Life on a strange planet

It must have been quite a shock when Thomas “Grif” Taylor’s Antarctic exploration team first spotted Blood Falls in 1911. The blood-red falls were certainly a surprise in the snowy, icy terrain. What could possibly cause a red waterfall in Antarctica?

A few million years ago, the Taylor Glacier (which now bears the explorer’s name) moved above a pool of salty water, trapping the pool under 400 meters of ice. The harsh environment in the resulting enclosed subglacial sea could hardly seem more hostile to life. It is extremely cold; there is no light and virtually no oxygen; and salt concentrations are several times higher than seawater. In short, it is not a place one might expect to find a diverse living ecosystem.

Some water is able to seep out of this subglacial sea. This water is stained a dark, rusty red, and it spills from the head of Taylor Glacier to form Blood Falls. Taylor speculated that red algae might account for the red coloration, but in the 1960s geologist Robert Black discovered that the water’s color arises from iron oxides that come from the underlying bedrock. With the methods then available, biologists could not detect any living organisms in the cold, saline, iron-rich water.

A half-century later, biologists were better equipped to study microscopic life in strange places. By then it was also possible to amplify and sequence genes from single microbes, and to place these gene sequences into the framework of the tree of life to identify and classify the microbes. Microbiologist Jill Mikucki and her colleagues used these techniques on water samples from Blood Falls, and reported in 2009 that the falls contain an unusual ecosystem of at least 17 different species of bacteria. The bacteria survive by metabolizing minute amounts of organic matter trapped in the subglacial sea, using sulfate and iron ions as catalysts and electron acceptors.

The presence of living organisms in Blood Falls confirms that it is hard to find a place on or even near the surface of the Earth that does not contain populations of prokaryotes. There are prokaryotes in volcanic vents, in the clouds, in environments as acidic as battery acid or as alkaline as household ammonia. There are species that can survive below the freezing point and above the boiling point.

A Splash of Color in a Frozen World of White

Antarctica’s Blood Falls is the outflow of a subglacial sea that contains an unusual ecosystem of bacteria that rely on sulfate and iron ions for metabolism.
How Did the Living World Begin to Diversify?

You may think that you have little in common with unicellular prokaryotes. But multicellular eukaryotes like yourself actually share many attributes with Bacteria and Archaea. For example, all three of you:

- conduct glycolysis
- use DNA as the genetic material that encodes proteins
- produce those proteins by transcription and translation using a similar genetic code
- replicate DNA semiconservatively
- have plasma membranes and ribosomes in abundance

These features support the conclusion that all living organisms are related to one another. If life had multiple origins, there would be little reason to expect all organisms to use overwhelmingly similar genetic codes or to share structures as unique as ribosomes. Furthermore, similarities in DNA sequences of universal genes (such as those that encode the structural components of ribosomes) confirm the monophyly of life.

Despite the commonalities found across all three domains, major differences have evolved as well. Let’s first distinguish between Eukarya and the two prokaryotic domains. Note that “domain” is a subjective term used for the largest groups of life. There is no objective definition of a domain, any more than there is of a kingdom or a family.

The three domains differ in significant ways

Prokaryotes are by far the most numerous organisms on Earth. Late in the twentieth century, it became apparent to microbiologists that all prokaryotes are not most closely related to one another. Two prokaryotic lineages diverged early in life’s evolution: Bacteria and Archaea. An early merging between members of these two groups is thought to have given rise to the eukaryotic lineage, which includes humans.

Prokaryotes Can Take the Heat

Entire ecosystems of prokaryotes create the beauty of Morning Glory Pool, a hot spring in Yellowstone National Park. Cyanobacteria impart the “morning glory blue” color. Archaea live in the intensely hot regions of the pool’s interior.

of water. There are more prokaryotes living on and inside our bodies than we have human cells. The prokaryotes are masters of metabolic ingenuity, having developed more ways to obtain energy from the environment than the eukaryotes have. They have been around much longer than other organisms, too.

Prokaryotes are by far the most numerous organisms on Earth. Late in the twentieth century, it became apparent to microbiologists that all prokaryotes are not most closely related to one another. Two prokaryotic lineages diverged early in life’s evolution: Bacteria and Archaea. An early merging between members of these two groups is thought to have given rise to the eukaryotic lineage, which includes humans.

IN THIS CHAPTER we will discuss the distribution of prokaryotes and examine their remarkable metabolic diversity. We will describe the difficulties involved in determining evolutionary relationships among the prokaryotes and will survey the surprising diversity of organisms in each domain. We will discuss how prokaryotes can have enormous influence on their environments. Finally, we will discuss the evolutionary origin and diversity of viruses and their relationship to the rest of life.
Prokaryotes have none of the membrane-enclosed cytoplasmic organelles—mitochondria, Golgi apparatus, and others—that are found in most eukaryotes. However, the cytoplasm of a prokaryotic cell may contain a variety of infoldings of the plasma membrane and photosynthetic membrane systems not found in eukaryotes.

A glance at Table 26.1 will show you that there are also major differences (most of which cannot be seen even under an electron microscope) between the two prokaryotic domains. In some ways archaea are more like eukaryotes; in other ways they are more like bacteria. (Note that we use lowercase when referring to the members of these domains and uppercase when referring to the domains themselves.) The structures of prokaryotic and eukaryotic cells are compared in Chapter 5. The basic unit of an archaeon (the term for a single archaeal organism) or bacterium (a single bacterial organism) is the prokaryotic cell. Each single-celled organism contains a full complement of genetic and protein-synthesizing systems, including DNA, RNA, and all the enzymes needed to transcribe and translate the genetic information into proteins. The prokaryotic cell also contains at least one system for generating the ATP it needs.

Genetic studies clearly indicate that all three domains had a single common ancestor. For a major portion of their genome, eukaryotes share a more recent common ancestor with Archaea than they do with Bacteria (Figure 26.1). However, the mitochondria of eukaryotes (as well as the chloroplasts of photosynthetic eukaryotes, such as plants) originated through the endosymbiosis of a bacterium, as described in Section 5.5. Some biologists prefer to view the origin of eukaryotes as a fusion of two equal partners (one ancestor that was related to modern archaea, and another that was more closely related to modern bacteria). Others view the divergence of the early eukaryotes from the archaea as a separate and earlier event than the later endosymbiosis of the bacterium (the origin of mitochondria). In either case, some genes of eukaryotes are more closely related to those of archaea, while others are more closely related to those of bacteria. The tree of life therefore contains some merging of lineages, as well as the predominant diverging of lineages.

The common ancestor of all three domains had DNA as its genetic material, and its machinery for transcription and translation produced RNAs and proteins, respectively. This ancestor probably had a circular chromosome.

Three shapes are particularly common among the bacteria: spheres, rods, and curved or helical forms (Figure 26.2). A spherical bacterium is called a *coccus* (plural *cocci*). Cocci may live singly or may associate in two- or three-dimensional arrays.

### Table 26.1

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>BACTERIA</th>
<th>ARCHAEA</th>
<th>EUKARYA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane-enclosed nucleus</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Membrane-enclosed organelles</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Peptidoglycan in cell wall</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Membrane lipids</td>
<td>Ester-linked</td>
<td>Ester-linked</td>
<td>Ester-linked</td>
</tr>
<tr>
<td>Ribosomes(^a)</td>
<td>70S</td>
<td>70S</td>
<td>80S</td>
</tr>
<tr>
<td>Initiator tRNA</td>
<td>Formylmethionine</td>
<td>Methionine</td>
<td>Methionine</td>
</tr>
<tr>
<td>Operons</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Plasmids</td>
<td>Yes</td>
<td>Yes</td>
<td>Rare</td>
</tr>
<tr>
<td>RNA polymerases</td>
<td>One</td>
<td>One(^b)</td>
<td>Three</td>
</tr>
<tr>
<td>Ribosomes sensitive to chloramphenicol and streptomycin</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ribosomes sensitive to diphtheria toxin</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

\(^a\)70S ribosomes are smaller than 80S ribosomes.

\(^b\)Archaeal RNA polymerase is similar to eukaryotic polymerases.

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*Prokaryotes have none of the membrane-enclosed cytoplasmic organelles—mitochondria, Golgi apparatus, and others—that are found in most eukaryotes. However, the cytoplasm of a prokaryotic cell may contain a variety of infoldings of the plasma membrane and photosynthetic membrane systems not found in eukaryotes.*
The prokaryotes were alone on Earth for a very long time, adapting to new environments and to changes in existing environments. They have survived to this day, in massive numbers and incredible diversity, and they are found everywhere.
Earth (see Figure 25.4). Stromatolites still form today in some parts of the world.

Biofilms are the subject of much current research. For example, some biologists are studying the chemical signals that bacteria in biofilms use to communicate with one another. By blocking the signals that lead to the production of the matrix polysaccharides, researchers may be able to prevent biofilms from forming.

A team of bioengineers and chemical engineers recently devised a sophisticated technique that enables them to monitor biofilm development in extremely small populations of bacteria, cell by cell. They developed a tiny chip housing six separate growth chambers, or “microchemostats” (Figure 26.4). The techniques of microfluidics use microscopic tubes and computer-controlled valves to direct fluid flow through complex “plumbing circuits” in the growth chambers.

26.4 The Microchemostat

Using techniques from microfluidic engineering, biologists can monitor the dynamics of extremely small bacterial populations. The photograph shows six microchemostats on a chip. Each of the six is equipped with input ports for growth and flushing media, and a number of output ports (diagram). Tiny valves, controlled by a computer, direct flow. Samples are removed through the output ports and are analyzed to record changes in the bacterial population.

In continuous circulation mode, medium containing cells is pumped around the growth chamber loop (green) as the cells multiply.

Valves can be adjusted to admit fresh growth medium and collect cells at an output port.
Prokaryotes have distinctive cell walls

Many prokaryotes have a thick and relatively stiff cell wall. It is quite different from the cell walls of land plants and algae, which contain cellulose and other polysaccharides, and from those of fungi, which contain chitin. The cell walls of almost all bacteria contain peptidoglycan (a cross-linked polymer of amino sugars), which produces a meshlike structure around the cell. Archaeal cell walls are of differing types, but most contain significant amounts of protein. One group of archaea has pseudopeptidoglycan in its cell wall; as you can probably guess from the prefix pseudo, pseudopeptidoglycan is similar to, but distinctly different from, the peptidoglycan of bacteria. The monomers making up pseudopeptidoglycan differ from and are differently linked than those of peptidoglycan. Peptidoglycan is a substance unique to bacteria; its absence from the walls of archaea is a key difference between the two prokaryotic domains.

To appreciate the complexity of some bacterial cell walls, consider the reactions of bacteria to a simple staining process. A test called the Gram stain separates most types of bacteria into two distinct groups, Gram-positive and Gram-negative. A smear of cells on a microscope slide is soaked in a violet dye and treated with iodine; it is then washed with alcohol and counterstained with a red dye (safranine). Gram-positive bacteria retain the violet dye and appear blue to purple (Figure 26.5A). The alcohol washes the violet stain out of Gram-negative cells; these cells then pick up the safranine counterstain, so Gram-negative bacteria appear pink to red (Figure 26.5B).

For most bacteria, the Gram-staining results are determined by the chemical structure of the cell wall. A Gram-negative cell wall usually has a thin peptidoglycan layer, and outside the peptidoglycan layer the cell is surrounded by a second, outer membrane quite distinct in chemical makeup from the plasma membrane (see Figure 26.5B). Between the inner (plasma) and outer membranes of Gram-negative bacteria is a periplasmic space. This space contains proteins that are important in digesting some materials, transporting others, and detecting chemical gradients in the environment.

A Gram-positive cell wall usually has about five times as much peptidoglycan as a Gram-negative wall. This thick peptidoglycan layer is a meshwork that may serve some of the same purposes as the periplasmic space of the Gram-negative cell wall.

The consequences of the different features of prokaryotic cell walls are numerous and relate to the disease-causing characteristics of some bacteria. Indeed, the cell wall is a favorite target in medical combat against pathogenic bacteria because it has no counterpart in eukaryotic cells. Antibiotics such as penicillin and ampicillin, as well as other agents that specifically interfere with the synthesis of peptidoglycan-containing cell walls, tend to have little, if any, effect on the cells of humans and other eukaryotes.

Prokaryotes have distinctive modes of locomotion

Although many prokaryotes cannot move, others are motile. These organisms move by one of several means. Some helical bacteria, called spirochetes, use a corkscrew-like motion made possible by modified flagella, called axial filaments, running along the axis of the cell beneath the outer membrane (Figure 26.6A). Many cyanobacteria and a few other groups of bacteria use various poorly understood gliding mechanisms, including rolling. Various aquatic prokaryotes, including some cyanobacteria, can move slowly up and down in the water by adjusting the amount of gas in gas vesicles (Figure 26.6B). By far the most common type of locomotion in prokaryotes, however, is that driven by flagella.

Prokaryotic flagella are slender filaments that extend singly or in tufts from one or both ends of the cell or are distributed all around it (Figure 26.7). A prokaryotic flagellum consists of a single fibril made of the protein fla-
gellin, projecting from the cell surface, plus a hook and basal body responsible for motion (see Figure 5.5). In contrast, the flagellum of eukaryotes is enclosed by the plasma membrane and usually contains a circle of nine pairs of microtubules surrounding two central microtubules, all containing the protein tubulin, along with many other associated proteins. The prokaryotic flagellum rotates about its base, much like a propeller, rather than beating in a whiplike manner, as a eukaryotic flagellum or cilium does.

Prokaryotes reproduce asexually, but genetic recombination can occur

Prokaryotes reproduce by binary fission, an asexual process (see Figure 11.2). Recall, however, that there are also processes—transformation, conjugation, and transduction—that allow the exchange of genetic information between some prokaryotes without reproduction occurring. So prokaryotes can exchange and recombine their DNA with other individuals (this is sex in the genetic sense of the word), but this genetic exchange is not directly linked to reproduction as it is in most eukaryotes. If conditions are favorable, some prokaryotes can multiply very rapidly. The shortest known prokaryote generation times are about 10 minutes, although these rapid rates of replication usually are not maintained for long. Under less optimal conditions, generation times often extend to many hours or even several days. Bacteria living deep in Earth’s crust may suspend their growth for more than a century without dividing, then multiply for a few days before once again suspending growth.

Prokaryotes can communicate

Prokaryotes can send and receive signals from one another and from other organisms. One communication channel they employ is chemical. Another is physical, with light as the medium. Bacteria release chemical substances that are sensed by other bacteria of the same species. They can announce their availability for conjugation, for example, by means of such signals. They can also monitor the density of their population. As the density of bacteria in a particular region increases, the concentration of a chemical signal builds up. When the bacteria sense that their population has become sufficiently dense, they can commence activities that smaller densities could not manage, such as forming a biofilm (see Figure 26.3). This density-sensing technique is called quorum sensing.

Like fireflies and many other organisms, some bacteria can emit light by a process called bioluminescence. A complex, enzyme-catalyzed reaction requiring ATP causes the emission of light but not heat. Often such bacteria luminesce only when a quorum has been sensed. The bioluminescent spots present in some deep-sea fishes are produced by colonies of biolumines-
Prokaryotes have amazingly diverse metabolic pathways

Bacteria and archaea outdo the eukaryotes in terms of metabolic diversity. Although much more diverse in size and shape, eukaryotes draw on fewer metabolic mechanisms for their energy needs. In fact, much of the eukaryotes’ energy metabolism is carried out in organelles—mitochondria and chloro-

plasts—that are endosymbiotic descendants of bacteria, as described in Section 5.5.

The long evolutionary history of bacteria and archaea, during which they have had time to explore a wide variety of habitats, has led to the extraordinary diversity of their metabolic “lifestyles”—their use or nonuse of oxygen, their energy sources, their sources of carbon atoms, and the materials they release as waste products.

ANAEROBIC VERSUS AEROBIC METABOLISM Some prokaryotes can live only by anaerobic metabolism because molecular oxygen is poisonous to them. These oxygen-sensitive organisms are called obligate anaerobes. Other prokaryotes can shift their metabolism between anaerobic and aerobic modes (see Chapter 9) and thus are called facultative anaerobes. Many facultative anaerobes alternate between anaerobic metabolism (such as fermentation) and cellular respiration as conditions dictate. Aerotolerant anaerobes cannot conduct cellular respiration but are not damaged by oxygen when it is present. By definition, an anaerobe does not use oxygen as an electron acceptor for its respiration.

At the other extreme from the obligate anaerobes, some prokaryotes are obligate aerobes, unable to survive for extended periods in the absence of oxygen. They require oxygen for cellular respiration.

NUTRITIONAL CATEGORIES All living organisms face the same nutritional challenges: they must synthesize energy-rich compounds such as ATP to power their life-sustaining metabolic reactions, and they must obtain carbon atoms to build their own organic molecules. Biologists recognize four broad nutritional categories of organisms: photoautotrophs, photoheterotrophs, chemolithotrophs, and chemoheterotrophs. Prokaryotes are represented in all four groups (Table 26.2).

Photoautotrophs perform photosynthesis. They use light as their energy source and carbon dioxide (CO₂) as their carbon source. Like green plants and other photosynthetic eukaryotes, the cyanobacteria, a group of photoautotrophic bacteria, use chlorophyll a as their key photosynthetic pigment and produce

| TABLE 26.2 How Organisms Obtain Their Energy and Carbon |
|-------------------------------|---------------|-----------------|
| NUTRITIONAL CATEGORY | ENERGY SOURCE | CARBON SOURCE |
| Photoautotrophs (found in all three domains) | Light | Carbon dioxide |
| Photoheterotrophs (some bacteria) | Light | Organic compounds |
| Chemolithotrophs (some bacteria, many archaea) | Inorganic substances | Carbon dioxide |
| Chemoherotrophs (found in all three domains) | Organic compounds | Organic compounds |
oxygen gas (O₂) as a by-product of noncyclic electron transport (see Section 10.1).

There are other photosynthetic groups among the bacteria, but these use bacteriochlorophyll as their key photosynthetic pigment, and they do not release O₂. Indeed, some of these photosynthesizers produce particles of pure sulfur, because hydrogen sulfide (H₂S) rather than H₂O is their electron donor for photophosphorylation (see Section 10.2). Bacteriochlorophyll molecules absorb light of longer wavelengths than the chlorophyll molecules used by all other photosynthesizing organisms. As a result, bacteria using this pigment can grow in water under fairly dense layers of algae, using light of wavelengths that are not absorbed by the algae (Figure 26.9).

**Phototrophs** use light as their energy source but must obtain their carbon atoms from organic compounds made by other organisms. Their “food” consists of organic compounds such as carbohydrates, fatty acids, and alcohols. For example, compounds released from plant roots (as in rice paddies) or from decomposing photosynthetic bacteria in hot springs are taken up by phototrophs and metabolized to form building blocks for other compounds; sunlight provides the necessary ATP through photophosphorylation. The purple nonsulfur bacteria, among others, are phototrophs.

**Chemolithotrophs** (also called chemoautotrophs) obtain their energy by oxidizing inorganic substances, and they use some of that energy to fix CO₂. Some chemolithotrophs use reactions identical to those of the typical photosynthetic cycle, but others use alternative pathways to fix CO₂. Some bacteria oxidize ammonia or nitrite ions to form nitrate ions. Others oxidize hydrogen gas, hydrogen sulfide, sulfur, and other materials. Many archaea are chemolithotrophs.

Deep-sea hydrothermal vent ecosystems are dependent on chemolithotrophic prokaryotes that are incorporated into large communities of crabs, mollusks, and giant worms, all living at a depth of 2,500 meters—below any hint of sunlight. These bacteria obtain energy by oxidizing hydrogen sulfide and other substances released in the near-boiling water flowing from volcanic vents in the ocean floor.

Finally, **chemoheterotrophs** obtain both energy and carbon atoms from one or more complex organic compounds that have been synthesized by other organisms. Most known bacteria and archaea are chemoheterotrophs—as are all animals and fungi and many protists.

**NITROGEN AND SULFUR METABOLISM** Key metabolic reactions in many prokaryotes involve nitrogen or sulfur. For example, some bacteria carry out respiratory electron transport without using oxygen as an electron acceptor. These organisms use oxidized inorganic ions such as nitrate, nitrite, or sulfate as electron acceptors. Examples include the **denitrifiers**, bacteria that release nitrogen to the atmosphere as nitrogen gas (N₂). These normally aerobic bacteria, mostly species of the genera *Bacillus* and *Pseudomonas*, use nitrate (NO₃⁻) as an electron acceptor in place of oxygen if they are kept under anaerobic conditions:

$$2 \text{NO}_3^- + 10 e^- + 12 H^+ \rightarrow N_2 + 6 H_2O$$

**Nitrogen fixers** convert atmospheric nitrogen gas into a chemical form (ammonia) usable by the nitrogen fixers themselves as well as by other organisms, especially land plants:

$$N_2 + 6 H \rightarrow 2 NH_3$$

All organisms require nitrogen in order to build proteins, nucleic acids, and other important compounds. Nitrogen fixation is thus vital to life as we know it. This all-important biochemical process is carried out by a wide variety of archaea and bacteria (including cyanobacteria) but by no other organisms, so we depend on these prokaryotes for our very existence. We describe the details of nitrogen fixation in Chapter 36.

Ammonia is oxidized to nitrate in soil and in seawater by chemolithotrophic bacteria called **nitrifiers**. Bacteria of two genera, *Nitrosomonas* and *Nitrosococcus*, convert ammonia to nitrite ions (NO₂⁻), and *Nitrobacter* oxidizes nitrite to nitrate (NO₃⁻).

What do the nitrifiers get out of these reactions? Their metabolism is powered by the energy released by the oxidation of ammonia or nitrite. For example, by passing the electrons from nitrite through an electron transport chain (see Section 9.3), *Nitrobacter* can make ATP, and using some of this ATP, can also make NADH. With this ATP and NADH, the bacterium can convert CO₂ and H₂O to glucose.
26.3 How Can We Resolve Prokaryote Phylogeny?

As detailed in Chapter 22, classification schemes serve three primary purposes: to identify organisms, to reveal evolutionary relationships, and to provide universal names. Classifying bacteria and archaea is of particular importance to humans because scientists and medical technologists must be able to identify bacteria quickly and accurately; when the bacteria are pathogenic, lives may depend on it. In addition, many emerging biotechnologies (see Chapter 18) depend on a thorough knowledge of prokaryote biochemistry, and understanding an organism’s phylogeny allows biologists to make predictions about the distribution of biochemical processes across the wide diversity of prokaryotes.

The small size of prokaryotes has hindered our study of their phylogeny

Until about 300 years ago, nobody had even seen an individual prokaryote; these organisms remained invisible to humans until the invention of the first simple microscope. Prokaryotes are so small that even the best light microscopes don’t reveal much about them. It took the advanced microscopic equipment and techniques of the twentieth century (see Figure 5.3) to open up the microbial world.

Until recently, taxonomists based prokaryote classification on observable phenotypic characters such as shape, color, motility, nutritional requirements, antibiotic sensitivity, and reaction to the Gram stain. When biologists learned how to grow bacteria in pure culture on nutrient media, they learned a great deal about the genetics, nutrition, and metabolism of those species that could be cultured. However, these species represent probably less than 1 percent of living prokaryote species. Furthermore, this work provided little insight into how prokaryotic organisms evolved—a question of great interest to microbiologists and evolutionary biologists. Only recently have systematists developed the appropriate tools to produce classification schemes that make sense in evolutionary terms.

The nucleotide sequences of prokaryotes reveal their evolutionary relationships

Analyses of nucleotide sequences of ribosomal RNA (rRNA) genes provided the first comprehensive evidence of evolutionary relationships among prokaryotes. For several reasons, rRNA is particularly useful for evolutionary studies of living organisms:

- rRNA is evolutionarily ancient, as it was found in the common ancestor of life.
- No free-living organism lacks rRNA, so rRNA genes can be compared throughout the tree of life.
- rRNA plays a critical role in translation in all organisms, so lateral transfer of rRNA genes among distantly related species is unlikely.
- rRNA has evolved slowly enough that gene sequences can be aligned and analyzed among even distantly related species.

Comparisons of rRNA genes from a great many organisms have revealed the probable phylogenetic relationships from throughout the tree of life. Databases such as GenBank contain rRNA gene sequences from hundreds of thousands of species—more than any other gene sequences.

Although these data are helpful, it is clear that even distantly related prokaryotes sometimes exchange genetic material. In some groups of prokaryotes, analyses of multiple gene sequences have suggested several different phylogenetic patterns. How could such differences among different gene sequences arise?

Lateral gene transfer can lead to discordant gene trees

As noted earlier, prokaryotes reproduce by binary fission. If we could follow these divisions back through evolutionary time, we would be tracing the path of the complete tree of life for bacteria and archaea. This underlying tree of relationships, represented in highly abbreviated form in Appendix A, is called the organismal (or species) tree. Because whole genomes are replicated during asexual binary fission divisions, we expect phylogenetic trees constructed from most gene sequences to reflect these same relationships (see Chapter 22).

From early in evolution to the present day, however, some genes have been moving “sideways” from one prokaryotic species to another, a phenomenon known as lateral gene transfer. Mechanisms of lateral gene transfer include transfer by plasmids and viruses and uptake of DNA from the environment by transformation. Lateral gene transfers are well documented, especially among closely related species; some have been documented even across the three primary domains of life.
Consider, for example, the genome of *Thermotoga maritima*, a bacterium that can survive extremely high temperatures. In comparing the 1,869 gene sequences of *T. maritima* against sequences for the same proteins in other species, investigators found that some of this bacterium’s genes have their closest relationships not with those of other bacterial species, but with the genes of archaean species that live in similar environments.

When genes involved in lateral transfer events are sequenced and analyzed phylogenetically, the resulting individual gene trees will not match the organismal phylogeny in every respect (Figure 26.10). Individual gene trees will vary because the history of lateral gene transfer events is different for each gene. Biologists reconstruct the underlying organismal phylogeny by comparing multiple genes (to produce a consensus tree), or by concentrating on genes that are unlikely to be involved in lateral gene transfer events. For example, genes that are involved in fundamental cell processes (such as the rRNA genes discussed above) are unlikely to be replaced by the same genes from other species, since functional, locally adapted copies of these genes are already present.

What kinds of genes are most likely to be involved in lateral gene transfer? Genes that result in a new, adaptive function that will convey higher fitness to a recipient species are most likely to be transferred repeatedly among species. For example, genes that produce antibiotic resistance are often transferred on plasmids among many bacterial species, especially under the strong selective conditions of antibiotic medication by humans. This selection for antibiotic resistance is why informed physicians are now more careful in prescribing antibiotics. Improper or frequent use of antibiotics can lead to selection for resistant strains of bacteria, which are then much harder to treat effectively.

It is debatable whether lateral gene transfer has seriously complicated our attempts to resolve the tree of prokaryotic life. Recent work suggests that it has not—while it complicates studies in some individual species, it need not present problems at higher levels. It is now possible to make nucleotide sequence comparisons involving entire genomes, and these studies are revealing a stable core of crucial genes that are uncomplicated by lateral gene transfer. Gene trees based on this stable core more accurately reveal relationships of the organismal phylogeny (see Figure 26.10). The problem remains, however, that only a very small proportion of the prokaryotic world has been described and studied.

**The great majority of prokaryote species have never been studied**

Most prokaryotes have defied all attempts to grow them in pure culture, causing biologists to wonder how many species, and possibly even important clades, we might be missing. A window onto this problem was opened with the introduction of a new way to look at nucleic acid sequences. Unable to work with the whole genome of a single species, biologists instead examine sequences in individual genes collected from a random sample of the environment.

Norman Pace of the University of Colorado isolated individual rRNA gene sequences from extracts of environmental samples such as soil and seawater. Comparing such sequences with previously known ones revealed an extraordinary number of new sequences, implying that they came from previously unrecognized species. Biologists have described only about 10,000 species of bacteria and only a few hundred species of archaean (see Figure 1.10). The results of Pace’s and similar studies suggest that there may be millions, perhaps hundreds of millions, of prokaryote species on Earth. Other biologists put the estimate much lower, and argue that the high dispersal ability of many bacterial species greatly reduces local endemism (geographically restricted species). Only the magnitude of these estimates differ; however, all sides agree that we have just begun to uncover Earth’s bacterial and archaean diversity.
The study of prokaryote phylogeny and diversity has been inhibited by the organisms’ small size, our inability to grow some of them in pure culture, and lateral gene transfer. However, nucleotide sequences of essential genes are providing a much clearer picture of bacterial and archaeal evolutionary relationships.

- How did biologists classify bacteria before it became possible to determine nucleotide sequences? See p. 545
- Explain why nucleotide sequences of rRNA genes are useful for evolutionary studies. See p. 545
- How does lateral gene transfer complicate evolutionary studies? See p. 545–546 and Figure 26.10

With the advent of sequencing techniques, biologists have made rapid progress in understanding the phylogeny of prokaryotes. In the next section, we identify the characteristics and life history of the major groups.

### 26.4 What Are the Major Known Groups of Prokaryotes?

Here we use a widely accepted classification scheme that has considerable support from nucleotide sequence data. More than a dozen major clades have been proposed under this scheme, just a few of which we discuss here. We pay the closest attention to six groups that have received the most study: the spirochetes, chlamydias, high-GC Gram-positives, cyanobacteria, low-GC Gram-positives, and proteobacteria (Figure 26.11).

First, however, a few words about the origins of the prokaryotes are in order.

Several of the earliest branching lineages of bacteria and archaea are thermophiles (Greek, “heat-lovers”). This observation is in line with the hypothesis that the first living organisms were thermophiles, given that most environments on early Earth were much hotter than those of today. While additional evidence continues to support this hypothesis, some researchers believe that the various thermophilic groups evolved more recently than did the lineages leading to the spirochetes and chlamydias.

**Spirochetes move by means of axial filaments**

*Spirochetes* are Gram-negative, motile, chemoheterotrophic bacteria characterized by unique structures called axial filaments, which are modified flagella running through the periplasmic space (see Figure 26.6A). The cell body is a long cylinder coiled into a helix (Figure 26.12). The axial filaments begin at either end of the cell and overlap in the middle. Protein motors connect the axial filaments to the cell wall, enabling rotation of these structures as they do in other prokaryotic flagella. Many spirochetes live in humans as parasites; a few are pathogens, including those that cause syphilis and Lyme disease. Others live free in mud or water.
Chlamydiases are extremely small parasites

Chlamydiases are among the smallest bacteria (0.2–1.5 μm in diameter). They can live only as parasites in the cells of other organisms. It was once believed that this obligate parasitism resulted from an inability of chlamydiases to produce ATP—that chlamydiases were “energy parasites.” However, genome sequencing from the end of the twentieth century indicates that chlamydiases have the genetic capability to produce at least some ATP. They can augment this capacity by using an enzyme called a translocase, which allows them to take up ATP from the cytoplasm of their host in exchange for ADP from their own cells.

These tiny, Gram-negative cocci are unique prokaryotes because of their complex life cycle, which involves two different forms of cells, elementary bodies and reticulate bodies (Figure 26.13). In humans, various strains of chlamydiases cause eye infections (especially trachoma), sexually transmitted diseases, and some forms of pneumonia.

Some high-GC Gram-positives are valuable sources of antibiotics

High-GC Gram-positives, also known as actinobacteria, derive their name from the relatively high ratio of G-C to A-T nucleotide base pairs in their DNA. These bacteria develop an elaborately branched system of filaments (Figure 26.14) and can resemble the filamentous growth habit of fungi, albeit at a reduced scale. Some high-GC Gram-positives reproduce by forming chains of spores at the tips of the filaments. In species that do not form spores, the branched, filamentous growth ceases, and the structure breaks up into typical cocci or bacilli, which then reproduce by binary fission.

The high-GC Gram-positives include several medically important bacteria. Mycobacterium tuberculosis causes tuberculosis, which kills 3 million people each year. Genetic data suggest that this bacterium arose 3 million years ago in East Africa, making it the oldest known human bacterial affliction. Streptomyces produce streptomycin as well as hundreds of other antibiotics. We derive most of our antibiotics from members of the high-GC Gram-positives.

Cyanobacteria are important photoautotrophs

Cyanobacteria, sometimes called blue-green bacteria because of their pigmentation, are photoautotrophs that require only water, nitrogen gas, oxygen, a few mineral elements, light, and carbon dioxide to survive. They use chlorophyll a for photosynthesis and release oxygen gas; many species also fix nitrogen. Their photosynthesis was the basis of the “oxygen revolution” that transformed Earth’s atmosphere (see Section 25.3).

Cyanobacteria carry out the same type of photosynthesis that is characteristic of eukaryotic photosynthesizers. They contain elaborate and highly organized internal membrane systems called photosynthetic lamellae. The chloroplasts of photosynthetic eukaryotes are derived from an endosymbiotic cyanobacterium.

Cyanobacteria may live free as single cells or associate in colonies. Depending on the species and on growth conditions, colonies may range from flat sheets one cell thick to filaments to spherical balls of cells.

Some filamentous colonies of cyanobacteria differentiate into three cell types: vegetative cells, spores, and heterocysts (Figure 26.15). Vegetative cells photosynthesize, spores are resting stages that can survive harsh environmental conditions and eventually develop into new filaments, and heterocysts are cells specialized for nitrogen fixation. All of the known cyanobacteria with heterocysts fix nitrogen. Heterocysts also have a role in reproduction: when filaments break apart to reproduce, the heterocyst may serve as a breaking point.
The low-GC Gram-positives include the smallest cellular organisms

As their name suggests, the low-GC Gram-positives have a lower ratio of G-C to A-T nucleotide base pairs than do the high-GC Gram-positives. Some of the low-GC Gram-positives are in fact Gram-negative, and some have no cell wall at all. Despite these differences among the various species, phylogenetic analyses of DNA sequences support the monophyly of this clade.

Some endospores can be reactivated after more than 1,000 years of dormancy. There are even credible claims of reactivation of Bacillus endospores after millions of years. Members of this endospore-forming group of low-GC Gram-positives include the many species of Clostridium and Bacillus.

Dormant endospores of Bacillus anthracis are the source of an exotoxin (see page 555) that causes anthrax. The spores germinate when they sense specific molecules (macrophages; see Chapter 42) in the cytoplasm of mammalian blood cells. However, endospores of other, nonpathogenic Bacillus species do not germi-
nate in this environment. *B. anthracis* has been used as a bioterrorism agent because large quantities of its endospores are relatively easy to transport and spread among human populations, where they may be inhaled or ingested.

The genus *Staphylococcus*—the staphylococci—includes low-GC Gram-positives that are abundant on the human body surface; they are responsible for boils and many other skin problems (Figure 26.17). *Staphylococcus aureus* is the best-known human pathogen in this genus; it is found in 20 to 40 percent of normal adults (and in 50 to 70 percent of hospitalized adults). In addition to skin diseases, it can cause respiratory, intestinal, and wound infections.

Another interesting group of low-GC Gram-positives, the mycoplasmas, lack cell walls, although some have a stiffening material outside the plasma membrane. The mycoplasmas include the smallest cellular creatures known (Figure 26.18)—even smaller than chlamydas. The smallest mycoplasmas capable of multiplying by fission have a diameter of about 0.2 μm. They are small in another crucial sense as well: they have less than half as much DNA as most other prokaryotes. It has been speculated that the amount of DNA in a mycoplasma, which codes for fewer than 500 proteins, may be the minimum amount required to encode the essential properties of a living cell.

The proteobacteria are a large and diverse group

By far the largest group of bacteria, in terms of number of described species, is the proteobacteria. The proteobacteria include many species of Gram-negative, bacteriochlorophyll-containing, sulfur-using photoautotrophs—as well as dramatically diverse bacteria that bear no phenotypic resemblance to the photoautotrophic species. Genetic and morphological evidence indicates that the mitochondria of eukaryotes were derived from a proteobacterium by endosymbiosis (see Section 27.2).

No characteristic demonstrates the diversity of the proteobacteria more clearly than their metabolic pathways (Figure 26.19). There are five groups of proteobacteria: alpha, beta, gamma, delta, and epsilon. The common ancestor of all the proteobacteria was a photoautotroph. Early in evolution, two groups of proteobacteria lost their ability to photosynthesize and have been chemotrophs ever since. The other three groups still have photoautotrophic members, but in each group some evolutionary lines have abandoned photoautotrophy and taken up other modes of nutrition. There are chemolithotrophs and chemoheterotrophs in all three groups. Why? One possibility is that each of the trends shown in Figure 26.19 was an evolution-
ary response to selective pressures encountered as these bacteria colonized new habitats that presented new challenges and opportunities. Lateral gene transfer may have played a role in these responses.

Among the proteobacteria are some nitrogen-fixing genera, such as *Rhizobium* (see Figure 36.9), and other bacteria that contribute to the global nitrogen and sulfur cycles. *Escherichia coli*, one of the most studied organisms on Earth, is a proteobacterium. So, too, are many of the most famous human pathogens, such as *Yersinia pestis* (which causes bubonic plague), *Vibrio cholerae* (cholera), and *Salmonella typhimurium* (gastrointestinal disease).

Although fungi cause most plant diseases, and viruses cause others, about 200 known plant diseases are of bacterial origin. *Crown gall*, with its characteristic tumors (Figure 26.20), is caused by the proteobacterium *Agrobacterium tumefaciens*, a proteobacterium that harbors a plasmid used in recombinant DNA studies as a vehicle for inserting genes into new plant hosts (see Section 18.2).

We have discussed six clades of bacteria in some detail. Other bacterial clades are well known, and there are probably dozens more waiting to be discovered. This estimate is conservative because so few bacteria have been cultured and studied in the laboratory.

**Archaea differ in several important ways from bacteria**

The separation of Archaea from Bacteria and Eukarya was originally based on phylogenetic relationships determined from sequences of rRNA genes. This conclusion was supported when biologists sequenced the first archaeal genome. It consisted of 1,738 genes, more than half of which were unlike any genes ever found in the other two domains. Archaea are well known for living in extreme habitats such as those with high salinity (salt content), low oxygen concentrations, high temperatures, or high or low pH (Figure 26.21). However, many archaea are not extremeophiles but live in moderate habitats; they are common in

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**INVESTIGATING LIFE**

**26.21 What Is the Highest Temperature Compatible with Life?**

Can any organism thrive at temperatures above 120°C?

This is the temperature used for sterilization, known to destroy all previously described organisms. Kazem Kashefi and Derek Lovley isolated an unidentified prokaryote from water samples taken near a hydrothermal vent and found it survived and even grew at 121°C. The organism was dubbed “Strain 121,” and its gene sequencing results indicate that it is an archaeal species.

**HYPOTHESIS**

Some prokaryotes survive and even multiply at temperatures above the 120°C threshold of sterilization.

**METHOD**

1. Seal samples of unidentified, iron-reducing, thermal vent prokaryotes in tubes with a medium containing Fe$^{3+}$ as an electron acceptor. Control tubes contain Fe$^{3+}$ but no organisms.
2. Hold both tubes in a sterilizer at 121°C for 10 hours. If the iron-reducing organisms are metabolically active, they will reduce the Fe$^{3+}$ to Fe$^{2+}$ (as magnetite, which can be detected with a magnet).
3. Isolate any surviving organisms and test for growth at various temperatures.

**RESULTS**

![Graph showing the generation time (hours) of Strain 121 at different temperatures.](image)

The iron-containing solids were attracted to a magnet only in those tubes that contained living cells.

Cells multiplied most rapidly at about 105°C but divided about once a day even at 121°C.

**CONCLUSION**

Some prokaryotic organisms can survive and grow at temperatures above the previously defined sterilization limit.

**FURTHER INVESTIGATION:** Note that Strain 121 did not grow during a 2-hour exposure to 130°C, but it did not die, either. How would you demonstrate that it was still alive?

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soil, for example. Perhaps the largest number of archaea live in the ocean depths.

One current classification scheme divides Archaea into two principal groups, Euryarchaeota and Crenarchaeota. Less is known about two more recently discovered groups, Korarchaeota and Nanoarchaeota. In fact, we know relatively little about the phylogeny of archaea, in part because the study of archaea is still in its early stages.

Two characteristics shared by all archaea are the absence of peptidoglycan in their cell walls and the presence of lipids of distinctive composition in their cell membranes (see Table 26.1). The unusual lipids in the membranes of archaea are found in all archaea and in no bacteria or eukaryotes. Most bacterial and eukaryotic membrane lipids contain unbranched long-chain fatty acids connected to glycerol molecules by ester linkages:

\[ \text{H} \quad \text{O} \quad \text{C} \quad \text{O} \quad \text{C} \quad \text{H} \]

In contrast, some archaeal membrane lipids contain long-chain hydrocarbons connected to glycerol molecules by ether linkages:

\[ \text{H} \quad \text{C} \quad \text{O} \quad \text{C} \quad \text{H} \]

These ether linkages are a synapomorphy of archaea. In addition, the long-chain hydrocarbons of archaea are branched. One class of these lipids, with hydrocarbon chains 40 carbon atoms in length, contains glycerol at both ends of the hydrocarbons (Figure 26.22). This lipid monolayer structure, unique to archaea, still fits in a biological membrane because the lipids are twice as long as the typical lipids in the bilayers of other membranes. Lipid monolayers and bilayers are both found among the archaea. The effects, if any, of these structural features on membrane performance are unknown. In spite of this striking difference in their membrane lipids, the membranes seen in all three domains have similar overall structures, dimensions, and functions.

Most Crenarchaeota live in hot and/or acidic places

Most known Crenarchaeota are either thermophilic (heat loving), acidophilic (acid loving), or both. Members of the genus Sulfolobus live in hot sulfur springs at temperatures of 70°C to 75°C. They become metabolically inactive at 55°C (131°F). Hot sulfur springs are also extremely acidic. Sulfolobus grows best in the range from pH 2 to pH 3, but some members of this genus readily tolerate pH values as low as 0.9. Most acidophilic thermophiles maintain an internal pH of 5.5 to 7 (close to neutral) in spite of their acidic environment. These and other thermophiles thrive where very few other organisms can even survive. The archaea living in the volcanic vent shown in the opening of this chapter are examples of such thermophiles.

Euryarchaeota are found in surprising places

Some species of Euryarchaeota share the property of producing methane (CH₄) by reducing carbon dioxide. All of these methanogens are obligate anaerobes, and methane production is the key step in their energy metabolism. Comparison of rRNA gene sequences has revealed a close evolutionary relationship among these methanogenic species, which were previously assigned to several different bacterial groups.

Methanogens release approximately 2 billion tons of methane gas into Earth’s atmosphere each year, accounting for 80 to 90 percent of the methane in the atmosphere, including the methane produced in some mammalian digestive systems. Approximately a third of this methane comes from methanogens living in the guts of grazing herbivores such as cattle, sheep, and deer, and another large fraction comes from the methanogens that live in the guts of termites and cockroaches. Methane is increasing in Earth’s atmosphere by about 1 percent per year and contributes to the greenhouse effect. Part of the increase is due to increases in cattle and rice farming and the methanogens associated with both.

One methanogen, Methanopyrus, lives on the ocean bottom near hot hydrothermal vents. Methanopyrus can survive and grow at 122°C. It grows best at 98°C and not at all at temperatures below 84°C.

Another group of Euryarchaeota, the extreme halophiles (salt lovers), lives exclusively in very salty environments, such as the water of Blood Falls described in the opening of this chapter. Because they contain pink carotenoid pigments, halophiles are sometimes easy to see (Figure 26.23). Halophiles grow in the Dead Sea and in brines of all types: the reddish pink spots that can occur on pickled fish are colonies of halophilic archaea. Few other organisms can live in the saltiest of the homes that the extreme halophiles occupy; most would “dry” to death, losing too much water to the hypertonic environment. Extreme halophiles have been found in lakes with pH values as high as 11.5—the most alkaline environment inhabited by living organisms, and almost as alkaline as household ammonia.

Some of the extreme halophiles have a unique system for trapping light energy and using it to form ATP—without using any form of chlorophyll—when oxygen is in short supply. They use the pigment retinal (also found in the vertebrate eye) co-

26.22 Membrane Architecture in Archaea The long-chain hydrocarbons of many archael membranes have glycerol molecules at both ends, so that the membranes consist of a lipid monolayer. In contrast, the membranes of other archaea, bacteria, and eukaryotes consist of a lipid bilayer.
Correlated with a protein to form a light-absorbing molecule called bacteriorhodopsin, and they form ATP by a chemiosmotic mechanism of the kind described in Figure 9.9.

Another member of the Euryarchaeota, Thermoplasma, has no cell wall. It is thermophilic and acidophilic, its metabolism is aerobic, and it lives in coal deposits. It has the smallest genome among the archaea, and among the smallest (along with the mycoplasmas) of any free-living organism—1,100,000 base pairs.

Korarchaeota and Nanoarchaeota are less well known. The Korarchaeota are known only by evidence derived from DNA isolated directly from hot springs. No korarchaeote has been successfully grown in pure culture.

Another distinctive archaeal lineage has been discovered at a deep-sea hydrothermal vent off the coast of Iceland. It is the first representative of a group christened Nanoarchaeota because of their minute size. This organism lives attached to cells of Ignicoccus, a crenarchaeote. Because of their association, the two species can be grown together in culture (Figure 26.24).

26.5 How Do Prokaryotes Affect Their Environments?

Prokaryotes live in and exploit all kinds of environments and are part of all ecosystems. In this section we examine the roles of prokaryotes that live in soils, in water, and even in other organisms, where they may exist in a neutral, beneficial, or parasitic relationship with their host’s tissues. The roles of some prokaryotes living in extreme environments have yet to be determined.

Remember that in spite of our frequent mention of prokaryotes as human pathogens, only a small minority of the known prokaryotic species are pathogenic. Many more prokaryotes play positive roles in our lives and in the biosphere. We make direct use of many bacteria and a few archaea in such diverse applications as cheese production, sewage treatment, and the industrial production of an amazing variety of antibiotics, vitamins, organic solvents, and other chemicals.

Prokaryotes are important players in element cycling

Many prokaryotes are decomposers—organisms that metabolize organic compounds in dead organisms and other organic material and return the products to the environment as inorganic substances. Prokaryotes, along with fungi, return tremen-
dous quantities of organic carbon to the atmosphere as carbon dioxide, thus carrying out a key step in the carbon cycle. Prokaryotic decomposers also return inorganic nitrogen and sulfur to the environment.

Animals depend on plants and other photosynthetic organisms for their food, directly or indirectly. But plants depend on other organisms—prokaryotes—for their own nutrition. The extent and diversity of life on Earth would not be possible without nitrogen fixation by prokaryotes. Nitrifiers are crucial to the biosphere because they convert the products of nitrogen fixation into nitrate ions, the form of nitrogen most easily used by many plants (see Figure 36.11). Plants, in turn, are the source of nitrogen compounds for animals and fungi. Denitrifiers also play a key role in keeping the nitrogen cycle going. Without denitrifiers, which convert nitrate ions back into nitrogen gas, all forms of nitrogen would leach from the soil and end up in lakes and oceans, making life on land impossible. Other prokaryotes—both bacteria and archaea—contribute to a similar cycle of sulfur.

In the ancient past, the cyanobacteria had an equally dramatic effect on life: their photosynthesis generated oxygen, converting Earth’s atmosphere from an anaerobic to an aerobic environment (see Section 25.3). A major result was the wholesale loss of obligate anaerobic species that could not tolerate the oxygen generated by the cyanobacteria. Only those anaerobes that adapted to aerobic conditions or colonized environments that remained anaerobic survived. However, this transformation to aerobic environments made possible the evolution of cellular respiration and the subsequent explosion of eukaryotic life.

Prokaryotes live on and in other organisms

Prokaryotes work together with eukaryotes in many ways. As we have seen, mitochondria and chloroplasts are descended from what were once free-living bacteria. Much later in evolutionary history, some plants became associated with bacteria to form cooperative nitrogen-fixing nodules on their roots (see Figure 36.9). Many animals harbor a variety of bacteria and archaea in their digestive tracts. Cattle depend on prokaryotes to perform important steps in digestion. Like most animals, cattle cannot produce cellulase, the enzyme needed to start the digestion of the cellulose that makes up the bulk of their plant food. However, bacteria living in a special section of the gut, called the rumen, produce enough cellulase to process the daily diet for the cattle.

Humans use some of the metabolic products—especially vitamins B₁₂ and K—of bacteria living in our large intestine. These and other bacteria and archaea line our intestines with a dense biofilm that is in intimate contact with the mucosal lining of the gut. This biofilm facilitates nutrient transfer from the intestine into the body and induces immunity to the gut contents. The biofilm in the gut is a major part of an “organ” consisting of prokaryotes that is essential to our health. Its makeup varies from time to time and from region to region of the intestinal tract, and it has a complex ecology that scientists have just begun to explore in detail—including the possibility that the species composition of an individual’s prokaryote gut fauna may contribute to obesity (or the resistance to it).

We are heavily populated inside and out by bacteria. A 2009 study of bacteria that live on human skin identified more than 1,000 species living on the outside of our bodies, and many of these are thought to be critical to maintaining the skin’s health. Although only a small percentage of bacterial species are agents of disease, popular notions of bacteria as “germs” and fear of the consequences of infection arouse our curiosity about those few.

A small minority of bacteria are pathogens

The late nineteenth century was a productive era in the history of medicine—a time when bacteriologists, chemists, and physicians proved that many diseases are caused by microbial agents. During this time the German physician Robert Koch laid down a set of four rules for establishing that a particular microorganism causes a particular disease:

- The microorganism is always found in individuals with the disease.
- The microorganism can be taken from the host and grown in pure culture.
- A sample of the culture produces the same disease when injected into a new, healthy host.
- The newly infected host yields a new, pure culture of microorganisms identical to those obtained in the second step.

These rules, called Koch’s postulates, were very important in a time when it was not widely understood that microorganisms cause disease. Although medical science today has more powerful diagnostic tools, the postulates remain useful on occasion. For example, physicians were taken aback in the 1990s when stomach ulcers—long accepted and treated as the result of excess stomach acid—were shown by Koch’s postulates to be caused by the bacterium Helicobacter pylori (see Figure 51.14).

Only a tiny percentage of all prokaryotes are pathogens, and of those that are known, all are in the domain Bacteria. For an organism to be a successful pathogen, it must:

- arrive at the body surface of a potential host;
- enter the host’s body;
- evade the host’s defenses;
- multiply inside the host; and finally
- infect a new host.

Failure to successfully complete any of these steps ends the reproductive career of a pathogenic organism. However, in spite of the many defenses available to potential hosts (see Chapter 42), some bacteria are very successful pathogens.

For the host, the consequences of a bacterial infection depend on several factors. One is the invasiveness of the pathogen—its ability to multiply in the host’s body. Another is its toxigenicity—its ability to produce toxins, chemical substances that are harmful to the host’s tissues. Corynebacterium diphtheriae, the agent that causes diphtheria, has low invasiveness and multiplies only in the throat, but its toxigenicity is so great that the entire body is affected. In contrast, Bacillus anthracis, which causes anthrax (a disease primarily of cattle and sheep, but
which is also sometimes fatal in humans), has low toxigenicity but is so invasive that the entire bloodstream ultimately teems with the bacteria.

There are two general types of bacterial toxins: exotoxins and endotoxins. **Endotoxins** are released when certain Gram-negative bacteria grow or lyse (burst). Endotoxins are lipopolysaccharides (complexes consisting of a polysaccharide and a lipid component) that form part of the outer bacterial membrane. Endotoxins are rarely fatal; they normally cause fever, vomiting, and diarrhea. Among the endotoxin producers are some strains of the gamma-proteobacteria *Salmonella* and *Escherichia*.

**Exotoxins** are usually soluble proteins released by living, multiplying bacteria, and they may travel throughout the host's body. They are highly toxic—often fatal—to the host, but they do not produce fevers. Human diseases induced by bacterial exotoxins include tetanus (*Clostridium tetani*), cholera (*Vibrio cholerae*), and bubonic plague (*Yersinia pestis*). Anthrax results from three exotoxins produced by *Bacillus anthracis*. Botulism is caused by exotoxins produced by *Clostridium botulinum* that are among the most poisonous ever discovered. The lethal dose of the botulinum A exotoxin for humans is about one-millionth of a gram (1 μg). Nonetheless, much smaller doses of this exotoxin, marketed under various trade names, are used to treat muscle spasms and also for cosmetic purposes (temporary wrinkle reduction in skin).

Pathogenic bacteria are often surprisingly difficult to combat, even with today's arsenal of antibiotics. One source of this difficulty is the ability of prokaryotes to form biofilms.

### 26.5 Recap

Prokaryotes play key roles in the cycling of Earth's elements. Many prokaryotes are beneficial and even necessary to other forms of life; others are pathogens.

- Describe the roles of bacteria in the nitrogen cycle. [See p. 554]
- What are some of the challenges facing a pathogen? [See p. 554]

Before moving on to discuss the diversity of eukaryotic life, it is appropriate to consider how viruses are related to the rest of life. Although they are not cellular, viruses are numerically among the most abundant organisms on Earth. Their effects on other organisms are enormous. Where did viruses come from, and how do they fit into the tree of life? Biologists are still working to answer these questions.

### 26.6 Where Do Viruses Fit into the Tree of Life?

Some biologists do not think of viruses as living organisms, primarily because they are not cellular and must depend on cellular organisms for basic life functions such as replication and metabolism. But viruses are derived from the cells of other living organisms. They use the same essential forms of genetic storage and transmission as do cellular organisms. Viruses infect all cellular forms of life, including bacteria, archaea, and eukaryotes. They replicate, mutate, evolve, and interact with other organisms, often causing serious diseases when they infect their hosts. They are also numerically among the most abundant organisms on the planet. And, finally, viruses clearly evolve independently of other organisms, so it is almost impossible not to treat them as a part of life.

Several factors make virus phylogeny difficult to resolve. The tiny size of many viral genomes restricts the phylogenetic analyses that can be conducted to relate viruses to cellular organisms. The rapid mutational rate, which results in rapid evolution of viral genomes, tends to cloud evolutionary relationships across long periods of time. There are no known viral fossils (viruses are too small and delicate to fossilize), so the paleontological record offers no clues as to viral origins. Finally, viruses are highly diverse (see Figure 26.25), and several lines of evidence support the hypothesis that viruses have evolved repeatedly within each of the major groups of life.

**Many RNA viruses probably represent escaped genomic components**

Although viruses are now obligate parasites of cellular species, they may once have been cellular components involved in basic cellular functions—that is, they may be “escaped” components of cellular life that now evolve independently of their hosts.

**Negative-Sense Single-Stranded RNA Viruses** A case in point is a class of viruses whose genome is composed of single-stranded RNA that is the complement (negative-sense) of the mRNA needed for protein translation. Many of these negative-sense single-stranded RNA viruses have only a few genes, including an RNA-dependent RNA polymerase that allows them to make mRNA from their negative-sense RNA genome. Modern cellular organisms cannot generate mRNA in this manner (at least in the absence of viral infections), but scientists speculate that single-stranded RNA genomes may have been common in the distant past, before DNA became the primary molecule for genetic information storage.

A self-replicating RNA polymerase gene that begins to replicate independently of a cellular genome could conceivably acquire a few additional protein-coding genes through recombination with its host’s DNA. If one or more of these genes were to foster the development of a protein coat, the virus might then survive outside the host and infect new hosts. It is believed that this scenario has been repeated many times independently across the tree of life, given that many of the negative-sense single-stranded RNA viruses that infect organisms from bacteria to humans are not closely related to one another. In other words, negative-sense single-stranded RNA viruses do not represent a distinct taxonomic group, but exemplify a particular process of cellular escape that probably happened many different times.

Examples of familiar negative-sense single-stranded RNA viruses include the viruses that cause measles, mumps, rabies, and influenza ([Figure 26.25A](#)).
The genome of another type of single-stranded RNA viruses is composed of positive-sense RNA. Positive-sense genomes are already set for translation; unlike in negative-sense RNA, no replication of the genome into the complement strand is needed before protein translation can take place. Positive-sense single-stranded RNA viruses are the most abundant and diverse class of viruses (Figure 26.25B). Most of the viruses that cause diseases in crop plants are in this group. When they infect plants, these viruses kill patches of cells in the leaves or stems of the plants, leaving live cells amid a patchwork of discolored dead plant tissue (giving them the name of mosaic or mottle viruses; Figure 26.26). Other viruses in this group infect bacteria, fungi, and animals. Human diseases caused by positive-sense single-stranded RNA viruses include polio, hepatitis C, and the common cold. As is true of the other functionally defined groups of viruses, these viruses appear to have evolved multiple times across the tree of life from different groups of cellular ancestors.

**RNA RETROVIRUSES** The RNA retroviruses are best known as the group that includes the human immunodeficiency viruses (HIV; Figure 26.25C). Like the previous two categories of viruses, RNA retroviruses have genomes composed of single-stranded RNA and likely evolved as escaped cellular components.
Retroviruses are so named because they regenerate themselves by reverse transcription. When the retroviruses enter the nucleus of their vertebrate host, viral reverse transcriptase produces cDNA from the viral RNA genome and then replicates the single-stranded cDNA to produce double-stranded DNA. Another virally encoded enzyme called integrase catalyzes the integration of the new piece of double-stranded viral DNA into the host’s genome. The viral genome is then replicated along with the host cell’s DNA; the integrated retroviral DNA is known as a provirus. Many components of cellular species (such as retrotransposons; see Section 17.3) resemble components of retroviruses.

Retroviruses are only known to infect vertebrates, although genomic elements that resemble portions of these viruses are a component of the genomes of a wide variety of organisms including bacteria, plants, and many animals. Several retroviruses are associated with the development of various forms of cancer, as cells infected with these viruses are likely to undergo uncontrolled replication.

DOUBLE-STRANDED RNA VIRUSES Double-stranded RNA viruses may have evolved repeatedly from single-stranded RNA ancestors—or perhaps vice versa. These viruses, which are not closely related to one another, infect organisms from throughout the tree of life. For example, many important plant diseases are caused by double-stranded RNA viruses, whereas other viruses of this type cause many cases of infant diarrhea in humans.

Some DNA viruses may have evolved from reduced cellular organisms

Another class of viruses is composed of those that have a double-stranded DNA genome (Figure 26.25D–F). This group is also almost certainly polyphyletic (with many independent origins). Many of the common phage that infect bacteria (bacteriophage) are double-stranded DNA viruses, as are the viruses that cause smallpox and herpes in humans.

Some biologists think that at least some of the DNA viruses may represent highly reduced parasitic organisms that have lost their cellular structure as well as their ability to survive as freeliving species. For example, some of the largest DNA viruses are the mimiviruses (see Figure 26.25F), which have a genome in excess of a million base pairs of DNA. This is double the genome size of some parasitic bacteria such as *Mycoplasma genitalium*. Phylogenetic analyses of these DNA viruses suggest that they have evolved repeatedly from cellular organisms. Furthermore, recombination among different viruses may have allowed the exchange of various genetic modules, further complicating the history and origins of viruses.

26.6 RECAP

Viruses are highly diverse and appear to have evolved independently from many different groups of cellular organisms from throughout the tree of life. Some viruses appear to have evolved from escaped components of cellular organisms, whereas other viruses may have evolved from parasitic cellular ancestors.

- What are some the reasons that it is difficult to place viruses precisely within the phylogeny of cellular organisms? See p. 555
- Explain the two main hypotheses of viral origins. See p. 555–557

It appears that the enormous diversity of viruses is, at least in part, a result of their multiple origins from many different cellular organisms. Some may have arisen as escaped genetic components, while others may represent reduced and specialized parasites. Some appear to be derived from bacterial species, others to be derived from eukaryotic organisms. It may be best to view viruses as spinoffs from the various branches on the tree of life—sometimes evolving independently of cellular genomes, sometimes recombining with them. One way to think of viruses is as the “bark” on the tree of life: certainly an important component all across the tree, but not quite like the main branches.
26.1 How Did the Living World Begin to Diversify?
- Two of life’s three domains, Bacteria and Archaea, are prokaryotic. They are distinguished from Eukarya in several ways, including their lack of membrane-enclosed organelles. Review Table 26.1
- Eukaryotes are related to both Archaea and Bacteria, and appear to have formed through a merging of members of these two lineages. The last common ancestor of all three domains probably lived 2 to 3 billion years ago. Review Figure 26.1, ANIMATED TUTORIAL 26.1
- Prokaryotes may be single or may form chains or clusters. The three most common bacterial morphologies are cocci (spheres), bacilli (rods), and helices (spirals). Review Figure 26.2
- The cells of some bacteria aggregate, forming filaments and other structures.

26.2 What Are Some Keys to the Success of Prokaryotes?
- Prokaryotes are the most numerous organisms on Earth. They occur in an enormous variety of habitats, including inside other organisms and deep in Earth’s crust.
- Prokaryotes form complex communities, some of which become dense films called biofilms. Review Figure 26.3
- Most prokaryotes have cell walls containing molecules important in transport, digestion, and sensing the environment. Almost all bacterial cell walls contain peptidoglycan. The cell walls of archaea lack peptidoglycan and instead include pseudopeptidoglycan or proteins. Review Figure 26.5, WEB ACTIVITY 26.1
- Bacteria can be placed into two groups by the Gram stain. Gram-negative bacteria have a periplasmic space between the inner (plasma) and outer membranes. In Gram-positive bacteria, the cell wall usually has about five times as much peptidoglycan as a Gram-negative wall.
- Prokaryotes move by a variety of means, including axial filaments, gas vesicles, and flagella. Review Figures 26.6 and 26.7
- Prokaryotes reproduce asexually but may undergo genetic recombination. Reproduction and genetic recombination are not directly linked in prokaryotes.
- Prokaryote metabolism is very diverse. Some prokaryotes are anaerobic, others are aerobic, and yet others can shift between these modes. Prokaryotes are classified as photoautotrophs, photoheterotrophs, chemolithotrophs, or chemoheterotrophs. Review Table 26.2
- The metabolic pathways of some prokaryotes involve sulfur or nitrogen. Nitrogen fixers convert nitrogen into forms that can be used by plants, and denitrifiers ensure that nitrogen is returned to the atmosphere.

26.3 How Can We Resolve Prokaryote Phylogeny?
- Early attempts to classify prokaryotes were hampered by the organisms’ small size and difficulties growing them in pure culture. Phylogenetic classification of prokaryotes is now based on nucleotide sequences of rRNA and other slowly evolving genes.
- Although lateral gene transfer has occurred throughout prokaryotic evolutionary history, elucidation of prokaryote phylogeny is still possible. Review Figure 26.10
- Only a tiny percentage of all prokaryote species have been described.

26.4 What Are the Major Known Groups of Prokaryotes?
- Several major taxonomic groups of prokaryotes have been recognized. The members of a prokaryote clade often differ profoundly from one another. Review Figures 26.11 and 26.19
- Of the major taxonomic groups of Bacteria, the proteobacteria embrace the largest number of species. Other important groups include the cyanobacteria, spirochetes, chlamydias, and low-GC Gram-positives. Some high-GC Gram positives produce important antibiotics.
- The low-GC Gram-positives include the mycoplasmas, which are among the smallest cellular organisms ever discovered.
- Ether linkages in the branched long-chain hydrocarbons of cell membranes are a synapomorphy of archaea.
- The best-studied groups of Archaea are Euryarchaeota and Crenarchaeota. Some species of Euryarchaeota are methanogens; extreme halophiles are also found among the Euryarchaeota. Most known Crenarchaeota are both thermophilic and acidophilic.

26.5 How Do Prokaryotes Affect their Environments?
- Prokaryotes play key roles in the cycling of elements such as nitrogen, oxygen, sulfur, and carbon. One such role is as decomposers of dead organisms.
- Nitrogen-fixing bacteria fix the nitrogen needed by all other organisms. Nitrifiers convert that nitrogen into forms that can be used by plants, and denitrifiers ensure that nitrogen is returned to the atmosphere.
- Oxygen production by early photosynthetic cyanobacteria reconfigured Earth’s atmosphere, which made aerobic forms of life possible.
- Prokaryotes inhabiting the guts of many animals help them digest their food.
- Koch’s postulates establish the criteria by which an organism may be classified as a pathogen. Relatively few bacteria—and no archaea—are known to be pathogenic.

26.6 Where Do Viruses Fit Into the Tree of Life?
- Viruses have evolved many times from many different groups of cellular organisms. They do not represent a single taxonomic group.
- Some viruses are probably derived from escaped genetic elements of cellular species; others are thought to have evolved as highly reduced parasites.
- Viruses are categorized by the nature of their genomes. Review Figure 26.25
SELF-QUIZ

1. Most prokaryotes
   - a. are agents of disease.
   - b. lack ribosomes.
   - c. evolved from the most ancient eukaryotes.
   - d. lack a cell wall.
   - e. are chemoheterotrophs.

2. The division of the living world into three domains
   - a. is based on the number of cells in organisms of each group.
   - b. is based mostly on the major morphological differences between archaea and bacteria.
   - c. emphasizes the greater importance of eukaryotes.
   - d. was proposed by the early microscopists.
   - e. is based on phylogenetic relationships determined from nucleotide sequences of rRNA and other genes.

3. Which statement about archaeal genomes is true?
   - a. They are typically organized in a circular chromosome, like bacterial genomes.
   - b. They include no rRNA genes.
   - c. They are always much smaller than bacterial genomes.
   - d. They are housed in the nucleus of the archaeal cell.
   - e. No archaeal genome has yet been sequenced.

4. Which statement about nitrogen metabolism is not true?
   - a. Certain prokaryotes reduce atmospheric N₂ to ammonia.
   - b. Some nitrifiers are soil bacteria.
   - c. Denitrifiers are obligate anaerobes.
   - d. Nitrifiers obtain energy by oxidizing ammonia and nitrite.
   - e. Without nitrifiers, terrestrial organisms would lack a nitrogen supply.

5. All photosynthetic bacteria
   - a. use chlorophyll a as their photosynthetic pigment.
   - b. use bacteriochlorophyll as their photosynthetic pigment.
   - c. release oxygen gas.
   - d. produce particles of sulfur.
   - e. are photoautotrophs.

6. Gram-negative bacteria
   - a. appear blue to purple following Gram staining.
   - b. are the most abundant of the bacterial groups.
   - c. are all either bacilli or cocci.
   - d. contain no peptidoglycan in their cell walls.
   - e. are all photosynthetic.

7. Endospores
   - a. are produced by viruses.
   - b. are reproductive structures.
   - c. are very delicate and easily killed.
   - d. are resting structures.
   - e. lack cell walls.

8. Chlamydias
   - a. are among the smallest archaea.
   - b. live on the surface of human skin.
   - c. are never pathogenic to humans.
   - d. are Gram-negative.
   - e. have a very simple life cycle.

9. Archaea
   - a. have cytoskeletons.
   - b. have distinctive lipids in their plasma membranes.
   - c. survive only at moderate temperatures and near neutrality.
   - d. all produce methane.
   - e. have substantial amounts of peptidoglycan in their cell walls.

10. Genetic evidence suggests that viruses
    - a. are most closely related to Bacteria.
    - b. are most closely related to Archaea.
    - c. are most closely related to Eukarya.
    - d. have evolved multiple times from many different cellular species.
    - e. evolved from the fusion of a bacterial and an archaeal species.

FOR DISCUSSION

1. Why do systematic biologists find rRNA sequence data more useful than data on metabolism or cell structure for classifying prokaryotes?
2. How can lateral gene transfer mislead studies about prokaryote phylogeny? How could this issue be addressed?
3. Differentiate among the members of the following sets of related terms:
   - a. prokaryotic/eukaryotic
   - b. obligate anaerobe/facultative anaerobe/obligate aerobe
   - c. photoautotroph/phototroph/chemolithotroph/chemoautotroph
   - d. Gram-positive/Gram-negative
4. Why are the endospores of low-GC Gram-positives not considered to be reproductive structures?
5. Originally, the cyanobacteria were called “blue-green algae” and were not grouped with the bacteria. Suggest several reasons for this (abandoned) tendency to separate the cyanobacteria from the bacteria. Why are the cyanobacteria now grouped with the other bacteria?
6. The high-GC Gram-positives are of great commercial interest. Why?
7. Thermophiles are of great interest to molecular biologists and biochemists. Why? What practical concerns might motivate that interest?
8. How can biologists discuss the Korarchaeota when they have never seen one?
9. Do you consider viruses to be living organisms? Why or why not?

ADDITIONAL INVESTIGATION

Kashefi and Lovley (see Figure 26.21) were able to grow an unnamed archaeal species at temperatures above 120°C only because they used Fe³⁺ as an electron acceptor—no other electron acceptor they tried allowed growth. How might you explore the same or other high-temperature environments for other hyperthermophilic organisms not detected by Kashefi and Lovley using Fe³⁺?