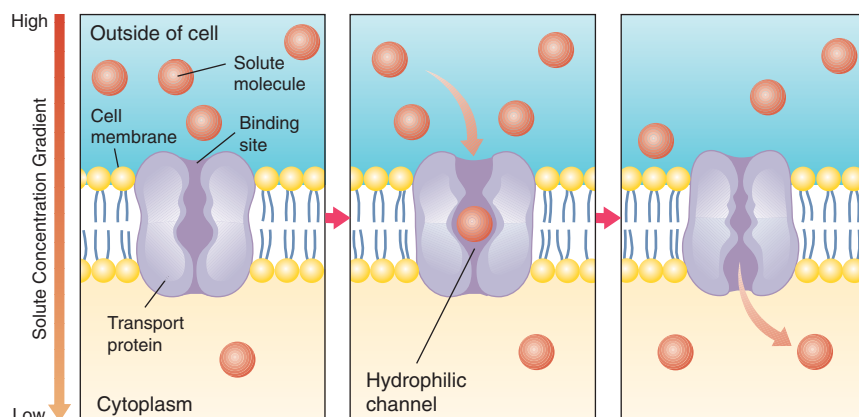
FIGURE 4.16 **Facilitated Transport Through a Membrane Protein.** Many transport proteins facilitate the diffusion of nutrients across the lipid bilayer. The transport protein forms a hydrophilic channel through which a specific solute can diffuse. »» Why would a solute move through a membrane protein rather than simply across the lipid bilayer?

Archaeal Membranes Are Structurally Unique

Besides the differences in gene sequences for ribosomal RNA in the domain Archaea, another major difference used to separate the archaeal organisms into their own domain is the chemical nature of the cell membrane.

The manner in which the hydrophobic lipid tails are attached to the glycerol is different in the Archaea. The tails are bound to the glycerol by "ether linkages" rather than the "ester linkages" found in the domains Bacteria and Eukarya (FIGURE 4.17A).

Also, typical fatty acid tails are absent from the membranes; instead, repeating five-carbon units are linked end-to-end to form lipid tails longer than the fatty acid tails. The result can be a lipid bilayer or **monolayer** (FIGURE 4.17B). A monolayer provides an advantage to the hyperthermophiles by preventing a peeling in two of the membrane, which would occur with a typical bilayer structure under high temperature conditions.

CONCEPT AND REASONING CHECKS 5

a. Penicillin and lysozyme primarily affect peptidoglycan synthesis in gram-positive bacterial cells. Why are these agents less effective against gram-negative bacterial cells?

b. Distinguish between peptidoglycan and pseudopeptidoglycan cell walls.

c. Justify the necessity for phospholipids and proteins in the cell membrane.

d. What is unique about archaeal membrane structure?
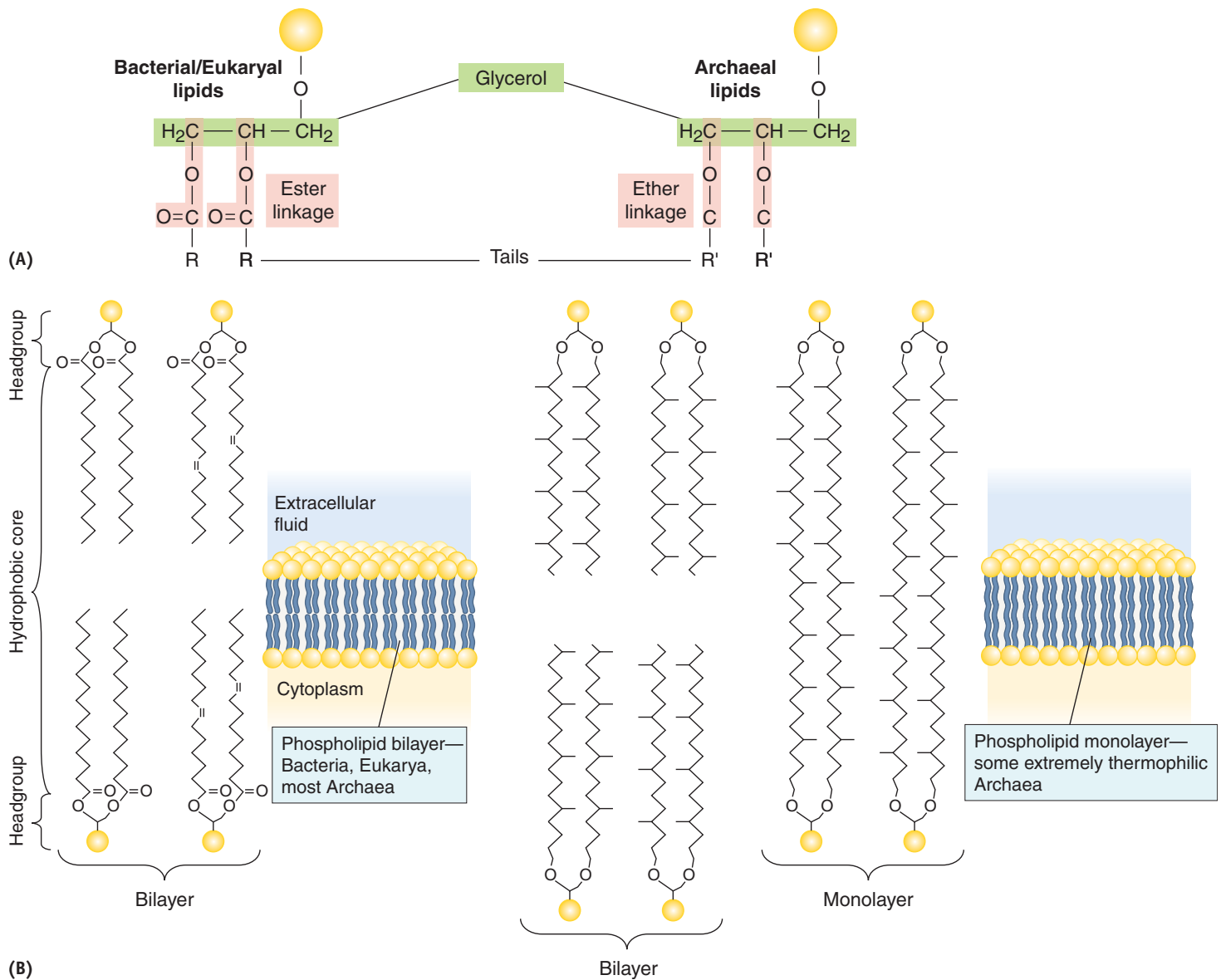
FIGURE 4.17   Structure of Cell Membranes. (A) Bacterial and eukaryotic cell membranes involve an ester linkage joining the glycerol to the fatty acid tails (R) while archaeal membranes have an ether linkage to the isoprenoid tails (R′). (B) Bacterial and eukaryal membranes form a bilayer while archaeal membranes may be a bilayer or a monolayer. »» What identifies an ester linkage from an ether linkage?

KEY CONCEPT 4.6   The Cell Cytoplasm Is Packed with Internal Structures

The cell membrane encloses the **cytoplasm**, which is the compartment within which most growth and metabolism occurs. The cytoplasm consists of the **cytosol**, a semifluid mass of proteins, amino acids, sugars, nucleotides, salts, vitamins, and ions—all dissolved in water—and several bacterial structures, subcompartments, and filaments, each with a specific function.

The Nucleoid Represents a Subcompartment Containing the Chromosome

The chromosome region in bacterial and archaeal cells appears as a diffuse mass termed the **nucleoid** (FIGURE 4.18). The nucleoid is not surrounded by a membrane envelope; rather, it represents a
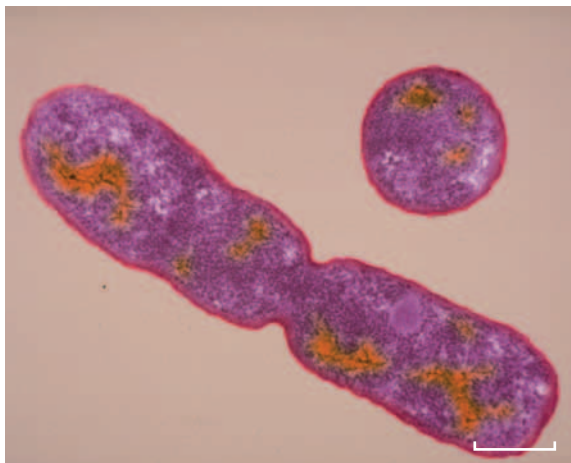
FIGURE 4.18 **The Bacterial Nucleoid.** In this false-color transmission electron micrograph of *Escherichia coli,* nucleoids (orange) occupy a large area in this dividing bacterial cell. Both longitudinal (center cells) and cross sections (cell upper right) of *E. coli* are visible. (Bar = 0.5 μm.) © Dennis Kunkel Microscopy, Inc./Visuals Unlimited/Corbis. »» How does a nucleoid differ from the eukaryotic cell nucleus described in the previous chapter?

subcompartment in the cytoplasm where the DNA aggregates and ribosomes are absent. Usually there is a single chromosome per cell and, with few exceptions, exists as a closed loop of DNA and protein.

The DNA contains the essential hereditary information for cell growth, metabolism, and reproduction. Because most cells only have one chromosome, the cells are genetically haploid. Unlike eukaryotic microorganisms and other eukaryotes, the nucleoid and chromosome do not undergo mitosis and having but the one set of genetic information cannot undergo meiosis.

The complete set of genes in an organism or virus is called the **genome**. For example, the genome of *E. coli,* a typical bacterial species in the mid-size range, contains about 4,300 genes. In all cases, these genes determine what proteins and enzymes the cell can make; that is, what metabolic reactions and activities can be carried out. For *E. coli,* this equates to some 2,000 different proteins.

Plasmids Are Found in Many Bacterial and Archaeal Cells

Besides a nucleoid, many bacterial and archaeal cells also contain smaller molecules of DNA called **plasmids**. About a tenth the size of the chromosome, these stable, extrachromosomal DNA molecules exist as closed loops containing 5 to 100 genes. There can be one or more plasmids in a cell and these may contain similar or different genes. Plasmids replicate independently of the chromosome and can be transferred between cells during recombination. They also represent important vectors in industrial technologies that use genetic engineering.

Although plasmids may not be essential for cellular growth, they provide a level of genetic flexibility. For example, some plasmids possess genes for disease-causing toxins and many carry genes for chemical or antibiotic resistance. For this latter reason, these genetic elements often are called **R plasmids** (R for resistance).

Other Subcompartments Exist in the Cell Cytoplasm

For a long time the cytoplasm was looked at as a bag enclosing the genetic machinery and biochemical reactions. As more studies were carried out with the electron microscope and with biochemical techniques, it became evident that the cytoplasm contained "more than meets the eye."

Ribosomes. One of the universal structures in all cells is the **ribosome**. There are thousands of these nearly spherical particles in the cell cytoplasm, which gives it a granular appearance when viewed with the electron microscope (FIGURE 4.19A). In addition, there also are ribosomes loosely associated with the cell membrane. Their relative size is measured by how fast they settle when spun in a centrifuge. Measured in Svedberg units (S), bacterial and archaeal ribosomes represent 70S particles.

The ribosomes are built from RNA and protein and are composed of a small subunit (30S) and a large subunit (50S) (FIGURE 4.19B). For proteins to be synthesized, the two subunits come together to form a 70S functional ribosome. Some antibiotics, such as streptomycin and tetracycline, prevent bacterial and archaeal ribosomes from carrying out protein synthesis.

The free ribosomes make soluble proteins that are used in the cell, while the membrane-associated ones produce proteins for the cell envelope and for secretion.

Microcompartments. Recently, some bacterial species have been found to contain **microcompartments**. The microcompartments appear to be unique to the Bacteria and consist of a polyprotein shell 100–200 nm in diameter (FIGURE 4.20).

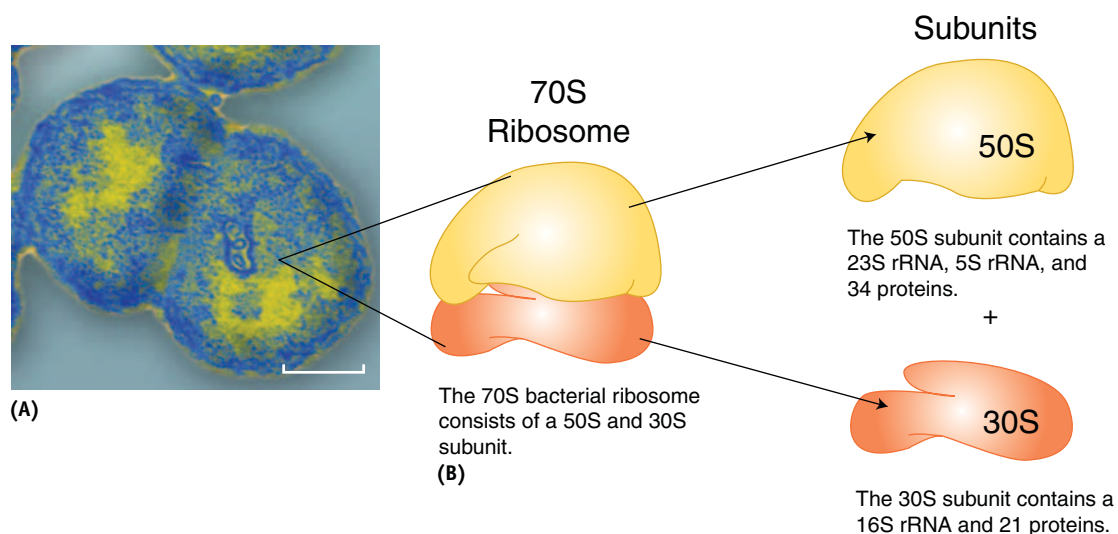■ Vector: A genetic element capable of incorporating and transferring genetic information.

■ Haploid: Having a single set of genetic information.

■ Centrifuge: An instrument that spins particles suspended in liquid at high speed.

Subunits

70S Ribosome

50S

The 50S subunit contains a 23S rRNA, 5S rRNA, and 34 proteins.

+

The 70S bacterial ribosome consists of a 50S and 30S subunit.

(A)

(B)

30S

The 30S subunit contains a 16S rRNA and 21 proteins.

FIGURE 4.19 The Bacterial Ribosome. (**A**) A false-color transmission electron micrograph of *Neisseria gonorrhoeae,* showing the nucleoid (yellow) and ribosomes (blue). (Bar = 1 µm.) © Science Source/Photo Researchers, Inc. (**B**) The functional 70S ribosome is assembled from a small (30S) and large (50S) subunit. »» How many rRNA molecules and proteins construct a 70S ribosome?
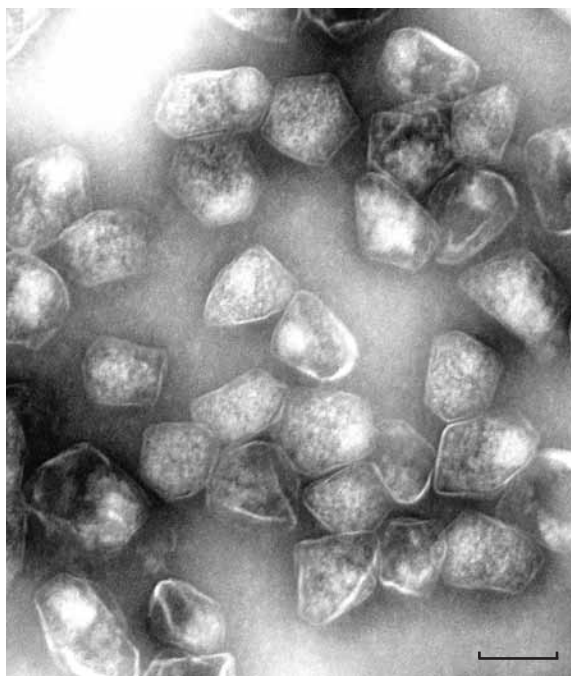
FIGURE 4.20 Microcompartments. Purified bacterial microcompartments from *Salmonella enterica* are composed of a complex protein shell that encases metabolic enzymes. (Bar = 100 nm.) Reprinted with permission from the American Society for Microbiology (Microbe, January, 2006, p. 20-24). Photo courtesy of Dr. Thomas A. Bobik, Department of Biochemistry, Biophysics and Molecular, Iowa State University. »» How do these bacterial microcompartments differ structurally from a eukaryotic organelle?

In the cyanobacteria, microcompartments called "carboxysomes" function to enhance carbon dioxide fixation and in some non-photosynthetic species, microcompartments limit diffusion of volatile or toxic metabolic products. In general, microcompartments represent localized areas where enzymes can more directly interact with their substrates or potentially sequester harmful reaction products.

Inclusions. Cytoplasmic structures, called **inclusions**, can be found in the cytoplasm. Many of these bodies store nutrients or the monomers for cellular structures. For example, some inclusions function as nutrient reserves. They consist of aggregates or granules of polysaccharides (glycogen), globules of elemental sulfur, or lipid. Other inclusions can serve as important identification characters for bacterial pathogens. One example is the diphtheria bacilli that contain **metachromatic granules**, also called **volutin granules**, which are deposits of polyphosphate (long chains of inorganic phosphate) along with calcium and other ions. These granules stain with dyes such as methylene blue. Similar organelles called "acidocalcisomes" are found in the protists.

Some aquatic and marine forms achieve buoyancy using **gas vacuoles**, cytoplasmic compartments built from a water-tight protein shell. These vacuoles are filled with a gas to decrease the density of the cell, which generates and regulates their buoyancy.

The **magnetosome**, another type of inclusion or subcompartment, is described in MicroFocus 4.3.

MICROFOCUS 4.3: Environmental Microbiology
Magnetic Navigation

To get from place to place, humans often require the assistance of maps, GPS systems, or gas station attendants. In the microbial world, life is generally more simple, and traveling is no exception.

In the early 1980s, Richard P. Blakemore and his colleagues at the University of New Hampshire observed mud-dwelling bacterial cells gathering at the north end of water droplets. On further study, they discovered each cell had a chain of aligned magnetic particles acting like a compass directing the organism's movements (magnetotaxis). Additional interdisciplinary investigations by microbiologists and physicists have shown the magnetotactic bacteria contain a linear array of 15–20 membrane-bound vesicles along the cell's long axis (see figure). Each vesicle, called a magnetosome, is an invagination of the cell membrane and contains the protein machinery to nucleate and grow a magnetic crystal of magnetite (Fe_3O_4) or greigite (Fe_3S_4). The chain of magnetosomes is organized by filaments that are similar to eukaryotic actin. By running parallel to the magnetosome chain, the filaments organize the vesicles into a chain. As each vesicle accumulates the magnetite or greigite crystal to form a magnetosome, magnetostatic interactions between vesicles stabilizes the linear aggregation. As an aside, magnetite is also found in migrating animals such as butterflies, homing pigeons, and dolphins.

To date, all magnetotactic bacterial cells are motile, gram-negative cells common in aquatic and marine habitats, including sediments where oxygen is absent. This last observation is particularly noteworthy because it explains why these organisms have magnetosomes—they act as a compass needle to orient the cell.

Recent studies have shown that magnetotactic bacteria prefer low concentrations of oxygen. So, a current theory is that both magnetotaxis and aerotaxis work together to allow cells to "find" the optimal oxygen concentration, permitting the bacteria cells to reach a sort of biological nirvana and settle in for a life of environmental bliss.
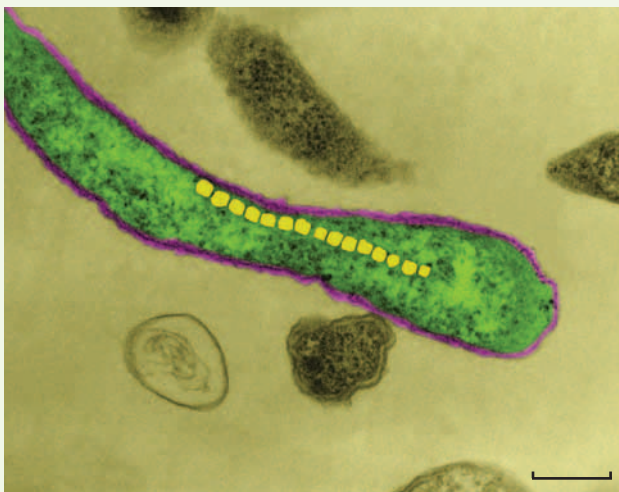
Bacterial magnetosomes (yellow) are seen in this false-color transmission electron micrograph of a magnetotactic marine spirillum. (Bar = 1 µm.) © Dennis Kunkel Microscopy, Inc./Visuals Unlimited/Corbis.

These inclusions, found in some aquatic bacterial species, are invaginations of the cell membrane, which are coordinated and positioned by cytoskeletal filaments similar to eukaryotic microfilaments.

Cytoskeletal Proteins Regulate Cell Division and Help Determine Cell Shape

Until recently, the dogma was that bacterial and archaeal cells lacked a cytoskeleton, which is a common feature in eukaryotic cells. However, it is now clear that cytoskeletal proteins that resemble those in the eukaryotic cytoskeleton are present in bacterial and archaeal cells.

The first protein discovered was a homolog of the eukaryotic protein tubulin, which forms filaments that assemble into microtubules. The homolog forms filaments similar to those in microtubules but the filaments do not assemble into microtubules. These tubulin-like proteins have been found in all bacterial and archaeal cells examined and appear to function in the regulation of cell division. During this process, the protein localizes around the neck of the dividing cell where it recruits other proteins needed for the deposition of a new cell wall between the dividing cells (MICROFOCUS 4.4).

Protein homologs remarkably similar in three-dimensional structure to eukaryotic microfilaments assemble into filaments that help determine cell shape in *E. coli* and *Bacillus subtilis*. These homologs have been found in most nonspherical cells where they form a helical network beneath the cell membrane to guide the proteins involved

■ Homolog:
An entity with similar attributes due to shared ancestry.

MICROFOCUS 4.4: Public Health

The Wall-less Cytoskeleton

Sometimes the lack of something can speak loudly. Take for example the mycoplasmas such as *Mycoplasma pneumoniae* that causes primary atypical pneumonia (walking pneumonia). This species, as well as other *Mycoplasma* species, lack a cell wall. How then can they maintain a defined cell shape (see figure)?

Transmission electron microscopy has revealed that mycoplasmal cells contain a very complex cytoskeleton and further investigations indicate the cytoskeletal proteins are very different from the typical cytoskeletal homologs found in other groups of the Firmicutes. For example, *Spiroplasma citri,* which causes infections in other animals, has a fibril protein cytoskeleton that is laid down as a helical ribbon. This fibril protein has not been found in any other organisms. Because the cells are spiral shaped, the ribbon probably is laid down in such a way to determine cell shape, suggesting that shape does not have to be totally dependent on a cell wall.

In *Mycoplasma genitalium,* which is closely related to *M. pneumoniae* and causes human urethral infections, a eukaryotic-like tubulin homolog has been identified, but none of the other proteins have been identified that it recruits at the division neck for cell division. Surprising? Not really. Important? Immensely! Because mycoplasmas do not have a cell wall, why would they require those proteins that lay down a peptidoglycan cross wall between cells? So the lack of something (wall-forming proteins) tells us what those proteins must do in their gram-positive relatives that do have walls.
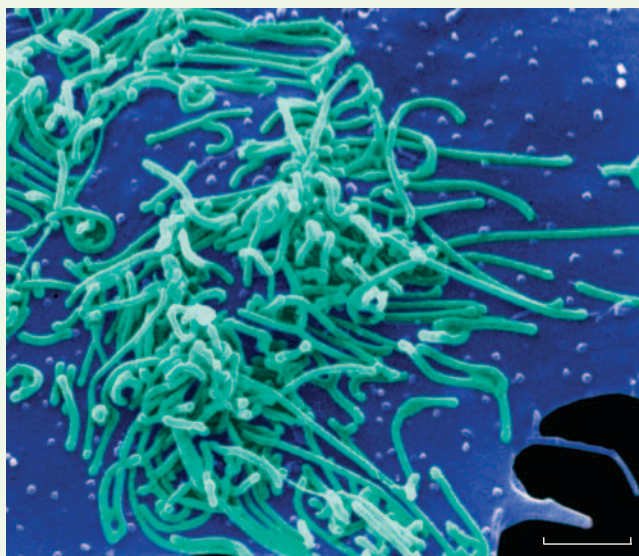
False-color scanning electron micrograph of *Mycoplasma pneumoniae* cells. (Bar = 2.5 µm.) © Don W. Fawcett/Photo Researchers, Inc.

in cell wall formation (FIGURE 4.21A). The homologs also are involved with chromosome segregation during cell division and magnetosome formation.

Intermediate filaments (IF), another component of the eukaryotic cytoskeleton in some metazoans, have a homolog as well. The protein, called crescentin, helps determine the characteristic crescent shape of *Caulobacter crescentus* cells. In older cells that become filamentous, crescentin maintains the helical shape of the cells by aligning with the inner cell curvature beneath the cytoplasmic membrane (FIGURE 4.21B).

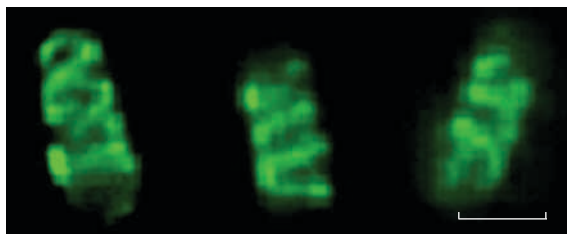Even though the evolutionary relationships are quite distant between bacterial/archaeal and eukaryotic cytoskeletal proteins based on protein sequence data, the similarity of their three-dimensional structure and function is strong evidence supporting homologous cytoskeletons. (TABLE 4.3) summarizes the structural features of bacterial and archaeal cells.

Metazoan: A member of the vertebrates, nematodes, and mollusks.

CONCEPT AND REASONING CHECKS 6

a. Why do we say that the bacterial chromosome contains the "essential hereditary information"?
b. What properties distinguish the bacterial chromosome from a plasmid?
c. Provide the roles for the subcompartments found in bacterial cells.
d. Evaluate the relationship between the eukaryotic cytoskeleton and the cytoskeletal protein homologs in bacterial and archaeal cells.

(A)

FIGURE 4.21 **A Bacterial Cytoskeleton.** Bacterial cells have proteins similar to those that form the eukaryotic cytoskeleton. (**A**) Microfilament-like proteins form helical filaments that curve around the edges of these cells of *Bacillus subtilis*. (Bar = 1.5 µm.) Courtesy of Rut Carballido-López, University of Oxford. (**B**) Three-dimensional model of a helical *Caulobacter crescentus* cell (green) with a helical cytoskeletal filament of crescentin (pink). Reprinted from *Cell*, vol 115, Jacobs-Wagner, C., cover, copyright 2003, with permission from Elsevier. Photo courtesy of Christine Jacobs-Wagner, Yale University. »» What would be the shape of these cells without the cytoskeletal proteins?

(B)

TABLE

4.3 **A Summary of the Structural Features of Bacterial and Archaeal Cells**

Structure	Chemical Composition	Function	Comment
External Structures			
Pilus	Protein	Attachment to surfaces Genetic transfer	Found primarily in gram-negative bacteria
Flagellum	Protein	Motility	Present in many rods and spirilla; few cocci; vary in number and placement
Glycocalyx	Polysaccharides and small proteins	Buffer to environment Attachment to surfaces	Capsule and slime layer Contributes to disease development Found in plaque bacteria and biofilms
Cell Envelope			
Cell wall		Cell protection Shape determination Cell lysis prevention	
Bacterial	Gram positives: thick peptidoglycan and teichoic acid Gram negatives: little peptidoglycan and an outer membrane		Site of activity of penicillin and lysozyme Gram-negative bacteria release endotoxins
Archaeal	Pseudopeptidoglycan Protein		S-layer
Cell membrane			
Bacterial	Protein and phospholipid	Cell boundary	Lipid bilayer
Archaeal		Transport into/out of cell Site of enzymatic reactions	Lipid bilayer/monolayer

TABLE

4.3 **A Summary of the Structural Features of Bacterial and Archaeal Cells (continued)**

Structure	Chemical Composition	Function	Comment
Internal Structures			
Nucleoid	DNA	Site of essential genes	Exists as single, closed loop chromosome
Plasmid	DNA	Site of nonessential genes	R plasmids
Ribosome	RNA and protein	Protein synthesis	Inhibited by certain antibiotics
Microcompartment	Various metabolic enzymes	Carbon dioxide fixation Retention of volatile or toxic metabolites	Enzymes are enclosed in a protein shell
Inclusion	Glycogen, sulfur, lipid	Nutrient storage	Used as nutrients during starvation periods
Metachromatic granule	Polyphosphate	Storage of polyphosphate and calcium ions	Found in diphtheria bacilli
Gas vacuole	Protein shell	Buoyancy	Helps cells remain buoyant
Magnetosome	Magnetite/greigite	Cell orientation	Helps locate preferred habitat
Cytoskeleton	Proteins	Cell division, chromosomal segregation, cell shape	Functionally similar to eukaryotic cytoskeletal proteins

Chapter Challenge C

Congratulations! You have made it to the end of the chapter on bacterial and archaeal cell structure. In these last three sections you discovered the exterior structures, studied the organization of the cell envelope, and surveyed the cytoplasmic structures.

QUESTION C:
The chapter challenge was: "Studying the diversity of life only accentuates life's unity." Summarize your opinion as to the validity of this statement, using cell structure as your reference.

Answers can be found on the Student Companion Website in **Appendix D**.

■ **In conclusion**, one of the take-home lessons from the discussions of cell structure and function explored in this chapter is the ability of bacterial and archaeal organisms to carry out the "complex" metabolic and biochemical processes typically associated with eukaryotic cells—usually without the need for elaborate membrane-enclosed subcompartments.

However, there is now clear evidence for an intricate and sophisticated subcellular architecture in the domains Bacteria and Archaea. But what about other major cellular processes such as making proteins? This requires two processes, that of tran-

scription and translation. In eukaryotic cells, these processes are spatially separated into the cell nucleus (transcription) and the cytoplasm (translation).

In bacterial and archaeal cells, there also can be spatial separation between transcription and translation (**FIGURE 4.22**). The RNA polymerase molecules needed for transcription are localized to a region separate from the ribosomes and other proteins that perform translation. In fact, studies indicate that transcription is localized to the periphery of the nucleoid. So, even without a
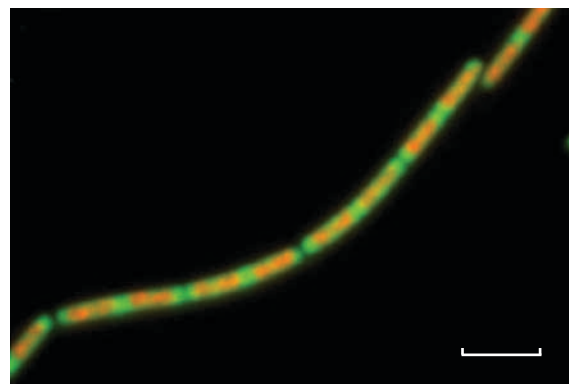
FIGURE 4.22 **Spatial Separation of Transcription and Translation.** In these cells of *Bacillus subtilis,* fluorescence microscopy was used to identify RNA polymerase (transcription) using a red fluorescent protein and ribosomes (translation) using a green fluorescent protein. Separate subcompartments are evident. (Bar = 3 μm.) Courtesy of Dr. Peter Lewis; School of Environmental and Life Sciences, University of Newcastle. »» What does the spatial separation indicate concerning compartmentation?

MICRO**O**INQUIRY 4

The Prokaryote/Eukaryote Model

"It is now clear that among organisms there are two different organizational patterns of cells, which Chatton . . . called, with singular prescience, the eukaryotic and prokaryotic type. The distinctive property of bacteria and blue-green algae is the prokaryotic nature of their cells. It is on this basis that they can be clearly segregated from all other protists (namely, other algae, protozoa, and fungi), which have eukaryotic cells."

<div align="right">

Stanier and van Niel (1962)
—*The concept of a bacterium*

</div>

The idea of a tree of life extends back centuries and originates not with scientific thinking, but rather with folklore and culture, and often focused on immortality or fertility (see figure).

The development of the three domains in tree of life, on the other hand, represents the evolutionary relationships between species. Its development has made a profound change in biology. Instead of two kinds of organisms—prokaryotes and eukaryotes—there are three: Bacteria, Archaea, and Eukarya.

In 2006, Norm Pace, a molecular biologist turned evolutionist at the University of Colorado, Boulder, suggested that the massive data bank of gene sequences identified since 1995 shows just how different archaeal organisms are from bacterial organisms and, in some ways, the archaeal ones are more similar to eukaryotic organisms. Therefore, Pace says, "we need to reassess our understanding of the course of evolution at the most fundamental level." Among items needing reassessment is the prokaryotic/eukaryotic paradigm—the tradition (folklore) if you will—that if an organism is not a eukaryote, it must be a prokaryote.

Glass mosaic of tree of life on a wall of the 16th century Sim Wat Xiang Thong Luang Prabang UNESCO World Heritage Site, Laos.

The quote at the top of the page refers to Edouard Chatton who coined the terms "prokaryotic" and "eukaryotic." Interestingly, neither he, nor Stanier and van Niel, ever really made mention as to what a prokaryotic cell is. Yet if you look in any introductory biology textbook, prokaryote is defined as a group of organisms that lack a cell nucleus. According to Pace, "the prokaryote/eukaryote model for biological diversity and evolution is invalid." How can all organisms without a cell nucleus be called "prokaryotic," especially because the eukaryotic cell nucleus appears to be descended from as ancient a line of cells as the Archaea? Yes, the concept of a nuclear membrane (or not) is important, but no more important than other cellular properties. And the problem is that the word "prokaryote" is so engrained in the culture of biology and in the scientific mind of biologists—and students—that inappropriate inferences about organisms are made using this term. Pace does not buy the argument that the term "prokaryote" can be used to identify organisms that are not eukaryotes because the Bacteria are very different from the Archaea and, therefore, should not be put under the umbrella of "prokaryote."

Pace believes that saying prokaryotes lack a cell nucleus is a scientifically invalid description; although open to debate, he says no one can define what a prokaryote is—only what it is not (e.g., no nucleus, no mitochondria, no chloroplasts, no endomembrane system, etc.). Therefore, lumping the Bacteria and Archaea conceptually dismisses the fundamental and important differences between these two kinds of organisms and reinforces an incorrect understanding of biological organization and evolution.

Pace believes it is time to delete the term prokaryote as a term for bacterial and archaeal organisms. Because it has long been used by all biology texts, including this one (although in many cases "bacteria" and "archaea" have replaced the term prokaryote), Pace says he realizes "it is hard to stop using the word prokaryote."

Discussion Point

There is no doubt that bacterial and archaeal organisms are very different entities. So, if "prokaryote" is to be deleted from the biological vocabulary, what can we call the Bacteria and Archaea? Can you think of some positive characters that would define both bacterial and archaeal cells? If so, then how about inventing a common noun and adjective for both? Or do we simply speak of the bacteria, archaea, and eukaryotes separately?

Answers (and comments) can be found on the Student Companion Website in **Appendix D**.

nuclear membrane, these cells can separate the process involved in making cellular proteins, in a manner similar to eukaryotic cells.

Traditionally, a prokaryote was an organism without a cell nucleus; that is, without a membrane surrounding the DNA or chromosome. But is that a fair way to describe all bacterial and archaeal organisms? In this chapter, you have learned that there are some differences between bacterial and archaeal cells, yet do we lump them together just because they both lack a cell nucleus? Some scientists say no—the terms "prokaryote" and "prokaryotic" are not appropriate for these two domains of life.

So, to finish this chapter, take a look at MICRO INQUIRY 4, which discusses the prokaryotic/eukaryotic model for living organisms.

■ SUMMARY OF KEY CONCEPTS

4.1 There Is Tremendous Diversity Among the Bacteria and Archaea

1. The **phylogenetic tree** of life contains many bacterial phyla and groups, including the **Proteobacteria, Gram-positive bacteria, Cyanobacteria, Chlamydiae**, and **Spirochaetes**. (Fig. 4.2)

2. Many organisms in the domain Archaea live in extreme environments. The **Euryarchaeota** (**methanogens, extreme halophiles, hyperthermophiles**, and the **thermoacidophiles**) and the **Crenarchaeota** are the two phyla.

4.2 Prokaryotes Can Be Distinguished by Their Cell Shape and Arrangements

3. **Bacilli** have a cylindrical shape and can remain as single cells or be arranged into **diplobacilli** or chains (**streptobacilli**). **Cocci** are spherical and form a variety of arrangements, including the **diplococcus, streptococcus**, and **staphylococcus**. The spiral-shaped bacteria can be curved rods (**vibrios**) or spirals (**spirochetes** and **spirilla**). Spirals generally appear as single cells. (Fig. 4.5)

4.3 An Overview to Bacterial and Archaeal Cell Structure

4. Cell organization is centered on three specific processes: sensing and responding to environmental changes, compartmentalizing metabolism, and growing and reproducing. (Figs. 4.6, 4.7)

4.4 External Cell Structures Interact with the Environment

5. **Pili** are short hair-like appendages found on many gram-negative bacteria to facilitate attachment to a surface. **Conjugation pili** are used for genetic transfer of DNA.

6. One or more **flagella**, found on many rods and spirals, provide for cell motility. Each flagellum consists of a **basal body** attached to the flagellar filament. In nature, flagella propel bacterial cells toward nutrient sources (**chemotaxis**). Spirochetes have **endoflagella**. (Figs. 4.10, 4.11)

7. The **glycocalyx** is a sticky layer of polysaccharides that protects the cell against desiccation, attaches it to surfaces, and helps evade immune cell attack. The glycocalyx can be thick and tightly bound to the cell (**capsule**) or thinner and loosely bound (**slime layer**).

4.5 Most Bacterial and Archaeal Cells Have a Cell Envelope

8. The **cell wall** provides structure and protects against cell lysis. Gram-positive bacteria have a thick wall of **peptidoglycan** strengthened with **teichoic acids**. Gram-negative cells have a single layer of peptidoglycan and an **outer membrane** containing **lipopolysaccharide** and **porin proteins**. (Fig. 4.14)

9. Archaeal cell walls lack peptidoglycan but may have either a **pseudopeptidoglycan** or **S-layer**.

10. The **cell membrane** represents a permeability barrier and the site of transfer for nutrients and metabolites into and out of the cell. The cell membrane reflects the **fluid mosaic model** for membrane structure in that the lipids are fluid and the proteins are a mosaic that can move laterally in the bilayer. (Fig. 4.15)

11. The archaeal cell membrane links lipids through an ether linkage and the lipid tails may be bonded together into a single **monolayer**. (Fig. 4.17)

4.6 The Cell Cytoplasm Is Packed with Internal Structures

12. The DNA (**bacterial chromosome**), located in the **nucleoid**, is the essential genetic information and represents most of the organism's **genome**.

13. Bacterial and archaeal cells may contain one or more **plasmids**, circular pieces of nonessential DNA that replicate independently of the chromosome.

14. **Ribosomes** carry out protein synthesis, **microcompartments** sequester species-specific processes, while **inclusions** store nutrients or structural building blocks.

15. The **cytoskeleton**, containing protein homologs to the cytoskeletal proteins in eukaryotic cells, helps determine cell shape, regulates cell division, and controls chromosomal segregation during cell division.

■ CHAPTER SELF-TEST

For **STEPS A–D**, answers to even-numbered questions and problems can be found in **Appendix C** on the Student Companion Website at **http://microbiology.jbpub.com/10e**. In addition, the site features eLearning, an online review area that provides quizzes and other tools to help you study for your class. You can also follow useful links for in-depth information, read more MicroFocus stories, or just find out the latest microbiology news.

■ STEP A: REVIEW OF FACTS AND TERMS

Multiple Choice

Read each question carefully, then select the **one** answer that best fits the question or statement.

1. Which one of the following is NOT a genus within the gram-positive bacteria?
   A. *Staphylococcus*
   B. Methanogens
   C. *Mycoplasma*
   D. *Bacillus* and *Clostridium*

2. The domain Archaea includes all the following groups *except* the _____.
   A. mycoplasmas
   B. extreme halophiles
   C. Crenarchaeota
   D. Euryarchaeota

3. Spherical bacterial cells in chains would be referred to as a _____ arrangement.
   A. vibrio
   B. streptococcus
   C. staphylococcus
   D. tetrad

4. Intracellular organization in bacterial and archaeal species is centered around _____.
   A. compartmentation of metabolism
   B. growth and reproduction
   C. sensing and responding to environment
   D. All the above (A–C) are correct.

5. Which one of the following statements does NOT apply to pili?
   A. Pili are made of protein.
   B. Pili allow for attachment to surfaces.
   C. Pili facilitate nutrient transport.
   D. Pili contain adhesins.

6. Flagella are _____.
   A. made of carbohydrate and lipid
   B. found on all bacterial cells
   C. shorter than pili
   D. important for chemotaxis

7. Capsules are similar to pili because both _____.
   A. contain DNA
   B. are made of protein
   C. contain dextran fibers
   D. permit attachment to surfaces

8. Gram-negative bacterial cells would stain _____ with the Gram stain and have _____ in the wall.
   A. orange-red; teichoic acid
   B. orange-red; lipopolysaccharide
   C. purple; peptidoglycan
   D. purple; teichoic acid

9. The cell membrane of archaeal hyperthermophiles contains _____.
   A. a monolayer
   B. sterols
   C. ester linkages
   D. All the above (A–C) are correct.

10. The movement of glucose into a cell occurs by _____.
    A. facilitated diffusion
    B. active transport
    C. simple diffusion
    D. phospholipid exchange

11. When comparing bacterial and archaeal cell membranes, only archaeal cell membranes _____.
    A. have three layers of phospholipids
    B. have a phospholipid bilayer
    C. are fluid
    D. have ether linkages

12. Which one of the following statements about the nucleoid is NOT true?
    A. It contains a DNA chromosome.
    B. It represents a nonmembranous subcompartment.
    C. It represents an area devoid of ribosomes.
    D. It contains nonessential genetic information.

13. Plasmids _____.
    A. replicate with the bacterial chromosome
    B. contain essential growth information
    C. may contain antibiotic resistance genes
    D. are as large as the bacterial chromosome

14. Which one of the following is NOT a structure or subcompartment found in bacterial cells?
    A. Microcompartments
    B. Volutin
    C. Ribosomes
    D. Mitochondria

15. The bacterial cytoskeleton _____.
    A. transports vesicles
    B. helps determine cell shape
    C. is organized identical to its eukaryotic counterpart
    D. centers the nucleoid

Label Identification

16. Identify and label the structure on the accompanying bacterial cell from each of the following descriptions. Some separate descriptions may apply to the same structure.

Descriptions

a. Is involved with nutrient storage.
b. An essential structure for chemotaxis, aerotaxis, or phototaxis.
c. Contains nonessential genetic information that provides genetic variability.
d. The structure that synthesizes proteins.
e. The protein structures used for attachment to surfaces.
f. Contains essential genes for metabolism and growth.
g. Prevents cell desiccation.
h. A 70S particle.
i. Contains peptidoglycan.
j. Regulates the passage of substances into and out of the cell.
k. Extrachromosomal loops of DNA.
l. Represents a capsule or slime layer.
m. The semifluid mass of proteins, amino acids, sugars, salts, and ions dissolved in water.
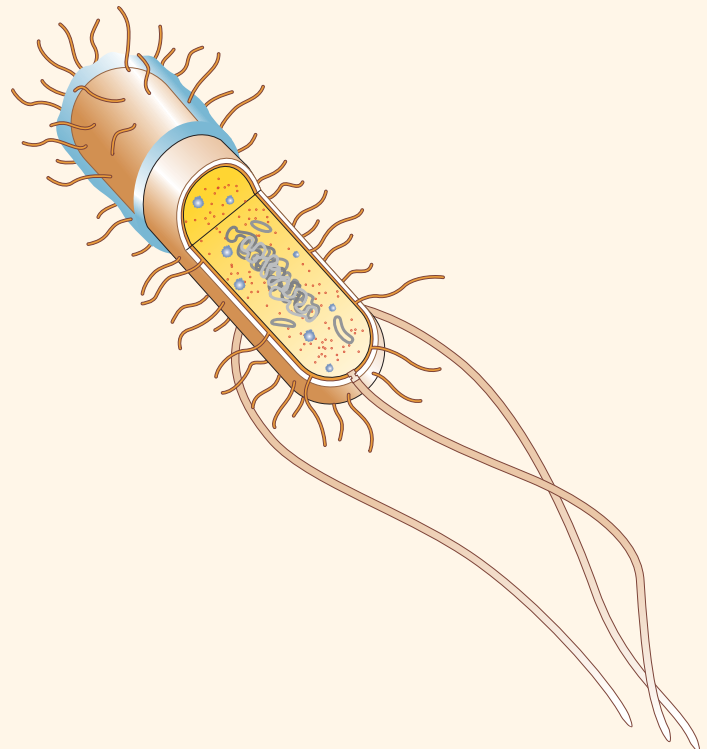
■ STEP B: CONCEPT REVIEW

17. Construct a concept map for the **domain Bacteria** using the following terms. (**Key Concept 1**)

Actinobacteria	Hyperthermophiles
Bacillus	*Mycoplasma*
Blooms	Proteobacteria
Chlamydiae	Rickettsiae
Cyanobacteria	Spirochaetes
Escherichia	*Staphylococcus*
Firmicutes	*Streptomyces*
Gram-negative species	*Treponema*
Gram-positive species	

18. Compare the various shapes and arrangements of bacterial and archaeal cells. (**Key Concept 2**)

19. Summarize how the processes of sensing and responding to the environment, compartmentation of metabolism, and growth and metabolism are linked to cell structure. (**Key Concept 3**)

20. Assess the role of the three external cell structures to cell function and survival. (**Key Concept 4**)

21. Construct a concept map for the **Cell Envelope** using the following terms. (**Key Concept 5**)

Active transport	Membrane proteins
Cell membrane	NAG
Cell wall	NAM
Endotoxin	Outer membrane
Facilitated transport	Peptidoglycan
Fluid-mosaic model	Periplasm
Gram-negative wall	Phospholipids
Gram-positive wall	Polysaccharide
Lipid A	Porin proteins
Lipopolysaccharide (LPS)	Teichoic acid

22. Justify the need for a **cell membrane** surrounding all bacterial and archaeal cells. (**Key Concept 5**)

23. Describe the function of the nucleoid and plasmids to cell metabolism and cell survival. (**Key Concept 6**)

24. Explain the roles for subcellular compartments in the bacterial cell. (**Key Concept 6**)

■ STEP C: APPLICATIONS AND PROBLEMS

25. A bacterial species has been isolated from a patient and identified as a gram-positive rod. Knowing that it is a human pathogen, what structures would it most likely have? Explain your reasons for each choice.

26. Another patient has a blood infection caused by a gram-negative bacterial species. Why might it be dangerous to prescribe an antibiotic to treat the infection?

27. In the research lab, the gene for the cytoskeletal protein similar to eukaryotic tubulin is transferred into the DNA chromosome of a coccus-shaped bacterium. When this cell undergoes cell division, predict what shape the daughter cells will exhibit. Explain your answer.

■ STEP D: QUESTIONS FOR THOUGHT AND DISCUSSION

28. In reading a story about a bacterial species that causes a human disease, the word "bacillus" is used. How would you know if the article is referring to a bacterial shape or a bacterial genus?

29. Suppose this chapter on the structure of bacterial and archaeal cells had been written in 1940, before the electron microscope became available. Which parts of the chapter would probably be missing?

30. Why has it taken so long for microbiologists to discover microcompartments and a cytoskeleton in bacterial and archaeal cells?

31. Apply the current understanding of the bacteria/eukaryote paradigm to the following statement: "Studying the diversity of life only accentuates life's unity."