fungi and bacteria.

Biofilms are present almost everywhere: they can cause the clogging of pipes and readily colonize surfaces in industrial settings. In recent, large-scale outbreaks of bacterial contamination of food, biofilms have played a major role. They also colonize household surfaces, such as kitchen counters, cutting boards, sinks, and toilets, as well as places on the human body, such as the surfaces of our teeth.

Interactions among the organisms that populate a biofilm, together with their protective *exopolysaccharidic (EPS)* environment, make these communities more robust than free-living, or planktonic, prokaryotes. The sticky substance that holds bacteria together also excludes most antibiotics and disinfectants, making biofilm bacteria hardier than their planktonic counterparts. Overall, biofilms are very difficult to destroy because they are resistant to many common forms of sterilization.

visual CONNECTION

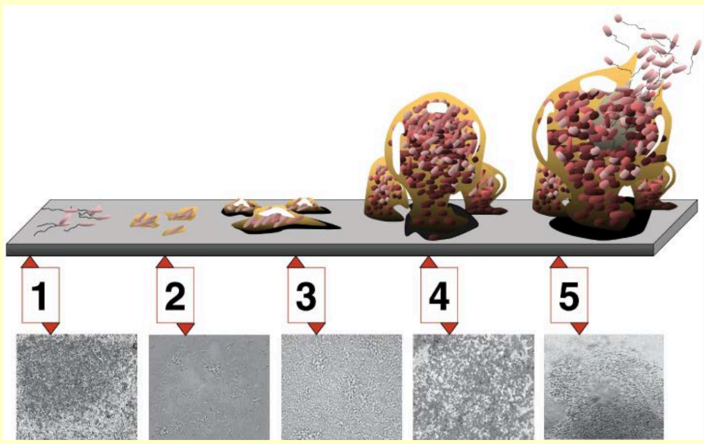


Figure 22.8 Development of a biofilm. Five stages of biofilm development are shown. During stage 1, initial attachment, bacteria adhere to a solid surface via weak *van der Waals interactions* (forces produced by induced electrical interactions between atoms). During stage 2, irreversible attachment, hairlike appendages called *pili* permanently anchor the bacteria to the surface. During stage 3, maturation I, the biofilm grows through cell division and recruitment of other bacteria. An extracellular matrix composed primarily of polysaccharides holds the biofilm together. During stage 4, maturation II, the biofilm continues to grow and takes on a more complex shape. During stage 5, dispersal, the biofilm matrix is partly broken down, allowing some bacteria to escape and colonize another surface. Micrographs of a *Pseudomonas aeruginosa* biofilm in each of the stages of development are shown. (credit: D. Davis, Don Monroe, PLoS)

Compared to free-floating bacteria, bacteria in biofilms often show increased resistance to antibiotics and detergents. Why do you think this might be the case?

22.2 | Structure of Prokaryotes: Bacteria and Archaea

By the end of this section, you will be able to do the following:

- Describe the basic structure of a typical prokaryote
- Describe important differences in structure between Archaea and Bacteria

There are many differences between prokaryotic and eukaryotic cells. The name "prokaryote" suggests that prokaryotes are defined by exclusion—they are not eukaryotes, or organisms whose cells contain a nucleus and other internal membrane-bound organelles. However, all cells have four common structures: the plasma membrane, which functions as a barrier for the cell and separates the cell from its environment; the cytoplasm, a complex solution of organic molecules and salts inside the cell; a double-stranded DNA genome, the informational archive of the cell; and ribosomes, where protein synthesis takes place. Prokaryotes come in various shapes, but many fall into three categories: *cocci* (spherical), *bacilli* (rod-shaped), and *spirilli* (spiral-shaped) (**Figure 22.9**).

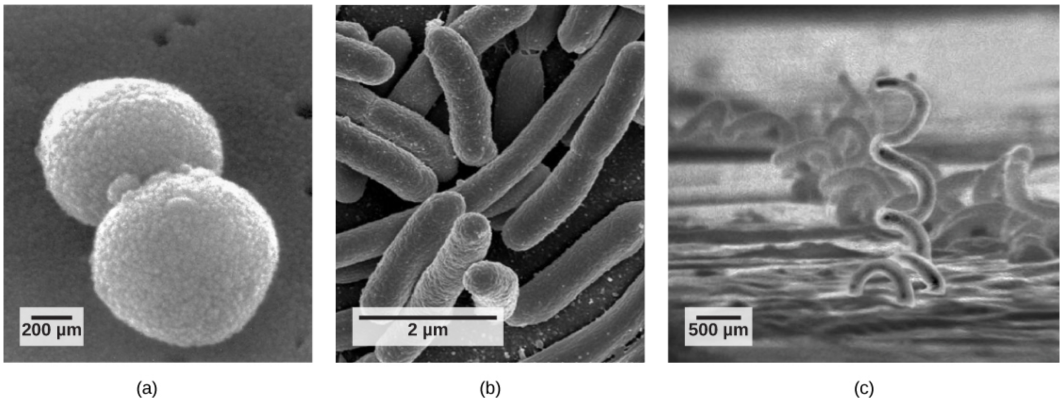


Figure 22.9 Common prokaryotic cell types. Prokaryotes fall into three basic categories based on their shape, visualized here using scanning electron microscopy: (a) cocci, or spherical (a pair is shown); (b) bacilli, or rod-shaped; and (c) spirilli, or spiral-shaped. (credit a: modification of work by Janice Haney Carr, Dr. Richard Facklam, CDC; credit c: modification of work by Dr. David Cox; scale-bar data from Matt Russell)

The Prokaryotic Cell

Recall that prokaryotes are unicellular organisms that lack membrane-bound organelles or other internal membrane-bound structures (**Figure 22.10**). Their chromosome—usually single—consists of a piece of circular, double-stranded DNA located in an area of the cell called the **nucleoid**. Most prokaryotes have a cell wall outside the plasma membrane. The **cell wall** functions as a protective layer, and it is responsible for the organism's shape. Some bacterial species have a capsule outside the cell wall. The capsule enables the organism to attach to surfaces, protects it from dehydration and attack by phagocytic cells, and makes pathogens more resistant to our immune responses. Some species also have flagella (singular, flagellum) used for locomotion, and pili (singular, pilus) used for attachment to surfaces including the surfaces of other cells. Plasmids, which consist of extra-chromosomal DNA, are also present in many species of bacteria and archaea.

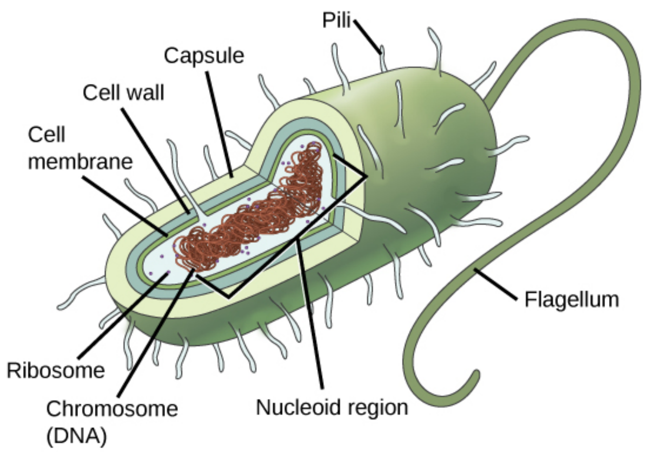


Figure 22.10 The features of a typical prokaryotic cell. Flagella, capsules, and pili are not found in all prokaryotes.

Recall that prokaryotes are divided into two different domains, Bacteria and Archaea, which together with Eukarya, comprise the three domains of life (**Figure 22.11**).

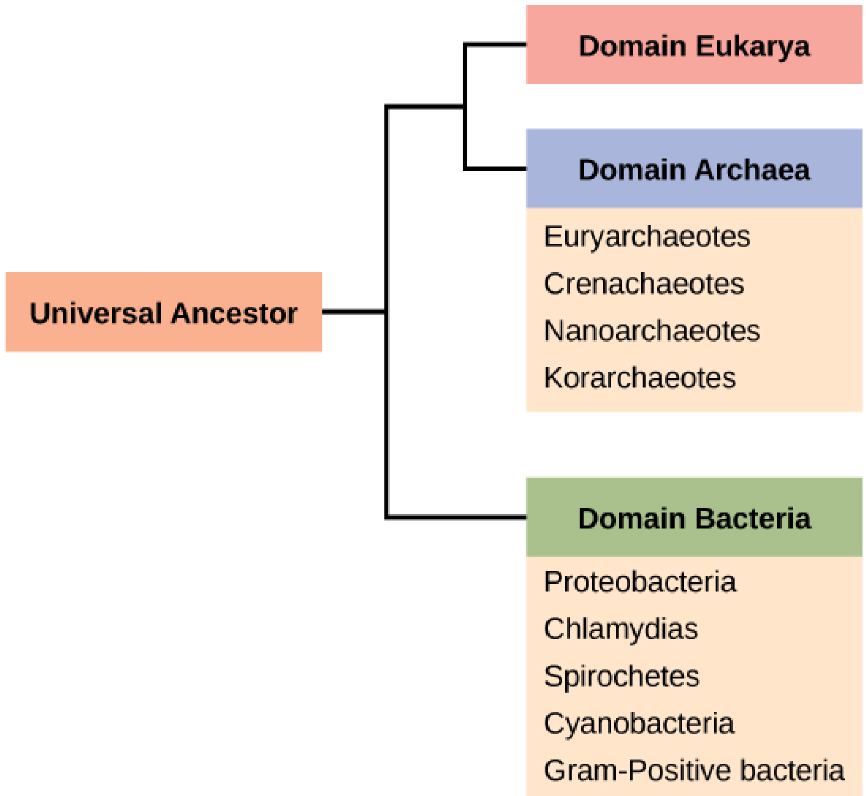


Figure 22.11 The three domains of living organisms. Bacteria and Archaea are both prokaryotes but differ enough to be placed in separate domains. An ancestor of modern Archaea is believed to have given rise to Eukarya, the third domain of life. Major groups of Archaea and Bacteria are shown.

Characteristics of bacterial phyla are described in **Figure 22.12** and **Figure 22.13**. Major bacterial phyla include the Proteobacteria, the Chlamydias, the Spirochaetes, the photosynthetic Cyanobacteria, and the Gram-positive bacteria. The Proteobacteria are in turn subdivided into several classes, from the Alpha- to the Epsilon proteobacteria. Eukaryotic mitochondria are thought to be the descendants of alphaproteobacteria, while eukaryotic chloroplasts are derived from cyanobacteria. Archaeal phyla are described in **Figure 22.14**.

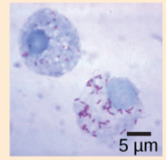
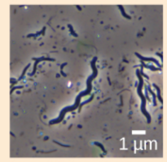
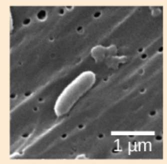
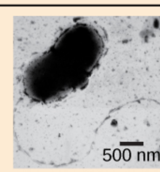
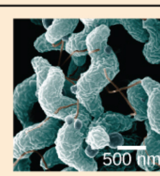
Bacteria of Phylum Proteobacteria		
Class	**Representative organisms**	**Representative micrograph**
Alpha Proteobacteria Some species are photoautotrophic but some are symbionts of plants and animals and others are pathogens. Eukaryotic mitochondria are thought be derived from bacteria in this group.	*Rhizobium* Nitrogen-fixing endosymbiont associated with the roots of legumes *Rickettsia* Obligate intracellular parasite that causes typhus and Rocky Mountain Spotted Fever (but not rickets, which is caused by Vitamin C deficiency)	 *Rickettsia rickettsia,* stained red, grow inside a host cell.
Beta Proteobacteria This group of bacteria is diverse. Some species play an important role in the nitrogen cycle.	*Nitrosomas* Species from this group oxidize ammonia into nitrite. *Spirillum minus* Causes rat-bite fever	 *Spirillum minus*
Gamma Proteobacteria Many are beneficial symbionts that populate the human gut, but others are familiar human pathogens. Some species from this subgroup oxidize sulfur compounds.	*Escherichia coli* Normally beneficial microbe of the human gut, but some strains cause disease *Salmonella* Certain strains cause food poisoning or typhoid fever *Yersinia pestis* Causative agent of Bubonic plague *Psuedomonas aeruginosa* Causes lung infections *Vibrio cholera* Causative agent of cholera *Chromatium* Sulfur-producing bacteria that oxidize sulfur, producing H_2S	 *Vibrio cholera*
Delta Proteobacteria Some species generate a spore-forming fruiting body in adverse conditions. Others reduce sulfate and sulfur.	*Myxobacteria* Generate spore-forming fruiting bodies in adverse conditions *Desulfovibrio vulgaris* Aneorobic, sulfate-reducing bacterium	 *Desulfovibrio vulgaris*
Epsilon Proteobacteria Many species inhabit the digestive tract of animals as symbionts or pathogens. Bacteria from this group have been found in deep-sea hydrothermal vents and cold seep habitats.	*Campylobacter* Causes blood poisoning and intestinal inflammation *Heliobacter pylori* Causes stomach ulcers	 *Campylobacter*

Figure 22.12 The Proteobacteria. Phylum *Proteobacteria* is one of up to 52 bacteria phyla. *Proteobacteria* is further subdivided into five classes, Alpha through Epsilon. (credit "Rickettsia rickettsia": modification of work by CDC; credit "Spirillum minus": modification of work by Wolframm Adlassnig; credit "Vibrio cholera": modification of work by Janice Haney Carr, CDC; credit "Desulfovibrio vulgaris": modification of work by Graham Bradley; credit "Campylobacter": modification of work by De Wood, Pooley, USDA, ARS, EMU; scale-bar data from Matt Russell)

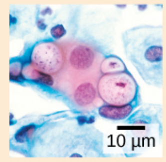
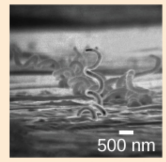
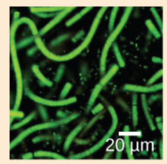
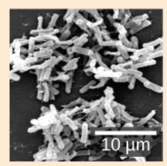
Bacteria: Chlamydia, Spirochaetae, Cyanobacteria, and Gram-positive		
Phylum	**Representative organisms**	**Representative micrograph**
Chlamydias All members of this group are obligate intracellular parasites of animal cells. Cells walls lack peptidoglycan.	*Chlamydia trachomatis* Common sexually transmitted disease that can lead to blindness	 In this pap smear, *Chlamydia trachomatis* appear as pink inclusions inside cells.
Spirochetes Most members of this species, which has spiral-shaped cells, are free-living aneaerobes, but some are pathogenic. Flagella run lengthwise in the periplasmic space between the inner and outer membrane.	*Treponema pallidum* Causative agent of syphilis *Borrelia burgdorferi* Causative agent of Lyme disease	 *Treponema pallidum*
Cyanobacteria Also known as blue-green algae, these bacteria obtain their energy through photosynthesis. They are ubiquitous, found in terrestrial, marine, and freshwater environments. Eukaryotic chloroplasts are thought be derived from bacteria in this group.	*Prochlorococcus* Believed to be the most abundant photosynthetic organism on earth; responsible for generating half the world's oxygen	 *Phormidium*
Gram-positive Bacteria Soil-dwelling members of this subgroup decompose organic matter. Some species cause disease. They have a thick cell wall and lack an outer membrane.	*Bacillus anthracis* Causes anthrax *Clostridium botulinum* Causes Botulism *Clostridium difficile* Causes diarrhea during antibiotic therapy *Streptomyces* Many antibiotics, including streptomyocin, are derived from these bacteria. *Mycoplasmas* These tiny bacteria, the smallest known, lack a cell wall. Some are free-living, and some are pathogenic.	 *Clostridium difficile*

Figure 22.13 Other bacterial phyla. Chlamydia, Spirochetes, Cyanobacteria, and Gram-positive bacteria are described in this table. Note that bacterial shape is not phylum-dependent; bacteria within a phylum may be cocci, rod-shaped, or spiral. (credit *"Chlamydia trachomatis"*: modification of work by Dr. Lance Liotta Laboratory, NCI; credit *"Treponema pallidum"*: modification of work by Dr. David Cox, CDC; credit *"Phormidium"*: modification of work by USGS; credit *"Clostridium difficile"*: modification of work by Lois S. Wiggs, CDC; scale-bar data from Matt Russell)

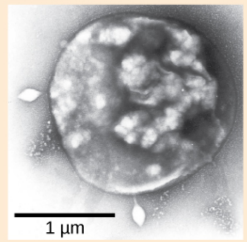
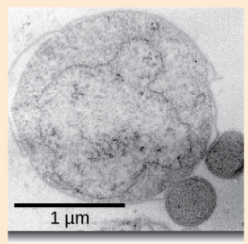
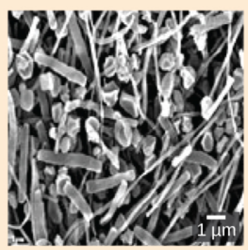
Archaea		
Phylum	**Representative organisms**	**Representative micrograph**
Euryarchaeota This phylum includes methanogens, which produce methane as a metabolic waste product, and halobacteria, which live in an extreme saline environment.	*Methanogens* Methane production causes flatulence in humans and other animals. *Halobacteria* Large blooms of this salt-loving archaea appear reddish due to the presence of bacterirhodopsin in the membrane. Bacteriorhodopsin is related to the retinal pigment rhodopsin.	 *Halobacterium* strain NRC-1
Crenarchaeota Members of the ubiquitous phylum play an important role in the fixation of carbon. Many members of this group are sulfur-dependent extremophiles. Some are thermophilic or hyperthermophilic.	*Sulfolobus* Members of this genus grow in volcanic springs at temperatures between 75° and 80°C and at a pH between 2 and 3.	 *Sulfolobus* being infected by bacteriophage
Nanoarchaeota This group currently contains only one species, *Nanoarchaeum equitans*.	*Nanoarchaeum equitans* This species was isolated from the bottom of the Atlantic Ocean and from a hydrothermal vent at Yellowstone National Park. It is an obligate symbiont with *Ignicoccus*, another species of archaea.	 *Nanoarchaeum equitans* (small dark spheres) are in contact with their larger host, *Ignicoccus*.
Korarchaeota Members of this phylum, considered to be one of the most primitive forms of life, have only been found in the Obsidian Pool, a hot spring at Yellowstone National Park.	No members of this species have been cultivated.	 This image shows a variety of korarchaeota species from the Obsidian Pool at Yellowstone National Park.

Figure 22.14 Archaeal phyla. Archaea are separated into four phyla: the Korarchaeota, Euryarchaeota, Crenarchaeota, and Nanoarchaeota. (credit *"Halobacterium"*: modification of work by NASA; credit *"Nanoarchaeotum equitans"*: modification of work by Karl O. Stetter; credit "Korarchaeota": modification of work by Office of Science of the U.S. Dept. of Energy; scale-bar data from Matt Russell)

The Plasma Membrane of Prokaryotes

The prokaryotic plasma membrane is a thin lipid bilayer (6 to 8 nanometers) that completely surrounds the cell and separates the inside from the outside. Its selectively permeable nature keeps ions, proteins, and other molecules within the cell and prevents them from diffusing into the extracellular environment, while other molecules may move through the membrane. Recall that the general structure of a cell membrane is a phospholipid bilayer composed of two layers of lipid molecules. In archaeal cell membranes, *isoprene (phytanyl) chains* linked to glycerol replace the fatty acids linked to glycerol in bacterial membranes. Some archaeal membranes are lipid monolayers instead of bilayers (**Figure 22.15**).

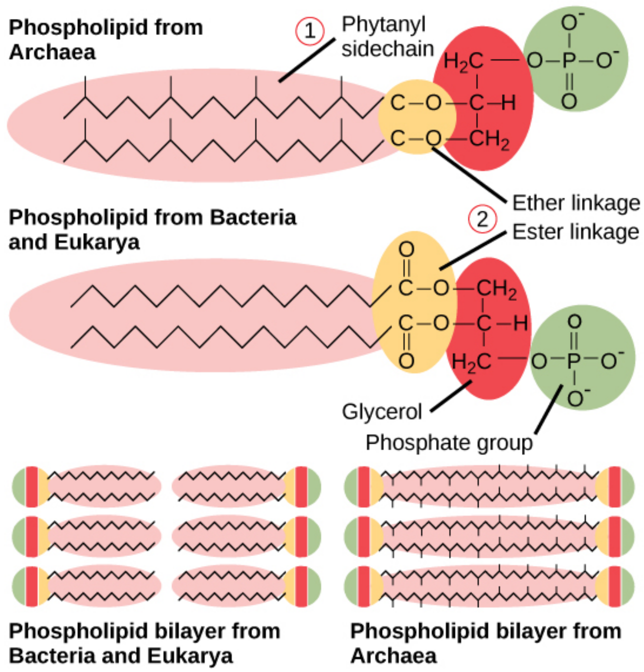


Figure 22.15 Bacterial and archaeal phospholipids. Archaeal phospholipids differ from those found in Bacteria and Eukarya in two ways. First, they have branched phytanyl sidechains instead of linear ones. Second, an ether bond instead of an ester bond connects the lipid to the glycerol.

The Cell Wall of Prokaryotes

The cytoplasm of prokaryotic cells has a high concentration of dissolved solutes. Therefore, the osmotic pressure within the cell is relatively high. The cell wall is a protective layer that surrounds some cells and gives them shape and rigidity. It is located outside the cell membrane and prevents *osmotic lysis* (bursting due to increasing volume). The chemical composition of the cell wall varies between Archaea and Bacteria, and also varies between bacterial species.

Bacterial cell walls contain **peptidoglycan**, composed of polysaccharide chains that are cross-linked by unusual peptides containing both L- and D-amino acids including D-glutamic acid and D-alanine. (Proteins normally have only L-amino acids; as a consequence, many of our antibiotics work by mimicking D-amino acids and therefore have specific effects on bacterial cell-wall development.) There are more than 100 different forms of peptidoglycan. *S-layer (surface layer) proteins* are also present on the outside of cell walls of both Archaea and Bacteria.

Bacteria are divided into two major groups: **Gram positive** and **Gram negative**, based on their reaction to Gram staining. Note that all Gram-positive bacteria belong to one phylum; bacteria in the other phyla (Proteobacteria, Chlamydias, Spirochetes, Cyanobacteria, and others) are Gram-negative. The Gram staining method is named after its inventor, Danish scientist Hans Christian Gram (1853–1938). The different bacterial responses to the staining procedure are ultimately due to cell wall structure. *Gram-positive organisms typically lack the outer membrane found in Gram-negative organisms* (**Figure 22.16**). Up to 90 percent of the cell-wall in Gram-positive bacteria is composed of peptidoglycan, and most of the rest is composed of acidic substances called *teichoic acids*. Teichoic acids may be covalently linked to lipids in the plasma membrane to form *lipoteichoic acids*. Lipoteichoic acids anchor the cell wall to the cell membrane. Gram-negative bacteria have a relatively thin cell wall composed of a few layers of peptidoglycan (only 10 percent of the total cell wall), surrounded by an outer envelope containing lipopolysaccharides (LPS) and lipoproteins. This outer envelope is sometimes referred to as a second lipid bilayer. The chemistry of this outer envelope is very different, however, from that of the typical lipid bilayer that forms plasma membranes.

visual CONNECTION

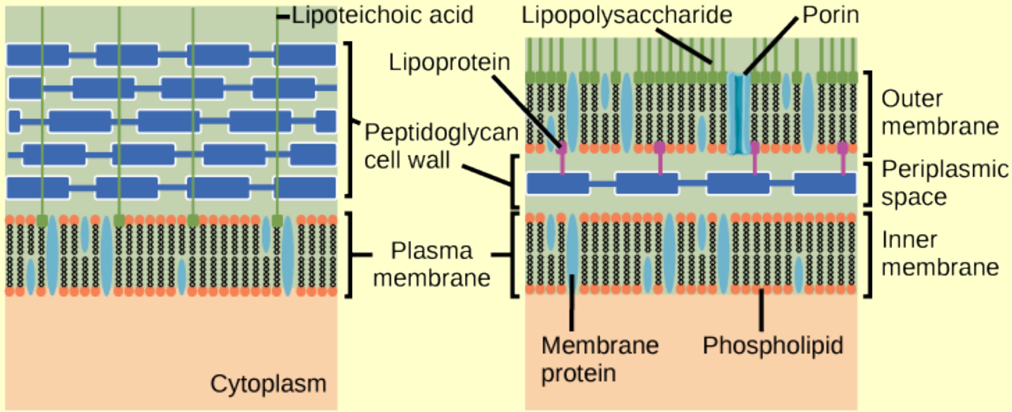


Figure 22.16 Cell walls in Gram-positive and Gram-negative bacteria. Bacteria are divided into two major groups: Gram positive and Gram negative. Both groups have a cell wall composed of peptidoglycan: in Gram-positive bacteria, the wall is thick, whereas in Gram-negative bacteria, the wall is thin. In Gram-negative bacteria, the cell wall is surrounded by an outer membrane that contains lipopolysaccharides and lipoproteins. Porins are proteins in this cell membrane that allow substances to pass through the outer membrane of Gram-negative bacteria. In Gram-positive bacteria, lipoteichoic acid anchors the cell wall to the cell membrane. (credit: modification of work by "Franciscosp2"/Wikimedia Commons)

Which of the following statements is true?

a. Gram-positive bacteria have a single cell wall anchored to the cell membrane by lipoteichoic acid.
b. Porins allow entry of substances into both Gram-positive and Gram-negative bacteria.
c. The cell wall of Gram-negative bacteria is thick, and the cell wall of Gram-positive bacteria is thin.
d. Gram-negative bacteria have a cell wall made of peptidoglycan, whereas Gram-positive bacteria have a cell wall made of lipoteichoic acid.

Archaean cell walls do not have peptidoglycan. There are four different types of archaean cell walls. One type is composed of **pseudopeptidoglycan**, which is similar to peptidoglycan in morphology but contains different sugars in the polysaccharide chain. The other three types of cell walls are composed of polysaccharides, glycoproteins, or pure protein. Other differences between Bacteria and Archaea are seen in Table 22.2. Note that features related to DNA replication, transcription and translation in Archaea are similar to those seen in eukaryotes.

Differences and Similarities between Bacteria and Archaea

Structural Characteristic	Bacteria	Archaea
Cell type	Prokaryotic	Prokaryotic
Cell morphology	Variable	Variable
Cell wall	Contains peptidoglycan	Does not contain peptidoglycan
Cell membrane type	Lipid bilayer	Lipid bilayer or lipid monolayer
Plasma membrane lipids	Fatty acids-glycerol ester	Phytanyl-glycerol ethers
Chromosome	Typically circular	Typically circular
Replication origins	Single	Multiple

Table 22.2

Differences and Similarities between Bacteria and Archaea

Structural Characteristic	Bacteria	Archaea
RNA polymerase	Single	Multiple
Initiator tRNA	Formyl-methionine	Methionine
Streptomycin inhibition	Sensitive	Resistant
Calvin cycle	Yes	No

Table 22.2

Reproduction

Reproduction in prokaryotes is *asexual* and usually takes place by binary fission. (Recall that the DNA of a prokaryote is a single, circular chromosome.) Prokaryotes do not undergo mitosis; instead, the chromosome is replicated and the two resulting copies separate from one another, due to the growth of the cell. The prokaryote, now enlarged, is pinched inward at its equator and the two resulting cells, which are *clones*, separate. Binary fission does not provide an opportunity for genetic recombination or genetic diversity, but prokaryotes can share genes by three other mechanisms.

In **transformation**, the prokaryote takes in DNA shed by other prokaryotes into its environment. If a nonpathogenic bacterium takes up DNA for a toxin gene from a pathogen and incorporates the new DNA into its own chromosome, it too may become pathogenic. In **transduction**, bacteriophages, the viruses that infect bacteria, may move short pieces of chromosomal DNA from one bacterium to another. Transduction results in a *recombinant organism*. Archaea also have viruses that may translocate genetic material from one individual to another. In **conjugation**, DNA is transferred from one prokaryote to another by means of a *pilus*, which brings the organisms into contact with one another, and provides a channel for transfer of DNA. The DNA transferred can be in the form of a plasmid or as a composite molecule, containing both plasmid and chromosomal DNA. These three processes of DNA exchange are shown in Figure 22.17.

Reproduction can be very rapid: a few minutes for some species. This short generation time coupled with mechanisms of genetic recombination and high rates of mutation result in the rapid evolution of prokaryotes, allowing them to respond to environmental changes (such as the introduction of an antibiotic) very quickly.

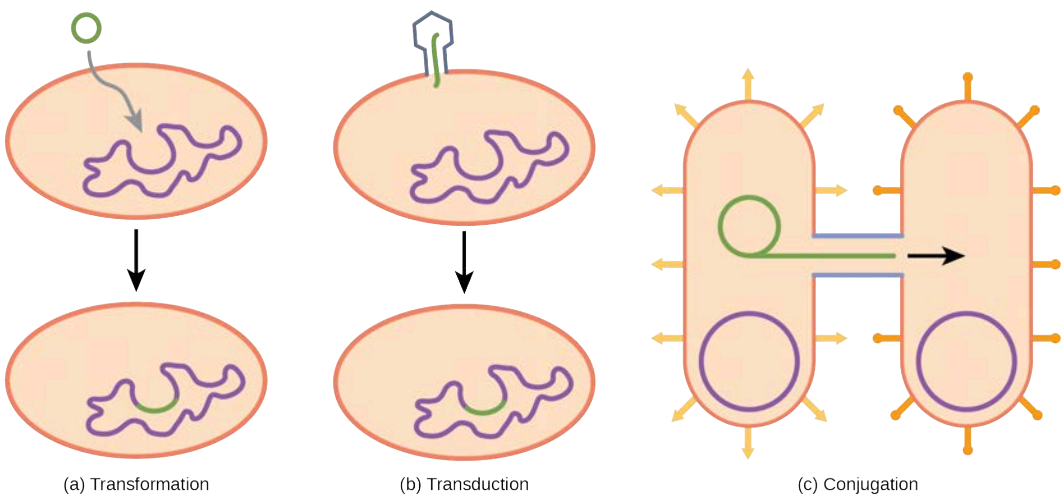


Figure 22.17 Gene transfer mechanisms in prokaryotes. There are three mechanisms by which prokaryotes can exchange DNA. In (a) transformation, the cell takes up prokaryotic DNA directly from the environment. The DNA may remain separate as plasmid DNA or be incorporated into the host genome. In (b) transduction, a bacteriophage injects DNA into the cell that contains a small fragment of DNA from a different prokaryote. In (c) conjugation, DNA is transferred from one cell to another via a mating bridge, or pilus, that connects the two cells after the sex pilus draws the two bacteria close enough to form the bridge.

evolution CONNECTION

The Evolution of Prokaryotes

How do scientists answer questions about the evolution of prokaryotes? Unlike with animals, artifacts in the fossil record of prokaryotes offer very little information. Fossils of ancient prokaryotes look like tiny bubbles in rock. Some scientists turn to genetics and to the principle of the molecular clock, which holds that the more recently two species have diverged, the more similar their genes (and thus proteins) will be. Conversely, species that diverged long ago will have more genes that are dissimilar.

Scientists at the NASA Astrobiology Institute and at the European Molecular Biology Laboratory collaborated to analyze the molecular evolution of 32 specific proteins common to 72 species of prokaryotes.[2] The model they derived from their data indicates that three important groups of bacteria—Actinobacteria, *Deinococcus*, and Cyanobacteria (collectively called *Terrabacteria* by the authors)—were the first to colonize land. Actinobacteria are a group of very common Gram-positive bacteria that produce branched structures like fungal mycelia, and include species important in decomposition of organic wastes. You will recall that *Deinococcus* is a genus of bacterium that is highly resistant to ionizing radiation. It has a thick peptidoglycan layer in addition to a second external membrane, so it has features of both Gram-positive and Gram-negative bacteria.

Cyanobacteria are photosynthesizers, and were probably responsible for the production of oxygen on the ancient earth. The timelines of divergence suggest that bacteria (members of the domain Bacteria) diverged from common ancestral species between 2.5 and 3.2 billion years ago, whereas the Archaea diverged earlier: between 3.1 and 4.1 billion years ago. Eukarya later diverged from the archaean line. The work further suggests that stromatolites that formed prior to the advent of cyanobacteria (about 2.6 billion years ago) photosynthesized in an anoxic environment and that because of the modifications of the Terrabacteria for land (resistance to drying and the possession of compounds that protect the organism from excess light), photosynthesis using oxygen may be closely linked to adaptations to survive on land.

22.3 | Prokaryotic Metabolism

By the end of this section, you will be able to do the following:

- Identify the macronutrients needed by prokaryotes, and explain their importance
- Describe the ways in which prokaryotes get energy and carbon for life processes
- Describe the roles of prokaryotes in the carbon and nitrogen cycles

Prokaryotes are metabolically diverse organisms. In many cases, a prokaryote may be placed into a species clade by its defining metabolic features: Can it metabolize lactose? Can it grow on citrate? Does it produce H_2S? Does it ferment carbohydrates to produce acid and gas? Can it grow under anaerobic conditions? Since metabolism and metabolites are the product of enzyme pathways, and enzymes are encoded in genes, the metabolic capabilities of a prokaryote are a reflection of its genome. There are many different environments on Earth with various energy and carbon sources, and variable conditions to which prokaryotes may be able to adapt. Prokaryotes have been able to live in every environment from deep-water volcanic vents to Antarctic ice by using whatever energy and carbon sources are available. Prokaryotes fill many niches on Earth, including involvement in nitrogen and carbon cycles, photosynthetic production of oxygen, decomposition of dead organisms, and thriving as parasitic, commensal, or mutualistic organisms inside multicellular organisms, including humans. The very broad range of environments that prokaryotes occupy is possible because they have diverse metabolic processes.

2. Battistuzzi, FU, Feijao, A, and Hedges, SB. A genomic timescale of prokaryote evolution: Insights into the origin of methanogenesis, phototrophy, and the colonization of land. *BioMed Central: Evolutionary Biology* 4 (2004): 44, doi:10.1186/1471-2148-4-44.