13 Microbial Evolution and Systematics

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Lokiarchaeota and the Origin of *Eukarya*

The domain *Eukarya* includes plants, animals, fungi, and a tremendous diversity of microorganisms. The plants, animals, and fungi are relative newcomers on the scene, as their evolutionary origins occurred some 400–600 million years ago. In contrast, the first eukaryotic microbes originated well over a billion years ago. The evolutionary origin of the eukaryotic cell remains enigmatic and we still do not know when or how the domain *Eukarya* was formed.

Genomic analyses clearly reveal *Eukarya* to be genetic chimeras. Eukaryotic genomes contain a mixture of genes that originated either within the *Bacteria* or within the *Archaea*, as well as many genes that are unique to *Eukarya*. Most evidence suggests that *Eukarya* share an ancestor with the domain *Archaea*, but *Eukarya* contain numerous "signature genes" not found in the *Archaea*. These unique eukaryotic genes encode proteins associated with the distinctive cell biology of *Eukarya* and were likely essential for the origins of multicellularity, a property widespread in the eukaryotic world.

The recent discovery of *Lokiarchaeota*—a new phylum of *Archaea*—has provided fresh insights into the origin of *Eukarya*. *Lokiarchaeota* were discovered through metagenomic analyses of microbial communities that inhabit deep marine sediments near a hydrothermal vent system known as Loki's Castle (see photo), located along the Mid-Atlantic Ridge between Greenland and Norway. Remarkably, the genomes of *Lokiarchaeota* contain a number of eukaryotic signature genes, and in particular, genes associated with membrane remodeling and the development of a cytoskeleton. The presence of a cytoskeleton and the ability to remodel intracellular membranes would have facilitated membrane invagination in primitive eukaryotic cells, and this would have allowed bacterial endosymbionts to be acquired and provided new nutritional strategies, such as phagocytosis.

These results suggest that features uniquely associated with the eukaryotic cell may actually have their origins in the domain *Archaea*. The discovery of the *Lokiarchaeota* also indicates that, rather than emerging as a sister group to the *Archaea*, the earliest eukaryotic cells emerged from within the *Archaea* following the endosymbiotic acquisition of the bacteria that gave rise to the eukaryotic cell's respiratory organelle, the mitochondrion. Hence, the first steps toward the origins of cellular complexity may have occurred within the domain *Archaea*.

Source: Sprang, A., et al. 2015. Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature 521:* 173–179.

Evolution is a topic that pervades all of biology, and microbiology in particular because microorganisms were the first life forms on Earth. In this chapter we tackle the topic of evolution and describe experimental methods for unraveling evolutionary relationships. We will also see how these powerful genetic methods along with phenotypic observations underlie the robust systematics of the microbial world that will unfold in the following five chapters.

I • Early Earth and the Origin and Diversification of Life

In these first sections, we consider the possible conditions under which life arose, the earliest evidence for cellular life, and its divergence into three evolutionary lineages: ***Bacteria***, ***Archaea***, and ***Eukarya***. Although much about these events and processes remains speculative, geological and molecular evidence has combined to build a plausible scenario for the earliest events in the **evolution** of life and for the fundamental impacts that microbes have had on the history of our Earth.

13.1 Formation and Early History of Earth

The Earth of 4 billion years ago would be foreign and inhospitable to human eyes, but this sterile wasteland of blasted rock and boiling seas was the incubator from which all life sprang. Few fossils exist to tell the story of the early Earth, and most of what we know is inferred from chemical and isotopic analyses of ancient rocks and minerals. The story of life begins not long after the dawn of our solar system with the formation of Earth itself.

Origin of Earth

Earth formed about 4.5 billion years ago (Figure 13.1), based on analyses of slowly decaying radioactive isotopes. Our planet and the other planets of our solar system arose from materials making up a disc-shaped nebular cloud of dust and gases released by the supernova of a massive old star. As a new star—our sun—formed within this cloud, it began to compact, undergo nuclear fusion, and release large amounts of energy in the form of heat and light. Materials left in the nebular cloud began to clump and fuse due to collisions and gravitational attractions, forming tiny accretions that gradually grew larger to form clumps that eventually coalesced into planets. Energy released in this process heated the emerging Earth as it formed, as did energy released by radioactive decay within the condensing materials, forming a planet Earth of fiery hot magma. As Earth cooled over time, a metallic core, rocky mantle, and a lower-density, thin surface crust formed.

The inhospitable conditions of early Earth, characterized by a molten surface under intense bombardment by asteroids and other objects from space, are thought to have persisted for over 500 million years. Water on Earth originated from volcanic outgassing of the planet's interior and from innumerable collisions with icy comets and asteroids. Given Earth's heat at the time, water would have been present only as water vapor. The intense heat and the absence of liquid water indicate that early Earth was certainly a sterile planet. No rocks dating to the origin of Earth have yet been discovered, presumably because they have undergone geological metamorphosis. However, ancient crystals of the mineral zircon ($ZrSiO_4$) have been discovered that were formed on the early Earth, and these materials have given us a glimpse of conditions on Earth prior to the origin of life.

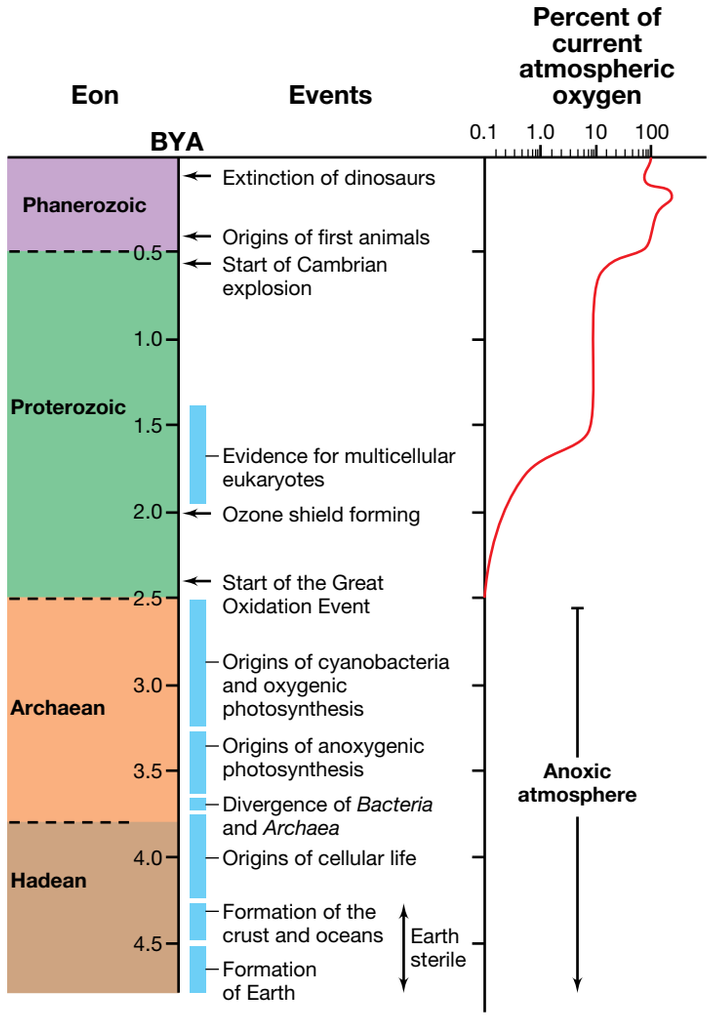


Figure 13.1 Major landmarks in biological evolution, Earth's changing geochemistry, and microbial metabolic diversification. The oldest date for the origin of life is fixed by the time of Earth's origin, and the minimum time for the origin of oxygenic photosynthesis is fixed by the Great Oxidation Event, about 2.4 billion years ago (BYA). Note how the oxygenation of the atmosphere from cyanobacterial metabolism was a gradual process, occurring over a period of about 2 billion years. Compare this figure with the introduction to the antiquity of life on Earth shown in Figure 1.5.

Liquid water is a requirement for life, and its presence on Earth made possible the origin of life. Analyses of ancient zircon crystals, including impurities trapped in these crystals and their oxygen isotope ratios (we discuss the use of isotopic analyses as indications of living processes in Section 19.10) indicate the presence of solid crust and liquid water as early as 4.3 billion years ago (Figure 13.1). Furthermore, graphite inclusions discovered within ancient zircon minerals have carbon isotope ratios that suggest biogenic origin, providing evidence for life on Earth 4.1 billion years ago. Among the oldest surviving sedimentary rocks are those found in southwestern Greenland. Sedimentary rocks are formed within bodies of water. The oldest known sedimentary rocks date to 3.86 billion years ago, indicating that oceans were present at the

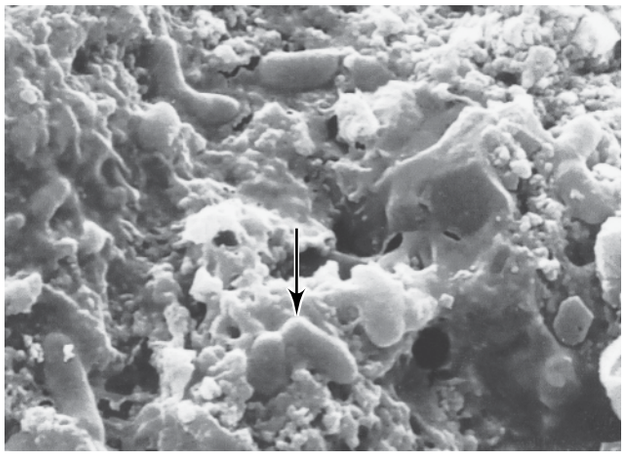


Figure 13.2 Ancient microbial life. Scanning electron micrograph of microfossil bacteria from 3.45-billion-year-old rocks of the Barberton Greenstone Belt, South Africa. Note the rod-shaped bacteria (arrow) attached to particles of mineral matter. The cells are about 0.7 μm in diameter.

time these rocks formed. These rocks contain fossilized remains of what appear to be cells (**Figure 13.2**), and carbon with isotopic ratios that provide further evidence for ancient microbial life.

Origin of Cellular Life

The origin of life on Earth remains the greatest of mysteries, obscured by the depths of time. Few rocks survive unaltered to testify about this period of Earth's history. Experimental evidence indicates that organic molecules such as RNA nucleotides, amino acids, and lipids can form spontaneously under conditions that were present on the early Earth, providing the preconditions needed for the first living systems. However, conditions on Earth's surface more than 4 billion years ago, in particular the extremely hot temperatures and levels of ultraviolet radiation, were likely hostile to the origin of life as we know it.

One hypothesis holds that life may have originated at hydrothermal systems on the ocean floor (**Figure 13.3**). Deep on the ocean floor, conditions would have been less hostile and more stable than on Earth's surface. A steady and abundant supply of energy in the form of reduced inorganic compounds—hydrogen (H_2) and hydrogen sulfide (H_2S), for example—would have been available at these hydrothermal systems. The unique geochemistry of these sites can support the abiotic production of molecules critical for the emergence of life. For example, molecules such as amino acids, lipids, sugars, and nucleotide bases can all be formed under conditions found at certain hydrothermal systems. Furthermore, mineral structures that form in these systems can produce compartmentalized structures that may have been necessary for conserving energy prior to the emergence of biological membranes. Whether on the seafloor or elsewhere, some form of prebiotic chemistry must have facilitated the development of the first self-replicating systems, the precursors to cellular life.

Molecules of RNA were likely a central component of the first self-replicating systems and it is possible that life began in an *RNA world* (**Figure 13.4**). RNA is a component of certain essential cofactors and molecules found in all cells (such as ATP, NADH, and coenzyme A); it can bind small molecules (such as ATP, other

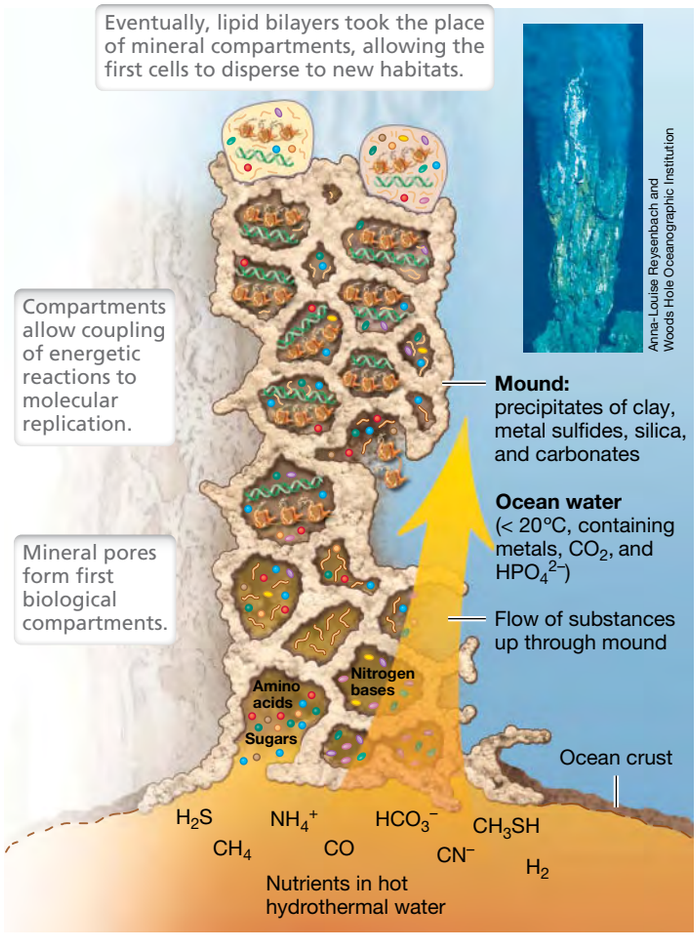


Figure 13.3 Submarine mounds and their possible link to the origin of life. Model of the interior of a hydrothermal mound with hypothesized transitions from prebiotic chemistry to cellular life depicted. Inset: photo of an actual hydrothermal mound. Hot, mineral-rich hydrothermal fluid mixes with cooler, more oxidized ocean water, forming precipitates of Fe and S compounds, clays, silicates, and carbonates. Mineral precipitates form pores that could have served as energy-rich compartments that facilitated the evolution of precellular forms of life.

nucleotides, and amino acids); it can possess catalytic activity; and it is known to catalyze protein synthesis through the activities of rRNA, tRNA, and mRNA (Section 4.10). It is thus possible to imagine that certain RNA molecules once had the ability to catalyze their own synthesis; these earliest forms of life may have had little or no need for DNA or protein. Indeed, comparative genomics studies of viruses suggest that the earliest viruses might have evolved from primitive cell-like structures that contained RNA (rather than DNA) genomes (Section 10.2).

Eventually, proteins replaced the catalytic role of RNAs in very primitive cells and at some point DNA, a molecule that is inherently more stable than RNA and therefore a better repository of genetic (coding) information, assumed the role of genome and template for all RNA synthesis (Figure 13.4). The earliest cellular forms of life likely possessed elements of this three-part system of DNA, RNA, and protein, in addition to a membrane system capable of conserving energy (see Figure 13.5). The *last universal common ancestor* (*LUCA*) of all extant life likely existed at 3.8–3.7 billion

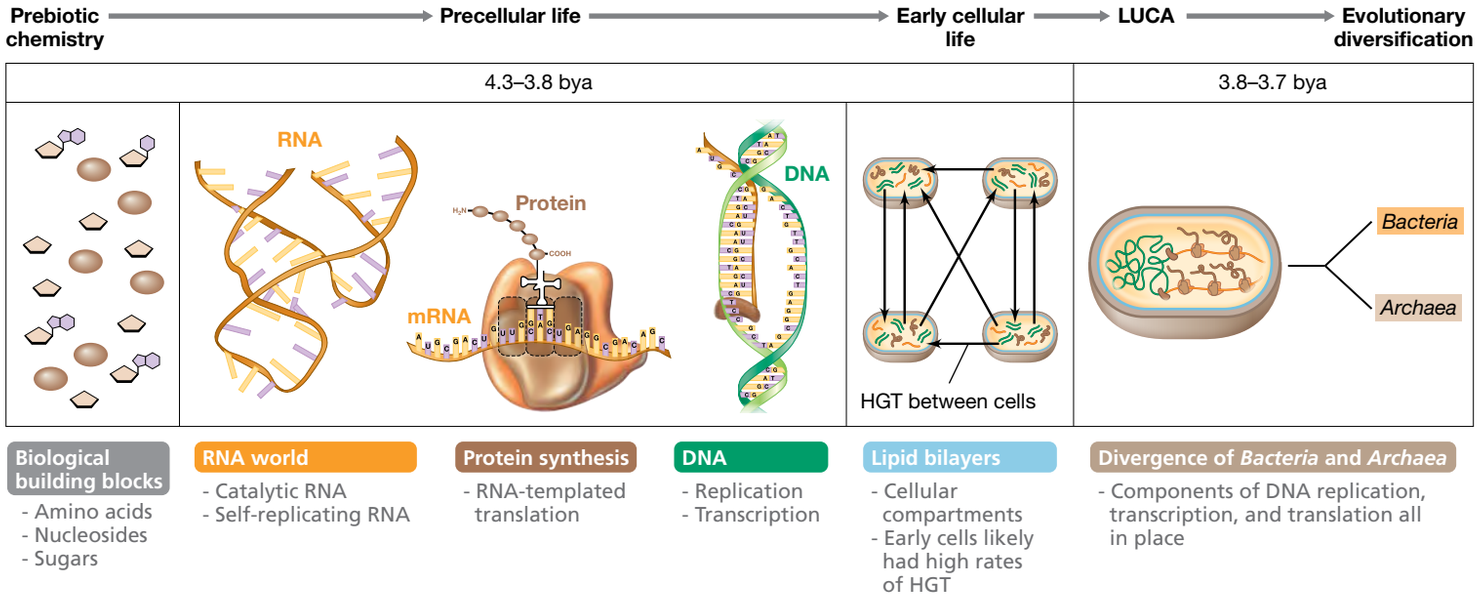


Figure 13.4 Events hypothesized to precede the origin of cellular life. The earliest self-replicating biological systems may have been based on catalytic RNA. At some point RNA enzymes evolved the capability to synthesize proteins, and proteins became the main catalytic molecules. Conversion from RNA- to DNA-based genomes required the evolution of DNA and RNA polymerases. The lipid bilayer is the site of electron transport and the evolution of this structure was likely important for energy conservation, in addition to containing and protecting biomolecules. The last universal common ancestor (LUCA), which preceded the divergence of *Bacteria* and *Archaea* (see Figure 13.9), was a cellular organism that had a lipid bilayer and used DNA, RNA, and protein. Horizontal gene transfer (HGT) may have allowed rapid transfer of beneficial genes among early forms of life.

years ago, the point at which *Bacteria* and *Archaea* became distinct and diverged and life began to diversify into the forms we recognize today. One can envision a time of intensive biochemical innovation and experimentation in which much of the structural and functional machinery of these earliest self-replicating systems evolved and was refined by natural selection.

Metabolic Diversification: Consequences for Earth's Biosphere

Following the origin of cells, microbial life experienced a long period of metabolic diversification, exploiting the various resources available on Earth. For much of Earth's history the planet, including all of its oceans, was anoxic (Figure 13.1). Thus, the energy-generating metabolism of primitive cells would have been exclusively anaerobic. During this era CO_2 may have been the major source of carbon for cells (autotrophy, Section 14.5) because abiotic sources of organic carbon would quickly have become limiting as life became more and more abundant on Earth. Likewise, abiotic sources of fixed nitrogen would have become limiting on the early Earth, and microbes evolved the ability to use atmospheric N_2 as a source of nitrogen (nitrogen fixation, Section 14.6) as early as 3.2 billion years ago, as indicated by isotopic ratios of nitrogen found in ancient sedimentary rocks. The capacity for both autotrophy and nitrogen fixation remain widespread among microorganisms today.

It is widely thought that H_2 was a major fuel for energy metabolism of early cells. This hypothesis is also supported by the tree of life (see Figure 13.9), in that virtually all of the earliest branching organisms in the *Bacteria* and *Archaea* use H_2 as an electron donor in energy metabolism and are also autotrophs. Elemental sulfur (S^0) may have been one of the earliest electron acceptors, as the reduction of S^0 to yield H_2S is exergonic and would likely have required relatively few enzymes (Figure 13.5). Moreover, because of the abundance of H_2 and sulfur compounds on early Earth, this scheme

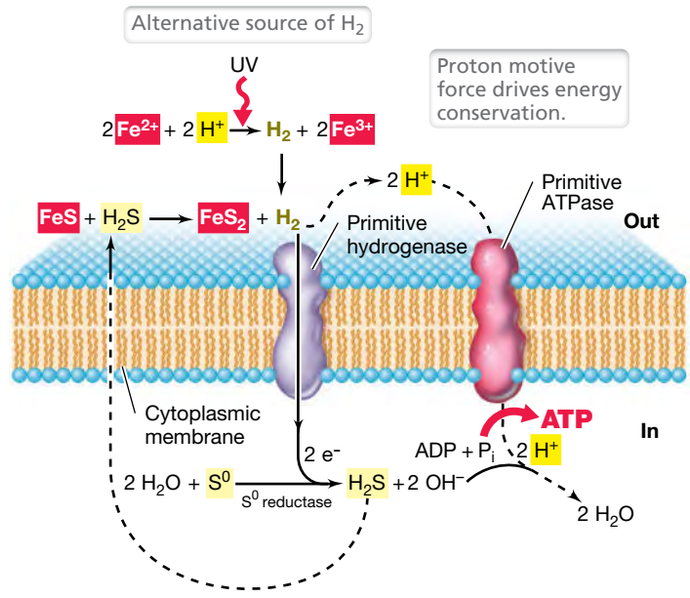


Figure 13.5 A possible energy-conserving scheme for primitive cells. Formation of pyrite (FeS_2) leads to H_2 production and S^0 reduction, which fuels a primitive ATPase. Note how H_2S plays only a catalytic role; the net substrates would be FeS and S^0. Also note how few different proteins would be required. $\Delta G^{0\prime} = -42$kJ for the reaction $FeS + H_2S \rightarrow FeS_2 + H_2$.

would have provided cells with a nearly limitless supply of energy. Other early microorganisms may have used H_2 and CO_2 to produce acetate (Section 14.16) or methane (Section 14.17). These early forms of chemolithotrophic metabolism driven by H_2 would likely have supported the production of large amounts of organic compounds from autotrophic CO_2 fixation. Over time, these organic materials would have accumulated and could have provided the conditions needed for the evolution of new chemoorganotrophic bacteria with diverse metabolic strategies to conserve energy from the oxidation of organic compounds.

Eventually Earth became a highly oxic planet, with its characteristic high levels of O_2 that we breathe today (Figure 13.1). This key geochemical transition in Earth's history was catalyzed by microbes and we explore this topic now.

MINIQUIZ

- What characteristics would have made the surface of Earth inhospitable to the formation of life 4.5 billion years ago?
- How do we know when oceans were first present on Earth? Why is the presence of oceans significant to the origins and diversification of life?
- What lines of reasoning support the hypothesis that the first self-replicating systems were based on RNA molecules?

13.2 Photosynthesis and the Oxidation of Earth

The evolution of photosynthesis was a biological breakthrough that revolutionized the chemistry of Earth. Phototrophic organisms use energy from the sun to oxidize molecules such as H_2S, S^0, or H_2O and to synthesize complex organic molecules from carbon dioxide or simple organic molecules (Section 14.5). Over time, the products of photosynthesis accumulated in the biosphere, stimulating the further diversification of microbial life. Earth's first phototrophs were anoxygenic (cells that do not produce O_2, Sections 14.3 and 15.4–15.7), but from these evolved the *Cyanobacteria*, the earliest O_2-producing (oxygenic phototrophs) (Figure 13.1, Section 15.3).

Fossilized microbial formations called **stromatolites** can be found in rocks that are 3.5 billion years old, providing the earliest conclusive evidence of life on Earth (Figure 13.6). Stromatolites, or "layered rocks," are formed when certain kinds of microbial mats cause the deposition of carbonate or silicate minerals that promote fossilization (we discuss microbial mats in Section 20.5). Stromatolites were diverse and common on Earth between 2.8 and 1 billion years ago, but declined dramatically in abundance over the last billion years. Stromatolites are largely gone from Earth today, and yet modern examples of this ancient microbial ecosystem can still be found in certain shallow marine basins (Figure 13.6*c, e*) or in hot springs (Figure 13.6*d*). Phototrophic bacteria, such as cyanobacteria (Section 15.3) and the green nonsulfur bacterium *Chloroflexus* (Section 15.7), play a central role in the formation of modern stromatolites. Ancient stromatolites contain microfossils that appear remarkably similar to modern species of phototrophic bacteria (Figure 13.7*a*). Hence, the earliest phototrophic organisms may have evolved in the *Bacteria* more than 3.5 billion years ago, giving rise to the stromatolites we observe in the fossil record.

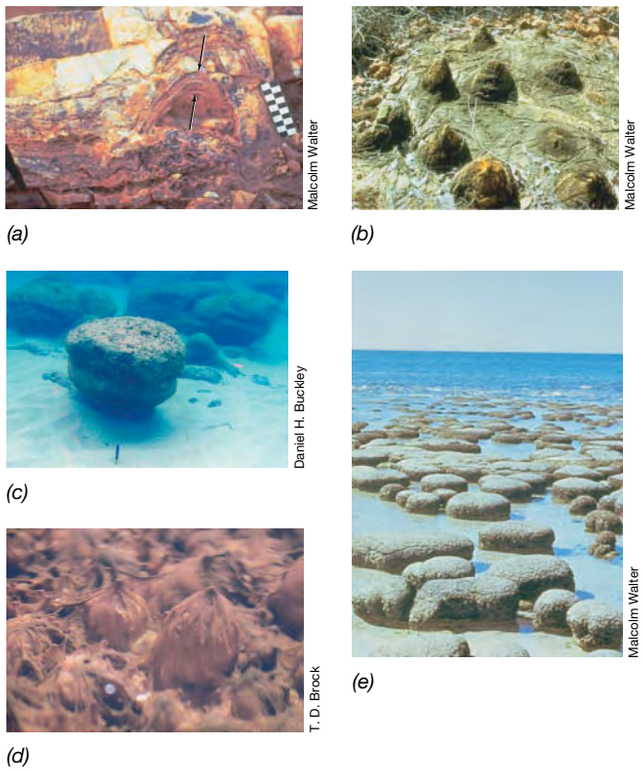
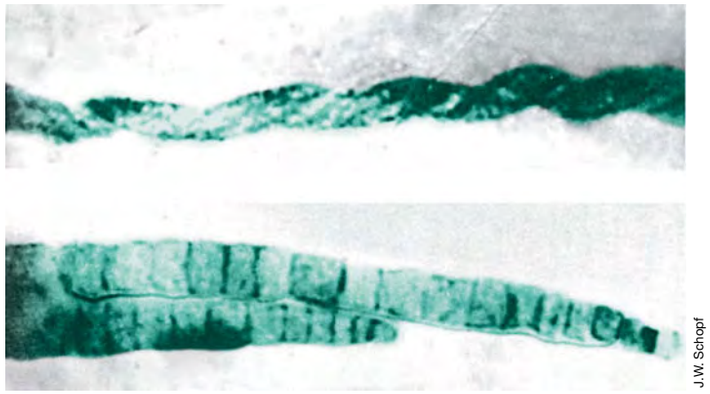


Figure 13.6 Ancient and modern stromatolites. *(a)* The oldest known stromatolite, found in a rock about 3.5 billion years old from the Warrawoona Group in Western Australia. Shown is a vertical section through the laminated structure preserved in the rock. Arrows point to the laminated layers. *(b)* Stromatolites of conical shape from 1.6-billion-year-old dolomite rock from northern Australia. *(c)* Modern stromatolites, Darby Island, Bahamas. The large stromatolite in the foreground is about 1 m in diameter. *(d)* Modern stromatolites composed of thermophilic cyanobacteria growing in a thermal pool in Yellowstone National Park, USA. Each structure is about 2 cm high. *(e)* Modern stromatolites from Shark Bay, Australia. Individual structures are 0.5–1 m in diameter.

The first phototrophs were anoxygenic, using electron donors such as H_2S for CO_2 fixation and generating elemental sulfur (S^0) as a waste product (Section 14.3). The ability to use solar radiation as an energy source allowed phototrophs to diversify extensively. By 2.5–3.3 billion years ago, the cyanobacterial lineage evolved a photosystem capable of oxygenic photosynthesis (Section 14.4) in which H_2O supplanted H_2S as the reductant for CO_2, thereby generating O_2 as a waste product. The origin of oxygenic photosynthesis and the rise of O_2 in Earth's atmosphere caused the greatest change ever in the history of our biosphere and set the stage for the evolution of even newer forms of life that evolved to exploit the energy available from O_2 respiration. We look at the evidence for and consequences of this great oxidation event now.

The Rise of Oxygen: Banded Iron Formations

In the absence of O_2, most of Earth's iron would have been present in reduced forms (Fe^0 and Fe^{2+}) and large amounts of iron would have been dissolved in Earth's anoxic oceans. Molecular and chemical evidence indicates that oxygenic photosynthesis first appeared on Earth several hundred million years before significant levels of O_2 appeared



(a)

(b)

Figure 13.7 More recent fossil bacteria and eukaryotes. *(a)* One-billion-year-old microfossils from central Australia that resemble modern filamentous cyanobacteria. Cell diameters, 5–7 µm. *(b)* Microfossils of eukaryotic cells from the same rock formation. The cellular structure is similar to that of certain modern green algae, such as *Chlorella* species. Cell diameter, about 15 µm. Color was added to make cell form more apparent.

in the atmosphere. However, the O_2 that cyanobacteria produced could not accumulate in the atmosphere because it reacted spontaneously with the reduced iron minerals in the oceans to make iron oxides. By 2.4 billion years ago, O_2 levels had risen to one part per million, a tiny amount by present-day standards, but enough to initiate what has come to be called the *Great Oxidation Event* (Figure 13.1).

The metabolism of cyanobacteria yielded O_2 that oxidized reduced minerals containing Fe^{2+} to iron oxides containing Fe^{3+}. These iron oxide minerals became a prominent marker in the geological record. Iron oxides are poorly soluble in water and would have precipitated in the oceans, raining down onto the seafloor and forming sedimentary structures we see today as **banded iron formations** (Figure 13.8), laminated sedimentary rocks formed in deposits of iron- and silica-rich materials. Much of the iron in rocks of Precambrian origin (>0.5 billion years ago, see Figure 13.1) exists in such banded formations, and today these minerals are mined as a major source of iron ore. During this span of Earth history, lasting more than 1.5 billion years, the presence of precipitating iron minerals could have caused the oceans to appear brown, or black, or even red rather than the blue color we know today. Only after the abundant Fe^{2+} on Earth was oxidized could O_2 begin to accumulate in the atmosphere, and not until 600–900 million years ago did atmospheric O_2 reach present-day levels (~21%, Figure 13.1).

As O_2 accumulated on Earth, the atmosphere gradually changed from anoxic to oxic (Figure 13.1). Species of *Bacteria* and *Archaea* unable to adapt to this change were increasingly restricted to anoxic habitats because of the toxicity of O_2 and because it chemically

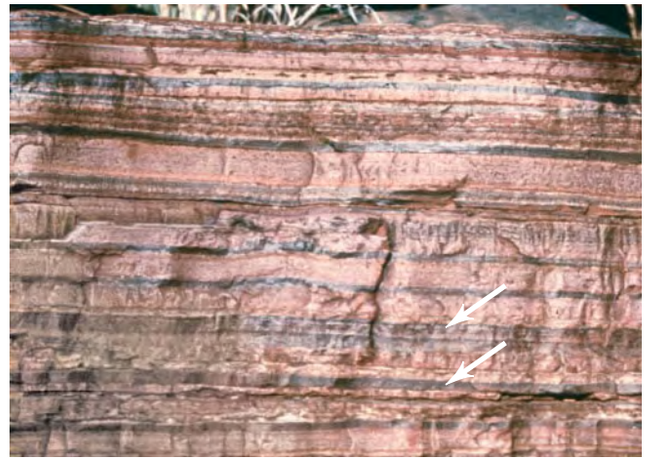


Figure 13.8 Banded iron formations. An exposed cliff made of sedimentary rock about 10 m in height in Western Australia contains layers of iron oxides (arrows) interspersed with layers containing iron silicates and other silica materials. The iron oxides contain iron in the ferric (Fe^{3+}) form produced from ferrous iron (Fe^{2+}) primarily by the oxygen released by cyanobacterial photosynthesis.

oxidized the reduced substances upon which their metabolisms depend. However, the oxic atmosphere also created conditions for the evolution of various new metabolic schemes, such as sulfide oxidation, nitrification, and the various other aerobic chemolithotrophic processes (Chapter 14). Microbes that evolved the capacity to respire O_2 gained a tremendous energetic advantage because of the high reduction potential of the O_2/H_2O couple (Section 3.6), and with more energy at their disposal, aerobes could reproduce far more rapidly than anaerobes.

The Ozone Shield

An important consequence of O_2 for the evolution of life was the formation of *ozone* (O_3). The sun bathes Earth in intense amounts of ultraviolet (UV) radiation, which is lethal to cells and can cause severe DNA damage (Section 11.4). When O_2 is subject to UV radiation from the sun, it is converted to ozone, which strongly absorbs UV radiation in wavelengths up to 300 nm. The conversion of O_2 to O_3 creates an *ozone shield*, a barrier that protects the surface of Earth from much of the UV radiation from the sun. Prior to the generation of the ozone shield, the punishing UV irradiation from the sun would have made Earth's surface fairly inhospitable for life, restricting life to environments that provided protection from UV radiation, such as in the oceans or in the subsurface. However, as Earth developed an ozone shield, organisms could range over the terrestrial surface of Earth, exploiting new habitats and evolving ever-greater diversity. Figure 13.1 summarizes some landmarks in biological evolution and Earth's geochemistry as Earth transitioned from an anoxic to a highly oxic planet.

MINIQUIZ

- Why is the origin of cyanobacteria considered a critical step in evolution?
- What caused the development of banded iron formations?
- What lines of evidence indicate that microbial life was present on Earth 3.5 billion years ago?

13.3 Living Fossils: DNA Records the History of Life

The evolutionary origins of microorganisms remained a mystery until it was discovered that certain molecular sequences are a record of evolutionary history (Section 1.13). Mutations occur at random and accumulate over time, causing heritable changes in the sequence of DNA (Chapter 11); this ultimately results in evolution. Organisms that share a recent ancestor have similar DNA sequences, and organisms that are more distantly related have more dissimilar DNA sequences. Hence, we can reconstruct the evolutionary history, or **phylogeny**, of a set of related DNA sequences by analyzing similarities in their nucleotide sequences (Section 13.7).

Carl Woese and the Tree of Life

Carl Woese was the first to construct a **universal tree of life** that he inferred from nucleotide sequence similarity in the **ribosomal RNA (rRNA)** genes of diverse organisms (Section 1.13).

The universal tree of life (Figure 13.9) is a genealogy of life on Earth. It depicts the evolutionary history of all cells and clearly reveals the three-domain concept in which all cells can be classified as *Bacteria*, *Archaea*, or *Eukarya*. The root of the universal tree represents a point in time when all extant life on Earth shared a common ancestor, the last universal common ancestor, LUCA (Figures 13.4 and 13.9). The universal tree of life shows that the first living things were microorganisms, and that microbes were the dominant life form for most of the history of life on Earth.

Genomics (Chapter 9) supports the three-domain concept through phylogenetic analysis of most genes central to cellular function. For example, at least 60 genes (including rRNA genes) are shared by nearly all extant cells and these genes must have been present in the universal ancestor. Most of these genes encode core functions in transcription, translation, and DNA replication. Across these conserved genes, those of *Eukarya* show greater sequence similarity to those of *Archaea* than to those of *Bacteria*. These and other data support the conclusion that the *Bacteria* and *Archaea* diverged prior to the origin of eukaryotic cells (Figure 13.9).

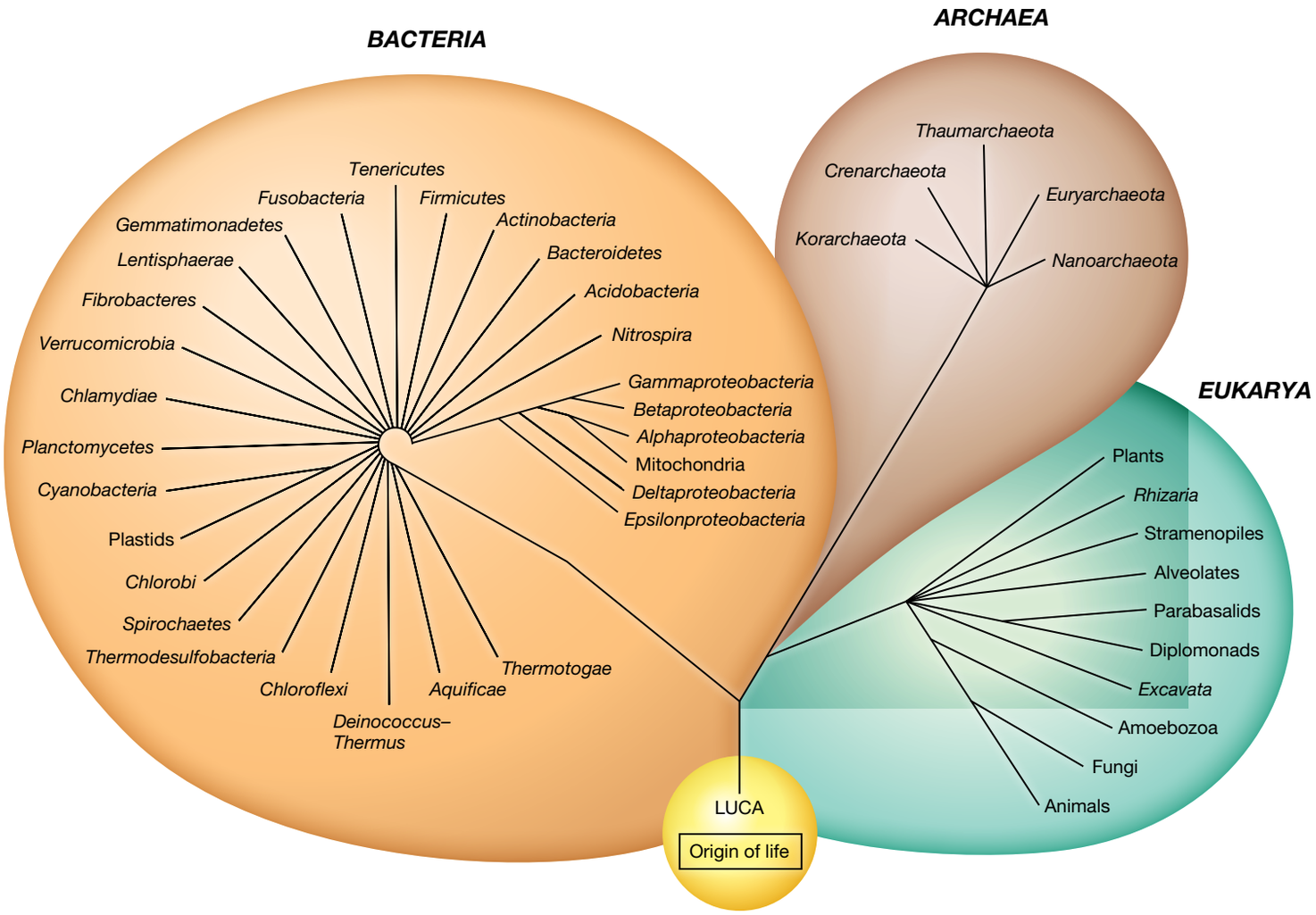


Figure 13.9 Universal phylogenetic tree as supported by comparative SSU rRNA gene sequence analysis. Only a few key organisms or lineages are shown in each domain. The branch lengths in this tree are arbitrary and nodes have been collapsed to reflect phylogenetic uncertainty. At least 84 phyla of *Bacteria* have now been identified, although many of these have not yet been cultured. LUCA, last universal common ancestor (Figure 13.4).

Hence, phylogenetic analyses of genome sequences suggest that LUCA had a prokaryotic cell structure along with a DNA-based genome and the ability to transcribe genes and synthesize proteins (Figure 13.9).

Other Influences Affecting Phylogeny

The manner in which the three domains were established remains a topic of debate. It is clear that the three domains represent the major evolutionary cell lineages that exist on Earth, and it is also clear that *Bacteria* and *Archaea* diverged prior to the divergence of *Eukarya* from *Archaea*. However, there are many examples of genes shared by any two of the three domains. One hypothesis is that early in the history of life, before the primary domains had diverged, **horizontal gene transfer** (↔ Chapter 11) was extensive. During this time, any genes that provided a strong benefit may have been transferred rapidly among early forms of life (Figure 13.4). As the domains continued to diverge over time, barriers to unrestricted horizontal gene transfer likely evolved in order to maintain genomic stability (↔ Section 11.12). As a result, the previously promiscuous cell population began to slowly sort out into the primary lines of evolutionary descent (Figures 13.4 and 13.9).

It seems likely that the domains *Bacteria* and *Archaea* had already diverged by about 3.7 billion years ago (Figure 13.1). Following this, there was a further bifurcation, perhaps 1.2 to 2.7 billion years ago, at which time the *Eukarya* diverged from *Archaea* to form a distinct domain. As each domain continued to evolve, certain traits became fixed within each group, giving rise to a multitude of genetic, physiological, and structural differences (Chapters 14–18). After nearly 4 billion years of microbial evolution, we see the grand result: three domains of cellular life that are each evolutionarily distinct and yet share certain features that reveal their common descent from a universal cellular ancestor.

Before we turn our attention to the evolutionary process per se, we focus briefly on the *Eukarya*, a phylogenetically distinct group whose eukaryotic cell structure contrasts with the prokaryotic cell structure of the *Bacteria* and *Archaea*. However, the eukaryotic cell we know today (↔ Sections 2.14–2.16) has been shaped in part by prokaryotic cells, and we now explore how this might have occurred.

MINIQUIZ

- What kinds of evidence support the three-domain concept of life?
- What is LUCA and what are some of its characteristics?
- Which of the three domains is the least ancient?

13.4 Endosymbiotic Origin of Eukaryotes

The divergence of the *Eukarya* from the domain *Archaea* marked a major milestone in cellular evolution as the origin of a membrane-enclosed nucleus and organelles gave rise to eukaryotic cell structure. Here we consider the origin of the *Eukarya* and show how eukaryotes are genetic chimeras containing genes from at least two different phylogenetic domains. A recent hypothesis for the origin of eukaryotes from within the *Archaea* was presented on page 399.

Endosymbiosis

As Earth became more oxic, organelle-containing eukaryotic microorganisms arose, and the rise in O_2 likely had a major impact on their evolution. While the exact origins of the eukaryotic cell remain unclear, the oldest microfossils that have recognizable nuclei are about 2 billion years old. Multicellular and increasingly complex microfossils of algae are evident from 1.9 to 1.4 billion years ago (Figure 13.7*b*). By 0.6 billion years ago, with O_2 near present-day levels, large multicellular organisms, the Ediacaran fauna, were present in the sea (Figure 13.1). In a relatively short time, multicellular eukaryotes diversified into the ancestors of modern-day algae, plants, fungi, and animals.

A well-supported explanation for the origin of organelles in the eukaryotic cell is the **endosymbiotic hypothesis** (Figure 13.10). This hypothesis, for which supporting evidence today is so strong that its basic tenets can be considered more like a theory than a hypothesis, states that the mitochondrion of modern-day eukaryotes arose from the stable incorporation of a bacterium capable of aerobic respiration into the cytoplasm of early eukaryotic cells. Endosymbiotic mitochondria were beneficial to these early eukaryotic cells because they increased the cell's respiratory capacity, and thus these early mitochondria-containing cells proceeded to become the ancestors of all living *Eukarya*. Virtually all eukaryotic cells have mitochondria, though these structures were subsequently lost in certain lineages of anaerobic microbial eukaryotes (Chapter 18).

There was a second endosymbiotic event that also had a major impact on the evolution of life. Chloroplasts arose from the stable incorporation of a cyanobacterium-like cell into the cytoplasm of a eukaryotic lineage, and this endosymbiotic event triggered the origin of photosynthesis within *Eukarya* (Figure 13.10). All phototrophic eukaryotes, including plants and algae, have descended from the lineage of cells that acquired endosymbiotic chloroplasts. Atmospheric oxygen is intimately associated with the endosymbiotic origins of organelles, being consumed by the ancestor of the mitochondrion and being produced by the ancestor of the chloroplast. These endosymbiotic events diversified the metabolism of early eukaryotic cells, with mitochondria providing aerobic respiration and chloroplasts providing the ability to exploit sunlight for energy. The endosymbiotic origin of organelles set the stage for the diversification of *Eukarya* into the forms we know today.

The overall physiology and metabolism of mitochondria and chloroplasts and the sequence and structures of their genomes support the endosymbiotic hypothesis (↔ Section 9.4). For example, both mitochondria and chloroplasts contain ribosomes the size of those in *Bacteria* and *Archaea* (70S), including a 16S ribosomal RNA (**16S rRNA**) molecule. The 16S rRNA gene sequences of mitochondria and chloroplasts are also characteristic of those from *Bacteria*, and sequence analyses place the ancestor of mitochondria in the **phylum** *Alphaproteobacteria*, and the ancestor of chloroplasts in the phylum *Cyanobacteria* (Figure 13.9). Moreover, the same antibiotics that inhibit ribosome function in free-living *Bacteria* inhibit ribosome function in these organelles. Mitochondria and chloroplasts also contain small amounts of DNA arranged in a covalently closed, circular form, which is typical of *Bacteria*,

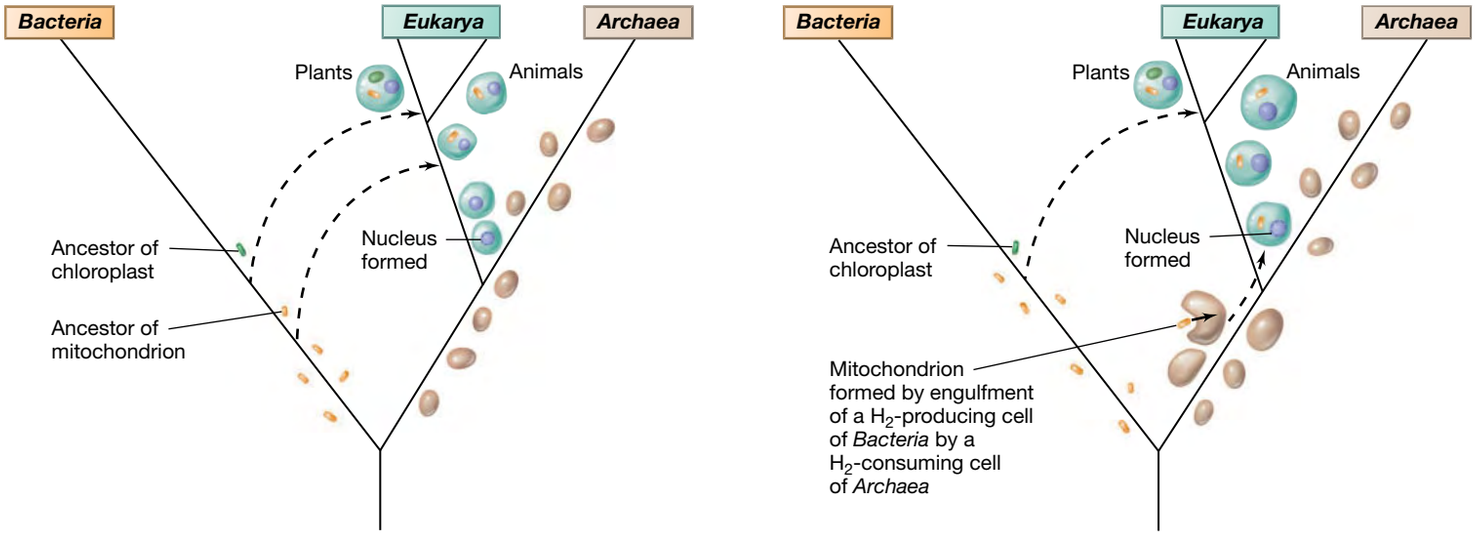


Figure 13.10 Endosymbiotic models for the origin of the eukaryotic cell. *(a)* The serial endosymbiosis hypothesis proposes that the eukaryotic ancestor diverged from the archaeal line and possessed a nucleus and other features of eukaryotic cells prior to endosymbiosis with the bacterial ancestor of the mitochondrion. A later endosymbiosis with the cyanobacterial ancestor of the chloroplast gave rise to the eukaryotic ancestor of all plants and all other photosynthetic eukaryotes. *(b)* The hydrogen hypothesis (which is a version of the symbiogenesis hypothesis) proposes that the eukaryotic cell evolved from a symbiotic relationship between H_2-producing cells of *Bacteria* and H_2-consuming cells of *Archaea*. The bacterial partner was eventually engulfed by its archaeal partner and evolved over time into the mitochondrion. The nucleus and other features of the eukaryotic cell evolved after establishment of endosymbiosis. A later endosymbiosis with the cyanobacterial ancestor of the chloroplast gave rise to the eukaryotic ancestor of all plants and all other photosynthetic eukaryotes. Note the position of the mitochondrion and plastids (chloroplasts are a type of plastid) on the universal phylogenetic tree (Figure 13.9).

and the phylogeny of these gene sequences indicates a bacterial ancestry. Indeed, these and many other telltale signs of *Bacteria* are present in organelles from modern eukaryotic cells.

Formation of the Eukaryotic Cell

The exact pathway by which the eukaryotic cell emerged remains a major unresolved question in evolution; however, it seems clear that the modern eukaryotic cell genome is a genetic chimera, made up of genes from both *Bacteria* and *Archaea*. In eukaryotes, most of the genes that encode information-processing machinery resemble those of *Archaea*, while most metabolic genes resemble those of *Bacteria*. For example, eukaryotic cells share with *Archaea* molecular features of transcription and translation (Chapter 4), while features they share with *Bacteria* include their ester-linked membrane lipids (Chapter 2) and their glycolytic pathway (Chapter 3). As we have seen, there is strong support for the endosymbiotic origin of mitochondria and chloroplasts from *Bacteria*, and it is clear that certain genes from these endosymbionts were transferred to the cell nucleus (Section 9.4).

Two major hypotheses have been put forward to explain the formation of the eukaryotic cell (Figure 13.10). In the *serial endosymbiosis hypothesis*, eukaryotes arose as a nucleus-bearing cell line that split from the *Archaea* and later acquired mitochondria and chloroplasts by endosymbiosis (Figure 13.10*a*). Endosymbiosis is posited to have occurred when this cell line engulfed a bacterial cell that, rather than being destroyed, managed to survive and replicate as an endosymbiont within the cytoplasm of the host cell. According to this hypothesis, eukaryotic genes that resemble those of *Bacteria* were acquired in gene transfers from the endosymbiont to the nuclear genome. A major problem with this hypothesis is that it does not easily account for the fact that *Bacteria* and *Eukarya* have structurally similar membrane lipids, in contrast to the lipids of *Archaea* (Section 2.6).

The second hypothesis, called the *symbiogenesis hypothesis*, proposes that the eukaryotic cell arose from a symbiotic relationship between cells of *Bacteria* and *Archaea* that ultimately resulted in engulfment of the bacterial partner to form mitochondria. One version of this hypothesis is the *hydrogen hypothesis*, which proposes that the eukaryotic cell arose from an association between a H_2-producing species of *Bacteria* and a H_2-consuming species of *Archaea* (Figure 13.10*b*). In the hydrogen hypothesis, the bacterial cell was a facultative aerobe and a chemoorganotroph that could grow by aerobic respiration or by syntrophic production of H_2 (Section 14.23). The archaeal partner was an anaerobic chemolithotroph that required H_2 for growth. These symbiotic partners could have coevolved for an extended period prior to bacterial engulfment and formation of the mitochondria. The nucleus arose after endosymbiosis as genes for lipid biosynthesis were transferred from the symbiont to the host. The transfer of these bacterial genes allowed the host to synthesize lipids containing fatty acids, lipids that may have been conducive to the formation of internal membranes, such as the nuclear membrane system (Section 2.14).

The origin of the nucleus was critical to the evolution of the eukaryotic cell, yet it remains unclear whether the nucleus appeared before or after the endosymbiotic origin of mitochondria. One hypothesis for the origin of the nucleus is that its formation is associated with the evolution of RNA processing in

Eukarya. In contrast to the genes of *Bacteria* and *Archaea*, eukaryotic genes frequently contain introns, which must be removed prior to translation (Section 4.6). For proper gene expression to occur in *Eukarya*, RNA splicing must occur prior to translation. Eukaryotic cells have a molecular complex called the *spliceosome* that performs RNA splicing and operates in the cell nucleus. Hence, the nuclear membrane may have evolved in *Eukarya* as a mechanism to separate spliceosomes in the nucleus from ribosomes in the cytoplasm.

In the next section we trace the evolutionary path of both eukaryotic and prokaryotic cells in detail. Analyses of molecular evolution provide direct evidence of the evolutionary history of cells, leading to the modern "tree of life."

MINIQUIZ

- What evidence supports the idea that the mitochondrion and chloroplast were once free-living members of the domain *Bacteria*?
- In what ways are modern eukaryotes a combination of attributes of *Bacteria* and *Archaea*?
- Describe the different hypotheses for the formation of the eukaryotic cell.

II • Microbial Evolution

While many of the basic principles of evolution are conserved across all domains of life, certain aspects of microbial evolution are uncommon in plants and animals. For example, *Bacteria* and *Archaea* are generally haploid and asexual, they have several mechanisms for horizontal gene transfer that result in the asymmetrical exchange of genetic material uncoupled from reproduction, and their genomes can be remarkably heterogeneous and highly dynamic. We now consider the processes that cause the diversification of microbial lineages and how these forces affect the evolution of microbial genomes.

13.5 The Evolutionary Process

In its simplest form, evolution is a change in **allele** frequencies in a population of organisms over time resulting in descent with modification. Alleles are alternate versions of a given gene. New alleles arise as a result of mutation and recombination, and changes in allele frequencies can occur through a variety of processes. We see here how these simple mechanisms give rise to the origin and divergence of microbial species.

Origins of Genetic Diversity

As we have seen, **mutations** are random changes in DNA sequence that accumulate over time, and they are a fundamental source of the natural variation that drives the evolutionary process. Most mutations are neutral or deleterious, though some can be beneficial. Mutations take several forms including *substitutions, deletions, insertions*, and *duplications* (Chapter 11). Duplication events produce a redundant copy of a gene that can be modified by further mutation without losing the function encoded by the original gene (Section 9.5). Hence, duplications allow for the diversification of gene function.

Recombination is a process by which segments of DNA are broken and rejoined to create new combinations of genetic material (Section 11.5). Recombination can cause reassortment of genetic material already present in a genome and is also required for the integration into the genome of DNA acquired through horizontal gene transfer. Recombination can be broadly classified as either *homologous* or *nonhomologous*. Homologous recombination requires short segments of highly similar DNA sequence flanking the region of DNA being transferred (Section 11.5). By contrast, nonhomologous recombination is mediated by several mechanisms that share in common the fact that they do not require high levels of sequence similarity to initiate successful DNA integration.

Selection and Genetic Drift

New alleles result when mutation and recombination cause variation in gene sequences. Evolution occurs when different alleles change in frequency in a population over a span of many generations. Evolutionary biologists have described many different mechanisms that can govern this evolutionary process, but chief among them are the forces of *selection* and *genetic drift*.

Selection is defined on the basis of **fitness**, the ability of an organism to produce progeny and contribute to the genetic makeup of future generations. Most mutations are *neutral* with respect to fitness and they have no effect on the cell, as a result of the degeneracy of the genetic code (Section 4.9). These mutations generally accumulate in DNA over time. Some mutations are *deleterious*; these decrease the fitness of an organism by disrupting gene function. Deleterious mutations are generally purged from populations over time by natural selection. Some mutations can be *beneficial*, increasing the fitness of an organism, and these mutations are favored by natural selection, increasing in frequency in a population over time. An example of a beneficial mutation would be a mutation that induces antibiotic resistance in a pathogenic bacterium infecting a person undergoing antibiotic therapy. It is important to remember that all mutations occur by chance; the selective nature of the environment does not *cause* adaptive mutations but simply *selects* for the growth and reproduction of those organisms that have incurred mutations that provide a fitness advantage.

While Darwin proposed natural selection as the mechanism by which gene frequencies change over time, evolutionary change can occur through mechanisms other than selection. A chief example is **genetic drift** (Figure 13.11), a random process that can cause gene frequencies to change over time, resulting in evolution in the absence of natural selection. Genetic drift occurs because some members of a population will have more offspring than others simply as a result of chance; over time these chance events can result in evolutionary change in the absence of selection. Genetic drift is most powerful in small populations and in populations that experience frequent "bottleneck" events. The latter occur when a population experiences a severe reduction in population size followed by regrowth from the cells that remain. For example, genetic drift can be very important in the evolution of pathogens because each new infection is caused by a small number of cells colonizing a new host. Hence, pathogen populations can change rapidly as a result of random genetic drift (Figure 13.11).

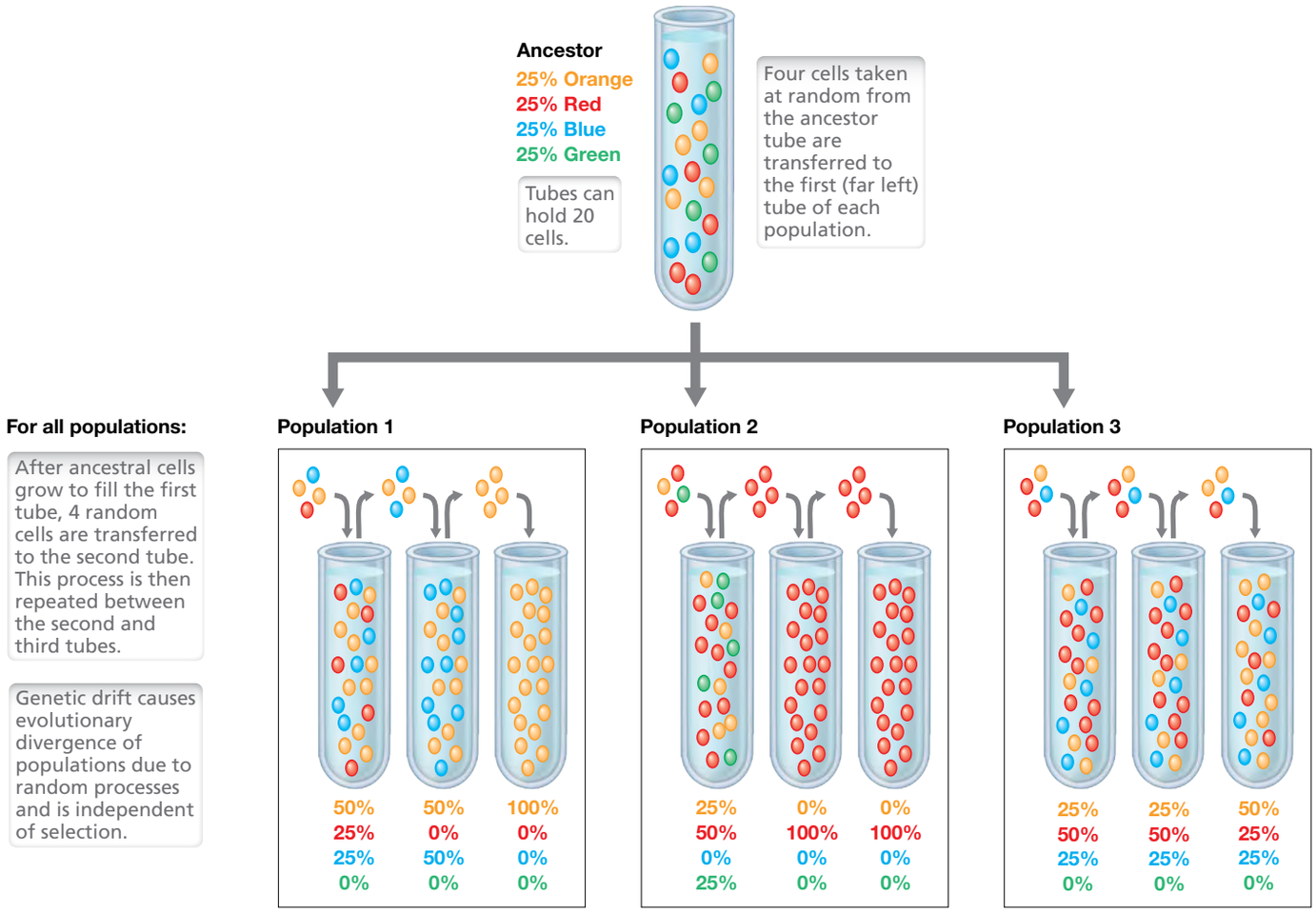


Figure 13.11 Genetic drift. Genetic drift is a random process that can cause gene frequencies in a population to change over time, causing evolution without natural selection. In this example, a population containing four different bacterial genotypes (indicated by different colors), each at equal frequency, is present in the ancestor tube. Four cells at random are then transferred to each of three new tubes and the cells allowed to grow to fill each tube. There is no difference in fitness between the cells and so they grow equally. Cells taken at random are then transferred in two successive rounds. Striking differences in genotype frequencies between the populations are observed after only three rounds of transfers.

New Traits Can Evolve Quickly in Microorganisms

A change in the environment or the introduction of cells to a new environment can cause rapid evolutionary changes in microbial populations. Microorganisms typically form large populations and can reproduce quickly, producing a new generation in as few as 20 minutes for some species, and thus evolutionary events in microbial populations can often be observed in the laboratory on relatively short time scales. The heritable variation already present in a population provides the raw material upon which natural selection acts following such a change in the selective environment. Here we consider two examples of rapid evolutionary change in bacteria, one involving the rapid loss of a characteristic trait in *Rhodobacter*, and one involving the acquisition of a new trait in *Escherichia coli*.

Rhodobacter is a phototrophic purple bacterium that carries out anoxygenic photosynthesis (↬ Section 14.3) in illuminated anoxic environments in nature. When cultured anaerobically in either the light or the dark, the cells synthesize bacteriochlorophyll and carotenoids because it is the absence of O_2, not the presence of light, that triggers pigment synthesis. In the light these pigments participate in photosynthetic reactions that lead to ATP synthesis, but in darkness, the pigments provide no benefit to the cell. Random mutations occasionally generate *Rhodobacter* cells that produce either reduced levels of photopigments or no photopigments at all. In nature, the ability to carry out photosynthesis is of significant value and such pigment mutants cannot compete with wild-type cells. However, when cultured in constant darkness, wild-type cells have no such advantage. In darkness, mutants that produce reduced levels of photopigments gain the advantage because they do not waste energy and resources synthesizing photopigments that provide them no benefit. As a result, photopigment mutants quickly take over a *Rhodobacter* culture that is maintained in darkness for several generations because the mutants quickly become the fittest organisms in the population and therefore enjoy the greatest reproductive success (**Figure 13.12**). Mutations affecting photosynthesis occur at the same rate in the light as in the dark, but in the light the selection for the wild-type phototrophic

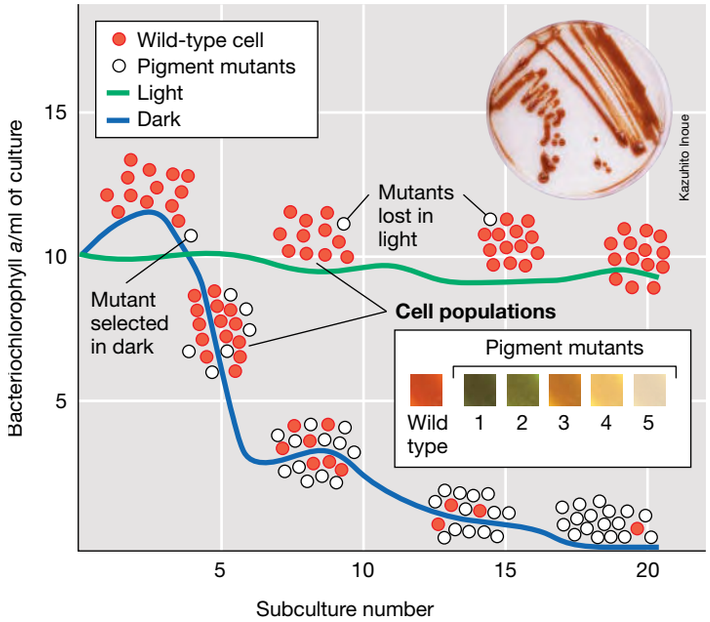
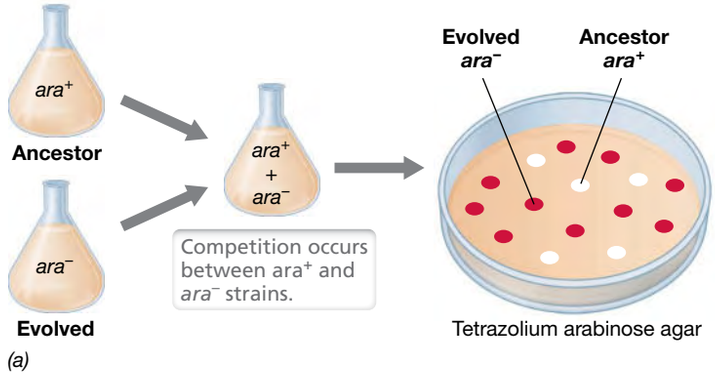


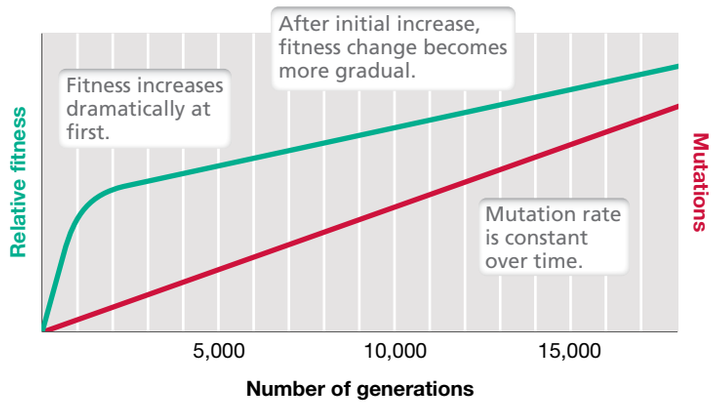
Figure 13.12 Survival of the fittest and natural selection in a population of phototrophic purple bacteria. Serial subculture of the purple bacterium *Rhodobacter capsulatus* in the light (green line) provides a benefit to wild-type phototrophic strains and they outcompete nonphototrophic mutants. In the dark (blue line), however, phototrophy provides no benefits and nonphototrophic mutants quickly outcompete wild-type cells still making bacteriochlorophyll and carotenoids. Photos: top, plate culture showing colonies of phototrophic cells of *R. capsulatus*; bottom, close-up photos showing the color of colonies of wild type and five pigment mutants (1–5) obtained during serial dark subculture. Wild-type cells are reddish-brown from their assortment of carotenoid pigments. The color of mutant colonies reflects the absence (or reduced synthesis) of one or more carotenoids. Mutant strain 5 lacked bacteriochlorophyll and was no longer able to grow phototrophically. Mutant strains 1–4 could grow phototrophically but at reduced growth rates from the wild type. Data adapted from Madigan, M.T., et al. 1982. *J. Bacteriol. 150:* 1422–1429.

phenotype is so strong that photopigment mutants are quickly lost from the population.

Experimental evolution is a growing field of study enabled by the rapid growth of bacterial populations and the ability to preserve bacteria indefinitely by freezing. The latter makes it possible to maintain a living "fossil record" of ancestral organisms that can be thawed later and compared to evolved strains. For example, the *E. coli* long-term evolution experiment (LTEE), which has been running since 1988, has tracked the evolution of 12 parallel lines of *E. coli* through more than 50,000 generations. The *E. coli* LTEE cultures have been grown aerobically on a minimal medium with glucose as a sole source of carbon and energy. *E. coli* is typically propagated in a rich medium that contains an excess of all the nutrients cells need to grow, and so the minimal glucose medium used in the LTEE represents a new adaptive environment in which *E. coli* can evolve over time.

In the LTEE, both the ancestor and the evolved lines were genetically engineered to contain a neutral marker that made their colonies either red or white. The marker made it possible to measure the fitness of evolved strains relative to the ancestor by competing them against one another (**Figure 13.13*a***). Genome sequencing during the experiment revealed that mutations accumulated randomly over time in the evolved lines. However, the relative fitness of the evolved lines on minimal glucose medium increased dramatically over the first 500 generations as a result of selection acting on mutations beneficial in this new environment (Figure 13.13*b*). The fitness of the evolved lines continued to increase,





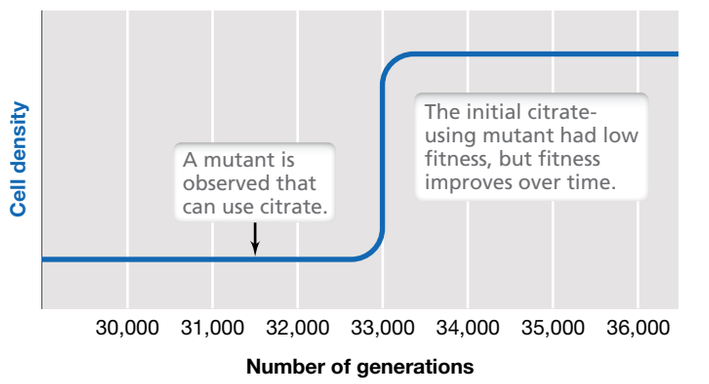


Figure 13.13 Long-term evolution of *Escherichia coli*. *(a)* In the *E. coli* long-term evolution experiment (LTEE), ancestral and derived lines differ in a mutation that affects their ability to use arabinose, allowing them to be differentiated by their colony color when grown on tetrazolium arabinose agar. *(b)* Competition experiments between evolved and ancestral strains show that relative fitness in minimal glucose media increases dramatically for evolved lines. *(c)* The ability to use citrate aerobically evolved in one of 12 LTEE lines. Cells growing on minimal glucose typically grow to low cell density, but the ability to use both glucose and citrate allowed the mutant cell line to reach significantly higher cell densities. Relative fitness is a measure of the growth rate of the evolved strain to that of the ancestral strain.

albeit at a reduced rate, as a result of further selection over the course of the experiment. Most remarkably, after 31,500 generations, one of the evolved lines obtained the ability to use citrate as an energy source (Figure 13.13*c*). Citrate was present as a pH buffer in the growth medium and was not considered a potential carbon source for *E. coli* because the inability to grow aerobically on citrate is a diagnostic trait for *E. coli*. However, the random accumulation of mutations in this one evolved line modified preexisting genes in such a way as to allow for the evolution of a new adaptive trait. The diverged strains can now exploit a new resource that was unavailable to the ancestral population. Since they can now catabolize both citrate and glucose, these cells grow to higher cell density than the ancestor (Figure 13.13*c*). The fact that only one of the 12 parallel lines evolved the ability to grow on citrate demonstrates the chance nature of evolution.

The transitions shown in these experiments remind us of how quickly evolutionary pressures can shift even major properties (such as metabolic strategies) of a microbial cell population. In the case of *Rhodobacter*, a mutation that is deleterious in the wild provides a selective advantage when the organism is grown in the laboratory in a continuously dark environment. Under this new condition, evolution causes *Rhodobacter* to lose unneeded metabolic machinery. In the case of *E. coli*, the accumulation of random mutations allows for the accumulation of genetic diversity in a population. Billions of different mutations were sampled by the population over thousands of generations and some rare combination of mutations, by chance, gave the cells the ability to exploit citrate as a resource. Natural variation caused by chance mutation generated a new trait, the ability to use citrate, and since the environment in which the cells were grown happened to contain citrate, this mutation provided a selective advantage to those cells. In the absence of citrate, these mutations would still occur at the same rate. However, in the absence of a selective benefit, cells able to use citrate would likely disappear from the population over time.

Speciation of Microorganisms Can Take a Long Time

Microbial species can possess a wide variety of individuals with diverse traits. As we discussed above, microorganisms can evolve new traits with remarkable speed, and as a result, microbial species can be genetically and phenotypically diverse. Sequence changes can be used as a **molecular clock** to estimate the time since two lineages have diverged. Major assumptions of the molecular clock approach are that nucleotide changes accumulate in a sequence in proportion to time, that such changes are generally neutral and do not interfere with gene function, and that they are random.

Molecular clock estimates are most reliable when they can be calibrated with evidence from the geological record. Molecular clock estimates can be calibrated using obligate bacterial symbionts of insects (Section 23.6) for which the insect host provides a suitable fossil record to date evolutionary events. From such calculations it is possible to estimate the divergence of microbial species based on dissimilarity in their gene sequences. For example, molecular clock estimates indicate that two well-characterized strains of *E. coli*, the harmless strain K-12 and the foodborne pathogenic strain O157:H7 (Section 32.11), diverged about 4.5 million years ago. Likewise, it is estimated that the closely related species *E. coli* and *Salmonella enterica* (*typhimurium*), which have 2.8% dissimilarity in their 16S rRNA genes, last shared a common ancestor some 100–140 million years ago. Hence, although microbes can evolve new traits rapidly, microbial speciation appears to take a very long time.

MINIQUIZ

- What are the different processes that give rise to genetic variation?
- What is the difference between selection and genetic drift and how do they promote evolutionary change?
- In the experiment of Figure 13.12, why did the dark cell population lose its pigments?

13.6 The Evolution of Microbial Genomes

The dynamic nature of microbial genomes was revealed in dramatic fashion when the first genomes were sequenced from multiple strains of a single species. Genome sequencing of *Escherichia coli* strain K-12 and two pathogenic strains showed that only 39% of their genes were shared among all three genomes (**Figure 13.14**)!

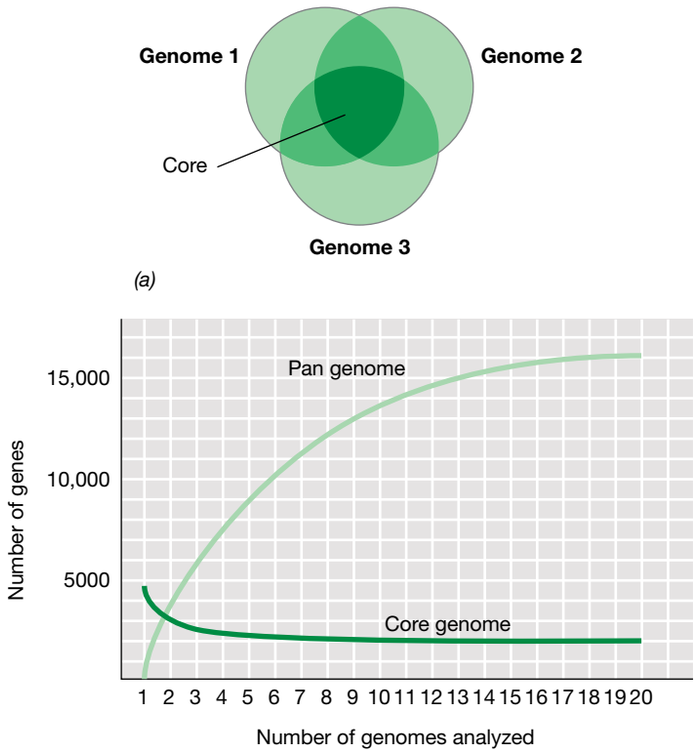


Figure 13.14 The core and pan genome concept. Microbial genomes are dynamic and heterogeneous. The first three genomes sequenced from different strains of *Escherichia coli* were found to have only 39% of their genes in common. The core genome is considered the set of genes that are shared by all members of a species (darkest green in part *a*), while the pan genome is the totality of all genes found in the different strains of a species (all the genes in genomes 1–3 in part *a*). The size of the core and pan genome can vary between species. In *E. coli* the core genome is composed of approximately 1976 genes *(b)*. The size of the pan genome in *E. coli* is not fixed, as each different strain has a unique complement of genes acquired from horizontal gene exchange. Data adapted from Touchon, M., et al. 2009. *PLoS Genetics 5:*(1) e1000344.

The three genomes varied in size by more than a million base pairs in length and each contained a unique and diverse complement of genes acquired through horizontal gene transfer (Figure 9.20).

Genomes of many microbial species have now been examined in this way and the studies have revealed that genes from different strains of a single microbial species can be placed into two classes: the **core genome**, genes shared by all strains of a species, and the **pan genome**, the core genome plus genes that are not shared by all strains of a species and which are often acquired through horizontal gene transfer (see Figure 13.21). We previously introduced the core/pan genome concept (Section 9.7) and here we consider the forces that drive these patterns of genome evolution.

The Dynamic Nature of the *Escherichia coli* Genome

Escherichia coli genomes have on average 4721 genes, with individual strains having as few as 4068 or as many as 5379 total genes. The core genome consists of only 1976 genes present in all strains, accounting for less than half the genes present in the average *E. coli* genome. The size of the core genome can be expected to decrease as the evolutionary distance of strains increases. Taking this to its extreme, only about 60 genes are predicted to be universally present in all species of *Bacteria* and *Archaea* (Section 13.3).

The number of unique genes observed in *E. coli*, and the size of its pan genome, continues to increase indefinitely as each new strain of *E. coli* has its genome sequenced. For example, a survey of genomes from 20 *E. coli* strains revealed a total of 17,838 unique genes (Figure 13.14*b*). Subtracting the contribution from the core genome, this indicates more than 15,862 genes not shared by all strains. A great many of these genes have clearly been obtained from horizontal gene transfers rather than through vertical patterns of inheritance (from mother cell to daughter cell). Patterns of gene exchange appear to be governed by phylogenetic distance, with rates of gene exchange declining as the phylogenetic distance increases between genomes. In the core genome of *E. coli*, for example, most horizontal gene transfer takes place between close relatives and occurs by homologous recombination of DNA segments of 50 to 500 base pairs in length.

Genome analysis reveals that the core and pan genome concept is a general feature of microbial genomes, though the relative number of genes present in each pool can vary widely between species. The dramatic change in genome size and gene content between strains of a single species indicates that microbial genomes are highly dynamic; that is, genomes can shrink or enlarge relatively quickly over time. Moreover, the existence of a pan genome suggests that prokaryotic cells regularly sample genes from other microbes in their environment through horizontal gene transfers. Variations between genomes then arise through the forces of mutation and recombination, with the evolutionary dynamics of genomes being governed by selection and genetic drift.

Gene Deletions in Microbial Genomes

Gene deletions play an important role in microbial genome dynamics (see Explore the Microbial World, "The Black Queen Hypothesis"). Deletions occur with far greater frequency than insertions in microbial genomes, and this bias toward deletions is the force that maintains the small size of many microbial genomes. Selection is the main force that counters the effect of deletions, preserving those genes that provide a fitness benefit to the cell.

It is common in prokaryotic cells for nonessential and nonfunctional materials to be deleted over evolutionary time (Figure 13.12), which is why the genomes of *Bacteria* and *Archaea* are tightly packed with genes and contain relatively few noncoding sequences (Section 9.3). Most genes acquired by horizontal gene transfer, like most mutations in general, can be expected to be neutral or deleterious to the cell. Hence, it is likely that of the new genes acquired from the environment, those that do not convey a fitness benefit are deleted from the genome over time. In addition, genetic drift (Figure 13.11) can promote deletion events when population sizes are small or when populations pass through a bottleneck. Deletions are also thought to be a driving force for the tiny genomes observed in many obligate intracellular symbionts and bacterial pathogens (Sections 9.3 and 23.6). The genomes of these bacteria can afford to be streamlined because several of the key metabolites each needs are provided to them by their host organisms.

MINIQUIZ

- What is the difference between the core and pan genomes of a given species?
- What kind of recombination might have the greatest impact on the core genome?
- What effects do deletions have on the evolution of microbial genomes?

III • Microbial Phylogeny and Systematics

Systematics is the study of the diversity of organisms and their relationships. It links phylogeny with **taxonomy**, the science in which organisms are characterized, named, and classified according to defined criteria.

Bacterial taxonomy has changed substantially in the past few decades, embracing a combination of methods for the identification of bacteria and description of new species. This *polyphasic approach* to taxonomy uses three kinds of methods—*phenotypic, genotypic*, and *phylogenetic*—for the identification and description of bacteria. Phenotypic analysis examines the morphological, metabolic, physiological, and chemical characteristics of the cell. Genotypic analysis considers characteristics of the genome. These two kinds of analysis categorize organisms based on similarities. They are complemented by phylogenetic analysis, which seeks to place organisms within an evolutionary framework using molecular sequence data. We begin by exploring how molecular sequences can provide phylogenetic insight.

13.7 Molecular Phylogeny: Making Sense of Molecular Sequences

Molecular sequences provide a record of past evolutionary events and can be used to build **phylogenetic trees**, which are diagrams that depict evolutionary history. All cells contain DNA as

THE BLACK QUEEN HYPOTHESIS

It is a common misconception that evolution inevitably causes organisms to increase in complexity over time. In reality, evolution is both a give and a take proposition. Fitness changes are completely dependent on the environment, and fitness in some environments may actually be improved by a loss, rather than a gain, of specific genes.

The *Black Queen hypothesis*[1] posits a mechanism and a rationale for this loss of function whose end result is the evolution of mutual dependence in microbial communities. The term *Black Queen* refers to the card game Hearts in which there are two winning strategies. One winning strategy is to avoid getting stuck with the queen of spades. In this strategy each player seeks to lose as many contests ("tricks") as possible so as not to be forced to collect the black queen. The second winning strategy is to "shoot the moon" by collecting all of the trump cards including the black queen. In its microbial context, the Black Queen hypothesis embraces these card game strategies by proposing that some organisms optimize fitness (i.e., "win") by the selective loss of specific genes while others optimize fitness by keeping them all.

The Black Queen hypothesis proposes that certain microbial genes encode extracellular products, such as metabolites or enzymes, which can be used by all or most members of the community. If an organism remains in the community, then selection will be relaxed on genes that encode the synthesis of products that are provided by other members of the community. The presence of such shared products in the community renders genes with similar functions nonessential for some community members (**Figure 1**). The mutation bias toward deletions can then cause these genes to be lost from the genome (see Section 13.6).

The fitness of organisms that lose functions and develop dependencies will actually increase in the community since these organisms no longer bear the costs of production. Such organisms will remain competitive as long as they remain within the community, but they may be unable to grow if separated from the community in which they coevolved. In this way, mutual dependencies accumulate within microbial communities over time. The Black Queen hypothesis also explains the not-uncommon observation that some microorganisms can only be grown in the laboratory in coculture with one or more other species from their environment.

In contrast to the gene loss strategy, organisms that preserve all essential functions (those that shoot the moon in the Hearts analogy) bear the costs of maintaining all gene functions, which puts them at a disadvantage to mutually dependent competitors when competing in the native community. However, cells that maintain their ability to grow independently still have a winning strategy because, unlike their mutually dependent competitors, they retain the option of dispersing to new habitats and growing outside of the native community.

Finally, in addition to describing how microbial community interdependencies might come about, the Black Queen hypothesis also reminds us of how interwoven microbial communities actually are. We will see in later chapters that several molecular tools are available to unwind this complexity and reveal both the diversity of the community and its genetic and metabolic potential.

[1]Morris, J.J., R.E. Lenski, and E.R. Zinser. 2013. The Black Queen hypothesis: Evolution of dependencies through adaptive gene loss. *mBio 3:* e00036-13.

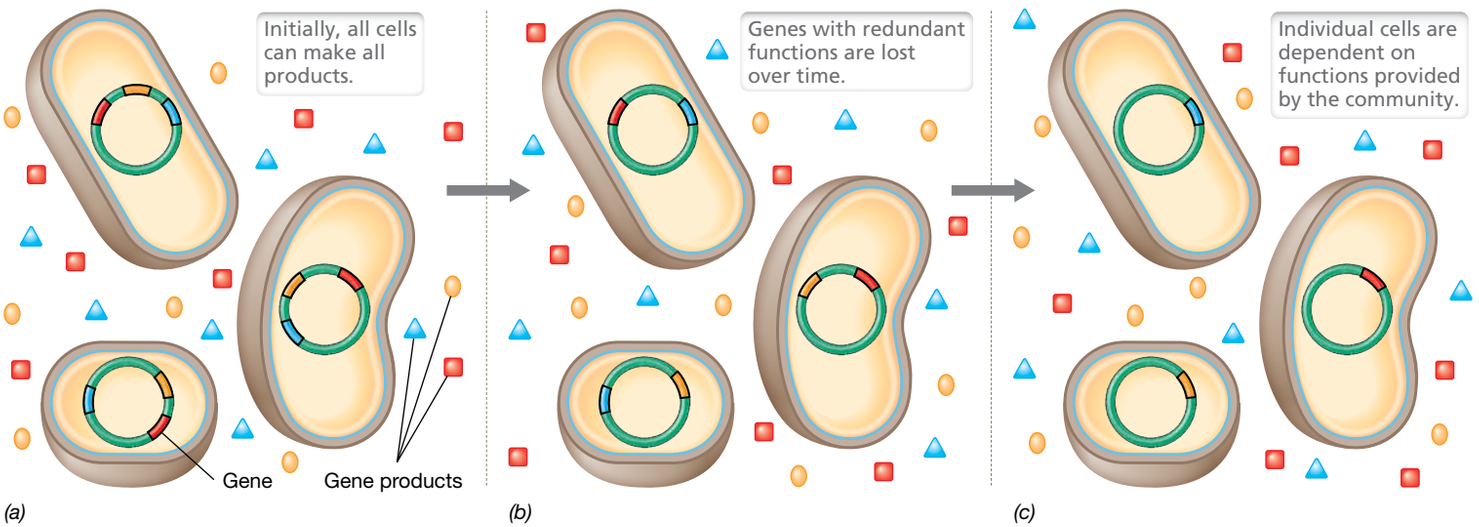


Figure 1 The Black Queen hypothesis and the evolution of dependence in microbial communities. *(a)* Three species in a community each have three different genes that make extracellular products that benefit the whole community (a gene and its product are shown in the same color). *(b)* Over time, random mutations cause functions to be lost from the genomes. *(c)* As long as some members of the community continue to make each product, there will be no fitness cost when a single species loses a single gene. Over time, the three species thus become mutually dependent.

their genetic material, and mutations accumulate in DNA sequences over time. These mutations occur at random and they are a major source of the natural variation that makes evolution possible (Section 13.5). Hence, the difference in nucleotide sequence between the DNA of any two organisms will be a function of the number of mutations that have accumulated since they shared a common ancestor. As a result, differences in DNA sequences can be used to infer evolutionary relationships. In this section we will learn how DNA sequences are used in the phylogenetic analysis of microbial life.

Obtaining DNA Sequences

The analysis of molecular phylogeny begins by determining the sequence of macromolecules such as DNA, RNA, or protein. Here we will focus on analysis of DNA sequence, which is used widely for determining phylogenetic relationships between organisms.

Obtaining DNA from a microorganism is relatively easy if the organism can be cultivated in isolation in the laboratory. In this case, genomic DNA is isolated and either the genome can be sequenced directly, or the genomic DNA can be used to amplify one or more specific genes, using the polymerase chain reaction (PCR, Section 12.1). Advances in DNA sequencing technology (Section 9.2) have made genome sequencing a standard tool employed in analyses of microbial phylogeny. However, sequence analysis of small subunit (SSU) ribosomal RNA (rRNA) genes, which encode the rRNA molecule found within the small subunit of the ribosome (Section 4.10), remains a cornerstone of molecular phylogeny in microbiology because **SSU rRNA** genes are highly conserved, present in all cellular organisms, and easily sequenced and analyzed.

PCR primers can be designed to target any region of DNA from any organism. Standard primers exist for many highly conserved genes, such as the SSU rRNA gene (**Figure 13.15**). Primers for the SSU

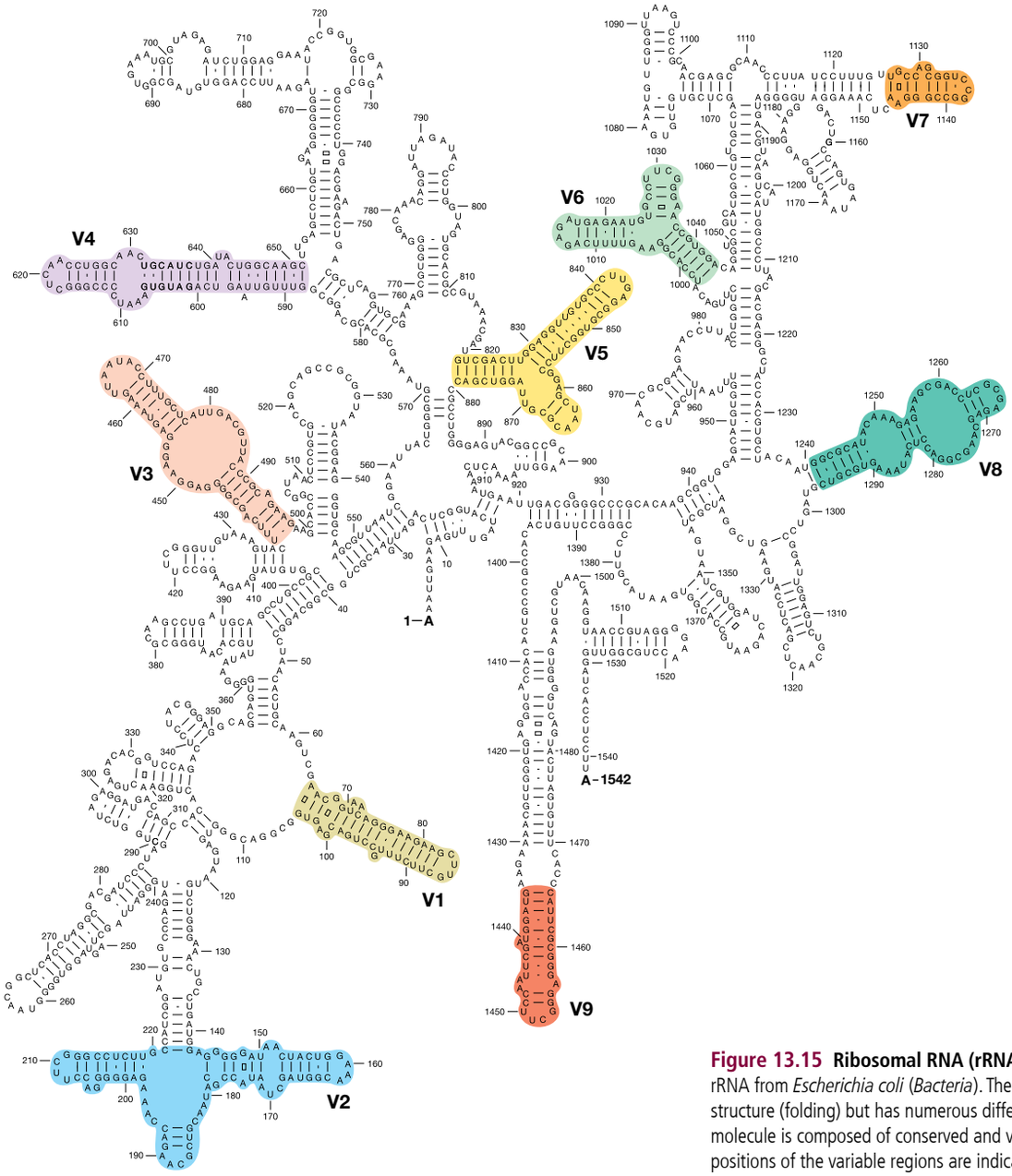


Figure 13.15 Ribosomal RNA (rRNA). Primary and secondary structure of 16S rRNA from *Escherichia coli* (*Bacteria*). The 16S rRNA from *Archaea* is similar in secondary structure (folding) but has numerous differences in primary structure (sequence). The molecule is composed of conserved and variable regions (V1–V9). The approximate positions of the variable regions are indicated in color.

rRNA gene can have different levels of phylogenetic specificity to target discrete species, genera, phyla, or domains, and there are even "universal" primers that will amplify the SSU rRNA gene from any organism. PCR products are visualized by agarose gel electrophoresis, excised from the gel, extracted and purified from the agarose, and then sequenced, often using the same oligonucleotides as primers for the sequencing reactions (Figure 13.16). Instead of starting with a microbial culture, it is also possible to amplify SSU rRNA genes from DNA extracted directly from an environmental sample or to sequence environmental DNA without amplification using a metagenomic approach (Sections 9.8 and 19.8). These latter approaches are widely used to characterize microorganisms that are difficult to grow in laboratory culture. Once sequences are obtained, they must be aligned and analyzed, issues we turn to now.

Sequence Alignment

Phylogeny can be inferred only from genes that have **homology**, that is, *genes that have been inherited from a common ancestor.* Thus homology is a binary trait; sequences are either homologous or they are not. The concept of homology is often confused with that of sequence similarity. The latter is a continuous trait defined as a percentage of nucleotide positions shared between any two sequences. Sequence similarity is used to infer homology, but a similarity value can be calculated between any two sequences regardless of their function or evolutionary relationship. Thus, the terms similarity and homology are not interchangeable. Genes that have homology can be either **orthologs**, if they have the same function and originate from a single ancestral gene in a common ancestor, or **paralogs**, if they have evolved to have different functions as a result of gene duplication (Section 9.5). Phylogenetic analyses typically focus on analysis of orthologous genes that have similar function.

Phylogenetic analyses estimate evolutionary changes from the number of sequence differences across a set of homologous nucleotide positions. Some mutations introduce nucleotide insertions or deletions, and these cause gene sequences to differ in length, making it necessary to *align* nucleotide positions prior to phylogenetic analysis of gene sequences. The purpose of **sequence alignment** is to add gaps to molecular sequences in order to establish positional homology, that is, to be sure that each position in the sequence was inherited from a common ancestor of all organisms under consideration (Figure 13.17). Proper sequence alignment is critical to phylogenetic analysis because the assignment of mismatches and gaps caused by deletions is in effect an explicit hypothesis of how the sequences have diverged from a common ancestral sequence.

Phylogenetic Trees: Composition and Construction

A phylogenetic tree is a diagram that depicts the evolutionary history of an organism and bears some resemblance to a family tree. Most microbes have not left fossils and so their ancestors are unknown, but ancestral relationships can be inferred from the DNA sequences of organisms that are alive today. Organisms that share a recent ancestor are likely to share characteristics, and thus phylogenetic trees allow us to make hypotheses about an organism's characteristics. Phylogenetic trees are also of great use in taxonomy and species identification, as we will discuss later (Section 13.9).

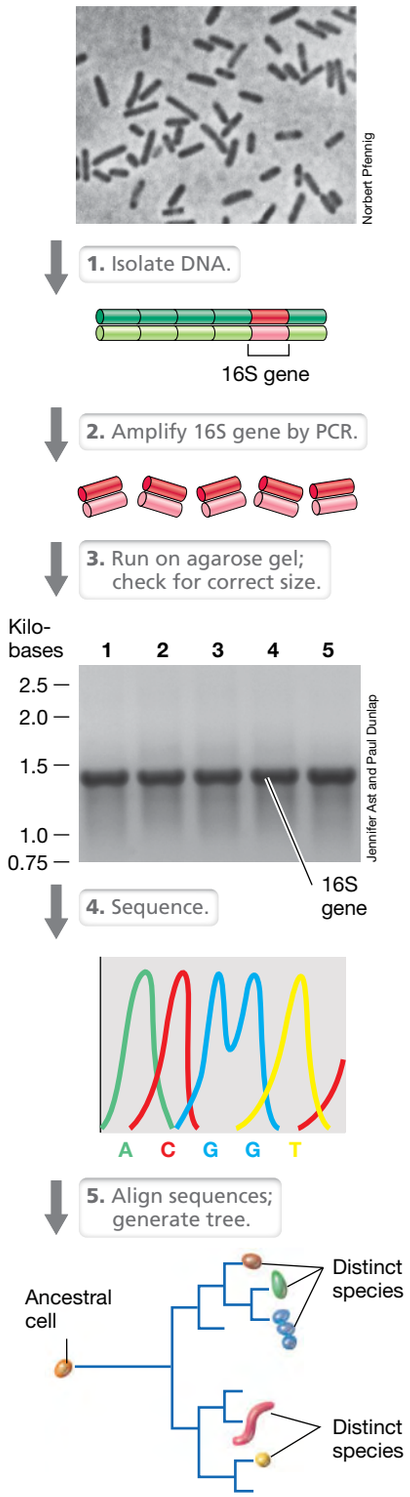


Figure 13.16 PCR amplification of the 16S rRNA gene. Following DNA isolation, primers complementary to the ends of the 16S rRNA (see Figure 13.15) are used to PCR-amplify the 16S rRNA gene from the genomic DNA of five different unknown bacterial strains, and the products are run on an agarose gel (second photo). The bands of amplified DNA are approximately 1465 nucleotides in length. Positions of DNA kilobase size markers are indicated at the left. Excision from the gel and purification of these PCR products is followed by sequencing and analysis to identify the bacteria.

Sequences before alignment

1 GGA CCT AAA TTT ATA CCC
2 GGA AAA GGG CCC AAA CGC
3 GGA GGG CCT TTT ATA CCC

Sequences after alignment

1 GGA --- --- CCT AAA TTT ATA CCC
2 GGA AAA GGG CCC --- --- AAA CGC
3 GGA --- GGG CCT --- TTT ATA CCC

(a)

Sequence differences

	1	2	3
1	–	–	–
2	11	–	–
3	6	11	–

	1	2	3
1	–	–	–
2	3	–	–
3	0	3	–

(b)

Figure 13.17 Alignment of DNA sequences. *(a)* Sequences for a hypothetical region of a gene are shown for three species before alignment and after alignment. A sequence alignment should display homologous positions in vertical columns. Sequence alignment is achieved by adding gaps, indicated by hyphens, to maximize local sequence similarity between the species in the alignment. *(b)* The distance matrices show the number of sequence differences that would be inferred for each species pair both before and after alignment.

A phylogenetic tree is composed of *nodes* and *branches* (**Figure 13.18**). The tips of the branches in a phylogenetic tree represent species that exist today. Phylogenetic trees can be constructed that are either *rooted trees* or *unrooted trees*. Rooted trees show the position of the ancestor of all organisms being examined. Unrooted trees depict the relative relationships among the organisms under study but do not provide evidence of the most ancestral node in the tree. The nodes represent a past stage of evolution where an ancestor diverged into two new lineages. The branch length represents the number of changes that have occurred along that branch. In a phylogenetic tree, only the position of nodes and the branch lengths are informative; rotation around nodes has no effect on the tree's topology (Figure 13.18*b*).

There is only one correct phylogenetic tree that most accurately depicts the evolutionary history of a group of gene sequences, but inferring this tree from sequence data can be a challenging task. The complexity of the problem is revealed by considering the total number of different trees that can be formed from a random set of sequences. For example, only three possible trees can be drawn for any four arbitrary sequences. But if one doubles to eight the number of sequences, now 10,395 trees are possible. This complexity continues to expand exponentially such that 2×10^{182} different trees can be drawn to represent 100 arbitrary sequences. Phylogenetic analysis uses molecular sequence data in an attempt to identify the one correct tree that accurately represents the evolutionary history of a set of sequences.

A variety of methods are available for inferring phylogenetic trees from molecular sequence data. The structure of a phylogenetic tree is inferred by applying either an *algorithm* or some set of

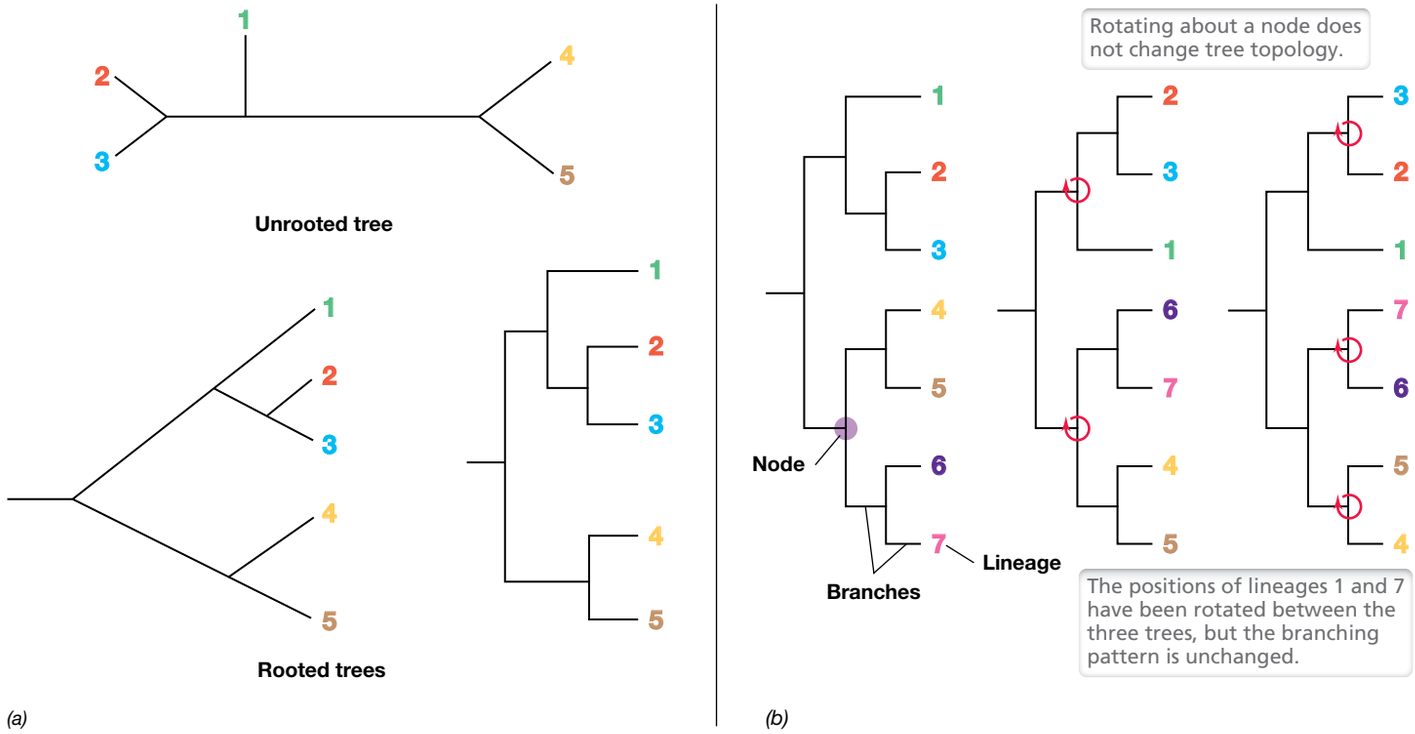


Figure 13.18 Phylogenetic trees and their interpretation. *(a)* Unrooted and rooted examples of phylogenetic trees. The tips of the branches are species (or strains) and the nodes are ancestors. Ancestral relationships are revealed by the branching order in rooted trees. *(b)* Three equivalent versions of the same phylogenetic tree are shown. The only difference between the trees is that their nodes have been rotated at the points indicated by red arrows. The vertical position of species is different between the trees but the pattern of ancestry (the nodes shared by each species) remains unchanged.

optimality criteria. An algorithm is a programmed series of steps designed to construct a single tree (Figure 13.19). Algorithms used to build phylogenetic trees include the *unweighted pair group method with arithmetic mean* (UPGMA) and the *neighbor-joining method*. Alternatively, phylogenetic methods that employ optimality criteria include *parsimony, maximum likelihood*, and *Bayesian* analyses. These latter methods evaluate many possible trees and select the one tree that has the best optimality score, that is, they select the tree that best fits the sequence data given a discrete model of molecular evolution. Optimality scores are calculated on the basis of evolutionary models that describe how molecular sequences change over time. For example, evolutionary models can account for variation in substitution rates and base frequencies between sequence positions.

Limitations of Phylogenetic Trees

Molecular phylogeny provides powerful insights into evolutionary history, but it is important to consider the limitations of building and interpreting phylogenetic trees. For example, it can be difficult to choose the true tree based on available sequence data if several different trees fit the data equally well. *Bootstrapping*, a statistical method in which information is resampled at random, is an approach used to deal with uncertainty in phylogenetic trees. Bootstrap values indicate the *percentage of the time* that a given node in a phylogenetic tree is supported by the sequence data. High bootstrap values indicate that a node in the tree is likely to be correct, while low bootstrap values indicate that the placement of a node cannot be accurately determined given the available data.

Homoplasy, also known as *convergent evolution*, occurs when organisms share a trait that was not inherited from a common ancestor. An example is the evolution of wings in insects and birds. These traits evolved separately and do not indicate that a winged ancestor was shared among insects and birds. Homoplasy occurs in molecular sequences as well, when similar sequence positions result from recurrent mutation rather than inheritance from a common ancestor (Figure 13.20). The problem of homoplasy in molecular phylogeny then increases in proportion to evolutionary time (Figure 13.20*b*). As a result of homoplasy, the reconstruction of accurate phylogenetic trees gets more difficult when sequence divergence between organisms is very high.

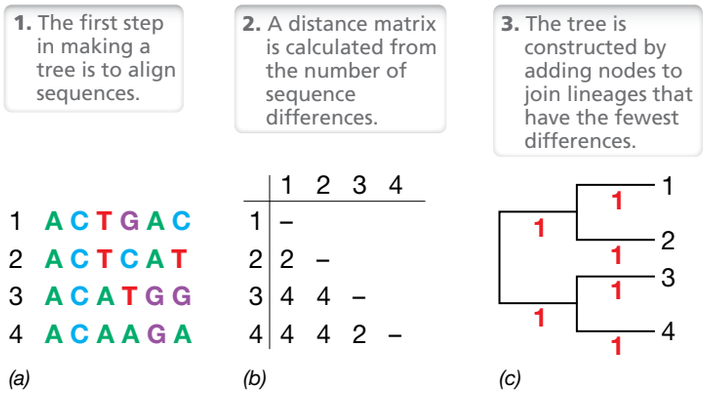


Figure 13.19 Building phylogenetic trees. The number of nucleotide differences between gene sequences can be used to build a phylogenetic tree. In the sequence alignment *(a)* we can count the number of differences between each pair of sequences to build a distance matrix *(b)*. This distance matrix can be used to build a tree *(c)* where the cumulative lengths of the horizontal branches (labeled with a red "1") between any two species in the tree are proportional to the number of nucleotide differences between these species.

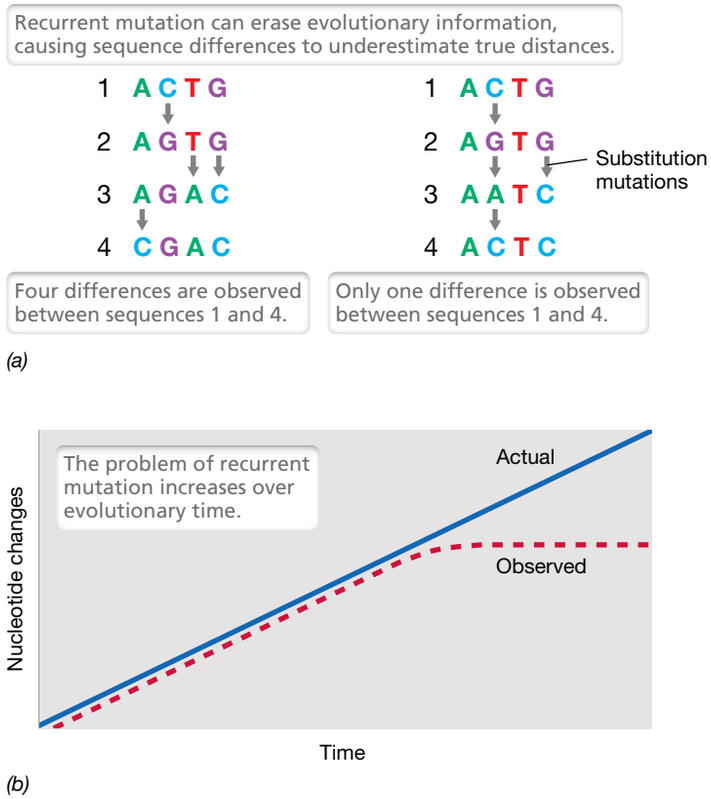


Figure 13.20 The problem of homoplasy due to recurrent mutation. It is possible for recurrent mutation to obscure the true number of mutations that have occurred since a pair of sequences have shared a common ancestor. *(a)* Two series of mutations during the evolution of a gene sequence are compared. On the left side, the number of mutations is equal to the number observed between species 1 and 4. However, if there is recurrent mutation (right side), the number of mutations observed between species 1 and 4 can be less than the number that actually occurred. *(b)* The likelihood of recurrent mutation increases as more and more mutations accumulate over time.

The prevalence of horizontal gene transfer (Chapter 11) also creates complications when considering the evolutionary history of microorganisms. When the sequence of a gene is used to infer the phylogeny of an organism, it must be assumed that the gene is inherited in a *vertical* fashion—from mother to daughter—throughout the evolutionary history of the organism. The *horizontal* exchange of genes between unrelated organisms violates this assumption (Figure 13.21). Hence, it is important to consider the difference between a *gene phylogeny*, which depicts the evolutionary history of an individual gene, and an *organismal phylogeny*, which depicts the evolutionary history of the cell.

In general, genes encoding SSU rRNAs appear to be transferred horizontally at very low frequencies, and rRNA gene phylogenies agree largely with those prepared from other genes that encode genetic informational functions. Thus, SSU rRNA gene sequences are generally considered to provide an accurate record of organismal phylogeny. Nevertheless, many microbial genomes contain genes that have been acquired by horizontal gene transfer at some point in

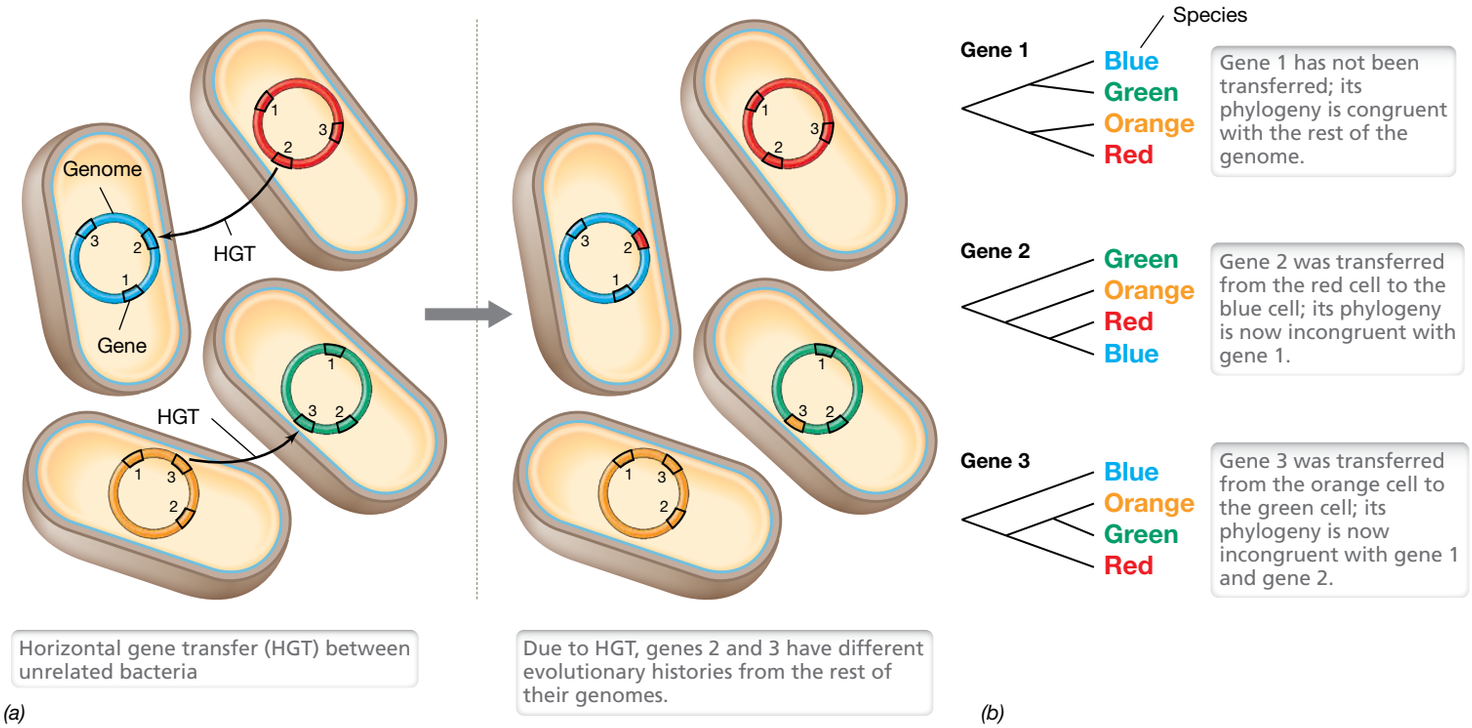


Figure 13.21 Horizontal gene transfer. The horizontal transfer of a gene will cause it to have a different evolutionary history from the rest of the genome. *(a)* Genes are transferred horizontally between distantly related microorganisms. Colors are used to match microorganisms with their genomes. *(b)* As a result of the horizontal transfer events in part *a*, different phylogenetic trees for gene 1, gene 2, and gene 3 are obtained. Only the gene tree for gene 1, which was not transferred, remains congruent with the organismal phylogeny.

their evolutionary history, and this has important implications for microbial evolution (Section 13.6 and Chapter 11).

MINIQUIZ

- How are DNA sequences obtained for phylogenetic analysis?
- What does a phylogenetic tree depict?
- Why is sequence alignment critical to phylogenetic analysis?

13.8 The Species Concept in Microbiology

Species are the fundamental units of biological diversity, and how we distinguish and classify species in microbiology greatly affects our ability to explain and assess the diversity of the microbial world. At present, there is no universally accepted concept of a microbial species. Microbial systematics combines phenotypic, genotypic, and sequence-based phylogenetic data within a framework of standards and guidelines for describing and identifying microorganisms in a taxonomic framework, but the issue of what actually constitutes a *species* remains controversial. However, a working definition of a microbial species has been developed and widely used, and we consider this here.

A Phylogenetic Species Concept for *Bacteria* and *Archaea*

From a taxonomic standpoint, all members of a species should be genetically and phenotypically cohesive, and their traits should be distinct from those described for other species. In addition, a species should be **monophyletic**, that is, the strains composing the species should all share a recent common ancestor to the exclusion of other species. The working definition of a microbial species tries to incorporate these principles and is best described as a *phylogenetic species concept*. The phylogenetic species concept defines a microbial species pragmatically as a group of strains that share certain characteristic traits and which are genetically cohesive and share a unique recent common ancestor. This species concept requires that a majority of genes in the species have congruent phylogenies and share a recent common ancestor. The phylogenetic species concept is not based on an evolutionary model of speciation, and thus species described in this way do not necessarily reflect meaningful units in terms of ecological or evolutionary processes. The phylogenetic species concept was developed to facilitate taxonomy, and species justifications derived from this concept are based largely on the expert judgment of taxonomists.

Under the phylogenetic species concept, species of *Bacteria* and *Archaea* are defined operationally as a group of strains sharing a high degree of similarity in many traits and sharing a recent common ancestor for their SSU rRNA genes. Species characterization employs a polyphasic approach that considers a range of different traits in making taxonomic judgments. Traits currently considered most important for identifying species include genomic similarity based on DNA hybridization and comparisons of SSU rRNA sequences.

The degree of **DNA–DNA hybridization** between the genomes of two organisms provides a measure of their genomic similarity (Section 12.1). In a hybridization experiment (Figure 13.22), *probe DNA* obtained from one organism is labeled with a fluorescent or radioactive label, sheared into small pieces, and heated to separate the two DNA strands. The probe is then added to single-stranded and sheared *target DNA* from a second organism and the mixture cooled to allow the DNA strands to reanneal. The genomic similarity between the two organisms is calculated as a percentage of probe hybridized to target relative to a control (probe DNA hybridized to target DNA from the same organism).

A value of 70% or less genomic hybridization and a difference in SSU rRNA gene sequence of 3% or more between two organisms is taken as evidence that the two are distinct species. Experimental data suggest that these criteria are valid, reliable, and consistent in identifying new microbial species for taxonomic purposes (Figure 13.23). On the basis of this phylogenetic species concept, over 10,000 species of *Bacteria* and *Archaea* have been formally recognized. The criteria that should be used to define a genus, the next highest taxon (see Table 13.2), is more a matter of judgment, but discrete genera typically have greater than 5% dissimilarity in their SSU rRNA gene sequences. There are no consensus criteria for defining taxonomic ranks above the level of genera.

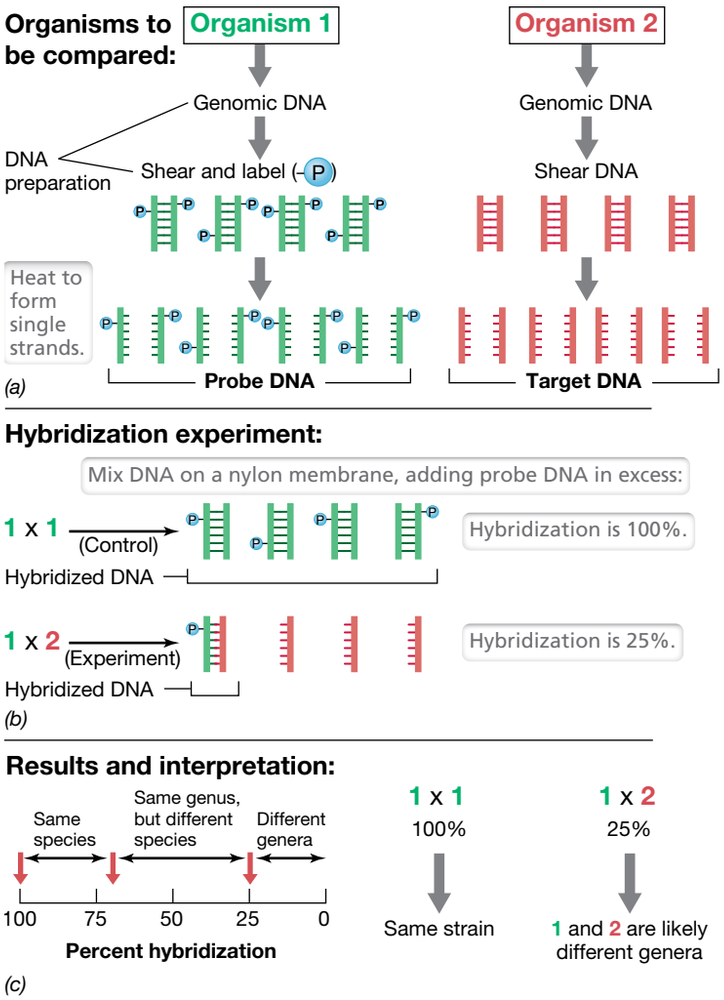


Figure 13.22 Genomic hybridization as a taxonomic tool. *(a)* Genomic DNA is isolated from the organisms to be compared and then sheared and denatured. Probe DNA is prepared from organism 1 by shearing, denaturing, and labeling the DNA (shown here as radioactive phosphate). *(b)* Sheared single-stranded target DNA from each genome is immobilized on a membrane and then hybridized with the labeled probe DNA from organism 1. Radioactivity in the hybridized DNA is measured. *(c)* Radioactivity in the control (organism 1 DNA hybridizing to itself) is taken as the 100% hybridization value.

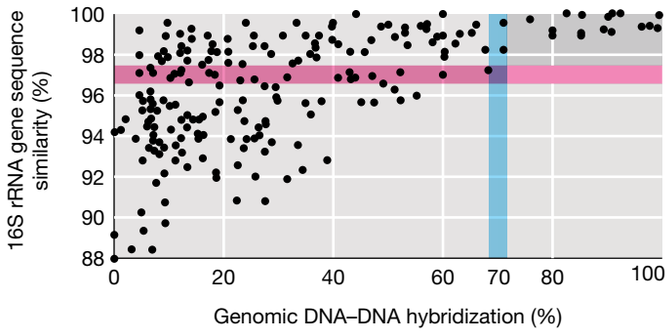


Figure 13.23 Relationship between 16S rRNA gene sequence similarity and genomic DNA–DNA hybridization for pairs of organisms. Pairs of microorganisms are compared on the basis of their 16S rRNA similarities and DNA–DNA hybridization values. Points in the upper right region represent pairs of strains that share greater than 97% 16S rRNA gene sequence similarity and 70% genomic hybridization values, and thus are likely members of the same species. Adapted from Rosselló-Mora, R., and R. Amann. 2001. *FEMS Microbiol. Rev. 25*: 39–67, and Stackebrandt, E., and J. Ebers. 2006. *Microbiology Today*. *11:* 153–155.

How Many Species of *Bacteria* and *Archaea* Exist?

The result of nearly 4 billion years of evolution is the microbial world we see today (Figure 13.9). Microbial taxonomists agree that no firm estimate of the number of bacterial and archaeal species can be given at present, in part because of uncertainty about what defines a species in these domains. However, they also agree that in the final analysis, this number will be very large. The diversity of bacterial and archaeal species on Earth is unquestionably higher than that of all plant and animal species combined, and their total species numbers of *Bacteria* and *Archaea* are likely several orders of magnitude higher than the 10,000 species that have already been characterized.

Every environment on Earth contains a diverse community of microorganisms. For example, analyses of environmental SSU rRNA gene sequences (Section 19.6) using the phylogenetic species concept indicate that over 10,000 different species can coexist in a single gram of soil! Since 1977 more than 3.3 million SSU rRNA sequences have been generated and used to characterize the vast diversity of the microbial world. The Ribosomal Database Project (RDP; http://rdp.cme.msu.edu) contains an ever-growing collection of these sequences and provides computational programs for their analysis and for the construction of phylogenetic trees. While we cannot yet know the biodiversity of microbial life, we do know that nearly all plant and animal species have microbiomes (Chapter 23) that contain countless numbers of unique microbes. Thus, microorganisms are not only the oldest but also unquestionably the most diverse forms of life on our planet. We will see this diversity in action over the next five chapters.

MINIQUIZ

- What is the difference between taxonomy and phylogeny?
- What are some key criteria from the phylogenetic species concept used to determine whether two strains belong to the same species?
- How many species of *Bacteria* and *Archaea* have been named? How many likely exist?

13.9 Taxonomic Methods in Systematics

A *polyphasic approach*, that is, an approach that uses many different methods in combination, is used to identify and name species of *Bacteria* and *Archaea* in accordance with the currently accepted phylogenetic species concept. In this section we describe methods commonly used for characterizing microbial, and primarily prokaryotic, species.

Gene Sequence Analyses

As we have described, gene sequences are commonly determined from PCR-amplified fragments of DNA, and the sequences are analyzed using phylogenetic analyses (Section 13.7). However, SSU rRNA gene sequences are highly conserved, and while they provide valuable phylogenetic information, they are not always useful for distinguishing closely related species. By contrast, other highly conserved genes, such as *recA*, which encodes a recombinase protein (Sections 11.4 and 11.5), and *gyrB*, which encodes DNA gyrase (Section 4.1), can be useful for distinguishing bacteria at the species level. The DNA sequences of protein-encoding genes accumulate mutations more rapidly than rRNA genes; for this reason, sequences from such genes can often distinguish prokaryotic species that cannot be resolved by rRNA gene sequence analyses alone (Figure 13.24).

Multilocus Sequence Typing

Multilocus sequence typing (MLST) is a method in which several different "housekeeping" genes from several related organisms are sequenced and the sequences are used collectively to distinguish the organisms. Housekeeping genes encode essential functions in cells and are always located on the chromosome rather than on a plasmid. For each gene, an approximately 450-base-pair sequence is amplified and then sequenced. The alleles of each gene (variants that differ by at least one nucleotide) are each assigned a number. The strain being studied is then assigned an allelic profile, or multilocus sequence type, consisting of a series of numbers representing its particular combination of alleles (Figure 13.25). In MLST, strains with identical sequences for a given gene have the same allele number for that gene, and two strains with identical sequences for all the genes have the same allelic profile (and would be considered identical by this method). The relatedness between each allelic profile is expressed in a dendrogram of genetic distances that vary from 0 (strains are identical) to 1 (strains are only distantly related, if at all).

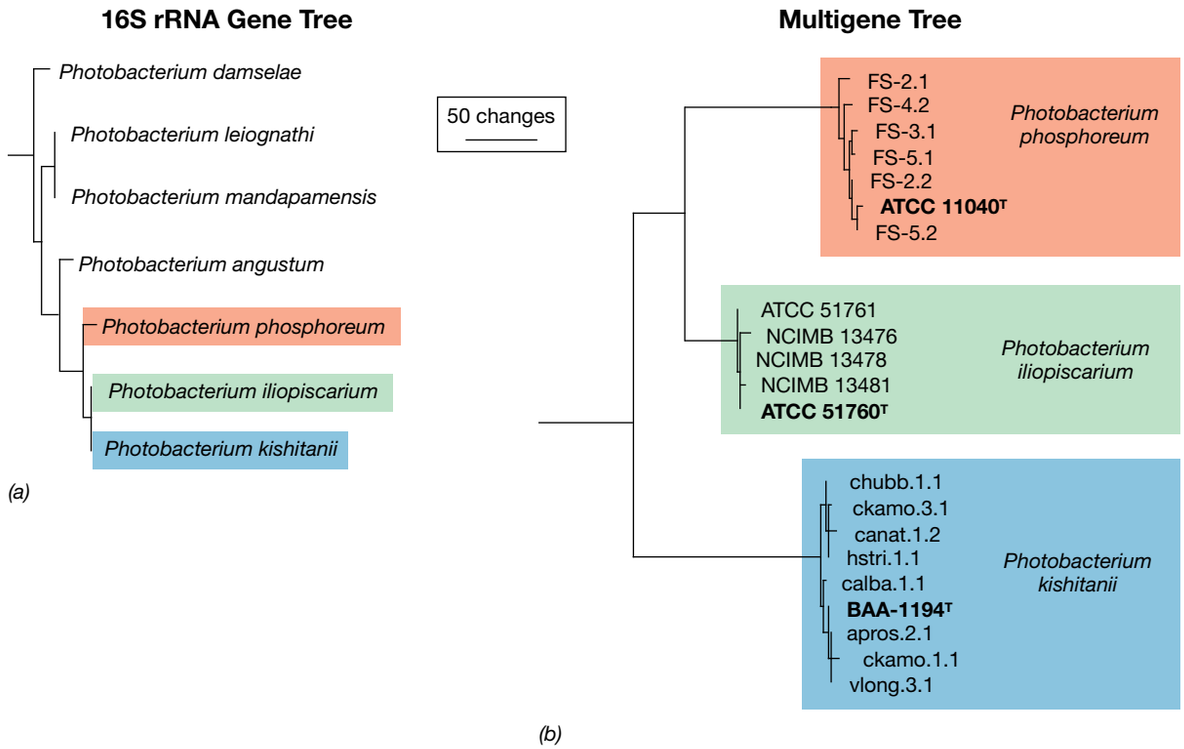


Figure 13.24 Multigene phylogenetic analysis. A phylogeny is shown for species in the genus *Photobacterium*. *(a)* 16S rRNA gene tree, showing the species to be poorly resolved. *(b)* Multigene analysis based on combined analysis of the 16S rRNA gene and *gyrB* and *luxABFE* genes in 21 isolates from three *Photobacterium* species. Multigene analysis clearly resolves the strains into three distinct phylogenetic species, *P. phosphoreum*, *P. iliopiscarium*, and *P. kishitanii*. The scale bar indicates the branch length equal to a total of 50 nucleotide changes. The type strain (Section 13.10) of each species is listed in bold. (All abbreviations are part of strain designations.) Phylogenetic analyses courtesy of Tory Hendy and Paul V. Dunlap, University of Michigan.

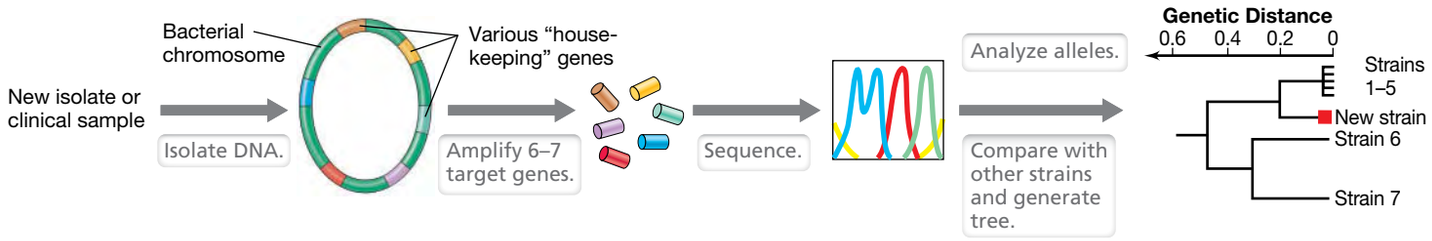


Figure 13.25 Multilocus sequence typing. Steps in MLST leading to a similarity phenogram are shown. Strains 1–5 are virtually identical, whereas strains 6 and 7 are distinct from one another and from both strains 1–5 and the new strain.

MLST has sufficient resolving power to distinguish among even very closely related strains of a given species. In practice, strains can be discriminated on the basis of a single nucleotide change in just one of the analyzed genes. MLST has found its greatest use in clinical microbiology, where it has been used to differentiate strains of various pathogens. This is important because some strains within a species—*Escherichia coli* K-12, for example—may be harmless, whereas others, such as *E. coli* strain O157:H7, can cause serious and even fatal infections (Section 32.11). MLST is also widely used in epidemiological studies to track a virulent strain of a bacterial pathogen as it moves through a population and in environmental studies to define the geographic distributions of strains.

Genome Fingerprinting

Genome fingerprinting is a rapid approach for evaluating polymorphisms between strains of a species. The fingerprints are generally fragments of DNA generated from individual genes or whole genomes. **Ribotyping** is a form of genome fingerprinting based on the localization of SSU rRNA genes on genome fragments. In this method, genomic DNA from an organism is digested by a restriction enzyme (Section 12.2) and the fragments are separated by gel electrophoresis, transferred to a nylon membrane, and labeled with an SSU rRNA gene probe (**Figure 13.26**). Different microbial species can have different numbers of rRNA operons, ranging from 1 to 15, and the number of rRNA operons present in a microbial genome is a conserved feature of all strains of a species. In addition, changes in genome sequence between strains can cause the endonuclease enzyme to cut in different locations, producing variation in the lengths of the restriction fragments that are visualized. Hence, the size and number of bands detected generates a specific pattern, a kind of genome fingerprint called a *ribotype*, and this pattern can be compared with patterns of reference organisms in a computer database.

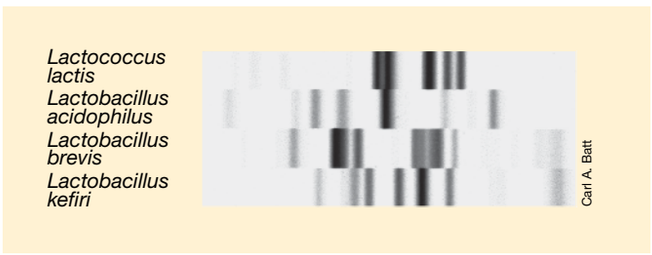


Figure 13.26 Ribotyping. Ribotype results for four different lactic acid bacteria. DNA was taken from a strain of each bacterium, digested into fragments by restriction enzymes, separated by gel electrophoresis, and then probed with a 16S rRNA gene probe. Variations in position and intensity of the bands are important in identification.

The ribotype of a particular organism can be unique and diagnostic, allowing rapid identification of different species and even different strains of a species. For these reasons, ribotyping has found many applications in clinical diagnostics and the microbial analyses of food, water, and beverages. Other common genome fingerprinting methods include *repetitive extragenic palindromic PCR* (*rep-PCR*) and *amplified fragment length polymorphism* (*AFLP*). The rep-PCR method is based on the presence of highly conserved repetitive DNA elements interspersed randomly around the bacterial chromosome. The number and positions of these elements differ between strains of a species. Oligonucleotide primers designed to be complementary to these elements enable PCR amplification of genomic fragments found between the repeated elements. These PCR products can be visualized using gel electrophoresis to reveal a pattern of bands that can be used as a fingerprint (**Figure 13.27**). AFLP is based on the digestion of genomic DNA with one or two restriction enzymes and selective PCR amplification of the resulting fragments, which are then separated by agarose gel electrophoresis. Strain-specific banding patterns similar to those of rep-PCR or other DNA fingerprinting methods are generated, with the large number of bands giving a high degree of discrimination between strains within a species.

Multigene and Whole Genome Analyses

The use of multiple genes and entire genomes for the identification and description of bacteria is becoming increasingly common as DNA sequencing capacities improve and costs decline (Section 9.2). A wide range of sequence analyses can be performed on entire genomes, providing insights into microbial physiology and microbial evolution. These analyses have also provided important insights into the large role that horizontal gene exchange has played in microbial evolution and on the highly dynamic nature of microbial genomes (Section 13.6).

Shared *orthologs* (homologous genes that share the same function, Section 13.7) can be aligned and examined using phylogenetic methods to determine the **average nucleotide identity** of these genes. Different microbial species typically have less than 95% average nucleotide identity for their shared orthologous genes. Comparative analyses of gene content (presence or absence of genes), *synteny* (the order of genes in the genome), and genome GC content provide further insights into relationships between

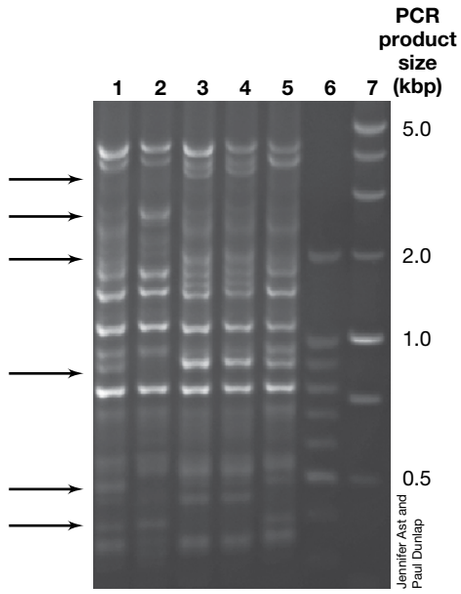


Figure 13.27 DNA fingerprinting with rep-PCR. Genomic DNAs from five strains (1–5) of a single species of bacteria were PCR-amplified using specific primers called *rep* (repetitive extragenic *p*alindromic). The PCR products were separated in an agarose gel on the basis of size to generate DNA fingerprints. Arrows indicate some of the differing bands. Lanes 6 and 7 are 100-bp and 1-kbp DNA size markers, respectively, used for estimating sizes of the DNA fragments.

strains. Entire genome sequences can also be used for metabolic reconstruction and characterization of a cell's genetic capacities. Several methods in comparative genomics and population genomics (Chapter 9) have been developed for use in systematic analyses.

Phenotypic Analysis

The observable characteristics—the **phenotype**—of a bacterium provide many traits that can be used to differentiate species. Typically, several phenotypic traits are determined routinely when describing a new microorganism. These phenotypic results are then compared to the phenotypes of organisms that have been described previously. The phenotypic traits that are determined will depend on the type of organism being described. For example, in applied situations, such as clinical diagnostic microbiology, where timely identification is important, a well-defined subset of traits can be tested to rapidly discriminate between different types of microorganisms. Table 13.1 lists general categories and examples of some phenotypic traits used in identifications and species descriptions, and we examine one of these traits here.

The types and proportions of fatty acids present in cytoplasmic membrane lipids and the outer membrane lipids of gram-negative bacteria are phenotypic traits often used in taxonomic analyses. The technique for identifying these fatty acids has been nicknamed **FAME**, for *f*atty *a*cid *m*ethyl *e*ster, and is in widespread use in clinical, public health, and food- and water-inspection laboratories where pathogens routinely must be identified. The fatty acid composition of *Bacteria* varies from species to species in chain length and in the presence or absence

TABLE 13.1 Some phenotypic characteristics of taxonomic value

Category	*Characteristics*
Morphology	Colony morphology; Gram reaction; cell size and shape; pattern of flagellation; presence of spores, inclusion bodies (e.g., PHB,[a] glycogen, or polyphosphate granules, gas vesicles, magnetosomes); capsules, S-layers, or slime layers; stalks or appendages; fruiting body formation
Motility	Nonmotile; gliding motility; swimming (flagellar) motility; swarming; motile by gas vesicles
Metabolism	Mechanism of energy conservation (phototroph, chemoorganotroph, chemolithotroph); utilization of individual carbon, nitrogen, or sulfur compounds; fermentation of sugars; nitrogen fixation; growth factor requirements
Physiology	Temperature, pH, and salt ranges for growth; response to oxygen (aerobic, facultative, anaerobic); presence of catalase or oxidase; production of extracellular enzymes
Cell lipid chemistry	Fatty acids;[b] polar lipids; respiratory quinones
Cell wall chemistry	Presence or absence of peptidoglycan; amino acid composition of cross-links; presence or absence of cross-link interbridge
Other traits	Pigments; luminescence; antibiotic sensitivity; serotype; production of unique compounds, for example, antibiotics

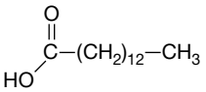
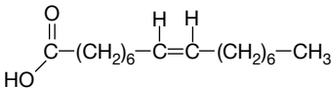
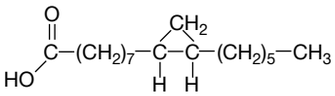
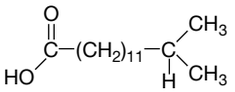
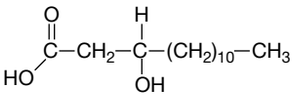
[a]PHB, poly-β-hydroxybutyric acid (Section 2.8).
[b]Figure 13.28.

of double bonds, rings, branched chains, or hydroxy groups (Figure 13.28). In FAME analyses, fatty acids extracted from cells grown in culture under standardized conditions are analyzed by gas chromatography. A chromatogram showing the types and amounts of fatty acids from the unknown bacterium is then compared with a database containing the fatty acid profiles of thousands of reference bacteria grown under the same conditions.

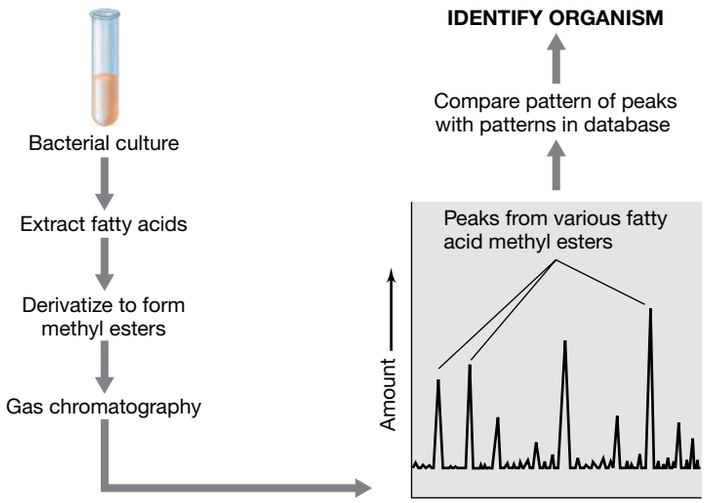
Fatty acid profiles of an organism, like many other phenotypic traits, can vary as a function of temperature, growth phase (exponential versus stationary), and growth medium. Hence, for comparative results to be valid, it is necessary to grow the unknown organism on a specific medium and at a specific temperature. For many organisms this is impossible, of course, limiting the applicability of FAME analyses as a taxonomic tool. In addition, the extent of variation in FAME profiles among strains of a species, a necessary consideration in studies to discriminate between species, is a work in progress.

Phenotypic characteristics of strains are generally highly dependent on growth conditions, and phenotypes observed in the laboratory environment may not well represent phenotypes present in the natural environment; thus care must be taken in using phenotypic characteristics in systematic analyses. In addition, the value of different phenotypic characteristics for reaching a firm systematics conclusion can vary with the taxonomic groups being examined.

Classes of Fatty Acids in *Bacteria*

Class/Example	Structure of example
I. ***Saturated:*** tetradecanoic acid	
II. ***Unsaturated:*** omega-*7-cis* hexadecanoic acid	
III. ***Cyclopropane:*** *cis-7,8*-methylene hexadecanoic acid	
IV. ***Branched:*** 13-methyltetradecanoic acid	
V. ***Hydroxy:*** 3-hydroxytetradecanoic acid	

(a)



(b)

Figure 13.28 Fatty acid methyl ester (FAME) analysis in bacterial identification. *(a)* Classes of fatty acids in *Bacteria*. Only a single example is given of each class; however, more than 200 structurally distinct fatty acids are known from bacterial sources. A methyl ester contains a methyl group (CH_3) in place of the proton on the carboxylic acid group (COOH) of the fatty acid. *(b)* Procedure. Each peak from the gas chromatograph is due to one particular fatty acid methyl ester, and the peak height is proportional to the amount.

MINIQUIZ

- What class of genes is used in MLST analyses?
- How is ribotyping different from rep-PCR?
- What is FAME analysis?

13.10 Classification and Nomenclature

We conclude our treatment of microbial evolution and systematics with a brief description of how *Bacteria* and *Archaea* are classified and named—the science of *taxonomy*. Information is also presented on culture collections, which are repositories for scientific deposition of live microbial cultures; on some key taxonomic resources available for microbiology; and on the procedures for naming new microbial species. The formal description of a new microbial species and the deposition of cultures into a culture collection form an important foundation of the systematics of prokaryotic species.

Taxonomy and Describing New Species

Classification is the organization of organisms into progressively more inclusive groups on the basis of either phenotypic similarity or evolutionary relationship. A species is made up of one to several strains, and similar species are grouped into genera (singular, genus). Similar genera are grouped into families, families into orders, orders into classes, up to the domain, the highest-level taxon based on a collection of phenotypic and genotypic information. This hierarchical scheme is illustrated in Table 13.2.

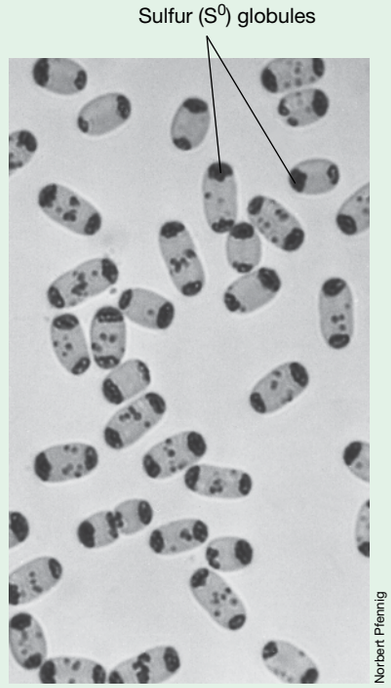
Nomenclature is the actual naming of organisms and follows the **binomial system** of nomenclature devised by the Swedish medical doctor and botanist Carl Linnaeus and used throughout biology; organisms are given *genus names* and *species epithets*. The names are Latin or Latinized Greek derivations, often descriptive of some key property of the organism, and are printed in *italics*. By classifying organisms into groups and naming them, we order the natural microbial world and make it possible to communicate effectively about all aspects of particular organisms, including their behavior, ecology, physiology, pathogenesis, and evolutionary relationships. The creation of new taxa of *Bacteria* and *Archaea* must follow the rules described in the *International Code of Nomenclature of Bacteria* (the Bacteriological Code). This source presents the formal framework by which *Bacteria* and *Archaea* are to be officially named and the procedures by which existing names can be changed, for example, when new data warrant taxonomic rearrangements.

When a new isolate of *Bacteria* or *Archaea* is isolated from nature and thought to be unique, a decision must be made as to whether it is sufficiently different from established taxa to be described as a new taxon. To achieve formal validation of taxonomic standing as a new genus or species, a detailed description of the organism's characteristics and distinguishing traits, along with its proposed name, must be published, and viable cultures of the organism must be deposited in at least two international culture collections (Table 13.3). The manuscript describing and naming a new taxon undergoes peer review before publication. A major vehicle for the description of new taxa is the *International Journal of Systematic and Evolutionary Microbiology* (*IJSEM*), the official publication of record for the taxonomy and classification of *Bacteria, Archaea*, and microbial eukaryotes. In each issue, the *IJSEM* also publishes an approved list of newly validated names. By providing validation of newly proposed names, publication in *IJSEM* paves the way for their inclusion in taxonomic reference sources. Two websites provide listings of valid, approved bacterial names: List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.net), and Prokaryotic Nomenclature Up-to-Date (http://www.dsmz.de).

It is possible to use molecular and genomic techniques (Chapters 9 and 19) to characterize the phenotypic and genotypic characteristics of microorganisms without the need for the

TABLE 13.2 Taxonomic hierarchy for the purple sulfur bacterium *Allochromatium warmingii*

Taxon	*Name*	*Properties*	*Confirmed by*
Domain	*Bacteria*	Bacterial cells; rRNA gene sequences typical of *Bacteria*	Microscopy; 16S rRNA gene sequence analysis; presence of unique biomarkers, for example, peptidoglycan
Phylum	*Proteobacteria*	rRNA gene sequence typical of *Proteobacteria*	16S rRNA gene sequence analysis
Class	*Gammaproteobacteria*	Gram-negative bacteria; rRNA sequence typical of *Gammaproteobacteria*	Gram-staining, microscopy
Order	*Chromatiales*	Phototrophic purple bacteria	Characteristic pigments (Figures 14.2, 14.3, and 14.9)
Family	*Chromatiaceae*	Purple sulfur bacteria	Ability to oxidize H_2S and store S^0 within cells; microscopic observation of S^0 (see photo); 16S rRNA gene sequence
Genus	*Allochromatium*	Rod-shaped purple sulfur bacteria; <95% 16S rRNA gene sequence identity with other genera	Microscopy (see photo)
Species	*warmingii*	Cells 3.5–4.0 μm × 5–11 μm; storage of sulfur mainly in poles of cell (see photo); <97% 16S rRNA gene sequence identity with other species	Cell size measured microscopically with a micrometer; observation of polar position of S^0 globules in cells (see photo); 16S rRNA gene sequence



Cells of *A. warmingii*

cultivation of an isolated strain. However, in the absence of an isolate that can be deposited in two international culture collections, it is not possible to validly name a new species of microorganism under the Bacteriological Code. However, when an organism is well characterized but not yet cultured or not yet obtained in pure culture, a provisional taxonomic name can be applied. The moniker *Candidatus* is appended to candidate taxonomic ranks. For example, "*Candidatus* Pelagibacter ubique" is a globally widespread and well-characterized marine bacterium that is difficult to grow in laboratory media (Section 19.3) and so has not been formally named under the Bacteriological Code. By contrast, the bacterium "*Candidatus* Heliomonas lunata" can be grown in laboratory culture but the culture is not pure so this bacterium also retains *Candidatus* status.

The International Committee on the Systematics of Prokaryotes (ICSP) oversees the nomenclature and taxonomy of *Bacteria* and *Archaea*. The ICSP also oversees the publication of *IJSEM* and the *International Code of Nomenclature of Bacteria* and gives guidance to several subcommittees that meet to establish and revise standards for the description of new species in the different groups of *Bacteria* and *Archaea*.

Bergey's Manual and *The Prokaryotes*

Because taxonomy is to some degree a matter of scientific judgment, there is no "official" classification of *Bacteria* and *Archaea*. However, the classification system most widely accepted by microbiologists is that of *Bergey's Manual of Systematic Bacteriology*, a major taxonomic treatment of *Bacteria* and *Archaea*. Widely used, *Bergey's Manual* has served microbiologists since 1923 as a compendium of information on all recognized prokaryotic species. Each chapter, written by experts, contains tables, photos, figures, and other systematic information useful for identification purposes.

A second major source describing the physiology, ecology, phylogeny, enrichment, isolation, and cultivation of *Bacteria* and *Archaea* is *The Prokaryotes*. This work is available online by subscription through university libraries. Collectively, *Bergey's Manual* and *The Prokaryotes* offer microbiologists both the concepts and the details of the biology of *Bacteria* and *Archaea* as we know them today and are the primary resources for microbiologists characterizing newly isolated organisms.

Culture Collections

National microbial culture collections (Table 13.3) are an important foundation of microbial systematics. These permanent collections catalog and store microorganisms and provide cultures upon request (for a fee) to researchers in academia, medicine, and industry. The collections play an important role in protecting microbial biodiversity, just as museums do in preserving plant and animal specimens for future study. However, unlike museums, which maintain collections of chemically preserved or dried, *dead* specimens, microbial culture collections store microorganisms as *viable cultures*, typically frozen or in a freeze-dried state. These storage methods maintain the cells indefinitely in a living state and prevent genetic changes that might occur if the organisms were continually subcultured.

TABLE 13.3 Some international microbial culture collections

Collection	*Name*	*Location*	*Web address*
ATCC	American Type Culture Collection	Manassas, Virginia	http://www.atcc.org
BCCM/LMG	Belgian Coordinated Collection of Microorganisms	Ghent, Belgium	http://bccm.belspo.be
CIP	Collection of the Institut Pasteur	Paris, France	http://www.pasteur.fr
CBS	Centraalbureau voor Schimmelcultures	Utrecht, The Netherlands	http://www.cbs.knaw.nl
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen	Braunschweig, Germany	http://www.dsmz.de
JCM	Japan Collection of Microorganisms	Saitama, Japan	http://jcm.brc.riken.jp/
NCCB	Netherlands Culture Collection of Bacteria	Utrecht, The Netherlands	http://www.cbs.knaw.nl
NCIMB	National Collection of Industrial, Marine and Food Bacteria	Aberdeen, Scotland	http://www.ncimb.com
NRRL	United States Department of Agriculture, Agricultural Research Service Culture Collection	Peoria, Illinois	http://nrrl.ncaur.usda.gov

A related and key role of culture collections is as repositories for *type strains*. When a new species of bacteria is described in a scientific journal, a strain is designated as the **type strain** and this strain serves as the nomenclatural type of the taxon for future taxonomic comparison with other strains of that species (see Figure 13.24 for a visual representation of this). Deposition of this type strain in the national culture collections of at least two countries—thereby making the strain publicly available internationally—is a prerequisite for validation of the new species name. Some of the large national culture collections are listed in Table 13.3. Their websites contain searchable databases of strain holdings together with information on the environmental sources of strains and their descriptions.

MINIQUIZ

- What roles do culture collections play in microbial systematics?
- What is the *IJSEM* and what taxonomic function does it fulfill?
- Why might viable cell cultures be of more use in microbial taxonomy than preserved specimens?

Chapter Review

I • Early Earth and the Origin and Diversification of Life

13.1 Planet Earth is about 4.5 billion years old. After its formation, Earth was hot and sterile. Gradual cooling of the Earth allowed formation of liquid water, a requirement for the origin of life. The earliest evidence of life comes from isotopic analysis of ancient sedimentary rocks and zircon minerals indicating that life was present on Earth 3.86–4.1 billion years ago.

Q What is LUCA, and what is a plausible explanation for the origin of cellular life?

13.2 In rocks 3.5 billion years old or younger, microbial formations called stromatolites are abundant and show extensive microbial diversification. The evolution of oxygenic photosynthesis caused O_2 to accumulate 2.4 billion years ago, eventually leading to the formation of banded iron formations, the ozone shield, and an oxygenated atmosphere, which set the stage for rapid diversification of metabolic types and the evolution of multicellularity.

Q Why was the origin of cyanobacteria of such importance to the evolution of life on Earth?

13.3 Ribosomal RNA genes have been used to construct a universal tree of life revealing that life on Earth evolved along three major lineages forming the domains *Bacteria*, *Archaea*, and *Eukarya*. The universal tree of life shows that the domains *Bacteria* and *Archaea* diverged billions of years ago, that *Eukarya* split from *Archaea* later in the history of life, and that complex multicellular eukaryotes only began to diverge within the last 600 million years.

Q What is the three-domain concept of the tree of life? How was the tree of life constructed?

13.4 The eukaryotic cell developed from endosymbiotic events. The modern eukaryotic cell is a chimera with genes and characteristics from both *Bacteria* and *Archaea*. SSU rRNA sequence analyses indicate the ancestors of mitochondria

are found in the phylum *Proteobacteria* and those of chloroplasts are found in the phylum *Cyanobacteria*.

Q What is the endosymbiotic hypothesis for the origin of mitochondria and chloroplasts? What evidence supports this hypothesis?

II • Microbial Evolution

13.5 Evolution is defined as a change in allele frequencies in a population of organisms over time resulting in descent with modification. New alleles are created through the processes of mutation and recombination. Mutations occur at random and most mutations are neutral or deleterious, but some are beneficial. Natural selection and genetic drift are two mechanisms that cause allele frequencies to change in a population over time.

Q What is fitness? To what degree does fitness depend on the environment in which organisms live?

13.6 Microbial genomes are dynamic, and genome size and gene content can vary considerably between strains of a species. The core genome is defined as the set of all genes shared by a species, while the pan genome is defined as the core genome plus genes whose presence varies among strains of a species.

Q What are some processes that influence the content of the pan genome?

III • Microbial Phylogeny and Systematics

13.7 Molecular sequences accumulate random mutations over time, and molecular phylogenetic analysis examines differences in molecular sequences to determine the evolutionary history of life. A phylogenetic tree is a diagram that depicts the evolutionary history of a set of genes or organisms.

Q What is the difference between a gene tree and an organismal tree?

13.8 At present, species in *Bacteria* and *Archaea* are defined operationally based on shared genetic and phenotypic traits. The dynamic nature of microbial genomes and the abundance of genes acquired through horizontal gene transfer have raised questions about the nature of microbial species.

Q What is the "species problem" and why is the concept of microbial species difficult to resolve?

13.9 Systematics is the study of the diversity and relationships of living organisms. Polyphasic taxonomy is based on phenotypic, genotypic, and phylogenetic information. Bacterial species can be distinguished genotypically on the basis of DNA–DNA hybridization, DNA fingerprinting, MLST, or multigene or whole genome analyses. Phenotypic traits useful in taxonomy include morphology, motility, metabolism, and cell chemistry, especially lipid analyses.

Q What are the criteria used in phylogenetic analysis to designate a new microbial species?

13.10 Nomenclature in microbiology follows the binomial system used in all of biology. Formal recognition of a new species of *Bacteria* or *Archaea* requires depositing a sample of the organism in culture collections and publishing the new species name and description.

Q Is it possible to provide a formal name for a microorganism that has not been cultivated in isolation? What kind of name might be used if a microorganism is well characterized but cannot yet be cultivated in isolation?

Application Questions

1. Compare and contrast the physical and chemical conditions on Earth at the time life first arose with conditions today. From a physiological standpoint, discuss at least two reasons why *animals* could not have existed on early Earth. In what ways has microbial metabolism altered Earth's biosphere? How might life on Earth be different if oxygenic photosynthesis had not evolved?
2. For the following sequences, construct the phylogenetic tree that best depicts their evolutionary relationships.

   Taxon 1: GTTCCCTTA
   Taxon 2: GTTCGGTAT
   Taxon 3: GAAAAACCCTAT
   Taxon 4: CTTCCCTTT
   Taxon 5: GTAAAACCCGAT
3. Imagine that you have been given several bacterial strains from various countries around the world and that all the strains are thought to cause the same gastrointestinal disease and to be genetically identical. Upon carrying out a DNA fingerprint analysis of the strains, you find that four different strain types are present. What methods could you use to test whether the different strains are actually members of the same species?
4. Imagine that you have discovered a new form of microbial life, one that appears to represent a fourth domain. How would you go about characterizing the new organism and determining if it actually is evolutionarily distinct from *Bacteria, Archaea*, and *Eukarya*?

Chapter Glossary

Allele a sequence variant of a given gene

Archaea a domain of life consisting of microorganisms that have prokaryotic cell structure and are distinct from *Bacteria*

Average nucleotide identity a genome-wide measure of genetic similarity between two microorganisms based on aligning all orthologous gene pairs across the two genomes and determining the percentage of matching nucleotides (see also *orthologs*)

Bacteria a domain of life consisting of microorganisms that have prokaryotic cell structure and are distinct from *Archaea*

Banded iron formation iron oxide–rich ancient sedimentary rocks containing zones of oxidized iron (Fe^{3+}) formed by oxidation of Fe^{2+} by O_2 produced by cyanobacteria

Binomial system the system devised by the Swedish scientist Carl Linnaeus for naming living organisms in which an organism is given a genus name and a species epithet

Core genome those genes found in common in the genomes of all strains of a species

DNA–DNA hybridization the experimental determination of genomic similarity by measuring the extent of hybridization of DNA from one organism with that of another

Endosymbiotic hypothesis the idea that a chemoorganotrophic bacterium and a cyanobacterium were stably incorporated into another cell type to give rise, respectively, to the mitochondria and chloroplasts of modern-day eukaryotes

Eukarya a domain of life consisting of organisms that have eukaryotic cell structure; includes algae, protists, fungi, slime molds, plants, and animals

Evolution a change in allele frequencies in a population of organisms over time, with new alleles arising due to mutation and recombination, resulting in descent with modification

FAME fatty acid methyl ester; a technique for identifying microorganisms by their fatty acids

Fitness the capacity of an organism to survive and reproduce as compared to that of competing organisms

Genetic drift a process that results in a change in allele frequencies in a population as a result of random changes in the number of offspring from each individual over time

Homology having shared ancestry

Homoplasy when two organisms have the same trait as a result of recurrent mutation or convergent evolution

Horizontal gene transfer the asymmetrical and unidirectional transfer of DNA from one cell to another

Molecular clock a DNA sequence, such as a gene for rRNA, that can be used as a comparative temporal measure of evolutionary divergence

Monophyletic in phylogeny, a group descended from a single shared ancestor

Multilocus sequence typing (MLST) a taxonomic tool for classifying organisms on the basis of gene sequence variations in several housekeeping genes

Mutation a heritable change in DNA sequence

Orthologs genes that are homologous (that is, they are descended from a common ancestor) and share the same function (see also *paralog*)

Pan genome the totality of the genes present in the different strains of a species

Paralogs genes that are homologous (that is, they are descended from a common ancestor) but which have diverged to have different functions; typically, paralogous genes are the result of gene duplication (see also *ortholog*)

Phenotype the physical and chemical characteristics of an organism that can be observed or measured

Phylogenetic tree a diagram that depicts the evolutionary history of an organism; consists of nodes and branches

Phylogeny evolutionary history

Phylum a major lineage of cells in one of the three domains of life

Recombination resorting or rearrangement of DNA fragments resulting in a new sequence

Ribosomal RNA (rRNA) RNA molecules found in the small and large subunits of the ribosome

Ribotyping a means of identifying microorganisms from analysis of DNA fragments generated from restriction enzyme digestion of the genes encoding their ribosomal RNA

Selection in an evolutionary context, a process that results in a change in allele frequencies in a population when individuals that are favored in a given environment are able to produce more offspring and make a greater contribution to the gene content of future generations

Sequence alignment the insertion of gaps into a set of molecular sequences organized in rows so that homologous positions are organized in vertical columns. Alignment is necessary prior to phylogenetic analysis because deletion and insertion mutations cause variations in the length of molecular sequences

16S rRNA the type of SSU rRNA found in *Bacteria* and *Archaea*; its eukaryotic counterpart is 18S rRNA (see also *SSU rRNA*)

Species defined in microbiology as a collection of strains that all share the same major properties and differ in one or more significant properties from other collections of strains; defined phylogenetically as a monophyletic, exclusive group based on DNA sequence

SSU rRNA (small subunit rRNA) the rRNA molecule found in the small subunit of the ribosome, comprised of 16S rRNA, which occurs in the 30S ribosomal subunit of *Bacteria* and *Archaea*, or its orthologous counterpart 18S rRNA, which occurs in the 40S ribosomal subunit of *Eukarya*. SSU rRNA genes are conserved in all forms of cellular life and this gene is often used in phylogenetic analysis of microorganisms

Stromatolite a laminated microbial mat which can become fossilized and is typically built from layers of filamentous microorganisms

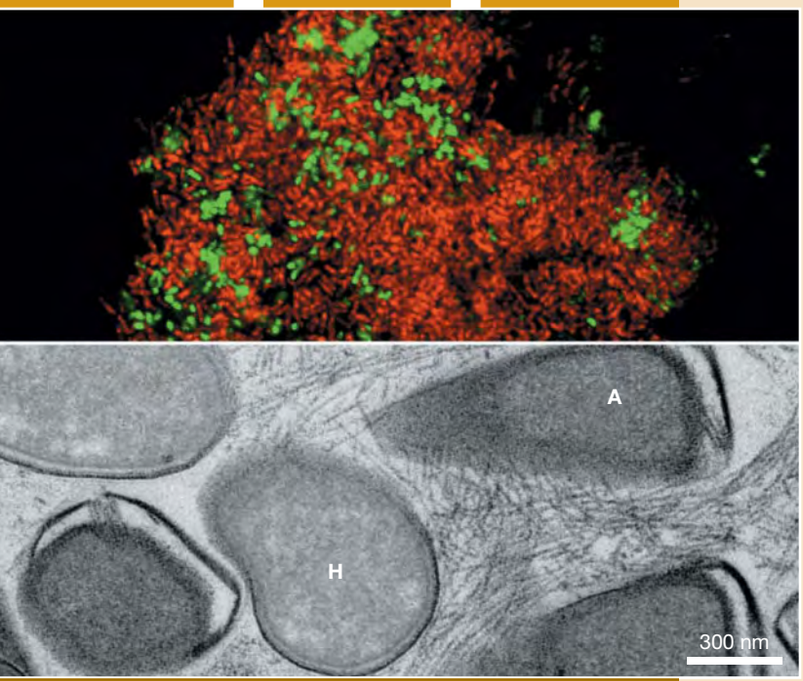
Systematics the study of the diversity of organisms and their relationships; includes taxonomy and phylogeny

Taxonomy the science of identification, classification, and nomenclature

Type strain the strain chosen to represent the nomenclatural type of a species; type strains are deposited in microbial culture collections and are used to represent the typical characteristics of their species

Universal tree of life a phylogenetic tree that shows the positions of representatives of all domains of cellular life

14 Metabolic Diversity of Microorganisms



microbiologynow

Microbes That Plug into the Matrix

Living things conserve energy by moving electrons around. Most microbes transfer electrons to soluble electron acceptors, such as O_2 or NO_3^- that have been taken up by the cell. Some microbes, such as iron-reducing bacteria, transfer electrons to insoluble electron acceptors, such as metallic minerals. Recent discoveries, however, show that certain microbes are actually able to conserve energy by plugging into other cells. Such direct interspecies electron transfer was recently discovered at the heart of an exceptional symbiosis between anaerobic methanotrophic *Archaea* (ANME) and sulfate-reducing bacteria (SRB).

Methane is a powerful greenhouse gas that is abundant in the deep sea as methane hydrates. Methane seeps occur where these hydrates meet ocean water, creating enormous potential for methane to bubble into the atmosphere. However, this methane never reaches the atmosphere because it is consumed by methane-eating *Archaea* that live in marine sediments. ANME live within aggregates that also contain SRB, their symbiotic partners (see upper photo of part of an aggregate where ANME-1 [red] and SRB HotSeep-1 [green] are identified using fluorescent probes and confocal laser microscopy). ANME catalyze the anaerobic oxidation of methane, but in the absence of a suitable electron acceptor, this process alone does not conserve energy. Here is where the SRB partners help out. The SRB take electrons from ANME and use them to reduce sulfate, thereby conserving enough energy to support both symbiotic partners.

The mechanism that underlies this symbiotic association has long been a mystery. In most syntrophic partnerships, H_2 is the "currency" of electron transfer, being produced by one partner and consumed by the other. However, H_2 is not exchanged between ANME and SRB. Instead, ANME and SRB are connected by "nanowires" up to 1000 nm long (see lower photo where ANME-1 [A], SRB HotSeep-1 [H], and their nanowires are visible in an electron micrograph). These structures are produced by the SRB and connect to ANME cells. Both partners have large, multiheme cytochrome c proteins, which enable them to donate (ANME) or accept (SRB) electrons via the nanowires. Hence, the organisms in this intimate symbiosis survive by plugging into an extracellular matrix of tiny wirelike structures that allow electrons to be transferred between species.

Source: Wegener, G., et al. 2015. Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria. *Nature 526:* 587–590.

A major theme of microbiology is the great *phylogenetic diversity* of microbial life on Earth. We got a taste of this in the last chapter and will explore microbial diversity in detail in the following four chapters. In this chapter we focus on the *metabolic diversity* of microorganisms, with special emphasis on the processes and mechanisms that underlie this diversity. We will then return to the organisms themselves and unveil the phylogenetic breadth of the microbial world in the context of metabolic diversity.

I • Phototrophy

Phototrophy—the use of light energy—is widespread in the microbial world. In this first part of the chapter, we examine the properties and energy-conserving strategies of phototrophic microorganisms and see how these support a lifestyle based on the use of CO_2 as carbon source.

14.1 Photosynthesis and Chlorophylls

The most important biological process on Earth is **photosynthesis**, the conversion of light energy to chemical energy. Organisms that carry out photosynthesis are called **phototrophs**. Photosynthetic organisms are also **autotrophs**, capable of growing with CO_2 as the sole carbon source. Energy from light is used in the reduction of CO_2 to organic compounds (*photoautotrophy*). Some phototrophs can also use organic carbon as their carbon source; this lifestyle is called *photoheterotrophy*.

Photosynthesis originated within the *Bacteria*, and a wide diversity of bacterial species can harvest energy from light. No less than six different photosynthetic systems have evolved within *Bacteria*, and these various systems are found in the *Heliobacteria*, *Acidobacteria*, green sulfur bacteria, purple bacteria, filamentous anoxygenic phototrophs (green nonsulfur bacteria), and *Cyanobacteria*. Ultimately, photosynthesis also evolved within the *Eukarya* as a result of the endosymbiotic origin of chloroplasts from cyanobacterial relatives (↻ Section 13.4). These photosynthetic systems all differ in characteristic ways but they all reveal similar underlying principles, which we review in the sections that follow.

Photoautotrophy is comprised of two distinct sets of reactions that operate in parallel: (1) *light reactions* that produce ATP and (2) light-independent *dark reactions* that reduce CO_2 to cell material for autotrophic growth. We will discuss the light reactions in Sections 14.3 and 14.4 and the light-independent dark reactions in Section 14.5. Reduction of CO_2 by the light-independent dark reactions requires both energy, in the form of ATP, and electrons, in the form of NADH (or NADPH). The generation of NADH (or NADPH) from reduction of $NAD(P)^+$ requires an electron donor supplied from the environment. Water (H_2O) is the electron donor for photosynthesis in green plants, algae, and cyanobacteria. By contrast, phototrophic bacteria such as the *Heliobacteria*, *Acidobacteria*, green sulfur bacteria, purple bacteria, and filamentous anoxygenic phototrophs can use diverse electron donors but cannot use water. For example, in green and purple sulfur bacteria the donor could be a reduced sulfur compound such as hydrogen sulfide (H_2S), or even molecular hydrogen (H_2).

The oxidation of H_2O produces molecular oxygen (O_2) as a waste product; because of this, the photosynthetic process in cyanobacteria (and chloroplasts) is called **oxygenic photosynthesis**. However, in all other phototrophic bacteria O_2 is *not* produced, and thus the process is called **anoxygenic photosynthesis** (Figure 14.1). Oxygen produced by cyanobacteria billions of years ago converted Earth from an anoxic to an oxic world and set the stage for an explosion of eukaryotic microbial diversity that eventually gave rise to plants and animals.

Photosynthesis requires light-sensitive pigments, the *chlorophylls*—present in plants, algae, and the cyanobacteria—and *bacteriochlorophylls*, present in anoxygenic phototrophs. Absorption of light energy by chlorophylls and bacteriochlorophylls begins the process of photosynthetic energy conversion, and the net result is chemical energy, ATP.

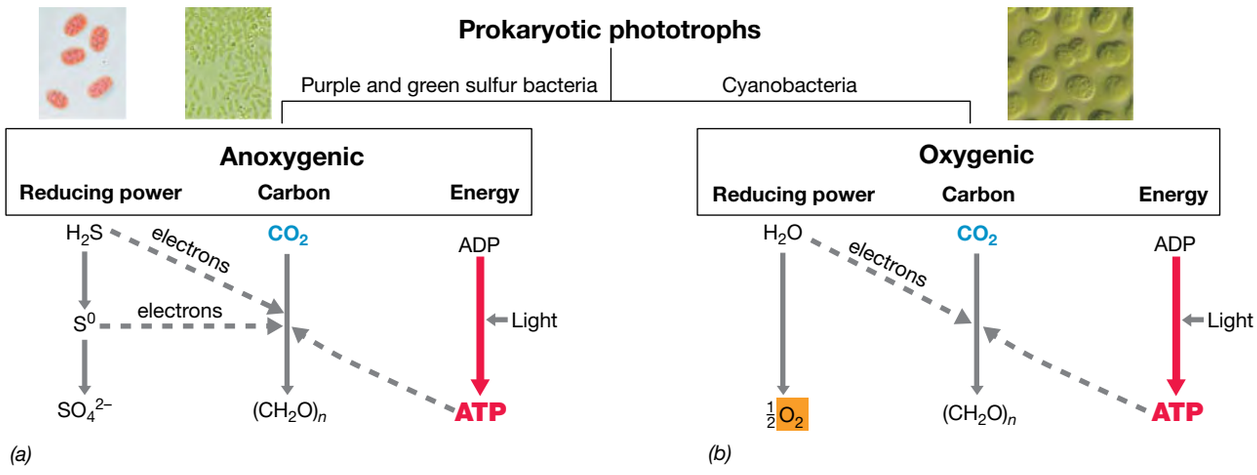


Figure 14.1 Patterns of photosynthesis. Energy and reducing power synthesis in *(a)* anoxygenic and *(b)* oxygenic phototrophs. Note that oxygenic phototrophs produce O_2, while anoxygenic phototrophs do not. Insets: Left, light photomicrographs of cells of a purple sulfur bacterium (*Chromatium*, cells 5 μm in diameter) and a green sulfur bacterium (*Chlorobium*, cells 0.9 μm in diameter). Note the sulfur globules inside or outside the cells produced from the oxidation of H_2S. Right, interference-contrast photomicrograph of cells of a coccoid-shaped cyanobacterium.

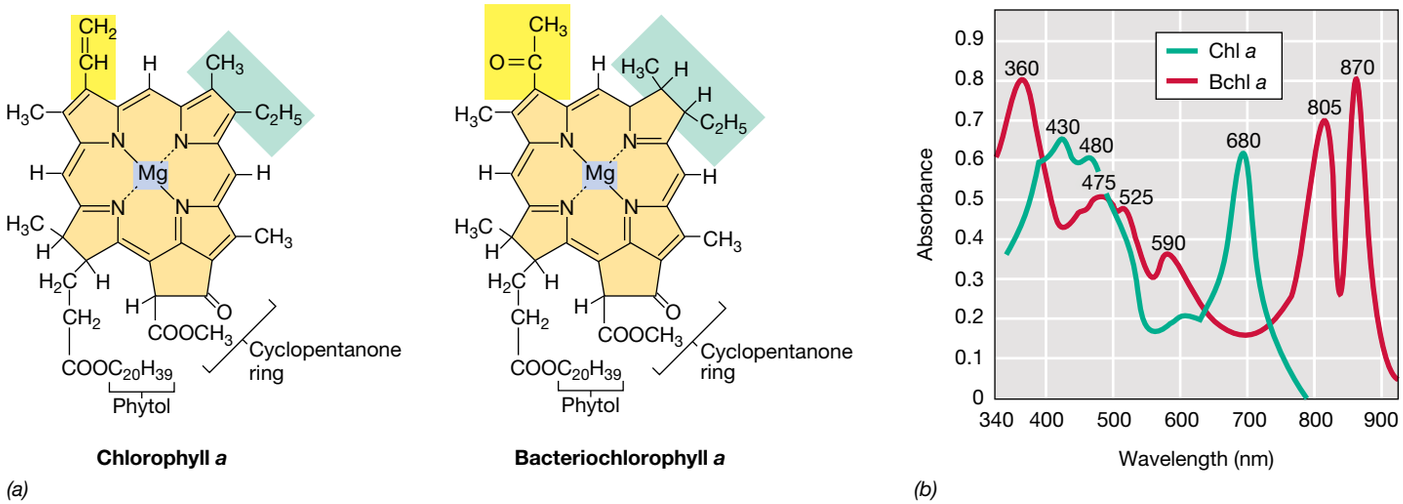


Figure 14.2 Structures and spectra of chlorophyll *a* and bacteriochlorophyll *a*. *(a)* The two molecules are identical except for those portions contrasted in yellow and green. *(b)* Absorption spectrum (green curve) of cells of the green alga *Chlamydomonas*. The peaks at 680 and 430 nm are due to chlorophyll *a*, and the peak at 480 nm is due to carotenoids. Absorption spectrum (red curve) of cells of the phototrophic purple bacterium *Rhodopseudomonas palustris*. Peaks at 870, 805, 590, and 360 nm are due to bacteriochlorophyll *a*, and peaks at 525 and 475 nm are due to carotenoids.

Chlorophyll and Bacteriochlorophyll

Chlorophyll and **bacteriochlorophyll** are tetrapyrroles that are related to the parent structure of the cytochromes. But unlike cytochromes, chlorophylls contain *magnesium* instead of *iron* at the center of the ring. Chlorophylls also contain specific substituents on the tetrapyrrole ring and a hydrophobic alcohol that helps anchor the chlorophyll into photosynthetic membranes. The structure of chlorophyll *a*, the principal chlorophyll of oxygenic phototrophs, is shown in Figure 14.2*a*. Chlorophyll *a* is green because it *absorbs* red and blue light and *transmits* green light; its absorption spectrum shows strong absorbance near 680 nm and 430 nm (Figure 14.2*b*). Several structurally distinct chlorophylls are known, each distinguished by its unique *absorption spectrum*. Cyanobacteria contain chlorophyll *a* (a few species contain chlorophyll *d*), while their relatives the prochlorophytes contain chlorophylls *a* and *b*.

Anoxygenic phototrophs produce one or more bacteriochlorophylls. Bacteriochlorophyll *a* (Figure 14.3), present in most purple bacteria (Sections 15.4 and 15.5), absorbs maximally between 800 and 925 nm (different purple bacteria synthesize slightly different photocomplexes, and the absorption maxima of bacteriochlorophyll *a* in any given organism depend to some degree on how proteins in the photocomplexes are arranged in the photosynthetic membrane; see Figure 14.6). Other bacteriochlorophylls, whose distribution runs along phylogenetic lines, absorb in other regions of the visible and near infrared spectrum (Figure 14.3).

The existence of different forms of chlorophyll or bacteriochlorophyll that absorb light of different wavelengths allows phototrophs to make better use of the available energy in the electromagnetic spectrum. By employing different pigments with distinct absorption properties, different phototrophs can coexist in the same habitat, each absorbing wavelengths of light that others cannot. Thus, pigment diversity has *ecological* significance for the successful coexistence of different phototrophs in the same habitat.

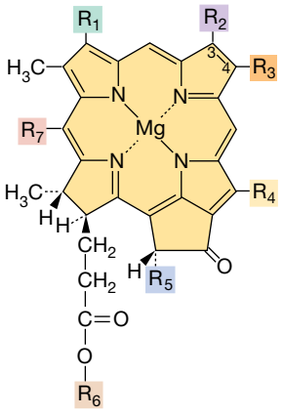
Reaction Centers and Antenna Pigments

In oxygenic phototrophs and in purple anoxygenic phototrophs, chlorophyll/bacteriochlorophyll molecules do not exist freely in the cell but are attached to proteins and housed within membranes to form *photocomplexes* consisting of anywhere from 50 to 300 chlorophyll/bacteriochlorophyll molecules. A small number of these pigment molecules are present within photosynthetic **reaction centers** (Figure 14.4), the complex macromolecular structures that participate directly in the reactions that lead to energy conservation. Photosynthetic reaction centers are surrounded by larger numbers of light-harvesting chlorophylls/bacteriochlorophylls. These so-called **antenna pigments** (also called *light-harvesting pigments*) function to absorb light and funnel some of the energy to the reaction center (Figure 14.4). At the low light intensities that are often found in nature, this arrangement for concentrating energy allows reaction centers to receive light energy that would otherwise be missed.

Photosynthetic Membranes, Chloroplasts, and Chlorosomes

The chlorophyll pigments and all the other components of the light-gathering apparatus exist within membranes in the cell. The location of these photosynthetic membranes differs between prokaryotic and eukaryotic phototrophs. In eukaryotic phototrophs, photosynthesis takes place in intracellular organelles, the *chloroplasts*, which contain sheetlike photosynthetic membrane systems

Pigment/Absorption maxima (in vivo)	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Bchl *a* (purple bacteria)/ 805, 830–890 nm	$-C(=O)-CH_3$	$-CH_3$[a]	$-CH_2-CH_3$	$-CH_3$	$-C(=O)-O-CH_3$	P/Gg[b]	$-H$
Bchl *b* (purple bacteria)/ 835–850, 1020–1040 nm	$-C(=O)-CH_3$	$-CH_3$[c]	$=C(H)-CH_3$	$-CH_3$	$-C(=O)-O-CH_3$	P	$-H$
Bchl *c* (green sulfur bacteria)/745–755 nm	$-C(H)(OH)-CH_3$	$-CH_3$	$-C_2H_5$ $-C_3H_7$[d] $-C_4H_9$	$-C_2H_5$ $-CH_3$	$-H$	F	$-CH_3$
Bchl c_s (green nonsulfur bacteria)/740 nm	$-C(H)(OH)-CH_3$	$-CH_3$	$-C_2H_5$	$-CH_3$	$-H$	S	$-CH_3$
Bchl *d* (green sulfur bacteria)/705–740 nm	$-C(H)(OH)-CH_3$	$-CH_3$	$-C_2H_5$ $-C_3H_7$ $-C_4H_9$	$-C_2H_5$ $-CH_3$	$-H$	F	$-H$
Bchl *e* (green sulfur bacteria)/719–726 nm	$-C(H)(OH)-CH_3$	$-C(=O)-H$	$-C_2H_5$ $-C_3H_7$ $-C_4H_9$	$-C_2H_5$	$-H$	F	$-CH_3$
Bchl *g* (heliobacteria)/ 670, 788 nm	$-C(H)=CH_2$	$-CH_3$[a]	$-C_2H_5$	$-CH_3$	$-C(=O)-O-CH_3$	F	$-H$



[a]No double bond between C_3 and C_4; additional H atoms are in positions C_3 and C_4.

[b]P, Phytyl ester ($C_{20}H_{39}O-$); F, farnesyl ester ($C_{15}H_{25}O-$); Gg, geranylgeraniol ester ($C_{10}H_{17}O-$); S, stearyl alcohol ($C_{18}H_{37}O-$).

[c]No double bond between C_3 and C_4; an additional H atom is in position C_3.

[d]Bacteriochlorophylls *c*, *d*, and *e* consist of isomeric mixtures with the different substituents on R_3 as shown.

Figure 14.3 Structure of all known bacteriochlorophylls (Bchl). The different substituents present in the positions R_1 to R_7 in the structure at the right are listed. Absorption properties can be determined by suspending intact cells of a phototroph in a viscous liquid such as 60% sucrose (this reduces light scattering and smooths out spectra) and running absorption spectra as shown in Figure 14.2*b*. In vivo absorption maxima are the physiologically relevant absorption peaks. The spectrum of bacteriochlorophylls extracted from cells and dissolved in organic solvents is often quite different.

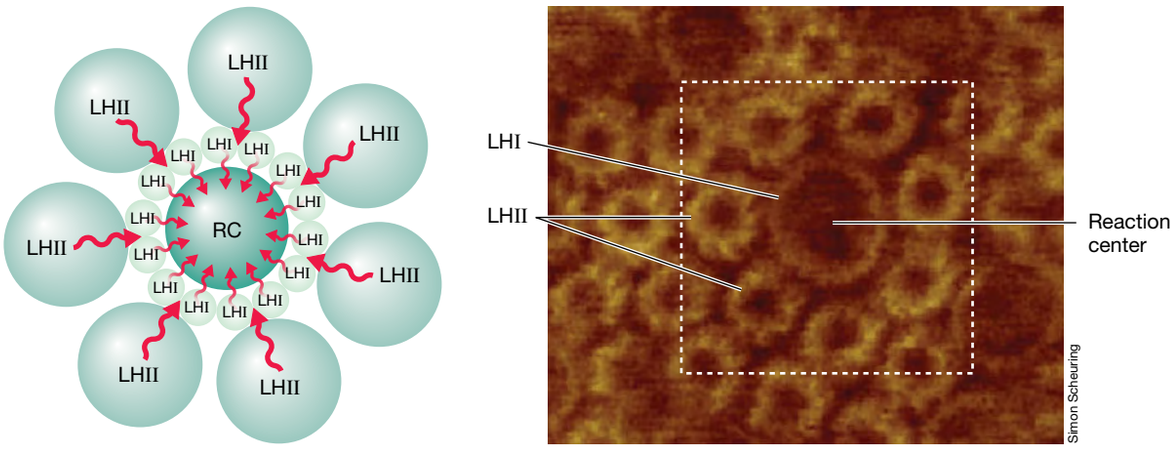


Figure 14.4 Arrangement of light-harvesting chlorophylls/bacteriochlorophylls and reaction centers within a photosynthetic membrane. *(a)* Light energy absorbed by light-harvesting (LH) molecules (light green) is transferred to the reaction centers (dark green, RC) where photosynthetic electron transport reactions begin. Pigment molecules are secured within the membrane by specific pigment-binding proteins. Compare this figure to Figure 14.12*b*. *(b)* Atomic force micrograph of photocomplexes of the purple bacterium *Phaeospirillum molischianum*. This organism has two types of light-harvesting complexes, LHI and LHII. LHII complexes transfer energy to LHI complexes, and these transfer energy to the reaction center (see Figure 14.11*b*).

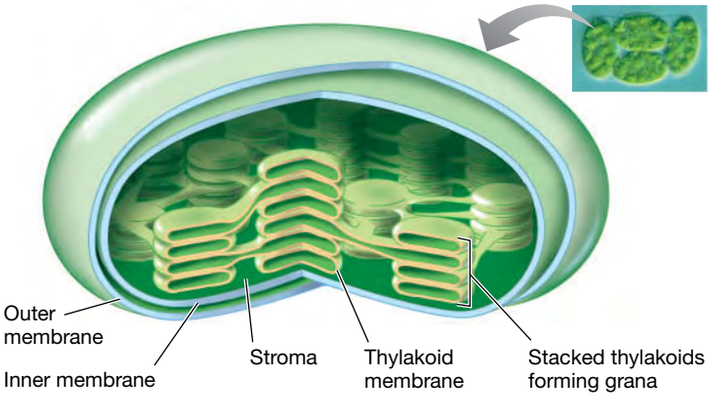


Figure 14.5 The chloroplast. Details of chloroplast structure, showing how the convolutions of the thylakoid membranes define an inner space called the stroma and form membrane stacks called grana. Inset: Photomicrograph of cells of the green alga *Makinoella*. Each of the four cells in a cluster contains several chloroplasts.

called **thylakoids** (Figure 14.5); stacks of thylakoids within the chloroplast form *grana*. The thylakoids are arranged so that the chloroplast is divided into two regions, the matrix space that surrounds the thylakoids, called the *stroma*, and the inner space within the thylakoid array, called the *lumen*. This arrangement makes possible the generation of a light-driven proton motive force that is used to synthesize ATP (Section 14.4).

Chloroplasts are absent from prokaryotic phototrophs. In purple bacteria, the photosynthetic pigments are integrated into internal membrane systems that arise from invagination of the cytoplasmic membrane. Membrane vesicles called *chromatophores* or membrane stacks called *lamellae* are common membrane arrangements in purple bacteria (Figure 14.6). In cyanobacteria, photosynthetic pigments reside in lamellar membranes (see Figure 14.10) also called *thylakoids* because of their resemblance to the thylakoids in the chloroplasts of algae (Figure 14.5).

The ultimate structure for capturing energy from low light intensities is the **chlorosome** (Figure 14.7). Chlorosomes are present in the anoxygenic green sulfur bacteria (*Chlorobium*, Figure 14.1 and Section 15.6), filamentous anoxygenic phototrophs (green nonsulfur bacteria; *Chloroflexus*, Section 15.7), and photosynthetic *Acidobacteria* (*Chloracidobacterium*, Section 15.8). Chlorosomes function as giant antenna systems, but unlike the antennae of purple bacteria or cyanobacteria, bacteriochlorophyll molecules in the chlorosome are not attached to proteins. Chlorosomes contain bacteriochlorophyll *c*, *d*, or *e* (Figure 14.3) arranged in dense arrays running along the long axis of the structure. Light energy absorbed by these antenna pigments is transferred to bacteriochlorophyll *a* in the reaction center in the cytoplasmic membrane through a small protein called the *FMO protein* (Figure 14.7).

Green bacteria can grow at the lowest light intensities of all known phototrophs and are often found in the deepest waters of lakes, inland seas, and other anoxic aquatic habitats where light levels are too low to support other phototrophs. Green nonsulfur bacteria are major components of microbial mats, thick biofilms that form in hot springs and highly saline environments (Section 20.5). Microbial mats experience a steep light gradient, with light levels even a few millimeters into the mat approaching darkness. Hence, chlorosomes allow green nonsulfur bacteria to grow phototrophically with only the minimal light intensities available.

MINIQUIZ

- What is the fundamental difference between an oxygenic and an anoxygenic phototroph?
- What is the purpose of chlorophyll and bacteriochlorophyll molecules? In what ways do they resemble cytochromes and in what ways to they differ?
- Why can phototrophic green bacteria grow at light intensities that will not support purple bacteria?

14.2 Carotenoids and Phycobilins

Although chlorophyll/bacteriochlorophyll is required for photosynthesis, phototrophic organisms contain other pigments as well. These pigments include, in particular, the *carotenoids* and *phycobilins*.

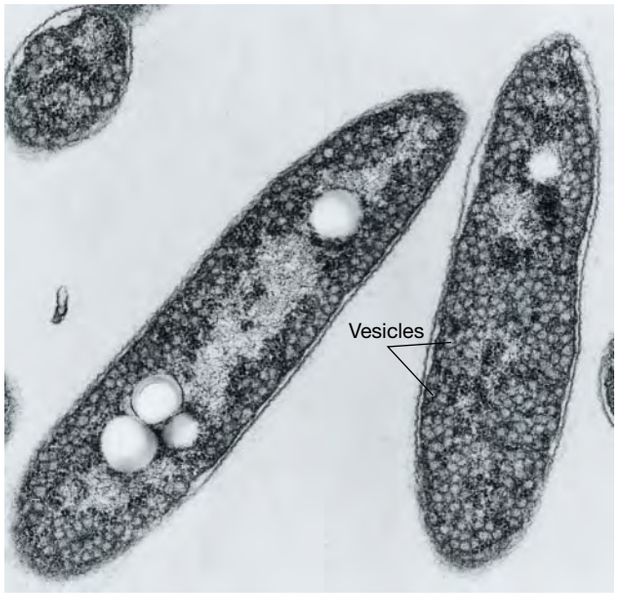
Carotenoids

The most widespread accessory pigments in phototrophs are the **carotenoids**. Carotenoids are hydrophobic pigments that are firmly embedded in the photosynthetic membrane. Figure 14.8 shows the structure of a common carotenoid, *β-carotene*. Carotenoids are typically yellow, red, brown, or green and absorb light in the blue region of the spectrum. The major carotenoids of anoxygenic phototrophs are shown in Figure 14.9. Because they tend to mask the color of bacteriochlorophylls, carotenoids are responsible for the brilliant colors of red, purple, pink, green, yellow, or brown that are observed in different species of anoxygenic phototrophs (Figure 15.12).

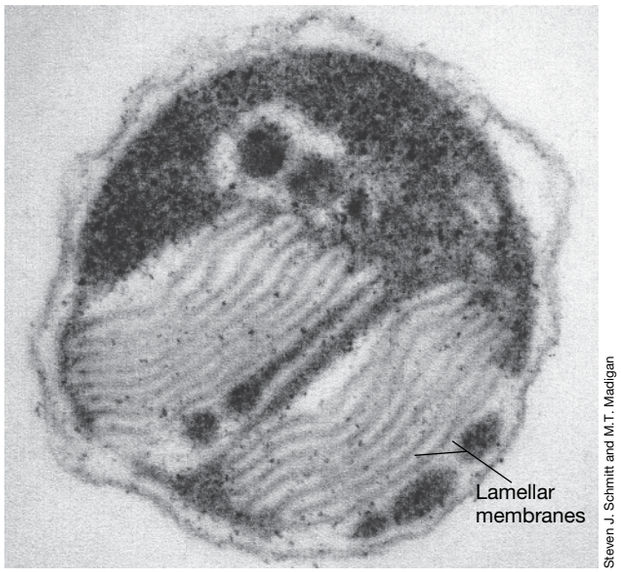
Carotenoids are closely associated with chlorophyll or bacteriochlorophyll in photosynthetic complexes, and some of the energy absorbed by carotenoids can be transferred to the reaction center. However, carotenoids function primarily as photoprotective agents. Bright light can be harmful to cells because it can catalyze photooxidation reactions that can produce toxic forms of oxygen, such as singlet oxygen ($^1O_2$). Like superoxide and other forms of toxic oxygen (Section 5.14), singlet oxygen can spontaneously oxidize photocomplexes, rendering them nonfunctional. Carotenoids quench toxic oxygen species by absorbing much of this harmful light and in this way prevent these dangerous photooxidations. Because phototrophic organisms by their very nature must live in the light, the photoprotection conferred by carotenoids is clearly advantageous.

Phycobiliproteins and Phycobilisomes

Cyanobacteria and the chloroplasts of red algae (which are descendants of cyanobacteria, Section 18.1) contain pigments called



(a)



(b)

Figure 14.6 Membranes in anoxygenic phototrophs. *(a)* Chromatophores. Section through a cell of the purple bacterium *Rhodobacter* showing vesicular photosynthetic membranes. The vesicles are continuous with and arise by invagination of the cytoplasmic membrane. A cell is about 1 μm wide. *(b)* Lamellar membranes in the purple bacterium *Ectothiorhodospira*. A cell is about 1.5 μm wide. These membranes are also continuous with and arise from invagination of the cytoplasmic membrane, but instead of forming vesicles, they form membrane stacks.

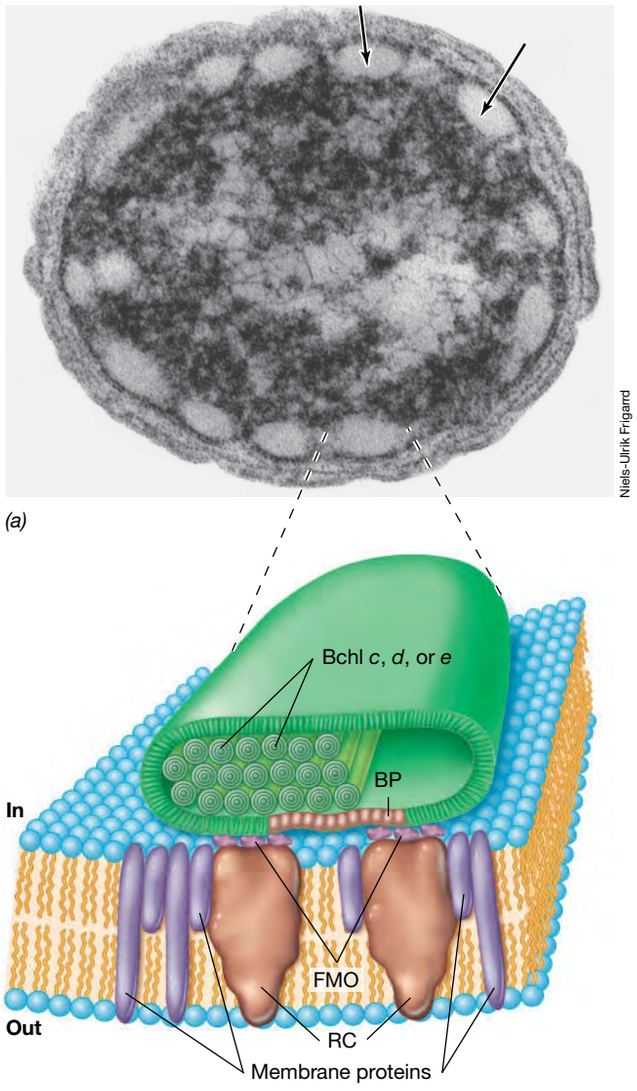


Figure 14.7 The chlorosome of green sulfur and green nonsulfur bacteria. *(a)* Transmission electron micrograph of a cross section of a cell of the green sulfur bacterium *Chlorobaculum tepidum*. Note the chlorosomes (arrows). *(b)* Model of chlorosome structure. The chlorosome (green) lies appressed to the inside surface of the cytoplasmic membrane. Antenna bacteriochlorophyll (Bchl) molecules are arranged in tubelike arrays inside the chlorosome, and energy is transferred from these to reaction center (RC) Bchl *a* in the cytoplasmic membrane through a protein called FMO. Base plate (BP) proteins function as connectors between the chlorosome and the cytoplasmic membrane.

phycobiliproteins, which are the main light-harvesting systems of these phototrophs. Phycobiliproteins consist of red or blue-green linear tetrapyrroles, called *bilins*, bound to proteins, and give cyanobacteria and red algae their characteristic colors (**Figure 14.10**). The red phycobiliprotein, called *phycoerythrin*, absorbs most strongly at wavelengths around 550 nm, whereas the blue phycobiliprotein, *phycocyanin* (Figure 14.10*b*), absorbs most

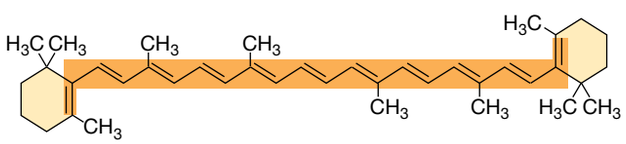


Figure 14.8 Structure of β-carotene, a typical carotenoid. The conjugated double-bond system is highlighted in orange.

UNIT 4

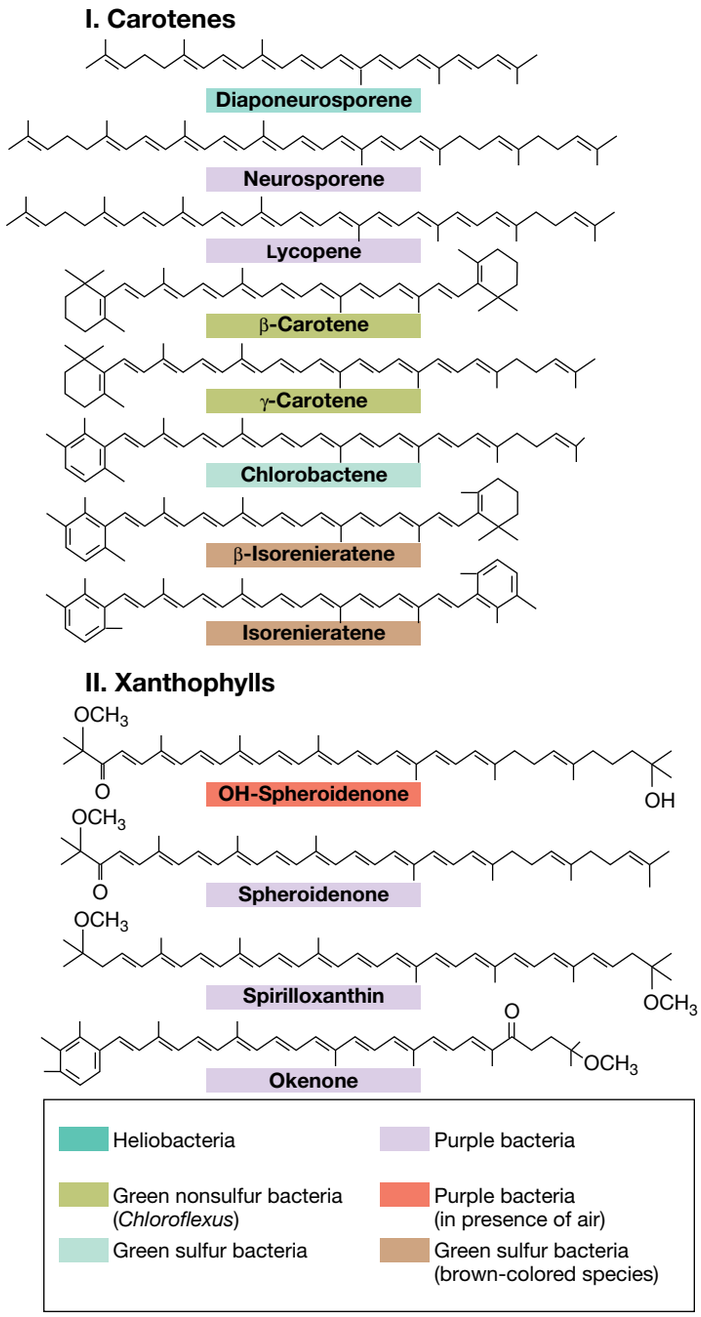


Figure 14.9 Structures of some common carotenoids found in anoxygenic phototrophs. Carotenes are hydrocarbon carotenoids, and xanthophylls are oxygenated carotenoids. Compare the structure of β-carotene shown in Figure 14.8 with how it is drawn here. For simplicity in the structures shown here, methyl (CH_3) groups are designated by bond only.

strongly at 620 nm. A third phycobiliprotein, called *allophycocyanin*, absorbs at about 650 nm.

Phycobiliproteins assemble into aggregates called **phycobilisomes** that attach to cyanobacterial thylakoids (Figure 14.10*c*). Phycobilisomes are arranged such that the allophycocyanin molecules are in direct contact with the photosynthetic membrane. Allophycocyanin is surrounded by phycocyanin or phycoerythrin (or both, depending on the organism). Phycocyanin and phycoerythrin absorb light of shorter wavelengths (higher energy) and transfer some energy to allophycocyanin, which is positioned closest to the reaction center chlorophyll and transfers energy to it (Figure 14.10*b*). Thus, in a fashion similar to how antenna bacteriochlorophyll systems function in anoxygenic phototrophs (Figure 14.4), energy transfer proceeds "downhill" from phycobilisomes to the reaction center. Phycobilisomes thus facilitate energy transfer to cyanobacterial reaction centers, allowing cyanobacteria to grow at lower light intensities than would otherwise be possible.

MINIQUIZ

- In which phototrophs are carotenoids found? Phycobiliproteins?
- How does the structure of a phycobilin compare with that of a chlorophyll?
- Phycocyanin is blue-green. What color of light does it absorb?

14.3 Anoxygenic Photosynthesis

In the photosynthetic light reactions, electrons travel through an electron transport chain whose components are arranged in a photosynthetic membrane in order of their increasingly more electropositive reduction potential (E_0'). This generates a proton motive force that drives ATP synthesis. Key parts of this process include photosynthetic reaction centers and photosynthetic membranes (Section 14.1).

Photosynthetic reaction centers are complex macromolecular structures localized within photosynthetic membranes. They are composed of multiple protein subunits and cofactors (including chlorophylls or bacteriochlorophylls), and they interact with both antenna pigments and components of the electron transport chain. Photosynthetic pigments funnel light energy to the reaction center to excite a special pair of chlorophylls (or bacteriochlorophylls), thereby generating high-potential electrons that can be donated to subsequent electron transport reactions. Several different types of reaction centers have been described but all fall into one of two classes; they are either of a *quinone type* (Q-type) or *iron–sulfur type* (FeS-type) depending on the electron acceptor in the reaction center.

Electron Flow in Purple Bacteria

Purple bacteria use a Q-type reaction center which contains three polypeptides, designated L, M, and H. These proteins, along with a molecule of cytochrome *c*, are firmly embedded in the photosynthetic membrane (Figure 14.6) and wind through the membrane several times (**Figure 14.11**). The L, M, and H polypeptides bind two molecules of bacteriochlorophyll *a*, called the *special pair*, two additional bacteriochlorophyll *a* molecules that function in photosynthetic electron flow, two molecules of bacteriopheophytin *a* (bacteriochlorophyll *a* minus its magnesium atom), two molecules of quinone (Section 3.10), and one carotenoid molecule (Figure 14.11).

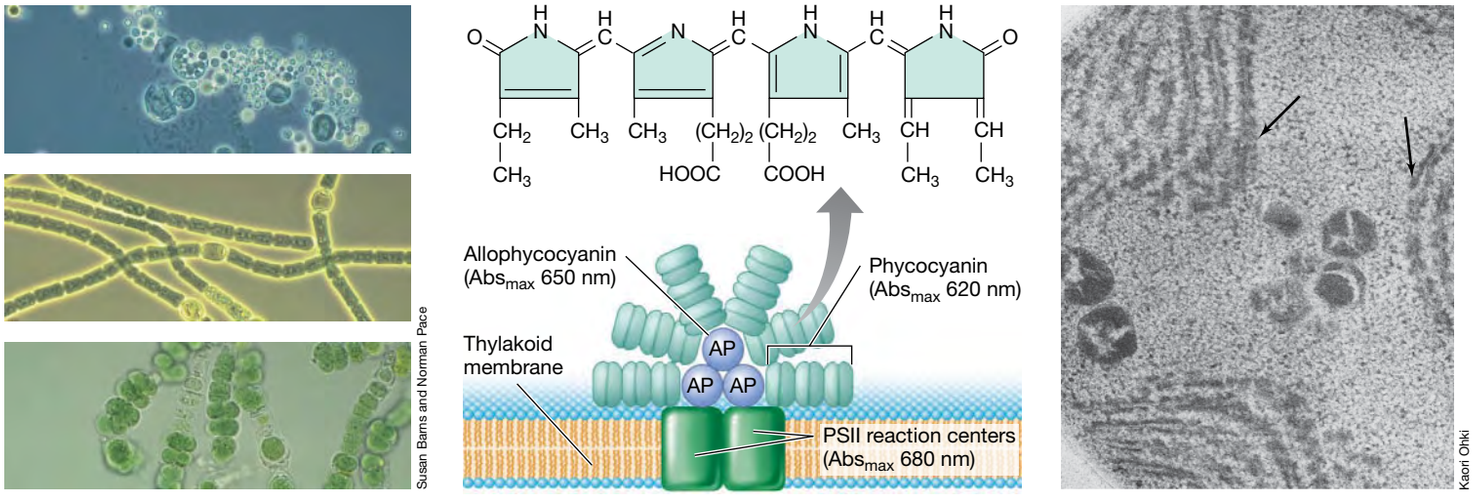


Figure 14.10 Phycobiliproteins and phycobilisomes. *(a)* Light photomicrographs of cells of the cyanobacteria (top to bottom) *Dermocarpa*, *Anabaena*, and *Fischerella*, showing the typical blue-green color of cells due to phycobiliproteins. *(b)* Structure of phycocyanin (top) and a phycobilisome. Phycocyanin absorbs at higher energies (shorter wavelengths) than allophycocyanin. Chlorophyll *a* absorbs at longer wavelengths (lower energies) than allophycocyanin. Energy flow is thus phycocyanin → allophycocyanin → chlorophyll *a* of PSII. *(c)* Electron micrograph of a thin section of the cyanobacterium *Synechocystis*. Note the darkly staining ball-like phycobilisomes (arrows) attached to the lamellar membranes.

Photosynthetic light reactions begin when light energy absorbed by the antenna systems is transferred to the special pair of bacteriochlorophyll *a* molecules (Figure 14.11*a*). This excites the special pair, converting it from a relatively weak to a very strong electron donor (very electronegative E_0', Section 3.6). Once this strong donor has been produced, the remaining steps in photosynthetic electron flow are highly reminiscent of those we have seen before in respiration (Section 3.10 and Figure 3.22); that is, electrons flow through a membrane from carriers of low E_0' to those of high E_0', generating a proton motive force in the process (**Figure 14.12**).

Before excitation, the purple bacterial reaction center, which is called *P870*, has an E_0' of about +0.5 V; after excitation, it has a potential of about −1.0 V (Figure 14.12*a*). An excited electron

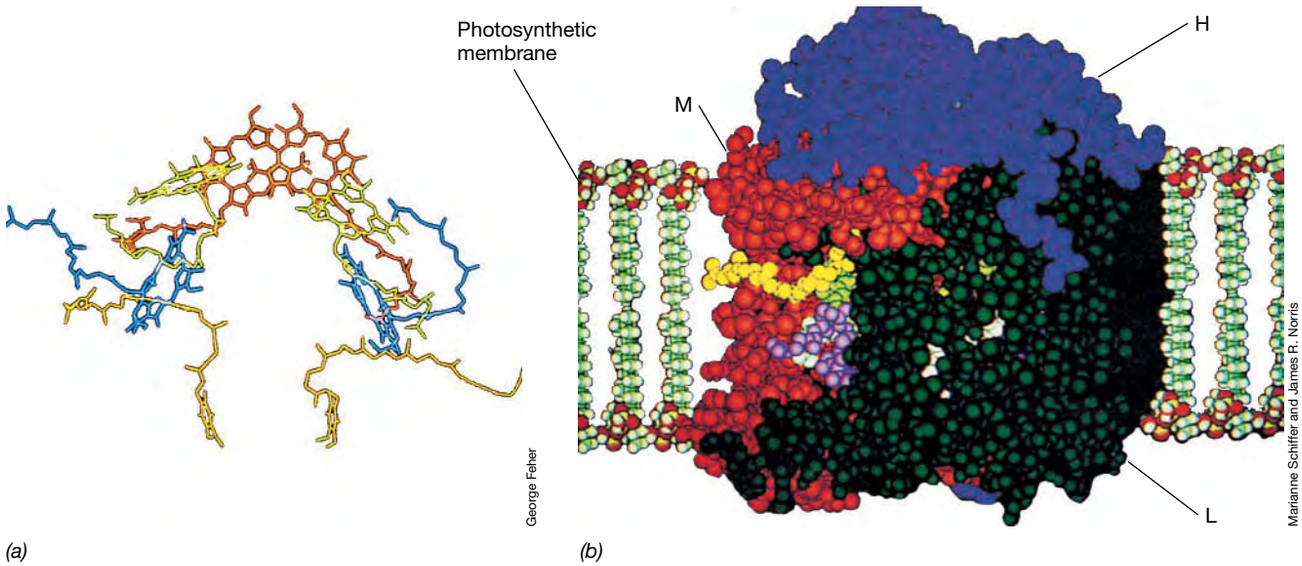
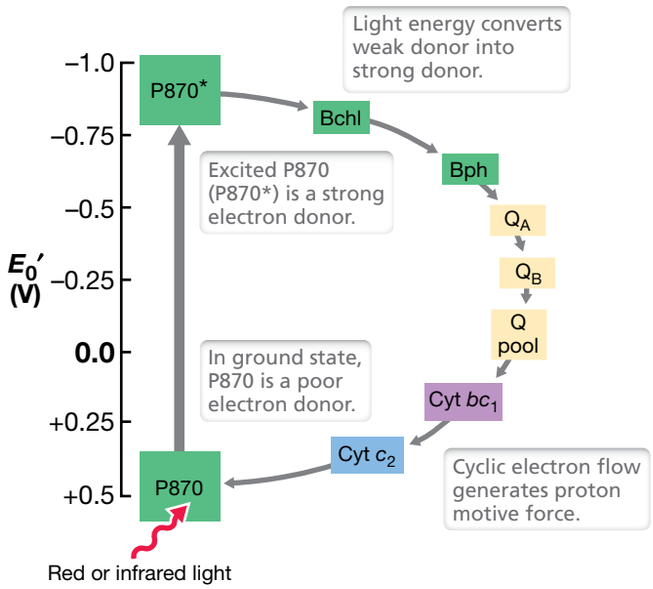
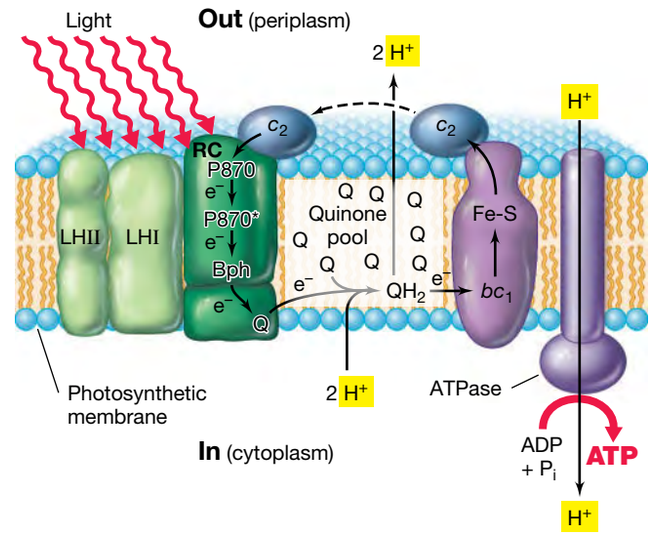


Figure 14.11 Structure of the reaction center of a purple phototrophic bacterium. *(a)* Arrangement of pigment molecules in the reaction center. The "special pair" of bacteriochlorophyll molecules (orange) overlap and occur at the top of the reaction center structural diagram; adjacent to and below the special pair in the diagram are a pair of accessory bacteriochlorophylls (light yellow). Bacteriopheophytin molecules (blue) are arranged below the bacteriochlorophylls, and quinones (dark yellow) are present at the bottom of the structural model. Compare the structure of these molecules in the reaction center to their role in electron transfer (see Figure 14.12). *(b)* Molecular model of the protein structure of the reaction center. The pigments described in part *a* are bound to membranes by protein H (blue), protein M (red), and protein L (green). The reaction center pigment–protein complex is integrated into the lipid bilayer.



(a) **Electron flow in anoxygenic photosynthesis**



(b) **Arrangement of protein complexes in the purple bacterium reaction center**

Figure 14.12 Electron flow in anoxygenic photosynthesis in a purple bacterium. *(a)* Schematic of electron flow in a purple bacterium. Bchl, bacteriochlorophyll; Bph, bacteriopheophytin; Q_A, Q_B, intermediate quinones; Q pool, quinone pool in membrane; Cyt, cytochrome. *(b)* Arrangement of protein complexes in the purple bacterium reaction center leading to proton motive force (photophosphorylation) by ATPase. Two protons are translocated for every electron that passes from the reaction center to Cyt bc_1. LH, light-harvesting bacteriochlorophyll complexes; RC, reaction center; Bph, bacteriopheophytin; Q, quinone; FeS, iron–sulfur protein; bc_1, cytochrome bc_1 complex; c_2, cytochrome c_2. For a description of ATPase function, see Section 3.11.

within P870 proceeds to reduce a molecule of bacteriochlorophyll *a* within the reaction center (Figures 14.11*a* and 14.12*a*). This transition takes place incredibly fast, taking only about three-trillionths (3×10^{-12}) of a second. Once reduced, bacteriochlorophyll *a* proceeds to reduce bacteriopheophytin *a*, and the latter reduces quinone molecules within the membrane (Figure 14.12). These transitions are also very fast, taking less than one-billionth of a second. From the quinone, electrons are transported through the membrane more slowly (on a millisecond scale) through a series of iron–sulfur proteins and cytochromes (Figure 14.12), eventually returning to the reaction center.

Figure 14.12*b* shows electron flow within the actual context of the photosynthetic membrane. Key electron transport proteins include many that also participate in respiratory electron flow (↔ Figure 3.22)—cytochrome bc_1 and cytochrome c_2, in particular (Figure 14.12). Cytochrome c_2 is a periplasmic cytochrome (recall that the periplasm is the region between the cytoplasmic membrane and the outer membrane in gram-negative bacteria, ↔ Section 2.5) that functions as an electron shuttle between the membrane-bound bc_1 complex and the reaction center (Figure 14.12*b*). Electron flow is completed when cytochrome c_2 donates an electron to the special pair to return it to its original ground-state reduction potential. The reaction center can then absorb new light energy and repeat the process.

ATP is synthesized during photosynthetic electron flow from the activity of ATPase that couples the proton motive force to ATP synthesis (↔ Section 3.11). This mechanism of ATP synthesis is called **photophosphorylation**, specifically *cyclic photophosphorylation*, because electrons move within a closed loop. However, unlike respiration, where there is a net consumption of electrons, in cyclic photophosphorylation there is no net input or consumption of electrons; electrons simply travel a circuitous route, returning from whence they came (Figure 14.12).

Generation of Reducing Power

For a purple bacterium to grow as a photoautotroph, the formation of ATP is not enough. Reducing power (NADH) is also necessary to reduce CO_2 to cell material. Reducing power for purple bacteria can come from many sources, in particular reduced sulfur compounds such as H_2S. When H_2S is the electron donor in purple sulfur bacteria, globules of S^0 are stored inside the cells (Figure 14.1). When S^0 is formed, electrons end up in the "quinone pool" (Figure 14.12). However, the E_0' of quinone (about 0 V) is insufficiently electronegative to reduce NAD^+ (−0.32 V). Hence, electrons from the quinone pool must be forced backwards (against the electrochemical gradient) to reduce NAD^+ to NADH (see Figure 14.13). This energy-requiring process, called **reverse electron transport**, is driven by the energy of the proton motive force. We will see later that reverse electron flow is also the mechanism by which chemolithotrophs obtain reducing power for CO_2 fixation; in many of these cases, the electrons come from electron donors of quite positive E_0' (Sections 14.7–14.15). However, as we will see shortly, reverse electron flow is not necessary in other anoxygenic phototrophs.

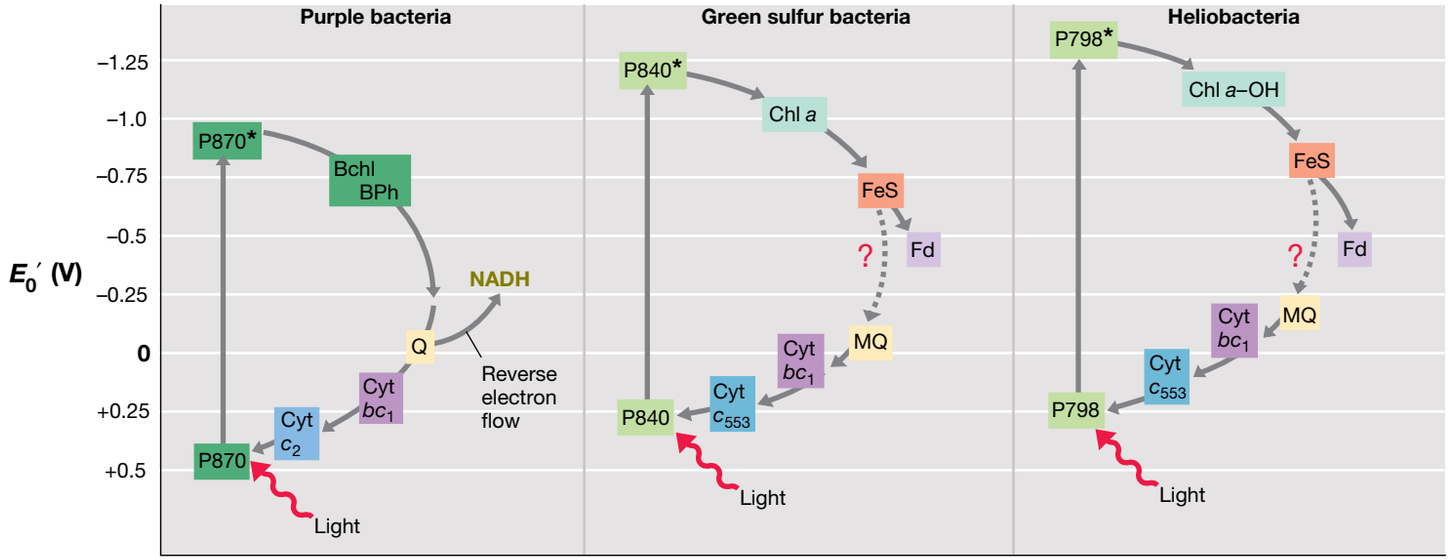


Figure 14.13 A comparison of electron flow in purple bacteria, green sulfur bacteria, and *Heliobacteria*. Reverse electron flow in purple bacteria is necessary to produce NADH because the primary acceptor (quinone, Q) is more positive in potential than the NAD^+/NADH couple. In green sulfur bacteria and *Heliobacteria*, ferredoxin (Fd), whose E_0' is more negative than that of NADH, is produced by light-driven reactions for reducing power needs. Cyclic electron flow in green sulfur bacteria and *Heliobacteria* would require electron transfer from the FeS-type photosystem to the menaquinone pool, but evidence for this mechanism is limited, suggesting noncyclic electron flow in these phototrophs. Bchl, bacteriochlorophyll; BPh, bacteriopheophytin; Q, quinone; MQ, menaquinone. P870 and P840 are reaction centers of purple and green bacteria, respectively, and consist of Bchl *a*. The reaction center of *Heliobacteria* (P798) contains Bchl *g*, and the reaction center of *Chloroflexus* is of the purple bacterial type. Note that forms of chlorophyll *a* are present in the reaction centers of green bacteria and heliobacteria.

Photosynthetic Electron Flow in Other Anoxygenic Phototrophs

Thus far we have focused on electron flow in purple bacteria. Although analogous membrane-associated reactions drive photophosphorylation in other anoxygenic phototrophs, there are significant differences in the details. Both filamentous anoxygenic phototrophs and purple bacteria employ structurally similar Q-type reaction centers, but by contrast the green sulfur bacteria, *Acidobacteria*, and *Heliobacteria* all employ FeS-type reaction centers and this is reflected in differences in electron flow.

Figure 14.13 contrasts photosynthetic electron flow in purple bacteria, green sulfur bacteria, and the *Heliobacteria*. Note that in green sulfur bacteria and *Heliobacteria* the excited state of the reaction center bacteriochlorophylls is significantly *more* electronegative than in purple bacteria and that actual chlorophyll *a* (green sulfur bacteria) or a structurally modified form of chlorophyll *a* (hydroxychlorophyll *a* in *Heliobacteria*) is present in the reaction center. Thus, unlike in purple bacteria, where the first stable acceptor molecule (quinone) has an E_0' of about 0 V (Figure 14.12*a*), the acceptors in green sulfur bacteria and *Heliobacteria* are FeS-proteins that have a much more electronegative E_0' than does NADH. Hence, reverse electron flow is unnecessary in green sulfur bacteria or *Heliobacteria*. The highly electronegative electrons generated by the FeS-type reaction centers are ultimately transferred to a protein called *ferredoxin* ($E_0' = -0.4$ V). In green sulfur bacteria ferredoxin is the direct electron donor for CO_2 fixation (Section 14.5), and electrons from ferredoxin can also pass to ferredoxin–NAD oxidoreductase for the production of NADH.

It remains unclear whether electron transfer in green sulfur bacteria and *Heliobacteria* is cyclic or noncyclic. It has been proposed that the FeS-type reaction centers in these phototrophs can transfer electrons directly to menaquinone, thereby generating a proton motive force resulting in cyclic photophosphorylation as seen in purple bacteria (Figure 14.13). However, little evidence for cyclic photophosphorylation has been observed in green sulfur bacteria and *Heliobacteria*. Alternatively, these phototrophs may employ noncyclic electron flow whereby electrons from external electron donors, such as H_2S, enter at the level of the menaquinone pool. These electrons would be transferred through the reaction center and then to ferredoxin where they would ultimately be channeled into biosynthetic reactions.

MINIQUIZ

- What parallels exist in the processes of photophosphorylation and oxidative phosphorylation?
- What is reverse electron flow and why is it necessary? Which phototrophs need to use reverse electron flow?
- What is the difference between cyclic and noncyclic photophosphorylation?

14.4 Oxygenic Photosynthesis

In contrast to photosynthetic electron flow in *anoxygenic* phototrophs, which have either FeS-type or Q-type photosynthetic reaction centers, *oxygenic* phototrophs have both types of reaction centers. In oxygenic phototrophs, electrons flow through

two distinct photosystems called *photosystem I* (*PSI*, or *P700*), which has an FeS-type reaction center, and *photosystem II* (*PSII*, or *P680*), which has a Q-type reaction center. PSI and PSII interact in the "Z scheme" of photosynthesis, so named because the pathway resembles the letter Z turned on its side (**Figure 14.14**). As in anoxygenic photosynthesis, the light reactions in oxygenic photosynthesis occur in photocomplexes embedded in specialized photosynthetic membranes. In eukaryotic cells, the membranes are in the chloroplast (Figure 14.5), whereas in cyanobacteria, the membranes are arranged in stacks within the cytoplasm (Figure 14.10*c*).

Electron Flow and ATP Synthesis in Oxygenic Photosynthesis

PSII performs the first—and most distinctive—step in oxygenic photosynthesis, the splitting of water into oxygen and electrons (Figure 14.14). Upon absorbing light energy, the P680 chlorophyll *a* molecule in PSII is excited to a very electronegative reduction potential that allows it to donate an electron to pheophytin *a* (chlorophyll *a* minus its magnesium atom), a molecule with an E_0' of about −0.5 V. This creates a charge separation that causes P680 to become so strongly electropositive that it can accept electrons from H_2O. The oxidation of water by PSII occurs at the *water-oxidizing complex* (**Figure 14.15**) and is catalyzed by a Mn_4Ca cluster, which binds 2 molecules of H_2O. P680 removes one electron from the Mn_4Ca cluster of the water-oxidizing complex for each photon absorbed. In this way 4 electrons are sequentially removed from the 2 H_2O molecules bound to the Mn_4Ca cluster, resulting in the production of O_2 and 4 H^+. Each electron transferred to pheophytin travels through several other proteins, including Q_A and Q_B, within the PSII photocomplex. Two electrons from the PSII photocomplex are then used to reduce plastoquinone (PQ) to PQH_2, a step that allows for the generation of the proton motive force.

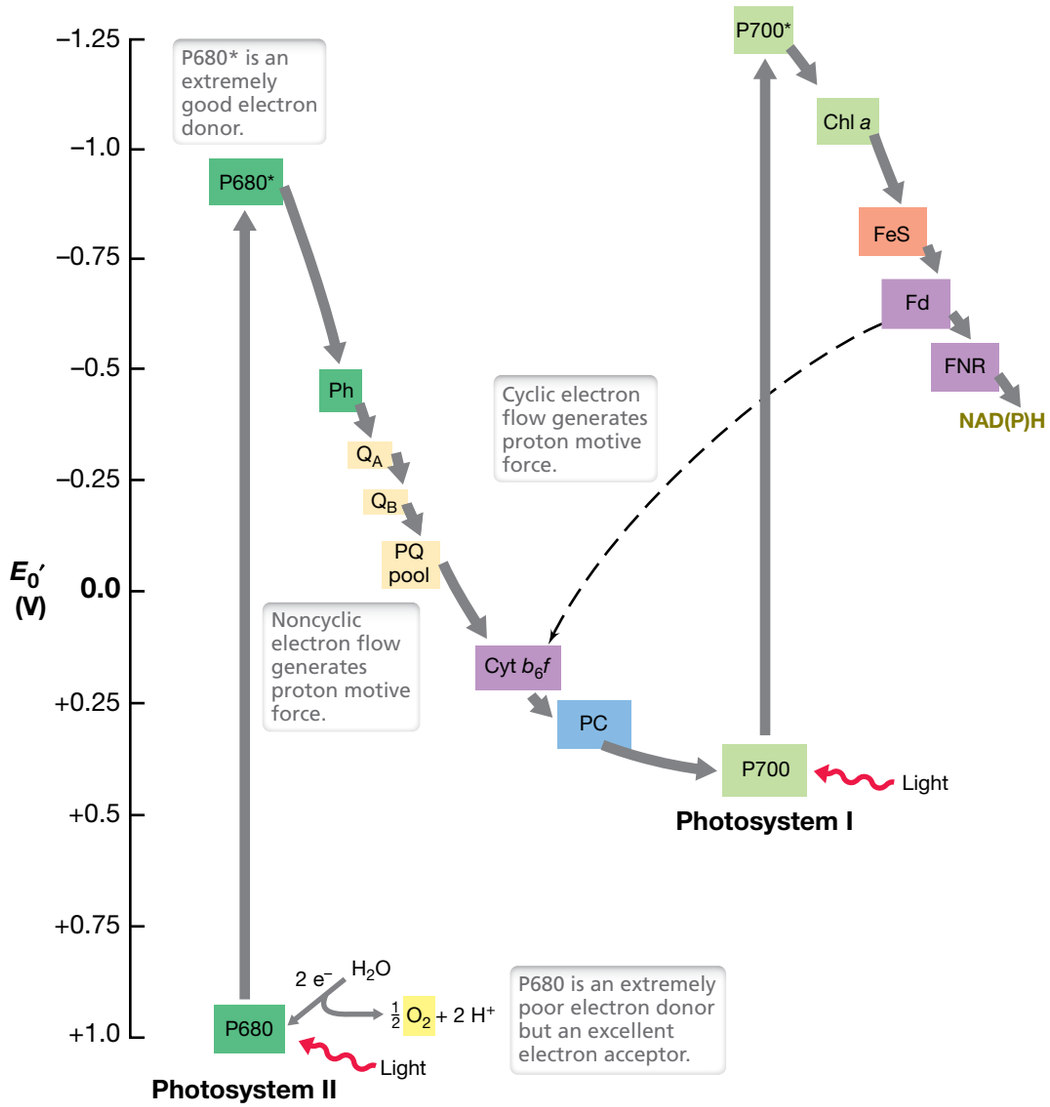


Figure 14.14 Electron flow in oxygenic photosynthesis, the "Z" scheme. Electrons flow through two photosystems, PSI and PSII. Ph, pheophytin; PQ, plastoquinone; Chl, chlorophyll; Cyt, cytochrome; PC, plastocyanin; FeS, nonheme iron–sulfur protein; Fd, ferredoxin; FNR, ferredoxin–NADP oxidoreductase; P680 and P700 are the reaction center chlorophylls of PSII and PSI, respectively. Compare with Figure 14.12*a*.

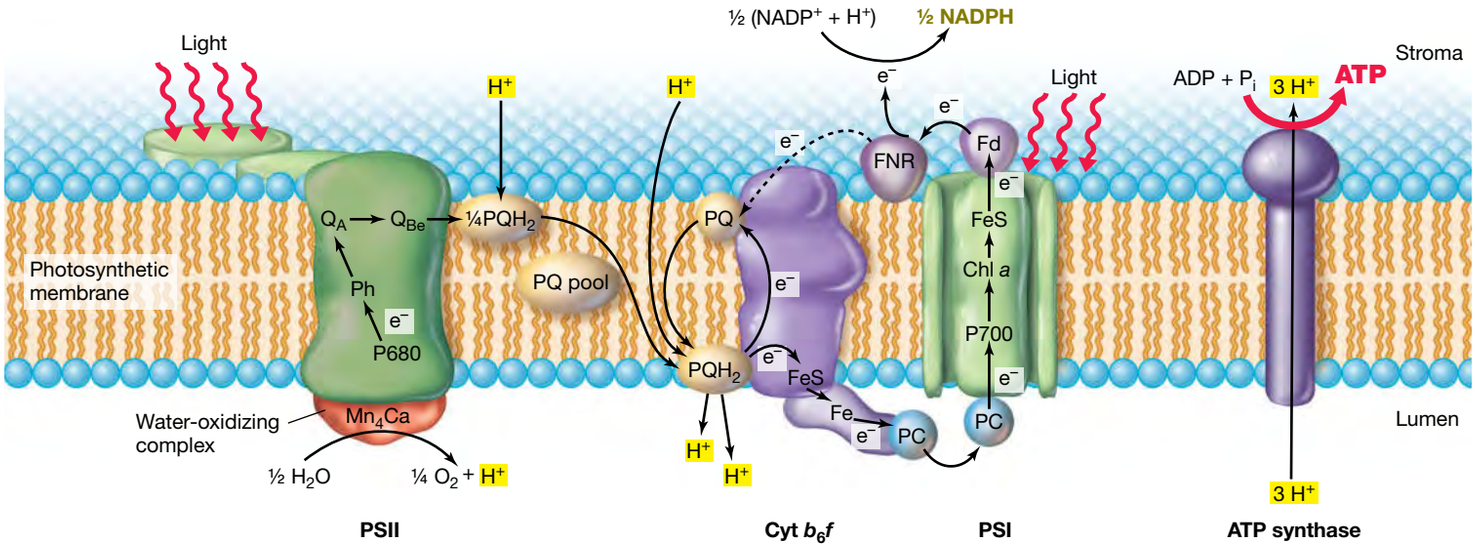


Figure 14.15 Electron transport in oxygenic photosynthesis. Photosystem II (PSII) is activated by photons, causing H_2O to be oxidized on the Mn_4Ca cluster of the water-oxidizing complex. Electrons are transferred from PSII to the plastoquinone pool (PQ/PQH_2). Protons are exchanged across the membrane when plastoquinone is oxidized by cytochrome b_6f. Electrons are then transferred to plastocyanin (PC), which carries them to photosystem I (PSI). Upon activation by light, PSI reduces ferredoxin (Fd), with sequential reduction of ferredoxin: $NADP^+$ oxidoreductase (FNR), and then $NADP^+$. The ATP and NADPH produced by the light reactions are used in CO_2 fixation by the Calvin cycle (see Section 14.5). Cyclic photophosphorylation occurs when FNR donates electrons to cytochrome b_6f instead of to $NADP^+$. During cyclic photophosphorylation, more ATP and less NADPH are produced than during noncyclic photophosphorylation.

The proton motive force is generated in oxygenic photosynthesis by electron transport through quinones and cytochromes of increasingly positive reduction potential. These electron transport reactions are similar to those encountered during our discussion of aerobic respiration (Section 3.11). Electrons from PQH_2 are transferred through cytochrome b_6f and through a copper-containing protein called *plastocyanin* before being donated to the PSI reaction center (Figure 14.15). The absorption of light by P700 of PSI allows it to accept electrons donated from plastocyanin. Electrons travel through several intermediates in PSI terminating with the reduction of $NADP^+$ to NADPH (Figure 14.14). Two protons are generated for each water molecule that is split by PSII and four protons are translocated across the membrane for every two electrons transferred through the electron transport chain, resulting in a total of 12 protons translocated for every molecule of O_2 produced. This proton motive force is then used by ATP synthase to produce ATP.

Oxygenic photosynthesis results in *noncyclic photophosphorylation* because electrons do not cycle back to reduce the oxidized P680, but instead are used in the reduction of $NADP^+$. However, when the cell requires less NADPH, oxygenic phototrophs can perform *cyclic photophosphorylation*. This occurs when, instead of reducing $NADP^+$, electrons from PSI are returned to the electron transport chain that connects PSII to PSI. When this happens, the recycled electrons can be used to generate a proton motive force that supports additional ATP synthesis (dashed line in Figures 14.14 and 14.15).

Anoxygenic Photosynthesis in Oxygenic Phototrophs

Photosystems I and II normally function in tandem in oxygenic photosynthesis. However, if PSII activity is blocked, some oxygenic phototrophs can perform photosynthesis using only PSI. Under these conditions, cyclic photophosphorylation (Figure 14.14) occurs exclusively, and reducing power for CO_2 reduction comes from sources other than water. In effect, this is anoxygenic photosynthesis occurring in oxygenic phototrophs.

Many cyanobacteria can use H_2S as an electron donor under these conditions and many green algae can use H_2. When H_2S is used, it is oxidized to elemental sulfur (S^0), and sulfur granules similar to those produced by green sulfur bacteria (Figure 14.1) are deposited outside the cyanobacterial cells. **Figure 14.16** shows this in the filamentous cyanobacterium *Oscillatoria limnetica*. This organism lives in anoxic salt ponds where it oxidizes sulfide and carries out anoxygenic photosynthesis along with green and purple bacteria.

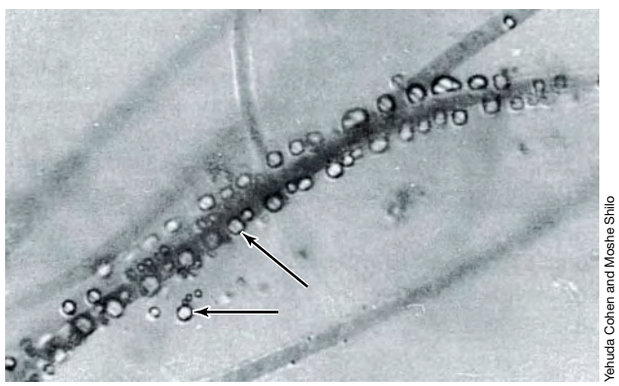


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Figure 14.16 Oxidation of H_2S by *Oscillatoria limnetica*. Note the globules of S^0 (arrows), the oxidation product of H_2S, formed outside the cells. *O. limnetica* carries out oxygenic photosynthesis, but cells revert to the anoxygenic process in the presence of H_2S.

From an evolutionary standpoint, the process of cyclic photophosphorylation in both oxygenic and anoxygenic phototrophs is one of many indications of their close relationship. Further evidence of evolutionary relationships among phototrophs can be found in the fact that the structure of the purple bacterial and green nonsulfur photosynthetic reaction centers resembles that of PSII, whereas the structure of the reaction centers of green sulfur bacteria and heliobacteria resembles that of PSI.

Because the evidence is strong that purple and green bacteria preceded cyanobacteria on Earth by perhaps as many as 0.5 billion years (Section 13.2), it is clear that anoxygenic photosynthesis was the first form of photosynthesis on Earth. The key evolutionary inventions of cyanobacteria were to connect the two forms of reaction centers (as PSI and PSII) and evolve the capacity to use H_2O as a photosynthetic electron donor. The latter was a seminal event in Earth's history since it not only oxygenated the planet but also allowed photoautotrophs to tap an inexhaustible supply of electrons.

MINIQUIZ

- Differentiate between cyclic and noncyclic electron flow in oxygenic photosynthesis.
- What is the key role of light energy in the initial step of the photosynthetic light reactions?
- What evidence is there that anoxygenic and oxygenic photosynthesis are related processes?

II • Autotrophy and N_2 Fixation

All cells require a source of carbon and nitrogen to form cell biomass (Section 3.1). The atmosphere contains a large reservoir of inorganic carbon, as CO_2, and nitrogen, as N_2. However, these gases must be chemically reduced before they can be assimilated into cell material. The reductive processes associated with the assimilation of CO_2 and N_2 are called *CO_2 fixation* and

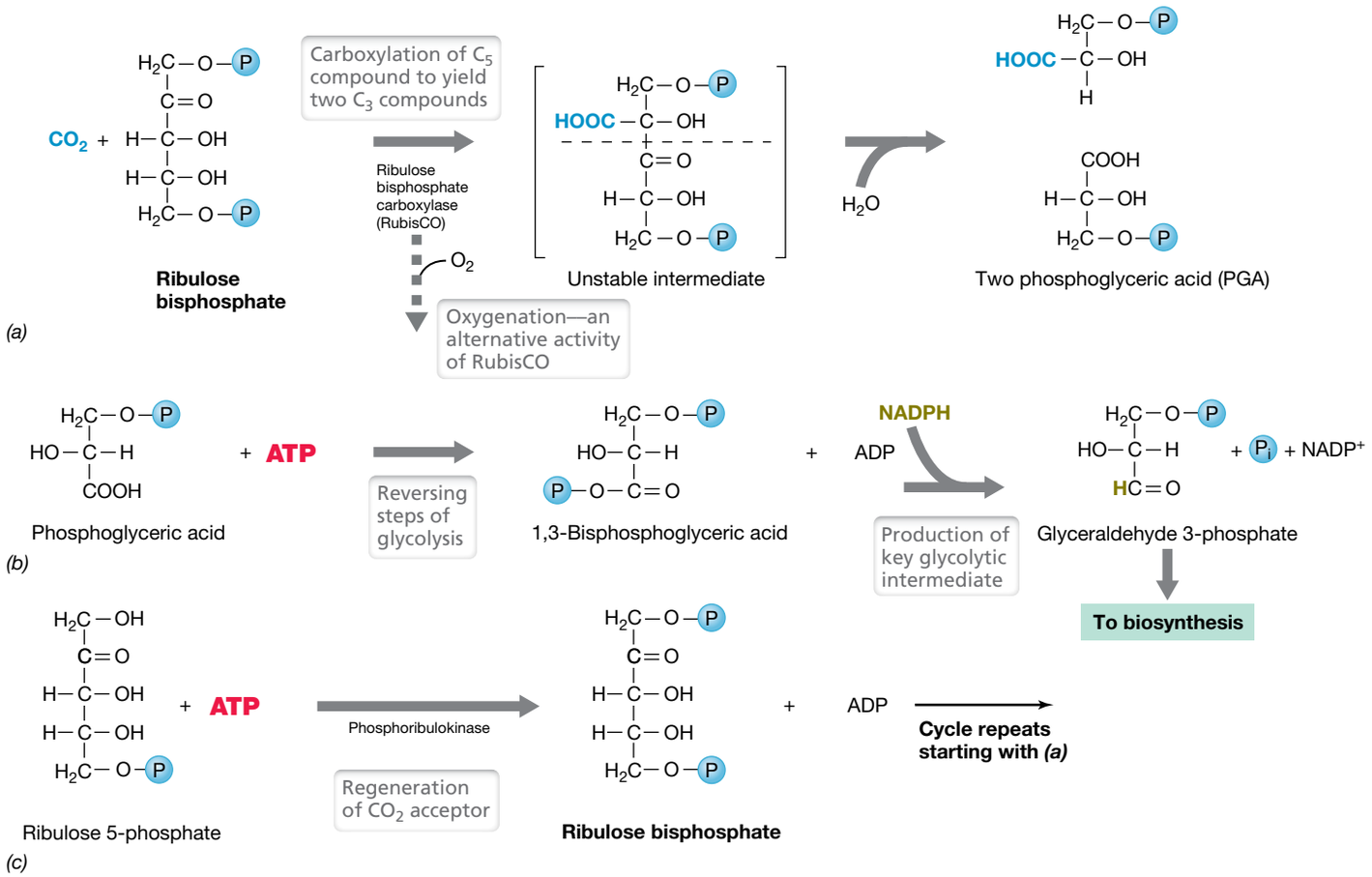


Figure 14.17 Key reactions of the Calvin cycle. *(a)* Reaction of the enzyme ribulose bisphosphate carboxylase. *(b)* Steps in the conversion of 3-phosphoglyceric acid (PGA) to glyceraldehyde 3-phosphate. Note that both ATP and NADPH are required. *(c)* Conversion of ribulose 5-phosphate to the CO_2 acceptor molecule ribulose 1,5-bisphosphate by the enzyme phosphoribulokinase.

N_2 fixation, respectively. Both of these pathways require substantial amounts of energy from the cell in the form of ATP and reducing power. These processes evolved very early in the history of life and are widely distributed among species of *Bacteria* and *Archaea*.

14.5 Autotrophic Pathways

Autotrophy is the process by which an energy-poor and highly oxidized form of carbon—CO_2—is reduced and assimilated into cell material. Many microbes are autotrophic, including virtually all phototrophs and chemolithotrophs. In photoautotrophic organisms, the assimilatory reactions responsible for CO_2 fixation are often called the light-independent *dark reactions* of photosynthesis, because they are not inhibited by the absence of light (see Section 14.1).

In oxygenic photosynthesis, CO_2 is reduced to the level of glyceraldehyde 3-phosphate by the **Calvin cycle**. Although the Calvin cycle is the most widespread and important pathway of CO_2 fixation in the biosphere, many autotrophic *Bacteria* and *Archaea* have evolved alternative pathways for fixing CO_2. These alternative autotrophic pathways all ultimately reduce CO_2 to the level of acetyl-coenzyme A (acetyl-CoA), a central metabolite that feeds into all major biosynthetic pathways (↔ Section 3.9). We begin our discussion of autotrophy by focusing on the Calvin cycle.

The Calvin Cycle

The Calvin cycle is present in purple bacteria, cyanobacteria, algae, green plants, most chemolithotrophic *Bacteria*, and a few *Archaea*. The cycle requires CO_2, a CO_2-acceptor molecule, NADPH, ATP, and two key enzymes, *ribulose bisphosphate carboxylase* and *phosphoribulokinase*. The first step in the Calvin cycle is catalyzed by the enzyme ribulose bisphosphate carboxylase, **RubisCO** for short. RubisCO catalyzes the formation of two molecules of 3-phosphoglyceric acid (PGA) from ribulose bisphosphate and CO_2 as shown in Figure 14.17*a*. The PGA is then phosphorylated and reduced to a key intermediate of glycolysis, glyceraldehyde 3-phosphate. From this, glucose can be formed by reversal of the early steps in glycolysis (↔ Figure 3.14).

Instead of focusing on the incorporation of a single molecule of CO_2, it is easiest to consider Calvin cycle reactions based on the incorporation of 6 molecules of CO_2, as this is what is required to make one hexose ($C_6H_{12}O_6$). For RubisCO to incorporate 6 molecules of CO_2, 6 molecules of ribulose bisphosphate (total, 30 carbons) are required; carboxylation of these yields 12 molecules of PGA (total, 36 carbon atoms) (Figure 14.18). These then form the carbon skeletons for the eventual synthesis of 6 molecules of ribulose bisphosphate (total, 30 carbons) plus one hexose (6 carbons) for cell biosynthesis. A series of biochemical rearrangements between various sugars follow, resulting in 6 molecules of ribulose 5-phosphate (30 carbons). The final step in the Calvin cycle is the phosphorylation of each of these by the enzyme phosphoribulokinase (Figures 14.17*c* and 14.18) to regenerate 6 molecules of the acceptor molecule, ribulose bisphosphate. All totaled, *12 NADPH and 18 ATP* are required to synthesize one glucose from 6 CO_2 by the Calvin cycle.

Many Calvin cycle autotrophs produce polyhedral cell inclusions called **carboxysomes**. These inclusions, about 100 nm in

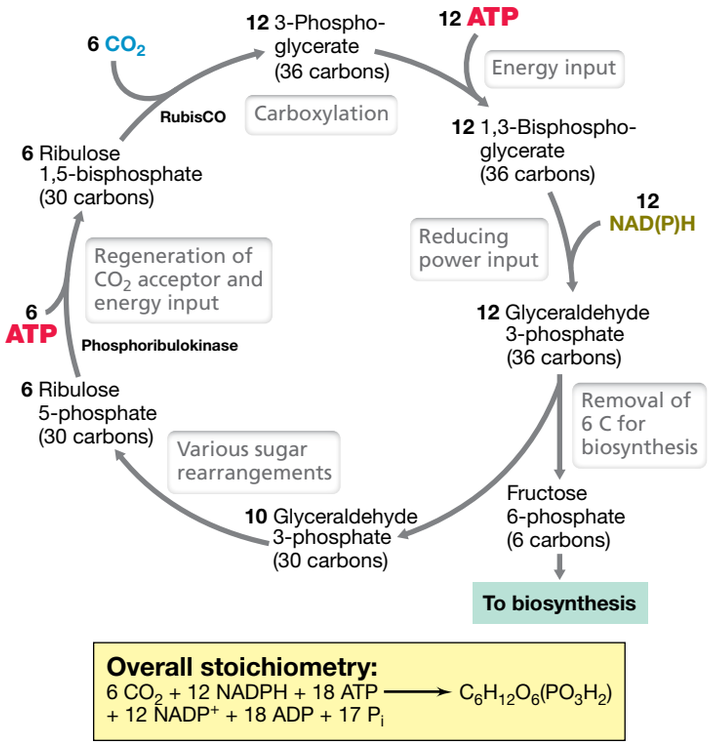


Figure 14.18 The Calvin cycle. Shown is the production of one hexose molecule from CO_2. For each six molecules of CO_2 incorporated, one fructose 6-phosphate is produced. In phototrophs, ATP comes from photophosphorylation and NAD(P)H from light or reverse electron flow.

diameter, are surrounded by a thin, protein shell and consist of a crystalline array of RubisCO (Figure 14.19); about 250 RubisCO molecules are present per carboxysome. Carboxysomes function to improve the efficiency of RubisCO. Though CO_2 is the actual substrate for RubisCO, inorganic carbon is first incorporated into the cell as bicarbonate (HCO_3^-); the latter diffuses into carboxysomes and is converted to CO_2 by the enzyme *carbonic anhydrase*. CO_2 cannot escape the carboxysome and can be maintained at high concentration therein, and this increases the efficiency of CO_2 fixation. RubisCO can react with either CO_2 or O_2, and O_2 competes with CO_2 for access to the enzyme (Figure 14.17*a*), lowering the efficiency of CO_2 fixation. However, the protein

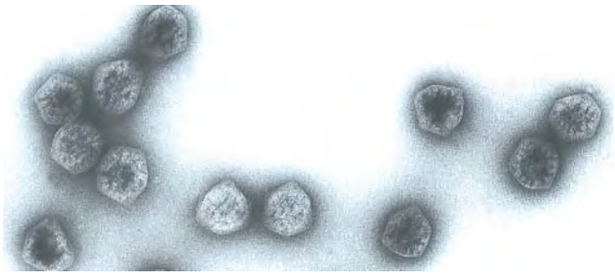


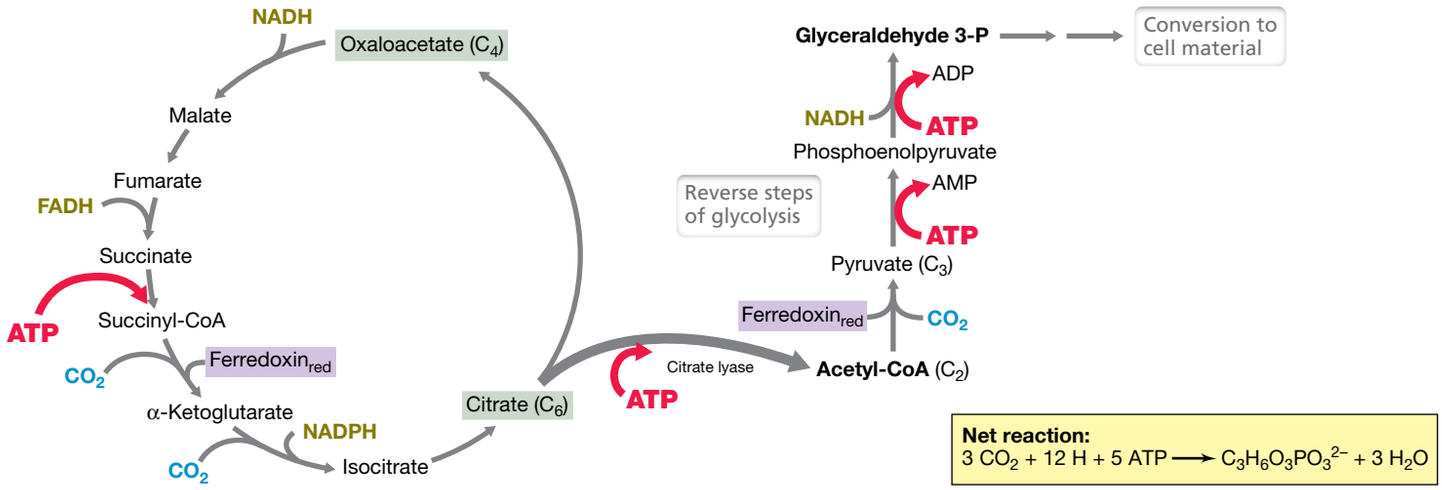
Figure 14.19 Crystalline Calvin cycle enzymes: Carboxysomes. Electron micrograph of carboxysomes purified from the chemolithotrophic sulfur oxidizer *Halothiobacillus neapolitanus*. The structures are about 100 nm in diameter. Carboxysomes are present in a wide variety of obligately autotrophic aerobic *Bacteria*.

shell of the carboxysome protects RubisCO from O_2 and maintains CO_2 at the high levels necessary for efficient CO_2 fixation. Plants lack carboxysomes and RubisCO in plants is instead concentrated in the stroma of chloroplasts (Figure 14.5). The stroma does not provide protection from O_2, however, and so the efficiency of CO_2 fixation in plants declines at high rates of O_2 production.

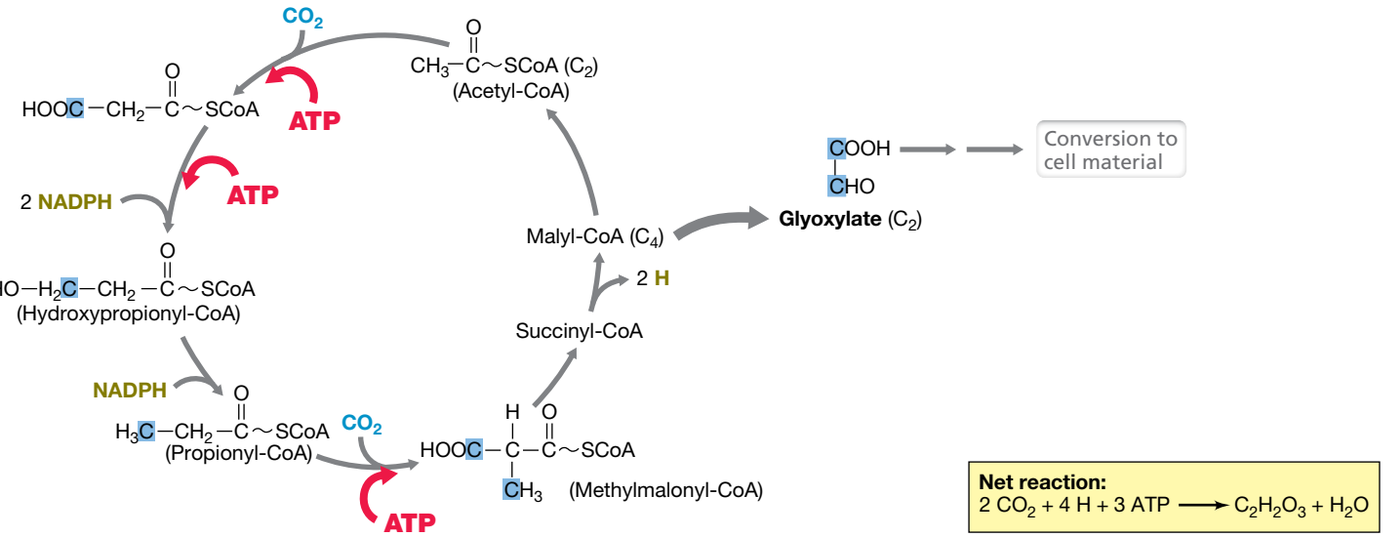
The Reverse Citric Acid Cycle

Not all phototrophic organisms rely on the Calvin cycle for CO_2 fixation. The **reverse citric acid cycle** (also called the *reductive TCA cycle*) is a pathway of CO_2 fixation that is used by green sulfur bacteria such as *Chlorobium* (Figure 14.1). In the reverse citric acid cycle, CO_2 is reduced by a reversal of steps in the citric acid cycle (↔ Section 3.9) (**Figure 14.20*a***). The reverse citric acid cycle is more efficient than the Calvin cycle, requiring only 4 NADH, 2 reduced ferredoxins, and 10 ATP to synthesize one glucose from 6 CO_2 in contrast to the 18 ATP required by the Calvin cycle.

As the name implies, most of the reactions of the reverse citric acid cycle are catalyzed by reverse reactions of enzymes of the citric acid cycle. However, the cycle requires the activity of several unique enzymes. These include in particular the enzymes α-ketoglutarate synthase and pyruvate synthase, which catalyze the reductive fixation of CO_2 using electrons supplied by reduced ferredoxin. Ferredoxin is an iron–sulfur protein of very electronegative E_0', about −0.4 V, and is produced in the light reactions of green sulfur bacteria (Figure 14.13). These two ferredoxin-linked reactions are (1) the carboxylation of succinyl-CoA to α-ketoglutarate, and



(a) **Reverse citric acid cycle**



(b) **Hydroxypropionate pathway**

Figure 14.20 Unique autotrophic pathways in phototrophic green bacteria. *(a)* The reverse citric acid cycle is the mechanism of CO_2 fixation in green sulfur bacteria. Ferredoxin$_{red}$ indicates carboxylation reactions requiring reduced ferredoxin (2 H each). Starting from oxaloacetate, each turn of the cycle results in three molecules of CO_2 being incorporated and pyruvate as the product. *(b)* The hydroxypropionate pathway is the autotrophic pathway in the green nonsulfur bacterium *Chloroflexus*. Acetyl-CoA is carboxylated twice to yield methylmalonyl-CoA. This intermediate is rearranged to yield a new acetyl-CoA acceptor molecule and a molecule of glyoxylate, which is converted to cell material.

(2) the carboxylation of acetyl-CoA to pyruvate (Figure 14.20*a*). The reverse citric acid cycle also replaces the enzyme *citrate synthase* from the citric acid cycle (Figure 3.16) with the enzyme *citrate lyase* (an ATP-dependent enzyme that cleaves citrate into acetyl-CoA and oxaloacetate), and the enzyme *succinate dehydrogenase* from the citric acid cycle by *fumarate reductase* in the reverse cycle (Figure 14.20*a*).

The reverse citric acid cycle operates in certain nonphototrophic autotrophs as well. For example, the hyperthermophilic chemolithotrophs *Thermoproteus* and *Sulfolobus* (*Archaea*; Section 17.9) and *Aquifex* (*Bacteria*; Section 16.19) use the reverse citric acid cycle, as do certain mesophilic sulfur chemolithotrophic *Bacteria*, such as *Sulfurimonas*. Thus, this pathway, originally discovered in green sulfur bacteria, is likely distributed among several groups of autotrophic microbes.

Other Pathways of CO_2 Fixation

In addition to the Calvin cycle and the reverse citric acid cycle, at least four other pathways of CO_2 fixation are known. The filamentous anoxygenic phototroph *Chloroflexus* (Section 15.7) grows autotrophically with either H_2 or H_2S as electron donor. However, neither the Calvin cycle nor the reverse citric acid cycle operates in this organism. Instead, two molecules of CO_2 are reduced to glyoxylate by the **3-hydroxypropionate bi-cycle**. This cycle is so named because hydroxypropionate, a three-carbon compound, is a key intermediate and it couples two cycles, one that fixes two molecules of bicarbonate into one molecule of glyoxylate and a second that adds a third molecule of bicarbonate to ultimately yield pyruvate, which can then be funneled into biosynthetic reactions (Figure 14.20*b*). The 3-hydroxypropionate bi-cycle is somewhat less efficient than the reverse citric acid cycle, requiring 16 ATP per glucose generated.

In phototrophic bacteria, the 3-hydroxypropionate bi-cycle is found in *Chloroflexus*, thought to be one of the earliest phototrophs on Earth. This suggests that the hydroxypropionate pathway may have been one of the earliest mechanisms, if not *the* earliest, for autotrophy in anoxygenic phototrophs. In addition to *Chloroflexus*, the hydroxypropionate bi-cycle operates in several hyperthermophilic *Archaea*, including *Metallosphaera, Acidianus*, and *Sulfolobus*. These are all chemolithotrophs that lie near the base of the phylogenetic tree of *Archaea* (Chapter 17). The evolutionary roots of the hydroxypropionate pathway may thus be very deep, and it is possible that this pathway was nature's first attempt at autotrophy.

Other routes of CO_2 fixation include the **3-hydroxypropionate/4-hydroxybutyrate cycle** and the **dicarboxylate/4-hydroxybutyrate cycle**. These pathways of CO_2 fixation are found among diverse autotrophic species of *Archaea*. These cycles each include two connected pathways in which bicarbonate and/or CO_2 is converted into acetyl-CoA for use in biosynthesis. These pathways are named for their key intermediates, which include 3-hydroxypropionate, 4-hydroxybutyrate, and C_4 dicarboxylic acids.

The final pathway of CO_2 fixation is the **reductive acetyl-coenzyme A pathway**. This pathway is found in obligate anaerobes including methanogenic *Archaea*, diverse acetogens, and *Planctomyces* that carry out the anammox reaction (all of these are described in this chapter). The reductive acetyl-coenzyme A pathway is the most efficient of all the CO_2 fixation pathways and requires only 6–8 molecules of ATP per 6 molecules of CO_2 fixed. It is also the only pathway of CO_2 fixation that can be coupled directly to energy conservation and we will consider it in detail in Section 14.16.

MINIQUIZ

- What reaction(s) does the enzyme RubisCO carry out?
- How much NADPH and ATP is required to make one hexose molecule by the Calvin cycle?
- Contrast autotrophy in the following phototrophs: cyanobacteria; purple and green sulfur bacteria; *Chloroflexus*.

14.6 Nitrogen Fixation

In addition to carbon, cells need a significant amount of nitrogen to synthesize proteins, nucleic acids, and many other organic molecules. Most microbes obtain this nitrogen from "fixed" forms of N in their environment, such as ammonia (NH_3) or nitrate (NO_3^-). However, many *Bacteria* and *Archaea* can form ammonia from gaseous dinitrogen (N_2), a process called **nitrogen fixation**. The ammonia produced is then assimilated into organic form. The ability to fix nitrogen frees an organism from a dependence on fixed nitrogen and confers a significant ecological advantage when fixed nitrogen is limiting. The process of nitrogen fixation is also of enormous agricultural importance, as it supports the nitrogen needs of key crops, such as soybeans and alfalfa.

Only certain species of *Bacteria* and *Archaea* can fix nitrogen, and a list of some important nitrogen-fixing organisms is given in Table 14.1. Some nitrogen-fixing bacteria are *free-living* and carry

TABLE 14.1 Some nitrogen-fixing organisms[a]

Free-living aerobes		
Chemoorganotrophs	***Phototrophs***	***Chemolithotrophs***
Azotobacter *Azomonas* *Azospirillum* *Klebsiella*[b] *Methylomonas*	Cyanobacteria (e.g., *Anabaena, Nostoc, Gloeothece, Aphanizomenon*)	*Alcaligenes* *Acidithiobacillus*
Free-living anaerobes		
Chemoorganotrophs	***Phototrophs***	***Chemolithotrophs***[c]
Clostridium *Desulfotomaculum*	Purple bacteria (e.g., *Chromatium, Methanococcus, Rhodobacter*) Green sulfur bacteria (e.g., *Chlorobium*) Heliobacteria	*Methanosarcina* *Methanocaldococcus*
Symbiotic		
With leguminous plants	***With nonleguminous plants***	
Soybeans, peas, clover, etc. with *Rhizobium, Bradyrhizobium, Sinorhizobium*	Alder, bayberry, autumn olive, many other bushy plants, with the actinomycete *Frankia*	

[a]Only some common genera are listed in each category; many other genera are known.
[b]Nitrogen fixation occurs only under anoxic conditions.
[c]All are *Archaea*.

UNIT 4

out the process completely independently. By contrast, others are *symbiotic* and fix nitrogen only in association with certain plants (Section 23.3). However, in symbiotic nitrogen fixation, it is the bacterium, not the plant, that fixes N_2; no eukaryotic organisms are known to fix nitrogen.

Nitrogenase

Nitrogen fixation is catalyzed by an enzyme complex called **nitrogenase**. Nitrogenase consists of two proteins, *dinitrogenase* and *dinitrogenase reductase*. Both proteins contain iron, and dinitrogenase contains molybdenum as well. The iron and molybdenum in dinitrogenase are part of the enzyme cofactor called the *iron–molybdenum cofactor* (*FeMo-co*), and reduction of N_2 occurs at this site. The composition of FeMo-co is $MoFe_7S_8$•homocitrate (**Figure 14.21**). Two "alternative" nitrogenases are known that lack molybdenum. These contain either vanadium (V) plus iron or iron-only in their cofactors and are made by certain nitrogen-fixing bacteria when molybdenum is limiting in their environment (Section 15.12).

Nitrogen fixation is inhibited by oxygen (O_2) because dinitrogenase reductase is irreversibly inactivated by O_2. Nevertheless, many nitrogen-fixing bacteria are obligate aerobes. In these organisms, nitrogenase is protected from oxygen inactivation by a combination of the rapid removal of O_2 by respiration and the production of O_2-retarding slime layers (**Figure 14.22**). In heterocystous cyanobacteria, nitrogenase is protected by its localization in a differentiated cell called a *heterocyst* (Figure 14.22*c*; Section 15.3). Inside the heterocyst, conditions are anoxic, while in neighboring vegetative cells, conditions are just the opposite because oxygenic photosynthesis is occurring. Oxygen production is shut down in the heterocyst, thus protecting it as a dedicated site for N_2 fixation (Section 7.8).

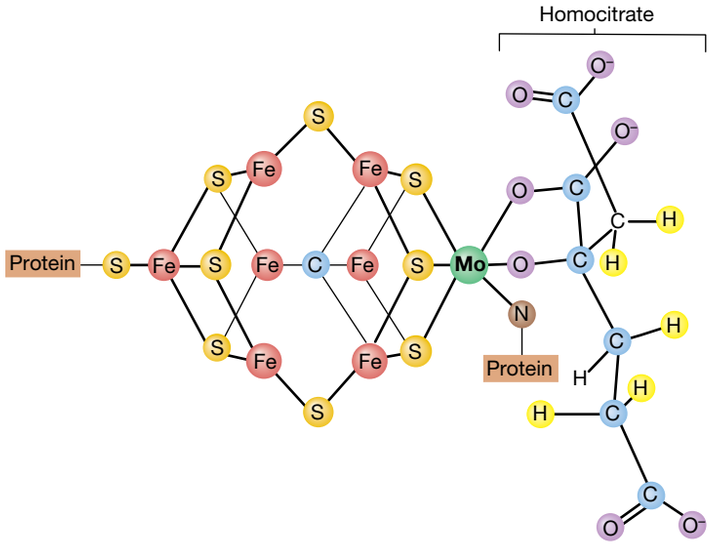
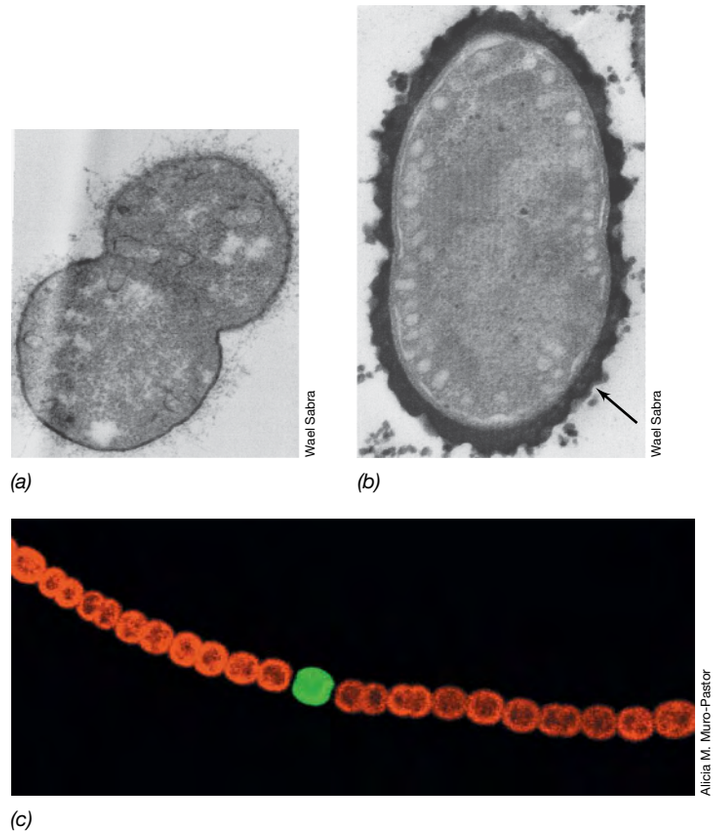


Figure 14.21 FeMo-co, the iron–molybdenum cofactor from nitrogenase. On the left is the Fe_7S_8 cube that binds to Mo along with O atoms from homocitrate (right, all O atoms shown in purple) and N and S atoms from dinitrogenase.



(a) (b) (c)

Figure 14.22 Two ways of protecting nitrogenase from O_2. Induction of slime formation by O_2 is demonstrated by comparing transmission electron micrographs of *(a)* nitrogen-fixing *Azotobacter vinelandii* cells grown with 2.5% O_2 and showing very little slime with *(b) A. vinelandii* cells grown in air (21% O_2) and showing an extensive darkly staining slime layer (arrow). The slime retards diffusion of O_2 into the cell, thus preventing nitrogenase inactivation by O_2. A single cell of *A. vinelandii* is about 2 μm in diameter. *(c)* Fluorescence photomicrograph of cells of the filamentous cyanobacterium *Anabaena* showing a single heterocyst (green). The heterocyst is a differentiated cell that specializes in nitrogen fixation and protects nitrogenase from O_2 inactivation.

Electron Flow in Nitrogen Fixation

Owing to the stability of the triple bond in N_2, its activation and reduction is very energy demanding. Six electrons are needed to reduce N_2 to NH_3, and the successive reduction steps occur directly on nitrogenase with no free intermediates accumulating (**Figure 14.23**). Although only *six* electrons are necessary to reduce N_2 to two NH_3, *eight* electrons are actually consumed in the process, two electrons being lost as H_2 for each mole of N_2 reduced. For unknown reasons, H_2 evolution is an obligatory step in nitrogen fixation and occurs in the first round of the nitrogenase reduction cycle. Following this, N_2 is reduced in successive steps, and ammonia is the released product (Figure 14.23).

The sequence of electron transfer in nitrogenase is as follows: electron donor → dinitrogenase reductase → dinitrogenase → N_2. The electrons for N_2 reduction are transferred to dinitrogenase reductase from the low-potential iron–sulfur proteins ferredoxin or flavodoxin (Section 3.10). In addition to electrons, ATP is required for nitrogen fixation. ATP binds to dinitrogenase reductase, and, following its hydrolysis to ADP, lowers

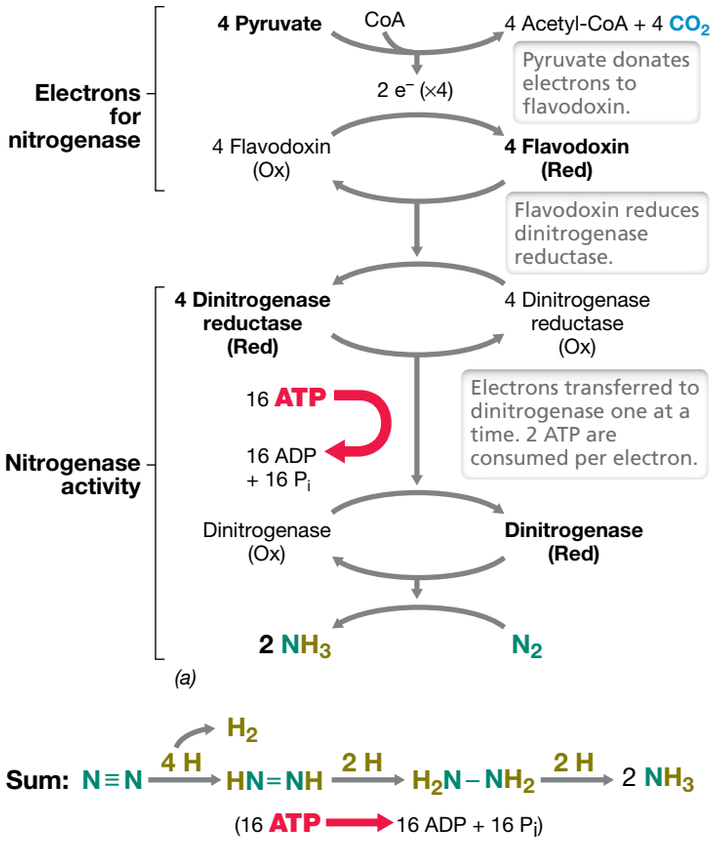


Figure 14.23 Biological nitrogen fixation by nitrogenase. The nitrogenase complex is composed of dinitrogenase and dinitrogenase reductase. Electrons from reduced ferredoxin are used to reduce dinitrogenase reductase and these electrons in turn reduce dinitrogenase at the expense of ATP. Ditrogenase ultimately donates these electrons to N_2 at the active site of the enzyme, resulting in the formation of 2 NH_3. The iron-molybdenum cofactor (FeMo-co, Figure 14.21) is part of dinitrogenase.

the reduction potential of the protein. This allows dinitrogenase reductase to interact with and reduce dinitrogenase. Electrons are transferred from dinitrogenase reductase to dinitrogenase one at a time, and each cycle of reduction requires two ATP. Thus a total of 16 ATP are required for the reduction of N_2 to 2 NH_3 (Figure 14.23).

Assaying Nitrogenase: Acetylene Reduction

Nitrogenases are not entirely specific for N_2 and also reduce other triply bonded compounds, such as acetylene (HC≡CH). The reduction of acetylene by nitrogenase is only a two-electron process, and *ethylene* ($H_2C{=}CH_2$) is the final product. However, the reduction of acetylene to ethylene provides a simple, sensitive, and rapid method for measuring nitrogenase activity (Figure 14.24). This technique, known as the *acetylene reduction assay*, is widely used in microbiology to detect and quantify nitrogen fixation.

Although the reduction of acetylene is taken as strong proof of N_2 fixation, definitive proof requires an isotope of nitrogen, $^{15}N_2$, as a tracer. If a culture or natural sample is enriched with $^{15}N_2$ and incubated, the production of $^{15}NH_3$ is firm evidence of nitrogen fixation. Nevertheless, acetylene reduction is a more rapid and sensitive method for measuring N_2 fixation and can easily be used in laboratory studies of pure cultures or ecological studies of nitrogen-fixing bacteria directly in their habitat. To do this, a sample, which may be soil, water, or a culture, is incubated in a vessel with HC≡CH and the gas phase is later analyzed by gas chromatography for the production of $H_2C{=}CH_2$ (Figure 14.24).

MINIQUIZ

- Write a balanced equation for the reaction catalyzed by nitrogenase.
- What is FeMo-co and what does it do?
- How is acetylene useful in studies of nitrogen fixation?

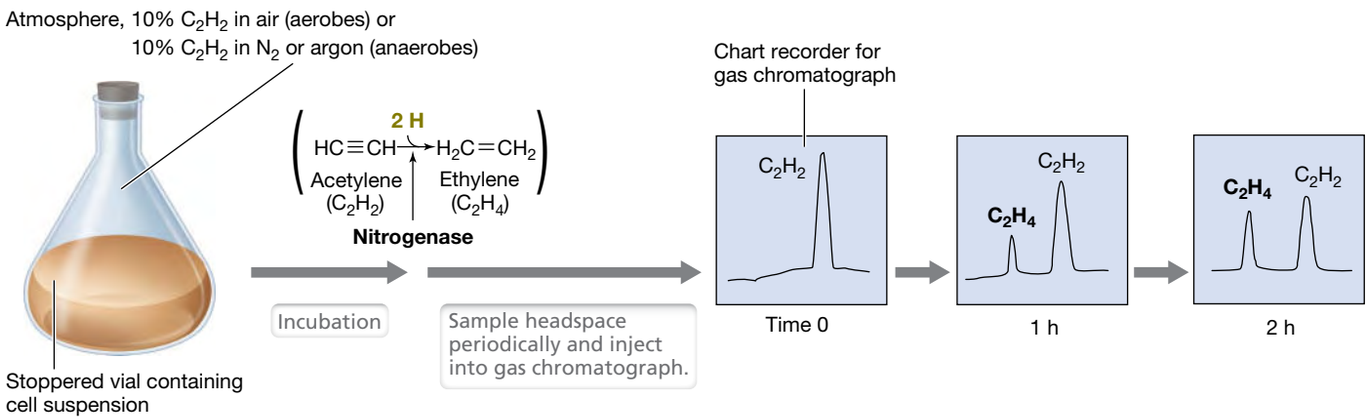


Figure 14.24 The acetylene reduction assay of nitrogenase activity in nitrogen-fixing bacteria. The results show no ethylene (C_2H_4) at time 0 but increasing production of C_2H_4 as the assay proceeds. As C_2H_4 is produced, a corresponding amount of C_2H_2 is consumed.

III • Respiratory Processes Defined by Electron Donor

As we learned in Chapter 3, energy is conserved in respiration by redox reactions that transfer electrons from an initial electron donor to a final electron acceptor. A tremendous diversity of respirations exist and the microbes that carry them out are typically characterized by the nature of their electron donors and/or electron acceptors. Some of these microbes oxidize organic compounds (*chemoorganotrophs*), whereas others oxidize inorganic compounds (*chemolithotrophs*) and obtain their carbon from CO_2. We first review some basic bioenergetics that apply to all forms of respiration.

14.7 Principles of Respiration

In all forms of respiration, low-potential electron donors (those that are more electronegative) are oxidized and the resulting electrons are driven through electron transport chains by their affinity for a high-potential electron acceptor (those that are more electropositive); the latter are ultimately reduced. Electron transport chains include components such as cytochromes, quinones, and iron–sulfur proteins (Section 3.10); collectively, these proteins harness the flow of electrons to generate a proton motive force. Finally, ATP synthesis is driven by an ion gradient through the activity of ATP synthase (Section 3.11).

Energetics of Respiration

Recall from Chapter 3 that the tendency of a substance to donate or accept electrons is defined by its reduction potential (E_0') and that reactions in which electrons are transferred are called *redox reactions*. Respiration can be understood as the coupling of two redox half reactions. Redox half reactions are reversible, and in a coupled reaction, the more electronegative (lower reduction potential) half reaction will proceed as an oxidation (the electron donor) while the more electropositive (higher reduction potential) half reaction will proceed as a reduction (the electron acceptor). The farther apart the two half reactions are in terms of the E_0' of their redox couples, the greater the amount of energy released (Figure 3.10). Based on this simple principle, a wide diversity of organic or inorganic electron donors can be coupled to terminal electron acceptors in the various forms of respiration (**Figure 14.25** and **Table 14.2**). These reactions can support growth provided that sufficient energy is released for the production of ATP (the energy-rich phosphate bond of ATP has a free energy of −31.8 kJ/mol).

Aerobic and Anaerobic Respiration

Respiration can occur under both oxic and anoxic conditions. *Anaerobic respirations* are those that have electron acceptors other than oxygen. Anaerobic respirations are distinct from fermentations because fermentations do not require an external electron acceptor and they generate ATP as a result of substrate-level phosphorylation, whereas respirations generate ATP by harnessing an ion motive force. From Figure 14.25 we can see that almost any half reaction can serve as an electron acceptor provided it is coupled with a sufficiently electronegative electron donor (that is, an electron donor with a lower reduction potential). The energy released from the oxidation of an electron donor using O_2 as electron acceptor is greater than if the same compound is oxidized with an alternate electron acceptor (Figure 3.10). These energy differences are dictated by the reduction potentials of each acceptor (Figure 14.25).

Because the O_2/H_2O couple is most electropositive, more energy is available from aerobic respiration than from anaerobic respiration. This means that for a given electron donor, aerobic organisms will always be able to conserve more energy—and will therefore outcompete—anaerobic organisms, and this is why aerobic respiration has been the dominant form of respiration on Earth ever since the Great Oxidation Event (Section 13.2). However, because oxygen is such a good electron acceptor, and because it is poorly soluble in water, it can be rapidly consumed; hence, anoxic habitats remain widespread in nature and are habitats for anaerobic microbes.

TABLE 14.2 Energy yields from the oxidation of various inorganic electron donors[a]

Electron donor	*Chemolithotrophic reaction*	*Group of chemolithotrophs*	*E_0' of couple (V)*	*$\Delta G^{0'}$ (kJ/reaction)*	*Number of electrons/ reaction*	*$\Delta G^{0'}$ (kJ/2 e^-)*
Phosphite[b]	$4\,HPO_3^{2-} + SO_4^{2-} + H^+ \rightarrow 4\,HPO_4^{2-} + HS^-$	Phosphite bacteria	−0.69	−364	8	−91
Hydrogen[b]	$H_2 + \frac{1}{2}O_2 \rightarrow H_2O$	Hydrogen bacteria	−0.42	−237.2	2	−237.2
Sulfide[b]	$HS^- + H^+ + \frac{1}{2}O_2 \rightarrow S^0 + H_2O$	Sulfur bacteria	−0.27	−209.4	2	−209.4
Sulfur[b]	$S^0 + 1\frac{1}{2}O_2 + H_2O \rightarrow SO_4^{2-} + 2\,H^+$	Sulfur bacteria	−0.20	−587.1	6	−195.7
Ammonium[c]	$NH_4^+ + 1\frac{1}{2}O_2 \rightarrow NO_2^- + 2\,H^+ + H_2O$	Nitrifying bacteria	+0.34	−274.7	6	−91.6
Nitrite[b]	$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^-$	Nitrifying bacteria	+0.43	−74.1	2	−74.1
Ferrous iron[b]	$Fe^{2+} + H^+ + \frac{1}{4}O_2 \rightarrow Fe^{3+} + \frac{1}{2}H_2O$	Iron bacteria	+0.77	−32.9	1	−65.8

[a]Data are from G_f^0 values in Table 3.2 (or references therein) and Figure 3.10, and from bioenergetics calculations as described in Sections 3.4 and 3.6; E_0' values for Fe^{2+} are for pH 2, and others are for pH 7. At pH 7, the E_0' for the Fe^{3+}/Fe^{2+} couple is about +0.2 V.
[b]Except for phosphite, all reactions are shown coupled to O_2 as electron acceptor. The only known phosphite oxidizer couples to SO_4^{2-} as electron acceptor. H_2 and most sulfur compounds can be oxidized anaerobically using one or more electron acceptors, and Fe^{2+} can be oxidized at neutral pH with NO_3^- as electron acceptor. For other chemolithotrophic reactions of sulfur compounds, see Table 14.3.
[c]Ammonium can also be oxidized with NO_2^- as electron acceptor (anammox, Section 14.12).

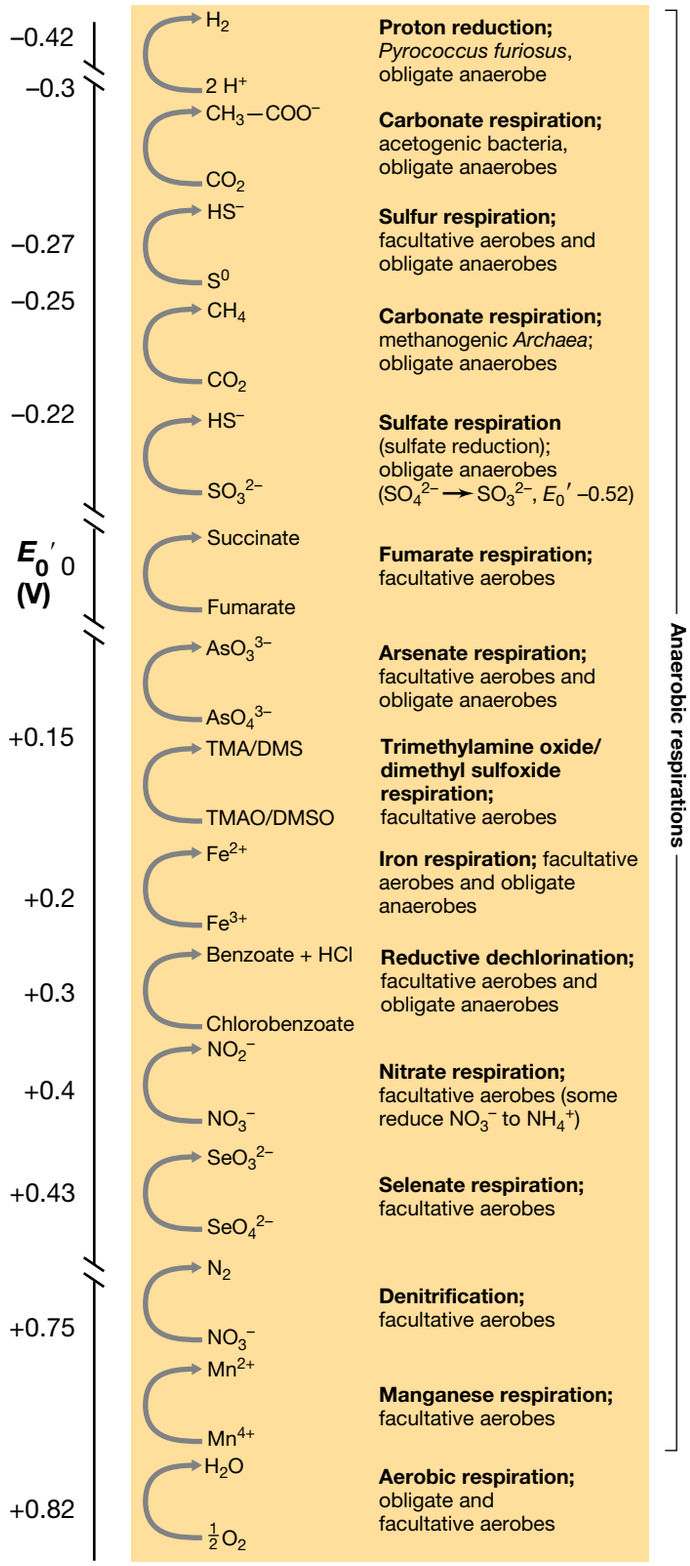


Figure 14.25 Major forms of anaerobic respiration. The redox couples are arranged in order from most electronegative E_0' (top) to most electropositive E_0' (bottom) assuming neutral pH. See Figure 3.10 to compare how the energy yields of some of these anaerobic respirations vary. The E_0' of the Fe^{3+}/Fe^{2+} couple at pH 2 is +0.77 V.

An organism's relationship to oxygen often defines its relationship to other electron acceptors. Organisms that are facultative aerobes (Section 5.14) can switch to alternative electron acceptors when oxygen is limiting, but will switch back to using oxygen as soon as it becomes available. Alternative electron acceptors used by facultative organisms include those that are fairly near the O_2/H_2O couple, such as many inorganic and organic compounds, and metals such as Fe^{3+} and Mn^{4+} (Figure 14.25). Microorganisms that use more electronegative electron acceptors often employ enzymes that are inhibited by oxygen and hence are typically obligate anaerobes. Organisms that use electronegative electron acceptors such as sulfate (SO_4^{2-}), elemental sulfur (S^0), and carbon dioxide (CO_2) are thus locked into an anaerobic lifestyle.

Assimilative and Dissimilative Reductions

Biosynthetic reactions, such as CO_2 fixation, require both ATP and reducing power. Reducing power in the cell is typically in the form of NADH ($E_0' = -0.32$ V), though some obligate anaerobes also require reducing power in the form of reduced ferredoxin (Fd^{2-}_{red}, $E_0' = -0.37$ to -0.5 V). In the case of chemoorganotrophs, NADH is readily generated during the oxidation of organic molecules (Sections 3.8 and 3.9). Reducing power is used to reduce inorganic compounds such as NO_3^-, SO_4^{2-}, and CO_2 so that they can be used as sources of N, S, and C in new cell material. The end products of such reductions are the amino groups (—NH_2) of amino acids and other nitrogenous substances, the sulfhydryl groups (—SH) of several sulfur-containing compounds in the cell, and the organic carbon found in all cell constituents, respectively. When NO_3^-, SO_4^{2-}, or CO_2 is reduced for these purposes, it is said to be *assimilated*, and the reduction process is called *assimilative* reduction. Assimilative metabolism is conceptually and physiologically quite different from the reduction of NO_3^-, SO_4^{2-}, and CO_2 during energy conservation in anaerobic respiration. To distinguish these two kinds of reductions, use of these compounds as electron acceptors for energy purposes is called *dissimilative* reduction.

Assimilative and dissimilative metabolisms differ markedly. In assimilative metabolism, *energy is consumed*, and so only enough of the compound (NO_3^-, SO_4^{2-}, or CO_2) is reduced to satisfy the needs for biosynthesis; the products of reduction are then converted to cell material in the form of macromolecules and other biomolecules. By contrast, in dissimilative metabolism, *energy is conserved*, a large amount of the electron acceptor is reduced, and the reduced product remains a small molecule (N_2, H_2S, or CH_4, for example), which is then excreted from the cell. Most microbes can perform a variety of assimilative reductions, whereas dissimilative reductions are only characteristic of organisms carrying out anaerobic respirations.

MINIQUIZ

- In a coupled reaction, how can you tell the electron donor half reaction from the electron acceptor half reaction?
- How does aerobic respiration differ from anaerobic respiration, and why does aerobic respiration repress anaerobic respiration?
- Describe the major differences between assimilative and dissimilative reductions.

14.8 Hydrogen (H_2) Oxidation

Chemolithotrophs are microbes that conserve energy from the oxidation of inorganic electron donors (Section 3.12). Most chemolithotrophs are also autotrophs. However, a few chemolithotrophs lack autotrophic capacities and grow as **mixotrophs**, meaning that they use their inorganic electron donor for energy conservation but assimilate organic carbon as their carbon source.

A simple consideration of bioenergetics tell us which kinds of chemolithotrophs should be expected in nature (Table 14.2), and the reactions of these highly diverse microbes form the heart of the major nutrient cycles (Chapter 21). We begin with perhaps the simplest of all chemolithotrophs, the hydrogen bacteria. Hydrogen (H_2) is a common product of microbial metabolism, especially of some fermentations (Sections 14.19–14.23), and the classical "hydrogen bacteria" respire H_2 aerobically, forming water and ATP as the final products.

Hydrogenase and the Energetics of H_2 Oxidation

Synthesis of ATP during H_2 oxidation by O_2 is the result of electron transport reactions that generate a proton motive force. The overall reaction

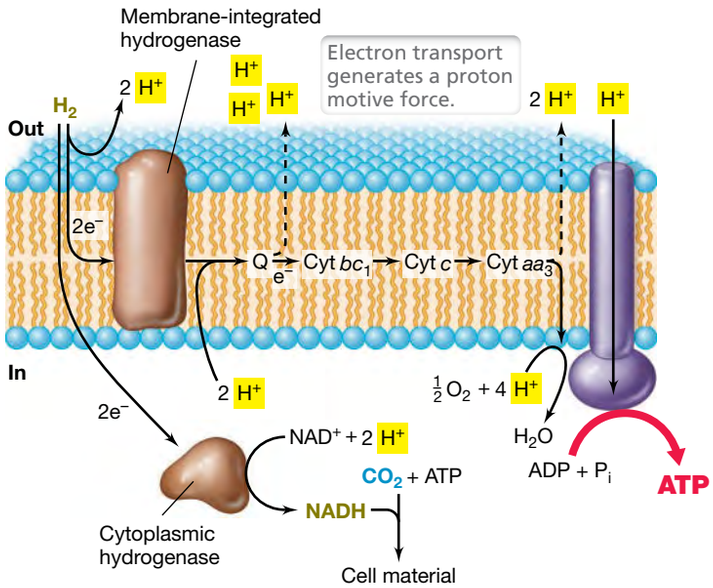
$$H_2 + \tfrac{1}{2}O_2 \rightarrow H_2O \qquad \Delta G^{0\prime} = -237 \text{ kJ}$$

is highly exergonic and can be coupled to the synthesis of ATP. In this reaction, which is catalyzed by the enzyme **hydrogenase**, the electrons from H_2 are initially transferred to a quinone acceptor. From there electrons travel through a series of cytochromes to generate a proton motive force and eventually reduce O_2 to water (Figure 14.26*a*).

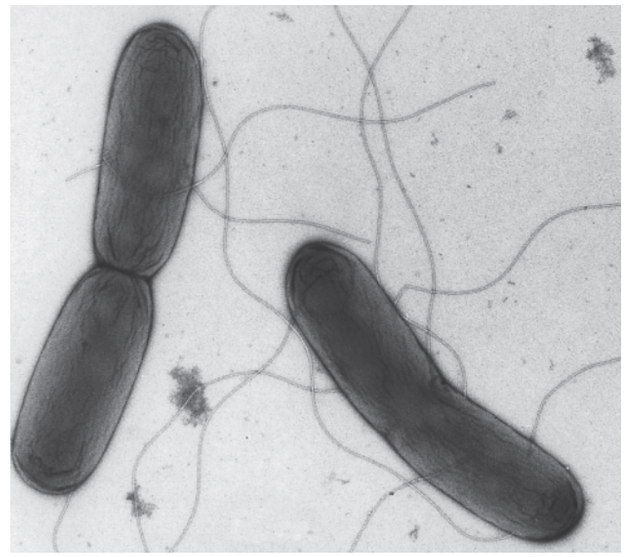
Some hydrogen bacteria synthesize two distinct hydrogenases, one cytoplasmic and one membrane-integrated. The latter enzyme participates in energetics, whereas the soluble hydrogenase has a different function. Instead of binding H_2 for use as an electron donor in energy metabolism, the cytoplasmic hydrogenase binds H_2 and catalyzes the reduction of NAD^+ to NADH directly (the reduction potential of H_2 is sufficiently electronegative that reverse electron flow reactions are unnecessary). The gammaproteobacterium *Ralstonia eutropha* (Figure 14.26*b*) has been a model for studying aerobic H_2 oxidation by species that make two hydrogenases. This gram-negative relative of *Pseudomonas* has well-developed genetic systems and grows robustly on H_2 as sole electron donor. Species that synthesize only one hydrogenase make only the membrane-integrated form of the enzyme, and it functions in both energy conservation and autotrophy. To generate reducing power for CO_2 reduction, single-hydrogenase H_2 bacteria must back up electrons from quinone to form NADH in the energy-requiring process of reverse electron transport (Section 14.3).

Autotrophy in H_2 Bacteria

Although most hydrogen bacteria can also grow as chemoorganotrophs, when growing chemolithotrophically, they fix CO_2 by the Calvin cycle (Section 14.5). However, when readily usable organic compounds such as glucose are present, synthesis of Calvin cycle and hydrogenase enzymes by H_2 bacteria is repressed. Thus, H_2 bacteria are *facultative* chemolithotrophs. This flexibility undoubtedly has ecological value. In nature, H_2 levels in oxic environments are fleeting for at least two reasons: (1) Most biological



(a)



Frank Mayer

(b)

Figure 14.26 Bioenergetics and function of the two hydrogenases of aerobic hydrogen bacteria. *(a)* In *Ralstonia eutropha*, two hydrogenases are present; the membrane-bound hydrogenase participates in energetics, whereas the cytoplasmic hydrogenase makes NADH for the Calvin cycle. Some hydrogen bacteria have only the membrane-bound hydrogenase, and in these organisms reducing power is synthesized by reverse electron flow from Q back to NAD^+ to form NADH. Cyt, cytochrome; Q, quinone. *(b)* Transmission electron micrograph of negatively stained cells of the hydrogen-oxidizing chemolithotroph *Ralstonia eutropha*. A cell is about 0.6 μm in diameter and contains several flagella.

H_2 production is the result of fermentations, which are anoxic processes, and (2) H_2 can be utilized by several different anaerobic *Bacteria* and *Archaea* and thus is exhausted before it reaches oxic regions of a habitat. Hence, aerobic hydrogen bacteria must have a backup metabolism to H_2 oxidation, and in nature they likely shift between chemoorganotrophic and chemolithotrophic lifestyles as nutrients in their habitats allow. Moreover, many aerobic H_2 bacteria grow best microaerobically and are probably most competitive as H_2 bacteria in oxic–anoxic interfaces where H_2 may be in greater and more continuous supply than in fully oxic habitats.

MINIQUIZ

- What enzyme is required for hydrogen bacteria to grow as H_2 chemolithotrophs?
- Why is reverse electron flow unnecessary in H_2 bacteria that contain two hydrogenases?

14.9 Oxidation of Sulfur Compounds

Many reduced sulfur compounds can be electron donors for the colorless sulfur bacteria, called *colorless* to distinguish them from the pigmented green and purple bacteria discussed earlier in this chapter (Figure 14.1 and Section 14.3). Historically, the concept of chemolithotrophy emerged in the late nineteenth century from studies of the sulfur bacteria by the Russian microbiologist Sergei Winogradsky (Section 1.11) and was a radically new idea at the time. However, as our understanding of metabolic diversity has improved, it has become clear that chemolithotrophy, and in particular sulfur chemolithotrophy, is a major metabolic lifestyle of many *Bacteria* and *Archaea*.

Energetics of Sulfur Oxidation

The most common sulfur compounds used as electron donors are hydrogen sulfide (H_2S), elemental sulfur (S^0), and thiosulfate ($S_2O_3^{2-}$); sulfite (SO_3^{2-}) can also be oxidized (Table 14.2 and Table 14.3). In most cases, the final oxidation product is sulfate (SO_4^{2-}). Sulfide oxidation occurs in stages, with the first oxidation step yielding elemental sulfur, S^0. Some sulfide-oxidizing bacteria, such as *Beggiatoa*, deposit this elemental sulfur inside the cell (Figure 14.27*a*), where the sulfur exists as a potential energy (electron) reserve. When the supply of sulfide has been depleted, additional energy can then be conserved from the oxidation of sulfur to sulfate. When S^0 is present externally, the organism must attach itself to the sulfur particle because elemental sulfur is rather insoluble (Figure 14.27*b*).

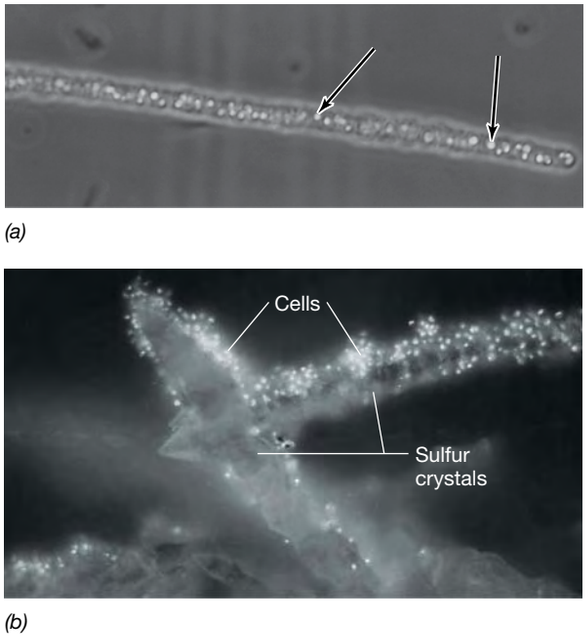


Figure 14.27 Sulfur bacteria. *(a)* Internal sulfur granules in *Beggiatoa* (arrows). *(b)* Attachment of cells of the sulfur-oxidizing archaeon *Sulfolobus acidocaldarius* to a crystal of elemental sulfur. Cells are visualized by fluorescence microscopy after being stained with the dye acridine orange. The sulfur crystal does not fluoresce.

One product of the oxidation of reduced sulfur compounds is protons (Tables 14.2 and 14.3). Consequently, one result of sulfur chemolithotrophy is acidification of the environment. Because of this, many sulfur bacteria have evolved to be acid-tolerant or even acidophilic. *Acidithiobacillus thiooxidans*, for example, grows best at a pH between 2 and 3.

Biochemistry of Sulfur Oxidation: The Sox System

There are diverse pathways for conserving energy from the oxidation of sulfur compounds. One of the best characterized is the *Sox* (for sulfur *ox*idation) *system* (Figure 14.28), which has been described in *Paracoccus pantotrophus*. The Sox system contains over 15 genes encoding various cytochromes and other proteins necessary for the oxidation of reduced sulfur compounds directly to sulfate. Elements of the Sox system are found in diverse sulfur chemolithotrophs and also in some phototrophic sulfur bacteria,

TABLE 14.3 Comparison of the energetics of oxidation of some common reduced sulfur compounds

Chemolithotrophic reaction	Electrons	Stoichiometry[a]	Energetics (kJ/electron)[a]
Sulfide to sulfate	8	$H_2S + 2\ O_2 \rightarrow SO_4^{2-} + 2\ H^+$	$\Delta G^{0\prime} = -798.2$ kJ/reaction (-99.75 kJ/e$^-$)
Sulfite to sulfate	2	$SO_3^{2-} + \frac{1}{2} O_2 \rightarrow SO_4^{2-}$	$\Delta G^{0\prime} = -258$ kJ/reaction (-129 kJ/e$^-$)
Thiosulfate to sulfate	8	$S_2O_3^{2-} + H_2O + 2\ O_2 \rightarrow 2\ SO_4^{2-} + 2\ H^+$	$\Delta G^{0\prime} = -818.3$ kJ/reaction (-102 kJ/e$^-$)

[a]All reactions are balanced, both atomically and electrically. See Table 3.2 and Sections 3.4 and 3.6 for details of calculations. For the reaction and energetics of the oxidation of sulfide to sulfur and sulfur to sulfate, see Table 14.2.

organisms that oxidize sulfide to obtain reducing power for CO_2 fixation rather than for energy conservation. The fact that this biochemical system is distributed among bacteria that oxidize sulfide for very different reasons is a good indication that the genes that encode Sox have been transferred between species by horizontal gene flow (Section 9.6 and Chapter 11).

There are four key proteins in the Sox system: *SoxXA, SoxYZ, SoxB*, and *SoxCD*. All of these proteins are present in the periplasm. The pathway begins when the enzyme SoxXA forms a heterodisulfide bond between the sulfur compound to be oxidized (which can be HS^-, S^0, or $S_2O_3^{2-}$) and the carrier protein, SoxYZ (Figure 14.28). The sulfur compound remains bound to the carrier throughout the pathway, being ultimately released as sulfate through the activity of SoxB. The enzyme SoxCD (sulfur dehydrogenase) is the key enzyme that mediates the removal of 6 electrons from the sulfur compound bound to the carrier (Figure 14.28). Electrons from the Sox system are funneled into the electron transport chain (see later), while the protons generated in the periplasm are released to and acidify the external environment.

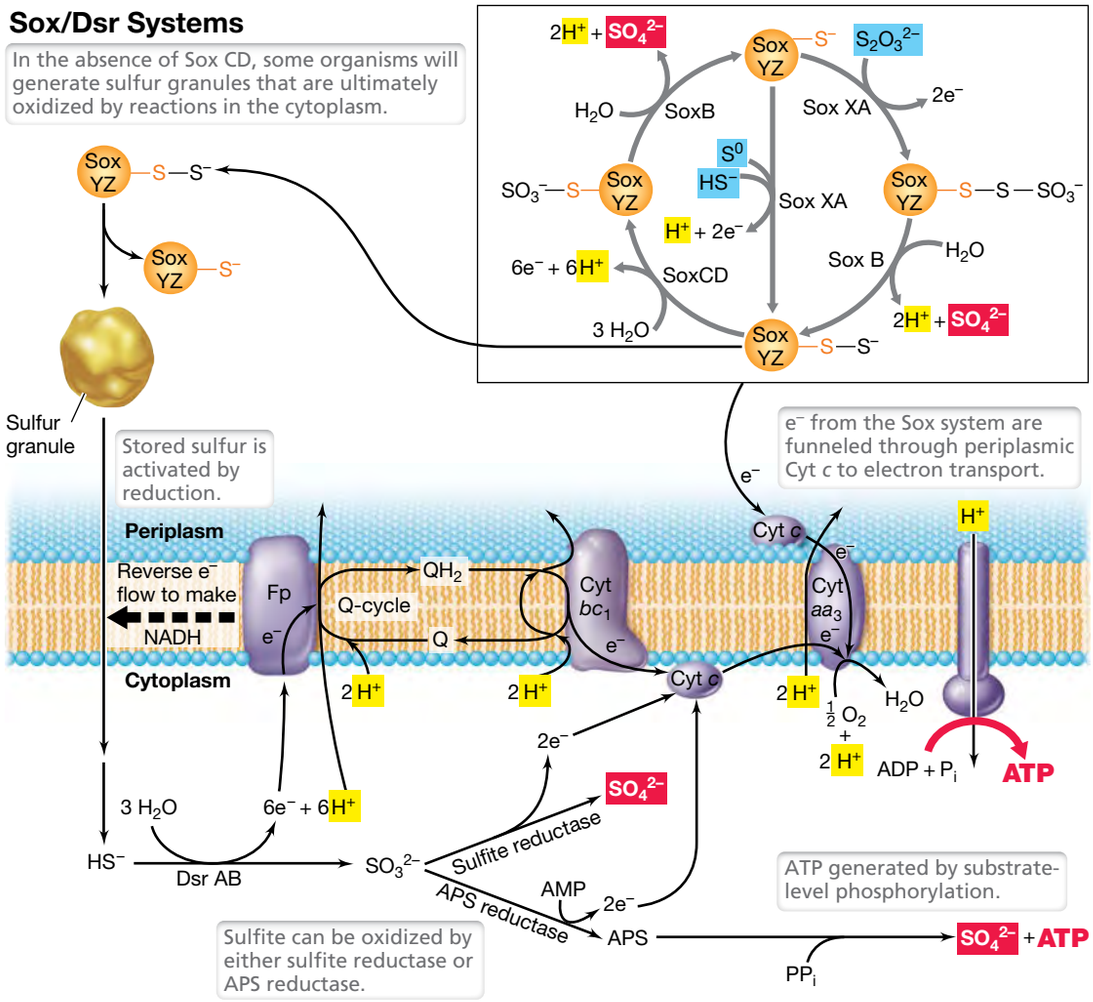


Figure 14.28 Oxidation of reduced sulfur compounds by sulfur chemolithotrophs. There are several different pathways for conserving energy through the oxidation of sulfide (H_2S), thiosulfate ($S_2O_3^-$), and elemental sulfur (S^0). In the Sox system (sulfur *ox*idation), SoxXA attaches a reduced sulfur compound to the carrier protein SoxYZ. The protein SoxCD, sulfur dehydrogenase, catalyzes removal of 6 e^- from the bound sulfur atom, and is a key enzyme for bacteria that use the complete Sox system for sulfur oxidation (such as *Paracoccus pantotrophus*). Sulfate (SO_4^{2-}) is released by the action of SoxB. In contrast, bacteria that form sulfur granules, such as *Beggiatoa* (Figure 14.27*a*), lack SoxCD and instead oxidize sulfur compounds using the enzymes DsrAB, dissimilatory sulfite reductase, and APS reductase (see Section 14.14). In sulfur oxidation, these enzymes are run backwards to oxidize sulfur compounds. In certain sulfur oxidizers, APS reductase is replaced by sulfite reductase. Reactions of the Sox cycle take place in the periplasm and electrons enter the electron transport chain through the activity of a periplasmic *c*-type cytochrome (Cyt *c*), while reactions of Sox/Dsr systems take place instead in the cytoplasm and electrons can enter electron transport at either the level of flavoproteins (Fp) or *c*-type cytochromes.

Other Aspects of Chemolithotrophic Sulfur Oxidation

Sulfur-oxidizing microbes that store sulfur granules (see Figure 14.27*a*) also use components of the Sox system but lack the key enzyme sulfur dehydrogenase (SoxCD). In the absence of SoxCD, a sulfur atom bound to SoxYZ is added to a growing sulfur granule in the periplasm (Figure 14.28). The sulfur in the granule can be reductively activated and transported to the cytoplasm where it is eventually oxidized to sulfite (SO_3^{2-}) by the reverse activity of DsrAB (an enzyme homologous to the enzyme sulfite reductase found in sulfate-reducing bacteria, Section 14.14). The sulfite is then oxidized to sulfate plus two electrons through one of two different pathways. The most widespread system employs the reverse activity of the cytoplasmic enzyme *sulfite reductase*. This enzyme oxidizes sulfite and transfers the electrons to the electron transport chain. By contrast, some sulfur chemolithotrophs oxidize SO_3^{2-} to SO_4^{2-} via a reversal of the activity of the enzyme adenosine phosphosulfate reductase (an enzyme essential for the metabolism of sulfate-reducing bacteria, see Section 14.14 and Figure 14.37). The oxidation of SO_3^{2-} to SO_4^{2-} yields an energy-rich phosphate bond by substrate-level phosphorylation when AMP is converted to ATP (Figure 14.28).

Electrons from the oxidation of reduced sulfur compounds eventually reach the electron transport chain, as shown in Figure 14.28. Though the exact details remain unknown, electrons are likely to enter at the flavoprotein or cytochrome *c* ($E_0' = +0.3$ V) levels and are transported through the chain to O_2, generating

a proton motive force that triggers ATP synthase activity (Figure 14.28). Electrons for CO_2 fixation come from reverse electron transport (Section 14.3), eventually yielding NADH, and autotrophy is driven by reactions of the Calvin cycle or some other autotrophic pathway (Section 14.5). Although the sulfur chemolithotrophs are primarily an aerobic group, some species can grow by anaerobic respiration using nitrate as an electron acceptor. The sulfur bacterium *Thiobacillus denitrificans* is a classic example, reducing nitrate to dinitrogen gas (the process of denitrification, Section 14.13).

MINIQUIZ

- How many electrons are available from the oxidation of H_2S if S^0 or SO_4^{2-} is the final product?
- In terms of intermediates, how does the Sox system differ from other sulfide-oxidizing systems?

14.10 Iron (Fe^{2+}) Oxidation

The aerobic oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) supports growth of the chemolithotrophic "iron bacteria" (Section 15.15). At acidic pH, only a small amount of energy is available from this reaction (Table 14.2), and for this reason the iron bacteria must oxidize large amounts of iron in order to produce only tiny amounts of cell material. The ferric iron produced becomes hydrated to form insoluble ferric hydroxide ($Fe^{3+} + 3\,H_2O \rightarrow Fe(OH)_3 + 3\,H^+$) and other iron precipitates in aquatic environments, and this drives down the pH (Figure 14.29). This inevitable chemical reaction probably explains why many iron-oxidizing bacteria have evolved to be strongly acidophilic.

Iron-Oxidizing Bacteria

The best-known iron bacteria, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*, can both grow autotrophically using ferrous iron (Figure 14.29) as electron donor at pH values as low as 1; growth is optimal at pH 2–3. These bacteria are common in acid-polluted environments such as coal-mining runoff waters (Figure 14.29*a*). *Ferroplasma*, a species of *Archaea*, is an extremely acidophilic iron oxidizer and can grow at pH values below 0 (Section 17.3). We discuss the role of all of these organisms in acid mine pollution and mineral oxidation in Sections 21.4, 22.1, and 22.2.

At neutral pH, Fe^{2+} spontaneously oxidizes to Fe^{3+}, so opportunities for the iron bacteria in neutral habitats are restricted to locations where Fe^{2+} is transitioning from anoxic to oxic conditions. For example, anoxic groundwater often contains dissolved Fe^{2+}, and when it is released, as in iron-rich spring water, it becomes exposed to O_2. At such interfaces, iron bacteria oxidize Fe^{2+} to Fe^{3+} before it oxidizes spontaneously. *Gallionella ferruginea, Sphaerotilus natans*, and *Leptothrix discophora* are examples of bacteria that live at these interfaces. They are typically seen mixed in with the characteristic ferric iron deposits they form (Figures 15.36 and 21.14).

Energy from Iron Oxidation

The bioenergetics of ferrous iron oxidation by *Acidithiobacillus ferrooxidans* and other acidophilic iron oxidizers are of considerable

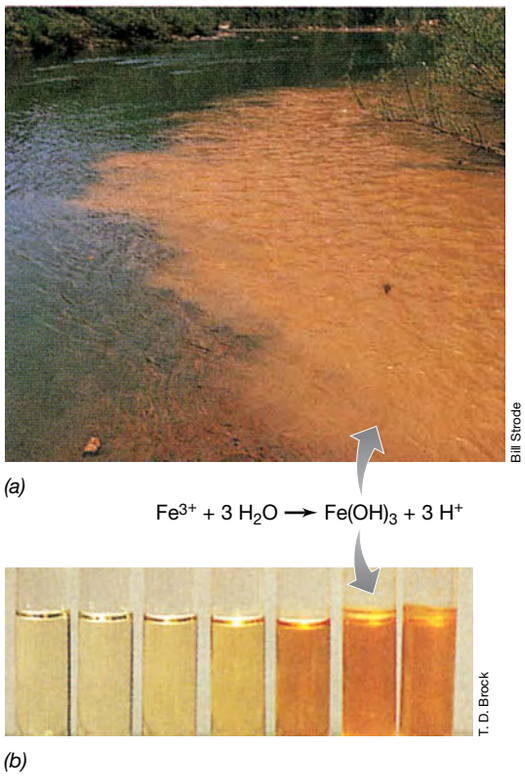


Figure 14.29 Iron-oxidizing bacteria. *(a)* Acid mine drainage, showing the confluence of a normal river and a creek draining a coal-mining area. At low pH values, Fe^{2+} does not oxidize spontaneously in air, but *Acidithiobacillus ferrooxidans* carries out the oxidation; insoluble $Fe(OH)_3$ and complex ferric salts precipitate. *(b)* Cultures of *A. ferrooxidans*. Shown is a dilution series, with no growth in the tube on the left and increasing amounts of growth from left to right. Growth is evident from the production of $Fe(OH)_3$.

interest because of the very electropositive reduction potential of the Fe^{3+}/Fe^{2+} couple at acidic pH ($E_0' = +0.77$ V at pH 2). The respiratory chain of *A. ferrooxidans* contains cytochromes of the *c* and aa_3 types and a periplasmic copper-containing protein called *rusticyanin* (Figure 14.30). There is also an iron-oxidizing protein located in the outer membrane of this gram-negative bacterium.

Because the reduction potential of the Fe^{3+}/Fe^{2+} couple is so high, steps in electron transport to oxygen ($\frac{1}{2}O_2/H_2O$, $E_0' = +0.82$ V) can obviously be few. Iron oxidation begins in the outer membrane where the organism contacts either soluble Fe^{2+} or insoluble ferrous iron minerals. Fe^{2+} is oxidized to Fe^{3+}, a one-electron transition (Table 14.2), by an outer membrane cytochrome *c* that transfers electrons into the periplasm where rusticyanin ($E_0' = +0.68$ V) is the electron acceptor. This thermodynamically slightly unfavorable reaction is thought to be pulled forward by the immediate consumption of Fe^{3+} in $Fe(OH)_3$ formation (Figure 14.30). Rusticyanin then reduces a periplasmic cytochrome *c*, which transfers electrons to cytochrome aa_3, and it is the latter protein that reduces O_2 to H_2O; ATP is synthesized by ATPase in the usual fashion (Figure 14.30).

The nature of the proton motive force in *A. ferrooxidans* is of interest. In a highly acidic environment, a large gradient of protons already exists across the *A. ferrooxidans* cytoplasmic membrane (the periplasm is pH 1–2, whereas the cytoplasm is

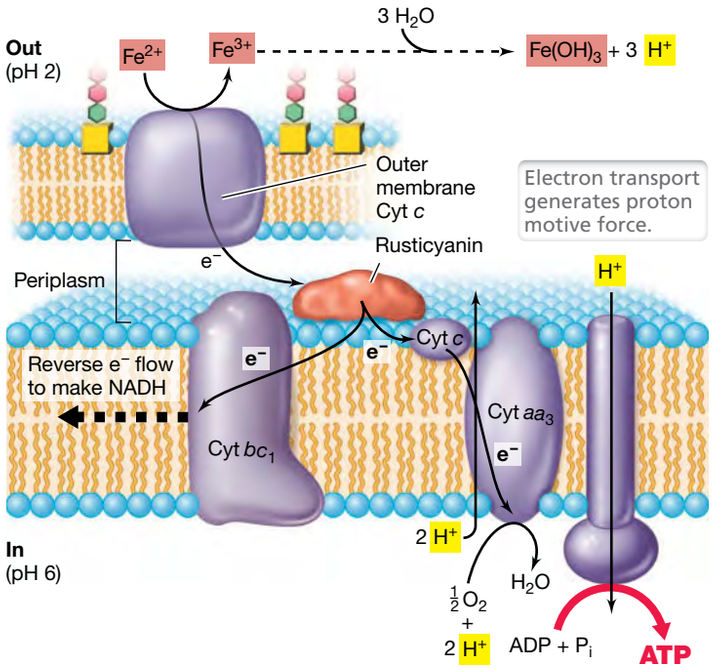
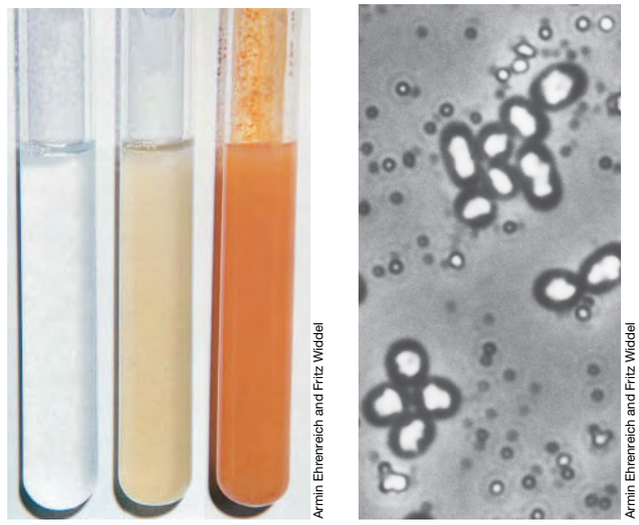


Figure 14.30 Electron flow during Fe^{2+} oxidation by the acidophile *Acidithiobacillus ferrooxidans*. The periplasmic copper-containing protein rusticyanin receives electrons from Fe^{2+} oxidized by a *c*-type cytochrome located in the outer membrane. From here, electrons travel a short electron transport chain, resulting in the reduction of O_2 to H_2O. Reducing power comes from reverse electron flow. Note the steep pH gradient across the membrane.



(a) (b)

Figure 14.31 Fe^{2+} oxidation by anoxygenic phototrophic bacteria. *(a)* Oxidation in anoxic tube cultures. Left to right: Sterile medium, inoculated medium, a growing culture showing $Fe(OH)_3$. *(b)* Phase-contrast photomicrograph of an Fe^{2+}-oxidizing purple bacterium. The bright refractile areas within cells are gas vesicles. The granules outside the cells are iron precipitates. This organism is phylogenetically related to the purple sulfur bacterium *Chromatium*.

pH 5.5–6, Figure 14.30). Although one might think that with this gradient *A. ferrooxidans* could make ATP at no energetic cost, this is not the case; the organism cannot make ATP from this preformed proton motive force in the absence of an electron donor. This is because H^+ ions that enter the cytoplasm via ATPase must be consumed in order to maintain the internal pH within acceptable limits. Proton consumption occurs during the reduction of O_2 in the electron transport chain and this reaction requires electrons; the latter come from the oxidation of Fe^{2+} to Fe^{3+} (Figure 14.30).

Autotrophy in *A. ferrooxidans* is supported by the Calvin cycle (Section 14.5), and because of the high potential of the electron donor, much energy must be consumed in reverse electron flow reactions to obtain the reducing power (NADH) necessary to drive CO_2 fixation. NADH is formed by reduction of NAD^+ by electrons obtained from Fe^{2+} that are forced backwards through cytochrome bc_1 and the quinone pool at the expense of the proton motive force (Figure 14.30).

The relatively poor energetic yield from ferrous iron oxidation coupled with the large energetic demands of the Calvin cycle (Figure 14.18) means that *A. ferrooxidans* must oxidize large amounts of Fe^{2+} to produce even a very small amount of cell material. Thus, in environments where acidophilic iron-oxidizing bacteria thrive, their presence is signaled not by the formation of high cell numbers but by the presence of the extensive ferric iron precipitates they have generated (Figure 14.29). We consider the ecology of iron bacteria in Chapters 21 and 22.

Ferrous Iron Oxidation under Anoxic Conditions

Ferrous iron can be oxidized under *anoxic* conditions by certain chemolithotrophs and phototrophic purple and green bacteria (**Figure 14.31**). In these cases, Fe^{2+} is used either as an electron donor in energy metabolism (chemolithotrophs) and/or as a reductant for CO_2 fixation (phototrophs). An important point to consider here is that at neutral pH where these organisms thrive, the E_0' of the Fe^{3+}/Fe^{2+} couple is significantly more electronegative than at acidic pH (+0.2 V versus +0.77 V, respectively, Figure 14.25). Hence, electrons from Fe^{2+} can reduce cytochrome *c* to initiate electron transport reactions. For chemolithotrophs, the electron acceptor is nitrate (NO_3^-), with either nitrite (NO_2^-) or dinitrogen gas (N_2) being the final product of this anaerobic respiration. For Fe^{2+}-oxidizing purple and green bacteria, either soluble Fe^{2+} or iron sulfide (FeS) can be used as electron donor. With FeS, both Fe^{2+} and S^{2-} are oxidized, Fe^{2+} to Fe^{3+} (one electron) and HS^- to SO_4^{2-} (eight electrons).

MINIQUIZ

- Why is only a very small amount of energy available from the oxidation of Fe^{2+} to Fe^{3+} at acidic pH?
- What is the function of rusticyanin and where is it found in the cell?
- How can Fe^{2+} be oxidized under anoxic conditions?

14.11 Nitrification

The reduced inorganic nitrogen compounds ammonia (NH_3) and nitrite (NO_2^-) are oxidized aerobically by the chemolithotrophic *nitrifying bacteria* in the process of **nitrification**

(↩ Section 15.13). Nitrifying bacteria are widely distributed in soils, water, wastewaters, and the oceans. Nitrification consists of two different sets of reactions; the first set of reactions catalyze oxidation of ammonia to nitrite, and the second set catalyze oxidation of nitrite to nitrate (NO_3^-). Most nitrifying microbes are only able to catalyze one set of these reactions. For example, *Bacteria* such as *Nitrosomonas* and *Archaea* such as *Nitrosopumilus* oxidize NH_3 only to nitrite, and we call these organisms *ammonia oxidizers*. The full nitrification pathway is ultimately completed when other *Bacteria* such as *Nitrobacter* oxidize NO_2^- to NO_3^-, and we call these organisms *nitrite oxidizers*. As far as is known, only certain bacteria in the genus *Nitrospira* can catalyze both sets of reactions, oxidizing NH_3 all the way to NO_3^-.

Bioenergetics and Enzymology of Ammonia and Nitrite Oxidation

The bioenergetics of nitrification is based on the same principles that govern other chemolithotrophic reactions: Electrons from reduced inorganic substrates (in this case, reduced nitrogen compounds) enter an electron transport chain, and electron transport reactions establish a proton motive force that drives ATP synthesis. The complete oxidation of NH_3 to NO_3^- involves an eight-electron transfer, and the electron donors for the nitrifying bacteria are not particularly strong. The E_0' of the NO_2^-/NH_3 couple (the first step in the oxidation of NH_3) is +0.34 V, and the E_0' of the NO_3^-/NO_2^- couple is even more positive, about +0.43 V. By necessity, these reduction potentials force the nitrifying bacteria to donate electrons to rather high-potential electron acceptors, and this of course limits the amount of energy that can be conserved (Section 14.7).

Several key enzymes participate in the oxidation of reduced nitrogen compounds. In ammonia-oxidizing bacteria such as *Nitrosomonas*, NH_3 is oxidized by *ammonia monooxygenase* (monooxygenases are discussed in Section 14.24), producing hydroxylamine (NH_2OH) and H_2O (**Figure 14.32**). A second key enzyme, *hydroxylamine oxidoreductase*, then oxidizes NH_2OH to NO_2^-, removing four electrons in the process. Ammonia monooxygenase is an integral membrane protein, whereas hydroxylamine oxidoreductase is periplasmic (Figure 14.32). In the reaction carried out by ammonia monooxygenase,

$$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$$

two electrons and protons are needed to reduce one molecule of oxygen (O_2) to H_2O. These electrons originate from the oxidation of hydroxylamine and are supplied to ammonia monooxygenase from hydroxylamine oxidoreductase via cytochrome *c* and ubiquinone (Figure 14.32). Thus, for every *four* electrons generated from the oxidation of NH_3 to NO_2^-, only *two* actually reach cytochrome aa_3, the terminal oxidase that interacts with O_2 to form H_2O (Figure 14.32).

Nitrite-oxidizing bacteria such as *Nitrobacter* oxidize NO_2^- to NO_3^- by the enzyme *nitrite oxidoreductase*, with electrons traveling a very short electron transport chain (because of the high potential of the NO_3^-/NO_2^- couple) to the terminal oxidase (**Figure 14.33**). Cytochromes of the *a* and *c* types are present in the

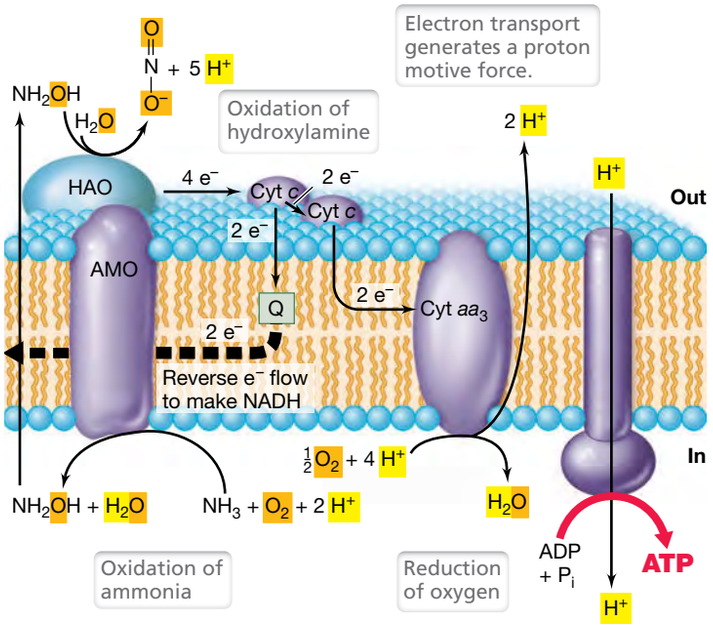


Figure 14.32 Oxidation of NH_3 and electron flow in ammonia-oxidizing bacteria. The reactants and the products of this reaction series are highlighted. The cytochrome *c* (Cyt *c*) in the periplasm is a different form of Cyt *c* than that in the membrane. AMO, ammonia monooxygenase; HAO, hydroxylamine oxidoreductase; Q, ubiquinone.

electron transport chain of nitrite oxidizers, and the activity of cytochrome aa_3 generates a proton motive force (Figure 14.33). As is the case with the iron bacteria (Section 14.10), only small amounts of energy are available from nitrite oxidation. Hence, minimal amounts of cell material are obtained even though large amounts of nitrite may be oxidized.

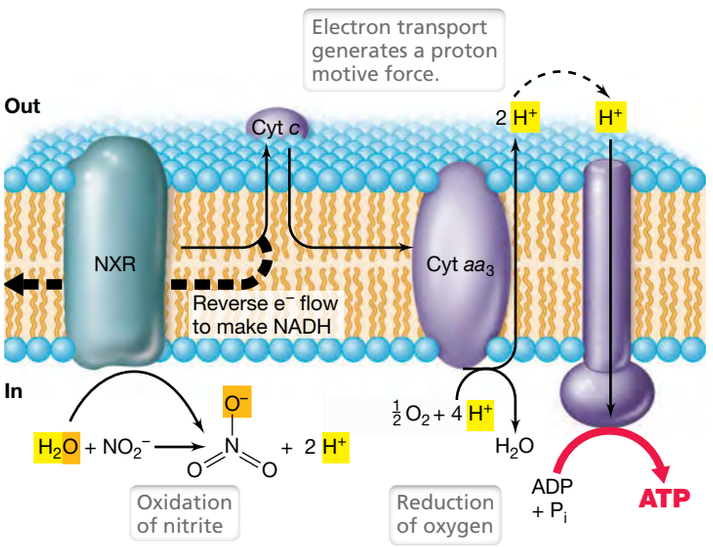


Figure 14.33 Oxidation of NO_2^- to NO_3^- by nitrifying bacteria. The reactants and products of this reaction series are highlighted to show the reaction clearly. NXR, nitrite oxidoreductase.

Carbon Metabolism and Ecology of Nitrifying Bacteria

Like sulfur- and iron-oxidizing chemolithotrophs (Sections 14.9 and 14.10), aerobic nitrifying *Bacteria* employ the Calvin cycle for CO_2 fixation. The ATP and reducing power requirements of the Calvin cycle place additional burdens on an energy-generating system that already has a relatively low yield (NADH to drive the Calvin cycle in nitrifiers is formed by reverse electron flow, Figures 14.32 and 14.33). The energetic constraints are particularly severe for nitrite oxidizers, and it is perhaps for this reason that most of these organisms have alternative energy-conserving mechanisms, being able to grow chemoorganotrophically on glucose and a few other organic substrates. By contrast, species of ammonia-oxidizing bacteria are either obligate chemolithotrophs or mixotrophs (Section 14.8). Autotrophy in ammonia-oxidizing *Archaea* is supported by a variation of the hydroxypropionate cycle (Section 14.5).

Nitrifying microbes play key ecological roles in the nitrogen cycle, converting ammonia into nitrate, a key plant nutrient. Nitrifiers are also important in sewage and wastewater treatment, removing toxic amines and ammonia and releasing less toxic nitrogen compounds (Sections 22.6 and 22.7). Nitrifiers play a similar role in the water column of lakes, where ammonia produced in the sediments from the decomposition of organic nitrogenous compounds is oxidized to nitrate, a more usable fixed nitrogen source for algae and cyanobacteria.

MINIQUIZ

- What are the substrates for the enzyme ammonia monooxygenase?
- What is the difference between ammonia oxidation and nitrite oxidation and in what types of organisms are these reactions found?

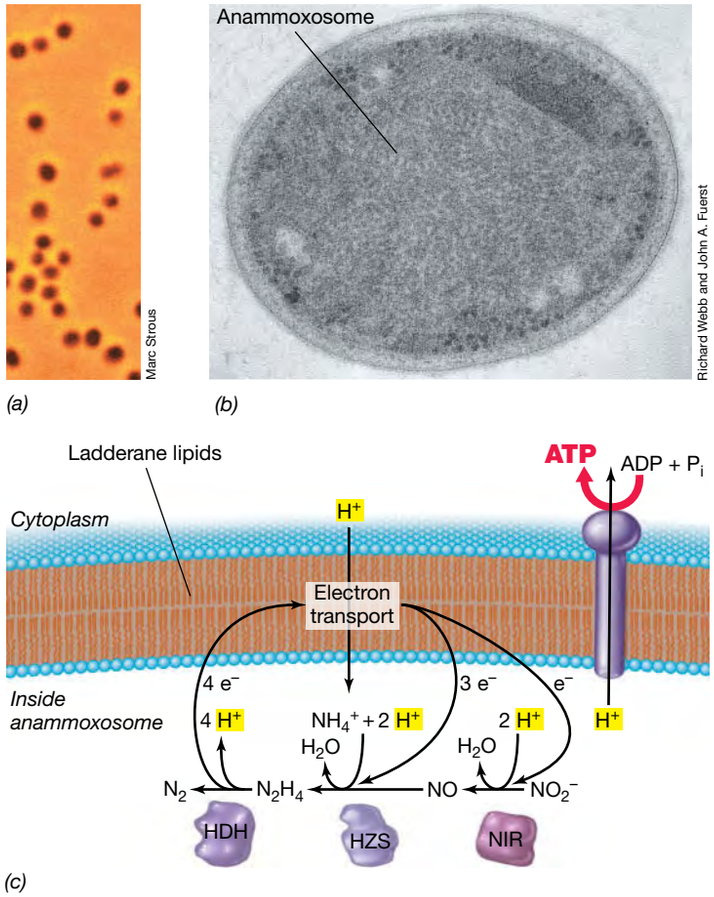


Figure 14.34 Anammox. *(a)* Phase-contrast photomicrograph of cells of *Brocadia anammoxidans*. A single cell is about 1 μm in diameter. *(b)* Transmission electron micrograph of a cell; note the membrane-enclosed compartments including the large fibrillar anammoxosome. *(c)* Reactions in the anammoxosome. NIR, nitrite reductase, HZS, hydrazine synthase; HDH, hydrazine dehydrogenase.

14.12 Anaerobic Ammonia Oxidation (Anammox)

Although the ammonia-oxidizing microbes just discussed are strict *aerobes*, NH_3 can also be oxidized under anoxic conditions. This process is called **anammox** (for *an*aerobic *amm*onia *ox*idation) and is catalyzed by an unusual group of obligately anaerobic *Bacteria*.

Ammonia is oxidized in the anammox reaction using NO_2^- as the electron acceptor to yield N_2:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\,H_2O \qquad \Delta G^{0\prime} = -357\ \text{kJ}$$

A major anammox organism, *Brocadia anammoxidans*, is a species of the *Planctomycetes* phylum of *Bacteria* (Section 16.16). *Planctomycetes* are unusual *Bacteria* in that their cytoplasm can contain membrane-enclosed compartments of various types (Figure 14.34). In cells of *B. anammoxidans*, this compartment is the *anammoxosome*, and it is within this structure that the anammox reaction occurs (Figure 14.34*c*). In addition to *Brocadia*, several other genera of anammox bacteria are known, including *Kuenenia, Anammoxoglobus, Jettenia*, and *Scalindua*, all of which are related to *Brocadia* and also contain anammoxosomes. Like aerobic ammonia oxidizers, anammox bacteria are also autotrophs, but they do not fix CO_2 using the pathways employed by aerobic ammonia oxidizers. Instead, anammox bacteria fix CO_2 by way of the reductive acetyl-CoA pathway, an autotrophic pathway widespread among some obligately anaerobic autotrophic *Bacteria* and *Archaea* (see Section 14.16).

The Anammoxosome and Its Reactions

The anammoxosome is a unit membrane–enclosed structure (Figure 14.34*b*) and in this respect is technically an organelle in the eukaryotic sense of the term. Lipids that form the anammoxosome membrane are not the typical lipids of *Bacteria* but instead consist of fatty acids constructed of multiple cyclobutane (C_4) rings bonded to glycerol by both ester and ether bonds. These *ladderane lipids*, as they are called, aggregate in the membrane to form an unusually dense membrane structure that prevents diffusion of substances from the anammoxosome into the cytoplasm.

The sturdy anammoxosome membrane is required to protect the cell from toxic intermediates produced during anammox reactions. These include, in particular, the compound *hydrazine* (N_2H_4),

a very strong reductant. In the anammox reaction, NO_2^- is first reduced to nitric oxide (NO) by nitrite reductase, and then NO reacts with ammonium (NH_4^+) to yield N_2H_4 by activity of the enzyme hydrazine synthase (Figure 14.34*c*). N_2H_4 is then oxidized to N_2 plus electrons by the enzyme hydrazine dehydrogenase, and the electrons are funneled into the electron transport chain where they are used to reduce nitrite and nitric oxide earlier in the pathway. In this way, anammox generates a *cyclical* series of electron transfer reactions in order to generate a proton motive force; ATP is formed from the latter by ATPases in the anammoxosome membrane (Figure 14.34*c*).

Reducing power for CO_2 fixation by anammox bacteria is derived from reverse electron transport, but because electron transfer reactions are cyclic, the electrons needed for reverse electron transport derive from an independent set of reactions that oxidize nitrite to nitrate by a nitrite oxidoreductase, a reaction also present in *Nitrobacter* (Figure 14.33). Interestingly, then, nitrite serves two different purposes for anammox bacteria: The *reduction* of nitrite is required to generate ATP by chemiosmosis, and the *oxidation* of nitrite is required to generate reducing power for CO_2 fixation.

Ecology of Anammox

In nature, the source of NO_2^- for the anammox reaction is presumably aerobic ammonia-oxidizing *Bacteria* and *Archaea*. These organisms coexist with anammox bacteria in ammonia-rich habitats such as sewage and other wastewaters. The suspended particles that form in these habitats contain both oxic and anoxic zones in which ammonia oxidizers of different physiologies can coexist in close association. In mixed laboratory cultures, high levels of oxygen inhibit anammox and favor classic nitrification, and thus it is likely that in nature, the fraction of ammonia oxidation catalyzed by anammox bacteria is governed by the concentration of O_2 in the habitat.

From an environmental standpoint, anammox is a very beneficial process in the treatment of wastewaters. The anoxic removal of NH_3 and amines by the formation of N_2 (Figure 14.34*c*) helps reduce the input of fixed nitrogen from wastewater treatment effluents that flow into rivers and streams, thereby maintaining higher water quality than would otherwise be possible. Also, marine anammox bacteria are likely responsible for the large amount of NH_3 that is known to disappear during mineralization processes in anoxic marine sediments. At least some ammonia-rich freshwater lake sediments also support anammox, and thus it appears that anammox can occur in any anoxic environment in which NH_3 and NO_2^- coexist.

MINIQUIZ

- What are the electron donor and acceptor in the anammox process?
- What does electron transport in anammox bacteria have in common with electron transport in purple sulfur bacteria?
- Compare CO_2 fixation in anammox bacteria and purple sulfur bacteria. What characteristics do these processes share and how are they different?

IV • Respiratory Processes Defined by Electron Acceptor

We examined the process of aerobic respiration in Chapter 3 and reviewed the bioenergetics of respiration in Section 14.7. Here we consider the details of **anaerobic respiration** in the many variations it is found in the microbial world. A wide variety of compounds function as electron acceptors in anaerobic respirations (Figure 14.25), and each acceptor is typically linked to a specific group or groups of microbes. We begin with a common form of anaerobic respiration in which nitrate functions as electron acceptor.

14.13 Nitrate Reduction and Denitrification

Inorganic nitrogen compounds are some of the most common electron acceptors in anaerobic respiration. Table 14.4 summarizes the relevant forms of inorganic nitrogen with their oxidation states. One of the most common alternative electron acceptors for dissimilative purposes is nitrate (NO_3^-), which can be reduced with two electrons to nitrite (NO_2^-), or reduced further to nitric oxide (NO), nitrous oxide (N_2O), or dinitrogen (N_2). Because NO, N_2O, and N_2 are all gases, they can be lost from the environment, and their biological production is called **denitrification** (Figure 14.35).

Some nitrate reducers, for example *Escherichia coli*, are not true denitrifiers, but only carry out the first step (nitrate to nitrite) in the process. Moreover, some organisms can reduce NO_2^- to ammonia (NH_3) in a dissimilative process. But it is the production of gaseous products—*denitrification*—that is of greatest global significance because it consumes fixed nitrogen and produces some polluting gases.

Denitrifying Microorganisms and Their Ecological Activities

Many denitrifying *Bacteria* are phylogenetically *Proteobacteria* and physiologically facultative aerobes. *Pseudomonas* species, for example, are typically strong denitrifiers. Aerobic respiration occurs when O_2 is present, even if NO_3^- is also present in the medium. Many denitrifying bacteria also reduce other electron acceptors anaerobically, such as Fe^{3+} and certain organic electron acceptors (Figure 14.25), and some denitrifiers can even ferment. Thus, denitrifying bacteria are metabolically diverse in terms

TABLE 14.4 Oxidation states of key nitrogen compounds

Compound	*Oxidation state of N atom*
Organic N (—NH_2)	−3
Ammonia (NH_3)	−3
Nitrogen gas (N_2)	0
Nitrous oxide (N_2O)	+1 (average per N)
Nitric oxide (NO)	+2
Nitrite (NO_2^-)	+3
Nitrogen dioxide (NO_2)	+4
Nitrate (NO_3^-)	+5

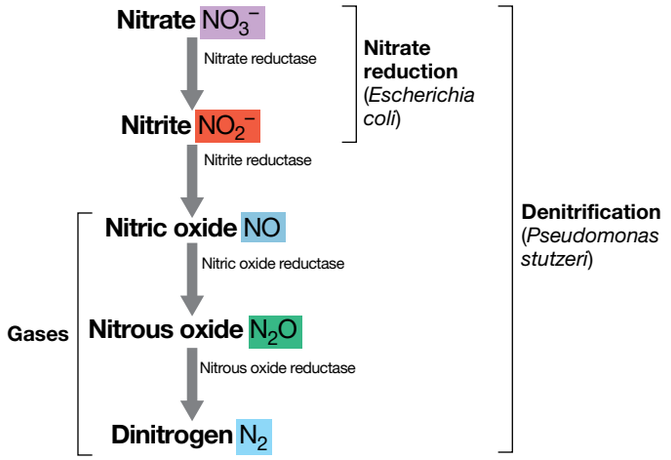
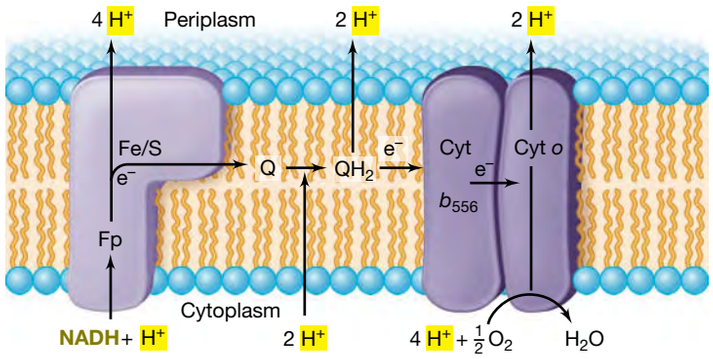


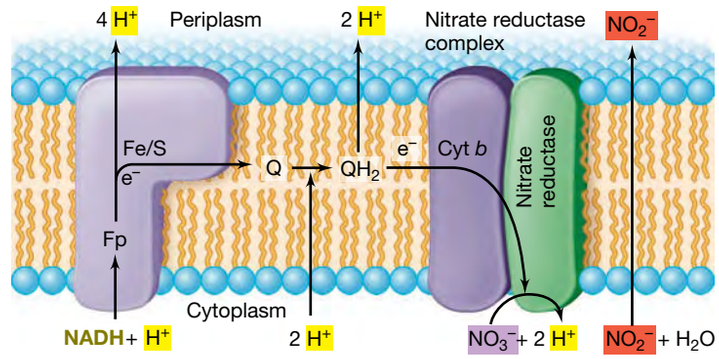
Figure 14.35 Steps in the dissimilative reduction of nitrate. Some organisms can carry out only the first step. All enzymes involved are derepressed by anoxic conditions. Also, some bacteria are known that can reduce NO_3^- to NH_4^+ in dissimilative metabolism. Note that colors used here match those used in Figure 14.36.

of alternative energy-generating mechanisms. Some species of *Archaea* can grow anaerobically by nitrate reduction to nitrite, and several archaeans can also denitrify. Interestingly, at least one eukaryote has also been shown to be a denitrifier. The protist *Globobulimina pseudospinescens*, a shelled amoeba (a foraminiferan, ↔ Section 18.6), can denitrify and likely employs this form of metabolism in its habitat, anoxic marine sediments.

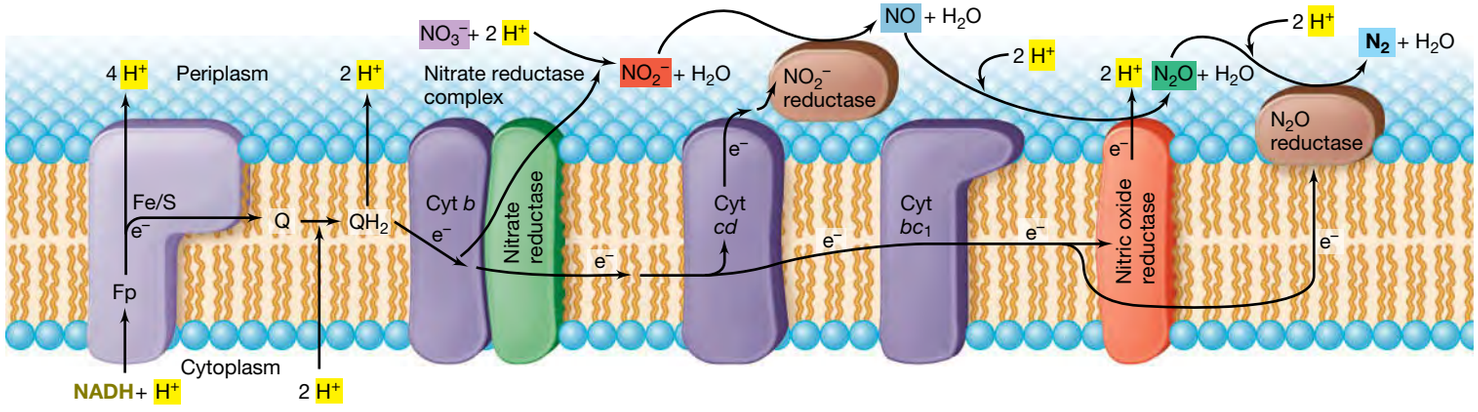
Denitrification is a process with significant ecological ramifications. For agricultural purposes, denitrification is a detrimental process, as it removes nitrate—often intentionally added by grain and other crop farmers as potassium nitrate fertilizer—from the soil. Gaseous products of denitrification other than N_2 (N_2O and NO) are also of significant environmental concern. N_2O is a strong greenhouse gas (contributing to climate change) and can also be converted to NO by sunlight; NO reacts with and consumes ozone (O_3) in the upper atmosphere to form NO_2^-. When it rains, NO_2^- returns to Earth as nitrous acid (HNO_2) in *acid rain*. In contrast to these environmentally harmful processes, for a desirable process like sewage treatment, denitrification (as well as anammox, see earlier) is beneficial because it removes fixed nitrogen, a major trigger of algal growth if a nitrate-rich sewage effluent is released into rivers or lakes (↔ Sections 20.8, 22.6, and 22.7).



(a) **Aerobic respiration**



(b) **Nitrate reduction**



(c) **Denitrification**

Figure 14.36 Respiration and nitrate-based anaerobic respiration. Electron transport processes in the membrane of *Escherichia coli* when *(a)* O_2 or *(b)* NO_3^- is used as an electron acceptor and NADH is the electron donor. Fp, flavoprotein; Q, ubiquinone. Under high-oxygen conditions, the sequence of carriers is Cyt b_{556} ⟶ Cyt *o* ⟶ O_2. However, under low-oxygen conditions (not shown), the sequence is Cyt b_{568} ⟶ Cyt *d* ⟶ O_2. Note how more protons are translocated per two electrons oxidized aerobically during electron transport reactions than anaerobically with NO_3^- as electron acceptor, because the aerobic terminal oxidase (Cyt *o*) pumps two protons. *(c)* Scheme for electron transport in membranes of *Pseudomonas stutzeri* during denitrification. Nitrate and nitric oxide reductases are integral membrane proteins, whereas nitrite and nitrous oxide reductases are periplasmic enzymes.

Biochemistry of Dissimilative Nitrate Reduction

The electron transport pathways of aerobic respiration, nitrate respiration, and denitrification are compared in **Figure 14.36**. The enzyme that catalyzes the first step of dissimilative nitrate reduction is *nitrate reductase*, a molybdenum-containing, membrane-integrated enzyme whose synthesis is repressed by O_2. All subsequent enzymes of the pathway are coordinately regulated and thus also repressed by O_2. But, in addition to anoxic conditions, nitrate must also be present before these enzymes are fully expressed.

The biochemistry of dissimilative nitrate reduction has been studied in detail in *E. coli*, in which NO_3^- is reduced only to NO_2^-, and *Paracoccus denitrificans* and *Pseudomonas stutzeri*, in which denitrification occurs. The *E. coli* nitrate reductase accepts electrons from a *b*-type cytochrome, and a comparison of the electron transport chains in aerobic versus nitrate-respiring cells of *E. coli* is shown in Figure 14.35*a, b*. Because of the reduction potential of the NO_3^-/NO_2^- couple (+0.43 V), fewer protons are pumped during nitrate reduction than in aerobic respiration (O_2/H_2O, +0.82 V). In *P. denitrificans* and *P. stutzeri*, nitrogen oxides are formed from NO_2^- by the enzymes nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. NO and N_2O are gaseous intermediates that are free to escape from the cell, and N_2O in particular is a major product of denitrification, though these intermediates are often reduced all the way to N_2. During these electron transport reactions, a proton motive force is established (Figure 14.36*c*), and ATPase couples this to the synthesis of ATP.

MINIQUIZ

- For *Escherichia coli*, why is more energy released in aerobic respiration than during NO_3^- reduction?
- How do the products of NO_3^- reduction differ between *E. coli* and *Pseudomonas*?
- Where is the dissimilative nitrate reductase found in the cell? What unusual metal does it contain?

14.14 Sulfate and Sulfur Reduction

Several inorganic sulfur compounds are important electron acceptors in anaerobic respiration, and **Table 14.5** lists the oxidation states of the key compounds. Sulfate (SO_4^{2-}), the most oxidized form of sulfur, is reduced by the *sulfate-reducing bacteria*, a highly diverse group of obligately anaerobic bacteria widely distributed in nature. The end product of sulfate reduction is hydrogen sulfide, H_2S, an important natural product that participates in many biogeochemical processes (Sections 21.4, 22.11, and 22.12). Species in the genus *Desulfovibrio*, in particular *D. desulfuricans*, have been widely studied, and the general properties of sulfate-reducing bacteria are discussed in Section 15.9.

As with nitrate (Section 14.13), it is necessary to distinguish between assimilative and dissimilative sulfate metabolism. Most microbes can incorporate sulfate for biosynthetic purposes to make cysteine, methionine, and many other organosulfur compounds; this is *assimilative* sulfate metabolism. By contrast, the ability to use sulfate as an electron acceptor for energy conservation requires its large-scale reduction and is restricted to the sulfate-reducing bacteria. H_2S is produced on a very large scale by these organisms and is excreted from the cell, free to be oxidized by air, used by other organisms, or combined with metals to form metal sulfides.

TABLE 14.5 Sulfur compounds for sulfate reduction

Compound	*Oxidation state of S atom*
Organic S (R—SH)	−2
Sulfide (H_2S)	−2
Elemental sulfur (S^0)	0
Thiosulfate (—S–SO_3^{2-})	−2/+6
Sulfur dioxide (SO_2)	+4
Sulfite (SO_3^{2-})	+4
Sulfate (SO_4^{2-})	+6

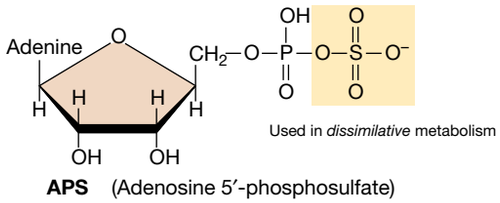
Biochemistry and Energetics of Sulfate Reduction

As was shown in Figure 14.25, SO_4^{2-} is an energetically much less favorable electron acceptor than is O_2 or NO_3^-. However, sufficient free energy to make ATP is available from sulfate reduction when an electron donor is oxidized that yields NADH or FADH. Hydrogen (H_2) is used by virtually all species, whereas the use of organic electron donors is more restricted. For example, lactate and pyruvate are widely used by species found in freshwater anoxic environments while acetate and longer-chain fatty acids are widely used by marine sulfate-reducing bacteria. Many morphological and physiological types of sulfate-reducing bacteria are known, and with the exception of *Archaeoglobus* (Section 17.4), a genus of *Archaea*, all known sulfate reducers are *Bacteria* (Section 15.9).

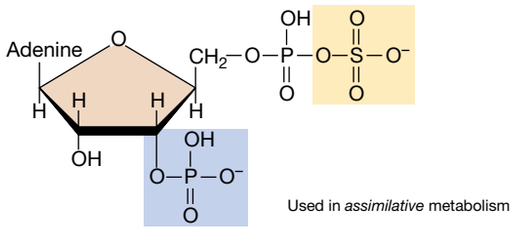
The reduction of SO_4^{2-} to H_2S requires eight electrons and proceeds through a number of intermediate stages. The reduction of SO_4^{2-} requires that it first be *activated* in a reaction requiring ATP. The enzyme ATP sulfurylase catalyzes the attachment of SO_4^{2-} to a phosphate of ATP, forming *adenosine phosphosulfate* (*APS*) as shown in **Figure 14.37*a***. Activation raises the extremely electronegative E_0' of the SO_4^{2-}/SO_3^{2-} couple (–0.52 V) to near 0 V, making reduction of the sulfate moiety possible with electron donors such as NADH (–0.32 V).

In *dissimilative* sulfate reduction, the SO_4^{2-} in APS is reduced directly to sulfite (SO_3^{2-}) by the enzyme APS reductase with the release of AMP. In *assimilative* reduction, by contrast, a second phosphate is added to APS to form *phosphoadenosine phosphosulfate* (*PAPS*) (Figure 14.37*a*), and only then is the SO_4^{2-} reduced. However, in both cases the product of sulfate reduction is sulfite (SO_3^{2-}), and once SO_3^{2-} is formed, it is reduced to H_2S by activity of the enzyme sulfite reductase (Figure 14.37*b*).

During dissimilative sulfate reduction, electron transport reactions generate a proton motive force and this drives ATP synthesis by ATPase. A major electron carrier in this process is *cytochrome* c_3, a periplasmic low-potential cytochrome (**Figure 14.38**). Cytochrome c_3 accepts electrons from a periplasmic hydrogenase and transfers these electrons to a membrane-associated protein complex. This complex, called *Hmc*, carries the electrons across the

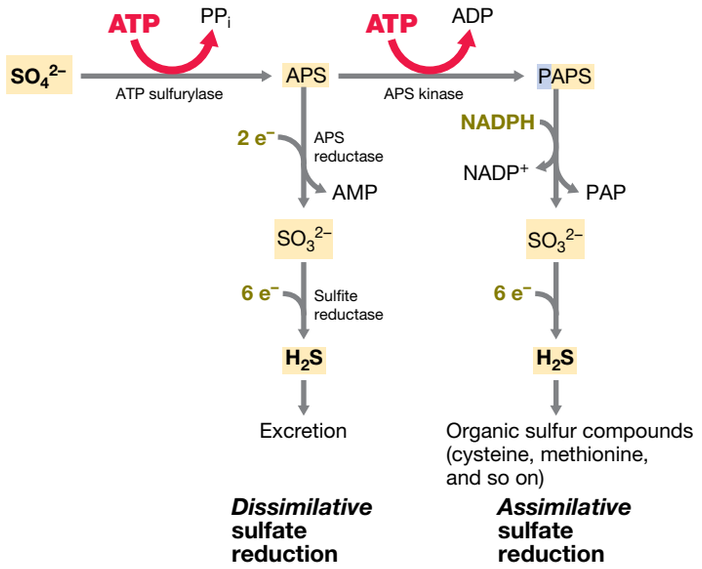


APS (Adenosine 5′-phosphosulfate)



PAPS (Phosphoadenosine 5′-phosphosulfate)

(a)



(b)

Figure 14.37 Biochemistry of sulfate reduction: Activated sulfate. *(a)* Two forms of active sulfate can be made, adenosine 5′-phosphosulfate (APS) and phosphoadenosine 5′-phosphosulfate (PAPS). Both are derivatives of adenosine diphosphate (ADP), with the second phosphate of ADP being replaced by SO_4^{2-}. *(b)* Schemes of assimilative and dissimilative sulfate reduction.

cytoplasmic membrane and transfers them to APS reductase and sulfite reductase, cytoplasmic enzymes that generate sulfite and sulfide, respectively (Figure 14.38).

The enzyme hydrogenase plays a central role in sulfate reduction whether *Desulfovibrio* is growing on H_2, per se, or on an organic compound such as lactate. This is because lactate is converted through pyruvate to acetate (much of the latter is either excreted or assimilated into cell material because *Desulfovibrio* cannot oxidize acetate to CO_2) with the production of H_2. This H_2 crosses the cytoplasmic membrane and is oxidized by the periplasmic hydrogenase to electrons, which are fed back into the system, and protons, which establish the proton motive force

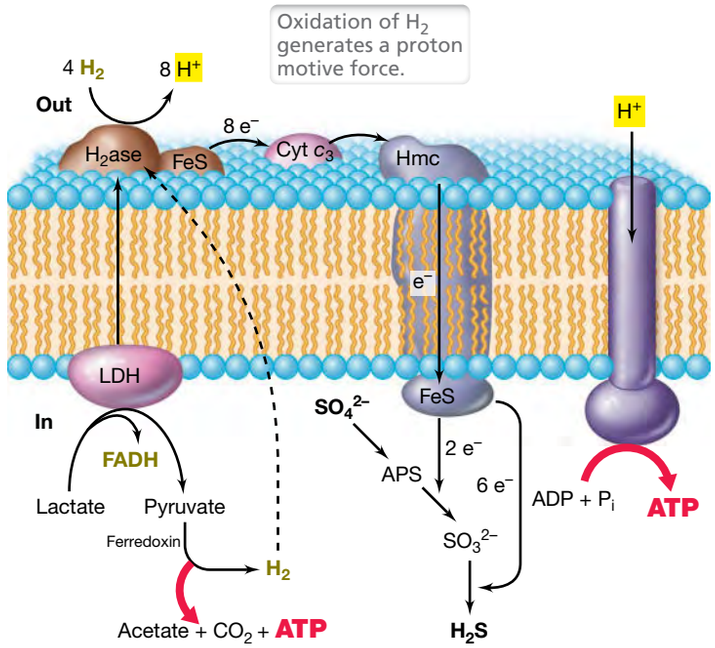


Figure 14.38 Electron transport and energy conservation in sulfate-reducing bacteria. In addition to external H_2, H_2 originating from the catabolism of organic compounds such as lactate and pyruvate can fuel hydrogenase. The enzymes hydrogenase (H_2ase), cytochrome (cyt) c_3, and a cytochrome complex (Hmc) are periplasmic proteins. A separate protein shuttles electrons across the cytoplasmic membrane from Hmc to a cytoplasmic iron–sulfur protein (FeS) that supplies electrons to APS reductase (forming SO_3^{2-}) and sulfite reductase (forming H_2S, Figure 14.37*b*). LDH, lactate dehydrogenase.

(Figure 14.38). A net of one ATP is produced for each SO_4^{2-} reduced to HS^- by H_2, and the reaction is

$$4\,H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\,H_2O \qquad \Delta G^{0\prime} = -152\,kJ$$

When lactate or pyruvate is the electron donor, ATP is produced not only from the proton motive force but also by substrate-level phosphorylation during the oxidation of pyruvate to acetate plus CO_2 (Figure 14.38).

Marine but not freshwater species of sulfate-reducing bacteria can couple sulfate reduction and the oxidation of acetate (and longer-chain fatty acids) to CO_2:

$$CH_3COO^- + SO_4^{2-} + 3\,H^+ \rightarrow 2\,CO_2 + H_2S + 2\,H_2O$$

$$\Delta G^{0\prime} = -57.5\,kJ$$

The mechanism for acetate oxidation in most of these species is the *acetyl-CoA pathway*, a series of reversible reactions used by many anaerobes for acetate synthesis or acetate oxidation (Section 14.16). A few sulfate-reducing bacteria can also grow autotrophically with H_2. Under these conditions, the organisms use the acetyl-CoA pathway for making acetate as a carbon source. Such species can be cultured in a completely organic-free medium containing only mineral salts, sulfate, CO_2, and H_2.

Special Metabolisms of Sulfate-Reducing Bacteria

Certain species of sulfate-reducing bacteria can catalyze unusual reactions not characteristic of all species. These include *disproportionation, phosphite oxidation*, and *sulfur reduction*.

Disproportionation is a process in which one molecule of a substance is oxidized while a second molecule is reduced, ultimately forming two different products. For example, *Desulfovibrio sulfodismutans* can disproportionate thiosulfate ($S–SO_3^{2-}$) as follows:

$$S—SO_3^{2-} + H_2O \rightarrow H_2S + SO_4^{2-} \qquad \Delta G^{0\prime} = -21.9\,kJ/reaction$$

Note that in this reaction, the right-hand sulfur atom of $S–SO_3^{2-}$ is oxidized (forming SO_4^{2-}), while the left-hand atom is reduced (forming H_2S). The free energy available from the oxidation of thiosulfate by *D. sulfodismutans* is insufficient to couple to substrate-level phosphorylation and so instead is coupled to a proton "pump" that uses the minimal energy available in the reaction to establish a proton motive force. Other reduced sulfur compounds such as sulfite (SO_3^{2-}) and sulfur (S^0) can also be disproportionated. These forms of sulfur metabolism allow sulfate-reducing bacteria to recover energy from sulfur intermediates produced from the oxidation of H_2S by sulfur chemolithotrophs that coexist with them in nature and also from intermediates generated in their own metabolism during SO_4^{2-} reduction (Figure 14.37*b*).

At least one sulfate-reducing bacterium can couple phosphite (HPO_3^-) oxidation to SO_4^{2-} reduction. This chemolithotrophic reaction yields phosphate and sulfide:

$$4\,HPO_3^- + SO_4^{2-} + H^+ \rightarrow 4\,HPO_4^{2-} + HS^- \qquad \Delta G^{0\prime} = -364\,kJ$$

This bacterium, *Desulfotignum phosphitoxidans*, is an autotroph and also a strict anaerobe, which by necessity it must be because phosphite spontaneously oxidizes in air. The natural sources of phosphite are likely to be organophosphorous compounds called *phosphonates* that are generated from the anoxic degradation of nucleic acids, phospholipids, and other cellular sources. Along with sulfur disproportionation (also a chemolithotrophic process) and H_2 utilization, phosphite oxidation underscores the diversity of chemolithotrophic reactions carried out by sulfate-reducing bacteria.

Sulfur Reduction

Besides sulfate, most sulfate-reducing bacteria can also conserve energy from the reduction of elemental sulfur to sulfide ($S^0 + 2\,H \rightarrow H_2S$). In addition, however, a variety of non-sulfate-reducing microbes can also reduce sulfur in anaerobic respiration. These are the *sulfur reducers*, a large group of *Bacteria* and *Archaea* that coexist with sulfate-reducing bacteria in anoxic, sulfur-rich habitats in nature.

The electrons for sulfur reduction come from H_2 or any of a number of organic compounds. For example, *Desulfuromonas acetoxidans* can oxidize acetate or ethanol to CO_2 coupled to the reduction of S^0 to H_2S. Sulfur reducers lack the capacity to activate sulfate to APS (Figure 14.37), and presumably this is what excludes them from using SO_4^{2-} as an electron acceptor. *Desulfuromonas* contains several cytochromes, including an analog of cytochrome c_3, a key electron carrier in sulfate-reducing bacteria. In culture some sulfur reducers including *Desulfuromonas* can also use Fe^{3+} as an electron acceptor in place of sulfur, but sulfur is probably the major electron acceptor used in nature. Indeed, it is the reduction of oxidized sulfur compounds and the production of H_2S that connects the sulfur- and sulfate-reducing bacteria in an ecological sense.

MINIQUIZ

- How is SO_4^{2-} converted to SO_3^{2-} during dissimilative sulfate reduction? Physiologically, how does *Desulfuromonas* differ from *Desulfovibrio*?
- Contrast the growth of *Desulfovibrio* on H_2 versus lactate as electron donors.
- Give an example of sulfur disproportionation.

14.15 Other Electron Acceptors

In addition to the electron acceptors for anaerobic respiration discussed thus far, several metals, metalloids, and halogenated and unhalogenated organic compounds are important electron acceptors for bacteria in nature (Figure 14.25). In addition to these, even protons can be used by a very few strict anaerobes. We consider these forms of anaerobic respiration here.

Metal and Metalloid Reduction

Several metals and metalloids can be reduced in anaerobic respirations. Ferric iron (Fe^{3+}) and manganic ion (Mn^{4+}) are the most important metals reduced. The reduction potential of the Fe^{3+}/Fe^{2+} couple is +0.2 V (at pH 7), and that of the Mn^{4+}/Mn^{2+} couple is +0.8 V; thus, several electron donors can couple to Fe^{3+} and Mn^{4+} reduction. In these reactions, electrons typically travel from the donor through an electron transport chain that generates a proton motive force and terminates in a metal reductase system, reducing Fe^{3+} to Fe^{2+} or Mn^{4+} to Mn^{2+}. The gram-negative bacteria *Shewanella* and *Geobacter* are major species here. Other inorganic substances can function as electron acceptors for anaerobic respiration, including the metalloids selenium, tellurium, and arsenic, and various oxidized chlorine compounds (some of these are shown in Figure 14.25). Several chlorate and perchlorate-reducing bacteria have also been isolated and are likely responsible for the removal of these toxic compounds from nature; the typical end product of these reactions is chloride (Cl^-).

The sulfate-reducing bacterium *Desulfotomaculum* can reduce both AsO_4^{3-} to AsO_3^{3-} and sulfate to sulfide (Figure 14.25), precipitating the yellow mineral orpiment (As_2S_3) in the process (**Figure 14.39**). This is an example of *biomineralization*, the formation of a mineral by bacterial activity. As_2S_3 formation also functions as a means of detoxifying what would otherwise be a toxic compound (arsenic), and thus such microbial activities may have practical applications for the cleanup of arsenic-containing toxic wastes and groundwater.

Organic Electron Acceptors

Several organic compounds can be electron acceptors in anaerobic respirations. Of those listed in Figure 14.25, the compound that has been most extensively studied is *fumarate*, a citric acid cycle intermediate (Figure 3.16), which is reduced to succinate. The role of fumarate as an electron acceptor for anaerobic respiration derives from the fact that the fumarate/succinate couple has a reduction potential near 0 V, which allows coupling of fumarate reduction to the oxidation of

Figure 14.39 Biomineralization during arsenate reduction by the sulfate-reducing bacterium *Desulfotomaculum auripigmentum*. Left, appearance of culture bottle after inoculation. Right, following growth for 2 weeks and biomineralization of arsenic trisulfide, As_2S_3. Center, synthetic sample of As_2S_3.

NADH, FADH, or H_2. Many facultatively aerobic bacteria can grow anaerobically on fumarate as electron acceptor, including *Escherichia coli*.

Trimethylamine oxide (TMAO) and dimethyl sulfoxide (DMSO) (Figure 14.25) are important organic electron acceptors. TMAO is a product of marine fish, and several bacteria can reduce it to trimethylamine (TMA), which has a strong odor and flavor (the odor of spoiled seafood is due primarily to TMA produced by bacterial action). Dimethyl sulfoxide (DMSO), which is reduced to dimethyl sulfide (DMS), is a common natural product and is found in both marine and freshwater environments. The reduction potentials of the TMAO/TMA and DMSO/DMS couples are about the same (near +0.15 V, Figure 14.25) and so the electron transport chains that terminate with TMAO or DMSO reductases typically contain cytochromes of the *b* type.

Several halogenated organic compounds function as electron acceptors in **reductive dechlorination** (also called *dehalorespiration*). For example, the sulfate-reducing bacterium *Desulfomonile* grows anaerobically with H_2 or organic electron donors and chlorobenzoate as an electron acceptor that is reduced to benzoate and hydrochloric acid (HCl):

$$C_7H_4O_2Cl^- + 2\,H \rightarrow C_7H_5O_2^- + HCl$$

Several other anaerobic bacteria can reductively dechlorinate, and some are even restricted to chlorinated compounds as electron acceptors for anaerobic respiration. For example, *Dehalobacter* and *Dehalococcoides* oxidize H_2 and reduce tetrachloroethylene to dichloroethylene and ethene, respectively. *Dehalococcoides* can also reduce polychlorinated biphenyls (PCBs). PCBs are widespread organic pollutants that contaminate freshwater environments, where they accumulate in fish and other aquatic life. However, removal of the chlorine groups from these molecules greatly reduces their toxicity and hence reductive dechlorination is not only a form of anaerobic respiration but also an environmentally significant process of bioremediation.

Proton Reduction

Perhaps the simplest of all anaerobic respirations is one carried out by the hyperthermophile *Pyrococcus furiosus*. *P. furiosus* is a species of *Archaea* that grows optimally at 100°C (Chapter 17) on sugars and small peptides as electron donors and protons as electron acceptors. This is possible because of a unique biochemical feature of the glycolytic pathway of *P. furiosus*.

During glycolysis, the oxidation of glyceraldehyde 3-phosphate forms 1,3-bisphosphoglyceric acid, an intermediate with two energy-rich phosphate bonds; this compound is then converted to 3-phosphoglyceric acid plus ATP (Figure 3.14). However, in *P. furiosus* this glycolytic step is bypassed and instead, 3-phosphoglyceric acid is formed directly from glyceraldehyde 3-phosphate (**Figure 14.40**). This prevents *P. furiosus* from making ATP by substrate-level phosphorylation at this step, but this problem is compensated for by the fact that glyceraldehyde 3-phosphate oxidation is coupled to the production of *ferredoxin* rather than NADH; ferredoxin has a more negative E_0' (–0.42 V) than does NAD^+/NADH (–0.32 V). This highly negative E_0' allows for the coupling of ferredoxin oxidation to the reduction of 2 H^+ to H_2, and this reaction pumps a proton across the membrane (Figure 14.40). Proton pumping by hydrogenase is analogous to proton pumping by terminal electron carriers in other respirations. Additional ATP is produced by *P. furiosus* by substrate-level phosphorylations in the conversions of phosphoenolpyruvate to pyruvate and acetyl-CoA to acetate (Figure 14.40).

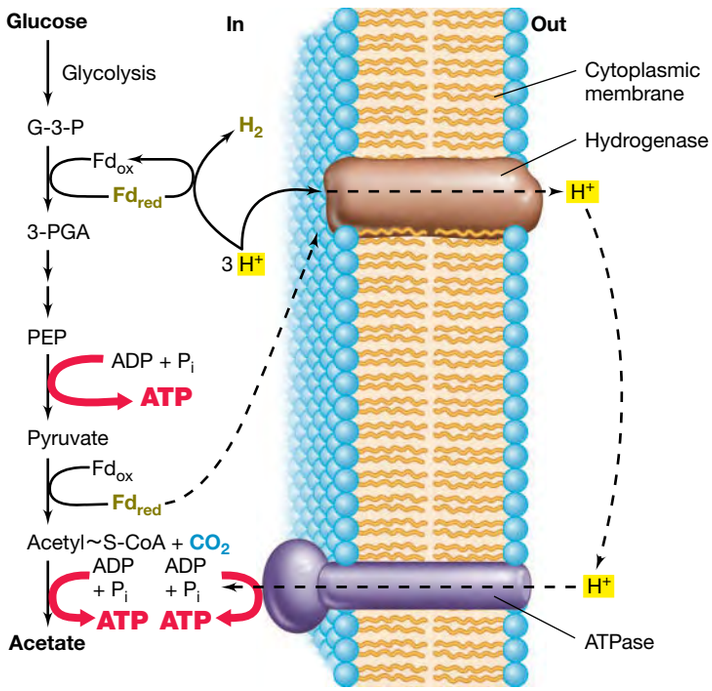


Figure 14.40 Modified glycolysis and proton reduction in anaerobic respiration in the hyperthermophile *Pyrococcus furiosus*. Hydrogen (H_2) production is linked to H^+ pumping by a hydrogenase that receives electrons from reduced ferredoxin (Fd_{red}). All intermediates from G-3-P downward in the pathway are present in two copies. Compare this figure with classical glycolysis in Figure 3.14. G-3-P, glyceraldehyde 3-phosphate; 3-PGA, 3-phosphoglycerate; PEP, phosphoenolpyruvate.

MINIQUIZ

- With H_2 as electron donor, why is reduction of Fe^{3+} a more favorable reaction than reduction of fumarate?
- What is reductive dechlorination and why is it environmentally relevant?
- How does anaerobic glucose catabolism differ in *Lactobacillus* and *Pyrococcus furiosus*?

V • One-Carbon (C_1) Metabolism

Carbon dioxide (CO_2) and methane (CH_4) are abundant in many anoxic habitats, and a wide diversity of microbes have evolved metabolic pathways that conserve energy from either the reduction of CO_2 or the oxidation of CH_4. A number of the enzymatic reactions in the metabolism of one-carbon compounds (C_1 metabolism) are unique to this kind of metabolism. In this section we consider the metabolism of organisms that perform C_1 metabolism of one sort or the other, highlighting the major similarities and differences.

14.16 Acetogenesis

Two major groups of strictly anaerobic microbes use CO_2 as an electron acceptor for energy conservation. One of these is the *acetogens*, and we discuss them here. The other group, the *methanogens*, are considered in the next section. Hydrogen (H_2) is a major electron donor for both of these organisms, and an overview of their energy metabolism, **acetogenesis** and **methanogenesis**, is shown in Figure 14.41. Both processes are linked to ion pumps, of either protons (H^+) or sodium ions (Na^+), as the mechanism of energy conservation, and these pumps fuel ATP synthases in the membrane. The pathway of acetogenesis also conserves energy in a substrate-level phosphorylation reaction.

Organisms and Pathway

Acetogens carry out the reaction

$$4\,H_2 + H^+ + 2\,HCO_3^- \rightarrow CH_3COO^- + 4\,H_2O \qquad \Delta G^{0\prime} = -105\ kJ$$

In addition to H_2, electron donors for acetogenesis include various C_1 compounds such as methanol, several methoxylated aromatic compounds, sugars, organic and amino acids, alcohols, and certain nitrogen bases, depending on the organism. Many acetogens can also reduce nitrate (NO_3^-) and thiosulfate ($S_2O_3^{2-}$) in dissimilative metabolisms. However, CO_2 reduction is the major reaction of ecological relevance.

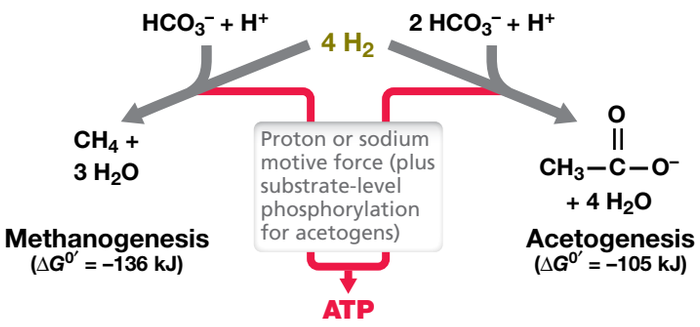


Figure 14.41 The contrasting processes of methanogenesis and acetogenesis. Note the difference in free energy released in the reactions.

A major unifying thread among acetogens is the pathway of CO_2 reduction. Acetogens reduce CO_2 to acetate by the reductive acetyl-CoA pathway (also called the *Wood–Ljungdahl pathway*), the major pathway in obligate anaerobes for the production of acetate (see Figure 14.42). The reactions of the reductive acetyl-CoA pathway are reversible, and some microbes reverse this pathway to oxidize acetate. Table 14.6 lists the groups that either produce or oxidize acetate by way of the acetyl-CoA pathway.

Acetogens such as *Acetobacterium woodii* and *Clostridium aceticum* can grow either chemoorganotrophically by fermentation of sugars (reaction A) or chemolithotrophically and autotrophically through the reduction of CO_2 to acetate with H_2 as electron donor (reaction B). In either case, the sole product is *acetate*:

$$(A)\ C_6H_{12}O_6 \rightarrow 3\,CH_3COO^- + 3\,H^+$$

$$(B)\ 2\,HCO_3^- + 4\,H_2 + H^+ \rightarrow CH_3COO^- + 4\,H_2O$$

When growing on glucose, acetogens use glycolysis (Figure 3.14) to oxidize the glucose into two molecules of pyruvate and two molecules of NADH. The pyruvate is then further oxidized to produce two molecules of acetate:

$$(C)\ 2\,Pyruvate^- \rightarrow 2\,acetate^- + 2\,CO_2 + 2\,NADH$$

The CO_2 generated in reaction (C) is then used as a terminal electron acceptor in the reductive acetyl-CoA pathway. The NADH generated during glycolysis and pyruvate oxidation is used as an

TABLE 14.6 Organisms employing the acetyl-CoA pathway

I. Pathway drives acetate synthesis for energy purposes
Acetoanaerobium noterae
Acetobacterium woodii
Acetobacterium wieringae
Acetogenium kivui
Acetitomaculum ruminis
Clostridium aceticum
Clostridium formicaceticum
Clostridium ljungdahlii
Moorella thermoacetica
Desulfotomaculum orientis
Sporomusa paucivorans
Eubacterium limosum (also produces butyrate)
Treponema primitia (from termite hindguts)
II. Pathway drives acetate synthesis for cell biosynthesis
Acetogens
Methanogens
Sulfate-reducing bacteria
III. Pathway drives acetate oxidation for energy purposes
Reaction: Acetate + H^+ + 2 H_2O → 2 CO_2 + 8 H
Group II sulfate reducers (other than *Desulfobacter*)
Reaction: Acetate + H^+ → CO_2 + CH_4
Acetotrophic methanogens (*Methanosarcina, Methanosaeta*)

UNIT 4

electron donor in CO_2 reduction. Starting from pyruvate, then, the overall production of acetate can be written as

$$2\,\text{Pyruvate}^- + 4\,\text{H} \rightarrow 3\,\text{acetate}^- + \text{H}^+$$

Most acetogenic bacteria that produce acetate in energy metabolism are gram-positive *Bacteria*, and many are species of the genera *Clostridium* or *Acetobacterium* (Table 14.6). A few other gram-positive and many different gram-negative *Bacteria* and *Archaea* use the reductive acetyl-CoA pathway for autotrophic purposes, reducing CO_2 to acetate as a source of cell carbon; these include autotrophic sulfate-reducing bacteria (Section 14.14), anammox bacteria (Section 14.12), and methanogens (Section 14.17). And finally, some microbes run the acetyl-CoA pathway in the *reverse direction* as a means of oxidizing acetate to CO_2; these include acetate-utilizing methanogens and sulfate-reducing bacteria. The acetyl-CoA pathway is thus a metabolically highly versatile series of reactions.

The Reductive Acetyl-CoA Pathway and Energy Conservation in Acetogenesis

Unlike other autotrophic pathways (Section 14.5), the reductive acetyl-CoA pathway of CO_2 fixation is not a cycle. Instead, it catalyzes the reduction of CO_2 along two linear pathways, with one molecule of CO_2 being reduced to the methyl group of acetate (the methyl branch of the pathway) and the other to the carbonyl group of acetate (the carbonyl branch of the pathway). These two C_1 units are then combined to form acetyl-CoA (**Figure 14.42**).

A key enzyme of the acetyl-CoA pathway is *carbon monoxide* (*CO*) *dehydrogenase*. CO dehydrogenase contains Ni, Zn, and Fe as cofactors and catalyzes the reaction

$$CO_2 + H_2 \rightarrow CO + H_2O$$

The CO produced by CO dehydrogenase ends up as the *carbonyl* carbon of acetate (Figure 14.42). The methyl group of acetate originates from the reduction of CO_2 by a series of reactions in which the coenzyme *tetrahydrofolate* plays a major role (Figure 14.42). The methyl group is then transferred from tetrahydrofolate to a cobalt- and iron-containing *corrinoid iron–sulfur protein* (CoFeSP) coenzyme. In the final step of the pathway, the methyl group is combined with CO by the activity of both CO dehydrogenase and acetyl-CoA synthase to form acetyl-CoA. Conversion of

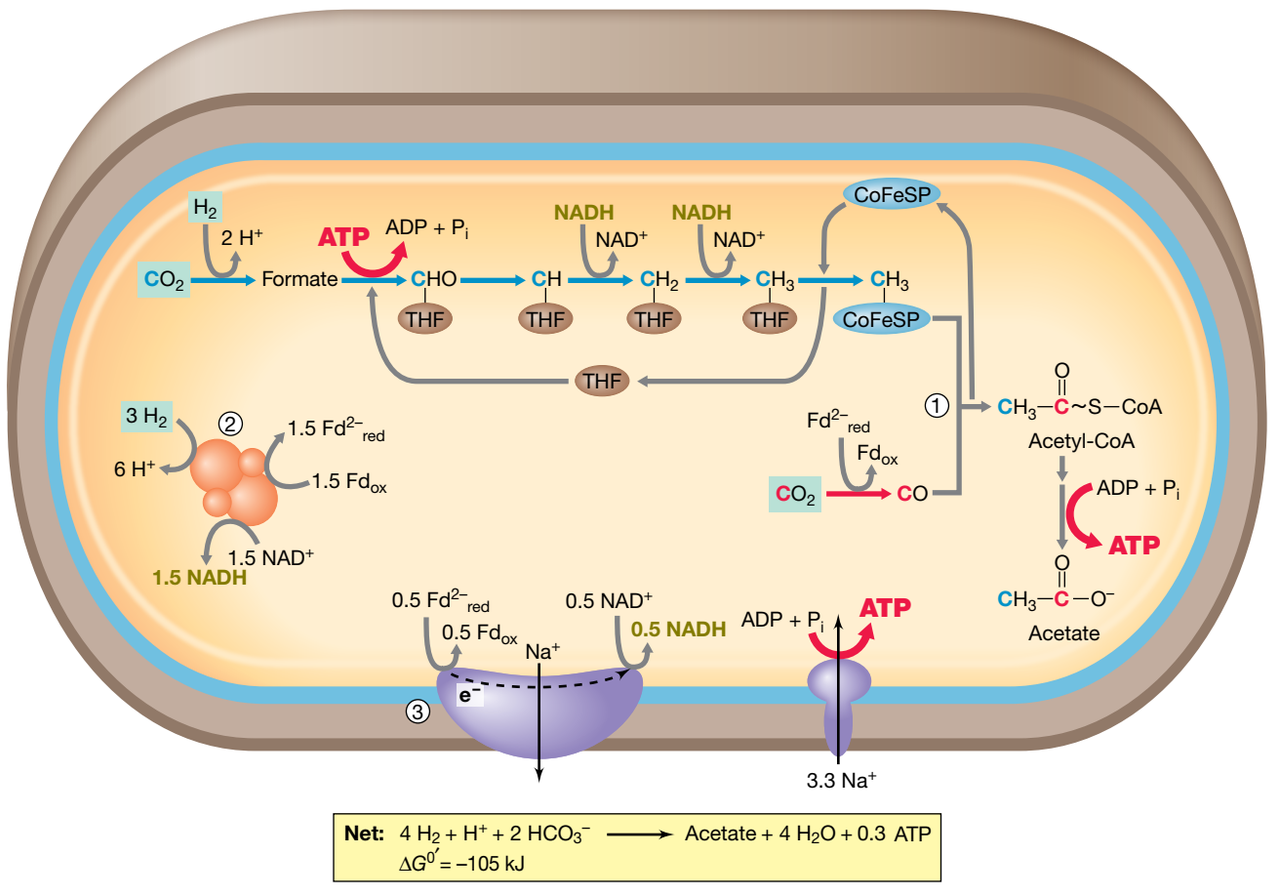


Figure 14.42 Reactions of acetogenesis from H_2 and CO_2 in *Acetobacterium woodii*. The reductive acetyl-CoA pathway is used to reduce CO_2 to acetate. The pathway has a methyl branch (blue arrows) and a carbonyl branch (red arrows). Carbon monoxide from the carbonyl branch and a methyl group from the methyl branch are combined into acetyl-CoA by carbon monoxide dehydrogenase (1), a key enzyme for the pathway. Note that the reductive acetyl-CoA pathway does not conserve energy. An electron-bifurcating hydrogenase (2) (see also Figure 14.43) is used to reduce ferredoxin (Fd^{2-}_{red}), and energy is conserved at the Rnf complex (3), which generates a Na^+ motive force. THF, tetrahydrofolate; CoFeSP, Co/Fe-containing corrinoid iron–sulfur protein.

acetyl-CoA to acetate is the last step in the pathway, generating one ATP by substrate-level phosphorylation (Figure 14.42, see Table 14.7). However, this ATP is consumed in the first step of the acetyl-CoA pathway. Considering this, how then do acetogens get their ATP?

Acetogens conserve energy by the generation of an ion motive force. In *Acetobacterium woodii* the ion motive force is generated by activity of the *Rnf complex* (or in some acetogens a related complex called the *Ech complex*); these enzymes use reduced ferredoxin (Fd^{2-}_{red}) as electron donor and NAD^+ as electron acceptor. The Rnf complex pumps one Na^+ across the membrane for each electron exchanged, thereby generating a Na^+ motive force, which can be used to make ATP using a Na^+-dependent ATP synthase (Figure 14.42). Alternatively, in other acetogens such as *Clostridium ljungdahlii*, the Rnf complex instead pumps H^+ instead of Na^+ and has a typical H^+-dependent ATP synthase. Ultimately, only 0.3 ATP are produced for every 4 H_2 and 2 CO_2 that are consumed by the acetogens, making these organisms minimalists in terms of the energy that can be conserved from their metabolism.

Flavin-Based Electron Bifurcation

The reduced ferredoxin (Fd^{2-}_{red}) that donates electrons to the Rnf cluster has a reduction potential of –0.45 V, but H_2 has a reduction potential of –0.414 V. How is it possible for acetogens to generate an ion motive force when the reduction of ferredoxin by H_2 is thermodynamically unfavorable? The answer is a process called *electron bifurcation* (Figure 14.43).

Electron bifurcation is an application of a basic concept we considered in Chapter 3: Endergonic reactions can be driven forward by coupling them to exergonic reactions. In electron bifurcation the endergonic reduction of Fd^{2-}_{red} by a hydrogenase is coupled to the exergonic reduction of NAD^+/NADH ($E_0' = -0.32$ V) by the same hydrogenase. The electron-bifurcating hydrogenase contains a flavin coenzyme. The flavin accepts two electrons at a time and donates one of these electrons to a higher-potential electron acceptor (NAD^+) in order to drive the unfavorable reduction of ferredoxin by the other electron (Figure 14.43). Hence, a total of 2 H_2 must be oxidized to generate one Fd^{2-}_{red} and one NADH.

Flavin-based electron bifurcation is used by many obligate anaerobes to generate the Fd^{2-}_{red} that they need. In particular, we will see shortly that flavin-based electron bifurcation is essential to energy conservation in methanogens that lack cytochromes.

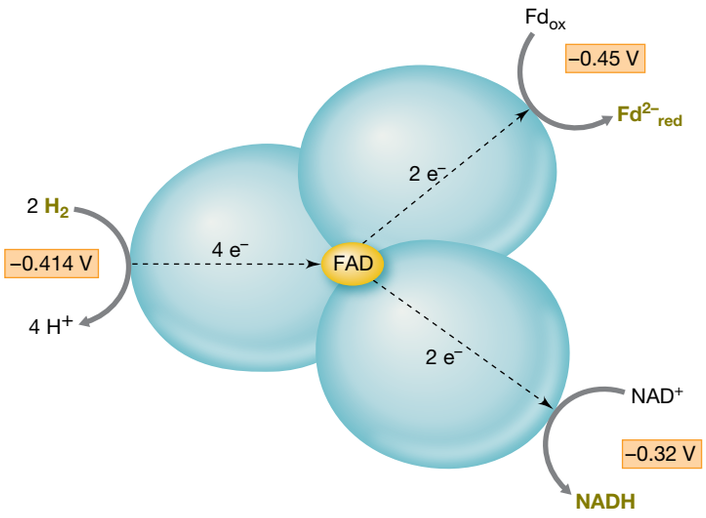


Figure 14.43 The reaction scheme for flavin-based electron bifurcation. Many obligate anaerobes require reduced ferredoxin as an electron donor (see Figure 14.42), but lack external electron donors that are sufficiently electronegative to reduce ferredoxin. In flavin-based electron bifurcation, two electrons from an electron donor (such as H_2) are transferred to a flavin (FAD), and one electron is used to reduce a favorable electron acceptor (such as NAD^+), making it possible to drive the second electron to an unfavorable electron acceptor (such as Fd_{ox}). A total of 4 electrons from H_2 are bifurcated to produce reduced ferredoxin (Fd^{2-}_{red}) and NADH.

MINIQUIZ

- What is the purpose of CO dehydrogenase?
- If acetogens conserve energy using the Rnf complex, then what is the purpose of the reductive CoA pathway?
- What is electron bifurcation and what role does it play in acetogens?

14.17 Methanogenesis

The biological production of methane—*methanogenesis*—is catalyzed by a group of strictly anaerobic *Archaea* called the **methanogens**. These organisms are present in freshwater sediments (Figure 14.44), sewage sludge digesters (Section 22.6) and other bioreactors, and the intestines of warm-blooded animals, including humans. The reduction of CO_2 by H_2 to form methane

Figure 14.44 Methanogenesis. Methane is collected in a funnel from swamp sediments where it was produced by methanogens and then ignited in a demonstration experiment at Woods Hole, Massachusetts (USA).

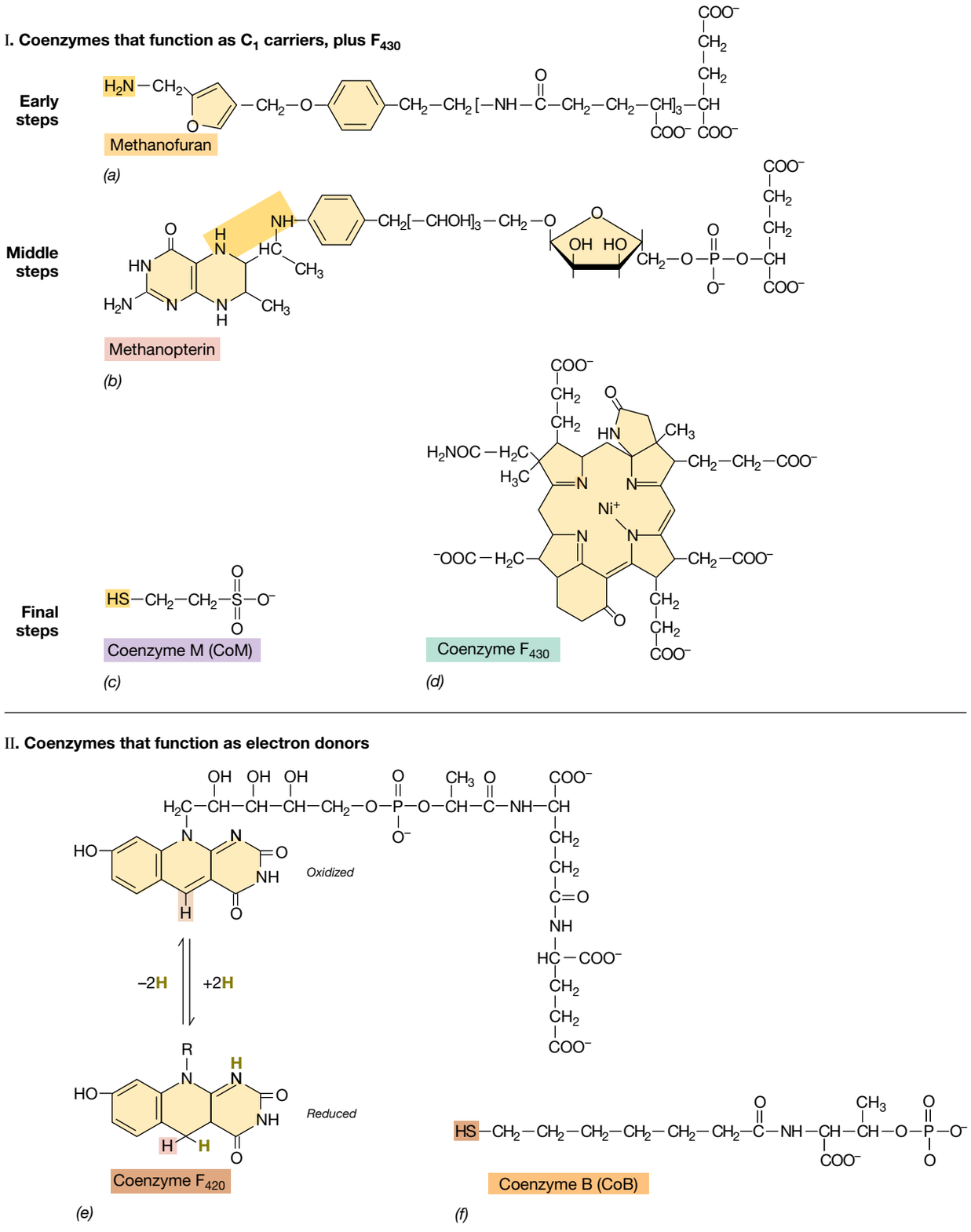


Figure 14.45 Coenzymes of methanogenesis. The atoms shaded in brown or yellow are the sites of oxidation–reduction reactions (brown in F_{420} and CoB) or the position to which the C_1 moiety is attached during the reduction of CO_2 to CH_4 (dark yellow in methanofuran, methanopterin, and coenzyme M). The same colors used to highlight a particular coenzyme (CoB is orange, for example) are also used in Figures 14.47 and 14.48 to follow the reactions in each figure. Coenzyme F_{430} participates in the terminal step of methanogenesis catalyzed by the enzyme methyl reductase, with the methyl group binding to Ni^+ in F_{430} prior to its reduction to CH_4.

(CH_4) is a major pathway of methanogenesis and is a form of anaerobic respiration. We consider the basic properties, phylogeny, and taxonomy of the methanogens in Section 17.2. Here we focus on their bioenergetics and unique biochemistry.

C_1 Carriers in Methanogenesis

Methanogenesis from CO_2 requires eight electrons, and these electrons are added two at a time. This leads to intermediary oxidation states of the carbon atom from +4 (CO_2) to −4 (CH_4). Several novel coenzymes participate in methanogenesis and can be divided into two classes: (1) those that carry the C_1 unit along its path of enzymatic reduction (*C_1 carriers*) and (2) those that donate electrons (*redox coenzymes*) (**Figure 14.45**). We consider the C_1 carriers first.

The coenzyme *methanofuran* is required for the first step of methanogenesis. Methanofuran contains the five-membered furan ring and an amino nitrogen atom that binds CO_2 (Figure 14.45*a*). *Methanopterin* (Figure 14.45*b*) is a methanogenic coenzyme that resembles the vitamin folic acid and plays a role analogous to that of tetrahydrofolate (a coenzyme that participates in C_1 transformations; see Figure 14.42) by carrying the C_1 unit in the intermediate steps of CO_2 reduction to CH_4. *Coenzyme M* (CoM) (Figure 14.45*c*) is required for the terminal step of methanogenesis, the reduction of the methyl group (CH_3) to CH_4. Although not a C_1 carrier, the nickel (Ni^{2+})-containing tetrapyrrole *coenzyme F_{430}* (Figure 14.45*d*) also participates in the terminal step of methanogenesis as part of the methyl reductase enzyme complex (discussed later).

Redox Coenzymes

The coenzymes F_{420} and *7-mercaptoheptanoylthreonine phosphate* (also called coenzyme B, CoB) are electron donors in methanogenesis. Coenzyme F_{420} (Figure 14.45*e*) is a flavin derivative, structurally resembling the flavin coenzyme FMN (Figure 3.18). F_{420} participates in methanogenesis as the electron donor in several steps of CO_2 reduction (see Figure 14.47). Coenzyme F_{420} takes its name from the fact that its oxidized form absorbs light at 420 nm and fluoresces blue-green. Such fluorescence is useful for the microscopic identification of a methanogen (**Figure 14.46**). CoB is required for the terminal step of methanogenesis catalyzed by the *methyl reductase enzyme complex*. As shown in Figure 14.45*f*, the structure of CoB resembles the vitamin pantothenic acid, which is part of acetyl-CoA (Figure 3.13).

Methanogenesis from CO_2 + H_2

Electrons for the reduction of CO_2 to CH_4 typically come from H_2, but a few other substrates can also supply the electrons in some methanogens. **Figure 14.47** shows the steps in CO_2 reduction by H_2:

1. CO_2 is activated by a methanofuran-containing enzyme and reduced to the formyl level. The immediate electron donor is ferredoxin, a strong reductant with a reduction potential (E_0') near −0.4 V.
2. The formyl group is transferred from methanofuran to an enzyme containing methanopterin (MP in Figure 14.47). It is subsequently dehydrated and reduced in two separate steps (total of 4 H) to the methylene and methyl levels. The immediate electron donor here is reduced F_{420}.

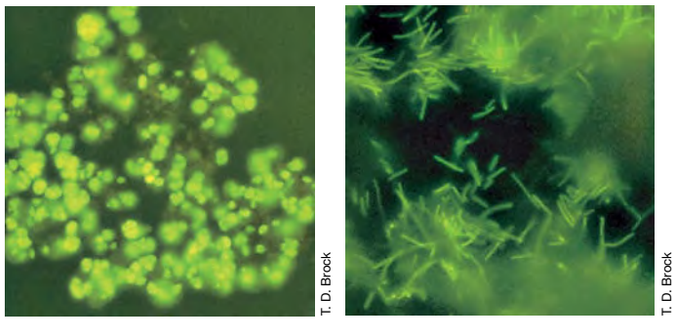


Figure 14.46 Fluorescence due to the methanogenic coenzyme F_{420}. *(a)* Autofluorescence in cells of the methanogen *Methanosarcina barkeri* due to the presence of the unique electron carrier F_{420}. A single cell is about 1.7 μm in diameter. The organisms were made visible by excitation with blue light in a fluorescence microscope. *(b)* F_{420} fluorescence in cells of the methanogen *Methanobacterium formicicum*. A single cell is about 0.6 μm in diameter.

3. The methyl group is transferred from methanopterin to an enzyme containing CoM by the enzyme methyl transferase. This reaction is highly exergonic and linked to the pumping of Na^+ across the membrane from inside to outside the cell.

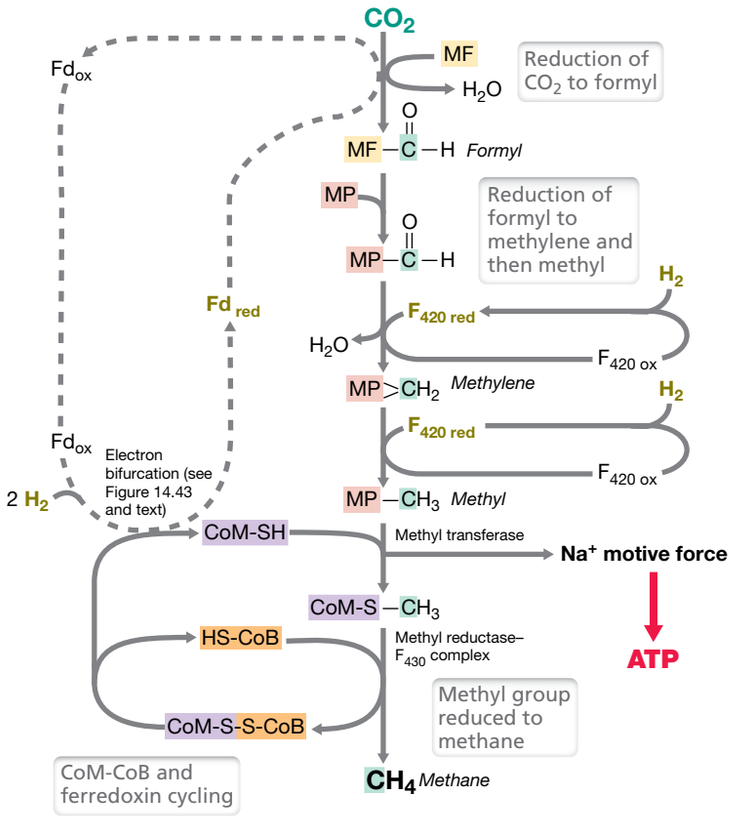


Figure 14.47 Methanogenesis from CO_2 plus H_2. The carbon atom reduced is highlighted in green, and the source of electrons is highlighted in brown. See Figure 14.45 for the structures of the coenzymes. MF, methanofuran; MP, methanopterin; CoM, coenzyme M; $F_{420\ red}$, reduced coenzyme F_{420}; F_{430}, coenzyme F_{430}; Fd, ferredoxin; CoB, coenzyme B.

4. Methyl-CoM is reduced to methane by methyl reductase. In this reaction, F_{430} and CoB are required. Coenzyme F_{430} removes the CH_3 group from CH_3–CoM, forming a Ni^+–CH_3 complex. This complex is reduced by CoB, generating CH_4 and a disulfide complex of CoM and CoB (CoM-S—S-CoB).
5. Free CoM and CoB are regenerated by the reduction of CoM-S—S-CoB ($E_0' = 0.14$) through a flavin-based electron-bifurcation reaction (see Figure 14.43) with H_2 (–0.414 V) as the electron donor. The high reduction potential of CoM-S—S-CoB is used to drive electrons to reduce ferredoxin ($E_0' = -0.45$ V). The Fd^{2-}_{red} is then used for CO_2 reduction in the first step of the pathway (Figure 14.47).

Methanogenesis from Methyl Compounds and Acetate

We will learn in Section 17.2 that methanogens can form CH_4 from certain methylated compounds such as methanol and acetate, as well as from $H_2 + CO_2$. Methanogens that reduce methylated compounds, such as *Methanosarcina*, typically have cytochromes, a feature that distinguishes them from methanogens that use only $H_2 + CO_2$. Methanol is catabolized by donating methyl groups to an enzyme containing a corrinoid coenzyme to form CH_3–corrinoid. Corrinoids are the parent structures of compounds such as vitamin B_{12} and contain a porphyrin-like ring with a central cobalt atom. The CH_3–corrinoid complex then transfers the methyl group to CoM, yielding CH_3–CoM (Figure 14.48*a*) from which methane is formed in the same way as in the terminal step of CO_2 reduction. If H_2 is unavailable to drive the terminal step, some of the methanol must be oxidized to CO_2 to yield electrons for this purpose. This occurs by reversal of steps in methanogenesis (Figures 14.47 and 14.48*a*).

When acetate is the substrate for methanogenesis, it is first activated to acetyl-CoA, which interacts with CO dehydrogenase from the acetyl-CoA pathway (Section 14.16 and Figure 14.42). The methyl group of acetate is then transferred to the corrinoid enzyme to yield CH_3–corrinoid, and from there it follows the CoM-mediated terminal step of methanogenesis. Simultaneously, the CO group is oxidized to yield CO_2 and electrons (Figure 14.48*b*).

Autotrophy

Autotrophy in methanogens is supported by the reductive acetyl-CoA pathway (Section 14.16). As we have just seen, parts of this pathway are already integrated into the catabolism of methanol and acetate by methanogens (Figure 14.48). However, methanogens lack the tetrahydrofolate-driven series of reactions of the acetyl-CoA pathway that lead to the production of a methyl group (Figure 14.42). But this is not a problem because methanogens either derive methyl groups directly from their electron donors (Figure 14.48) or make methyl groups during methanogenesis from $H_2 + CO_2$ (Figure 14.47). Thus methanogens have access to

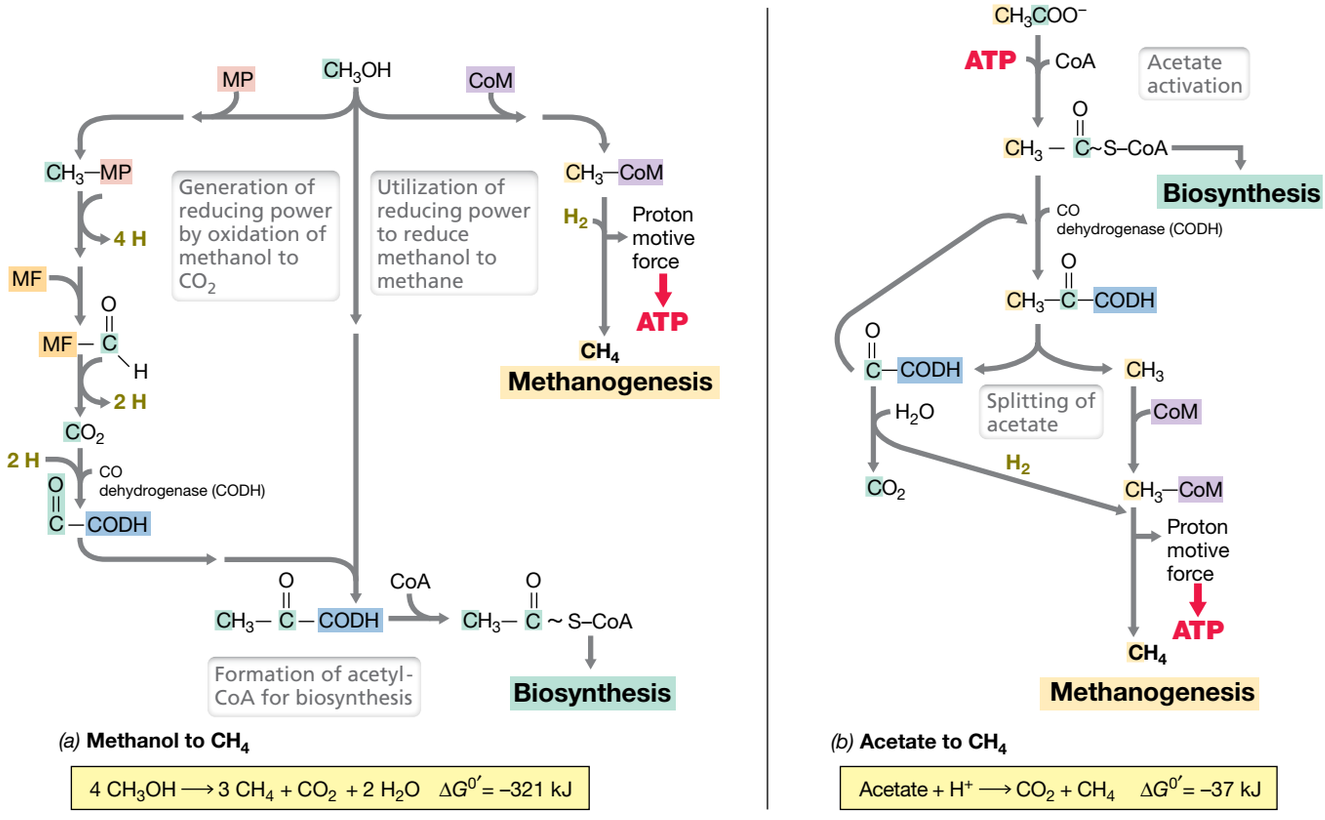


Figure 14.48 Methanogenesis from methanol and acetate. Both reaction series contain parts of the acetyl-CoA pathway. *(a)* For growth on methanol (CH_3OH), most CH_3OH carbon is converted to CH_4, and a smaller amount is converted to either CO_2 or, via formation of acetyl-CoA, is assimilated into cell material. *(b)* Acetate is split into CH_4 and CO_2. Abbreviations and color-coding are as in Figures 14.45 and 14.47; CODH, carbon monoxide dehydrogenase.

abundant methyl groups, and the removal of a portion for biosynthesis is of little consequence. The carbonyl group of the acetate produced during autotrophic growth of methanogens is derived from the activity of carbon monoxide dehydrogenase, and the terminal step in acetate synthesis is as described for acetogens (Section 14.16 and Figure 14.42).

Energy Conservation in Methanogenesis

Under standard conditions, the free energy of methanogenesis from $H_2 + CO_2$ is −131 kJ/mol. Energy conservation in methanogenesis occurs at the expense of a proton or sodium motive force, depending on the substrate used; substrate-level phosphorylation (Sections 3.8 and 14.19) does not occur. When methane is formed from $H_2 + CO_2$, ATP is produced from the sodium motive force generated during methyl transfer from MP to CoM by the enzyme methyl transferase (Figure 14.47). This energized state of the membrane then drives the synthesis of ATP, probably by way of an H^+-linked ATPase following conversion of the sodium motive force into a proton motive force by exchange of Na^+ for H^+ across the membrane. The ATP yield per CH_4 produced is about 0.5.

In some methanogens, such as *Methanosarcina*, a nutritionally versatile organism that can make methane from acetate or methanol as well as from $CO_2 + H_2$, a different mechanism of energy conservation occurs from acetate or methanol, since the methyl transferase reaction cannot be coupled to the generation of a sodium motive force under these conditions. Instead, in acetate- and methanol-grown cells energy conservation is linked to the terminal step in methanogenesis, the methyl reductase step (Figures 14.47, 14.48, and Figure 14.49). In this reaction, the interaction of CoB with CH_3–CoM and methyl reductase forms CH_4 and a heterodisulfide product, CoM-S—S-CoB. The latter is reduced by H_2 to regenerate CoM-SH and CoB-SH (Figure 14.49). This reduction, carried out by the enzyme *heterodisulfide reductase*, is exergonic and is coupled to the pumping of H^+ across the membrane (Figure 14.49). Electrons from H_2 flow to the heterodisulfide reductase through a membrane-associated electron carrier called *methanophenazine*. This compound is reduced by F_{420} and subsequently oxidized by a *b*-type cytochrome; the latter is the electron donor to the heterodisulfide reductase (Figure 14.49). Cytochromes and methanophenazine are absent in methanogens that can use only $H_2 + CO_2$ for methanogenesis, and $H_2 + CO_2$ methanogens instead regenerate CoM-SH and CoB-SH by using an electron-bifurcation reaction (Figure 14.43).

In methanogens we thus see at least two mechanisms for energy conservation: (1) a proton motive force linked to the methyl reductase reaction and used to drive ATP synthesis in acetate- or methanol-grown cells, and (2) a sodium motive force (which is likely converted to a proton motive force) during methanogenesis from $H_2 + CO_2$.

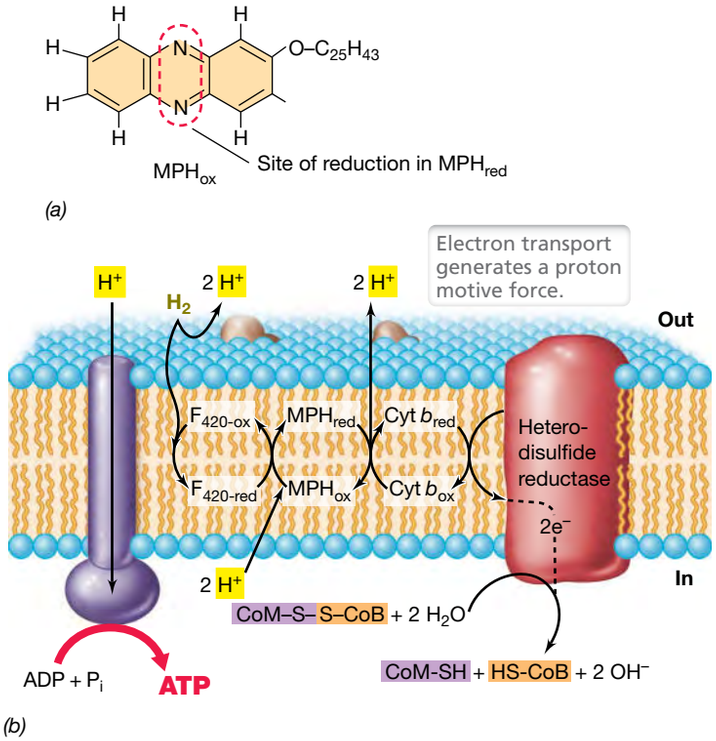


Figure 14.49 Energy conservation in methanogenesis from methanol or acetate. *(a)* Structure of methanophenazine (MPH in part *b*), an electron carrier in the electron transport chain leading to ATP synthesis; the central ring of the molecule can be alternately reduced and oxidized. *(b)* Steps in electron transport. Electrons originating from H_2 reduce F_{420} and then methanophenazine. The latter, through a cytochrome of the *b* type, reduces heterodisulfide reductase with the extrusion of H^+ to the outside of the membrane. In the final step, heterodisulfide reductase reduces CoM-S—S-CoB to HS-CoM and HS-CoB. See Figure 14.45 for the structures of CoM and CoB.

MINIQUIZ

- Which coenzymes function as C_1 carriers in methanogenesis? Which function as electron donors?
- In methanogens growing on $H_2 + CO_2$, how is carbon obtained for cell biosynthesis?
- How is ATP made in methanogenesis when the substrates are $H_2 + CO_2$? Acetate?

14.18 Methanotrophy

Methane (CH_4) and many other C_1 organic compounds can be catabolized both aerobically and anaerobically. In this section, we consider the oxidation of these compounds by **methylotrophs**, organisms that use organic compounds that lack C—C bonds as electron donors and carbon sources. The oxidations of CH_4 and methanol (CH_3OH) have been the best-studied reactions, and we focus here on the oxidation of CH_4 as an example of a methylotrophic lifestyle.

Aerobic Methane Oxidation

The steps in CH_4 oxidation to CO_2 can be summarized as

$$CH_4 \rightarrow CH_3OH \rightarrow CH_2O \rightarrow HCOO^- \rightarrow CO_2$$

Not all methylotrophs can use methane. **Methanotrophs** are those methylotrophs that can use CH_4, and methanotrophy has been especially well studied in the gram-negative bacterium *Methylococcus capsulatus*. Methanotrophs assimilate either all or one-half of their cell carbon (depending on the pathway used) from the C_1 compound formaldehyde (CH_2O).

The initial step in the *aerobic* oxidation of CH_4 is catalyzed by the enzyme *methane monooxygenase* (MMO). Monooxygenases

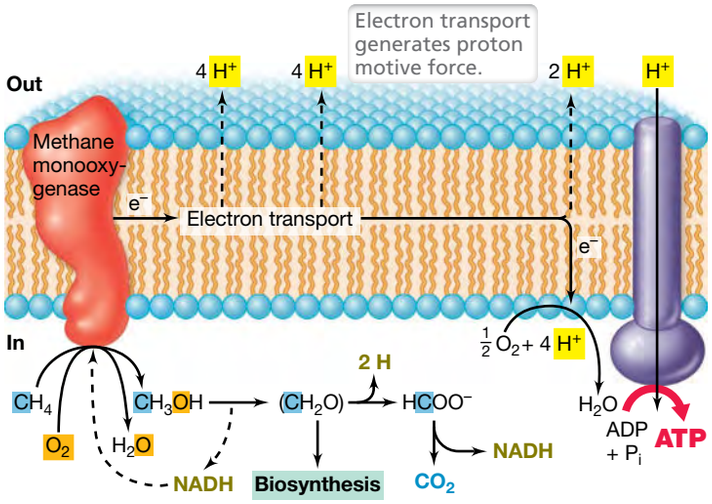
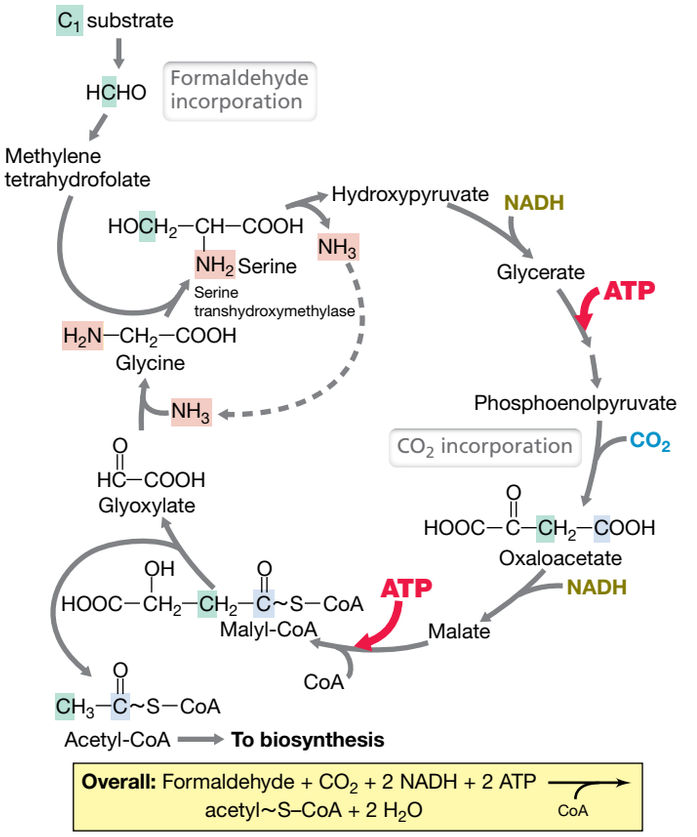


Figure 14.50 Oxidation of methane by methanotrophic bacteria. CH_4 is oxidized to CH_3OH by the membrane-integrated enzyme methane monooxygenase (MMO). A proton motive force is established from electron flow in the membrane, and this fuels ATPase. Note how carbon for biosynthesis comes from CH_2O.

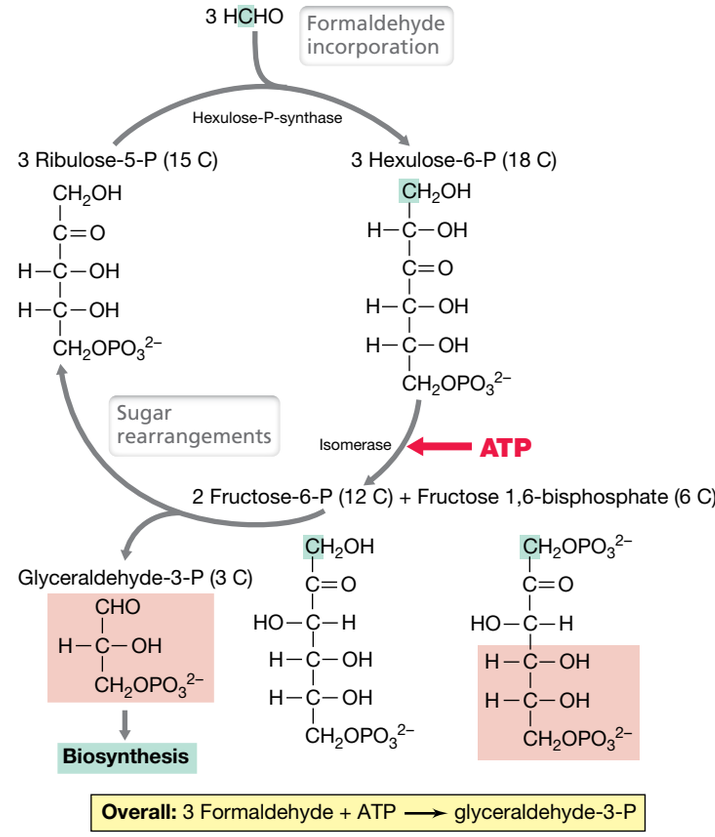
incorporate one oxygen atom from O_2 into a carbon compound (see Section 14.24 and Figure 14.64*a*). *M. capsulatus* contains two MMOs, one cytoplasmic (soluble MMO, sMMO) and the other membrane-integrated (particulate MMO, pMMO). In the MMO reaction, an atom of oxygen is introduced into CH_4, forming CH_3OH, and the second atom of O is reduced to form H_2O (Figure 14.50). CH_3OH is oxidized by an alcohol dehydrogenase, yielding formaldehyde (CH_2O) and NADH, and the CH_2O is either oxidized to CO_2 or used to make new cell material.

C_1 Assimilation by Aerobic Methanotrophs

At least two distinct pathways exist for the incorporation of C_1 units into cell material in methanotrophs. The **serine pathway** is outlined in Figure 14.51*a*. In this pathway, acetyl-CoA is synthesized from one molecule of CH_2O (produced from the oxidation of CH_3OH, Figure 14.50) and one molecule of CO_2. The serine pathway requires reducing power and energy in the form of two molecules each of NADH and ATP, respectively, for each acetyl-CoA synthesized. The serine pathway employs a number of enzymes of the citric acid cycle and one enzyme, *serine transhydroxymethylase*, unique to the pathway (Figure 14.51*a*).



(a) **The serine pathway**



(b) **The ribulose monophosphate pathway**

Figure 14.51 The serine and ribulose monophosphate pathways for the assimilation of C_1 units into cell material by methylotrophic bacteria. *(a)* Serine pathway. The product, acetyl-CoA, is used as the starting point for making new cell material. The key enzyme of the pathway is serine transhydroxymethylase. *(b)* Ribulose monophosphate pathway. Three molecules of CH_2O are required, with the product being glyceraldehyde 3-phosphate. The key enzyme of this pathway is hexulosephosphate synthase. The sugar rearrangements require enzymes of the pentose phosphate pathway (Figure 3.26).

An alternative pathway for C_1 incorporation is the **ribulose monophosphate pathway** (Figure 14.51*b*). This pathway is more energy efficient than the serine pathway because *all* of the carbon for cell material is derived from CH_2O. Because CH_2O is at the same oxidation level as cell material, no reducing power is needed for its incorporation. Hence, all of the NADH from the oxidation of methane can be oxidized in the electron transport chain.

The ribulose monophosphate pathway consumes one molecule of ATP for each molecule of glyceraldehyde 3-phosphate (G-3-P) synthesized (Figure 14.51*b*); two G-3-Ps can then be converted into glucose by reversal of the glycolytic pathway (Figure 3.14). The enzymes *hexulosephosphate synthase*, which condenses one molecule of formaldehyde with one molecule of ribulose 5-phosphate, and *hexulose 6-P isomerase* (Figure 14.51*b*) are unique to the ribulose monophosphate pathway. The remaining enzymes of this pathway are enzymes of intermediary metabolism widely distributed in bacteria.

Anaerobic Oxidation of Methane (AOM)

The *anaerobic* oxidation of methane uses a variety of enzymes and cofactors common to other forms of C_1 metabolism; however, anaerobic methanotrophy shows some novel features, as well. Methane can be oxidized anaerobically by an association (called a *consortium*) of two organisms, a sulfate-reducing bacterium (SRB) plus a species of *Archaea* phylogenetically related to methanogens. These consortia thrive in anoxic marine sediments and are responsible for oxidizing more than 90% of the methane produced there. The components of the consortium coexist in spatially structured aggregates (**Figure 14.52**). The archaeal component, called ANME (*an*aerobic *me*thanotroph), of which there are several different types, oxidizes CH_4 as an electron donor. Electrons from methane oxidation are then transferred to the sulfate reducer, which uses them to reduce SO_4^{2-} to H_2S (Figure 14.52*b*).

ANME *Archaea* oxidize CH_4 to CO_2 by reversing the steps of methanogenesis (Figure 14.47). This process is endergonic but is made possible by the SRB partner organism, which consumes electrons from ANME, thereby making the oxidation CH_4 to CO_2 energetically favorable. Remarkably, electrons are transferred between the ANME and SRB partners by *direct electron transfer* (Figure 14.52*b*). Cells of ANME make electrically conductive multiheme cytochromes that span their outer cell layer and transfer electrons from the cytoplasmic membrane to the outside of the cell. The SRB have similar large electrically conductive cytochromes as well as pili (Section 2.7) that serve as electrically conductive "nanowires." These pili can be more than 1 µm in length and electrically connect the cells within aggregates to facilitate the two microbes' metabolisms (see page 428 for more on this). The presence of direct electron transfer may explain why some ANME can also use insoluble metals such as iron (Fe^{3+}) and manganese oxides (Mn^{4+}) as terminal electron acceptors (Section 14.15) in CH_4 oxidation.

AOM is not limited to consortia of ANME and SRB. ANME *Archaea* include *Methanoperedens nitroreducens*, which uses nitrate as a terminal electron acceptor for the anaerobic oxidation of CH_4. This organism couples reverse methanogenesis to the reduction of NO_3^- to NO_2^-. *Methanoperedens nitroreducens* can be found in consortia with denitrifying bacteria that then use NO_2^- as an electron acceptor. These consortia are active in anoxic environments where CH_4 and NO_3^- coexist, such as certain freshwater sediments.

Intra-Aerobic Methanotrophy

The methanotrophic denitrifying bacterium *Methylomirabilis oxyfera* is an obligate anaerobe that catalyzes AOM linked to NO_2^- as an electron acceptor. *M. oxyfera* can grow on CH_4 in pure culture and has a highly unusual polygonal morphology (**Figure 14.53**). Analysis of the *M. oxyfera* genome reveals all of the genes required for the *aerobic* oxidation of CH_4 to CO_2. *M. oxyfera* is also able to reduce NO_2^- to N_2 and it has most of the genes required for denitrification although it lacks nitric oxide reductase and nitrous oxide reductase (Section 14.13 and Figure 14.36). This leads to the interesting question of why an *anaerobic* bacterium would use *aerobic* pathways for CH_4 oxidation.

The answer to this conundrum is that *M. oxyfera* reduces nitrite in a novel way. The organism reduces NO_2^- to nitric oxide (NO) like a normal denitrifying bacterium would, but then *M. oxyfera* does something remarkable: The organism generates O_2 through the reaction $2\ NO \rightarrow N_2 + O_2$ and proceeds to use this O_2 as the electron acceptor for CH_4 oxidation. Because *M. oxyfera* produces its own O_2, its methanotrophic metabolism has been termed *intra-aerobic methanotrophy*. As it turns out, O_2 is toxic for the anaerobic *M. oxyfera*. However, if the O_2 is consumed (by its reduction to H_2O

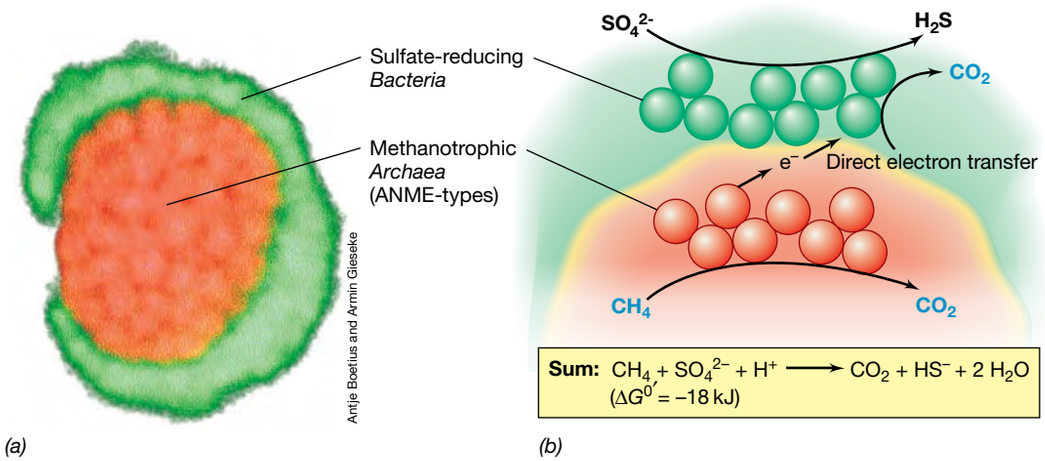
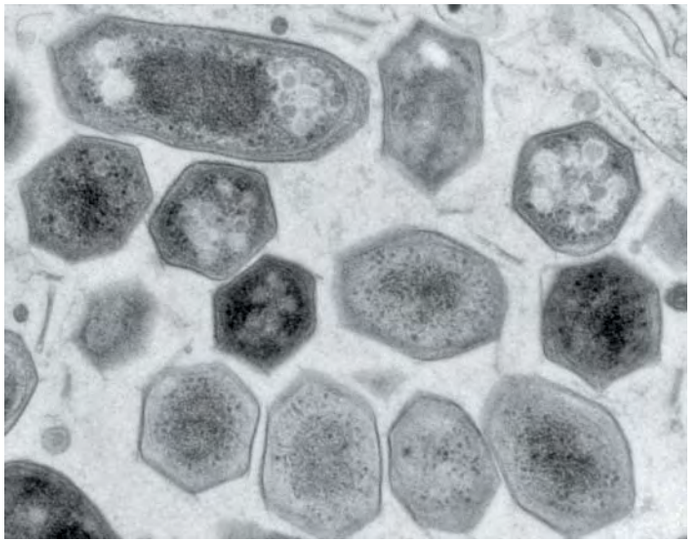


Figure 14.52 Anaerobic oxidation of methane. *(a)* Methane-oxidizing cell aggregates from marine sediments. The aggregates contain methanotrophic *Archaea* (red) surrounded by sulfate-reducing bacteria (green). Each cell type has been stained by a different FISH probe (Section 19.5). The aggregate is about 30 µm in diameter. *(b)* Mechanism for the cooperative degradation of CH_4. An organic compound or some other carrier of reducing power transfers electrons from methanotroph to sulfate reducer. ANME, anaerobic methanotroph. See pages 173 and 428 for more on the ANME consortium.



Laura Van Niftrik and Mingliang Wu

Figure 14.53 The cell morphology of *Methylomirabilis oxyfera*. The denitrifying methanotroph *M. oxyfera* has a unique polygonal morphology as revealed in this transmission electron micrograph of cells from a microbial community grown in a bioreactor. The scale bar is 0.5 μm.

using electrons from the oxidation of CH_4) as soon as it is produced, O_2 never accumulates, and the organism's environment remains anoxic.

MINIQUIZ

- When using CH_4 as electron donor, why is *Methylococcus capsulatus* an obligate aerobe?
- In which two ways does the ribulose monophosphate pathway save energy over reactions of the serine pathway?
- What is unique about methanotrophy in *Methylomirabilis oxyfera*?

VI • Fermentation

Thus far we have considered phototrophy and many types of aerobic and anaerobic respiration; these processes are unified by the fact that they all conserve energy at the expense of an ion gradient (H^+ or Na^+) that fuels oxidative phosphorylation. Here we turn our focus to *fermentations*, metabolisms in which energy conservation occurs at the expense of other reactions.

14.19 Energetic and Redox Considerations

If terminal electron acceptors, such as sulfate (SO_4^{2-}), nitrate (NO_3^-), and ferric iron (Fe^{3+}), are absent from anoxic habitats, then organic compounds are catabolized by **fermentation**. Recall from Chapter 3 that we emphasized how redox balance is achieved in fermentations by having the substrate serve as both electron donor and electron acceptor and that ATP is synthesized by *substrate-level phosphorylation*. We pick up on these two essential features of fermentation here (Figure 14.54).

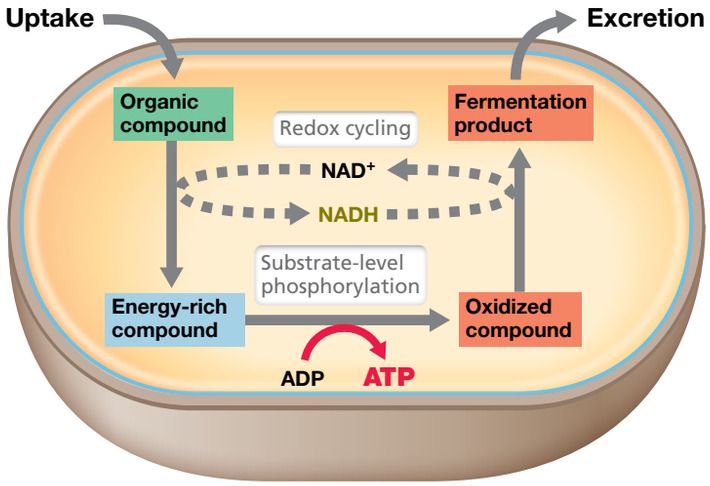


Figure 14.54 The essentials of fermentation. The fermentation product is excreted from the cell, and only a relatively small amount of the original organic compound is used for biosynthesis.

Energy-Rich Compounds and Substrate-Level Phosphorylation

Energy can be conserved by substrate-level phosphorylation from many different compounds. However, central to an understanding of substrate-level phosphorylation is the concept of *energy-rich compounds*. These are organic compounds that contain an energy-rich phosphate bond or a molecule of coenzyme A. The bond is "energy-rich" because its hydrolysis is highly exergonic. Table 14.7 lists some energy-rich compounds formed during metabolism; the hydrolysis of most of these yields sufficient free energy to be coupled to ATP synthesis ($\Delta G^{0\prime} = -31.8$ kJ/mol). If an organism can form one of these compounds during fermentative metabolism, it can make ATP by transferring the phosphate bond from the energy-rich compound to ADP to form ATP—substrate-level phosphorylation.

Redox Balance and H_2 and Acetate Production

In any fermentation there must be atomic and redox balance. That is, the total number of each type of atom and electrons in the products of the reaction must balance those in the reactants (the substrates). Redox balance is achieved in fermentations by the excretion from the cell of *fermentation products*, reduced substances such as acids or alcohols that are produced as end products of the catabolism of the original fermentable substance (Figure 14.54).

In several fermentations, redox balance is facilitated by the production of molecular hydrogen (H_2). The production of H_2 is associated with the activity of the iron–sulfur protein *ferredoxin*, a very low-potential electron carrier, and is catalyzed by the enzyme *hydrogenase*. H_2 can also be produced from the C_1 fatty acid formate (Figure 14.55). Although the H_2 can no longer be used by the fermenter and is thus excreted, H_2 is a very powerful electron donor and can be oxidized by many different *Bacteria* and *Archaea*. Indeed, with its very electronegative E_0' (making it suitable as an electron donor for any form of respiration), H_2 is never wasted in microbial ecosystems.

TABLE 14.7 Energy-rich compounds that can couple to substrate-level phosphorylation[a]

Compound	Free energy of hydrolysis, $\Delta G^{0\prime}$ (kJ/mol)[b]
Acetyl-CoA	−35.7
Propionyl-CoA	−35.6
Butyryl-CoA	−35.6
Caproyl-CoA	−35.6
Succinyl-CoA	−35.1
Acetyl phosphate	−44.8
Butyryl phosphate	−44.8
1,3-Bisphosphoglycerate	−51.9
Carbamyl phosphate	−39.3
Phosphoenolpyruvate	−51.6
Adenosine phosphosulfate (APS)	−88
N^{10}-Formyltetrahydrofolate	−23.4
Energy of hydrolysis of ATP (ATP ⟶ ADP + P_i)	−31.8

[a]Data from Thauer, R.K., K. Jungermann, and K. Decker. 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev. 41:* 100–180.
[b]The $\Delta G^{0\prime}$ values shown here are for "standard conditions," which are not necessarily those of cells. Including heat loss, the energy costs of making an ATP are more like 60 kJ than 32 kJ, and the energy of hydrolysis of the energy-rich compounds shown here is thus likely higher. But for simplicity and comparative purposes, the values in this table will be taken as the actual energy released per reaction.

Many anaerobic bacteria produce acetate or other fatty acids as a major or minor fermentation product. The production of these is energy conserving because it offers the organism the opportunity to make ATP by substrate-level phosphorylation. The key intermediate generated is the coenzyme-A derivative of each fatty acid, since these are energy-rich compounds (Table 14.7). For example, acetyl-CoA can be converted to acetyl phosphate (Figure 14.55) and the phosphate group subsequently transferred to ADP, yielding ATP. Fatty acid production is common in fermentations and if the fatty acid is metabolized through a CoA intermediate, the potential for ATP synthesis by substrate-level phosphorylation is a possibility.

With these foundational principles of fermentative bioenergetics firmly in hand, we explore the metabolic diversity of

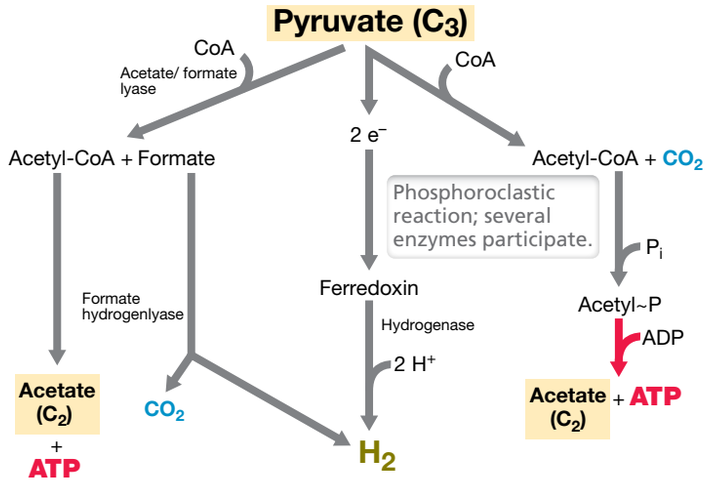


Figure 14.55 Production of H_2 and acetate from pyruvate. At least two mechanisms are known, one that produces H_2 directly and the other that makes formate as an intermediate. When acetate is produced, ATP synthesis is possible (Table 14.7).

fermentations beginning with species that produce acidic fermentation products, common and widespread bacteria in most anoxic environments.

MINIQUIZ

- Why is H_2 produced during many types of fermentation?
- Why is acetate formation in fermentation energetically beneficial to the cell?

14.20 Lactic and Mixed-Acid Fermentations

Fermentations are classified by either the substrate fermented or the products formed. Table 14.8 lists some major fermentations classified on the basis of the products formed, such as alcohol, lactic acid, propionic acid, mixed acid, butyric acid, and acetate. Other fermentations are classified by the substrate

TABLE 14.8 Common fermentations and their energetics and example organisms

Type	Reaction	Energy yield ($\Delta G^{0\prime}$, kj/mol)	Organisms
Alcoholic	Hexose ⟶ 2 ethanol + 2 CO_2	−239	Yeast, *Zymomonas*
Homolactic	Hexose ⟶ 2 lactate$^-$ + 2 H^+	−196	*Streptococcus,* some *Lactobacillus*
Heterolactic	Hexose ⟶ lactate$^-$ + ethanol + CO_2 + H^+	−216	*Leuconostoc,* some *Lactobacillus*
Propionic acid	3 Lactate$^-$ ⟶ 2 propionate$^-$ + acetate$^-$ + CO_2 + H_2O	−170	*Propionibacterium, Clostridium propionicum*
Mixed acid	Hexose ⟶ ethanol + 2,3-butanediol + succinate^{2-} + lactate$^-$ + acetate$^-$ + formate$^-$ + H_2 + CO_2	Depends on product ratio	Enteric bacteria including *Escherichia, Salmonella, Shigella, Klebsiella, Enterobacter*
Butyric acid	Hexose ⟶ butyrate$^-$ + 2 H_2 + 2 CO_2 + H^+	−264	*Clostridium butyricum*
Butanol	2 Hexose ⟶ butanol + acetone + 5 CO_2 + 4 H_2	−468	*Clostridium acetobutylicum*
Caproate/ Butyrate	6 Ethanol + 3 acetate$^-$ ⟶ 3 butyrate$^-$ + caproate$^-$ + 2 H_2 + 4 H_2O + H^+	−183	*Clostridium kluyveri*
Acetogenic	Fructose ⟶ 3 acetate$^-$ + 3 H^+	−276	*Clostridium aceticum*

fermented rather than the fermentation product; for instance, amino acid, purine/pyrimidine, or the succinate fermentation. Some anaerobes even ferment aromatic compounds and other unusual substrates (Table 14.9). Clearly, a wide variety of organic compounds can be fermented, and in a few cases, only a very restricted group of anaerobes can carry out the fermentation. Many of these are metabolic specialists, having evolved the capacity to ferment a substrate not catabolized by other bacteria (Table 14.9).

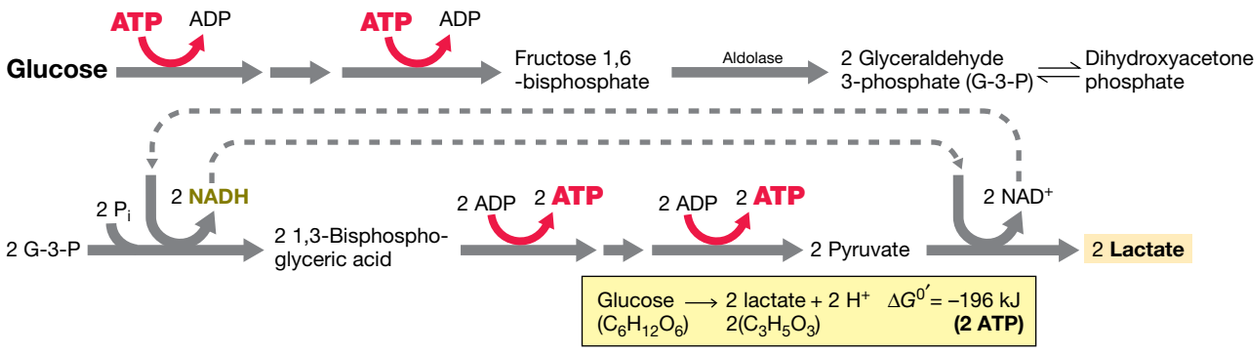
We begin with two very common fermentations of sugars in which lactic acid is the sole or major product.

TABLE 14.9 Some unusual bacterial fermentations

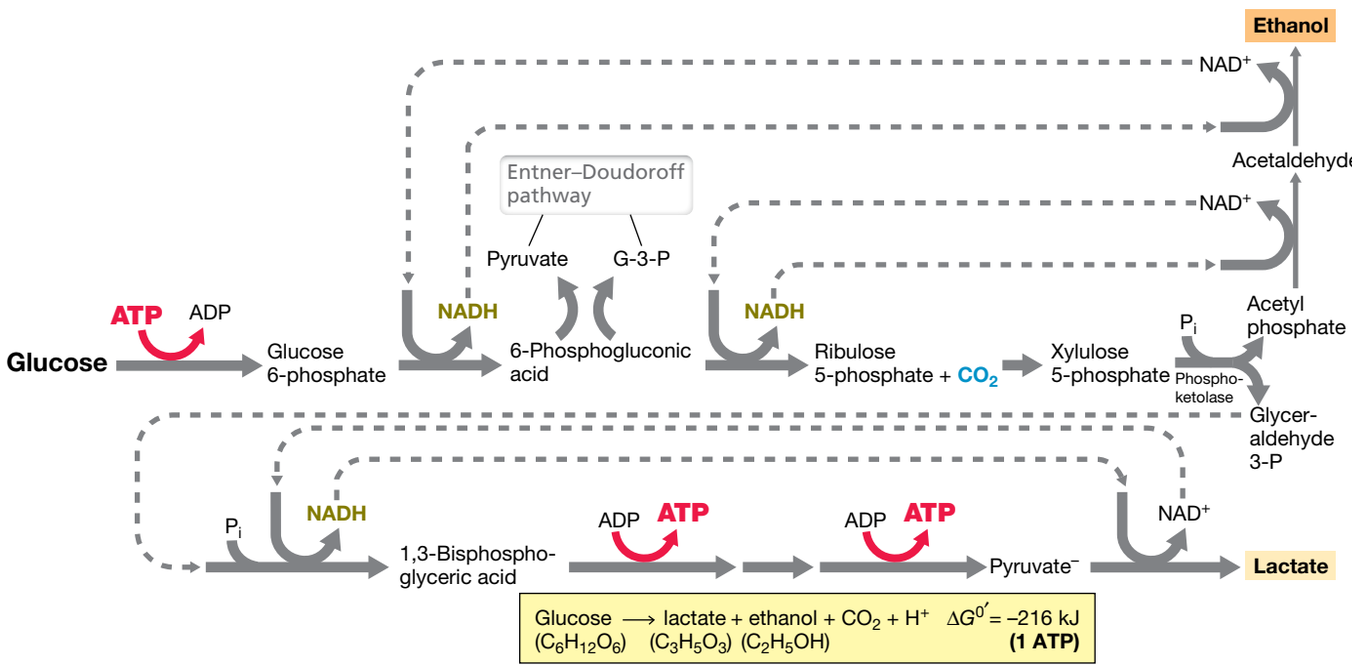
Type	Reaction	Organisms
Acetylene	$2\ C_2H_2 + 3\ H_2O \rightarrow$ ethanol + acetate$^-$ + H^+	*Pelobacter acetylenicus*
Glycerol	4 Glycerol + 2 $HCO_3^- \rightarrow$ 7 acetate$^-$ + 5 H^+ + 4 H_2O	*Acetobacterium* spp.
Phloroglucinol (aromatic)	$C_6H_6O_3 + 3\ H_2O \rightarrow 3$ acetate$^-$ + 3 H^+	*Pelobacter massiliensis* *Pelobacter acidigallici*
Putrescine	$10\ C_4H_{12}N_2 + 26\ H_2O \rightarrow$ 6 acetate$^-$ + 7 butyrate$^-$ + 20 NH_4^+ + 16 H_2 + 13 H^+	Unclassified gram-positive nonsporulating anaerobes
Citrate	Citrate^{3-} + 2 $H_2O \rightarrow$ formate$^-$ + 2 acetate$^-$ + HCO_3^- + H^+	*Bacteroides* spp.
Benzoate (aromatic)	2 Benzoate$^- \rightarrow$ cyclohexane carboxylate$^-$ + 3 acetate$^-$ + HCO_3^- + 3 H^+	*Syntrophus aciditrophicus*

Lactic Acid Fermentation

Lactic acid bacteria are gram-positive nonsporulating bacteria that produce lactic acid as a major or sole fermentation product from the fermentation of sugars (Section 16.6). Two fermentative patterns are observed. One, called **homofermentative**,



(a) **Homofermentative**



(b) **Heterofermentative**

Figure 14.56 The fermentation of glucose in *(a)* homofermentative and *(b)* heterofermentative lactic acid bacteria. Note that no ATP is made in reactions leading to ethanol formation in heterofermentative organisms.

yields a single fermentation product, lactic acid. The other, called **heterofermentative**, yields products in addition to lactate, mainly ethanol plus CO_2.

Figure 14.56 summarizes pathways for the fermentation of glucose by homofermentative and heterofermentative lactic acid bacteria. The differences observed can be traced to the presence or absence of the enzyme *aldolase*, a key enzyme of glycolysis (Figure 3.14). Homofermentative lactic acid bacteria contain aldolase and produce *two* molecules of lactate from glucose by the glycolytic pathway (Figure 14.56*a*). Heterofermenters lack aldolase and thus cannot break down fructose bisphosphate to triose phosphate. Instead, they oxidize glucose 6-phosphate to 6-phosphogluconate and then decarboxylate this to pentose phosphate. The latter compound is then converted to triose phosphate and acetyl phosphate by the key enzyme *phosphoketolase* (Figure 14.56*b*). The early steps in catabolism by heterofermentative lactic acid bacteria are those of the pentose phosphate pathway (Figure 3.26).

In heterofermenters, triose phosphate is converted to lactic acid with the production of ATP (Figure 14.56*b*). However, to achieve redox balance the acetyl phosphate produced is used as an electron acceptor and is reduced by NADH (generated during the production of pentose phosphate) to ethanol. This occurs without ATP synthesis because the energy-rich CoA bond is lost during ethanol formation. Because of this, heterofermenters produce only *one* ATP/glucose instead of the *two* ATP/glucose produced by homofermenters. In addition, because heterofermenters decarboxylate 6-phosphogluconate, they produce CO_2 as a fermentation product; homofermenters do not produce CO_2. Thus an easy way to differentiate a homofermenter from a heterofermenter is to observe for the production of CO_2 in laboratory cultures.

Entner–Doudoroff Pathway

The initial stages of glucose fermentation (Section 3.8) often rely on glycolysis or a variant of the glycolytic pathway called the *Entner–Doudoroff pathway*. In the Entner–Doudoroff pathway, glucose 6-phosphate is oxidized to 6-phosphogluconic acid and NADPH; the 6-phosphogluconic acid is dehydrated and split into pyruvate and glyceraldehyde 3-phosphate (G-3-P), a key intermediate of the glycolytic pathway. G-3-P is then catabolized as in glycolysis, generating NADH and two ATP, and used as an electron acceptor to balance redox reactions (Figure 14.56*a*).

Because pyruvate is formed directly in the Entner–Doudoroff pathway and cannot yield ATP as can G-3-P (Figure 14.56*a*), the Entner–Doudoroff pathway yields only half the ATP of the glycolytic pathway. Organisms using the Entner–Doudoroff pathway therefore share this physiological characteristic with heterofermentative lactic acid bacteria (Figure 14.56*b*). *Zymomonas*, an obligately fermentative gram-negative bacterium, and *Pseudomonas*, a strictly respiratory bacterium (Section 16.4), are major genera that employ the Entner–Doudoroff pathway for glucose catabolism.

Mixed-Acid Fermentations

In *mixed-acid fermentations* (Table 14.8), characteristic of enteric bacteria (Section 16.3), three different acids—*acetic, lactic*, and *succinic*—are formed from the fermentation of glucose or other sugars that can be converted into glucose. Ethanol, CO_2, and H_2 are also typically formed as fermentation products. Glycolysis is the pathway used by mixed-acid fermenters, such as *Escherichia coli*, and we detailed the steps in that pathway in Figure 3.14.

Some enteric bacteria produce acidic products in lower amounts than *E. coli* and balance redox in their fermentations by producing larger amounts of neutral products. One key neutral product is the four-carbon alcohol *butanediol*. In this variation of the mixed-acid fermentation, the main products observed are butanediol, ethanol, CO_2, and H_2 (Figure 14.57). In the mixed-acid fermentation of *E. coli*, equal amounts of CO_2 and H_2 are produced, whereas in a

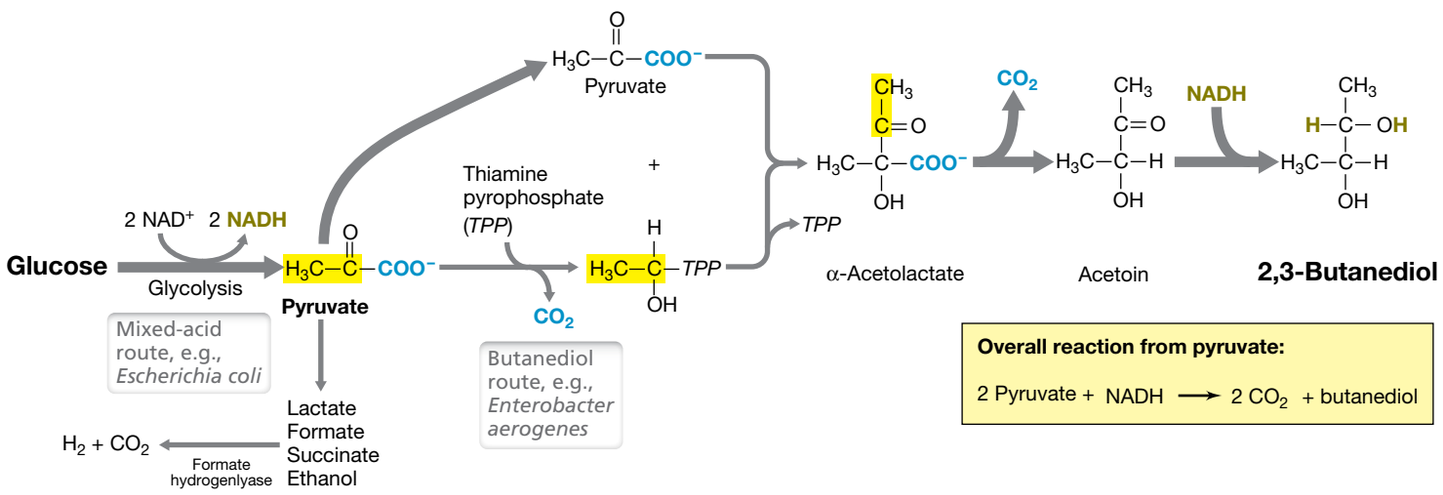


Figure 14.57 Butanediol production and mixed-acid fermentations. Note how only one NADH but two molecules of pyruvate are used to make one butanediol. This leads to redox imbalance and the production of more ethanol by butanediol producers than by mixed-acid fermenters.

UNIT 4

butanediol fermentation, considerably more CO_2 than H_2 is produced. This is because mixed-acid fermenters produce CO_2 only from formic acid by means of the enzyme *formate hydrogenlyase* (Figure 14.55):

$$HCOOH \rightarrow H_2 + CO_2$$

By contrast, butanediol producers, such as *Enterobacter aerogenes*, produce CO_2 and H_2 from formic acid but also produce two additional molecules of CO_2 during the formation of each molecule of butanediol. However, because butanediol production consumes only one-half of the NADH generated in glycolysis, more ethanol is produced by these organisms than by non-butanediol fermenters in order to achieve redox balance (Figure 14.57).

MINIQUIZ

- How can homo- and heterofermentative metabolism be differentiated in pure cultures of lactic acid bacteria?
- Butanediol production leads to greater ethanol production than in the mixed-acid fermentation of *Escherichia coli*. Why?

14.21 Clostridial and Propionate Fermentations

Species of the genus *Clostridium* are obligately fermentative anaerobes (Section 16.7). Different clostridia ferment sugars, amino acids, purines and pyrimidines, and a few other compounds. In all cases ATP synthesis is linked to substrate-level phosphorylations either in the glycolytic pathway or from the hydrolysis of a CoA intermediate (Table 14.7). We begin with sugar-fermenting (called *saccharolytic*) clostridia.

Sugar Fermentation by *Clostridium* Species

A number of clostridia ferment sugars, producing *butyric acid* as a major fermentation product. Some species also produce the neutral products acetone and butanol; *Clostridium acetobutylicum* is a classic example of this pattern. The biochemical steps in the formation of butyric acid and neutral products from sugars are shown in Figure 14.58.

In saccharolytic clostridia, glucose is converted to pyruvate and NADH via the glycolytic pathway, and pyruvate is split to yield acetyl-CoA, CO_2, and H_2 (through ferredoxin) by the phosphoroclastic reaction (Figure 14.55). Most of the acetyl-CoA is then reduced to butyrate or other fermentation products using NADH derived from glycolytic reactions as electron donor. The actual products observed are influenced by the duration and the conditions of the fermentation. During the early stages of the butyric fermentation, butyrate and a small amount of acetate and ethanol are produced. But as the pH of the medium drops, acid production decreases and acetone and butanol begin to appear. If the pH of the medium is kept neutral by buffering, there is very little formation of acetone and butanol; instead, butyric acid production continues, and this is for a good reason.

When *C. acetobutylicum* synthesizes butyrate, extra ATP is produced (Figure 14.58) and so the organism will continue to make butyrate unless conditions become overly acidic. However,

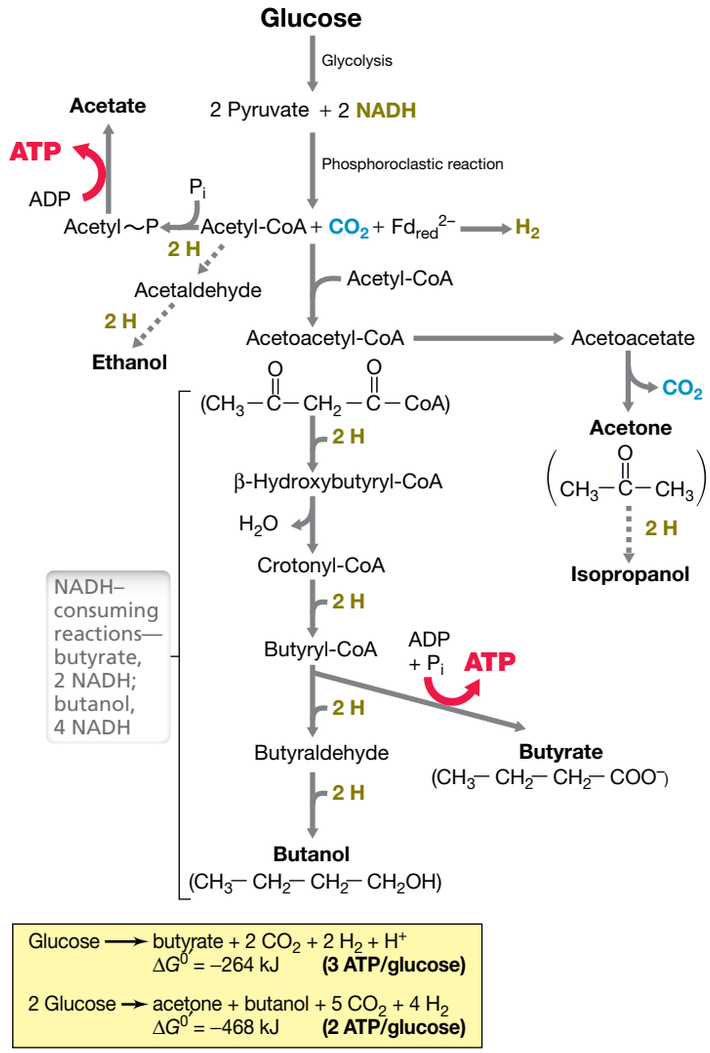


Figure 14.58 The butyric acid and butanol/acetone fermentation. All fermentation products from glucose are shown in bold (dashed lines indicate minor products). Note how the production of acetate and butyrate lead to additional ATP by substrate-level phosphorylation. By contrast, formation of butanol and acetone reduces the ATP yield because the butyryl-CoA to butyrate step is bypassed. 2 H, NADH; Fd_{red}^{2-}, reduced ferredoxin.

C. acetobutylicum is acid-sensitive, and if the pH drops below about pH 5, genes encoding enzymes that make neutral products are derepressed and the fermentation shifts to acetone/butanol production. Interestingly, the production of butanol is a consequence of the production of acetone. For each acetone that is made, two NADH produced during glycolysis are not reoxidized as they would be if butyrate were produced. To achieve redox balance, the cell then uses butyrate as an electron acceptor with butanol being the final fermentation product (Figure 14.58). Previously excreted butyrate can also be reincorporated by the cell and reduced to butanol and then excreted again. Although neutral product formation helps *C. acetobutylicum* keep its environment from becoming too acidic, there is an energetic price to pay for this. In producing butanol, the cell loses the opportunity to convert butyryl-CoA to butyrate and gain ATP (Figure 14.58).

Amino Acid Fermentation by *Clostridium* Species and the Stickland Reaction

Some *Clostridium* species ferment amino acids. These are the *proteolytic* clostridia, organisms that degrade proteins released from dead organisms. Some of these, such as the animal pathogen *Clostridium tetani* (tetanus), are strictly proteolytic, while other species are both saccharolytic and proteolytic.

Depending on the species, some proteolytic clostridia ferment individual amino acids, typically glutamate, glycine, alanine, cysteine, histidine, serine, or threonine. The biochemistry behind these fermentations is quite complex, but the metabolic strategy is simple. In virtually all cases, the amino acids are catabolized in such a way as to eventually yield a fatty acid–CoA derivative, typically acetyl (C_2), butyryl (C_4), or caproyl (C_6). From these, ATP is produced by substrate-level phosphorylation (Table 14.7). Other typical products of amino acid fermentation include ammonia (NH_3) and CO_2.

Some clostridia ferment only an amino acid *pair*. In this situation one amino acid functions as the electron donor and is oxidized, whereas the other amino acid is the electron acceptor and is reduced. This *coupled* amino acid fermentation is called a **Stickland reaction**, named for the scientist who discovered it. For example, *Clostridium sporogenes* ferments glycine and alanine, and in this reaction, alanine is the electron donor and glycine is the electron acceptor (**Figure 14.59**). The products of the Stickland reaction are invariably NH_3, CO_2, and a carboxylic acid with one fewer carbons than the amino acid that was oxidized (Figure 14.59).

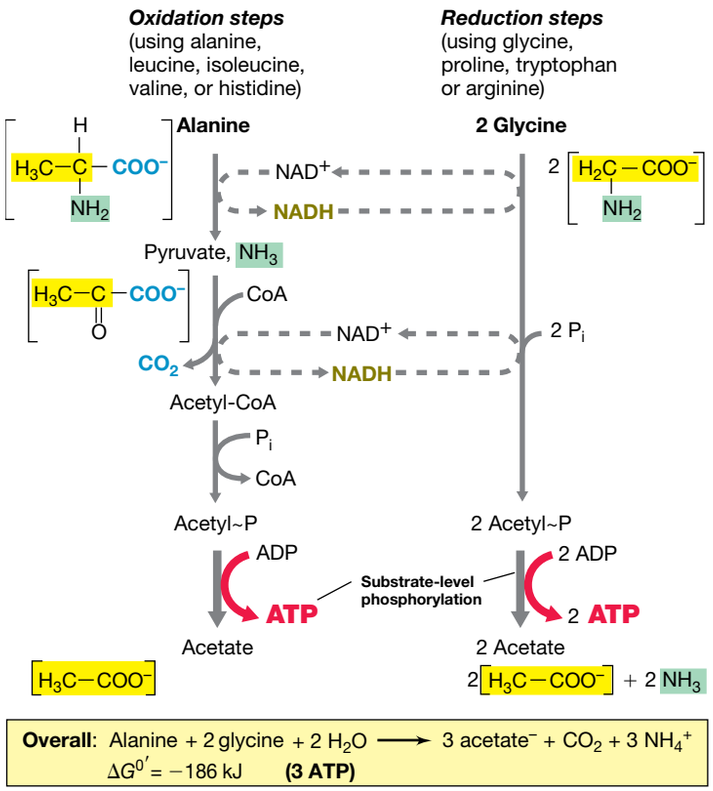


Figure 14.59 The Stickland reaction. This example shows the co-catabolism of the amino acids alanine and glycine. The structures of key substrates, intermediates, and products are shown in brackets to allow the chemistry of the reaction to be followed. Note how in the reaction shown, alanine is the electron donor and glycine is the electron acceptor.

Many of the products of amino acid fermentation by clostridia are foul-smelling substances, and the odor that results from putrefaction is mainly a result of clostridial activity. In addition to fatty acids, other odoriferous compounds produced include hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH, derived from sulfur-containing amino acids), cadaverine (from lysine), putrescine (from ornithine, see Table 14.9), and NH_3. Purines and pyrimidines, released from the degradation of nucleic acids, lead to many of the same fermentation products and yield ATP by substrate-level phosphorylation from the hydrolysis of fatty acid–CoA derivatives (Table 14.7) produced in their respective fermentative pathways.

Clostridium kluyveri Fermentation

Another species of *Clostridium* also ferments a mixture of substrates in which one is the donor and one is the acceptor, as in the Stickland reaction. However, this organism, *C. kluyveri*, ferments not amino acids but instead *ethanol plus acetate*. In this fermentation, ethanol is the electron donor and acetate is the electron acceptor. The overall reaction is shown in Table 14.8.

The ATP yield in the caproate/butyrate fermentation is low, 1 ATP/6 ethanol fermented. However, *C. kluyveri* has a selective advantage over all other fermenters in its apparently unique ability to oxidize a highly reduced fermentation product of other anaerobes (ethanol) and couple it to the reduction of another common fermentation product (acetate), reducing the latter to longer-chain fatty acids, reactions that consume NADH. The single ATP produced per 6 ethanol oxidized comes from substrate-level phosphorylation during conversion of a fatty acid–CoA derivative formed during the fermentation. The fermentation of *C. kluyveri* is an example of a **secondary fermentation**, which can be viewed as a fermentation of fermentation products. We see another example of this next.

Propionic Acid Fermentation

The gram-positive bacterium *Propionibacterium* and some related bacteria produce *propionic acid* as a major fermentation product from either glucose or lactate. Lactate, a fermentation product of the lactic acid bacteria (Section 14.20), is probably the major substrate for propionic acid bacteria in nature, where these two groups live in close association. *Propionibacterium* is an important agent in the ripening of Emmental (Swiss) cheese, which gets its unique bitter and nutty taste from the propionic and acetic acids produced, and the CO_2 produced during the fermentation forms bubbles that leave the characteristic holes (eyes) in the cheese.

Figure 14.60 shows the reactions leading from lactate to propionate. When glucose is the starting substrate, it is first catabolized to pyruvate by the glycolytic pathway. Then pyruvate, produced either from glucose or from the oxidation of lactate, is converted to acetate plus CO_2 or carboxylated to form methylmalonyl-CoA; the latter is converted into oxaloacetate and, eventually, propionyl-CoA (Figure 14.60). Propionyl-CoA reacts with succinate in a step catalyzed by the enzyme CoA transferase, producing succinyl-CoA and propionate. This results in a lost opportunity for ATP production from propionyl-CoA (Table 14.7) but avoids the energetic costs of having to activate succinate with ATP to form succinyl-CoA. The succinyl-CoA is

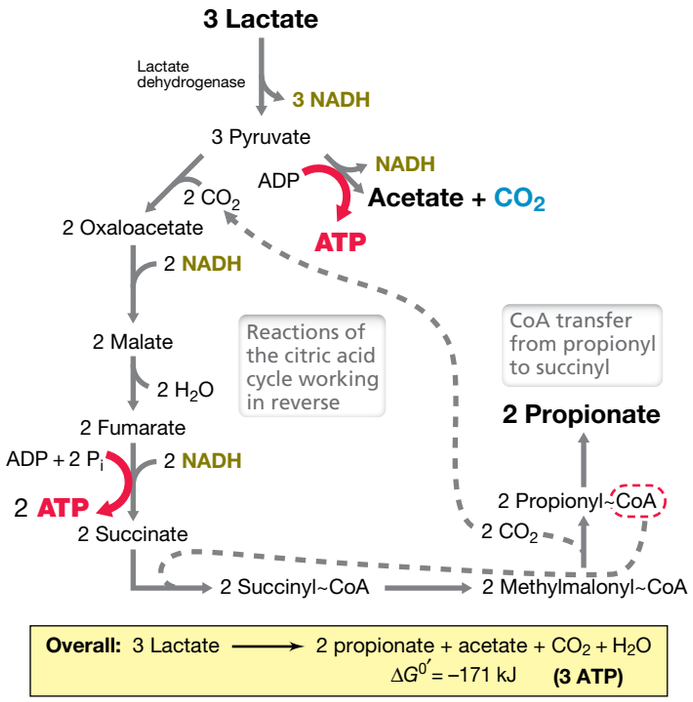


Figure 14.60 The propionic acid fermentation of *Propionibacterium*. Products are shown in bold. The four NADH made from the oxidation of three lactate are reoxidized in the reduction of oxaloacetate and fumarate, and the CoA group from propionyl-CoA is exchanged with succinate during the formation of propionate.

then isomerized to methylmalonyl-CoA and the cycle is complete; propionate is formed and CO_2 regenerated (Figure 14.60).

NADH is oxidized in the steps between oxaloacetate and succinate. The reduction of fumarate to succinate (Figure 14.60) is linked to electron transport reactions and the formation of a proton motive force; this yields one ATP by oxidative phosphorylation. The propionate pathway also converts some lactate to acetate plus CO_2, which allows for additional ATP to be made by substrate-level phosphorylation (Figure 14.60). Thus, in the propionate fermentation, both substrate-level *and* oxidative phosphorylation occur.

Propionate is also formed in the fermentation of succinate by the bacterium *Propionigenium*, but by a completely different mechanism than that described here for *Propionibacterium*. *Propionigenium*, to be considered next, is phylogenetically and ecologically unrelated to *Propionibacterium*, but aspects of its energy metabolism are of considerable interest from the standpoint of metabolic diversity and the energetic limits to life.

MINIQUIZ

- Compare the mechanisms for energy conservation in *Clostridium acetobutylicum* and *Propionibacterium*.
- What type of substrates are fermented by saccharolytic clostridia? By proteolytic clostridia?
- What are the substrates for the *Clostridium kluyveri* fermentation? In nature, where do these come from?

14.22 Fermentations That Lack Substrate-Level Phosphorylation

Certain fermentations yield insufficient energy to synthesize ATP by substrate-level phosphorylation (that is, less than –32 kJ, Table 14.7), yet still support anaerobic growth without added electron acceptors. In these cases, catabolism of the compound is linked to ion pumps that establish an ion gradient across the cytoplasmic membrane. Examples of these include the fermentation of succinate by *Propionigenium modestum* and the fermentation of oxalate by *Oxalobacter formigenes*.

Propionigenium modestum

Propionigenium modestum was first isolated in anoxic enrichment cultures lacking electron acceptors and fed succinate as an electron donor. *Propionigenium* inhabits marine and freshwater sediments and can also be isolated from the human oral cavity. The organism is a gram-negative short rod and, phylogenetically, is a species of *Fusobacteria*. During studies of the physiology of *P. modestum*, it was shown to require sodium chloride (NaCl) for growth and to catabolize succinate under strictly anoxic conditions:

$$\text{Succinate}^{2-} + H_2O \rightarrow \text{propionate}^{-} + HCO_3^{-} \qquad \Delta G^{0\prime} = -20.5\ \text{kJ}$$

This decarboxylation releases insufficient free energy to support ATP synthesis by substrate-level phosphorylation (Table 14.7) but sufficient free energy to pump a sodium ion (Na^+) from the cytoplasm to the periplasm across the cytoplasmic membrane. Energy conservation in *Propionigenium* is then linked to the resulting *sodium motive force*; a sodium-translocating (instead of proton-translocating) ATPase exists in the membrane of this organism that uses the sodium motive force to drive ATP synthesis (**Figure 14.61*a***).

In a related decarboxylation reaction, the bacterium *Malonomonas* decarboxylates the C_3 dicarboxylic acid malonate, forming acetate plus CO_2. As for *Propionigenium*, energy metabolism in *Malonomonas* is linked to Na^+ and a sodium-driven ATPase. But the free energy available from malonate fermentation by *Malonomonas* (–17.4 kJ) is even less than that of succinate fermentation by *P. modestum*. *Sporomusa*, an endospore-forming bacterium and also an acetogen (Section 14.16), is also capable of fermenting malonate, as are a few other *Bacteria*.

Oxalobacter formigenes

Oxalobacter formigenes is a bacterium present in the intestinal tract of animals, including humans. It catabolizes the C_2 dicarboxylic acid oxalate, producing formate plus CO_2. Oxalate degradation by *O. formigenes* is thought to be important in the human colon for preventing the accumulation of oxalate, a substance that can form calcium oxalate kidney stones. *O. formigenes* is a gram-negative strict anaerobe that carries out the following reaction:

$$\text{Oxalate}^{2-} + H_2O \rightarrow \text{formate}^{-} + HCO_3^{-} \qquad \Delta G^{0\prime} = -26.7\ \text{kJ}$$

As in the catabolism of succinate by *P. modestum*, insufficient energy is available from this reaction to drive ATP synthesis by substrate-level phosphorylation (Table 14.7). However, the reaction supports growth of the organism because the decarboxylation

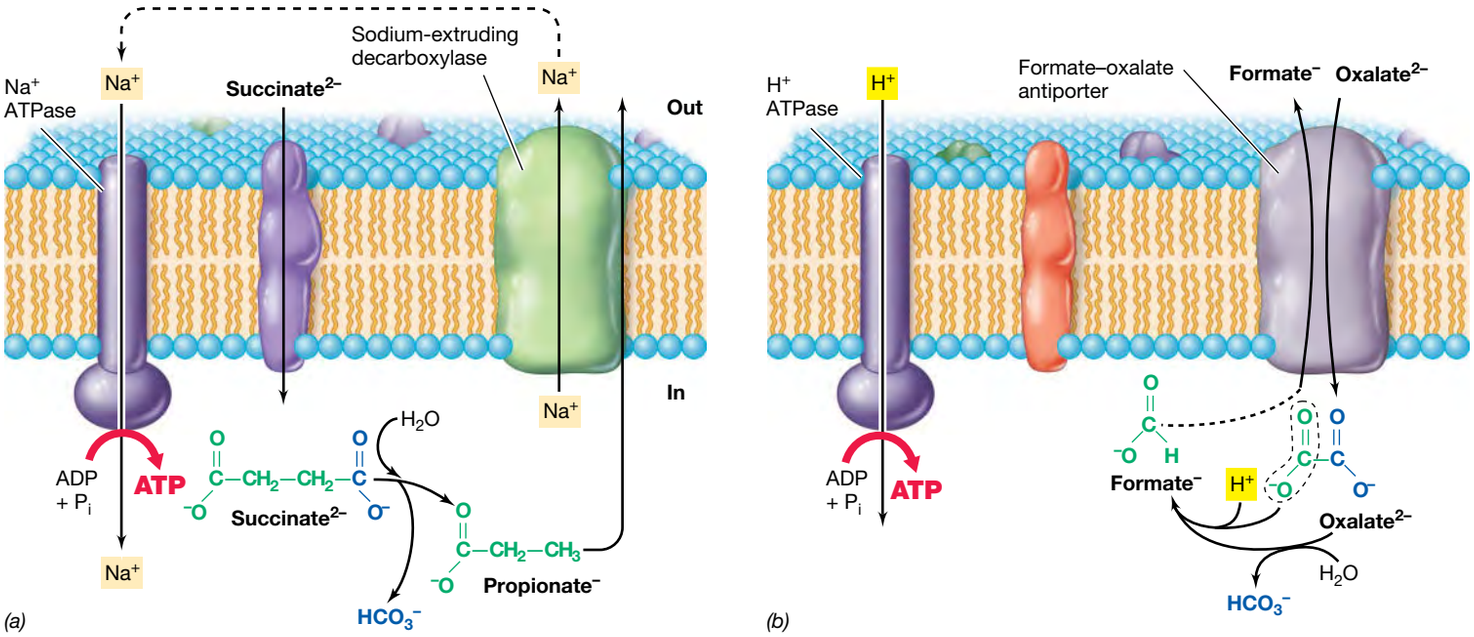


Figure 14.61 The unique fermentations of succinate and oxalate. *(a)* Succinate fermentation by *Propionigenium modestum*. Sodium export is linked to the energy released by succinate decarboxylation, and a sodium-translocating ATPase produces ATP. *(b)* Oxalate fermentation by *Oxalobacter formigenes*. Oxalate import and formate export by a formate–oxalate antiporter (Figure 3.3) consume cytoplasmic protons. ATP synthesis is linked to a proton-driven ATPase. All substrates and products are shown in bold.

of oxalate is exergonic and forms formate, which is excreted from the cell. This is because the internal consumption of protons during the oxidation of oxalate and production of formate is, in effect, a proton pump; a divalent molecule (oxalate) enters the cell while a univalent molecule (formate) is excreted. The continued exchange of oxalate for formate establishes a proton motive force that is coupled to ATP synthesis by the proton-translocating ATPase in the membrane (Figure 14.61*b*).

What Can Be Learned from the Decarboxylations of Succinate and Oxalate?

The unique aspect of decarboxylation-type fermentations is that ATP is made without substrate-level phosphorylation *or* oxidative phosphorylation fueled by electron transport. Instead, ATP synthesis is driven by ion pumps coupled to the small amount of energy released from the decarboxylation reaction. Organisms such as *Propionigenium*, *Malonomonas*, or *Oxalobacter* thus offer an important lesson in microbial bioenergetics: ATP synthesis from reactions that yield less than −32 kJ is still possible if the reaction can be coupled to an ion pump.

At a minimum, then, an energy-conserving reaction must yield sufficient free energy to pump at least one ion. This energy requirement is estimated to be near −12 kJ. Reactions that release less free energy than this should not be able to drive ion pumps and should therefore not be potential energy-conserving reactions. However, as we will see in the next section, bacteria are known that push this theoretical limit even lower, and their energetics, consequently, are still incompletely understood. These are the syntrophs, bacteria living on the energetic margin of existence.

MINIQUIZ

- Why does *Propionigenium modestum* require sodium for growth?
- Of what benefit is the organism *Oxalobacter* to human health?
- How can a fermentation that yields insufficient free energy to make an ATP still support growth?

14.23 Syntrophy

There are many examples in microbiology of **syntrophy**, a situation in which two different microbes cooperate to degrade a substance that neither can degrade alone. Most syntrophic reactions are secondary fermentations in which organisms ferment the fermentation products of other anaerobes. We will see in Chapter 21 how syntrophy is often a key step in the anoxic catabolism that leads to the production of methane in nature. Here we consider the microbiology and energetic aspects of syntrophy.

H_2 Consumption in Syntrophy: The Metabolic Link

Table 14.10 lists some major groups of syntrophs and the compounds they degrade. Many organic compounds can be degraded syntrophically, including even aromatic and aliphatic hydrocarbons. But the major compounds of interest in syntrophic environments are fatty acids and alcohols. The heart of syntrophic reactions is *interspecies H_2 transfer*—H_2 *production* by one partner, the syntroph, linked to H_2 *consumption* by the other. The H_2 consumer can be any one of a number of physiologically distinct organisms: denitrifying bacteria, ferric iron–reducing bacteria, sulfate-reducing bacteria, acetogens, or methanogens, groups we have already considered.

TABLE 14.10 Properties of major syntrophic bacteria[a]

Genus	*Number of known species*	*Phylogeny*[b]	*Substrates fermented in coculture*[c]
Syntrophobacter	4	*Deltaproteobacteria*	Propionate (C_3), lactate; some alcohols
Syntrophomonas	8	*Firmicutes*	C_4–C_{18} saturated/unsaturated fatty acids; some alcohols
Pelotomaculum	5	*Firmicutes*	Propionate, lactate, several alcohols; some aromatic compounds
Syntrophus	3	*Deltaproteobacteria*	Benzoate and several related aromatic compounds; some fatty acids and alcohols

[a]All syntrophs are obligate anaerobes.
[b]See Chapters 15 and 16.
[c]Not all species can use all substrates listed.

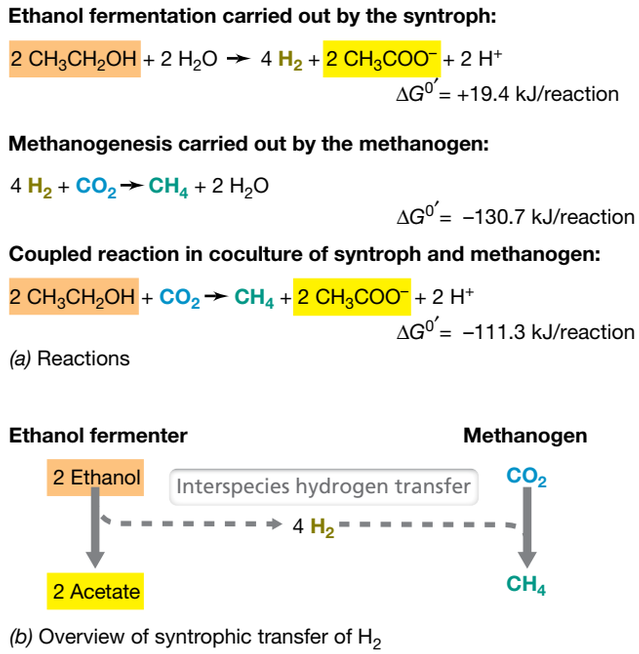


Figure 14.62 Syntrophy: Interspecies H_2 transfer. Shown is the fermentation of ethanol to methane and acetate by syntrophic association of an ethanol-oxidizing syntroph and a H_2-consuming partner (in this case, a methanogen). *(a)* Reactions involved. The two organisms share the energy released in the coupled reaction. *(b)* Overview of the syntrophic transfer of H_2.

As an example of syntrophy, consider the fermentation of ethanol to acetate plus H_2 by the syntroph *Pelotomaculum* coupled to the production of methane (**Figure 14.62**). As can be seen, the syntroph carries out a reaction whose standard free-energy change ($\Delta G^{0\prime}$) is positive. Hence, in pure culture, the organism will not grow. However, the H_2 produced by *Pelotomaculum* can be used as an electron donor by a methanogen to produce methane, an exergonic reaction. When the two reactions are summed, the overall reaction is exergonic (Figure 14.62), and when *Pelotomaculum* and a methanogen are cultured together (cocultured), both organisms grow luxuriously.

A second example of syntrophy is the oxidation of a fatty acid such as butyrate to acetate plus H_2 by the fatty acid–oxidizing bacterium *Syntrophomonas* (**Figure 14.63*a***):

$$\text{Butyrate}^- + 2\,H_2O \rightarrow 2\,\text{acetate}^- + H^+ + 2\,H_2 \qquad \Delta G^{0\prime} = +48.2\,\text{kJ}$$

The free-energy change of this reaction is even more unfavorable than that of ethanol oxidation (Figure 14.63*a*), and in pure culture *Syntrophomonas* will obviously not grow on butyrate. However, as with ethanol fermentation by *Pelotomaculum*, if the H_2 produced by *Syntrophomonas* is consumed by a partner organism, *Syntrophomonas* will grow on butyrate in coculture with the H_2-consuming partner. How does this occur?

Energetics of H_2 Transfer

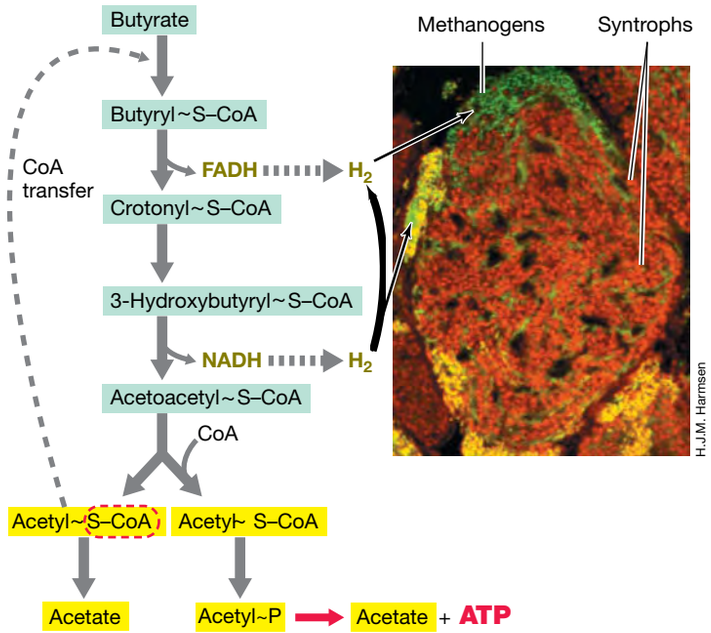
In a syntrophic relationship, the removal of H_2 by a partner organism shifts the equilibrium of the entire reaction and pulls it in the direction of product formation; this can greatly affect the energetics of the reaction. Recall from our consideration of the principles of free energy (↔ Section 3.4) that the concentration of reactants and products in a reaction can have a major effect on the reaction's energetics. This is usually not the case for most fermentation products because they are not consumed to extremely low levels. H_2, by contrast, is an exception and can be consumed to levels near zero; at these tiny H_2 concentrations, the energetics of a reaction whose product is H_2 is dramatically affected.

For convenience, the $\Delta G^{0\prime}$ of a reaction is calculated on the basis of *standard conditions*—one molar concentration of products and reactants (↔ Section 3.4). By contrast, the related term ΔG is calculated on the basis of the *actual concentrations* of products and reactants present. At near-zero levels of H_2, the energetics of the oxidation of ethanol or fatty acids to acetate plus H_2, reactions that are endergonic under standard conditions, become exergonic. For example, if the concentration of H_2 is kept extremely low from consumption by the partner organism, ΔG for the oxidation of butyrate by *Syntrophomonas* yields −18 kJ (Figure 14.63*a*).

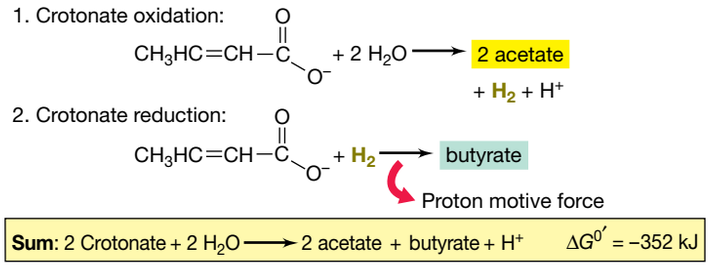
While H_2 transfer is characteristic of many syntrophic associations, particularly those associated with fermentation, it is also possible to have syntrophic associations in which *only electrons* are transferred. In direct electron transfer, such as the anoxic consumption of CH_4 by the ANME–sulfate reducer consortium (Section 14.18 and Figure 14.52), we learned that some respiratory syntrophs can use electrically conductive proteins (large multiheme cytochromes and "nanowires") to transfer electrons directly between cells separated by significant distances. In such syntrophic reactions, direct electron transfer does not depend on diffusion rates or H_2 concentrations (see also page 428).

Energetics in Syntrophs

Energy conservation in syntrophs is grounded in both substrate-level and oxidative phosphorylations. From biochemical studies



(a) **Syntrophic culture**



(b) **Pure culture**

Figure 14.63 Energetics of growth of *Syntrophomonas* in syntrophic culture and in pure culture. *(a)* In syntrophic culture, growth requires a H_2-consuming organism, such as a methanogen. H_2 production is driven by reverse electron flow because the E_0' values of the FADH and NADH couples are more electropositive than that of 2 H^+/H_2. *(b)* In pure culture, energy conservation is linked to anaerobic respiration with crotonate reduction to butyrate. Inset: photomicrograph of cells of a fatty acid–degrading syntrophic bacterium (red) in association with a methanogen (green-yellow).

of syntrophic butyrate catabolism, substrate-level phosphorylation has been shown to occur during the conversion of acetyl-CoA to acetate (Figure 14.63*a*) although the −18 kJ of energy released (ΔG) is in theory insufficient for this. However, the energy released is sufficient to produce *a fraction* of an ATP, so it is possible that in some way *Syntrophomonas* can couple two or more rounds of butyrate oxidation to the synthesis of one ATP by substrate-level phosphorylation.

Besides a syntrophic lifestyle, many syntrophs can also carry out anaerobic respirations in pure culture by the disproportionation of unsaturated fatty acids (disproportionation is a process in which one molecule of a substrate is oxidized while another is reduced). For example, crotonate, an intermediate in syntrophic butyrate metabolism (Figure 14.63*a*), supports growth of pure cultures of *Syntrophomonas*. Under these conditions some of the crotonate is oxidized to acetate and some is reduced to butyrate (Figure 14.63*b*). Because crotonate reduction by *Syntrophomonas* is coupled to the formation of a proton motive force, as occurs in other anaerobic respirations that employ organic electron acceptors (such as fumarate reduction to succinate, Section 14.15), it is possible that some step(s) in syntrophic metabolism generate a proton motive force as well. Pumping protons or some other ion would almost certainly be required for benzoate- and propionate-fermenting syntrophs, whose free energy yield (ΔG) is vanishingly low, only about −5 kJ per reaction.

Regardless of how ATP is made during syntrophic growth, an additional energetic problem burdens syntrophs. During syntrophic metabolism, syntrophs produce H_2 ($E_0' = -0.42$ V) from more electropositive electron donors such as FADH ($E_0' = -0.22$ V) and NADH ($E_0' = -0.32$ V), generated during fatty acid oxidation reactions (Figure 14.63*a*); it is unlikely that this occurs without an energy input. Thus, some fraction of the meager ATP generated by *Syntrophomonas* during syntrophic growth is probably consumed to drive reverse electron flow reactions (Section 14.3) to produce H_2. Combining this energy drain with the inherently poor energetic yields of syntrophic reactions, it should be obvious that syntrophic bacteria thrive on a very marginal energy economy.

Ecology of Syntrophs

Ecologically, syntrophic bacteria are key links in the anoxic steps of the carbon cycle (Section 21.1). Syntrophs consume highly reduced fermentation products and release a key product, H_2, for anaerobic respirations. Without syntrophs, a bottleneck would develop in anoxic environments in which electron acceptors (other than CO_2) were limiting. By contrast, when conditions are oxic or alternative electron acceptors are abundant, syntrophic relationships are unnecessary. For example, if O_2 or NO_3^- is available as an electron acceptor, the energetics of the respiration of a fatty acid or an alcohol is so favorable that syntrophic relationships are unnecessary. Thus, syntrophy is characteristic of anoxic catabolism in which primarily methanogenesis or acetogenesis are the terminal processes in the ecosystem. Methanogenesis is a major process in anoxic wastewater biodegradation, and microbiological studies of sludge granules that form in such systems have shown the close physical relationship that develops between H_2 producer and H_2 consumer in such habitats (Figure 14.63*a* inset).

MINIQUIZ

- Give an example of interspecies H_2 transfer. Why can it be said that both organisms benefit from this process?
- Why can a pure culture of *Syntrophomonas* grow on crotonate but not butyrate?

VII • Hydrocarbon Metabolism

Hydrocarbons, molecules that contain only carbon and hydrogen atoms, are widely used by microbes as electron donors, and we wrap up our coverage of metabolic diversity with a consideration of this process. Unlike methane, hydrocarbons containing

two or more carbons typically have to be oxygenated before they can be catabolized. We first consider the aerobic catabolism of aliphatic and aromatic hydrocarbons, where this oxygenation involves O_2. However, we then proceed with a consideration of anoxic hydrocarbon metabolism, a situation where oxygenation of the hydrocarbon is still necessary, but where O_2 obviously plays no role.

14.24 Aerobic Hydrocarbon Metabolism

We previously discussed the role of molecular oxygen (O_2) as an *electron acceptor* in energy-generating reactions. By contrast, O_2 also plays an important role as a *reactant* in the catabolism of hydrocarbons, and oxygenase enzymes are key players in the process.

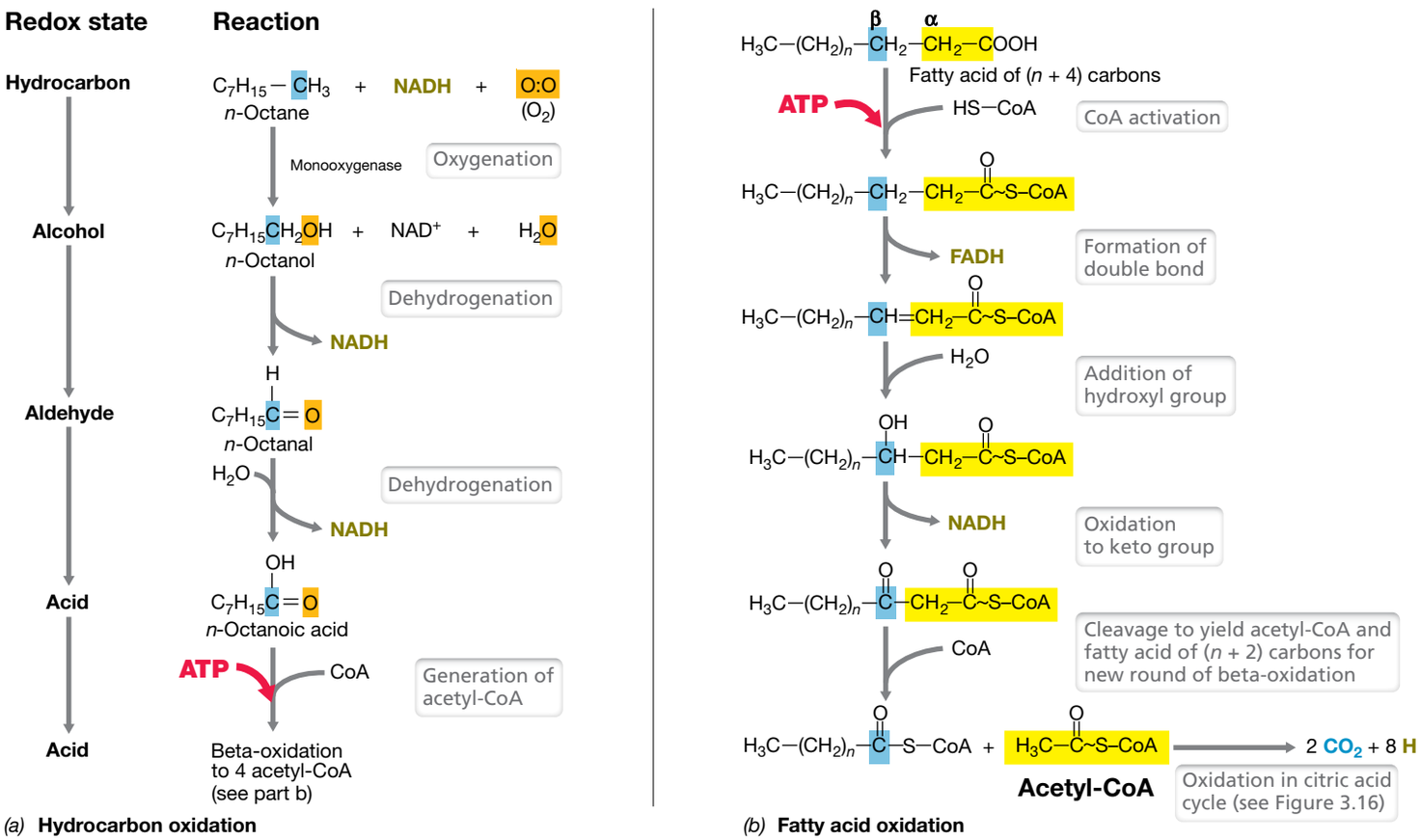
Oxygenases and Aliphatic Hydrocarbon Oxidation

Oxygenases are enzymes that catalyze the incorporation of O_2 into organic compounds and in some cases, inorganic compounds (Section 14.11). There are two classes of oxygenases: *dioxygenases*, which catalyze the incorporation of *both atoms* of O_2 into the molecule, and *monooxygenases*, which catalyze the incorporation of *only one* of the two oxygen atoms of O_2 into an organic compound with the second atom of O_2 being reduced to H_2O. For most monooxygenases, the required electron donor is NADH or NADPH.

In the initial oxidation step of a saturated aliphatic hydrocarbon, one of the atoms of O_2 is incorporated, typically at a terminal carbon atom. This reaction is catalyzed by a monooxygenase, and a typical reaction sequence is shown in **Figure 14.64*a***. The end product of the reaction sequence is a fatty acid of the same length as the original hydrocarbon. The fatty acid is then oxidized by *beta-oxidation*, a series of reactions in which two carbons of the fatty acid are split off at a time (Figure 14.64*b*). During beta-oxidation, NADH is formed and is oxidized in the electron transport chain for energy conservation purposes. A single round of beta-oxidation releases acetyl-CoA plus a new fatty acid that is two carbon atoms shorter than the original fatty acid. The process of beta-oxidation is then repeated, and another acetyl-CoA molecule is released. The acetyl-CoA formed by beta-oxidation is either oxidized through the citric acid cycle (Figure 3.16) or used to make new cell material. With the exception of how the hydrocarbon is oxygenated, much of the biochemistry of anoxic hydrocarbon catabolism is the same as that shown for aerobic catabolism (Figure 14.64), with beta-oxidation reactions being of prime importance in both cases.

Aromatic Hydrocarbon Oxidation

Many aromatic hydrocarbons can also be used as electron donors aerobically by microorganisms. The metabolism of these



(a) **Hydrocarbon oxidation** (b) **Fatty acid oxidation**

Figure 14.64 Monooxygenase activity and beta-oxidation. *(a)* Steps in the oxidation of an aliphatic hydrocarbon, the first of which is catalyzed by a monooxygenase. *(b)* Fatty acid oxidation by beta-oxidation leads to the successive formation of acetyl-CoA.

compounds, some of which contain multiple rings, such as naphthalene or biphenyls, typically has as its initial stage the formation of catechol or a structurally related compound via catalysis by oxygenase enzymes, as shown in Figure 14.65. Once catechol is formed it can be cleaved and further degraded into compounds that can enter the citric acid cycle, such as succinate, acetyl-CoA, and pyruvate.

Several steps in the aerobic catabolism of aromatic hydrocarbons require oxygenases. Figure 14.65*a–c* shows four different oxygenase-catalyzed reactions, one using a monooxygenase, two using a ring-cleaving dioxygenase, and one using a ring-hydroxylating dioxygenase. As in aerobic aliphatic hydrocarbon catabolism (Figure 14.64), aromatic compounds, whether single- or multi-ringed, are typically oxidized completely to CO_2, with electrons entering an electron transport chain or used to make new cell material.

MINIQUIZ

- How do monooxygenases differ in function from dioxygenases?
- What is the final product of catabolism of a hydrocarbon?
- What is meant by the term beta-oxidation?

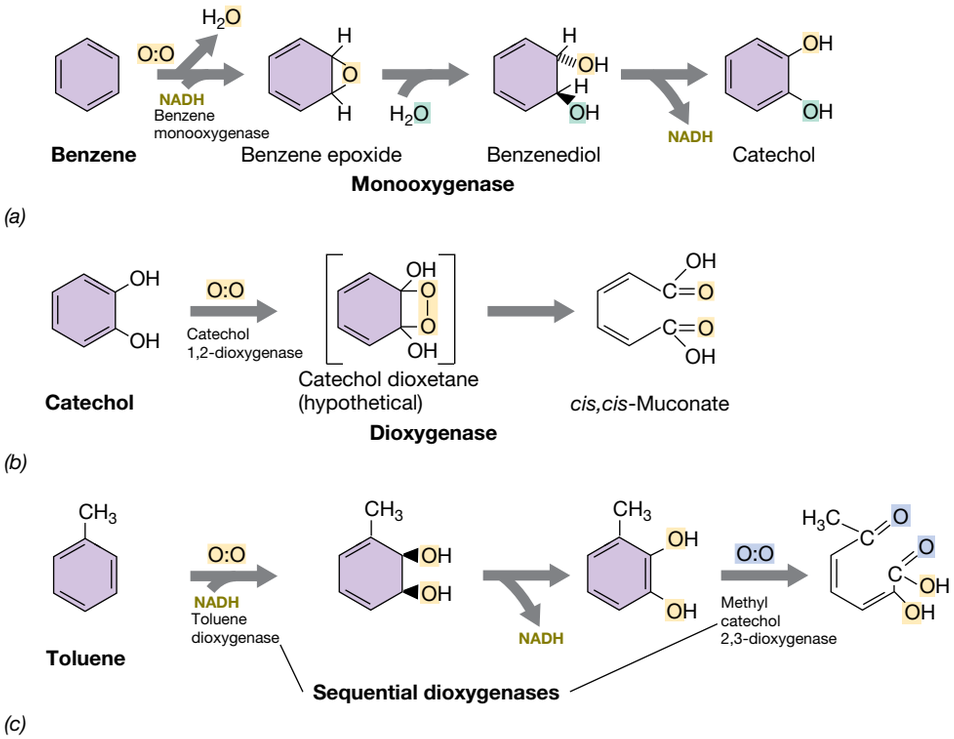


Figure 14.65 Roles of oxygenases in catabolism of aromatic compounds. Monooxygenases introduce one atom of oxygen from O_2 into a substrate, whereas diooxygenases introduce both atoms of oxygen. *(a)* Hydroxylation of benzene to catechol by a monooxygenase in which NADH is an electron donor. *(b)* Cleavage of catechol to *cis*, *cis*-muconate by an intradiol ring-cleavage dioxygenase. *(c)* The activities of a ring-hydroxylating dioxygenase and an extradiol ring-cleavage dioxygenase in the degradation of toluene. The oxygen atoms that each enzyme introduces are distinguished by different colors. Compare aerobic toluene catabolism to anoxic toluene catabolism shown in Figure 14.66*b*.

14.25 Anaerobic Hydrocarbon Metabolism

Although aerobic hydrocarbon oxidation is a major process in nature, anaerobic hydrocarbon oxidation linked to the reduction of nitrate, sulfate, or ferric iron as electron acceptors in anaerobic respirations is also possible. And, as for the aerobic process, both aliphatic and aromatic hydrocarbons can be degraded anaerobically.

Aliphatic Hydrocarbons

Aliphatic hydrocarbons are straight-chain saturated or unsaturated organic compounds, and many are substrates for denitrifying and sulfate-reducing bacteria. Saturated aliphatic hydrocarbons as long as C_{20} support growth, although shorter-chain hydrocarbons are more soluble and therefore more readily catabolized. The mechanism of anoxic hydrocarbon degradation has been well studied for hexane (C_6H_{14}) metabolism in denitrifying bacteria (NO_3^- as electron acceptor). However, the mechanism appears to be the same for the anoxic catabolism of longer-chain hydrocarbons and for anoxic hydrocarbon oxidation linked to other electron acceptors, and so we focus on the hexane/nitrate system here.

In anoxic hexane metabolism, hexane is modified on carbon atom 2 by attachment of a molecule of *fumarate*, a C_4 intermediate of the citric acid cycle (Figure 3.16), forming the compound *1-methylpentylsuccinate* (Figure 14.66*a*). The enzymatic addition of fumarate to hexane effectively oxygenates the hexane and allows the molecule to be further catabolized anaerobically. Following the addition of coenzyme A, a series of reactions occurs that includes beta-oxidation (Figure 14.64*b*) and regeneration of fumarate. The electrons released during beta-oxidation generate a proton motive force and are consumed in nitrate or sulfate reduction (Sections 14.13 and 14.14, respectively).

Aromatic Hydrocarbons

Aromatic hydrocarbons can be degraded anaerobically by some nitrate, ferric iron, and sulfate-reducing bacteria. For anoxic catabolism of the aromatic hydrocarbon toluene, oxygen needs to be added to the compound to begin catabolism, and this occurs by the addition of fumarate, as in aliphatic hydrocarbon catabolism (Figure 14.66*a*). The reaction series eventually yields benzoyl-CoA, which is further degraded by ring reduction (Figure 14.66*b*). Benzene (C_6H_6) can also be catabolized anaerobically, likely by a similar mechanism. Multi-ringed aromatic hydrocarbons such as naphthalene ($C_{10}H_8$) can be degraded by certain sulfate-reducing and denitrifying bacteria. In contrast to other hydrocarbons, the oxygenation of multi-ringed hydrocarbons occurs by the addition of CO_2 to the ring to form a carboxylic acid derivative rather than by fumarate

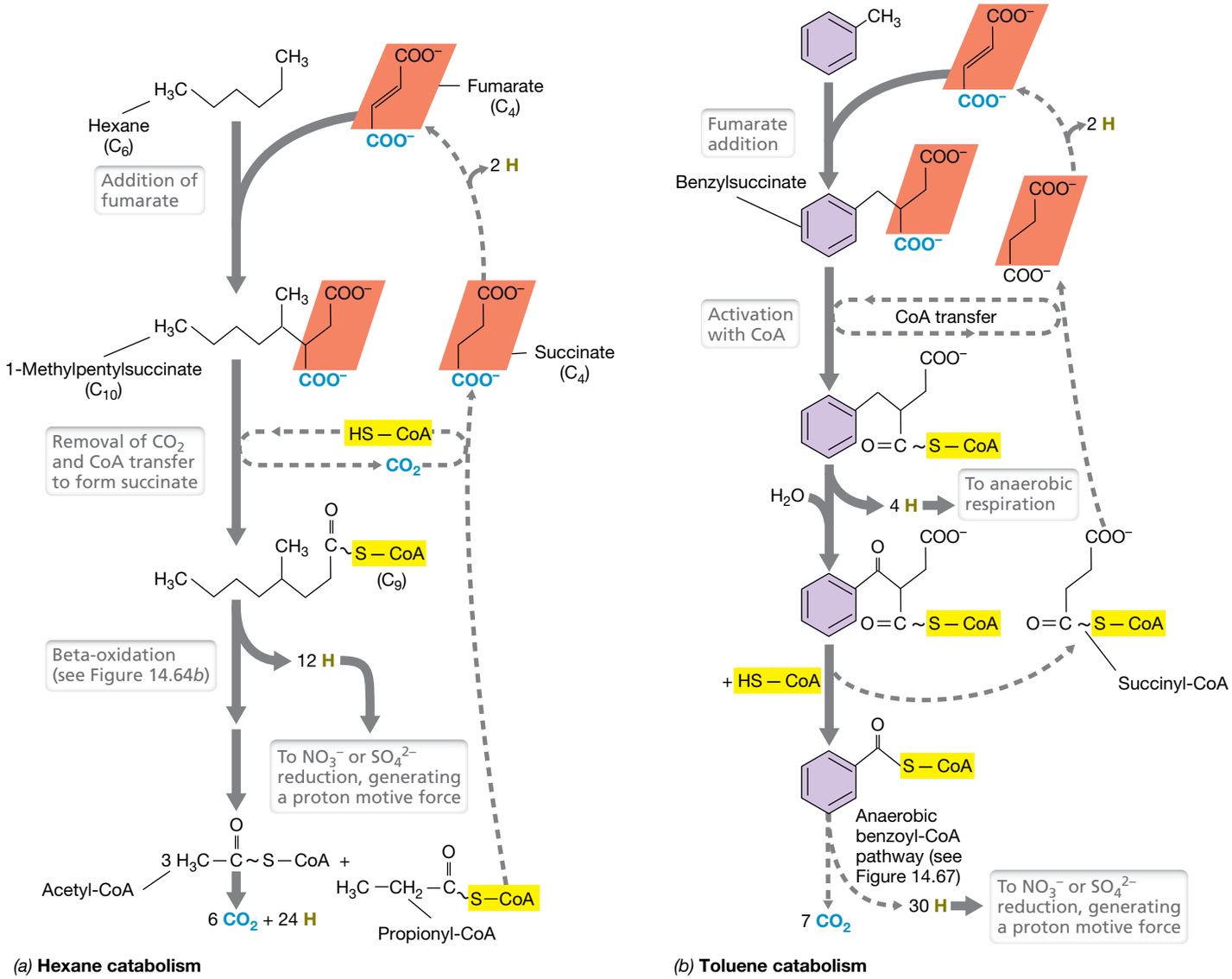


Figure 14.66 Anoxic catabolism of two hydrocarbons. *(a)* In anoxic catabolism of the aliphatic hydrocarbon hexane, the addition of fumarate provides the oxygen atoms necessary to form a fatty acid derivative that can be catabolized by beta-oxidation (see Figure 14.64) to yield acetyl-CoA. Electrons (H) generated from hexane catabolism are used to reduce sulfate or nitrate in anaerobic respirations. *(b)* Fumarate addition during the anoxic catabolism of the aromatic hydrocarbon toluene forms benzylsuccinate.

addition. But this carboxylation reaction serves the same purpose as oxygenase reactions (Figures 14.64*a* and 14.65) or the addition of fumarate (Figure 14.66); an O atom becomes part of the hydrocarbon and facilitates its catabolism.

Many bacteria can catabolize certain aromatic hydrocarbons anaerobically, including even fermentative and phototrophic bacteria. However, except for toluene, only aromatic compounds that already contain an O atom are degraded, typically by a common mechanism. In contrast to aerobic catabolism that occurs by way of ring *oxidation* (Figure 14.65), anaerobic catabolism proceeds by ring *reduction*. The anaerobic degradation of aromatic hydrocarbons is often facilitated by their conversion into benzoate followed by aromatic ring cleavage and benzoate catabolism performed by the *benzoyl-CoA pathway* (**Figure 14.67**). Benzoate catabolism in this pathway begins by forming the coenzyme A derivative followed by ring cleavage to yield fatty or dicarboxylic acids that can be further catabolized to intermediates of the citric acid cycle (Figure 14.67).

As we reach the end of our survey of metabolic diversity, we need not keep in mind all of the metabolic details, for they are numerous and formidable, but instead, we need to see the "metabolic big picture" formed by the major themes of this chapter: phototrophy and chemolithotrophy, CO_2 and N_2 fixations, respirations by electron donor or electron acceptor, C_1 metabolisms, fermentations, and hydrocarbon metabolism. These overarching principles will guide us through the next four

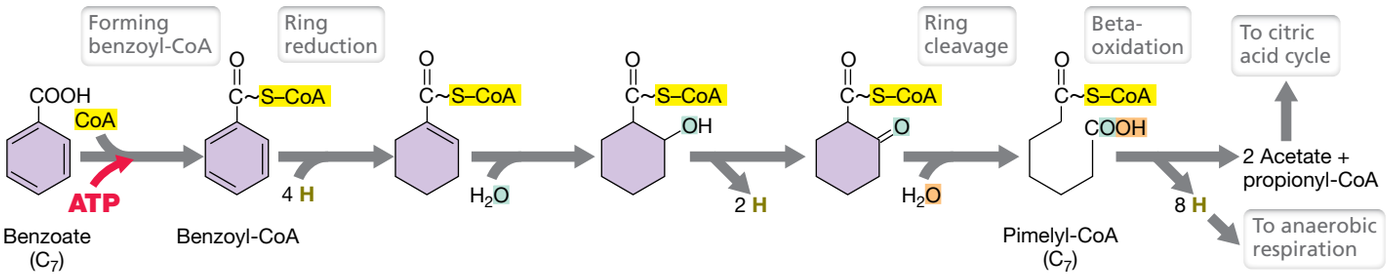


Figure 14.67 Anoxic degradation of benzoate by the benzoyl-CoA pathway. This pathway operates in the purple phototrophic bacterium *Rhodopseudomonas palustris* and many other facultative bacteria, both phototrophic and chemotrophic. Note that all intermediates of the pathway are bound to coenzyme A. The acetate produced is further catabolized in the citric acid cycle.

chapters where our focus will be on the diversity of the microbes themselves rather than their metabolisms. Chapters 15–17 in particular will cover many of the *Bacteria* and *Archaea* whose metabolisms we have described here, and we will witness there the many cases in which metabolic diversity and prokaryotic diversity are inextricably linked.

MINIQUIZ

- What is the benzoyl-CoA pathway, and how might it participate in the anaerobic degradation of toluene?
- How is hexane oxygenated during anoxic catabolism?

Chapter Review

I • Phototrophy

14.1 In photosynthesis, ATP is generated from light and then consumed in the reduction of CO_2. Two forms of photosynthesis are known: oxygenic, where O_2 is produced (for example, in cyanobacteria), and anoxygenic, where it is not (for example, in purple and green bacteria). Photosynthetic reaction centers and photosynthetic pigments reside in membranes where the light reactions of photosynthesis are carried out.

Q What are the functions of light-harvesting and reaction center chlorophylls?

14.2 Accessory pigments including carotenoids and phycobilins absorb light and transfer the energy to reaction center chlorophyll, thus broadening the wavelengths of light usable in photosynthesis. Carotenoids also play an important photoprotective role in preventing photooxidative damage to cells.

Q What accessory pigments are present in phototrophs, and what are their functions?

14.3 Electron transport reactions occur in the photosynthetic reaction center of anoxygenic phototrophs, forming a proton motive force and ATP.

Q What is reverse electron transport and why is it necessary in purple sulfur bacteria?

14.4 In oxygenic photosynthesis, H_2O donates electrons to drive CO_2 fixation, and O_2 is a by-product. There are two separate but interconnected photosystems in oxygenic phototrophs, PSI and PSII, whereas anoxygenic phototrophs contain a single photosystem.

Q How does the reduction potential (E_0') of chlorophyll *a* in PSI and PSII differ? Why must the reduction potential of PSII chlorophyll *a* be so highly electropositive?

II • Autotrophy and N_2 Fixation

14.5 Autotrophy is supported in most phototrophic and chemolithotrophic bacteria by the Calvin cycle, in which the enzyme RubisCO plays a key role. The reverse citric acid and hydroxypropionate cycles are autotrophic pathways in green sulfur and green nonsulfur bacteria, respectively.

Q What is a carboxysome, and what is its role in CO_2 fixation? Plants do not have carboxysomes; how might this affect the efficiency with which they fix CO_2?

14.6 The reduction of N_2 to NH_3 is called nitrogen fixation and is catalyzed by the enzyme nitrogenase. Nitrogenase can be assayed using the triply bonded compound acetylene as a surrogate of N_2; nitrogenase reduces acetylene to ethylene.

Q Is assaying for nitrogenase using acetylene reduction a definitive proof of nitrogen fixation?

III • Respiratory Processes Defined by Electron Donor

14.7 During respiration, energy is conserved by chemiosmotic ion gradients generated by the transport of electrons through the electron transport chain, and ATP is synthesized by ATP synthase. Anaerobic respiration yields less energy than aerobic respiration but can proceed in environments where O_2 is absent but alternative electron acceptors are present.

Q Why is NO_3^- a better electron acceptor for anaerobic respiration than is SO_4^{2-}?

14.8 The chemolithotrophic hydrogen bacteria use H_2 as an electron donor, reducing O_2 to H_2O. The enzyme hydrogenase is required to oxidize H_2, and H_2 also supplies reducing power for the fixation of CO_2 in these autotrophs.

Q Which inorganic electron donors are used by the organisms *Ralstonia*, *Thiobacillus*, and *Acidithiobacillus*?

14.9 Reduced sulfur compounds such as H_2S, $S_2O_3^{2-}$, and S^0 are electron donors for energy conservation in sulfur chemolithotrophs. Electrons from these substances enter electron transport chains, yielding a proton motive force. Sulfur chemolithotrophs are also autotrophs and fix CO_2 by the Calvin cycle.

Q Compare and contrast the utilization of H_2S by a purple phototrophic bacterium and by a colorless sulfur bacterium such as *Beggiatoa*. What role does H_2S play in the metabolism of each organism?

14.10 Chemolithotrophic iron bacteria oxidize Fe^{2+} as an electron donor. Most iron bacteria grow at an acidic pH and are often associated with acidic pollution from mineral and coal mining. A few chemolithotrophic and phototrophic bacteria can oxidize Fe^{2+} to Fe^{3+} anaerobically.

Q Why do most iron-oxidizing bacteria grow at an acidic pH?

14.11 The ammonia-oxidizing *Bacteria* and *Archaea* produce nitrite from ammonia, which is then oxidized by nitrite-oxidizing *Bacteria* to nitrate.

Q Are there any types of *Bacteria* or *Archaea* that are able to oxidize ammonia to nitrate?

14.12 Anoxic ammonia oxidation (anammox) consumes both ammonia and nitrite, forming N_2. The anammox reaction occurs within a membrane-enclosed compartment called the anammoxosome.

Q Contrast classical nitrification with anammox in terms of oxygen requirements, organisms involved, and the need for monooxygenases.

IV • Respiratory Processes Defined by Electron Acceptor

14.13 Nitrate is a common electron acceptor in anaerobic respiration. Nitrate reduction is catalyzed by the enzyme nitrate reductase, reducing NO_3^- to NO_2^-. Many bacteria that use NO_3^- in anaerobic respiration produce gaseous nitrogen compounds (NO, N_2O, or N_2) as final end products of reduction (denitrification).

Q What are the steps in nitrate reduction as carried out by *Pseudomonas* species? What is this metabolic process called?

14.14 Sulfate-reducing bacteria are obligately anaerobic bacteria that reduce SO_4^{2-} to H_2S in a process in which SO_4^{2-} must first be activated to adenosine phosphosulfate (APS). Disproportionation is an additional energy-yielding strategy for certain species. Some organisms, such as *Desulfuromonas*, cannot reduce SO_4^{2-} but can reduce S^0 to H_2S.

Q Why is the enzyme hydrogenase useful to *Desulfovibrio* even when it is not grown on H_2 as electron donor?

14.15 Besides inorganic nitrogen and sulfur compounds and CO_2, several other substances can function as electron acceptors for anaerobic respiration. These include Fe^{3+}, Mn^{4+}, fumarate, certain organic and chlorinated organic compounds, and even protons.

Q Compare and contrast ferric iron reduction with reductive dechlorination in terms of (1) product of the reduction and (2) environmental significance.

V • One-Carbon (C_1) Metabolism

14.16 Acetogens are strict anaerobes that reduce CO_2 to acetate, usually with H_2 as electron donor. The mechanism of acetate formation is the acetyl-CoA pathway, a pathway widely distributed in obligate anaerobes for either autotrophic purposes or acetate oxidation.

Q Compare and contrast acetogens with methanogens in terms of (1) substrates and products of their energy metabolism, (2) ability to use organic compounds as electron donors in energy metabolism, and (3) phylogeny.

14.17 Methanogenesis is the production of CH_4 from $CO_2 + H_2$ or from acetate or methanol by strictly anaerobic methanogenic *Archaea*. Several unique coenzymes are required for methanogenesis, and energy conservation is linked to either a proton motive or sodium motive force.

Q What are the major differences in the conservation of energy by methanogens that only use $H_2 + CO_2$ versus those that can use methyl compounds?

14.18 Methanotrophy is the use of CH_4 as both carbon source and electron donor, and the enzyme methane monooxygenase is a key enzyme in the aerobic

catabolism of methane. In methanotrophs, C_1 units are assimilated into cell material as formaldehyde or formaldehyde plus CO_2 by the ribulose monophosphate or serine pathways, respectively. The anaerobic oxidation of methane can be performed by ANME *Archaea*, which are related to methanogens, but which conserve energy by running the pathway for methanogenesis in reverse. The anaerobe *Methylomirabilis oxyfera* performs intra-aerobic methane oxidation in which O_2 is formed and then consumed.

Q How does a methano*troph* differ from a methano*gen*? Which pathway for C_1 assimilation found in aerobic methanotrophs is most energetically efficient and why? In what ways does *M. oxyfera* resemble denitrifiers and aerobic methanotrophs, and in what important ways does it differ?

VI • Fermentation

14.19 In the absence of external electron acceptors, organic compounds can be catabolized anaerobically only by fermentation. Most fermentations require that an energy-rich organic compound be formed that can yield ATP by substrate-level phosphorylation. Redox balance is achieved by the production of fermentation products.

Q Define the term substrate-level phosphorylation: How does it differ from oxidative phosphorylation? What compound(s) do fermentative bacteria need to synthesize in order to make ATP by substrate-level phosphorylation?

14.20 The lactic acid fermentation is carried out by homofermentative species, where lactate is the sole product, and heterofermentative species, where lactate, ethanol, and CO_2 are produced. The mixed-acid fermentation typical of enteric bacteria yields various acids plus neutral products (ethanol, butanediol), depending on the organism.

Q What are the major fermentation products of *Lactobacillus* and *Escherichia*?

14.21 Clostridia ferment sugars, amino acids, and other organic compounds, with butyric acid being a major product. Butyrate production allows for an additional ATP to be produced. *Propionibacterium* produces propionate, acetate, and CO_2 in a secondary fermentation of lactate.

Q How does *Propionibacterium* use secondary fermentation to conserve energy?

14.22 Energy conservation in *Propionigenium*, *Oxalobacter*, and *Malonomonas* is linked to decarboxylation reactions that pump Na^+ or H^+ across the membrane; ATPases use the energy in the ion gradient to form ATP. The reactions catalyzed by these organisms yield insufficient free energy to make ATP by substrate-level phosphorylation.

Q Give an example of a fermentation that does not employ substrate-level phosphorylation.

14.23 In syntrophy, two organisms cooperate to degrade a compound that neither can degrade alone. In this process H_2 produced by one organism is consumed by the partner. H_2 consumption affects the energetics of the reaction carried out by the H_2 producer, allowing it to make ATP where it otherwise could not.

Q Why is syntrophy also called "interspecies H_2 transfer"? When does syntrophy not involve H_2 transfer?

VII • Hydrocarbon Metabolism

14.24 In addition to its role as a terminal electron acceptor, O_2 can also be a substrate. In aerobic metabolism, oxygenases introduce atoms of oxygen from O_2 into hydrocarbons. Once oxygenated, aliphatic hydrocarbons can be further degraded by beta-oxidation and aromatic hydrocarbons by ring splitting and oxidation.

Q How do monooxygenases differ from dioxygenases in terms of the reactions they catalyze? Why are oxygenases necessary for the aerobic catabolism of hydrocarbons?

14.25 Hydrocarbons can be oxidized under anoxic conditions following addition of the dicarboxylic acid fumarate. Aromatic compounds are catabolized anaerobically by ring reduction and cleavage to form intermediates that can be catabolized in the citric acid cycle.

Q How do denitrifying and sulfate-reducing bacteria degrade hydrocarbons anaerobically and without oxygenases?

Application Questions

1. The growth rate of the phototrophic purple bacterium *Rhodobacter* is about twice as fast when the organism is grown *phototrophically* in a medium containing malate as the carbon source as when it is grown with CO_2 as the carbon source (with H_2 as the electron donor). Discuss the reasons why this is true, and list the nutritional class in which we would place *Rhodobacter* when growing under each of the two different conditions.
2. Although physiologically distinct, aerobic chemolithotrophs and chemoorganotrophs share a number of features with respect to the production of ATP. Discuss these common features along with reasons why the growth yield (grams of cells per mole of substrate consumed) of a chemoorganotroph respiring glucose is so much higher than for a chemolithotroph respiring sulfur.
3. A fatty acid such as butyrate cannot be fermented in pure culture, although its anaerobic catabolism under other conditions occurs readily. How do these conditions differ, and why does the latter allow for butyrate catabolism? How then can butyrate be fermented in mixed culture?

4. When methane is made from CO_2 (plus H_2) or from methanol (in the absence of H_2), various steps in the metabolic pathways are shared in common. Compare and contrast methanogenesis from these two substrates, highlighting the similarities and differences in the processes.

Chapter Glossary

Acetogenesis energy metabolism in which acetate is produced from either H_2 plus CO_2 or from organic compounds

Anaerobic respiration use of an electron acceptor other than O_2 in an electron transport–based oxidation leading to a proton motive force

Anammox anoxic ammonia oxidation

Anoxygenic photosynthesis photosynthesis in which O_2 is not produced

Antenna pigments light-harvesting chlorophylls or bacteriochlorophylls in photocomplexes that funnel energy to the reaction center

Autotroph an organism that uses CO_2 as its sole carbon source

Bacteriochlorophyll the chlorophyll pigment of anoxygenic phototrophs

Calvin cycle the biochemical pathway for CO_2 fixation in many autotrophic organisms

Carboxysomes crystalline inclusions of RubisCO

Carotenoid a hydrophobic accessory pigment present along with chlorophyll in photosynthetic membranes

Chlorophyll a light-sensitive, Mg-containing porphyrin of phototrophic organisms that initiates the process of photophosphorylation

Chlorosome a cigar-shaped structure present in the periphery of cells of green sulfur and green nonsulfur bacteria and containing the antenna bacteriochlorophylls (*c*, *d*, or *e*)

Denitrification anaerobic respiration in which NO_3^- or NO_2^- is reduced to nitrogen gases, primarily N_2

Dicarboxylate/4-hydroxybutyrate cycle an autotrophic pathway found in certain *Archaea*

Fermentation anaerobic catabolism of an organic compound in which the compound serves as both an electron donor and an electron acceptor and in which ATP is usually produced by substrate-level phosphorylation

Heterofermentative producing a mixture of products, typically lactate, ethanol, and CO_2, from the fermentation of glucose

Homofermentative producing only lactic acid from the fermentation of glucose

Hydrogenase an enzyme, widely distributed in anaerobic microorganisms, capable of oxidizing or evolving H_2

3-Hydroxypropionate bi-cycle an autotrophic pathway found in *Chloroflexus* and a few *Archaea*

3-Hydroxypropionate/4-hydroxybutyrate cycle an autotrophic pathway found in certain *Archaea*

Methanogen a methane-producing member of the *Archaea*

Methanogenesis the biological production of CH_4

Methanotroph an organism that oxidizes CH_4

Methylotroph an organism capable of growth on compounds containing no C—C bonds; some methylotrophs are methanotrophic

Mixotroph an organism in which an inorganic compound serves as the electron donor in energy metabolism and organic compounds serve as the carbon source

Nitrification the microbial oxidation of ammonia to nitrate

Nitrogenase the enzyme complex required to reduce N_2 to NH_3 in biological nitrogen fixation

Nitrogen fixation the reduction of N_2 to NH_3 by the enzyme nitrogenase

Oxygenase an enzyme that catalyzes the incorporation of oxygen from O_2 into organic or inorganic compounds

Oxygenic photosynthesis photosynthesis carried out by cyanobacteria and green plants in which O_2 is evolved

Photophosphorylation the production of ATP in photosynthesis

Photosynthesis the series of reactions in which ATP is synthesized by light-driven reactions and CO_2 is fixed into cell material

Phototroph an organism that uses light as an energy source

Phycobiliprotein the antenna pigment complex in cyanobacteria that contains phycocyanin and allophycocyanin or phycoerythrin coupled to proteins

Phycobilisome an aggregate of phycobiliproteins

Reaction center a photosynthetic complex containing chlorophyll or bacteriochlorophyll and several other components; the initial electron transfer reactions of photosynthetic electron flow occur here

Reductive acetyl-coenzyme A (acetyl-CoA) pathway a pathway used for acetogenesis, autotrophic CO_2 fixation, and acetate oxidation (when run in the reverse direction) widespread in obligate anaerobes including methanogens, acetogens, and sulfate-reducing bacteria

Reductive dechlorination an anaerobic respiration in which a chlorinated organic compound is used as an electron acceptor, usually with the release of Cl^-

Reverse citric acid cycle a mechanism for autotrophy in green sulfur bacteria and a few other autotrophic *Bacteria*, and also in some *Archaea*

Reverse electron transport the energy-dependent movement of electrons against the thermodynamic gradient to form a strong reductant from a weaker electron donor

Ribulose monophosphate pathway a reaction series in certain methylotrophs in which formaldehyde is assimilated into cell material using ribulose monophosphate as the C_1 acceptor molecule

RubisCO the acronym for ribulose bisphosphate carboxylase, a key enzyme of the Calvin cycle

Secondary fermentation a fermentation in which the substrates are the fermentation products of other organisms

Serine pathway a reaction series in certain methylotrophs in which CH_2O plus CO_2 are assimilated into cell material by way of the amino acid serine

Stickland reaction the fermentation of an amino acid pair

Syntrophy a process whereby two or more microorganisms cooperate to degrade a substance neither can degrade alone

Thylakoids membrane stacks in cyanobacteria or in the chloroplast of eukaryotic phototrophs

Functional Diversity of Microorganisms

15

microbiologynow

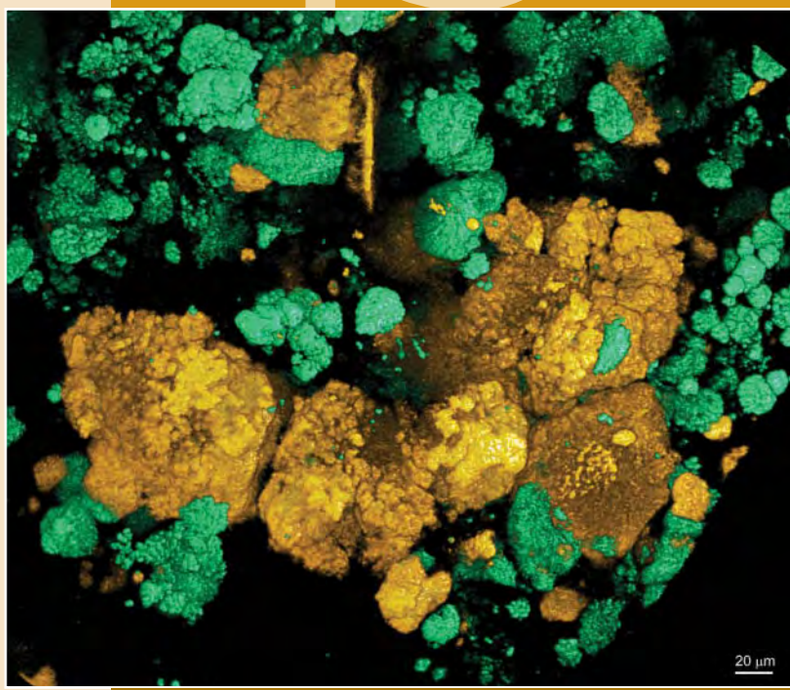
New Discoveries Have Redefined the Global Nitrogen Cycle

Nitrification, the aerobic oxidation of ammonia to nitrate, is a critical component of the global nitrogen cycle and is mediated exclusively by microorganisms. The Russian microbiologist Sergei Winogradsky was the first to describe the microbial basis of nitrification when he isolated chemolithotrophic bacteria that oxidized ammonia to nitrite over a century ago. Since then, the process of nitrification has been considered a two-step process that requires two physiological classes of chemolithotrophs: (1) microbes that oxidize ammonia to nitrite, and (2) microbes that oxidize nitrite to nitrate. Ammonia-oxidizing *Bacteria* and *Archaea* typically associate closely with nitrite-oxidizing *Bacteria*, and it has long been thought that nitrification absolutely required the coordinated activities of both of these functional groups. That is, only by working together could these microbes catalyze the overall nitrification process.

Recent discoveries continue to redefine our understanding of the nitrogen cycle, and sometimes these discoveries come from unexpected sources, such as a microbial biofilm found growing in an oil well. This biofilm was used to inoculate an enrichment culture designed to grow nitrifying bacteria. Over time the culture was found to oxidize ammonia all the way to nitrate, but the microbes in this culture did not match any known nitrifying organisms. Since the bacteria could not be grown in isolation, metagenomic sequencing was used to reconstruct the genomes of the species present. One of the dominant genomes belonged to the phylum *Nitrospira* (the photo shows clusters of fluorescently stained *Nitrospira* cells [yellow] in a 3D reconstruction of a biofilm observed by confocal laser microscopy). Bacteria from this phylum were already known to oxidize nitrite to nitrate (the second step of nitrification), but this microbe was different. It also contained the genes required for ammonia oxidation, most notably the gene encoding the key enzyme ammonia monooxygenase.

This novel bacterium, named "*Candidatus* Nitrospira inopinata," is the first microbe shown to perform both steps of nitrification. Now that they have been identified, unique gene sequences from these complete ammonia-oxidizing bacteria—nicknamed "comammox" bacteria for their dual metabolic activities—have been found in freshwater, soil, and engineered environments worldwide. The discovery of comammox bacteria once again emphasizes the fact that new microbial metabolisms are typically just "one culture away."

Source: Daims, H., et al. 2015. Complete nitrification by *Nitrospira* bacteria. *Nature 528:* 504–509.



I • Functional Diversity as a Concept

Microbial diversity can be understood in terms of both phylogenetic diversity and functional diversity. We begin by defining and contrasting the concepts of phylogenetic diversity and functional diversity.

15.1 Making Sense of Microbial Diversity

Phylogenetic diversity is the component of microbial diversity that deals with evolutionary relationships between microorganisms. Most fundamentally, phylogenetic diversity deals with the diversity of evolutionary lineages such as phyla, genera, and species. At its broadest, phylogenetic diversity encompasses the genetic and genomic diversity of evolutionary lineages and so can be defined on the basis of either genes or organisms (↔ Section 13.7). Most commonly, though, phylogenetic diversity is defined on the basis of ribosomal RNA gene phylogeny, which is thought to reflect the phylogenetic history of the entire organism (↔ Section 13.7). Phylogenetic diversity is the overarching theme of our coverage of microbial diversity in Chapters 16–18.

Functional diversity is the component of microbial diversity that deals with diversity in form and function as it relates to microbial physiology and ecology. It is useful to consider microbial diversity in terms of functional groupings because organisms with common traits and common genes often share physiological characteristics and have similar ecological roles. In many cases, functional traits align with phylogenetic groups (for example, with the organisms described in Sections 15.3, 15.4, 15.6, 15.7, 15.19). Microbial functional diversity, however, often does not correspond with phylogenetic diversity as defined by the 16S ribosomal RNA gene. We will see many examples in this chapter where functional traits are widely distributed among the *Bacteria* and *Archaea* (Figure 15.1).

At least three reasons can account for why a functional trait is shared between divergent organisms with dissimilar 16S ribosomal RNA gene sequences. The first is *gene loss*, a situation where a trait present in the common ancestor of several lineages is subsequently lost in some lineages but retained in others that over evolutionary time became quite divergent. The second is **convergent evolution**, in which a trait has evolved independently in two or more lineages and is not encoded by homologous genes shared by these lineages. The third is **horizontal gene transfer**

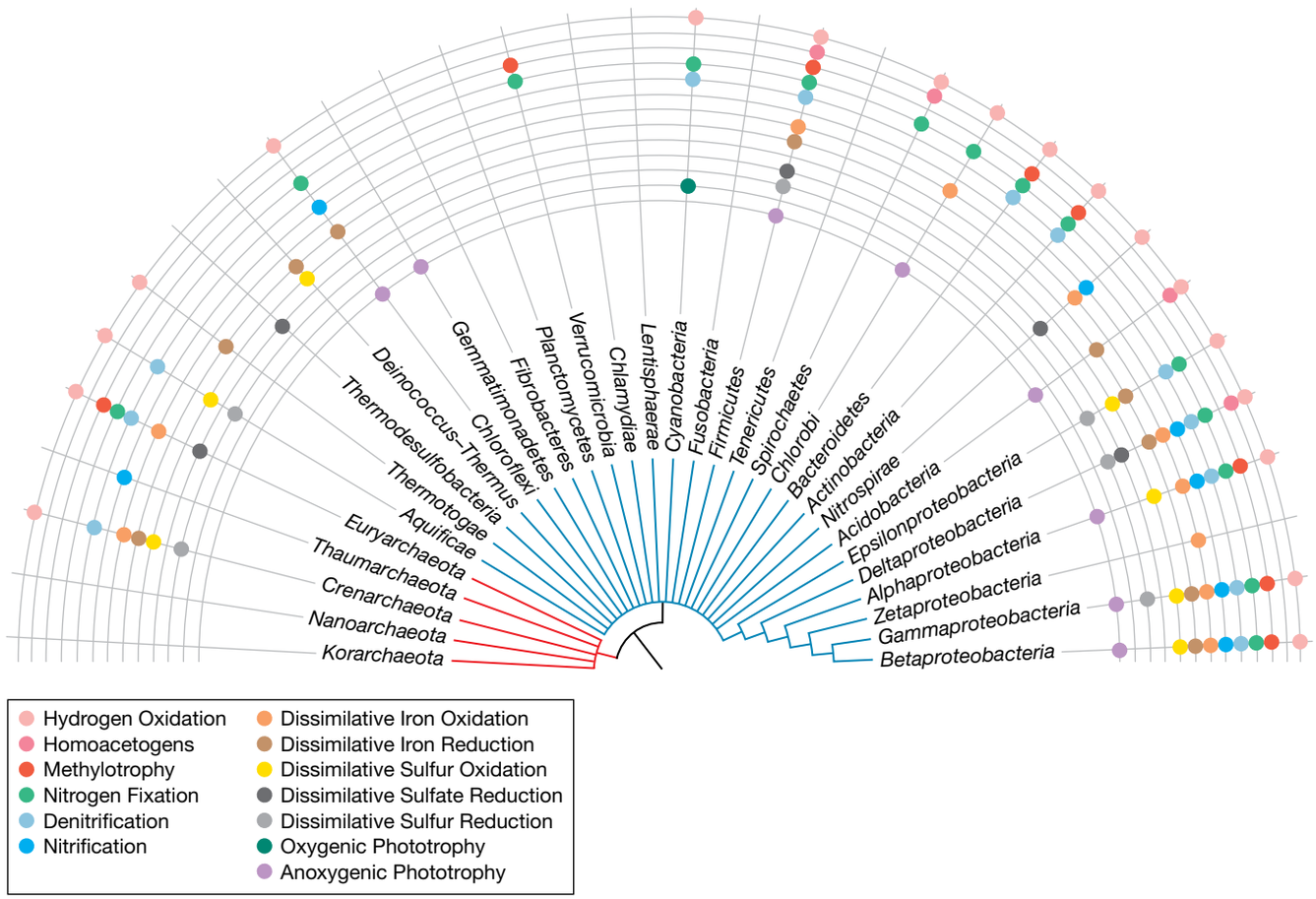


Figure 15.1 Major functional traits mapped across major phyla of *Bacteria* and *Archaea*.
The dendrogram shows relationships between microbial phyla as inferred by analysis of 16S ribosomal RNA gene sequences. Blue branches are used to denote phyla of *Bacteria* and red branches phyla of *Archaea*. Colored circles indicate phyla that contain at least one species with a functional trait indicated in the color key.

(↔ Sections 9.6 and 13.7), a situation where genes that confer a particular trait are homologous and have been exchanged between distantly related lineages.

Functional diversity can be further defined in terms of physiological diversity, ecological diversity, and morphological diversity. *Physiological diversity* relates to the functions and activities of microorganisms. Physiological diversity is most commonly described in terms of microbial metabolism and cellular biochemistry (Chapter 14). *Ecological diversity* relates to relationships between organisms and their environments. Organisms with similar physiological characteristics can have different ecological strategies (Section 15.11). Causes and consequences of ecological diversity will also be considered when we consider the science of microbial ecology in Chapters 19 and 20. *Morphological diversity* relates to the outward appearance of an organism, as cell shape and cellular structures often have ecological significance for microorganisms (Sections 15.19–15.22). In some cases, the morphology of a group is so distinctive that the group is essentially defined by this property, for example, with the spirochetes (Section 15.19).

The concepts of physiological, ecological, and morphological diversity are often intertwined. The examples provided in this chapter are meant to be illustrative and not exhaustive, and we will consider other organisms with important ecological functions in Chapters 16–18 and 20–23.

MINIQUIZ

- Why is it necessary to consider microbial diversity in terms of phylogenetic diversity and functional diversity?
- What are three reasons that functional traits might not correspond with distinct phylogenetic groups as defined by 16S ribosomal RNA gene sequences?

II • Diversity of Phototrophic *Bacteria*

In this section we consider the diversity of phototrophic microorganisms, those microbes that conserve energy from light. We will see that phototrophy is widespread within the domain *Bacteria* and that several distinct types of phototrophs can be defined on the basis of their physiological traits.

15.2 Overview of Phototrophic Bacteria

The ability to conserve energy from light evolved early in the history of life, when the Earth was anoxic (↔ Section 13.2). Photosynthesis originated within the *Bacteria*, and the first phototrophic organisms were *anoxygenic phototrophs*, organisms that do not generate O_2 as a product of photosynthesis (↔ Section 14.3). Instead of H_2O, these early phototrophs likely used H_2, ferrous iron (Fe^{2+}), or H_2S as the electron donor for photosynthesis. Anoxygenic photosynthesis is present in six bacterial phyla: the *Proteobacteria, Chlorobi, Chloroflexi, Firmicutes, Acidobacteria*, and *Gemmatimonadetes*. Oxygenic photosynthesis, by contrast, is known only within the *Cyanobacteria* (Figure 15.1). There is extensive metabolic diversity among the anoxygenic phototrophs, which are found in a wide range of habitats. It is clear that horizontal gene exchange has had a major impact on the evolution of photosynthesis and on the distribution of photosynthetic genes across the phylogenetic tree of *Bacteria*.

Phototrophic bacteria have several common features. All phototrophic bacteria use chlorophyll-like pigments and various accessory pigments to harvest energy from light and transfer this energy to a membrane-bound reaction center where it is used to drive electron transfer reactions that ultimately result in the production of ATP (↔ Sections 14.1–14.4). There are two different types of photosynthetic reaction centers: type I reaction centers (FeS-type), which are found in photosystem I of oxygenic phototrophs, and type II reaction centers (quinone-type, or Q-type), which are found in photosystem II of oxygenic phototrophs (↔ Sections 14.1–14.4). Both types of reaction centers are present in *Cyanobacteria* (↔ Section 14.4), whereas only one type or the other is present in anoxygenic phototrophs. In some cases photosynthetic pigments are found in the cytoplasmic membrane, but often they are present in intracellular photosynthetic membrane systems that originate from invaginations of the cytoplasmic membrane. These internal membranes allow phototrophic bacteria to increase the amount of pigment they contain for better use of light of low intensities.

Many phototrophic bacteria couple light energy to carbon fixation through a variety of different mechanisms (↔ Section 14.5), but not all phototrophs fix CO_2; some instead either prefer or require organic sources of carbon to support growth. We will see that many of the characteristics of phototrophic bacteria, including their membrane systems and photosynthetic pigments, have evolved as a result of niche adaptation for the light environment.

MINIQUIZ

- What form of photosynthesis was most likely the first to appear on Earth?

15.3 *Cyanobacteria*

KEY GENERA: *Prochlorococcus, Crocosphaera, Synechococcus, Trichodesmium, Oscillatoria, Anabaena*

Cyanobacteria comprise a large, morphologically and ecologically heterogeneous group of oxygenic, phototrophic *Bacteria*. As we saw in Section 13.2, these organisms were the first oxygen-evolving phototrophic organisms on Earth, and over billions of years converted the once anoxic atmosphere of Earth to the oxygenated atmosphere we see today.

Phylogeny and Classification of *Cyanobacteria*

The morphological diversity of the *Cyanobacteria* is impressive (**Figure 15.2**). Both unicellular and filamentous forms are known, and there is considerable variation within these morphological types. Cyanobacterial cells range in size from 0.5 µm in diameter to cells as large as 100 µm in diameter. *Cyanobacteria* can be divided into five morphological groups: (1) *Chroococcales* are unicellular,

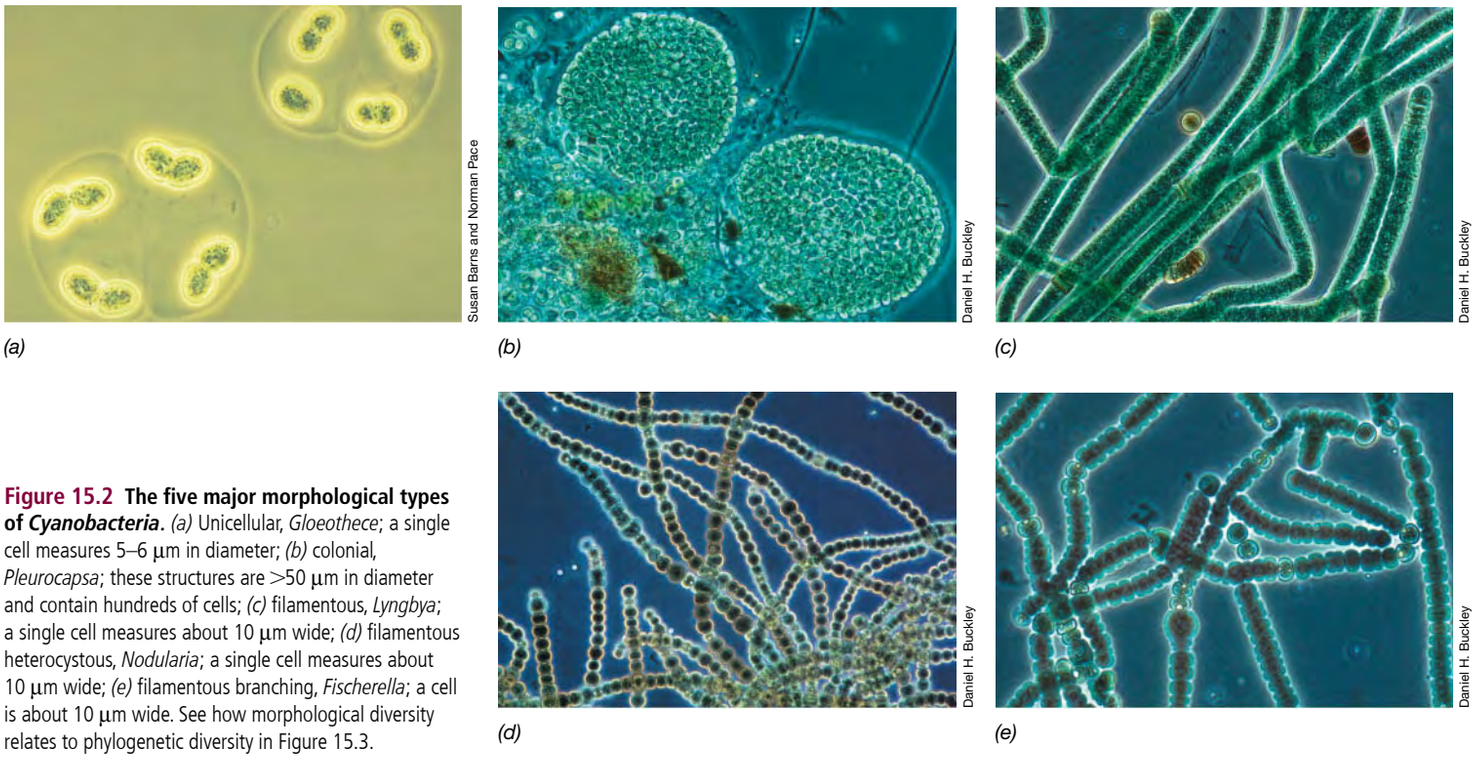


Figure 15.2 The five major morphological types of *Cyanobacteria*. *(a)* Unicellular, *Gloeothece*; a single cell measures 5–6 μm in diameter; *(b)* colonial, *Pleurocapsa*; these structures are >50 μm in diameter and contain hundreds of cells; *(c)* filamentous, *Lyngbya*; a single cell measures about 10 μm wide; *(d)* filamentous heterocystous, *Nodularia*; a single cell measures about 10 μm wide; *(e)* filamentous branching, *Fischerella*; a cell is about 10 μm wide. See how morphological diversity relates to phylogenetic diversity in Figure 15.3.

dividing by binary fission (Figure 15.2*a*); (2) *Pleurocapsales* are unicellular, dividing by multiple fission (colonial) (Figure 15.2*b*); (3) *Oscillatoriales* are filamentous nonheterocystous forms (Figure 15.2*c*); (4) *Nostocales* are filamentous, divide along a single axis, and are capable of cellular differentiation (Figure 15.2*d*); and (5) *Stigonematales* are morphologically similar to *Nostocales* except that cells divide in multiple planes, forming branching filaments (Figure 15.2*e*). Finally, the **prochlorophytes** are a lineage of unique unicellular *Cyanobacteria* once thought to be distinct but now classified within the *Chroococcales*.

Some of the major morphological classifications of *Cyanobacteria* correspond to coherent phylogenetic groups, but others do not (**Figure 15.3**). Species of *Pleurocapsales* form a coherent group within the cyanobacteria, indicating that reproduction by multiple fission arose only once in the evolutionary history of *Cyanobacteria* (Figure 15.3). Likewise, species of the *Nostocales* and *Stigonematales* share a common ancestor and form a coherent phylogenetic group indicating a single origin of cellular differentiation within the *Cyanobacteria* (Figure 15.3). All *Stigonematales* share a single ancestor within the clade composed of *Nostocales* and *Stigonematales*, indicating that the capacity to form branching filaments arose only once within the lineage of *Cyanobacteria* capable of cellular differentiation (Figure 15.3). In contrast, unicellular and simple filamentous *Cyanobacteria* (*Chroococcales* and *Oscillatoriales*, respectively) are dispersed in the cyanobacterial phylogeny, and these morphological groups do not represent coherent evolutionary lineages (Figure 15.3).

Physiology and Photosynthetic Membranes

Cyanobacteria are oxygenic phototrophs and therefore have both FeS-type and Q-type photosystems. All species are able to fix CO_2 by the Calvin cycle, many can fix N_2, and most can synthesize their own vitamins. Cells harvest energy from light and fix CO_2 during the day. During the night, cells generate energy by fermentation or aerobic respiration of carbon storage products such as glycogen. While CO_2 is the predominant source of carbon for most species, some *Cyanobacteria* can assimilate simple organic compounds such as glucose and acetate if light is present, a process called *photoheterotrophy*. A few *Cyanobacteria*, mainly filamentous species, can also grow in the dark on glucose or sucrose, using the sugar as both carbon and energy source. Finally, when sulfide concentrations are high, some *Cyanobacteria* are able to switch from oxygenic photosynthesis to anoxygenic photosynthesis using hydrogen sulfide rather than water as electron donor for photosynthesis (↻ Figure 14.16).

Cyanobacteria have specialized membrane systems called *thylakoids* that increase the ability of cells to harvest light energy (↻ Figure 14.10). The cell wall of cyanobacteria contains peptidoglycan and is structurally similar to that of other gram-negative bacteria. Photosynthesis takes place in the thylakoid membrane, a complex and multilayered photosynthetic membrane system containing photopigments and proteins that mediate photosynthesis (↻ Sections 14.1 and 14.2). In most unicellular *Cyanobacteria*, the thylakoid membranes are arranged in regular concentric circles around the periphery of the cytoplasm (**Figure 15.4**). *Cyanobacteria* produce chlorophyll *a*, and most also have characteristic pigments called **phycobilins** (↻ Figure 14.10), which function as accessory pigments in photosynthesis. One class of phycobilins, *phycocyanins*, are blue and, together with the green chlorophyll *a*, are responsible for the blue-green color of most cyanobacteria. Some *Cyanobacteria* produce *phycoerythrin*, a red phycobilin, and species producing phycoerythrin are red or brown. Photopigments are

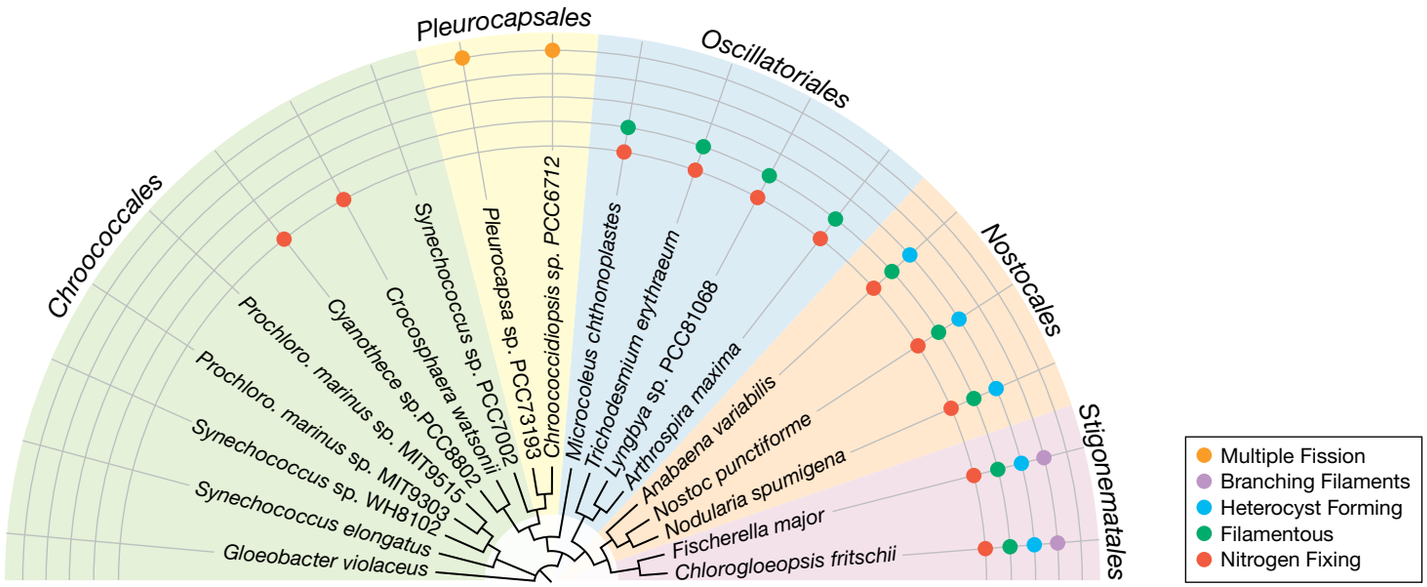


Figure 15.3 Taxonomically informative traits mapped onto the phylogeny of *Cyanobacteria*. The dendrogram depicts phylogenetic relationships inferred from analysis of conserved protein families in cyanobacterial genomes. Colored circles are used to indicate species traits as indicated by the key. Color shading is used to indicate taxonomic groupings. "*Prochloro.*" is used to indicate *Prochlorococcus*, which is a distinct group within the *Chroococcales*. Note that the *Chroococcales* and *Oscillatoriales* are not monophyletic in origin, meaning that these traits have arisen independently on multiple occasions in the phylogeny.

fluorescent and emit light when visualized using a fluorescence microscope; chlorophyll *a*, for example, fluoresces bright red (Figure 15.5). Prochlorophytes, such as *Prochlorococcus* and *Prochloron*, are unique among *Cyanobacteria* in that all members of this group contain chlorophyll *a* and *b* but do not contain phycobilins.

Motility and Cellular Structures

Cyanobacteria possess several mechanisms for motility. Many cyanobacteria exhibit gliding motility (Section 2.12). Gliding occurs only when a cell or filament is in contact with a solid surface or with another cell or filament. In some *Cyanobacteria*, gliding is not a simple translational movement but is accompanied by rotations, reversals, and flexing of filaments. Most gliding species exhibit directional movement toward light (phototaxis), and chemotaxis (Section 2.13) may occur as well. *Synechococcus* exhibits an unusual form of swimming motility that does not require flagella or any other extracellular organelle. The cell surface of *Synechococcus* has specialized proteins that provide direct thrust by way of a mechanism that has yet to be resolved. Gas vesicles (Section 2.9) are also found in a variety of aquatic *Cyanobacteria* and are important in positioning cells in the water column. The function of gas vesicles is to regulate cell buoyancy such that cells can remain in a position in the water column where light intensity is optimal for photosynthesis.

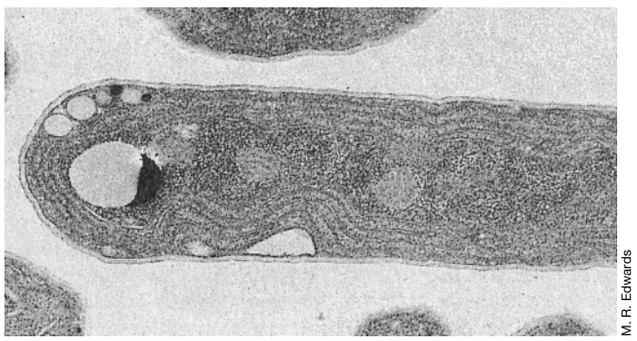


Figure 15.4 Thylakoids in *Cyanobacteria*. Electron micrograph of a thin section of the cyanobacterium *Synechococcus lividus*. A cell is about 5 μm in diameter. Note thylakoid membranes running parallel to the cell wall.

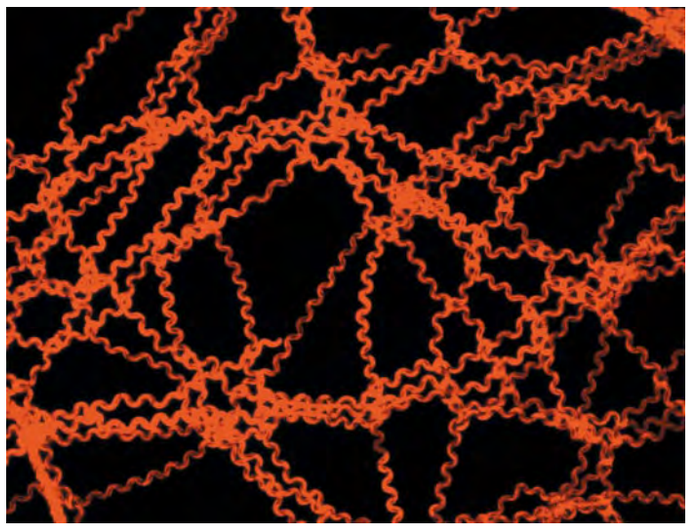


Figure 15.5 Phycocyanin fluorescence in *Cyanobacteria*. Fluorescence micrograph of *Spirulina*. Filaments consist of chains of helical cells with each cell approximately 5 μm wide.

Cyanobacteria are able to form a variety of structures associated with energy storage, reproduction, and survival. Many *Cyanobacteria* produce extensive mucilaginous envelopes, or sheaths, that bind groups of cells or filaments together (Figure 15.2*a*). Some filamentous cyanobacteria form *hormogonia* (**Figure 15.6**), short, motile filaments that break off from longer filaments to facilitate dispersal in times of stress. Some species also form resting structures called *akinetes* (Figure 15.6*c*), which protect the organism during periods of darkness, desiccation, or cold. Akinetes are cells with thickened outer walls. When conditions improve, akinetes germinate by breaking down their outer wall and initiating growth of a new vegetative filament. Many *Cyanobacteria* also form a structure called *cyanophycin*. This structure is a copolymer of aspartic acid and arginine and is a nitrogen storage product; when nitrogen in the environment becomes deficient, cyanophycin is broken down and used as a cellular nitrogen source. Many species of the *Nostocales* and *Stigonematales* are also able to form heterocysts, as discussed next.

Heterocysts and Nitrogen Fixation

Many *Cyanobacteria* are capable of nitrogen fixation (Figure 15.3). The nitrogenase enzyme, however, is inhibited by oxygen and thus nitrogen fixation cannot occur along with oxygenic photosynthesis (Section 14.6). *Cyanobacteria* have evolved several regulatory mechanisms for separating nitrogenase activity from photosynthesis (Section 7.8). For example, many unicellular *Cyanobacteria*, such as *Cyanothece* and *Crocosphaera* (**Figure 15.7*a***), fix nitrogen only at night when photosynthesis does not occur. In contrast, the filamentous cyanobacteria *Trichodesmium* (Figure 15.7*b*) fixes nitrogen only during the day through a mechanism that remains somewhat unclear, but appears to require transient suppression of photosynthetic activity within filaments. Finally, many filamentous *Cyanobacteria* of the *Nostocales* and *Stigonematales* facilitate nitrogen fixation by forming specialized cells called *heterocysts*, either on the ends of filaments (**Figure 15.8*a, b***) or along the filament (Figure 15.8*c, d*).

Heterocysts arise from differentiation of vegetative cells and are the sites of nitrogen fixation in heterocystous *Cyanobacteria*. Heterocysts are surrounded by a thickened cell wall that slows the diffusion of O_2 into the cell and permits nitrogenase activity to occur in an anoxic environment. Heterocysts lack photosystem II, the oxygen-evolving photosystem that generates reducing power from H_2O (Section 14.4), and thus do not fluoresce as strongly as vegetative cells (Figure 15.8). Without photosystem II, heterocysts are unable to fix CO_2 and thus lack the necessary electron donor (pyruvate) for nitrogen fixation. However, heterocysts have intercellular connections with adjacent vegetative cells that allow for mutual exchange of materials between these cells. Fixed carbon is imported by the heterocyst from adjacent vegetative cells, and this is oxidized to yield electrons for nitrogen fixation. The products of photosynthesis move from vegetative cells to heterocysts, and fixed nitrogen moves from heterocysts to vegetative cells (Figure 7.17).

Ecology of *Cyanobacteria*

Cyanobacteria are of central importance to the productivity of the oceans. Small unicellular *Cyanobacteria*, such as *Synechococcus*

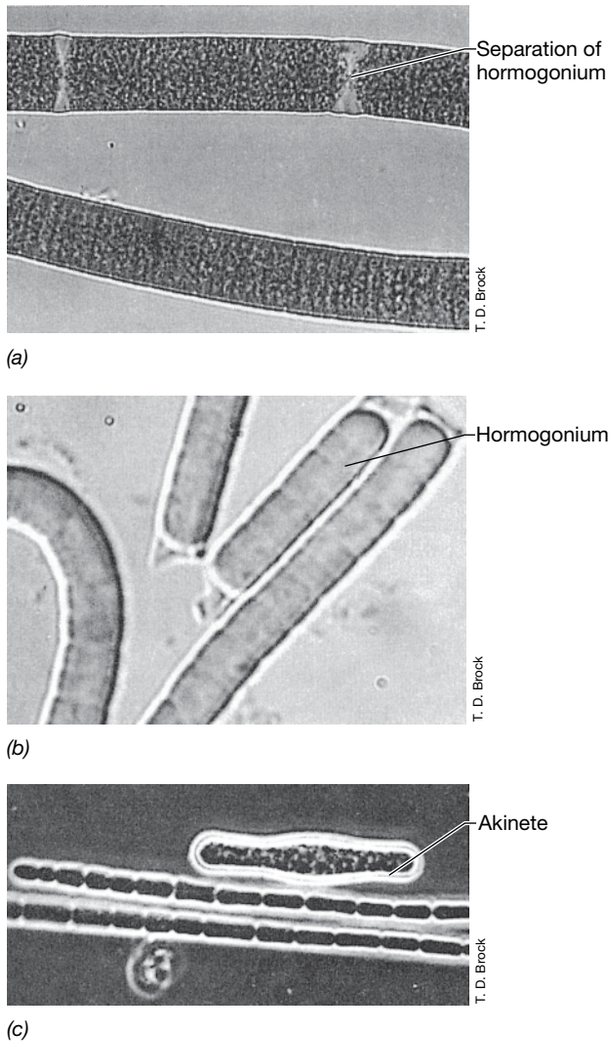


Figure 15.6 Structural differentiation in filamentous *Cyanobacteria*. *(a)* Initial stage of hormogonium formation in *Oscillatoria*. Notice the empty spaces where the hormogonium is separating from the filament. *(b)* Hormogonium of a smaller *Oscillatoria* species. Notice that the cells at both ends are rounded. Cells are about 10 μm wide. Differential interference contrast microscopy. *(c)* Akinete (resting spore) of *Anabaena* in a phase-contrast micrograph, cells about 5 μm wide.

and *Prochlorococcus* (Section 20.10), are the most abundant phototrophs in the oceans. Together these organisms contribute 80% of marine photosynthesis and 35% of all photosynthetic activity on Earth.

Cyanobacterial nitrogen fixation represents the dominant input of new nitrogen into vast segments of Earth's oceans, particularly in oligotrophic tropical and subtropical waters. Marine nitrogen fixation is dominated by two groups of *Cyanobacteria*, the unicellular species, such as *Crocosphaera*, and the filamentous *Trichodesmium*. *Crocosphaera* (Figure 15.7*a*) and relatives dominate nitrogen fixation in most of the Pacific Ocean and are widespread in tropical and subtropical habitats. *Trichodesmium* is the dominant nitrogen-fixer in the North Atlantic Ocean and parts of the Pacific where dissolved iron concentrations are elevated. *Trichodesmium* forms macroscopically visible tufts of filaments (Figure 15.7*b*) and relies on gas vesicles to remain suspended in the photic

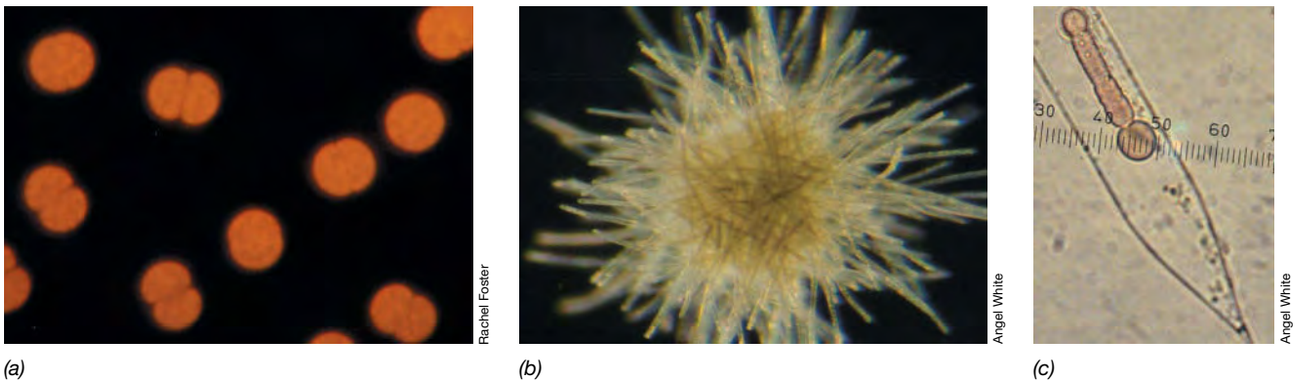


Figure 15.7 Marine *Cyanobacteria* that fix N_2. *(a)* Unicellular *Crocosphaera*-like cells in the process of dividing; cells are approximately 5 μm diameter. *(b)* Colonial "tuft" of *Trichodesmium*. The tuft is composed of many attached, undifferentiated, unbranched filaments and has a diameter of approximately 100 μm. *(c)* A diatom containing the cyanobacterial symbiont *Richelia* (scale in micrometers). The *Richelia* symbiont is an unbranched filament with a terminal heterocyst; cells are about 5 μm wide.

zone where it is often observed in dense masses of cells called *blooms*. In addition, other marine nitrogen-fixers including species of *Calothrix* and *Richelia* form symbiotic associations with diatoms (Figure 15.7*c*); these symbiotic associations are often observed in tropical and subtropical oceans. Finally, heterocystous cyanobacteria such as *Nodularia* (Figure 15.2*d*) and *Anabaena* can sometimes dominate nitrogen fixation in cold waters of the Northern Hemisphere and are often observed in the Baltic Sea.

Cyanobacteria are also widely found in terrestrial and freshwater environments. In general, they are more tolerant of environmental extremes, particularly extremes of desiccation, than are eukaryotic algae. *Cyanobacteria* are often the dominant or sole oxygenic phototrophic organisms in hot springs, saline lakes, desert soils, and other extreme environments. In some of these environments, cyanobacterial mats of variable thickness may form (↔ Figure 20.7). Freshwater lakes, especially those rich in inorganic nutrients, often develop blooms of *Cyanobacteria*, especially in late summer when temperatures are warmest (↔ Figures 20.1 and 20.17). A few *Cyanobacteria* are symbionts of liverworts, ferns, and cycads, and a number are phototrophic

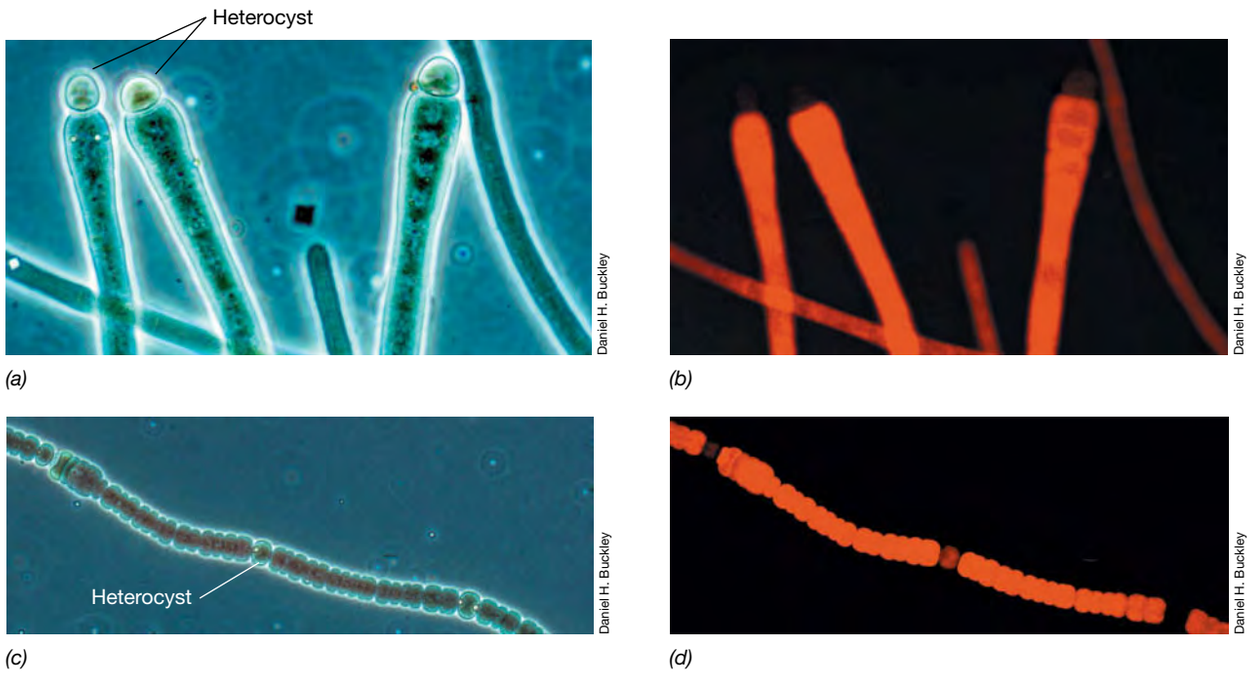


Figure 15.8 Heterocysts. Differentiation of heterocysts causes the loss of photopigments and inability to carry out photosynthesis. *(a)* Phase-contrast micrograph of *Calothrix* with terminal heterocysts. *(b)* Fluorescence micrograph of the same *Calothrix* filaments; cells are about 10 μm wide. *(c)* Phase-contrast micrograph of *Fischerella*. *(d)* Fluorescence micrograph of the same *Fischerella* filaments; cells are about 10 μm wide. See how heterocyst formation is regulated at the genetic level in the well-studied cyanobacterium *Anabaena* in Figure 7.17.

components of lichens, a symbiosis between a phototroph and a fungus (Section 23.1).

Several metabolic products of *Cyanobacteria* are of considerable practical importance. Some *Cyanobacteria* produce potent neurotoxins, and toxic blooms may form when massive accumulations of *Cyanobacteria* develop. Animals ingesting water containing these toxic products may be killed. Many *Cyanobacteria* are also responsible for the production of earthy odors and flavors in some freshwater, and if such waters are used as drinking water sources, aesthetic problems may arise. The major compound produced is geosmin, a substance also produced by many actinomycetes (Section 16.12).

MINIQUIZ

- What are the differentiating properties of the five major morphological groups of *Cyanobacteria*?
- What is a heterocyst and what is its function?

15.4 Purple Sulfur Bacteria

KEY GENERA: *Chromatium, Ectothiorhodospira*

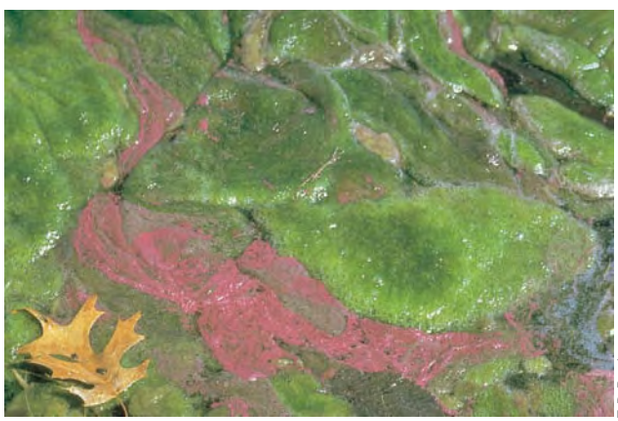
Purple sulfur bacteria are anoxygenic phototrophs that use hydrogen sulfide (H_2S) as an electron donor for photosynthesis (Figure 14.1). Purple sulfur bacteria are a phylogenetically coherent group found within the order *Chromatiales* in the *Gammaproteobacteria*.

Purple sulfur bacteria are generally found in illuminated anoxic zones where H_2S is present. Such habitats occur commonly in lakes, marine sediments, and "sulfur springs," where H_2S produced geochemically or biologically can support the growth of purple sulfur bacteria (**Figure 15.9**). Purple sulfur bacteria are also commonly found in microbial mats (Section 20.5) and in salt marsh sediments. The characteristic color of purple sulfur bacteria comes from their carotenoids, accessory pigments involved in light harvesting (Section 14.2). These bacteria use a Q-type photosystem (Figure 14.12), contain either bacteriochlorophyll *a* or *b*, and carry out CO_2 fixation by the Calvin cycle (Section 14.5).

During autotrophic growth of purple sulfur bacteria, H_2S is oxidized to elemental sulfur (S^0), which is deposited as sulfur granules (**Figure 15.10**). When sulfide is limiting, the sulfur is used as an electron donor for photosynthesis, resulting in the oxidation of S^0 to sulfate (SO_4^{2-}). Many purple sulfur bacteria can also use other reduced sulfur compounds as photosynthetic electron donors; for example, thiosulfate ($S_2O_3^{2-}$) is commonly used to grow laboratory cultures.

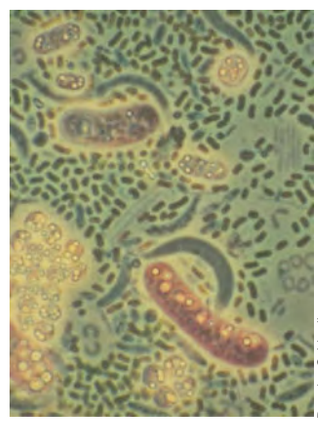
The purple sulfur bacteria form two families: the *Chromatiaceae* and the *Ectothiorhodospiraceae*. Species of the two families are readily distinguished by the location of sulfur granules and by their photosynthetic membranes. *Chromatiaceae*, including the genera *Chromatium* and *Thiocapsa*, store S^0 granules *inside* their cells (in the periplasmic space) and have vesicular intracellular photosynthetic membrane systems (**Figure 15.11*b***). These organisms are common in stratified lakes containing sulfide and in the anoxic sediments of salt marshes. *Ectothiorhodospiraceae*, including the two main genera *Ectothiorhodospira* and *Halorhodospira*, oxidize H_2S to S^0 that is deposited *outside* the cell (Figure 15.10*d*) and have lamellar intracellular photosynthetic membrane systems (Figure 15.11*a*). These genera are also interesting because many species are extremely halophilic (salt loving) or alkaliphilic (alkalinity loving) and are among the most extreme in these characteristics of all known *Bacteria*. These organisms are typically found in saline lakes, soda lakes, and salterns, where abundant levels of SO_4^{2-} support sulfate-reducing bacteria (Section 21.4 and Section 15.9), the organisms that produce H_2S.

Purple sulfur bacteria are often observed in high density in meromictic (permanently stratified) lakes. Meromictic lakes form layers because they have denser (usually saline) water on the bottom and less dense (usually freshwater) water nearer the surface.



(a)

(b)



(c)

Figure 15.9 Blooms of purple sulfur bacteria. *(a) Lamprocystis roseopersicina* in a sulfide spring. The bacteria grow near the bottom of the spring pool and float to the top (by virtue of their gas vesicles) when disturbed. The purple color is from the photopigments of the purple sulfur bacteria and the green color is from cells of the alga *Spirogyra*. *(b)* Sample of water from a depth of 7 m in Lake Mahoney, British Columbia; the major phototroph is the purple sulfur bacterium *Amoebobacter purpureus*. *(c)* Phase-contrast photomicrograph of layers of purple sulfur bacteria from a small, stratified lake in Michigan. The purple sulfur bacteria include *Chromatium* species (large rods) and *Thiocystis* (small cocci). The small green-colored rods are green sulfur bacteria such as *Chlorobium* (Section 15.6).

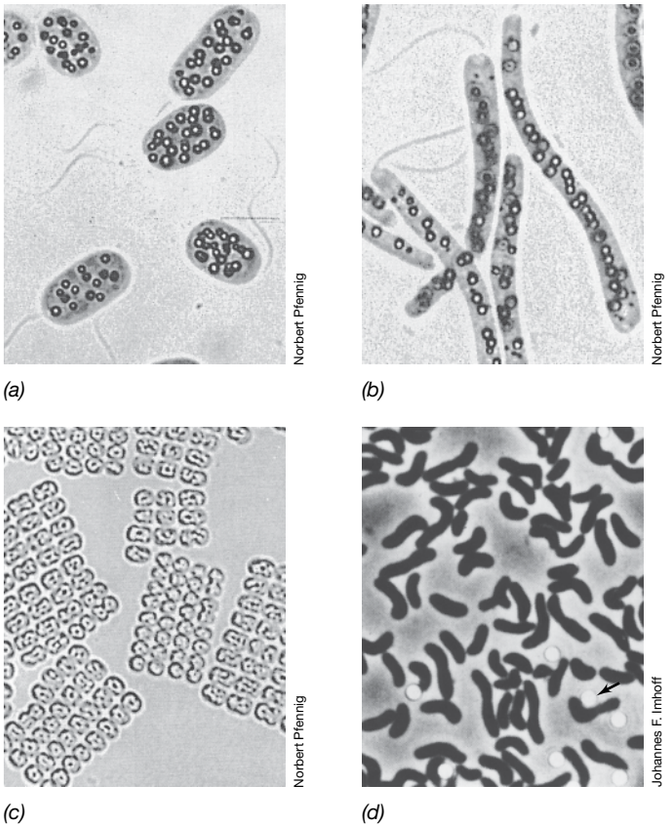


Figure 15.10 Bright-field and phase-contrast photomicrographs of purple sulfur bacteria. *(a) Chromatium okenii*; cells are about 5 μm wide. Note the globules of elemental sulfur inside the cells. *(b) Thiospirillum jenense*, a very large, polarly flagellated spiral; cells are about 30 μm long. Note the sulfur globules. *(c) Thiopedia rosea*; cells are about 1.5 μm wide. *(d)* Phase-contrast micrograph of cells of *Ectothiorhodospira mobilis*; cells are about 0.8 μm wide. Note external sulfur globules (arrow).

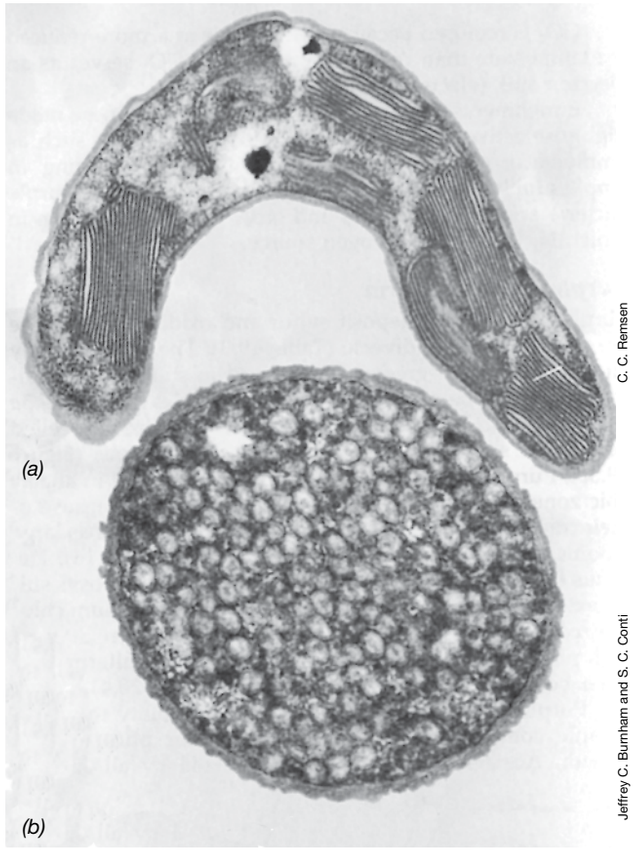


Figure 15.11 Membrane systems of phototrophic purple bacteria as revealed by transmission electron microscopy. *(a) Ectothiorhodospira mobilis*, showing the photosynthetic membranes in flat sheets (lamellae). *(b) Allochromatium vinosum*, showing the membranes as individual, spherical vesicles.

If sufficient sulfate is present to support sulfate reduction, sulfide is produced in the sediments and diffuses upward into the anoxic bottom waters. The presence of sulfide and light in the anoxic layers of the lake allow purple sulfur bacteria to form dense cell masses (Figure 15.9*b*), usually in association with green phototrophic bacteria.

MINIQUIZ

- What is the source of the purple color from which the purple sulfur bacteria get their name?
- Where would you expect to find purple sulfur bacteria in nature?

15.5 Purple Nonsulfur Bacteria and Aerobic Anoxygenic Phototrophs

Purple Nonsulfur Bacteria

KEY GENERA: *Rhodospirillum, Rhodoferax, Rhodobacter*

The **purple nonsulfur bacteria** are the most metabolically versatile of all microbes. Despite their name, they are not always purple; these organisms synthesize an array of carotenoids (Section 14.2) that can lend them a variety of spectacular colors (**Figure 15.12**). Together, these pigments give purple bacteria their colors, usually purple, red, or orange. Purple nonsulfur bacteria are typically photoheterotrophs (a condition where light is the energy source and an organic compound is the carbon source), and species are able to use a wide range of carbon sources and electron donors for photosynthesis, including organic acids, amino acids, alcohols, sugars, and even aromatic compounds like benzoate or toluene. Like purple sulfur bacteria, purple nonsulfur bacteria use a Q-type photosystem, and contain either bacteriochlorophyll *a* or *b*. The purple nonsulfur bacteria are phylogenetically and morphologically diverse (**Figure 15.13**) and reside within the *Alphaproteobacteria* (e.g., *Rhodospirillum, Rhodobacter, Rhodopseudomonas*) or *Betaproteobacteria* (e.g., *Rubrivivax, Rhodoferax*).

Purple nonsulfur bacteria can conserve energy through a variety of metabolic processes. For example, some species can grow photoautotrophically using H_2, low levels of H_2S, or even ferrous iron (Fe^{2+}) as the electron donor for photosynthesis with CO_2 fixation carried out by the Calvin cycle. Most species are also able to grow in darkness by using aerobic respiration of organic or even some inorganic compounds; synthesis of the photosynthetic machinery is typically repressed by O_2. Finally, some species can

Figure 15.12 Photograph of liquid cultures of phototrophic purple bacteria showing the color of species with various carotenoid pigments. All species contain bacteriochlorophyll *a*. The blue culture is a carotenoidless mutant strain of *Rhodospirillum rubrum* showing that bacteriochlorophyll *a* is actually blue. The bottle on the far right (*Rhodobacter sphaeroides* strain G) lacks one of the carotenoids of the wild type and thus is less red and more green.

also grow by fermentation or anaerobic respiration using a variety of electron donors and acceptors.

Enrichment and isolation of purple nonsulfur bacteria is easy using a mineral salts medium supplemented with an organic acid as carbon source. Such media, inoculated with a mud, lake water, or sewage sample and incubated anaerobically in the light, invariably select for purple nonsulfur bacteria. Enrichment cultures can be made even more selective by omitting fixed nitrogen sources (for example, ammonia) or organic nitrogen sources (for example, yeast extract or peptone) from the medium and supplying a gaseous headspace of N_2. Virtually all purple nonsulfur bacteria can fix N_2 (↔ Section 14.6) and will thrive under such conditions, rapidly outcompeting other bacteria.

Aerobic Anoxygenic Phototrophs

KEY GENERA: *Roseobacter, Erythrobacter*

The **aerobic anoxygenic phototrophs** are obligatory aerobic heterotrophs that use light as a supplemental source of energy to support growth. Like purple nonsulfur bacteria, aerobic anoxygenic phototrophs are phylogenetically diverse and are *Alphaproteobacteria* or *Betaproteobacteria*. The primary physiological difference with the purple nonsulfur bacteria is that aerobic anoxygenic phototrophs are strict heterotrophs and employ anoxygenic photosynthesis only under *oxic* conditions as a supplemental source of energy. Aerobic anoxygenic phototrophs contain bacteriochlorophyll *a* and a Q-type photosystem, but are unable to fix CO_2 and thus rely on organic forms of carbon as their carbon source. Carotenoids of various types lend colors of yellow, orange, or pink to cultures.

Aerobic anoxygenic phototrophs are only able to photosynthesize when grown on a day/night cycle. Under these conditions, bacteriochlorophyll *a* is made only in the dark and then used to conserve energy by photophosphorylation when the light returns. Aerobic anoxygenic phototrophs can account for as much as a quarter of the microbial community inhabiting coastal marine waters and 5% of gross photosynthesis in such systems

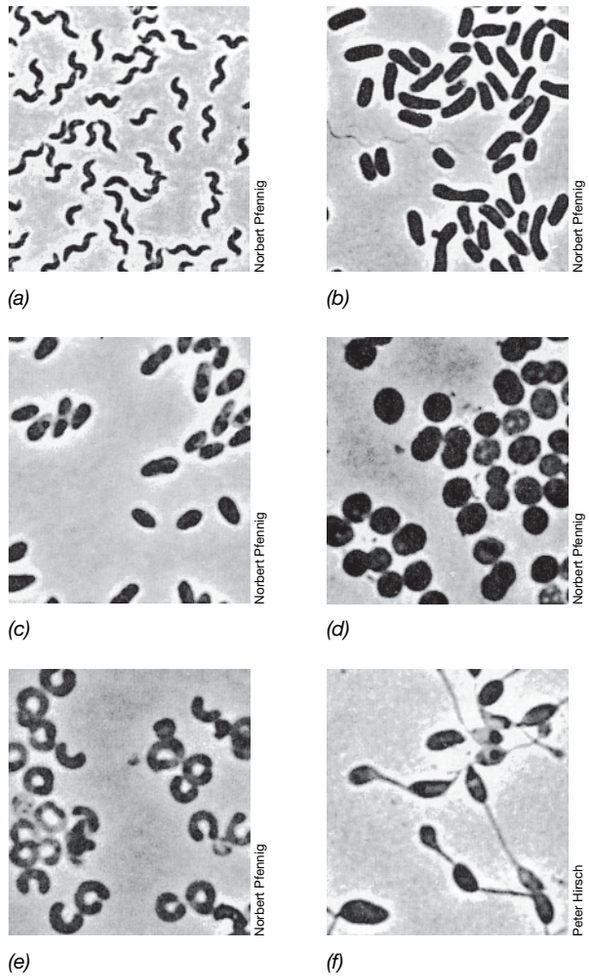


Figure 15.13 Representatives of several genera of purple nonsulfur bacteria. *(a) Phaeospirillum fulvum*; cells are about 3 μm long. *(b) Rhodoblastus acidophilus*; cells are about 4 μm long. *(c) Rhodobacter sphaeroides*; cells are about 1.5 μm wide. *(d) Rhodopila globiformis*; cells are about 1.6 μm wide. *(e) Rhodocyclus purpureus*; cells are about 0.7 μm in diameter. *(f) Rhodomicrobium vannielii*; cells are about 1.2 μm wide.

(↔ Section 20.10). Common genera found in coastal marine habitats include *Roseobacter* and *Erythrobacter*.

MINIQUIZ

- What are some similarities between purple nonsulfur bacteria and aerobic anoxygenic phototrophs? What are the differences between these two groups?
- Where would you expect to find aerobic anoxygenic phototrophs?

15.6 Green Sulfur Bacteria

KEY GENERA: *Chlorobium, Chlorobaculum, "Chlorochromatium"*

Green sulfur bacteria are a phylogenetically coherent group of anoxygenic phototrophs that forms the phylum *Chlorobi*. Green sulfur bacteria have little metabolic versatility and they are typically nonmotile and strictly anaerobic anoxygenic phototrophic

bacteria. The group is also morphologically restricted and includes primarily short to long rods (Figure 15.14).

Like purple sulfur bacteria, green sulfur bacteria oxidize hydrogen sulfide (H_2S) as an electron donor for autotrophic growth, oxidizing it first to sulfur (S^0) and then to sulfate (SO_4^{2-}). But unlike most purple sulfur bacteria, the S^0 produced by green sulfur bacteria is deposited only outside the cell (Figure 15.14*a*). Autotrophy is supported not by the reactions of the Calvin cycle, as in purple bacteria, but instead by a reversal of steps in the citric acid cycle (Section 14.5 and Figure 14.20*a*), a unique means of autotrophy in phototrophic bacteria.

Pigments and Ecology

Green sulfur bacteria contain bacteriochlorophyll *c, d*, or *e* and house these pigments in unique structures called **chlorosomes** (Figure 15.15). A small amount of bacteriochlorophyll *a* is present in the reaction center and FMO protein, the latter of which connects the chlorosome to the cytoplasmic membrane (Figure 14.7*b*). Chlorosomes are oblong bacteriochlorophyll-rich bodies bounded by a thin, nonunit membrane and attached to the cytoplasmic membrane in the periphery of the cell (Figure 15.15 and Figure 14.7). Chlorosomes function to funnel energy into the photosystem, and this eventually leads to ATP synthesis. Unlike purple anoxygenic phototrophs, green sulfur bacteria use an FeS-type photosystem. Both green- and brown-colored species of green sulfur bacteria are known, the brown-colored species containing bacteriochlorophyll *e* and carotenoids that turn dense cell suspensions brown (Figure 15.16).

Like purple sulfur bacteria (Section 15.4), green sulfur bacteria live in anoxic, sulfidic, illuminated aquatic environments. However, the chlorosome is a very efficient light-harvesting structure, which allows green sulfur bacteria to grow at light intensities much lower than those required by other phototrophs. Green sulfur bacteria also tend to have a greater tolerance of H_2S than do other anoxygenic phototrophs. As a result, green sulfur bacteria are typically found at the greatest depths of all phototrophic microorganisms in lakes or microbial mats, where light intensities are low and H_2S levels the highest.

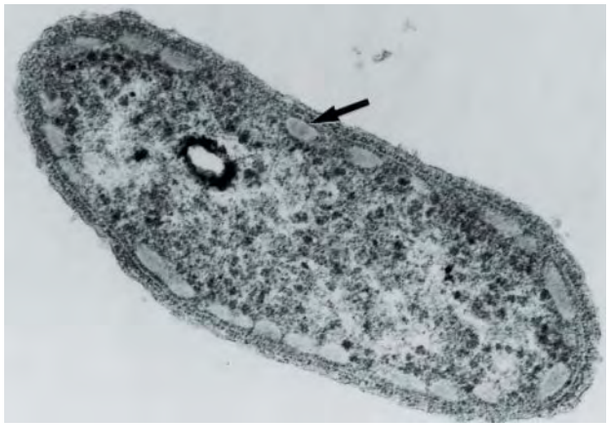
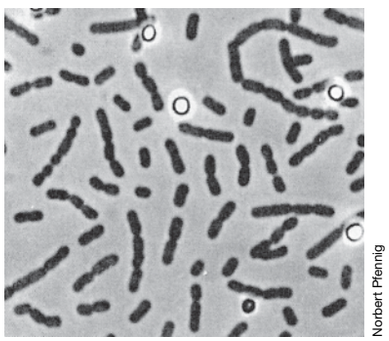


Figure 15.15 The thermophilic green sulfur bacterium *Chlorobaculum tepidum*. Transmission electron micrograph. Note chlorosomes (arrow) in the cell periphery. A cell is about 0.7 μm wide.

As an example, a species of green sulfur bacteria isolated from a deep-sea hydrothermal vent (Section 20.14) was found to be growing phototrophically on the weak glow of infrared radiation emitted from the geothermally heated rock. One species, *Chlorobaculum tepidum* (Figure 15.15), is thermophilic and forms dense microbial mats in high-sulfide hot springs. *C. tepidum* also grows rapidly and is amenable to genetic manipulation by both conjugation and transformation (Chapter 11). Because of these features, *C. tepidum* has become the model organism for studying the molecular biology of green sulfur bacteria.

Green Sulfur Bacteria Consortia

Certain species of green sulfur bacteria form an intimate two-membered association, called a **consortium**, with a chemoorganotrophic bacterium. In the consortium, each organism benefits, and thus a variety of such consortia containing different phototrophic and chemotrophic components probably exist in nature. The phototrophic component, called the *epibiont*, is physically attached to the nonphototrophic central



(a) (b)

Figure 15.14 Phototrophic green sulfur bacteria. *(a) Chlorobium limicola*; cells are about 0.8 μm wide. Note the spherical sulfur granules deposited extracellularly. *(b) Chlorobium clathratiforme*, a bacterium forming a three-dimensional network; cells are about 0.8 μm wide.

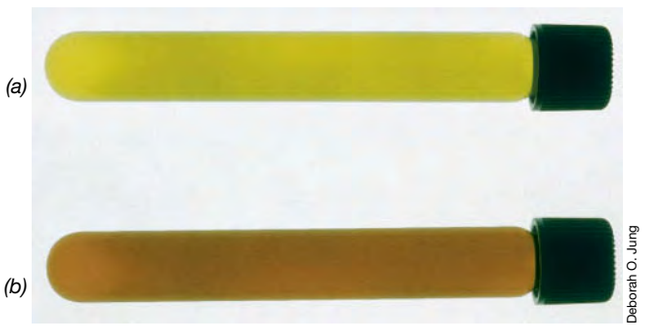


Figure 15.16 Green and brown chlorobia. Tube cultures of *(a) Chlorobaculum tepidum* and *(b) Chlorobaculum phaeobacteroides*. Cells of *C. tepidum* contain bacteriochlorophyll *c* and green carotenoids, and cells of *C. phaeobacteroides* contain bacteriochlorophyll *e* and isorenieratene, a brown carotenoid. The structures of bacteriochlorophylls *c* and *e* and green bacteria carotenoids were shown in Figures 14.3 and 14.9.

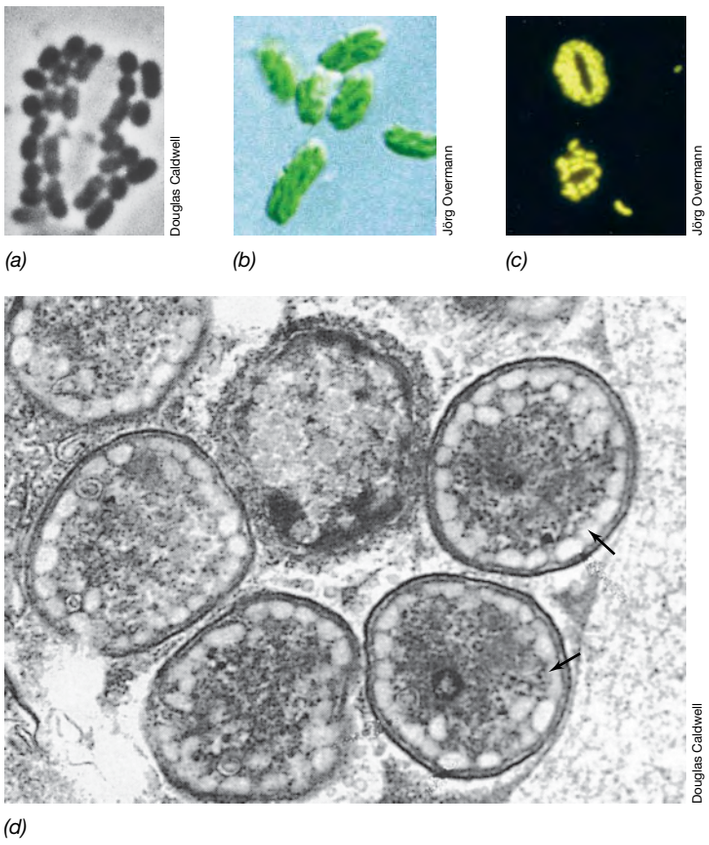


Figure 15.17 "*Chlorochromatium aggregatum*." Consortia of green sulfur bacteria and a chemoorganotroph. *(a)* In a phase-contrast micrograph, the nonphototrophic central organism is lighter in color than the pigmented phototrophic bacteria. *(b)* Green carotenoids lend their color to the phototrophs in a differential interference contrast micrograph. *(c)* A fluorescence micrograph shows the cells stained with a fluorescent probe specific for green sulfur bacteria. *(d)* Transmission electron micrograph of a cross section through a single consortium; note the chlorosomes (arrows) in the epibionts. The entire consortium is about 3 μm in diameter.

cell (**Figure 15.17**) and communicates with it in various ways (Section 23.2).

The name "*Chlorochromatium aggregatum*" (not a formal name because this is a mixed culture) has been used to describe a commonly observed green-colored consortium that is green because the epibionts are green sulfur bacteria that contain green-colored carotenoids (Figure 15.17*b*). Evidence that the epibionts are indeed green sulfur bacteria comes from pigment analyses, the presence of chlorosomes (Figure 15.17*d*), and phylogenetic staining (Figure 15.17*c*). A structurally similar consortium called "*Pelochromatium roseum*" is brown because its epibionts produce brown-colored carotenoids (Figures 23.3 and 23.4). We examine the symbiotic nature of the *Chlorochromatium* consortium in more detail in Section 23.2.

MINIQUIZ

- Which pigments are present in the chlorosome?
- What evidence exists that the epibionts of green bacterial consortia are truly green sulfur bacteria?

15.7 Green Nonsulfur Bacteria

KEY GENERA: *Chloroflexus, Heliothrix, Roseiflexus*

Green nonsulfur bacteria, which are also called *filamentous anoxygenic phototrophs*, are anoxygenic phototrophs of the phylum *Chloroflexi*. The latter contains several distinct lineages, one of which, the class *Chloroflexi*, contains green nonsulfur bacteria. The remainder of the phylum contains metabolically diverse organisms including both aerobic and anaerobic chemoorganotrophs as well as the *Dehalococcoidetes*, a group of dehalogenating bacteria that use halogenated organic compounds as electron acceptors in anaerobic respiration (Section 14.15). Analyses of 16S ribosomal RNA sequences from environmental samples (Section 19.6) indicate that species of the phylum *Chloroflexi* are widespread and that most species in the phylum have yet to be cultivated in isolation; thus the metabolic diversity of this phylum remains poorly characterized.

All cultured representatives of the green nonsulfur bacteria are filamentous bacteria that are capable of gliding motility. *Chloroflexus*, one of the most studied of the green nonsulfur bacteria, forms thick microbial mats in neutral to alkaline hot springs along with thermophilic cyanobacteria (**Figure 15.18**; Figure 20.7*b*). Green nonsulfur bacteria grow best as photoheterotrophs using simple carbon sources as electron donors for photosynthesis. However, growth also occurs photoautotrophically using H_2 or H_2S as electron donors for photosynthesis. The 3-hydroxypropionate bi-cycle, a pathway of CO_2 incorporation unique to only a few *Bacteria* and *Archaea*, supports autotrophic growth (Section 14.5). Most green nonsulfur bacteria also grow well in the dark by aerobic respiration of a wide variety of carbon sources.

The photosynthetic features of the green nonsulfur bacteria form a "hybrid" between those of both green sulfur bacteria (Section 15.6) and purple phototrophic bacteria (Sections 15.4, 15.5). Green nonsulfur bacteria have reaction centers that contain bacteriochlorophyll *a* and chlorosomes that contain bacteriochlorophyll *c* (Figure 15.15) and in this way are similar to green sulfur bacteria. However, in contrast to green sulfur bacteria, green nonsulfur bacteria contain a Q-type photosynthetic reaction center and in this respect resemble purple bacteria.

Other *Chloroflexi*

In addition to *Chloroflexus*, other phototrophic green nonsulfur bacteria include the thermophile *Heliothrix* and the large-celled mesophiles *Oscillochloris* (Figure 15.18*b*) and *Chloronema* (Figure 15.18*c*). *Oscillochloris* and *Chloronema* form rather large cells, 2–5 μm wide and up to several hundred micrometers long (Figure 15.18*c*). Species of both genera inhabit freshwater lakes containing H_2S. *Roseiflexus* and *Heliothrix* are similar to *Chloroflexus* in their filamentous morphology and thermophilic lifestyle, but differ in a major photosynthetic property. *Roseiflexus* and *Heliothrix* lack bacteriochlorophyll *c* and chlorosomes and thus more closely resemble purple phototrophic bacteria (Sections 15.4, 15.5) than *Chloroflexus*. This can be seen in cultures of *Roseiflexus* that are yellow-orange instead of green from their extensive carotenoid pigments and lack of bacteriochlorophyll *c* (Figure 15.18*d*).

Thermomicrobium is a nonphototrophic genus of *Chloroflexi* and a strictly aerobic, gram-negative rod, growing optimally in

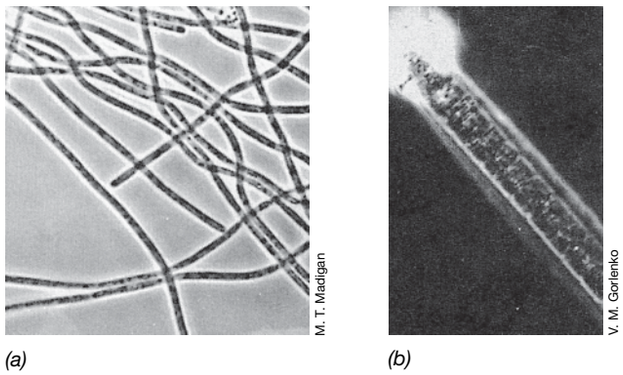


Figure 15.18 Green nonsulfur bacteria. *(a)* Phase-contrast micrograph of the anoxygenic phototroph *Chloroflexus aurantiacus*; cells are about 1 μm in diameter. *(b)* Phase-contrast micrograph of the large phototroph *Oscillochloris*; cells are about 5 μm wide. The brightly contrasting material on the top is a holdfast, used for attachment. *(c)* Phase-contrast micrograph of filaments of a *Chloronema* species; the cells are wavy filaments and about 2.5 μm in diameter. *(d)* Tube cultures of *C. aurantiacus* (right) and *Roseiflexus* (left). *Roseiflexus* is yellow because it lacks bacteriochlorophyll *c* and chlorosomes.

complex media at 75°C. Besides its phylogenetic properties, *Thermomicrobium* is also of interest because of its membrane lipids (**Figure 15.19**). Recall that the lipids of *Bacteria* and *Eukarya* contain fatty acids esterified to *glycerol* (↔ Section 2.3). By contrast, the lipids of *Thermomicrobium* are formed on *1,2-dialcohols* instead of glycerol, and have neither ester *nor* ether linkages (Figure 15.19; ↔ Section 2.3). In addition, cells of *Thermomicrobium* contain only small amounts of peptidoglycan, and the cell wall is composed primarily of protein.

MINIQUIZ

- In what ways do *Chloroflexus* and *Roseiflexus* resemble *Chlorobium*? *Rhodobacter*?
- What is unique about *Thermomicrobium*?

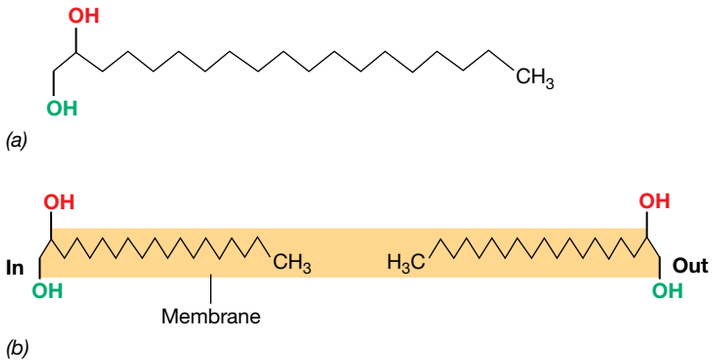


Figure 15.19 The unusual lipids of *Thermomicrobium*. *(a)* Membrane lipids from *Thermomicrobium roseum* contain long-chain diols like the one shown here (1,2-nonadecanediol). Note that unlike the lipids of other *Bacteria* or of *Archaea*, neither ester- nor ether-linked side chains are present. *(b)* To form a bilayer membrane, dialcohol molecules oppose each other at the methyl groups, and the —OH groups are the inner and outer hydrophilic surfaces. Small amounts of the diols have fatty acids esterified to the secondary —OH group (shown in red), whereas the primary —OH group (shown in green) can bond a hydrophilic molecule like phosphate.

15.8 Other Phototrophic *Bacteria*

KEY GENERA: *Heliobacterium, Chloracidobacterium*

Heliobacteria

Heliobacteria are a phylogenetically coherent group of phototrophic gram-positive *Bacteria* found within the phylum *Firmicutes*. The heliobacteria are anoxygenic phototrophs that have an FeS-type photosystem and that produce a unique pigment, bacteriochlorophyll *g* (↔ Figure 14.3). Heliobacteria grow photoheterotrophically using a narrow range of organic compounds including pyruvate, lactate, acetate, or butyrate, and the group contains five genera: *Heliobacterium, Heliophilum, Heliorestis, Heliomonas*, and *Heliobacillus*. All known heliobacteria form rod-shaped or filamentous cells (**Figure 15.20**), although *Heliophilum* is unusual because its cells form into bundles (Figure 15.20*b*) that are motile as a unit.

Heliobacteria are strict anaerobes, but in addition to phototrophic growth, they can grow chemotrophically in darkness by pyruvate fermentation (as can many clostridia, close relatives of the heliobacteria). Heliobacteria produce endospores, the highly resistant structures produced by certain gram-positive bacteria (↔ Section 2.10). Like the endospores of *Bacillus* or *Clostridium* species, the endospores of heliobacteria (Figure 15.20*c*) contain elevated calcium (Ca^{2+}) levels and the signature molecule of the endospore, *dipicolinic acid*. Heliobacteria reside in soil, especially paddy (rice) field soils, where their nitrogen fixation activities (↔ Section 14.6) may benefit rice productivity. A large diversity of heliobacteria have also been found in highly alkaline environments, such as soda lakes and surrounding alkaline soils.

Phototrophic *Acidobacteria*

A novel group of anoxygenic phototrophs has been discovered growing in photosynthetic microbial mats of certain thermal springs in Yellowstone National Park. *Chloracidobacterium thermophilum* is a thermophilic oxygen-tolerant anoxygenic

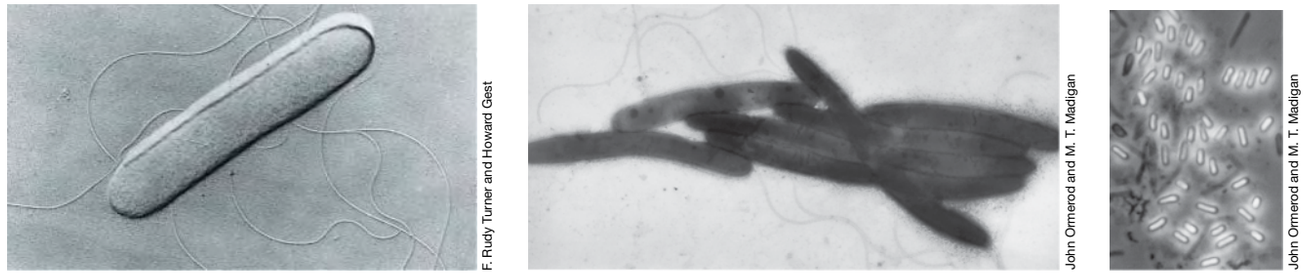


Figure 15.20 Cells and endospores of heliobacteria. *(a)* Electron micrograph of *Heliobacillus mobilis*, a peritrichously flagellated species. *(b) Heliophilum fasciatum* cell bundles as observed by electron microscopy. *(c)* Phase-contrast micrograph of endospores from *Heliobacterium gestii*. Most heliobacteria cells are about 1–2 μm in diameter.

phototroph of the phylum *Acidobacteria* (Section 16.21). Similar to green sulfur bacteria, *C. thermophilum* produces bacteriochlorophyll *a* and *c*, the latter in chlorosomes (Figure 15.21), and uses an FeS-type photosystem. However, unlike green sulfur bacteria, *C. thermophilum* can also grow aerobically, as is true for the aerobic anoxygenic phototrophs (Section 15.5). In terms of its carbon metabolism, *C. thermophilum* is a photoheterotroph that uses short-chain fatty acids as carbon sources, but unlike green sulfur or green nonsulfur bacteria, it is incapable of autotrophy.

Phototrophic *Gemmatimonadetes*

Another novel group of anoxygenic phototrophs has been discovered in a freshwater lake in the western Gobi desert (China and Mongolia). *Gemmatimonas phototrophica* is an aerobic facultative photoheterotroph of the phylum *Gemmatimonadetes*. It gains most of its energy through the aerobic respiration of organic compounds, whether in the light or in the dark. However, in the light, *G. phototrophica* uses photophosphorylation to supplement energy generated by aerobic respiration. *G. phototrophica* cannot grow as an obligate phototroph, it cannot fix CO_2, and it cannot grow anaerobically. *G. phototrophica* contains a photosynthetic gene cluster that resembles those of aerobic anoxygenic phototrophs (Section 15.5), and it produces bacteriochlorophyll *a* and a Q-type reaction center, both of which are characteristic properties of purple bacteria (Section 14.3). It thus seems likely that *G. phototrophica* acquired its photosynthetic gene cluster and the ability to perform photophosphorylation as the result of an ancient horizontal gene transfer event.

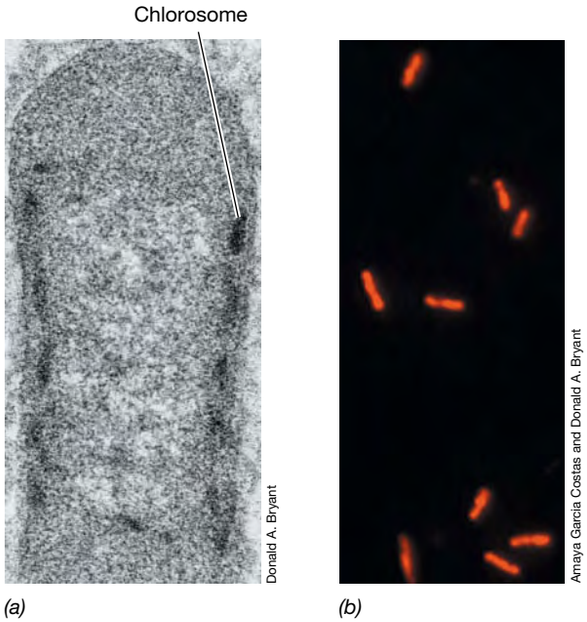


Figure 15.21 Chlorosomes in *Chloracidobacterium thermophilum*, a phototrophic member of the phylum *Acidobacteria*. *(a)* Electron micrograph of *C. thermophilum* showing chlorosomes. (*b*) Fluorescence photomicrograph of *C. thermophilum*. The red color is the fluorescence of bacteriochlorophyll *c* present in chlorosomes. A cell of *C. thermophilum* is about 0.8 μm wide.

MINIQUIZ

- What types of anoxygenic phototrophs contain chlorosomes?
- What kind of phototrophic bacteria make endospores?

III • Microbial Diversity in the Sulfur Cycle

Sulfur metabolism may have fueled the earliest forms of life on our planet (Section 13.1), and the sulfur cycle (Section 21.4) continues to support an enormous diversity of microorganisms. In this section we consider the diversity of organisms capable of *dissimilative sulfur metabolism*; that is, organisms that conserve energy through the oxidation or reduction of sulfur compounds (Sections 14.9 and 14.14).

The remarkable diversity of *Bacteria* and *Archaea* capable of dissimilative sulfur metabolism is in part a function of the chemical diversity in which sulfur occurs in the biosphere. Sulfur has eight oxidation states that range from its most oxidized form, sulfate (SO_4^{2-}, oxidation state of +6), to thiosulfate ($S_2O_3^{2-}$, oxidation state of +2), to elemental sulfur (S^0, oxidation state of 0), and finally to hydrogen sulfide (H_2S, oxidation state of −2), its most reduced form. In addition, sulfur compounds can take on diverse chemical forms including inorganic sulfur compounds, organosulfur compounds, and metal sulfides.

In this part of the chapter we will focus on the diversity of **dissimilative sulfate-reducers, dissimilative sulfur-reducers**, and **dissimilative sulfur-oxidizers**. Anoxygenic phototrophs, such as the purple and green sulfur bacteria discussed in Sections 15.4–15.6, are also important links in the sulfur cycle. However, here we restrict our focus to chemotrophic dissimilative sulfur metabolisms.

15.9 Dissimilative Sulfate-Reducers

KEY GENERA: *Desulfovibrio, Desulfobacter*

Sulfate-reducing bacteria gain energy by coupling the oxidation of H_2 or organic compounds to the reduction of SO_4^{2-} (anaerobic respiration). There are more than 30 known genera of sulfate reducers found across five phyla of *Bacteria* and *Archaea* (**Figure 15.22**). Most sulfate reducers reside in the *Deltaproteobacteria*, though sulfate reducers are also found in the *Firmicutes* (e.g., *Desulfotomaculum* and *Desulfosporosinus*), *Thermodesulfobacteria* (e.g., *Thermodesulfobacterium)*, and *Nitrospirae* (e.g., *Thermodesulfovibrio*). Sulfate reduction also occurs in *Archaeoglobus*, a genus of the archaeal phylum *Euryarchaeota*.

Physiology of Sulfate-Reducing Bacteria

Sulfate-reducing bacteria are morphologically and biochemically diverse. The biochemistry of sulfate reduction was discussed in Section 14.14, so here we consider some of the more general physiological properties of this group. Sulfate reducers are generally obligate anaerobes, and strict anoxic techniques must be used in their cultivation (**Figure 15.23*g***).

Sulfate reducers use H_2 or organic compounds as electron donors for growth, and the range of organics used is fairly broad. Lactate and pyruvate are almost universally used, and many species also oxidize short-chain alcohols (ethanol, propanol, and butanol) as electron donors. Some species, such as *Desulfosarcina and Desulfonema*, grow chemolithotrophically and autotrophically with H_2 as an electron donor, SO_4^{2-} as an electron acceptor, and CO_2 as the sole carbon source. A few sulfate reducers can oxidize hydrocarbons as electron donors (Section 14.25).

There are two physiological types of dissimilative sulfate-reducers, the *complete oxidizers*, which can oxidize acetate and other fatty acids completely to CO_2, and the *incomplete oxidizers*, which are unable to oxidize acetate to CO_2. The latter group includes the best studied of the sulfate-reducing bacteria, *Desulfovibrio* (Figure 15.23*a*), along with *Desulfomonas, Desulfotomaculum*, and *Desulfobulbus* (Figure 15.23*c*). The acetate oxidizers include *Desulfobacter* (Figure 15.23*d*), *Desulfococcus, Desulfosarcina* (Figure 15.23*e*), and *Desulfonema* (Figure 15.23*b*), among many others. These bacteria specialize in the oxidation of fatty acids (in particular acetate) to CO_2, and reducing SO_4^{2-} to H_2S. These two physiological groups are not phylogenetically coherent but instead are distributed widely across the phylogeny of sulfate-reducing bacteria (Figure 15.22).

Some sulfate-reducing bacteria can exploit alternative metabolic pathways. In addition to SO_4^{2-} or S^0, some sulfate reducers can also reduce nitrate and sulfonates (such as isethionate, $HO—CH_2—CH_2—SO_3^-$). Certain organic compounds can also be fermented by sulfate-reducing bacteria. The most common of these is pyruvate, which is fermented by way of the phosphoroclastic reaction to acetate, CO_2, and H_2 (Figure 14.55). Moreover, although generally obligate anaerobes, a few sulfate-reducing bacteria are quite O_2-tolerant (primarily strains that coexist with O_2-producing cyanobacteria in microbial mats). At least one species, *Desulfovibrio oxyclinae*, can actually grow with O_2 as the electron acceptor under microaerophilic conditions.

Ecology of Sulfate-Reducing Bacteria

Sulfate reducers are widespread in aquatic and terrestrial environments that contain SO_4^{2-} and become anoxic as a result of microbial decomposition. Sulfate reducers are abundant in marine sediments, and the H_2S they generate is responsible for the pungent smell (like that of rotten eggs) often encountered in decaying vegetation near coastal ecosystems. *Desulfotomaculum*, phylogenetically a species of *Firmicutes* (gram-positive *Bacteria*), consists of endospore-forming rods found primarily in soil. Growth and reduction of SO_4^{2-} by *Desulfotomaculum* in certain

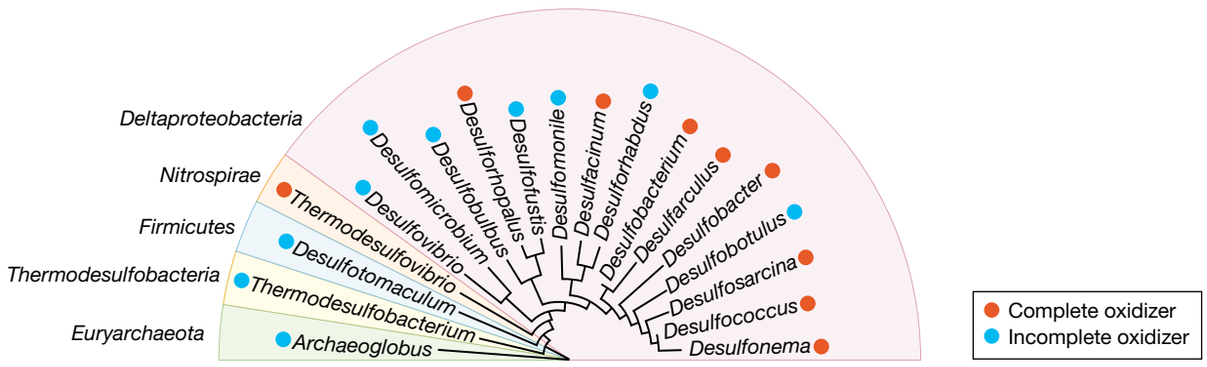


Figure 15.22 Dissimilative sulfate-reducers. The dendrogram depicts phylogenetic relationships among some genera of sulfate reducers as inferred by analysis of their 16S ribosomal RNA gene sequences. Color shading is used to differentiate the five main phyla that contain genera of sulfate reducers. Colored circles indicate whether species are complete oxidizers, which are able to oxidize acetate to CO_2, or incomplete oxidizers, which cannot oxidize acetate. The physiology of sulfate-reducing bacteria is considered in Section 14.14, and their role in the sulfur cycle in Section 21.4.

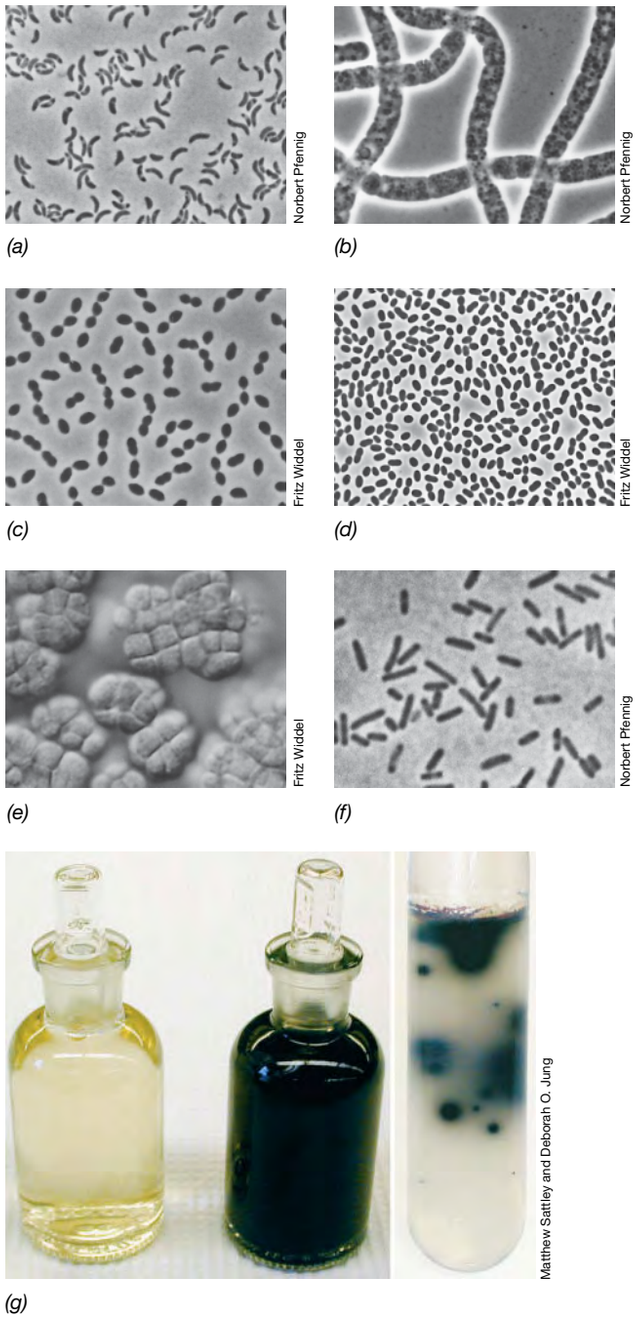


Figure 15.23 Representative sulfate-reducing and sulfur-reducing bacteria. *(a) Desulfovibrio desulfuricans*; cell diameter about 0.7 μm. *(b) Desulfonema limicola*; cell diameter 3 μm. *(c) Desulfobulbus propionicus*; cell diameter about 1.2 μm. *(d) Desulfobacter postgatei*; cell diameter about 1.5 μm. *(e) Desulfosarcina variabilis*; cell diameter about 1.25 μm. *(f) Desulfuromonas acetoxidans*; cell diameter about 0.6 μm. *(g)* Enrichment culture of sulfate-reducing bacteria. Left, sterile medium; center, a positive enrichment showing black FeS; right, colonies of sulfate-reducing bacteria in a dilution tube (Sections 19.1 and 19.2). Photos *a–d* and *f* are phase-contrast photomicrographs; part *e* is an interference contrast micrograph.

canned foods leads to a type of spoilage called *sulfide stinker*. Species of *Thermodesulfobacterium, Thermodesulfovibrio*, and *Archaeoglobus* (an archaeon) are all thermophilic and found in geothermally heated environments such as hot springs, hydrothermal vents, and oil reserves. The remaining genera of sulfate reducers are indigenous to anoxic marine and freshwater environments and can occasionally be isolated from the mammalian gut.

The enrichment of *Desulfovibrio* species is straightforward in an anoxic lactate–sulfate medium containing ferrous iron (Fe^{2+}). A reducing agent, such as thioglycolate or ascorbate, is required to achieve a low reduction potential (E_0')in the medium. When sulfate-reducing bacteria grow, the H_2S they form combines with the ferrous iron to form black, insoluble ferrous sulfide (Figure 15.23*g*). Purification can be accomplished by diluting the culture in molten agar tubes (Section 19.2 and Figure 19.3*b*). Upon solidification, individual cells of sulfate-reducing bacteria become distributed throughout the agar and grow to form black colonies (Figure 15.23*g*) that can be removed aseptically to yield pure cultures.

MINIQUIZ

- What are the typical electron donors used by dissimilative sulfate-reducers?
- What bacterial phyla are known to contain dissimilative sulfate-reducers?

15.10 Dissimilative Sulfur-Reducers

KEY GENERA: *Desulfuromonas, Wolinella, Sulfolobus*

Here we consider the dissimilative *sulfur*-reducers, microorganisms that are able to use the respiratory reduction of S^0 to conserve energy. Dissimilative sulfur-reducing bacteria can reduce S^0 and other oxidized forms of sulfur (such as SO_3^{2-}) to H_2S but are unable to reduce SO_4^{2-}. There are more than 25 genera of dissimilative sulfur-reducers spread across five bacterial and archaeal phyla (Figure 15.1).

Most sulfur-reducing bacteria are *Proteobacteria*, primarily *Deltaproteobacteria* (e.g., *Desulfuromonas, Pelobacter, Desulfurella, Geobacter*), with some genera residing in the *Epsilonproteobacteria* (e.g., *Wolinella* and *Sulfurospirillum*) and *Gammaproteobacteria* (e.g., *Shewanella* and *Pseudomonas mendocina*). Other sulfur-reducing bacteria are species of *Firmicutes* (e.g., *Desulfitobacterium* and *Ammonifex*), *Aquificae* (e.g., *Desulfurobacterium* and *Aquifex*), *Synergistetes* (e.g., *Dethiosulfovibrio*), or *Deferribacteres* (e.g., *Geovibrio*). The sulfur-reducing *Archaea*—of which there are many—are all genera of the phylum *Crenarchaeota* (e.g., *Acidianus, Sulfolobus, Pyrodictium*, and *Thermodiscus*).

Physiology and Ecology of Sulfur-Reducing Bacteria

The physiology of sulfur reducers is more diverse than that of sulfate reducers. Most sulfur reducers are obligate anaerobes, but facultatively aerobic species are also common. Sulfur reducers are often able to reduce electron acceptors such as nitrate, ferrous iron, or thiosulfate as alternatives to S^0. Like sulfate reducers (Section 15.9), the physiology of sulfur reducers is characterized by whether they completely oxidize acetate and other fatty acids to CO_2. Species of *Desulfuromonas* (Figure 15.23*f*) are complete oxidizers that grow anaerobically by coupling the oxidation of acetate, succinate, ethanol, or propanol to the reduction of S^0.

In contrast, *Sulfospirillum* and *Wolinella* are incomplete oxidizers and cannot use acetate as an electron donor. *Sulfospirillum* can reduce S^0 using either H_2 or formate as electron donor.

Dissimilative sulfur-reducing bacteria reside in many of the same habitats as dissimilative sulfate-reducing bacteria and often form associations with bacteria that oxidize H_2S to S^0, such as green sulfur bacteria (Section 15.6). The S^0 produced from H_2S oxidation is then reduced back to H_2S during metabolism of the sulfur reducer, completing an anoxic sulfur cycle (↔ Section 21.4).

MINIQUIZ

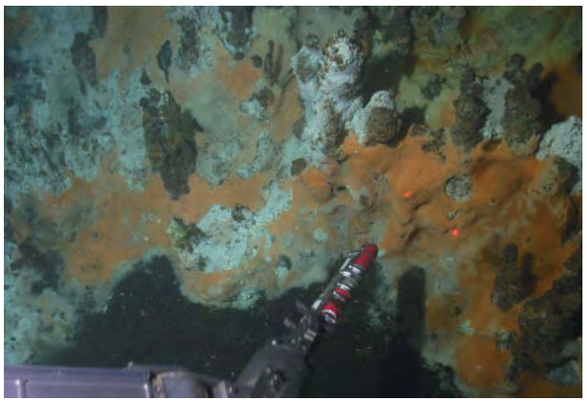
- What are the typical electron donors used by dissimilative sulfur-reducers?
- What bacterial phyla contain dissimilative sulfur-reducers?

15.11 Dissimilative Sulfur-Oxidizers

KEY GENERA: *Thiobacillus, Achromatium, Beggiatoa*

Dissimilative sulfur-oxidizers are **chemolithotrophs** that oxidize reduced sulfur compounds such as H_2S, S^0, thiosulfate, or thiocyanate ($^-$SCN) as electron donors in energy conservation, typically with O_2 as electron acceptor. These organisms are common in environments such as marine sediments, sulfur springs, and hydrothermal systems where H_2S produced by sulfate- or sulfur-reducing bacteria (Sections 15.9, 15.10), or abiotically by geothermal reactions, is released into oxygenated waters (**Figure 15.24**). The sulfur oxidizers are found in three phyla of *Bacteria* (*Proteobacteria, Aquificae, Deinococcus–Thermus*) and one of *Archaea* (*Crenarchaeota*) (Figure 15.1). Most sulfur-oxidizing bacteria are *Beta-* (*Thiobacillus*), *Gamma-* (*Achromatium, Beggiatoa*), or *Epsilonproteobacteria* (*Thiovulum, Sulfurimonas*).

(a)



(b)

Figure 15.24 Habitats of sulfur oxidizers. *(a)* A sulfidic artesian spring in Florida (USA). The outside of the spring is coated with a mat of *Thiothrix* (see Figure 15.26*b*). The mat is about 1.5 m in diameter. *(b)* Hydrothermal chimneys at Cathedral Hill in the Guaymas Basin (Mexico), 2000-m depth. Sulfide-rich waters vent from the chimneys, which are covered by mats composed of orange, white, and yellow cells of *Beggiatoa*.

Physiological Diversity of Sulfur-Oxidizing Bacteria

The morphological and physiological diversity of sulfur oxidizers is significant. Cells can be less than 1 micrometer in diameter (e.g., *Sulfurimonas denitrificans*) or as large as 750 micrometers in diameter (e.g., *Thiomargarita namibiensis*). Most sulfur oxidizers are obligate aerobes; however, species of *Thiomargarita* and *Sulfurimonas* can also reduce NO_3^- in denitrification (↔ Section 14.13 and Section 15.13). Many species oxidize H_2S to elemental sulfur (S^0), which they deposit as either intracellular or extracellular granules for later use as an electron donor (↔ Figure 14.27) if H_2S becomes limiting.

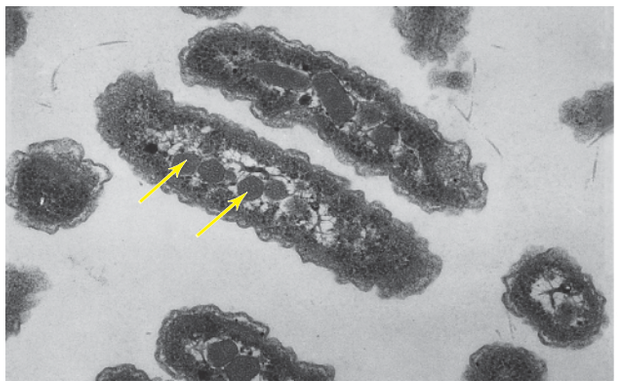
Some sulfur chemolithotrophs are *obligate chemolithotrophs*, locked into a lifestyle of using inorganic instead of organic compounds as electron donors. When growing in this fashion, they are also autotrophs, converting CO_2 into cell material by reactions of the Calvin cycle. **Carboxysomes** are often present in cells of obligate chemolithotrophs (**Figure 15.25*a***). These structures contain high levels of Calvin cycle enzymes and probably increase the rate at which these organisms fix CO_2 (↔ Section 14.5).

Other sulfur chemolithotrophs are *facultative chemolithotrophs*, facultative in the sense that they can grow *either* chemolithotrophically (and thus, also as autotrophs) *or* chemoorganotrophically. Most species of *Beggiatoa* can obtain energy from the oxidation of inorganic sulfur compounds but lack enzymes of the Calvin cycle. They thus require organic compounds as carbon sources. Organisms that use a mix of carbon and energy sources, for example those that simultaneously assimilate carbon from both CO_2 and organic sources, are called **mixotrophs**.

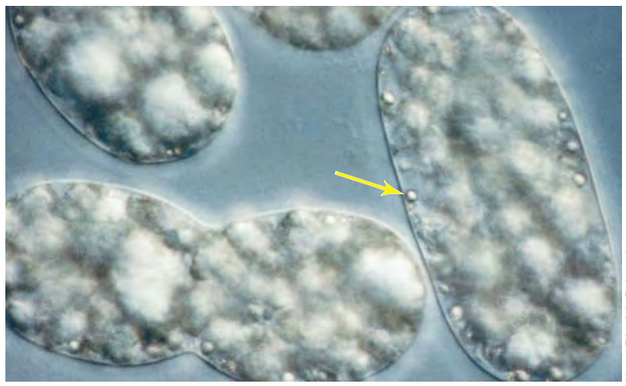
Thiobacillus and *Achromatium*

The genus *Thiobacillus* and related genera include several gram-negative, rod-shaped *Betaproteobacteria* (Figure 15.25*a*) that are the best studied of the sulfur chemolithotrophs. The oxidation of H_2S, S^0, or thiosulfate by *Thiobacillus* generates sulfuric acid (H_2SO_4), and thus thiobacilli are often acidophilic. One highly acidophilic species, *Acidithiobacillus ferrooxidans*, can also grow chemolithotrophically by the oxidation of Fe^{2+} and is a major biological agent for the oxidation of this metal. Iron pyrite (FeS_2) is a major natural source of ferrous iron as well as of sulfide. The oxidation of FeS_2, especially in mining operations, can be both beneficial (because leaching of the ore releases the iron from the sulfide mineral) and ecologically disastrous (the environment can become acidic and contaminated with toxic metals such as aluminum, cadmium, and lead) (↔ Sections 22.1 and 22.2).

Achromatium is a spherical sulfur-oxidizing chemolithotroph that is common in freshwater sediments of neutral pH containing H_2S. Cells of *Achromatium* are large cocci that can have diameters



(a)



(b)

Figure 15.25 Nonfilamentous sulfur chemolithotrophs. *(a)* Transmission electron micrograph of cells of the chemolithotrophic sulfur-oxidizer *Halothiobacillus neapolitanus*. A single cell is about 0.5 µm in diameter. Note the polyhedral bodies (carboxysomes) distributed throughout the cell (arrows) (Figure 14.19). *(b) Achromatium*. Cells photographed by differential interference contrast microscopy. The small globular structures near the periphery of the cells (arrow) are elemental sulfur, and the large granules are calcium carbonate. A single *Achromatium* cell is about 25 µm in diameter.

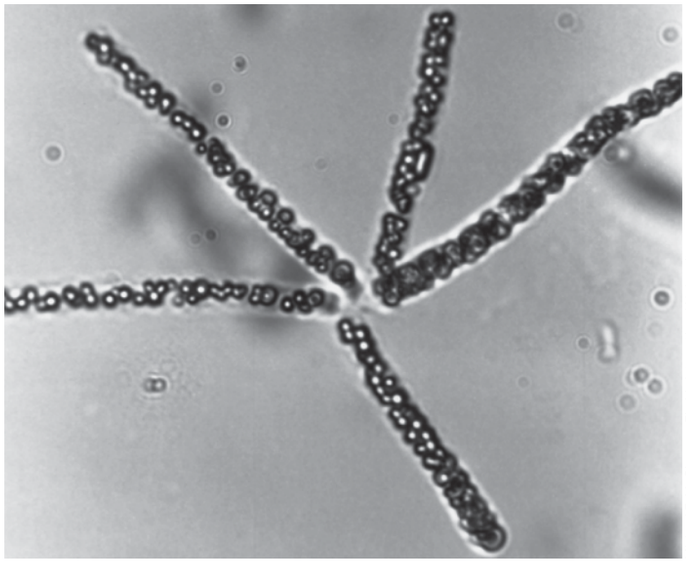
of 10–100 µm (Figure 15.25*b*). *Achromatium* is a species of *Gammaproteobacteria* and is specifically related to purple sulfur bacteria, such as its phototrophic counterpart *Chromatium* (Section 15.4 and Figure 15.10*a*). Like *Chromatium*, cells of *Achromatium* store S^0 internally (Figure 15.25*b*); the granules later disappear as S^0 is oxidized to SO_4^{2-}. Cells of *Achromatium* also store large granules of calcite ($CaCO_3$) (Figure 15.25*b*), possibly as a carbon source (in the form of CO_2) for autotrophic growth. The physiology of chemolithotrophic sulfur-oxidizers is discussed in Section 14.9.

Ecological Diversity and Strategies of Sulfide-Oxidizing Bacteria

Aerobic sulfide-oxidizers provide a case study that demonstrates the degree of ecological diversification that can occur among microbes that share the same basic metabolic features. The chemical oxidation of H_2S to H_2SO_4 is spontaneous and rapid in the presence of O_2. Hence, aerobic H_2S-oxidizers have evolved diverse ecological strategies that allow them to metabolize two molecules that otherwise react with each other spontaneously. We consider here six different strategies used by aerobic sulfide-oxidizers to cope with the chemical instability of H_2S in the presence of O_2.

1. *Thiothrix* is a filamentous sulfur chemolithotroph that forms filaments that group together at their ends by way of a holdfast to form cell arrangements called *rosettes* (**Figure 15.26**). The ecological strategy of *Thiothrix* is to use its holdfast to position itself in high-flow environments downstream from a source of H_2S. Such environments are common near sulfur springs and in creeks draining sulfidic salt marshes where abundant H_2S is produced and carried away in waters rich with O_2 (Figure 15.26*a*). Physiologically, *Thiothrix* is an obligately aerobic mixotroph, and in this and most other respects it resembles *Beggiatoa*.
2. *Beggiatoa* are filamentous, gliding, sulfur-oxidizing bacteria that are usually large in both diameter and length, consisting of many short cells attached end to end (**Figure 15.27*a***). Filaments can flex and twist so that many filaments become intertwined to form a complex tuft. *Beggiatoa* is found primarily in microbial mats, sediments, sulfur springs, and hot springs. The ecological strategy of *Beggiatoa* is to use gliding motility to position itself at the point where H_2S and O_2 co-occur in an environment. For example, *Beggiatoa* in microbial mats can move vertically by as much as several centimeters per day in response to cyanobacterial O_2 production, moving up to obtain O_2 when photosynthesis ceases at night and down during the day when cyanobacterial O_2 production at the mat surface causes H_2S to be found deeper in the mat.
3. The genus *Thiomargarita* contains some of the largest bacteria yet observed, with diameters that can be as much as 0.75 millimeter (**Figure 15.28**). *Thiomargarita* is nonmotile, and its ecological strategy is to separate in time the oxidation of H_2S from the reduction of O_2. To accomplish this, *Thiomargarita* contains a giant vacuole (Figure 15.28*b*) that it fills with high concentrations of nitrate (NO_3^-). This vacuole can fill almost the entire volume of the cell. Cells live in sulfide-rich marine sediments that are mixed occasionally with O_2-rich waters, such as that in salt marshes and in ocean upwelling zones. When buried in sediment, cells oxidize H_2S to S^0 anaerobically by reducing NO_3^- stored in the vacuole to ammonium (NH_4^+). They then store the S^0 as intracellular granules (Figure 15.28*a*). When turbulent waters mix the cells into the water column where H_2S is lacking, they switch to the aerobic oxidation of stored S^0. The energy they gain from S^0 oxidation is used to refill their vacuole with NO_3^- from the water column so they will be able to survive the next period of anoxia.
4. *Thioploca* are large filamentous bacteria that use a strategy similar to that of *Thiomargarita*. *Thioploca* also have intracellular S^0 granules and large vacuoles filled with NO_3^- (Figure 15.27*b*). However, filaments of *Thioploca* are motile by gliding and they occur in large sheaths that can be filled with many parallel filaments (Figure 15.27*b*). Sheaths are arranged vertically in the sediments and filaments glide up and down in the sheaths, going down to anaerobically respire H_2S using stored NO_3^- as electron acceptor and going up to aerobically respire S^0 and to refill their vacuoles with NO_3^- (Figure 20.8).

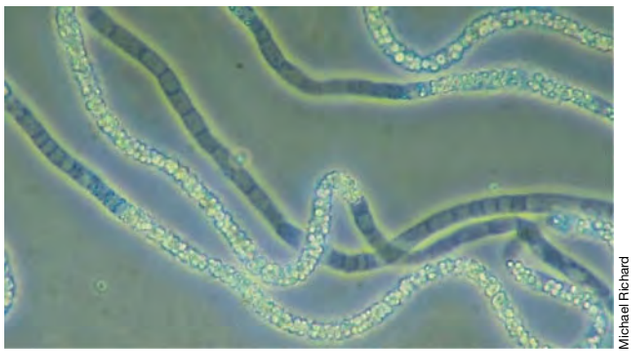
(a)



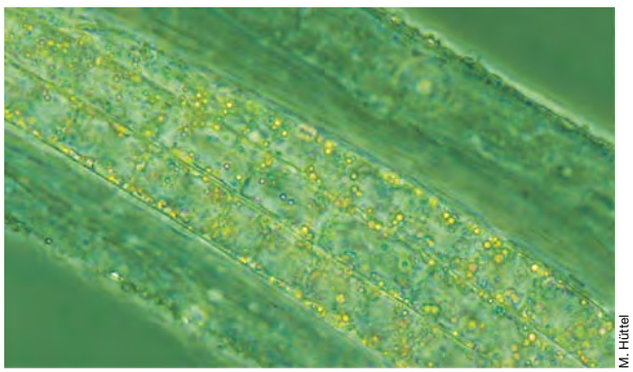
(b)

Figure 15.26 *Thiothrix.* *(a)* Filaments of *Thiothrix* attached to plant material found in the outwash stream of a sulfidic cave in Frasassi, Italy. From the plant branch point, the longest branch is about 4 mm long. *(b)* Phase-contrast photomicrograph of a rosette of cells of *Thiothrix* isolated from the sulfide-containing artesian spring shown in Figure 15.24*a*. Note the internal sulfur globules produced from the oxidation of sulfide. Each filament is about 4 μm in diameter.

5. *Thiovulum* are found in freshwater and marine habitats in which sulfide-rich muds interface with oxic zones (**Figure 15.29**). *Thiovulum* cells are fairly large (10–20 μm) cocci, and when motile, they swim at exceptionally high speed, perhaps the fastest of all known bacteria (~0.6 mm/sec). The ecological strategy of *Thiovulum* is to actually control the flow of nutrients to cells. *Thiovulum* cells secrete a slime that links cells together in a veil-like structure that can be centimeters in diameter (Figure 15.29*a*). The veils, composed of many *Thiovulum* cells, are formed over a source of H_2S. Cells have long flagella that attach to the veil and to solid surfaces. Since the terminal end of the flagellum is attached and immobile, flagellar rotation causes cells to rotate along their flagellar axis. The simultaneous unidirectional rotation of all of the *Thiovulum* cells in the veil creates a flow of water through the veil, allowing the cells to generate and regulate the gradients of H_2S and O_2 they require to generate energy.



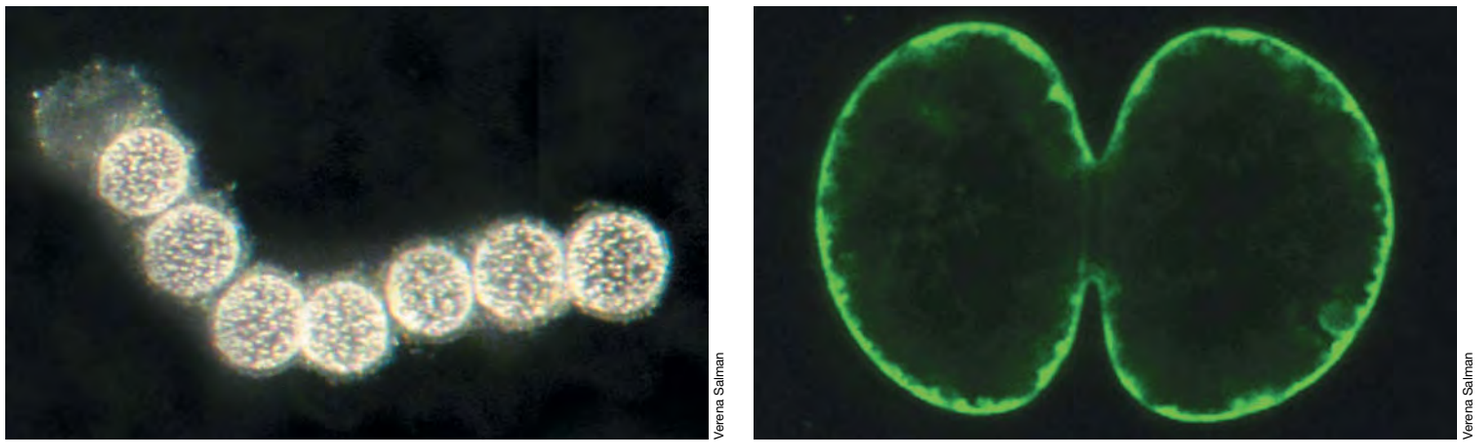
(a)



(b)

Figure 15.27 Filamentous sulfur-oxidizing bacteria. *(a)* Phase-contrast photomicrograph of a *Beggiatoa* species isolated from a sewage treatment plant. Note the abundant elemental sulfur granules in some of the cells. *(b)* Cells of a large marine *Thioploca* species. Cells contain sulfur granules (yellow) and are about 40–50 μm wide.

6. The final ecological strategy of sulfur chemolithotrophs is for the organism to form a symbiotic association with a eukaryote. There are diverse symbiotic associations in which the host provides a mechanism for regulating H_2S and O_2 levels and the sulfide-oxidizing symbiont fixes CO_2 and provides a source of carbon and energy to the host. The best example is the tube worm *Riftia*, which contains sulfide-oxidizing endosymbionts and lives at deep-sea hydrothermal vents (Section 23.9). A variety of other such symbiotic associations are present at hydrothermal vent ecosystems, including symbionts living in the gill tissue of the giant clam *Calyptogena magnifica* and on the surface of the yeti crab, which farms sulfide-oxidizing bacteria by waving its claws over sulfide-rich vent fluid. Symbioses involving invertebrates are also common in the sulfide-rich marine sediments of shallow coastal systems. For example, bivalves in the family *Solemyidae* burrow into sulfide-rich sediments and pump sulfide- and oxygen-rich water over gills that contain sulfide-oxidizing bacteria.



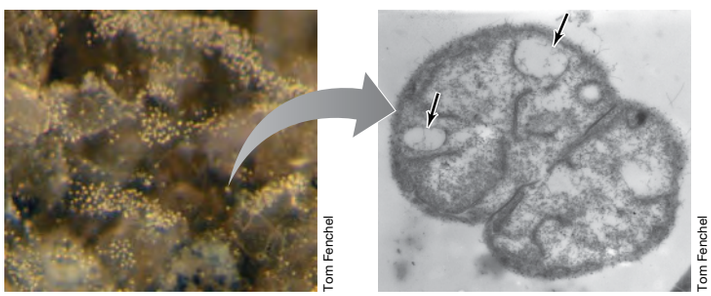
(a) (b)

Figure 15.28 The giant sulfide-oxidizing bacterium, *Thiomargarita*. *(a) Thiomargarita namibiensis* recovered from the Namibian upwelling (off the Namibian coast, southwest Africa). Cells are about 100 μm in diameter. *(b)* Dividing cells of vacuole-containing sulfide-oxidizers recovered from the same location. The fluorescence micrograph shows ribosomes of *Thiomargarita* stained with a fluorescent nucleic acid probe. Ribosomes are found in the cytoplasm, which is present as a thin layer along the outer edge of the cells. The cytoplasm is squeezed between the cell wall and the large central vacuole, which appears dark in the image. Cells are about 50 μm wide.

From these examples it should be clear how ecological diversity drives bacteria that carry out the same energy metabolism—in this case sulfide oxidation—to best exploit the different environments they inhabit. In each case, the goal of the organism is the same, to obtain the electron donor and acceptor it needs. But also in each case, the strategy to accomplish this is unique and the best fit to both the properties of the organism and the habitat it exploits.

MINIQUIZ

- Describe the energy and carbon metabolism of *Thiobacillus* in terms of how ATP and new cell material are made.
- What are some ecological strategies that sulfur oxidizers use to compete with the spontaneous (chemical) oxidation of H_2S?



(a) (b)

Figure 15.29 The sulfur-oxidizing *Thiovulum*. *(a)* Macrophotograph of cells of *Thiovulum* (yellow dots) that formed a thin veil in marine sand containing H_2S (large, irregular structures are sand grains). The *Thiovulum* veil is employed as a strategy for regulating nutrient flow, in particular for obtaining H_2S and O_2 for energy needs. *(b)* Transmission electron micrograph of a dividing cell of *Thiovulum*. Sulfur (S^0) globules are shown with arrows. Single cells of *Thiovulum* are typically 10–20 μm in diameter.

IV • Microbial Diversity in the Nitrogen Cycle

All forms of life must assimilate nitrogen for growth and thus all organisms must catalyze certain nitrogen transformations. The *Bacteria* and *Archaea*, however, are the only domains in which representatives exist that can conserve energy from the transformation of inorganic nitrogen species. In this section we will consider the diversity of three physiological groups of bacteria that participate in the nitrogen cycle: *diazotrophs, nitrifiers*, and *denitrifiers*. The physiology of these groups was considered in Sections 14.6, 14.11, and 14.13. We start our tour of microbial diversity in the nitrogen cycle by considering those microbes that reduce atmospheric nitrogen: the nitrogen fixers.

15.12 Diversity of Nitrogen Fixers

KEY GENERA: *Mesorhizobium, Desulfovibrio, Azotobacter*

Diazotrophs are microorganisms that fix dinitrogen gas (N_2) into NH_3 that can be assimilated as a source of nitrogen for cells. Nitrogen fixation is an assimilative process and requires ATP and the enzyme nitrogenase (↻ Section 14.6). Diazotrophs typically fix N_2 only when other forms of N are absent, and nitrogenase expression is inhibited when NH_3 is available to cells. Nitrogenase is irreversibly inhibited by O_2 and this is one cause of ecological diversification among diazotrophs; we will see that different organisms have evolved different solutions to protecting nitrogenase from O_2.

Nitrogen fixation is widespread among *Bacteria* and is also found in a few *Archaea*, and it is thought that the last universal common ancestor (↻ Section 13.1) possessed a primitive nitrogenase. The *nifH* gene encodes the dinitrogenase reductase component of nitrogenase (↻ Section 14.6) and can be used as a measure

of diazotroph diversity. More than 30,000 unique *nifH* gene sequences have been described spanning nine bacterial phyla and one archaeal phylum (Figure 15.1). The phylogenetic distribution of nitrogenase in the tree of life has been influenced strongly by horizontal gene exchange. As a result, the phylogeny of *nifH* is largely inconsistent with the 16S ribosomal RNA gene phylogeny (**Figure 15.30**). We consider here the diversity of both symbiotic and free-living diazotrophic *Bacteria*.

Symbiotic Diazotrophs

Diazotrophs form several symbiotic relationships with plants, animals, and fungi. These relationships are generally defined by the host providing a hospitable environment, including a source of carbon and energy and a system for regulating oxygen concentrations, and the microbial symbiont providing in return a supply of fixed nitrogen to the host.

The symbiosis between rhizobia and leguminous plants is one of the best-characterized nitrogen-fixing symbiotic associations (Section 23.3). Root-nodule-forming bacteria are *Alphaproteobacteria* (e.g., *Mesorhizobium, Bradyrhizobium, Sinorhizobium*), *Betaproteobacteria* (e.g., *Burkholderia*), or *Actinobacteria* (e.g., *Frankia*). Other genera of symbiotic diazotrophs are found in association with shipworms (*Teredinibacter*), termite guts (*Treponema*) (Section 23.7), endomycorrhizal fungi (*Glomeribacter*) (Sections 18.11 and 23.4), and several fungi, algae, and plants (*Cyanobacteria*) (Sections 23.1 and 23.3). These different symbioses have evolved independently multiple times as a result of convergent evolution (Figure 15.30).

Free-Living Diazotrophs

Free-living diazotrophs need a mechanism for protecting nitrogenase from oxygen (Sections 14.6 and 7.8). The simplest solution to this problem is to grow only in anoxic environments. The origin of nitrogen fixation predates the origin of oxygenic photosynthesis and thus the first nitrogen-fixing organisms were free-living anaerobes. Obligately anaerobic free-living diazotrophs are common in anoxic environments including marine and freshwater sediments and microbial mats. Obligately anaerobic free-living diazotrophs are found in the bacterial phyla *Firmicutes* (e.g., *Clostridium*), *Chloroflexi* (e.g., *Oscillochloris*), *Chlorobi* (e.g., *Chlorobium*), *Spirochaetes* (e.g., *Spirochaeta*), and *Proteobacteria* (e.g., *Desulfovibrio, Chromatium*) and in the archaeal phylum *Euryarchaeota* (e.g., *Methanosarcina*). *Desulfovibrio* occur in anoxic salt marsh sediments dominated by *Spartina* grass, and their N_2 fixation is an important nitrogen source to plants that live in this ecosystem.

Other simple mechanisms for protecting nitrogenase from oxygen include fixing N_2 only at times when oxygen is absent or present in low concentration. For example, facultative aerobes will often fix N_2 only while growing anaerobically (e.g., *Klebsiella*). Some aerobic nitrogen-fixers are *microaerophiles*; these organisms fix nitrogen only in environments where oxygen is present at low concentration (typically less than 2%). However, some organisms have evolved more complex mechanisms for protecting nitrogenase from oxygen and are able to grow in the presence of air.

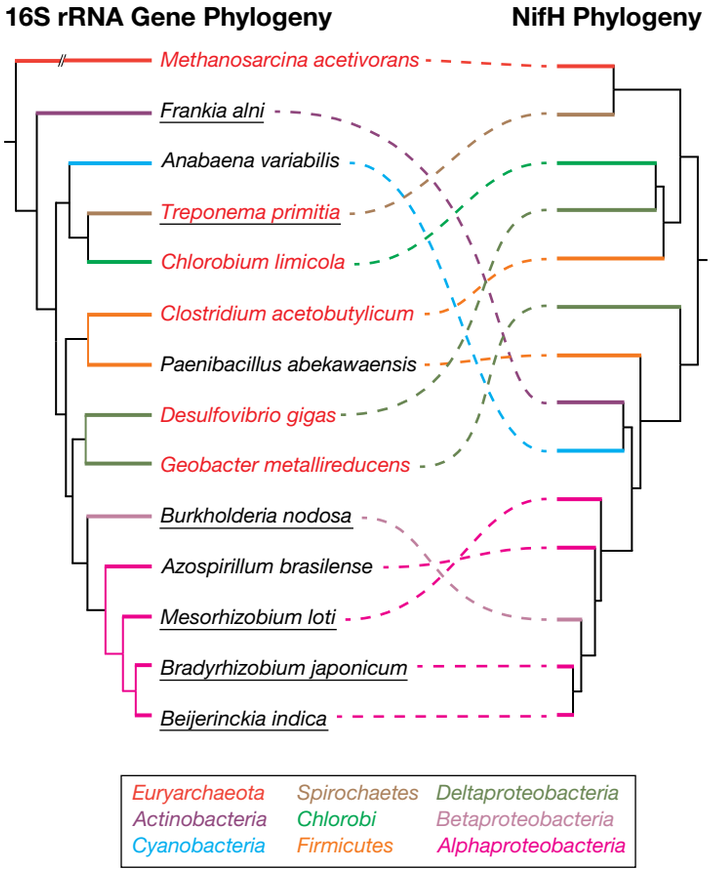
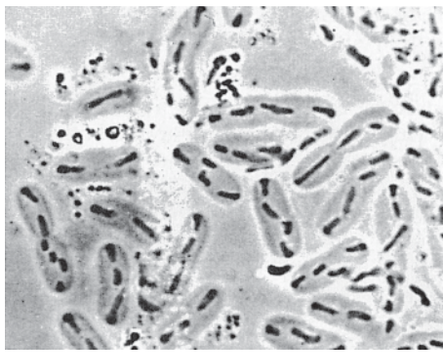


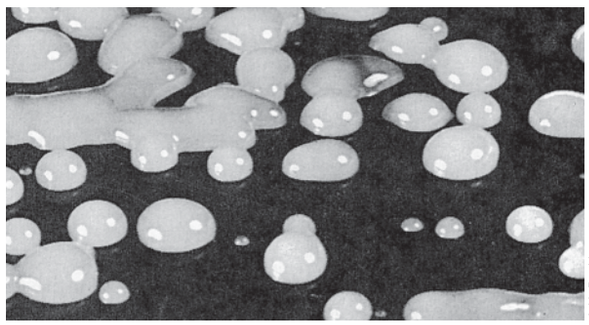
Figure 15.30 Relationships among diazotrophic (nitrogen-fixing) bacteria as inferred from 16S ribosomal RNA gene sequences and NifH amino acid sequences. Branches in each tree are colored to indicate phyla. The dashed lines indicate branches shared between the two trees. The incongruence between the two trees has resulted from multiple horizontal transfer events of the *nifH* gene. Red text denotes obligate anaerobes and underlined text indicates species that form symbioses with *Eukarya*.

Obligately aerobic free-living diazotrophs include the *Cyanobacteria*, which have evolved a variety of mechanisms of protecting nitrogenase from oxygen (Section 15.3), as well as a variety of unicellular free-living chemoorganotrophic bacteria. Obligately aerobic free-living diazotrophs include *Azotobacter, Azospirillum*, and *Beijerinckia*. *Azotobacter* cells are large rods or cocci with diameters of 2–4 μm or more. When they are growing on N_2 as a nitrogen source, extensive capsules or slime layers are typically produced (**Figure 15.31** and Figures 2.17 and 14.22*a, b*). It is thought that the high respiratory rate characteristic of *Azotobacter* cells and the abundant capsular slime they produce help protect nitrogenase from O_2. *Azotobacter* is able to grow on many different carbohydrates, alcohols, and organic acids, and metabolism is strictly oxidative.

Azotobacter can form resting structures called *cysts* (**Figure 15.32*b***). Like bacterial endospores, *Azotobacter* cysts show negligible endogenous respiration and are resistant to desiccation, mechanical disintegration, and ultraviolet and ionizing radiation. In contrast to endospores, however, cysts are not very



(a)



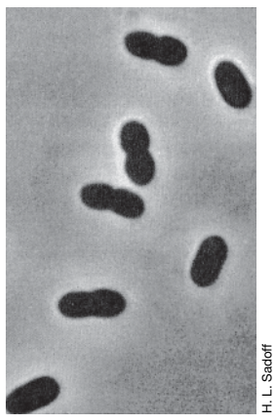
(b)

Figure 15.31 Examples of slime production by free-living N_2-fixing bacteria. *(a)* Cells of *Derxia gummosa* encased in slime. Cells are about 1–1.2 μm wide. *(b)* Colonies of *Beijerinckia* species growing on a carbohydrate-containing medium. Note the raised, glistening appearance of the colonies due to abundant capsular slime.

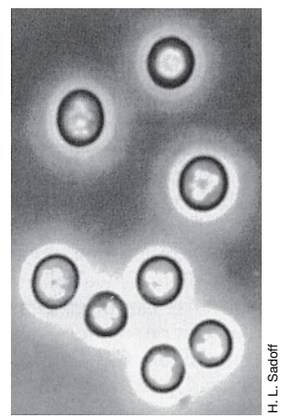
heat resistant, and they are not completely dormant because they rapidly oxidize carbon sources if supplied.

Azotobacter and Alternative Nitrogenases

We considered the important process of biological N_2 fixation in Section 14.6 and discussed the central importance of the metals molybdenum (Mo) and iron (Fe) to the enzyme nitrogenase.



(a)



(b)

Figure 15.32 *Azotobacter vinelandii.* *(a)* Vegetative cells and *(b)* cysts visualized by phase-contrast microscopy. A cell measures about 2 μm in diameter and a cyst about 3 μm.

The species *Azotobacter chroococcum* was the first nitrogen-fixing bacterium shown to grow on N_2 in the absence of molybdenum. This is because either of two "alternative nitrogenases" are formed when Mo limitation prevents the MoFe nitrogenase from being synthesized. These nitrogenases are less efficient than the MoFe nitrogenase and contain either vanadium (V) or Fe in place of Mo. The three different types of nitrogenase (MoFe, VFe, and FeFe) are encoded by paralogous genes and likely arose as the result of gene duplications (↻ Sections 9.5 and 13.7). Subsequent investigations of other nitrogen-fixing bacteria have shown that these genetically distinct "backup" nitrogenases are widely distributed among nitrogen-fixing microbes, in particular in the *Cyanobacteria* and *Archaea*.

MINIQUIZ

- What mechanisms do free-living diazotrophs use to protect nitrogenase from oxygen?
- Where might you expect to find nitrogen-fixing bacteria?

15.13 Diversity of Nitrifiers and Denitrifiers

Microorganisms that grow by the anaerobic respiration of inorganic nitrogen (NO_3^-, NO_2^-) to the gaseous products NO, N_2O, and N_2 are called **denitrifiers** (↻ Section 14.13). These organisms are typically facultative aerobes and chemoorganotrophs that use organic carbon as both carbon source and electron donor.

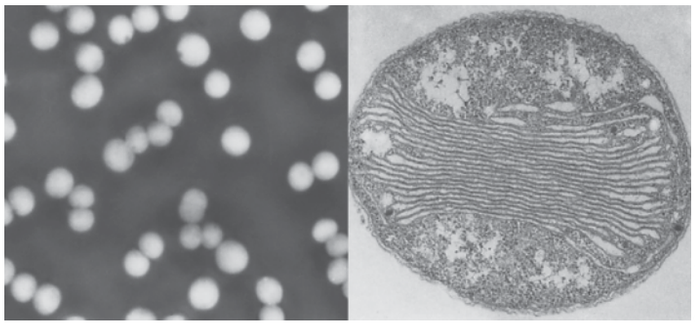
Microorganisms able to grow chemolithotrophically at the expense of reduced inorganic nitrogen compounds (NH_3, NO_2^-) are called **nitrifiers** (Figure 15.33) (↻ Section 14.11). These organisms are typically obligate aerobes that can also grow autotrophically; most species fix CO_2 by the Calvin cycle. A few species have also been shown to grow mixotrophically by assimilating organic carbon in addition to CO_2.

Physiology of Nitrifying *Bacteria* and *Archaea*

Nitrification often results from the sequential activities of two physiological groups of organisms, the *ammonia oxidizers* (which oxidize NH_3 to nitrite, NO_2^-) (Figure 15.33*a*), and the *nitrite oxidizers*, the actual nitrate-producing microorganisms, which oxidize NO_2^- to NO_3^- (Figure 15.33*b*). Ammonia oxidizers typically have genus names beginning in *Nitroso-*, whereas genus names of nitrate producers begin with *Nitro-*. However, certain microbes within the genus *Nitrospira* are able to carry out both ammonia oxidation and nitrite oxidation, and are therefore able to oxidize ammonia all the way to nitrate (see page 487 for more on these bacteria).

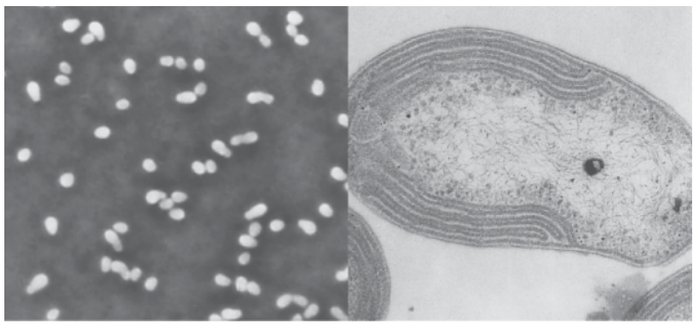
Many species of nitrifiers have internal membrane stacks (Figure 15.33) that closely resemble the photosynthetic membranes found in their close phylogenetic relatives, the purple phototrophic bacteria (Section 15.4) and the methane-oxidizing (methanotrophic) bacteria (Section 15.16). The membranes are the location of key enzymes in nitrification: *ammonia monooxygenase*, which oxidizes NH_3 to hydroxylamine (NH_2OH), and *nitrite oxidoreductase*, which oxidizes NO_2^- to NO_3^- (↻ Section 14.11).

Enrichment cultures of nitrifying bacteria can be achieved using mineral salts media containing NH_3 or NO_2^- as electron



Reaction: $NH_3 + 1\frac{1}{2}\ O_2 \longrightarrow NO_2^- + H^+ + H_2O$

(a)



Reaction: $NO_2^- + \frac{1}{2}\ O_2 \longrightarrow NO_3^-$

(b)

Figure 15.33 Nitrifying bacteria. *(a)* Phase-contrast photomicrograph (left) and electron micrograph (right) of the ammonia-oxidizing bacterium *Nitrosococcus oceani*. A single cell is about 2 μm in diameter. *(b)* Phase-contrast photomicrograph (left) and electron micrograph (right) of the nitrite-oxidizing bacterium *Nitrobacter winogradskyi*. A cell is about 0.7 μm in diameter. Beneath each panel is the chemolithotrophic reaction that each organism catalyzes. The distinct internal membranes of each species are sites of key enzymes of nitrification.

donors and bicarbonate (HCO_3^-) as the sole carbon source. Because these organisms produce very little ATP from their electron donors (Section 14.11), visible turbidity may not develop in cultures even after extensive nitrification has occurred. An easy means of monitoring growth is thus to assay for the production of NO_2^- (with NH_3 as electron donor) or NO_3^- (with NO_2^- as electron donor).

Nitrifying *Bacteria* and *Archaea*: Ammonia Oxidizers

KEY GENERA: *Nitrosomonas, Nitrosospira, Nitrosopumilus*

Ammonia oxidizers are found in the *Beta-* (e.g., *Nitrosomonas, Nitrosospira, Nitrosolobus, Nitrosovibrio*) and *Gammaproteobacteria* (*Nitrosococcus*), in the phylum *Nitrospirae*, and in the archaeal phylum *Thaumarchaeota* (*Nitrosopumilus, Nitrosocaldus, Nitrosoarchaeum, Nitrososphaera*).

Ammonia oxidizers are widespread in soil and water. Bacterial ammonia-oxidizers are present in highest numbers in habitats where NH_3 is abundant, such as sites with extensive protein decomposition (ammonification), and also in sewage treatment facilities (Sections 22.6 and 22.7). Nitrifying bacteria develop especially well in lakes and streams that receive inputs of sewage or other wastewaters because these are frequently high in NH_3. *Nitrosomonas* is often observed in the activated sludge present in aerobic wastewater treatment facilities. Bacterial ammonia-oxidizers are also common in soils (e.g., *Nitrosospira, Nitrosovibrio*) and in the oceans (e.g., *Nitrosococcus*).

Archaeal ammonia-oxidizers (Section 17.5) appear to be most common in habitats where NH_3 is present in low concentration. These organisms are thought to be the dominant ammonia-oxidizers in the oceans where ammonia levels are very low (Sections 20.9 and 20.11). Archaeal ammonia-oxidizers are also common in soils, and in some soils they outnumber bacterial ammonia-oxidizers by several orders of magnitude. The availability of NH_3 relative to NH_4^+ declines with pH, and thus acid soils (pH < 6.5), which are common, may favor organisms able to grow at low NH_3 concentration.

Nitrifying Bacteria: Nitrite Oxidizers

KEY GENERA: *Nitrospira, Nitrobacter*

Nitrite oxidizers are found in the classes *Alpha-* (*Nitrobacter*), *Beta-* (*Nitrotoga*), *Gamma-* (*Nitrococcus*), and *Deltaproteobacteria* (*Nitrospina*), as well as in the phylum *Nitrospirae* (genus *Nitrospira*) (Section 16.21).

Like nitrite-oxidizing *Proteobacteria, Nitrospira* oxidizes nitrite (NO_2^-) to nitrate (NO_3^-) and grows autotrophically (**Figure 15.34**). However, *Nitrospira* lacks the extensive internal membranes found in species of nitrifying *Proteobacteria*. Nevertheless, *Nitrospira* inhabits many of the same environments as nitrite-oxidizing *Proteobacteria* such as *Nitrobacter*, so it has been suggested that its capacity for NO_2^- oxidation may have been acquired by horizontal gene flow from nitrifying *Proteobacteria* (or vice versa). As we know, this mechanism for acquiring physiological traits has been widely exploited in the bacterial world (Chapter 11 and Section 13.7). However, environmental surveys for the presence of nitrifying bacteria in nature have shown *Nitrospira* to be much more abundant than *Nitrobacter*; thus most of the NO_2^- oxidized in natural environments is probably due to the activities of *Nitrospira*.

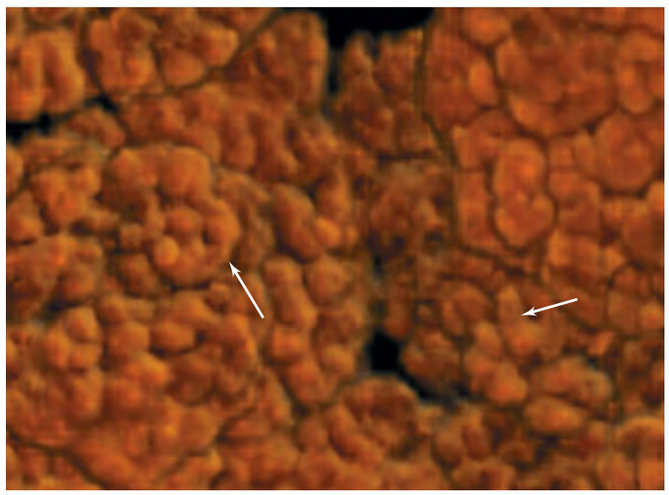


Figure 15.34 The nitrifying bacterium *Nitrospira*. An aggregate of *Nitrospira* cells enriched from activated sludge from a wastewater treatment facility. Individual cells are curved (arrows) and group into tetrads in the aggregate. A single cell of *Nitrospira* is about 0.3 × 1–2 μm (see page 487 for a metabolically unique *Nitrospira*).

UNIT 4

Denitrifying *Bacteria* and *Archaea*

KEY GENERA: *Paracoccus, Pseudomonas*

Denitrifiers are capable of growth by the anaerobic respiration of NO_3^- or NO_2^- to the gaseous products NO, N_2O, and N_2 (Section 14.13). Nearly all denitrifiers are chemoorganotrophs that use organic carbon as both carbon source and electron donor. Exceptions include the denitrifying sulfur-oxidizers discussed in Section 15.11. Denitrifiers are typically facultative aerobes and in nearly all cases will grow preferentially as aerobes if O_2 is present. Denitrifiers are of great importance in agricultural soils where they cause the loss of nitrogen fertilizers and the production of N_2O, which is a dominant component of greenhouse gases produced by agricultural soils (Section 21.8).

Denitrifiers are phylogenetically and metabolically diverse and include two archaeal phyla and six bacterial phyla, including five classes of *Proteobacteria* (Figure 15.1). One of the best-characterized denitrifiers is *Paracoccus denitrificans* (*Alphaproteobacteria*). Denitrification of NO_3^- to N_2 requires several key enzymatic steps (Section 14.13), and the genes that encode these enzymes are present throughout the tree of life, indicating the strong influence of horizontal gene exchange. However, many nitrate reducers possess only part of the denitrification pathway and are thus unable to reduce NO_3^- completely to N_2, producing final products such as NO_2^-, NO, or N_2O.

MINIQUIZ

- Under what conditions would you expect microorganisms to grow as a result of denitrification?
- Which traits are shared among ammonia oxidizers and nitrite oxidizers?

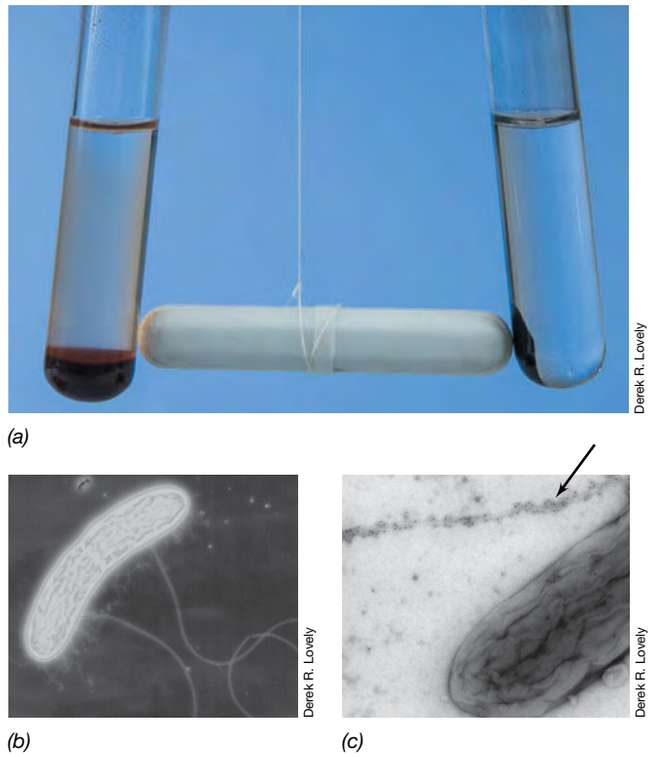


Figure 15.35 The dissimilative iron-reducing bacterium *Geobacter*. *(a)* The uninoculated tube (left) contains an anoxic medium that includes acetate and ferrihydrite, a poorly magnetic iron oxide. Following growth of *Geobacter* (right tube) the ferrihydrite is reduced to magnetite, which is magnetic. *(b)* Transmission electron micrograph of *Geobacter sulfurreducens* showing flagella and pili. The cell is about 0.7 × 3.5 μm. *(c)* Transmission electron micrograph of *G. sulfurreducens* showing immunogold labeling of cytochrome OmcS on the pili (arrow).

V • Other Distinctive Functional Groupings of Microorganisms

We continue our focus on functional groups whose physiological and ecological traits span different phyla as a result of convergent evolution or horizontal gene transfer. From a physiological standpoint, all groups here are chemotrophs—either chemolithotrophs or chemoorganotrophs—that contribute to specific steps in the carbon cycle or that metabolize hydrogen or metals.

15.14 Dissimilative Iron-Reducers

KEY GENERA: *Geobacter, Shewanella*

Dissimilative iron-reducers couple the reduction of oxidized metals or metalloids to cellular growth. These organisms need to overcome the fundamental obstacle of using an insoluble solid material as an electron acceptor in respiration. A variety of microorganisms are able to enzymatically reduce metals as a consequence of either fermentation reactions or sulfur or sulfate reduction, but such organisms do not conserve energy from metal reduction. In contrast, dissimilative iron-reducers carry out metal respiration by coupling the oxidation of H_2 or organic compounds to the reduction of ferric iron (Fe^{3+}) (**Figure 15.35*a***) or manganese (Mn^{6+}).

Dissimilative iron-reducers are phylogenetically diverse (Figure 15.1). Iron-reducing bacterial genera are found in the *Proteobacteria* (*Geobacter, Shewanella*), *Acidobacteria* (*Geothrix*), *Deferribacteres* (*Geovibrio*), *Deinococcus–Thermus* (*Thermus*), *Thermotogae* (*Thermotoga*), and *Firmicutes* (*Bacillus, Thiobacillus*), while archaeal genera are found in the *Crenarchaeota* (*Pyrobaculum*). Iron respiration likely evolved early in the history of life and its wide distribution may be due to its presence in the universal ancestor coupled with subsequent gene loss in some lineages and horizontal gene transfer to others.

Physiology

Dissimilative iron-reducers specialize in using insoluble external electron acceptors, and these organisms are typically extremely versatile at anaerobic respiration. Dissimilative iron-reducers are unusual in that they possess outer membrane cytochromes that facilitate electron transfer with insoluble minerals. Most species are able to use either iron oxides or manganese oxides as electron acceptors, and various species are also able to use nitrate, fumarate, and oxidized inorganic sulfur, cobalt, chromium, uranium, tellurium, selenium, arsenic, and humic compounds (Section 14.15). Most genera of iron-reducing bacteria are obligate anaerobes, but some, such as *Shewanella* and relatives, are facultative aerobes. Electron donors are typically organic compounds such as fatty acids, alcohols, sugars, and in certain cases, even aromatic

compounds. Many species are also able to use H_2 as an electron donor, but they are generally unable to grow autotrophically, requiring a source of organic carbon to support growth.

The family *Geobacteraceae* in the *Deltaproteobacteria* contains four genera of dissimilative iron-reducing bacteria (*Geobacter, Desulfuromonas, Desulfuromusa, Pelobacter*) that aptly demonstrate the physiological diversity of the obligately anaerobic metal reducers. *Geobacter, Desulfuromonas*, and *Desulfuromusa* can all use acetate as an electron donor as well as a diversity of other small organics, and they oxidize these substrates completely to CO_2. These genera typically specialize in anaerobic respiration. *Geobacter* in particular can use a wide range of electron donors and acceptors. *Geobacter* produce pili (Figure 15.35*b*) that contain cytochromes (Figure 15.35*c*), and these pili facilitate electron transfer to the surface of iron oxide minerals. *Pelobacter*, in contrast, are primarily fermentative organisms having a more limited respiratory capacity. For example, *Pelobacter carbinolicus* can only use lactate as the electron donor and can only use ferric iron or S^0 as the electron acceptors. *Pelobacter* species are unable to oxidize their carbon substrates completely to CO_2.

Shewanella and its relatives *Ferrimonas* and *Aeromonas* in the *Gammaproteobacteria* are facultative aerobes and will grow aerobically when O_2 is available. *Shewanella* are able to use a wide diversity of electron donors and acceptors in addition to ferric iron and manganese. However, like *Pelobacter* species, they are unable to oxidize their carbon substrates completely to CO_2 and are unable to oxidize acetate as an electron donor for anaerobic respiration.

Ecology

Dissimilative iron-reducers are common in anoxic freshwater and marine sediments. These organisms are thought to play an important role in organic matter oxidation in many anoxic habitats. Dissimilative iron-reducers are also common in the deep subsurface, found in shallow aquifers as well as in the deep subsurface environment (↔ Section 20.7). In addition, several thermophilic and hyperthermophilic iron-reducing species are known (e.g., *Thermus, Thermotoga*) and are often found in hot springs and other geothermally heated systems, including the deep subsurface.

MINIQUIZ

- In what phylogenetic groups are *Geobacter* and *Shewanella* found?
- Which genera of dissimilative iron-reducers contain facultative aerobes?

15.15 Dissimilative Iron-Oxidizers

KEY GENERA: *Acidithiobacillus, Gallionella*

The ability to couple the oxidation of ferrous iron (Fe^{2+}) to cell growth is widespread in the tree of life and thought to be a trait that evolved early in Earth's history. Genera capable of using ferrous iron as an electron donor to support growth are spread across five bacterial and two archaeal phyla (Figure 15.1).

Aerobic iron-oxidizer diversity and distribution are influenced strongly by pH and O_2. Ferrous iron oxidizes spontaneously to form insoluble precipitates in the presence of O_2 at neutral to alkaline pH (pH > 7) but is stable either under anoxic conditions or aerobically at acidic pH (pH < 4). Iron oxidizers can be divided into four functional groups on the basis of their physiology: acidophilic aerobic iron-oxidizers, neutrophilic aerobic iron-oxidizers, anaerobic chemotrophic iron-oxidizers, and anaerobic phototrophic iron-oxidizers.

Acidophilic Aerobic Iron-Oxidizing *Bacteria*

The growth of iron-oxidizing bacteria is favored in iron-rich acidic environments where soluble ferrous iron is present. Aerobic iron-oxidizers are often abundant in acid mine drainage generated from abandoned coal or iron mines or from mine tailings (↔ Sections 22.1 and 22.2). Acidophilic aerobic iron-oxidizers also inhabit iron-rich acidic springs in volcanic areas. In these environments, sulfur is often present along with ferrous iron, and many acidophilic aerobic iron-oxidizers are able to oxidize both elemental sulfur and ferrous iron. Species can be either autotrophic or heterotrophic, and commonly observed genera include *Acidithiobacillus* (*Gammaproteobacteria*), *Leptospirillum* (*Nitrospirae*), and *Ferroplasma* (*Euryarchaeota*). Other acidophilic aerobic iron-oxidizers can be found in the *Actinobacteria* and *Firmicutes*.

Neutrophilic Aerobic Iron-Oxidizing *Bacteria*

Neutrophilic aerobic iron-oxidizers are organisms adapted to a specialized niche (↔ Section 14.10). This is because ferrous iron is relatively insoluble at neutral pH and its chemical oxidation is spontaneous and rapid in the presence of air. Furthermore, at neutral pH, iron oxidation at the cell surface causes the formation of an iron oxide crust that can effectively entomb growing cells. Neutrophilic aerobic iron-oxidizers therefore thrive where iron-rich anoxic waters are exposed to air. Such habitats are common near wetlands or soils where anoxic groundwater forms a spring, but iron oxidizers also inhabit the rhizosphere of wetland plants and certain submarine hydrothermal systems.

Few genera of neutrophilic aerobic iron-oxidizers have been described and they all belong to the *Proteobacteria*. Those species found in freshwater habitats belong to a set of closely related genera in the *Betaprotobacteria*, while species found in marine habitats belong to the *Zetaproteobacteria*. The metabolism of these organisms is fairly narrow. Species are typically microaerophiles and obligate chemolithotrophs, though in certain cases mixotrophy has been observed. The genera *Leptothrix* and *Sphaerotilus* are exceptions (Section 15.21). *Leptothrix* and *Sphaerotilus* are common in freshwater environments containing neutrophilic aerobic iron-oxidizers. They catalyze the oxidation of both iron and manganese but do not appear to conserve energy from these reactions, conserving energy instead from the oxidation of organic matter.

Characteristic species of neutrophilic aerobic iron-oxidizers are found in the genus *Gallionella* (freshwater) and the marine genus *Mariprofundus* (marine). Species of *Gallionella* and *Mariprofundus* each form a twisted stalklike structure containing $Fe(OH)_3$ from the oxidation of ferrous iron (**Figure 15.36**). The iron-encrusted stalk contains an organic matrix on which $Fe(OH)_3$ accumulates as it is excreted from the cell surface. Stalk formation is presumably an adaptation that prevents cells from becoming entombed in an iron oxide crust.

Gallionella is common in the waters draining bogs, iron springs, and other habitats where ferrous iron is present. *Mariprofundus*

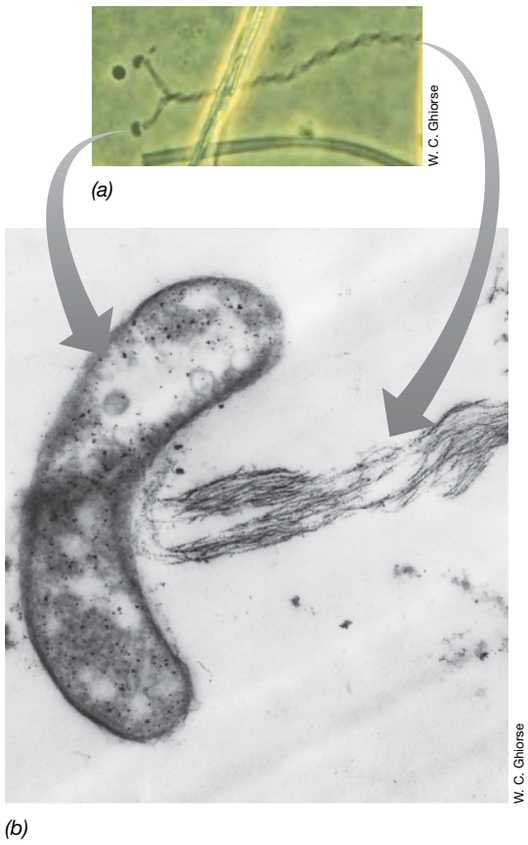


Figure 15.36 The neutrophilic ferrous iron-oxidizer *Gallionella ferruginea*, from an iron seep near Ithaca, New York. *(a)* Photomicrograph of two bean-shaped cells with stalks that combine to form one twisted mass. *(b)* Transmission electron micrograph of a thin section of a *Gallionella* cell with stalk. Cells are about 0.6 µm wide.

was first isolated from Lō'ihi Seamount, a submarine volcano near Hawaii (Section 21.5 and Figure 21.14). *Gallionella* and *Mariprofundus* are both autotrophic chemolithotrophs containing enzymes of the Calvin cycle (Section 14.5).

Anaerobic Iron-Oxidizing *Bacteria*

Anaerobic ferrous iron oxidation can be mediated by both chemotrophic and phototrophic bacteria. These groups are common in anoxic sediments and wetlands. Anoxic conditions promote the solubility of ferrous iron across a wide range of pH and so, unlike the aerobic iron-oxidizing bacteria, growth of anaerobic iron-oxidizers is not strictly limited to neutral pH. These groups contain organisms that are metabolically diverse and able to grow by using a variety of different electron donors and acceptors.

Phototrophic iron oxidation occurs in select species of purple nonsulfur bacteria of the *Alphaproteobacteria* (e.g., *Rhodopseudomonas palustris*), select species of purple sulfur bacteria of the *Gammaproteobacteria* (Figure 14.31), and select species of green sulfur bacteria found in the *Chlorobi* (*Chlorobium ferrooxidans*). In all cases ferrous iron is one of several compounds that these organisms can use as an electron donor in photosynthesis.

Anaerobic chemotrophic iron-oxidizers couple the oxidation of ferrous iron to nitrate reduction, producing either NO_2^- or nitrogen gases (denitrification). These organisms are *Alpha-, Beta-, Gamma-*, or *Deltaproteobacteria*, and most are also able to use various organic electron donors in nitrate reduction; many can also grow aerobically. The bacterial genera *Acidovorax, Aquabacterium*, and *Marinobacter* all contain anaerobic iron-oxidizers. While most species are mixotrophs when growing with ferrous iron as electron donor, species such as *Marinobacter aquaeolei* and *Thiobacillus denitrificans* are able to grow autotrophically as iron-oxidizing chemolithotrophs.

MINIQUIZ

- What habitat characteristics govern the diversity and distribution of iron oxidizers?
- How do aerobic neutrophilic iron-oxidizers keep their cells from becoming entombed in a crust of iron?

15.16 Methanotrophs and Methylotrophs

Methylotrophs are organisms that grow using organic compounds lacking C—C bonds as electron donors in energy metabolism and as carbon sources. Methylotrophy occurs in the bacterial phyla *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Verrucomicrobia*, and in the archaeal phylum *Euryarchaeota* (Figure 15.1). **Methanotrophs** are a subset of methylotrophs defined by their ability to use methane as a substrate for growth (Section 14.18).

Aerobic methylotrophs are common in soil and aquatic environments where O_2 is present. Anaerobic methylotrophs are common in anoxic environments, particularly in marine sediments. Many anaerobic methylotrophs are methanogenic *Archaea*. In addition, a consortium of methanogenic *Archaea* and sulfate-reducing bacteria combine to oxidize methane from gas hydrates found in deep-sea sediments (Section 14.18). We consider here only the aerobic methylotrophs.

Aerobic Facultative Methylotrophs

KEY GENERA: *Hyphomicrobium, Methylobacterium*

Aerobic facultative methylotrophs are unable to use methane but can use many other methylated compounds. They are species of *Alpha-, Beta-*, and *Gammaproteobacteria, Actinobacteria*, and *Firmicutes*. Facultative methylotrophs are metabolically diverse and, in addition to methylated substrates, most species can grow aerobically using other organic compounds, such as organic acids, ethanol, and sugars. When growing as methylotrophs, most species can grow aerobically with methanol and some can also metabolize methylated amines, methylated sulfur compounds, and halomethanes. Most are obligate aerobes, though some species are capable of denitrification.

The genus *Hyphomicrobium* provides an example of the metabolic versatility of the aerobic facultative methylotrophs. Certain species of *Hyphomicrobium* can grow as aerobic methylotrophs using methanol, methylamine, or dimethyl sulfide. Species of *Hyphomicrobium* can also grow as anaerobic methylotrophs using methanol as an electron donor coupled to denitrification. Finally, *Hyphomicrobium* can grow aerobically on a range of C_2 and C_4 compounds.

TABLE 15.1 Some characteristics of methanotrophic *Bacteria*

Organism	*Morphology*	*Phylogenetic group*[a]	*Internal membranes*[b]	*Carbon assimilation pathway*[c]	*N_2 fixation*
Methylomonas	Rod	*Gamma*	I	Ribulose monophosphate	No
Methylomicrobium	Rod	*Gamma*	I	Ribulose monophosphate	No
Methylobacter	Coccus to ellipsoid	*Gamma*	I	Ribulose monophosphate	No
Methylococcus	Coccus	*Gamma*	I	Ribulose monophosphate and Calvin cycle	Yes
Methylosinus	Rod or vibrioid	*Alpha*	II	Serine	Yes
Methylocystis	Rod	*Alpha*	II	Serine	Yes
Methylocella[d]	Rod	*Alpha*	II	Serine	Yes
Methylacidiphilum[d]	Rod	*Verrucomicrobiaceae*[d]	Membrane vesicles	Serine and Calvin cycle	Yes

[a]All except for *Methylacidiphilum* are *Proteobacteria*.
[b]Internal membranes: type I, bundles of disc-shaped vesicles distributed throughout the organism; type II, paired membranes running along the periphery of the cell. See Figure 15.37.
[c]See Figure 14.51.
[d]Acidophiles. For the properties of *Verrucomicrobiaceae*, see Section 16.17.

Aerobic Methanotrophs

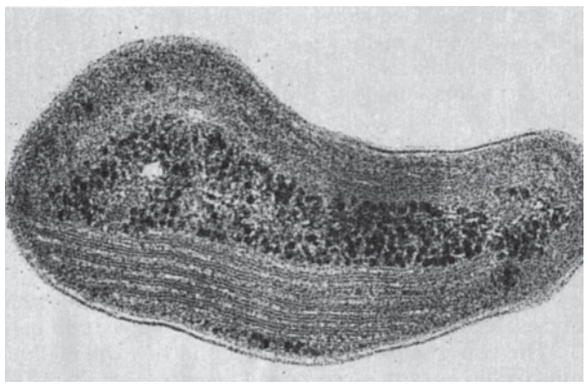
KEY GENERA: *Methylomonas, Methylosinus*

Aerobic methanotrophs are methylotrophs that can use methane as an electron donor and typically can use it as a carbon source as well. Table 15.1 gives a taxonomic overview of the methanotrophs. Most methanotrophs are *Proteobacteria* and are classified into two major groups based on their internal cell structure, phylogeny, and carbon assimilation pathway. *Type I methanotrophs* assimilate one-carbon compounds via the ribulose monophosphate cycle and are *Gammaproteobacteria*. By contrast, *type II methanotrophs* assimilate C_1 intermediates via the serine pathway and are *Alphaproteobacteria* (Table 15.1). We discussed the biochemical details of these pathways in Section 14.18. Most methanotrophs are metabolically specialized for aerobic growth on methane, though some can grow on either methane or methanol. Methanotrophs are typically obligate methylotrophs; however, the methanotrophic genus *Methylocella* contains species that can also grow on acetate or organic acids such as pyruvate and succinate.

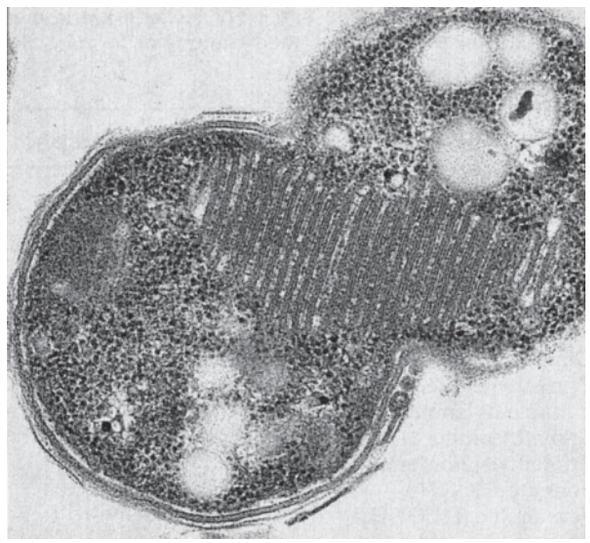
In addition to the proteobacterial methanotrophs described above, the phylum *Verrucomicrobia* contains the bacterium *Methylacidiphilum*. Genome analysis has shown that species of *Methylacidiphilum* lack key enzymes of both the ribulose monophosphate and serine pathways. Instead, *Methylacidiphilum* uses the Calvin cycle to assimilate carbon from CO_2.

Physiology

Methanotrophs possess a key enzyme, *methane monooxygenase*, which catalyzes the incorporation of an atom of oxygen from O_2 into CH_4, forming methanol (CH_3OH, ⇄ Section 14.18). The requirement for O_2 as a reactant in the initial oxygenation of CH_4 explains why these methanotrophs are obligate aerobes. Methane monooxygenase is located in extensive internal membrane systems that are the site of methane oxidation. Membranes in type I methanotrophs are arranged as bundles of disc-shaped vesicles distributed throughout the cell (**Figure 15.37*b***). Type II species possess paired membranes running


D. W. Ribbons

(a)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(b)

Figure 15.37 Methanotrophs. *(a)* Electron micrograph of a cell of *Methylosinus*, illustrating a type II membrane system. Cells are about 0.6 μm in diameter. *(b)* Electron micrograph of a cell of *Methylococcus capsulatus*, illustrating a type I membrane system. Cells are about 1 μm in diameter. Compare with Figure 15.33.

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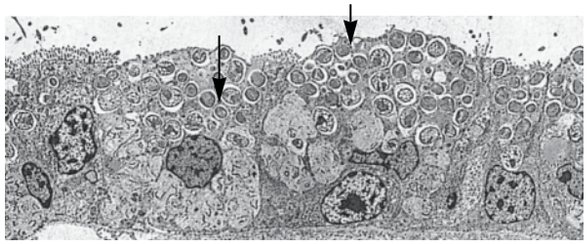
along the periphery of the cell (Figure 15.37*a*). Verrucomicrobial methanotrophs possess membrane vesicles. Methylotrophs unable to use methane lack these internal membrane arrays.

Methanotrophs are virtually unique among bacteria in possessing relatively large amounts of sterols. Sterols are rigid planar molecules found in the cytoplasmic and other membranes of eukaryotes but are absent from most bacteria. Sterols may be an essential part of the complex internal membrane system for methane oxidation (see Figure 15.37). The only other group of bacteria in which sterols are widely distributed is the mycoplasmas, bacteria that lack cell walls and thus probably require a tougher cytoplasmic membrane (Section 16.9). Many methylotrophs contain various carotenoid pigments and high levels of cytochromes in their membranes, and these features often render colonies of aerobic methylotrophs pink.

Ecology

Aerobic methylotrophs are found in the open ocean, soils, in association with plant roots and leaf surfaces, and at the oxic interface of many anoxic environments. Methanol is produced during the breakdown of plant pectin and this is likely an important substrate for methylotrophs in terrestrial ecosystems. In addition, soils contain aerobic methanotrophs that consume atmospheric methane and are an important biological sink for atmospheric methane. Aerobic methanotrophs are also common at the oxic interface of anoxic environments found in lakes, sediments, and wetlands where methanogens provide a constant source of methane. These methanotrophs play an important role in the global carbon cycle by oxidizing CH_4 and converting it into cell material and CO_2 before it reaches the atmosphere (CH_4 is a strong greenhouse gas).



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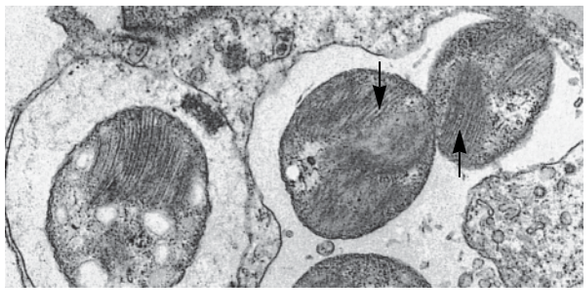


(b)

Figure 15.38 Methanotrophic symbionts of marine mussels. *(a)* Electron micrograph of a thin section at low magnification of gill tissue from a marine mussel living near hydrocarbon seeps in the Gulf of Mexico. Note the symbiotic methanotrophs (arrows) in the tissues. *(b)* High-magnification view of gill tissue showing methanotrophs with type I membrane bundles (arrows). Cells of the methanotrophs are about 1 μm in diameter. Compare with Figure 15.37*b*.

Methanotrophs also form a variety of symbioses with eukaryotic organisms. For example, some marine mussels live in the vicinity of hydrocarbon seeps on the seafloor, places where CH_4 is released in substantial amounts. Methanotrophic symbionts reside within the animal's gill tissue (**Figure 15.38**), which ensures effective gas exchange with seawater. Assimilated CH_4 is distributed throughout the animal by the excretion of organic compounds by the methanotrophs. These methanotrophic symbioses are therefore conceptually similar to those that develop between sulfide-oxidizing chemolithotrophs and hydrothermal vent tube worms and giant clams (Section 23.9).

Methylomirabilis oxyfera is a methanotroph isolated from anoxic waters in the Black Sea, and was the first isolate obtained from the unique bacterial phylum NC-10. *M. oxyfera* is an obligate anaerobe; however, it uses the O_2-dependent enzyme of aerobic methanotrophs (methane monooxygenase) to oxidize methane to CO_2. *M. oxyfera* accomplishes this by reducing nitrite to nitric oxide (NO), which is then dismutated to N_2 and O_2 ($2\ NO \rightarrow N_2 + O_2$). The O_2 produced by this pathway is then consumed by methane monooxygenase during the oxidation of CH_4 (Section 14.18). Like the methanotroph *Methylacidiphilum, M. oxyfera* assimilates C_1 units as CO_2, probably by the Calvin cycle.

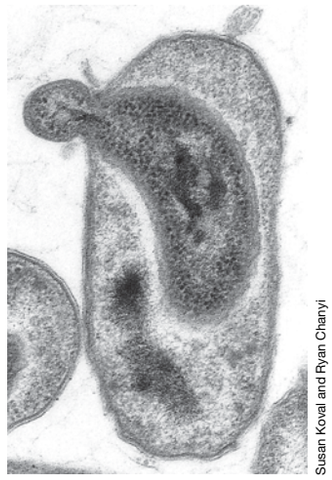
MINIQUIZ

- What is the difference between a methanotroph and a methylotroph?
- What is unique about the methanotroph *Methylomirabilis*?

15.17 Microbial Predators

KEY GENERA: *Bdellovibrio, Myxococcus*

Some bacteria are predators that consume other bacteria. Known bacterial predators reside among several classes of *Proteobacteria* and in the *Bacteroidetes* and *Cyanobacteria*. Several different



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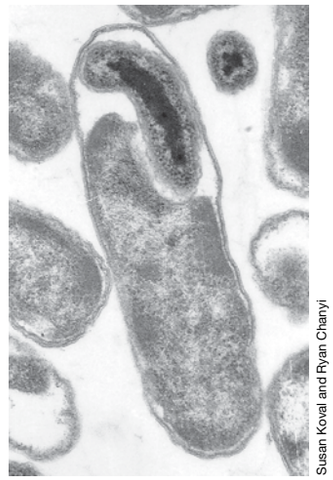
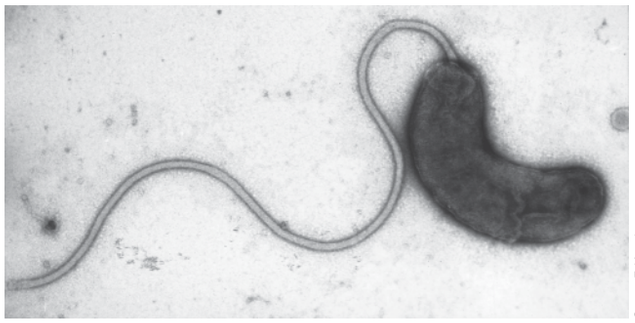


(b)

Figure 15.39 Attack on a prey cell by *Bdellovibrio*. Thin-section electron micrographs of *Bdellovibrio* attacking a cell of *Delftia acidovorans*. *(a)* Entry of the predator cell. *(b) Bdellovibrio* cell inside the host. The *Bdellovibrio* cell is enclosed in the bdelloplast and replicates in the periplasmic space. A *Bdellovibrio* cell measures about 0.3 μm in diameter.



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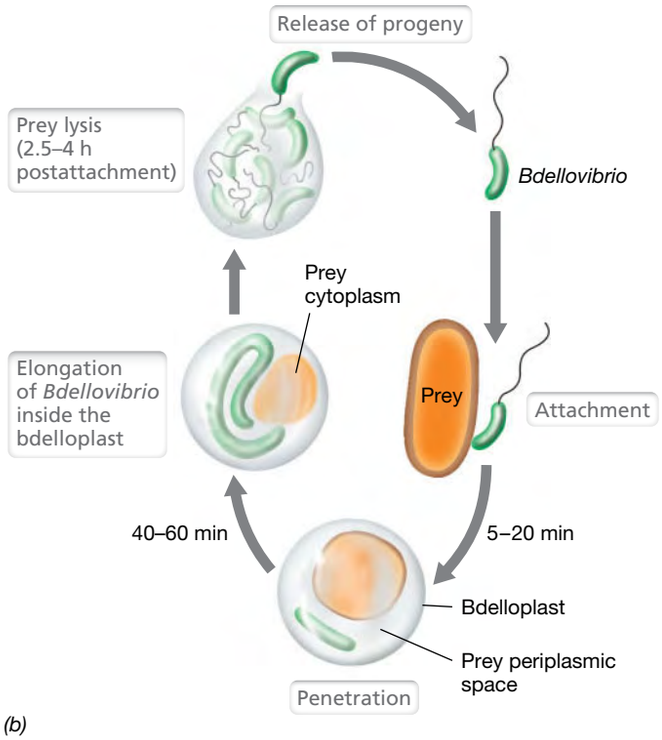


(b)

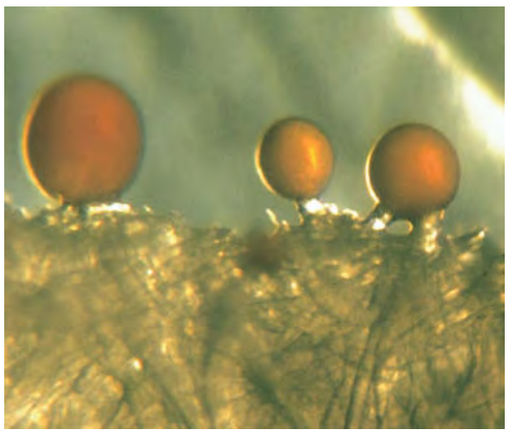
Figure 15.40 Developmental cycle of the bacterial predator *Bdellovibrio bacteriovorus*. *(a)* Electron micrograph of a cell of *Bdellovibrio bacteriovorus*; note the thick flagellum. A cell is 0.3 μm wide. *(b)* Events in predation. Following primary contact with a gram-negative bacterium, the highly motile *Bdellovibrio* cell attaches to and penetrates into the prey periplasmic space. Once inside the periplasmic space, *Bdellovibrio* cells elongate and within 4 h progeny cells are released. The number of progeny cells released varies with the size of the prey; 5–6 bdellovibrios are released from *Escherichia coli* and 20–30 for a larger prey cell, such as *Aquaspirillum*.

methods of predation have been observed. Some predators, such as *Vampirococcus* (phylogeny unknown), *Micavibrio* (*Alphaproteobacteria*), and the green algal predator *Vampirovibrio* (related to *Cyanobacteria*, Section 9.3 and Figure 9.9) are *epibiotic predators*; they attach to the surface of their prey and acquire nutrients from its cytoplasm or periplasm. Other predators, such as *Daptobacter* (*Epsilonproteobacteria*), are *cytoplasmic predators*, as they invade their host cells and replicate in the cytoplasm, consuming their prey from the inside out. *Bdellovibrio* have a similar lifestyle as *periplasmic predators*; they invade and replicate within the periplasmic space of their prey cells. Finally, predators such as *Lysobacter* (*Gammaproteobacteria*) and *Myxococcus* (*Deltaproteobacteria*) are *social predators*. These gliding bacteria use swarming behavior to find prey, which they lyse and feed upon collectively. *Bdellovibrio* and *Myxococcus* are the most thoroughly described genera of bacterial predators.

Bdellovibrio

Bdellovibrio are small, highly motile and curved bacteria that prey on other bacteria, using the cytoplasmic constituents of their hosts as nutrients (*bdello* is a prefix meaning "leech"). After attachment of a *Bdellovibrio* cell to its prey, the predator penetrates the cell wall of the prey and replicates in the periplasmic space, eventually forming a spherical structure called a *bdelloplast*. Two stages of penetration are shown in electron micrographs in Figure 15.39 and diagrammatically in Figure 15.40. A wide variety of gram-negative prey bacteria can be attacked by *Bdellovibrio*, but gram-positive cells are not attacked.

Bdellovibrio is an obligate aerobe, obtaining its energy from the oxidation of amino acids and acetate. In addition, *Bdellovibrio* assimilates nucleotides, fatty acids, peptides, and even some intact



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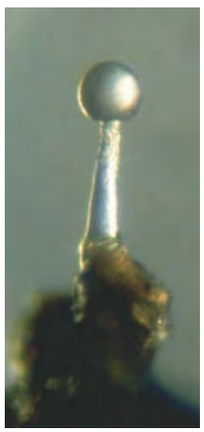

(b)

(c)

Figure 15.41 Fruiting bodies of three species of fruiting myxobacteria. *(a) Myxococcus fulvus* (125 μm high). *(b) Myxococcus stipitatus* (170 μm high). *(c) Chondromyces crocatus* (560 μm high).

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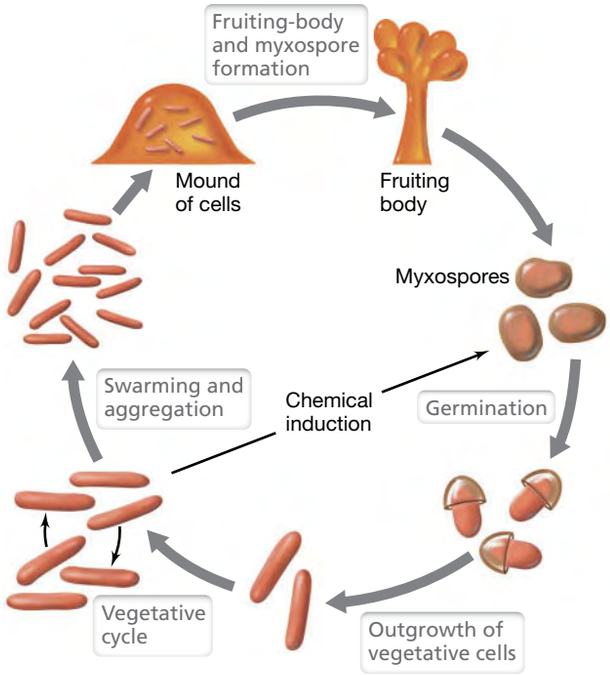

Figure 15.42 Life cycle of *Myxococcus xanthus*. Aggregation assembles vegetative cells that then undergo fruiting body formation, within which some vegetative cells undergo morphogenesis to form resting cells called myxospores. The myxospores germinate under favorable nutritional and physical conditions to yield vegetative cells.

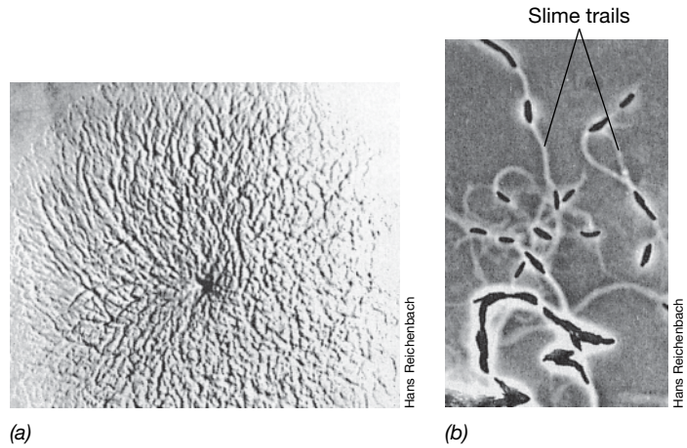

Figure 15.44 Swarming in *Myxococcus*. *(a)* Photomicrograph of a swarming colony (5-mm radius) of *Myxococcus xanthus* on agar. *M. xanthus* has been used as a model for developmental events in myxobacteria. *(b)* Single cells of *Myxococcus fulvus* from an actively gliding culture, showing the characteristic slime trails on the agar. A cell of *M. fulvus* is about 0.8 μm in diameter.

proteins directly from its host without first hydrolyzing them. Prey-independent derivatives of *Bdellovibrio* can be isolated and grown on complex media, however, showing that predation is not an obligatory lifestyle.

Phylogenetically, bdellovibrios are *Deltaproteobacteria*, and they are widespread in aquatic habitats. Procedures for their isolation are similar to those used to isolate bacterial viruses (Section 8.4). Prey bacteria are spread on the surface of an agar plate forming a lawn, and the surface is inoculated with a small amount of soil suspension that has been filtered through a membrane filter; the filter retains most bacteria but allows the small *Bdellovibrio* cells to pass. On incubation of the agar plate, plaques analogous to bacteriophage plaques (Figure 8.9*b*) are formed at locations where *Bdellovibrio* cells are multiplying. Pure cultures of *Bdellovibrio* can then be isolated from these plaques. *Bdellovibrio* are widely distributed, as cultures have been obtained from many soils and from sewage.

Myxobacteria

Myxobacteria exhibit the most complex behavioral patterns of all known bacteria. The life cycle of myxobacteria results in the formation of multicellular structures called *fruiting bodies*. The fruiting bodies are often strikingly colored and morphologically elaborate (**Figure 15.41**), and these can often be seen with a hand lens on moist pieces of decaying wood or plant material. The fruiting myxobacteria are classified on morphological grounds using characteristics of the vegetative cells, the myxospores, and fruiting body structure.

The life cycle of a typical myxobacterium is shown in **Figure 15.42**. The vegetative cells of the myxobacteria are simple,

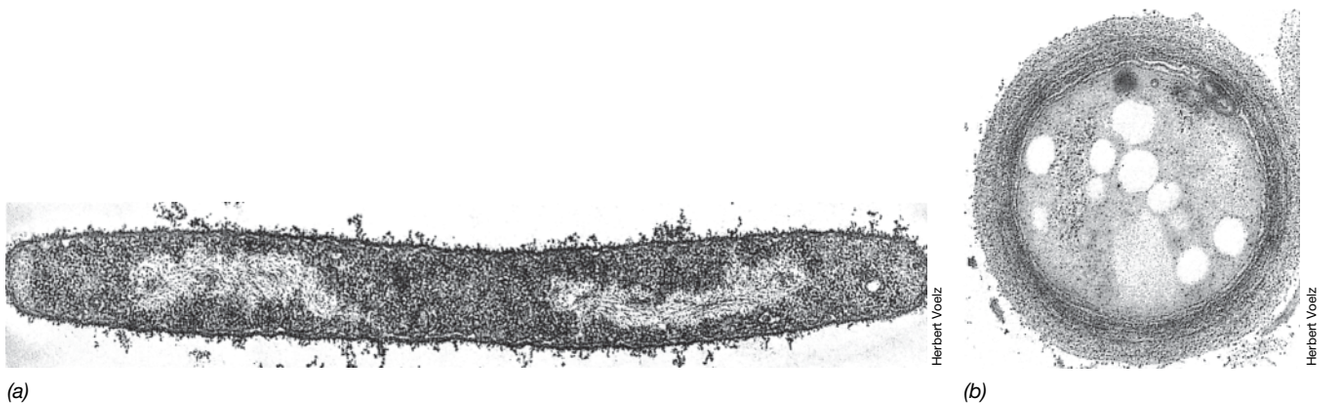

Figure 15.43 *Myxococcus*. *(a)* Electron micrograph of a thin section of a vegetative cell of *Myxococcus xanthus*. A cell measures about 0.75 μm wide. *(b)* Myxospore of *M. xanthus*, showing the multilayered outer wall. Myxospores measure about 2 μm in diameter.

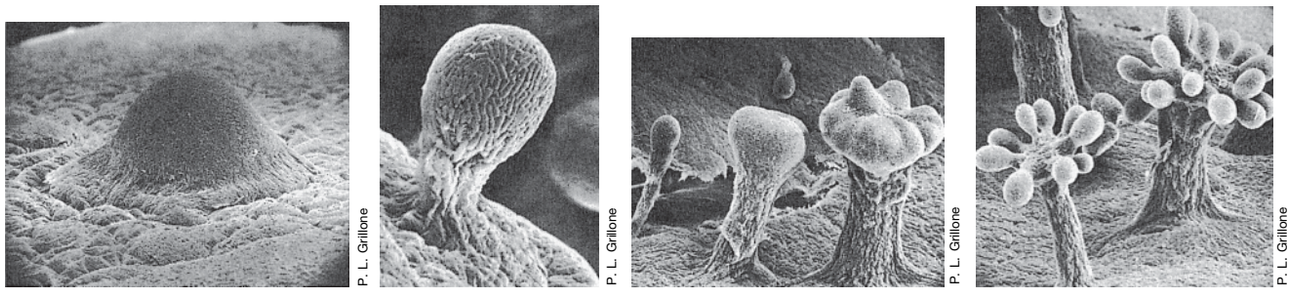

Figure 15.45 Scanning electron micrographs of fruiting body formation in *Chondromyces crocatus*. *(a)* Early stage, showing aggregation and mound formation. *(b)* Initial stage of stalk formation. Slime formation in the head has not yet begun and so the cells that compose the head are still visible. *(c)* Three stages in head formation. Note that the diameter of the stalk also increases. *(d)* Mature fruiting bodies. The entire fruiting structure is about 600 μm in height (compare with Figure 15.41*c*).

nonflagellated, gram-negative rods (**Figure 15.43**) that glide across surfaces and obtain their nutrients primarily by using extracellular enzymes to lyse other bacteria and use the released nutrients. A vegetative cell excretes slime, and as it moves across a solid surface, it leaves behind a slime trail (**Figure 15.44**). The vegetative cells form a swarm that exhibits self-organizing behavior, and this allows them to behave as a single coordinated entity in response to environmental cues.

Upon nutrient exhaustion, vegetative cells of myxobacteria begin to migrate toward each other, aggregating together in mounds or heaps (**Figure 15.45**). Aggregation is likely mediated by chemotactic or quorum-sensing responses (Sections 6.7 and 6.8). As the cell masses become higher, they begin to differentiate into fruiting bodies (**Figure 15.46**) containing *myxospores*. Myxospores are specialized cells that are resistant to drying, ultraviolet radiation, and heat, but the degree of heat resistance is much less than that of the bacterial endospore (Section 2.10). Fruiting bodies can be simple, consisting of masses of myxospores embedded in slime, or complex, consisting of a stalk and heads (Figure 15.46). The fruiting body stalk is composed of slime within which a few cells are trapped. The majority of the cells migrate to the fruiting body head, where they undergo differentiation into myxospores (Figure 15.42).

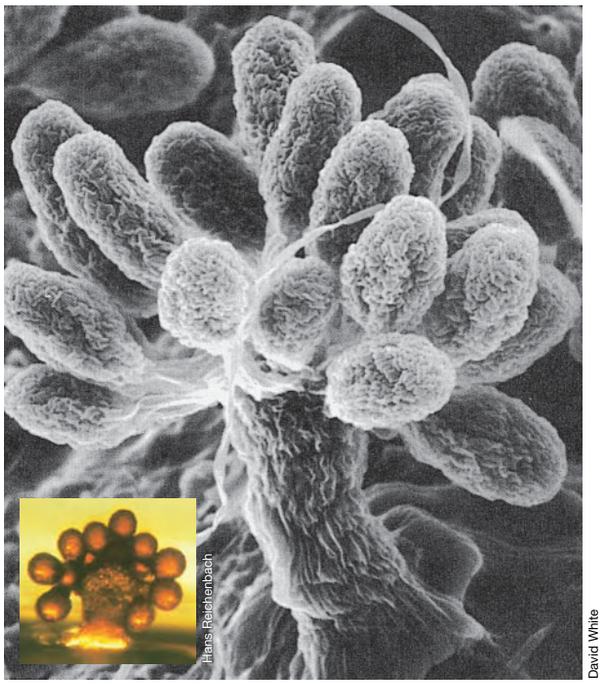

Figure 15.46 The myxobacterium *Stigmatella aurantiaca*. Scanning electron micrograph of a fruiting body growing on a piece of wood. Note the individual cells visible in each fruiting body. Inset: Phase-contrast photomicrograph of a single fruiting body about 150 μm high. The color is due to the production of glucosylated carotenoid pigments.

MINIQUIZ

- What environmental conditions trigger fruiting body formation in myxobacteria?
- What are the different ways in which species of *Myxococcus* and *Bdellovibrio* kill their prey?

15.18 Microbial Bioluminescence

KEY GENERA: *Vibrio, Aliivibrio, Photobacterium*

Several species of bacteria can emit light, a process called **bioluminescence** (**Figure 15.47**). Most bioluminescent bacteria are classified in the genera *Photobacterium, Aliivibrio*, and *Vibrio*, but a few species reside in *Shewanella*, a genus of primarily marine bacteria, and in *Photorhabdus*, a genus of terrestrial bacteria (all *Gammaproteobacteria*).

Most bioluminescent bacteria inhabit the marine environment, and some species colonize specialized *light organs* of certain marine fishes and squids, producing light that the animal uses for signaling, avoiding predators, and attracting prey (Figure 15.47 *c–f* and Section 23.8). When living symbiotically in light organs of fish and squids, or saprophytically, for example on the skin of a dead fish, or parasitically in the body of a crustacean, luminous bacteria can be recognized by the light they produce.

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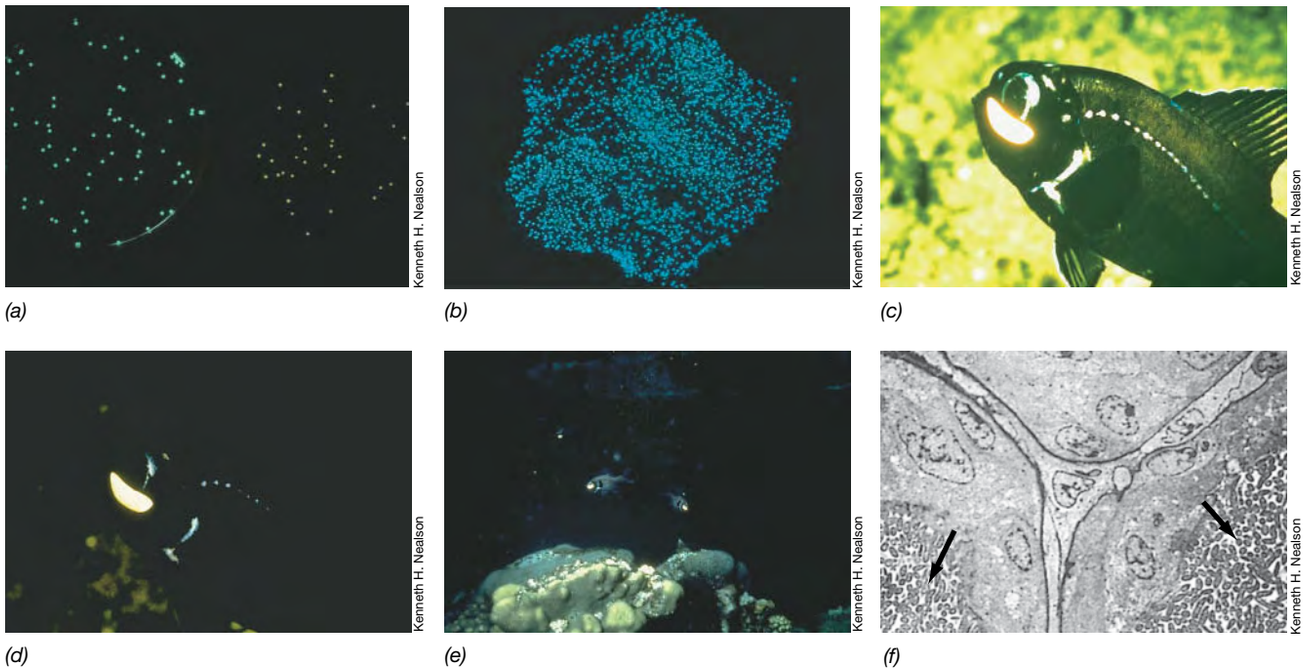

Figure 15.47 Bioluminescent bacteria and their role as light organ symbionts in the flashlight fish. *(a)* Two Petri plates of luminous bacteria photographed by their own light. Note the different colors. Left, *Aliivibrio fischeri* strain MJ-1, blue light, and right, strain Y-1, green light. *(b)* Colonies of *Photobacterium phosphoreum* photographed by their own light (Figure 1.2). *(c)* The flashlight fish *Photoblepharon palpebratus*; the bright area is the light organ containing bioluminescent bacteria. *(d)* Same fish photographed by its own light. *(e)* Underwater photograph taken at night of *P. palpebratus*. *(f)* Electron micrograph of a thin section through the light-emitting organ of *P. palpebratus* showing the dense array of bioluminescent bacteria (arrows).

Mechanism and Ecology of Bioluminescence

Although *Photobacterium, Aliivibrio*, and *Vibrio* isolates are facultative aerobes, they are bioluminescent only when O_2 is present. Luminescence in bacteria requires the genes *luxCDABE* (Section 6.8) and is catalyzed by the enzyme *luciferase*, which uses O_2, a long-chain aliphatic aldehyde (RCHO) such as tetradecanal, and reduced flavin mononucleotide ($FMNH_2$) as substrates:

$$FMNH_2 + O_2 + RCHO \xrightarrow{\text{Luciferase}} FMN + RCOOH + H_2O + \text{light}$$

The light-generating system constitutes a metabolic route for shunting electrons from $FMNH_2$ to O_2 directly, without employing other electron carriers such as quinones and cytochromes.

Luminescence in many luminous bacteria only occurs at high population density. The enzyme luciferase and other proteins of the bacterial luminescence system exhibit a population density–responsive induction, called **autoinduction**, in which transcription of the *luxCDABE* genes is controlled by a regulatory protein, LuxR, and an inducer molecule, acyl homoserine lactone (AHL, Section 6.8 and Figure 6.20). During growth, cells produce AHL, which can rapidly cross the cytoplasmic membrane in either direction, diffusing in and out of cells. Under conditions in which a high local population density of cells of a given species is attained, as in a test tube, a colony on a plate, or in the light organ of a fish or squid (Section 23.8), AHL accumulates. Only when it reaches a certain concentration in the cell is AHL bound by LuxR, forming a complex that activates transcription of *luxCDABE*; cells then become luminous (Figure 15.47*b*, Figure 1.2). This gene regulatory mechanism is also called *quorum sensing* because of the population density–dependent nature of the phenomenon (Section 6.8).

The strategy of population-density-responsive induction of luminescence ensures that luminescence develops only when population densities are high enough to allow the light produced to be visible to animals. The bacterial light can then attract animals to feed on the luminous material, thereby bringing the bacteria into the animal's nutrient-rich gut for further growth. Alternatively, the luminous material may function as a light source in symbiotic light organ associations.

Quorum sensing is a form of regulation that has also been found in many different nonluminous bacteria, including several animal and plant pathogens. Quorum sensing in these bacteria controls activities such as the production of extracellular enzymes and expression of virulence factors for which a high population density is beneficial if the bacteria are to have a biological effect.

MINIQUIZ

- What substrates and enzyme are required for an organism such as *Aliivibrio* to emit visible light?
- What is quorum sensing and how does it control bioluminescence?

VI • Morphologically Diverse *Bacteria*

15.19 Spirochetes

KEY GENERA: *Spirochaeta, Treponema, Cristispira, Leptospira, Borrelia*

Spirochetes are morphologically unique bacteria found only within the bacterial phylum *Spirochaetes*. Spirochetes are gram-negative, motile, tightly coiled *Bacteria*, typically slender and flexuous in shape (Figure 15.48). Spirochetes are widespread in aquatic sediments and in animals. Some cause diseases, including syphilis, an important human sexually transmitted disease. Spirochetes are classified into eight genera (Table 15.2) primarily on the basis of habitat, pathogenicity, phylogeny, and morphological and physiological characteristics.

Spirochetes have an unusual mode of motility conveyed by their unusual morphology. Spirochetes contain *endoflagella*, which resemble normal flagella but are found in the cell periplasm (Figure 15.49). The endoflagella are anchored at the cell poles and extend back along the length of the cell. Both the endoflagella and the protoplasmic cylinder are surrounded by a flexible membrane called the *outer sheath* (Figure 15.49*b*). Endoflagella rotate, as do typical bacterial flagella. However, when both endoflagella rotate in the same direction, the protoplasmic cylinder rotates in the opposite direction, placing torsion on the cell (Figure 15.49*b*). This torsion causes the spirochete cell to flex, resulting in a corkscrew-like motion that allows cells to burrow through viscous materials or tissues.

Spirochetes are often confused with spirilla. **Spirilla** are helically curved rod-shaped cells, usually motile by means of polar flagella (Figure 15.50). The word *spirillum* refers to a general cell shape that is widespread among *Bacteria* and *Archaea*. The number of helical turns in a single spirillum may vary from less than one complete turn (in which case the organism looks like a vibrio) to many turns. In addition, spirilla that divide terminally, such as the cyanobacterium *Spirulina* (Figure 15.5), can form long helical filaments that superficially resemble spirochetes. Spirilla, however, lack the outer sheath, endoflagella, and corkscrew-like motility of spirochetes. In addition, spirilla are typically fairly rigid cells while spirochetes are highly flexible and quite thin (<0.5 μm).

Spirochaeta and *Cristispira*

The genus *Spirochaeta* includes free-living, anaerobic, and facultatively aerobic spirochetes. These organisms, of which several species are known, are common in aquatic environments such as freshwater and sediments, and also in the oceans. *Spirochaeta plicatilis* (Figure 15.48*b*) is a large spirochete found in sulfidic freshwater and marine habitats. The 20 or so endoflagella inserted at each pole of *S. plicatilis* are arranged in a bundle that winds around the coiled protoplasmic cylinder. Another species, *Spirochaeta stenostrepta* (Figure 15.48*a*), is an obligate anaerobe commonly found in H_2S-rich black muds. It ferments sugars to ethanol, acetate, lactate, CO_2, and H_2.

Cristispira (Figure 15.51) is a unique spirochete found in nature only in the crystalline style of certain molluscs, such as clams and oysters. The crystalline style is a flexible, semisolid rod seated in a sac and rotated against a hard surface of the digestive tract, thereby mixing and grinding the small particles of food taken in by the animal. *Cristispira* lives in both freshwater and marine molluscs, but not all species of molluscs possess this organism. Unfortunately, *Cristispira* has not been cultured, and so the

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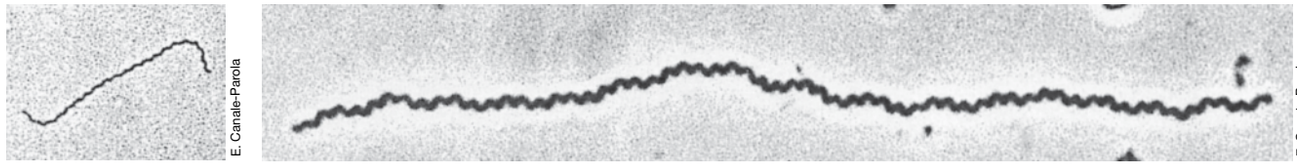
TABLE 15.2 Genera of spirochetes and their characteristics

Genus	Dimensions (μm)	General characteristics	Number of endoflagella	Habitat	Diseases
Cristispira	30–150 × 0.5–3.0	3–10 complete coils; bundle of endoflagella visible by phase-contrast microscopy	~100	Digestive tract of molluscs; has not been cultured	None known
Spirochaeta	5–250 × 0.2–0.75	Anaerobic or facultatively aerobic; tightly or loosely coiled	2–40	Aquatic, free-living, freshwater and marine	None known
Treponema	5–15 × 0.1–0.4	Microaerophilic or anaerobic; helical or flattened coil amplitude up to 0.5 μm	2–32	Commensal or parasitic in humans, other animals	Syphilis, yaws, swine dysentery, pinta
Borrelia	8–30 × 0.2–0.5	Microaerophilic; 5–7 coils of approximately 1 μm amplitude	7–20	Humans and other mammals, arthropods	Relapsing fever, Lyme disease, ovine and bovine borreliosis
Leptospira	6–20 × 0.1	Aerobic, tightly coiled, with bent or hooked ends; requires long-chain fatty acids	2	Free-living or parasitic in humans, other mammals	Leptospirosis
Leptonema	6–20 × 0.1	Aerobic; does not require long-chain fatty acids	2	Free-living	None known
Brachyspira	7–10 × 0.35–0.45	Anaerobe	8–28	Intestine of warm-blooded animals	Causes diarrhea in chickens and swine
Brevinema	4–5 × 0.2–0.3	Microaerophile; forms deep branch in spirochete lineage as assessed by 16S rRNA sequence analysis	2	Blood and tissue of mice and shrews	Infectious for laboratory mice



(a) (b)

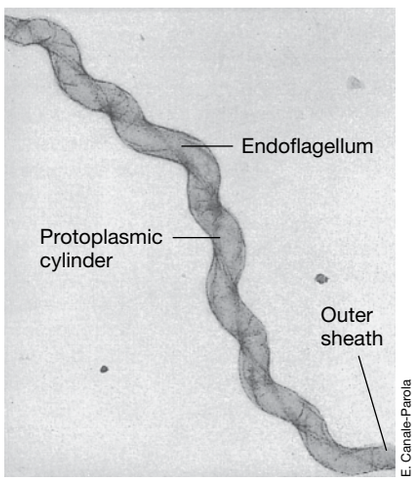
Figure 15.48 Morphology of spirochetes. Two spirochetes at the same magnification, showing the wide size range in the group. *(a) Spirochaeta stenostrepta,* by phase-contrast microscopy. A single cell is 0.25 μm in diameter. *(b) Spirochaeta plicatilis*. A single cell is 0.75 μm in diameter and can be up to 250 μm (0.25 mm) in length.

physiological rationale for its restriction to this unique habitat is unknown.

Treponema and *Borrelia*

Anaerobic or microaerophilic host-associated spirochetes that are commensals or pathogens of humans and animals reside in the genus *Treponema*. *T. pallidum*, the causal agent of syphilis (Section 30.13), is the best-known species of *Treponema*. It differs in morphology from other spirochetes in that the *Treponema* cell is not helical but flat and wavy. The *T. pallidum* cell is remarkably thin, measuring only 0.2 μm in diameter. Because of this, dark-field microscopy has long been used to examine exudates from suspected syphilitic lesions (Figure 30.37).

Other species of *Treponema* are also often found as commensals in humans and other animals. For example, *Treponema denticola* is common in the human oral cavity and is associated with gum disease. It ferments amino acids such as cysteine and serine, forming acetate as the major fermentation acid, as well as CO_2, NH_3, and H_2S. Spirochetes are also common in the rumen, the digestive forestomach of ruminant animals (Section 23.13). For instance, *Treponema saccharophilum* (**Figure 15.52*a***) is a large, pectinolytic spirochete found in the bovine rumen where it ferments pectin, starch, inulin, and other plant polysaccharides. *Treponema primitia* can be found in the hindgut of certain termites. In the termite gut, fermentation of cellulose causes production of H_2 and CO_2. *T. primitia* is



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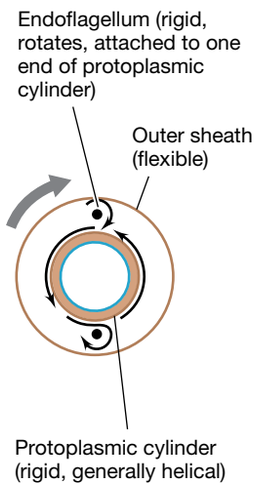
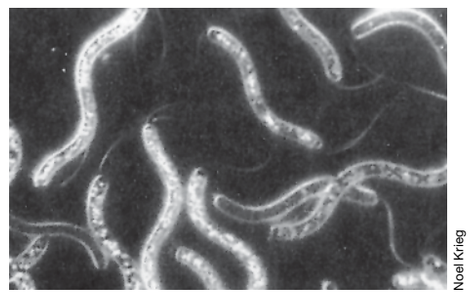


(b)

Figure 15.49 Motility in spirochetes. *(a)* Electron micrograph of a negatively stained cell of *Spirochaeta zuelzerae,* showing the position of the endoflagellum; the cell is about 0.3 μm in diameter. *(b)* Diagram of a spirochete cell, showing the arrangement of the protoplasmic cylinder, endoflagella, and external sheath, and how rotation of the endoflagellum generates rotation of both the protoplasmic cylinder and the external sheath.



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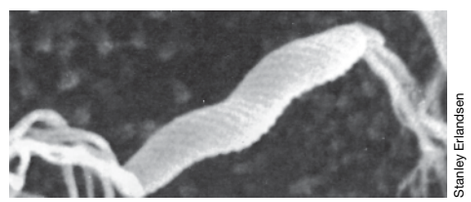

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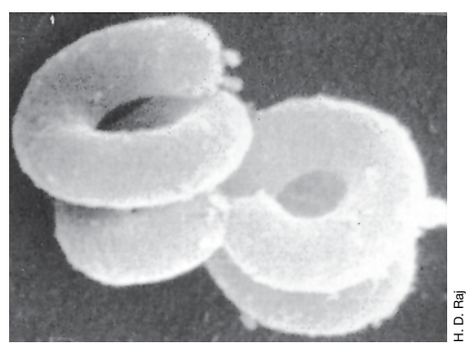


(c)

Figure 15.50 Spirilla. *(a) Spirillum volutans*, visualized by dark-field microscopy, showing flagellar bundles and volutin (polyphosphate) granules. Cells are about 1.5 × 25 μm. *(b)* Scanning electron micrograph of an intestinal spirillum. Note the polar flagellar tufts and the spiral structure of the cell surface. *(c)* Scanning electron micrograph of cells of *Ancylobacter aquaticus*. Cells are about 0.5 μm in diameter.

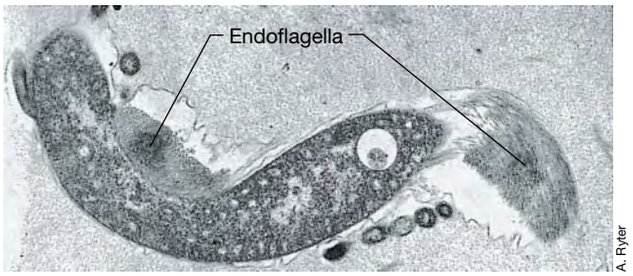
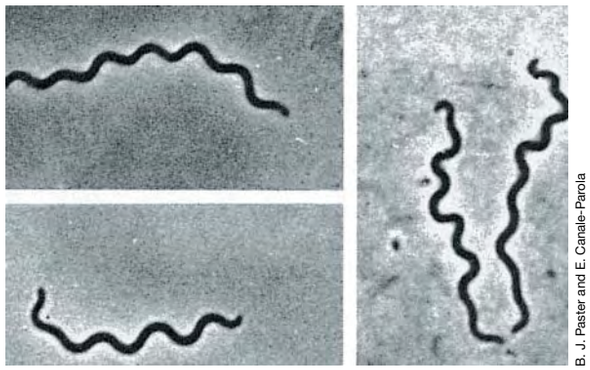


Figure 15.51 ***Cristispira.*** Electron micrograph of a thin section of a cell of *Cristispira*. This large spirochete is about 2 μm in diameter. Notice the numerous endoflagella.

an acetogen (Section 14.16) that grows on H_2 plus CO_2, forming acetate, which is an important component of the insect's nutrition. *Treponema azotonutricium* is also found in the termite hindgut and is capable of nitrogen fixation (Section 14.6).

The majority of species of *Borrelia* are animal or human pathogens. *Borrelia burgdorferi* (Figure 15.52*b*) is the causative agent of the tickborne *Lyme disease*, which infects humans and animals (Section 31.4). *B. burgdorferi* is also of interest because it is one of the few known bacteria that has a linear (as opposed to a circular) chromosome (Sections 4.2 and 9.3). Other species of *Borrelia* are primarily of veterinary importance, causing diseases in cattle, sheep, horses, and birds. In most cases, the bacterium is transmitted to the animal host from the bite of a tick.



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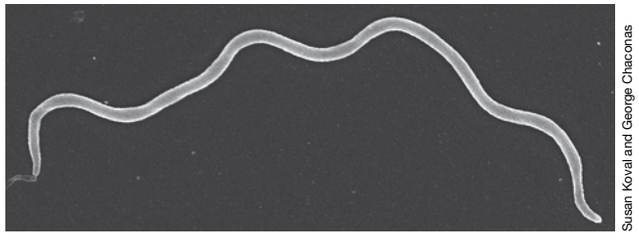


(b)

Figure 15.52 ***Treponema* and *Borrelia.*** *(a)* Phase-contrast micrographs of *Treponema saccharophilum*, a large pectinolytic spirochete from the bovine rumen. A cell measures about 0.4 μm in diameter. Left, regularly coiled cells; right, irregularly coiled cells. *(b)* Scanning electron micrograph of a cell of *Borrelia burgdorferi*, the causative agent of Lyme disease.

Leptospira and *Leptonema*

The genera *Leptospira* and *Leptonema* contain strictly aerobic spirochetes that oxidize long-chain fatty acids (for example, the C_{18} fatty acid oleic acid) as electron donors and carbon sources. With few exceptions, these are the only substrates utilized for growth. Leptospiras are thin, finely coiled, and usually bent at each end into a semicircular hook. At present, several species are recognized in this group, some free-living and many parasitic. Two major species of *Leptospira* are *L. interrogans* (parasitic) and *L. biflexa* (free-living). Strains of *L. interrogans* are parasitic for humans and animals. Rodents are the natural hosts of most leptospiras, although dogs and pigs are also important carriers of certain strains.

In humans the most common leptospiral syndrome is *leptospirosis*, a disorder in which the organism localizes in the kidneys and can cause renal failure or even death. Leptospiras ordinarily enter the body through the mucous membranes or through breaks in the skin during contact with an infected animal. After a transient multiplication in various parts of the body, the organism localizes in the kidneys and liver, causing nephritis and jaundice. Domestic animals such as dogs are vaccinated against leptospirosis with a killed virulent strain in the combined distemper–leptospira–hepatitis vaccine.

MINIQUIZ

- What are the major differences between spirochetes and spirilla?
- Name two diseases of humans caused by spirochetes.

15.20 Budding and Prosthecate/Stalked Microorganisms

KEY GENERA: *Hyphomicrobium, Caulobacter*

The growth of most bacteria is coupled to cell division by the well-known process of binary fission (Section 5.1 and Figure 5.1). In this section, we consider organisms that grow and divide in different ways, including budding and the formation of appendages. Budding and appendaged species often have life cycles that are distinct among bacteria.

Budding Division

As we learned in Section 5.1, budding bacteria divide as a result of unequal cell growth. Cell division in stalked and budding bacteria forms a totally new daughter cell, with the mother cell retaining its original identity (Figure 5.3). In contrast, binary fission produces two equivalent cells.

A fundamental difference between budding bacteria and bacteria that divide by binary fission is the formation of new cell wall material from a single point (polar growth) rather than throughout the whole cell (intercalary growth) as in binary fission (Chapter 7). Several genera not normally considered to be budding bacteria show polar growth without differentiation of cell size (Figure 5.3). An important consequence of polar growth is that internal structures, such as membrane complexes, are not partitioned in the cell division process and must be formed de novo. However, this has an advantage in that more complex internal structures can

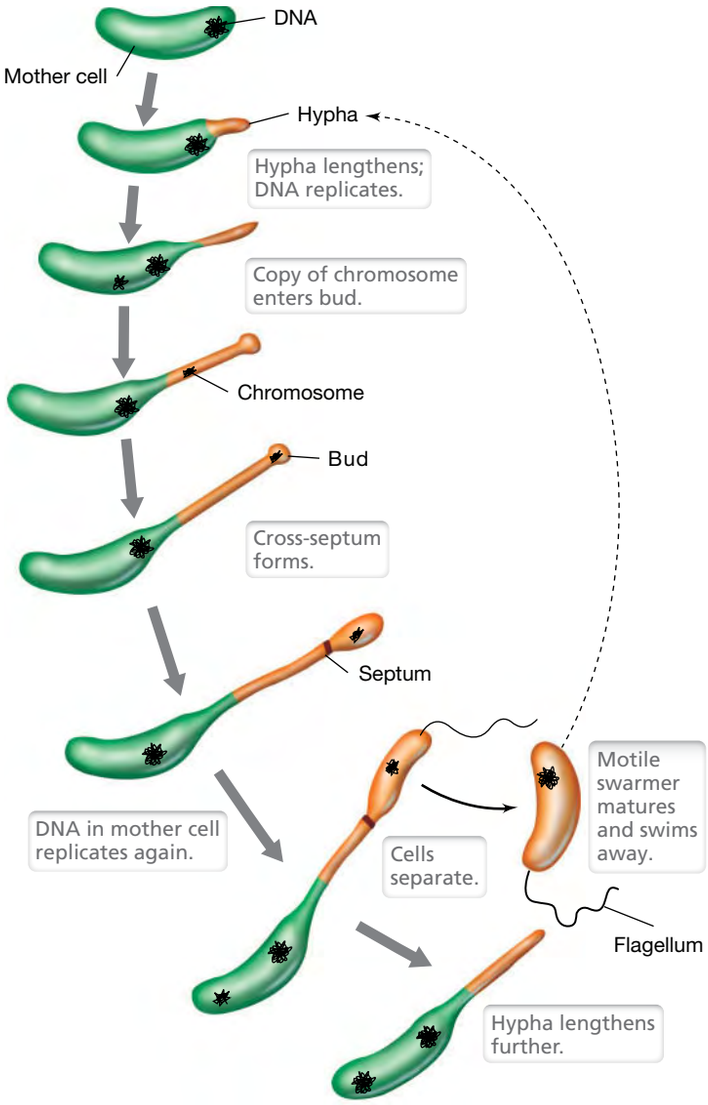


Figure 15.53 Stages in the *Hyphomicrobium* cell cycle. The single chromosome of *Hyphomicrobium* is circular.

be formed in budding cells than in cells that divide by binary fission, since the latter cells would have to partition these structures between the two daughter cells. Not coincidentally, many budding bacteria, particularly phototrophic and chemolithotrophic species, contain extensive internal membrane systems.

Budding Bacteria: *Hyphomicrobium*

Two well-studied budding bacteria are closely related *Alphaproteobacteria: Hyphomicrobium* (**Figure 15.53**), which is chemoorganotrophic, and *Rhodomicrobium*, which is phototrophic. These organisms release buds from the ends of long, thin hyphae. The hypha is a direct cellular extension and contains cell wall, cytoplasmic membrane, and ribosomes, and can contain DNA.

Figure 15.53 shows the life cycle of *Hyphomicrobium*. The mother cell, which is often attached by its base to a solid substrate, forms a thin outgrowth that lengthens to become a hypha. At the end of the hypha, a bud forms. This bud enlarges, forms a flagellum,

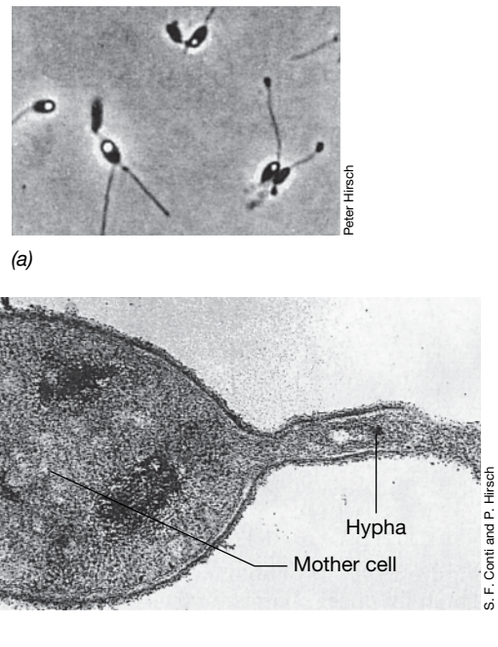


Figure 15.54 Morphology of *Hyphomicrobium*. *(a)* Phase-contrast micrograph of cells of *Hyphomicrobium*. Cells are about 0.7 μm wide. *(b)* Electron micrograph of a thin section of a single *Hyphomicrobium* cell. The hypha is about 0.2 μm wide.

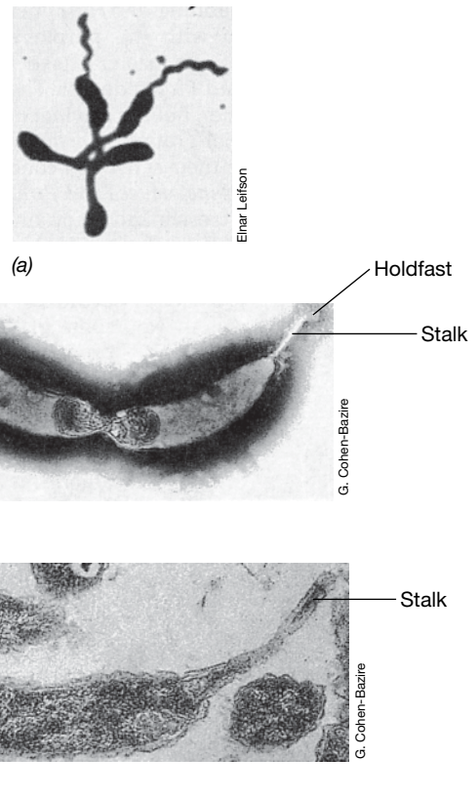


Figure 15.55 Stalked bacteria. *(a)* A *Caulobacter* rosette. A single cell is about 0.5 μm wide. The five cells are attached by their stalks, which are also prosthecae. Two of the cells have divided, and the daughter cells have formed flagella. *(b)* Negatively stained preparation of a *Caulobacter* cell in division. *(c)* A thin section of *Caulobacter* showing that cytoplasm is present in the stalk. Parts *b* and *c* are transmission electron micrographs.

breaks loose from the mother cell, and swims away. Later, the daughter cell loses its flagellum and after a period of maturation forms a hypha and buds. More buds can also form at the hyphal tip of the mother cell, leading to arrays of cells connected by hyphae. In some cases, a bud begins to form directly from the mother cell without the intervening formation of a hypha, whereas in other cases a single cell forms hyphae from each end (Figure 15.54). Nucleoid replication events occur before the bud emerges, and then once a bud has formed, a copy of the chromosome moves down the hypha and into the bud. A cross-septum then forms, separating the still-developing bud from the hypha and mother cell (Figure 15.54).

Physiologically, *Hyphomicrobium* is a methylotrophic bacterium (Sections 14.18 and 15.16), and it is widespread in freshwater, marine, and terrestrial habitats. Preferred carbon sources are methanol (CH_3OH), methylamine (CH_3NH_2), formaldehyde (CH_2O), and formate ($HCOO^-$). A fairly specific enrichment procedure for *Hyphomicrobium* is to use CH_3OH as an electron donor with nitrate (NO_3^-) as an electron acceptor in a dilute medium incubated under anoxic conditions. The only rapidly growing denitrifying bacterium known that uses CH_3OH as an electron donor is *Hyphomicrobium*, and so this procedure can select this organism out of a wide variety of environments.

Prosthecate and Stalked *Bacteria*

A variety of bacteria are able to produce cytoplasmic extrusions including *stalks* (Figure 15.55), *hyphae*, and *appendages* (Table 15.3). Extrusions of these kinds, which are smaller in diameter than the mature cell and contain cytoplasm and a cell wall, are collectively called **prosthecae** (Figure 15.56). Prosthecae allow organisms to attach to particulate matter, plant material, or other microorganisms in aquatic habitats. In addition, prosthecae can be used to increase the ratio of surface area to cell volume. Recall that the high surface-to-volume ratio of prokaryotic cells in general confers an increased ability to take up nutrients and expel wastes (Section 2.2). The unusual morphology of appendaged bacteria (Figure 15.56) carries this theme to an extreme, and may be an evolutionary adaptation to life in oligotrophic (nutrient-poor) waters where these organisms are most commonly found.

Prosthecae may also function to reduce cell sinking. Because these organisms are aquatic and their metabolism is typically aerobic, prosthecae may keep cells from sinking into anoxic zones in their aquatic environments where they would be unable to respire. Some prosthecate bacteria produce gas vesicles (Section 2.9) (Table 15.3), which would also help prevent sinking.

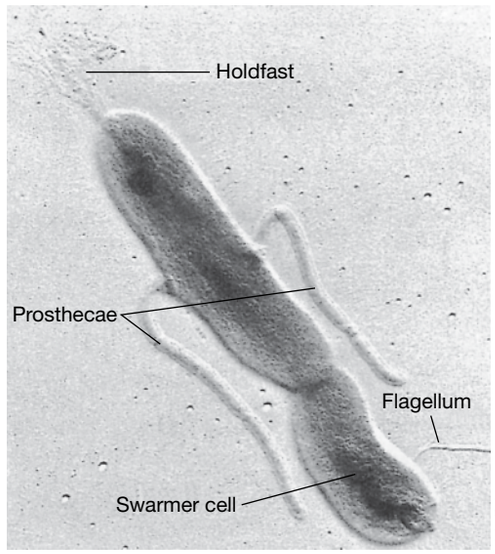
Caulobacter

Two common stalked bacteria are *Caulobacter* (Figure 15.55) and *Gallionella* (Figure 15.36). The former is a chemoorganotroph that produces a cytoplasm-filled stalk, that is, a prostheca, while the latter is a chemolithotrophic iron-oxidizing bacterium whose stalk is composed of ferric hydroxide [$Fe(OH)_3$] (Section 15.15). *Caulobacter* cells are often seen on surfaces in aquatic environments with the stalks of several cells attached to form *rosettes* (Figure 15.55*a*). At the end of the stalk

TABLE 15.3 Characteristics of major genera of stalked, appendaged (prosthecate), and budding *Bacteria*

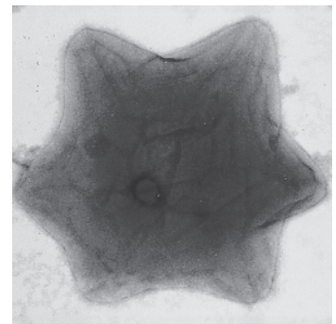
Characteristics	*Genus*	*Phylogenetic group*[a]
Stalked bacteria		
Stalk an extension of the cytoplasm and involved in cell division	*Caulobacter*	*Alpha*
Stalked, fusiform-shaped cells	*Prosthecobacter*	*Verrucomicrobiaceae*[b]
Stalked, but stalk is an excretory product not containing cytoplasm:		
Stalk depositing iron, cell vibrioid	*Gallionella*	*Beta*
Laterally excreted gelatinous stalk not depositing iron	*Nevskia*	*Gamma*
Appendaged (prosthecate) bacteria		
Single or double prosthecae	*Asticcacaulis*	*Alpha*
Multiple prosthecae:		
Short prosthecae, multiply by fission, some with gas vesicles	*Prosthecomicrobium*	*Alpha*
Flat, star-shaped cells, some with gas vesicles	*Stella*	*Alpha*
Long prosthecae, multiply by budding, some with gas vesicles	*Ancalomicrobium*	*Alpha*
Budding bacteria		
Phototrophic, produce hyphae	*Rhodomicrobium*	*Alpha*
Phototrophic, budding without hyphae	*Rhodopseudomonas*	*Alpha*
Chemoorganotrophic, rod-shaped cells	*Blastobacter*	*Alpha*
Chemoorganotrophic, buds on tips of slender hyphae:		
Single hypha from parent cell	*Hyphomicrobium*	*Alpha*
Multiple hyphae from parent cell	*Pedomicrobium*	*Alpha*

[a] All but *Prosthecobacter* are *Proteobacteria*.
[b] See Section 16.17.



(a)

(b)



(c)

Figure 15.56 Prosthecate bacteria. *(a)* Electron micrograph of a shadow-cast preparation of *Asticcacaulis biprosthecum*, illustrating the location and arrangement of the prosthecae, the holdfast, and a swarmer cell. The swarmer cell breaks away from the mother cell and begins a new cell cycle. Cells are about 0.6 μm wide. *(b)* Negatively stained electron micrograph of a cell of *Ancalomicrobium adetum*. The prosthecae are bounded by the cell wall, contain cytoplasm, and are about 0.2 μm in diameter. *(c)* Electron micrograph of the star-shaped prosthecate bacterium *Stella*. Cells are about 0.8 μm in diameter.

is a structure called a *holdfast* by which the stalk anchors the cell to a surface.

The *Caulobacter* cell division cycle (**Figure 15.57**; ⇄ Section 7.7 and Figure 7.16) is unique because cells undergo unequal binary fission. A stalked cell of *Caulobacter* divides by elongation of the cell followed by binary fission, and a single flagellum forms at the pole opposite the stalk. The flagellated cell so formed, called a *swarmer*, separates from the nonflagellated mother cell and eventually attaches to a new surface, forming a new stalk at the flagellated pole; the flagellum is then lost. Stalk formation is a necessary precursor of cell division and is coordinated with DNA synthesis (Figure 15.57). The cell division cycle in *Caulobacter* is thus more complex than simple binary fission or budding division because the stalked and swarmer cells are structurally different and the growth cycle must include both forms.

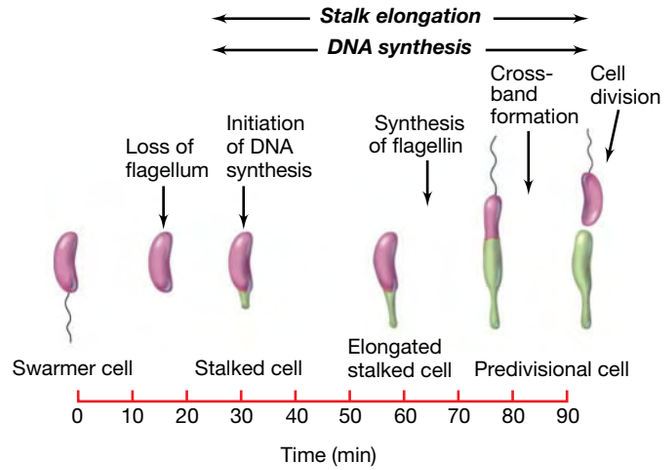


Figure 15.57 Growth of *Caulobacter*. Stages in the *Caulobacter* cell cycle, beginning with a swarmer cell. Compare with Figure 7.16.

MINIQUIZ

- How does budding division differ from binary fission? How does binary fission differ from the division process in *Caulobacter*?
- What advantage might a prosthecate organism have in a very nutrient-poor environment?

15.21 Sheathed Microorganisms

KEY GENERA: *Sphaerotilus, Leptothrix*

Bacteria in many phyla form sheaths made of polysaccharide or protein that encase one or many cells. Sheaths often function to bind cells together into long multicellular filaments (Sections 15.3 and 15.11). *Sphaerotilus* and *Leptothrix* are filamentous bacteria that grow within a sheath and have a unique life cycle. Under favorable conditions, the cells grow vegetatively, leading to the formation of long, cell-packed sheaths. Flagellated swarmer cells form within the sheath under unfavorable growth conditions, and the swarmer cells break out and are dispersed to new environments, leaving behind the empty sheath.

Sphaerotilus and *Leptothrix* are common in freshwater habitats that are rich in organic matter, such as wastewaters and polluted streams. Because they are typically found in flowing waters, they are also abundant in trickling filters and activated sludge digesters in sewage treatment plants (⇄ Section 22.6). In habitats in which reduced iron (Fe^{2+}) or manganese (Mn^{2+}) is present, the sheaths may become coated with ferric hydroxide [$Fe(OH)_3$] or manganese oxides from the oxidation of these metals.

Leptothrix

The ability of *Sphaerotilus* and *Leptothrix* to precipitate iron oxides on their sheaths is well established, and when sheaths become iron encrusted, as occurs in iron-rich waters, they can frequently be seen microscopically (**Figure 15.58**). Iron precipitates form when ferrous iron (Fe^{2+}), chelated to organic materials such as humic or tannic acids, is oxidized. These chemoorganotrophic bacteria use the organic materials as a carbon or energy source and, when no longer chelated, the ferrous iron becomes oxidized and precipitates

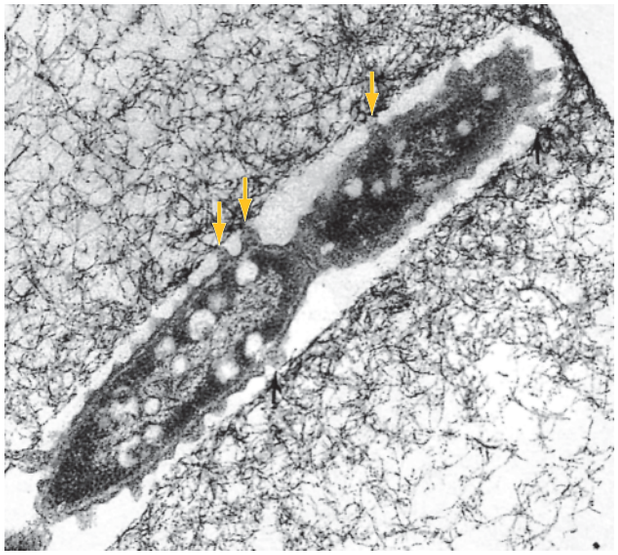


Figure 15.58 ***Leptothrix* and iron precipitation.** Transmission electron micrograph of a thin section of *Leptothrix* growing in a ferromanganese film in a swamp in Ithaca, New York. A single cell measures about 0.9 µm in diameter. Note the protuberances of the cell envelope that contact the sheath (arrows).

on the sheath. Iron oxidation is fortuitous and though these organisms are closely related to dissimilative iron-oxidizers (Section 15.15), the organism does not gain energy from iron oxidation. In a similar way, *Leptothrix* can also oxidize manganese.

Sphaerotilus

The *Sphaerotilus* filament is composed of a chain of rod-shaped cells enclosed in a closely fitting sheath. This thin, transparent structure is difficult to see when it is filled with cells, but when it is partially empty, the sheath can more easily be resolved (**Figure 15.59*a***). Individual cells are 1–2 × 3–8 µm in dimensions and stain gram-negatively. The cells within the sheath (Figure 15.59*b*) divide by binary fission, and the new cells synthesize new sheath material at the tips of the filaments. Eventually, motile swarmer cells are liberated from the sheaths (Figure 15.59*c*) and then migrate, attach to a solid surface, and begin to grow, with each swarmer being the forerunner of a new filament. The sheath, which is devoid of peptidoglycan, consists of protein and polysaccharide.

Sphaerotilus species are nutritionally versatile and use simple organic compounds as carbon and energy sources; one species can grow mixotrophically with thiosulfate as electron donor. Befitting its habitat in flowing waters, *Sphaerotilus* is an obligate aerobe. Large masses (blooms) of *Sphaerotilus* often occur in the fall of the year in streams and brooks when leaf litter causes a temporary increase in the organic content of the water. In addition, its filaments are the main component of a microbial complex that wastewater engineers call "sewage fungus," a filamentous slime found on the rocks in streams receiving sewage pollution. In activated sludge of sewage treatment plants (Section 22.6), *Sphaerotilus* is often responsible for a condition called *bulking*, where the tangled masses of *Sphaerotilus* filaments so increase the bulk of the sludge that it remains suspended and does not settle as it should. This has a negative effect on the oxidation of organic matter and the recycling of inorganic nutrients and leads to treatment plant discharges with high nitrogen and carbon loads.

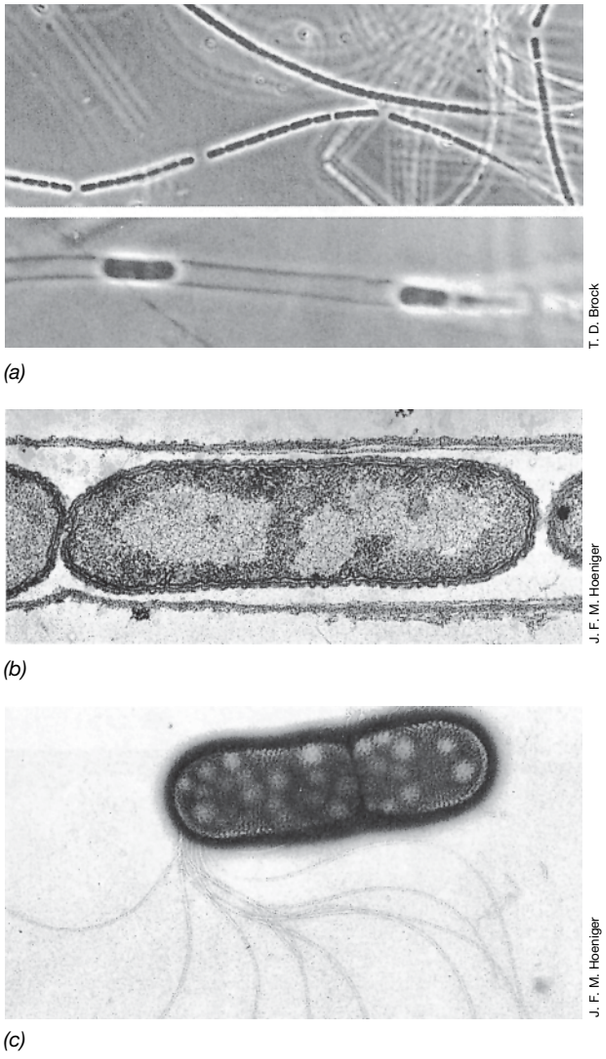


Figure 15.59 ***Sphaerotilus natans.*** A single cell is about 2 µm wide. *(a)* Phase-contrast photomicrographs of material collected from a polluted stream. Active growth stage (above) and swarmer cells leaving the sheath. *(b)* Electron micrograph of a thin section through a filament, clearly showing the sheath. *(c)* Electron micrograph of a negatively stained swarmer cell. Notice the polar flagellar tuft.

MINIQUIZ

- Describe how a sheathed bacterium such as *Sphaerotilus* grows.
- List two metals that are oxidized by sheathed bacteria.

15.22 Magnetic Microbes

KEY GENERA: *Magnetospirillum*

In a magnetic field, magnetic bacteria demonstrate a dramatic directed movement called *magnetotaxis*. Within these cells are structures called *magnetosomes*, which consist of chains of magnetic particles made of magnetite (Fe_3O_4) or greigite (Fe_3S_4)

(↔ Section 2.8 and Figure 2.24). Magnetosomes are localized within invaginations of the cell membrane that are organized in a linear conformation by a protein scaffold. Magnetic bacteria orient along the north–south magnetic moment of a magnetic field, aligning parallel to the field lines in much the same manner as a compass needle. Magnetic bacteria are typically microaerophilic or anaerobic and are most often found near the oxic–anoxic interface in sediments or stratified lakes. The magnetosomes of aerobic species typically contain the mineral magnetite while those of anaerobes contain exclusively greigite.

Although the ecological role of bacterial magnets is unclear, the ability to orient in a magnetic field may be of selective advantage in maintaining these organisms in zones of low O_2 concentration. Generally, the concentration of O_2 decreases with depth through sediments or the water column of stratified lakes. Since Earth is spherical, its magnetic field lines have a strong vertical component in the Northern and Southern Hemispheres. Thus, bacteria that orient along these field lines can preferentially swim down and away from O_2. The magnetosome functions like a compass needle to "point" the bacterium in the right direction; rotation of the flagellum, by contrast, is controlled by a chemotactic response to O_2 (↔ Section 2.13).

Magnetic bacteria display one of two magnetic polarities depending on the orientation of magnetosomes within the cell. Cells in the Northern Hemisphere have the north-seeking pole of their magnetosomes forward with respect to their flagella and thus move in a northward direction (which in the Northern Hemisphere is downward). Cells in the Southern Hemisphere have the opposite polarity and move southward.

Most of the magnetic bacteria that have been described are species of *Alphaproteobacteria*, but species have also been observed in the *Gammaproteobacteria*, the *Deltaproteobacteria*, and the *Nitrospira* group. One of the best-characterized species is *Magnetospirillum magnetotacticum* (**Figure 15.60**), which is a chemoorganotrophic microaerophile that can also grow anaerobically by reducing NO_3^- or N_2O. In contrast, the species *Desulfovibrio magneticus* is a sulfate reducer and an obligate anaerobe. In addition, magnetosomes have been observed in a few species of sulfur oxidizers and purple nonsulfur bacteria. Multicellular magnetotactic bacteria are also known. These are *Deltaproteobacteria* that form multicellular aggregates of 10–20 cells organized as a hollow sphere. While multicellular magnetotactic bacteria are obligate anaerobes, the basis of their metabolism has not yet been determined.

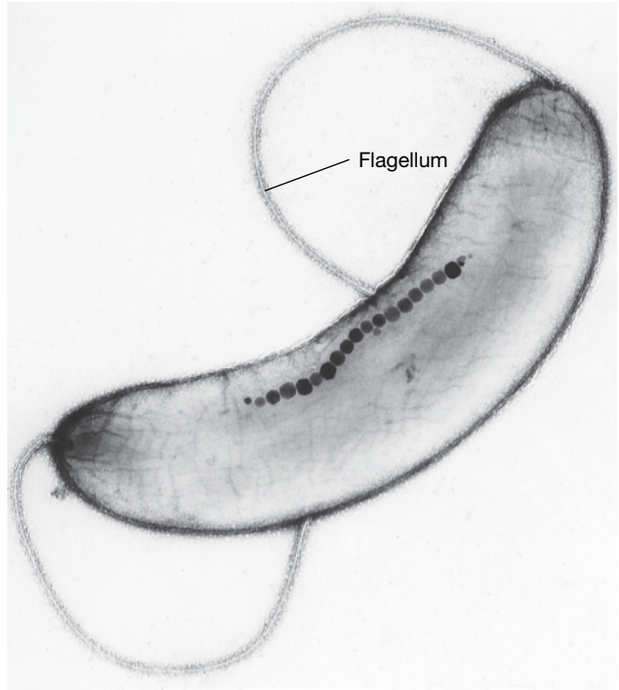


Figure 15.60 A magnetotactic spirillum. Electron micrograph of a single cell of *Magnetospirillum magnetotacticum*; a cell measures 0.3 × 2 μm. The cell contains particles of magnetosomes made of Fe_3O_4 arranged in a chain.

We transition now from viewing *Bacteria* from a functional diversity perspective to consider some other important phyla in a phylogenetic perspective in Chapter 16. We then conclude our coverage of prokaryotic microbes with Chapter 17 dedicated to the *Archaea*.

MINIQUIZ

- What benefit do magnetic bacteria accrue from having magnetosomes?
- Would you expect to find greigite or magnetite in the magnetosomes of *Desulfovibrio magneticus*?

Chapter Review

I • Functional Diversity as a Concept

15.1 Phylogenetic diversity is that component of microbial diversity that deals with evolutionary relationships between microorganisms. By contrast, functional diversity deals with diversity in form and function as it relates to microbial physiology and ecology. Incongruence between phylogeny and the functional traits of microorganisms can result from patterns of gene loss, horizontal gene transfer, and/or convergent evolution.

Q What is convergent evolution and how is it different from horizontal gene transfer?

II • Diversity of Phototrophic *Bacteria*

15.2 Anoxygenic phototrophs, which do not produce oxygen, were the first phototrophic organisms to evolve. The evolution of photosynthesis has been affected strongly by patterns of horizontal gene transfer.

Q Which phyla of *Bacteria* contain anoxygenic phototrophs? Which phylum contains purple sulfur bacteria? Which phylum contains green sulfur bacteria?

15.3 *Cyanobacteria* is the only bacterial phylum that contains oxygenic phototrophs. All species of cyanobacteria can fix CO_2 and many can also fix N_2, making these organisms important primary producers in many ecosystems.

Q Which morphological groups can *Cyanobacteria* be divided into?

15.4 Purple sulfur bacteria are anoxygenic phototrophic *Gammaproteobacteria*. Purple sulfur bacteria use H_2S and S^0 as electron donors and fix CO_2 by the Calvin cycle. These phototrophs have bacteriochlorophylls *a* or *b* and use a Q-type reaction center.

Q Compare and contrast the metabolism, morphology, and phylogeny of purple sulfur and purple nonsulfur bacteria.

15.5 Purple nonsulfur bacteria are anoxygenic phototrophic *Alpha-* and *Betaproteobacteria*. Purple nonsulfur bacteria are metabolically diverse, growing best as photoheterotrophs, and can also grow in darkness. These phototrophs have bacteriochlorophylls *a* or *b* and use type II photosystems with Q-type reaction centers. Aerobic anoxygenic phototrophs have type II photosystems but only possess bacteriochlorophyll *a*.

Q In what metabolic ways are aerobic anoxygenic phototrophs and purple nonsulfur bacteria similar? How do they differ?

15.6 Green sulfur bacteria are anoxygenic phototrophs of the phylum *Chlorobi*. Green sulfur bacteria use H_2S or S^0 as electron donors and fix CO_2 by the reverse citric acid cycle. These phototrophs contain bacteriochlorophylls *c, d*, or *e* localized in chlorosomes and bacteriochlorophyll *a* localized in their FeS-type photosynthetic reaction centers.

Q Compare and contrast the metabolism, morphology, and phylogeny of green sulfur and purple sulfur bacteria.

15.7 Green nonsulfur bacteria, also known as filamentous anoxygenic phototrophs, are anoxygenic phototrophs of the phylum *Chloroflexi* and grow best as photoheterotrophs. These phototrophs contain bacteriochlorophyll *c* in chlorosomes and bacteriochlorophyll *a* in their Q-type photosynthetic reaction centers.

Q What traits do green nonsulfur bacteria share with green sulfur bacteria and purple sulfur bacteria?

15.8 Heliobacteria are anoxygenic phototrophic *Firmicutes* that grow as photoheterotrophs or in darkness as chemotrophs. Heliobacteria produce bacteriochlorophyll *g* and have FeS-type reaction centers. *Chloracidobacterium thermophilum* is an anoxygenic phototrophic acidobacterium that grows photoheterotrophically, possesses bacteriochlorophyll *a* and *c* as well as chlorosomes, and has an FeS-type reaction center.

Q In what ways is *Chloracidobacterium thermophilum* similar to green sulfur bacteria, and in what ways is it different?

III • Microbial Diversity in the Sulfur Cycle

15.9 Dissimilative sulfate-reducers are obligate anaerobes that grow by reducing SO_4^{2-} with H_2 or various organic compounds as electron donors. Most sulfate reducers belong to the *Deltaproteobacteria*.

Q With respect to sulfate-reducing bacteria and acetate as substrate, which genera contain "incomplete oxidizers" and which genera contain "complete oxidizers"?

15.10 Dissimilative sulfur-reducers are metabolically and phylogenetically diverse organisms that grow by reducing S^0 and other oxidized sulfur compounds (other than SO_4^{2-}) as electron acceptors.

Q What are the physiological traits that can differentiate sulfur-reducing bacteria from sulfate-reducing bacteria?

15.11 Sulfur chemolithotrophs, most of which are species of *Proteobacteria*, oxidize H_2S and other reduced sulfur compounds as electron donors with O_2 or NO_3^- as electron acceptors and use either CO_2 or organic compounds as carbon sources. Sulfur chemolithotrophs use a variety of ecological strategies to conserve energy from H_2S and O_2, substances that otherwise react together spontaneously.

Q What are some ecological strategies that aerobic sulfide-oxidizers use to compete with the chemical oxidation of H_2S by atmospheric O_2?

IV • Microbial Diversity in the Nitrogen Cycle

15.12 Diazotrophs are bacteria that assimilate N_2 through activity of the enzyme nitrogenase. Diazotrophs are metabolically and phylogenetically diverse and employ various adaptations to protect nitrogenase from oxygen inactivation.

Q What are some ways that diazotrophs protect nitrogenase from O_2?

15.13 Nitrifying bacteria are aerobic chemolithotrophs that oxidize NH_3 to NO_2^- (genus prefix *Nitroso-*) or NO_2^- to NO_3^- (genus prefix *Nitro-*). Ammonia oxidizers are *Proteobacteria* or *Thaumarchaeota*, while nitrite oxidizers are *Proteobacteria* or *Nitrospirae*. Denitrifiers are metabolically and phylogenetically diverse facultative aerobes and chemoorganotrophs that reduce NO_3^- to the gaseous products NO, N_2O, and N_2.

Q Compare and contrast the nitrogen metabolism of nitrifiers with that of denitrifiers.

V • Other Distinctive Functional Groupings of Microorganisms

15.14 Dissimilative iron-reducers reduce insoluble electron acceptors in anaerobic respirations. Most species can grow anaerobically by reducing ferric iron using H_2 or simple organic compounds as electron donor. The best-characterized genera include *Geobacter*, which contains exclusively obligate anaerobes, and *Shewanella*, which contains facultative aerobes.

Q In what ways are the dissimilative iron-reducing bacteria *Shewanella* and *Geobacter* similar, and in what ways are they different?

15.15 Dissimilative iron-oxidizers conserve energy from the aerobic oxidation of ferrous iron. These microbes use several ecological strategies to cope with the chemical instability of ferrous iron in oxic habitats at neutral pH. Iron oxidizers are found in four physiological groups: aerobic acidophiles, aerobic neutrophiles, anaerobic chemotrophs, and anaerobic phototrophs.

Q Which group of dissimilative iron-oxidizers is the least diverse and in what way is this related to oxygen and pH?

15.16 Methylotrophs grow on organic compounds that lack carbon–carbon bonds. Some methylotrophs are also methanotrophs, organisms able to catabolize methane. Most methanotrophs are *Proteobacteria* that contain extensive internal membranes and incorporate carbon by either the serine or ribulose monophosphate pathways.

Q What are the differences between type I and type II methanotrophs?

15.17 Bacterial predators such as *Bdellovibrio* and *Myxococcus* consume other microorganisms. Myxobacteria have a complex developmental cycle that involves the formation of fruiting bodies that contain myxospores.

Q Compare and contrast the life cycle of *Myxococcus* with that of *Bdellovibrio*.

15.18 *Vibrio, Aliivibrio*, and *Photobacterium* species are marine bacteria, some of which are pathogenic and bioluminescent. Bioluminescence, catalyzed by the enzyme luciferase, is controlled by a quorum-sensing mechanism that ensures that light is not emitted until a large cell population has been attained.

Q Describe the manner in which cell density regulates light production in luminescent bacteria.

VI • Morphologically Diverse *Bacteria*

15.19 The phylum *Spirochaetes* contains helically shaped bacteria that show a novel form of motility that allows them to "corkscrew" through viscous materials. These organisms are common in anoxic habitats and are the cause of many well-known human diseases, such as syphilis.

Q Contrast the motility of spirochetes with that of spirilla.

15.20 Prosthecate bacteria, such as *Hyphomicrobium, Caulobacter*, and *Gallionella*, are appendaged cells that form stalks or prosthecae used for attachment or nutrient absorption, and are primarily aquatic. Some prosthecate bacteria, such as *Hyphomicrobium*, have a complex life cycle in which new cells form by budding from hyphae.

Q Contrast the life cycle of *Hyphomicrobium* with that of *Caulobacter*.

15.21 Sheathed bacteria are filamentous *Proteobacteria* in which individual cells form chains within an outer layer called the sheath. *Sphaerotilus* and *Leptothrix* are major genera of sheathed bacteria and can oxidize metals, such as Fe^{2+} and Mn^{2+}.

Q In what environment might you expect to find *Leptothrix*?

15.22 Magnetosomes are specialized magnetic structures present in magnetotactic bacteria. Magnetosomes orient cells along the magnetic field lines of Earth, and this allows cells to use their normal chemotactic response to move vertically in a directed fashion in sediments or stratified aquatic systems.

Q In what way does a magnetosome contribute to the fitness of microaerophilic bacteria in sediments?

Application Questions

1. Describe a key physiological feature of the following *Bacteria* that would differentiate each from the others: *Acetobacter, Methylococcus, Azotobacter, Photobacterium, Desulfovibrio*, and *Spirillum*.
2. Describe the metabolism for each of the following *Bacteria* and state whether the organism is an aerobe or an anaerobe: *Thiobacillus, Nitrosomonas, Methylomonas, Pseudomonas, Acetobacter*, and *Gallionella*.
3. Using an example from each of the morphologically diverse groups of *Bacteria* (Sections 15.19–15.22), describe how you could distinguish them from each other using only microscopy. How do the habitats of your example organisms differ from each other? Could you find any of these organisms in or on the human body? Despite their ability to oxidize inorganic electron donors, why are *Sphaerotilus* and *Leptothrix* not considered chemolithotrophs?

Chapter Glossary

Aerobic anoxygenic phototroph an organism that is an aerobic heterotroph that uses anoxygenic photosynthesis as a supplemental source of energy

Autoinduction a gene regulatory mechanism involving small, diffusible signal molecules that are produced in larger amounts as population size increases

Bioluminescence the enzymatic production of visible light by living organisms

Carboxysome a polyhedral cellular inclusion of crystalline ribulose bisphosphate carboxylase (RubisCO), the key enzyme of the Calvin cycle

Chemolithotroph an organism able to oxidize inorganic compounds (such as H_2, Fe^{2+}, S^0, or NH_4^+) as energy sources (electron donors)

Chlorosome a cigar-shaped structure bounded by a nonunit membrane and containing the light-harvesting bacteriochlorophyll (*c, d*, or *e*) in green sulfur bacteria and *Chloroflexus*

Consortium a two- or more-membered association of bacteria, usually living in an intimate symbiotic fashion

Convergent evolution a circumstance where a trait or set of traits that are similar in form and/or function between two organisms are not inherited from a shared ancestor (that is, traits that are similar but not homologous)

Cyanobacteria prokaryotic oxygenic phototrophs containing chlorophyll *a* and phycobilins

Denitrifier an organism that carries out anaerobic respiration with NO_3^- or NO_2^-, reducing it to the gaseous products NO, N_2O, and N_2

Diazotroph an organism that can assimilate N_2 into biomass by activity of the enzyme nitrogenase

Dissimilative sulfate-reducer an anaerobic microorganism that conserves energy through the reduction of SO_4^{2-}

Dissimilative sulfur-oxidizer a microorganism that gains energy for growth through oxidation of reduced sulfur compounds

Dissimilative sulfur-reducer an anaerobic or facultatively aerobic microorganism that conserves energy through the reduction of S^0 but cannot reduce SO_4^{2-}

Functional diversity the component of biological diversity that deals with the forms and functions of organisms as they relate to differences in physiology and ecology

Green nonsulfur bacteria anoxygenic phototrophs containing chlorosomes, Q-type photosynthetic reaction centers, bacteriochlorophylls *a* and *c* as light-harvesting chlorophylls, and typically growing best as photoheterotrophs

Green sulfur bacteria anoxygenic phototrophs containing chlorosomes, FeS-type photosynthetic reaction centers, bacteriochlorophylls *c, d*, or *e* as antenna bacteriochlorophylls, and typically growing with H_2S as an electron donor

Heliobacteria anoxygenic phototrophs containing bacteriochlorophyll *g* and FeS-type reaction centers

Horizontal gene transfer a unidirectional transfer of genes between unrelated organisms; can cause homologous genes to be dispersed in a phylogeny

Methanotroph an organism capable of oxidizing methane (CH_4) as an electron donor in energy metabolism

Methylotroph an organism capable of oxidizing organic compounds that do not contain carbon–carbon bonds; if able to oxidize CH_4, also a methanotroph

Mixotroph an organism that conserves energy from the oxidation of inorganic compounds but requires organic compounds as a carbon source

Nitrifier a chemolithotroph capable of carrying out the oxidation of NH_3 or NO_2^-

Phycobilin a protein containing the pigment phycocyanin or phycoerythrin that functions as a photosynthetic accessory pigment in cyanobacteria

Prochlorophyte a bacterial oxygenic phototroph that contains chlorophylls *a* and *b* but lacks phycobilins

Prosthecae extrusions of cytoplasm, often forming distinct appendages, bounded by the cell wall

Purple nonsulfur bacteria a group of phototrophic bacteria that contain bacteriochlorophyll *a* or *b* and Q-type reaction centers, and that grow best as photoheterotrophs

Purple sulfur bacteria a group of phototrophic bacteria that contain bacteriochlorophylls *a* or *b*, Q-type reaction center, and can oxidize H_2S as photosynthetic electron donor

Spirilla (singular, spirillum) spiral-shaped cells

Spirochete a slender, tightly coiled, gram-negative bacterium of the phylum *Spirochaetes* characterized by possession of endoflagella used for motility

16 Diversity of *Bacteria*

microbiologynow

The Mystery of the Missing Peptidoglycan

Planctomycetes have many enigmatic features. Unlike most *Bacteria* they lack the protein FtsZ, which is required for binary fission; they divide by budding much like yeast; and they appear capable of endocytosis, a feature uniquely associated with eukaryotic cells. What is more, *Planctomycetes* were long thought to lack peptidoglycan, and transmission electron micrographs (TEM) suggested the presence of a membrane-enclosed nucleus in some species. Even more incredible, some *Planctomycetes* actually have membrane-enclosed organelles (anammoxosomes). This unique mix of prokaryotic and eukaryotic features has for years led many microbiologists to argue that *Planctomycetes* are a missing link in cellular evolution. However, the mysteries of the missing peptidoglycan and the nature of the "nucleus"—like many other microbial mysteries—were ultimately solved by advances in microbiological techniques.

Cryo-electron tomography (CET) is revolutionizing our understanding of cellular structures. CET generates detailed three-dimensional images with nanometer-scale resolution. For example, CET scans of *Planctomycetes* revealed that what appeared to be a nuclear membrane in two-dimensional transmission electron micrographs was actually a system of deep invaginations of the cytoplasmic membrane. Two-dimensional slices through this three-dimensional system of membranes gave the false appearance of a nuclear membrane. Furthermore, these images, such as the CET scan of *Planctopirus limnophila* shown here, clearly reveal that cells of *Planctomycetes* have a gram-negative cell envelope composed of an outer membrane (green), a peptidoglycan layer (orange), and the cytoplasmic membrane (blue).

The *Planctopirus* cytoplasmic membrane is heavily invaginated, and when viewed in three dimensions, these invaginations can be seen to wrap around both the DNA containing nucleoids (yellow) and ribosomes (white) present in the cytoplasm. Genomic analyses of *Planctomycetes* revealed that key genes required for the biosynthesis of peptidoglycan were present in the genome, and biochemical experiments confirmed that the major constituents of peptidoglycan (*N*-acetylglucosamine, *N*-acetylmuramic acid, and 2,6-diaminopimelic acid) were present.

For every mystery solved in science, another is often revealed. While we now know that cells of *Planctomycetes* contain both peptidoglycan and a gram-negative cell envelope, an understanding of the remarkable membrane invaginations that appear in CET scans of cells of *Planctomycetes* and the unusual crateriform structures (magenta) that stud the surface of their outer membranes remains elusive and beyond our current understanding of prokaryotic cell biology.

Source: Jeske, O., et al. 2015. *Planctomycetes* do possess a peptidoglycan cell wall. *Nature Communications 6:* 7116.

In the last chapter we examined the *functional* diversity of microorganisms. In this and the next two chapters we shift our focus to the *phylogenetic* diversity of microorganisms. We discussed the difference between functional diversity and phylogenetic diversity in Section 15.1. In this chapter we examine the major lineages of *Bacteria* (**Figure 16.1a**) and we focus on the *Archaea* and microbial *Eukarya* in Chapters 17 and 18, respectively.

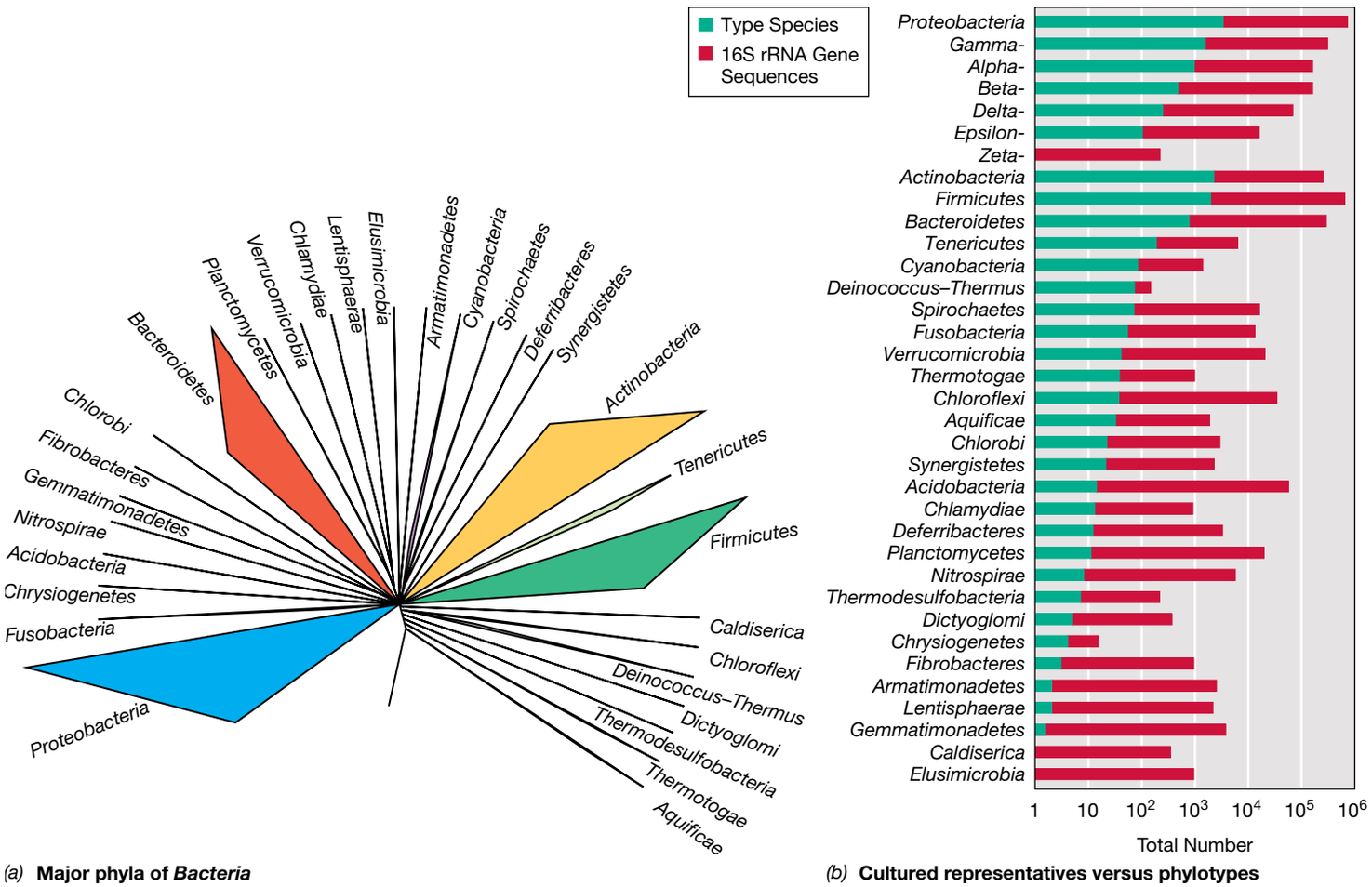
Including phyla of *Bacteria* known only from 16S ribosomal RNA (rRNA) gene sequences retrieved from the environment (Section 19.6), over 80 phyla can be distinguished. However, fewer than half of these contain species that have been characterized in laboratory culture (Figure 16.1*b*). Remarkably, more than 90% of characterized genera and species of *Bacteria* originate in only four phyla: *Proteobacteria, Actinobacteria, Firmicutes*, and *Bacteroidetes* (Figure 16.1*b*).

With more than ten thousand species of bacteria described, we obviously cannot consider them all. Therefore, using phylogenetic trees to focus our discussion, we will explore some of the best-known species from a broad diversity of phyla. In this chapter we will consider species from more than 20 bacterial phyla, focusing on those with the largest numbers of characterized species. We begin our tour of the *Bacteria* with the phylum *Proteobacteria*, a hotbed of cultured species in this domain.

Proteobacteria

The ***Proteobacteria*** are by far the largest and most metabolically diverse phylum of *Bacteria* (**Figure 16.2**). More than a third of characterized species of *Bacteria* originate within this group (Figure 16.1*b*), and *Proteobacteria* constitute the majority of known bacteria of medical, industrial, and agricultural significance.

As a group, the *Proteobacteria* are all gram-negative bacteria. They show an exceptionally wide diversity of energy-generating mechanisms, with chemolithotrophic, chemoorganotrophic, and phototrophic species (Figure 16.2). Indeed, we have already encountered diverse representatives of this group in Chapters 14 and 15 where we considered the tremendous metabolic and functional diversity of the *Proteobacteria*. The *Proteobacteria* are equally



(a) **Major phyla of *Bacteria***

(b) **Cultured representatives versus phylotypes**

Figure 16.1 Some major phyla of *Bacteria* based on 16S ribosomal RNA gene sequence comparisons. *(a)* Depicted are the major phyla of *Bacteria* that have cultivated species. Analyses of 16S rRNA gene sequences from natural environments suggest there are more than 80 bacterial phyla. *(b)* Numbers of cultured and characterized species (green bars) and known 16S rRNA gene sequences (phylotypes, red bars) for each of the 29 major bacterial phyla that have at least one characterized species in pure culture. Also shown are related data for the different classes of *Proteobacteria*. Differences between the size of the red and green bars indicate the degree to which members of each group are common in natural environments but difficult to cultivate in isolation. Note that the abscissa is a log scale.

UNIT 4

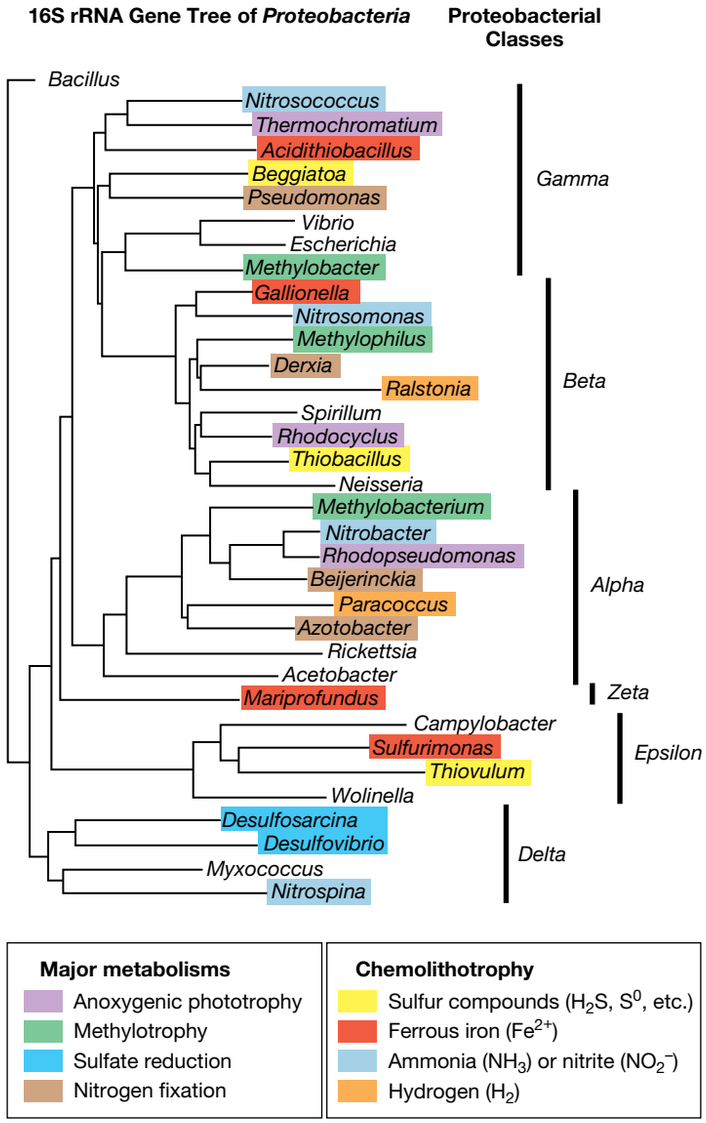


Figure 16.2 Phylogenetic tree and metabolic links of some key genera of *Proteobacteria*. Phylogeny of representative genera of *Proteobacteria* as revealed by analysis of 16S rRNA gene sequences. Note how identical metabolisms are often distributed in phylogenetically distinct genera, suggesting that horizontal gene flow has been extensive in the *Proteobacteria*. Some organisms listed may have multiple properties; for example, some sulfur chemolithotrophs are also iron or hydrogen chemolithotrophs, and several of the organisms listed can fix nitrogen. Phylogenetic analyses were performed and the phylogenetic tree constructed by Marie Asao, Ohio State University.

diverse in terms of their relationship to oxygen (O_2), with anaerobic, microaerophilic, and facultatively aerobic species known. Morphologically, they also exhibit a wide range of cell shapes, including straight and curved rods, cocci, spirilla, and filamentous, budding, and appendaged forms.

As we proceed through this chapter, we will need to recall the descending hierarchy of microbial systematics: domain (*Bacteria* or *Archaea*)→phylum→class→order→family→genus→species. We will use these terms frequently in this chapter. Based on 16S rRNA gene sequences, the phylum *Proteobacteria* can be divided into six classes: *Alpha-, Beta-, Gamma-, Delta-, Epsilon-*, and *Zetaproteobacteria*. Each class contains many genera with the exception of the *Zetaproteobacteria*, which is composed of a single known species, the marine iron-oxidizing bacterium *Mariprofundus ferrooxydans* (Section 15.15).

Despite the phylogenetic breadth of the *Proteobacteria*, species in different classes often have similar metabolisms. For example, phototrophy and methylotrophy occur in three different classes of *Proteobacteria*, and nitrifying bacteria span four classes of *Proteobacteria* (Figure 15.1). This suggests that horizontal gene flow (Chapter 11 and Section 13.7) has played a major role in shaping the metabolic diversity of the *Proteobacteria*. The sharing of metabolic traits in the different classes of *Proteobacteria* is also a good reminder that phenotype and phylogeny often provide different views of prokaryotic diversity (Section 15.1).

16.1 *Alphaproteobacteria*

With about one thousand described species, the *Alphaproteobacteria* are the second largest class of *Proteobacteria* (Figure 16.1*b*). The *Alphaproteobacteria* contain extensive functional diversity (Figure 16.2, Figure 15.1), and many genera in this group have already been considered in Chapter 14. Most species are obligate aerobes or facultative aerobes and many are **oligotrophic**, preferring to grow in environments that have low nutrient concentration. A total of 10 orders have been described within the *Alphaproteobacteria*, but the vast majority of species fall within the *Rhizobiales, Rickettsiales, Rhodobacterales, Rhodospirillales, Caulobacterales*, and *Sphingomonadales* (**Figure 16.3**, **Table 16.1**).

Rhizobiales

KEY GENERA: *Bartonella, Methylobacterium, Pelagibacter, Rhizobium, Agrobacterium*

The *Rhizobiales* (Figure 16.3) are the largest and most metabolically diverse order of *Alphaproteobacteria* and contain phototrophs (e.g., *Rhodopseudomonas*), chemolithotrophs (e.g., *Nitrobacter*), symbionts (e.g., rhizobia), free-living nitrogen-fixing bacteria (e.g., *Beijerinckia*), a few pathogens of plants and animals, and diverse chemoorganotrophs. The group gets its name from the *rhizobia*, a *polyphyletic* collection of genera that form root nodules and fix nitrogen in symbiotic association with leguminous plants (Section 23.00).

Among the *Rhizobiales* are nine genera that contain rhizobia: *Bradyrhizobium, Ochrobactrum, Azorhizobium, Devosia, Methylobacterium, Mesorhizobium, Phyllobacterium, Sinorhizobium*, and *Rhizobium*. These are typically chemoorganotrophs and obligate aerobes, and the genes that convey the ability to form root nodules have clearly been distributed among these genera by horizontal gene transfer. Each rhizobial genus has a distinct range of plant hosts that can be colonized (Table 23.1). Rhizobia can be isolated by crushing nodules and spreading their contents on nutrient-rich solid media; colonies typically produce copious amounts of exopolysaccharide slime (**Figure 16.4**).

The organism *Agrobacterium tumefaciens* (also called *Rhizobium radiobacter*) is closely related to root nodule *Rhizobium* species but is a plant pathogen that causes crown gall disease (Section 23.5). *A. tumefaciens* is unable to form root nodules, and the genes that encode gall formation are unrelated to those that mediate nodule formation.

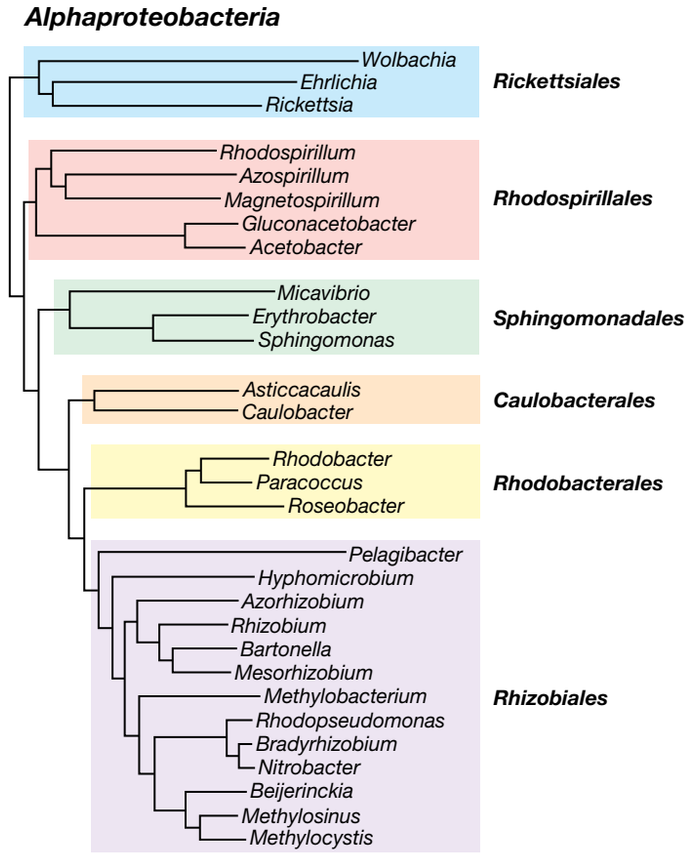


Figure 16.3 **Major orders of *Proteobacteria* in the class *Alphaproteobacteria*.** The phylogenetic tree was constructed using 16S rRNA gene sequences from representative genera of *Alphaproteobacteria*. Order names are shown in bold.

The genus *Methylobacterium* is one of the largest in the *Rhizobiales*. These species are often called "pink-pigmented facultative methylotrophs" (Section 15.16) because of the pink color of their colonies and their good growth on methanol. Species are commonly found on the surface of plants and in soils and freshwater systems. These organisms are also commonly encountered in toilets and baths where their growth on shower curtains, caulk, and in toilet bowls results in the formation of pink-pigmented biofilms. Species of *Methylobacterium* are readily isolated by pressing the surface of a plant leaf onto an agar Petri plate containing methanol as the sole source of carbon.

Bartonella is another notable genus of *Rhizobiales*. These organisms, once classified with the *Rickettsiales*, are intracellular pathogens of humans. Species of *Bartonella* can cause a variety of diseases in humans and other vertebrate animals. *Bartonella quintana* is the causative agent of *trench fever*, a disease that decimated troops in World War I. Other species of *Bartonella* can cause bartonellosis, cat scratch disease, and a variety of inflammatory diseases. Disease transmission is mediated by arthropod vectors including fleas, lice, and sand flies (Chapter 31). Species of *Bartonella* are fastidious and difficult to cultivate, and isolation is most commonly achieved using blood agar. When growing in tissue culture, cells of *Bartonella* grow on the outside surface of the eukaryotic host cells rather than within the cytoplasm or the nucleus.

TABLE 16.1 **Notable genera of *Alphaproteobacteria***

Family	*Genus*	*Notable characteristics*
Caulobacterales	*Caulobacter*	Asymmetric cell division and formation of prosthecae
Rickettsiales	*Rickettsia*	Obligate intracellular parasites, transmitted by arthropods
	Wolbachia	Live within arthropods and affect their reproduction
Rhizobiales	*Bartonella*	Obligate intracellular parasites, transmitted by arthropods
	Bradyrhizobium	Form root nodules with soybean and other legumes
	Brucella	Facultative intracellular parasites of animals, zoonotic pathogen
	Hyphomicrobium	Stalked cells, metabolically versatile
	Mesorhizobium	Form root nodules with bird's-foot trefoil and other legumes
	Methylobacterium	Methylotroph found on plants and in soil
	Nitrobacter	Nitrifying bacterium that oxidizes NO_2^- to NO_3^-
	Pelagibacter	Oligotrophic chemoorganotroph; high abundance in ocean surface
	Rhodopseudomonas	Metabolically versatile purple nonsulfur bacterium
Rhodobacterales	*Paracoccus*	Species used as a model for studying denitrification
	Rhodobacter	Metabolically versatile purple nonsulfur bacteria
	Roseobacter	Aerobic anoxygenic phototroph
Rhodospirillales	*Acetobacter*	Used industrially for producing acetic acid
	Azospirillum	Obligately aerobic diazotroph
	Gluconobacter	Used industrially for producing acetic acid
	Magnetospirillum	Magnetotactic bacterium
Sphingomonadales	*Sphingomonas*	Aerobic degradation of aromatic organics, biodegradation
	Zymomonas	Ferments sugars into ethanol, potential for biofuel production

Finally, the genus *Pelagibacter* also belongs to the *Rhizobiales*. *Pelagibacter ubique* is an oligotroph and an obligately aerobic chemoorganotroph that inhabits the photic zone of Earth's oceans. This organism can make up 25% of the bacterial cells found at the ocean's surface, and its numbers can reach 50% of cells in temperate

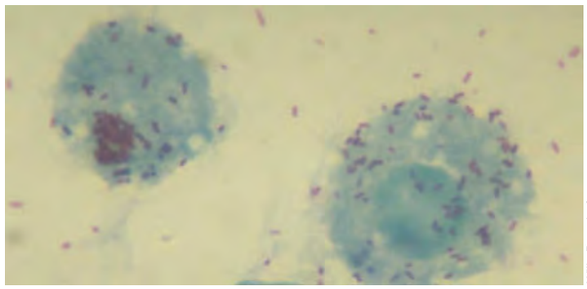
Figure 16.4 Colonies of *Rhizobium mongolense*. Colonies of rhizobia often produce copious exopolysaccharide slime. These colonies of *Rhizobium mongolense* were grown on a medium low in nitrogen with sucrose as carbon source.

waters in the summer; as a consequence, *Pelagibacter ubique* is likely the most abundant bacterial species on Earth (Section 20.11).

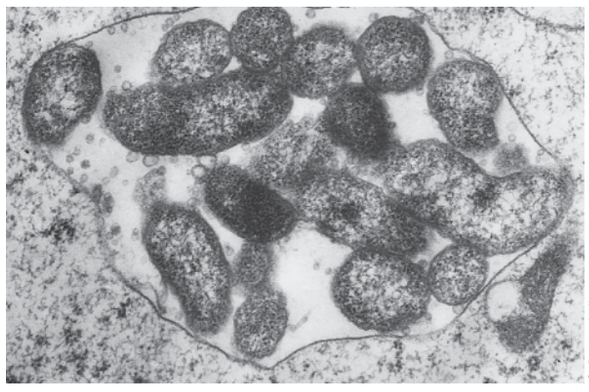
Rickettsiales

KEY GENERA: *Rickettsia, Wolbachia*

Rickettsiales (Figure 16.3) are all obligate intracellular parasites or mutualists of animals. Species in this order have not yet been cultivated in the absence of host cells (**Figure 16.5**) and must be grown in chicken eggs or in host cell tissue culture. Typically, *Rickettsiales* are closely associated with arthropods. Those genera that cause disease such as *Rickettsia* and *Ehrlichia* are transmitted by arthropod bites; other genera such as *Wolbachia* are obligate parasites or mutualists of insects and other arthropods.



(a)



(b)

Figure 16.5 Rickettsias growing within host cells. *(a) Rickettsia rickettsii* in tissue culture. Cells are about 0.3 μm in diameter. *(b)* Electron micrograph of cells of *Rickettsiella popilliae* within a blood cell of its host, the beetle *Melolontha melolontha*. The bacteria grow inside a vacuole within the host cell.

Species of the genus *Rickettsia* are the causative agents of several human diseases, including typhus (*Rickettsia prowazekii*) and spotted fever rickettsiosis, commonly called Rocky Mountain spotted fever (*Rickettsia rickettsii*) (Section 31.3). These organisms are closely associated with arthropod vectors and can be transmitted by ticks, fleas, lice, and mites. Most rickettsias are metabolically specialized, able to oxidize only the amino acids glutamate or glutamine and unable to oxidize glucose or organic acids. Rickettsias are unable to synthesize certain metabolites and must instead obtain them from host cells. Rickettsias do not survive long outside their hosts, and this may explain why they must be transmitted from animal to animal by arthropod vectors.

Electron micrographs of thin sections of rickettsial cells show a typical prokaryotic morphology including a cell wall (Figure 16.5*b*). The penetration of a host cell by a rickettsial cell is an active process, requiring both host and parasite to be viable and metabolically active. Once inside the host cell, the bacteria multiply primarily in the cytoplasm and continue replicating until the host cell is loaded with parasites (Figure 16.5; Figure 31.6). The host cell then bursts and liberates the bacterial cells.

The genus *Wolbachia* contains intracellular parasites of many insects (**Figure 16.6**), a huge group that constitutes 70% of all known arthropod species. *Wolbachia* species can have any of several effects on their insect hosts. These include inducing parthenogenesis (development of unfertilized eggs), the killing of males, and feminization (the conversion of male insects into females).

Wolbachia pipientis is the best-studied species in the genus. Cells of *W. pipientis* colonize the insect egg (Figure 16.6), where they multiply in vacuoles of host cells surrounded by a membrane of host origin. Cells of *W. pipientis* are passed from an infected female to her offspring through this egg infection. *Wolbachia*-induced parthenogenesis occurs in a number of species of wasps. In these insects, males normally arise from unfertilized eggs (which contain only one set of chromosomes), while females arise from fertilized eggs (which contain two sets of chromosomes). However, in unfertilized eggs infected with

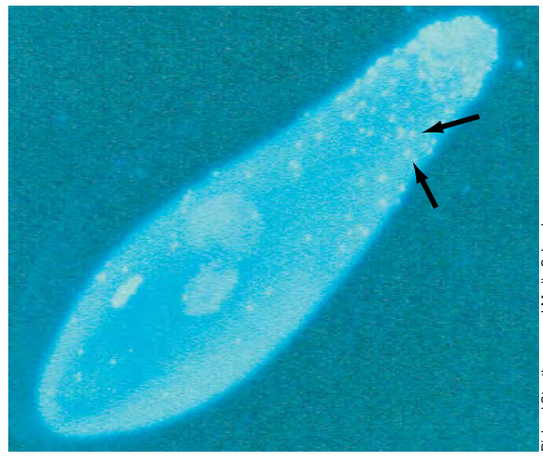


Figure 16.6 *Wolbachia*. Photomicrograph of a DAPI-stained egg of the parasitoid wasp *Trichogramma kaykai* infected with *Wolbachia pipientis*, which induces parthenogenesis. The *W. pipientis* cells are primarily located in the narrow end of the egg (arrows).

Wolbachia, the organism somehow triggers a doubling of the chromosome number, thus yielding only females. Predictably, if female insects are fed antibiotics that kill *Wolbachia*, parthenogenesis ceases.

Other Groups of *Alphaproteobacteria*

KEY GENERA: *Rhodobacter, Acetobacter, Caulobacter, and Sphingomonas*

The orders *Rhodobacterales* and *Rhodospirillales* (Figure 16.3) contain metabolically diverse organisms that have been discussed previously, including purple nonsulfur bacteria (*Rhodobacter* and *Rhodospirillum*, Section 15.5), aerobic anoxygenic phototrophs (*Roseobacter*, Section 15.5), nitrogen-fixing bacteria (*Azospirillum*, Section 15.12), denitrifiers (*Paracoccus*, Section 15.13), methylotrophs (*Methylobacterium*, Section 15.16), and magnetotactic bacteria (*Magnetospirillum*, Section 15.22), among others.

The *Caulobacterales* are typically oligotrophic and strictly aerobic chemoorganotrophs. Species typically form prosthecae or stalks (Section 15.20), and many species display asymmetric forms of cell division. The characteristic genus is *Caulobacter*, which has a characteristic life cycle that we have discussed previously (Sections 7.7 and 15.20).

The *Sphingomonadales* include diverse aerobic and facultatively aerobic chemoorganotrophs as well as species of aerobic anoxygenic phototrophs (*Erythrobacter*) and a few obligate anaerobes. The characteristic genus is *Sphingomonas*, which consists of obligately aerobic and nutritionally versatile species. Sphingomonads are widespread in aquatic and terrestrial environments and are notable for their ability to metabolize a wide range of organic compounds including many aromatic compounds that are common environmental contaminants (e.g., toluene, nonylphenol, dibenzo-*p*-dioxin, naphthalene, and anthracene, among others). As a consequence, sphingomonads have been widely studied as potential agents of bioremediation (Section 22.4). These organisms are typically easy to cultivate and grow well on a variety of complex culture media.

MINIQUIZ

- What are some ways in which *Wolbachia* species can affect insects?
- What organisms might form the pink scum you find on the edge of a bathtub? How might you try to cultivate these organisms?

16.2 *Betaproteobacteria*

With about 500 described species, the *Betaproteobacteria* are the third largest class of *Proteobacteria* (Figure 16.1). The *Betaproteobacteria* contain an immense amount of functional diversity (Figure 16.2 and Figure 15.1), and many species in this group have already been considered in Chapter 15. A total of six orders of *Betaproteobacteria* have many characterized species: *Burkholderiales, Hydrogenophilales, Methylophilales, Neisseriales, Nitrosomonadales*, and *Rhodocyclales* (Figure 16.7), and we focus on these here.

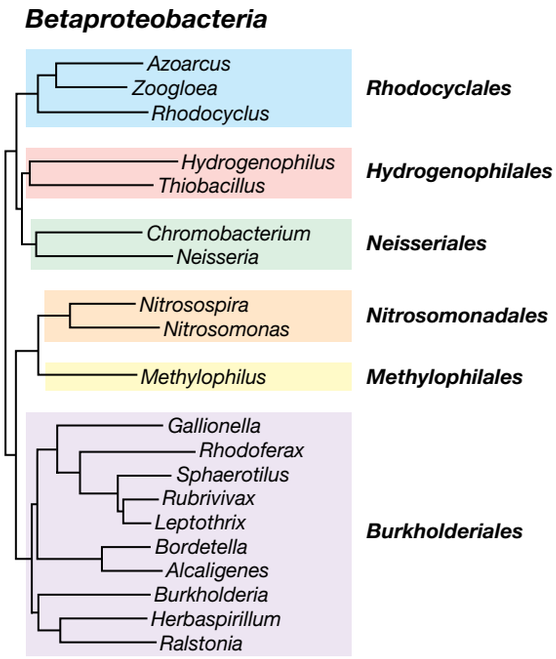


Figure 16.7 Major orders of *Proteobacteria* in the class *Betaproteobacteria*. The phylogenetic tree was constructed using 16S rRNA gene sequences from representative genera of *Betaproteobacteria*. Order names are shown in bold.

Burkholderiales

KEY GENERA: *Burkholderia*

The *Burkholderiales* contain species with a wide range of metabolic and ecological characteristics. Species include strictly aerobic, facultatively aerobic, and obligately anaerobic chemoorganotrophs, anoxygenic phototrophs, obligate and facultative chemolithotrophs, free-living nitrogen fixers, and pathogens of plants, animals, and humans.

Burkholderia is the type genus for the *Burkholderiales*. The genus *Burkholderia* includes diverse species of chemoorganotrophs with strictly respiratory metabolism. All species can grow aerobically, some also grow anaerobically with nitrate as the electron acceptor, and many strains are able to fix N_2. The metabolic versatility of *Burkholderia* species with respect to organic compounds, and aromatic compounds in particular, has led to interest in their use in bioremediation (Section 22.5). Certain strains of *Burkholderia* have also been shown to promote plant growth. However, many species are potentially pathogenic for plant or animals. One of the best known of the pathogenic species is *Burkholderia cepacia*.

B. cepacia is primarily a soil bacterium but also an opportunistic pathogen (Figure 16.8). Often found in the rhizosphere of plants, *B. cepacia* can produce both anti-fungal and anti-nematodal compounds, and thus its ability to colonize plant roots can provide disease protection and promote plant growth. However, *B. cepacia* is also known as a plant pathogen in certain circumstances, and it is a major cause of soft rot in onions. *B. cepacia* has also emerged as an opportunistic hospital-acquired infection in humans, as it is a hardy organism that is difficult to eradicate from the clinical setting. *B. cepacia* can form secondary lung infections in patients who are immunocompromised or have pneumonia or cystic fibrosis.

Figure 16.8 Colonies of *Burkholderia*. Photograph of colonies of *Burkholderia cepacia* on an agar plate.

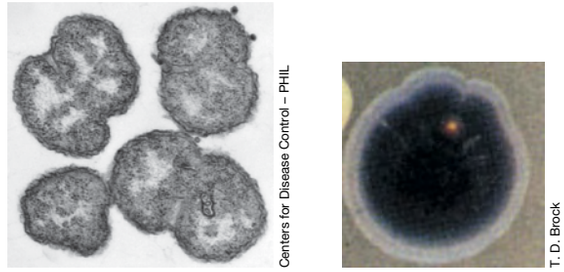


Figure 16.9 *Neisseria* and *Chromobacterium*. *(a)* Transmission electron micrograph of cells of *Neisseria gonorrhoeae* showing the typical diplococcus cell arrangements. *(b)* A large colony of *Chromobacterium violaceum*.

Its ability to form biofilms in the lung and its natural resistance to many antibiotics has made this organism particularly dangerous for patients with cystic fibrosis (Sections 7.9 and 20.4).

Rhodocyclales

KEY GENERA: *Rhodocyclus, Zoogloea*

Like the *Burkholderiales*, the order *Rhodocyclales* contains species with diverse metabolic and ecological characteristics. The type genus for the *Rhodocyclales* is *Rhodocyclus*, a purple nonsulfur bacterium (Section 15.5). Like most purple nonsulfur bacteria, *Rhodocyclus* species grow best as photoheterotrophs but most can also grow as photoautotrophs with H_2 as electron acceptor. Species can also grow by respiration in darkness, but they are typically found in illuminated anoxic environments where organic matter is present.

Zoogloea is another important genus of the *Rhodocyclales*. *Zoogloea* species are aerobic chemoorganotrophs that are distinctive for producing a thick gelatinous capsule that binds cells together into a complex matrix with branching, fingerlike projections. This gelatinous matrix can cause *flocculation*, the formation of macroscopic particles that settle out of solution. *Zoogloea ramigera* is of particular importance in aerobic wastewater treatment (Section 22.6), where it degrades much of the organic carbon in the waste stream and promotes flocculation and settling, crucial steps in water purification.

Neisseriales

KEY GENERA: *Chromobacterium, Neisseria*

The order *Neisseriales* contains at least 29 genera of diverse chemoorganotrophs. The best-characterized species are in the genera *Neisseria* and *Chromobacterium*. Species of *Neisseria* are commonly isolated from animals, and some of them are pathogenic. *Neisseria* species are always cocci (**Figure 16.9*a***). Some *Neisseria* are free-living saprophytes and reside in the oral cavity and other moist areas on the animal body. Others are serious pathogens, such as *Neisseria meningitidis*, which can cause a potentially fatal inflammation of the membranes lining the brain (meningitis, Section 30.5). We discuss the clinical microbiology of *Neisseria gonorrhoeae*—the causative agent of the sexually transmitted disease gonorrhea—in Section 28.3, and the pathogenesis of gonorrhea itself in Section 30.13.

Chromobacterium is a close phylogenetic relative of *Neisseria* but is rod-shaped in morphology. The best-known *Chromobacterium* species is *C. violaceum*, a purple-pigmented organism (Figure 16.9*b*) found in soil and water and occasionally in pus-forming wounds of humans and other animals. *C. violaceum* and a few other chromobacteria produce the purple pigment *violacein* (Figure 16.9*b*), a water-insoluble pigment with both antimicrobial and antioxidant properties. *Chromobacterium* is a facultative aerobe, growing fermentatively on sugars and aerobically on various carbon sources.

Hydrogenophilales, Methylophilales, and *Nitrosomonadales*

KEY GENERA: *Hydrogenophilus, Thiobacillus, Methylophilus, Nitrosomonas*

These three orders contain organisms that have fairly specialized metabolic capabilities including chemolithotrophs and methylotrophs; most species are obligate aerobes and many are autotrophic. *Hydrogenophilus thermoluteolus* is an obligate aerobe that can grow as a chemolithotroph using H_2 as an electron donor for respiration (Section 14.8) and the Calvin cycle to fix CO_2. This species is a facultative chemolithotroph, and can also grow as a chemoorganotroph on simple carbon sources. *Thiobacillus* is another important genus of *Hydrogenophilales*. Species of *Thiobacillus* can be chemoorganotrophs or chemolithotrophs. Chemolithotrophic species of *Thiobacillus* are sulfur bacteria (Sections 14.9 and 15.11) that oxidize reduced sulfur compounds as electron donors and grow by aerobic respiration or denitrification (Sections 14.13 and 15.13). Species of *Thiobacillus* can also fix CO_2 using the Calvin cycle and are commonly found in soils, sulfur springs, marine habitats, and other locales where reduced sulfur compounds are available.

The *Methylophilales* and *Nitrosomonadales* contain metabolically specialized organisms. *Methylophilus* species are obligate and facultative methylotrophs (Section 14.18) that grow on methanol and other C_1 compounds, but not on CH_4. Facultative species can grow as chemoorganotrophs through aerobic respiration of simple sugars. The order *Nitrosomonadales* contains obligately chemolithotrophic ammonia-oxidizing bacteria, the key genera being *Nitrosomonas* and *Nitrosospira* (Section 15.13).

MINIQUIZ

- List three species of *Betaproteobacteria* that are known to be human pathogens.
- List three genera of *Betaproteobacteria* that contain chemolithotrophic species.

16.3 *Gammaproteobacteria: Enterobacteriales*

KEY GENERA: *Enterobacter, Escherichia, Klebsiella, Proteus, Salmonella, Serratia, Shigella*

The *Gammaproteobacteria* are the largest and most diverse class of *Proteobacteria*, containing nearly half of all characterized species in the phylum. The class contains more than 1500 characterized species among its 15 orders (**Figure 16.10**, Figure 16.1*b*). Its species have diverse metabolic and ecological characteristics (Figure 16.2 and Figure 15.1) and include many well-known human pathogens. Species can be phototrophic (including the purple sulfur bacteria, Section 15.4), chemoorganotrophic, or chemolithotrophic, and can have either respiratory or fermentative metabolisms. Members of this group often develop quickly in laboratory media and can be isolated from a wide diversity of habitats. In this section we consider the *Enterobacteriales*, one of the largest and best-known orders within the *Gammaproteobacteria*.

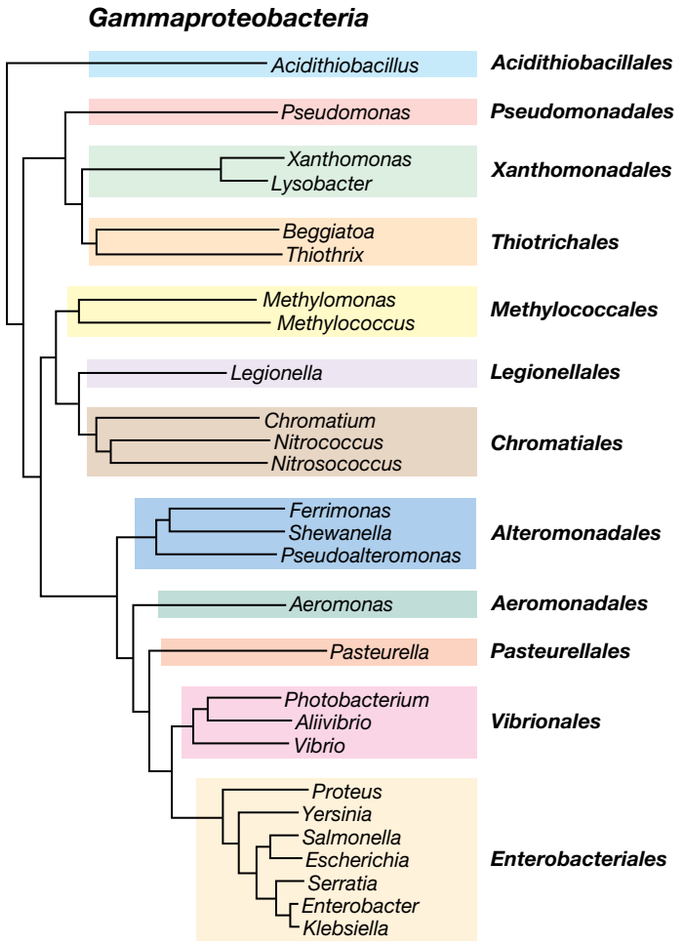


Figure 16.10 Major orders of *Proteobacteria* in the class *Gammaproteobacteria*. The phylogenetic tree was constructed using 16S rRNA gene sequences from representative genera of *Gammaproteobacteria*. Order names are shown in bold.

The *Enterobacteriales*, commonly called the **enteric bacteria**, comprise a relatively homogeneous phylogenetic group within the *Gammaproteobacteria* and consist of facultatively aerobic, gram-negative, nonsporulating rods that are either nonmotile or motile by peritrichous flagella (**Figure 16.11**). The *oxidase test* and the *catalase test* are common assays used to characterize bacteria (Section 28.3), and these tests can be used to discriminate enteric bacteria from many other *Gammaproteobacteria*. The oxidase test is an assay for the presence of cytochrome *c* oxidase, an enzyme present in many respiring bacteria. The catalase test assays for the enzyme catalase, which detoxifies hydrogen peroxide and is commonly found in bacteria able to grow in the presence of oxygen (Section 5.14 and Figure 5.29). Enteric bacteria are oxidase-negative and catalase-positive. They also produce acid from glucose and reduce nitrate but only to nitrite. Enteric bacteria have relatively simple nutritional requirements and ferment sugars to a variety of end products.

Among the enteric bacteria are many species pathogenic to humans, other animals, or plants, as well as other species of industrial importance. *Escherichia coli*, the best known of all organisms, is the classic enteric bacterium. Because of the medical importance of many enteric bacteria, an extremely large number have been characterized, and numerous genera and species have been defined, largely for ease in identification purposes in clinical microbiology. However, because enteric bacteria are genetically very closely related, their positive identification often presents considerable difficulty. In clinical laboratories, identification is typically based on the combined analysis of a large number of diagnostic tests carried out using miniaturized rapid diagnostic media kits along with immunological and genomic analyses to identify signature proteins or genes of particular species (Chapter 28).

Fermentation Patterns in Enteric Bacteria

One major taxonomic characteristic separating the various genera of enteric bacteria is the type and proportion of fermentation products generated from the fermentation of glucose. Two broad

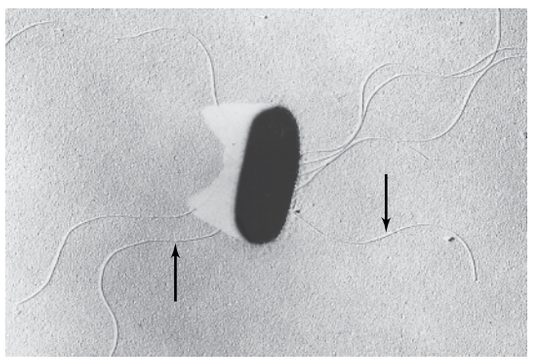


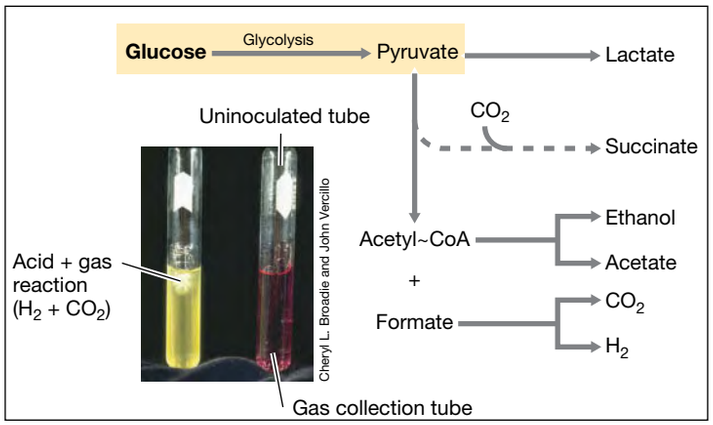
Figure 16.11 A butanediol-producing enteric bacterium. Electron micrograph of a shadow-cast preparation of a cell of the butanediol-producing bacterium *Erwinia carotovora*. The cell is about 0.8 μm wide. Note the peritrichously arranged flagella (arrows), typical of enteric bacteria.

patterns are recognized, the *mixed-acid fermentation* and the *2,3-butanediol fermentation* (Figure 16.12).

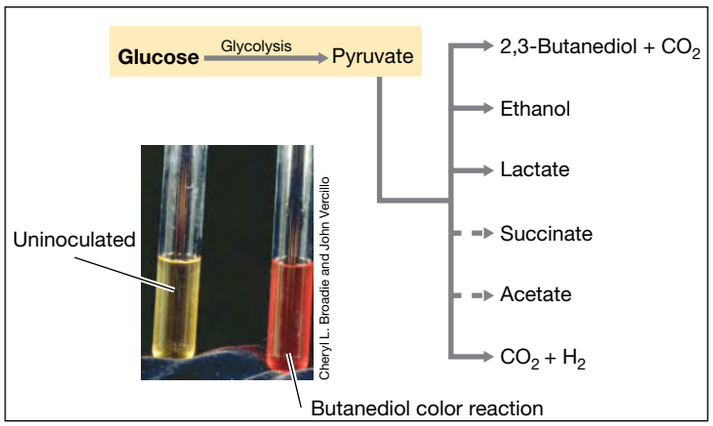
In the mixed-acid fermentation, three acids are formed in significant amounts: acetic, lactic, and succinic. Ethanol, CO_2, and H_2 are also formed, but not butanediol. In the butanediol fermentation, smaller amounts of acids are formed, and butanediol, ethanol, CO_2, and H_2 are the main products (Figure 14.57). As a result of mixed-acid fermentation, equal amounts of CO_2 and H_2 are produced, whereas in the butanediol fermentation, considerably more CO_2 than H_2 is produced. This is because mixed-acid fermenters produce CO_2 only from formic acid by means of the enzyme formate hydrogenlyase:

$$HCOOH \rightarrow H_2 + CO_2$$

This reaction results in equal amounts of CO_2 and H_2. The butanediol fermenters also produce CO_2 and H_2 from formic acid, but they produce two additional molecules of CO_2 during the formation of each molecule of butanediol (Figure 16.12*b*). Butanediol fermentation is characteristic of *Enterobacter, Klebsiella, Erwinia*, and *Serratia*, whereas mixed-acid fermentation is observed in *Escherichia, Salmonella, Shigella, Citrobacter, Proteus*, and *Yersinia*.



(*a*) **Mixed-acid fermentation** (for example, *Escherichia coli*)



(*b*) **Butanediol fermentation** (for example, *Enterobacter aerogenes*)

Figure 16.12 Enteric fermentations. Distinction between *(a)* mixed-acid and *(b)* butanediol fermentation in enteric bacteria (Figure 14.57). The solid arrows indicate reactions leading to major products. Dashed arrows indicate minor products. *(a)* The photo shows the production of acid (yellow) and gas (in the inverted Durham tube) in a culture of *Escherichia coli* carrying out a mixed-acid fermentation (purple tube was uninoculated). *(b)* The photo shows the pink-red color in the Voges–Proskauer (VP) test, which indicates butanediol production, following growth of *Enterobacter aerogenes*. The left (yellow) tube was not inoculated. Note that the mixed-acid fermentation produces less CO_2 but more acid products from glucose than does the butanediol fermentation.

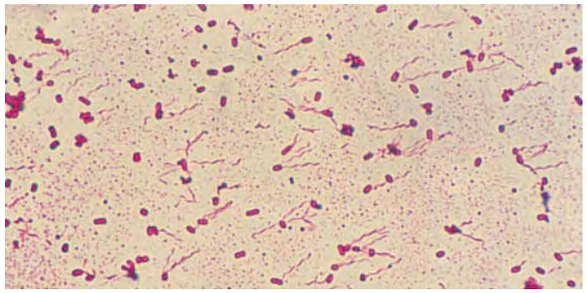
Mixed-Acid Fermenters: *Escherichia, Salmonella, Shigella,* and *Proteus*

Species of *Escherichia* are almost universal inhabitants of the intestinal tract of humans and other warm-blooded animals, although they are by no means the dominant organisms in this habitat. *Escherichia* may play a nutritional role in the intestinal tract by synthesizing vitamins, particularly vitamin K. As a facultative aerobe, this organism probably also helps consume O_2, thus rendering the large intestine anoxic. Wild-type *Escherichia* strains rarely show any growth-factor requirements and are able to grow on a wide variety of carbon and energy sources such as sugars, amino acids, organic acids, and so on.

Some strains of *Escherichia* are pathogenic and have been implicated in diarrheal diseases, especially in infants; diarrheal diseases are a major public health problem in developing countries (Sections 32.1, 32.3, 32.10, and 32.11). *Escherichia* is also a major cause of urinary tract infections in women. Enteropathogenic *E. coli* strains are becoming more frequently implicated in gastrointestinal infections and generalized fevers. Some strains, such as enterohemorrhagic *E. coli*, an important representative of which is strain O157:H7, can cause sporadic outbreaks of severe foodborne disease. Infection occurs primarily through consumption of contaminated foods, such as raw or undercooked ground beef, unpasteurized milk, or contaminated water. In a small percentage of cases, *E. coli* O157:H7 causes a life-threatening complication related to its production of a very potent enterotoxin.

Salmonella and *Escherichia* are quite closely related. However, in contrast to *Escherichia*, species of *Salmonella* are almost always pathogenic, either to humans or to other warm-blooded animals (*Salmonella* is also found in the intestines of cold-blooded animals, such as turtles and lizards). In humans the most common diseases caused by salmonellas are typhoid fever and gastroenteritis (Sections 32.5 and 32.10). The shigellas are also genetically very closely related to *Escherichia*. Genomic analyses strongly suggest that *Shigella* and *Escherichia* have exchanged a significant number of genes by horizontal gene flow. In contrast to most *Escherichia*, however, species of *Shigella* are typically pathogenic to humans, causing a rather severe gastroenteritis called *bacillary dysentery*. *Shigella dysenteriae*, transmitted by food- and waterborne routes, is a good example of this. The bacterium, which contains endotoxin, invades intestinal epithelial cells, where it excretes a neurotoxin that causes acute gastrointestinal distress.

The genus *Proteus* typically contains highly motile cells (Figure 16.13) that produce the enzyme *urease*. Unlike *Salmonella* and *Shigella, Proteus* shows only a distant relationship to *E. coli*. *Proteus* is a frequent cause of urinary tract infections in humans and probably benefits in this regard from its ready ability to degrade urea by urease. Because of the rapid motility of *Proteus* cells, colonies growing on agar plates often exhibit a characteristic *swarming* phenotype (Figure 16.13*b*). Cells at the edge of the growing colony are more rapidly motile than those in the center of the colony. The former move a short distance away from the colony in


D. E. Snyder

(a)


James Shapiro

(b)

Figure 16.13 Swarming in *Proteus*. *(a)* Cells of *Proteus mirabilis* stained with a flagella stain; the peritrichous flagella of each cell form into a bundle to rotate in synchrony. *(b)* Photo of a swarming colony of *Proteus vulgaris*. Note the concentric rings.

a mass and then undergo a reduction in motility, settle down, and divide, forming a new population of motile cells that again swarm. As a result, the mature colony appears as a series of concentric rings, with higher concentrations of cells alternating with lower concentrations (Figure 16.13*b*).

Butanediol Fermenters: *Enterobacter, Klebsiella*, and *Serratia*

The butanediol fermenters are genetically more closely related to each other than to the mixed-acid fermenters, a finding that is in agreement with the observed physiological differences (Figure 16.12). *Enterobacter aerogenes* is a common species in water and sewage as well as the intestinal tract of warm-blooded animals and is an occasional cause of urinary tract infections. One species of *Klebsiella, K. pneumoniae*, occasionally causes pneumonia in humans, but klebsiellas are most commonly found in soil and water. Most *Klebsiella* strains also fix nitrogen (↪ Sections 14.6 and 15.12), a property not characteristic of other enteric bacteria.

The genus *Serratia* forms a series of red pyrrole-containing pigments called *prodigiosins* (Figure 16.14). Prodigiosin is produced in stationary phase as a secondary metabolite and is of interest

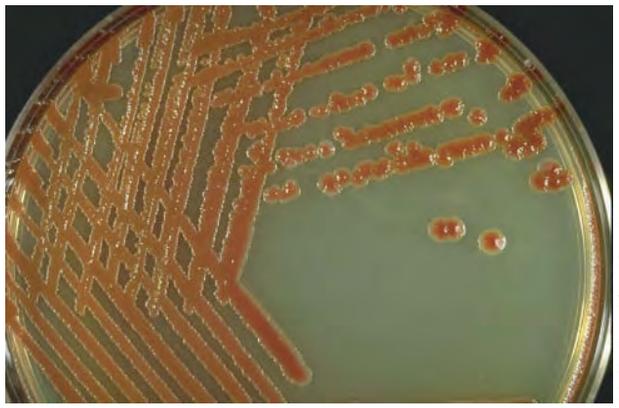

John Vercillo and Cheryl Broadie

Figure 16.14 Colonies of *Serratia marcescens*. The orange-red pigmentation is due to the pyrrole-containing pigment prodigiosin.

because it contains the pyrrole ring also found in the pigments for energy transfer: porphyrins, chlorophylls, bacteriochlorophylls, and phycobilins (↪ Sections 14.1–14.3). However, it is unclear if prodigiosin plays any role in energy transfer, and its exact function is unknown. Species of *Serratia* can be isolated from water and soil as well as from the gut of various insects and vertebrates and occasionally from the intestines of humans. *Serratia marcescens* is also a human pathogen that can cause infections in many body sites. It has been implicated in infections caused by some invasive medical procedures and is an occasional contaminant in intravenous fluids.

MINIQUIZ

- What is a mixed-acid fermentation, and of what significance is this trait to enteric bacteria?
- What characteristics would you use to distinguish between *Escherichia coli* and *Klebsiella pneumoniae*?

16.4 *Gammaproteobacteria: Pseudomonadales* and *Vibrionales*

KEY GENERA: *Aliivibrio, Pseudomonas, Vibrio*

The phylogenetic and metabolic diversity of the *Gammaproteobacteria* makes it difficult to select the many notable species in this class of *Proteobacteria*. We focus here on the *Pseudomonadales* and *Vibrionales*, since these groups (along with the *Enterobacteriales*) represent three of the most abundant and most commonly encountered orders of *Gammaproteobacteria* (Figure 16.10).

Pseudomonadales

The *Pseudomonadales* contain exclusively chemoorganotrophs that carry out respiratory metabolisms. All species can grow as aerobes and are typically oxidase- and catalase-positive, but some are also capable of anaerobic respiration with nitrate as the electron acceptor. Most species are able to use a wide diversity of organic compounds as sources of carbon and energy for growth. These organisms are ubiquitous in soil and aquatic systems, and many species cause diseases of plants and animals, including humans.

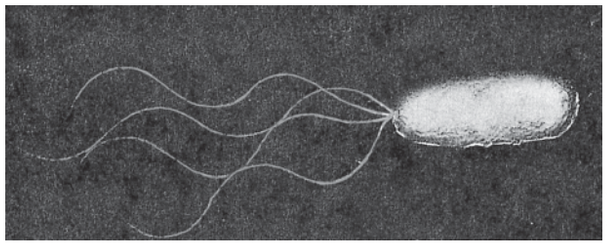


Figure 16.15 Cell morphology of pseudomonads. Shadow-cast transmission electron micrograph of a *Pseudomonas* cell. The cell measures about 1 μm in diameter.

The term **pseudomonad** is often used to describe any gram-negative, polarly flagellated, aerobic rod that is able to use diverse carbon sources. Pseudomonads can be found in several different groups of *Proteobacteria*, but here we consider only those organisms in the order *Pseudomonadales*. The type genus for this order is *Pseudomonas*.

Several species of *Pseudomonas* are pathogenic. Among these, *Pseudomonas aeruginosa* (Figure 16.15) is frequently associated with infections of the urinary and respiratory tracts in humans. *P. aeruginosa* is not an obligate pathogen. Instead, the organism is an opportunist, initiating infections in individuals with weakened immune systems. *P. aeruginosa* is a common cause of hospital-acquired (nosocomial) infections from catheterizations, tracheostomies, lumbar punctures, and intravenous infusions, and often emerges in patients given prolonged treatment with immunosuppressive agents. *P. aeruginosa* is also a common pathogen in patients receiving treatment for severe burns or other traumatic skin damage and in people suffering from cystic fibrosis. In addition to localized infections, *P. aeruginosa* can also cause systemic infections, usually in individuals who have experienced extensive skin damage.

P. aeruginosa is naturally resistant to many widely used antibiotics, so treatment of infections is often difficult. Resistance is typically due to a resistance transfer plasmid (R plasmid) (Sections 4.2 and 28.12), which is a plasmid whose genes encode proteins that detoxify various antibiotics or pump them out of the cell. Polymyxin, an antibiotic not ordinarily used in human therapy because of its toxicity, is effective against *P. aeruginosa* and is used in critical medical situations.

Certain species of *Pseudomonas*, such as *Pseudomonas syringae*, are well-known plant pathogens (phytopathogens). Phytopathogens frequently inhabit nonhost plants (in which disease symptoms are inapparent) and from there become transmitted to host plants and initiate infection. Disease symptoms vary considerably, depending on the particular phytopathogen and host plant. The pathogen releases plant toxins, lytic enzymes, plant growth factors, and other substances that destroy or distort plant tissue, releasing nutrients for use by the bacterium. In many cases the disease symptoms help identify the phytopathogen. Thus, *Pseudomonas syringae* is typically isolated from leaves showing chlorotic (yellowing) lesions, whereas *Pseudomonas marginalis*, a "soft-rot" pathogen, infects stems and shoots but rarely leaves.

Vibrionales

The *Vibrionales* contain facultatively aerobic rods and curved rods that employ a fermentative metabolism. One key difference between the *Vibrio* group and enteric bacteria is that *Vibrio* are oxidase-positive whereas enteric bacteria are oxidase-negative. Although *Pseudomonas* species are also oxidase-positive, they are not fermentative and so are clearly distinct from *Vibrio* species. The best-known genera in this group are *Vibrio, Aliivibrio*, and *Photobacterium*, which contain several species that are bioluminescent (Section 15.18).

Most vibrios and related bacteria are aquatic, being found in marine, brackish, or freshwater habitats. *Vibrio cholerae* is the cause of the disease cholera in humans (Sections 29.8 and 32.3); the organism does not normally cause disease in other hosts. Cholera is one of the most common human infectious diseases in developing countries and is transmitted almost exclusively via water.

Vibrio parahaemolyticus inhabits the marine environment and is a major cause of gastroenteritis in Japan, where raw fish is widely consumed; the organism has also been implicated in outbreaks of gastroenteritis in other parts of the world, including the United States. *V. parahaemolyticus* can be isolated from seawater itself or from shellfish and crustaceans, and its primary habitat is probably marine animals, with humans being an accidental host.

MINIQUIZ

- What species of *Pseudomonas* is a common cause of lung infection in cystic fibrosis patients?
- What major characteristic could be used to differentiate strains of *Pseudomonas* from those of *Vibrio*?

16.5 *Deltaproteobacteria* and *Epsilonproteobacteria*

These classes of *Proteobacteria* contain fewer species and less functional diversity than we have encountered in the *Alpha-, Beta-*, and *Gammaproteobacteria* (Figure 16.2 and Figure 15.1). The *Deltaproteobacteria* are primarily sulfate- and sulfur-reducing bacteria (Sections 14.14, 15.9, and 15.10), dissimilative iron-reducers (Section 15.14), and bacterial predators (Section 15.17). *Epsilonproteobacteria*, by contrast, contain many species that oxidize the H_2S produced by the sulfate and sulfur reducers. The final class of *Proteobacteria*, the *Zetaproteobacteria*, contains only one characterized species (the iron oxidizer *Mariprofundus ferrooxydans*) and was considered earlier (Section 15.15).

Deltaproteobacteria

KEY GENERA: *Bdellovibrio, Myxococcus, Desulfovibrio, Geobacter, Syntrophobacter*

Eight orders have been characterized within the *Deltaproteobacteria* (Figure 16.16). The *Myxococcales* and *Bdellovibrionales* contain notable genera of bacterial predators (Section 15.17). In contrast, the *Desulfuromonadales* contains diverse species of metal- and sulfur-reducing genera such as *Geobacteria* (Sections 15.10 and 15.14). Indeed, like the *Desulfuromonadales*, many genera from the *Deltaproteobacteria* are associated with the reduction of sulfur compounds.

The largest and most common order containing sulfate reducers is the *Desulfovibrionales*. These organisms are readily cultivated

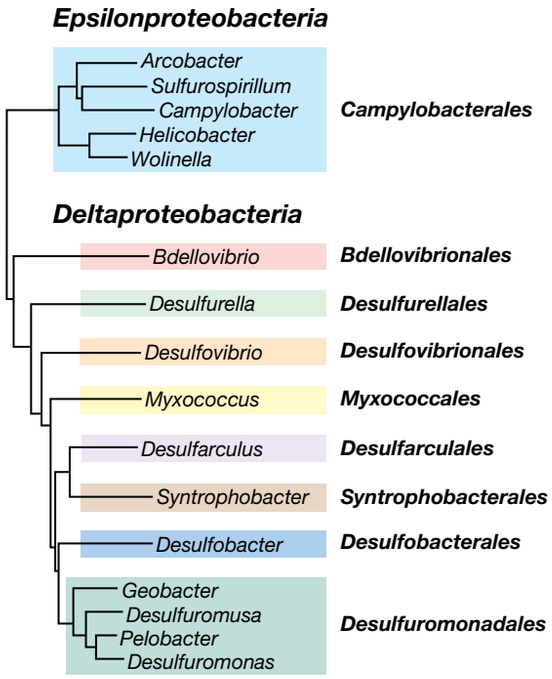


Figure 16.16 Major orders of *Proteobacteria* in the classes *Deltaproteobacteria* and *Epsilonproteobacteria*. The phylogenetic tree was constructed using 16S rRNA gene sequences from representative genera in the *Delta-* and *Epsilonproteobacteria*. Order names are shown in bold.

from marine sediments and nutrient-rich anoxic environments that contain sulfate. Species of *Desulfovibrionales* are typically incomplete oxidizers (Section 15.9). All use sulfate as the terminal electron acceptor, and all require small organic compounds such as lactate as a source of carbon and energy for growth. Species within the orders *Desulfobacterales* and *Desulfarculales* also typically reduce sulfate; however, in contrast to the *Desulfovibrionales*, these species can be complete or incomplete acetate oxidizers (Section 15.9). In addition to sulfate, some species in these three orders can also reduce sulfite, thiosulfate, or nitrate, and some are capable of certain fermentations.

The final order containing sulfate reducers is the *Syntrophobacterales*. Some but not all species of the *Syntrophobacterales* are able to reduce sulfate. In nature, however, species of *Syntrophobacterales* primarily interact with H_2-consuming bacteria in a metabolic partnership called *syntrophy* (Section 14.23). For example, syntrophic species such as *Syntrophobacter wolinii* oxidize propionate, producing acetate, CO_2, and H_2. However, such growth is only possible when a H_2-consuming partner is present. If sulfate is present, *S. wolinii* can grow as a sulfate reducer without the need for a partner. *S. wolinii* can also grow without a partner organism by fermenting pyruvate, fumarate, or malate.

Epsilonproteobacteria

KEY GENERA: *Campylobacter, Helicobacter*

The *Epsilonproteobacteria* (Figure 16.16) were initially defined by only a few pathogenic bacteria; in particular, by species of *Campylobacter* and *Helicobacter*. However, environmental studies of marine and terrestrial microbial habitats have shown that a diversity of *Epsilonproteobacteria* exist in nature, and their numbers and metabolic capabilities suggest they play important ecological roles (**Table 16.2**). Species of *Epsilonproteobacteria* are especially abundant at oxic–anoxic interfaces in sulfur-rich environments, and play major roles in the oxidation of sulfur compounds in nature.

Campylobacter and *Helicobacter*

These two genera of *Epsilonproteobacteria* share a number of characteristics. *Campylobacter* and *Helicobacter* species are gram-negative, oxidase- and catalase-positive, motile spirilla, and most species are pathogenic to humans or other animals (Table 16.2). These organisms are also microaerophilic (Section 5.14) and must therefore be cultured from clinical specimens at low (3–15%) O_2 and high (3–10%) CO_2.

TABLE 16.2 Characteristics of key genera of *Epsilonproteobacteria*

Genus	*Habitat*	*Descriptive characters*	*Physiology and metabolism*
Campylobacter	Reproductive organs, oral cavity, and intestinal tract of humans and other animals; pathogenic	Slender, spirally curved rods; corkscrew-like motility by single polar flagellum	Microaerophilic; chemoorganotrophic
Arcobacter	Diverse habitats (freshwater, sewage, saline environments, animal reproductive tract, plants); some species pathogenic for humans and other animals	Slender, curved rods; motile by single polar flagellum	Microaerophilic; aerotolerant or aerobic; chemoorganotrophic; oxidation of sulfide to elemental sulfur (S^0) by some species; nitrogen fixation in one species
Helicobacter	Intestinal tract and oral cavity of humans and other animals; pathogenic	Rods to tight spiral; some species with tightly coiled periplasmic fibers	Microaerophilic, chemoorganotrophic; produce high levels of urease (nitrogen assimilation)
Sulfurospirillum	Freshwater and marine habitats containing sulfur	Vibrioid to spiral-shaped cells; motile by polar flagella	Microaerophilic; reduces elemental sulfur (S^0)
Thiovulum	Freshwater and marine habitats containing sulfur; not yet in pure culture (Figure 15.29)	Cells contain orthorhombic S^0 granules; rapid motility by peritrichous flagella	Microaerophilic; chemolithotrophic, oxidizing H_2S
Wolinella	Bovine rumen	Rapidly motile by polar flagellum; single species known: *W. succinogenes*	Anaerobe; anaerobic respiration using fumarate, nitrate, or other compounds as terminal electron acceptor, and with H_2 or formate as electron donor

Campylobacter species, over a dozen of which have been described, cause acute gastroenteritis that typically results in a bloody diarrhea. Pathogenesis is due to several factors, including an enterotoxin that is related to cholera toxin. *Helicobacter pylori*, also a pathogen, causes both chronic and acute gastritis, leading to the formation of peptic ulcers. We consider these diseases, including their modes of transmission and clinical symptoms, in more detail in Section 30.10.

Sulfurospirillum and *Wolinella*

Species of *Sulfurospirillum*, a *Campylobacter* relative, are nonpathogenic, free-living microaerophiles found in freshwater and marine habitats (Table 16.2). These bacteria also carry out anaerobic respirations using elemental sulfur (S^0), selenate, or arsenate as electron acceptors (Section 14.15).

Wolinella is an anaerobic bacterium isolated from the bovine rumen (Table 16.2; Section 23.13). Unlike other *Epsilonproteobacteria*, the single known species, *W. succinogenes*, grows best as an anaerobe and can catalyze anaerobic respirations using fumarate or nitrate as electron acceptors with H_2 or formate as electron donors. Although *W. succinogenes* has thus far been found only in the rumen, its genome shows significant homologies to both the *Campylobacter* and *Helicobacter* genomes and contains additional genes that encode nitrogen fixation, extensive cell signaling mechanisms, and virtually complete metabolic pathways, absent from closely related genomes. This suggests that *Wolinella* inhabits its diverse environments outside of the rumen.

Environmental *Epsilonproteobacteria*

In addition to cultured representatives of the genera mentioned above, and many additional species and genera not considered here, there are large groups within this class that are known only from 16S ribosomal RNA gene sequences obtained from the environment (Section 19.6). Through environmental sequencing studies and ongoing cultivation efforts, species of *Epsilonproteobacteria* are now becoming recognized as ubiquitous in marine and terrestrial environments where sulfur-cycling activities are ongoing, particularly in deep-sea hydrothermal vent habitats where sulfide-rich and oxygenated waters mix (Section 20.14). Also, living attached to the surface of animals such as the tube worm *Alvinella* and the shrimp *Rimicaris* that reside near hydrothermal vents, a large variety of uncultured *Epsilonproteobacteria* may, through their sulfur metabolism, detoxify H_2S that would otherwise be deleterious to their animal hosts, allowing the animals to thrive in a chemically hostile environment (Section 23.9). Further exploration of the phylogeny, metabolic activities, and ecological roles of *Epsilonproteobacteria* will likely uncover exciting new aspects of prokaryotic diversity.

MINIQUIZ

- What four metabolic traits are most common in species of *Deltaproteobacteria*?
- Why is *Wolinella* physiologically unusual among the *Epsilonproteobacteria*?

II • *Firmicutes*, *Tenericutes*, and *Actinobacteria*

We continue our tour of phylogenetic bacterial diversity with the gram-positive bacteria of the phyla *Actinobacteria* and *Firmicutes*, and the closely related phylum *Tenericutes* (Figure 16.17). These three phyla contain nearly half of all characterized species of *Bacteria* (Figure 16.1*b*).

The *Actinobacteria* include the actinomycetes, a huge group of primarily filamentous soil bacteria. One distinguishing feature of the *Actinobacteria* is that their genomic DNA typically has a high GC content, and as a result they are also called the **high GC gram-positive bacteria**. The *Tenericutes* include cells that lack a cell wall, and the *Firmicutes* include the endospore-forming bacteria, lactic acid bacteria, and several other groups. In contrast to the *Actinobacteria*, the genomes of *Firmicutes* generally have a low GC content, and as a result, they are also called the **low GC gram-positive bacteria**.

We begin by examining *Firmicutes* that do not form endospores.

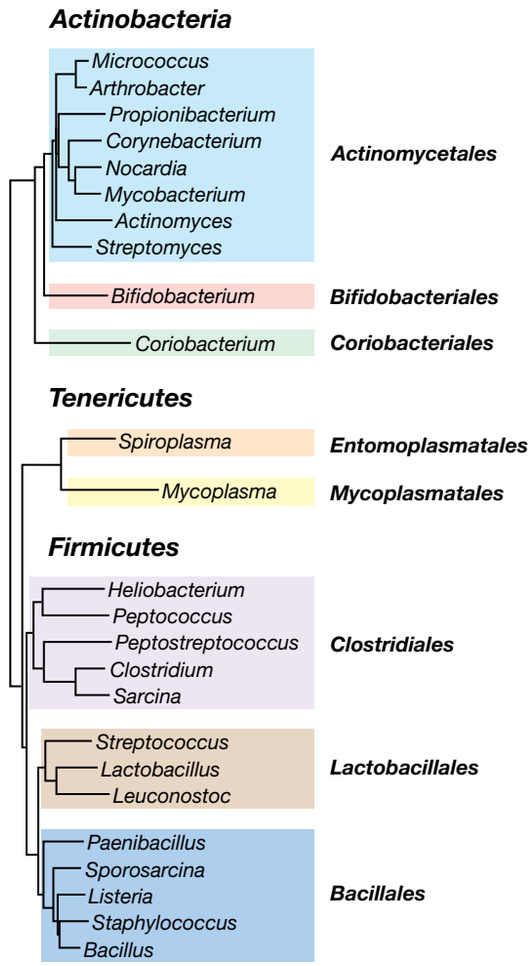


Figure 16.17 Major orders of gram-positive bacteria and relatives. The phylogenetic tree was constructed from 16S rRNA gene sequences of representative genera of *Actinobacteria, Firmicutes,* and *Tenericutes*. Order names are shown in bold.

16.6 *Firmicutes: Lactobacillales*

KEY GENERA: *Lactobacillus, Streptococcus*

The order *Lactobacillales* contains the **lactic acid bacteria**, fermentative organisms that produce lactic acid as a major end product of metabolism. These organisms are used widely in food production and preservation. Lactic acid bacteria are nonsporulating, oxidase- and catalase-negative rods or cocci that show an exclusively fermentative metabolism. All lactic acid bacteria produce lactic acid as a major or sole fermentation product. Members of this group lack porphyrins and cytochromes, do not carry out oxidative phosphorylation, and hence obtain energy only by substrate-level phosphorylation. Unlike many anaerobes, however, most lactic acid bacteria are not sensitive to oxygen (O_2) and can grow in its presence; thus they are called *aerotolerant anaerobes*.

Most lactic acid bacteria obtain energy only from the metabolism of sugars and therefore are usually restricted to habitats in which sugars are present. They typically have limited biosynthetic abilities, and their complex nutritional requirements include needs for amino acids, vitamins, purines, and pyrimidines (for example, Table 5.1 for *Leuconostoc mesenteroides*). One important difference between subgroups of the lactic acid bacteria lies in the pattern of products formed from the fermentation of sugars. One group, called **homofermentative**, produces a single fermentation product, *lactic acid*. The other group, called **heterofermentative**, produces other products, mainly ethanol and CO_2, as well as lactate (Section 14.20 provides additional coverage of homofermentative and heterofermentative pathways).

Lactobacillus

Lactobacilli are typically rod-shaped and grow in chains, varying from long and slender to short, bent rods (**Figure 16.18**), and most are homofermentative. Lactobacilli are common in dairy products, and some strains are used in the preparation of fermented milk products. For instance, *Lactobacillus acidophilus* (Figure 16.18*a*) is used in the production of acidophilus milk; *Lactobacillus delbrueckii* (Figure 16.18*c*) is used in the preparation of yogurt; and other species are used in the production of sauerkraut, silage, and pickles (Section 32.6).

Lactobacilli are typically more resistant to acidic conditions than are other lactic acid bacteria and are able to grow well at pH values as low as 4. Because of this, they can be selectively enriched from dairy products and fermenting plant material on acidic carbohydrate-containing media. The acid resistance of the lactobacilli enables them to continue growing during natural lactic fermentations, even when the pH value has dropped too low for other lactic acid bacteria to grow. The lactobacilli are therefore typically responsible for the final stages of most lactic acid fermentations. They are rarely, if ever, pathogenic.

Streptococcus and Other Cocci

The genera *Lactococcus* and *Streptococcus* (**Figure 16.19**) contain homofermentative species of coccoid-shaped lactic acid bacteria with quite distinct habitats and activities that are of considerable practical importance to humans. Some species are pathogenic to humans and animals (Section 30.2). *Streptococcus* species (Figure 16.19*a*) have a characteristic cell morphology of cocci in chains or tetrads

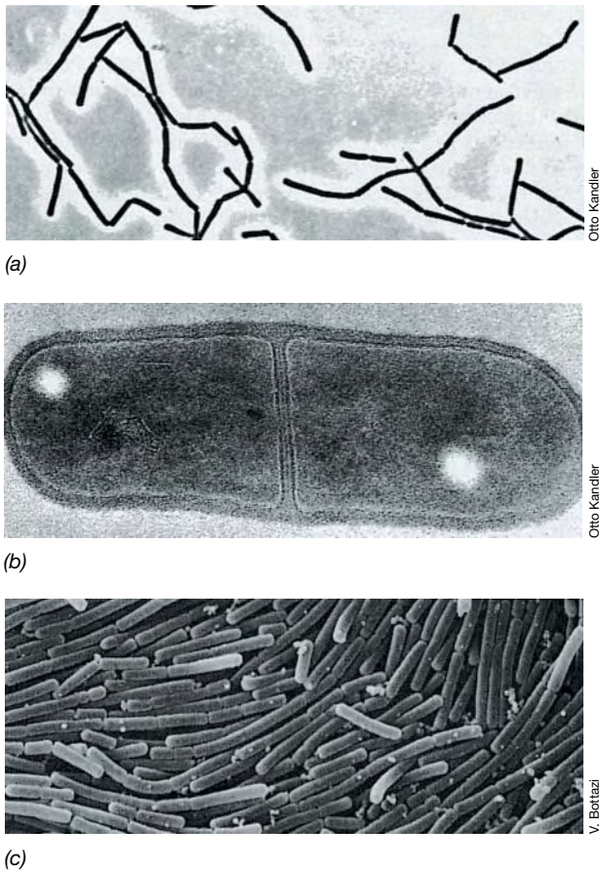


Figure 16.18 *Lactobacillus* species. *(a) Lactobacillus acidophilus,* phase-contrast. Cells are about 0.75 μm wide. *(b) Lactobacillus brevis,* transmission electron micrograph. Cells measure about 0.8 × 2 μm. *(c) Lactobacillus delbrueckii,* scanning electron micrograph. Cells are about 0.7 μm in diameter.

and so are readily resolved from the rod-shaped lactobacilli. As producers of lactic acid, other streptococci play important roles in the production of buttermilk, silage, and other fermented products (Section 32.6), and certain species play a major role in the formation of dental caries (Sections 24.3 and 25.2).

There are several other genera of homofermentative cocci. The genus *Lactococcus* (Figure 16.19*b*) contains those streptococci of

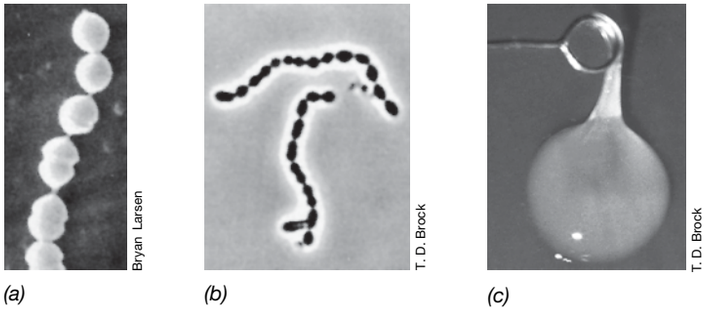


Figure 16.19 Gram-positive cocci. *(a) Streptococcus* sp., scanning electron micrograph. *(b) Lactococcus lactis*, phase-contrast micrograph. Cells in both photos are 0.5–1 μm in diameter. *(c)* Colony of *Leuconostoc mesenteroides* showing the extensive dextran slime produced by cells grown on sucrose.

dairy significance, whereas the genus *Enterococcus* includes streptococci that are primarily of fecal origin and can be human pathogens. Species of the genera *Peptococcus* and *Peptostreptococcus* are obligate anaerobes that ferment proteins rather than sugars.

Streptococci have been divided into two groups of related species: the *pyogenes subgroup*, characterized by *Streptococcus pyogenes*, the cause of strep throat (Section 30.2), and the *viridans subgroup*, characterized by *Streptococcus mutans*, the major cause of dental caries (Sections 24.3 and 25.2). Hemolysis on blood agar is of considerable importance in the subdivision of the genus into species. For example, species that produce the virulence factors streptolysin O or S form colonies surrounded by a large zone of complete red blood cell hemolysis when plated on blood agar, a condition called *β-hemolysis* (Figure 25.16*a*). β-hemolysis is diagnostic for streptococci in the pyogenes subgroup. In contrast, streptococci in the viridans subgroup cause incomplete hemolysis on blood agar, a condition that leads to greening of the agar under colonies. Streptococci are also divided into immunological groups (designated by the letters A, B, C, F, G), based on the presence of specific carbohydrate antigens (antigens are substances that elicit an immune response). Those β-hemolytic streptococci found in humans usually contain the group A antigen, whereas enterococci contain the group D antigen.

Heterofermentative lactococci reside in the genus *Leuconostoc*. Strains of *Leuconostoc* also produce the flavoring ingredients diacetyl and acetoin from the catabolism of citrate; they have been used as starter cultures in dairy fermentations. Some strains of *Leuconostoc* produce large amounts of glucose or fructose polysaccharide slimes, especially when cultured on sucrose as the carbon and energy source (Figure 16.19*c*), and some of these polymers have found medical use as plasma extenders in blood transfusions.

MINIQUIZ

- How do heterofermentative and homofermentative bacteria differ physiologically?
- How can *Streptococcus pyogenes* be distinguished from *Streptococcus mutans*?

16.7 *Firmicutes:* Nonsporulating *Bacillales* and *Clostridiales*

KEY GENERA: *Listeria, Staphylococcus, Sarcina*

Firmicutes that form endospores reside in the orders *Bacillales* and *Clostridiales*. However, numerous *Bacillales* and *Clostridiales* are unable to form endospores, and we consider some of these here.

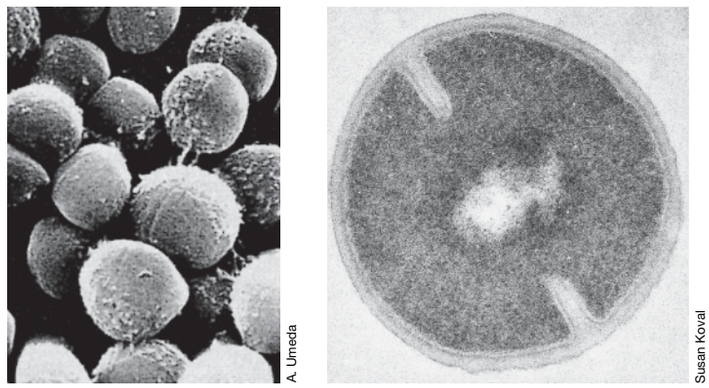
Listeria

The order *Bacillales* typically contains aerobic and facultatively aerobic chemoorganotrophs. Members of this group are widespread and particularly common in soils. For example, *Listeria* is found widely in soils and is an opportunistic pathogen and a common cause of foodborne illness. *Listeria* are gram-positive, catalase-positive, rod-shaped, facultatively aerobic chemoorganotrophs. Although several species of *Listeria* are known, the species *Listeria monocytogenes* is most noteworthy because it causes a major foodborne illness, *listeriosis* (Section 32.13). The organism is transmitted in contaminated, usually ready-to-eat foods such as cheese and sausages, and can cause anything from a mild illness to a fatal form of meningitis. Species of *Listeria* often grow well at low temperatures, allowing growth in refrigerated foods.

Staphylococcus

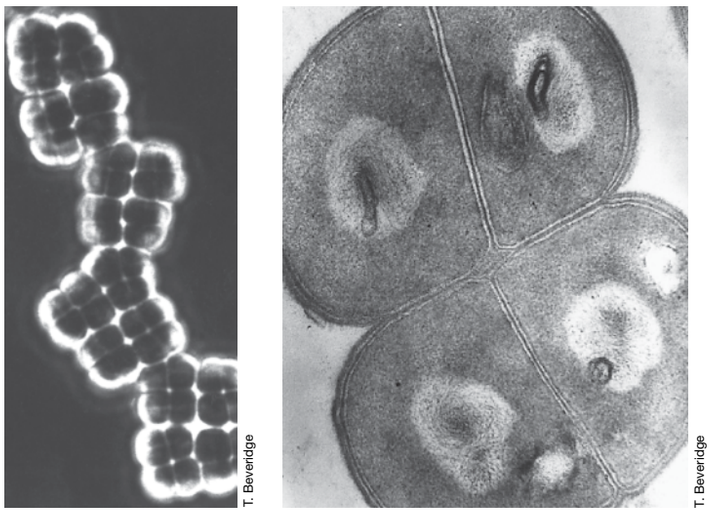
Staphylococcus (**Figure 16.20**) is a facultative aerobe that shows a typical respiratory metabolism but can also grow fermentatively. Cells typically grow in clusters and produce acid from glucose both aerobically and anaerobically. *Staphylococcus* species are catalase-positive, and this permits their distinction from *Streptococcus* and some other genera of lactic acid bacteria. Staphylococci are relatively resistant to reduced water potential and tolerate drying and high salt (NaCl) fairly well. Their ability to grow in media containing salt provides a selective means for isolation. For example, if an appropriate inoculum such as a skin swab, dry soil, or room dust is spread on a rich-medium agar plate containing 7.5% NaCl and the plate is incubated aerobically, gram-positive cocci often form the predominant colonies. Many species are pigmented, and this provides an additional aid in selecting gram-positive cocci.

Staphylococci are common commensals and parasites of humans and animals, and they occasionally cause serious infections. In humans, there are two major species, *Staphylococcus epidermidis*, a nonpigmented, nonpathogenic organism usually found on the skin or mucous membranes, and *Staphylococcus aureus* (Figure 16.20), a yellow-pigmented species that is most commonly associated with pathological conditions including boils, pimples, pneumonia, osteomyelitis, meningitis, and arthritis. Some *S. aureus* strains are resistant to multiple antibiotics (so-called *MRSA* strains) and are fierce pathogens that can cause extensive tissue damage (Figure 30.29). We discuss the pathogenesis of MRSA and other strains of *S. aureus* and staphylococcal diseases in Sections 24.5, 28.2, and 30.9.



(a) (b)

Figure 16.20 ***Staphylococcus.*** *(a)* Scanning electron micrograph of typical *Staphylococcus aureus* cells, showing the irregular arrangement of the cell clusters. Individual cells are about 0.8 μm in diameter. *(b)* Transmission electron micrograph of a dividing cell of *S. aureus*. Note the thick gram-positive cell wall.



(a) (b)

Figure 16.21 ***Sarcina.*** *(a)* Phase-contrast photomicrograph of cells of a typical gram-positive coccus *Sarcina*. A single cell is about 2 µm in diameter. *(b)* Electron micrograph of a thin section from *Sarcina ventriculi*. The outermost layer of the cell consists of cellulose.

Sarcina

The genus *Sarcina* groups obligate anaerobes that are catalase-negative within the order *Clostridiales*. *Sarcina* species divide in three perpendicular planes to yield packets of eight or more cells and are notable for this morphology (**Figure 16.21**). *Sarcina* are also extremely acid-tolerant, being able to ferment sugars and grow in environments at a pH as low as 2. Cells of one species, *Sarcina ventriculi*, contain a thick, fibrous layer of cellulose surrounding the cell wall (Figure 16.21*b*). The cellulose layers of adjacent cells become attached, and this functions as a cementing material to hold together packets of *S. ventriculi* cells.

Sarcina species can be isolated from soil, mud, feces, and stomach contents. Because of its extreme acid tolerance, *S. ventriculi* is one of only a few bacteria that can inhabit and grow in the stomach of humans and other monogastric animals. Rapid growth of *S. ventriculi* is observed in the stomach of humans suffering from certain gastrointestinal disorders, such as pyloric ulcerations. These pathological conditions retard the flow of food to the intestine and often require surgery to correct.

MINIQUIZ

- How could species of *Staphylococcus* be differentiated from *Streptococcus*?
- What characteristics differentiate *Sarcina* from *Staphylococcus*?

16.8 *Firmicutes:* Sporulating *Bacillales* and *Clostridiales*

KEY GENERA: *Bacillus, Clostridium, Sporosarcina*

All endospore-forming bacteria are gram-positive species of *Bacillales* or *Clostridiales*. The ability to form endospores evolved only once in a common ancestor of the *Bacillales, Clostridiales*, and *Lactobacillales* (Figure 16.17). However, many *Bacillales* and *Clostridiales* and the entire order *Lactobacillales* are unable to form endospores. The capacity to make endospores requires many genes (↔ Sections 2.10 and 7.6) and has not been acquired by horizontal gene transfer. It thus appears that the phylogenetic distribution of endospores has seen many cases where the capacity to form endospores has been lost during the course of evolution.

Endospore-forming bacteria are distinguished on the basis of cell morphology, shape and cellular position of the endospore (**Figure 16.22**), relationship to O_2, and energy metabolism. The two genera about which most is known are *Bacillus*, species of which are aerobic or facultatively aerobic, and *Clostridium*, which contains species that are obligately anaerobic and fermentative. All endospore-forming bacteria are ecologically related because they are found in nature primarily in soil. Even those species that are pathogenic to humans or other animals are primarily saprophytic soil organisms and infect animals only incidentally. Indeed, the ability to produce endospores should be advantageous for a soil microorganism because soil is a highly variable environment in terms of nutrient levels, temperature, and water activity.

Endospore-forming bacteria can be selectively isolated from soil, food, dust, and other materials by heating the sample to 80°C for 10 min, a treatment that effectively kills vegetative cells while any endospores present remain viable. Streaking such heat-treated samples on plates of the appropriate medium and incubating either aerobically or anaerobically selectively yields species of *Bacillus* or *Clostridium*, respectively.

Bacillus and *Paenibacillus*

Species of *Bacillus* and *Paenibacillus* grow well on defined media containing any of a number of carbon sources. Many bacilli produce extracellular hydrolytic enzymes that break down complex polymers such as polysaccharides, nucleic acids, and lipids, permitting the organisms to use these products as carbon sources and electron donors. Many bacilli produce antibiotics, including bacitracin, polymyxin, tyrocidine, gramicidin, and circulin. In most cases the antibiotics are released when the culture enters the stationary phase of growth and is committed to sporulation.

Several bacilli, most notably *Paenibacillus popilliae* and *Bacillus thuringiensis*, produce toxic insecticidal proteins. *P. popilliae* causes a fatal condition called milky disease in Japanese beetle larvae and larvae of closely related beetles of the family *Scarabaeidae*. *B. thuringiensis* causes a fatal disease of many different groups of insects. Both of these insect pathogens form a crystalline protein during sporulation called the *parasporal body*, which is deposited within the sporangium but outside the endospore proper (**Figure 16.23**). In *B. thuringiensis*, the parasporal body is a protoxin that is converted to a toxin in the insect gut. The toxin binds to specific receptors in the intestinal epithelial cells of certain insects and induces pore formation that causes leakage of the host cell cytoplasm followed by lysis. Diverse strains of *B. thuringiensis* can make different types of toxin that have specificity for different groups of insects. Endospore preparations derived from *B. thuringiensis* and *P. popilliae* are commercially available as biological insecticides.

The *cry* genes that encode crystal proteins have been isolated from several *B. thuringiensis* strains. The genes for the *B. thuringiensis* crystal protein (known commercially as "Bt toxin") have been

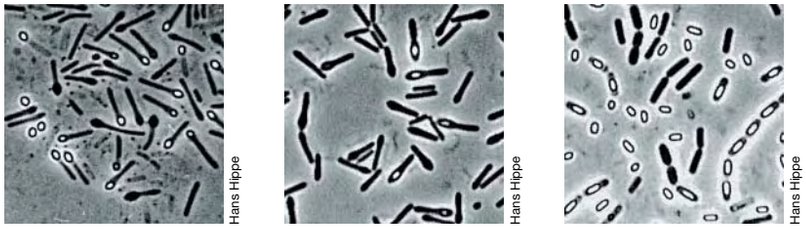


Figure 16.22 *Clostridium* species and endospore location. *(a) Clostridium cadaveris,* terminal endospores. Cells are about 0.9 μm wide. *(b) Clostridium sporogenes,* subterminal endospores. Cells are about 1 μm wide. *(c) Clostridium bifermentans,* central endospores. Cells are about 1.2 μm wide. All are phase-contrast micrographs.

introduced into genetically modified crops (e.g., maize, soybeans, and cotton) to render the plants resistant to insects. These genetically modified "Bt crops" are used widely around the world. Genetically altered Bt toxins have also been developed by genetic engineering to help increase toxicity and reduce resistance (Section 12.7).

Clostridium

Clostridia lack a respiratory chain, and so unlike *Bacillus* species, they obtain ATP by substrate-level phosphorylation. Many anaerobic energy-yielding mechanisms are known in the clostridia (Section 14.21). Indeed, the separation of the genus *Clostridium* into subgroups is based primarily on these properties and on the fermentable substrate used. A number of clostridia are *saccharolytic* and ferment sugars, producing butyric acid as a major end product. Some of these also produce acetone and butanol, such as *Clostridium pasteurianum*, which is also a vigorous nitrogen-fixing bacterium (Section 14.6).

One group of clostridia including the species *C. thermocellum, C. cellulolyticum*, and *C. cellulovorans* ferments cellulose with the formation of acids and alcohols. These species are likely the major organisms decomposing cellulose in anoxic environments such as the rumen and sediments. Cellulolytic clostridia possess *cellulosomes*, a complex multienzyme structure found on the outer surface of the cell wall. The cellulosome binds insoluble cellulose and degrades it into soluble products that are transported into the cytoplasm and metabolized by the cell. This cellulosome mechanism is common to bacteria that are able to degrade cellulose anaerobically.

Another group of clostridia are *proteolytic* and conserve energy from the fermentation of amino acids. Some species ferment individual amino acids, but others ferment only amino acid pairs. The products of amino acid fermentation are typically acetate, butyrate, CO_2, and H_2. The coupled catabolism of an amino acid pair is called a *Stickland reaction*; for example, *Clostridium sporogenes* ferments glycine plus alanine. In the Stickland reaction, one amino acid functions as the electron donor and is oxidized, whereas the other is the electron acceptor and is reduced (Figure 14.59). Many of the products of amino acid fermentation by clostridia are foul-smelling substances, and the odor that results from putrefaction is mainly the result of clostridial action. In addition to butyric acid, other odoriferous compounds produced are isobutyric acid, isovaleric acid, caproic acid, hydrogen sulfide, methyl mercaptan (from sulfur amino acids), cadaverine (from lysine), putrescine (from ornithine), and ammonia.

The main habitat of clostridia is the soil, where they live primarily in "pockets" made anoxic by facultative or obligately aerobic bacteria. In addition, a number of clostridia inhabit the anoxic environment of the mammalian intestinal tract. Several clostridia are capable of causing severe diseases in humans, as will be discussed in Sections 24.8, 31.9, and 32.9. For example, botulism is caused by *Clostridium botulinum*, tetanus by *Clostridium tetani*, and gas gangrene by *Clostridium perfringens* and a number of other clostridia, both sugar and amino acid fermenters. These pathogenic clostridia seem in no way unusual metabolically but are distinct in that they produce specific toxins or, in those causing gas gangrene, a group of toxins. *C. perfringens* and related species can also cause gastroenteritis in humans and domestic animals (Section 32.9), and botulism outbreaks are not uncommon in birds such as ducks and a variety of other animals.

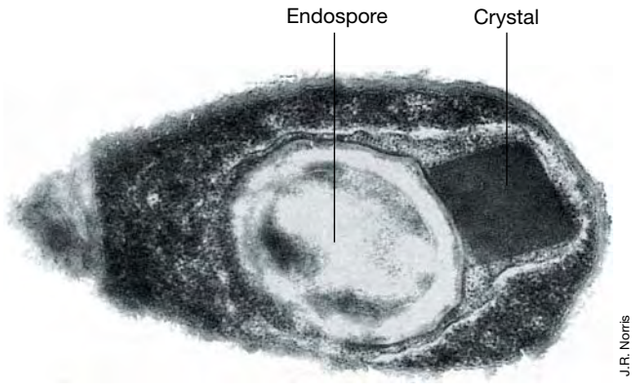


Figure 16.23 The toxic parasporal crystal in the insect pathogen *Bacillus thuringiensis*. Electron micrograph of a thin section of a sporulating cell. The crystalline protein (Bt toxin) is toxic to certain insects by causing lysis of their intestinal cells.

Sporosarcina

The genus *Sporosarcina* (**Figure 16.24**) is unique among endospore formers because cells are cocci instead of rods. *Sporosarcina* consists of strictly aerobic spherical to oval cells that divide in two or three perpendicular planes to form tetrads or packets of eight or more cells. The major species is *Sporosarcina ureae*. This bacterium can be enriched from soil by plating dilutions of a pasteurized soil sample on alkaline nutrient agar supplemented with 8% urea and incubating in air. Most soil bacteria are strongly inhibited by as little as 2% urea. However, *S. ureae* tolerates this, catabolizing urea to CO_2 and ammonia (NH_3), which dramatically raises the pH. *S. ureae* is remarkably alkaline-tolerant and can be grown in media up to pH 10, and this feature can be used to advantage in its enrichment from soil.

MINIQUIZ

- What is the major physiological distinction between *Bacillus* and *Clostridium* species?
- What is the crystalline protein made by *Bacillus thuringiensis* and what is its significance to agriculture?

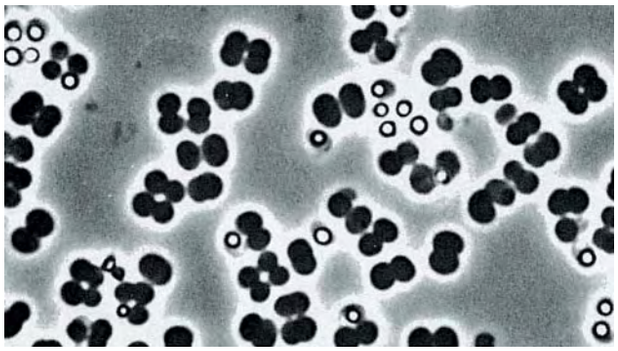


Figure 16.24 ***Sporosarcina ureae.*** Phase-contrast micrograph. A single cell is about 2 μm wide. Note bright refractile endospores. Most cell packets contain eight cells.

16.9 *Tenericutes:* The Mycoplasmas

KEY GENERA: *Mycoplasma, Spiroplasma*

The *Tenericutes*, which contain the single class *Mollicutes*, are bacteria that lack cell walls (*mollis* is Latin for "soft") and are some of the smallest organisms known. This group is often called the *mycoplasmas* because *Mycoplasma*, a notable genus containing human pathogens, is the best-characterized genus in the phylum (Table 16.3).

Although they do not stain gram-positively (because they lack cell walls), mycoplasmas are phylogenetically related to the *Firmicutes*. Mycoplasmas typically live in close association with animal and plant hosts and this may eliminate the need for a gram-positive cell wall. These organisms also have small genomes (ranging in size from 0.6 to 2.2 megabase pairs [Mbp]), a characteristic common in obligate symbionts (Sections 9.3 and 23.6).

Properties of Mycoplasmas

The absence of cell walls in mycoplasmas has been confirmed by electron microscopy and chemical analyses, which show that peptidoglycan is absent. Mycoplasmas resemble protoplasts (bacteria treated to remove their cell walls), but they are more resistant to osmotic lysis and are able to survive conditions under which protoplasts lyse. This ability to resist osmotic lysis is at least partially determined by the presence of sterols, which make the cytoplasmic membrane of mycoplasmas more stable than that of other bacteria. Indeed, some mycoplasmas require sterols in their growth media, and this sterol requirement can aid in the classification of mycoplasmas (Table 16.3).

In addition to sterols, certain mycoplasmas contain compounds called *lipoglycans* (Table 16.3). Lipoglycans are long-chain heteropolysaccharides covalently linked to membrane lipids and embedded in the cytoplasmic membrane of many mycoplasmas. Lipoglycans in some ways resemble the lipopolysaccharides in the outer membrane of gram-negative bacteria, except that they lack the lipid A backbone (Section 2.5). Lipoglycans function to help stabilize the cytoplasmic membrane and have also been identified as facilitating attachment of mycoplasmas to cell surface receptors of animal cells.

Growth of Mycoplasmas

Mycoplasmas can be grown in the laboratory and are small and pleomorphic cells. A single culture may exhibit small coccoid elements; larger, swollen forms; and filamentous forms, often highly branched (Figure 16.25). The small coccoid elements (0.2–0.3 μm in size) are among the smallest of free-living cells (Section 2.2). The mode of growth of mycoplasmas differs in liquid and agar cultures. On agar the organisms tend to grow so that they become embedded in the medium. These colonies show a characteristic "fried-egg" appearance consisting of a dense central core that penetrates downward into the agar, surrounded by a circular spreading area that is lighter in color (Figure 16.26). As would be expected of cells lacking cell walls, growth of *Mollicutes* is not inhibited by antibiotics that inhibit cell wall synthesis. However,

TABLE 16.3 ***Major characteristics of mycoplasmas***

Genus	*Properties*	*Genome size (megabase pairs)*	*Presence of lipoglycans*
Require sterols			
Mycoplasma	Many pathogenic; facultative aerobe (Figure 16.25)	0.66–1.35	+
Anaeroplasma	May or may not require sterols; obligate anaerobes; degrade starch, producing acetic, lactic, and formic acids plus ethanol and CO_2; found in the bovine and ovine rumen	1.5–1.6	+
Spiroplasma	Spiral to corkscrew-shaped cells; associated with various phytopathogenic (plant disease) conditions; facultative aerobe	0.94–2.2	–
Ureaplasma	Coccoid cells; occasional clusters and short chains; growth optimal at pH 6; strong urease reaction; associated with certain urinary tract infections in humans; microaerophile	0.75	–
Entomoplasma	Facultative aerobe; associated with insects and plants	0.79–1.14	Unknown
Do not require sterols			
Acholeplasma	Facultative aerobe	1.5	+
Asteroleplasma	Obligate anaerobe; isolated from the bovine or ovine rumen	1.5	+
Mesoplasma	Phylogenetically and ecologically related to *Entomoplasma*; facultative aerobe	0.87–1.1	Unknown

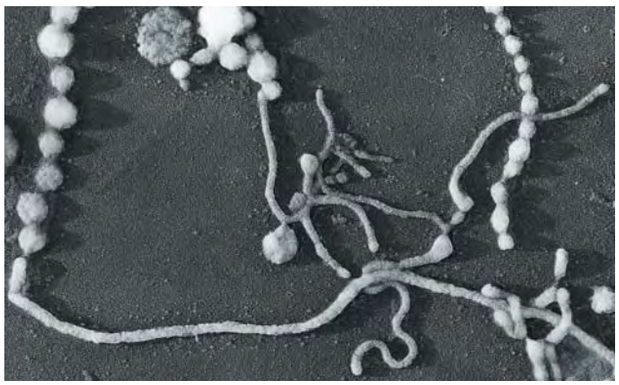


Figure 16.25 ***Mycoplasma mycoides*.** Metal-shadowed transmission electron micrograph. Note the coccoid and hyphae-like elements. The average diameter of cells in chains is about 0.5 μm.

mycoplasmas are as sensitive as most *Bacteria* to antibiotics whose targets are other than the cell wall.

Media for the culture of mycoplasmas are typically quite complex. For many species, growth is poor or absent even in complex yeast extract–peptone–beef heart infusion media. Fresh serum or ascitic fluid (peritoneal fluid) is needed as well to provide unsaturated fatty acids and sterols. Some mycoplasmas can be cultivated on relatively simple culture media, however, and even defined media have been developed for some species. Most mycoplasmas use carbohydrates as carbon and energy sources and require vitamins, amino acids, purines, and pyrimidines as growth factors. The energy metabolism of mycoplasmas is variable; some species are strictly aerobic, whereas others are facultative aerobes or obligate anaerobes (Table 16.3).

Spiroplasma

The genus *Spiroplasma* consists of helical or spiral-shaped *Mollicutes*. Amazingly, although they lack a cell wall and flagella, spiroplasmas are motile by means of a rotary (screw) motion or a slow undulation. Intracellular fibrils that are thought to play a role in motility have been demonstrated. The organism has been isolated from ticks, the hemolymph (Figure 16.27) and gut of insects, vascular plant fluids and insects that feed on these fluids, and the surfaces of flowers and other plant parts. For example, *Spiroplasma citri* has been isolated from the leaves of citrus plants, where it causes a disease called citrus stubborn disease, and from corn plants suffering from corn stunt disease. A number of other

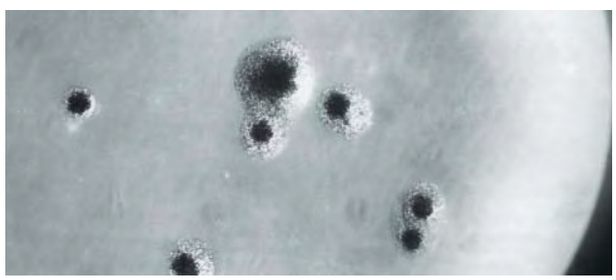


Figure 16.26 **Colonies of a *Mycoplasma* species on agar.** Note the typical "fried-egg" appearance. The colonies are about 0.5 mm in diameter.

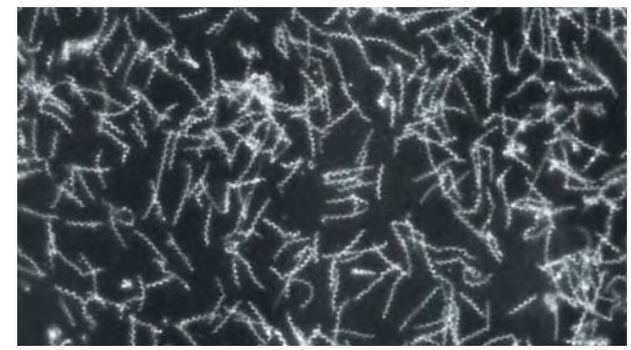


Figure 16.27 **"Sex-ratio" spiroplasma from the hemolymph of the fly *Drosophila pseudoobscura*.** Dark-field micrograph. Female flies infected with the sex-ratio spiroplasma bear only female progeny. Cells are about 0.15 μm in diameter.

mycoplasma-like organisms have been detected in diseased plants by electron microscopy, which indicates that a large group of plant-associated *Mollicutes* may exist. Some species of *Spiroplasma* are known that cause insect diseases, such as honeybee spiroplasmosis and lethargy disease of the beetle *Melolontha*.

MINIQUIZ

- Why do mycoplasmas need to have stronger cytoplasmic membranes than other bacteria?
- Motile spiroplasmas cannot contain a normal bacterial flagellum; why?

16.10 *Actinobacteria:* Coryneform and Propionic Acid Bacteria

KEY GENERA: *Arthrobacter, Corynebacterium, Propionibacterium*

The other major group of gram-positive bacteria is the *Actinobacteria*, which form their own phylum within the *Bacteria*. The *Actinobacteria* contain rod-shaped to filamentous and primarily aerobic bacteria that are common inhabitants of soil and plant materials. For the most part they are harmless commensals, species of *Mycobacterium* (for example, *Mycobacterium tuberculosis*) being notable exceptions. Some are of great economic value in either the production of antibiotics or certain fermented dairy products. While there are nine orders of *Actinobacteria*, the vast majority of species belong to the order *Actinomycetales* (Figure 16.17). We consider here the coryneform bacteria, species of *Actinomycetales* that have an unusual method of cell division, and the propionic acid bacteria, important agents in the ripening of Swiss cheese.

Coryneform Bacteria

Coryneform bacteria are gram-positive, aerobic, nonmotile, rod-shaped organisms that form irregular-shaped, club-shaped, or V-shaped cell arrangements during growth. V-shaped cells arise as a result of an abrupt movement that occurs just after cell division, a process called *snapping division* (Figure 16.28). Snapping division occurs because the cell wall consists of two layers; only the inner layer participates in cross-wall formation, and so after the cross-wall is formed, the two daughter cells remain attached by the

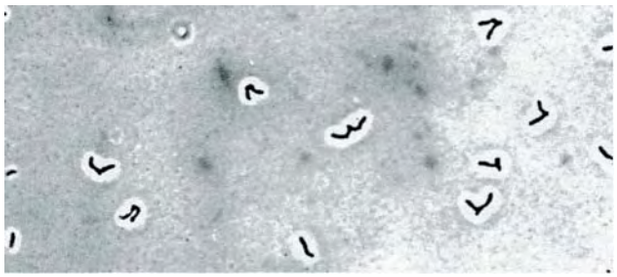


Figure 16.28 Snapping division in *Arthrobacter*. Phase-contrast micrograph of characteristic V-shaped cell groups in *Arthrobacter crystallopoietes* resulting from snapping division. Cells are about 0.9 μm in diameter.

outer layer of the cell wall. Localized rupture of this outer layer on only one side of the cell results in a bending of the two cells away from the ruptured side (**Figure 16.29**) and thus development of V-shaped forms.

The main genera of coryneform bacteria are *Corynebacterium* and *Arthrobacter*. The genus *Corynebacterium* consists of an extremely diverse group of bacteria, including animal and plant pathogens and saprophytes. Some species, such as *Corynebacterium diphtheriae*, are pathogenic (diphtheria, Section 30.3). The genus *Arthrobacter*, consisting primarily of soil organisms, is distinguished from *Corynebacterium* on the basis of a developmental cycle involving conversion from rod to coccus and back to rod again (**Figure 16.30**). However, some coryneform bacteria are pleomorphic and form coccoid cells during growth, and so the distinction between the two genera on the basis of life cycle is not absolute. The *Corynebacterium* cell frequently has a swollen end, so it has a club-shaped appearance, whereas *Arthrobacter* species are less commonly club-shaped.

Along with the *Acidobacteria* (Section 16.21), species of *Arthrobacter* are among the most common of all soil bacteria. They are remarkably resistant to desiccation and starvation, despite the fact that they do not form spores or other resting cells. Arthrobacters are a heterogeneous group that have considerable nutritional versatility, and strains have been isolated that decompose herbicides, caffeine, nicotine, phenols, and other unusual organic compounds.

Propionic Acid Bacteria

The **propionic acid bacteria** (genus *Propionibacterium*) were first discovered in Swiss (Emmentaler) cheese, where their fermentative production of CO_2 produces the characteristic holes and the propionic acid they produce is at least partly responsible for

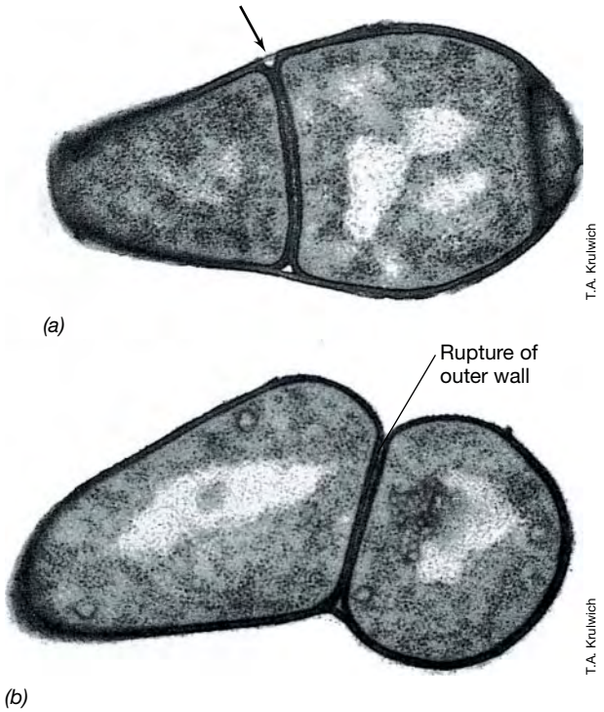


Figure 16.29 Cell division in *Arthrobacter*. Transmission electron micrograph of cell division in *Arthrobacter crystallopoietes,* illustrating how snapping division and V-shaped cell groups arise. *(a)* Before rupture of the outer cell wall layer (arrow). *(b)* After rupture of the outer layer on one side. Cells are 0.9–1 μm in diameter.

the unique flavor of the cheese. The bacteria in this group are gram-positive anaerobes that ferment lactic acid, carbohydrates, and polyhydroxy alcohols, producing primarily propionic acid, acetic acid, and CO_2 (Section 14.21).

The fermentation of lactate is of interest because lactate itself is a fermentation product of many bacteria (Section 16.6). The starter culture in Swiss cheese manufacture consists of a mixture of homofermentative streptococci and lactobacilli, plus propionic acid bacteria. The homofermentative organisms carry out the initial fermentation of lactose to lactic acid during formation of the curd (protein and fat). After the curd has been drained, the propionic acid bacteria develop rapidly. The eyes (or holes) characteristic of Swiss cheese are formed by the accumulation of CO_2, the gas diffusing through the curd and gathering at weak points. The propionic acid bacteria are thus able to obtain energy anaerobically

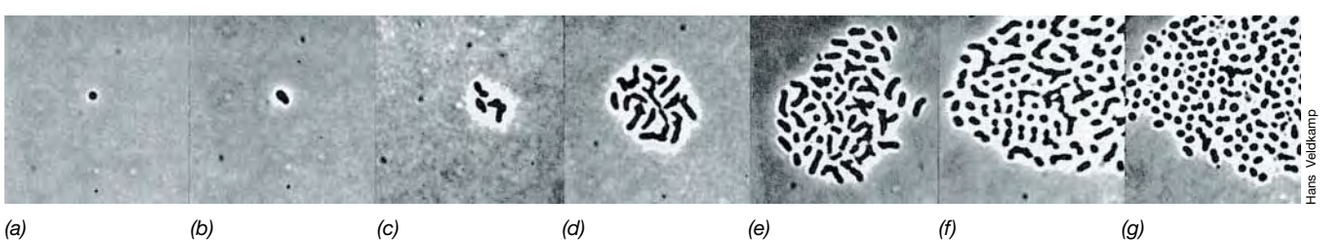


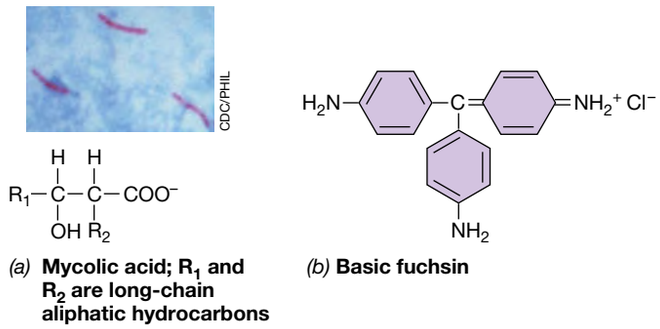
Figure 16.30 Stages in the life cycle of *Arthrobacter globiformis* as observed in slide culture.
(a) Single coccoid element; *(b–e)* conversion to rod and growth of a microcolony consisting predominantly of rods; *(f–g)* conversion of rods to coccoid forms. Cells are about 0.9 μm in diameter.

from a product that other bacteria have produced by fermentation. This metabolic strategy is called a *secondary fermentation*.

Propionate is also formed in the fermentation of succinate by the bacterium *Propionigenium*. This organism is phylogenetically and ecologically unrelated to *Propionibacterium*, but energetic aspects of its fermentation are of considerable interest. We discussed the mechanism of the *Propionigenium* fermentation in Section 14.22.

MINIQUIZ

- What is snapping division and what organism exhibits it?
- What organism is involved in the production of Swiss cheese, and what products does it make that help to flavor the cheese and make the holes?



(a) **Mycolic acid; R_1 and R_2 are long-chain aliphatic hydrocarbons** *(b)* **Basic fuchsin**

Figure 16.31 Acid-fast staining. Structure of *(a)* mycolic acid and *(b)* basic fuchsin, the dye used in the acid-fast stain. The fuchsin dye combines with mycolic acids in the cell wall via ionic bonds between COO^- and NH_2^+. Inset: Acid-fast stain of cells (red) of *Mycobacterium tuberculosis* present in a sputum sample from a tuberculosis patient.

16.11 *Actinobacteria: Mycobacterium*

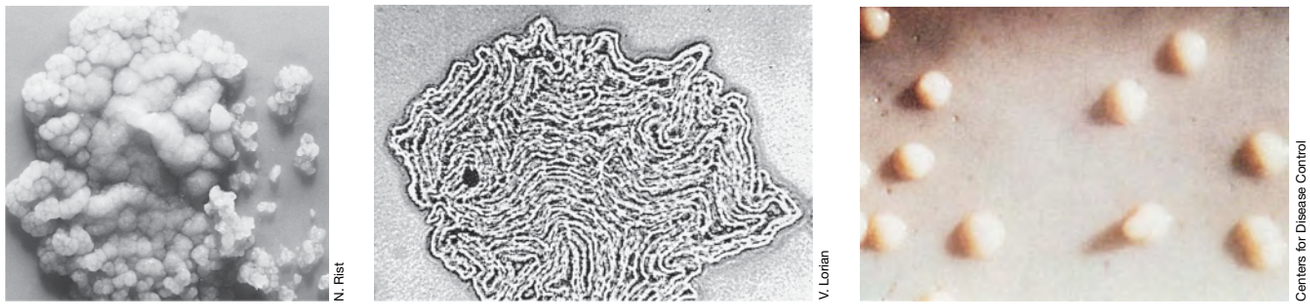
KEY GENUS: *Mycobacterium*

Mycobacteria are common in soils and most are harmless, but the genus *Mycobacterium* contains several notable human pathogens, chief among them *Mycobacterium tuberculosis*, the cause of tuberculosis (Section 30.4). Species are rod-shaped bacteria that at some stage of their growth cycle possess the distinctive staining property called **acid-fastness**. This property is due to the presence of unique lipids called *mycolic acids*, found only in species of the genus *Mycobacterium*, on the surface of the mycobacterial cell. Mycolic acids are a group of complex branched-chain hydroxylated lipids (**Figure 16.31***a*) covalently bound to peptidoglycan in the cell wall; the complex gives the cell surface a waxy, hydrophobic consistency.

Because of their waxy surface, mycobacteria do not stain well with Gram stain. A mixture of the red dye basic fuchsin and phenol is used in the acid-fast (Ziehl–Neelsen) stain. The stain is driven into the cells by slow heating, and the role of the phenol is to enhance penetration of the fuchsin into the lipids. After washing in distilled water, the preparation is decolorized with acid alcohol and counterstained with methylene blue. Cells of acid-fast organisms stain red (Figure 16.31 inset), whereas the background and non-acid-fast organisms appear blue (Figure 30.15*a*).

Mycobacteria are somewhat pleomorphic and may undergo branching or even filamentous growth. However, in contrast to the filaments of the actinomycetes (Section 16.12), the filaments of the mycobacteria do not form a true mycelium. Mycobacteria can be separated into two major groups: slow-growing species (e.g., *M. tuberculosis, M. avium, M. bovis*, and *M. gordonae*) and fast-growing species (e.g., *M. smegmatis, M. phlei, M. chelonae, M. parafortuitum*). *Mycobacterium tuberculosis* is a typical slow grower, and visible colonies are produced from dilute inoculum only after days to weeks of incubation. When growing on solid media, mycobacteria form tight, compact, often wrinkled colonies (**Figure 16.32**). This colony morphology is probably due to the high lipid content and hydrophobic nature of the cell surface, which facilitates cells sticking together.

For the most part, mycobacteria have relatively simple nutritional requirements. Most species can grow aerobically in a simple mineral salts medium with ammonium as the nitrogen source and glycerol or acetate as the sole carbon source and electron donor. Growth of *M. tuberculosis* is more difficult and is stimulated by lipids and fatty acids. The virulence of *M. tuberculosis* cultures has been correlated with the formation of long, cordlike structures (Figure 16.32*b*) that form as a result of side-to-side aggregation and intertwining of long chains of bacteria. Growth in cords reflects the presence of a characteristic glycolipid, the *cord factor*, on the cell surface (**Figure 16.33**).



(a) *(b)* *(c)*

Figure 16.32 Characteristic colony morphology of mycobacteria. *(a) Mycobacterium tuberculosis,* showing the compact, wrinkled appearance of the colony. The colony is about 7 mm in diameter. *(b)* A colony of virulent *M. tuberculosis* at an early stage, showing the characteristic cordlike growth. Individual cells are about 0.5 μm in diameter. (See also the historic drawings of *M. tuberculosis* cells made by Robert Koch, Figure 1.31). *(c)* Colonies of *Mycobacterium avium* from a strain of this organism isolated as an opportunistic pathogen from an AIDS patient.

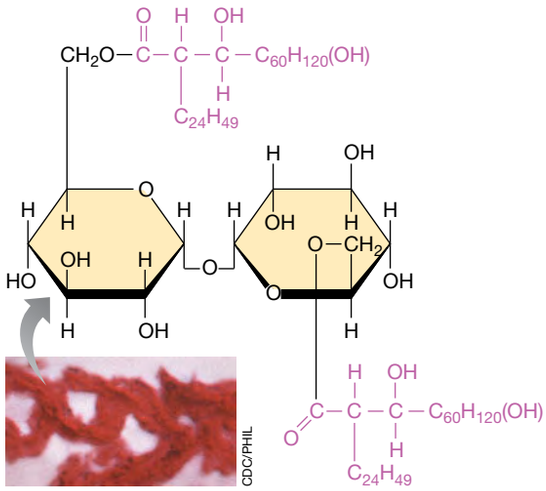


Figure 16.33 Structure of cord factor, a mycobacterial glycolipid: 6,6′-di-*O*-mycolyl trehalose. The two identical long-chain dialcohol groups are shown in purple. Inset: Photomicrograph of acid-fast stained cells of *Mycobacterium tuberculosis* (Figure 16.31) that have formed cords.

The pathogenesis of tuberculosis, along with the related mycobacterial disease leprosy, is discussed in Section 30.4.

Some mycobacteria produce yellow carotenoid pigments (Figure 16.32*c*), and pigmentation can aid in identification. Mycobacteria can either be nonpigmented (e.g., *M. tuberculosis, M. bovis, M. smegmatis, M. chelonae*); or can form pigment only when cultured in light, a property called *photochromogenesis* (e.g., *M. parafortuitum*); or can form pigment even when cultured in the dark, a property called *scotochromogenesis* (e.g., *M. gordonae, M. phlei*). Photochromogenesis is triggered by the blue region of the visible spectrum and is characterized by the photoinduction of one of the early enzymes in carotenoid biosynthesis. As with other carotenoid-containing bacteria, it is likely that carotenoids protect mycobacteria against oxidative damage from singlet oxygen (Section 5.14).

MINIQUIZ

- What is mycolic acid, and what properties does this substance confer on mycobacteria?

16.12 Filamentous *Actinobacteria: Streptomyces* and Relatives

KEY GENERA: *Streptomyces, Actinomyces, Nocardia*

The **actinomycetes** are a large group of phylogenetically related, filamentous and aerobic gram-positive *Bacteria* common in soils. Many actinomycetes have a characteristic developmental cycle that culminates in the production of desiccation-resistant spores. Filaments elongate from their ends and form branching *hyphae*. Hyphal growth results in a network of filaments called a *mycelium* (**Figure 16.34**), analogous to that formed by filamentous fungi (Section 18.8). When nutrients are depleted, the mycelium forms aerial hyphae that differentiate into spores that allow for survival and dispersal. We focus here on the genus *Streptomyces*, the most important genus in this group.

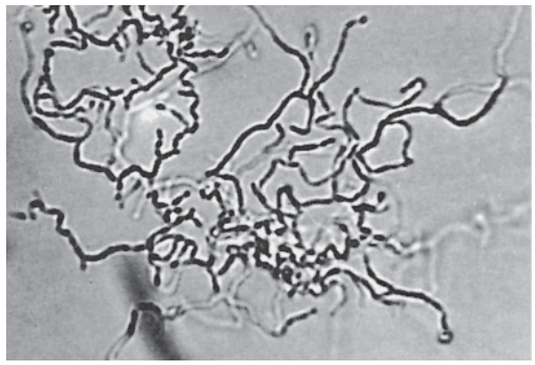
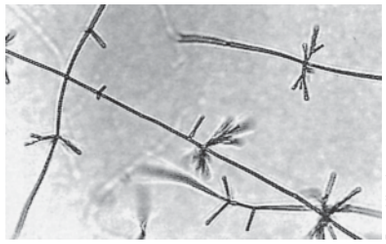


Figure 16.34 *Nocardia*. A young colony of an actinomycete of the genus *Nocardia*, showing typical filamentous cellular structure (mycelium). Each filament is about 0.8–1 μm in diameter.

Streptomyces

Over 500 species of *Streptomyces* are recognized. *Streptomyces* filaments are typically 0.5–1.0 μm in diameter and of indefinite length, and often lack cross-walls in the vegetative phase. *Streptomyces* grow at the tips of the filaments and may branch often. Thus, the vegetative phase consists of a complex, tightly woven matrix, resulting in a compact, convoluted mycelium and subsequent colony. As the colony ages, characteristic aerial filaments called *sporophores* are formed, which project above the surface of the colony and give rise to spores (**Figure 16.35**).



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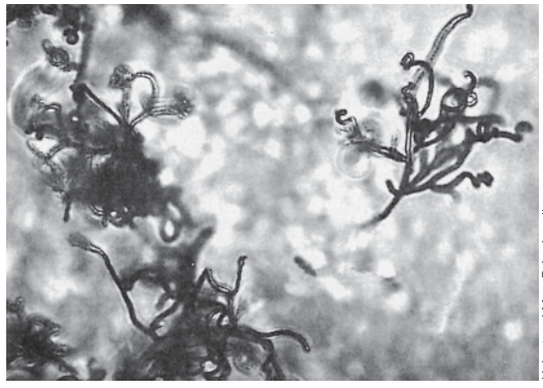


(b)

Figure 16.35 Spore-bearing structures of actinomycetes. Phase-contrast micrographs. Compare these photos with the art in Figure 16.37. *(a) Streptomyces,* a monoverticillate type. *(b) Streptomyces,* a closed spiral type. Filaments are about 0.8 μm wide in both types.

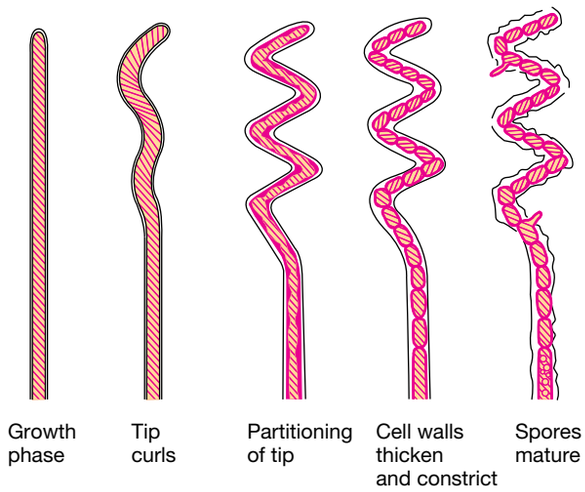


Figure 16.36 Spore formation in *Streptomyces*. Diagram of stages in the conversion of an aerial hypha (sporophore) into spores (conidia).

Streptomyces spores, called *conidia*, are quite distinct from the endospores of *Bacillus* and *Clostridium*. Unlike the elaborate cellular differentiation that leads to the formation of an endospore, conidia are produced by the formation of cross-walls in the multinucleate sporophores followed by separation of the individual cells directly into spores (**Figure 16.36**). Differences in the shape and arrangement of aerial filaments and spore-bearing structures of various species are among the fundamental features used in classifying the *Streptomyces* species (**Figure 16.37**). The conidia and sporophores are often pigmented and contribute a characteristic color to the mature colony (**Figure 16.38**). The dusty appearance of the mature colony, its compact nature, and its color make detection of *Streptomyces* colonies on agar plates relatively easy (Figure 16.38*b*).

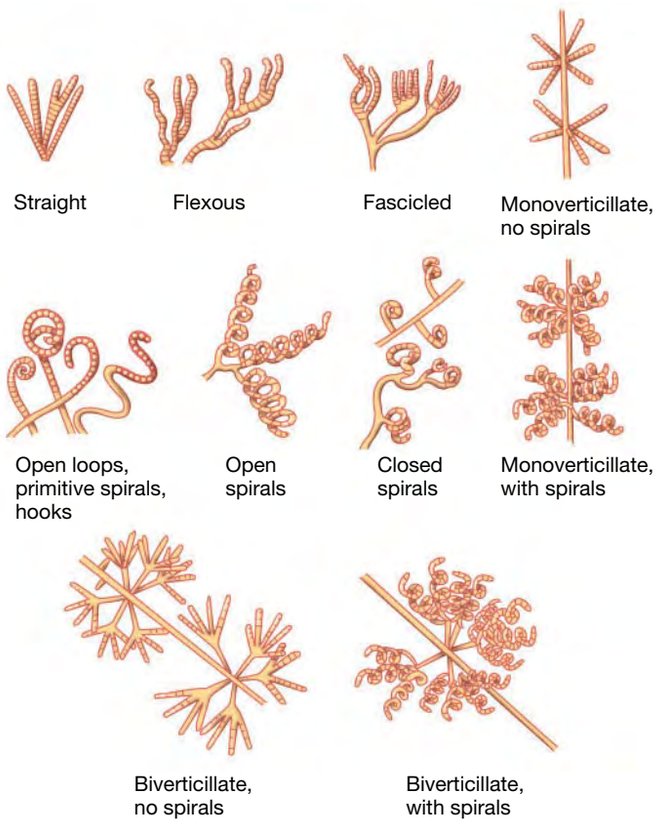


Figure 16.37 Morphologies of spore-bearing structures in the streptomycetes. A given species of *Streptomyces* produces only one morphological type of spore-bearing structure. The term "verticillate" means "whorls."

Ecology and Isolation of *Streptomyces*

Although a few streptomycetes are aquatic, they are primarily soil organisms. In fact, the characteristic earthy odor of soil is caused by the production by streptomycetes of a series of complex metabolites all called *geosmin*. Alkaline to neutral soils are more favorable for the development of *Streptomyces* than are acid soils. Moreover, higher numbers of *Streptomyces* are found in well-drained soils (such as sandy loams or soils covering limestone), where conditions are more likely to be aerobic, than in waterlogged soils, which quickly become anoxic.

Isolation of *Streptomyces* from soil is relatively easy: A suspension of soil in sterile water is diluted and spread on selective agar medium, and the plates are incubated aerobically at 25°C (Figure 16.38). Media selective for *Streptomyces* contain mineral salts plus polymeric substances such as starch or casein as organic nutrients. Streptomycetes typically produce extracellular hydrolytic enzymes that permit utilization of polysaccharides (starch, cellulose, and hemicellulose), proteins, and fats, and some

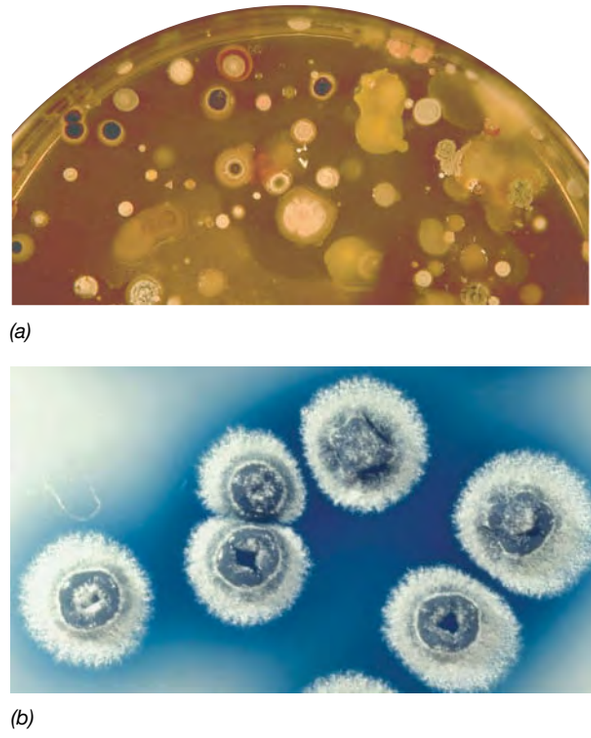


Figure 16.38 Streptomycetes. *(a)* Colonies of *Streptomyces* and other soil bacteria derived from spreading a soil dilution on a casein–starch agar plate. The *Streptomyces* colonies are of various colors (several black *Streptomyces* colonies are near the top of the plate) but can easily be identified by their opaque, rough, nonspreading morphology. *(b)* Close-up photo of colonies of *Streptomyces coelicolor*.

strains can use hydrocarbons, lignin, tannin, and other polymers. After incubation for 5–7 days in air, the plates are examined for the presence of the characteristic *Streptomyces* colonies (Figure 16.38), and spores from colonies can be restreaked to isolate pure cultures.

Antibiotics of *Streptomyces*

Perhaps the most striking physiological property of the streptomycetes is the extent to which they produce *antibiotics* (Table 16.4). Evidence for antibiotic production is often seen on the agar plates used in their initial isolation: Adjacent colonies of other bacteria show zones of inhibition (Figure 16.39*a*).

About 50% of all *Streptomyces* isolated have been found to be antibiotic producers. Over 500 distinct antibiotics are produced by streptomycetes and many more are suspected; most of these have been identified chemically. Some species produce more than one antibiotic, and often the several antibiotics produced by one organism are chemically unrelated. Although an antibiotic-producing organism is resistant to its own antibiotics, it usually remains sensitive to antibiotics produced by other streptomycetes. Many genes are required to encode the enzymes for antibiotic synthesis, and because of this, the genomes of *Streptomyces* species are typically quite large (8 Mbp and larger; Table 9.1). More than 60 streptomycete antibiotics have been used in human and veterinary medicine, and some of the most commonly used are listed in Table 16.4.

Ironically, despite the extensive research done on antibiotic-producing streptomycetes by the antibiotic industry and the fact that *Streptomyces* antibiotics are a multibillion-dollar-a-year industry, the ecology of *Streptomyces* remains poorly understood. The interactions of these organisms with other bacteria and the ecological rationale for antibiotic production remains an important topic about which we know very little. One hypothesis for why *Streptomyces* species produce antibiotics is that antibiotic production, which is linked to sporulation (a process itself triggered by nutrient depletion), might be a mechanism to inhibit the growth of other organisms competing with *Streptomyces* cells for limiting nutrients. This would allow the *Streptomyces* to complete the sporulation process and form a dormant structure that would increase their chances of survival.

MINIQUIZ

- Contrast spores and sporulation in *Streptomyces* and *Bacillus* species.
- Why might antibiotic production be of advantage to streptomycetes?

III • *Bacteroidetes*

The phylum *Bacteroidetes* contains more than 700 characterized species spread across four orders: *Bacteroidales, Cytophagales, Flavobacteriales*, and *Sphingobacteriales* (Figure 16.40). The *Bacteroidetes* are gram-negative nonsporulating rods; species are typically saccharolytic and can be aerobic or fermentative, including obligate aerobes, facultative aerobes, and obligate anaerobes. Gliding motility (Section 2.12) is widespread in the phylum, though many species are nonmotile and a few are motile by flagella. The genus *Bacteroides* has been particularly well studied as these organisms are a major component of the microbial community in the human gut.

16.13 *Bacteroidales*

KEY GENUS: *Bacteroides*

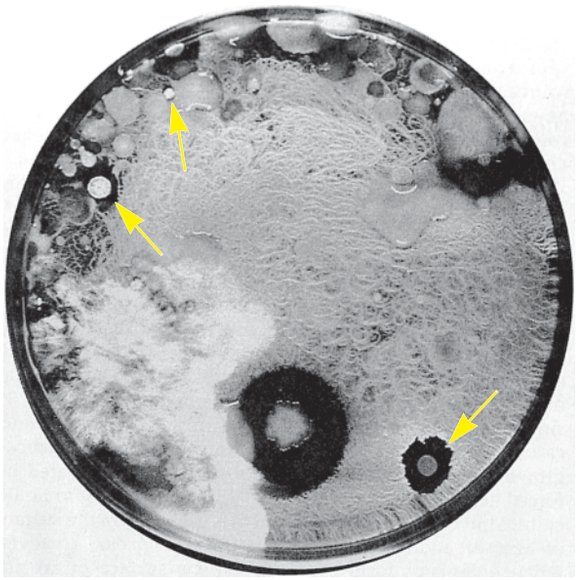
The order *Bacteroidales* primarily contains obligately anaerobic fermentative species. The type genus is *Bacteroides*, which contains species that are saccharolytic, fermenting sugars or proteins (depending on the species) to acetate and succinate as major fermentation products. *Bacteroides* are normally commensals, found in the intestinal tract of humans and other animals. In fact, *Bacteroides* species are the numerically dominant bacteria in the human large intestine, where measurements have shown that

UNIT 4

TABLE 16.4 Some common antibiotics synthesized by species of *Streptomyces* and related *Actinobacteria*

Chemical class	*Common name*	*Produced by*	*Active against*[a]
Aminoglycosides	Streptomycin	*S. griseus*[b]	Most gram-negative *Bacteria*
	Spectinomycin	*Streptomyces* spp.	*Mycobacterium tuberculosis,* penicillinase-producing *Neisseria gonorrhoeae*
	Neomycin	*S. fradiae*	Broad spectrum, usually used in topical applications because of toxicity
Tetracyclines	Tetracycline	*S. aureofaciens*	Broad spectrum, gram-positive and gram-negative *Bacteria*, rickettsias and chlamydias, *Mycoplasma*
	Chlortetracycline	*S. aureofaciens*	As for tetracycline
Macrolides	Erythromycin	*Saccharopolyspora erythraea*	Most gram-positive *Bacteria*, frequently used in place of penicillin; *Legionella*
	Clindamycin	*S. lincolnensis*	Effective against obligate anaerobes, especially *Bacteroides fragilis*, the major cause of anaerobic peritoneal infections
Polyenes	Nystatin	*S. noursei*	Fungi, especially *Candida* (a yeast) infections
	Amphotericin B	*S. nodosus*	Fungi
None	Chloramphenicol	*S. venezuelae*	Broad spectrum; drug of choice for typhoid fever

[a]Most antibiotics are effective against several different *Bacteria*. The entries in this column refer to the common clinical application of a given antibiotic. The structures and mode of action of many of these antibiotics are discussed in Sections 28.10–28.12.
[b]All species names beginning with an "*S.*" are species of *Streptomyces*.



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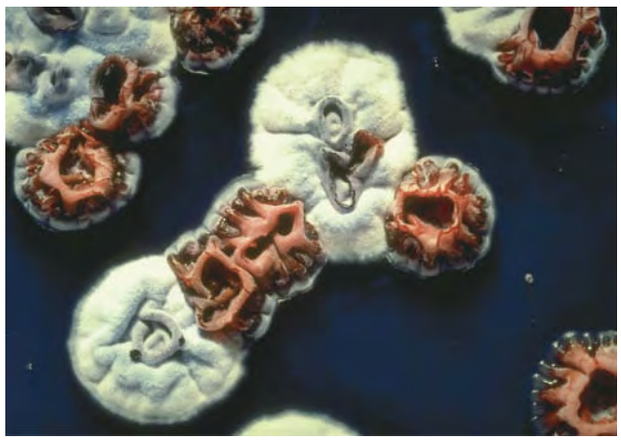


(b)

Figure 16.39 Antibiotics from *Streptomyces*. *(a)* Antibiotic action of soil microorganisms on a crowded agar plate. The smaller colonies surrounded by inhibition zones (arrows) are streptomycetes; the larger, spreading colonies are *Bacillus* species, some of which are also producing antibiotics. *(b)* The red-colored antibiotic undecylprodigiosin is being excreted by colonies of *S. coelicolor*.

about 10^{11} prokaryotic cells are present per gram of wet feces (↔ Section 24.2). However, species of *Bacteroides* can occasionally be pathogens and are the most important anaerobic bacteria associated with human infections such as *bacteremia* (bacteria in the blood).

Bacteroides thetaiotaomicron is one of the most prominent species of *Bacteroides* found in the lumen of the large intestine. *B. thetaiotaomicron* specializes in the degradation of complex polysaccharides. A majority of its genome is devoted to making enzymes that degrade polysaccharides. The diversity and number of genes for carbohydrate metabolism found in its genome far exceeds those found in any other bacterial species. *B. thetaiotaomicron* produces many enzymes that are not encoded by the human genome and thus it vastly increases the diversity of plant polymers that can be degraded in the human digestive tract.

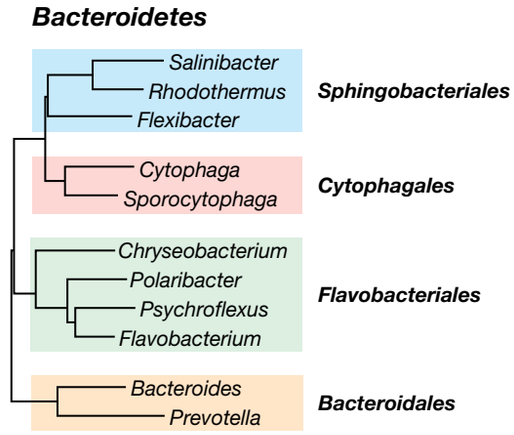


Figure 16.40 Major orders of *Bacteroidetes*. The phylogenetic tree was constructed from 16S rRNA gene sequences of representative genera of *Bacteroidetes*. Order names are shown in bold.

Species of *Bacteroides* are unusual in that they are one of the few groups of bacteria to synthesize a special type of lipid called *sphingolipid* (**Figure 16.41**), a collection of lipids characterized by the long-chain amino alcohol sphingosine in place of glycerol in the lipid backbone. Sphingolipids such as sphingomyelin, cerebrosides, and gangliosides are common in mammalian tissues, especially in the brain and other nervous tissues, but are rare in most bacteria. The production of sphingolipids can be found in a number of other genera in the phylum *Bacteroidetes* including *Flectobacillus, Prevotella, Porphyromonas*, and *Sphingobacterium*.

MINIQUIZ

- What is the role of *Bacteroides thetaiotaomicron* in the human gut?

16.14 *Cytophagales, Flavobacteriales,* and *Sphingobacteriales*

KEY GENERA: *Cytophaga, Flavobacterium, Flexibacter*

Cytophagales

The order *Cytophagales* (Figure 16.40) contains almost exclusively obligate aerobes, though some species have limited fermentative

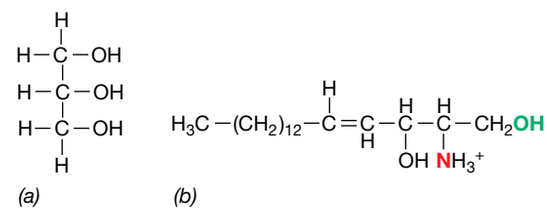


Figure 16.41 Sphingolipids. Comparison of *(a)* glycerol with *(b)* sphingosine. In sphingolipids, characteristic of *Bacteroides* species, sphingosine is the esterifying alcohol; a fatty acid is bonded by peptide linkage through the N atom (shown in red), and the terminal —OH group (shown in green) can be any of a number of compounds including phosphatidylcholine (sphingomyelin) or various sugars (cerebrosides and gangliosides).

capabilities. Cells are typically long, slender, gram-negative rods, often containing pointed ends, and move by gliding (**Figure 16.42**). Cytophagas specialize in the degradation of complex polysaccharides. They are widespread in toxic soils and freshwaters, where they probably account for much of the bacterial cellulose digestion. Cellulose decomposers can easily be isolated by placing small crumbs of soil on pieces of cellulose filter paper laid on the surface of mineral salts agar. The bacteria attach to and digest the cellulose fibers, forming spreading colonies (Figure 16.42*b*).

Cellulose degradation by cytophagas can proceed by two different mechanisms. The typical mechanism is the free cellulase mechanism in which cells secrete extracellular enzymes called *exoenzymes* that degrade insoluble cellulose outside of the cell. A complex mixture of enzymes is secreted including *processive endocellulases*, which cleave *internal* β-1,4 glycosidic bonds, and *processive exocellulases*, which cleave *terminal* β-1,4 glucosidic bonds, releasing cellobiose. These exoenzymes degrade insoluble cellulose into soluble polysaccharides and disaccharides that can be readily assimilated by cells. *Cytophaga hutchinsonii* does not produce processive cellulases, and its degradation of cellulose likely requires physical contact of cellulose fibers with cellulase enzymes located on the outer surface of its cell wall.

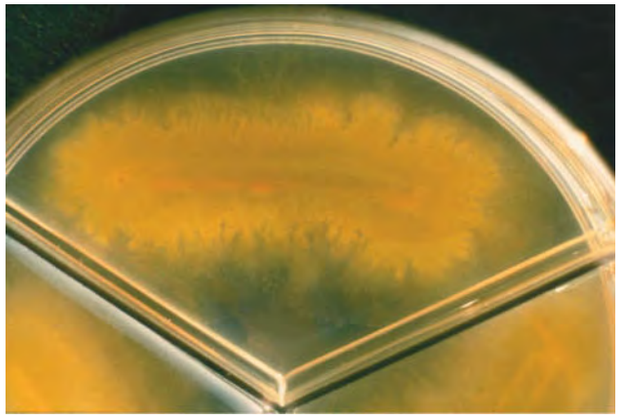
The genus *Cytophaga* contains species that can degrade not only cellulose (Figure 16.42*c*) but also agar (Figure 16.42*a*) and chitin. In pure culture *Cytophaga* can be grown on agar containing embedded cellulose fibers (Figure 16.42*b*). The related genus *Sporocytophaga* is similar to *Cytophaga* in morphology and physiology, but the cells form resting spherical structures called *microcysts* (Figure 16.42*d*), similar to those produced by some fruiting myxobacteria (Section 15.17).

Several species of *Cytophaga* are fish pathogens and can cause serious problems in the cultivated fish industry. Two of the most important diseases are *columnaris disease*, caused by *Cytophaga columnaris*, and *cold-water disease*, caused by *Cytophaga psychrophila*. Both diseases preferentially affect stressed fish, such as those living in waters receiving pollutant discharges or living in high-density confinement situations such as fish hatcheries and aquaculture facilities. Infected fish show tissue destruction, frequently around the gills, probably from proteolytic activities of the *Cytophaga* pathogen.

Flavobacteriales and *Sphingobacteriales*

Flavobacteriales and *Sphingobacteriales* (Figure 16.40) typically contain aerobic and facultatively aerobic chemoorganotrophs. Like most *Bacteroidetes*, these organisms are gram-negative rods, and are saccharolytic with many species motile by gliding. Species are found widely in soils and in aquatic habitats, where they typically degrade complex polysaccharides.

Flavobacteriales can be particularly abundant in marine waters including aquatic systems in polar environments. *Flavobacterium* species are primarily found in aquatic habitats, both freshwater and marine, as well as in foods and food-processing plants. Most species are obligate aerobes, though some species are able to reduce nitrate in an anaerobic respiration. Flavobacteria frequently produce yellow pigments and are generally saccharophilic; most can also degrade starch and proteins. Flavobacteria are rarely pathogenic; however, one species, *Flavobacterium meningosepticum*,



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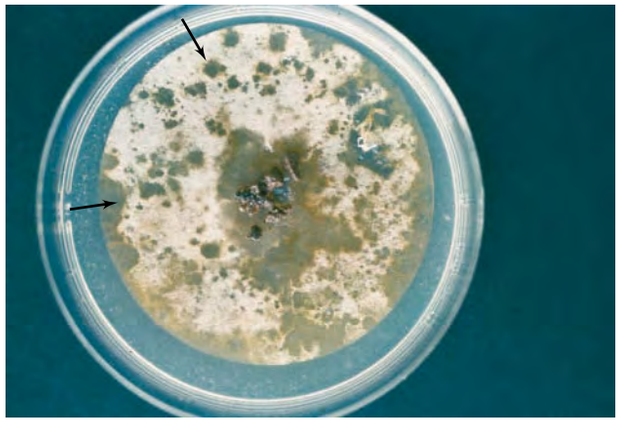

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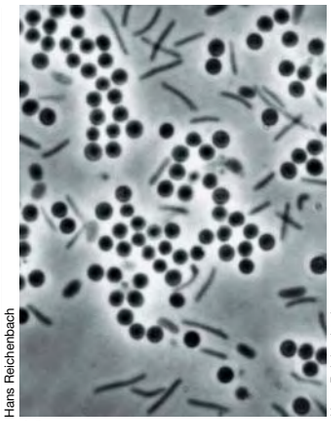


(d)

Figure 16.42 *Cytophaga* and *Sporocytophaga*. *(a)* Streak of an agarolytic marine *Cytophaga* hydrolyzing agar in a Petri dish. *(b)* Colonies of *Sporocytophaga* growing on cellulose. Note the clearing zones (arrows) where the cellulose has been degraded. *(c)* Phase-contrast micrograph of cells of *Cytophaga hutchinsonii* grown on cellulose filter paper (cells are about 1.5 μm in diameter). *(d)* Phase-contrast micrograph of the rod-shaped cells and spherical microcysts of *Sporocytophaga myxococcoides* (cells are about 0.5 μm and microcysts about 1.5 μm in diameter). Although *Sporocytophaga* microcysts are only slightly more heat-tolerant than vegetative cells, they are extremely resistant to desiccation and thus help the organism survive dry periods in soil. The genera *Cytophaga* and *Sporocytophaga* form a major clade within the phylum *Bacteriodetes* (Figure 16.40).

has been implicated in cases of infant meningitis, and several fish pathogens are also known.

Some *Flavobacteriales* are psychrophilic or psychrotolerant (↔ Section 5.10). These include, in particular, the genera *Polaribacter* and *Psychroflexus*, organisms commonly isolated from cold environments, especially permanently cold environments such as polar waters and sea ice. Many related genera are also capable of good growth below 20°C and can thus be agents of food spoilage. None are pathogenic.

Sphingobacteriales are phenotypically similar to many *Flavobacteriales*. In terms of physiology, species of *Sphingobacteriales* are generally able to degrade a greater breadth of complex polysaccharides than are *Flavobacteriales*, and in this regard they resemble species of *Cytophagales*. The genus *Flexibacter* is typical of many genera of *Sphingobacteriales*. Species of *Flexibacter* differ from those of *Cytophaga* in that they usually require complex media for good growth and are unable to degrade cellulose. Cells of some *Flexibacter* species also undergo changes in cell morphology from long, gliding, threadlike filaments lacking cross-walls to short, nonmotile rods. Many flexibacteria are pigmented due to carotenoids located in their cytoplasmic membrane, or from related pigments called *flexirubins*, located in the cell's outer membrane. *Flexibacter* species are common in soil and freshwaters where they degrade polysaccharides, and none have been identified as pathogens.

MINIQUIZ

- Describe a method for isolating *Cytophaga* species from nature.
- What characteristics are shared between the genera *Cytophaga* and *Bacteroides*, and in what ways do they differ?

IV • *Chlamydiae, Planctomycetes,* and *Verrucomicrobia*

The phyla *Chlamydiae, Planctomycetes*, and *Verrucomicrobia* share an ancestor and are more closely related to each other than to other bacterial phyla (**Figure 16.43**). These three groups contain organisms that can be found in a variety of habitats including soils, aquatic systems, and in association with eukaryotic hosts. We first consider the chlamydias, a group of small gram-negative bacteria that cause some serious human and animal diseases.

16.15 *Chlamydiae*

KEY GENERA: *Chlamydia, Chlamydophila, Parachlamydia*

The phylum *Chlamydiae* contains a single order, the *Chlamydiales*. The entire phylum consists of obligate intracellular parasites of eukaryotes. Though the species that are human pathogens have been characterized in most detail, the phylum contains diverse species that interact with a wide variety of eukaryotic hosts. Species are typically very small cocci, approximately 0.5 µm in diameter, and display a distinctive developmental cycle. Like many obligate parasites and symbionts, the genomes of *Chlamydiae* are typically reduced, ranging in size from 0.55–1 Mbp (↔ Section 9.3).

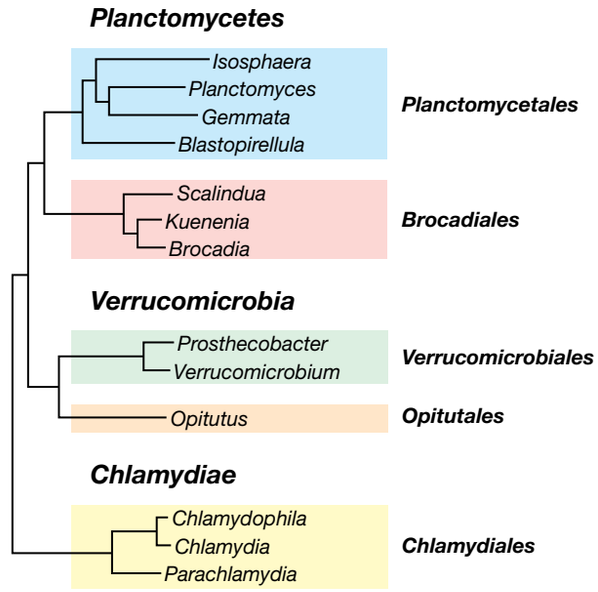


Figure 16.43 Major orders of *Chlamydiae, Planctomycetes*, and *Verrucomicrobia*. The phylogenetic tree was constructed from 16S rRNA gene sequences of representative genera of *Chlamydiae, Planctomycetes,* and *Verrucomicrobia*. Order names are shown in bold.

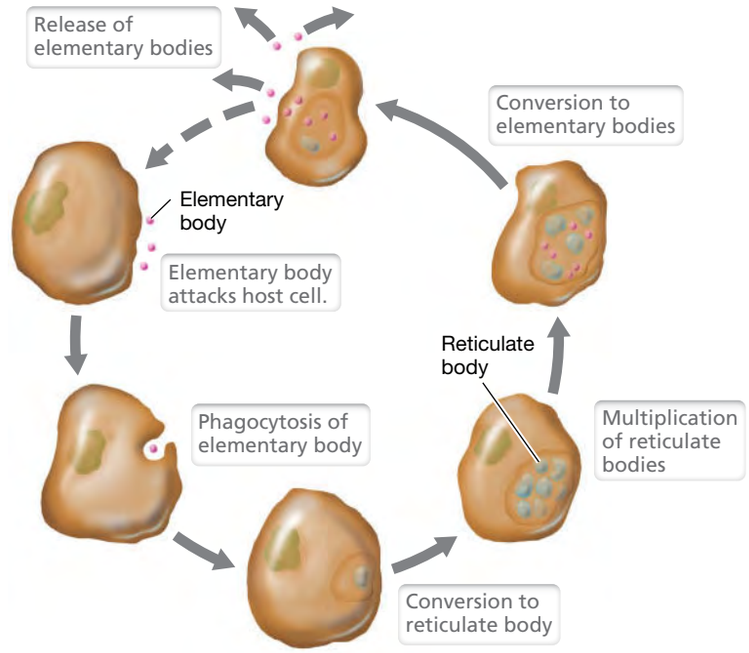
Life Cycle of *Chlamydiae*

All species of *Chlamydiae* demonstrate a unique chlamydial life cycle (**Figure 16.44**). Two types of cells are seen in the life cycle: (1) a small, dense cell, called an *elementary body*, which is relatively resistant to drying and is the means of dispersal, and (2) a larger, less dense cell, called a *reticulate body*, which divides by binary fission and is the vegetative form.

Elementary bodies are nonmultiplying cells specialized for infectious transmission. By contrast, reticulate bodies are noninfectious forms that function only to multiply inside host cells to form a large inoculum for transmission. Unlike the rickettsias, the chlamydias are not transmitted by arthropods but are primarily airborne invaders of the respiratory system—hence the significance of resistance to drying of the elementary bodies. A dividing reticulate body can be seen in **Figure 16.45**. After a number of cell divisions, these vegetative cells are converted into elementary bodies that are released when the host cell disintegrates (Figure 16.44*b*) and can then infect other nearby host cells. Generation times of 2–3 h have been measured for reticulate bodies, considerably faster than times found for the rickettsias (Section 16.1).

Notable Genera of *Chlamydiae*

Chlamydiae are particularly well adapted to invading and colonizing eukaryotic cells, and different species can infect a diverse array of eukaryotic hosts. The species *Parachlamydia acanthamoebae* infects free-living amoebae, particularly amoebae in the genus *Acanthamoeba*. *Parachlamydia* demonstrates the typical chlamydial life cycle during infection of amoebae (Figure 16.44). Most species of *Chlamydiae* can multiply or survive within free-living amoebae, and these hosts may be important for the survival and dispersal of *Chlamydiae* in nature. A diversity of 16S rRNA gene sequences from *Chlamydiae* can be detected in natural environments, suggesting



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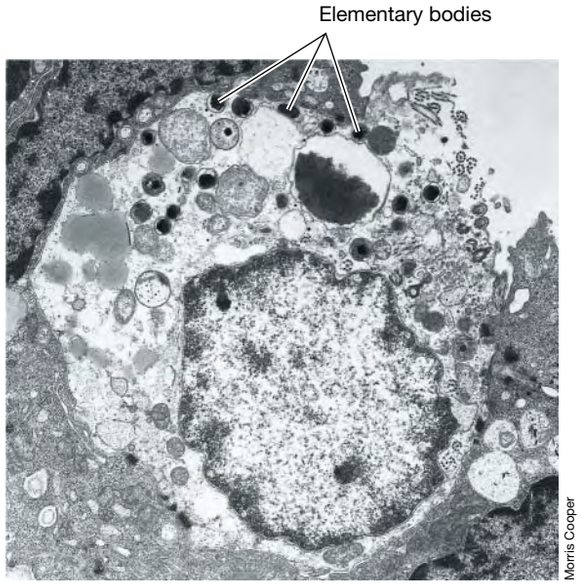


(b)

Figure 16.44 The infection cycle of a chlamydia. *(a)* Schematic diagram of the cycle: The entire cycle takes about 48 h. *(b)* Human chlamydial infection. Elementary bodies (~0.3 μm in diameter) are the infectious form and reticulate bodies (~1 μm in diameter) are the multiplying form. An infected fallopian tube cell is bursting, releasing mature elementary bodies.

that these organisms are widespread and that many of their natural hosts have yet to be identified. While free-living amoebae are the natural hosts for *P. acanthamoebae*, this species can also infect humans, although only weakly compared with *Chlamydiae* whose natural hosts are human.

The best-studied human pathogens are found in the genera *Chlamydia* and *Chlamydophila*. Several species are recognized within these genera: *Chlamydophila psittaci*, the causative agent of the disease psittacosis; *Chlamydia trachomatis*, the causative agent of trachoma and a variety of other human diseases; and *Chlamydophila pneumoniae*, the cause of some respiratory syndromes. Psittacosis is an epidemic disease of birds that is occasionally transmitted to humans and causes pneumonia-like symptoms. Trachoma, a debilitating disease of the eye characterized by vascularization and scarring of the cornea, is the leading cause of blindness in humans. Other strains of *C. trachomatis* infect the genitourinary tract, and chlamydial infections are currently one of the leading sexually transmitted diseases (Section 30.14).

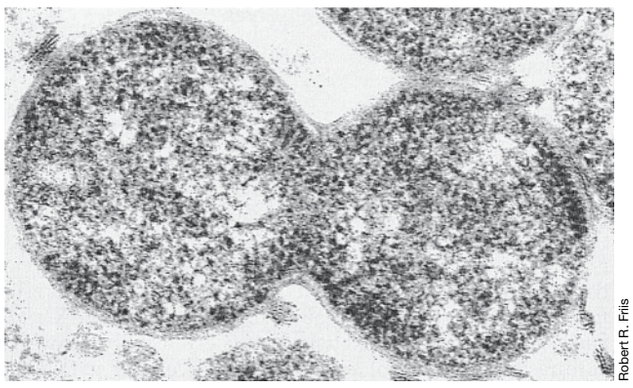


Figure 16.45 *Chlamydia*. Thin-section electron micrograph of a dividing reticulate body of *Chlamydophila psittaci* within a mouse tissue-culture cell. A single chlamydial cell is about 1 μm in diameter.

Molecular and Metabolic Properties

The chlamydias are among the most biochemically limited of all known *Bacteria*. Indeed, their genomes, approximately 1 Mbp in size, appear to be even more biosynthetically limited than those of the rickettsias, the other group of obligate intracellular parasites known among the *Bacteria* (Section 16.1). Interestingly, the *C. trachomatis* genome lacks a gene encoding the protein FtsZ, a key protein in septum formation during cell division (Section 7.3) and thought to be indispensable for growth of all prokaryotic cells. Also, some genes in *C. trachomatis* are distinctly *eukaryotic*, suggesting horizontal transfer from host to bacterium; these genes may encode functions that facilitate the pathogenic lifestyle of *C. trachomatis* (Section 30.14). In sum, the chlamydias appear to have evolved an efficient and effective survival strategy including parasitizing the resources of the host and producing resistant cell forms for transmission.

MINIQUIZ

- How are *Chlamydia* and *Mycoplasma* (Section 16.9) similar? How are they different?
- What is the difference between an elementary body and a reticulate body?

UNIT 4

16.16 *Planctomycetes*

KEY GENERA: *Planctomyces, Blastopirellula, Gemmata, Brocadia*

The phylum *Planctomycetes* contains several morphologically unique bacteria found primarily in two orders, *Planctomycetales* and *Brocadiales* (Figure 16.43).

Planctomycetes are gram-negative bacteria and many divide by budding. They often have stalks or appendages and their cells arranged in rosettes. *Planctomycetes* are unusual among bacteria because they can have an S-layer in their cell envelope (Section 2.6). Another remarkable feature of *Planctomycetes* is that they often contain intracellular compartments that resemble the organelles of eukaryotes.

Compartmentalization in *Planctomycetes*

We learned in Section 1.2 of the major structural differences between prokaryotic and eukaryotic cells. In particular, eukaryotes have a membrane-enclosed nucleus whereas in *Bacteria* and *Archaea*, DNA supercoils and compacts to form the nucleoid present in the cytoplasm. However, *Planctomycetes* are unique among all known bacteria in that they show evidence of cell compartmentalization.

All *Planctomycetes* produce a structure enclosed by a nonunit membrane and called a *pirellulosome*; this structure contains the nucleoid, ribosomes, and other necessary cytoplasmic components. But in some *Planctomycetes*, for example, in the bacterium *Gemmata* (**Figure 16.46**), the nucleoid itself is surrounded by invaginations of the cell membrane. DNA in *Gemmata* remains in a covalently closed, circular, and supercoiled form, typical of bacteria (Section 4.2), but it is highly condensed and remains partitioned from the remaining cytoplasm by a true unit membrane (Figure 16.46) (see page 530 for more on this).

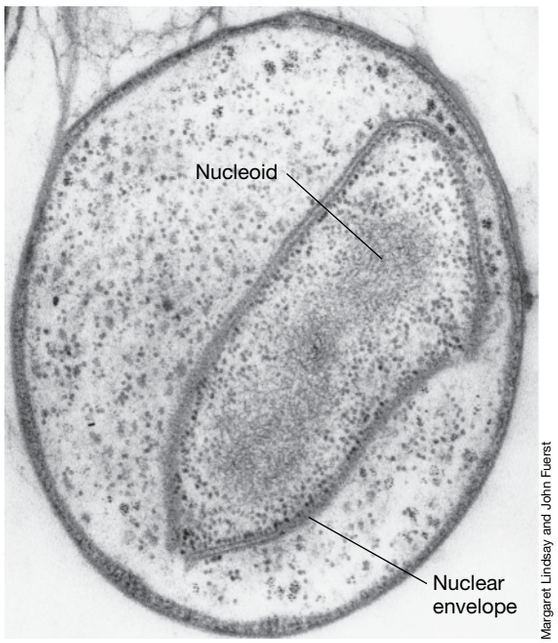


Figure 16.46 ***Gemmata:* a nucleated bacterium.** Thin-section electron micrograph of a cell of *Gemmata obscuriglobus* showing the nucleoid surrounded by a "nuclear envelope" (see page 530). The cell is about 1.5 μm in diameter.

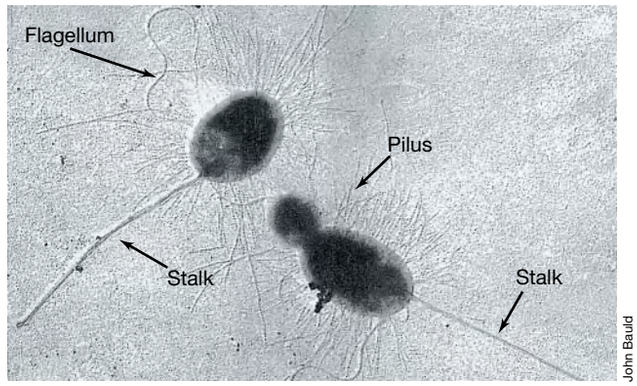


Figure 16.47 ***Planctomyces maris*.** Metal-shadowed transmission electron micrograph. A single cell is about 1–1.5 μm long. Note the fibrillar nature of the stalk. Pili are also abundant. Note also the flagella (curly appendages) on each cell and the bud that is developing from the nonstalked pole of one cell.

Another interesting compartment is the *anammoxosome*, found in species of the *Brocadiales* including *Brocadia anammoxidans*. This bacterium catalyzes the anaerobic oxidation of ammonia (NH_3) within the anammoxosome structure. The anammoxosome membrane is composed of unique lipids that form a tight seal, protecting cytoplasmic components from toxic intermediates produced during the anaerobic oxidation of ammonia (Section 14.12).

Planctomyces

Planctomyces is the best-characterized genus in the *Planctomycetes*. In Section 15.20 we considered the stalked proteobacterium *Caulobacter*. *Planctomyces* is also a stalked bacterium (**Figure 16.47**). However, unlike *Caulobacter*, the stalk of *Planctomyces* consists of protein and does not contain a cell wall or cytoplasm (compare Figure 16.47 with Figure 15.56). The *Planctomyces* stalk presumably functions in attachment, but it is a much narrower and finer structure than the prosthecal stalk of *Caulobacter*.

Like *Caulobacter* (Figures 7.16 and 15.56), *Planctomyces* is a budding bacterium that displays a life cycle. Its motile swarmer cells attach to a surface, grow a stalk from the attachment point, and generate a new cell from the opposite pole by budding. This daughter cell produces a flagellum, breaks away from the attached mother cell, and begins the cycle anew. Physiologically, *Planctomyces* species are facultatively aerobic chemoorganotrophs, growing either by fermentation or respiration of sugars.

The habitat of *Planctomyces* is primarily aquatic, both freshwater and marine, and the genus *Isosphaera* is a filamentous, gliding hot spring bacterium. The isolation of *Planctomyces* and relatives, like that of *Caulobacter*, requires dilute media.

MINIQUIZ

- How does the stalk of *Planctomyces* differ from the stalk of *Caulobacter*?
- What is unusual about the bacterium *Gemmata*?

16.17 *Verrucomicrobia*

KEY GENERA: *Verrucomicrobium, Prosthecobacter*

The phylum *Verrucomicrobia* contains at least four orders with characterized species, but most are found within the order *Verrucomicrobiales* (Figure 16.43). Species of *Verrucomicrobia* are aerobic or facultatively aerobic bacteria capable of fermenting sugars. An exception is the genus *Methylacidiphilum*, which contains aerobic methanotrophs (Section 15.16). In addition, some *Verrucomicrobia* form symbiotic associations with protists. *Verrucomicrobia* are widespread in nature, inhabiting freshwater and marine environments as well as forest and agricultural soils. The *Verrucomicrobia* can have membrane-bound intracellular structures similar to those found in the *Planctomycetes*. The *Verrucomicrobia* typically form cytoplasmic appendages called *prosthecae* (Section 15.20). *Verrucomicrobia* share with other prosthecate bacteria the presence of peptidoglycan in their cell walls and in this way are clearly distinct from *Planctomycetes*.

The genera *Verrucomicrobium* and *Prosthecobacter* produce two to several prosthecae per cell (**Figure 16.48**). Unlike cells of *Caulobacter* (Figures 7.16 and 15.56), which contain a single prostheca and produce flagellated and nonprosthecate swarmer cells, *Verrucomicrobium* and *Prosthecobacter* divide symmetrically, and both mother and daughter cells contain prosthecae at the time of cell division. The genus name *Verrucomicrobium* derives from Greek roots meaning "warty," which is an appropriate description of cells of *Verrucomicrobium spinosum* with their multiple projecting prosthecae (Figure 16.48).

Species of the genus *Prosthecobacter* contain two genes that show significant homology to the genes that encode tubulin in eukaryotic cells. Tubulin is the key protein that makes up the cytoskeleton of eukaryotic cells (Section 2.16). Although the important cell division protein FtsZ (Section 7.3) is also a tubulin homolog, the *Prosthecobacter* proteins are structurally more similar to eukaryotic tubulin than is FtsZ. The role of the tubulin proteins in *Prosthecobacter* is unknown since a eukaryotic-like cytoskeleton has not been observed in these organisms.

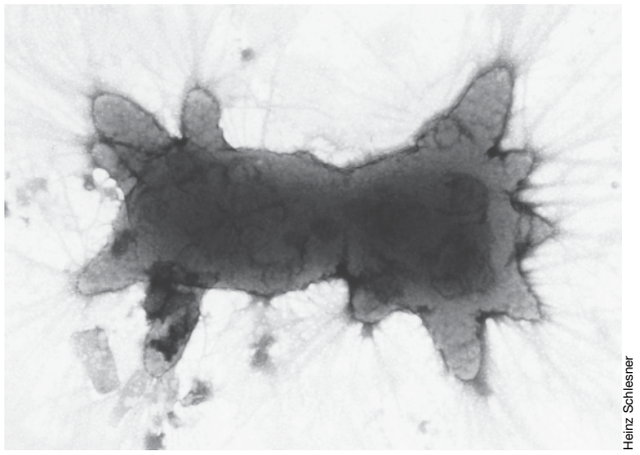


Figure 16.48 *Verrucomicrobium spinosum*. Negatively stained transmission electron micrograph. Note the wartlike prosthecae. A cell is about 1 μm in diameter.

MINIQUIZ

- Describe two ways that *Verrucomicrobia* differ from *Planctomycetes*.

V • Hyperthermophilic *Bacteria*

Three phyla of hyperthermophilic bacteria cluster deep in the phylogenetic tree of *Bacteria*, near the root (Figure 16.1). Each group consists of one or two major genera, and a key physiological feature of most species is *hyperthermophily*—optimal growth at temperatures above 80°C (Section 5.11). We begin with *Thermotoga* and *Thermodesulfobacterium*, each representative of its own lineage.

16.18 *Thermotogae* and *Thermodesulfobacteria*

KEY GENERA: *Thermotoga, Thermodesulfobacterium*

Thermotoga species are rod-shaped hyperthermophiles that form a sheathlike envelope (called a *toga*; thus the genus name) (**Figure 16.49*a***), stain gram-negatively, and are nonsporulating. *Thermotoga* species are fermentative anaerobes, catabolizing sugars or starch and producing lactate, acetate, CO_2, and H_2 as fermentation products. The organisms can also grow by anaerobic respiration using H_2 as an electron donor and ferric iron as an electron acceptor. Species of *Thermotoga* have been isolated from terrestrial hot springs as well as marine hydrothermal vents.

Despite being bacterial, the genome of *Thermotoga* contains many genes that show strong homology to genes from hyperthermophilic *Archaea*. In fact, over 20% of the genes of *Thermotoga*

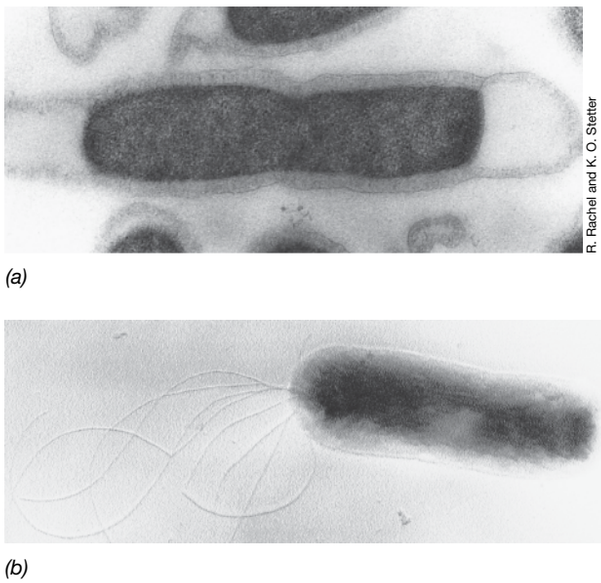
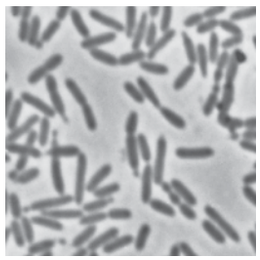


Figure 16.49 Hyperthermophilic *Bacteria*. Electron micrographs of two hyperthermophiles: *(a) Thermotoga maritima*—temperature optimum, 80°C. Note the outer covering, the toga. *(b) Aquifex pyrophilus*—temperature optimum, 85°C. Cells of *Thermotoga* measure 0.6 × 3.5 μm; cells of *Aquifex* measure 0.5 × 2.5 μm.



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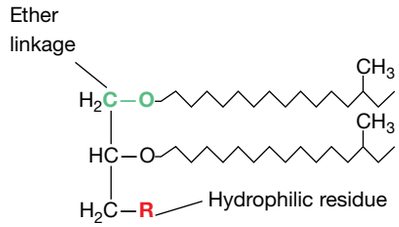


(b)

Figure 16.50 ***Thermodesulfobacterium.*** *(a)* Phase-contrast micrograph of cells of *Thermodesulfobacterium thermophilum*. *(b)* Structure of one of the lipids of *Thermodesulfobacterium mobile*. Note that although the two hydrophobic side chains are ether-linked, they are not phytanyl units, as in *Archaea*. The designation "R" is for a hydrophilic residue, such as a phosphate group.

probably originated from *Archaea* by horizontal gene transfers (Sections 9.6 and 13.7). Although a few archaea-like genes have been identified in the genomes of other *Bacteria* and vice versa, only in *Thermotoga* has such large-scale horizontal transfer of genes between domains been detected thus far.

Thermodesulfobacterium (**Figure 16.50**) is a thermophilic sulfate-reducing bacterium, positioned on the phylogenetic tree in a separate phylum after *Thermotoga* and *Aquifex* (Figure 16.1*a*). *Thermodesulfobacterium* is a strict anaerobe that uses compounds such as lactate, pyruvate, and ethanol (but not acetate) as electron donors, as do sulfate-reducing bacteria such as *Desulfovibrio* (Section 15.9), reducing SO_4^{2-} to H_2S.

An unusual biochemical feature of *Thermodesulfobacterium* is the production of *ether-linked lipids*. Recall that such lipids are a hallmark of the *Archaea* and that a polyisoprenoid C_{20} hydrocarbon (phytanyl) replaces fatty acids as the side chains in archaeal lipids (Section 2.3). However, the ether-linked lipids in *Thermodesulfobacterium* are unusual because the glycerol side chains are not phytanyl groups, as they are in *Archaea*, but instead are composed of unique C_{17} hydrocarbons along with some fatty acids (Figure 16.50*b*). Thus we see in *Thermodesulfobacterium* both a deep phylogenetic lineage (Figure 16.1) and a lipid profile that combines features of both the *Archaea* and the *Bacteria*. However, a few other *Bacteria* have also been found to contain ether-linked lipids, and thus these lipids may be more common among *Bacteria* than previously thought.

MINIQUIZ

- What is unique about the genome of *Thermotoga* and the lipids of *Thermodesulfobacterium*?

16.19 *Aquificae*

KEY GENERA: *Aquifex, Thermocrinis*

The genus *Aquifex* (Figure 16.49*b*) is an obligately chemolithotrophic and autotrophic hyperthermophile and is the most thermophilic of all known *Bacteria*. Various *Aquifex* species utilize H_2, sulfur (S^0), or thiosulfate ($S_2O_3^{2-}$) as electron donors and O_2 or nitrate (NO_3^-) as electron acceptors, and grow at temperatures up to 95°C. *Aquifex* can tolerate only very low O_2 concentrations (microaerophilic), and is unable to oxidize all tested organic compounds. *Hydrogenobacter*, a relative of *Aquifex*, shows most of the same properties as *Aquifex*, but is an obligate aerobe.

Aquifex and Autotrophy

Autotrophy in *Aquifex* occurs by way of the reverse citric acid cycle, a series of reactions previously detected only in green sulfur bacteria (Sections 14.5 and 15.6) within the domain *Bacteria*. The genome sequence of *Aquifex aeolicus* reveals that an entirely chemolithotrophic and autotrophic lifestyle is encoded by a genome of only 1.55 Mbp (one-third the size of the *Escherichia coli* genome). The discovery that so many hyperthermophilic species of *Archaea* and *Bacteria*, like *Aquifex*, are H_2 chemolithotrophs, coupled with the finding that they branch as very early lineages on their respective phylogenetic trees (Figure 16.1*a*), suggests that H_2 was a key electron donor for energy metabolism in primitive organisms that appeared on early Earth (Sections 13.1 and 17.13).

Thermocrinis

Thermocrinis (**Figure 16.51**) is a relative of *Aquifex* and *Hydrogenobacter*. This bacterium grows optimally at 80°C as a chemolithotroph oxidizing H_2, $S_2O_3^{2-}$, or S^0 as electron donors, with O_2 as electron acceptor. *Thermocrinis ruber*, the only known species, grows in the outflow of certain hot springs in Yellowstone National Park (Figure 16.51*a*) where it forms pink "streamers" consisting of a filamentous form of the cells attached to siliceous sinter (Figure 16.51*b*). In static culture, cells of *T. ruber* grow as individual rod-shaped cells (Figure 16.51*c*). However, when cultured in a flowing system in which growth medium is trickled over a solid glass surface to which cells can attach, *Thermocrinis* assumes the streamer morphology it forms in its constantly flowing habitat in nature.

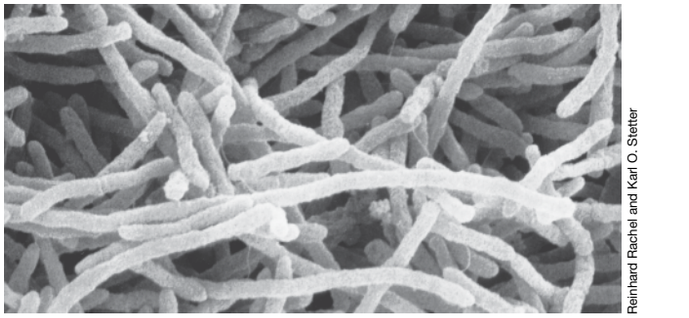
T. ruber is of historical significance in microbiology because it was one of the organisms discovered in the 1960s by Thomas Brock, a pioneer in the field of thermal microbiology and an author of the first seven editions of this textbook. The discovery by Brock that the pink streamers (Figure 16.51*b*) contained protein and nucleic acids clearly indicated that they were living organisms and not just mineral debris. Moreover, the presence of streamers in 80–90°C hot spring outflow waters but not those of lower temperatures supported Brock's hypothesis that these organisms actually *required* heat for growth and were therefore likely to be present in even boiling or superheated waters. Both of these conclusions were subsequently supported by the discovery by Brock and other microbiologists of dozens of genera of hyperthermophilic *Bacteria* and *Archaea* inhabiting hot springs, hydrothermal vents, and other thermal environments. More coverage of hyperthermophiles can be found in Section 5.11 and Chapter 17.

MINIQUIZ

- Of what evolutionary significance is the fact that organisms in the *Aquifex* lineage are both hyperthermophilic and H_2 chemolithotrophs?

(a)

(b)



(c)

Figure 16.51 ***Thermocrinis*.** *(a)* Octopus Spring, Yellowstone National Park (USA). The source water of this alkaline and siliceous hot spring is 92°C. *(b)* Cells of *Thermocrinis ruber* growing as filamentous streamers (arrow) attached to siliceous sinter in the outflow (85°C) of Octopus Spring. *(c)* Scanning electron micrograph of rod-shaped cells of *T. ruber* grown on a silicon-coated cover glass. A single cell of *T. ruber* is about 0.4 μm in diameter and from 1 to 3 μm long.

VI • Other *Bacteria*

Thus far in this chapter we have focused on phyla that have many described species (Figure 16.1). Beyond these mainstream bacterial phyla are many others that have but one or at most a handful of characterized species (Figure 16.1*b*). In addition, many more phyla are known only from community sampling of 16S rRNA genes from nature (Section 19.6). We cannot cover them all. So in this final part of the chapter we consider one phylum that has been well studied and then summarize some other phyla that are emerging into the mainstream of microbial diversity.

16.20 *Deinococcus–Thermus*

KEY GENERA: *Deinococcus, Thermus*

The *deinococci* group contains only a few characterized genera in two orders, the *Deinococcales* and the *Thermales*. Members of this phylum are typically aerobic chemoorganotrophs that metabolize sugars, amino and organic acids, or various complex mixtures. Though deinococci stain gram-positively, they have a gram-negative cell wall structure (**Figure 16.52**) made up of several layers, including an outer membrane, which is characteristic of gram-negative bacteria (Section 2.5). However, unlike the outer membrane of bacteria such as *Escherichia coli*, the outer membrane of deinococci lack lipid A. Deinococci also contain an unusual form of peptidoglycan in which ornithine replaces diaminopimelic acid in the *N*-acetylmuramic acid cross-links (Section 2.4).

Species of *Thermales* are typically thermophiles or hyperthermophiles and the type genus is *Thermus*. *Thermus aquaticus*, discovered in a Yellowstone National Park hot spring in the mid-1960s by Thomas Brock (Section 16.19), has been a model organism for studying life at high temperatures. *T. aquaticus* has subsequently been isolated from many geothermal systems, and is the source of *Taq* DNA polymerase. Because it is so heat-stable, *Taq* polymerase allowed the polymerase chain reaction (PCR) technique for amplifying DNA to be fully automated (Section 12.1), an advance that has revolutionized all of biology.

Radiation Resistance of *Deinococcus radiodurans*

Species of *Deinococcales* have the unusual property of being extremely radiation resistant, and *Deinococcus radiodurans* is the best-studied species in this regard. Most deinococci are red or pink due to carotenoids, and many are highly resistant to both radiation and desiccation. Resistance to ultraviolet (UV) radiation can be used to advantage in isolating deinococci. These remarkable organisms can be selectively isolated from soil, ground meat, dust, and filtered air following exposure of the sample to intense UV (or even gamma) radiation and plating on a rich medium containing tryptone and yeast extract. For example, *D. radiodurans* cells survive exposure to 15,000 grays (Gy) of ionizing radiation (1 Gy = 100 rad). This is sufficient to shatter the organism's chromosome into hundreds of fragments (by contrast, a human can be killed by exposure to less than 10 Gy) (Section 5.16).

In addition to impressive radiation resistance, *D. radiodurans* is resistant to the effects of many mutagenic agents. The only chemical mutagens that seem to work on *D. radiodurans* are agents such as nitrosoguanidine, which induces deletions in DNA. Deletions are apparently not repaired as efficiently as point mutations in this organism, and mutants of *D. radiodurans* can be isolated in this way.

DNA Repair in *Deinococcus radiodurans*

Studies of *D. radiodurans* have shown that it is highly efficient in repairing damaged DNA. Several different DNA repair enzymes exist in *D. radiodurans*. In addition to the DNA repair enzyme RecA (Sections 11.4 and 11.5), several RecA-independent DNA systems exist in *D. radiodurans* that can repair breaks in single- or

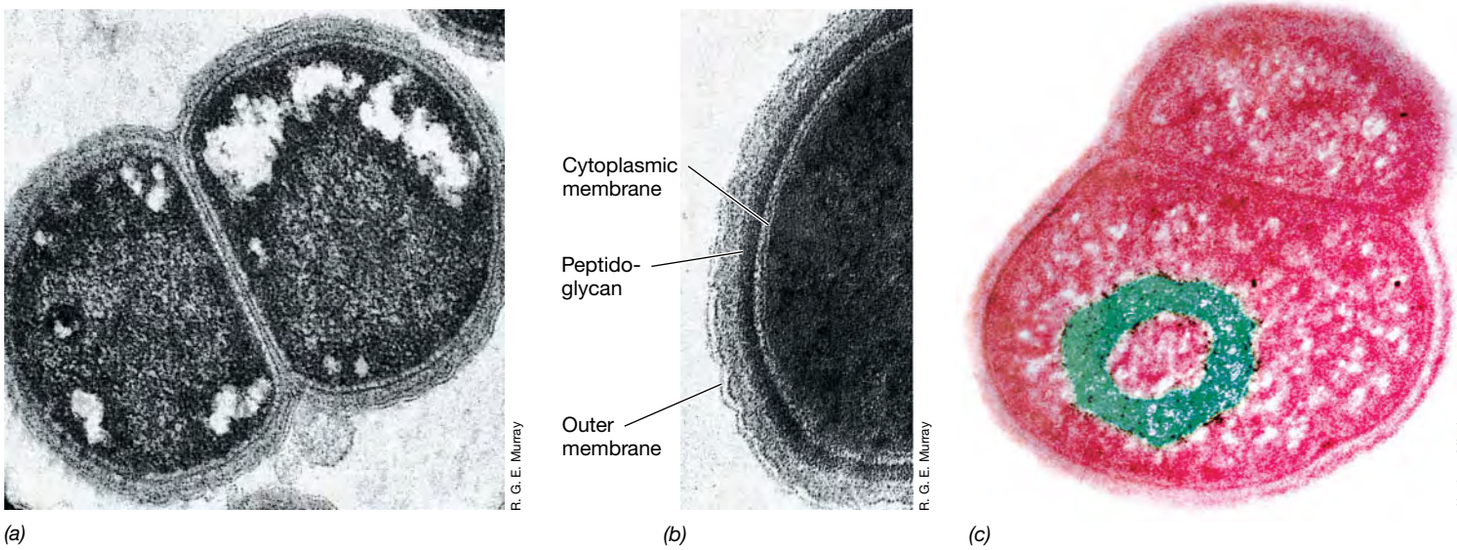


Figure 16.52 The radiation-resistant coccus *Deinococcus radiodurans*. An individual cell is about 2.5 µm in diameter. *(a)* Transmission electron micrograph of *D. radiodurans*. Note the outer membrane layer. *(b)* High-magnification micrograph of wall layer. *(c)* Transmission electron micrograph of cells of *D. radiodurans* colored to show the toroidal morphology of the nucleoid (green).

double-stranded DNA, and excise and repair misincorporated bases. In fact, repair processes are so effective that the chromosome can even be reassembled from a fragmented state.

It is also thought that the unique arrangement of DNA in *D. radiodurans* cells plays a role in radiation resistance. Cells of *D. radiodurans* always exist as pairs or tetrads (Figure 16.52*a*). Instead of scattering DNA within the cell as in a typical nucleoid, DNA in *D. radiodurans* is ordered into a toroidal (coiled, or stack of rings) structure (Figure 16.52*c*). Repair is then facilitated by the fusion of nucleoids from adjacent compartments, because their toroidal structure provides a platform for homologous recombination. From this extensive recombination, a single repaired chromosome emerges, and the cell containing this chromosome can then grow and divide.

MINIQUIZ

- Describe a commercial application of *Thermus aquaticus*.
- Describe an unusual biological feature of *Deinococcus radiodurans*.

16.21 Other Notable Phyla of *Bacteria*

The basic properties of seven other phyla of *Bacteria* are discussed briefly below. Although most of these have few cultured representatives (Figure 16.1*b*), many may well be of considerable ecological importance. If so, future research on their culture and ecological activities will supply the necessary proof. Until then, we paint a picture of these phyla with a broad brush to summarize their major characteristics in a general way.

Acidobacteria

Acidobacteria are widespread in the environment as revealed by analyses of 16S rRNA genes retrieved from environmental samples (Figure 16.1*b*). *Acidobacteria* are abundant in soils, particularly acid soils (pH < 6.0) where they often comprise a majority of some soil communities. *Acidobacteria* also inhabit freshwater, hot spring microbial mats, wastewater treatment reactors, and sewage sludge. There is evidence for as many as 25 major subgroups within the *Acidobacteria*, indicating substantial phylogenetic and metabolic diversity of the species in this phylum. Their abundance, widespread distribution, and likely metabolic diversity indicate they play important ecological roles, especially in soil. Unfortunately, while *Acidobacteria* are widespread in the environment, they have proven difficult to cultivate; as a result, few species have been isolated (Figure 16.1*b*) and only a handful of genera have been described.

The few species of *Acidobacteria* that have been characterized are metabolically diverse, including both chemoorganotrophs and photoheterotophs as well as obligate aerobes and obligately fermentative anaerobes. Three species of *Acidobacteria* have been well characterized, *Acidobacterium capsulatum, Geothrix fermentans*, and *Holophaga foetida*; all are gram-negative chemoorganotrophs. *A. capsulatum* is an acidophilic, encapsulated, obligately aerobic bacterium isolated from acid mine drainage; it utilizes various sugars and organic acids. *G. fermentans*, a strict anaerobe, oxidizes simple organic acids (acetate, propionate, lactate, fumarate) to CO_2 coupled to the reduction of ferric iron as electron acceptor (dissimilative iron reduction, Section 15.14), and can also ferment citrate to acetate plus succinate as products. *H. foetida* is a strictly anaerobic homoacetogen (Section 14.16) that grows by degrading methylated aromatic compounds to acetate. Some *Acidobacteria* degrade polymers such as cellulose and chitin, and at least one genus, *Chloracidobacterium*, is phototrophic (Section 15.8).

Nitrospirae, Deferribacteres, and *Chrysiogenetes*

The phylum *Nitrospirae* is named for the genus *Nitrospira*, a chemolithotroph that oxidizes nitrite to nitrate and grows

autotrophically (Section 15.13), as do species of the proteobacterium *Nitrobacter* (Section 15.13). *Nitrospira* inhabits many of the same environments as *Nitrobacter*. However, environmental surveys have shown that *Nitrospira* is much more abundant than *Nitrobacter* in nature, and thus most of the nitrite oxidized in nitrogen-rich environments such as wastewater treatment plants and ammonia-rich soils is probably due to *Nitrospira*. Some species of *Nitrospira* found widely in soils have also been observed to contain the complete pathway for nitrification, oxidizing ammonia all the way to nitrate (Sections 14.11 and 15.13). Other key *Nitrospirae* include *Leptospirillum*, an aerobic, acidophilic, iron-oxidizing chemolithotroph (Section 15.15) common in acid mine drainage associated with the mining of coal and iron (Section 22.2).

The phyla *Deferribacteres* and *Chrysiogenetes* (Figure 16.1) contain anaerobic chemoorganotrophs that display considerable metabolic diversity with respect to the electron acceptors used in anaerobic respirations (Chapter 14 and Figure 14.25). Most, though not all, species are able to grow through anaerobic respiration of nitrate to nitrite or ammonium. The *Deferribacteres* group is named for the genus *Deferribacter*, a thermophilic dissimilative ferric iron-reducer (Sections 14.15 and 15.14) that can also reduce nitrate and metal oxides. *Geovibrio* is a related genus that can also grow using elemental sulfur as an electron acceptor (Section 15.10). The bacterium *Chrysiogenes arsenatis* and its relatives are notable for the ability to couple the oxidation of acetate and a few other organic compounds to the reduction of arsenate as a terminal electron acceptor, reducing it to arsenite. In addition to arsenate, many species of *Chrysiogenetes* can reduce selenate, nitrite, nitrate, thiosulfate, and elemental sulfur in anaerobic respirations (Sections 14.14 and 14.15).

Synergistetes, Fusobacteria, Fibrobacteres

The phyla *Synergistetes, Fusobacteria*, and *Fibrobacteres* contain relatively few characterized species (Figure 16.1*b*), but those that have been cultured employ fermentative metabolisms. Species in these groups are often associated with the gastrointestinal tracts of animals and some have been associated with human disease.

Synergistetes are gram-negative nonsporulating rods found in association with animals and in anoxic environments in terrestrial and marine systems. Described species are typically obligate anaerobes that degrade proteins and are capable of fermenting amino acids. In animals they are most often found in the gastrointestinal tract; for example, *Synergistes jonesii* inhabits the rumen (Section 23.13). In humans, species of *Synergistetes* have been associated with certain soft tissue wounds and abscesses, dental plaque, and periodontal conditions.

Fusobacteria are gram-negative nonsporulating rods found in sediments and the gastrointestinal systems and oral cavities of animals. *Fusobacteria* are obligate anaerobes that ferment carbohydrates, peptides, and amino acids. Species of the genus *Fusobacterium* are common components of the human microbiome where they colonize mucous membranes. Different species can be found in the oral cavity, the gastrointestinal tract, and the vagina (Chapter 24). *Fusobacterium nucleatum* is often found in gingival crevices in the human oral cavity. Some fusobacteria may be human pathogens and *F. nucleatum* is often present in patients suffering from periodontal disease.

While 16S ribosomal RNA genes from *Fibrobacteres* can be recovered from a wide range of habitats, the only characterized species have come from either the rumen or gastrointestinal tracts of animals. The genus *Fibrobacter* contains gram-negative fermentative strict anaerobes. However, unlike most *Fusobacteria* and *Synergistetes*, species of *Fibrobacter* are unable to ferment proteins or amino acids and specialize instead in the fermentation of carbohydrates, including cellulose. In the rumen, cellulose is the major source of energy, and in such environments it supports not only cellulolytic bacteria such as *Fibrobacter* but many noncellulolytic anaerobes that use glucose released during cellulose degradation.

MINIQUIZ

- What is a major habitat for many species of *Acidobacteria*?
- How do *Nitrospira* and *Deferribacter* differ in terms of lifestyle and metabolism?
- What metabolic characteristics are shared by most *Synergistetes, Fusobacteria,* and *Fibrobacteres,* and what disease in humans has been correlated with the presence of *Synergistetes* and *Fusobacteria*?

Chapter Review

I • *Proteobacteria*

16.1 The *Alphaproteobacteria* are the second largest class of *Proteobacteria* and metabolically diverse. Key genera are: *Rhizobium, Rickettsia, Rhodobacter*, and *Caulobacter.*

Q Which genera of *Alphaproteobacteria* are known to form nitrogen-fixing nodules in plants?

16.2 The *Betaproteobacteria* are the third largest class of *Proteobacteria* and metabolically diverse. Key genera are *Burkholderia, Rhodocyclus, Neisseria*, and *Nitrosomonas*.

Q Which order of *Betaproteobacteria* does *Nitrosospira* belong to?

16.3 The *Gammaproteobacteria* are the largest and most diverse class of *Proteobacteria* and contain many human

pathogens. The *Enterobacteriales*, or enteric bacteria, are the most heavily studied of all bacteria. Key genera are *Escherichia* and *Salmonella*.

Q What is the catalase test? What catalase reaction would you expect from an obligate aerobe? What reaction would you expect from an obligate anaerobe?

16.4 The *Pseudomonadales* and *Vibrionales* are among the most common *Gammaproteobacteria*. Key genera are *Pseudomonas* and *Vibrio*.

Q Why is *P. aeruginosa* resistant to many widely used antibiotics?

16.5 The *Deltaproteobacteria* and *Epsilonproteobacteria* are smaller and less metabolically diverse classes of *Proteobacteria*. Key genera of *Deltaproteobacteria* are *Myxococcus, Desulfovibrio*, and *Geobacter*. Key genera of *Epsilonproteobacteria* are *Campylobacter* and *Helicobacter*.

Q Which environments do *Epsilonproteobacteria* typically inhabit? What role do they play in these environments?

II • *Firmicutes, Tenericutes,* and *Actinobacteria*

16.6 Lactic acid bacteria such as *Lactobacillus* and *Streptococcus* produce lactate as the primary end product of fermentation, and they have many roles in food production and preservation. The *Firmicutes* are one of the two main phyla of gram-positive bacteria.

Q How are lactic acid bacteria different from other anaerobes and why are they usually restricted to environments which contain sugar?

16.7 Many genera of *Firmicutes* in the orders *Bacillales* and *Clostridiales*, including *Staphylococcus, Listeria*, and *Sarcina*, are unable to form endospores.

Q What characteristics of *Listeria* make it a frequent cause of foodborne illness?

16.8 Production of endospores is a hallmark of the key genera *Bacillus* and *Clostridium* and is only found in the phylum *Firmicutes*.

Q What is a good strategy for isolating spore-forming bacteria from an environmental sample?

16.9 The phylum *Tenericutes* contains the mycoplasmas, organisms that lack cell walls and have very small genomes. Many species are pathogenic for humans, other animals, and plants. The key genus is *Mycoplasma*.

Q What two phyla are most closely related to the *Tenericutes*?

16.10 *Actinobacteria* are the second major phylum of gram-positive bacteria. *Corynebacterium* and *Arthrobacter* are common gram-positive soil bacteria. *Propionibacterium* ferments lactate to propionate and is the key agent responsible for the unique flavor and texture of Swiss cheese.

Q In what sort of environment would you expect to find large numbers of *Actinobacteria*?

16.11 Species of *Actinobacteria* in the genus *Mycobacterium* are mainly harmless soil saprophytes, but *Mycobacterium tuberculosis* causes the disease tuberculosis.

Q How does the cell wall of *Mycobacterium* influence its reaction to the Gram stain and the acid-fast stain?

16.12 The streptomycetes are a large group of filamentous, gram-positive bacteria that form spores at the end of aerial filaments and are found in the phylum *Actinobacteria*. Many clinically useful antibiotics such as tetracycline and neomycin have come from *Streptomyces* species.

Q How are the spores of streptomycetes different from endospores?

III • *Bacteroidetes*

16.13 The phylum *Bacteroidetes* includes gram-negative rods that do not form spores, many of which have gliding motility. Most species in the order *Bacteroidales* are obligate anaerobes that ferment carbohydrates in anoxic environments. The genus *Bacteroides* contains species that are common in the gastrointestinal tract of animals.

Q What species of *Bacteroidetes* is most abundant in the human gastrointestinal tract, and what role does this organism play in the human gut?

16.14 The *Cytophagales* and *Flavobacteriales* are orders in the *Bacteroidetes* that include aerobic bacteria able to degrade complex polysaccharides such as cellulose. These bacteria are important in organic matter decomposition.

Q What is an exoenzyme and why are these types of organisms important in the degradation of cellulose?

IV • *Chlamydiae, Planctomycetes,* and *Verrucomicrobia*

16.15 The phylum *Chlamydiae* includes small obligate intracellular parasites that are adept at invading eukaryotic cells. Many species cause various diseases in humans and other animals.

Q Describe the infection cycle of *Chlamydia*.

16.16 The *Planctomycetes* are a group of stalked, budding bacteria that form intracellular compartments of various types, and show extensive invaginations of the cytoplasmic membrane.

Q What are two types of intracellular compartments that have been observed within the *Planctomycetes*?

16.17 Species of *Verrucomicrobia* are distinguished by the multiple prosthecae on their cells and their unique phylogeny.

Q Verruca is a word that means "wart." How do you think the *Verrucomicrobia* got their name?

V • Hyperthermophilic *Bacteria*

16.18 *Thermotogae* and *Thermodesulfobacteria* form two deeply branching phyla within the *Bacteria*. These hyperthermophilic bacteria have proven that extensive horizontal gene transfer has occurred from *Archaea* to *Bacteria* (*Thermotoga*) and that ether-linked lipids are not limited to the *Archaea* (*Thermodesulfobacterium*).

Q Why are ether-linked lipids in *Thermodesulfobacterium* unusual?

16.19 The *Aquificae* phylum contains a group of hyperthermophilic, H_2-oxidizing bacteria that form the earliest branch on the tree of the domain *Bacteria*.

Q In what environment might you observe *Thermocrinis ruber,* and what role did this organism play in the discovery of hyperthermophiles?

VI • Other *Bacteria*

16.20 *Deinococcus* and *Thermus* are the major genera in a distinct phylum of *Bacteria*. *Thermus* is the source of the key enzyme in automated PCR, whereas *Deinococcus* is the most radiation-resistant bacterium known, exceeding even endospores in this regard.

Q What are some of the remarkable properties that allow *Deinococcus* to survive exposure to massive doses of radiation?

16.21 *Acidobacteria* are widespread in many environments, especially soils, and show various physiologies. The genus *Nitrospira* includes nitrite-oxidizing bacteria, while species of *Deferribacteres* and *Chrysiogenetes* specialize in various forms of anaerobic respiration. Species of *Synergistetes, Fusobacteria*, and *Fibrobacteres* are fermentative anaerobes that inhabit the gastrointestinal tract and other anoxic niches in animals.

Q What are four ways in which different species of *Acidobacteria* have been shown to generate energy?

Application Questions

1. Enteric bacteria, lactic acid bacteria, and propionic acid bacteria have distinctive metabolic traits that can be used to characterize and identify these organisms. Describe the metabolic characteristics of these organisms, name a genus that belongs to each group, and indicate in what way these organisms can be differentiated.
2. Microorganisms can have a variety of different relationships with oxygen. Describe the terms used to characterize a cell's response to oxygen, and give an example from this chapter of an organism that can be described by each of these terms.

Chapter Glossary

Acid-fastness a property of *Mycobacterium* species in which cells stained with the dye basic fuchsin resist decolorization with acidic alcohol

Actinomycetes a term used to refer to aerobic filamentous bacteria in the phylum *Actinobacteria*

Coryneform bacteria gram-positive, aerobic, nonmotile, rod-shaped organisms with the characteristic of forming irregular-shaped, club-shaped, or V-shaped cell arrangements, typical of several genera of unicellular *Actinobacteria*

Enteric bacteria a large group of gram-negative, rod-shaped *Bacteria* characterized by a facultatively aerobic metabolism and commonly found in the intestines of animals

Heterofermentative in reference to lactic acid bacteria, capable of making more than one fermentation product

High GC gram-positive bacteria a term that refers to bacteria in the *Actinobacteria*

Homofermentative in reference to lactic acid bacteria, producing only lactic acid as a fermentation product

Lactic acid bacteria fermentative bacteria that produce lactic acid, are found in the *Firmicutes*, and are important in the production and preservation of many foods

Low GC gram-positive bacteria a term that refers to bacteria in the *Firmicutes*

Oligotrophic a term that refers to organisms that grow best under low-nutrient conditions

Propionic acid bacteria gram-positive fermentative bacteria that generate propionate as a fermentation end product and are important in the production of cheese

Proteobacteria the largest and most metabolically diverse phylum of bacteria

Pseudomonad a term used to refer to any gram-negative, polarly flagellated, aerobic rod able to use a diverse suite of carbon sources

17 Diversity of *Archaea*

microbiologynow

The *Archaea* Just Under Your Feet

The domain *Archaea* is named for the Archaean eon, the period of geological history when life first spread across Earth. In the Archaean, high temperatures and an atmosphere thick in toxic gases enveloped Earth. *Archaea* were once thought to be remnants of this forgotten age since most isolates had been obtained from extreme environments such as volcanic systems or salt ponds. However, in the microbial world, things are not always as they seem.

Our knowledge of microbial diversity has changed dramatically in recent years. Now we can use molecular techniques to characterize an organism's DNA without needing to cultivate it first in the laboratory. One of the earliest discoveries made using molecular techniques was that *Archaea* are not restricted to extreme environments; in fact, they are plentiful in our oceans and soils. The vast majority of these *Archaea* belong to the phylum *Thaumarchaeota*, a diverse group of microbes that account for up to 20% of prokaryotic cells in the oceans and 1% of all microbes in soils. But while the discovery of this novel phylum relied on molecular techniques, puzzling out their purpose required cultivation of strains that could be analyzed in the laboratory.

Thaumarchaeota are ammonia oxidizers and critically important to the biosphere, being major players in the global nitrogen cycle. Prior to the discovery of *Thaumarchaeota*—for over 100 years in fact—microbiologists thought that ammonia oxidation was catalyzed only by *Bacteria*. The marine genus *Nitrosopumilus* was the first of the *Thaumarchaeota* to be isolated, and now *Nitrososphaera viennensis* (see photo) is the first species to be described from soil. *N. viennensis* was isolated from a backyard garden using clues derived from cultivation-independent molecular analyses. Cells of *N. viennensis* form irregular coccoids, and the organism is both mesophilic and neutrophilic. Physiologically, *N. viennensis* is a mixotroph (an organism that can fix CO_2 but which grows best with organic matter present) and conserves energy from either ammonia or urea as chemolithotrophic electron donors.

N. viennensis and related soil *Archaea* carry out nitrification in soils worldwide, a fact that reminds us that when we open our eyes to the microbial world we find that major discoveries are often "just underfoot."

Source: Stieglmeier, M., et al. 2014. *Nitrososphaera viennensis* gen. nov., sp. nov., an aerobic and mesophilic, ammonia-oxidizing archaeon from soil and a member of the archaeal phylum *Thaumarchaeota. IJSEM 64:* 2738.

The domain *Archaea* was once thought to contain ancient forms of microbial life that only survived in extreme habitats having environmental conditions similar to those of Earth during the Archaean age (from ~4.0 billion to 2.5 billion years before the present, Figure 13.1). We now know, however, that *Archaea* compose a considerable fraction of living matter in the biosphere and perform important biogeochemical reactions in soil, the oceans, wetlands, and even in the guts of animals. In this chapter we will learn about the enormous phylogenetic and physiological diversity found within the domain *Archaea*.

The *Archaea* are composed of five phyla: *Euryarchaeota, Crenarchaeota, Thaumarchaeota, Korarchaeota*, and *Nanoarchaeota* (**Figure 17.1**). The exact ancestry and number of archaeal phyla remains a contentious issue, and it is likely that many other phyla of *Archaea* remain to be discovered and described. Given the relationship of *Archaea* and *Eukarya* (Section 13.4), the exploration of genomic diversity within *Archaea* is essential to understanding the evolutionary origins of eukaryotic cells. Many *Archaea* are quite difficult to cultivate in isolation, however, and the use of metagenomics (Section 9.8) and single-cell genome sequencing (Section 9.12) continues to provide remarkable new insights into their phylogenetic and physiological diversity.

While all *Archaea* share certain features, this domain encompasses considerable physiological diversity. Common traits of all *Archaea* include ether-linked lipids, a lack of peptidoglycan in cell walls (Chapter 2), and structurally complex RNA polymerases that resemble those of *Eukarya* (Figure 4.20). Despite these shared features, *Archaea* display diverse metabolisms including various forms of chemoorganotrophy and chemolithotrophy that employ aerobic respiration, anaerobic respiration, or fermentation for energy conservation. For example, chemolithotrophy is well established in the *Archaea*, with H_2 being a common electron donor (Section 17.13) for species in many phyla and with ammonia oxidation widespread among species of *Thaumarchaeota*. Anaerobic respiration, especially forms employing elemental sulfur (S^0) as electron acceptor, is also prevalent among the *Archaea*, especially in *Crenarchaeota*. And finally, aerobic respiration also occurs widely in *Thaumarchaeota*, among some groups of *Euryarchaeota*, and among certain species of *Crenarchaeota*.

In addition, certain physiological capabilities are uniquely found within the domain *Archaea*. Methane production, for example, is a unique characteristic of **methanogens**, *Euryarchaeota* that conserve energy from the production of methane (Section 14.17). *Methanogenesis* is a globally important process that is uniquely archaeal (Sections 14.17, 21.1, and 21.2). *Archaea* are also well known for containing many examples of **extremophiles**, including **hyperthermophiles** (organisms with growth temperature optima above 80°C), as well as halophiles, acidophiles, and psychrophiles (Chapter 5).

With this brief background and the phylogeny of *Archaea* firmly in mind (Figure 17.1), we now consider the organismal diversity of this fascinating domain of life.

I • *Euryarchaeota*

Euryarchaeota comprise a large and physiologically diverse group of *Archaea*. This phylum includes methanogens as well as many genera of extremely halophilic (salt-loving) *Archaea*. As a study in physiological contrasts, these two groups are remarkable: Methanogens are the strictest of anaerobes while extreme halophiles are primarily obligate aerobes. Other groups of *Euryarchaeota* include the hyperthermophiles *Thermococcus* and

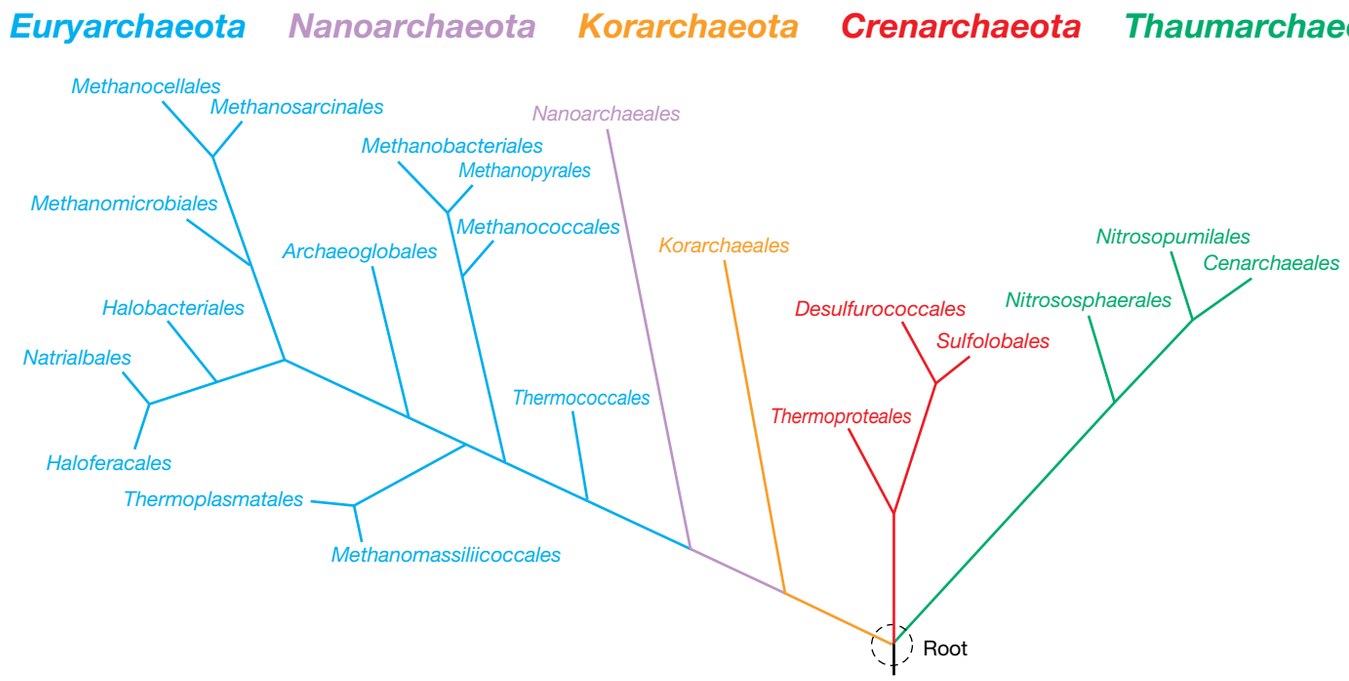


Figure 17.1 Schematic representation of the phylogeny of the major taxonomic orders within the domain *Archaea*. Each of the five archaeal phyla and their major orders are indicated in a different color.

Pyrococcus, the hyperthermophilic methanogen *Methanopyrus*, and the cell wall–less *Thermoplasma*, an organism phenotypically similar to the mycoplasmas (↔ Section 16.9). We begin our review of *Euryarchaeota* by reviewing the extremely halophilic *Archaea*.

17.1 Extremely Halophilic *Archaea*

KEY GENERA: *Halobacterium, Haloferax, Natronobacterium*

Extremely halophilic *Archaea*, often just called the "haloarchaea," are a diverse group that inhabits environments high in salt. These include naturally salty environments, such as solar salt evaporation ponds and salt lakes, and artificial saline habitats such as the surfaces of heavily salted foods, for example, certain fish and meats. Such salty habitats are called *hypersaline* (**Figure 17.2**). The term **extreme halophile** is used to indicate that these organisms are not only halophilic, but that their requirement for salt is very high, in some cases at levels near saturation (↔ Figure 5.24).

An organism is considered an extreme halophile if it requires 1.5 M (about 9%) or more sodium chloride (NaCl) for growth. Most species of extreme halophiles require 2–4 M NaCl (12–23%) for optimal growth and can grow at salinities as high as 5.5 M NaCl (32%, the limit of saturation for NaCl), although some species grow very slowly at this salinity. Some phylogenetic relatives of extremely halophilic *Archaea*, for example species of *Haloferax* and *Natronobacterium*, are able to grow at much lower salinities, such as at or near that of seawater (about 2.5% NaCl).

Hypersaline Environments: Chemistry and Productivity

Hypersaline habitats are common throughout the world, but extremely hypersaline habitats are rare. Most such environments are in hot, dry areas of the world. Salt lakes can vary considerably in ionic composition. The predominant ions in a hypersaline lake depend on the surrounding topography, geology, and general climatic conditions.


T. D. Brock

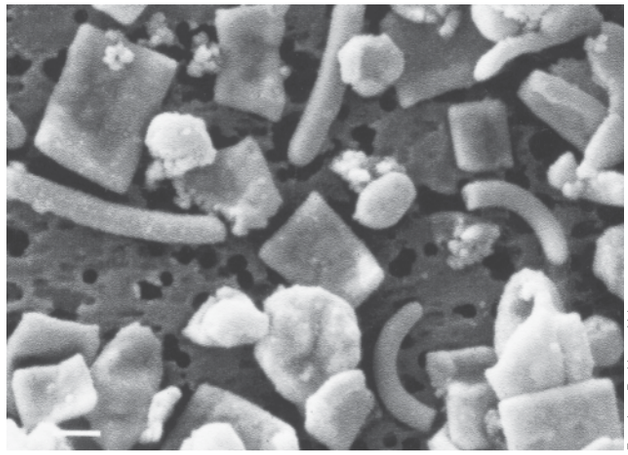
(a)


NASA

(b)


Michael T. Madigan

(c)


Francisco Rodriguez-Valera

(d)

Figure 17.2 Hypersaline habitats for halophilic *Archaea*. These organisms not only tolerate salt but *require* salt, and typically in large amounts. *(a)* The north arm of Great Salt Lake, Utah, a hypersaline lake in which the ratio of ions is similar to that in seawater, but in which absolute concentrations of ions are several times that of seawater. The green color is primarily from cells of cyanobacteria and green algae. *(b)* Aerial view near San Francisco Bay, California, of a series of seawater evaporating ponds where solar salt is prepared. The red-purple color is predominantly due to bacterioruberins and bacteriorhodopsin in cells of haloarchaea. *(c)* Lake Hamara, Wadi El Natroun, Egypt. A bloom of pigmented haloalkaliphiles is growing in this pH 10 soda lake. Note the deposits of trona ($NaHCO_3 \cdot Na_2CO_3 \cdot 2\ H_2O$) around the edge of the lake. *(d)* Scanning electron micrograph of halophilic bacteria including square *Archaea* present in a saltern in Spain.

Great Salt Lake in Utah (USA) (Figure 17.2*a*), for example, is essentially concentrated seawater. In this hypersaline lake the relative proportions of the various ions [e.g., sodium (Na^+), chloride (Cl^-), and sulfate (SO_4^{2-})] are those of seawater, although the overall concentration of ions is much higher. In addition, the pH of this hypersaline lake is slightly alkaline.

Soda lakes, in contrast, are highly alkaline hypersaline environments. The water chemistry of soda lakes resembles that of hypersaline lakes such as Great Salt Lake, but because high levels of carbonate minerals are also present in the surrounding strata, the pH of soda lakes is quite high. Waters of pH 10–12 are not uncommon in these environments (Figure 17.2*c*). In addition, calcium (Ca^{2+}) and Mg^{2+} are virtually absent from soda lakes because they precipitate out at high pH and carbonate concentrations.

The diverse chemistries of hypersaline habitats have selected for a large diversity of halophilic microorganisms. Some organisms are unique to one environment while others are widespread. Moreover, despite their extreme conditions, salt lakes can be highly productive ecosystems (the word *productive* here means high levels of autotrophic CO_2 fixation). *Archaea* are not the only microorganisms present. The eukaryotic alga *Dunaliella* (Figure 18.33*a*) is the major, if not the sole, oxygenic phototroph in most salt lakes. In highly alkaline soda lakes where *Dunaliella* is absent, anoxygenic phototrophic purple bacteria of the genera *Ectothiorhodospira* and *Halorhodospira* (Section 15.4) predominate. Organic matter originating from primary production by oxygenic or anoxygenic phototrophs sets the stage for growth of haloarchaea, which are chemoorganotrophic organisms. In addition, a few extremely halophilic chemoorganotrophic *Bacteria*, such as *Halanaerobium*, *Halobacteroides*, and *Salinibacter*, thrive in such environments.

Marine salterns are also habitats for extreme halophiles. Marine salterns are enclosed basins filled with seawater left to evaporate, eventually yielding solar sea salt (Figure 17.2*b*, *d*). As salterns approach the minimum salinity limits for haloarchaea, the waters turn a reddish purple color due to the massive growth—called a *bloom*—of cells (the red coloration apparent in Figure 17.2*b* and *c* is due to carotenoids and other pigments to be discussed later). Morphologically unusual *Archaea* are often present in salterns, including species with a square or cup-shaped morphology (Figure 17.2*d*). Extreme halophiles are also present in highly salted foods, such as certain types of sausages, marine fish, and salted pork.

Taxonomy and Physiology of Extremely Halophilic *Archaea*

The extremely halophilic *Archaea* are found within the orders *Halobacteriales, Natrialbales*, and *Haloferacales*, which share a common ancestor within the *Euryarchaeota* (Figure 17.1). These three orders constitute the haloarchaea, but they are also sometimes called "halobacteria" because the genus *Halobacterium* (**Figure 17.3**), the best-studied representative of the extreme halophiles, was discovered and characterized prior to the discovery of *Archaea*. Many genera of *Natrialbales*, including *Natronobacterium, Natronomonas*, and their relatives, differ from other extreme halophiles in being extremely alkaliphilic as well as halophilic. As befits their soda lake habitat (Figure 17.2*c*), these natronobacteria grow optimally at low Mg^{2+} concentrations and alkaline pH (9–11).

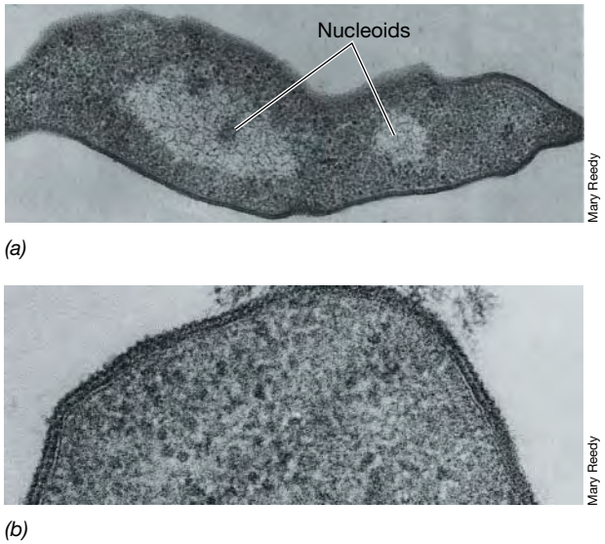


Figure 17.3 Electron micrographs of thin sections of the extreme halophile *Halobacterium salinarum*. A cell is about 0.8 µm in diameter. *(a)* Longitudinal section of a dividing cell showing the nucleoids. *(b)* High-magnification electron micrograph showing the glycoprotein subunit structure of the cell wall.

Haloarchaea stain gram-negatively, reproduce by binary fission, and do not form resting stages or spores. Cells of the various cultured genera are rod-shaped, cocci, or cup-shaped, but even cells that form squares are known (Figure 17.2*d*). Cells of *Haloquadratum* are square and only about 0.1 µm thick. *Haloquadratum* also forms gas vesicles (Section 2.9) that allow cells to float in its salty hypersaline habitat, probably as a means to be in contact with air since most extreme halophiles are obligate aerobes. Many other extremely halophilic *Archaea* also produce gas vesicles. A few strains of extreme halophiles are weakly motile by *archaella*, the archaeal analog of bacterial flagella, that rotate to propel the cell forward (Section 2.11), but most halophiles lack archaella. The genomes of *Halobacterium* and *Halococcus* are unusual in that large plasmids containing up to 30% of the total cellular DNA are present and the GC base ratio of these plasmids (near 60% GC) differs significantly from that of chromosomal DNA (66–68% GC). Plasmids from extreme halophiles are among the largest naturally occurring plasmids known.

Most haloarchaea use amino acids or organic acids as electron donors aerobically and require a number of growth factors such as vitamins for optimal growth. A few haloarchaea oxidize carbohydrates aerobically, but this capacity is rare; sugar fermentation does not occur. Electron transport chains containing cytochromes of the *a*, *b*, and *c* types are present in *Halobacterium*, and energy is conserved via a proton motive force arising from electron transport. Some haloarchaea can grow anaerobically, as growth by anaerobic respiration (Section 14.7) linked to the reduction of nitrate or fumarate has been demonstrated in certain species.

Water Balance in Extreme Halophiles

Extremely halophilic *Archaea* require large amounts of NaCl for growth. Detailed salinity studies of *Halobacterium* have shown that the requirement for Na^+ cannot be satisfied by any other ion,

even the chemically related ion potassium (K^+). However, cells of *Halobacterium* need *both* Na^+ and K^+ for growth because each plays an important role in maintaining osmotic balance.

As we learned in Section 5.13, microbes must withstand the osmotic forces they face in their habitats. To do so in a high-solute environment such as the salt-rich habitats of *Halobacterium*, organisms must either accumulate or synthesize solutes intracellularly. These solutes are called **compatible solutes**. These compounds counteract the tendency of the cell to become dehydrated under conditions of high osmotic strength by placing the cell in positive water balance with its surroundings. Cells of *Halobacterium*, however, do not synthesize or accumulate organic compounds but instead pump large amounts of K^+ from the environment into the cytoplasm. This ensures that the concentration of K^+ *inside* the cell is even greater than the concentration of Na^+ *outside* the cell (Table 17.1). This ionic condition maintains positive water balance.

The *Halobacterium* cell wall (Figure 17.3*b*) is composed of glycoprotein and is stabilized by Na^+. Sodium ions bind to the outer surface of the *Halobacterium* wall and are absolutely essential for maintaining cellular integrity. When insufficient Na^+ is present, the cell wall breaks apart and the cell lyses. This is a consequence of the exceptionally high content of the *acidic* (negatively charged) amino acids aspartate and glutamate in the glycoprotein of the *Halobacterium* cell wall. The negative charge on the carboxyl group of these amino acids is bound to Na^+; when Na^+ is diluted away, the negatively charged parts of the proteins tend to repel each other, leading to cell lysis.

Halophilic Cytoplasmic Components

Like cell wall proteins, cytoplasmic proteins of *Halobacterium* are highly acidic, but it is K^+, not Na^+, that is required for activity. This makes sense because K^+ is the predominant cation in the cytoplasm of cells of *Halobacterium* (Table 17.1). Besides having a high acidic amino acid composition, halobacterial cytoplasmic proteins typically contain lower levels of hydrophobic amino acids and lysine, a positively charged (basic) amino acid, than proteins of nonhalophiles. This is also to be expected because in a highly ionic cytoplasm, more polar proteins would tend to remain in solution whereas less polar proteins would tend to cluster and perhaps lose activity. The ribosomes of *Halobacterium* also require high KCl levels for stability, whereas ribosomes of nonhalophiles have no KCl requirement.

Extremely halophilic *Archaea* are thus well adapted to life in a highly ionic environment. Cellular components exposed to the external environment require high Na^+ for stability, whereas cytoplasmic components require high K^+. With the exception of a few extremely halophilic species of *Bacteria* that also use KCl as a compatible solute, in no other group of prokaryotic cells do we find this unique requirement for such high amounts of specific cations.

TABLE 17.1 Concentration of ions in cells of *Halobacterium salinarum*[a]

Ion	Concentration in medium (M)	Concentration in cells (M)
Na^+	4.0	1.4
K^+	0.032	4.6
Mg^{2+}	0.13	0.12
Cl^-	4.0	3.6

[a]Data from Christian, J.H.B, and Waltho, J.A. *Biochim. Biophys. Acta 65:* 506–508 (1962).

Bacteriorhodopsin and Light-Mediated ATP Synthesis in Haloarchaea

Certain species of haloarchaea can catalyze a light-driven synthesis of ATP. This form of phototrophy is not linked to CO_2 fixation, and does not require chlorophyll pigments, and so it is not photosynthesis in the traditional sense. However, other light-sensitive pigments are present, including red and orange carotenoids—primarily C_{50} pigments called *bacterioruberins*—and inducible pigments involved in energy conservation; we discuss these pigments here.

Under conditions of low aeration, *Halobacterium salinarum* and some other haloarchaea synthesize a protein called **bacteriorhodopsin** and insert it into their cytoplasmic membranes. Bacteriorhodopsin is so named because of its structural and functional similarity to rhodopsin, the visual pigment of the eye. Conjugated to bacteriorhodopsin is a molecule of retinal, a carotenoid-like molecule that can absorb light energy and pump a proton across the cytoplasmic membrane. The retinal gives bacteriorhodopsin a purple hue. Thus cells of *Halobacterium* that are switched from growth under high-aeration conditions to oxygen-limiting growth conditions (a trigger of bacteriorhodopsin synthesis) gradually change color from orange-red to purple-red as they synthesize bacteriorhodopsin and insert it into their cytoplasmic membranes.

Bacteriorhodopsin absorbs green light around 570 nm. Following absorption, the retinal of bacteriorhodopsin, which normally exists in a *trans* configuration (Ret_T), becomes excited and converts to the *cis* (Ret_C) form (Figure 17.4). This transformation is coupled to the translocation of a proton across the cytoplasmic membrane. The retinal molecule then decays to the *trans* isomer along with the uptake of a proton from the cytoplasm, and this completes the cycle. The proton pump is then ready to repeat the cycle (Figure 17.4). As protons accumulate on the outer surface of the membrane,

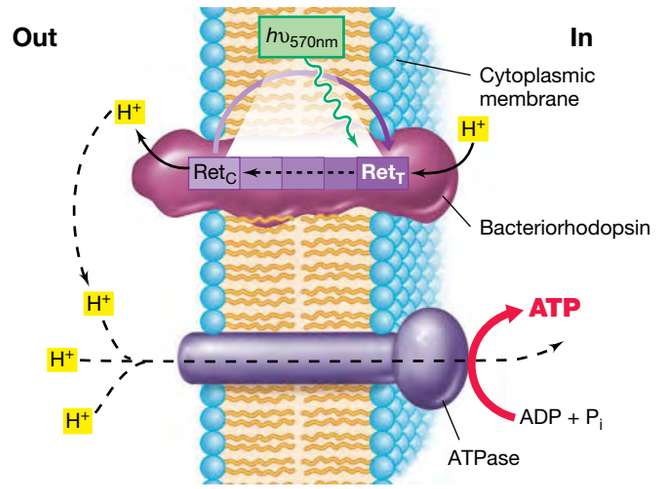


Figure 17.4 Model for the mechanism of bacteriorhodopsin. Light of 570 nm ($h\upsilon_{570nm}$) converts the protonated retinal of bacteriorhodopsin from the *trans* form (Ret_T) to the *cis* form (Ret_C), along with translocation of a proton to the outer surface of the cytoplasmic membrane, thus establishing a proton motive force. ATPase activity is driven by the proton motive force.

a proton motive force is generated that is coupled to ATP synthesis through the activity of a proton-translocating ATPase (Figure 17.4) (↩ Section 3.11).

Bacteriorhodopsin-mediated ATP production in *H. salinarum* supports slow growth of this organism under anoxic conditions. The light-stimulated proton pump of *H. salinarum* also functions to pump Na^+ out of the cell by activity of a Na^+–H^+ antiport system and to drive the uptake of nutrients, including the K^+ needed for osmotic balance. Amino acid uptake by *H. salinarum* is indirectly driven by light as well, because amino acids are cotransported into the cell with Na^+ by an amino acid–Na^+ symporter (↩ Section 3.2); removal of Na^+ from the cell occurs by way of the light-driven Na^+–H^+ antiporter.

Other Rhodopsins

Besides bacteriorhodopsin, at least three other rhodopsins are present in the cytoplasmic membrane of cells of *H. salinarum*. **Halorhodopsin** is a light-driven chloride (Cl^-) pump that brings Cl^- into the cell as the anion for K^+. The retinal of halorhodopsin binds Cl^- and transports it into the cell. Two other light sensors, called *sensory rhodopsins*, are present in *H. salinarum*. These light sensors control phototaxis (movement toward light, ↩ Section 2.13) by the organism. Through the interaction of a cascade of proteins similar to those in chemotaxis (↩ Section 6.7), sensory rhodopsins affect archaellar rotation, moving cells of *H. salinarum* toward light where bacteriorhodopsin can function to make ATP (Figure 17.4).

We will learn when we consider marine microbiology (↩ Sections 20.10 and 20.11) that diverse species of chemoorganotrophic *Bacteria* that inhabit the upper layers of the ocean contain bacteriorhodopsin-like proteins called *proteorhodopsins*. As far as is known, proteorhodopsin functions like bacteriorhodopsin except that different spectral forms exist, each form being tuned to the absorption of its own specific wavelengths of light. Although the energy generated from proteorhodopsin alone is insufficient to sustain growth, these marine bacteria use proteorhodopsin as a supplement to the ATP they generate from respiration. Proteorhodopsin as a mechanism for energy conservation in marine bacteria makes good ecological sense because levels of dissolved organic matter in the open oceans are typically very low, and thus a strictly chemoorganotrophic lifestyle would be difficult.

MINIQUIZ

- Since cells of *Halobacterium* require high levels of Na^+ for growth, why is this not true for the organism's cytoplasmic enzymes?
- What benefit does bacteriorhodopsin confer on *Halobacterium salinarum*?

17.2 Methanogenic *Archaea*

KEY GENERA: *Methanobacterium, Methanocaldococcus, Methanosarcina, Methanopyrus*

Many *Euryarchaeota* are methanogens, microorganisms that produce methane (CH_4) as an integral part of their energy metabolism (methane production is called *methanogenesis*). In Section 14.17 we considered the unique biochemistry of methanogenesis. Later, we will learn how methanogenesis is a major component of the global carbon cycle, serving as the terminal step in the biodegradation of organic matter in many anoxic habitats (↩ Sections 21.1 and 21.2). Methanogens are important in a wide range of anoxic habitats including freshwater sediments, wetlands, rice paddies, wastewater treatment plants, geothermal systems, the subsurface of the Earth's crust, and within the guts of many animals.

Diversity and Physiology of Methanogens

Methanogens occur in at least seven orders, including *Methanobacteriales, Methanococcales, Methanopyrales, Methanomassiliicoccales, Methanomicrobiales, Methanocellales*, and *Methanosarcinales* (Figure 17.1). Methanogens exhibit considerable morphological and physiological diversity (**Figure 17.5** and see Table 17.2). As can be seen in Figure 17.1, methanogens are spread widely within the *Euryarchaeota* and do not represent a single coherent phylogenetic group. We have already been introduced to the factors that cause inconsistency between phylogenetic diversity and functional diversity (↩ Section 15.1). In the case of methanogenesis it is most likely that the ability to reduce CO_2 to CH_4 evolved only once within the *Euryarchaeota*, and that gene loss caused lineages such as the haloarchaea and *Thermoplasmatales* to lose the capacity to produce methane. We will also see that *Archaeoglobus* (Section 17.4) still retains some of the genes encoding methanogenesis and can actually produce methane under certain growth conditions.

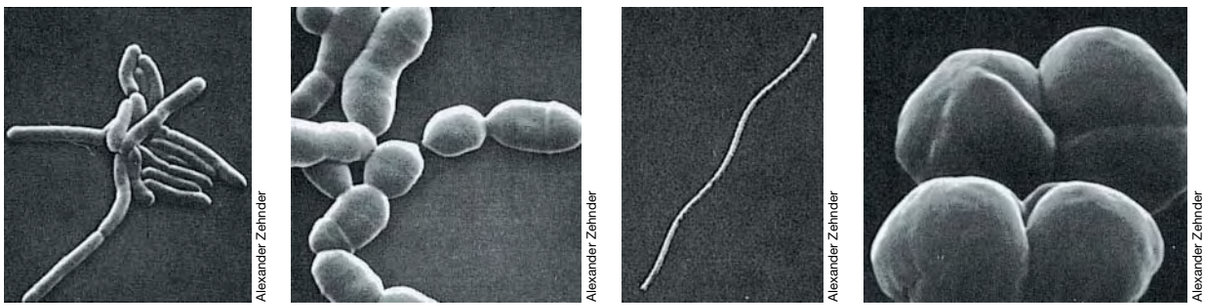


Figure 17.5 Scanning electron micrographs of cells of diverse species of methanogenic *Archaea*. *(a) Methanobrevibacter ruminantium*. A cell is about 0.7 μm in diameter. *(b) Methanobrevibacter arboriphilus*. A cell is about 1 μm in diameter. *(c) Methanospirillum hungatei*. A cell is about 0.4 μm in diameter. *(d) Methanosarcina barkeri*. A cell is about 1.7 μm wide.

Methanogens have diverse physiological characteristics, but they are united by their ability to produce methane and by their intolerance to oxygen. Several cofactors required by methanogenic pathways are inhibited by oxygen, and hence all methanogens are obligate anaerobes. As a result, strict anoxic techniques are necessary for their cultivation in isolation. While most methanogens are mesophilic and live in non-extreme environments, a diversity of species have been described including some that grow optimally at very high (see Figure 17.7) or very low temperatures, at very high salt concentrations, or at extremes of pH.

Methanogenesis can be performed through three different pathways (**Table 17.2**): methanogenesis by CO_2 reduction (↻ Figure 14.47), methylotrophic methanogenesis (↻ Figure 14.48*a*), and acetoclastic methanogenesis (↻ Figure 14.48*b*). Each of these pathways relies on *coenzyme M* with methane ultimately produced by reduction of methyl-CoM to methane (↻ Section 14.17). There is a limited set of substrates that methanogens can convert into methane through these pathways (Table 17.2). Interestingly, these substrates do not include such common compounds as glucose, organic acids, or fatty acids (other than acetate and pyruvate). Methanogens often form syntrophic associations with fermentative anaerobes (↻ Sections 14.23 and 21.2). In this way the fermentative organisms degrade a wide range of organic carbon molecules into H_2, CO_2, and acetate, which are ultimately used as substrates for methanogenesis.

The three methanogenic pathways are found in different phylogenetic groups of methanogens (**Table 17.3**). Methanogenesis by CO_2 reduction is found widely across the known diversity of methanogens, but not all methanogens can produce methane by CO_2 reduction. Notably, many species in the *Methanosarcinales*, species of *Methanosphaera*, and species in the *Methanomassiliicoccales* have lost the ability to produce methane by CO_2 reduction (Table 17.3). The CO_2 reduction pathway can also be used by some species to produce methane from formate, or carbon monoxide. In addition, while most species use H_2 to reduce CO_2, some can also reduce CO_2 by using electrons from pyruvate or certain alcohols (Tables 17.2 and 17.3).

TABLE 17.2 The three methanogenic pathways and their substrates

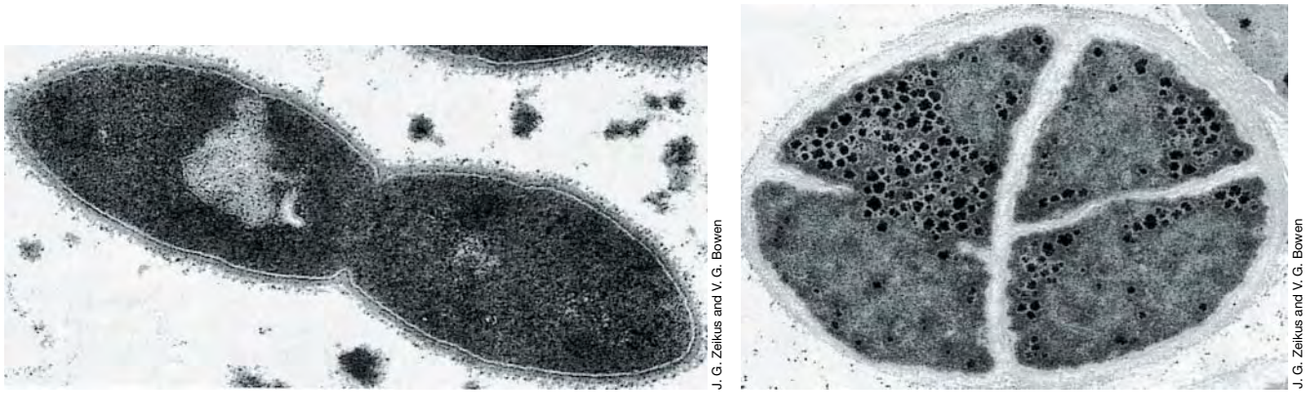
I.	**CO_2 reduction pathway**
	Carbon dioxide, CO_2 (with electrons derived from H_2, certain alcohols, or pyruvate)
	Formate, $HCOO^-$
	Carbon monoxide, CO
II.	**Methylotrophic pathway**
	Methanol, CH_3OH
	Methylamine, $CH_3NH_3^+$
	Dimethylamine, $(CH_3)_2NH_2^+$
	Trimethylamine, $(CH_3)_3NH^+$
	Methyl mercaptan, CH_3SH
	Dimethyl sulfide, $(CH_3)_2S$
III.	**Acetoclastic pathway**
	Acetate, CH_3COO^-
	Pyruvate, CH_3COCOO^-

The acetoclastic and methylotrophic pathways of methanogenesis are found mainly within the *Methanosarcinales* (Table 17.3). Methylated substrates include methanol (CH_3OH) and many others (Table 17.2). Many species in the *Methanosarcinales* will actually oxidize one molecule of CH_3OH to CO_2 to generate

TABLE 17.3 Characteristics of some methanogenic *Archaea*[a]

Order/Genus	*Substrates for methanogenesis*[b]
Methanobacteriales	
Methanobacterium	$H_2 + CO_2$, formate
Methanobrevibacter	$H_2 + CO_2$, formate
Methanosphaera	H_2 + methanol
Methanothermus	$H_2 + CO_2$
Methanothermobacter	$H_2 + CO_2$, formate, CO
Methanococcales	
Methanococcus	$H_2 + CO_2$, pyruvate + CO_2, formate
Methanothermococcus	$H_2 + CO_2$, formate
Methanocaldococcus	$H_2 + CO_2$
Methanotorris	$H_2 + CO_2$
Methanomicrobiales	
Methanomicrobium	$H_2 + CO_2$, formate
Methanogenium	$H_2 + CO_2$, formate
Methanospirillum	$H_2 + CO_2$, formate
Methanoplanus	$H_2 + CO_2$, formate
Methanocorpusculum	$H_2 + CO_2$, H_2 + alcohols, formate
Methanoculleus	$H_2 + CO_2$, H_2 + alcohols, formate
Methanofollis	$H_2 + CO_2$, formate
Methanolacinia	$H_2 + CO_2$, H_2 + alcohols
Methanosarcinales	
Methanosarcina	$H_2 + CO_2$, methanol, methylamines, acetate, CO
Methanolobus	Methanol, methylamines
Methanohalobium	Methanol, methylamines
Methanococcoides	Methanol, methylamines
Methanohalophilus	Methanol, methylamines, methyl sulfides
Methanosaeta	Acetate
Methanosalsum	Methanol, methylamines, dimethyl sulfide
Methanimicrococcus	H_2 + methanol, H_2 + methylamines
Methanopyrales	
Methanopyrus	$H_2 + CO_2$
Methanocellales	
Methanocella	$H_2 + CO_2$
Methanomassiliicoccales	
Methanomassiliicoccus	H_2 + methanol
Methanomethylophilus	H_2 + methanol

[a]Taxonomic orders are listed in bold. An order is a taxonomic rank that consists of several families; families consist of several genera (↻ Table 13.2).
[b]Methylamines can include the substrates methylamine ($CH_3NH_3^+$), dimethylamine ($(CH_3)_2NH_2^+$), and trimethylamine ($(CH_3)_3NH^+$); methyl sulfides can include dimethyl sulfide ($(CH_3)_2S$) and methyl mercaptan (CH_3SH).

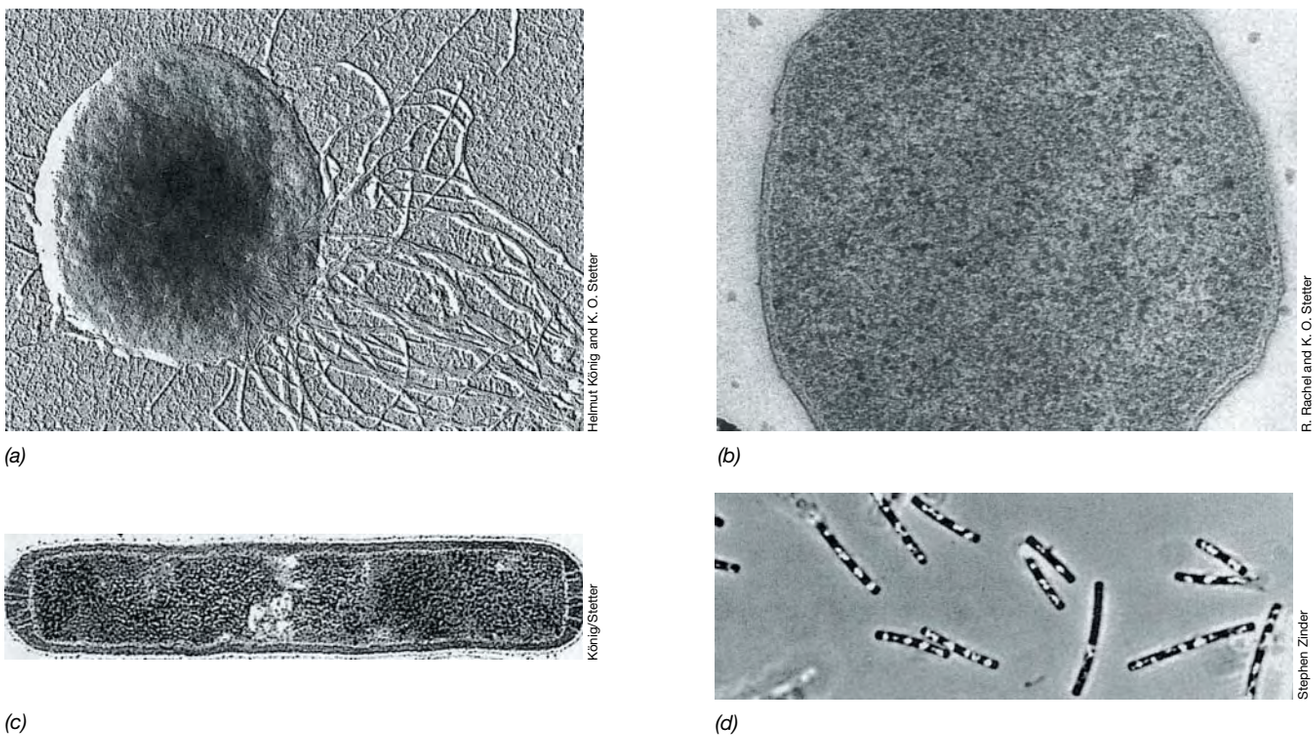


(a) *(b)*

Figure 17.6 Transmission electron micrographs of thin sections of methanogenic *Archaea*.
(a) Methanobrevibacter ruminantium. A cell is 0.7 μm in diameter. *(b) Methanosarcina barkeri*, showing the thick cell wall and the manner of cell segmentation and cross-wall formation. A cell is 1.7 μm in diameter.

the electrons needed to reduce three molecules of CH_3OH to CH_4 and H_2O (Figure 14.48*a*). However, other methylotrophic species lack this ability (Table 17.3) and require an external electron donor such as H_2 to reduce methylated compounds to CH_4. Only a few methanogens have been shown to use acetate as a substrate and they are all members of the *Methanosarcinales* (Table 17.3). Methanogenesis from acetate is a dominant source of methane production in a wide range of environments (Section 21.2).

Methanogens show a diversity of cell wall chemistries. These include the pseudomurein walls of *Methanobacterium* species and relatives (**Figure 17.6*a***), walls composed of methanochondroitin (so named because of its structural resemblance to chondroitin, the connective tissue polymer of vertebrate animals) in *Methanosarcina* and relatives (Figure 17.6*b*), the protein or glycoprotein walls of *Methanocaldococcus* (**Figure 17.7*a***) and *Methanoplanus* species, respectively, and the S-layer walls of *Methanospirillum* (Figure 17.5*c*) (Section 2.6).



(a) *(b)*

(c) *(d)*

Figure 17.7 Hyperthermophilic and thermophilic methanogens. *(a) Methanocaldococcus jannaschii* (temperature optimum, 85°C), shadowed preparation electron micrograph. A cell is about 1 μm in diameter. *(b) Methanotorris igneus* (temperature optimum, 88°C), thin section. A cell is about 1 μm in diameter. *(c) Methanothermus fervidus* (temperature optimum, 88°C), thin-sectioned electron micrograph. A cell is about 0.4 μm in diameter. *(d) Methanosaeta thermophila* (temperature optimum, 60°C), phase-contrast micrograph. A cell is about 1 μm in diameter. The refractile bodies inside the cells are gas vesicles.

Methanocaldococcus jannaschii as a Model Methanogen

The genomes of the hyperthermophilic methanogen *Methanocaldococcus jannaschii* (Figure 17.7*a*) and many other methanogens have been sequenced. The 1.66-megabase-pair (Mbp) circular genome of *M. jannaschii*, an organism that has been used as a model for the molecular study of methanogenesis and archaeal motility, contains about 1700 genes, and genes encoding enzymes of methanogenesis and several other key cell functions have been identified. Interestingly, the majority of *M. jannaschii* genes encoding functions such as central metabolic pathways and cell division are similar to those in *Bacteria*. By contrast, most of the *M. jannaschii* genes encoding core molecular processes such as transcription and translation more closely resemble those of eukaryotes. These findings reflect the various traits shared by organisms in the three cellular domains and are consistent with our understanding of how the three domains of cells evolved, as discussed in Chapter 13. However, analyses of the *M. jannaschii* genome also show that fully 40% of its genes have no counterparts in genes from either of the other domains. Some of these are genes that encode the enzymes needed for methanogenesis, of course, but many others likely encode novel cellular functions absent from cells in the other domains or may encode redundant functions carried out by classes of enzymes distinct from those found in *Bacteria* and *Eukarya*.

Methanopyrus, a Hyperthermophilic Methanogen

Methanopyrus (**Figure 17.8**), the only genus in the order *Methanopyrales*, is a rod-shaped hyperthermophilic methanogen that shares phenotypic properties with both the hyperthermophiles (see Section 17.13) and the methanogens. *Methanopyrus* was isolated from hot sediments near submarine hydrothermal vents and from the walls of "black smoker" hydrothermal vent chimneys (Section 17.10; Section 20.14). *Methanopyrus* produces CH_4 only from $H_2 + CO_2$ and grows rapidly for an autotrophic organism (generation time <1 h at 100°C). In special pressurized vessels, growth of one strain of *Methanopyrus* has been recorded at 122°C, the highest temperature yet shown to support microbial growth (Sections 17.11 and 17.12).

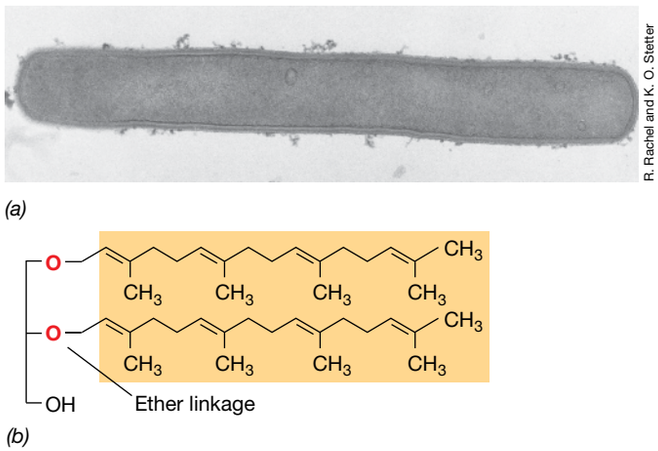


Figure 17.8 ***Methanopyrus.*** *Methanopyrus* grows optimally at 100°C and can make CH_4 only from $CO_2 + H_2$. *(a)* Electron micrograph of a cell of *Methanopyrus kandleri*, the most thermophilic of all known organisms (upper temperature limit, 122°C). This cell measures 0.5 × 8 μm. *(b)* Structure of the novel lipid of *M. kandleri*. This is the normal ether-linked lipid of the *Archaea* except that the side chains are an unsaturated form of phytanyl (geranylgeraniol).

Methanopyrus was once thought to be one of the most ancestral lineages of *Archaea*, but genome sequence analysis reveals it to be a relative to the *Methanobacteriales* and *Methanococcales* (Figure 17.1). *Methanopyrus* also has a pseudomurein cell wall, a trait that it shares with species of *Methanobacteriales*.

In addition to its remarkable tolerance to high temperature, *Methanopyrus* is also unusual because it contains membrane lipids found in no other known organism. Recall that in the lipids of *Archaea*, the glycerol side chains contain **phytanyl** rather than fatty acids bonded in ether linkage to the glycerol (Section 2.3). In *Methanopyrus*, this ether-linked lipid is an *unsaturated* form of the otherwise saturated biphytanyl tetraethers found in all other hyperthermophilic *Archaea* (Figure 17.8*b*). These unusual lipids may help stabilize the cytoplasmic membrane of *Methanopyrus* at its unusually high growth temperatures.

MINIQUIZ

- What are the three major pathways of methanogenesis and in what phylogenetic groups are they found?
- What physiological and structural features distinguish the *Methanosarcinales* from other methanogens?

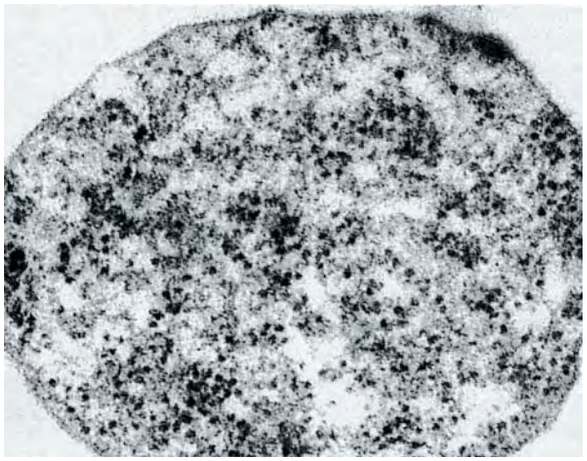
17.3 *Thermoplasmatales*

KEY GENERA: *Thermoplasma, Picrophilus, Ferroplasma*

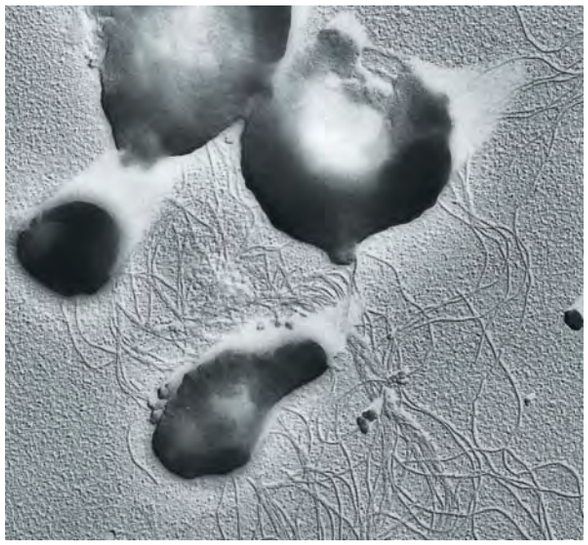
A phylogenetically distinct line of *Archaea* contains thermophilic and extremely acidophilic genera: *Thermoplasma, Ferroplasma*, and *Picrophilus*. These organisms are among the most acidophilic of all known microbes, with *Picrophilus* being capable of growth even below pH 0. Most are thermophilic as well. These genera also form their own taxonomic order within the *Euryarchaeota*, the *Thermoplasmatales* (Figure 17.1). We begin with a description of the mycoplasma-like organisms *Thermoplasma* and *Ferroplasma*.

Archaea Lacking Cell Walls

Thermoplasma and *Ferroplasma* lack cell walls, and in this respect they resemble the mycoplasmas (Section 16.9). *Thermoplasma* (**Figure 17.9**) is a chemoorganotroph that grows optimally at 55°C and pH 2 in complex media. Two species of *Thermoplasma* have been described, *Thermoplasma acidophilum* and *Thermoplasma volcanium*. Species of *Thermoplasma* are facultative aerobes, growing either aerobically or anaerobically by sulfur respiration (Section 14.14). Most strains of *T. acidophilum* have been obtained from self-heating coal refuse piles. Coal refuse contains coal fragments, pyrite (FeS_2), and other organic materials extracted from coal. When dumped into piles in surface mining operations, coal refuse heats as a result of microbial metabolism bringing it to combustion temperature (**Figure 17.10**). This sets the stage for growth of *Thermoplasma*, which likely metabolizes organic



(a)



(b)

Figure 17.9 *Thermoplasma* species. *(a) Thermoplasma acidophilum*, an acidophilic and thermophilic mycoplasma-like archaeon; electron micrograph of a thin section. The diameter of cells varies from 0.2 to 5 μm. The cell shown is about 1 μm in diameter. *(b)* Shadowed preparation of cells of *Thermoplasma volcanium* isolated from hot springs. Cells are 1–2 μm in diameter. Notice the abundant archaella and irregular cell morphology.

compounds leached from the hot coal refuse. The second species, *T. volcanium*, has been isolated in hot acidic soils throughout the world and is highly motile by multiple archaella (Figure 17.9*b*).

To survive the osmotic stresses of life without a cell wall and to withstand the dual environmental extremes of low pH and high temperature, *Thermoplasma* has evolved a unique cytoplasmic membrane structure. The membrane contains a lipopolysaccharide-like material called *lipoglycan*. This substance consists of glycolipids containing sugars such as mannose and glucose (**Figure 17.11**) and these glycolipids form a tetraether lipid monolayer membrane. The hydrophobic core of this glycolipid consists of a biphytanyl (↪ Section 2.3). In *Thermoplasma* and similar organisms such as *Sulfolobus*, this basic biphytanyl structure can be modified to include one to four cyclopentane rings, with the number of rings

Figure 17.10 A typical self-heating coal refuse pile, habitat of *Thermoplasma*. The pile, containing coal debris, pyrite, and other microbial substrates, self-heats as a result of microbial metabolism.

tending to increase in proportion to the temperature of the environment. These glycolipids constitute a major fraction of the total lipids of *Thermoplasma*. The membrane also contains glycoproteins and glycophospholipids, but not sterols. These molecules render the *Thermoplasma* membrane stable to hot, acidic conditions.

Like mycoplasmas (↪ Section 16.9), *Thermoplasma* contains a relatively small genome (1.5 Mbp). In addition, *Thermoplasma* DNA is complexed with a highly basic DNA-binding protein that organizes the DNA into globular particles resembling the nucleosomes of eukaryotic cells. This protein is homologous to the histone-like DNA-binding protein HU of *Bacteria*, which plays an important role in organization of the DNA in the cell. In contrast, several other *Euryarchaeota* contain basic proteins homologous to the DNA-binding histone proteins of eukaryotic cells.

Ferroplasma

Ferroplasma is a chemolithotrophic relative of *Thermoplasma*. *Ferroplasma* is a strong acidophile; however, it is not a thermophile, as it grows optimally at 35°C. *Ferroplasma* oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) to obtain energy (this reaction generates acid, Figure 17.18*d*) and uses CO_2 as its carbon source (autotrophy). *Ferroplasma* grows in mine tailings containing

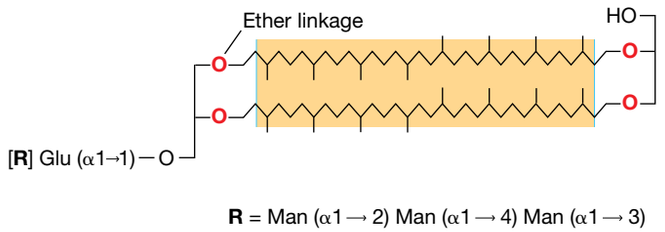


Figure 17.11 Structure of a tetraether glycolipid of *Thermoplasma acidophilum*. The dominant glycolipids of *T. acidophilum* have two polar head groups that are connected by ether linkages to a hydrophobic core. This structure causes them to form a thermostable lipid monolayer. One or both of the polar head groups typically contains a mono- or oligosaccharide that can be composed of glucose (Glu), mannose (Man), or other sugars. The hydrophobic core consists of caldarchaeol (shown), which can be modified to include one to four cyclopentane rings (not shown). The lipid shown contains an oligosaccharide linked to a single polar head group.

pyrite, which is its energy source. The extreme acidophily of *Ferroplasma* allows it to drive the pH of its habitat down to extremely acidic values. After moderate acidity is generated from Fe^{2+} oxidation by acidophilic organisms such as *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* (Section 21.5), *Ferroplasma* becomes active and subsequently generates the very low pH values typical of acid mine drainage. Acidic waters at pH 0 can be generated by the activities of *Ferroplasma*.

Picrophilus

A phylogenetic relative of *Thermoplasma* and *Ferroplasma* is *Picrophilus*. Although *Thermoplasma* and *Ferroplasma* are extreme acidophiles, *Picrophilus* is even more so, growing optimally at pH 0.7 and capable of growth at pH values lower than 0. *Picrophilus* also has a cell wall (an S-layer; Section 2.6) and a much lower DNA GC base ratio than does *Thermoplasma* or *Ferroplasma*. Although phylogenetically related, *Thermoplasma, Ferroplasma*, and *Picrophilus* have quite distinct genomes. Two species of *Picrophilus* have been isolated from acidic Japanese solfataras, and like *Thermoplasma*, both grow heterotrophically on complex media.

The physiology of *Picrophilus* is of interest as a model for extreme acid tolerance. Studies of its cytoplasmic membrane point to an unusual arrangement of lipids that forms a highly acid-impermeable membrane at very low pH. By contrast, at moderate acidities such as pH 4, the membranes of cells of *Picrophilus* become leaky and disintegrate. Obviously, this organism has evolved to survive only in highly acidic habitats.

MINIQUIZ

- In what ways are *Thermoplasma* and *Picrophilus* similar? In what ways do they differ?
- How does *Thermoplasma* strengthen its cytoplasmic membrane to survive without a cell wall?

17.4 *Thermococcales* and *Archaeoglobales*

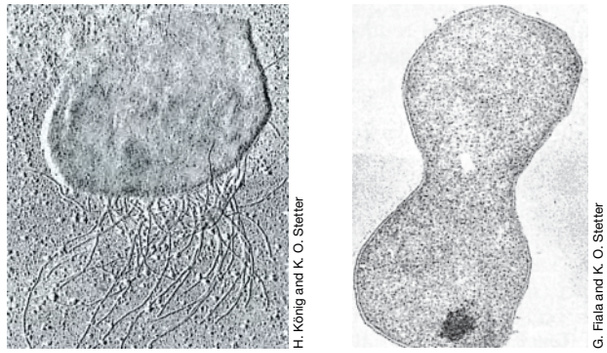
KEY GENERA: *Thermococcus, Pyrococcus, Archaeoglobus, Ferroglobus*

A few euryarchaeotes thrive in thermal environments and some are hyperthermophiles. We consider here the *Thermococcales* and *Archaeoglobales*, which are two orders of *Euryarchaeota* that contain hyperthermophilic species (Figure 17.1).

Thermococcus and *Pyrococcus*

Thermococcus and *Pyrococcus* are genera within the order *Thermococcales*. *Thermococcus* is a spherical hyperthermophilic euryarchaeote indigenous to anoxic thermal waters in various locations throughout the world. The spherical cells contain a tuft of polar archaella and are thus highly motile (**Figure 17.12*a***). *Thermococcus* is an obligately anaerobic chemoorganotroph that metabolizes proteins and other complex organic mixtures (including some sugars) with elemental sulfur (S^0) as electron acceptor at temperatures from 55 to 95°C.

Pyrococcus is morphologically similar to *Thermococcus* (Figure 17.12*b*). *Pyrococcus* differs from *Thermococcus* primarily by its higher temperature requirements; *Pyrococcus* grows between 70 and 106°C with an optimum of 100°C. *Thermococcus* and *Pyrococcus* are also metabolically quite similar. Proteins, starch, or maltose are oxidized as electron donors, and S^0 is the terminal electron acceptor and is reduced to hydrogen sulfide (H_2S). Both *Thermococcus* and *Pyrococcus* form H_2S when S^0 is present, but form H_2 when S^0 is absent (see Table 17.4).



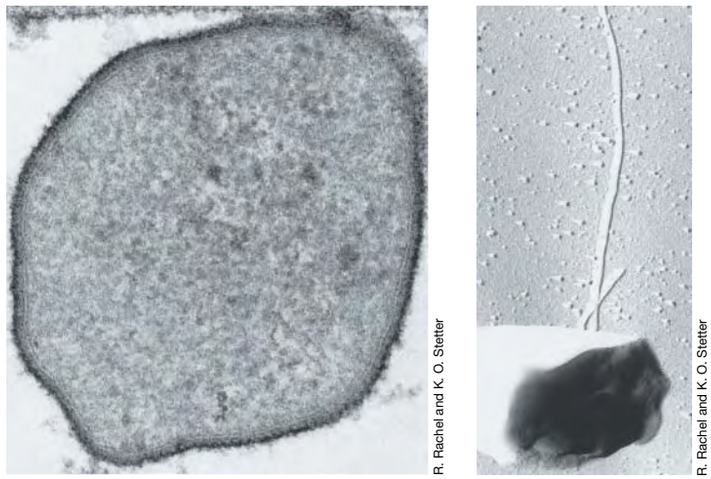
(a) (b)

Figure 17.12 Spherical hyperthermophilic *Euryarchaeota* from submarine volcanic areas. *(a) Thermococcus celer*; electron micrograph of shadowed cells (note tuft of archaella). *(b)* Dividing cell of *Pyrococcus furiosus*; electron micrograph of thin section. Cells of both organisms are about 0.8 μm in diameter.

Archaeoglobus and *Ferroglobus*

Archaeoglobus was isolated from hot marine sediments near hydrothermal vents. In its metabolism, *Archaeoglobus* couples the oxidation of H_2, lactate, pyruvate, glucose, or complex organic compounds to the reduction of SO_4^{2-} to H_2S. Cells of *Archaeoglobus* are irregular cocci (**Figure 17.13*a***) and grow optimally at 83°C.

Archaeoglobus and methanogens share some characteristics. We learned in Section 14.17 about the unique biochemistry of



(a) (b)

Figure 17.13 *Archaeoglobales*. *(a)* Transmission electron micrograph of the sulfate-reducing hyperthermophile *Archaeoglobus fulgidus*. The cell measures 0.7 μm in diameter. *(b)* Freeze-etched electron micrograph of *Ferroglobus placidus*, a ferrous iron–oxidizing, nitrate-reducing hyperthermophile. The cell measures about 0.8 μm in diameter.

methanogenesis. Briefly, this process requires a series of novel coenzymes, and with rare exceptions, these coenzymes have only been found in methanogens. *Archaeoglobus*, however, also contains many of these coenzymes, and cultures of this organism actually produce small amounts of CH_4. In addition, the genome of *Archaeoglobus*, which contains about 2400 genes, shares many genes in common with methanogens (Section 17.2). It seems likely that the ancestor of *Archaeoglobus* was a methanogen that lost many of the genes required for methanogenesis. Furthermore, genome analysis suggests that the ancestor of *Archaeoglobus* acquired genes for sulfate reduction as a result of horizontal gene transfer from sulfate-reducing bacteria within the *Deltaproteobacteria* (Section 15.9).

Ferroglobus (Figure 17.13*b*) is related to *Archaeoglobus* but is not a sulfate reducer. Instead, *Ferroglobus* is an iron-oxidizing chemolithotroph, conserving energy from the oxidation of Fe^{2+} to Fe^{3+} coupled to the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) (see Table 17.4). *Ferroglobus* grows autotrophically and can also use H_2 or H_2S as electron donors in its energy metabolism. *Ferroglobus* was isolated from a shallow marine hydrothermal vent and grows optimally at 85°C.

Ferroglobus is interesting for several reasons, but especially for its ability to oxidize Fe^{2+} to Fe^{3+} under anoxic conditions. This process might help explain the origin of some Fe^{3+} found in ancient rocks dated to before the predicted appearance of cyanobacteria on Earth (Section 13.2). With organisms like *Ferroglobus*, it would have been possible for Fe^{2+} oxidation to proceed without the need for molecular oxygen (O_2) as an electron acceptor. The metabolism of *Ferroglobus* thus has implications for dating the origin of cyanobacteria and the subsequent oxygenation of Earth. Certain anoxygenic phototrophic bacteria can also oxidize Fe^{2+} under anoxic conditions (Section 14.10), and so several anaerobic routes to ancient Fe^{3+} are possible. This makes it difficult to estimate when cyanobacteria first appeared on Earth and to what degree nonphototrophic organisms helped trigger the Great Oxidation Event (Figure 13.1).

MINIQUIZ

- How do *Thermococcus* and *Pyrococcus* make ATP?
- Compare the energy-yielding metabolisms of *Archaeoglobus* and *Ferroglobus*.

II • *Thaumarchaeota*, *Nanoarchaeota*, and *Korarchaeota*

Our understanding of *Archaea* has been revolutionized by the development of molecular phylogeny (Sections 1.13 and 13.7) and methods for studying microorganisms without the need for cultivation in laboratory cultures (Sections 19.4–19.8). The *Thaumarchaeota, Nanoarchaeota*, and *Korarchaeota* were all discovered and characterized initially with the aid of techniques for analysis of 16S ribosomal RNA genes. From these initial efforts, species representing each of these phyla were subsequently isolated or at least grown in enrichment cultures. We begin our consideration of these unusual phyla with the *Thaumarchaeota*.

17.5 *Thaumarchaeota* and Nitrification in *Archaea*

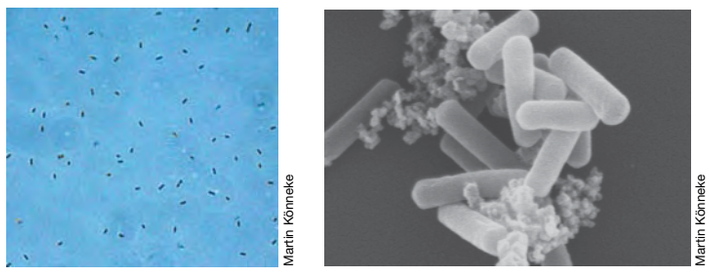
KEY GENERA: *Nitrosopumilus, Nitrososphaera*

Early surveys of 16S ribosomal RNA genes from open-ocean microbial communities resulted in the shocking conclusion that *Archaea* were abundant and widespread in the oceans. At the time, the archaeal domain was considered to contain only extremophiles and obligate anaerobes, and their presence in oxygen-rich temperate and even polar oceanic environments was something of a mystery. Even more remarkable, these novel *Archaea* were widespread and common in soils all over the world (see page 566).

Phylogenetic analyses of 16S ribosomal RNA gene sequences initially suggested that this novel group of *Archaea* was a deeply divergent lineage of the *Crenarchaeota*, a group of hyperthermophilic *Archaea* (Section 17.8). It was only after genome sequence analysis of the marine nitrifier *Nitrosopumilus maritimus* that it became clear that the ***Thaumarchaeota*** constitute a unique phylum of *Archaea* and that they diverged from the primary line of archaeal descent prior to the divergence of *Crenarchaeota* and *Euryarchaeota* (Figure 17.1).

Physiological Characteristics of *Thaumarchaeota*

The physiology of *Thaumarchaeota* remained a mystery until the isolation of *Nitrosopumilus maritimus* (**Figure 17.14**). *N. maritimus* grows chemolithotrophically by aerobically oxidizing ammonia (NH_3) to nitrite (NO_2^-), the first step in nitrification (Sections 14.11, 15.13, and 21.3). This organism uses CO_2 as its sole carbon source (autotrophy), as do nitrifying *Bacteria* (Section 15.13). However, unlike ammonia-oxidizing *Bacteria* such as *Nitrosomonas, N. maritimus* is adapted to life under extreme nutrient limitation, as would befit an organism indigenous to open ocean waters. *N. maritimus* can grow at NH_3 concentrations that are a hundred times lower than those required by bacterial nitrifiers, and their growth is actually inhibited at the higher NH_3 concentrations required to support growth of nitrifying species of *Bacteria*.



(a) *(b)*

Figure 17.14 ***Nitrosopumilus maritimus*, a nitrifying species of *Archaea*.** This organism can oxidize NH_3 present at the very low amounts typical of marine environments. *(a)* Phase-contrast photomicrograph. *(b)* Scanning electron micrograph. A single cell of *N. maritimus* is about 0.2 µm in diameter.

Several species of *Thaumarchaeota* have been isolated and characterized, revealing a number of properties common to this group. Species have been isolated from habitats including the oceans, marine sediment, an estuary, soil, and hot springs. All existing isolates are chemolithotrophic ammonia-oxidizers (Section 14.11), and most species, like *N. maritimus*, are able to grow at very low concentrations of NH_3. The membranes of all *Thaumarchaeota* also have a unique lipid called *crenarchaeol* (Figure 2.6*c*), a compound limited to species of this phylum. In addition, autotrophy in *Thaumarchaeota* is supported by the 3-hydroxypropionate/4-hydroxybutyrate cycle, a finding that further distinguishes archaeal nitrifiers from nitrifying *Bacteria* that employ the Calvin cycle for CO_2 fixation (Section 14.5). The 3-hydroxypropionate/4-hydroxybutyrate cycle also allows for the assimilation of organic carbon, and some archaeal nitrifiers have been shown to assimilate pyruvate during mixotrophic growth. Growth temperatures of *Thaumarchaeota* vary widely, as some species thrive in polar seas while others inhabit hot spring environments up to about 75°C.

Nitrososphaera viennensis, which represents a lineage of *Thaumarchaeota* found widely in soils (see page 566), can grow at a wide range of NH_3 concentrations. Like marine species of *Thaumarchaeota, N. viennensis* can grow at low concentrations of NH_3, but *N. viennensis* can also tolerate high levels (up to 10 mM) of ammonium at neutral pH. Hence, *N. viennensis*, and other archaeal nitrifiers, may be active in soils that have fairly high levels of ammonia, and in these environments they may compete directly with bacterial nitrifiers. In addition, several species of *Thaumarchaeota*, including *N. viennensis*, possess urease activity. *N. viennensis* can grow with urea, which is hydrolyzed to ammonia and subsequently used as an electron donor for energy conservation.

Environmental Distribution of *Thaumarchaeota*

Thaumarchaeota are ubiquitous in soils (see page 566) and found throughout the marine water column from the equator to the polar seas. Indeed, these *Archaea* are one of the most abundant and widespread phyla on Earth, and in surveys of soil or marine samples, thaumarchaea are often found to be the dominant group of *Archaea*. With the use of fluorescent phylogenetic probes (FISH, Section 19.5), thaumarchaea have been detected in oxic marine waters worldwide; they thrive even in waters and sea ice near Antarctica (**Figure 17.15**). Marine species are planktonic (suspended freely or attached to suspended particles in the water column, Figure 17.15*b*) and present in significant numbers (~10^4/ml) in waters that are both nutrient-poor and very cold (0–4°C in seawater and below 0°C in sea ice). Marine thaumarchaea are found throughout the oceans and may constitute 20% of picoplankton (very small prokaryotic cells) worldwide. They are particularly abundant in the deep ocean where they can also account for up to 40% of picoplankton (Section 20.11). The NH_3 concentration in marine waters is often at the threshold for archaeal nitrification, suggesting that *Thaumarchaeota* may play a major role in controlling the levels of NH_3 in the oceans.

Thaumarchaea are also common in soils, comprising as much as 1–2% of the total ribosomal RNA in soil microbial communities and, in some soils, outnumbering nitrifying *Bacteria* by 1000-fold. They are found in soils across a wide range of pH from 3.5 to 8.7. While widely present in soils, thaumarchaea may be particularly important in acid soils (pH < 5.5), which make up more than 30% of all soils. Nitrifiers oxidize NH_3, but at low pH, NH_4^+ predominates and is thus unavailable for nitrification. While nitrification occurs in acid soils, and often at high rates, bacterial nitrifiers have not been observed to grow below pH 6.3. In contrast, the thaumarchaeotal species *Nitrosotalea devanaterra*, isolated from acidic agricultural soil, grows optimally at pH 4–5. The ability of thaumarchaea to grow at low NH_3 concentrations explains how they can be successful in acidic soils where free NH_3 is present in very low concentration.

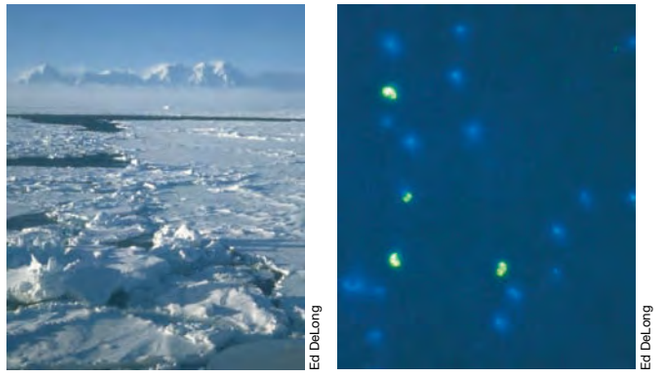


Figure 17.15 Cold-dwelling *Thaumarchaeota*. *(a)* Photo of the Antarctic Peninsula taken from shipboard. The frigid waters that lie under the surface ice shown here are habitats for cold-dwelling *Thaumarchaeota*. *(b)* Fluorescence photomicrograph of seawater treated with a fluorescent probe (Section 19.5) specific for species of *Thaumarchaeota* (green cells). Blue cells are stained with DAPI, a fluorescent DNA stain that stains all cells.

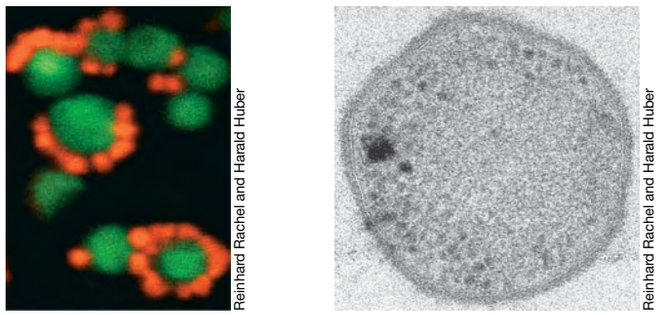
MINIQUIZ

- How does the organism *Nitrosopumilus maritimus* conserve energy and obtain carbon?
- In what environments might you expect to find species of *Thaumarchaeota*?

17.6 *Nanoarchaeota* and the "Hospitable Fireball"

KEY GENUS: *Nanoarchaeum*

The ***Nanoarchaeota*** are represented by a single isolated species, the highly unusual *Nanoarchaeum equitans* (**Figure 17.16**). *N. equitans* is one of the smallest cellular organisms known and has the smallest genome among species of *Archaea* (0.49 Mb). The coccoid cells of *N. equitans* are very small, about 0.4 µm in diameter, and have only about 1% of the volume of an *Escherichia coli* cell. They cannot grow in pure culture and replicate only when attached to the surface of their host organism, *Ignicoccus hospitalis* (Section 17.10), a hyperthermophilic species of *Crenarchaeota* whose name means "the hospitable fireball." *N. equitans* grows to 10 or more cells per *Ignicoccus* cell and lives an apparently parasitic lifestyle, making it the only known archaeal symbiont. Indeed, in agreement with its lifestyle, the species epithet *equitans* means "riding," as in "riding the fireball."



(a) (b)

Figure 17.16 ***Nanoarchaeum equitans***. *(a)* Fluorescence micrograph of cells of *N. equitans* (red) attached to cells of *Ignicoccus* (green). Cells were stained by FISH (Section 19.5) using specific nucleic acid probes targeted to each organism. *(b)* Transmission electron micrograph of a thin section of a cell of *N. equitans*. Note the distinct cell wall. Cells of *N. equitans* are about 0.4 μm in diameter.

Nanoarchaeum and Its Host

N. equitans and its host *Ignicoccus* were first isolated from a submarine hydrothermal vent (Section 20.14) off the coast of Iceland. However, environmental sampling of 16S ribosomal RNA genes (Section 19.6) indicates that organisms phylogenetically similar to *N. equitans* exist in other submarine hydrothermal vents and in terrestrial hot springs, so *Archaea* of this kind are probably distributed worldwide in suitable hot habitats. Like its host *Ignicoccus, N. equitans* grows at temperatures from 70 to 98°C and optimally at 90°C.

The metabolism of *Nanoarchaeum* is not fully understood, but it likely depends on its host for many metabolic functions. *Ignicoccus* is an autotroph that uses H_2 as an electron donor and S^0 as an electron acceptor and so probably supplies *N. equitans* with organic carbon. *N. equitans* is incapable of metabolizing H_2 and S^0 for energy, and whether it generates ATP from substances obtained from *Ignicoccus* or obtains its ATP directly from its host is unknown. The appearance of *N. equitans* cells is typical of *Archaea*, with a cell wall consisting of an S-layer (Section 2.6) that overlays what appears to be a periplasmic space (Figure 17.16*b*).

Although the sequence of its 16S ribosomal RNA gene clearly places *N. equitans* in the domain *Archaea*, the sequence differs at many sites from 16S ribosomal RNA gene sequences of other *Archaea*, even in regions of the molecule that are highly conserved. These differences initially led to the conclusion that *N. equitans* was an early-branching lineage of *Archaea*. However, more detailed phylogenetic analyses of genes encoding ribosomal proteins suggest that the divergence of *N. equitans* occurred at about the time that the *Euryarchaeota* were formed (Figure 17.1). Some analyses even suggest that *N. equitans* may be a species of *Euryarchaeota*. However, genomic analyses show the organism to lack several genes that encode information processing and cell division in *Euryarchaeota*. Conclusive phylogenetic placement of the *Nanoarchaeota* will ultimately require the discovery of more species from this group.

The *N. equitans* Genome

The sequence of the *N. equitans* genome provides insight into this organism's obligately parasitic lifestyle. Its single, circular genome is only 490,885 nucleotides long, one of the smallest cellular genomes yet sequenced (Table 9.1). Genes for several important metabolic functions are missing from the *N. equitans* genome, including those for the biosynthesis of amino acids, nucleotides, coenzymes, and lipids. Also missing are genes encoding proteins for widely distributed catabolic pathways, such as glycolysis. Presumably, all of these functions are carried out for *N. equitans* by its *Ignicoccus* host, with transfer of needed substances from *Ignicoccus* to the attached *N. equitans* cells. *N. equitans* also lacks some of the genes necessary to encode ATPase, and this indicates that it may not synthesize a functional ATPase. If true, this would be the only cellular organism that lacks ATPase. If no ATPase is present and substrate-level phosphorylation does not occur (because of the lack of glycolytic enzymes), then *N. equitans* would be dependent on *Ignicoccus* for energy as well as carbon.

With so many genes missing, which genes remain in the *N. equitans* genome? *N. equitans* contains genes encoding the key enzymes for DNA replication, transcription, and translation as well as genes for DNA repair enzymes. In addition to its small size, the genome of *N. equitans* is also among the most gene dense of any organism known as over 95% of the *N. equitans* chromosome encodes proteins—a value higher than that of most all other prokaryotic cells (Section 9.3).

MINIQUIZ

- Which aspects of the biology of *Nanoarchaeum equitans* make it especially interesting from an evolutionary point of view?
- Why can it be said that *N. equitans* is both a carbon and an energy parasite?

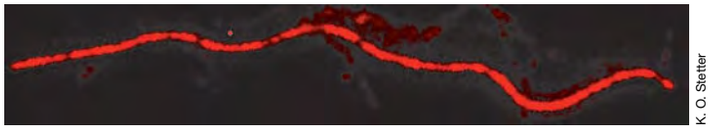
17.7 *Korarchaeota* and the "Secret Filament"

KEY GENUS: *Korarchaeum*

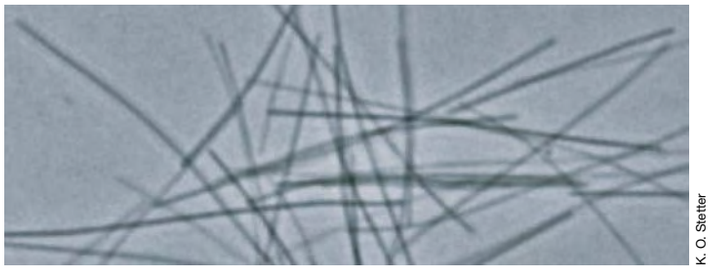
Ribosomal RNA sequences of ***Korarchaeota*** have been observed in a range of geothermal habitats, both submarine and terrestrial. However, *Korarchaeum cryptofilum*, whose name means "the secret filament of youth," is the only characterized species in the phylum *Korarchaeota*.

First observed as a 16S ribosomal RNA gene phylotype recovered from the hot spring named Obsidian Pool in Yellowstone National Park, USA, *K. cryptofilum* has yet to be grown in pure culture. However, its genome sequence has been determined from metagenomic analyses (see Sections 9.8 and 19.8) of an enrichment culture. *K. cryptofilum* is an obligately anaerobic chemoorganotroph and a hyperthermophile, growing at 85°C. Cells are long, thin (<0.2-μm diameter) filaments of variable length (**Figure 17.17*a–c***), with most filaments being around 15 μm long but some reaching as much as 100 μm. Filaments of *K. cryptofilum* have a tough paracrystalline S-layer (Figure 17.17*d*), which maintains cell integrity in its extremely hot habitat.

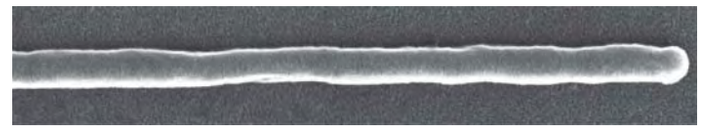
Though *K. cryptofilum* cannot be grown in isolation, its genome sequence provides clues about its lifestyle. *K. cryptofilum* lacks the ability to perform anaerobic respiration (with the possible exception of proton reduction, Section 14.15) and lives a fermentative lifestyle. Similar to other archaeal hyperthermophiles, *K. cryptofilum* grows by fermentation of peptides or amino acids (see Table 17.4).



(a)



(b)



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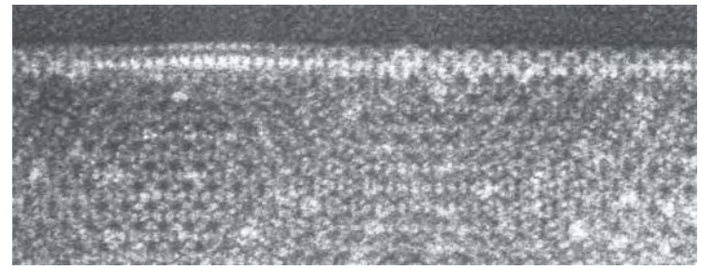

(d)

Figure 17.17 ***Korarchaeum cryptofilum.*** *(a)* Fluorescence in situ hybridization (FISH) was used to identify the morphology of *Korarchaeota* growing in an enrichment culture at 85°C. *(b)* Phase-contrast image of filaments of *K. cryptofilum*. *(c)* Scanning electron micrograph of a *K. cryptofilum* filament. *(d)* Transmission electron micrograph of the surface of a *K. cryptofilum* filament showing the paracrystalline S-layer (Section 2.6). Filaments of *K. cryptofilum* are about 0.17 μm wide and 15 μm long.

K. cryptofilum lacks many core genes in biosynthesis including the ability to synthesize purines, coenzyme A, and several essential cofactors. Presumably *K. cryptofilum* obtains these essential components from its environment. The inability of *K. cryptofilum* to synthesize molecules essential for its own growth may be explained by the evolution of mutual dependence as described by the Black Queen hypothesis (Explore the Microbial World, Chapter 13). This dependence on other members of the hot spring microbial community may explain why *K. cryptofilum* has proven difficult to obtain in pure culture.

As with the *Nanoarchaeota*, there is some uncertainty about the phylogenetic position of the *Korarchaeota*. The genome of *K. cryptofilum* includes some gene families that share affinity with *Euryarchaeota* and others that share affinity with *Crenarchaeota*. For example, phylogenetic analyses of ribosomal proteins, RNA polymerase subunits, and ribosomal RNA genes indicate affinity between *Crenarchaeota* and *Korarchaeota*. In contrast, genes for cell division, tRNA maturation, and DNA replication and repair indicate affinity between *Euryarchaeota* and *Korarchaeota.* The unique genetic composition of *K. cryptofilum* supports its placement near the base of the archaeal radiation (Figure 17.1), and future work on this interesting archaeon should clarify its actual phylogenetic position.

MINIQUIZ

- What is the most likely reason that *Korarchaeum cryptofilum* has been difficult to isolate in pure culture?

III • *Crenarchaeota*

Among *Archaea* in laboratory culture, the ***Crenarchaeota*** are mostly hyperthermophiles and include species growing optimally above the boiling point of water. Many hyperthermophiles are chemolithotrophic autotrophs, and because no phototrophs can survive such temperatures, these organisms are the sole primary producers in these habitats.

17.8 Habitats and Energy Metabolism

Most hyperthermophilic *Archaea* have been isolated from geothermally heated soils or waters containing S^0 and H_2S, and most species metabolize sulfur in one way or another. In terrestrial environments, sulfur-rich springs, boiling mud, and soils may have temperatures up to 100°C and are mildly to extremely acidic owing to the production of sulfuric acid (H_2SO_4) from the biological oxidation of H_2S and S^0 (Sections 14.9 and 21.4). Such hot, sulfur-rich environments, called **solfataras**, are found throughout the world (**Figure 17.18**), including Italy, Iceland, New Zealand, and Yellowstone National Park in Wyoming (USA). Depending on the surrounding geology, solfataras can be mildly acidic to slightly alkaline (pH 5–8) or extremely acidic, with pH values below 1. Hyperthermophilic crenarchaeotes have been obtained from all of these environments, but most inhabit neutral or weakly acidic thermal habitats.

Hyperthermophilic *Crenarchaeota* also inhabit undersea hot springs called **hydrothermal vents**. We discuss the geology and microbiology of these habitats in Section 20.14. Here we only note that submarine waters can be much hotter than surface waters because the water is under hydrostatic pressure. Indeed, all hyperthermophiles with growth temperature optima above 100°C originate from submarine sources. These sources include shallow (2–10 m depth) vents such as those off the coast of Vulcano, Italy, as well as deep (2000–4000 m depth) vents near ocean-spreading centers (see Figure 17.24). Deep hydrothermal vents are the hottest habitats so far known to yield viable life forms.

With a few exceptions, hyperthermophilic *Crenarchaeota* are obligate anaerobes. Their energy-yielding metabolism is either chemoorganotrophic or chemolithotrophic (or both, for example, in *Sulfolobus*) and is dependent on diverse electron donors and

(a)

(b)

(c)

(d)

Figure 17.18 Terrestrial habitats of hyperthermophilic *Archaea:* Yellowstone National Park.
(a) A typical solfatara; steam rich in H_2S rises to the surface. *(b)* Sulfur-rich hot spring, a habitat containing dense populations of *Sulfolobus*. The acidity in solfataras and sulfur springs comes from the oxidation of H_2S and S^0 to H_2SO_4 (sulfuric acid) by *Sulfolobus* and related sulfur-oxidizing microbes. *(c)* A typical neutral pH boiling spring, Imperial Geyser. Many different species of hyperthermophilic *Archaea* may reside in such a habitat. *(d)* An acidic iron-rich geothermal spring, another *Sulfolobus* habitat; here the oxidation of Fe^{2+} to Fe^{3+} generates acidity.

acceptors. Fermentation is rare and most bioenergetic strategies employ anaerobic respiration (Table 17.4). Energy is conserved during these respiratory processes by the same general mechanism widespread in *Bacteria*: electron transfer within the cytoplasmic membrane leading to the formation of a proton motive force from which ATP is made by way of proton-translocating ATPases (Section 3.11).

Many hyperthermophilic crenarchaeotes can grow chemolithotrophically under anoxic conditions, with H_2 as the electron donor and S^0 or NO_3^- as the electron acceptor; a few can also oxidize H_2 aerobically (Table 17.4). H_2 respiration with ferric iron (Fe^{3+}) as electron acceptor also occurs in several hyperthermophiles. Other chemolithotrophic lifestyles include the oxidation of S^0 and Fe^{2+} aerobically or Fe^{2+} anaerobically with NO_3^- as the acceptor (Table 17.4). Only one sulfate-reducing hyperthermophile is known (the euryarchaeote *Archaeoglobus*, Section 17.4). The only bioenergetic option apparently impossible is photosynthesis, a means of energy conservation that is apparently limited to temperatures below 74°C (see Figure 17.28).

MINIQUIZ

- What form of energy metabolism is widespread among hyperthermophiles?
- How might the temperature and pH tolerance of a hyperthermophile living in a solfatara differ from that of a hyperthermophile living in a hydrothermal vent?

17.9 *Crenarchaeota* from Terrestrial Volcanic Habitats

KEY GENERA: *Sulfolobus, Acidianus, Thermoproteus, Pyrobaculum*

Terrestrial volcanic habitats can have temperatures as high as 100°C and are thus suitable for hyperthermophilic *Archaea*. Two phylogenetically related organisms isolated from these environments are *Sulfolobus* and *Acidianus*. These genera form the heart of an order called the *Sulfolobales* (Table 17.5). In addition, *Sulfolobus* has been a model organism for molecular biology studies of *Archaea*.

TABLE 17.4 Energy-yielding reactions of hyperthermophilic *Archaea* by nutritional class

Energy-yielding reaction	*Metabolic type*[a]	*Example genera*[b]
Chemoorganotrophic		
Organic compound + $S^0 \rightarrow H_2S + CO_2$	AnR	*Thermoproteus, Thermococcus, Desulfurococcus, Thermofilum, Pyrococcus*
Organic compound + $SO_4^{2-} \rightarrow H_2S + CO_2$	AnR	*Archaeoglobus*
Organic compound + $O_2 \rightarrow H_2O + CO_2$	AeR	*Sulfolobus*
Organic compound $\rightarrow CO_2 + H_2$ + fatty acids	AnR	*Staphylothermus, Pyrodictium*
Organic compound + $Fe^{3+} \rightarrow CO_2 + Fe^{2+}$	AnR	*Pyrodictium*
Organic compound + $NO_3^- \rightarrow CO_2 + N_2$	AnR	*Pyrobaculum*
Pyruvate $\rightarrow CO_2 + H_2$ + acetate	AnR	*Pyrococcus*
Peptides $\rightarrow CO_2$ + acetate + butanol	F	*Hyperthermus, Korarchaeum*
Chemolithotrophic		
$H_2 + S^0 \rightarrow H_2S$	AnR	*Acidianus, Pyrodictium, Thermoproteus, Stygiolobus, Ignicoccus*
$H_2 + NO_3^- \rightarrow NO_2^- + H_2O$ (NO_2^- is reduced to N_2 by some species)	AnR	*Pyrobaculum*
$4\ H_2 + NO_3^- + H^+ \rightarrow NH_4^+ + 2\ H_2O + OH^-$	AnR	*Pyrolobus*
$H_2 + 2\ Fe^{3+} \rightarrow 2\ Fe^{2+} + 2\ H^+$	AnR	*Pyrobaculum, Pyrodictium, Archaeoglobus*
$2\ H_2 + O_2 \rightarrow 2\ H_2O$	AeR	*Acidianus, Sulfolobus, Pyrobaculum*
$2\ S^0 + 3\ O_2 + 2\ H_2O \rightarrow 2\ H_2SO_4$	AeR	*Sulfolobus, Acidianus*
$2\ FeS_2 + 7\ O_2 + 2\ H_2O \rightarrow 2\ FeSO_4 + 2\ H_2SO_4$	AeR	*Sulfolobus, Acidianus, Metallosphaera*
$2\ FeCO_3 + NO_3^- + 6\ H_2O \rightarrow 2\ Fe(OH)_3 + NO_2^- + 2\ HCO_3^- + 2\ H^+ + H_2O$	AnR	*Ferroglobus*
$4\ H_2 + SO_4^{2-} + 2\ H^+ \rightarrow 4\ H_2O + H_2S$	AnR	*Archaeoglobus*
$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$	AnR	*Methanopyrus, Methanocaldococcus, Methanothermus*

[a]AnR, anaerobic respiration; AeR, aerobic respiration; F, fermentation.
[b]Most are *Crenarchaeota*; Figure 17.1.

Sulfolobales

Sulfolobus grows in sulfur-rich acidic thermal areas (Figure 17.18) at temperatures up to 90°C and at pH values of 1–5. *Sulfolobus* is an aerobic chemolithotroph that oxidizes H_2S or S^0 to H_2SO_4 and fixes CO_2 as a carbon source. *Sulfolobus* can also grow chemoorganotrophically. Cells of *Sulfolobus* are more or less spherical but contain distinct lobes (**Figure 17.19*a***). Cells adhere tightly to sulfur crystals, where they can be seen with a microscope after staining with fluorescent dyes (Figure 14.27*b*). Besides the aerobic respiration of sulfur or organic compounds, *Sulfolobus* can also oxidize Fe^{2+} to Fe^{3+}, and this ability has been harnessed for the high-temperature leaching of iron and copper ores (Sections 21.5 and 22.1).

A facultative aerobe resembling *Sulfolobus* also lives in acidic solfataric springs. This organism, *Acidianus* (Figure 17.19*b*), differs from *Sulfolobus* most clearly by its ability to grow using S^0 both anaerobically as well as aerobically. Under aerobic conditions the organism uses S^0 as an electron *donor*, oxidizing S^0 to H_2SO_4, with O_2 as an electron acceptor. Anaerobically, *Acidianus* uses S^0 as an electron *acceptor* with H_2 as an electron donor, forming H_2S as the reduced product. Thus, the metabolic fate of S^0 in cultures of *Acidianus* depends on the presence or absence of O_2. Like *Sulfolobus, Acidianus* is roughly spherical in shape but is not as lobed (Figure 17.19*b*). It grows at temperatures from 65°C up to a maximum of 95°C, with an optimum of about 90°C. As a group, then, the *Sulfolobales* contain the most thermophilic of all highly acidophilic *Archaea*.

Thermoproteales

Key genera within the *Thermoproteales* are *Thermoproteus, Thermofilum*, and *Pyrobaculum*. The genera *Thermoproteus* and *Thermofilum* consist of rod-shaped cells that inhabit neutral or slightly acidic hot springs. Cells of *Thermoproteus* are rigid rods about 0.5 µm in diameter and highly variable in length, ranging from short cells of 1–2 µm (**Figure 17.20*a***) up to filaments 70–80 µm long. Filaments of *Thermofilum* are thinner, some as little as 0.17–0.35 µm wide, with filament lengths ranging up to 100 µm (Figure 17.20*b*).

Both *Thermoproteus* and *Thermofilum* are strict anaerobes that carry out an S^0-based anaerobic respiration (Table 17.4). Most *Thermoproteus* isolates can grow chemolithotrophically on H_2 or chemoorganotrophically on complex carbon substrates such as yeast extract, small peptides, starch, glucose, ethanol, malate, fumarate, or formate (Table 17.4). *Pyrobaculum* (Figure 17.20*c*) is a rod-shaped hyperthermophile but is physiologically distinct from other *Thermoproteales* in that some species of *Pyrobaculum* can respire aerobically. However, *Pyrobaculum* can also grow by anaerobic respiration with NO_3^-, Fe^{3+}, or S^0 as electron acceptors and H_2 as an electron donor (that is, they can grow chemolithotrophically and autotrophically). Other species of *Pyrobaculum* can grow anaerobically on organic electron donors, reducing S^0 to H_2S. The growth temperature optimum of *Pyrobaculum* is 100°C, and species of this organism have been isolated from terrestrial hot springs and from hydrothermal vents.

TABLE 17.5 Properties of some hyperthermophilic *Crenarchaeota*

Order/Genus[a]	Morphology	Relationship to O_2[b]	Temperature			
			Minimum	Optimum	Maximum	Optimum pH
Sulfolobales						
Sulfolobus	Lobed coccus	Ae	55	75	87	2–3
Acidianus	Coccus	Fac	60	88	95	2
Metallosphaera	Coccus	Ae	50	75	80	2
Stygiolobus	Lobed coccus	An	57	80	89	3
Sulfurisphaera	Coccus	Fac	63	84	92	2
Sulfurococcus	Coccus	Ae	40	75	85	2.5
Thermoproteales						
Thermoproteus	Rod	An	60	88	96	6
Thermofilum	Rod	An	70	88	95	5.5
Pyrobaculum	Rod	Fac	74	100	102	6
Caldivirga	Rod	An	60	85	92	4
Thermocladium	Rod	An	60	75	80	4.2
Desulfurococcales						
Desulfurococcus	Coccus	An	70	85	95	6
Aeropyrum	Coccus	Ae	70	95	100	7
Staphylothermus	Cocci in clusters	An	65	92	98	6–7
Pyrodictium	Disc-shaped with filaments	An	82	105	110	6
Pyrolobus	Lobed coccus	Fac	90	106	113	5.5
Thermodiscus	Disc-shaped	An	75	90	98	5.5
Ignicoccus	Irregular coccus	An	65	90	103	5
Hyperthermus	Irregular coccus	An	75	102	108	7
Stetteria	Coccus	An	68	95	102	6
Sulfophobococcus	Disc-shaped	An	70	85	95	7.5
Thermosphaera	Coccus	An	67	85	90	7
Strain 121[c]	Coccus	An	85	106	121	7

[a]The group names ending in "ales" are order names.
[b]Ae, aerobe; An, anaerobe; Fac, facultative.
[c]Also known by the unofficial taxonomic name of "*Geogemma barossii*."

MINIQUIZ

- What are the similarities and differences between *Sulfolobus* and *Pyrobaculum*?
- Among *Thermoproteales*, what is unusual about the metabolism of *Pyrobaculum*?

17.10 *Crenarchaeota* from Submarine Volcanic Habitats

KEY GENERA: *Pyrodictium, Pyrolobus, Ignicoccus, Staphylothermus*

We now consider the microbiology of submarine volcanic habitats, homes to the most thermophilic of all known *Archaea*. These habitats include both shallow-water thermal springs and deep-sea hydrothermal vents. We discuss the geology of these fascinating microbial habitats in Section 20.14 and the interesting animal communities that develop there in Section 23.9. The organisms to be described here constitute an order of *Archaea* called the *Desulfurococcales* (Table 17.5).

Pyrodictium and *Pyrolobus*

Pyrodictium and *Pyrolobus* are examples of microorganisms whose growth temperature optimum lies above 100°C; the optimum for *Pyrodictium* is 105°C and for *Pyrolobus* is 106°C. Cells of *Pyrodictium* are irregularly disc-shaped and grow in culture in a mycelium-like layer attached to crystals of S^0. The cell mass consists of a network of fibers to which individual cells are attached (**Figure 17.21a**). The fibers are hollow and consist of protein arranged in a fashion similar to that of bacterial flagella (↔ Section 2.11). However, the filaments do not function in motility but instead as organs of attachment. The cell walls of *Pyrodictium* are composed of glycoprotein. Physiologically, *Pyrodictium* is a strict anaerobe that grows chemolithotrophically on H_2 as an electron donor and S^0 as an electron acceptor (↔ Section 14.14) or chemoorganotrophically on complex mixtures of organic compounds (Table 17.4).

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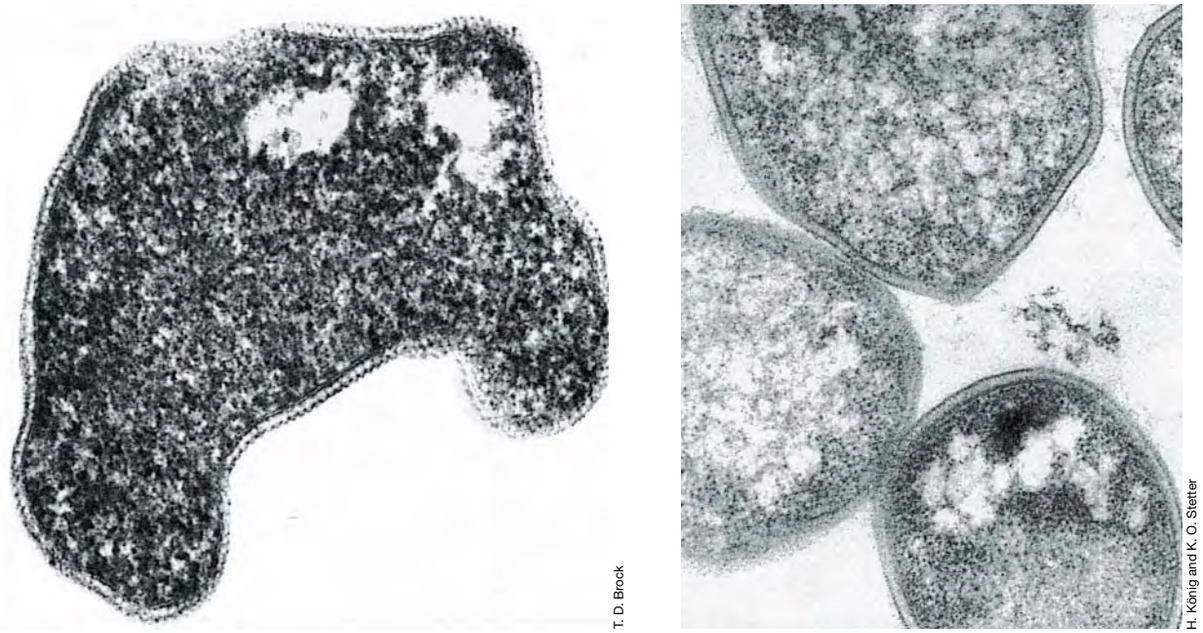


Figure 17.19 Acidophilic hyperthermophilic *Archaea*, the *Sulfolobales*. *(a) Sulfolobus acidocaldarius*; electron micrograph of a thin section. *(b) Acidianus infernus*; electron micrograph of a thin section. Cells of both organisms vary from 0.8 to 2 μm in diameter. *Sulfolobales* typically show temperature optima below 90°C (Table 17.5).

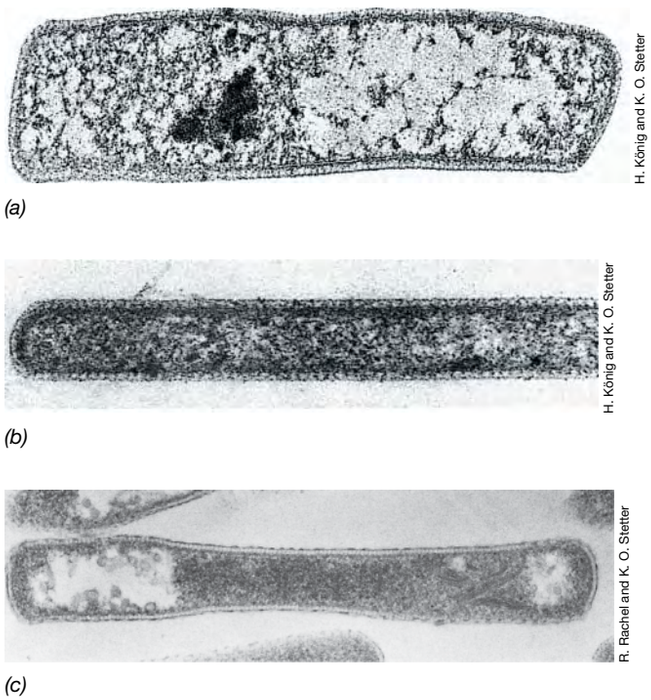


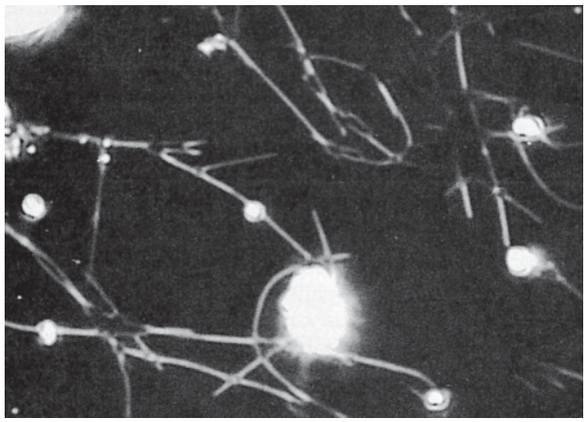
Figure 17.20 Rod-shaped hyperthermophilic *Archaea*, the *Thermoproteales*. *(a) Thermoproteus neutrophilus*; electron micrograph of a thin section. A cell is about 0.5 μm in diameter. *(b) Thermofilum librum*. A cell is about 0.25 μm in diameter. *(c) Pyrobaculum aerophilum*. Transmission electron micrograph of a thin section; a cell measures 0.5 × 3.5 μm. Although the temperature optimum of *P. aerophilum* is 100°C, optima for other *Thermoproteales* are all below 90°C (Table 17.5).

Pyrolobus fumarii (Figure 17.21*c*) is one of the most thermophilic of the hyperthermophiles. Its growth temperature maximum is 113°C (Table 17.5). *P. fumarii* lives in the walls of "black smoker" hydrothermal vent chimneys (Section 20.14 and Figures 20.37, 20.38, and 20.40) where its autotrophic abilities contribute organic carbon to this otherwise inorganic environment. *P. fumarii* cells are coccoid-shaped (Figure 17.21*c*), and the cell wall is composed of protein. The organism is an obligate H_2 chemolithotroph, growing by the oxidation of H_2 coupled to the reduction of NO_3^- to ammonium (NH_4^+), thiosulfate ($S_2O_3^{2-}$) to H_2S, or very low concentrations of O_2 to H_2O. Besides its extremely thermophilic nature, *P. fumarii* can withstand temperatures substantially above its growth temperature maximum. For example, cultures of *P. fumarii* survive autoclaving (121°C) for 1 h, a condition that even bacterial endospores (Section 2.10) cannot withstand.

Another organism in this group shares with *Pyrolobus* a growth temperature optimum of 106°C. However, "Strain 121," as this organism has been called, actually shows weak growth at 121°C, and cells remain viable for 2 h at 130°C. Only *Methanopyrus*, a hyperthermophilic methanogen, can grow at a higher temperature (122°C, Section 17.2). Strain 121 consists of coccoid cells with archaella (Figure 17.21*d*); the organism is also a strict anaerobe and grows chemolithotrophically and autotrophically with Fe^{3+} as electron acceptor and formate or H_2 as electron donors. It is thus clear that the *Pyrodictium*/*Pyrolobus* group collectively contains the most hyperthermophilic examples of all known microbes.

Desulfurococcus and *Ignicoccus*

Other notable members of the *Desulfurococcales* include *Desulfurococcus*, the genus for which the order is named (**Figure 17.22*a***), and



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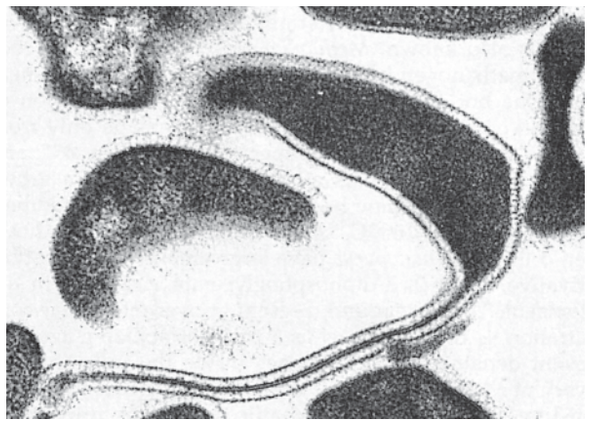

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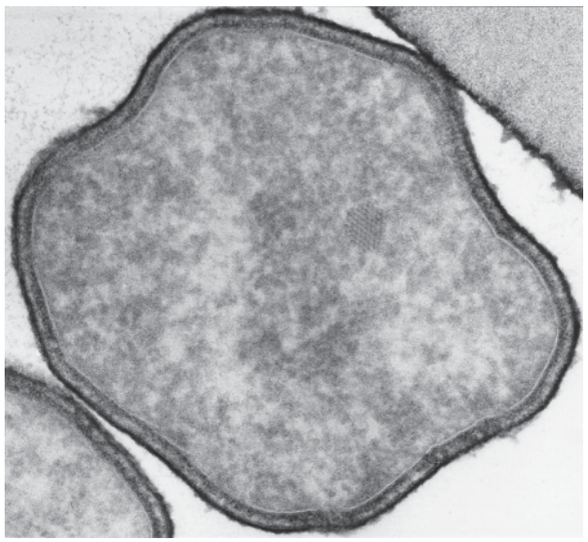

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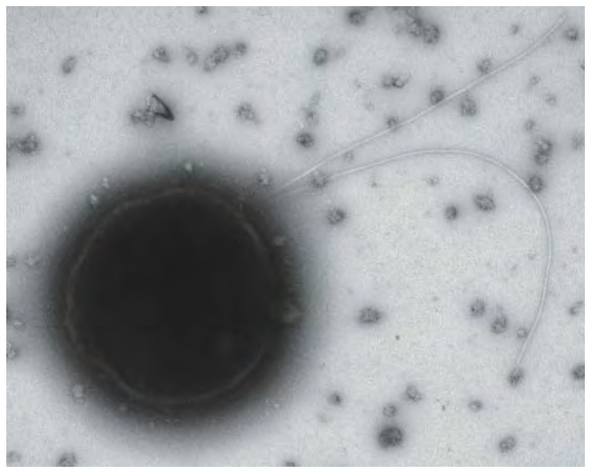


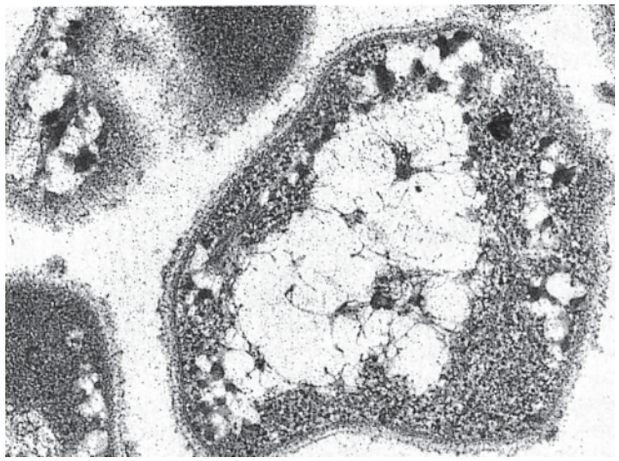
(d)

Figure 17.21 *Desulfurococcales* with growth temperature optima >100°C. *(a) Pyrodictium occultum* (growth temperature optimum, 105°C), dark-field micrograph. *(b)* Thin-section electron micrograph of *P. occultum*. Cells are highly variable in diameter from 0.3 to 2.5 μm. *(c)* Thin section of a cell of *Pyrolobus fumarii*, one of the most thermophilic organisms ever described (growth temperature optimum, 106°C); a cell is about 1.4 μm in diameter. *(d)* Negative stain of a cell of "Strain 121," capable of growth at 121°C; a cell is about 1 μm wide. Although the *Desulfurococcales* contain the greatest number of hyperthermophiles capable of growth above 100°C, the most thermophilic of all known *Archaea* is actually a euryarchaeote, *Methanopyrus* (Section 17.2).

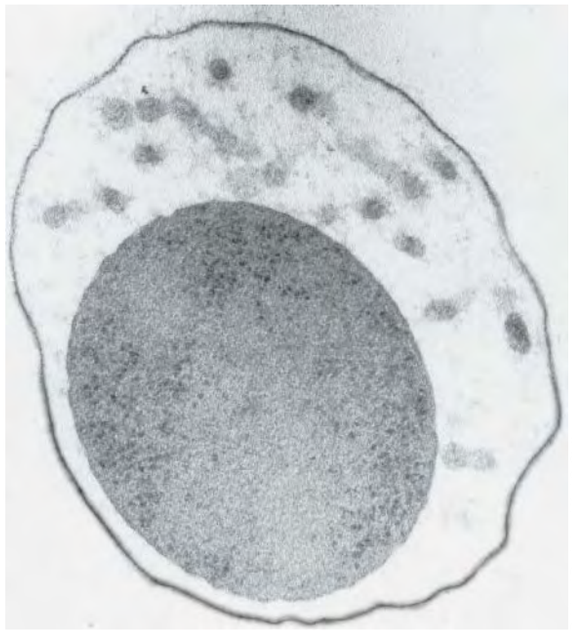
Ignicoccus. *Desulfurococcus* is a strictly anaerobic S^0-reducing organism like *Pyrodictium*, but differs from this organism in its phylogeny and the fact that it is much less thermophilic, growing optimally at about 85°C.

Ignicoccus grows optimally at 90°C, and its energy metabolism is based on H_2 as an electron donor and S^0 as an electron acceptor, as is that of so many hyperthermophilic *Archaea* (Table 17.4). Some *Ignicoccus* species are hosts to the small parasitic archaeon *Nanoarchaeum equitans* (Section 17.6). *Ignicoccus* (Figure 17.22*b*) has a novel cell structure that lacks an S-layer and possesses a unique *outer cellular membrane*. This outer cellular membrane is distinct in several ways from the outer membrane of gram-negative *Bacteria* (↻ Section 2.5). Most notably, the outer cellular membrane of *Ignicoccus* contains ATPase and is the site of energy conservation. *Ignicoccus* also contains an inner cellular membrane that contains the cytoplasm and the enzymes responsible for biosynthesis and information processing. In this way neither the outer nor inner cellular membrane satisfies the typical definition of a cytoplasmic membrane (↻ Section 2.3).

Between the inner and outer cellular membranes of *Ignicoccus* is a large *intermediate compartment* that is analogous to the periplasm of gram-negative *Bacteria* but is much larger in volume, representing some two to three times the volume of the cytoplasm (Figure 17.22*b*). The periplasm of *Ignicoccus* also contains membrane-bound vesicles (Figure 17.22*b*) that may function in exporting substances outside the cell. In this way, the cell structure of *Ignicoccus* resembles that of *Eukarya*. Hence, *Ignicoccus* has been proposed to be a modern descendant of the ancestral cell type that gave rise to the origin of eukaryotic cells (↻ Figure 13.9).



(a)



(b)

Figure 17.22 *Desulfurococcales* with growth temperature optima <100°C. *(a)* Thin section of a cell of *Desulfurococcus saccharovorans*; a cell is 0.7 µm in diameter. *(b)* Thin section of a cell of *Ignicoccus islandicus*; the cell proper is surrounded by an extremely large periplasmic compartment. The cell itself measures about 1 µm in diameter and the cell plus periplasm measures 1.4 µm.

Staphylothermus

A morphologically unusual member of the order *Desulfurococcales* is the genus *Staphylothermus* (**Figure 17.23**). Cells of *Staphylothermus* are spherical, about 1 µm in diameter, and form aggregates of up to 100 cells, much like its morphological counterpart among the *Bacteria, Staphylococcus* (Figures 16.20 and 30.28*a*). Unlike many hyperthermophiles, *Staphylothermus* is not a chemolithotroph but instead a chemoorganotroph, growing optimally at 92°C. Energy is obtained from the fermentation of peptides, producing the fatty acids acetate and isovalerate as fermentation products (Table 17.4).

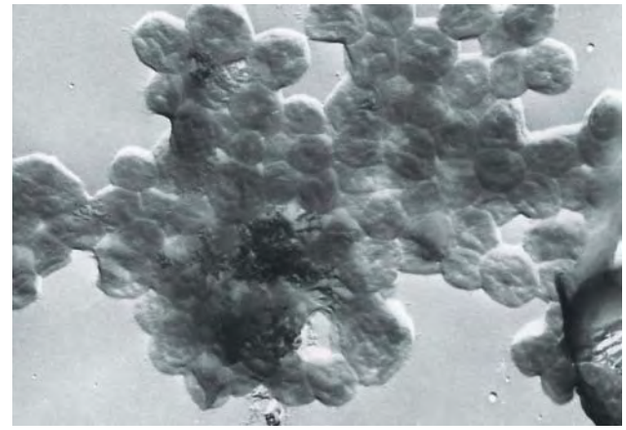


Figure 17.23 The hyperthermophile *Staphylothermus marinus*. Electron micrograph of shadowed cells. A single cell is about 1 µm in diameter.

Isolates of *Staphylothermus* have been obtained from both shallow marine hydrothermal vents and very hot black smokers (see Figure 17.24; Section 20.14). This organism is apparently widely distributed in submarine thermal areas, where it is likely to play a significant role in consuming proteins released from dead organisms.

MINIQUIZ

- What can we conclude about the *Pyrodictium/Pyrolobus* group in terms of life at high temperature?
- What unusual structural features are present in *Ignicoccus* and *Staphylothermus*?

IV • Evolution and Life at High Temperature

Most of the hyperthermophiles discovered so far are species of *Archaea* and some grow near to what may be the upper temperature limit for life. Here we consider the major factors that likely define the upper temperature limit for life and the biological adaptations of hyperthermophiles that permit them to exist at the exceptionally high temperatures of 100°C and higher. We end with a discussion of the importance of hydrogen (H_2) metabolism to the biology of hyperthermophiles.

17.11 An Upper Temperature Limit for Microbial Life

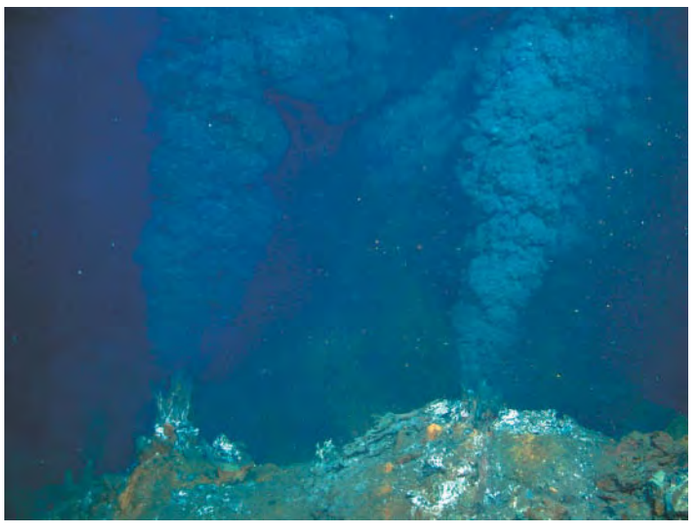
Habitats that contain liquid water—a prerequisite for cellular life—and that have temperatures higher than 100°C are only found where geothermally heated water flows out of vents or rifts in the ocean floor (Figures 13.3 and 20.37–20.40). The hydrostatic pressure that overlies the water keeps it from boiling, allowing it to reach temperatures of up to about 400°C in vents at several thousand meters' depth. In contrast, terrestrial hot springs can

boil and therefore only attain temperatures near 100°C. It is not surprising, then, that hydrothermal vents have been rich sources of hyperthermophilic *Archaea* with growth temperature optima above 100°C (Table 17.5).

Black smokers emit hydrothermal vent fluid at 250–350°C or higher. Metallic mounds or more upright structures called *chimneys* form from the metal sulfides that precipitate out of the hot fluid as it mixes with the surrounding, much cooler seawater (**Figure 17.24**). As far as is known, the superheated vent water itself is sterile. However, hyperthermophiles thrive in mounds or smoker chimney walls where temperatures are compatible with their survival and growth (Figure 20.40). By studying structures such as these, we can address the question, "What is the upper temperature for microbial (and presumably all forms of) life?"

What Is the Upper Temperature Limit for Life?

How high a temperature can hyperthermophiles withstand? Over the past several decades, the known upper temperature limit for life has been pushed higher and higher with the isolation and characterization of new species of thermophiles and hyperthermophiles (**Figure 17.25**). For some time the record holder was *Pyrolobus fumarii* (Figure 17.21*c*), with its upper temperature limit for growth of 113°C. The current record holder, *Methanopyrus* (Section 17.2 and Figure 17.8), however, has pushed the limit somewhat higher, with the ability to grow at 122°C and to survive substantial periods at even higher temperatures. Given the trend over the past several years (Figure 17.25), one can predict that *Archaea* even more hyperthermophilic than *Methanopyrus* may inhabit hydrothermal environments but have yet to be isolated. Indeed, many experts predict that the upper temperature limit for *Archaea* is likely to exceed 140°C, perhaps even 150°C, and that the maximum temperature allowing survival but not growth is even hotter yet.



Anna-Louise Reysenbach and Woods Hole Oceanographic Institution

Figure 17.24 Hydrothermal vents. Hydrothermal mound from the Rainbow vent field, Mid-Atlantic Ridge hydrothermal system. The hydrothermal fluid emitting from the two short chimneys is >300°C.

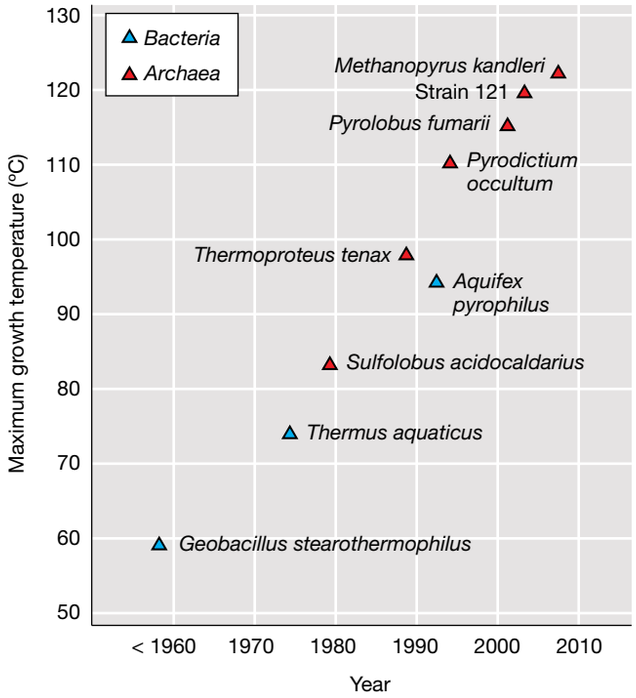


Figure 17.25 Thermophilic and hyperthermophilic *Bacteria* and *Archaea*. The graph gives the species that were, in turn, the record holders for growing at the highest temperature, from before 1960 to the present.

Biochemical Problems at Supercritical Temperatures

Whatever the upper temperature limit is for life, it is likely to be defined by one or more biochemical challenges that evolution has been unable to solve. There is obviously an upper limit, but we do not yet know what it is. Water samples taken directly from superheated (>250°C) hydrothermal vent discharges are devoid of measurable biochemical markers (DNA, RNA, and protein) that would signal life as we know it, while vents emitting water at temperatures below about 150°C yield evidence of macromolecules. These results are consistent with laboratory experiments on the stability of key biomolecules. For example, ATP is degraded almost instantly at 150°C. Thus, above 150°C, any life forms would have to deal with the heat lability of a molecule that is, as far as is known, universally distributed in cells. As a caveat, however, the stability of small molecules such as ATP may be significantly greater under cytoplasmic conditions of high levels of dissolved solutes than in pure solutions tested in the laboratory. Nevertheless, if life forms exist at temperatures above 150°C, they must be unique in many ways, either using a suite of novel small molecules absent from cells as we know them, or deploying special protection systems that maintain small molecules in a stable state such that biochemistry can proceed.

MINIQUIZ

- Where are the hottest potential microbial habitats located on Earth?
- Why would it be impossible for organisms to grow at 200 or 300°C?

17.12 Molecular Adaptations to Life at High Temperature

Because all cellular structures and activities are affected by heat, hyperthermophiles are likely to exhibit multiple adaptations to the exceptionally high temperatures of their habitats. Here we briefly examine some adaptations employed by hyperthermophiles to protect their proteins and nucleic acids at high temperatures.

Protein Folding and Thermostability

Because most proteins denature at high temperatures, much research has been done to identify the properties of thermostable proteins. Protein thermostability derives from the folding of the molecule itself, not because of the presence of any special amino acids. Perhaps surprisingly, however, the amino acid composition of thermostable proteins is not particularly unusual except perhaps in their slight bias for increased levels of amino acids that promote alpha-helical secondary structures. In fact, many enzymes from hyperthermophiles contain the same major structural features in both primary and higher-order structure (Section 4.7) as their heat-labile counterparts from organisms that grow best at much lower temperatures.

Thermostable proteins typically do display some structural features that likely improve their thermostability. These include having highly hydrophobic cores, which decrease the tendency of the protein to unfold in an ionic environment, and more ionic interactions on the protein surfaces, which also help hold the protein together and work against unfolding. Ultimately, it is the *folding* of the protein that most affects its heat stability, and noncovalent ionic bonds called *salt bridges* on a protein's surface likely play a major role in maintaining the biologically active structure. But, as previously stated, many of these changes are possible with only minimal changes in primary structure (amino acid sequence), as seen when thermostable and heat-labile forms of the same protein are compared.

Chaperones: Assisting Proteins to Remain in Their Native State

Earlier we discussed a class of proteins called *chaperones* (heat shock proteins; Section 4.11) that function to refold partially denatured proteins. Hyperthermophilic *Archaea* have special classes of chaperones that function only at the highest growth temperatures. In cells of *Pyrodictium abyssi* (**Figure 17.26**), for example, a major chaperone is the protein complex called the **thermosome**. This complex keeps other proteins properly folded and functional at high temperature, helping cells survive even at temperatures above their maximal growth temperature. Cells of *P. abyssi* grown near its maximum temperature (110°C) contain high levels of the thermosome. Possibly because of this, the cells can remain viable following a heat shock, such as a 1-h treatment in an autoclave (121°C). In cells experiencing such a treatment and then returned to the optimum temperature, the thermosome, which is itself quite heat resistant, is thought to refold sufficient copies of key denatured proteins that *P. abyssi* can once again begin to grow and divide. Thus, as a result of chaperone activity, the upper temperature limit at which many hyperthermophiles can *survive* is higher than the upper temperature at which they can *grow*. The "safety net" of chaperone activity probably ensures that cells in nature that briefly experience temperatures above their growth temperature maximum are not killed by the exposure.

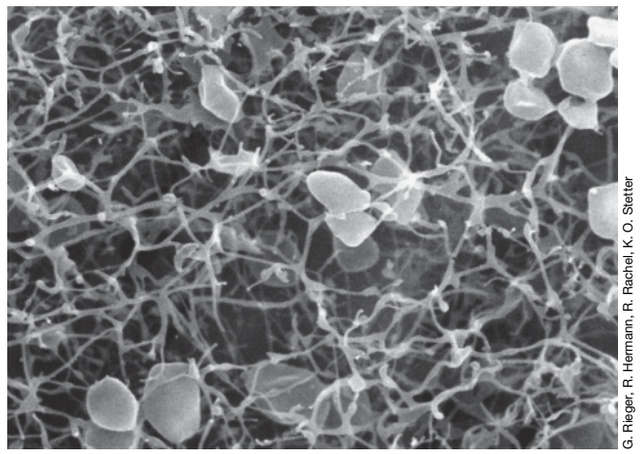


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Figure 17.26 ***Pyrodictium abyssi*, scanning electron micrograph.** *Pyrodictium* has been studied as a model of macromolecular stability at high temperatures. Cells are enmeshed in a sticky glycoprotein matrix that binds them together.

DNA Stability: Solutes, Reverse Gyrase, and DNA-Binding Proteins

What keeps DNA from melting at high temperatures? Various mechanisms are known to contribute. One such mechanism increases cellular solute levels, in particular potassium (K^+) or compatible organic compounds. For example, the cytoplasm of the hyperthermophilic methanogen *Methanopyrus* (Section 17.2) contains greater than 1 M potassium cyclic 2,3-diphosphoglycerate. This solute prevents chemical damage to DNA, such as depurination or depyrimidization (loss of a nucleotide base through hydrolysis of the glycosidic bond) from high temperatures, events that can lead to mutation (Section 11.2). This compound and other compatible solutes, such as potassium di-*myo*-inositol phosphate, which protects against osmotic stress, and the polyamines putrescine and spermidine, which stabilize both ribosomes and nucleic acids at high temperature, help maintain key cellular macromolecules in hyperthermophiles in their active forms.

A unique protein found *only* in hyperthermophiles is responsible for DNA stability in these organisms. All hyperthermophiles produce a special DNA topoisomerase called **reverse DNA gyrase**. This enzyme introduces positive supercoils into the DNA of hyperthermophiles (in contrast to the negative supercoils introduced by DNA gyrase present in *Bacteria* and most *Archaea*; Section 4.1). Positive supercoiling stabilizes DNA to heat and thereby prevents the DNA helix from spontaneously unwinding. The noticeable absence of reverse DNA gyrase in organisms whose growth temperature optima lie below 80°C strongly suggests a specific role for this enzyme in DNA stability at high temperatures.

Species of *Euryarchaeota* also contain highly basic (positively charged) DNA-binding proteins that are remarkably similar in amino acid sequence and folding properties to the core histones of the *Eukarya* (Figure 2.46). Archaeal histones from the hyperthermophilic methanogen *Methanothermus fervidus* (Figure 17.7*c*)

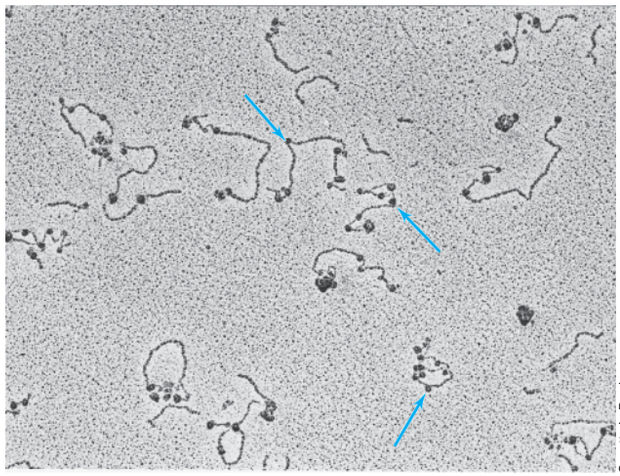


Figure 17.27 Archaeal histones and nucleosomes. Electron micrograph of linearized plasmid DNA wrapped around copies of archaeal histone Hmf (from the hyperthermophilic methanogen *Methanothermus fervidus*) to form the roughly spherical, darkly stained nucleosome structures (arrows).Compare this micrograph with an artist's depiction of the histones and nucleosomes of *Eukarya* shown in Figure 2.46*b*.

have been particularly well studied. These proteins wind and compact DNA into nucleosome-like structures (**Figure 17.27**) and maintain the DNA in a double-stranded form at very high temperatures. Archaeal histones are found in most *Euryarchaeota*, including extremely halophilic *Archaea*, such as *Halobacterium*. However, because the extreme halophiles are not thermophiles, archaeal histones may have other functions besides DNA stability, in particular in assisting in gene expression by opening the helix to allow transcriptional proteins to bind.

Lipid and Ribosomal RNA Stability

How have the lipids and the protein-synthesizing machinery of hyperthermophiles adjusted to high temperatures? Virtually all hyperthermophilic *Archaea* synthesize lipids of the biphytanyl tetraether type (Section 2.3). These lipids are naturally heat resistant because the phytanyl units forming each half of the membrane structure are covalently bonded to one another; this yields a *lipid monolayer* membrane instead of the normal lipid bilayer (Figure 2.6). This structure resists the tendency of heat to pull apart a lipid bilayer constructed of fatty acid or phytanyl side chains that are not covalently bonded.

A final point on molecular adaptations to life at high temperatures is the base composition of ribosomal RNAs. Ribosomal RNAs are key structural and functional components of the ribosome, the cell's protein-synthesizing apparatus (Section 4.10). Hyperthermophilic species of both *Bacteria* and *Archaea* show as much as a 15% greater proportion of GC base pairs in their small ribosomal subunit RNAs compared with organisms that grow at lower temperatures. GC base pairs form three hydrogen bonds compared to the two of AU base pairs (Figure 4.1*c*), and thus the higher GC content of the ribosomal RNAs should confer greater thermal stability on the ribosomes of these organisms and this should assist protein synthesis at high temperatures. By contrast to ribosomal RNAs, the GC content of genomic DNA of hyperthermophiles is often rather low, which suggests that the thermal stability of ribosomal RNA might be an especially significant factor for life under hyperthermophilic conditions.

MINIQUIZ

- How do hyperthermophiles keep proteins and DNA from being destroyed by high heat?
- How are the lipids and ribosomes of hyperthermophiles protected from heat denaturation?

17.13 Hyperthermophilic *Archaea*, H_2, and Microbial Evolution

When cellular life first arose on Earth nearly 4 billion years ago, temperatures almost certainly were far hotter than they are today. Thus, for hundreds of millions of years, Earth may have been suitable only for hyperthermophiles. Given the discussion above on the temperature limits to life, it has been hypothesized that biological molecules, biochemical processes, and the first cells arose on Earth around hydrothermal springs and vents on the seafloor as they cooled to temperatures compatible with biological molecules (Section 13.1 and Figures 13.3 and 13.4). The phylogeny of modern hyperthermophiles (Figure 17.1), as well as the similarities in their habitats and metabolism to those of early cells on Earth, suggests that hyperthermophiles may be the closest remaining descendants of ancient cells and are a living window into the biology of ancient microbial life.

Hyperthermophilic Habitats and H_2 as an Energy Source

The oxidation of H_2 linked to the reduction of Fe^{3+}, S^0, NO_3^-, or, rarely, O_2 is a widespread form of energy metabolism in hyperthermophiles (Table 17.4 and **Figure 17.28**). This, coupled with the likelihood that these hyperthermophiles best characterize early Earth phenotypes, points to the important role H_2 has played in the evolution of microbial life. Hydrogen metabolism may have evolved in primitive organisms because of the ready availability of H_2 and suitable inorganic electron acceptors in their primordial environments, but also because a H_2-based energy economy requires relatively few proteins (Figure 13.5). As chemolithotrophs, these organisms may have obtained all of their carbon from CO_2 or might have assimilated available organic compounds directly for biosynthetic needs. Either way it is likely that the oxidation of H_2 was the energetic driving force for maintaining life processes.

If one compares microbial energy conservation mechanisms as a function of temperature from data of cultured *Bacteria* and *Archaea*, only chemolithotrophic organisms are known at the hottest temperatures (Figure 17.28). Chemoorganotrophy occurs up to at least 110°C, as this is the upper temperature limit for growth of *Pyrodictium occultum*, an organism that can conserve energy and grow by fermentation and by chemolithotrophic growth on H_2 with S^0 as electron acceptor (Table 17.4). Photosynthesis is apparently the least heat-tolerant of all bioenergetic processes, with no hyperthermophilic representatives known and an upper temperature limit of 73°C. This is consistent with the conclusion that anoxygenic photosynthesis first appeared on Earth some

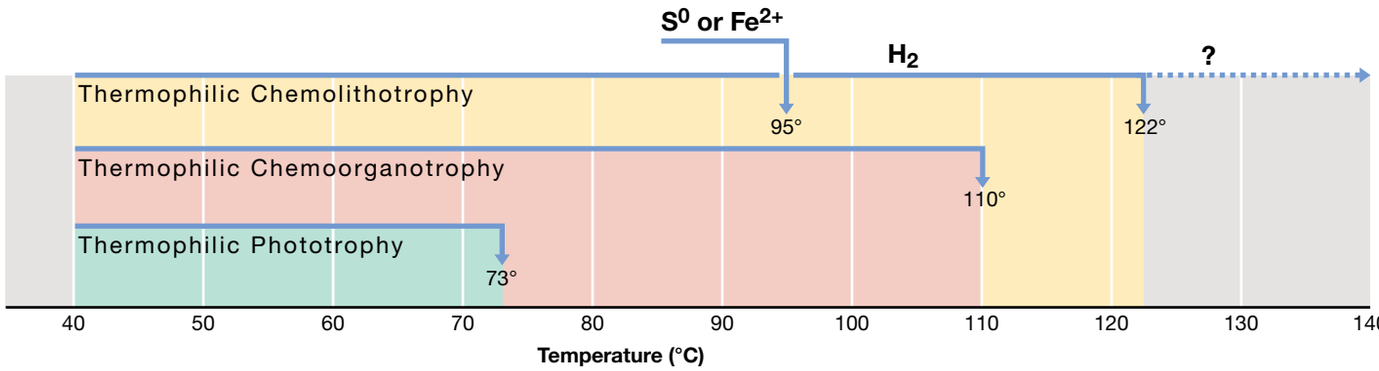


Figure 17.28 Upper temperature limits for energy metabolism. The record holder for phototrophy is *Synechococcus lividus* (*Bacteria*, cyanobacteria); for chemoorganotrophy, *Pyrodictium occultum* (*Archaea*); for chemolithotrophy with S^0 as electron donor, *Acidianus infernus* (*Archaea*); for chemolithotrophy with Fe^{2+} as electron donor, *Ferroglobus placidus* (*Archaea*); and for chemolithotrophy with H_2 as electron donor, *Methanopyrus kandleri* (*Archaea*, 122°C).

hundreds of millions of years after the first life forms are thought to have appeared (Figure 13.1).

Comparisons of bioenergetic options as a function of temperature (Figure 17.28) point to the H_2-oxidizing hyperthermophilic *Archaea* and *Bacteria* as the most likely extant examples of Earth's earliest cellular life forms. More so than any other microbes, these organisms retain the metabolic and physiological traits one would predict to be necessary for existence on a hot early Earth.

MINIQUIZ

- What phylogenetic and physiological evidence suggests that today's hyperthermophiles are the closest living links to Earth's earliest cells?
- Which mechanism of energy conservation is least heat tolerant?
- Which chemolithotrophic lifestyle seems best suited to life at the highest temperatures?

Chapter Review

I • *Euryarchaeota*

17.1 Extremely halophilic *Archaea* require large amounts of NaCl for growth and accumulate large levels of KCl in their cytoplasm as a compatible solute. These salts affect cell wall stability and enzyme activity. The light-mediated proton pump bacteriorhodopsin helps extreme halophiles make ATP.

Q Describe how the light-mediated proton motive force is established in *Halobacterium salinarum*.

17.2 Methanogenic *Archaea* are strict anaerobes whose metabolism is linked to the production of CH_4. Methane can be produced by CO_2 reduction by H_2, from various methyl substrates such as CH_3OH or CH_3NH_2, or from acetate.

Q When acetate is the methanogenic substrate, what are the two products of acetate catabolism?

17.3 *Thermoplasma, Ferroplasma*, and *Picrophilus* are extremely acidophilic thermophiles that form their own phylogenetic family of *Archaea*. Cells of *Thermoplasma* and *Ferroplasma* lack cell walls, resembling the mycoplasmas in this regard.

Q What two major physiological features unify species of *Thermoplasmatales*? Why does this allow some of them to successfully colonize coal refuse piles?

17.4 *Archaeoglobus* and *Ferroglobus* are related anaerobic *Archaea* that carry out different anaerobic respirations. *Archaeoglobus* is a sulfate reducer and *Ferroglobus* is a nitrate reducer that oxidizes ferrous iron.

Q What is physiologically unique about *Ferroglobus*?

II • *Thaumarchaeota, Nanoarchaeota,* and *Korarchaeota*

17.5 *Thaumarchaeota* are widespread and abundant in soils and marine environments. All cultivated species of thaumarchaea are autotrophic ammonia-oxidizers

and these organisms are important in the global nitrogen cycle.

Q What is physiologically unusual about the thaumarchaeotal species *Nitrosopumilus maritimus*?

17.6 *Nanoarchaeum equitans* is a hyperthermophile that forms its own phylum, the *Nanoarchaeota*, and is a parasite of the crenarchaeote *Ignicoccus*. *N. equitans* has a tiny, highly compact genome and depends on *Ignicoccus* for most of its cellular needs, including both carbon and energy.

Q How is *Nanoarchaeum* similar to other *Archaea*? How does it differ?

17.7 *Korarchaeum cryptofilum* forms its own phylum, the *Korarchaeota*, and is a hyperthermophile that lacks important biosynthetic pathways, obtaining key building blocks from its environment. *K. cryptofilum* has some genes that are similar to *Euryarchaeota* and other genes that are similar to *Crenarchaeota*.

Q Why is it difficult to determine the phylogenetic placement of *Nanoarchaeota* and *Korarchaeota*?

III • *Crenarchaeota*

17.8 A wide variety of chemoorganotrophic and chemolithotrophic energy metabolisms have been found in hyperthermophilic *Crenarchaeota*, including fermentation and anaerobic respirations. Strictly autotrophic lifestyles are common but photosynthesis is absent.

Q What forms of energy metabolism are present in *Crenarchaeota*? What form is not present?

17.9 Hyperthermophilic *Crenarchaeota* thrive in terrestrial hot springs of various chemistries. These include in particular organisms such as *Sulfolobus, Acidianus, Thermoproteus*, and *Pyrobaculum*.

Q What is unusual about the metabolism of S^0 by *Acidianus*?

17.10 In deep-sea hydrothermal systems, *Crenarchaeota* such as *Pyrolobus, Pyrodictium, Ignicoccus*, and *Staphylothermus* thrive. With the exception of the methanogen *Methanopyrus* (*Euryarchaeota*), species of these genera grow at the highest temperatures of all *Archaea*, in many cases well above the boiling point of water.

Q What is unusual about the organism *Pyrolobus fumarii*?

IV • Evolution and Life at High Temperature

17.11 Life as we know it is probably limited to temperatures below 150°C. Key small molecules, such as ATP, are quickly destroyed above this temperature.

Q What organism is the current record holder for the upper temperature limit for growth?

17.12 Macromolecules in hyperthermophiles are protected from heat denaturation by their heat-stable folding patterns (proteins), solutes and binding proteins (DNA), unique monolayer membrane architecture (lipids), and the high GC content of their ribosomal RNAs.

Q What is reverse DNA gyrase and why is it important to hyperthermophiles?

17.13 Hydrogen metabolism is likely to have been the driving force behind the energetics of the earliest cells on Earth. Chemolithotrophic metabolisms based on H_2 as an electron donor are found in the most heat-tolerant of all known *Bacteria* and *Archaea*.

Q Why might H_2 metabolism have evolved as a mechanism for energy conservation in the earliest organisms on Earth?

UNIT 4

Application Questions

1. Using the phylogenetic tree in Figure 17.1 as a guide, discuss what indicates that bacteriorhodopsin may have been a late evolutionary invention and that anaerobic respiration with S^0 as electron acceptor might have been an early evolutionary invention.
2. Defend or refute the following statement: The upper temperature limit to life is unrelated to the stability of proteins or nucleic acids.

Chapter Glossary

Bacteriorhodopsin a protein containing retinal that is found in the membranes of certain extremely halophilic *Archaea* and that is involved in light-mediated ATP synthesis

Compatible solute an organic or inorganic substance that is accumulated in the cytoplasm of a halophilic organism and maintains osmotic pressure

Crenarchaeota a phylum of *Archaea* that contains hyperthermophilic organisms

Euryarchaeota a phylum of *Archaea* that contains primarily methanogens, extreme halophiles, *Thermoplasma*, and some marine hyperthermophiles

Extreme halophile an organism whose growth is dependent on large concentrations (generally 9% or more) of NaCl

Extremophile an organism whose growth is dependent on extremes of temperature, salinity, pH, pressure, or radiation, which are generally inhospitable to most forms of life

Halorhodopsin a light-driven chloride pump that accumulates Cl^- within the cytoplasm

Hydrothermal vent a deep-sea hot spring emitting warm (~20°C) to superheated (>300°C) water

Hyperthermophile an organism with a growth temperature optimum of 80°C or greater

Korarchaeota a phylum of *Archaea* that contains the hyperthermophile *Korarchaeum cryptophilum*

Methanogen a CH_4-producing organism

Nanoarchaeota a phylum of *Archaea* that contains the hyperthermophilic parasite *Nanoarchaeum equitans*

Phytanyl a branched-chain hydrocarbon containing 20 carbon atoms and commonly found in the lipids of *Archaea*

Reverse DNA gyrase a protein universally present in hyperthermophiles that introduces positive supercoils into circular DNA

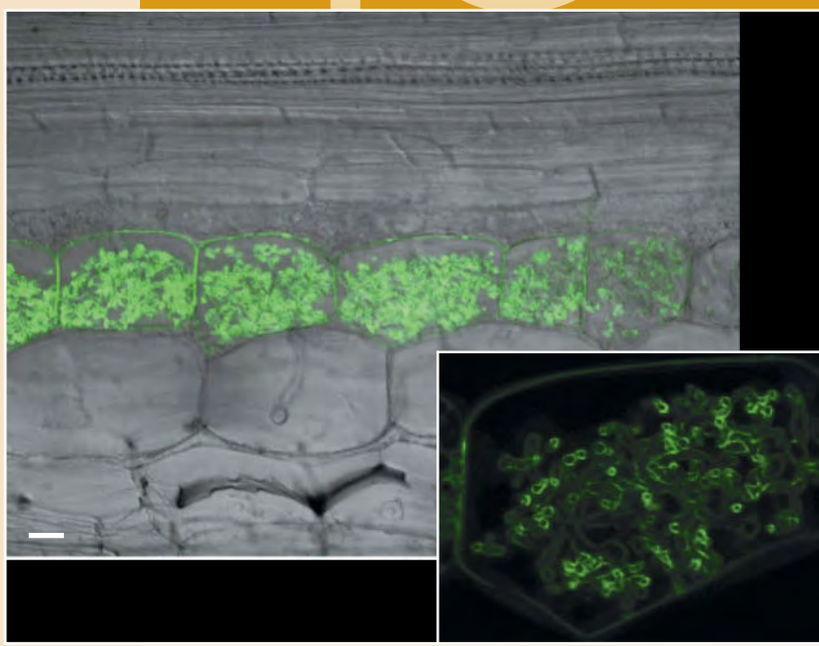
Solfatara a hot, sulfur-rich, generally acidic environment commonly inhabited by hyperthermophilic *Archaea*

Thaumarchaeota a phylum of *Archaea* that contains widespread species capable of aerobic ammonia oxidization

Thermosome a heat shock (chaperone) protein complex that functions to refold partially heat-denatured proteins in hyperthermophiles

18

Diversity of Microbial *Eukarya*



microbiologynow

Arbuscular Mycorrhizal Fungi: Intimate, Unseen, and Powerful

Fungi are common eukaryotic microbes in soil and their role in decomposition is of critical importance to the health of terrestrial ecosystems. Perhaps just as important, though less well recognized, is the role that fungi play in promoting plant productivity.

Mycorrhizae are fungi that form symbiotic associations with plant roots. These fungi acquire carbon and energy from their plant host. In exchange, the mycorrhizal fungi use their extensive mycelial networks to acquire mineral nutrients from the soil and then pass these nutrients along to their plant partners. Arbuscular mycorrhizal fungi (AMF) are one of the most important groups of mycorrhizae. AMF are found within the division *Glomeromycota*, an ancient fungal lineage that diverged early in the history of the fungal kingdom. AMF symbioses are both ancient and intimate. Indeed, fossil evidence suggests that the earliest terrestrial plants formed symbioses with AMF. More than two-thirds of plant species today can form AMF symbioses, including most flowering plants and many important crop species. And they do this for good reason: AMF can increase plant photosynthesis by 20%, a substantial increase in plant fitness.

AMF are obligate biotrophs, symbionts that require a live host. AMF exist as spores in the soil and then germinate and infect their hosts. Upon encountering a plant root, AMF form hyphae that penetrate the root epithelium. The fungi then colonize root cortical cells where they develop elaborately branched structures called arbuscules; the arbuscules are enveloped by a plant membrane that controls nutrient exchange between the plant and the fungus. Arbuscules of the fungus *Rhizophagus irregularis* are shown here within *Medicago truncatula* (barrel clover) cells where they express a green fluorescent marker in the plant cell membrane (the image shows a section of root colonized by AMF with an inset that shows a single cortical cell, scale bar 10 μm).

The 153-megabase genome of *R. irregularis* has been sequenced, revealing at least 23,561 genes. And, as one would expect considering the ecology of this fungus, *R. irregularis* genes encoding nutrient uptake systems are particularly highly expressed. Analyses of AMF, enabled by genomic techniques, may eventually yield discoveries that have tangible effects on agricultural productivity and shed light on the evolutionary origins of land plants.

Source: Tisserant, E., et al. 2013. Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. *PNAS 110:* 20117–20122.

I • Organelles and Phylogeny of Microbial *Eukarya*

In this chapter we consider the phylogeny and diversity of microbial eukaryotes. A tremendous diversity of microorganisms can be found within the domain *Eukarya*. Indeed, eukaryotic microorganisms are far more phylogenetically diverse than their macroscopic relatives. The majority of microbial eukaryotes are protists. **Protists** are *single-celled* eukaryotic microorganisms. While protists are found widely within the *Eukarya*, microbial eukaryotes can also be colonial or multicellular. Many of the microbial eukaryotes that have been discovered have unusual characteristics and lifestyles and we have only begun to describe their extensive diversity.

The exact evolutionary origin of the eukaryotic cell remains a mystery (↻ Section 13.4). It is now clear that the last eukaryotic common ancestor was a single-celled microorganism closely related to *Archaea*. This microorganism had certain features now shared by all eukaryotic cells, including a nucleus, a cytoskeleton, spliceosomes, a genome with spliceosomal introns (that is, those introns processed by spliceosomes), and mitochondria (↻ Sections 2.14–2.16 and 4.6). Once eukaryotic cell structure had evolved, it was remarkably successful and made possible the evolution of diverse microbial lineages in addition to complex multicellular organisms such as plants and animals.

It is clear that endosymbiosis has played a major role in the origin and diversification of *Eukarya*. It is thus fitting to begin our coverage of microbial eukaryotes by reviewing the properties of their organelles—the mitochondrion and the chloroplast—derived from endosymbiosis. The evolutionary history of these energy powerhouses is distinct from that of the eukaryotic cell itself (↻ Sections 2.15 and 13.4). However, once the mitochondrion and chloroplast were established as characteristic features of eukaryotic cells, they exercised a foundational role in the further evolution of *Eukarya*.

18.1 Endosymbioses and the Eukaryotic Cell

Initial speculation on the link between organelles and bacteria goes back over a century and was based on the fact that microscopically, mitochondria and chloroplasts "looked like" bacteria. Through the years this idea slowly gathered experimental support to yield the current view that mitochondria and chloroplasts are ancestors of respiratory or phototrophic *Bacteria*, respectively, that established residence inside another cell type to provide ATP in exchange for a safe and stable existence. This is the **endosymbiotic hypothesis** (↻ Section 13.4) and is a major tenet of modern biology.

Support for the Endosymbiotic Hypothesis

Several lines of evidence support the endosymbiotic hypothesis:

1. **Mitochondria and chloroplasts contain DNA.** Although most mitochondrial and chloroplast proteins are encoded by nuclear DNA, a few are encoded by a small genome residing within the organelle itself (↻ Section 9.4). These include proteins of the respiratory chain (mitochondrion) and photosynthetic apparatus (chloroplast) as well as ribosomal RNAs and transfer RNAs. Most mitochondrial DNA and all chloroplast DNA is of a covalently closed circular form like that of most *Bacteria* (↻ Sections 1.2, 4.2, and 9.3). Organellar DNA can be visualized in eukaryotic cells with special staining methods (**Figure 18.1**).

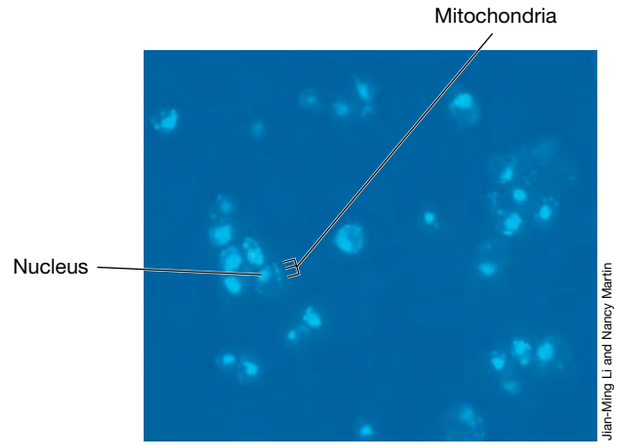


Figure 18.1 Organellar DNA. Cells of the yeast *Saccharomyces cerevisiae* have been stained with the fluorescent dye DAPI that binds to DNA. Each mitochondrion has two to four circular chromosomes that stain blue with the dye.

2. **The eukaryotic nucleus contains genes derived from *Bacteria*.** Genomic sequences of eukaryotic cells (↻ Section 9.4) have clearly shown that several nuclear genes encode functions specific to mitochondria and chloroplasts. Moreover, because these gene sequences more closely resemble those of *Bacteria* than those of *Archaea* or *Eukarya*, it is concluded that these genes were translocated to the nucleus from ancestors of mitochondria and chloroplasts during the transition from engulfed cells to dedicated organelles.
3. **Organellar ribosomes and their phylogeny.** Ribosomes are either 80S in size, typical of the cytoplasm of eukaryotic cells, or 70S, typical of *Bacteria* and *Archaea* (↻ Section 4.10). Mitochondria and chloroplasts contain 70S ribosomes, and phylogenetic analyses of their ribosomal RNA gene sequences (Chapter 13) along with genomic studies of organellar DNA (↻ Section 9.4) show unequivocally that these structures were originally *Bacteria*.
4. **Antibiotic specificity.** Several antibiotics (for example, streptomycin) kill or inhibit *Bacteria* by interrupting the protein synthesis functions of 70S ribosomes. These antibiotics also inhibit protein synthesis in mitochondria and chloroplasts.
5. **Hydrogenosomes.** Hydrogenosomes are membrane-enclosed organelles found in certain anaerobic eukaryotes that lack mitochondria. They supply the cell with ATP from fermentative reactions (↻ Figure 2.49). Like mitochondria, hydrogenosomes also contain their own DNA and ribosomes, and phylogenetic analyses of hydrogenosome ribosomal RNA have revealed their connection to *Bacteria*.

Secondary Endosymbioses

The mitochondrion, chloroplast, and hydrogenosome are structures that originated from *primary* endosymbiosis events. That is, these structures are derived from cells of *Bacteria*. Primary endosymbioses gave rise to the chloroplast in the common ancestor of green algae, red algae, and plants (**Figure 18.2** and see Figure 18.3). However, following this primary event, several unrelated groups of nonphototrophic microbial eukaryotes also acquired chloroplasts but by *secondary* rather than primary endosymbioses. The secondary events occurred when entire green algal or red algal cells were engulfed and their chloroplasts stably retained, thereby making the engulfing cell phototrophic.

Secondary endosymbioses of green algae account for the presence of chloroplasts in euglenids and chlorarachniophytes, while alveolates (ciliates, apicomplexans, and dinoflagellates) and stramenopiles obtained their chloroplasts through secondary endosymbioses with red algae (Figure 18.2 and see Figure 18.3). The ancestral red algal chloroplasts were apparently lost from some lineages, such as the ciliates, or became greatly reduced in size in others, such as the apicomplexans, where only molecular traces of chloroplasts remain. In some other organisms, such as the dinoflagellates, the red algal chloroplast was apparently replaced altogether with a chloroplast from different algae, including green algae.

These many examples of endosymbiotic events underscore the importance of endosymbiosis in the evolution and diversification of microbial eukaryotes. It is unlikely that primary endosymbiotic events occurred only once in evolutionary history—after all, trial and error is the essence of evolution—and secondary endosymbioses almost certainly occurred quite commonly (Figure 18.2). Even today there are many examples of nonphototrophic protists that engulf phototrophic protists, and the entrapped phototrophs carry out photosynthesis for extended periods (Section 23.11). Indeed, it appears that endosymbioses are a common and ongoing occurrence in the eukaryotic world.

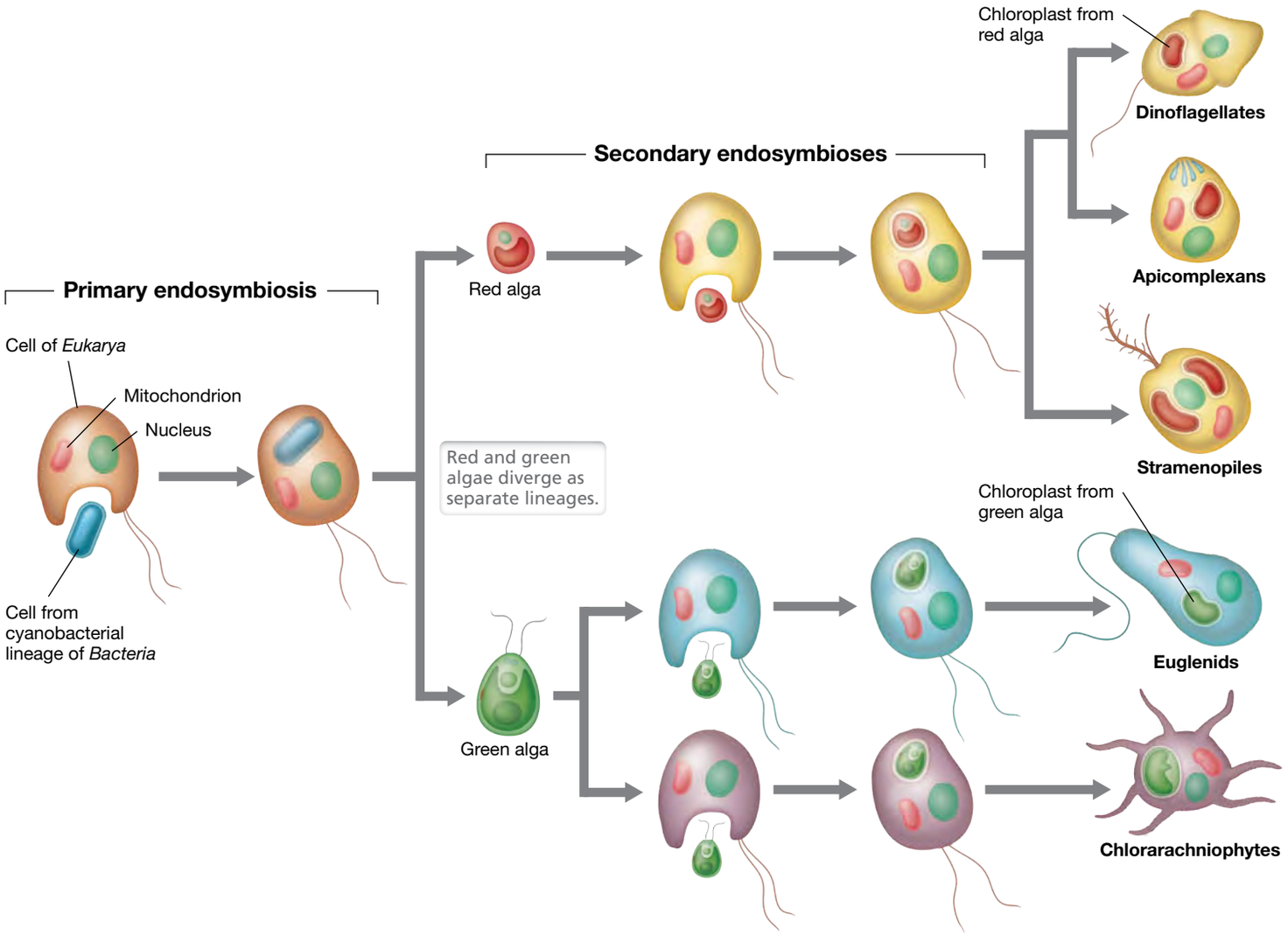


Figure 18.2 Endosymbioses. Following primary endosymbiotic association(s) leading to the mitochondrion, primary endosymbioses with phototrophic *Bacteria* led to the red and green algae. Secondary symbioses of green and red algae spread the property of photosynthesis to many independent lineages of protists.

MINIQUIZ

- What is the endosymbiotic hypothesis?
- Summarize the molecular evidence that supports the relationship of organelles to *Bacteria*.
- Distinguish between primary and secondary endosymbiosis.

18.2 Phylogenetic Lineages of *Eukarya*

Biologists agree that the eukaryotic cell is a genetic chimera. The main part of the eukaryotic cell including its cytoplasm (and likely its nucleus) can be traced to the *Eukarya* domain, whereas its energy-producing organelles—the mitochondria and chloroplasts—contain their own DNA and are clearly derived from *Bacteria* (Section 18.1). Many genes of bacterial and archaeal origin can be found in the nucleus of eukaryotic cells (Section 9.4), suggesting that extensive horizontal gene transfer took place early in the evolution of eukaryotic cells, and many of these gene transfer events are a direct consequence of primary endosymbiosis.

It appears likely that a major phylogenetic radiation took place early in eukaryote evolution, possibly triggered by the endosymbiotic acquisition of mitochondria. All extant *Eukarya* contain mitochondria, structures homologous to mitochondria (for example, hydrogenosomes), or some genetic trace of these structures. The mitochondrion would have provided the early eukaryotic cell with dramatic new metabolic capabilities. What promoted this primary endosymbiotic event is unknown, but quite possibly it was the accumulation of O_2 in the atmosphere from cyanobacterial photosynthesis (Figure 13.2). The ready availability of O_2 would have selected for bacterial cells that could carry out aerobic respiration and favored those eukaryotes that could stably incorporate them and employ them as energy organelles. Somewhat later in evolutionary time, the ancestor of the chloroplast was acquired in another primary endosymbiotic event, with further eukaryotic phototrophic diversity unfolding later through secondary endosymbioses (Section 18.1).

Eukaryotic Evolution: The Big Picture

Although phylogenies based on ribosomal RNA gene sequences (Chapter 13) confirm the three domains of life—*Bacteria, Archaea*, and *Eukarya*—our picture of eukaryotic evolution has changed dramatically with the incorporation of data from whole genome sequences. There are currently six recognized *supergroups* of *Eukarya* (a supergroup is not an official taxon but essentially equivalent to a kingdom in taxonomic hierarchy): *Archaeplastida, Rhizaria, Chromalveolata* (*Stramenopiles* and *Alveolata*), *Excavata, Amoebozoa*, and *Opisthokonta* (**Figure 18.3**).

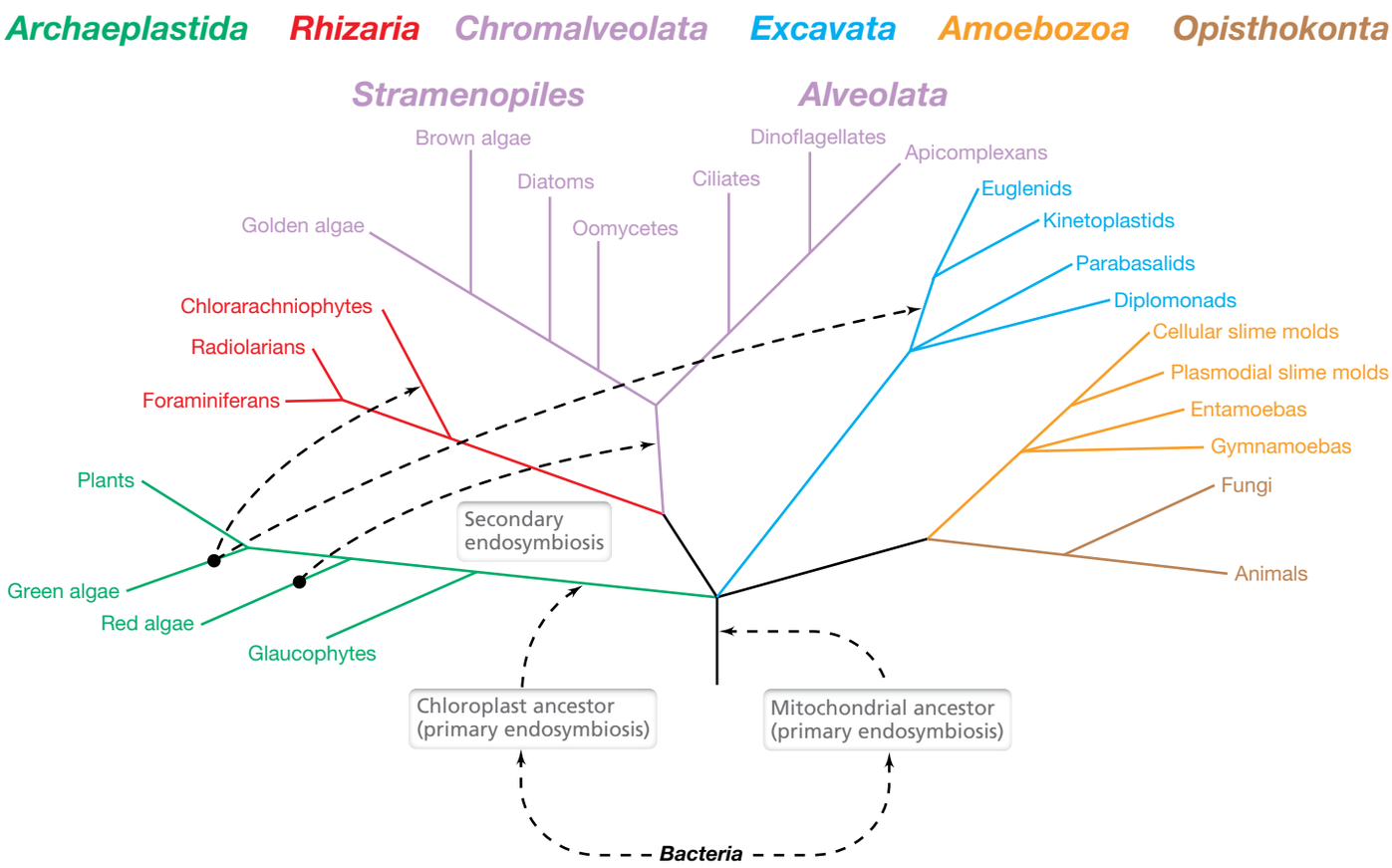


Figure 18.3 Phylogenetic tree of *Eukarya*. The tree diagram provides a schematic view of phylogenetic relationships for six of the best characterized supergroups within the *Eukarya*. Colors are used to identify the supergroups in the tree. Dashed lines indicate both primary endosymbioses of mitochondria and chloroplasts, and the secondary endosymbioses of red and green algae. Protists are common in all eukaryotic lineages except the *Opisthokonta* (*Fungi* and *Animalia*) and the plants.

The *Archaeplastida* include the entire plant kingdom as well as all red and green algae; these photosynthetic lineages resulted from the primary endosymbiosis of the ancestor of all chloroplasts. The *Stramenopiles, Alveolata*, and *Rhizaria* include highly diverse protists including both heterotrophic and phototrophic species. These three groups share an ancestor and they are classified together into a large phylogenetic cluster known as *SAR* (an acronym for *Stramenopiles, Alveolata*, and *Rhizaria*). Where it occurs, phototrophy within these lineages was acquired as a result of secondary endosymbiosis (Figure 18.3 and Section 18.1). The *Excavata* include diverse heterotrophic protists, many of which are anaerobic, and some of which have acquired phototrophy as a result of secondary endosymbiosis (Figure 18.3 and Section 18.1). The *Amoebozoa* include many forms of amoebae and slime molds, though cells with an amoeboid morphology occur in many other lineages of *Eukarya* as well. Finally, the *Opisthokonta* include the well-known kingdoms *Fungi* and *Animalia*.

The phylogenetic tree shown in Figure 18.3 should not be considered the final word on eukaryotic evolution. New genome sequences continue to be determined, new organisms continue to be discovered, and new aspects of eukaryotic biology continue to be revealed. With each new discovery we shed more light on eukaryotic phylogeny and it is likely that our understanding of eukaryotic phylogeny will continue to improve as our knowledge grows.

Phylogenetic Insights on Endosymbiosis

As we have seen, endosymbiosis has clearly been an important aspect of eukaryotic evolution, and the acquisition of the mitochondrion by primitive *Eukarya* was central to the evolutionary success of this domain. However, there are some parasitic microbial eukaryotes such as *Giardia* and *Microsporidia* that lack mitochondria. The *Microsporidia* were once thought to be ancient members of the *Eukarya*, descended from an ancestor of *Eukarya* that lacked mitochondria. We now know, however, that these amitochondriate eukaryotes are descended from eukaryotic ancestors that once had mitochondria (see the position of *Microsporidia* in Figure 18.23) but then lost them for some reason, perhaps while transitioning to an anaerobic lifestyle such as in *Giardia, Entamoeba*, and several other parasitic eukaryotes. However, amitochondriate eukaryotes typically retain a few genes of mitochondrial origin, and these molecular leftovers are strong evidence that the organisms once had mitochondria.

The tree of *Eukarya* (Figure 18.3) also shows how secondary endosymbioses account for the origin of chloroplasts in some unicellular phototrophic eukaryotes. Following primary endosymbiosis of the cyanobacterial ancestor of chloroplasts by early mitochondrion-containing eukaryotes, these now phototrophic eukaryotes diverged into red and green algae. Then, in secondary endosymbioses, ancestors of the euglenids, kinetoplastids, and chlorarachniophytes engulfed green algae while ancestors of the alveolates and stramenopiles engulfed red algae (Section 18.1). These secondary endosymbioses account for the great phylogenetic diversity of phototrophic eukaryotes, which we explore later in this chapter.

MINIQUIZ

- What does the endosymbiotic hypothesis propose?
- How does the composite tree of eukaryotes differ from the ribosomal RNA–based tree?
- How does secondary endosymbiosis help explain the diversity of phototrophic eukaryotes?

II • Protists

With the big picture of eukaryotic cell phylogeny in mind, we proceed to examine the major groups of eukaryotic microorganisms. We begin with protists other than the green and red algae. Protists include both phototrophic and nonphototrophic microbial eukaryotes. These organisms are widely distributed in nature, exhibit a wide range of morphologies, and show great phylogenetic diversity. Indeed, protists are common in all eukaryotic lineages except plants, fungi, and animals (Figure 18.3); thus they represent much of the diversity found in the domain *Eukarya*.

18.3 *Excavata*

KEY GENERA: *Giardia, Trichomonas, Trypanosoma, Euglena*

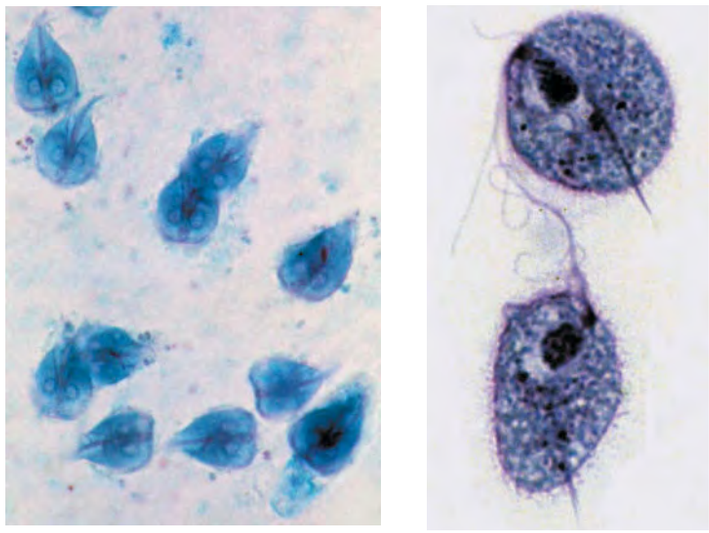
Excavata encompasses diverse protists including both chemoorganotrophs and phototrophs, with some species being anaerobic. We begin with the diplomonads and parabasalids, flagellated protists that lack mitochondria and chloroplasts. These microbes live in anoxic habitats, such as animal intestines, either symbiotically or as parasites, and conserve energy from fermentation. Some diplomonads cause serious and common diseases in fish, domestic animals, and humans, and one parabasalid causes a major sexually transmitted disease of humans. Both groups share a relatively recent common ancestor before they diverged to form separate phylogenetic lineages (Figure 18.3).

Diplomonads

Diplomonads (**Figure 18.4*a***) characteristically contain two nuclei of equal size, and also contain mitosomes, much reduced mitochondria lacking electron transport proteins and enzymes of the citric acid cycle. The diplomonad *Giardia* has a relatively small genome for a eukaryote, about 12 megabase pairs (Mbp). The genome is also quite compact, contains few introns, and lacks genes for many metabolic pathways, including the citric acid cycle (↔ Figure 3.16). These characteristics likely account for the organism's parasitic and anaerobic lifestyle. *Giardia intestinalis* (Figure 18.4*a*), also known as *Giardia lamblia*, causes giardiasis, one of the most common waterborne diarrheal diseases in the United States. We examine the disease giardiasis in Section 33.4.

Parabasalids

Parabasalids contain a *parabasal body* that, among other functions, gives structural support to the cell's Golgi complex. These anaerobic microbial eukaryotes lack mitochondria but contain hydrogenosomes (↔ Section 2.15). Parabasalids live in the intestinal and urogenital tract of vertebrates and invertebrates as parasites or as commensal symbionts (↔ Section 33.4).



(a) (b)

Figure 18.4 Diplomonads and parabasalids. *(a)* Light photomicrograph of cells of *Giardia intestinalis*, a typical diplomonad. Note the dual nuclei. Cells are about 10 μm wide. *(b)* Light photomicrograph of cells of the parabasalid *Trichomonas vaginalis*. Cells are about 6 μm wide. The spearlike structure (axostyle) is used to attach the cell to urogenital tissues.

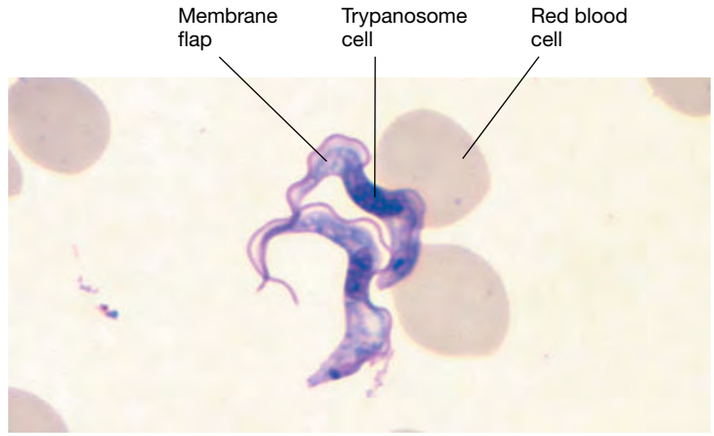


Figure 18.5 Trypanosomes. Photomicrograph of the flagellated kinetoplastids *Trypanosoma brucei*, the causative agent of African sleeping sickness. Blood smear preparation. The cells of *T. brucei* are about 3 μm wide.

The parabasalid *Trichomonas vaginalis* is motile by a tuft of flagella (Figure 18.4*b*) and causes a widespread sexually transmitted disease in humans.

The genomes of parabasalids are unique among eukaryotes in that most of them lack introns, the noncoding sequences characteristic of eukaryotic genes (↔ Sections 4.6 and 9.4). In addition, the genome of *T. vaginalis* is surprisingly huge for a parasitic organism, about 160 Mbp, and shows evidence of genes acquired from bacteria by horizontal gene transfer. Much of the genome of *T. vaginalis* contains repetitive DNA sequences and transposable elements (↔ Section 11.11), which has made genomic analyses difficult. But *Trichomonas* is still thought to contain nearly 60,000 genes, about twice that of the human genome and near the upper limit observed thus far for eukaryotic genomes.

Kinetoplastids

Kinetoplastids are a well-studied group of *Excavata* and are named for the presence of the *kinetoplast*, a mass of DNA present in their single, large mitochondrion. Kinetoplastids live primarily in aquatic habitats, where they feed on bacteria. Some species, however, are parasites of animals and cause serious diseases in humans and vertebrate animals. Cells of *Trypanosoma*, a genus infecting humans, are small, about 20 μm long, thin, and crescent-shaped. Trypanosomes have a single flagellum that originates in a basal body and folds back laterally across the cell where it is enclosed by a flap of cytoplasmic membrane (**Figure 18.5**). Both the flagellum and the membrane participate in propelling the organism, making effective movement possible even in viscous liquids, such as blood, where pathogenic trypanosomes are often found.

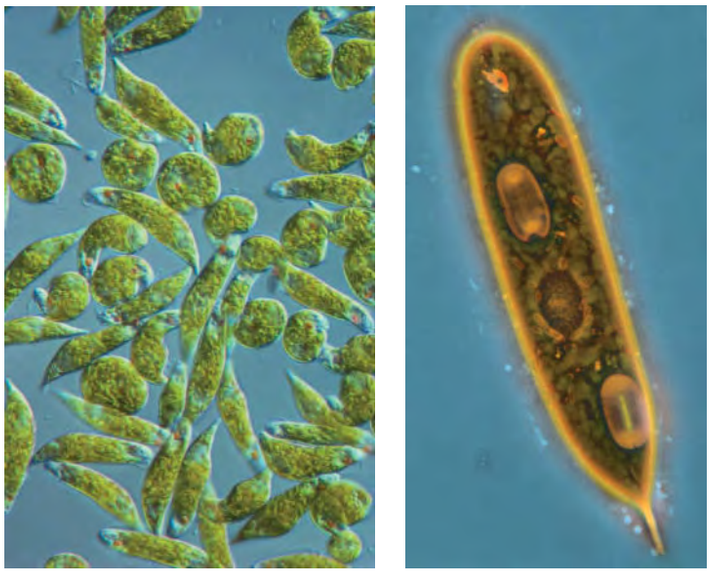
Trypanosoma brucei (Figure 18.5) causes *African sleeping sickness*, a chronic and usually fatal human disease. The parasite lives and grows primarily in the bloodstream, but in the later stages of the disease it invades the central nervous system, causing an inflammation of the brain and spinal cord that is responsible for the characteristic neurological symptoms of the disease. The parasite is transmitted from host to host by the tsetse fly, *Glossina* spp., a bloodsucking fly found only in certain parts of Africa. After moving from the human to the fly in a blood meal, the parasite proliferates in the intestinal tract of the fly and invades the insect's salivary glands and mouthparts, from which it is transferred to a new human host by a fly bite (↔ Section 33.6).

Other kinetoplastids that are human parasites include *Trypanosoma cruzi*, the causative agent of *Chagas disease*, and *Leishmania* species, the causative agents of cutaneous and systemic *leishmaniasis*. Chagas disease is spread by the bite of a blood-feeding insect called the "kissing bug." The disease is usually self-limiting, but it can become chronic and lead to a fatal infection. Leishmaniasis is a disease of tropical and subtropical regions transmitted to humans and other mammals by a bite from the sand fly. This potentially fatal disease can be localized to the skin surrounding a fly bite or can infect the spleen and liver and cause systemic infection. Both Chagas disease and leishmaniasis are covered in more detail in Section 33.6.

Euglenids

Another well-studied group of *Excavata* are the euglenids (**Figure 18.6**). Unlike the kinetoplastids, these motile microbial eukaryotes are nonpathogenic and are both chemotrophic *and* phototrophic. Most euglenids contain two flagella, dorsal and ventral, and their active motility allows the organisms to access both illuminated and dark habitats in their environment to support their alternate nutritional lifestyles.

Euglenids live exclusively in aquatic habitats, both freshwater and marine, and contain chloroplasts, which support phototrophic growth (Figure 18.6). In darkness, however, cells of *Euglena*, a typical euglenid, can lose their chloroplasts and exist as chemoorganotrophs. Many euglenids can also feed on bacterial cells via **phagocytosis**, a process of surrounding a particle with a portion of their flexible cytoplasmic membrane to engulf the particle and bring it into the cell where it is digested.



(a) (b)

Figure 18.6 *Euglena*, a euglenid. *(a)* This phototrophic protist, like other euglenids, is not pathogenic. A cell is about 15 μm wide. *(b)* High-magnification view.

MINIQUIZ

- Contrast the two nutritional options for *Euglena*.
- What diseases are caused by *Trypanosoma cruzi, Leishmania*, and *Giardia*?
- How do diplomonads obtain energy?

18.4 *Alveolata*

KEY GENERA: *Gonyaulax, Plasmodium, Paramecium*

The alveolates as a group are characterized by their *alveoli*, cytoplasmic sacs located just under the cytoplasmic membrane. Although the function of alveoli is unknown, they may help the cell maintain osmotic balance by controlling water influx and efflux, and in the dinoflagellates, they may function as armor plates (see Figure 18.9). Three phylogenetically distinct, although related, kinds of alveolates are known: the *ciliates*, which use cilia for motility; the *dinoflagellates*, which are motile by means of a flagellum; and the *apicomplexans*, which are parasites of humans and other animals (Figure 18.3).

Ciliates

Ciliates possess *cilia* (**Figure 18.7**) at some stage of their life cycle. Cilia are structures that function in motility and may cover the cell or form tufts or rows, depending on the species. Probably the best-known and most widely distributed ciliates are those of the genus *Paramecium* (Figure 18.7). Like many other ciliates, *Paramecium* uses cilia not only for motility but also to obtain food by ingesting particulate materials such as bacterial cells through a distinctive funnel-shaped oral groove. Cilia that line the oral groove move material down the groove to the cell mouth, also called the *gullet* (Figure 18.7*b*). Once in the gullet, the material is enclosed in a vacuole by phagocytosis. Digestive enzymes secreted into the vacuole then break down the material as a source of nutrients.

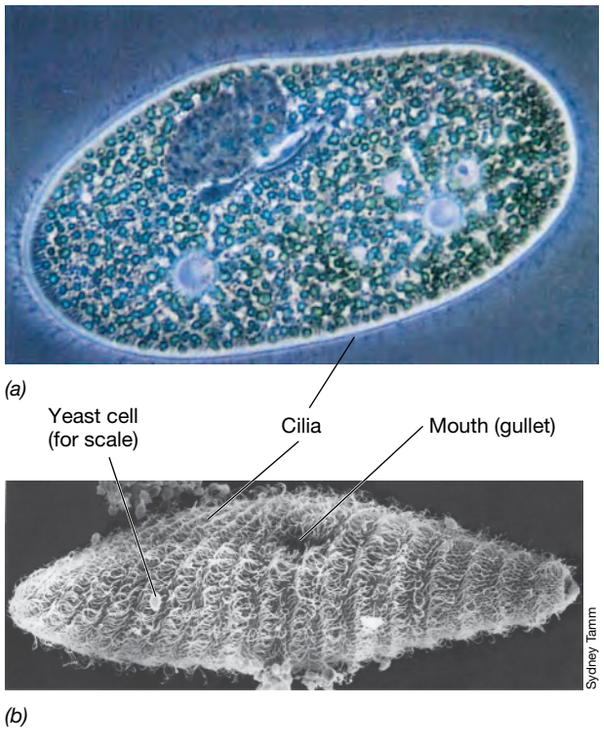


Figure 18.7 *Paramecium*, a ciliated protist. *(a)* Phase-contrast photomicrograph. *(b)* Scanning electron micrograph. Note the cilia in both micrographs. A single *Paramecium* cell is about 60 μm in diameter.

Ciliates are unique among protists in having two kinds of nuclei, *micronuclei* and *macronuclei*. Genes in the macronucleus regulate basic cellular functions, such as growth and feeding, whereas those of the micronucleus are involved in sexual reproduction, which occurs through a partial fusion of two *Paramecium* cells and exchange of micronuclei. The genome of *Paramecium* is huge, with macronuclear genes numbering about 40,000, nearly twice that of humans.

Many *Paramecium* species (as well as many other protists) are hosts for endosymbiotic *Bacteria, Archaea*, or eukaryotes, the latter usually green algae. These organisms may play a nutritional role, synthesizing vitamins or other growth factors used by the host cell. Several anaerobic ciliated protists contain endosymbionts. For example, ciliated protists in the termite hindgut contain endosymbiotic methanogens (*Archaea*) that consume H_2 plus CO_2 to form methane (CH_4). Ciliates themselves can also be symbiotic: Obligately anaerobic ciliates are present in the rumen, the forestomach of ruminant animals, and play an important role in the digestive and fermentative processes of the animal (Section 23.13).

In contrast to symbioses, some ciliates are animal parasites, although this lifestyle is less common in ciliates than in some other groups of protists. The species *Balantidium coli* (**Figure 18.8**), for example, is primarily an intestinal parasite of domestic animals but occasionally infects the intestinal tract of humans, producing dysentery-like symptoms. Cells of *B. coli* form cysts (Figure 18.8) that promote disease transmission in infected food or water.

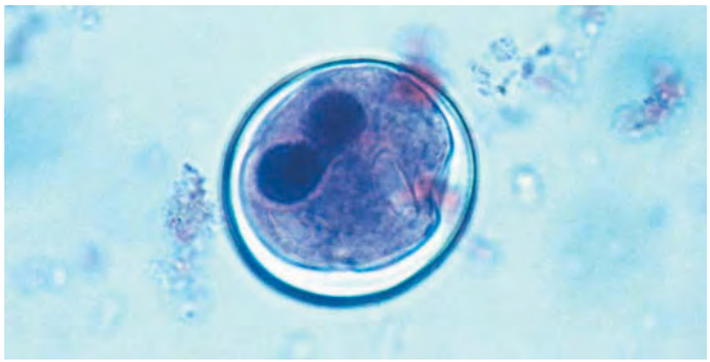
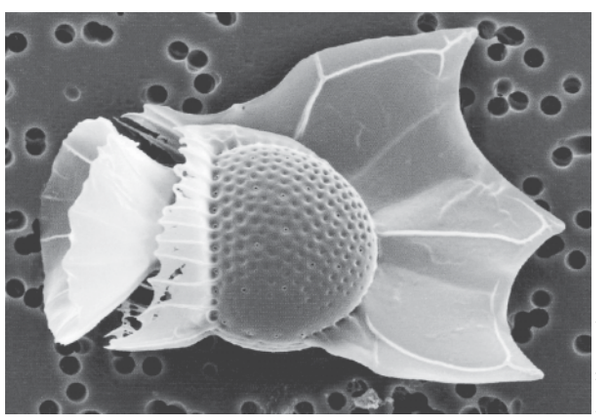


Figure 18.8 *Balantidium coli*, a ciliated protist that causes a dysentery-like disease in humans. The dark blue–stained lobed structure in this *B. coli* cyst obtained from swine intestine is a dividing macronucleus. The cell is about 50 μm wide.

Dinoflagellates

Dinoflagellates are a diverse group of marine and freshwater phototrophic alveolates (**Figure 18.9**) that acquired the capacity to photosynthesize through secondary endosymbioses (Figures 18.2 and 18.3). Flagella encircling the cell impart spinning movements that give dinoflagellates their name (*dinos* is Greek for "whirling"). Dinoflagellates have two flagella of different lengths and with different points of insertion into the cell, transverse and longitudinal. The transverse flagellum is attached laterally, whereas the longitudinal flagellum originates from the lateral groove of the cell and extends lengthwise (see Figure 18.10*b*). Some dinoflagellates are free-living, whereas others live a symbiotic existence with animals that form coral reefs, obtaining a sheltered and protected habitat in exchange for supplying phototrophically fixed carbon as a food source for the reef. A number of free-living species are capable of bioluminescence and emit light when disturbed at night; this bioluminescence results in a "sparkling" effect that can often be observed in coastal seas and bioluminescent bays.

Several species of dinoflagellates are toxic. For example, dense suspensions of *Gonyaulax* cells, called "red tides" (**Figure 18.10*a***) because of the red-colored pigments of this organism, can form in warm and typically polluted coastal waters. Such blooms are often

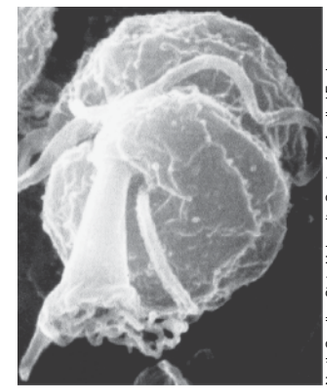


Irena Kaczmarska

Figure 18.9 The marine dinoflagellate *Ornithocercus magnificus* (an alveolate). The cell proper is the globular central structure; the attached ornate structures are called *lists*. A cell is about 30 μm wide.

Rita R. Colwell

(a)



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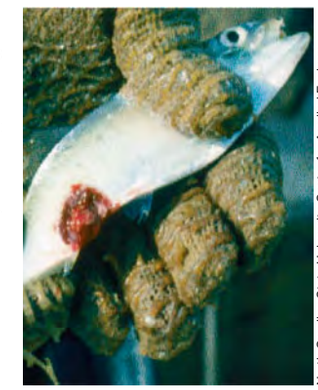

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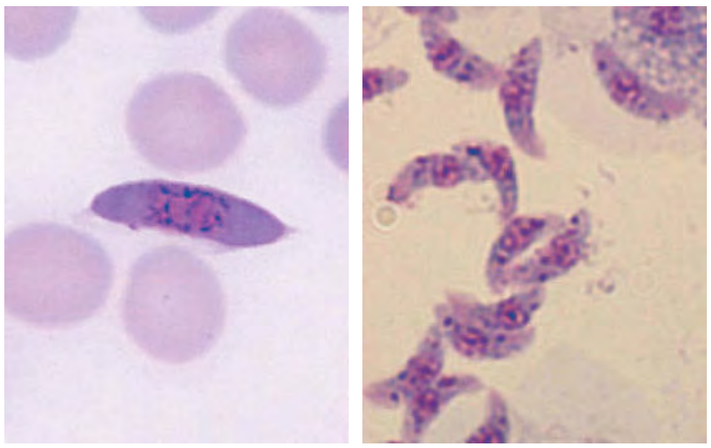
(c)

Figure 18.10 Toxic dinoflagellates (alveolates). *(a)* Photograph of a "red tide" caused by massive growth of toxin-producing dinoflagellates such as *Gonyaulax*. The toxin is excreted into the water and also accumulates in shellfish that feed on the dinoflagellates. *(b)* Scanning electron micrograph of a toxic spore of *Pfiesteria piscicida*; the structure is about 12 μm wide. *(c)* A fish killed by *P. piscicida*; note the lesions of decaying flesh.

associated with fish kills and poisoning in humans following consumption of mussels that have accumulated *Gonyaulax* through filter feeding. Toxicity results from a neurotoxin that can cause a condition called *paralytic shellfish poisoning* in humans and some marine animals, such as sea otters. Symptoms include numbness of the lips, dizziness, and difficulty breathing; in severe cases, death can result from respiratory failure. *Pfiesteria* is another toxic dinoflagellate. Toxic spores of *Pfiesteria piscicida* (Figure 18.10*b*) infect fish and eventually kill them by way of neurotoxins that affect movement and destroy skin. Lesions form on areas of the fish, allowing opportunistic bacterial pathogens to grow (Figure 18.10*c*). Symptoms of human toxemia from *Pfiesteria* poisoning include skin rashes and respiratory problems.

Apicomplexans

Apicomplexans are nonphototrophic obligate parasites that cause severe human diseases such as malaria (*Plasmodium* species, **Figure 18.11*a***), toxoplasmosis (*Toxoplasma*, Figure 18.11*b*), and coccidiosis (*Eimeria*). These organisms are characterized by nonmotile adult stages, and nutrients are taken up in soluble form across the cytoplasmic membrane as in bacteria and fungi.



(a) (b)

Figure 18.11 Apicomplexans. *(a)* A gametocyte of *Plasmodium falciparum* in a blood smear. The gametocyte is the stage in the malarial parasite life cycle that infects the mosquito vector. *(b)* Sporozoites of *Toxoplasma gondii*.

Apicomplexans produce structures called *sporozoites* (Figure 18.11*b*), which function in transmission of the parasite to a new host, and the name apicomplexan derives from the presence at one apex of the sporozoite of a complex of organelles that penetrate host cells. Apicomplexans also contain *apicoplasts*. These are degenerate chloroplasts that lack pigments and photosynthetic capacity but contain a few of their own genes. Apicoplasts catalyze fatty acid, isoprenoid, and heme biosyntheses, and export their products to the cytoplasm. It is hypothesized that apicoplasts are derived from red algal cells engulfed by apicomplexans in a secondary endosymbiosis (Figures 18.2 and 18.3). Over time, the chloroplast of the red algal cell degenerated to play a nonphototrophic role in the apicomplexan cell.

Both vertebrates and invertebrates can be hosts for apicomplexans. In some cases, an alternation of hosts takes place, with some stages of the life cycle linked to one host and some to another. Important apicomplexans are the coccidia, which are typically parasites of birds, and species of *Plasmodium* (malarial parasites) (Figure 18.11*a*). We reserve detailed discussion of malaria—a disease that throughout the course of history has killed more humans than any other disease—for Section 33.5.

MINIQUIZ

- How does the organism *Paramecium* move?
- What health problem is associated with the organism *Gonyaulax*?
- What are apicoplasts, which organisms have them, and which functions do they carry out?

18.5 *Stramenopiles*

KEY GENERA: *Phytophthora, Nitzschia, Ochromonas, Macrocystis*

The *Stramenopiles* include both chemoorganotrophic and phototrophic microorganisms as well as macroorganisms. Members of this group bear flagella with many short, hairlike extensions (Figure 18.2), and this morphological feature gives the group its name (from Latin *stramen* for "straw" and *pilus* for "hair"). The diatoms, oomycetes, golden algae, and brown algae are the major groups of *Stramenopiles* (Figure 18.3).

Diatoms

Diatoms include over 200 genera of unicellular, phototrophic, microbial eukaryotes, and are major components of the planktonic (suspended) phytoplankton microbial community in marine and freshwaters. Diatoms characteristically produce a cell wall made of silica to which protein and polysaccharide are added. The wall, which protects the cell against predation, exhibits widely different shapes in different species and can be highly ornate (**Figure 18.12**). The external structure formed by this wall, called a *frustule*, often remains after the cell dies and the organic materials have disappeared. Diatom frustules typically show morphological symmetry, including *pinnate symmetry* (having similar parts arranged on opposite sides of an axis, as in the common diatom *Nitzschia*, Figure 18.12*b*), and *radial symmetry*, as in the marine diatoms *Thalassiosira* and *Asterolampra* (Figure 18.12*c*, *d*). Because the diatom frustules, which are composed mainly of silica, are resistant to decay, these structures can remain intact for long periods of time and often sink and remain in the sediments for millions of years. Diatom frustules constitute some of the best-known unicellular eukaryotic fossils, and from dating of frustule samples, it has been shown that diatoms first appeared on Earth relatively recently, about 200 million years ago.

Oomycetes

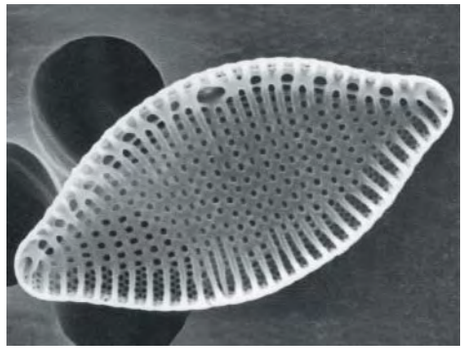
The oomycetes, also called *water molds*, were previously grouped with fungi based on their filamentous growth and the presence of **coenocytic** (that is, multinucleate) hyphae, morphological traits characteristic of fungi (Section 18.8). Phylogenetically, however, the oomycetes are distant from fungi and are closely related to other *Stramenopiles* (Figure 18.3). Oomycetes differ from fungi in other fundamental ways, as well. For example, the cell walls of oomycetes are typically made of cellulose instead of the chitin cell walls of fungi, and the water molds have flagellated cells, which are lacking in all but a few fungi. Nonetheless, oomycetes are ecologically similar to fungi in that they grow as a mass of hyphae decomposing dead plant and animal material in aquatic habitats.

Oomycetes have had a major impact on human society, as many species are plant pathogens (phytopathogens). The oomycete *Phytophthora infestans*, which causes late blight disease of potatoes, contributed to massive famines in Ireland in the mid-nineteenth century. The famines led to the death of a million Irish and triggered great waves of Irish immigration to North America. Other major phytopathogens include *Pythium*, a common pathogen of greenhouse seedlings, and *Albugo*, which causes "white rusts" on several agricultural crops.

Golden Algae and Brown Algae

Along with the diatoms, golden and brown algae form major lineages of *Stramenopiles*. Golden algae, also called *chrysophytes*, are primarily unicellular marine and freshwater phototrophs. Some

(a)



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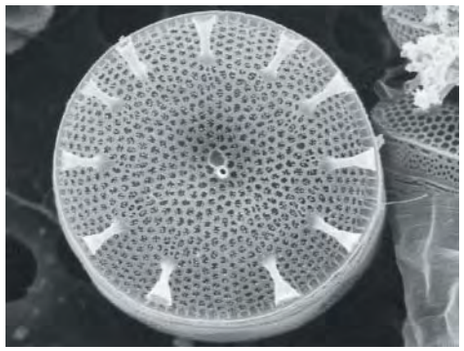
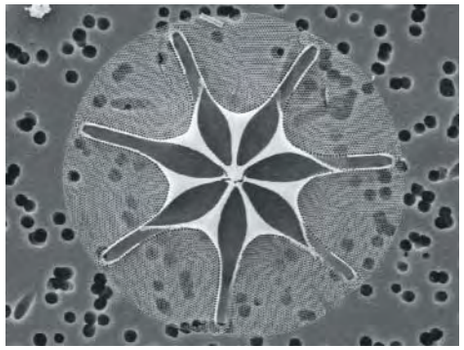


(c)



(d)

Figure 18.12 Diatom frustules. *(a)* Dark-field photomicrograph of a collage of frustules from different diatom species showing various forms of symmetry. *(b–d)* Scanning electron micrographs of diatom frustules showing pinnate (part *b*) or radial (parts *c*, *d*) symmetry. Diatoms vary considerably in size from very small species about 5 μm wide to larger species up to 200 μm wide.

species are chemoorganotrophs and feed either by phagocytosis or by transporting soluble organic compounds across the cytoplasmic membrane. Some golden algae, such as *Dinobryon* (**Figure 18.13*a***), found in freshwater, are colonial. However, most golden algae are unicellular and motile by the activity of two flagella of unequal length.

Golden algae are so named because of their golden-brown color (Figure 18.13*a*, *c*). This is due to chloroplast pigments dominated by the brown-colored carotenoid fucoxanthin. The major chlorophyll pigment in golden algae is chlorophyll *c* rather than chlorophyll *a*, and they lack the phycobiliproteins present in red algal chloroplasts (Section 18.14). Cells of the unicellular golden alga *Ochromonas*, the best-studied genus of this group, have only one or two chloroplasts (Figure 18.13*c*).

Brown algae are primarily marine and are multicellular and typically macroscopic. No unicellular brown algae are known. The kelps, such as the giant kelp *Macrocystis* (Figure 18.13*b*), which can grow up to 50 m in length, are perhaps the most widespread of brown algae. *Fucus*, another common seaweed of intertidal regions, can grow up to 2 m. As their name implies, brown algae are brown or green-brown in color depending on how much of the carotenoid pigment fucoxanthin they produce. Most marine "seaweeds" are brown algae and their rapid growth, especially in cold marine waters, can cause nuisance odor problems when they wash ashore and decay.

(a) (b) (c)

Figure 18.13 Golden and brown algae. *(a) Dinobryon*, a golden alga (family *Chrysophyceae*) that forms branched colonies. *(b) Macrocystis*, a marine kelp belonging to the brown algae (family *Phaeophyceae*). *(c) Ochromonas*, a unicellular chrysophyte. The golden or brown color of the chloroplasts of these algae is due to the pigment fucoxanthin.

MINIQUIZ

- What structure of diatoms accounts for their excellent fossil record?
- In what ways do oomycetes differ from and resemble fungi?
- Which chlorophyll pigment is found in golden and brown algae?

18.6 *Rhizaria*

Rhizaria form a diverse group of protists that includes the *Chlorarachniophyta, Radiolaria*, and *Foraminifera* (Figure 18.3). They are distinguished from other protists by their threadlike cytoplasmic extrusions (pseudopodia) by which they move and feed. Some *Rhizaria* have amoeboid morphology and were once mistakenly classified as *amoebae* because of this and their pseudopodia, but it is now known that many phylogenetically diverse organisms employ pseudopodia for motility and feeding purposes.

Chlorarachniophyta

Chlorarachniophytes are freshwater and marine amoeba-like phototrophs that develop a flagellum for dispersal. The group's acquisition of green algal chloroplasts is a prime example of a secondary endosymbiosis (Figure 18.2) and shows how extensively this process has molded several phylogenetically distinct lineages of microbial eukaryotes (Figure 18.3).

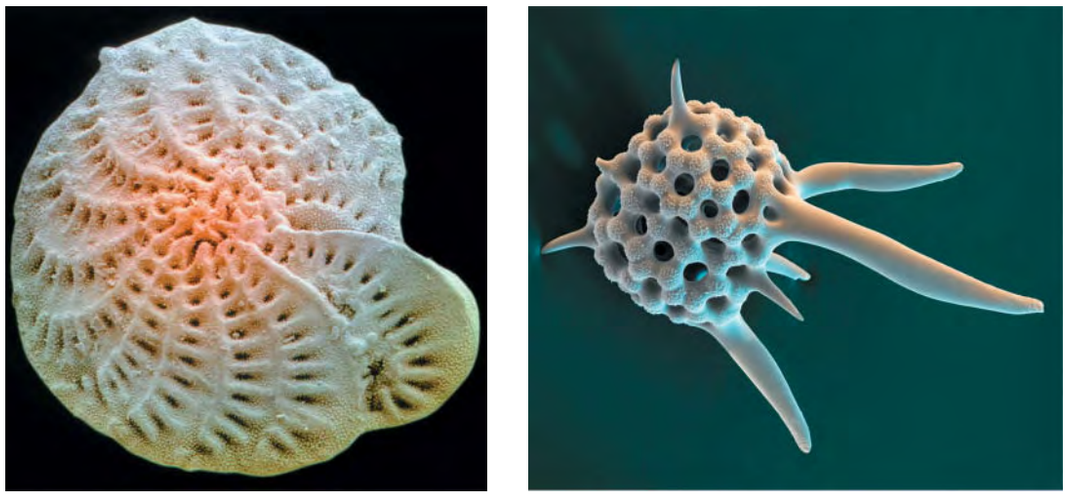
Chloroplasts typically have two membranes, derived from the inner and outer membranes of *Cyanobacteria*, which have a gram-negative cell envelope (Section 2.5). Chloroplasts in chlorarachniophytes, however, have four membranes. In addition, they have **nucleomorphs** tucked in between the two sets of chloroplast membranes. Nucleomorphs are remnant nuclei left over from when the algal endosymbionts were acquired during secondary endosymbiosis (Section 18.1). Both the nucleomorphs and the extra membranes were derived from this algal endosymbiont (Figure 18.2).

It is a testament to endosymbiosis to consider that chlorarachniophytes have merged a minimum of five different genomes: the host genome, the host mitochondrial genome, the algal endosymbiont genome (which has become the nucleomorph), the algal endosymbiont mitochondrial genome, and the algal endosymbiont chloroplast genome! Most nonessential or duplicate genes have been deleted over time and many genes from the mitochondria and chloroplasts have been transferred to the host genome. As a result, the majority of genes in a chlorarachniophyte are present in the nucleus of the host cell. The nucleomorph itself is greatly reduced in size relative to its ancestor and over time may be lost completely from the chloroplast.

Foraminifera

In contrast to chlorarachniophytes, foraminiferans are exclusively marine microbes and form shell-like structures called *tests*, which have distinctive characteristics and are often quite ornate (**Figure 18.14a**). Tests are typically made of organic materials reinforced with calcium carbonate. The test is not firmly attached to the cell, and the amoeba-like cell may extend partway out of the test during feeding. However, because of the weight of the test, the cell usually sinks to the bottom of the water column, and it is thought that the organisms feed on dissolved organic matter and particulate deposits, primarily bacteria, other protists, and the remains of dead organisms near the sediments. Foraminiferan cells can also host a variety of algae that form endosymbiotic relationships with the protist and supply it with organic carbon, probably in exchange for inorganic nutrients derived from the breakdown of dead organisms. Phototrophs are found primarily in planktonic foraminifera that remain suspended in the water column to provide their endosymbionts with sufficient sunlight.

Foraminiferan tests (Figure 18.14*a*) are relatively resistant to decay and are readily fossilized. These buried and preserved tests are quite useful to geologists. Because particular taxa of foraminifera are typically associated with particular strata in the geological record, fossilized foraminiferan tests in samples obtained from exploratory wells are used by oil industry paleontologists as a means to date and assess the petroleum potential of a given drill site.



(a) (b)

Figure 18.14 Foraminifera and radiolaria. *(a)* A foraminiferan. Note the ornate and multilobed test. The test is about 1 mm wide. *(b)* A spiked radiolarian of the *Nassellaria* group. A test is about 150 µm wide. Both *a* and *b* are colorized scanning electron micrographs.

Radiolaria

Radiolarians are chemoorganotrophic and mostly planktonic marine eukaryotes that reside in the upper 100 m of the ocean where they consume bacteria and particulate organic matter. Some species associate with algae that take on a symbiotic (but not endosymbiotic) role and supply nutrients to the radiolarian.

The name "radiolarian" comes from the radial symmetry of their tests, transparent or translucent mineral skeletons made of silica in one fused piece (Figure 18.14*b*). Along with the accumulation of lipid droplets and large cytoplasmic vacuoles, the needle-like pseudopodia of radiolarians probably help keep the organisms from sinking in their mainly open ocean (planktonic) habitats. However, when cells eventually die, their tests settle to the ocean floor and can build up over time into thick layers of slowly decaying cell material.

MINIQUIZ

- What structure distinguishes rhizaria from all other protists?
- How are chlorarachniophytes thought to have acquired the ability to photosynthesize?

18.7 *Amoebozoa*

KEY GENERA: *Amoeba, Entamoeba, Physarum, Dictyostelium*

The *Amoebozoa* are a large group of terrestrial and aquatic protists that use lobe-shaped pseudopodia for movement and feeding, in contrast to the threadlike pseudopodia of rhizaria. The major groups of *Amoebozoa* are the *gymnamoebas*, the *entamoebas*, and the *plasmodial* and *cellular slime molds*. Phylogenetically, the *Amoebozoa* diverged from a lineage that eventually led to the fungi and animals (Figure 18.3).

Gymnamoebas and Entamoebas

The gymnamoebas are free-living protists that inhabit aquatic and soil environments. They use pseudopodia to move by a process called *amoeboid movement* (Figure 18.15) and feed by phagocytosis on bacteria, other protists, and particulate organic materials. Amoeboid movement results from streaming of the cytoplasm as it flows forward at the less contracted and viscous cell tip, taking the path of least resistance. Cytoplasmic streaming is facilitated by microfilaments (Section 2.16), which exist in a thin layer just beneath the cytoplasmic membrane. *Amoeba* (Figure 18.15) is a common organism in pond waters, with species varying in size from 15 µm in diameter (clearly microscopic) to over 750 µm (visible with the naked eye).

In contrast to gymnamoebas, the entamoebas are parasites of vertebrates and invertebrates. Their usual habitat is the oral cavity or intestinal tract of animals. The anaerobe *Entamoeba histolytica* is pathogenic in humans and causes amebic dysentery, an ulceration of the intestinal tract that results in bloody diarrhea. This parasite forms cysts that are transmitted from person to person by fecal contamination of water, food, and eating utensils. In Section 33.3 we discuss the etiology and pathogenesis of amebic dysentery, an important cause of death from intestinal parasites in humans.

Slime Molds

The **slime molds** were previously grouped with fungi since they undergo similar life cycles and produce fruiting bodies with spores for dispersal. As protists, however, slime molds are motile and can move across a solid surface fairly quickly (see Figures 18.16–18.18). Slime molds are divided into two groups: *plasmodial slime molds*

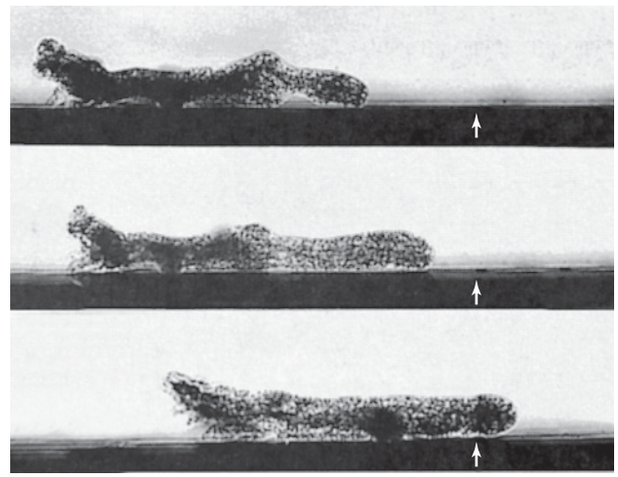


Figure 18.15 Time-lapse view of the amoebozoan *Amoeba proteus*. The time interval from top to bottom is about 6 sec. The arrows point to a fixed spot on the surface. A single cell is about 80 µm wide.

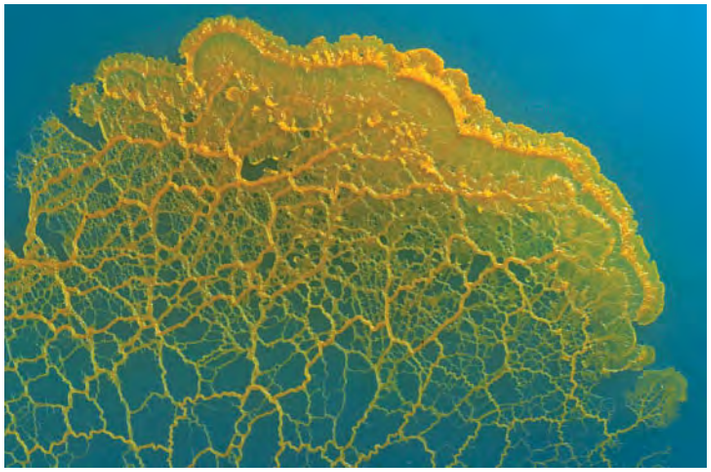


Figure 18.16 Slime mold. The plasmodial slime mold *Physarum* growing on an agar surface. The plasmodium is about 5 cm long and 3.5 cm wide.

(also called *acellular slime molds*), whose vegetative forms are masses of protoplasm of indefinite size and shape called plasmodia (**Figure 18.16**), and *cellular slime molds*, whose vegetative forms are single amoebae. Slime molds live primarily on decaying plant matter, such as leaf litter, logs, and soil, where they consume other microorganisms, especially bacteria. Slime molds can maintain themselves in a vegetative state for long periods but eventually form differentiated sporelike structures that can remain dormant and then germinate later to once again generate the active amoeboid state.

Plasmodial slime molds, such as *Physarum*, exist in the vegetative phase as an expanding single mass of protoplasm called the *plasmodium* that contains many diploid nuclei (Figure 18.16). The plasmodium is actively motile by amoeboid movement, and from this phase, a sporangium containing haploid spores can be produced; when conditions are favorable, the spores germinate to yield haploid flagellated swarm cells. The fusion of two swarm cells then regenerates a diploid plasmodium.

In contrast to their plasmodial relatives, cellular slime molds are individual haploid cells and form diploids only under certain conditions. The well-studied cellular slime mold *Dictyostelium discoideum* undergoes an asexual life cycle in which vegetative cells aggregate, migrate as a cell mass, and eventually produce fruiting bodies in which cells differentiate and form spores (**Figures 18.17** and **18.18**). When cells of *Dictyostelium* are starved, they aggregate and form a pseudoplasmodium; in this stage cells lose their individuality, but do not fuse. Aggregation is triggered by the production of cyclic adenosine monophosphate (cAMP). The first cells of *Dictyostelium* that produce this compound attract neighboring cells and eventually aggregate into motile masses of cells called *slugs*. Fruiting body formation begins when the slug becomes stationary and vertically oriented. The emerging structure differentiates into

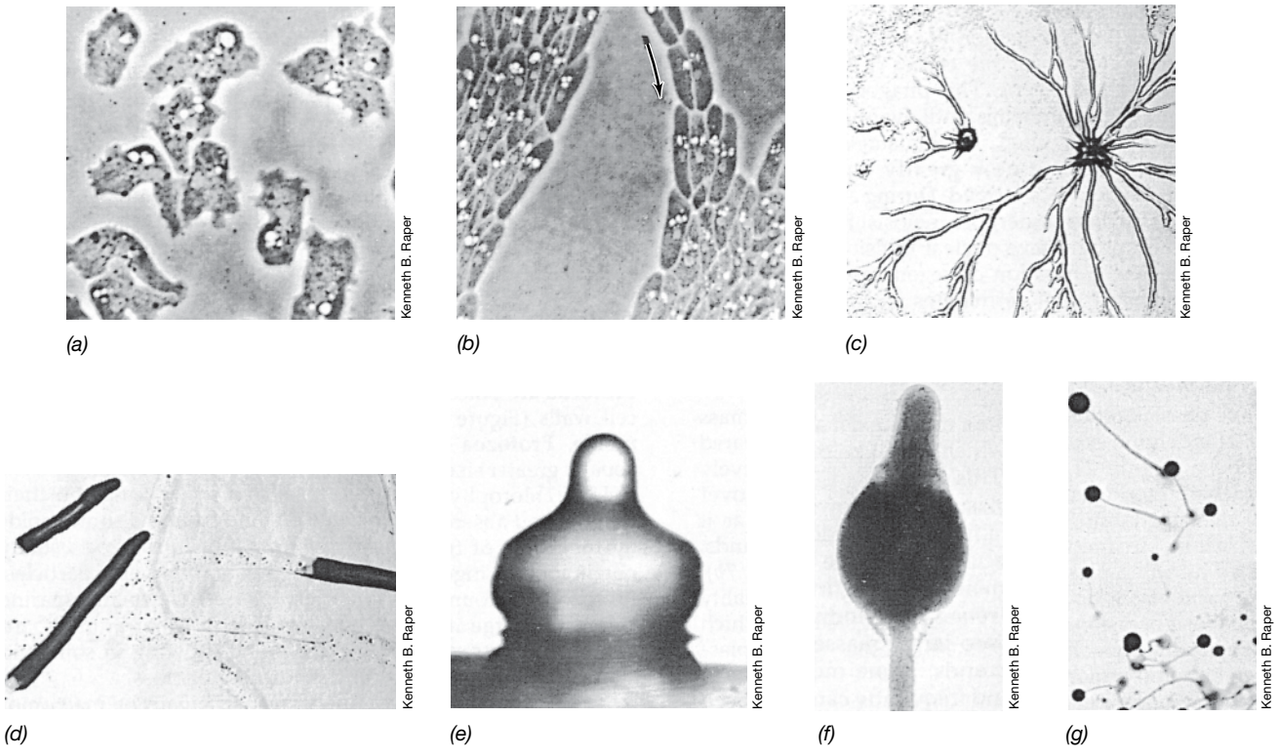


Figure 18.17 Photomicrographs of various stages in the life cycle of the cellular slime mold *Dictyostelium discoideum*. *(a)* Amoebae in preaggregation stage. *(b)* Aggregating amoebae. Amoebae are about 300 μm in diameter. *(c)* Low-power view of aggregating amoebae. *(d)* Migrating pseudoplasmodia (slugs) moving on an agar surface and leaving trails of slime behind. *(e, f)* Early stages of fruiting body. *(g)* Mature fruiting bodies. Figure 18.18 shows the sizes of these structures. *Dictyostelium* has long served as a model for development in multicellular organisms, and its genome of 12,500 genes is about half that of the human genome.

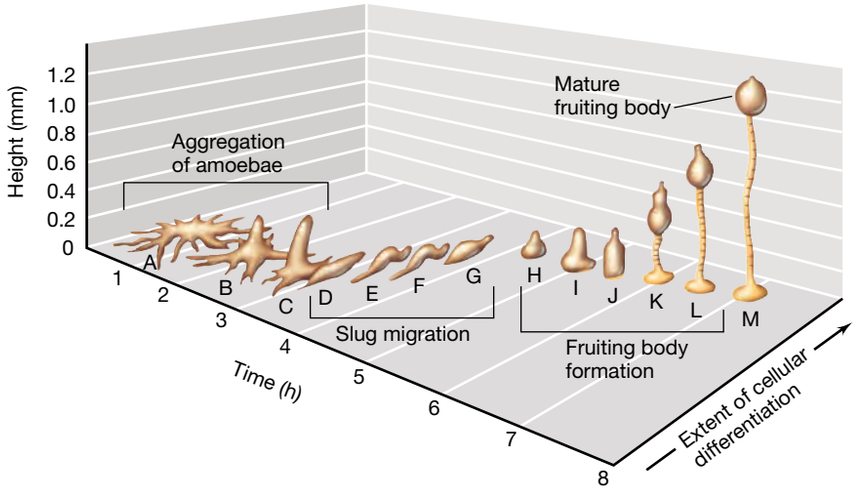


Figure 18.18 Stages in fruiting body formation in the cellular slime mold *Dictyostelium discoideum*. *(A–C)* Aggregation of amoebae. *(D–G)* Migration of the slug formed from aggregated amoebae. *(H–I)* Culmination of migration and formation of the fruiting body. *(M)* Mature fruiting body composed of stalk and head. Cells from the rear of the slug form the head and become spores. *Dictyostelium* also undergoes sexual reproduction (not shown) when two amoebae fuse to form a macrocyst; the fused nuclei in the macrocyst return to the haploid stage when meiosis forms new vegetative amoebae.

a stalk and a head. The stalk cells form cellulose, which provides the rigidity of the stalk, and the head cells differentiate into spores. Eventually, spores are released and dispersed, with each spore forming a new amoeba (Figures 18.17 and 18.18).

In addition to this asexual process, *Dictyostelium* can produce sexual spores. These form when two amoebae in an aggregate fuse to form a single giant amoeba. A thick cellulose wall develops around this cell to form a structure called the *macrocyst*, and this can remain dormant for long periods. Eventually, the diploid nucleus undergoes meiosis to form haploid nuclei that become integrated into new amoebae that can once again initiate the asexual cycle.

MINIQUIZ

- How can amoebozoans be distinguished from rhizaria?
- Compare and contrast the lifestyles of gymnamoebas and entamoebas.
- Describe the major steps in the life cycle of *Dictyostelium discoideum*.

III • Fungi

The **Fungi** are a large, diverse, and widespread group of organisms that includes such well-known groups as the *molds, mushrooms*, and *yeasts*. Approximately 100,000 fungal species have been described, and as many as 1.5 million species may exist. Fungi form a phylogenetic cluster distinct from other protists and are the microbial group most closely related to animals (Figure 18.3).

Most fungal species are microscopic and terrestrial. They inhabit soil or dead plant matter and play crucial roles in the mineralization of organic carbon. A large number of fungal species are plant pathogens, and a few cause diseases of animals, including humans. Certain species of fungi also establish symbiotic associations with plants, facilitating the plant's acquisition of minerals from soil, and many fungi benefit humans through fermentation and the synthesis of antibiotics.

18.8 Fungal Physiology, Structure, and Symbioses

In this section we describe some general features of fungi, including their physiology, cell structure, and the symbiotic associations they develop with plants and animals. In the following section we examine fungal reproduction and phylogeny.

Nutrition, Physiology, and Ecology

Fungi are chemoorganotrophs—typically displaying simple nutritional requirements—and most are aerobic. Fungi feed by secreting extracellular enzymes that digest polymeric materials, such as polysaccharides or proteins, into monomers that are assimilated as sources of carbon and energy. As decomposers, fungi digest dead animal and plant materials. As parasites of plants or animals, fungi use the same mode of nutrition but take up nutrients from the living cells of the plants and animals they invade rather than from dead organic materials.

A major ecological activity of fungi, especially the basidiomycetes, is the decomposition of wood, paper, cloth, and other products derived from these natural sources. Lignin, a complex polymer in which the building blocks are phenolic compounds, is an important constituent of woody plants, and in association with cellulose it confers rigidity on them. Lignin is decomposed in nature almost exclusively through the activities of certain basidiomycetes called *wood-rotting fungi*. Two types of wood rot are known: *brown rot*, in which the cellulose is attacked preferentially and the lignin left unmetabolized, and *white rot*, in which both cellulose and lignin are decomposed. The white rot fungi are of major ecological importance because they play such a key role in decomposing woody materials in forests.

Fungal Morphology, Spores, and Cell Walls

Most fungi are multicellular, forming a network of filaments called *hyphae* (singular, hypha) from which asexual spores are produced (Figure 18.19). Hyphae are tubular cell walls that surround the cytoplasmic membrane. Fungal hyphae are often septate, with cross-walls dividing each hypha into separate cells. In some cases, however, the vegetative cell of a fungal hypha contains more than one nucleus, and hundreds of nuclei can form as a result of repeated nuclear divisions without the formation of cross-walls, a condition called *coenocytic*. Each hyphal filament grows mainly at the tip by extension of the terminal cell (Figure 18.19).

Hyphae typically grow together across and above a surface to form a compact, macroscopically visible tuft called a *mycelium* (Figure 18.20*a*). From the mycelium, aerial hyphae reach up into the air above the surface, and spores called **conidia** are formed

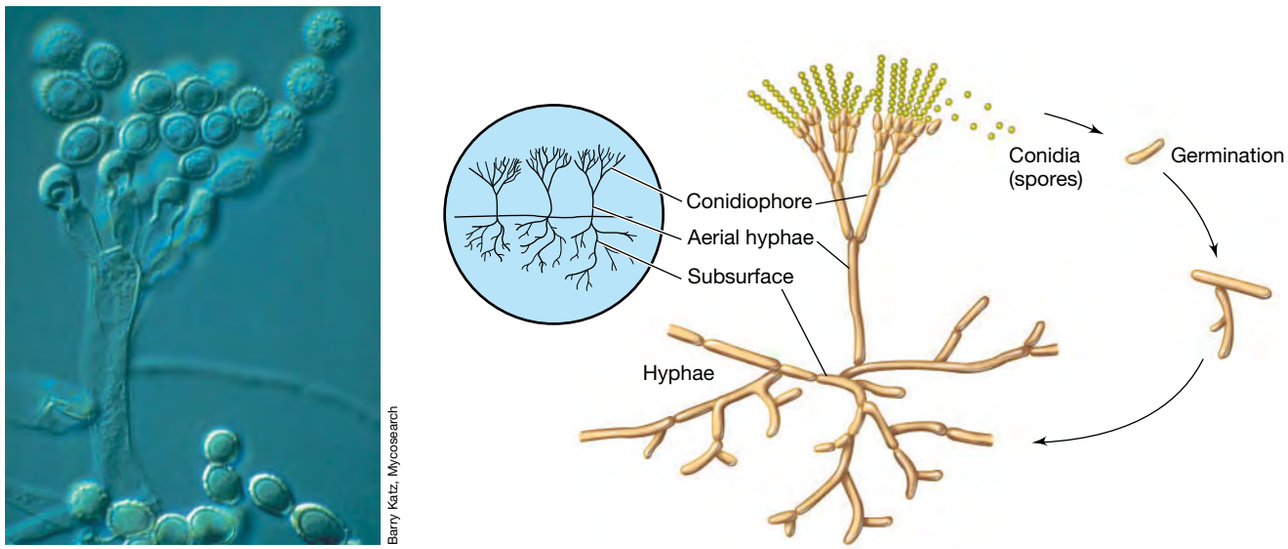


Figure 18.19 Fungal structure and growth. *(a)* Photomicrograph of a typical mold. Spherical structures at the ends of aerial hyphae are asexual spores (conidia). *(b)* Diagram of a mold life cycle. The conidia can be dispersed by either wind or animals and are about 2 μm wide.

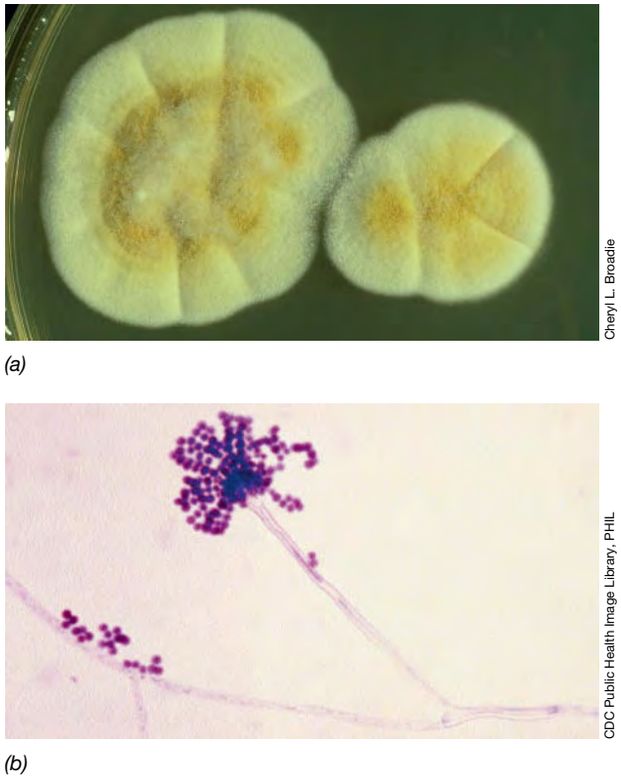


Figure 18.20 Hyphal fungi (molds). *(a)* Colonies of an *Aspergillus* species (ascomycete), growing on an agar plate. Note the masses of filamentous cells (mycelia) and asexual spores that give the colonies a dusty, matted appearance. *(b)* Conidiophore and conidia of *Aspergillus fumigatus* (see Figure 18.19*b*). The conidiophore is about 300 μm long and the conidia here are about 3 μm wide. These cells were stained to improve contrast. Besides being a common saprophyte, *Aspergillus* can be pathogenic, causing serious pulmonary and occasional systemic infections in humans and some domestic animals. Cancer patients and others with weakened immune systems are particularly susceptible to aspergillosis.

on their tips (Figure 18.20*b*). Conidia are asexual spores and they are often pigmented black, green, red, yellow, or brown (Figure 18.20). Conidia give the mycelium a dusty appearance (Figure 18.20*a*) and function to disperse the fungus to new habitats. Some fungi form macroscopic reproductive structures called *fruiting bodies* (**mushrooms** or puffballs, for example), in which millions of spores are produced that can be dispersed by wind, water, or animals (Figure 18.21). In contrast to mycelial fungi, some fungi grow as single cells; these are the **yeasts**.

Most fungal cell walls consist of **chitin**, a polymer of *N*-acetylglucosamine. Chitin is arranged in the walls in microfibrillar bundles, as is cellulose in plant cell walls, to form a thick, tough wall structure. Other polysaccharides such as mannans and galactosans, or even cellulose itself, replace or supplement chitin in some fungal cell walls. Fungal cell walls are typically 80–90% polysaccharide, with only small amounts of proteins, lipids, polyphosphates, and inorganic ions making up the wall-cementing matrix.

Symbioses and Pathogenesis

Most plants are dependent on certain fungi to facilitate their uptake of minerals from soil. These fungi form symbiotic associations with the plant roots called *mycorrhizae* (the word means, literally, "fungus roots"). Mycorrhizal fungi establish close physical contact with the roots and help the plant obtain phosphate and other minerals and also water from the soil. In return, the fungi obtain nutrients such as sugars from the plant root (↪ Figure 23.21). There are two kinds of mycorrhizal associations. One, *ectomycorrhizae*, typically forms between basidiomycetes (Section 18.13) and the roots of woody plants, while the second, *endomycorrhizae*, forms between glomeromycete fungi (Section 18.11) and many nonwoody plants. Some fungi also form associations with cyanobacteria or green algae. These are the *lichens*, the colorful and crusty growths often seen on the surfaces of trees and rocks.

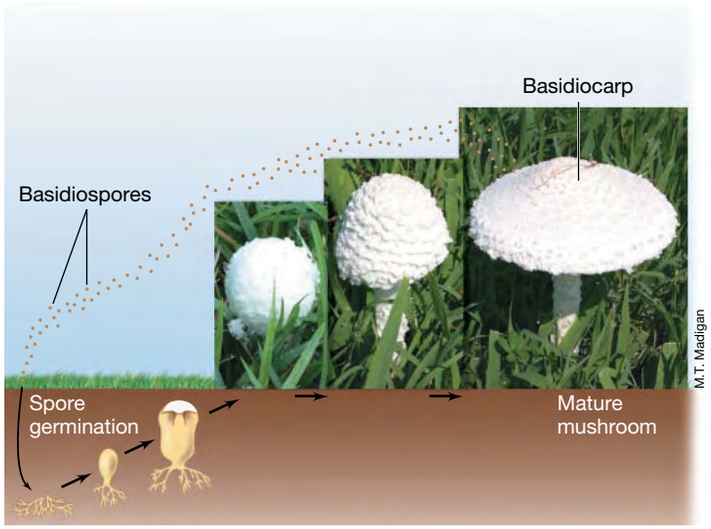


Figure 18.21 Mushroom life cycle. Mushrooms typically develop underground and then emerge on the surface rather suddenly (usually overnight), triggered by an influx of moisture. Photos of stages in formation of a common lawn mushroom (see also Section 18.13).

We explore the biology of lichens and mycorrhizae in more detail in Sections 23.1 and 23.4, respectively.

Fungi can invade and cause disease in plants and animals. Fungal plant pathogens cause widespread crop and plant damage worldwide, and fruit and grain crops in particular suffer significant yearly losses as a result of fungal infection. Human fungal diseases, called *mycoses*, range from relatively minor and easily cured conditions, such as athlete's foot and jock itch, to serious, life-threatening systemic mycoses, such as histoplasmosis. We consider some major fungal diseases of humans in Chapter 33.

MINIQUIZ

- What are conidia? How does a conidium differ from a hypha? From a mycelium?
- What is chitin and where is it present in fungi?
- Distinguish between mycorrhizae and lichens.

18.9 Fungal Reproduction and Phylogeny

Fungi reproduce by *asexual* means in one of three ways: (1) by the growth and spread of hyphal filaments; (2) by the asexual production of spores (conidia; Figures 18.19 and 18.20); or (3) by simple cell division, as in budding yeasts (**Figure 18.22**). Most fungi also form sexual spores, typically as part of an elaborate life cycle. Some fungi, such as the well-known mold *Penicillium* (the source of the antibiotic penicillin), were long thought to lack a sexual stage and reproduce only by way of conidia. But it has now been shown that *Penicillium* (and probably all fungi of its taxonomic class, the *Deuteromycetes*) go through a sexual stage in their life cycles.

Sexual Spores of Fungi

Some fungi produce spores as a result of sexual reproduction. The spores develop from the fusion of either unicellular gametes or specialized hyphae called *gametangia*. Alternatively, sexual spores can originate from the fusion of two haploid cells to yield a diploid cell; this then undergoes meiosis and mitosis to yield individual haploid spores.

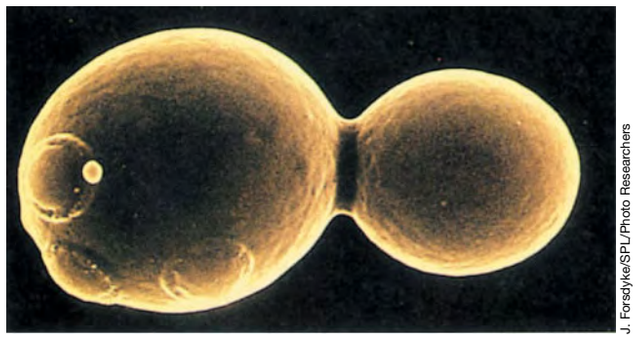


Figure 18.22 The common baker's and brewer's yeast *Saccharomyces cerevisiae* (*Ascomycota*). In this colorized scanning electron micrograph, note the budding division and scars from previous buds. A single cell is about 6 μm in diameter.

Depending on the group, different types of sexual spores are produced. Spores formed within an enclosed sac (ascus) are called *ascospores*. Many yeasts produce ascospores, and we consider sporulation in the common baker's yeast *Saccharomyces cerevisiae* in Section 18.12. Sexual spores produced on the ends of a club-shaped structure (basidium) are *basidiospores* (Figure 18.21 and see Figure 18.30*c*). *Zygospores*, produced by zygomycetous fungi such as the common bread mold *Rhizopus* (Section 18.11), are macroscopically visible structures that result from the fusion of hyphae and genetic exchange. Eventually the zygospore matures and produces asexual spores that are dispersed by air and germinate to form new fungal mycelia. Chytrid fungi produce sexual spores, motile by eukaryotic flagella, called *zoospores*.

Sexual spores of fungi are typically resistant to drying, heating, freezing, and some chemical agents. However, neither sexual nor asexual spores of fungi are as resistant to heat as bacterial endospores (Section 2.10). Both asexual and sexual spores of fungi can germinate and develop into a new hypha.

The Phylogeny of Fungi

Fungi share an ancestor with animals, and are more closely related to animals than any other eukaryotic group (Figure 18.3). The last common ancestor of all fungi likely existed sometime between 450 million and 1.5 billion years ago. One of the earliest fungal lineages is thought to be the *Chytridiomycota*, an unusual group of motile fungi that produce zoospores. Thus the lack of flagella in most fungi indicates that motility is a characteristic that has been lost at various times in different fungal lineages.

Some of the major groups of fungi are shown in the evolutionary tree in **Figure 18.23**. The phylogeny shown in this figure includes several distinct fungal groups: the *Microsporidia, Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota*, and *Basidiomycota*. The vast majority of described fungal species belong to the *Ascomycota* and *Basidiomycota*. The *Ascomycota* are a large and diverse group of fungi that includes the yeasts, such as *Saccharomyces* (Figure 18.22), and molds, such as *Aspergillus* (Figure 18.20). The *Basidiomycota* include fungi that form mushrooms (Figure 18.21 and see Figure 18.30), as well as many

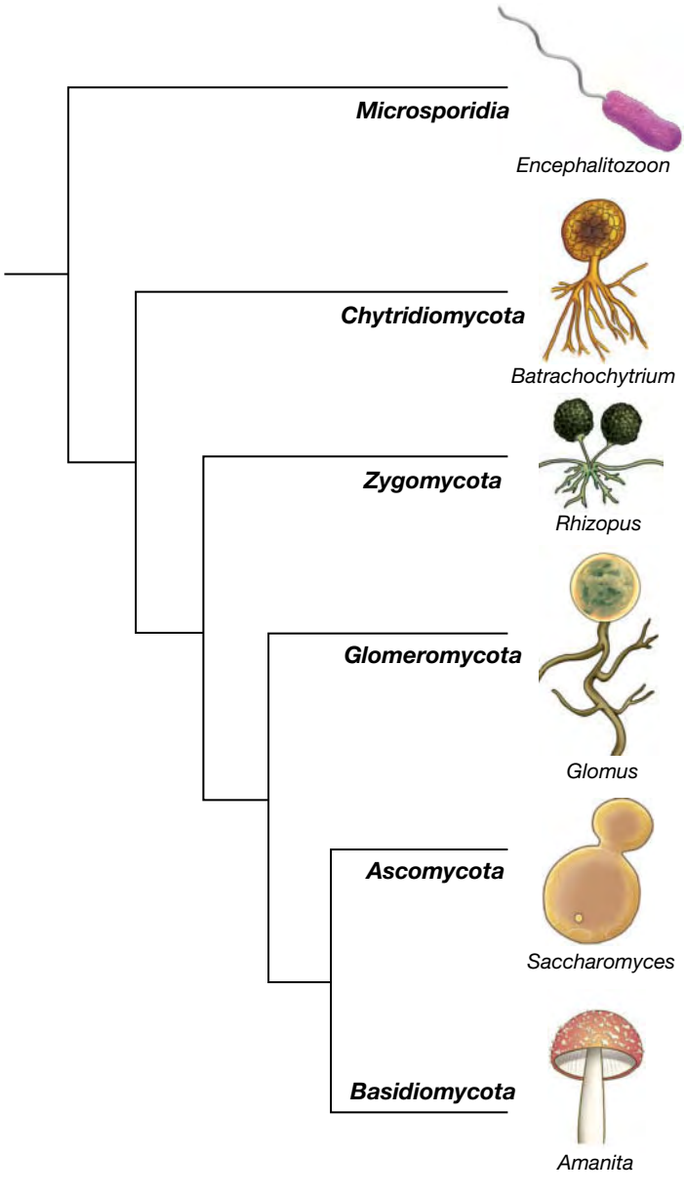


Figure 18.23 Phylogeny of fungi. This schematic phylogenetic tree depicts the relationships among the major groups (phyla) of fungi. A typical genus is listed for each group and depicted in the tree.

important plant pathogens such as rusts and smuts. While a tremendous diversity of fungal species have already been cultured and described, phylogenetic analyses of fungal DNA sequences recovered from environmental samples (Section 19.6) indicate that more than 90% of fungal species remain to be discovered. It is thus clear that we have much to learn about the biology and phylogeny of the fungi.

MINIQUIZ

- Why is the mold *Penicillium* economically important?
- What are the major differences between ascospores and conidia?
- To what major group of macroorganisms are fungi most closely related?

18.10 *Microsporidia* and *Chytridiomycota*

KEY GENERA: *Allomyces, Batrachochytrium, Encephalitozoon*

The *Microsporidia* and *Chytridiomycota* are ancient phylogenetic groups of parasitic or saprophytic fungi. Microsporidia species are obligate parasites of a wide variety of animal hosts including humans, whereas the *Chytridiomycota* are primarily aquatic fungi whose species are either parasites or saprophytes.

Microsporidia

Microsporidia are tiny (2–5 µm) unicellular parasites of animals and protists. Based on 18S ribosomal RNA gene sequencing and their lack of mitochondria, *Microsporidia* were once thought to form a very early-branching lineage of *Eukarya*. However, composite gene and protein sequencing has shown the microsporidians are fungi closely related to the *Chytridiomycota* (Figure 18.23). There remains debate as to whether the *Microsporidia* should be included as one of the most deeply divergent lineages within the fungi or whether they should instead be classified as a distinct lineage closely related to fungi.

Microsporidia have adapted to a parasitic lifestyle. They exist as spores when outside of the host. When near a host cell they extend a helical polar tubule that penetrates the host cytoplasmic membrane. The spore then injects its *sporoplasm* into the host cell. The sporoplasm replicates within the cytoplasm of the host cell, forming new spores as it completes its life cycle. The cell membrane of the host is disrupted and the spores are released in the surrounding environment, free to infect a new cell.

Like most obligate parasites, microsporidia have undergone significant genome reduction, losing many features that would allow them to live outside of a host cell. The microsporidium *Encephalitozoon* (**Figure 18.24a**), for example, lacks not only mitochondria and hydrogenosomes but also a Golgi complex (another key eukaryotic cell structure, Section 2.16). Moreover, *Encephalitozoon* contains a very small genome of only 2.9 Mbp and contains only about 2000 genes (this is 1.5 Mbp and 2600 genes *smaller* than that of the bacterium *Escherichia coli*). The *Encephalitozoon* genome lacks genes for major metabolic pathways, such as the citric acid cycle (Section 3.9), meaning that this pathogen must depend on its host for even the most basic of metabolites and metabolic processes.

In humans, *Encephalitozoon* causes chronic debilitating diseases of the intestine, lung, eye, muscle, and some internal organs but is uncommon among healthy adults with normal immune systems. However, microsporidial diseases have appeared with increasing frequency in immune-compromised individuals, such as those with HIV/AIDS or those on long-term administration of immune-suppressing drugs, such as those who have had organ transplants.

Chytridiomycota

Chytridiomycota, or *chytrids*, are the earliest diverging lineage of fungi (Figure 18.23), and their name refers to the structure of the fruiting body, which contains their *zoospores* (Section 18.9). These spores are unusual among fungal spores in being flagellated and motile, and are ideal for dispersal of these organisms in the aquatic environments, mostly freshwater and moist soils, where they are commonly found.

Many species of chytrids are known and some exist as single cells, whereas others form colonies with hyphae. They include both free-living forms that degrade organic material, such as *Allomyces*, and parasites of animals, plants, and protists.

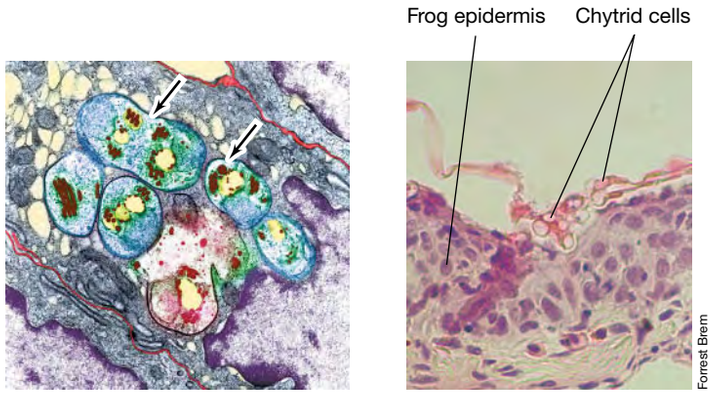


Figure 18.24 *Microsporidia* and *Chytridiomycota*. *(a)* Colorized transmission electron micrograph of thin sections of cells of the microsporidium *Encephalitozoon intestinalis* (arrows) growing in human intestinal cells. *(b)* Cells of the chytrid *Batrachochytrium dendrobatidis* stained pink growing on the surface of frog epidermis.

The chytrid *Batrachochytrium dendrobatidis* causes chytridiomycosis of frogs (Figure 18.24*b*), a condition in which the organism infects the frog's epidermal layers, leading to a loss of ions across the membrane and osmotic imbalance. Chytrids have been implicated in the massive die-off of frogs and some other amphibians worldwide, probably in response to increases in global temperatures that have stimulated chytrid proliferation and to increased animal susceptibility due to habitat loss and aquatic pollution.

Unresolved aspects of the phylogeny of chytrids suggest that this group is not monophyletic. That is, some organisms currently classified as chytrids may actually be more closely related to species of other fungal groups, such as the *Zygomycota*, to which we turn next. As is true for the protists, much about the evolution of the chytrids and other groups of fungi remains to be learned.

MINIQUIZ

- What animal group has been most affected by chytrids?
- What are some features of *Microsporidia* that distinguish them from chytrids?

18.11 *Zygomycota* and *Glomeromycota*

KEY GENERA: *Rhizopus, Glomus*

We consider two groups of fungi here, the *Zygomycota*, known primarily for their role in food spoilage, and the *Glomeromycota*, important fungi in certain mycorrhizal associations. *Zygomycota* are commonly found in soil and on decaying plant material, whereas *Glomeromycota* form symbiotic relationships with plant roots. All of these fungi are coenocytic (multinucleate), and a unifying feature is the formation of sexual spores called *zygospores* (Section 18.9).

Zygomycota

The common black bread mold *Rhizopus nigricans* (**Figure 18.25**) is a widespread zygomycete. This organism undergoes a complex life cycle that includes both asexual and sexual reproduction. In the asexual phase the mycelia form sporangia within which haploid spores are produced. Once released, spores disperse and eventually

Figure 18.25 *Zygomycota*. *(a)* Moldy bread from growth of the zygomycete *Rhizopus nigricans*. *(b)* Stained mycelium of *Rhizopus* showing the black aerial sporangia containing asexual spores.

germinate, giving rise to vegetative mycelia. In the sexual phase, mycelial gametangia of different mating types (analogous to male and female, see Section 18.12) fuse to yield a cell with two nuclei called a *zygosporangium*, which can remain dormant and resist dryness and other unfavorable conditions. When conditions are favorable, the different haploid nuclei fuse to form a diploid nucleus followed by meiosis to yield haploid spores. As in the asexual phase, the release of the spores, in this case genetically nonidentical spores, disperses the organism for vegetative hyphal growth.

Most species of *Rhizopus* and related zygomycetes are harmless saprophytes whose airborne spores land and form spreading colonies on stale bread (Figure 18.25*a*) and various moist surfaces in the home or on walls and crevices in buildings where moisture is trapped. However, some species are human pathogens. If inhaled in sufficient amounts, spores of pathogenic *Rhizopus* species can cause serious infections of the lungs, sinuses, eyes, nose, and mouth leading to swelling of facial features, asthma-like symptoms, and even fatal systemic fungal infections if the initial infection is not promptly treated.

Glomeromycota

The *Glomeromycota* are a relatively small and unique group of obligately symbiotic fungi in which all known species form associations with plants called *endomycorrhizae* (Section 18.8 and Section 23.4). As many as 80% or more of land plant species form these associations in which the fungal hyphae enter the plant cell and aid the plant's acquisition of phosphate from the soil in return for fixed carbon from the plant. Most of these *Glomeromycota* are *arbuscular mycorrhizae*, fungi that form structures called arbuscules that penetrate cells of their plant host and specialize in nutrient exchange (Section 23.4). As plant symbionts, glomeromycetes are thought to have played a pivotal role in the ability of early vascular plants to colonize land (see page 593).

As far as is known, glomeromycetes reproduce only asexually and are mostly coenocytic in their hyphal morphology. Asexual spores of *Glomus* (Figure 18.23), a major genus of endomycorrhizae, are collected from the roots of cultivated plants and used as an agricultural inoculant to promote vigorous symbiotic associations between plant and fungus. This natural approach to plant fertilization is a widespread practice in small, sustainable farming

operations and has been shown to increase both the growth and nutrient content of tomato, pepper, squash, bean, and several other small fruit and vegetable plants.

MINIQUIZ

- Contrast the habitats of *Zygomycota* and *Glomeromycota*.
- How does the fungus *Glomus* aid the acquisition of nutrients by plants?

18.12 *Ascomycota*

KEY GENERA: *Saccharomyces, Candida, Aspergillus*

The *Ascomycota*, also called the ascomycetes, are the largest and most diverse group of fungi and they range from single-celled species, such as the baker's yeast *Saccharomyces* (**Figure 18.26** and Figure 18.22), to species that grow as filaments, such as the common mold *Aspergillus* (Figure 18.20). Ascomycetes are found in aquatic and terrestrial environments and take their name from the production of *asci* (singular, ascus), cells in which two haploid nuclei from different mating types fuse to form a diploid nucleus that eventually undergoes meiosis to form haploid ascospores. In addition to ascospores, ascomycetes reproduce asexually by the production of conidia that form at the tips of specialized hyphae called *conidiophores* (Figure 18.20). Both saprophytic and pathogenic yeasts, such as *Candida albicans*, are common in nature. We focus here on the yeast *Saccharomyces* as a model ascomycete.

Saccharomyces cerevisiae

The cells of *Saccharomyces* and other single-celled ascomycetes are spherical, oval, or cylindrical, and cell division typically takes place by budding. In the budding process, a new cell forms as a small outgrowth of the old cell; the bud gradually enlarges and then separates from the parent cell (Figures 18.22 and 18.26).

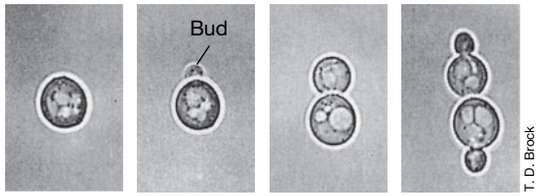


Figure 18.26 Growth by budding division in *Saccharomyces cerevisiae*. A time-lapse series of phase-contrast micrographs shows the budding division process starting from a single cell. Note the pronounced nucleus. A single cell of *S. cerevisiae* is about 6 μm in diameter.

Yeast cells are typically much larger than bacterial cells and can be distinguished from bacteria microscopically by their larger size and by the obvious presence of internal cell structures, such as the nucleus or cytoplasmic vacuoles (Figure 18.26). Yeasts flourish in sugar-rich habitats such as fruits, flowers, and the bark of trees. Yeasts are typically facultative aerobes, growing aerobically as well as by fermentation. Several yeasts live symbiotically with animals, especially insects, and a few species are pathogenic for animals and humans (Sections 33.1 and 33.2). The most important commercial yeasts are the baker's and brewer's yeasts, which are species of *Saccharomyces*. The yeast *S. cerevisiae* has been studied as a model eukaryote for many years and was the first eukaryote to have its genome completely sequenced (Section 9.4).

Mating Types and Sexual Reproduction in *Saccharomyces*

The yeast *Saccharomyces* can reproduce by sexual means in which two cells fuse. Within the fused cell, called a *zygote*, meiosis occurs and ascospores are eventually formed. The life cycle of *S. cerevisiae* is depicted in **Figure 18.27**. *S. cerevisiae* can grow vegetatively as either haploid or diploid cells. *S. cerevisiae* forms two different types of haploid cells called *mating types* designated α (alpha) and

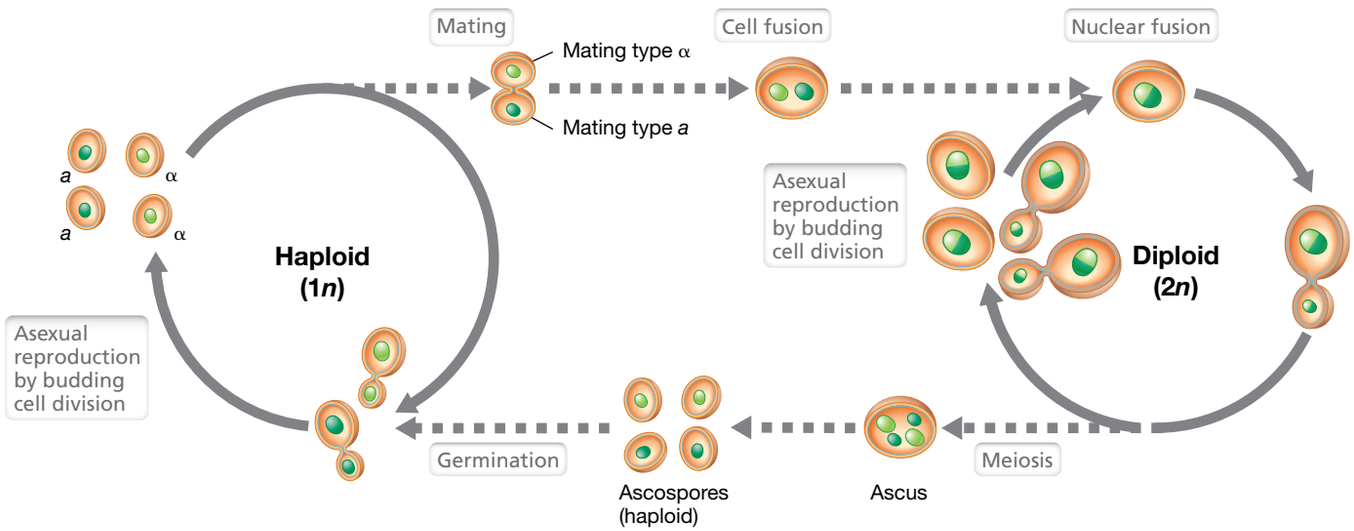
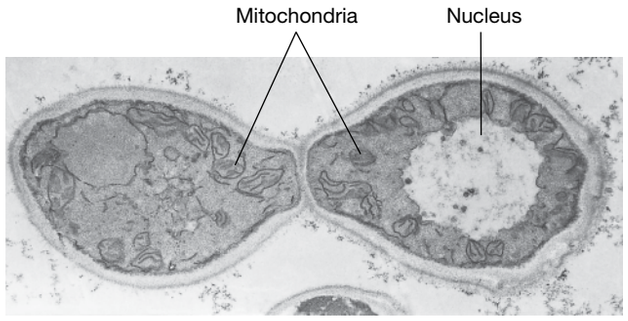
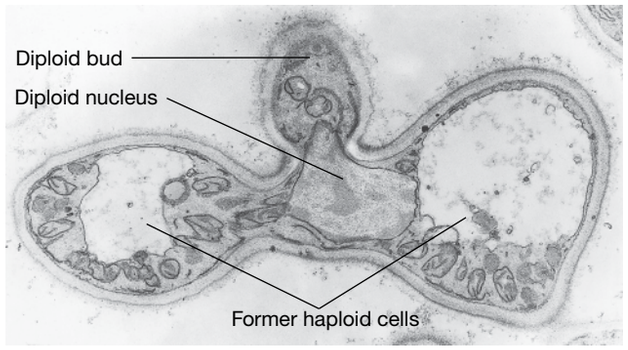


Figure 18.27 Life cycle of a typical ascomycete yeast, *Saccharomyces cerevisiae*. Cells can grow vegetatively for long periods as haploid cells or as diploid cells before life cycle events (dashed lines) generate the alternate genetic form.

UNIT 4



(a)



(b)

Figure 18.28 Electron micrographs of mating in the ascomycete yeast *Hansenula wingei*. *(a)* Two cells have fused at the point of contact. *(b)* Late stage of mating. The nuclei of the two cells have fused, and a diploid bud has formed at a right angle to the mating cells. This bud becomes the progenitor of a diploid cell line. A cell of *Hansenula* is about 10 μm in diameter.

a (encoded by genes α and *a*); these are analogous to male and female gametes. The α and *a* genes regulate the production of the peptide hormones *α factor* or *a factor*, which are excreted by yeast cells during mating. The hormones bind to cells of the opposite mating type and bring about changes in their cell surfaces that enable the cells to fuse; once mating has occurred, the nuclei fuse, forming a diploid zygote (**Figure 18.28**). The zygote undergoes vegetative growth by budding, but under starvation conditions it undergoes meiosis and generates ascospores (Figure 18.27).

Haploid strains of *S. cerevisiae* are genetically predisposed to be either *a* or α but are able to switch their mating type. This switch occurs when the active mating-type gene is replaced with one of two otherwise "silent" genes, as shown in **Figure 18.29**. There is a single location on one of the *S. cerevisiae* chromosomes called the *MAT* (for *mating type*) locus, at which either gene *a* or gene α can be inserted. At this locus, the *MAT* promoter controls transcription of whichever gene is present. If gene *a* is at that locus, then the cell is mating-type *a*, whereas if gene α is at that locus, the cell is mating-type α. Elsewhere in the yeast genome are copies of genes *a* and α that are not expressed, and these are the source of the inserted gene. In the switch (Figure 18.29), the appropriate gene, *a* or α, is copied from its silent site and inserted into the *MAT* location, replacing the gene already present. The old mating-type gene is excised and discarded, and the new gene is inserted. Whichever gene is inserted in the *MAT* locus is the one that will govern the

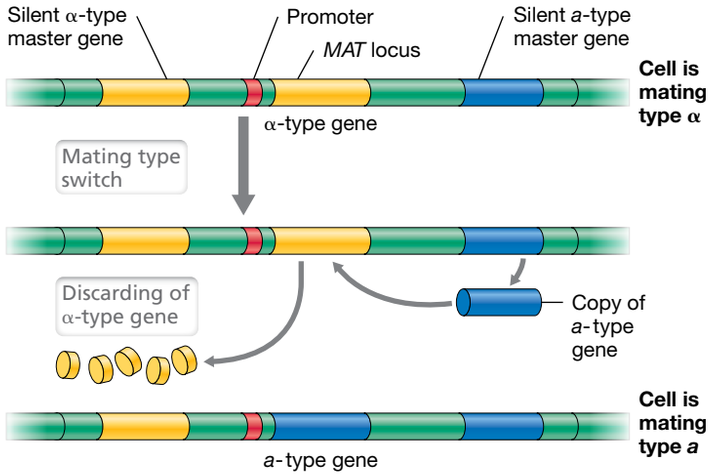


Figure 18.29 The cassette mechanism that switches an ascomycete yeast from mating type α to *a*. The cassette inserted at the *MAT* locus determines the mating type. The process shown is reversible, so type *a* can also revert to type α.

mating type of the strain. It is thus possible for cells from a pure culture of *S. cerevisiae* derived from a single cell to mate, following a mating-type switch in one or more cells in the culture.

MINIQUIZ

- Are ascospores haploid or diploid cells?
- Explain how a *single* haploid cell of *Saccharomyces* can eventually yield a diploid cell.

18.13 *Basidiomycota*

KEY GENERA: *Agaricus, Amanita*

Basidiomycota are a large group of fungi, with over 30,000 species described. Many are the commonly recognized mushrooms and toadstools, some of which are edible, such as the commercially grown mushroom *Agaricus*. Others, such as the mushroom *Amanita* (**Figure 18.30*a***), are highly poisonous. Other basidiomycetes include puffballs, smuts, rusts, and an important human fungal pathogen, *Cryptococcus* (Sections 33.1 and 33.2). The defining characteristic of the *Basidiomycota* is the *basidium* (plural, basidia), a structure in which haploid basidiospores are formed by meiosis. The basidium, a word that means "little pedestal" (Figure 18.30*c*), gives the group its name.

Mushroom Development

During most of its existence, a mushroom fungus lives as a simple haploid mycelium, growing vegetatively in soil, leaf litter, or decaying logs. It is the sexual reproductive phase of basidiomycetes that produces the visible mushroom structure (Figures 18.21 and 18.30). In this process, mycelia of different mating types fuse, and the faster growth of the dikaryotic (two nuclei per cell) mycelium formed from that fusion overgrows and crowds out the parental haploid mycelia. Then, when environmental conditions are favorable, usually following periods of wet and cool weather, the dikaryotic mycelium develops rapidly into the fruiting body.

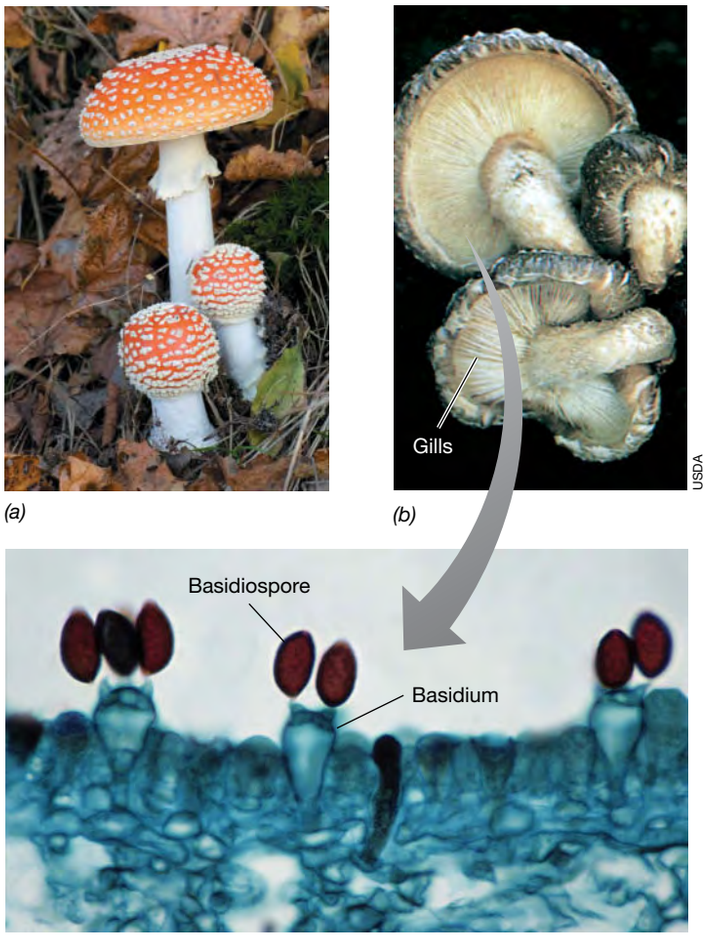


Figure 18.30 Mushrooms. *(a) Amanita*, a highly poisonous mushroom. *(b)* Gills on the underside of the mushroom fruiting body contain the spore-bearing basidia. *(c)* Light micrograph of basidia and basidiospores from the mushroom *Coprinus*.

The mushroom fruiting body, called a *basidiocarp*, begins as a mycelium that differentiates into a small button-shaped structure underground that then expands into the full-grown basidiocarp that we see aboveground, the mushroom (Figures 18.21 and 18.30). The dikaryotic basidia are borne on the underside of the basidiocarp on flat plates called *gills*, which are attached to the cap of the mushroom (Figure 18.30*b, c*). The basidia then undergo a fusion of the two nuclei, forming basidia with diploid nuclei. The two rounds of meiotic division generate four haploid nuclei in the basidia, and each of the nuclei becomes a basidiospore. The genetically distinct basidiospores can then be dispersed by wind to new habitats to begin the cycle again, germinating under favorable conditions and growing as haploid mycelia (Figure 18.21).

Pathogenic Basidiomycetes

The smuts and rusts are plant pathogenic basidiomycetes. Smuts are pathogens of cereal grains and other crops, and the genus *Ustilago* contains several species whose hosts include corn, sugarcane, wheat, and several other grains. The fungus targets the reproductive system of the plant, and in corn smut causes the developing kernels to form massive tumorlike kernels that give the cob a swollen and burned appearance.

Rust fungi attack rapidly growing plant tissues such as emerging shoots, leaves, and fruits. Rust fungi of the genus *Puccinia* can infect any of a wide variety of plants whereas other rust fungi are more host-restricted. Some common rusts include stem rust of wheat and white pine blister rust, a disease of several pine species that can trigger branch dieback and even death of the entire tree. Pine trees weakened by rust fungi are typically more susceptible to insect attack such as from infestations of the pine bark beetle; this highly destructive pest has destroyed major stands of various pine species in the western United States and central Europe in recent years. Hence, even if the rust fungus itself is not lethal, the damage it does to the overall vigor of the tree can set the stage for lethal attack by a secondary agent.

MINIQUIZ

- What are some distinguishing features of basidiospores and of zoospores (Section 18.9)?
- Are basidiospores haploid or diploid?

IV • *Archaeplastida*

We conclude our tour of eukaryotic microbial diversity with the **algae**. The kingdom *Archaeplastida* includes both the red and green algae as well as land plants. As we have previously discussed, only the red and green algae originated from primary endosymbiotic events, whereas other protists containing chloroplasts were the result of secondary endosymbioses (Figures 18.2 and 18.3). Here we focus on the red and green algae, a large and diverse group of eukaryotic organisms that contain chlorophyll and carry out oxygenic photosynthesis.

18.14 Red Algae

KEY GENERA: *Polysiphonia, Cyanidium, Galdiera*

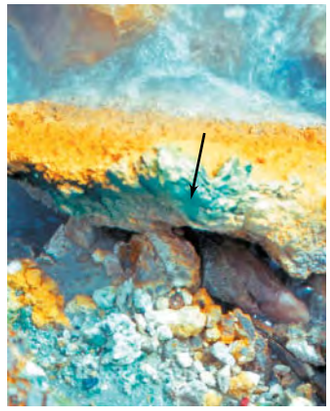
The red algae, also called *rhodophytes*, mainly inhabit the marine environment, but a few species are found in freshwater and terrestrial habitats. Both unicellular and multicellular species are known, and some of the latter are macroscopic.

Basic Properties

Red algae are phototrophic and contain chlorophyll *a*; their chloroplasts lack chlorophyll *b* but contain phycobiliproteins, the major light-harvesting pigments of the cyanobacteria (Section 14.2). The reddish color of many red algae (**Figure 18.31**) results from phycoerythrin, an accessory pigment that masks the green color of chlorophyll. This pigment is present along with phycocyanin and allophycocyanin in structures called *phycobilisomes*, the light-harvesting (antenna) components of cyanobacteria. At greater depths in aquatic habitats, where less light penetrates, cells compensate by producing more phycoerythrin and are a darker red, whereas shallow-dwelling species often have less phycoerythrin and can be green in color (see Figure 18.32).

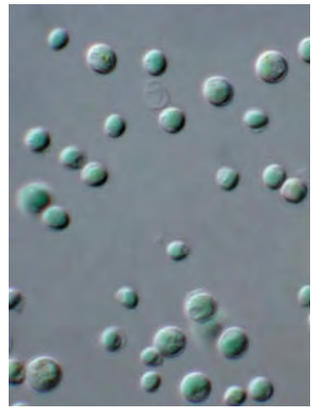
Most species of red algae are multicellular and lack flagella. Some are considered seaweeds and are the source of agar, the

Figure 18.31 *Polysiphonia*, a filamentous marine red alga. Light micrograph. *Polysiphonia* grows attached to the surfaces of marine plants. Cells are about 150 µm wide.



Christine Oesterhelt and Gerald Schönknecht

(a)



Richard W. Castenholz

(b)

Figure 18.32 *Galdieria*, a unicellular red alga. *(a) Galdieria* inhabits acidic and sulfidic hot springs where it often grows attached to mineral debris as shown here (arrow). *(b)* Cells of *Galdieria* are about 25 µm in diameter and are more blue-green than red in color because the phototroph contains mainly phycocyanin rather than phycoerythrin as its phycobilin (Section 14.2).

solidifying agent used in bacteriological culture media, and carrageenans, thickening and stabilizing agents used in the food industry. Other species of red algae, such as the genus *Porphyra*, are harvested, dried, and used as a wrap in sushi. Different species of red algae are filamentous, leafy, or, if they deposit calcium carbonate, *coralline* (coral-like) in morphology. Coralline red algae play an important role in the development of coral reefs and help strengthen reefs against wave damage (Section 23.11).

Polysiphonia (Figure 18.31) is a genus of filamentous and branched red algae found worldwide in marine environments. The organism is found primarily near shore where the cells grow attached to rocks, other algae, and man-made surfaces such as jetties, concrete abutments, retaining walls, and docks. Nearly 200 species of this genus are recognized, and the organism undergoes a complex life cycle in which an alternation of generations occurs. In this cycle, haploid male and female gametes released from a diploid multicellular organism develop into haploid male and female multicellular organisms. The male alga then releases haploid "sperm" cells that fuse with a specialized reproductive structure on the female alga to yield a diploid zygote; the latter develops a multicellular form and, following meiosis, releases male and female gametes to complete the life cycle.

Cyanidium, Galdieria, and Relatives

In addition to multicellular red algae like *Polysiphonia* (Figure 18.31), unicellular species are also known. One such group, members of the *Cyanidiales* that includes the genera *Cyanidium, Cyanidioschyzon*, and *Galdieria* (**Figure 18.32**), live in hot, acidic and metal-rich hot springs at temperatures from 30 to 60°C and acidic pH (0.5 to 4.0); under these extreme conditions, no other phototrophic microorganisms (including anoxygenic phototrophs) can exist. The unicellular red algae are unusual in other ways as well. For example, cells of *Cyanidioschyzon merolae* are unusually small (1–2 µm in diameter) for eukaryotes, and the genome of this species, approximately 16.5 Mbp, is one of the smallest genomes known for a phototrophic eukaryote.

Molecular analyses of the *Galdieria* genome have revealed the surprising result that this *eukaryotic* phototroph contains at least 75 genes acquired by horizontal transfer from various *prokaryotic* sources. Some of the key genes transferred encode protections against salt stress and metal toxicity and the construction of a toughened cytoplasmic membrane to withstand the heat and acidity of the *Galdieria* habitat (Figure 18.32*a*). The ability of *Galdieria* to grow in darkness—an unusual feature for an alga—has also been linked to horizontally transferred genes, in particular to genes encoding transport systems for various organic compounds. Although these genetic transfers are unusual in the sense that donor and recipient belong to different phylogenetic domains, they once again underscore the importance of horizontal gene transfer in molding microbial genomes (Sections 9.6 and 13.6).

MINIQUIZ

- What traits link cyanobacteria and red algae?
- What physiological properties would be necessary for *Galdieria* to live in its habitat?

18.15 Green Algae

KEY GENERA: *Chlamydomonas, Volvox*

The green algae, also called *chlorophytes*, have chloroplasts containing chlorophylls *a* and *b*, which give them their characteristic green color, but they lack phycobiliproteins and so do not develop the red or blue-green colors of red algae (Figures 18.31 and 18.32). In the composition of their photosynthetic pigments, green algae are similar to plants and are closely related to plants phylogenetically. There are two main groups of green algae, the *chlorophytes*, examples of which are the microscopic *Chlamydomonas* and *Dunaliella* (**Figure 18.33*a***), and the charophyceans such as *Chara* (Figure 18.33*b*), macroscopic organisms that often resemble land plants and are actually most closely related to land plants.

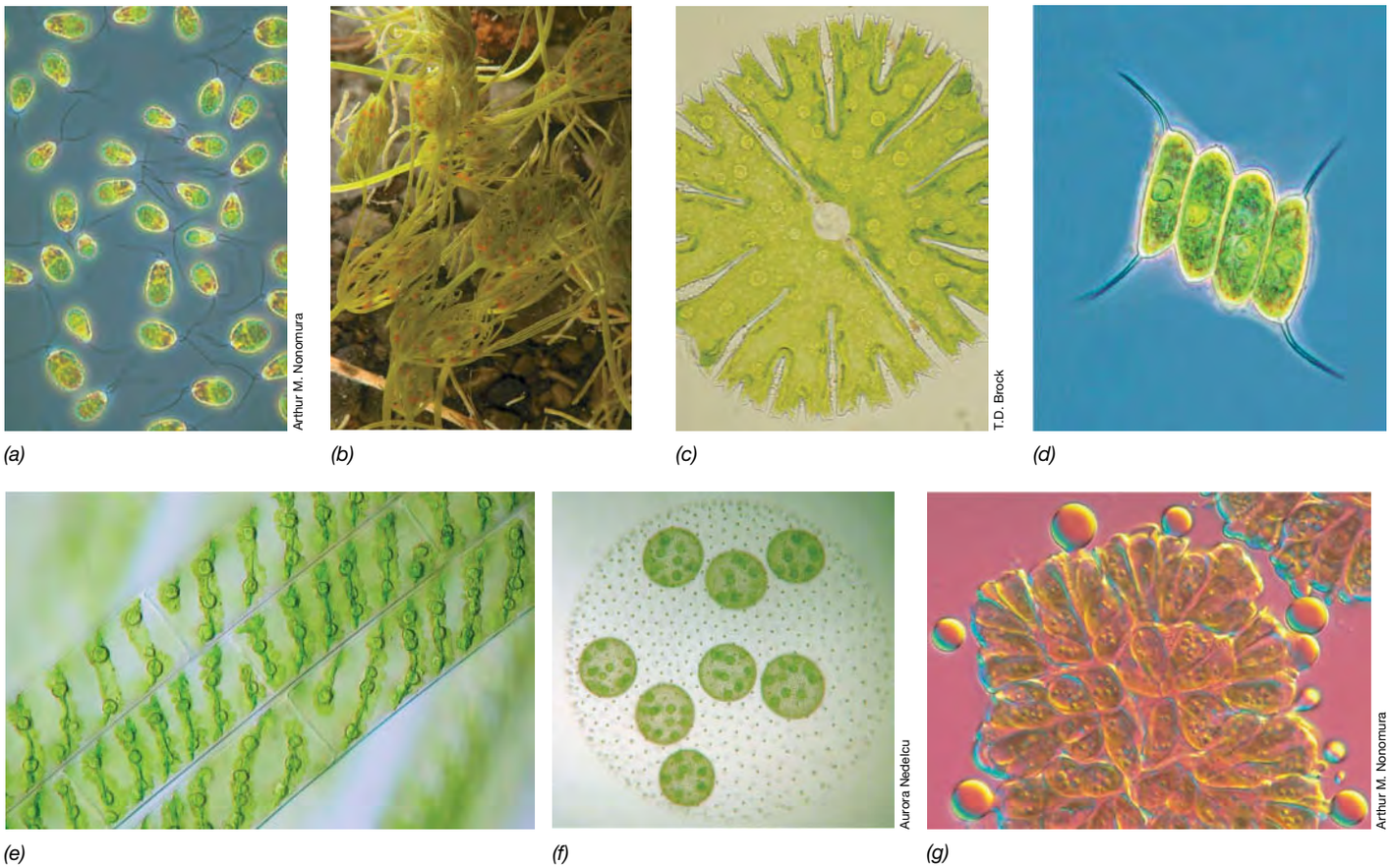


Figure 18.33 Green algae. *(a)* A single-celled, flagellated green alga, *Dunaliella*. A cell is about 5 μm wide. *(b)* The plantlike green alga *Chara*. *(c) Micrasterias*. This single multilobed cell is about 100 μm wide. *(d) Scenedesmus*, showing packets of four cells each. *(e) Spirogyra*, a filamentous alga with cells about 20 μm wide. Note the green spiral-shaped chloroplasts. *(f) Volvox carteri* colony with eight daughter colonies. *(g)* The petroleum-producing green alga *Botryococcus braunii*. Note the excreted oil droplets surrounding the cell.

Most green algae inhabit freshwater while others are found in moist soil or growing in snow, to which they impart a pink color (Figure 5.20). Other green algae live as symbionts in lichens (Section 23.1). The morphology of chlorophytes ranges from unicellular (Figure 18.33*a*, *c*) to filamentous, with individual cells arranged end to end (Figure 18.33*e*), to **colonial**, as aggregates of cells (Figure 18.33*f*). Even multicellular species exist, an example of which is the seaweed *Ulva*. Most green algae have a complex life cycle, with both sexual and asexual reproductive stages.

Very Small Green Algae and Colonial Green Algae

One of the smallest eukaryotes known is the green alga *Ostreococcus tauri*, a common unicellular species of marine phytoplankton (Section 20.10 and Figure 20.24*b*). Cells of *O. tauri* have a diameter of approximately 2 μm, and the organism contains the smallest genome of any known phototrophic eukaryote, approximately 12.6 Mbp. *Ostreococcus* has thus provided a model organism for research into the evolution of genome reduction and specialization in eukaryotes.

At the colonial level of organization in green algae is *Volvox* (Figure 18.33*f*). This alga forms colonies composed of several hundred flagellated cells, some of which are motile and primarily carry out photosynthesis, while others specialize in reproduction. Cells in a *Volvox* colony are interconnected by thin strands of cytoplasm that allow the entire colony to swim in a coordinated fashion. *Volvox* has been a long-term model for research on the genetic mechanisms controlling multicellularity and the distribution of functions among cells in multicellular organisms.

Some colonial green algae have potential as sources of biofuels. For example, the colonial green alga *Botryococcus braunii* excretes long-chain (C_{30}–C_{36}) hydrocarbons that have the consistency of crude oil (Figure 18.33*g*). About 30% of the *B. braunii* cell dry weight consists of this petroleum, and there has been heightened interest in using this and other oil-producing algae as renewable sources of petroleum. Evidence from biomarker studies have shown that some known petroleum reserves originated from green algae such as *B. braunii* that settled in lakebeds in ancient times. Hence, if the scale-up challenges for commercial algal petroleum production could ever be met, it is possible that some fraction of the world's oil supply could someday come from photosynthesis by green algae.

(a)

(b)

Figure 18.34 Endolithic phototrophs. *(a)* Photograph of a limestone rock from the McMurdo Dry Valleys region of Antarctica broken open to show the layer of endolithic green algae. *(b)* Light micrograph of cells of the green alga *Trebouxia*, a widespread endolithic alga in Antarctica.

Endolithic Phototrophs

Some green algae grow inside rocks. These *endolithic* (*endo* means "inside") phototrophs inhabit porous rocks, such as those containing quartz, and are typically found in layers near the rock surface (**Figure 18.34*a***). Endolithic phototrophic communities are most common in dry environments such as deserts or cold, dry environments such as Antarctica. For example, in the McMurdo Dry Valleys of Antarctica, where temperatures and humidity are extremely low (↔ Figure 5.19*d*, *e*), life within a rock has its advantages. Rocks in these harsh environments are heated by the sun, and water from snowmelt can be absorbed and retained for relatively long periods, supplying moisture needed for growth. Moreover, water absorbed by a porous rock makes the rock more transparent, thus funneling more light to the algal layers.

A wide variety of phototrophs can form endolithic communities, including cyanobacteria and various green algae (Figure 18.34*b*). In addition to being free-living phototrophs, green algae and cyanobacteria coexist with fungi in endolithic lichen communities (↔ Section 23.1 for discussion of the lichen symbiosis). Metabolism and growth of these internal rock microbial communities slowly weathers the rock, allowing gaps to develop where water can enter, freeze and thaw, and eventually crack the rock, producing new habitats for microbial colonization. The decomposing rock also forms a crude soil that can support development of plant and animal communities in environments where conditions (temperature, moisture, and so on) permit.

MINIQUIZ

- What phototrophic properties link green algae and plants?
- What is unusual about the green algae *Ostreococcus, Volvox*, and *Botryococcus*?
- What are endolithic phototrophs?

Chapter Review

I • Organelles and Phylogeny of Microbial *Eukarya*

18.1 Key metabolic organelles of eukaryotes are the chloroplast, which functions in photosynthesis, and the mitochondrion or hydrogenosome, which function in respiration or fermentation. These organelles were originally *Bacteria* that established permanent residence inside other cells (endosymbiosis).

Q Distinguish between a primary and a secondary endosymbiosis. Which groups of protists are derived from which form of endosymbiosis?

18.2 Ribosomal RNA gene sequences do not yield as reliable a phylogenetic tree of the *Eukarya* as do other genes and proteins. The modern, multigene tree of eukaryotes shows a major radiation of eukaryotic diversity emerging at some time following symbiotic events that led to the mitochondrion.

Q What are the six major supergroups within the *Eukarya*?

II • Protists

18.3 Diplomonads such as *Giardia* are unicellular, flagellated, nonphototrophic protists. Parabasalids such as *Trichomonas* are human pathogens and contain huge genomes that lack introns. Euglenids and kinetoplastids are unicellular, flagellated protists. Some are phototrophic. This group includes

some important human pathogens, such as *Trypanosoma*, and some well-studied nonpathogens, such as *Euglena*.

Q What morphological feature unites kinetoplastids and euglenids?

18.4 Three groups make up the alveolates: ciliates, dinoflagellates, and apicomplexans. Most ciliates and dinoflagellates are free-living organisms, whereas apicomplexans are obligate parasites of animals.

Q How are alveolates as a group characterized?

18.5 *Stramenopiles* are protists that bear a flagellum with fine, hairlike extensions. They include oomycetes, diatoms, and brown and golden algae.

Q In terms of their photosynthetic pigments, how are brown and golden algae similar?

18.6 The *Rhizaria* include diverse protists such as the phototrophic chlorarachniophytes and foraminiferans, as well as radiolarians, which are chemoorganotrophs.

Q How can *Rhizaria* be distinguished from other protists in their environment?

18.7 *Amoebozoa* are protists that use pseudopodia for movement and feeding. Within *Amoebozoa* are the gymnamoebas, entamoebas, and slime molds. Plasmodial slime molds form masses of motile protoplasm, whereas cellular slime molds are individual cells that aggregate to form fruiting bodies from which spores are released.

Q Where are gymnamoebas found in nature? How do they feed?

III • Fungi

18.8 Fungi include the molds, mushrooms, and yeasts. Other than phylogeny, fungi primarily differ from protists by their rigid cell wall, production of spores, and lack of motility.

Q How can fungi benefit both plants and humans?

18.9 A variety of sexual spores are produced by fungi, including ascospores, basidiospores, and zygospores. From a phylogenetic standpoint, fungi are the closest relatives of animals.

Q List the different types of sexual spores of fungi. Are conidia sexual or asexual spores?

18.10 *Chytridiomycota* and *Microsporidia* are basal to all other known fungal groups in the fungal phylogeny. Some chytrids are amphibian pathogens, while microsporidians are parasites of animals.

Q In what way do chytrids and microsporidians differ from other fungi?

18.11 *Zygomycota* form coenocytic hyphae and undergo both asexual and sexual reproduction, and the common bread mold *Rhizopus* is a good example. *Glomeromycota* are fungi that form endomycorrhizal associations with plants.

Q What is the major feature of the ecology of glomeromycetes?

18.12 The *Ascomycota* are a large and diverse group of mostly saprophytic fungi. Some, such as *Candida albicans*, can be pathogenic in humans. There are two mating types in the yeast *Saccharomyces cerevisiae*, and yeast cells can convert from one type to the other by a genetic switch mechanism.

Q How is the mating type of a yeast cell determined?

18.13 *Basidiomycota* include the mushrooms, puffballs, smuts, and rusts. Basidiomycetes undergo both vegetative reproduction as haploid mycelia and sexual reproduction via fusion of mating types and formation of haploid basidiospores.

Q What morphological feature unites the *Basidiomycota*, and where is this feature found?

IV • *Archaeplastida*

18.14 Red algae are mostly marine and range from unicellular to multicellular. Their reddish color is due to the pigment phycoerythrin, a key cyanobacterial pigment, present in their chloroplast.

Q In what kinds of habitats would one likely find red algae?

18.15 Green algae are common in aquatic environments and can be unicellular, filamentous, colonial, or multicellular. A unicellular green alga, *Ostreococcus*, has the smallest genome known for a phototrophic eukaryote, while the green alga *Volvox* is a model colonial phototroph.

Q What traits link green algae and plants?

UNIT 4

Application Questions

1. Explain why the process of endosymbiosis can be viewed as both an ancient event and a more recent event. What advantages could endosymbiosis give to both the endosymbiont and the host?
2. Summarize the evidence for endosymbiosis. How could the endosymbiotic hypothesis have originated before the era of molecular biology? How has molecular biology supported the theory?
3. Considering all of the groups of microbes covered in this chapter, which of them seem most similar to prokaryotic cells in cell structure? Discuss at least two lines of evidence that show that these microbes are not prokaryotic cells.

Chapter Glossary

Algae an informal term for phototrophic eukaryotes other than plants; algae are polyphyletic, being found in diverse groups of *Eukarya* as a result of secondary endosymbioses

Chitin a polymer of *N*-acetylglucosamine commonly found in the cell walls of fungi

Ciliate any protist characterized in part by rapid motility driven by numerous short appendages called cilia

Coenocytic the presence of multiple nuclei in fungal hyphae without septa

Colonial the growth form of certain protists and green algae in which several cells live together and cooperate for feeding, motility, or reproduction; an early form of multicellularity

Conidia the asexual spores of fungi

Endosymbiotic hypothesis the concept that a respiratory bacterium and a cyanobacterium were stably incorporated into another cell type to yield the mitochondria and chloroplasts, respectively, of eukaryotic cells

Fungi nonphototrophic eukaryotic microorganisms with rigid cell walls

Mushroom the aboveground fruiting body, or basidiocarp, of basidiomycete fungi

Nucleomorphs remnant nuclei found in certain algae; derived from an ancestral algal endosymbiont and associated with chloroplasts acquired by secondary endosymbiosis

Phagocytosis a mechanism for ingesting particulate material in which a portion of the cytoplasmic membrane surrounds the particle and brings it into the cell

Protist an informal term used to describe any unicellular eukaryotic microorganism whether heterotrophic or photosynthetic

Secondary endosymbiosis the endosymbiotic acquisition by a mitochondrion-containing eukaryotic cell of a red or green algal cell, which itself contains a chloroplast derived from primary endosymbiosis

Slime mold a nonphototrophic protist that lacks cell walls and that aggregates to form fruiting structures (cellular slime molds) or masses of protoplasm (acellular slime molds)

Yeast the single-celled growth form of various fungi

19

Taking the Measure of Microbial Systems

microbiologynow

The Vineyard Microbiome Revealed by Next-Generation Sequencing Technology

It has been recognized for millennia that the quality of wine produced from the same variety of grape can vary dramatically depending on where the grapevines are grown. The French refer to the influence of location on wine characteristics as *terroir* (from the French *terre*, meaning "land"). The influence of locale on wine quality is the primary basis for defining the appellation boundaries for vineyards within major growing regions. The Burgundy region in France, for example, has dozens of such appellations. It has long been considered that *terroir* is a function of climate and the physical and chemical characteristics of the local soil. However, a recent study using next-generation sequencing of 16S rRNA genes to determine the diversity and relative abundance of different microorganisms on grape plants and in soils from major wine-growing areas in the United States and France has revealed that *terroir* may result from a combination of microbial and abiotic factors.

Distinct microbial communities are imprinted on the grapes from different winegrowing regions, even for the same grape variety. Next-generation sequencing technology can unravel these microbial communities and define their species composition and abundance. Modern sequencing efforts have shown that the soil microbial community can vary significantly between appellations and that soil is the major source of the microbiota found on grapevine leaves and fruit. This suggests that *terroir* is influenced not only by soil physics and chemistry and the prevailing climate, but also by the local soil microbial community. Apart from a direct contribution soil microbes might make in wine fermentation by influencing the flavor, color, and quality of the final product, differences in the soil microbial community may also affect the physiology of the plant, for example, by controlling the production of specific plant compounds that alter grape chemistry.

These initial applications of next-generation sequencing to the study of *terroir* highlight the growing importance of modern microbial ecology in agriculture. As our understanding of plant–soil–microbial interactions develops, a soil microbial census could well become a valuable tool for improving soil quality and crop performance by ensuring that each crop plant has the best possible microbial associates.

Source: Zarraonaindia, I., et al. 2015. The soil microbiome influences grapevine-associated microbiota. *MBio:* 6 e02527-14.