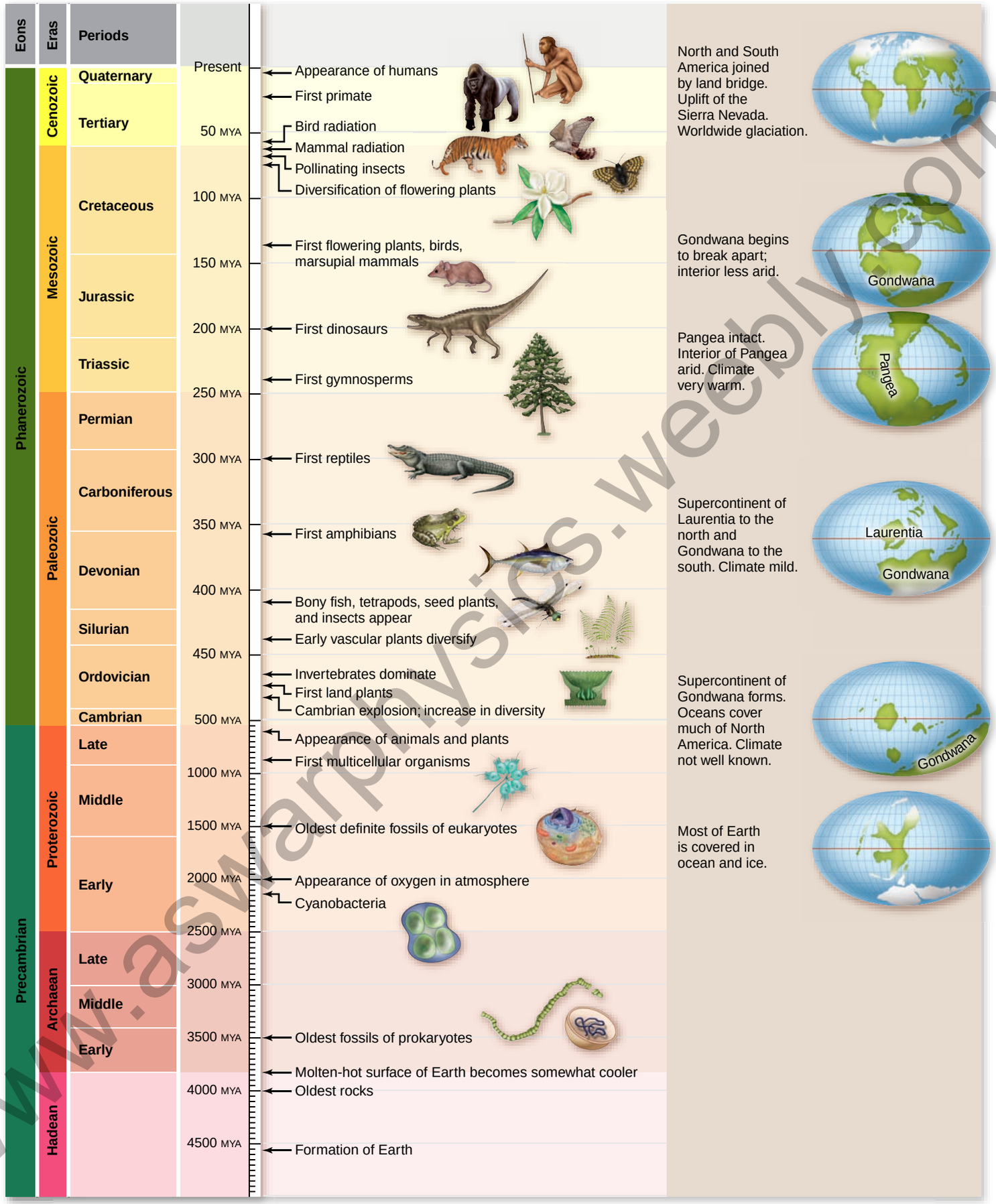


Figure 26.4 **Geological timescale and the evolution of life on earth.**

26.2 Classification of Organisms

Learning Outcomes

1. *Explain how taxonomists name and group organisms.*
2. *Evaluate the usefulness of taxonomic hierarchies in answering evolutionary questions.*

People have known from the earliest times that differences exist between organisms. Early humans learned that some plants could be eaten, but others were poisonous. Some animals could be hunted or domesticated; others were dangerous hunters themselves. In this section, we review formal scientific classification.

Taxonomy is a quest for identity and relationships

More than 2000 years ago, the Greek philosopher Aristotle formally categorized living things as either plants or animals. The Greeks and Romans expanded this simple system and grouped animals and plants into basic units such as cats, horses, and oaks. Eventually, these units began to be called genera (singular, *genus*), the Latin word for "groups." Starting in the Middle Ages, these names began to be systematically written down in Latin, the language used by scholars at that time. Thus, cats were assigned to the genus *Felis*, horses to *Equus*, and oaks to *Quercus*.

Linnaeus instituted the use of binomial names

Until the mid-1700s, whenever biologists wanted to refer to a particular kind of organism, which they called a species, they added a series of descriptive terms to the name of the genus; this was a polynomial, or "many names" system.

A much simpler system of naming organisms stemmed from the work of the Swedish biologist Carolus Linnaeus (1707–1778). In the 1750s, Linnaeus used the polynomial names *Apis pubescens, thorace subgriseo, abdomine fusco, pedibus posticis glabris utrinque margine ciliates* to denote the European honeybee. But as a kind of shorthand, he also included a two-part name for the honeybee; he designated it *Apis mellifera*. These two-part names, or **binomials,** have become our standard way of designating species. You have already encountered many binomial names in earlier chapters.

Taxonomy is the science of classifying living things. A group of organisms at a particular level in a classification system is called a *taxon* (plural, *taxa*). By agreement among taxonomists throughout the world, no two organisms can have the same scientific name. The scientific name of an organism is the same anywhere in the world and avoids the confusion caused by common names (figure 26.5).

Also by agreement, the first word of the binomial name is the genus to which the organism belongs. This word is always capitalized. The second word refers to the particular species and is not capitalized. The two words together are called the species name (or scientific name) and are written in italics—for example, *Homo sapiens*. Once a genus has been used in the body of a text, it is often abbreviated in later uses. For example, the dinosaur *Tyrannosaurus rex* becomes *T. rex*.

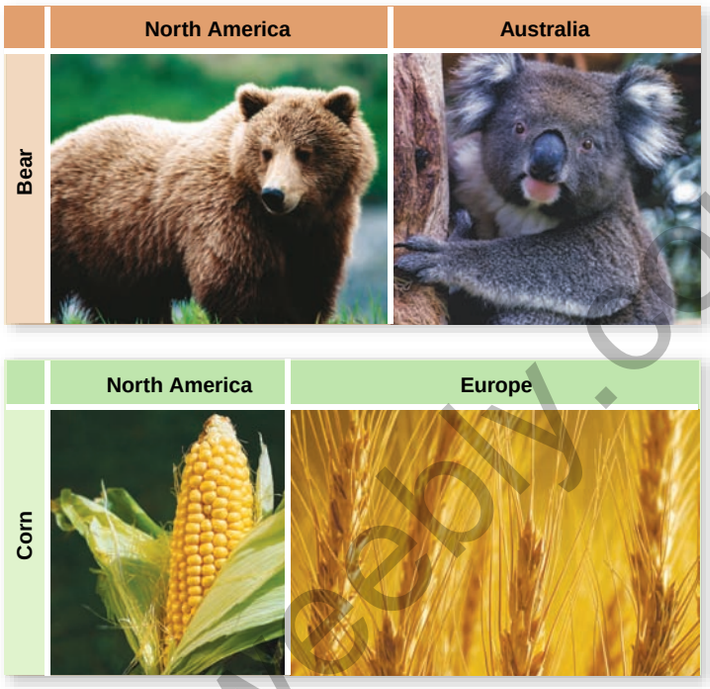


Figure 26.5 Common names make poor labels. In North America, the common names "bear" and "corn" bring clear images to our minds, but the images are very different for someone living in Europe or Australia.

Taxonomic hierarchies have limitations

Named species are organized into larger groups based on shared characteristics. As discussed in chapter 23, sound evolutionary hypotheses can be constructed when organisms are grouped based on derived characters, not ancestral characters. Early taxonomists were not aware that the distinction between derived and ancestral characters could make a difference; as a result, many hierarchies are now being re-examined. As the phylogenetic and systematic revolution continues, other limitations of the original levels of taxonomic organization, called the *Linnaean taxonomy*, are being revealed.

The Linnaean hierarchy

In the decades following Linnaeus, taxonomists began to group organisms into larger, more inclusive categories. Genera with similar characters were grouped into a cluster called a **family,** and similar families were placed into the same **order** (figure 26.6). Orders with common properties were placed into the same **class,** and classes with similar characteristics into the same **phylum** (plural, *phyla*). Finally, the phyla were assigned to one of several great groups, the **kingdoms.** These kingdoms include two kinds of prokaryotes (Archaea and Bacteria), a largely unicellular group of eukaryotes (Protista), and three multicellular groups (Fungi, Plantae, and Animalia). As you will see later in this chapter, the protists are not

Figure 26.6 The hierarchical system used in classifying an organism. The organism, in this case the eastern gray squirrel, is first recognized as a eukaryote (domain Eukarya). Within this domain, it is an animal (kingdom Animalia). Among the different phyla of animals, it is a vertebrate (phylum Chordata, subphylum Vertebrata). The organism's fur characterizes it as a mammal (class Mammalia). Within this class, it is distinguished by its gnawing teeth (order Rodentia). Next, because it has four front toes and five back toes, it is a squirrel (family Sciuridae). Within this family, it is a tree squirrel (genus *Sciurus*), with gray fur and white-tipped hairs on the tail (species *Sciurus carolinensis*, the eastern gray squirrel).

a monophyletic group, and the term kingdom is a bit of a misnomer for protists.

In addition, an eighth level of classification, called a **domain,** is frequently used. Biologists recognize three domains, which will be discussed in section 26.3. The names of the taxonomic units from the genus level and higher are capitalized.

The categories at the different levels may include many, a few, or only one taxon. For example, there is only one living genus of the family Hominidae (namely *Homo*), but several living genera of Fagaceae (the birch family). To someone familiar with classification or having access to the appropriate reference books, each taxon implies both a set of characteristics and a group of organisms belonging to the taxon.

To return to the example of the European honeybee, we can analyze the bee's taxonomic classification as follows:

1. **Species level:** *Apis mellifera*, meaning honey-bearing bee.
2. **Genus level:** *Apis*, a genus of bees.
3. **Family level:** Apidae, a bee family. All members of this

family are bees—some solitary, some living in colonies as *A. mellifera* does.

4. **Order level:** Hymenoptera, a grouping that includes bees, wasps, ants, and sawflies—all of which have wings with membranes.
5. **Class level:** Insecta, a very large class that comprises animals with three major body segments, three pairs of legs attached to the middle segment, and wings.
6. **Phylum level:** Arthropoda. Animals in this phylum have a hard exoskeleton made of chitin and jointed appendages.
7. **Kingdom level:** Animalia. The animals are multicellular heterotrophs with cells that lack cell walls.

Limitations of the hierarchy

In chapter 23, we discussed the modern phylogenetic approach, which distinguishes relationships between different species based on evolutionary history. Emerging phylogenies, frequently based on molecular data, reveal that the Linnaean hierarchy is inadequate for recognizing the hierarchical relationships among taxa that result naturally from a history of common ancestory and descent. New evolutionary hypotheses are developing.

One problem with the Linnaean system is that many higher taxonomic ranks are not monophyletic (for example, Reptilia) and therefore do not represent natural groups. A common ancestor and all of its descendants is a natural group that results from descent from a common ancestor, but any other type of group (paraphyletic or polyphyletic) is an artificial group created by taxonomists.

In addition, Linnaean ranks, as currently recognized, are not equivalent in any meaningful way. For example, two families may not represent clades that originated at the same time. One family may have diverged 70 million years before another family, and therefore these families have had vastly different amounts of time to diverge and develop evolutionary adaptations. Two groups that diverged from a common ancestor at the same time may be given different ranks. Thus, comparisons using Linnaean categories may be misleading. It is much better to use hypotheses of phylogenetic relationships in such instances.

One result of all these differences is that families demonstrate different degrees of biological diversity. Here's one example. It is difficult to say that the legume family with 16,000 species represents the same level of taxonomic organization as the cat family with only 36 species. The differences across a single rank, whether it is class, order, or family, limit the usefulness of taxonomic hierarchies in making evolutionary predictions.

Learning Outcomes Review 26.2

By convention, a species is given a binomial name. The first part of the name identifies the genus, and the second part the individual species. The Linnaean taxonomic hierarchy groups species into genera, then families, orders, classes, phyla, and kingdoms. Traditional classification systems are based on similar traits, but because they include a mix of derived and ancestral traits, they do not necessarily take into account evolutionary relationships.

- ***What can you infer about evolutionary relationships by comparing a taxonomic hierarchy for a squirrel and a fox (refer to figure 26.6)? What questions remain unanswered?***

26.3 *Grouping Organisms*

Learning Outcomes

1. ***List examples showing that the three domains of life are monophyletic, but the six kingdoms are not.***
2. ***Distinguish among the characteristics of Eukarya, Archaea, and Bacteria.***
3. ***Explain why biologists do not include viruses in the tree of life.***

In this section, we examine the largest groupings of organisms: kingdoms and domains. The earliest classification systems recognized only two kingdoms of living things: animals and plants. But as biologists discovered microorganisms and learned more about other multicellular organisms, they added kingdoms in recognition of certain fundamental differences. The six-kingdom system was first proposed by Carl Woese of the University of Illinois (figure 26.7*b*).

The six kingdoms are not necessarily monophyletic

In the six-kingdom system, four of the kingdoms consist of eukaryotic organisms. The two most familiar kingdoms, *Animalia* and *Plantae*, contain only organisms that are multicellular during most of their life cycle. The kingdom *Fungi* contains multicellular forms and single-celled yeasts.

Fundamental differences divide these three kingdoms. Plants are mainly stationary, but some have motile sperm; most fungi lack motile cells; animals are mainly motile or mobile. Animals ingest their food, plants manufacture it, and fungi digest and absorb it by means of secreted extracellular enzymes.

The large number of eukaryotes that do not fit in any of the three eukaryotic kingdoms are arbitrarily grouped into a single kingdom called **Protista** (see chapter 29). Most protists are unicellular or, in the case of some algae, have a unicellular phase in their life cycle. This kingdom reflects the current controversy between taxonomic and phylogenetic approaches. The protists are a paraphyletic group, containing several nonmonophyletic adaptive lineages with distinct evolutionary origins.

The remaining two kingdoms, **Archaea** and **Bacteria,** consist of prokaryotic organisms, which are vastly different from all other living things (see chapter 28). Archaea are a diverse group that includes the methanogens and extreme thermophiles, and its members differ from the other prokaryotes—Bacteria.

The three domains probably are monophyletic

As biologists have learned more about the Archaea, it has become increasingly clear that this group is very different from all other organisms. When the full genomic DNA sequences of an archaean and a bacterium were first compared in 1996,

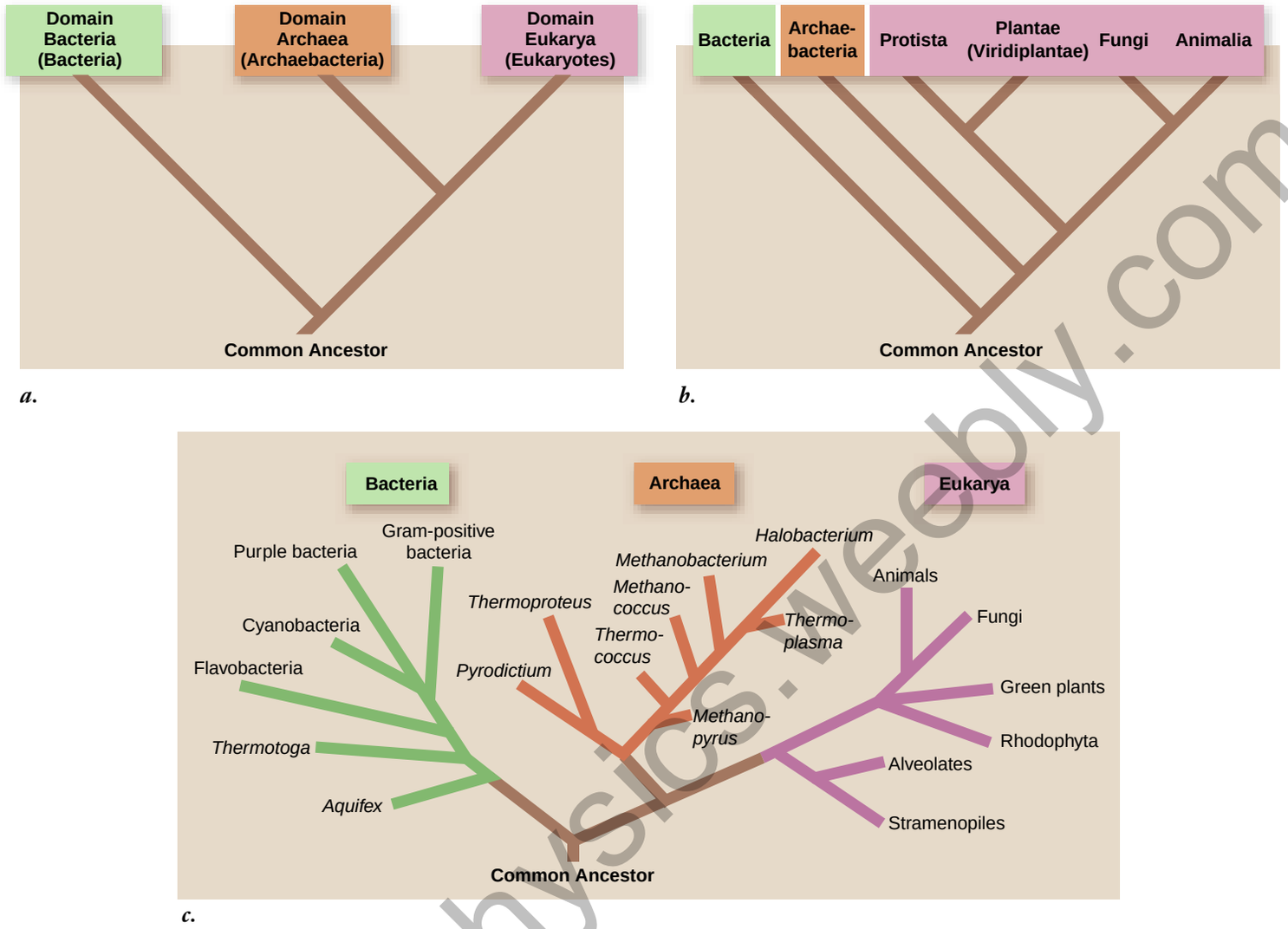


Figure 26.7 Different approaches to classifying living organisms. ***a.*** Bacteria and Archaea are so distinct that they have been assigned to separate domains distinct from the Eukarya. Members of the domain Bacteria are thought to have diverged early from the evolutionary line that gave rise to the archaea and eukaryotes. ***b.*** Eukarya are grouped into four kingdoms, but these, especially the protists, are not necessarily monophyletic groups. ***c.*** This phylogeny is prepared from rRNA analyses. The base of the tree was determined by examining genes that are duplicated in all three domains, the duplication presumably having occurred in the common ancestor. Archaea and eukaryotes diverged later than bacteria and are more closely related to each other than either is to bacteria. Bases of trees constructed with other traits are often less clear because of horizontal gene transfer (see chapter 24).

the differences proved striking. Archaea are as different from bacteria as bacteria are from eukaryotes.

Recognizing this, biologists are increasingly adopting a classification of living organisms that recognizes three **domains,** a taxonomic level higher than kingdom (figure 26.7*a*). Archaea are in one domain **(Domain Archaea),** bacteria in a second **(Domain Bacteria),** and eukaryotes in the third **(Domain Eukarya).** Phylogenetically each of these domains form a clade.

Inquiry question

? **Why would the Archaea be considered a clade?**

In the remainder of this section, we preview the major characteristics of the three domains and viruses. Our current understanding of the "tree of life" is presented in figure 26.7*c*. The oldest divergences represent the deepest rooted branches in the tree. The archaea and eukaryotes are more closely related to each other than to bacteria and are on a separate evolutionary branch of the tree.

Bacteria are more numerous than any other organism

The bacteria are the most abundant organisms on Earth. There are more living bacteria in your mouth than there are mammals living on Earth.

Although too tiny to see with the unaided eye, bacteria play critical roles throughout the biosphere. Some extract from the air all the nitrogen used by organisms, and they play key roles in cycling carbon and sulfur. Much of the world's

photosynthesis is carried out by bacteria. In contrast, certain bacteria are also responsible for many forms of disease. Understanding bacterial metabolism and genetics is a critical part of modern medicine.

Bacteria are highly diverse, and the evolutionary links among species are not well understood. Although taxonomists disagree about the details of bacterial classification, most recognize 12 to 15 major groups of bacteria. Comparisons of the nucleotide sequences of ribosomal RNA (rRNA) molecules are beginning to reveal how these groups are related to one another and to the other two domains.

Archaea may live in extreme environments

The archaea seem to have diverged very early from the bacteria and are more closely related to eukaryotes than to bacteria (figure 26.7*c*). This conclusion comes largely from comparisons of genes that encode ribosomal RNAs.

Horizontal gene transfer in microorganisms

Comparing whole-genome sequences from microorganisms has led evolutionary biologists to a variety of phylogenetic trees, some of which contradict each other. It appears that during their early evolution, microorganisms swapped genetic information via horizontal gene transfer (HGT), as you learned in chapter 24. The potential for gene transfer makes constructing phylogenetic trees for microorganisms very difficult.

Consider the archaean *Thermotoga*, a thermophile found on Vulcano Island off the coast of Italy. The sequence of one of its RNAs places it squarely within the bacteria near an ancient microbe called *Aquifex*. Recent DNA sequencing, however, fails to support any consistent relationship between the two microbes.

Over the next few years, we can expect to see considerable change in accepted viewpoints as more and more data are brought to bear.

Archaean characteristics

Although they are a diverse group, all archae share certain key characteristics (table 26.1). Their cell walls lack peptidoglycan (an important component of the cell walls of bacteria); the lipids in the cell membranes of archae have a different structure from those in all other organisms; and archae have distinctive ribosomal RNA sequences. Some of their genes possess introns, unlike those of bacteria. Both archae and eukaryotes lack the peptidoglycan cell wall found in bacteria.

The archae are grouped into three general categories—methanogens, extremophiles, and nonextreme archae—based primarily on the environments in which they live or on their specialized metabolic pathways. The word *extreme* refers to our current environment. When archae first appeared on the scene their now extreme habitats may have been typical.

Methanogens obtain their energy by using hydrogen gas (H_2) to reduce carbon dioxide (CO_2) to methane gas (CH_4). They are strict anaerobes, poisoned by even traces of oxygen. They live in swamps, marshes, and the intestines of mammals. Methanogens release about 2 billion tons of methane gas into the atmosphere each year.

TABLE 26.1 Features of the Domains of Life

	DOMAIN		
Feature	**Archaea**	**Bacteria**	**Eukarya**
Amino acid that initiates protein synthesis	Methionine	Formyl-methionine	Methionine
Introns	Present in some genes	Absent	Present
Membrane-bounded organelles	Absent	Absent	Present
Membrane lipid structure	Branched	Unbranched	Unbranched
Nuclear envelope	Absent	Absent	Present
Number of different RNA polymerases	Several	One	Several
Peptidoglycan in cell wall	Absent	Present	Absent
Response to the antibiotics streptomycin and chloramphenicol	Growth not inhibited	Growth inhibited	Growth not inhibited

Extremophiles are able to grow under conditions that seem extreme to us. There are several types of extremophiles:

- Thermophiles, which live in temperatures ranging from 60° to 80°C. Many of these are autotrophs with a sulfur-based metabolism.
- Cold-adapted, which live in glacier ice and alpine lakes.
- Halophiles, which live in very salty environments including the Great Salt Lake and the Dead Sea. These organisms require water with a salinity of 15 to 20%.
- pH-tolerant archaea, growing in highly acidic (pH = 0.7) or highly basic (pH = 11) environments.
- Pressure-tolerant archaea found in the ocean depths. These archaeans require at least 300 atmospheres (atm) of pressure to survive and tolerate up to 800 atm. To experience a pressure of 300 atm (300 times the pressure of our atmosphere) you would need to dive 3000 m below the surface of the ocean (not a good idea unless you were in a deep-sea submersible). The deepest recorded skin dive is 127 m and a 145-m record is reported for SCUBA diving.

Nonextreme archaea grow in the same environments bacteria do. As the genomes of archaea have become better known, microbiologists have been able to identify signature sequences of DNA present only in archaea. The newly discovered microbe *Nanoarchaeum equitens* was identified as an archaean based on a signature sequence. This odd Icelandic microbe may have the smallest known genome, only 500 bp.

Eukaryotes have compartmentalized cells

For at least 1 billion years, prokaryotes ruled the Earth. No other types of organisms existed to eat them or compete with them, and their tiny cells formed the world's oldest fossils. Members of the third great domain of life, the eukaryotes, appear in the fossil record much later, only about 2.5 BYA. But despite the metabolic similarity of eukaryotic cells to prokaryotic cells, their structure and function enabled these cells to be larger, and eventually, allowed multicellular life to evolve.

Endosymbiosis and the origin of eukaryotes

The hallmark of eukaryotes is complex cellular organization, highlighted by an extensive endomembrane system that subdivides the eukaryotic cell into functional compartments (chapter 4). Not all cellular compartments, however, are derived from the endomembrane system.

With few exceptions, modern eukaryotic cells possess the energy-producing organelles termed *mitochondria*, and photosynthetic eukaryotic cells possess *chloroplasts*, the energy-harvesting organelles. Mitochondria and chloroplasts are both believed to have entered early eukaryotic cells by a process called **endosymbiosis,** which is discussed in more detail in chapter 29 (figure 26.8).

Mitochondria are the descendants of relatives of purple sulfur bacteria and the parasitic *Rickettsia* that were incorporated into eukaryotic cells early in the history of the group. Chloroplasts are derived from cyanobacteria (figure 26.9). As shown in figures 26.8 and 26.9, the red and green algae

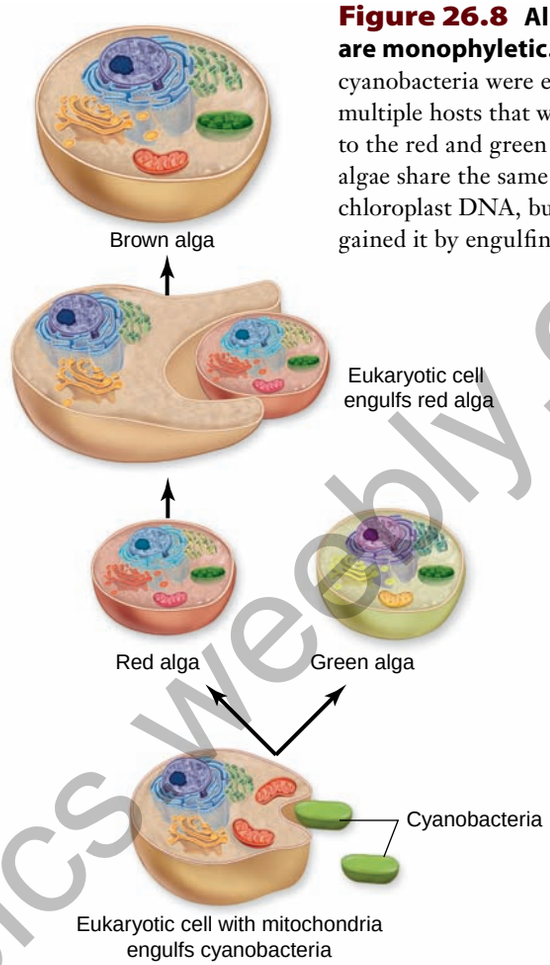


Figure 26.8 All chloroplasts are monophyletic. The same cyanobacteria were engulfed by multiple hosts that were ancestral to the red and green algae. Brown algae share the same ancestral chloroplast DNA, but most likely gained it by engulfing red algae.

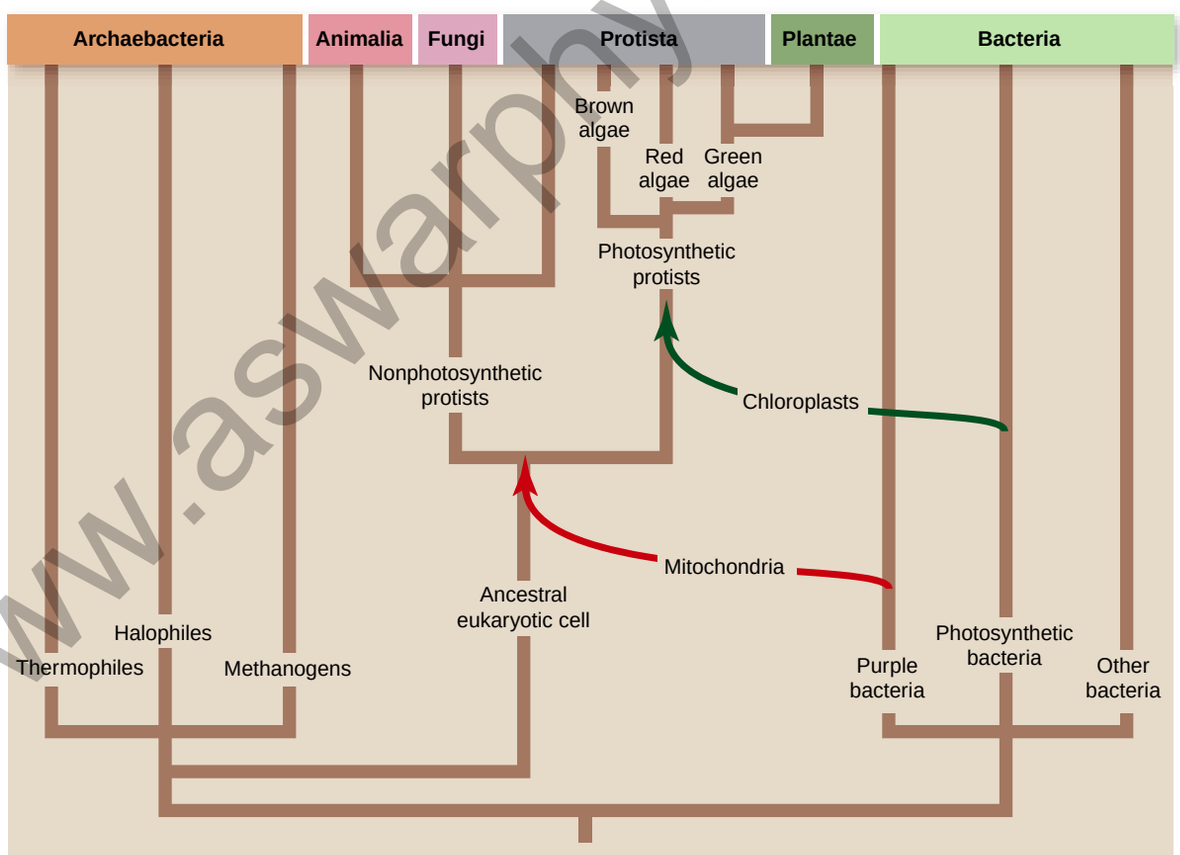


Figure 26.9 Hypothesis for evolutionary relationships among the six kingdoms of organisms. The colored lines indicate symbiotic events.

acquired their chloroplasts by directly engulfing a cyanobacterium. The brown algae most likely engulfed red algae to obtain chloroplasts.

The four kingdoms of eukaryotes

The first eukaryotes were unicellular organisms. A wide variety of unicellular eukaryotes exist today, grouped together in the kingdom Protista (along with some multicellular descendants) on the basis that they do not fit into any of the other three kingdoms of eukaryotes. Fungi, plants, and animals are largely multicellular kingdoms, each a distinct evolutionary line from a single-celled ancestor that would be classified in the kingdom Protista.

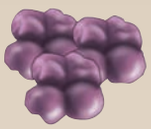
Because of the size and ecological dominance of plants, animals, and fungi, and because they are predominantly multicellular, we recognize them as kingdoms distinct from Protista, even though the amount of diversity among the protists is much greater than that within or between the fungi, plants, and animals.

Key characteristics of the eukaryotes

The characteristics of the six kingdoms are outlined in table 26.2; note that the archaea and bacteria are grouped in the same column. Although eukaryotic organisms are extraordinarily diverse, they share three characteristics that distinguish them from prokaryotes: compartmentalization; multicellularity in many, but not all, eukaryotes; and sexual reproduction.

Compartmentalization. Discrete compartments provide evolutionary opportunities for increased specialization

TABLE 26.2 Characteristics of the Six Kingdoms and Three Domains

	 Archaea and Bacteria	 Protista	 Plantae	 Fungi	 Animalia
Cell Type	Prokaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic
Nuclear Envelope	Absent	Present	Present	Present	Present
Transcription and Translation	Occur in same compartment	Occur in different compartments	Occur in different compartments	Occur in different compartments	Occur in different compartments
Histone Proteins Associated with DNA	Absent	Present	Present	Present	Present
Cytoskeleton	Absent	Present	Present	Present	Present
Mitochondria	Absent	Present (or absent)	Present	Present	Present
Chloroplasts	None (photosynthetic membranes in some types)	Present (some forms)	Present	Absent	Absent
Cell Wall	Noncellulose (polysaccharide plus amino acids)	Present in some forms, various types	Cellulose and other polysaccharides	Chitin and other noncellulose polysaccharides	Absent
Means of Genetic Recombination, if Present	Conjugation, transduction, transformation	Fertilization and meiosis	Fertilization and meiosis	Fertilization and meiosis	Fertilization and meiosis
Mode of Nutrition	Autotrophic (chemosynthetic, photosynthetic) or heterotrophic	Photosynthetic or heterotrophic, or combination of both	Photosynthetic, chlorophylls *a* and *b*	Absorption	Ingestion
Motility	Bacterial flagella, gliding or nonmotile	9 + 2 cilia and flagella; amoeboid, contractile fibrils	None in most forms; 9 + 2 cilia and flagella in gametes of some forms	Both motile and nonmotile	9 + 2 cilia and flagella, contractile fibrils
Multicellularity	Absent	Absent in most forms	Present in all forms	Present in most forms	Present in all forms
Nervous System	None	Primitive mechanisms for conducting stimuli in some forms	A few have primitive mechanisms for conducting stimuli	None	Present (except sponges), often complex

within the cell, as we see with chloroplasts and mitochondria. The evolution of a nuclear membrane, not found in prokaryotes, also accounts for increased complexity in eukaryotes. In eukaryotes, RNA transcripts from nuclear DNA are processed and transported across the nuclear membrane into the cytosol, where translation occurs. The physical separation of transcription and translation in eukaryotes adds additional levels of control to the process of gene expression.

Multicellularity. The unicellular body plan has been tremendously successful, with unicellular prokaryotes and eukaryotes constituting about half of the biomass on Earth. But a single cell has limits. The evolution of multicellularity allowed organisms to deal with their environments in novel ways through differentiation of cell types into tissues and organs.

True multicellularity, in which the activities of individual cells are coordinated and the cells themselves are in contact, occurs only in eukaryotes and is one of their major characteristics. Bacteria and many protists form colonial aggregates of many cells, but the cells in the aggregates have little differentiation or integration of function.

Other protists—the red, brown, and green algae, for example—have independently attained multicellularity. One lineage of multicellular green algae was the ancestor of the plants (see chapters 29 and 30), and most taxonomists now place its members in the green plant kingdom, the *Viridiplantae*.

The multiple origins of multicellularity are also seen in the fungi and the animals, which arose from unicellular protist ancestors with different characteristics. As you will see in subsequent chapters, the groups that seem to have given rise to each of these kingdoms are still in existence.

Sexual Reproduction. Another major characteristic of eukaryotic species as a group is sexual reproduction. Although some interchange of genetic material occurs in bacteria, it is certainly not a regular, predictable mechanism in the same sense that sex is in eukaryotes. Sexual reproduction allows greater genetic diversity through the processes of meiosis and crossing over, as you learned in chapter 13.

In many of the unicellular phyla of protists, sexual reproduction occurs only occasionally. The first eukaryotes were probably haploid; diploids seem to have arisen on a number of separate occasions by the fusion of haploid cells, which then eventually divided by mitosis.

Viruses are a special case

Viruses possess only a portion of the properties of organisms. Viruses are literally "parasitic" macromolecules, segments of DNA or RNA wrapped in a protein coat. They cannot reproduce on their own, and for this reason they are not considered alive by biologists. They can, however, reproduce within cells, often with disastrous results to the host.

Viruses are currently viewed as detached fragments of the genomes of organisms because of the high degree of similarity found in some viral and eukaryotic genes. Viruses thus present a special classification problem. Because they are not organisms, we cannot logically place them in any of the kingdoms.

Viruses vary greatly in appearance and size. The smallest are only about 17 nanometers (nm) in diameter, and the largest are up to 1000 nm (1 micrometer; μm) in their greatest dimension, barely visible with a light microscope (figure 26.10). Viral morphology is best revealed using the electron microscope.

Biologists first began to suspect the existence of viruses near the end of the 19th century. European scientists were attempting to isolate the infectious agent responsible for hoof-and-mouth disease in cattle, and they concluded that it was smaller than a bacterium. The true nature of viruses was discovered in 1933, when biologist Wendell Stanley prepared an extract of the tobacco mosaic virus (TMV) and attempted to purify it. To his great surprise, the purified TMV preparation precipitated in the form of crystals—the virus was acting like a chemical off the shelf rather than like an organism. Stanley

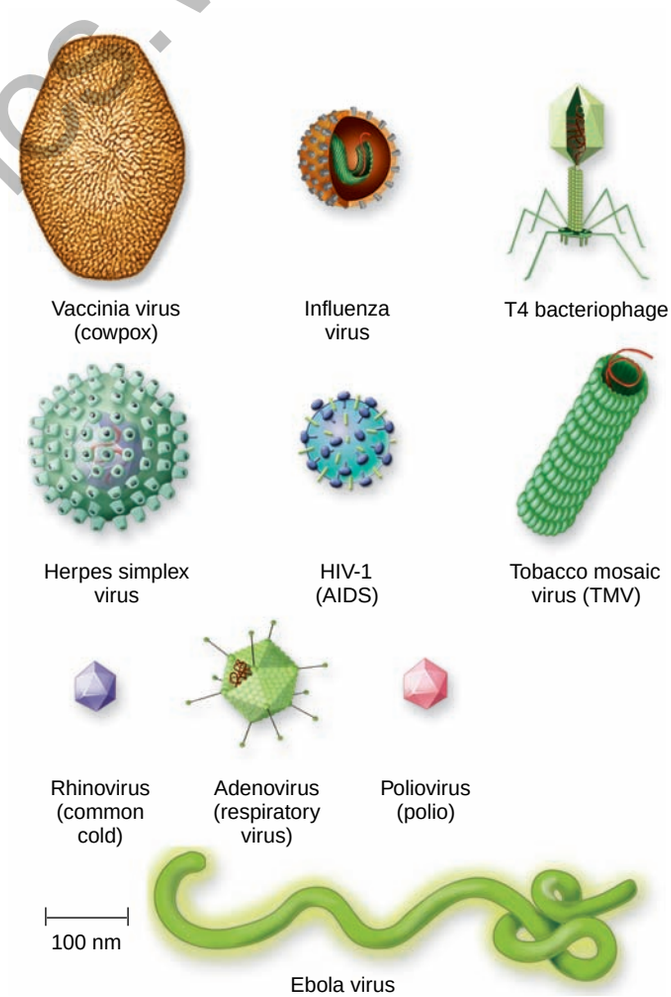


Figure 26.10 Viral diversity. Viruses exhibit extensive diversity in shape and size. At the scale these sample viruses are shown, a human hair would be nearly 8 m thick.

concluded that TMV is best regarded as just that—chemical matter rather than a living organism.

Within a few years, scientists disassembled the TMV virus and found that Stanley was right. TMV was not cellular but chemical. Each particle of TMV virus is in fact a mixture of two chemicals: RNA and protein. The TMV virus consists of a tube made of protein with an RNA core. If these two components are separated and then reassembled, the reconstructed TMV particles are fully able to infect healthy tobacco plants.

Because eukaryote diversity is so vast, we next take a brief look at the three kingdoms of the eukaryote domain.

Learning Outcomes Review 26.3

The six kingdoms are not necessarily based on common lineage; Kingdom Protista, for example, is not a monophyletic group. The three domains, however, do appear to be monophyletic. Bacteria and archaea are tiny but numerous unicellular organisms that lack internal compartmentalization. Eukaryotic cells are highly compartmentalized, and they have acquired mitochondria and chloroplasts by endosymbiosis. Viruses are not organisms classified in the kingdoms of life, but instead are chemical assemblies that can infect cells and replicate within them.

- ***What would be the outcome if a virus infected a cell and became a permanent resident in the cell's genome?***

26.4 Making Sense of the Protists

Learning Outcome

1. ***Describe the relationships among groups of protists.***

In reading this chapter and chapter 23, you may have sensed some tension between traditional classification systems and systems based on evolutionary relationships, such as cladistics and phylogenetic analysis. The kingdom Protista illustrates well the source of this tension. This kingdom is the weakest area of the six-kingdom classification system shown in figure 26.7.

Eukaryotes diverged rapidly in a world that was shifting from anaerobic to aerobic conditions. We may never be able to completely sort out the relationships among different lineages during this major evolutionary transition. Molecular systematics, however, clearly shows that the protists are a paraphyletic group (figure 26.11). Although biologists continue to use the term *protist* as a catchall for any eukaryote that is not a plant, fungus, or animal, this grouping is not based on evolutionary relationships.

The six main branchings of protists, shown at the base of figure 26.11, represent a current working hypothesis, although at least 60 protists do not seem to fit in any of the six groups. Choanoflagellates are most closely related to sponges, and indeed, to all animals. The green algae can be split into two

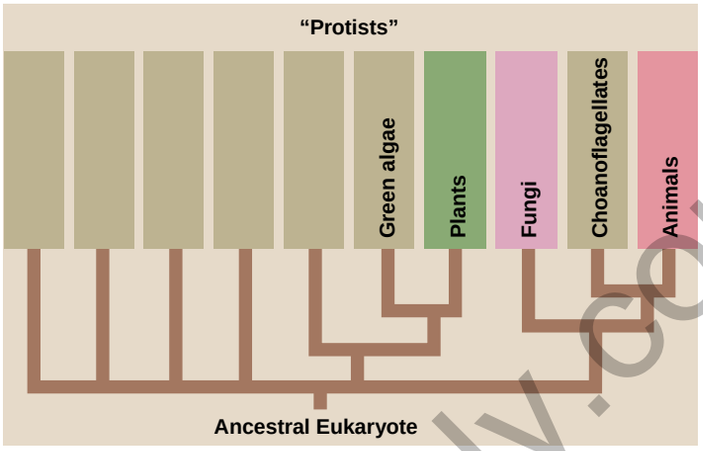


Figure 26.11 The fall of kingdom Protista. Systematists have shown that protists as a group are not monophyletic. Note how some lineages are actually more closely related to plants or animals than they are to other protists.

monophyletic groups, one of which gave rise to land plants. Many systematists are calling for a new kingdom called Viridiplantae, or the green plant kingdom, which would include all the green algae (not red or brown algae) and the land plants. Thus, the definition of a plant has been expanded beyond those species that made it onto land. Although the kingdom Protista is in ruins, our understanding of the evolutionary relationships among these early eukaryotes is growing exponentially.

Learning Outcome Review 26.4

Relationships among organisms in Kingdom Protista, a paraphyletic group, are being clarified by modern methods. Choanoflagellates are most closely related to animals, and green algae may belong in a new kingdom that would include plants. Systematics and cladistics continue to add to our knowledge of these relationships.

- ***How might life have evolved if photosynthesis had not produced atmospheric oxygen?***

26.5 Origin of Plants

Learning Outcomes

1. ***Describe the evolutionary relationship between algae and plants.***
2. ***Explain how moss genes have come to be found in the genome of a flowering plant.***

The origin of land plants from a green algal ancestor has long been recognized as a major evolutionary event. Molecular phylogenetics reveals that land plants arose from an ancestral green alga, and that the evolution of land plants occurred only once, an indication of the incredible challenges involved in the move onto land.

Molecular phylogenetics has identified the closest living relatives of land plants

The phylogenetic relationships among the algae and the first land plants have been fuzzy and subject to long debate. Cell biology, biochemistry, and molecular systematics have provided surprising new evolutionary hypotheses.

The green algae consist of two monophyletic groups, the *Chlorophyta* and the *Streptophyta* (chapter 30). Land plants are actually members of the Streptophyta, not a separate kingdom. This new phylogenetic information demoted the land plants from constituting a kingdom to being a branch within the algal group Streptophyta. The Streptophyta along with the sister green algal clade, Chlorophyta, are now considered by most to make up the kingdom Viridiplantae. The current phylogeny is shown in figure 26.12.

What was the earliest streptophyte? Conflicting answers have been obtained with different phylogenetic analyses, but growing evidence supports the hypothesis that the scaly, unicellular flagellate Mesostigma (order Mesostigmatales) represents the earliest streptophyte branch.

Which of the Streptophyta clades contains the closest living relative of land plants? The two contenders have been the Charales, with about 300 species, and the Coleochaetales, with about 30 species. Both lineages are freshwater algae, but the Charales are huge compared with the microscopic Coleochaetales. At the moment, the Charales appear to be the sister clade to land plants, with the Coleochaetales the next closest relatives. Charales fossils dating back 420 MYA indicate that the common ancestor of land plants was a relatively complex freshwater alga.

Horizontal gene transfer occurred in land plants

The shrub *Amborella trichopoda* is the closest living relative to the earliest flowering plants (angiosperms). Its clade is a sister clade to all other flowering plants, yet at least one copy of 20 out of 31 of its known mitochondrial protein genes hopped into the mitochondrial genome from other land plants through horizontal gene transfer (HGT). In addition, three different moss species contributed to the mix (figure 26.13).

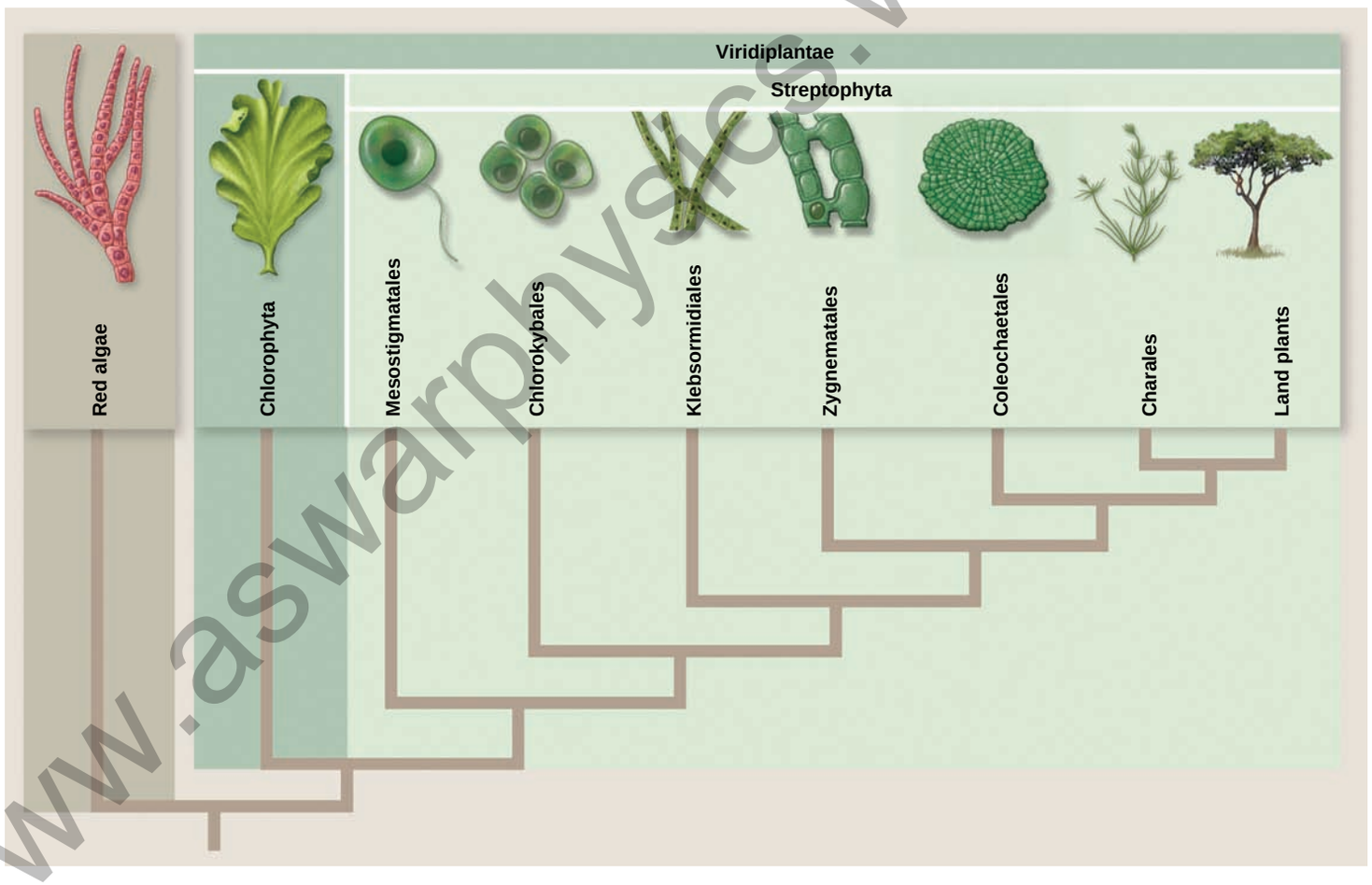
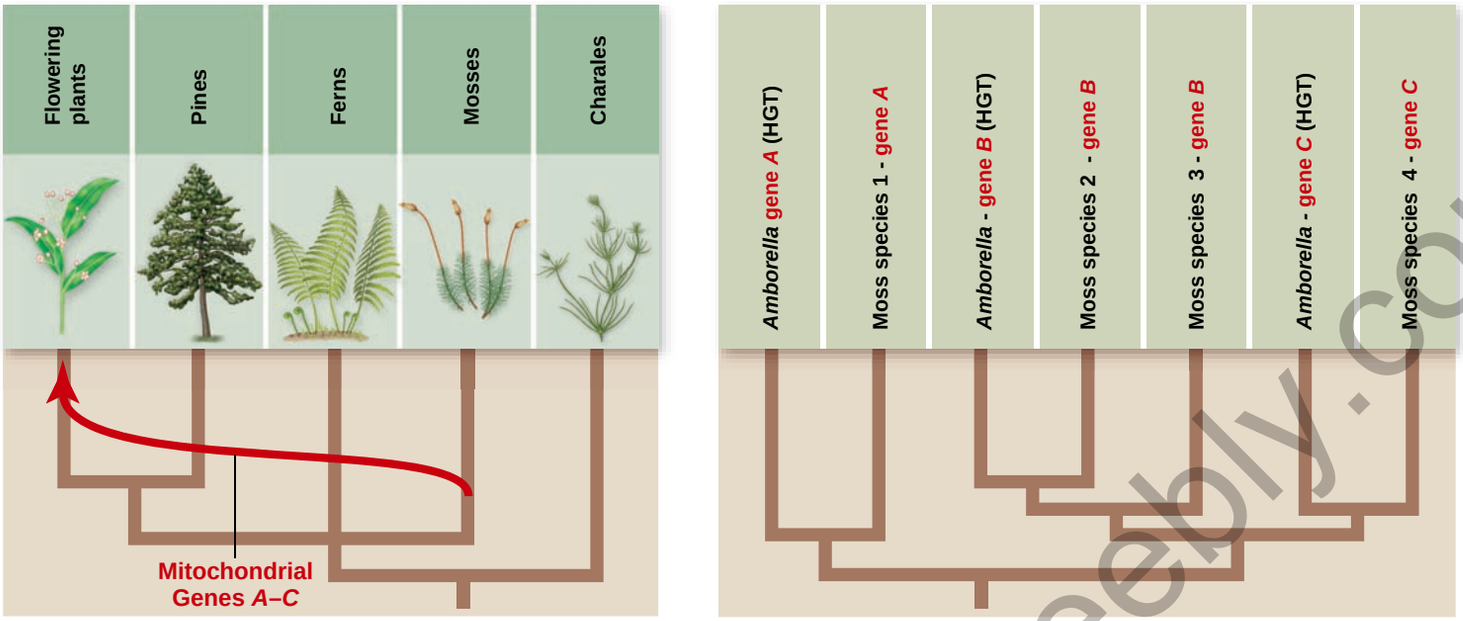


Figure 26.12 A new hypothesis for land plant evolution. Kingdom Plantae (land plants) has been reduced to a clade within the green algal branch Streptophyta, and a new kingdom, Viridiplantae, which includes the green algal branches Chlorophyta and Streptophyta, has been proposed. Within the Streptophyta, the relatively complex Charales are believed to be the sister clade to the land plants. Contrast this phylogeny with the one predicted by the six-kingdom system in figure 26.7b.



a. *b.*

Figure 26.13 The flowering plant *Amborella* acquired three moss genes through horizontal gene transfer. ***a.*** Phylogenetic relationship of *Amborella* to other land plants. As shown by the arrow connecting moss and the flowering plants, HGT is the only plausible explanation for the presence of moss mitochondrial genes in *Amborella*. ***b.*** Phylogenetic relationships among the horizontally transferred gene.

Inquiry question

Explain why a phylogenetic tree based on comparisons of a single gene could result in an inaccurate evolutionary hypothesis.

Amborella is not typical of most extant flowering plants. It is the only existing member of its genus and is native only to the tropical rain forests of New Caledonia, an island group east of Australia that has been isolated for some 70 million years and contains many ancient endemic species. Here parasitic plants called *epiphytes* (plants that derive nutrients from other plants) are common. Close contact with parasitic plants could increase the probability of HGT (figure 26.14).

An open question is whether the moss genes in *Amborella* have functions. About half the genes are intact and could be transcribed and translated into a protein. The protein would be similar to an existing protein in the plant, but its function, if any, remains to be determined.

Figure 26.14 Close contact between species can lead to HGT. Here moss are growing on the base of an *Amborella* leaf with lichens scattered on the rest of the leaf.

Inquiry question

How would you determine if a moss gene in *Amborella* had a function? (Hint: Refer to chapter 25.)

Learning Outcomes Review 26.5

Land plants are now thought to be most closely related to the Streptophyta group of green algae. The Charales appear to be the sister clade to land plants. Not all plant evolution is vertical, however; horizontal gene transfer has apparently mixed genes from distantly related species.

- ***How could genes from moss be transported into a flowering plant, other than via parasitic plants?***

26.6 *Sorting Out the Animals*

Learning Outcomes

1. ***Describe current ideas regarding the origin of segmentation in animals.***
2. ***Explain why insects are termed "flying crustaceans."***
3. ***State the evidence for a common ancestor between whales and hippos.***

Molecular systematics is leading to a revision of our understanding of evolutionary history in all kingdoms, including the

animals. Some phylogenies are changing, and others, including mammalian phylogenies, are actually being written for the first time. In this section, we explore three examples: the relationship between annelids and arthropods, relationships within the arthropods, and the discovery of phylogenetic relationships among mammals.

The origins of segmentation are puzzling

The arthropod phylum is a group of over one million described invertebrates that includes the insects and crustaceans; the annelid phylum, another invertebrate group, contains the segmented worms such as the earthworm. Morphological traits such as segmentation have been used in the past to group arthropods and annelids close together, but comparisons of rRNA sequences are raising questions about their relationship. As rRNA sequences are obtained, it is becoming increasingly clear that annelids and arthropods are more distantly related than taxonomists previously believed.

Evolutionary occurrences of segmentation

Distinctions can be made among eukaryote animals on the basis of timing of embryonic development of the mouth and anus. Annelids and arthropods belong to the **protostome** group, in which the mouth develops before the anus. Chordates, including humans, fall into the **deuterostome** group, in which the anus forms first. (You will learn more about these divisions in chapter 33.)

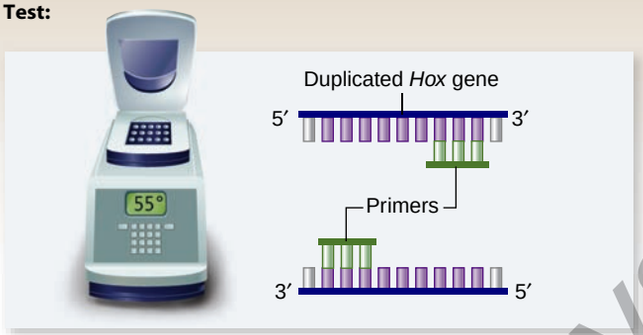
With the addition of newly available molecular traits, annelids and arthropods fell into two distinct protostome branches (figure 26.15): **lophotrochozoans** and **ecdysozoans.**

SCIENTIFIC THINKING

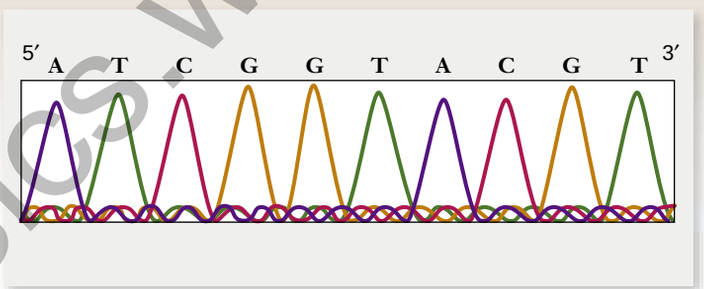
Hypothesis: *Origins of segmentation can be explained by the duplication of a* Hox *gene that regulates segmentation.*

Prediction: *Duplication of* Hox *genes will correlate with changes in segmentation patterns.*

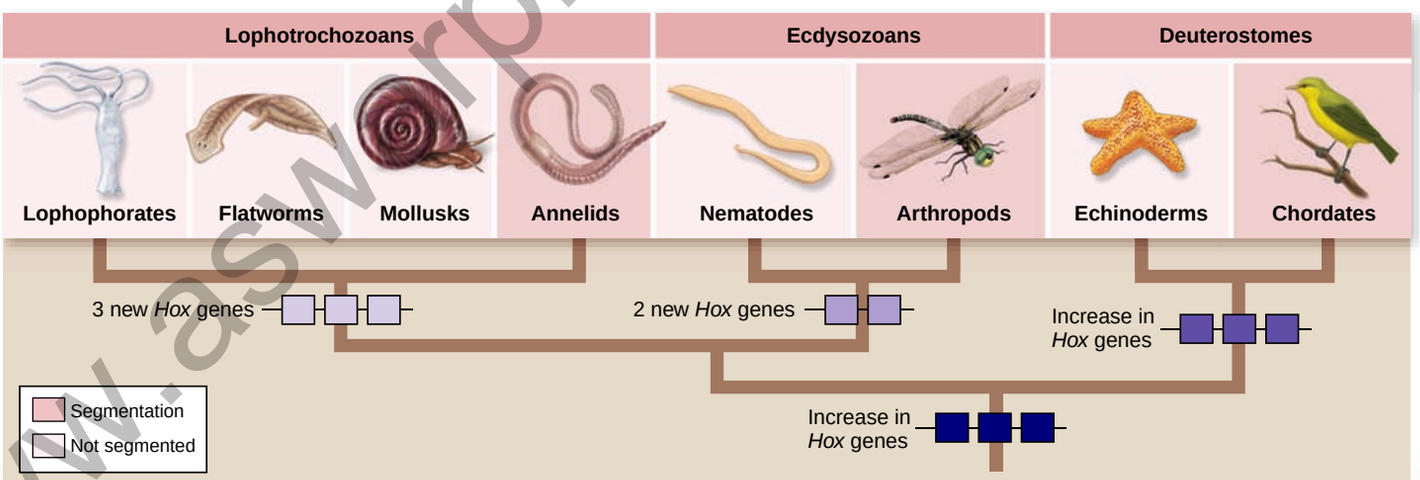
Test:



1. Using primers for the Hox *gene of interest and polymerase chain reaction (PCR), amplify the* Hox *gene of interest. Duplicated genes have nearly identical sequences and will be amplified with the same primer sets.*



2. Sequence the PCR products and group them based on sequence similarity.



3. Map the gene duplication findings on a phylogeny based on ribosomal RNA.

Result: *Four independent* Hox *gene duplication events were identified.*

Conclusion: *Three independent* Hox *gene duplication events correspond with the origins of segmentation.*

Further Research: *Explore where the duplicated* Hox *genes are expressed using in situ hybridization (refer to figure 19.13).*

Figure 26.15 **Multiple origins of segmentation.**

These two branches have been evolving independently since ancient times. Lophotrochozoans include flatworms, mollusks, and annelids. Two ecdysozoan phyla have been particularly successful: roundworms (nematodes) and arthropods.

In the new protostome phylogeny, annelids and arthropods do not constitute a monophyletic group, as they had in the past. The implication is that segmentation arose twice, not once, in the protostomes, as had been believed originally. Segmentation then arose independently once again in the deuterostomes, specifically in the chordates.

Molecular details of segmentation

The most likely explanation for the independent appearance of segmentation is that members of the same family of genes were co-opted at least three times. Segmentation is regulated by the *Hox* gene family that contains a homeodomain region (see chapter 19). The *Hox* ancestral genes predate the ecdysozoans and lophotrochozoans. The ancient ancestor of the lophotrochozoans, ecdysozoans, and deuterostomes most likely already had seven *Hox* genes. Some of these genes appear to have evolved a role in segmentation (see figure 26.15).

Insects and crustaceans are sister groups

Arthropods are the most diverse of all the animal phyla, composed of 80% of all described animal species. Within the arthropods, insects have traditionally been set apart from the crustaceans (such as shrimp, crabs, and lobsters), and grouped instead with the myriapods (centipedes and millipedes).

This phylogeny, still widely employed, dates back to benchmark work by Robert Snodgrass in the 1930s. He pointed out that insects, centipedes, and millipedes are united by several seemingly powerful attributes, including **uniramous** (single-branched) appendages. All crustacean appendages, by contrast, are basically **biramous,** or "two-branched" (figure 26.16), although some of these appendages have become single-branched by reduction in the course of their evolution.

Taxonomists have traditionally assumed a character such as two-branched appendages to be a fundamental one, conserved over the course of evolution, and thus suitable for making taxonomic distinctions. As molecular methods have been developed, however, this assumption has become questionable.

Hox *genes and appendages*

The patterning of appendages among arthropods is orchestrated by *Hox* genes. A single one of these *Hox* genes, called *Distal-less*, has been shown to initiate development of unbranched limbs in insects and branched limbs in crustaceans. The same *Distal-less* gene is found in many animal phyla, including the vertebrates.

Distal-less appears to be necessary to initiate limb development, and it turns on genes that are more directly involved in the development of the limb itself. Evolutionary changes in the genes that *Distal-less* acts on most likely account for differences in limb morphology.

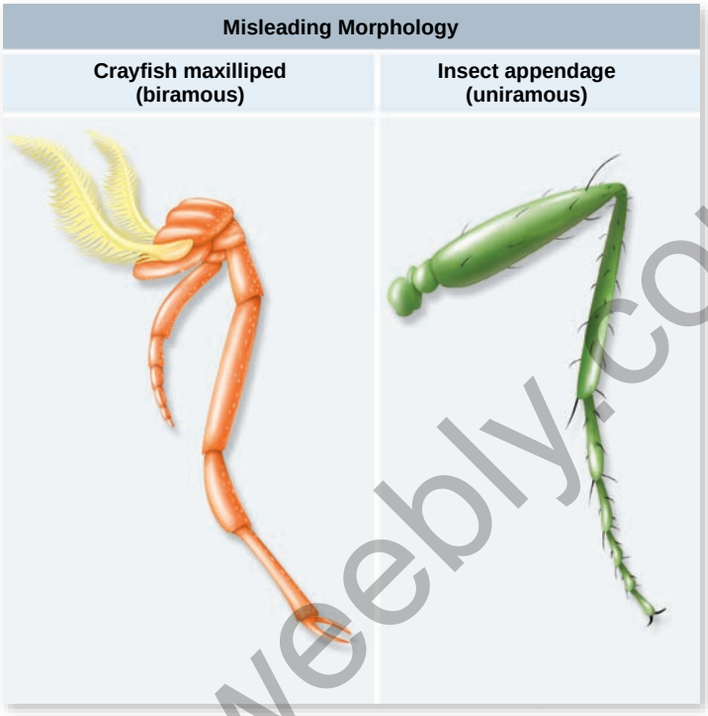


Figure 26.16 Branched and single appendages.
Development of a biramous leg in a crustacean (crayfish) and a uniramous leg in an insect are both initiated by the *Distal-less* gene even though their adult morphologies are distinct.

A change in taxonomic relationship?

In recent years, a mass of accumulating morphological and molecular data has led many taxonomists to suggest new arthropod phylogenies. Hexapods (insects) with their six legs and terrestrial habitat are the closest relatives of crustaceans, not the myriopods. Hexapods and crustaceans form a clade called pancrustacea. But, hexapods likely are not monophyletic, indicating that crustacean ancestors moved onto land multiple times. The relationships among the pancrustacea suggest that hexapods are "flying crustaceans."

These conclusions engender lively discussion since they are in conflict with 150 years of morphology-based phylogenetic inference.

The mammalian family tree is emerging

In the preceding arthropod examples, our interpretation of evolutionary history has been rewritten. In mammals, however, parts of the phylogeny are just emerging, based on molecular data.

The four groups of placental mammals

Among the vertebrate classes, mammals are unique because they have mammary glands to feed their young. The majority of mammals—over 90%—are **eutherians,** or **placental mammals** (see chapter 36). There are at least 18 extant orders of eutherians, which are now divided into four major groups (figure 26.17).

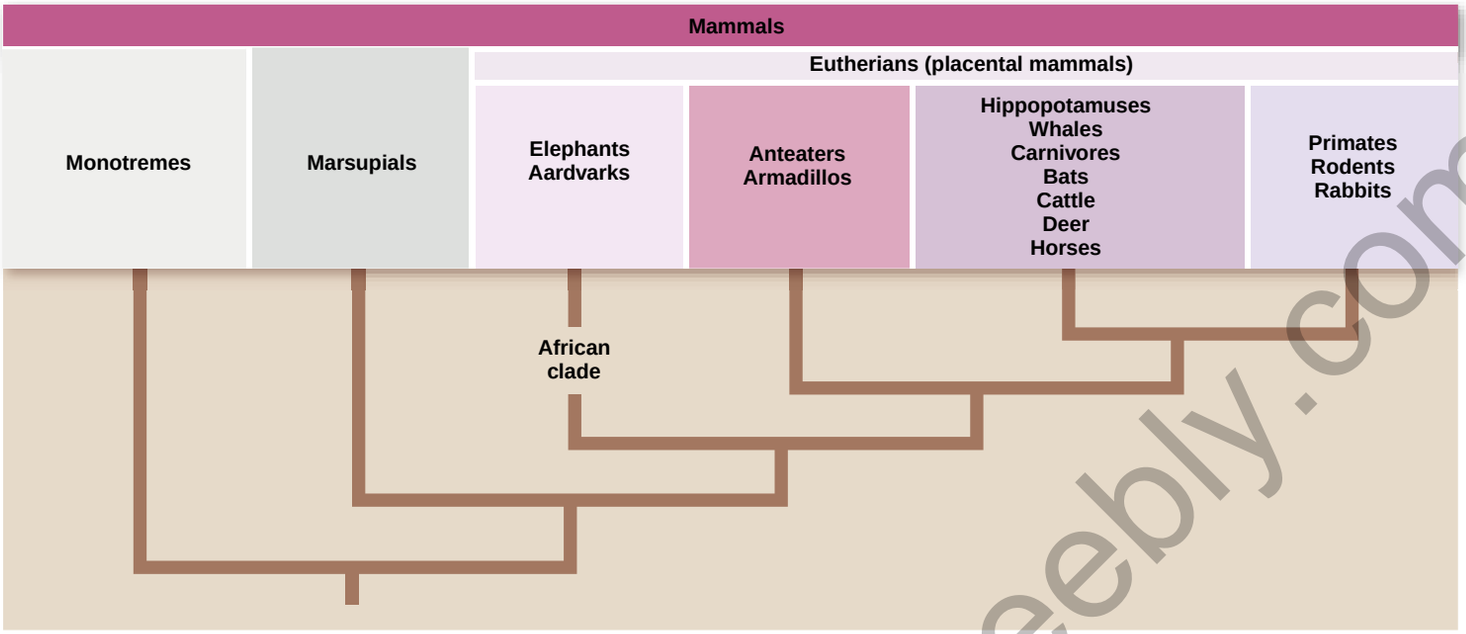


Figure 26.17 **Major groups of mammals.**

The first major split occurred between the African clade and the other placental mammals when South America and Africa separated about 100 MYA. Aardvarks and elephants are part of this African lineage, called the Afrotheria, a clade we did not even recognize a decade ago. In South America, anteaters and armadillos soon appeared. Then two other branches arose—one includes ungulates with an even number of toes (camels, llamas, and other artiodactyls), odd-toed ungulates (perissodactyls such as horses and rhinoceros), and carnivores, and the other, primates and rodents. Sorting out the relationships within these branches is an ongoing challenge.

Whales and hippos

The origin and relationships of whales has been debated for over 200 years. Whales were initially thought to be relatives of pigs based on morphological information from fossils and extant animals, primarily the bones of the skull and shape of the teeth.

DNA sequence data, however, revealed a particularly close relationship between whales and hippopotamuses, suggesting that whales were derived from within the group Artiodactyla. Whales and hippopotamuses appear to be much more closely related than, for example, hippopotamuses and cows. With this new phylogenetic information, the possibility arises that some adaptations to aquatic environments in both species had a common origin. Recent finds of fossil whales with hind limbs have confirmed the artiodactyl origin of whales. Prior to these recent discoveries, no fossil whales with hind limbs had been found, so the key character uniting whales and artiodactyls, the shape of a bone in the ankle, was not known. A careful analysis of 80 traits in fossil whales and hippos indicated a common, water-loving ancestor existed 50 to 60 MYA belonging to the anthracotheres. The anthracotheres split into two groups. One moved back to an aquatic environment. The other gave rise to at least 37 genera that left a single descendent, the hippopotamus. In this case, molecular data provided insight into whale origins that was later confirmed by fossil evidence.

Understanding evolutionary relationships among organisms does more than provide biologists with a sense of order and a logical way to name organisms. A phylogenetically based taxonomy allows researchers to ask important questions about physiology, behavior, and development using information already known about a related species. This information not only enriches our understanding of how biological complexity evolved, but also provides novel insights that lead to progress in our understanding of the history and origins of important features and functions.

Learning Outcomes Review 26.6

Molecular systematics has provided new insight into evolutionary relationships of animals. Segmentation likely arose more than once; hexapods are more closely related to crustaceans than to myriapods; and relationships among mammalian groups are still being revised based on molecular data and new fossil finds.

- ***Why might the most closely related living organisms have very different appearances?***

Chapter Review

26.1 Origins of Life

All organisms share fundamental properties of life.

Common properties of living things include cellular organization, sensitivity, growth and development, reproduction, regulation and homeostasis, and heredity.

Life may have had extraterrestrial origins.

The panspermia hypothesis proposes that the organic molecules of life came to Earth inside meteorites.

Life may have originated on early Earth.

Conditions on the early Earth were very different, with a reducing atmosphere that may have led to formation of carbon-rich, organic molecules.

Cells evolved from the functional assembly of organic molecules.

Complex molecules may have coalesced inside a space surrounded by a lipid or protein "bubble," a precursor of a cell membrane. Here, reactions could proceed more rapidly.

26.2 Classification of Organisms

Taxonomy is a quest for identity and relationships.

Taxonomy is the science of assigning organisms to a particular level of classification called a taxon. Taxonomic hierarchies are organized by domain, kingdom, phylum, class, order, family, genus, and species (see figure 26.6).

Linnaeus instituted the use of binomial names.

Carolus Linnaeus devised a system of giving individual species unique names, beginning with the capitalized genus, followed by a species name. These are italicized.

Taxonomic hierarchies have limitations.

Traditional classifications are limited because they are based on similar traits and do not take into account evolutionary relationships.

26.3 Grouping Organisms

The six kingdoms are not necessarily monophyletic.

The six kingdoms proposed by Woese are not necessarily monophyletic. In particular, Protista is paraphyletic (see figures 26.7 and 26.9).

The three domains probably are monophyletic.

Domain Eukarya contains the kingdoms Protista, Plantae, Fungi, and Animalia; the other two domains, Bacteria and Archaea, each contain only prokaryotes.

Bacteria are more numerous than any other organism.

Bacteria are the most abundant and diverse organisms on Earth; they consist of 12 to 15 major groups but their evolutionary links are unclear.

Archaea may live in extreme environments.

Archaea are prokaryotes that are more closely related to eukaryotes than to bacteria. They are grouped into methanogens, extremophiles, and nonextreme archaea.

Eukaryotes have compartmentalized cells.

Eukaryote cells have compartmentalized organelles and other structures. Eukaryotes acquired mitochondria and chloroplasts by endosymbiosis (see figure 26.8). Many eukaryotes are multicellular, and most undergo sexual reproduction.

Viruses are a special case.

Viruses are diverse chemical assemblies that cannot reproduce on their own (see figure 26.10).

26.4 Making Sense of the Protists

Protists are divided into six groups, but at least 60 known protists do not fit into any of these groups (see figure 26.11).

A new kingdom, Viridiplantae, has been suggested to include green algae and all aquatic and land plants.

26.5 Origin of Plants

Molecular phylogenetics has identified the closest living relatives of land plants (see figure 26.12).

Green algae consist of two monophyletic groups: Chlorophyta and Streptophyta. The latter group gave rise to land plants.

Horizontal gene transfer occurred in land plants.

Some land plants show evidence of horizontal gene transfer, such as between the flowering plant *Amborella* and mosses (see figure 26.13).

26.6 Sorting Out the Animals

The origins of segmentation are puzzling.

Phylogeny based on rRNA shows that segmentation in arthropods, annelids, and chordates arose independently at least three times. Evidence points to duplications in *Hox* genes that regulate segmentation as the cause (see figure 26.15).

Insects and crustaceans are sister groups.

Although previously based on appendages, classification of arthropods has now placed hexapods (insects) as close relatives of crustaceans based on molecular and genetic data.

The mammalian family tree is emerging.

Mammalian phylogeny continues to be refined as molecular data becomes available. Whales, for example, are most closely related to hippopotamuses, sharing a water-loving common ancestor.

Review Questions

UNDERSTAND

1. The Miller–Urey experiment demonstrated that
   a. life originated on Earth.
   b. organic molecules could have originated in the early atmosphere.
   c. the early genetic material on the planet was DNA.
   d. the early atmosphere contained large amounts of oxygen.
2. Which of the following properties of life would have to be significantly different for an organism that evolved on a planet located far from its sun?
   a. Homeostasis
   b. Reproduction
   c. Growth
   d. Sensitivity
3. Analyze a key limitation of the Linnaean system of classification.
4. Identify which of the following would not belong to the domain Eukarya.
   a. Photosynthetic plants
   b. Multicellular fungi
   c. Thermophilic archaea
   d. Multicellular animals
5. Explain which of the kingdoms presented the greatest challenge to the acceptance of a six-kingdom system.
6. Identify which of the following statements is false and correct the statement.
   a. Brown and red algae are not closely related phylogenetically.
   b. Chloroplasts in brown and red algae are monophyletic.
   c. Brown algae gained chloroplasts by engulfing green algae (endosymbiosis).
   d. None of the above statements are false.
7. Which of the following events occurred first in eukaryotic evolution?
   a. Endosymbiosis and mitochondria evolution
   b. Endosymbiosis and chloroplast evolution
   c. Compartmentalization and formation of the nucleus
   d. Formation of multicellular organisms
8. Given your understanding of phylogenetics, where would you place viruses in the tree of life?
   a. Archaea
   b. Fungi
   c. Bacteria
   d. None of the above

APPLY

1. As a researcher you discover a new species that is eukaryotic, motile, possesses a cell wall made of chitin, but lacks any evidence of a nervous system. Choose the kingdom of life that best aligns with this new species.
2. Kingdom Plantae is being replaced by a new kingdom named Viridiplantae. Choose the evidence that justifies this change.
   a. Molecular phylogenetics
   b. Newly discovered fossils
   c. Biochemical differences
   d. All of the above
3. Based on information in this chapter, choose which areas of research in plant biology have the greatest potential to increase our understanding of land plant evolution.
   a. Photosynthetic pigments
   b. Chloroplast endosymbiosis
   c. Horizontal gene transfer
   d. Changes in cell wall composition
4. You are given access to a database of *Hox* gene sequences from a very large number of animals. Choose which topic you could explore with your sequences from an evolutionary perspective.
   a. Multicellularity
   b. Sexual reproduction
   c. Cellular compartmentalization
   d. Segmentation
5. Molecular evidence and morphological evidence do not always produce the same evolutionary hypotheses. Consider the morphological evidence for arthropod evolution and evaluate how it aligns with the conclusions drawn from the molecular evidence. Choose the aspect of morphological evidence for arthropod evolution that has been refuted by molecular evidence.
   a. Protostome and deuterostome classification
   b. Limb morphology and development
   c. Metamorphosis
   d. Development of eyes
6. Choose the most compelling evidence supporting the claim that the whale is the closest relative of the hippopotamus.
   a. Morphological information from fossils
   b. Morphological information from hippopotamuses
   c. Morphological evidence from carnivores
   d. DNA sequence data

SYNTHESIZE

1. The conditions on Mars, Jupiter's moon Europa, and Saturn's moon Titan mimic those that are believed to have occurred on the early Earth. Yet, these places are also different from our early planet. For example, both Europa and Titan are located far from the Sun. Suppose that someday in the future scientists discover bacteria on these moons that are very similar biochemically to the early bacteria on Earth. Explain how this information would support the theory of panspermia. What if the life was biochemically different?
2. Construct a phylogeny that includes arthropods, hexapods, crustaceans, echinoderms, nematodes, mollusks, and annelids based on evidence in this chapter.
3. You are part of a research team that has recently discovered evidence of a single-celled prokaryotic organism on Mars. As you begin your study of the organism, you wish to use a species from Earth as a comparison. Propose which domain of life you should obtain your reference species from and defend your choice.
4. In the past, classification has relied primarily on the evolution of morphological characteristics. Modern approaches are relying more heavily on molecular analysis. Defend the claim that molecular approaches are very important in developing evolutionary hypotheses.

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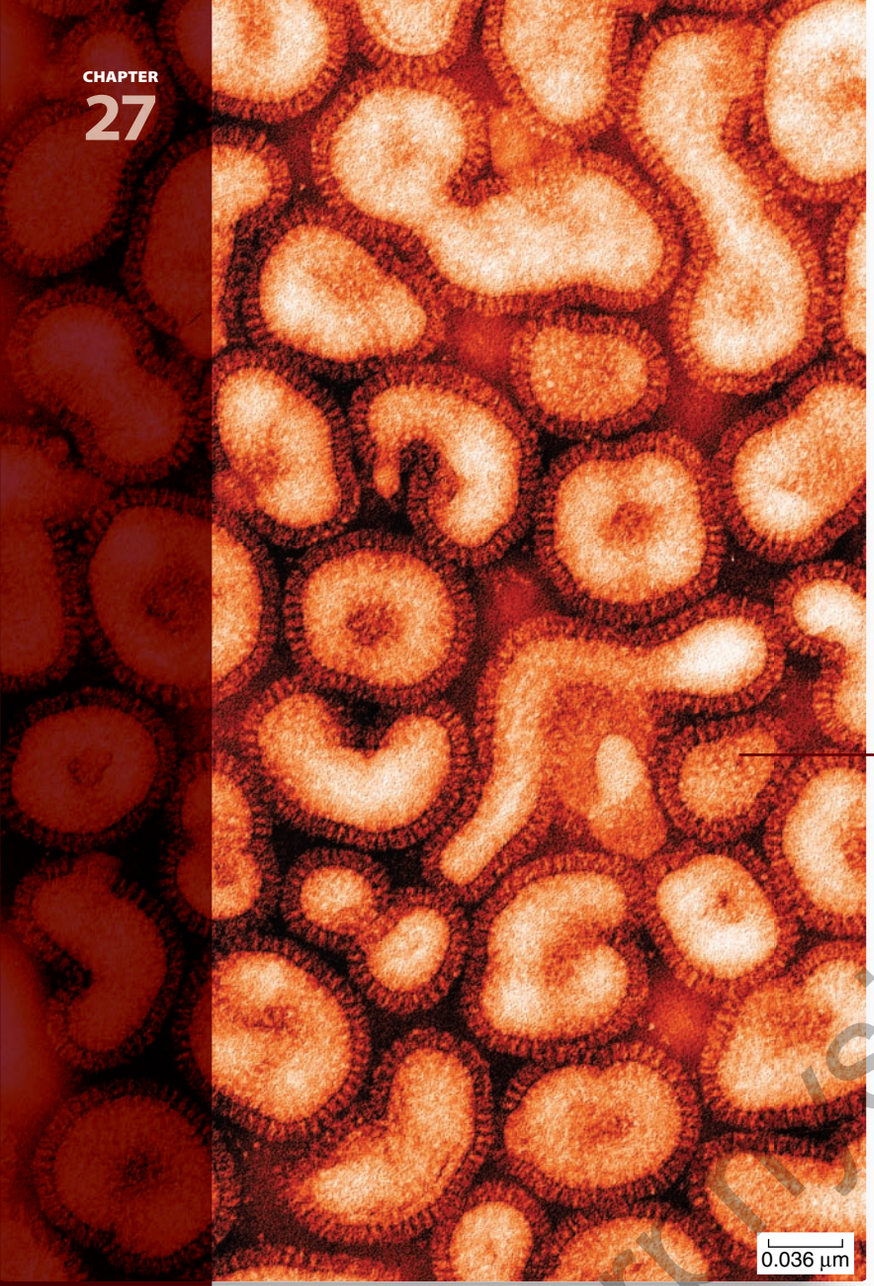


Chapter 27

Viruses

Chapter Outline

Introduction

We begin our exploration of the diversity of life with viruses. Viruses are genetic elements enclosed in protein; they are not considered organisms since they lack many of the features associated with life, including cellular structure, and independent metabolism or replication. For this reason viral particles are not called viral cells, but virions, *and they are generally not described as living or dead but as active or inactive. Because of their disease-producing potential, however, viruses are important biological entities. The virus particles pictured here are responsible for causing influenza—flu for short. In the flu season of 1918 to 1919, an influenza pandemic killed approximately 20 to 50 million people worldwide, twice as many as were killed in combat during World War I. Other viruses cause such diseases as AIDS, SARS, and hemorrhagic fever, and some cause certain forms of cancer.*

For more than four decades, viral studies have been thoroughly intertwined with those of genetics and molecular biology. Classic studies using viruses that infect bacteria (known as bacteriophage*) have led to the discovery of restriction enzymes and the identification of nucleic acid, not protein, as the hereditary material. Currently, viruses are one of the principal tools used to experimentally carry genes from one organism to another. Applications of this technology could include treating genetic illnesses and fighting cancer.*

27.1 The Nature of Viruses

Learning Outcomes

1. *Describe the different structures found in viruses.*
2. *Understand the basic mechanism of viral replication.*

All viruses have the same basic structure—a core of nucleic acid surrounded by protein. This structure lacks cytoplasm, and it is not a cell. Individual viruses contain only a single type of nucleic acid, either DNA or RNA. The DNA or RNA genome may be linear or circular; single-stranded or double-stranded.

RNA viruses may be segmented, with multiple RNA molecules within a virion, or nonsegmented, with a single RNA molecule. Viruses are classified, in part, by the nature of their genomes: RNA viruses, DNA viruses, or retroviruses.

Viruses are strands of nucleic acids encased in a protein coat

Nearly all viruses form a protein sheath, or **capsid,** around their nucleic acid core (figure 27.1). The capsid is composed of one to a few different protein molecules repeated many times. The repeating units are called capsomeres.

In several viruses, specialized enzymes are stored with the nucleic acid, inside the capsid. One example is reverse transcriptase, which is required for retroviruses to complete their cycle and is not found in the host. This enzyme is needed early in the infection process and is carried within each virion.

Many animal viruses have an *envelope* around the capsid that is rich in proteins, lipids, and glycoprotein molecules. The lipids found in the envelope are derived from the host cell; however, the proteins found in a viral envelope are generally virally encoded.

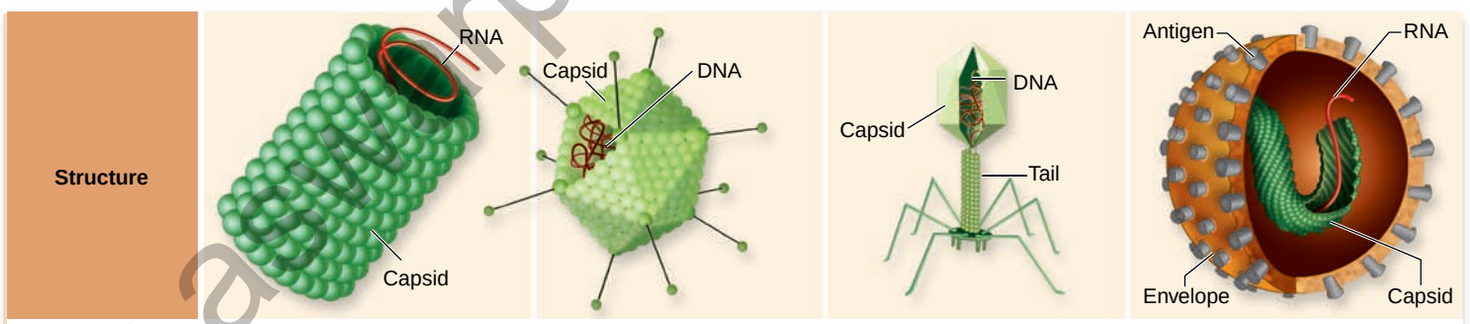
Viral hosts include virtually every kind of organism

Viruses occur as obligate intracellular parasites in every kind of organism that has been investigated for their presence. Viruses infect fungal cells, bacterial cells, and protists as well as cells of plants and animals; however, each type of virus can replicate in only a very limited number of cell types. A virus that infects bacteria would be ill-equipped to infect a human or plant cell.

The suitable cells for a particular virus are collectively referred to as its **host range.** Once inside a multicellular host, many viruses also exhibit **tissue tropism,** targeting only a specific set of cells. For example, rabies virus grows within neurons, and hepatitis virus replicates within liver cells. Once inside a host cell, some viruses, such as the highly dangerous Ebola virus, wreak havoc on the cells they infect; others produce little or no damage. Still other viruses remain dormant until a specific signal or event triggers their expression.

As one example, a person can get chicken pox as a child, recover, and develop the disease shingles decades later. Both chicken pox and shingles are caused by the same virus, varicella zoster. This virus can remain dormant, or *latent*, for years. Stresses to the immune system may trigger an outbreak of shingles in people who have had chicken pox in the past. This is caused by the same virus, but the infection may be called herpes zoster because the virus is actually a herpes virus.

Any given organism may often be susceptible to more than one kind of virus. This observation suggests that many more kinds of viruses may exist than there are kinds of organisms—perhaps trillions of different viruses. Only a few thousand viruses have been described at this point.



Structure	a.	b.	c.	d.
Virus	Plant virus (TMV)	Animal virus (Adenovirus)	Bacterial virus	Animal virus (Influenza)
Viral shape	Helical capsid	Icosahedral capsid	Icosahedral head: helical tail	Helical capsid within envelope

Figure 27.1 Structure of virions. Viruses are characterized as helical, icosahedral, binal, or polymorphic, depending on their symmetry. ***a.*** The capsid may have helical symmetry such as the tobacco mosaic virus (TMV). TMV infects plants and consists of 2130 identical protein molecules *(green)* that form a cylindrical coat around the single strand of RNA *(red)*. ***b.*** The capsid of icosahedral viruses has 20 facets made of equilateral triangles. These viruses can come in many different sizes all based on the same basic shape. ***c.*** Bacteriophage come in a variety of shapes, but binal symmetry is exclusively seen in phages such as the T4 phage of *E. coli.* This form of symmetry is characterized by an icosahedral head, which contains the viral genome, and a helical tail. ***d.*** Viruses can also have an envelope surrounding the capsid such as the influenza virus. This gives the virus a polymorphic shape. This virus has eight RNA segments, each within a helical capsid.

Viruses replicate by taking over host machinery

An infecting virus can be thought of as a set of instructions, not unlike a computer program. A cell is normally directed by chromosomal DNA-encoded instructions, just as a computer's operation is controlled by the instructions in its operating system. A virus is simply a set of instructions, the viral genome, that can trick the cell's replication and metabolic enzymes into making copies of the virus. Computer viruses get their name because they perform similar actions, taking over a computer and directing its activities. Like a computer with a virus, a cell with a virus is often damaged by infection.

Viruses can reproduce only when they enter cells. When they are outside of a cell, viral particles are called *virions* and are metabolically inert. Viruses lack ribosomes and the enzymes necessary for protein synthesis and most, if not all of the enzymes for nucleic acid replication. Inside cells, the virus hijacks the transcription and translation systems to produce viral proteins from *early genes*, which are the genes in the viral genome expressed first. This is followed by the expression of *middle genes* and eventually *late genes*. This cascade of gene expression leads to replication of viral nucleic acid and production of viral capsid proteins. The late genes generally code for proteins important in assembly and release of viral particles from a host cell.

Most viruses come in two simple shapes

Most viruses have an overall structure that is either *helical* or *icosahedral*. Helical viruses, such as the tobacco mosaic virus in figure 27.1*a*, have a rodlike or threadlike appearance. Icosahedral viruses have a soccer ball shape, the geometry of which is revealed only under the highest magnification with an electron microscope.

The **icosahedron** is a structure with 20 equilateral triangular facets. Most animal viruses are icosahedral in basic structure (figure 27.1*b*). The icosahedron is the basic design of the geodesic dome, and it is the most efficient symmetrical arrangement that subunits can take to form a shell with maximum internal capacity (figure 27.2).

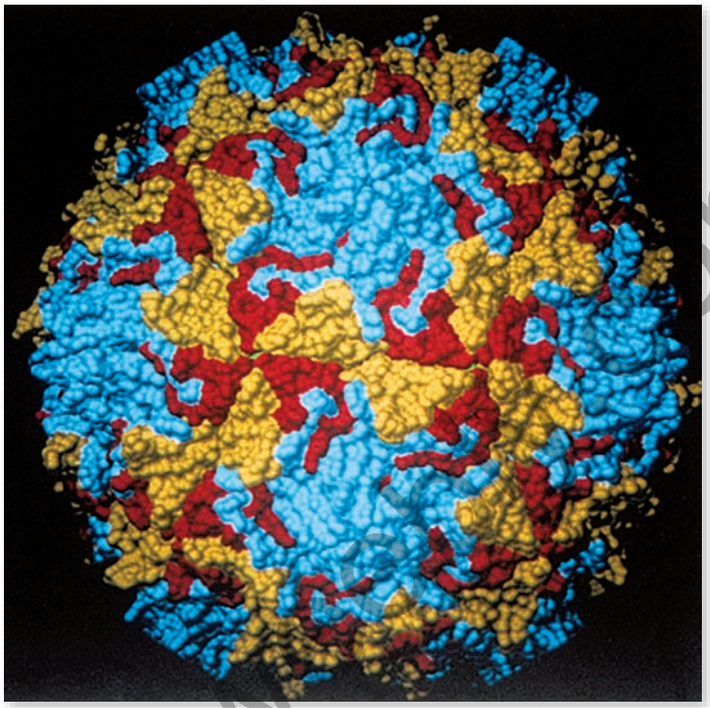
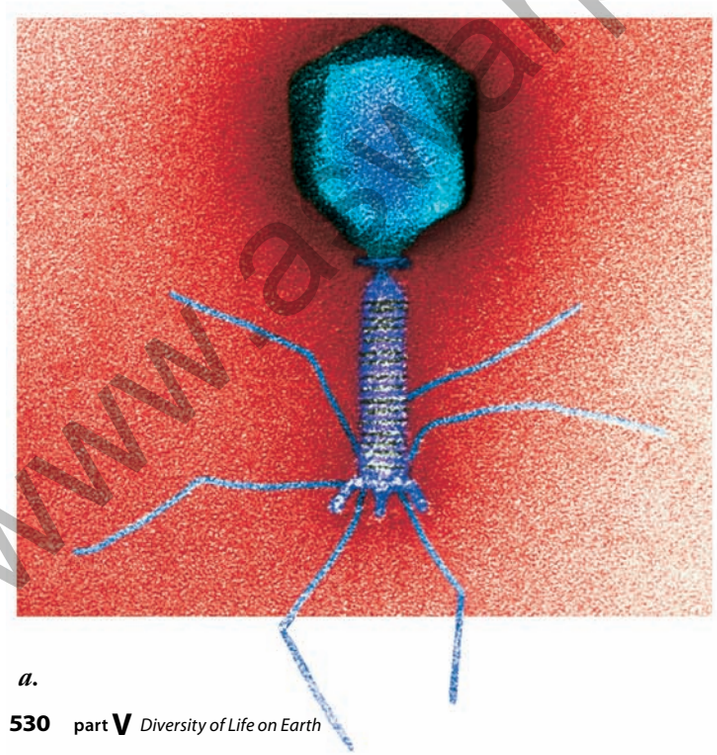
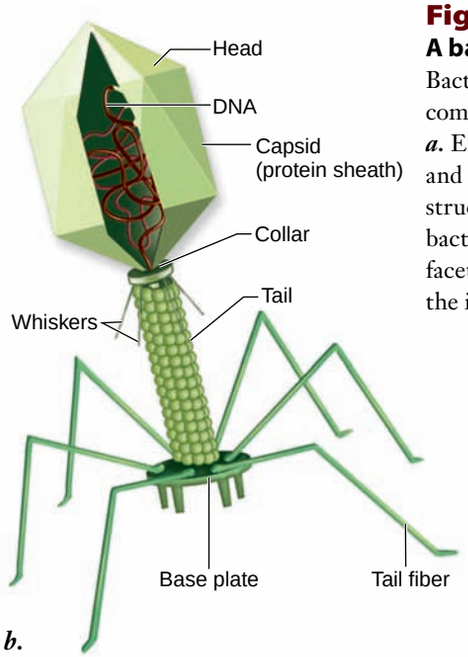


Figure 27.2 Icosahedral virion. The poliovirus has icosahedral symmetry. The capsid is formed from multiple copies of four different proteins shown in different colors. (One protein is internal and cannot be seen.)

Some viruses, such as the T-even bacteriophage shown in figure 27.3, are complex. Complex viruses have a *binal*, or



a.



b.

Figure 27.3
A bacterial virus. Bacteriophage exhibit a complex structure. ***a.*** Electron micrograph and ***(b)*** diagram of the structure of a T4 bacteriophage (some facets removed to reveal the interior).

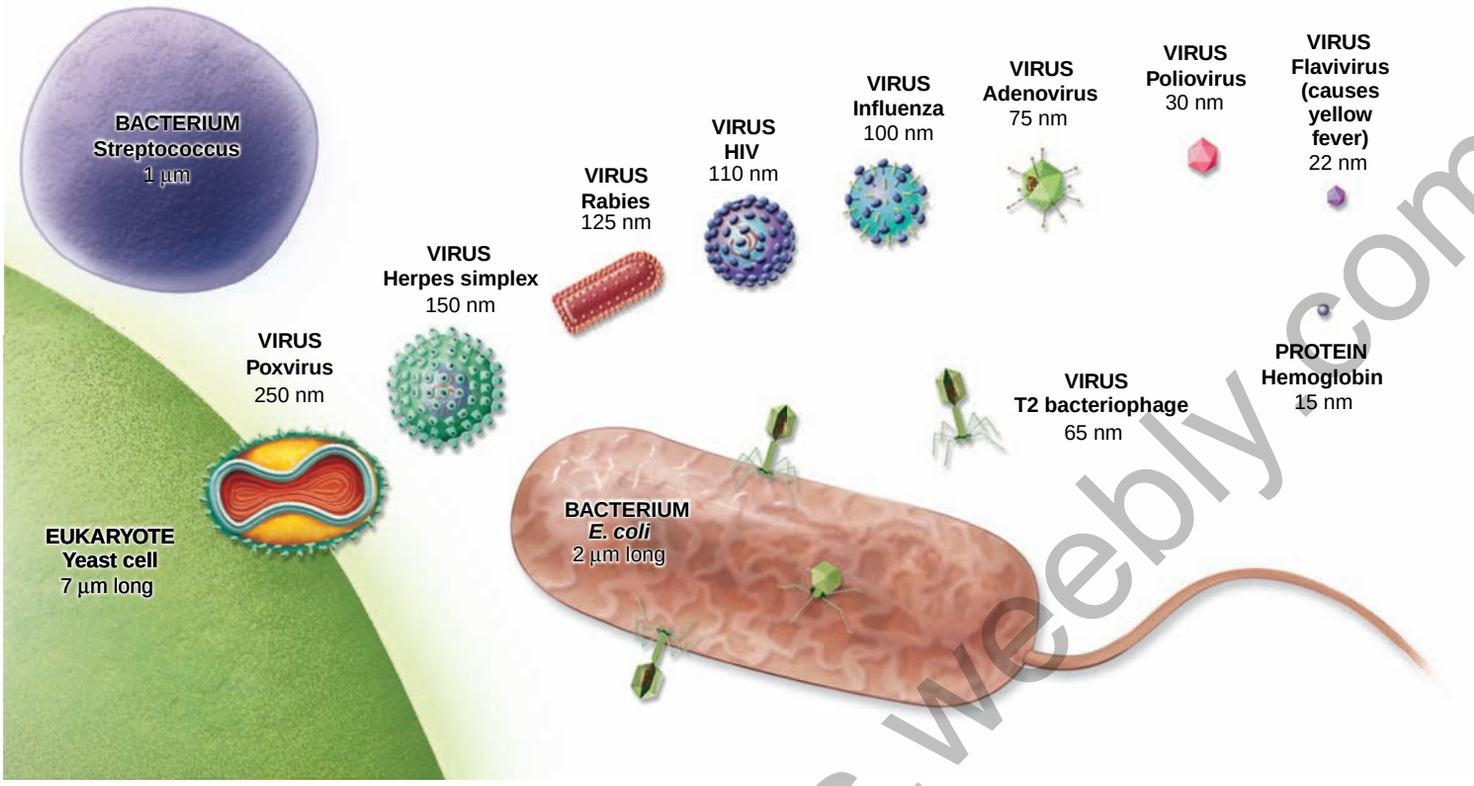


Figure 27.4 Viruses vary in size and shape. Note the dramatic differences in the size of a eukaryotic yeast cell, prokaryotic bacterial cells, and the many different viruses.

two-fold, symmetry that is not either purely icosahedral or helical. The T-even phage shown has a head structure that is an elongated icosahedron. A collar connects the head to a hollow tube with helical symmetry that ends in a complex baseplate with tail fibers. Although animal viruses do not have this binal symmetry, some, such as the poxviruses, do have a complex multilayered capsid structure. Some enveloped viruses, such as influenza, are *polymorphic*, having no distinctive symmetry.

Viruses also vary greatly in size. As shown in figure 27.4, the very smallest viruses, such as the poliovirus, have actually been synthesized in a lab using nothing more than sequence data and a machine capable of synthesizing nucleic acids from nucleotides. The larger viruses, such as the poxviruses, generally carry more genes, have more complex structures, and tend to have a very short cycle time between entry of viral particles and release of newly formed virions.

Viral genomes exhibit great variation

Viral genomes vary greatly in both type of nucleic acid and number of strands (table 27.1). Some viruses, including those that cause flu, measles, and AIDS, possess RNA genomes. Most RNA viruses are single-stranded and are replicated and assembled in the cytosol of infected eukaryotic cells. RNA virus replication is error-prone, leading to high rates of mutation. This makes them difficult targets for the host immune system, vaccines, and antiviral drugs.

In single-stranded RNA viruses, if the genome has the same base sequence as the mRNA used to produce viral proteins, then the genomic RNA can serve as the mRNA. Such viruses are called *positive-strand viruses*. In contrast, if the genome is complementary to the viral mRNA, then the virus is called a *negative-strand virus*.

A special class of RNA viruses, called *retroviruses*, have an RNA genome that is reverse-transcribed into DNA by the enzyme *reverse transcriptase*. The DNA fragments produced by reverse transcription are often integrated into a host's chromosomal DNA. *Human immunodeficiency virus (HIV)*, the agent that causes *acquired immune deficiency syndrome (AIDS)*, is a retrovirus. (We describe HIV in detail later on.)

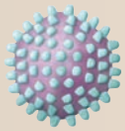
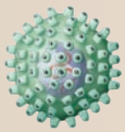
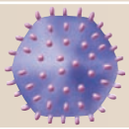
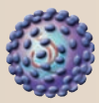
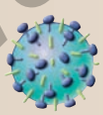
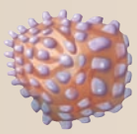
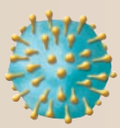
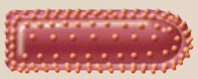
Other viruses, such as the viruses causing smallpox and herpes, have DNA genomes. Most DNA viruses are double-stranded, and their DNA is replicated in the nucleus of eukaryotic host cells.

Learning Outcomes Review 27.1

Viruses have a very simple structure that includes a nucleic acid genome encased in a protein coat. Viruses replicate by taking over a host's cell systems and are thus obligate intracellular parasites. Viruses show diverse genomes that are composed of DNA or RNA, which may be single- or double-stranded; most DNA viruses are double-stranded.

- ***Why can't viruses replicate outside of a cell?***

TABLE 27.1 Important Human Viral Diseases

Disease	Pathogen	Genome	Vector/Epidemiology
Chicken pox	Varicella-zoster virus 	Double-stranded DNA	Spread through contact with infected individuals. No cure. Rarely fatal. Vaccine approved in U.S. in early 1995. May exhibit latency leading to shingles.
Hepatitis B (viral)	Hepadnavirus 	Double-stranded DNA	Highly infectious through contact with infected body fluids. Approximately 1% of U.S. population infected. Vaccine available. No cure. Can be fatal.
Herpes	Herpes simplex virus 	Double-stranded DNA	Blisters; spread primarily through skin-to-skin contact with cold sores/blisters. Very prevalent worldwide. No cure. Exhibits latency—the disease can be dormant for several years.
Mononucleosis	Epstein–Barr virus 	Double-stranded DNA	Spread through contact with infected saliva. May last several weeks; common in young adults. No cure. Rarely fatal.
Smallpox	Variola virus 	Double-stranded DNA	Historically a major killer; the last recorded case of smallpox was in 1977. A worldwide vaccination campaign wiped out the disease completely.
AIDS	HIV 	(+) Single-stranded RNA (two copies)	Destroys immune defenses, resulting in death by opportunistic infection or cancer. For the year 2007, WHO estimated that 33.2 million people are living with AIDS, with an estimated 4.1 million new HIV infections and an estimated 2.8 million deaths.
Polio	Enterovirus 	(+) Single-stranded RNA	Acute viral infection of the CNS that can lead to paralysis and is often fatal. Prior to the development of Salk's vaccine in 1954, 60,000 people a year contracted the disease in the U.S. alone.
Yellow fever	Flavivirus 	(+) Single-stranded RNA	Spread from individual to individual by mosquito bites; a notable cause of death during the construction of the Panama Canal. If untreated, this disease has a peak mortality rate of 60%.
Ebola	Filoviruses 	(–) Single-stranded RNA	Acute hemorrhagic fever; virus attacks connective tissue, leading to massive hemorrhaging and death. Peak mortality is 50–90% if untreated. Outbreaks confined to local regions of central Africa.
Influenza	Influenza viruses 	(–) Single-stranded RNA (eight segments)	Historically a major killer (20–50 million died during 18 months in 1918–1919); wild Asian ducks, chickens, and pigs are major reservoirs. The ducks are not affected by the flu virus, which shuffles its antigen genes while multiplying within them, leading to new flu strains. Vaccines are available.
Measles	Paramyxoviruses 	(–) Single-stranded RNA	Extremely contagious through contact with infected individuals. Vaccine available. Usually contracted in childhood, when it is not serious; more dangerous to adults.
SARS	Coronavirus 	(–) Single-stranded RNA	Acute respiratory infection; an emerging disease, can be fatal, especially in the elderly. Commonly infected animals include bats, foxes, skunks, and raccoons. Domestic animals can be infected.
Rabies	Rhabdovirus 	(–) Single-stranded RNA	An acute viral encephalomyelitis transmitted by the bite of an infected animal. Fatal if untreated. Commonly infected animals include bats, foxes, skunks, and raccoons. Domestic animals can be infected.

27.2 Bacteriophage: Bacterial Viruses

Learning Outcomes

1. *Distinguish between lytic and lysogenic cycles in bacteriophage.*
2. *Describe how viruses can contribute DNA to their hosts.*

Bacteriophage (both singular and plural) are viruses that infect bacteria. They are diverse, both structurally and functionally, and are united solely by their occurrence in bacterial hosts. Many of these types of bacteriophage, called *phage* for short, are large and complex, with relatively large amounts of DNA and proteins.

E. coli-infecting viruses were among the first bacteriophage to be discovered and are still some of the best studied. Some of these viruses that infect *E. coli* have been named as members of a "T" series (T1, T2, and so forth); others have been given different types of names. To illustrate the diversity of these viruses, T3 and T7 phage are icosahedral and have short tails. In contrast, the so-called T-even phage (T2, T4, and T6) have an icosahedral head, a capsid that consists primarily of three proteins, a connecting neck with a collar and long "whiskers," a long tail, and a complex base plate (see figure 27.3).

Archaeal viruses have diverse morphologies

Archaeal viruses were initially thought to be similar to bacterial viruses, but recent evidence argues against this. Surveys of viruses in several extreme environments dominated by archaeal species have uncovered an unexpected diversity of viral forms. In addition to the viral types described in the previous section, viruses with a two-tailed structure, with a bottle-shaped structure, and with a spindle-shaped structure have all been observed. All of these viruses have double-stranded DNA genomes, and most appear to be unrelated to any bacteriophage. The characterization of these viruses is in the early stages, so we will not discuss them further.

Bacterial viruses exhibit two reproductive cycles

During the process of bacterial infection by phage T4, at least one of the tail fibers of the phage—they are normally held near the phage head by the "whiskers"—contacts proteins of the host bacterial cell wall. The other tail fibers set the phage perpendicular to the surface of the bacterium and bring the base plate into contact with the cell surface.

Contact with the host

Different phages may target different parts of the outer surface of a bacterial cell. This first step is called *attachment*, or *adsorption*. The next step, release of the phage genome into the host, is best understood in the binal phage, such as T4. Once contact is established, the tail contracts, and the tail tube passes through an opening that appears in the base plate, piercing the bacterial cell wall. The contents of the head, the DNA genome, are then injected into the host cytoplasm. This step is called *penetration*, or *injection*.

Once inside the bacterial cell a phage may immediately take over the cell's replication and protein synthesis enzymes to synthesize viral components. This is the *synthesis* phase. Once the components are made, they are assembled **(assembly)** and mature virus particles are *released*, either through the action of enzymes that lyse the host cell or by budding through the host cell wall.

The time between adsorption and the formation of new viral particles is called an *eclipse period* because if a cell is lysed at this point, few if any active virions can be released.

The lytic cycle

When a virus lyses the infected host cell in which it is replicating, the reproductive cycle is referred to as a **lytic cycle** (figure 27.5, *left*). The basic steps of a lytic bacteriophage cycle are similar to those of a nonenveloped animal virus. The T-series bacteriophage are all **virulent,** or **lytic, phage,** multiplying within infected cells and eventually lysing (rupturing) them.

The lysogenic cycle

In contrast to the rather simple lytic cycles, some bacteriophage do not immediately kill the cells they infect, instead they integrate their nucleic acid into the genome of the infected host cell. This integration gives them a distinct advantage; integration allows a virus to be replicated along with the host cell's DNA as the host divides. These viruses are called **temperate,** or **lysogenic, phage.** The DNA segment that is integrated into a host cell's genome is called a *prophage*, and the resulting cell is called a *lysogen*.

Among the bacteriophage that do this is the binal phage lambda (λ) of *E. coli*. Lambda may be the best studied biological particle; the complete sequence of its 48,502 bases has been determined. At least 23 proteins are associated with the development and maturation of phage λ, and other enzymes are involved in integrating this virus into the host genome.

When phage λ infects a cell, the early events constitute a genetic switch that will determine whether the virus will replicate and destroy the cell or become a lysogen and be passively replicated with the cell's genome. This lysis/lysogeny "decision" depends on the expression of early genes. Early on, two regulatory proteins are produced that will compete for binding to sites on the phage's DNA. Depending on which protein "wins" either the genes necessary for replication of the genome will be expressed beginning the lytic cycle, or the enzymes necessary for integrating the viral genome into the chromosome will be expressed and the **lysogenic cycle** initiated (figure 27.5, *right*).

A lysogenic phage has the expression of its genome repressed (see chapter 16) by one of the two viral regulatory proteins mentioned earlier. This is not a permanent state, however; in times of cell stress, the prophage can be derepressed, and the enzymes necessary for excision of the genome expressed. The viral genome then is in the same state as the initial stage of infection, and the lytic cycle can commence, leading to formation of viral particles and lysis of the cell.

The switch from a lysogenic prophage to a lytic cycle is called **i**nduction because it requires turning on the gene expression necessary for the lytic cycle. It can be stimulated in

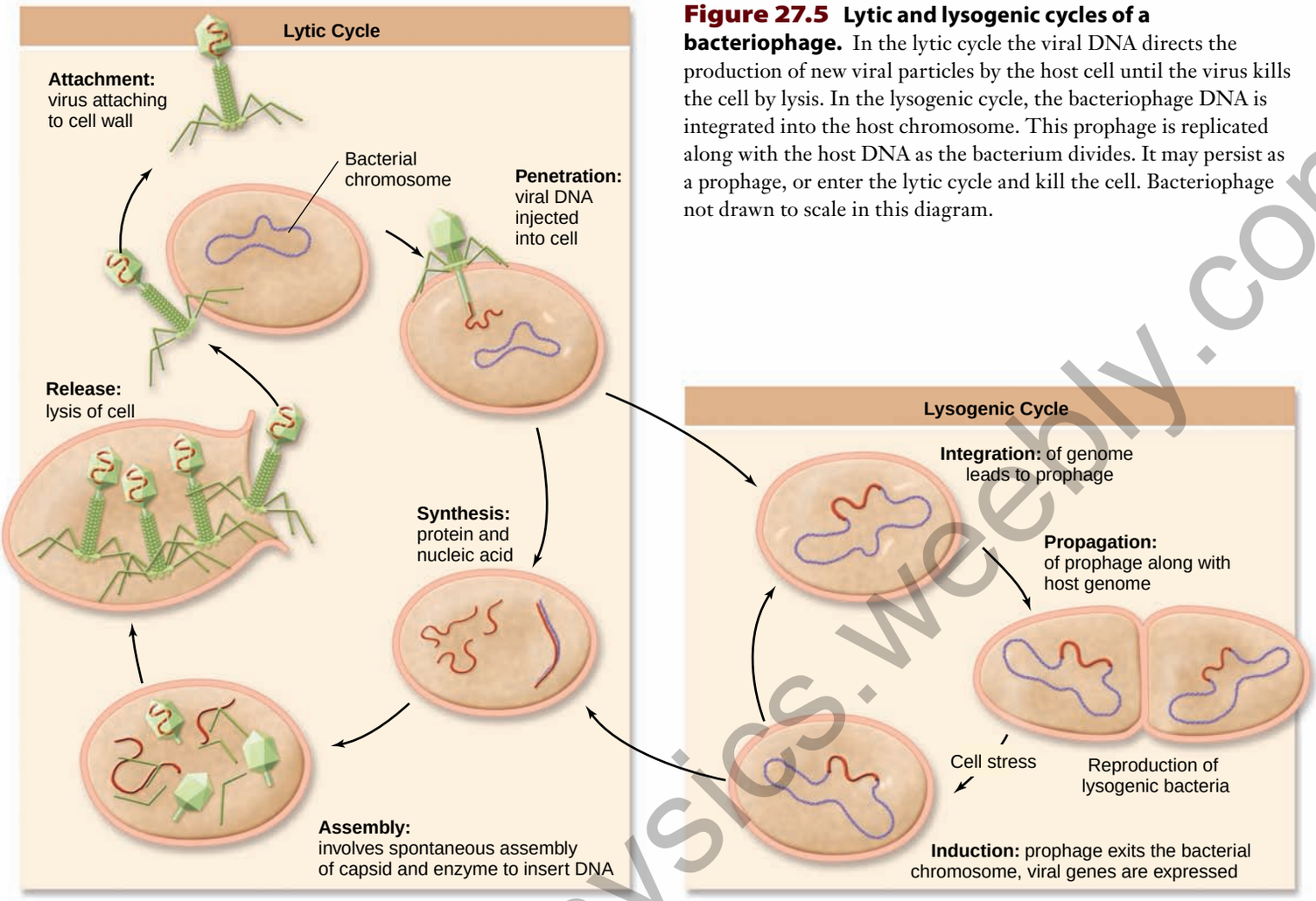


Figure 27.5 Lytic and lysogenic cycles of a bacteriophage. In the lytic cycle the viral DNA directs the production of new viral particles by the host cell until the virus kills the cell by lysis. In the lysogenic cycle, the bacteriophage DNA is integrated into the host chromosome. This prophage is replicated along with the host DNA as the bacterium divides. It may persist as a prophage, or enter the lytic cycle and kill the cell. Bacteriophage not drawn to scale in this diagram.

the laboratory by stressors such as starvation or ultraviolet radiation. The molecular events of induction take advantage of host proteins that respond to stress to produce a protease that can destroy the repressor protein that is keeping the viral genome silent. The normal function of this protease is to degrade a host repressor that controls DNA repair genes. The two repressor proteins are similar enough that both are degraded by the protease.

Bacteriophage can contribute genes to the host genome

During the integrated portion of a lysogenic reproductive cycle, a few viral genes may be expressed at the same time as host cell genes. Sometimes the expression of these genes has an important effect on the host cell, altering it in novel ways. When the phenotype or characteristics of the lysogenic bacterium is altered by the prophage, the alteration is called **phage conversion.**

Phage conversion of the cholera-causing bacterium

The bacterium *Vibrio cholerae* usually exists in a harmless form, but a second, disease-causing form also occurs. In this latter form, the bacterium is responsible for the deadly disease cholera, but how the bacteria changed from harmless to deadly was not known until recently.

Research now shows that a lysogenic bacteriophage that infects *V. cholerae* introduces into the host bacterial cell a gene that codes for the cholera toxin. This gene, along with the rest of the phage genome, becomes incorporated into the bacterial chromosome. The toxin gene is expressed along with the other host genes, thereby converting the benign bacterium to a disease-causing agent.

The receptors used by this toxin-encoding phage are pili (hairlike projections) found on the outer surface of *V. cholerae* (chapter 28); in recent experiments, it was determined that mutant bacteria that did not have pili were resistant to infection by the bacteriophage. This discovery has important implications in efforts to develop vaccines against cholera, which have been unsuccessful up to this point. Phage conversion could change any pili-expressing, nontoxigenic *V. cholerae* into a toxin-producing, potentially deadly form.

Another example involved in human disease is the toxin found in *Corynebacterium diphtheriae*. This toxin is the product of phage conversion, as are the changes to the outer surface of certain infectious *Salmonella* species.

Learning Outcomes Review 27.2

Bacteriophage are viruses that infect bacteria. They have two major types of life cycle: the lytic cycle that results in immediate death of the host, and the lysogenic cycle in which the virus becomes part of the host genome. This viral genome is then transmitted vertically by cell division. Under certain conditions the lysogenic phage can switch to the lytic cycle. Lysogenic phage contribute genes to the host, as is the case with *V. cholera*. The toxin responsible for the disease cholera came from a phage.

- ***What would be the result of a mutation in the λ repressor gene that resulted in a protein resistant to host protease?***

27.3 Human Immunodeficiency Virus (HIV)

Learning Outcomes

1. ***Explain how the HIV virus compromises the immune system.***
2. ***Describe the disease AIDS.***
3. ***Illustrate the different therapeutic options for AIDS.***

A diverse array of viruses occurs among animals. A good way to gain a general idea of the characteristics of these viruses is to look at one animal virus in detail. Here we examine the virus responsible for a comparatively new and fatal viral disease, *acquired immune deficiency syndrome (AIDS)*.

AIDS is caused by HIV

The disease now known as AIDS was first reported in the United States in 1981, although a few dozen people in the United States had likely died of AIDS prior to that time and had not been diagnosed. Frozen plasma samples and estimates based on evolutionary speed and current diversity of HIV strains trace the origins of HIV in the human population to Africa in the 1950s. It was not long before the infectious agent, a retrovirus, was identified by laboratories in France. Study of HIV revealed it to be closely related to a chimpanzee virus (simian immunodeficiency virus, SIV), suggesting a recent host expansion to humans from chimpanzees in central Africa.

Infected humans have varying degrees of resistance to HIV. Some have little resistance to infection and rapidly progress from having HIV-positive status to developing AIDS and eventually die. Others, even after repeated exposure, fail to become HIV-positive or may become HIV-positive without developing AIDS.

A relatively recent hypothesis to explain this great variability in susceptibility is genetic variation among these groups due to the selective pressure put on the human population by the smallpox virus (variola major) over the centuries. Because of successful vaccination and immunization, smallpox has been eradicated from the human population; however, before its eradication, it caused billions of deaths worldwide.

In order for smallpox to infect a cell, the cell must have a receptor protein in its plasma membrane that the virus can bind to. Individuals with mutated receptors would have been more resistant to smallpox and would have passed their genes on to their offspring. It has been suggested that one of the receptors used by HIV, CCR5, is also a receptor for smallpox. It is known that people resistant to HIV infection have a mutation in the CCR5 gene. The historical appearance and distribution of this mutation in human populations correlates with the historical distribution of smallpox. The AIDS epidemic is discussed further in chapter 52.

HIV infection compromises the host immune system

In AIDS patients, HIV primarily targets **$CD4^+$ cells,** particularly T-helper cells. **T-Helper cells** are responsible for mounting the immune response against foreign invaders, and their action is described more fully in chapter 52.

HIV infects and kills the $CD4^+$ cells until very few are left. Without these crucial immune system cells, the body cannot mount a defense against invading bacteria or viruses. AIDS patients die of infections that a healthy person could fight off. These diseases, called *opportunistic infections*, normally do not cause disease and are part of the progression from HIV infection to having AIDS.

Clinical symptoms typically do not begin to develop until after a long latency period, generally 8 to 10 years after the initial infection with HIV. Some individuals, however, may develop symptoms in as few as two years. During latency, HIV particles are not in circulation, but the virus can be found integrated within the genome of macrophages and $CD4^+$ T cells as a provirus (equivalent to a prophage in bacteria).

HIV testing

HIV tests do not test for the presence of circulating virus but rather for the presence of antibody against HIV. Because only those people exposed to HIV in their bloodstream at one time or another would have anti-HIV antibodies, this screening provides an effective way to determine whether further testing is needed to confirm HIV-positive status.

The spread of AIDS

Although carriers of HIV have no clinical symptoms during the long latency period, they are apparently fully infectious, which makes the spread of HIV very difficult to control. The reason HIV remains hidden for so long seems to be that its infection cycle continues throughout the 8- to 10-year latency period without doing serious harm to the infected person because of an effective immune response. Eventually, however, a random mutational event in the virus or a failure of the immune response allows the virus to quickly overcome the immune defense, beginning the course of AIDS.

HIV infects key immune-system cells

The way in which HIV infects humans provides a good example of how animal viruses replicate (figure 27.6). Most other viral infections follow a similar course, although the details of entry and replication differ in individual cases.

Attachment

When HIV is introduced into the human bloodstream, the virus particles circulate throughout the body but only infect $CD4^+$ cells. Most other animal viruses are similarly narrow in their requirements; hepatitis goes only to the liver, and rabies to the brain. This tissue tropism is determined by the proteins found on a cell surface and on a viral surface.

For example, the common cold virus uses the ICAM-1 membrane protein as a receptor to enter cells. ICAM-1 is a protein that is up-regulated (increased) in times of immune activation and stress. So, the more inflammation and stress in an area, the more receptors exist for the virus to enter a cell and continue the disease process.

How does a virus such as HIV recognize a target cell? Recall from chapter 4 that every kind of cell in the human body has a specific array of cell-surface glycoprotein markers that serve to identify them to other, similar cells. Invading viruses take advantage of this to bind to specific cell types. Each HIV particle possesses a glycoprotein called gp120 on its surface that precisely fits the cell-surface marker protein CD4 on the surfaces of the immune system macrophages and T cells. Macrophages, another type of white blood cell, are infected first. Because macrophages commonly interact with $CD4^+$ T cells, this may be one way that the T cells

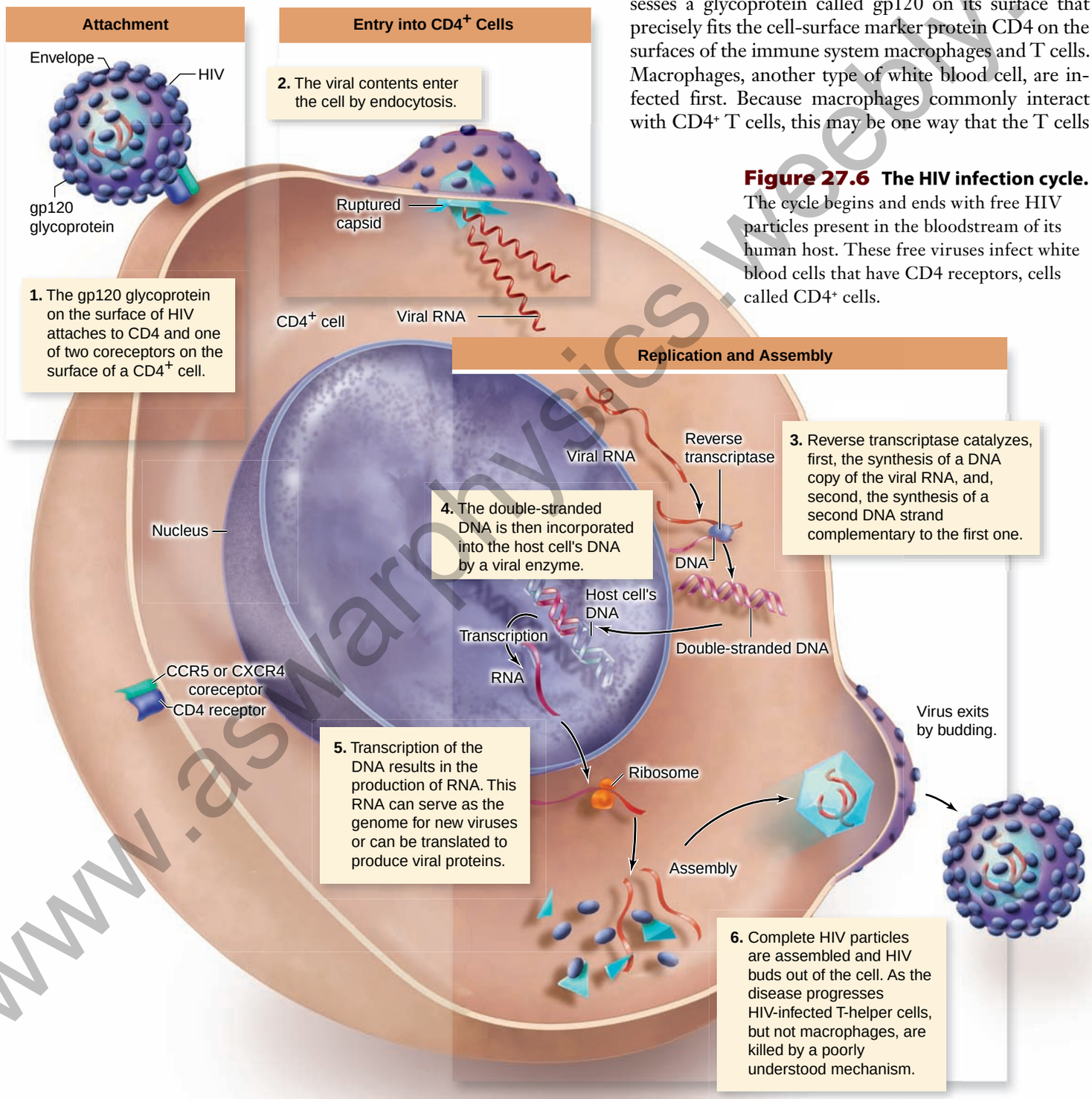


Figure 27.6 The HIV infection cycle. The cycle begins and ends with free HIV particles present in the bloodstream of its human host. These free viruses infect white blood cells that have CD4 receptors, cells called $CD4^+$ cells.

are infected. Several coreceptors also significantly affect the likelihood of viral entry into cells, including the CCR5 receptor, which is mutated in HIV-immune individuals.

Entry of virus

After docking onto the CD4 receptor of a cell, HIV requires a coreceptor such as CCR5, to pull itself across the cell membrane. After gp120 binds to CD4, it goes through a conformational change that allows it to then bind the coreceptor. Receptor binding is thought to ultimately result in fusion of the viral and target cell membranes and entry of the virus through a fusion pore. The coreceptor, CCR5, is hypothesized to have been used by the smallpox virus as was mentioned earlier.

Replication

Once inside the host cell, the HIV particle sheds its protective coat. This leaves viral RNA floating in the cytoplasm, along with the reverse transcriptase enzyme that was also within the virion. Reverse transcriptase synthesizes a double strand of DNA complementary to the virus RNA, often making mistakes and introducing new mutations. This double-stranded DNA then enters the nucleus along with a viral enzyme that incorporates the viral DNA into the host cell's DNA. After a variable period of dormancy the HIV provirus directs the host cell's machinery to produce many copies of the virus.

As is the case with most enveloped viruses, HIV does not directly rupture and kill the cells it infects. Instead, the new viruses are released from the cell by *budding*, a process much like exocytosis. HIV synthesizes large numbers of viruses in this way, challenging the immune system over a period of years. In contrast, naked viruses, those lacking an envelope, generally lyse the host cell in order to exit. Some enveloped viruses may produce enzymes that damage the host cell enough to kill it or may produce lytic enzymes as well.

Evolution of HIV during infection

During an infection, HIV is constantly replicating and mutating. The reverse transcriptase enzyme is less accurate than DNA polymerases, leading to a high mutation rate. Eventually, by chance, variants in the gene for gp120 arise that cause the gp120 protein to alter its second-receptor partner. This new form of gp120 protein will bind to a different second receptor, for example CXCR4, instead of CCR5. During the early phase of an infection, HIV primarily targets immune cells with the CCR5 receptor. Eventually the virus mutates to infect a broader range of cells. Ultimately, infection results in the destruction and loss of critical T-helper cells.

This destruction of T cells blocks the body's immune response and leads directly to the onset of AIDS, with cancers and opportunistic infections free to invade the defenseless victim. Most deaths due to AIDS are not a direct result of HIV, but are from other diseases that normally do not harm a host with a normal immune system.

AIDS treatment targets different phases of the HIV life cycle

The federal Food and Drug Administration (FDA) currently lists 32 antiretroviral drugs that are used in AIDS therapy. These target four aspects of the HIV life cycle: viral entry, genome replication, integration of viral DNA, and maturation of HIV proteins (figure 27.7). Of these, the vast majority are

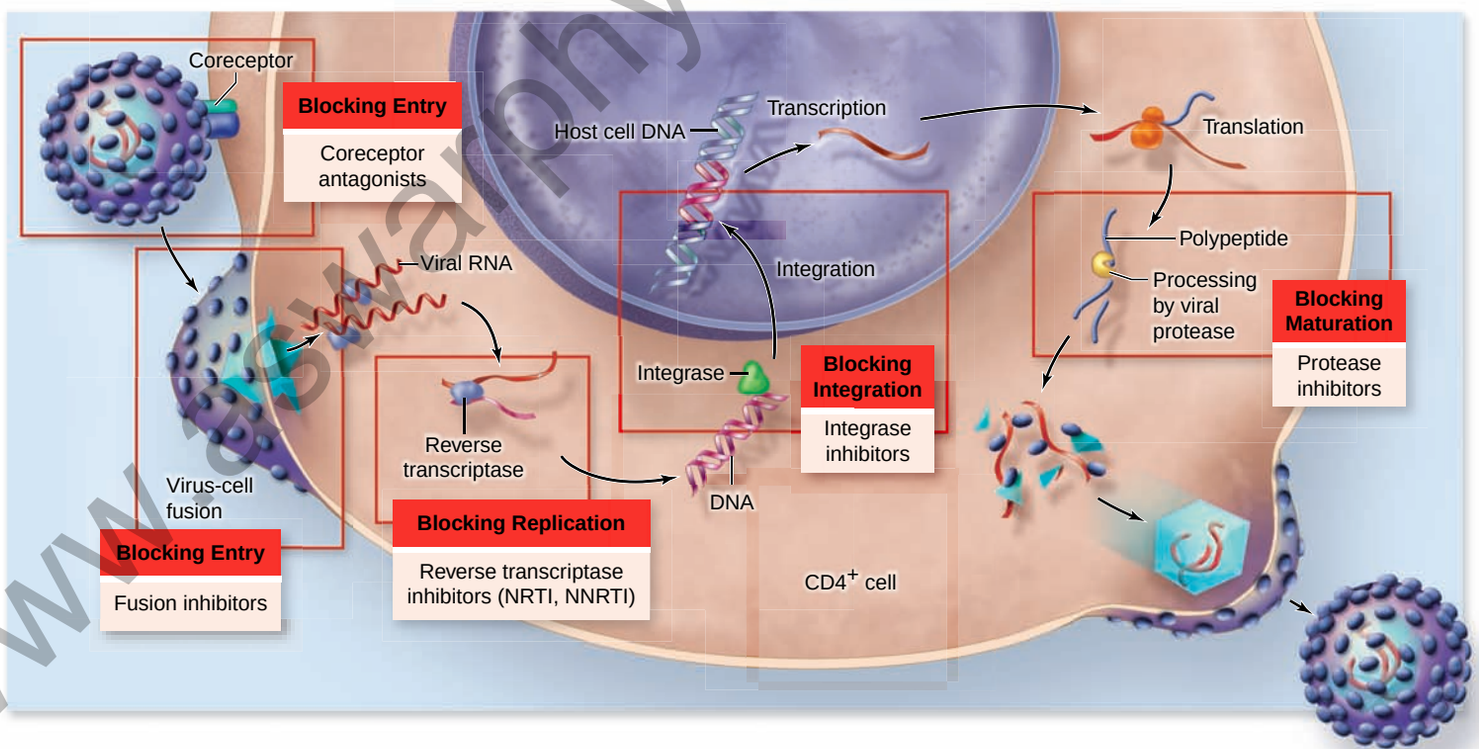


Figure 27.7 Viral targets for therapeutic drugs. A simplified version of the HIV infection cycle is shown with the steps targeted for drug intervention. Four parts of the life cycle have been targeted: viral entry, genome replication, integration of the genome, and maturation of viral proteins. NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor.

inhibitors of the replication enzyme, reverse transcriptase, and the protease that is involved in maturation of proteins. Only two drugs block viral entry: one that blocks the fusion of virus with the cell membrane, and one that blocks the chemokine coreceptor CCR5. A single drug has also been approved that targets the integrase protein that integrates the viral genome into a chromosome.

Reverse transcriptase inhibitors

The first drug licensed for clinical use was AZT, a reverse transcriptase inhibitor. This class of drugs falls into two categories: nucleotide or nucleoside reverse transcriptase inhibitors (NRTI) such as AZT, and nonnucleoside reverse transcriptase inhibitors (NNRTI). These drugs are selective for HIV (or other retroviruses) because reverse transcriptase is not a cellular enzyme. So although the NRTI drugs affect cellular enzymes, they inhibit reverse transcriptase at much lower doses. More than 17 RT inhibitors are currently approved by FDA.

Protease inhibitors

The second class of drugs discovered to be effective in AIDS treatment were the protease inhibitors. These drugs target a protease that cleaves a polyprotein into the smaller proteins necessary for viral replication and assembly. Some of these drugs are actually a good example of the concept of rational drug design. Drug designers started with the protease enzyme, then targeted drugs at transition state analogues of this enzyme. There are now more than 10 of these drugs that have received FDA approval.

Blocking viral entry

Two drugs have been approved by the FDA that block the entry of HIV into the cell. One of these, the fusion inhibitor, was approved in 2003. The drug blocks the fusion of the viral envelope with the plasma membrane of a target cell. This entry also requires recognizing the CD4 receptor protein, and a coreceptor such as CCR5. The coreceptor blocker is a new drug that was just approved in mid-2007.

Integrase inhibitors

A number of companies have been working on drugs targeting the viral integrase protein. This protein catalyzes the integration reaction of the viral genome. One of these was approved in late 2007. At least one other is currently in testing for approval.

Combination therapy

The most successful form of therapy has been to use combinations of the previously discussed drugs. The standard treatment regimen consists of a minimum of three active drugs: an NNRTI or protease inhibitor combined with two different NRTIs. This form of combination therapy has entirely eliminated the HIV virus from many patients' bloodstreams. All of these patients began to receive the combination drug therapy within three months of contracting the virus, before their bodies had an opportunity to develop tolerance to any single drug. Widespread use of this *combination therapy*, otherwise known as *highly active antiretroviral therapy (HAART)* has cut the U.S. AIDS death rate by three-fourths since its introduction in the mid-1990s.

Unfortunately, this sort of combination therapy does not appear to actually eliminate HIV from the body. Although the virus disappears from the bloodstream, traces of it can still be detected in the patient's lymph tissue. When combination therapy is discontinued, virus levels in the bloodstream once again rise.

In addition to the search for new drugs to add to the mix of HAART, much effort has been put into simplifying the drug regimens. The more complex the regimen of drugs, the less likely that patients will stick with it. Thus drugs have been developed that combine two NRTIs into one dose, for example.

Vaccine development for HIV has been unsuccessful

A large amount of effort has been put toward developing an anti-HIV vaccine. Thus far, this has been totally unsuccessful. A recent large-scale international HIV vaccine trial was halted when an early examination of data indicated that the vaccine was essentially useless for preventing infection or lowering viral load. This has led to a retooling of clinical trials to have periodic examination of data instead of waiting for endpoints, but has not helped in terms of the vaccine itself. This vaccine was a subunit vaccine in which a specific HIV protein was engineered into an adenovirus vector.

While the high mutation rate of HIV has always been seen as a problem for vaccine development, it appears that the reason for vaccine failures may be more basic, and harder to surmount. A vaccine needs to produce a strong cellular immune response, and thus far no trial HIV subunit vaccine has done this. The only type of vaccine in an animal system that has been shown to provide protection against infection was a vaccine made from attenuated SIV. Unfortunately over time, the attenuated virus was able to mutate into an infective virus, and the experimental animals eventually developed simian AIDS.

Learning Outcomes Review 27.3

HIV is a retrovirus that enters cells via membrane fusion. The virus primarily infects host $CD4^+$ T cells, ultimately resulting in massive death of these cells, which thereby compromises the host's immune system. Most deaths result from cancer and infections that typically do not harm hosts with normal immune systems. Combination drug therapy is a treatment modality in developed countries, and much research is being done to develop vaccines or to find agents that can prevent infection.

- ***Does combination therapy such as HAART represent a cure for AIDS?***

27.4 Other Viral Diseases

Learning Outcomes

1. *Explain why we need a new vaccine each year for influenza.*
2. *Illustrate where emerging viruses come from.*

Humans have known and feared diseases caused by viruses for thousands of years. Among the diseases that viruses cause (see table 27.1) are influenza, smallpox, hepatitis, yellow fever, polio, AIDS, and SARS. In addition, viruses have been implicated in some cancers including leukemias. Viruses not only cause many human diseases, but also cause major losses in agriculture, forestry, and the productivity of natural ecosystems.

The flu is caused by influenza virus

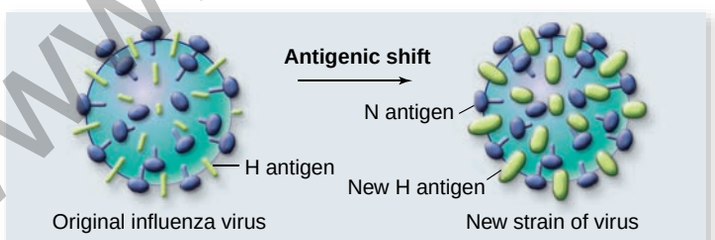
Perhaps the most lethal virus in human history has been the influenza virus. As mentioned earlier, some 20 to 50 million people worldwide died of flu within 18 months in 1918 and 1919.

Types and subtypes

Flu viruses are enveloped segmented RNA viruses that infect animals. An individual flu virus resembles a rod studded with spikes composed of two kinds of protein. The three general "types" of flu virus are distinguished by their capsid protein, which surrounds the viral RNA segments and is different for each type: **Type A flu virus** causes most of the serious flu epidemics in humans and also occurs in mammals and birds. Type B and type C viruses are restricted to humans and rarely cause serious health problems.

Different strains of flu virus, called subtypes, differ in their protein spikes. One of these proteins, hemagglutinin (H), aids the virus in gaining access to the cell interior. The other, neuraminidase (N), helps the daughter viruses break free of the host cell once virus replication has been completed.

Parts of the H molecule contain "hotspots" that display an unusual tendency to change as a result of mutation of the viral RNA during imprecise replication. Point mutations cause changes in these spike proteins in 1 of 100,000 viruses during the course of each generation. These highly variable segments of the H molecule are targets against which the body's antibodies are directed. These constantly changing H-molecule regions improve the reproductive capacity of the virus and hinder our ability to make effective vaccines.



Because of accumulating changes in the H and N molecules, different flu vaccines are required to protect against different subtypes. Type A flu viruses are currently classified into 13 distinct *H subtypes* and 9 distinct *N subtypes*, each of which requires a different vaccine to protect against infection. Thus, the type A virus that caused the 1918 influenza pandemic (that is, worldwide epidemic) has type 1H and type 1N and is designated A(H1N1).

The importance of recombination

The greatest problem in combating flu viruses arises not through mutation, but through recombination. Viral RNA segments are readily reassorted by genetic recombination when two different subtypes simultaneously infect the same cell. This may put together novel combinations of H and N spikes unrecognizable by human antibodies specific for the old configuration.

Viral recombination of this kind seems to have been responsible for the three major flu pandemics that occurred in the 20th century, by producing drastic shifts in H–N combinations. The "Spanish flu" of 1918, A(H1N1), killed 20–50 million people worldwide. The Asian flu of 1957, A(H2N2), killed over 100,000 Americans. The Hong Kong flu of 1968, A(H3N2), infected 50 million people in the United States alone, of whom 70,000 died.

Origin of new strains

It is no accident that new strains of flu usually originate in the Far East. The most common hosts of influenza virus are ducks, chickens, and pigs, which in Asia often live in close proximity to each other and to humans. Pigs are subject to infection by both bird and human strains of the virus, and individual animals are often simultaneously infected with multiple strains. This creates conditions favoring genetic recombination between strains, producing new combinations of H and N subtypes.

The Hong Kong flu, for example, arose from recombination between A(H3N8) from ducks and A(H2N2) from humans. The new strain of influenza, in this case A(H3N2), then passed back to humans, causing a pandemic in 1968-69.

In 1997, a form of avian influenza, A(H5N1), was discovered that could infect humans. Avian influenza, or "bird flu," is highly contagious and deadly among domestic bird populations, and it is now clear that this H5N1 strain is transmitted between domestic birds, which live in close contact with humans, and wild birds that have worldwide migratory patterns. This new influenza variant has caused much concern because, while the number of infections worldwide is low, the mortality rate in these rare infections has been around 50%. However, these infections all appear to be transmitted from birds to humans, and it does not appear to spread by human-to-human contact.

While H5N1 captured the interest of the press worldwide, another viral reassortment occurred that has resulted in an actual pandemic. An A(H1N1) virus was isolated that appears to be the result of reassortment of human A(H3N2) and pig A(H1N1). Epidemiological studies indicate that this virus first appeared in an outbreak of influenza in Veracruz, Mexico.

The virus has since spread worldwide with the WHO raising the event to pandemic status in May, 2009. As of September, 2009, more than 300,000 cases have been reported worldwide. Fortunately, as of this writing, the pathogenicity of this virus is low, with a mortality rate of around 1.5%.

New viruses emerge by infecting new hosts

Sometimes viruses that originate in one organism pass to another, thus expanding their host range. Often, this expansion is deadly to the new host. HIV, for example, is thought to have arisen in chimpanzees and relatively recently passed to humans. Influenza is fundamentally a bird virus. Viruses that originate in one organism and then pass to another and cause disease are called **emerging viruses.** They represent a considerable threat in an age when airplane travel potentially allows infected individuals to move about the world quickly, spreading an infection.

Hantavirus

An emerging virus caused a sudden outbreak of a deadly pneumonia in the southwestern United States in 1993. This disease was traced to a species of **hantavirus** and was called the *sin nombre*, or *no-name, virus.* Hantavirus is a single-stranded RNA virus associated with rodents. This virus was eventually traced to deer mice. The deer mouse hantavirus is transmitted to humans through fecal and urine contamination in areas of human habitation. Controlling the deer mouse population has limited the disease.

Hemorrhagic fever: Ebola

Sometimes the origin of an emerging virus is unknown, making an outbreak more difficult to control. Among the most lethal of emerging viruses are a collection of filamentous viruses arising in central Africa that cause severe hemorrhagic fever. With lethality rates in excess of 50%, these so-called **filoviruses** are among the most lethal infectious diseases known. One, **Ebola virus** (figure 27.8), has exhibited lethality rates in excess of 90% in isolated outbreaks in central Africa. The outbreak of Ebola virus in the summer of 1995 in Zaire killed 245 people out of 316 infected—a mortality rate of 78%. A recent (2004) outbreak of Ebola in Yambio, southern Sudan, caused 17 infections and 7 deaths. This outbreak was rapidly contained by isolating patients from family members as soon as symptoms appeared. The natural host of Ebola is unknown.

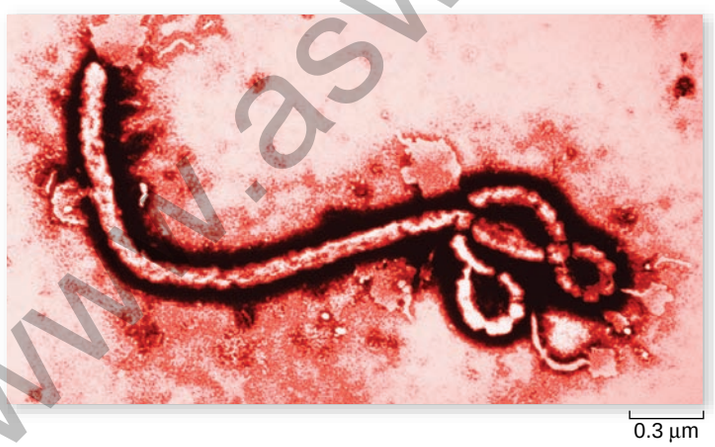


Figure 27.8 The Ebola virus. This virus, with a fatality rate that can exceed 90%, appears sporadically in central Africa. The natural host of the virus currently is unknown.

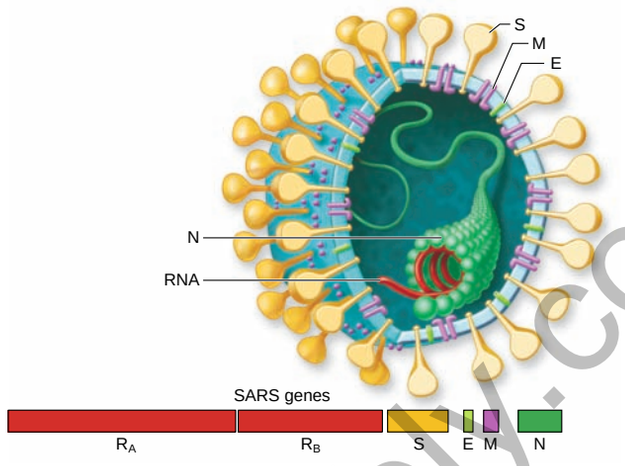


Figure 27.9 SARS coronavirus. The 29,751-nucleotide SARS genome is composed of RNA and contains six principal genes: R_A and R_B, replicases; S, spike proteins; E, envelope glycoproteins; M, membrane glycoprotein; N, nucleocapsid protein.

SARS

A recently emerged species of coronavirus (figure 27.9) was responsible for the 2003 worldwide outbreak of **severe acute respiratory syndrome (SARS),** a respiratory infection with pneumonia-like symptoms that is fatal in over 8% of cases. When the 29,751-nucleotide RNA genome of the SARS coronavirus was sequenced, it proved to be a completely new form of coronavirus, not closely related to any of the three previously described forms.

Virologists suspect that the SARS coronavirus most likely came from civets (a weasel-like mammal) and possibly other wild animals that live in China and are eaten as delicacies. If the SARS virus indeed exists in natural populations, future outbreaks will be difficult to prevent without an effective vaccine. Recent data have implicated bats as the natural reservoir for SARS virus. The significance of this finding for control of the virus is unclear at present.

Genome sequences have been analyzed from SARS patients at various stages of the outbreak, and these analyses indicate that the virus's mutation rate is low, in marked contrast to HIV, another RNA virus. The stable genome of the SARS virus should make development of a SARS vaccine practical. The lessons learned from developing antiviral agents against other RNA viruses, such as HIV and influenza have helped develop drugs to treat SARS. Several anti-SARS agents and vaccines are currently being tested in laboratories across the world.

Viruses can cause cancer

Through epidemiological studies and research, scientists have established a link between some viral infections and the subsequent development of cancer. Examples include the association between chronic hepatitis B infections and the development of liver cancer,

and the development of cervical carcinoma following infections with certain strains of human papillomaviruses (HPV).

Viruses may contribute to about 15% of all human cancer cases worldwide. They are capable of altering the growth properties of human cells they infect by triggering the expression of cancer-causing genes called oncogenes (see chapter 10). Changes in the normal function of these genes leads to cancer.

These changes can occur because viral proteins interfere with the regulation of oncogene expression. Alternatively, the integration of a viral genome into a host chromosome may disrupt a gene required to control the cell cycle. Viruses themselves may encode these oncogenes as well. Virus-induced cancer involves complex interactions with cellular genes and requires a series of events in order to develop. The association of viruses with some forms of cancer has led to research on vaccine development for the prevention of these cancers. In June 2006, the FDA approved the use of a new HPV vaccine in women and young girls from the age of 11 to prevent cervical cancer.

Learning Outcomes Review 27.4

Many types of viruses have caused disease in humans for as long as we have recorded history. Some of these, such as influenza, have caused pandemics responsible for millions of deaths worldwide. Recombination is common in the influenza virus, making natural immunity and development of vaccines problematic. Emerging diseases can occur when viruses switch hosts, that is, jump from another species to humans. Hantavirus, Ebola, and SARS all fall into this category. Virus infection has also been linked to the development of certain cancers.

- ***Why does the effectiveness of flu shots vary from year to year?***

27.5 Prions and Viroids: Subviral Particles

Learning Outcomes

1. ***Explain why prion replication was a "heretical" concept.***
2. ***Describe the mechanism of prion transmission.***

For decades, scientists have been fascinated by a peculiar group of fatal brain diseases. These diseases have an unusual property: Years and often decades pass after infection before the disease is detected in infected individuals. The brains of infected individuals develop numerous small cavities as neurons die, producing a marked spongy appearance. Called *transmissible spongiform encephalopathies (TSEs)*, these diseases include scrapie in sheep; bovine spongiform encephalopathy (BSE), or "mad cow" disease in cattle; chronic wasting disease in deer and elk; and kuru, Creutzfeldt–Jakob disease (CJD), and variant Creutzfeldt–Jakob disease (vCJD) in humans.

TSEs can be transmitted experimentally by injecting infected brain tissue into a recipient animal's brain. TSEs can also spread via tissue transplants and, apparently, tainted food. The disease kuru was once common in the Fore people of Papua New Guinea, because they practiced ritual cannibalism, eating the brains of infected individuals. Mad cow disease spread widely among the cattle herds of England in the 1990s because cows were fed bonemeal prepared from sheep and cattle carcasses to increase the protein content of their diet. Like the Fore, the British cattle were eating the tissue of cattle that had died of the disease.

In the years following the outbreak of BSE, there has been a significant increase in CJD incidence in England. Some cases appear to be genetic. Mysteriously, patients with no family history of CJD were being diagnosed with the disease. This led to the discovery of a new form of CJD called variant CJD, or vCJD, that is acquired from eating meat of BSE-infected animals. Concern exists that vCJD may be transmitted from person to person through blood products, similar to the transmission of HIV through blood and blood products.

Prion replication was a heretical suggestion

In the 1960s, British researchers Tikvah Alper and John Stanley Griffith noted that infectious TSE preparations remained infectious even after exposure to radiation that would destroy DNA or RNA. They suggested that the infectious agent was a protein. They speculated that the protein could sometimes misfold, and then catalyze other proteins to do the same, the misfolding spreading like a chain reaction. This "heretical" suggestion was not accepted by the scientific community, because it violated a key tenet of molecular biology: Only DNA or RNA act as hereditary material, transmitting information from one generation to the next.

Evidence has accumulated that prions cause TSEs

In the early 1970s, physician Stanley Prusiner began to study TSEs. Try as he might, Prusiner could find no evidence of nucleic acids or viruses in the infectious TSE preparations. He concluded, as Alper and Griffith had, that the infectious agent was a *protein*, which in a 1982 paper he named a **prion,** for "proteinaceous infectious particle."

Prusiner went on to isolate a distinctive prion protein, and to amass evidence that prions play a key role in triggering TSEs. Every host tested to date expresses a normal prion protein (PrP^c) in their cells. The disease-causing prions are the same protein, but folded differently (PrP^{sc}). These misfolded proteins have been shown in vitro to serve as a template for normal PrP to misfold. The misfolded PrP proteins are very resistant to degradation, making it possible for them to pass through the acidic digestive tract intact and therefore to be transmitted by ingesting tainted food.

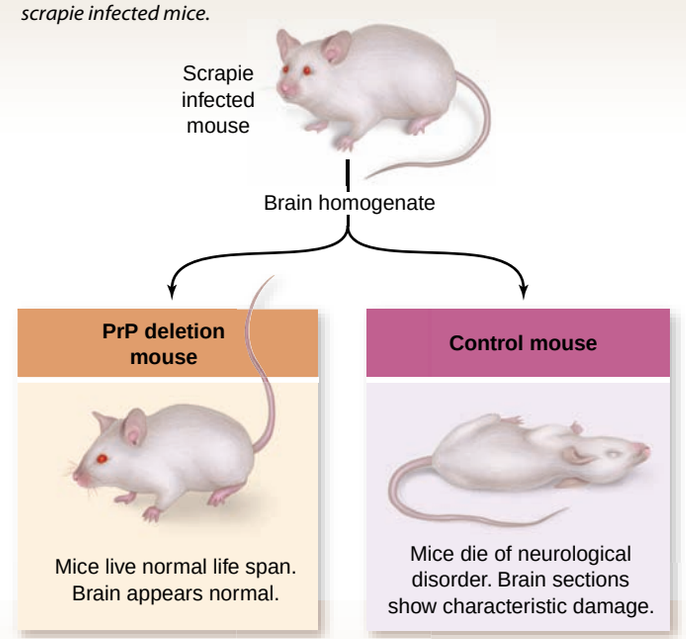
Experimental evidence has accumulated to support this idea. Injection of prions with different abnormal conformations into hosts leads to the same abnormal conformations as the parent prions. Mice genetically engineered to lack PrP^c are

SCIENTIFIC THINKING

Hypothesis: *Normal cellular PrP protein is required for scrapie infection.*

Prediction: *Infection of mice lacking PrP with scrapie agent should not lead to scrapie.*

Test: *Construct "knockout" mice with PrP deleted. Inject PrP knockout mice, and control mice with normal PrP, with brain homogenates from scrapie infected mice.*



Result: *All control mice died with scrapie like neurological damage. Periodic sacrifice and histological examination showed normal disease progress. None of the PrP knockout mice showed any sign of neurological disorder.*

Conclusion: *Normal PrP protein is necessary for productive infection by scrapie infectious particle.*

Further Experiments: *What kind of experiment would conclusively prove that the infectious agent of scrapie is a misfolded PrP protein?*

Figure 27.10 Demonstration that normal prion protein is necessary for scrapie infectivity.

immune to TSE infection (figure 27.10). If brain tissue with the prion protein is grafted into the mice, the grafted tissue—but not the rest of the brain—can then be infected with TSE. However, infectious PrP has not been generated in vitro in quantities that would produce disease in nature. The mechanism of pathogenesis also remains controversial.

Prions have also been found in yeast and other fungi. Three different "genes" that behave in a nonmendelian fashion have all been shown to be prions in yeast. There has been greater progress in the in vitro conversion of normal cellular proteins to the prion form in the yeast system.

Viroids are infectious RNA with no protein coat

Viroids are tiny, naked molecules of circular RNA, only a few hundred nucleotides long, that are important infectious disease agents in plants. A recent viroid outbreak killed over 10 million coconut palms in the Philippines. Despite their small size, viroids autonomously replicate without a helper virus, although they obviously use host proteins. Despite being nucleic acids, the important information they carry appears to be in their three-dimensional structure. There are also some intriguing hints that they might use the plant siRNA machinery to affect gene expression.

Learning Outcomes Review 27.5

Prions and viroids are smaller and simpler than viruses. Prions are infectious particles that do not seem to contain any nucleic acid. They appear to be misfolded proteins that cause related cellular proteins to also misfold. Prions are the causative agent of TSEs. Viroids are infectious RNAs that are implicated in some plant diseases.

- ***If prions are infectious proteins, then what is the form of genetic information that they carry?***

Chapter Review

27.1 The Nature of Viruses

Viruses are strands of nucleic acids encased in a protein coat.

Viral genomes can consist of either DNA or RNA and can be classified as DNA viruses, RNA viruses, or retroviruses.

Most viruses have a protein sheath or capsid around their nucleic acid core. Many animal viruses have an envelope around the capsid composed of proteins that are virally encoded, lipids from the host cell, and glycoproteins.

Some viruses also have enzymes inside their capsid that are important in early infection.

Viral hosts include virtually every kind of organism.

Each virus has a limited host range, and many also exhibit tissue tropism.

Viruses replicate by taking over host machinery.

Viruses are obligate intracellular parasites lacking ribosomes and proteins needed for replication. Viruses take over host machinery and direct their own nucleic acid and protein synthesis.

Most viruses come in two simple shapes.

Viruses vary in size and come in two simple shapes: helical (rodlike) or icosahedral (spherical) (see figures 27.1 and 27.4).

Viral genomes exhibit great variation.

The DNA or RNA viral genome may be linear or circular, single- or double-stranded. RNA viruses may have multiple RNA molecules (segmented), or only one RNA molecule (nonsegmented). Retroviruses contain RNA that is transcribed into DNA by reverse transcriptase.

27.2 Bacteriophage: Bacterial Viruses

Archaeal viruses have diverse morphologies.

Bacterial viruses exhibit two reproductive cycles.

The lytic cycle kills the host cell, whereas the lysogenic cycle incorporates the virus into the host genome as a prophage (see figure 27.5). A cell containing a prophage is called a lysogen.

The prophage can be induced by DNA damage and other environmental cues to reenter the lytic cycle.

For most phage, steps in infection include attachment, injection of DNA (penetration), macromolecular synthesis, assembly of new phage, and release of progeny phage.

Bacteriophage can contribute genes to the host genome.

Phage conversion occurs when foreign DNA is contributed to the host by a bacterial virus.

27.3 Human Immunodeficiency Virus (HIV)

AIDS is caused by HIV.

Human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS) (see figure 27.6).

HIV infection compromises the host immune system.

HIV specifically targets macrophages and $CD4^+$ cells, a type of helper T-lymphocyte cell. With the loss of these cells the body cannot fight off opportunistic infections, which ultimately lead to death.

HIV infects key immune-system cells.

The viral glycoprotein gp120 precisely fits on the cell-surface marker protein $CD4^+$ on macrophages and T cells. When HIV attaches to two receptors, $CD4^+$ and CCR5, receptor-mediated endocytosis is activated, bringing the HIV particle into the cell.

Once inside the cell, the protective coat is shed, releasing viral RNA and reverse transcriptase into the cytoplasm. Reverse transcriptase makes double-stranded DNA complementary to the viral RNA. This DNA may be incorporated into the host DNA as a provirus.

Replicated viruses are budded off the host cell by exocytosis.

HIV has a high mutation rate because the reverse transcriptase enzyme is much less accurate than DNA polymerases. Mutations lead to an altered glycoprotein gp120, which now binds instead to the CXCR4 receptor found only on the surface of $CD4^+$ cells. Incorporation of the altered HIV particle leads to a rapid decline in T cells and immune response.

AIDS treatment targets different phases of the HIV life cycle.

Drugs target reverse transcriptase, a protease involved in protein maturation, viral entry, and the integration of the genome. Most approved drugs are reverse transcriptase and protease inhibitors.

Combination drug therapy using nucleoside analogues and protease inhibitors eliminates HIV from the bloodstream but not totally from the body.

Vaccine development for HIV has been unsuccessful.

Successful vaccines must elicit a strong immune response, and attempts to create immunity against a specific HIV subunit have failed. Attenuated viruses in animal tests have also proved capable of mutating into infectious forms.

27.4 Other Viral Diseases

The flu is caused by influenza virus.

One of the most lethal viruses in human history is type A influenza virus. Influenza can also infect other mammals and birds.

Genes in influenza viruses undergo recombination frequently, so they are not recognized by antibodies against past infections. Each year the composition of flu vaccines must be changed.

New viruses emerge by infecting new hosts.

Viruses can extend their host range by jumping to another species. Examples include hantavirus, hemorrhagic fever, and SARS (see figure 27.9).

Viruses can cause cancer.

Viruses have been linked to formation of cancers, including liver cancer and cervical papillomas.

27.5 Prions and Viroids: Subviral Particles

Prion replication was a heretical suggestion.

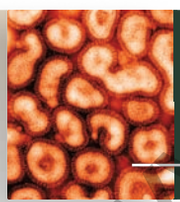
Prions are proteinaceous infectious particles consisting of a misfolded form of a protein. This misfolding catalyzes a chain reaction of misfolding in normal proteins, causing disease.

Evidence has accumulated that prions cause TSEs (see figure 27.10).

TSE disease-causing prions (PrP^{sc}) are the same protein as the normal version (PrP^{c}) but misfolded.

Viroids are infectious RNA with no protein coat.

Viroids are circular, naked molecules of RNA that infect plants. They use host protein to replicate.



Review Questions

UNDERSTAND

1. The reverse transcriptase enzyme is active in which class of viruses?
   a. Positive-strand RNA viruses
   b. Double-stranded DNA viruses
   c. Retroviruses
   d. Negative-strand RNA viruses

2. Which of the following is not part of a virus?
   a. Capsid
   b. Ribosomes
   c. Genetic material
   d. All of the above are found in viruses.

3. Which of the following is common in animal viruses but not in bacteriophage?
   a. DNA
   b. Capsid
   c. Envelope
   d. Icosahedral shape
4. Which of the following would not be part of the life cycle of a lytic virus?
   a. Macromolecular synthesis
   b. Attachment to host cell
   c. Assembly of progeny virus
   d. Integration into the host genome
5. A process by which a virus may change a benign bacteria into a virulent strain is called
   a. induction.
   b. phage conversion.
   c. lysogeny.
   d. replication.
6. Prior to entry, the _____ glycoprotein of the HIV virus recognizes the _____ receptor on the surface of the macrophage.
   a. CCR5; gp120
   b. CXCR4; CCR5
   c. CD4; CCR5
   d. gp120; CD4
7. The use of multiple drugs in HAART to treat AIDS has
   a. completely removed the virus from infected individuals.
   b. reduced the viral level in the bloodstream to undetectable levels.
   c. been a complete failure.
   d. been supplanted by the new HIV vaccine.

APPLY

1. The varying degrees of resistance to HIV in populations has been suggested to be related to the patterns of smallpox outbreaks over human history. This explanation hinges on
   a. the similarity in the genomes of the two viruses.
   b. the fact that both viruses use reverse transcriptase.
   c. both viruses using the same receptor to bind to host cells.
   d. the fact that both viruses compromise the immune system.
2. The idea of a protein that was an infectious agent was heretical because
   a. proteins are not that important in cells.
   b. proteins are not the informational molecules in cells.
   c. the function of proteins does not depend on their structure.
   d. proteins require nucleic acids for their function.
3. Bacterial viruses and animal viruses are similar in that they both
   a. have only DNA as genetic material.
   b. have only RNA as genetic material.
   c. require host functions for some aspect of their life cycle.
   d. do not require any host proteins.
4. The drugs used against HIV in AIDS therapy are not effective against the flu because
   a. HIV is an RNA virus and influenza is a DNA virus.
   b. HIV is a DNA virus and influenza is an RNA virus.
   c. the two viruses have different sized genomes.
   d. the proteins targeted by HIV drugs are not found in influenza.
5. Phage conversion in which viruses add genes to a bacterial cell can be considered to be a form of
   a. standard inheritance.
   b. horizontal gene transfer.
   c. vertical gene transfer.
   d. parasitism.
6. According to the prion hypothesis, the infectious agent for scrapie must have "genetic" information in
   a. the sequence of amino acids in the scrapie protein.
   b. the sequence of bases in the scrapie gene.
   c. the three-dimensional structure of the scrapie prion.
   d. all of the above.
7. The difficulty designing a single flu vaccine that will work forever is that influenza
   a. is an RNA virus.
   b. is a DNA virus.
   c. both mutates and can be recombined to form new viruses.
   d. infects only humans.
8. The SARS outbreak is an example of
   a. a virus jumping from one species to another.
   b. mutation of a virus that only infects humans.
   c. how viruses can disable the human immune system.
   d. two viruses combining to form a new virus.

SYNTHESIZE

1. *E. coli* lysogens derived from infection by phage λ can be induced to form progeny viruses by exposure to radiation. The inductive event is the destruction of a repressor protein that keeps the prophage genome unexpressed. What might be the normal role of the protein that recognizes and destroys the λ repressor?
2. Most biologists believe that viruses evolved following the origin of the first cells. Defend or critique this concept.
3. Much effort has been expended to produce a vaccine for HIV. To date, this has not been successful. Why has this been such a difficult task? Are any other viruses equally resistant to a vaccine, and are the reasons the same? Why do you think that we could make a vaccine against the smallpox virus that allowed us to completely eradicate this virus?
4. What do we mean by the term "emerging virus"? How is this a medical problem, and what is a recent example?
5. How might phage λ be used to transfer *E. coli* genes between different bacterial cells? Could this be used to transfer any gene?

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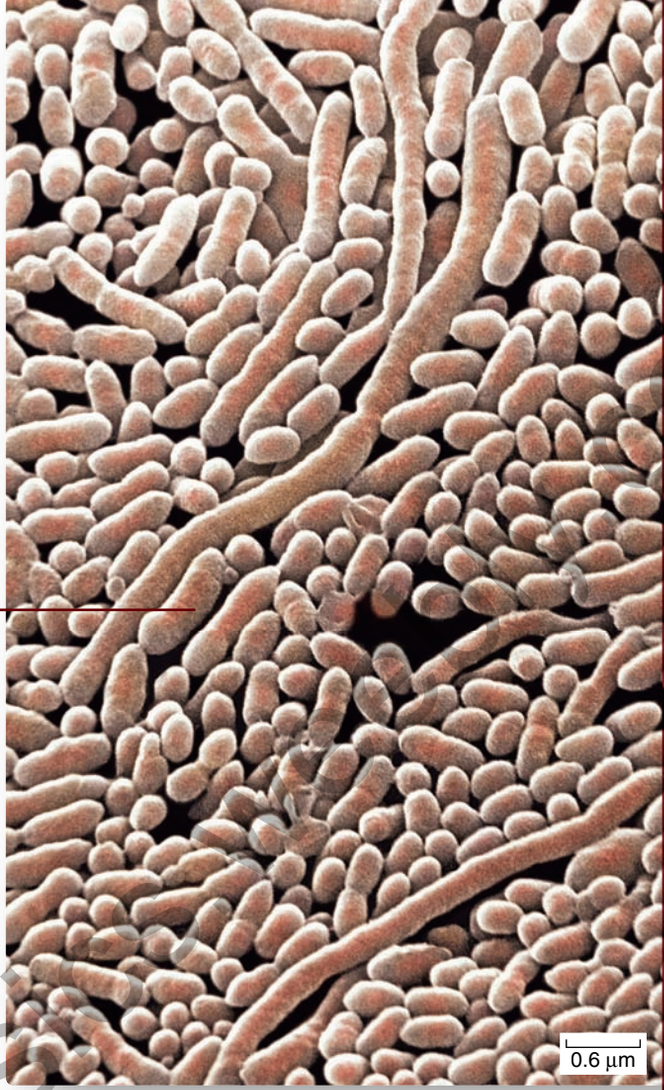
Chapter 28

Prokaryotes



Chapter Outline

Introduction

One of the hallmarks of living organisms is their cellular organization. You learned earlier that living things come in two basic cell types: prokaryotes and eukaryotes. To review, prokaryotes lack the membrane-bounded nucleus found in all eukaryotes, and they also have a much less complex cellular structure, lacking many of the organelles seen in eukaryotes (chapter 4). Prokaryotes are considerably smaller and more numerous than their eukaryotic counterparts. If we examined a human being closely, we would discover that for every single cell of the human body there are approximately 10 prokaryotic cells—and there are trillions of human cells.

Prokaryotic microbes play an important role in global ecology as well. Most biologists think prokaryotes were the first organisms to evolve. The diversity of eukaryotic organisms that currently live on Earth could not exist without prokaryotes because they make possible many of the essential functions of ecosystems. Prokaryotic photosynthesis, for example, is thought to have been the source for the oxygen in the ancient Earth's atmosphere, and it still contributes significantly to oxygen production today. An understanding of prokaryotes is essential to understanding all life on Earth, past and present.

28.1 The First Cells

Learning Outcomes

1. *Explain the evidence for the earliest cells.*
2. *Describe possible pathways of carbon fixation by early life forms.*

As no human was present at the formation of life, we are left with indirect evidence for the earliest life-forms. The most direct evidence we have are fossils, but these can be difficult to interpret, especially since we are looking for microscopic evidence of life. We also can analyze the composition of carbon-containing rocks to look for signs of life acting on organic material, as indicated by a change in isotopic ratios. Finally, we can look for the presence of organic chemicals, which are of biological origin.

Microfossils indicate that the first cells were probably prokaryotic

Evidence of life in the form of microfossils is difficult both to find and to interpret. Rocks older than 3 billion years are rarely unchanged by geologic action over time. Two main formations of 3.5- to 3.8-billion-year-old rocks have been found that are mostly intact: the Kaapvaal craton in South Africa and the Pilbara craton in western Australia. (A *craton* is a rock layer of undisturbed continental crust.) Structures have been found in each of these formations and others that are interpreted to be biological in origin. Although this interpretation has been controversial, the accumulation of evidence over time favors these structures as being true fossil cells.

Microfossils are fossilized forms of microscopic life. Many microfossils are small (1–2 μm in diameter) and appear to be single-celled, lack external appendages, and have little evidence of internal structure (figure 28.1). Thus, microfossils seem to resemble present-day prokaryotes.

The currently oldest microfossils are 3.5 billion years old. The claim that these microfossils are the remains of living organisms is supported by isotopic data (described shortly) and by spectroscopic analysis that indicates they do contain complex carbon molecules. Whether these microscopic structures are true fossil cells is still controversial, and the identity of the prokaryotic groups represented by the various microfossils is still unclear. Arguments have been made for various bacteria, including cyanobacteria (described later on) being the microfossils in question, but definitive interpretation is difficult.

Figure 28.1 Evidence of bacterial fossils. Rocks approximately 3.5 billion years old to 1 billion years old have tiny fossils resembling bacterial cells embedded within them.

Figure 28.2 Stromatolites. Mats of bacterial cells that trap mineral deposits and form the characteristic dome shapes seen here.

In addition to these microfossils, indirect evidence for ancient life can be found in the form of sedimentary deposits called **stromatolites.** These structures are commonly interpreted as a combination of sedimentary deposits and precipitated material that are held in place by mats of microorganisms. The microorganisms that make up the mats are thought to be cyanobacteria. Formations of stromatolites are as old as 2.7 billion years. Because relatively modern stromatolites are also known, the formation and biological nature of these structures is less contentious (figure 28.2).

Isotopic data indicate that carbon fixation is an ancient process

Another way to ask when life began is to look for the signature of living systems in the geological record. Living systems alter their environments, and sometimes this change can be detected. The most obvious change is that living systems are selective in the isotopes of carbon in compounds they use. Living organisms incorporate carbon-12 into their cells before any other carbon isotope, and thus they can alter the ratios of these isotopes in the atmosphere. They also have a higher level of carbon-12 in their fossilized bodies than does the nonorganic rock around them.

Much work has been done on dating and analyzing carbon compounds in the oldest rocks, looking for signatures of life. Although this work is controversial, it has been argued that carbon signatures indicate carbon fixation, the incorporation of inorganic carbon into organic form, was active as long as 3.8 BYA.

The ancient fixation of carbon happened via four possible pathways. The most common pathway for carbon fixation is the Calvin cycle (see chapter 8). This is the pathway used by cyanobacteria, algae, and modern land plants that perform oxygenic photosynthesis using two photosystems. The Calvin cycle is also active in green and purple sulfur bacteria that perform anoxygenic photosynthesis using a single photosystem. This anoxygenic form of photosynthesis could account for ancient carbon fixation.

To date, the entire Calvin cycle has not been demonstrated in the domain Archaea, although the key enzyme for this pathway has been identified in a few archaeal isolates. Instead, some archaea use a reductive version of the Krebs cycle (see chapter 7). This pathway of carbon fixation is also used by some lithotrophic bacteria, which derive energy from the oxidation of inorganic compounds, and by the green sulfur bacteria. Two other pathways may also occur in the lithotrophs, archaea, and the green nonsulfur bacteria. Evidence suggests that the ability to fix carbon has evolved multiple times over the course of evolution.

Some hydrocarbons found in ancient rocks may have biological origins

Another way to look for evidence of ancient life is to look for organic molecules, which are clearly of biological origin; such molecules are called *biomarkers*. Although the process sounds simple, it has proved difficult to find such markers. One type of biomarker molecule is hydrocarbons, which are derived from the fatty acid tails of lipids. These can be analyzed for their carbon isotope ratios to indicate biological origin. The analysis of extractable hydrocarbons from the Pilbara formation in Australia found lipids that are indicative of cyanobacteria as long ago as 2.7 billion years. The search for definitive chemical markers for living systems in the oldest rocks and in meteorites is an area of intense interest.

Arguments for the oldest microfossils have been supported by analysis of the carbon isotope ratios in carbonaceous material from the same formations. If these fossils indeed represent living cells, it implies that life was much more abundant 3.5 BYA than previously thought. Although much of this work is still being debated, it pushes the possible origin of life back well beyond 3.5 BYA.

Learning Outcomes Review 28.1

Evidence for the earliest cells exists in microfossils. The earliest microfossils are controversial, but they are at least 3.5 billion years old. Other evidence for early life includes isotopic ratios that are skewed by biological activity. The Calvin cycle and a reductive version of the Krebs cycle, as well as other pathways, appear to have led to carbon fixation in ancient life. Some hydrocarbons appear to be biomarkers and may therefore also indicate ancient life forms.

- ***When we are looking for life on Mars, what kind of evidence would be the most convincing?***

28.2 Prokaryotic Diversity

Learning Outcomes

1. ***Differentiate among archaea, bacteria, and eukarya.***
2. ***Describe the basic features of bacteria and archaea.***
3. ***Explain classification methods for prokaryotes.***

Although thousands of different kinds of prokaryotes are currently recognized, many thousands more await proper identification. New molecular techniques have allowed scientists to identify and study microorganisms without culturing them. As a result, microbiologists have discovered thousands of new species that were never discovered or characterized because they could not be maintained in culture.

It is estimated that only between 1 and 10% of all prokaryotic species are known and characterized, leaving between 90 and 99% unknown and undescribed. Every place microbiologists look, new species are being discovered, often altering the way we think about prokaryotes. In the 1970s and 1980s, a new type of prokaryote was identified and analyzed that eventually led to the division of prokaryotes into two groups: the *Archaea* (formerly called Archaebacteria) and the *Bacteria* (sometimes also called Eubacteria).

Archaea and bacteria are the oldest, structurally simplest, and most abundant forms of life. They are also the only organisms with prokaryotic cellular organization. Prokaryotes were abundant for over a billion years before eukaryotes appeared in the world. Early photosynthetic bacteria (cyanobacteria) altered the Earth's atmosphere by producing oxygen, which stimulated extreme bacterial and eukaryotic diversity.

Prokaryotes are ubiquitous and live everywhere eukaryotes do; they are also able to thrive in places no eukaryote could live. Bacteria and archaea have been found in deep-sea caves, volcanic rims, and deep within glaciers. Some of the extreme environments in which prokaryotes can be found would be lethal to any other life-form.

Many archaea are *extremophiles*. They live in hot springs that would cook other organisms, in hypersaline environments that would dehydrate other cells, and in atmospheres rich in otherwise-toxic gases such as methane or hydrogen sulfide. They have even been recovered living beneath 435 m of ice in Antarctica!

These harsh environments may be similar to the conditions present on the early Earth when life first began. It is likely that prokaryotes evolved to dwell in these harsh conditions early on and have retained the ability to exploit these areas as the rest of the atmosphere has changed.

Prokaryotes are fundamentally different from eukaryotes

Prokaryotes differ from eukaryotes in numerous important features. These differences represent some of the most fundamental distinctions that separate any groups of organisms.

Unicellularity. With a few exceptions prokaryotes are fundamentally single-celled). In some types, individual cells adhere to one another within a matrix and form filaments; however, the cells retain their individuality. Cyanobacteria, in particular, are likely to form such associations, but their cytoplasm is not directly interconnected, as is often the case in multicellular eukaryotes. These filaments do have a common cell wall, however, making it difficult to isolate single cells.

In their natural environments, most bacteria appear to be capable of forming a complex community of different species called a **biofilm.** Although not a multicellular organism, a biofilm is more resistant to antibiotics, dessication, and other environmental stressors than is a simple colony of a single type of microbe, such as a laboratory culture.

Cell size. As new species of prokaryotes are discovered, investigators are finding that the size of prokaryotic cells varies tremendously, by as much as five orders of magnitude. The largest bacterial cells currently characterized are from *Thiomargarita namibia*. A single cell from this species is up to 750 μm across, which is visible to the naked eye and is roughly the size of the eye of a bumblebee. Most prokaryotic cells, however, are only 1 μm or less in diameter, whereas most eukaryotic cells are well over 10 times bigger. This generality is misleading, however, because there are very small eukaryotes as well as very large prokaryotes.

Chromosomes. Eukaryotic cells have a membrane-bounded nucleus containing linear chromosomes made up of both nucleic acids and histone proteins. Prokaryotes do not have membrane-bounded nuclei; instead they usually have a single circular chromosome made up of DNA and histonelike proteins in a *nucleoid* region of the cell. An exception to this single chromosome includes *Vibrio cholerae*, which has two circular chromosomes. Prokaryotic cells often have accessory DNA molecules called plasmids as well. Plasmids are genetic elements that can sometimes be transferred between prokaryotic cells.

Cell division and genetic recombination. Cell division in eukaryotes takes place by mitosis and involves spindles made up of microtubules. Cell division in prokaryotes takes place mainly by binary fission (see chapter 10), which is also a form of asexual reproduction. True sexual reproduction occurs only in eukaryotes and involves the production of haploid gametes that fuse to form a diploid zygote that grows to adulthood, producing more gametes and starting the cycle over again (see chapter 11).

Despite their asexual mode of reproduction, prokaryotes do have mechanisms that lead to the transfer of genetic material and generation of genetic diversity. These mechanisms are collectively called *horizontal gene transfer* and are not a form of reproduction.

Internal compartmentalization. In eukaryotes, the enzymes for cellular respiration are packaged in mitochondria. In prokaryotes, the corresponding enzymes are not packaged separately, but instead are bound to the cell membranes or are in the cytosol. The cytoplasm of prokaryotes, unlike that of eukaryotes, contains no internal compartments and no membrane-bounded organelles. Ribosomes are found in both prokaryotes and eukaryotes, but differ significantly in structure. (See chapter 4 for a review of cell structure.)

Flagella. Prokaryotic flagella are simple in structure, composed of a single fiber of the protein flagellin. Eukaryotic flagella and cilia are complex, having a 9 + 2 structure of microtubules (see figure 4.23). Bacterial flagella also function differently, being rigid and spinning like propellers, whereas eukaryotic flagella have a whiplike motion (described in more detail later and in figure 28.8).

Metabolic diversity. Only one kind of photosynthesis occurs in eukaryotes, and it involves the release of oxygen. Photosynthetic bacteria have two basic patterns of photosynthesis: *oxygenic*, producing oxygen, and *anoxygenic*, nonoxygen producing. Anoxygenic photosynthesis involves the formation of products such as sulfur and sulfate instead of oxygen.

Prokaryotic cells can also be *chemolithotrophic*, meaning that they use the energy stored in chemical bonds of inorganic molecules to synthesize carbohydrates; eukaryotes are not capable of this metabolic process.

Despite similarities, bacteria and archaea differ fundamentally

Archaea and bacteria are similar in that both have a prokaryotic cellular structure, but they vary considerably at the biochemical and molecular levels. They differ in four key areas: plasma membranes, cell walls, DNA replication, and gene expression.

Plasma membranes. All prokaryotes have plasma membranes with a fluid mosaic architecture (see chapter 5). The plasma membranes of archaea differ from both bacteria and eukaryotes. Archaean membrane lipids are composed of glycerol linked to hydrocarbon chains by ether linkages, not the ester linkages seen in bacteria and eukaryotes (figure 28.3*a*). These hydrocarbons may also be branched, and they may be organized as tetraethers that form a monolayer instead of a bilayer (figure 28.3*b*).

In the case of some hyperthermophiles, the majority of the membrane may be this tetraether monolayer. This structural feature is part of what allows these archaeans to withstand high temperatures.

Cell wall. Both kinds of prokaryotes typically have cell walls covering the plasma membrane that strengthen the cell. The cell walls of bacteria are constructed, minimally, of **peptidoglycan,** which is formed from carbohydrate polymers linked together by peptide cross-bridges. The peptide cross-bridges also contain D-amino acids, which are never found in cellular protein. The cell walls of archaea lack peptidoglycan, although some have **pseudomurein,** which is similar to peptidogylcan in structure and function. This wall layer is also a carbohydrate polymer with peptide cross-bridges, but the carbohydrates are different, and the peptide cross-bridge

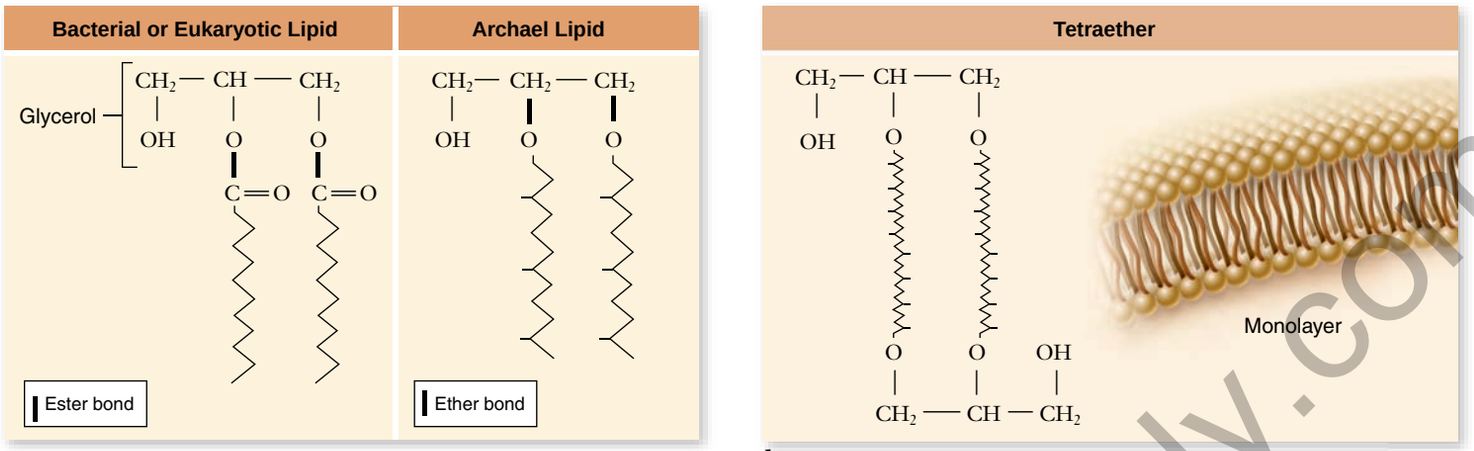


Figure 28.3 Archaea membrane lipids. ***a.*** Archaea membrane lipids are formed on a glycerol skeleton similar to bacterial and eukaryotic lipids, but the hydrocarbon chains are connected to the glycerol by ether linkages not ester linkages. The hydrocarbons can also be branched and even contain rings. ***b.*** These lipids can also form as tetraethers instead of diethers. The tetraether forms a monolayer as it includes two polar regions connected by hydrophobic hydrocarbons.

structure also differs. Other archaeal cell walls have been found to be composed of a variety of proteins and carbohydrates, making generalizations difficult.

DNA replication. Although both archaea and bacteria have a single replication origin, the nature of this origin and the proteins that act there are quite different. Archaeal initiation of DNA replication is more similar to that of eukaryotes (see chapter 14).

Gene expression. The machinery used for gene expression also differs between archaea and bacteria. The archaea may have more than one RNA polymerase, and these enzymes more closely resemble the eukaryotic RNA polymerases than they do the single bacterial RNA polymerase. Some of the translation machinery is also more similar to that of eukaryotes (see chapter 15).

Most prokaryotes have not been characterized

Prokaryotes are not easily classified according to their forms, and only recently has enough been learned about their biochemical and metabolic characteristics to develop a satisfactory overall classification scheme comparable to that used for other organisms.

Early classification characteristics

Early systems for classifying prokaryotes relied on differential stains such as the Gram stain and differences in the observable phenotype of the organism. Key characteristics once used in classifying prokaryotes were

1. photosynthetic or nonphotosynthetic
2. motile or nonmotile
3. unicellular or colony-forming or filamentous
4. formation of spores or division by transverse binary fission
5. importance as human pathogens or not

Molecular approaches to classification

With the development of genetic and molecular approaches, prokaryotic classifications may help reflect true evolutionary relatedness. Molecular approaches include

1. the analysis of the amino acid sequences of key proteins
2. the analysis of nucleic acid–base sequences by establishing the percent of guanine (G) and cytosine (C)
3. nucleic acid hybridization, which is essentially the mixing of single-stranded DNA from two species and determining the amount of base-pairing (closely related species will have more bases pairing)
4. gene and RNA sequencing, especially looking at ribosomal RNA
5. whole-genome sequencing

The three-domain, or Woese, system of phylogeny (figure 28.4) relies on all of these molecular methods, but emphasizes the comparison of rRNA sequences to establish the evolutionary relatedness of all organisms. The rRNA sequences were chosen for their high degree of evolutionary

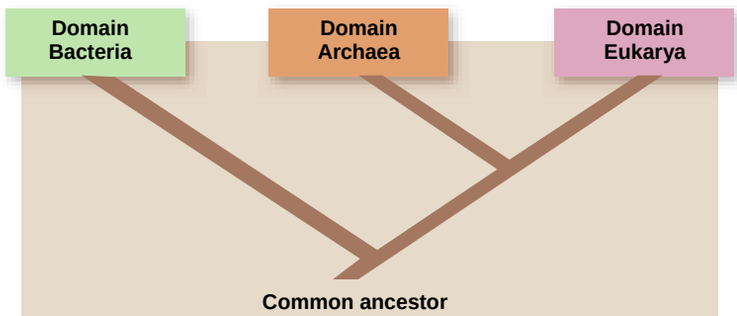


Figure 28.4 The three domains of life. The two prokaryotic domains, Archaea and Bacteria, are not closely related, though both are prokaryotes. In many ways (see text), archaea more closely resemble eukaryotes than bacteria. This tree is based on rRNA sequences.

conservation to ask questions about these most ancient splits in the tree of life.

Based on these sorts of molecular data, several groupings of prokaryotes have been proposed. The most widely accepted is that presented in *Bergey's Manual of Systematic Bacteriology*, second edition, which is being published in 5 volumes, 3 of which have been completed (figure 28.5). At the same time, large scale sequencing of randomly sampled collections of bacteria show an incredible amount of diversity. While it has always been challenging to assign bacteria to species, these new data indicate that the vast majority of bacteria have never been cultured and studied in any detail. The field is in a state of flux as attempts are made to define the nature of bacterial species.

Learning Outcomes Review 28.2

Compared with eukaryotyes, prokaryotes are distinctly different, lacking both a membrane-bounded nucleus and diverse organelles. Prokaryotes also reproduce by binary fission. Bacteria and archaea are clearly different from each other based on both structure and metabolism. Classification of prokaryotes had been based on physical characteristics, and it has now been aided by the use of DNA analysis; but a vast number of prokaryotes remain unidentified because they cannot be cultured.

- ***What features distinguish archaea from both bacteria and eukaryotes?***

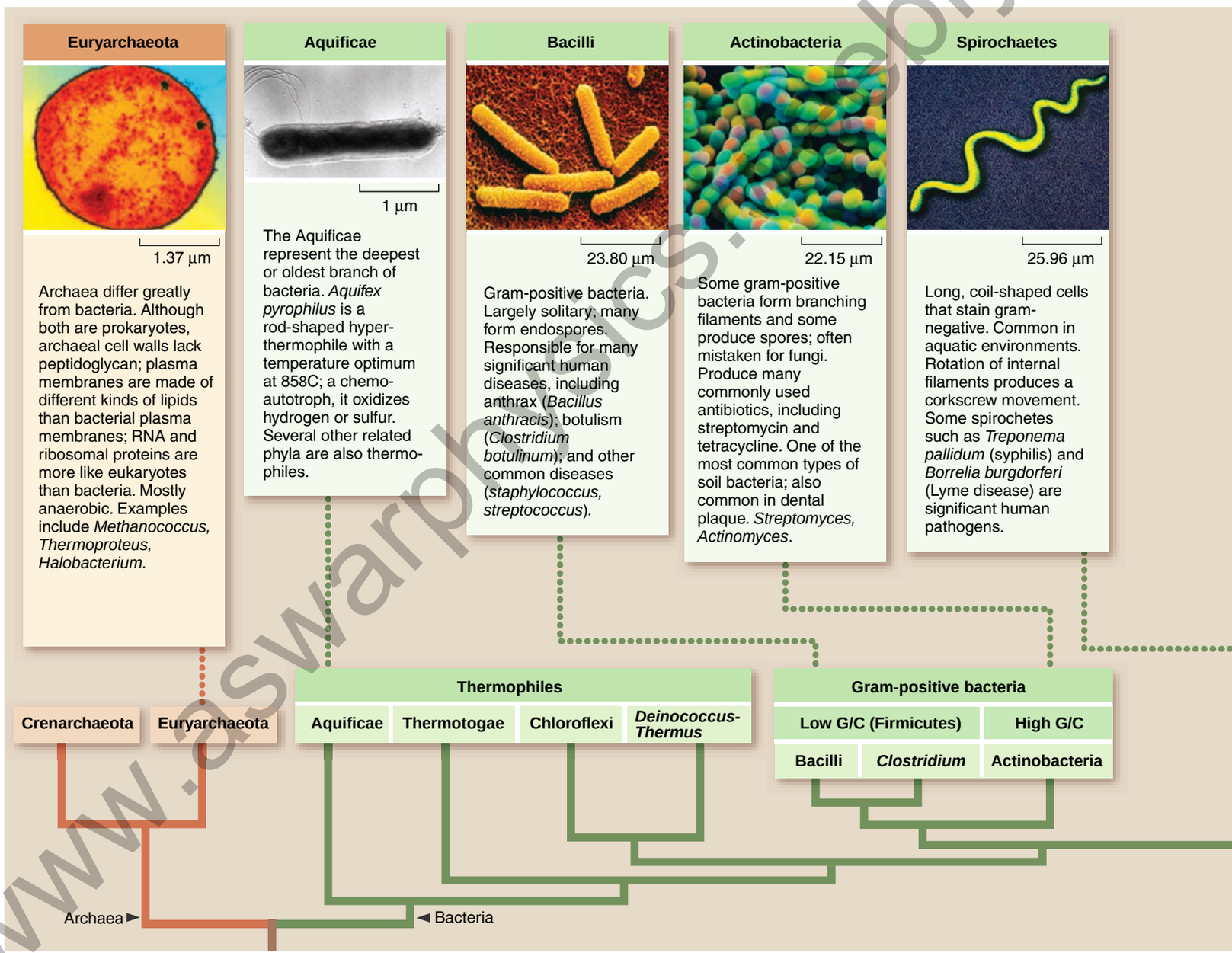


Figure 28.5 Some major clades of prokaryotes. The classification adopted here is that of *Bergey's Manual of Systematic Bacteriology*, second edition, 2001. G/C refers to %G/C in genome.

28.3 Prokaryotic Cell Structure

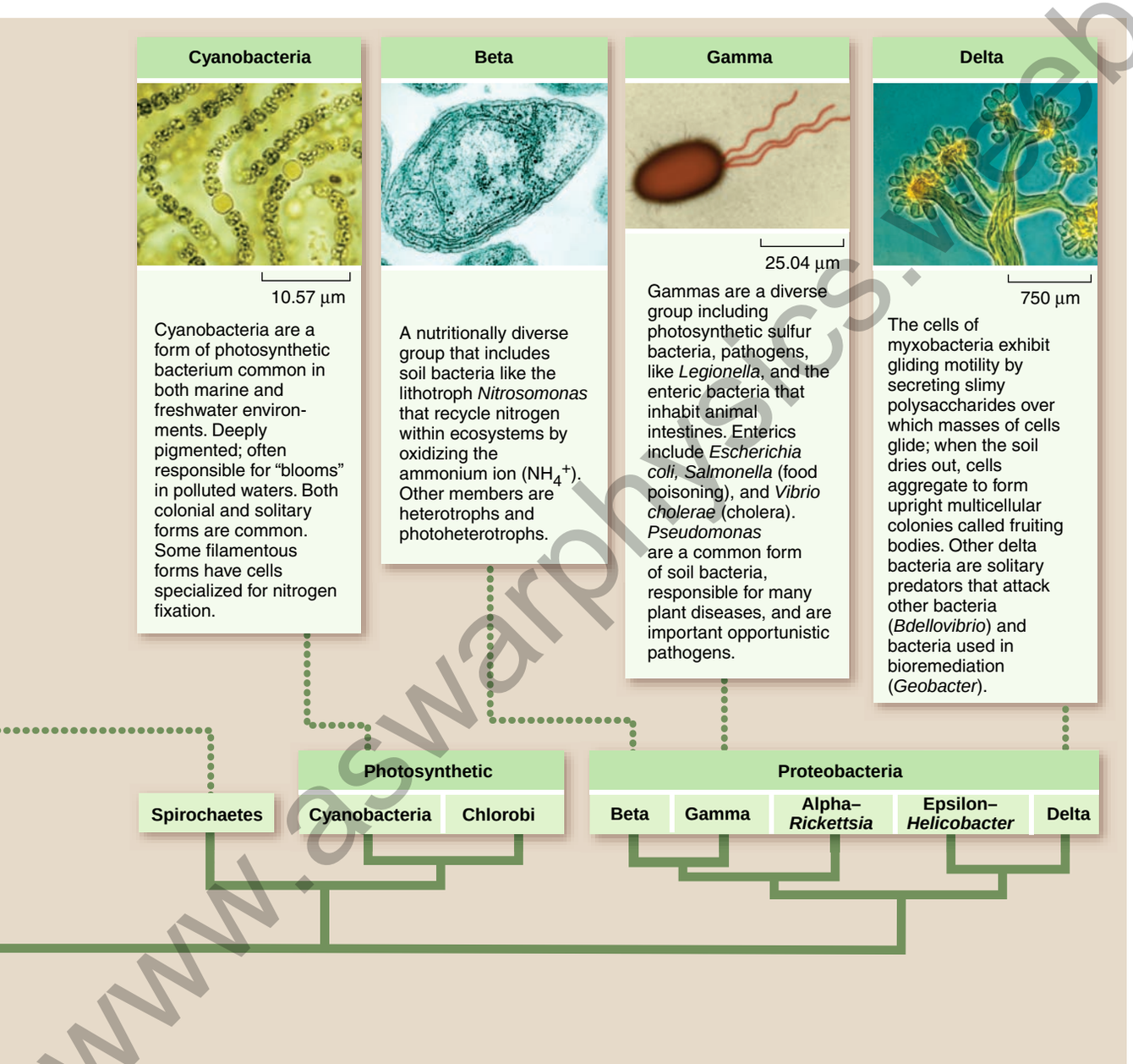
Learning Outcomes

1. *Describe the general features common to all prokaryotic cells.*
2. *Explain the differences between gram-positive and gram-negative bacterial cells.*
3. *Describe the features that distinguish different kinds of prokaryotic cells.*

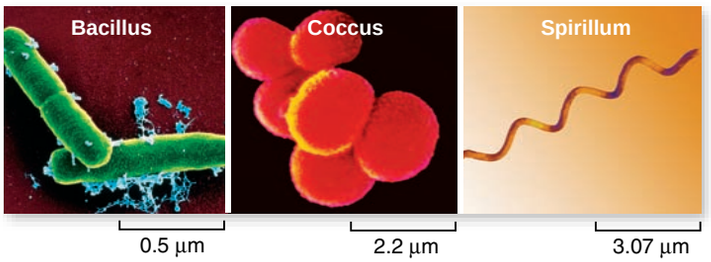
Prokaryotic cells are relatively simple, but they can be categorized based on cell shape. They also have some variations in structure that give them different staining properties for certain dyes. Other features are found in some types of cells but not in others.

Prokaryotes have three basic forms: rods, cocci, and spirals

Although it is an oversimplification, it is useful to divide bacteria based on easily definable morphologies. Most prokaryotes exhibit one of three basic shapes: rod-shaped, often called a



bacillus (plural, *bacilli*); *coccus* (plural, *cocci*), spherical- or ovoid-shaped; and *spirillum* (plural, *spirilla*), long and helical-shaped; these bacteria are also called *spirochetes*.



The bacterial cell wall is the single most important contributor to cell shape. Bacteria that normally lack cell walls, such as the mycoplasmas, do not have a set shape.

As diverse as their shapes may be, prokaryotic cells also have many different methods to move through their environment. A *flagellum* or several flagella may be found on the outer surface of many prokaryotic cells. These structures are used to propel the organisms in a fluid environment. Some rod-shaped and spherical bacteria form colonies, adhering end-to-end after they have divided, forming chains. Some bacterial cells change into stalked structures or grow long, branched filaments. Some filamentous bacteria are capable of a gliding motion on solid surfaces, often combined with rotation around a longitudinal axis.

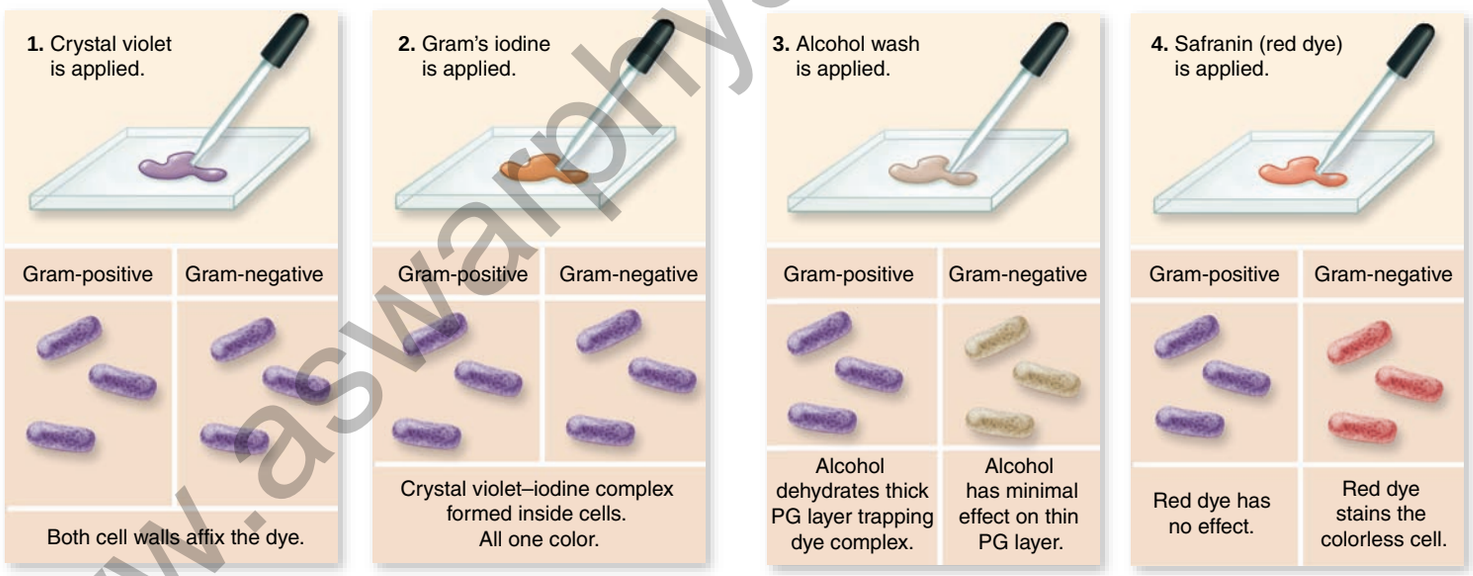
Prokaryotes have a tough cell wall and other external structures

The prokaryotic cell wall is often complex, consisting of many layers. Minimally it consists of peptidoglycan, a polymer unique to bacteria. This polymer forms a rigid network of polysaccharide strands cross-linked by peptide side chains. It is an important structure because it maintains the shape of the cell and protects the cell from swelling and rupturing in hypotonic solutions, which are most commonly found in the environment. The archaea do not possess peptidoglycan, but some have a similar structure called pseudomurein, or pseudopeptidoglycan.

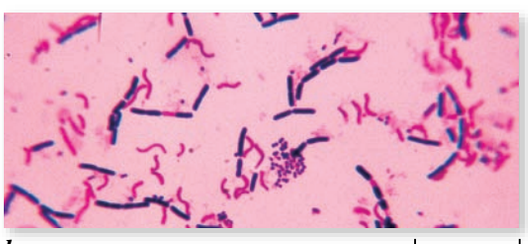
Gram-positive and gram-negative bacteria

Two types of bacteria can be identified using a staining process called the **Gram stain,** hence their names. **Gram-positive** bacteria have a thicker peptidoglycan wall and stain a purple color, whereas the more common **gram-negative** bacteria contain less peptidoglycan and do not retain the purple-colored dye. These gram-negative bacteria can be stained with a red counterstain and then appear dark pink (figure 28.6).

In the gram-positive bacteria, the peptidoglycan forms a thick, complex network around the outer surface of the cell. This network also contains lipoteichoic and teichoic acid, which protrudes from the cell wall. In the gram-negative bacteria, a thin layer of peptidoglycan is sandwiched between the plasma membranes and a second outer membrane (figure 28.7). The outer membrane contains large molecules



a.



b.

Figure 28.6 The Gram stain. ***a.*** The thick peptidoglycan (PG) layer encasing gram-positive bacteria traps crystal violet dye, so the bacteria appear purple in a gram-stained smear (named after Hans Christian Gram—Danish bacteriologist, 1853–1938—who developed the technique). Because gram-negative bacteria have much less peptidoglycan (located between the plasma membrane and an outer membrane), they do not retain the crystal violet dye and so exhibit the red counterstain (usually a safranin dye). ***b.*** A micrograph showing the results of a Gram stain with both gram-positive and gram-negative cells.

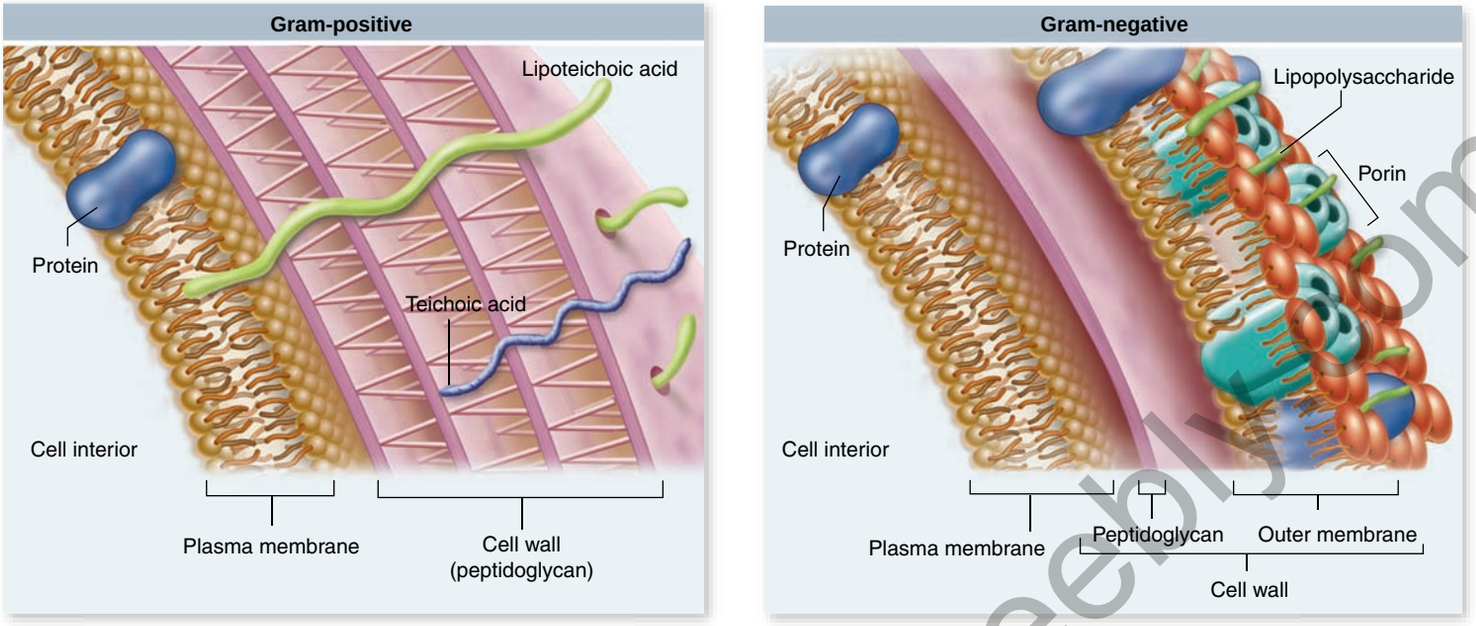


Figure 28.7 The structure of gram-positive and gram-negative cell walls. The gram-positive cell wall is much simpler, composed of a thick layer of cross-linked peptidoglycan chains. Molecules of lipoteichoic acid and teichoic acid are also embedded in the wall and exposed on the surface of the cell. The gram-negative cell wall is composed of multiple layers. The peptidoglycan layer is thinner than in gram-positive bacteria and is surrounded by an additional membrane composed of lipopolysaccharide. Porin proteins form aqueous pores in the outer membrane. The space between the outer membrane and peptidoglycan is called the periplasmic space.

of **lipopolysaccharide,** lipids with polysaccharide chains attached. The outer membrane layer makes gram-negative bacteria resistant to many antibiotics that interfere with cell-wall synthesis in gram-positive bacteria. For example, penicillin acts to inhibit the cross-linking of peptidoglycan in a gram-positive cell wall, killing growing bacterial populations.

S-layer

In some bacteria and archaea, an additional protein or glycoprotein layer forms a rigid paracrystalline surface called an *S-layer* outside of the peptidoglycan or outer membrane layers of gram-positive and gram-negative bacteria, respectively. Among the archaea, the S-layer is almost universal and can be found outside of a pseudopeptidoglycan layer or, in contrast to the bacteria, may be the only rigid layer surrounding the cell. The functions of S-layers are diverse and variable but often involve adhesion to surfaces or protection.

The capsule

In some bacteria, an additional gelatinous layer, the **capsule,** surrounds the other wall layers. A capsule enables a prokaryotic cell to adhere to surfaces and to other cells, and, most important, to evade an immune response by interfering with recognition by phagocytic cells. Therefore, a capsule often contributes to the ability of bacteria to cause disease.

Bacterial flagella and pili

Many kinds of prokaryotes have slender, rigid, helical flagella composed of the protein **flagellin** (figure 28.8). These flagella range from 3 to 12 μm in length and are very thin—only 10 to 20 nm thick. They are anchored in the cell wall and spin like a propeller, moving the cell through a liquid environment. Bacterial cells that have lost the genes for flagellin are not able to swim.

Pili (singular, *pilus*) are other hairlike structures that occur on the cells of some gram-negative prokaryotes. They are shorter than prokaryotic flagella and about 7.5 to 10 nm

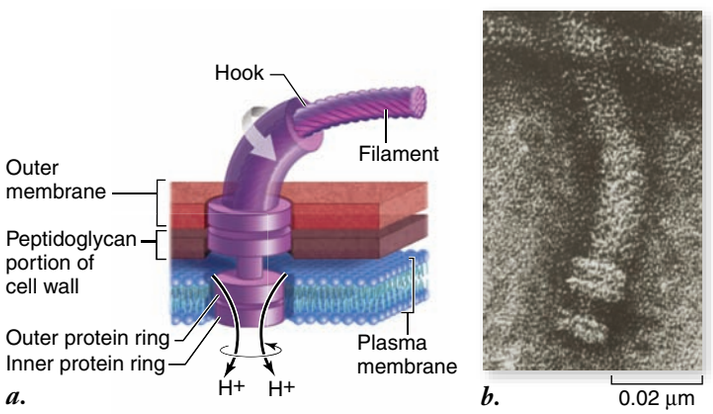


Figure 28.8 The flagellar motor of a gram-negative bacterium. ***a.*** A protein filament, composed of the protein flagellin, is attached to a protein rod that passes through a sleeve in the outer membrane and through a hole in the peptidoglycan layer to rings of protein anchored in the cell wall and plasma membrane, like rings of ball bearings. The rod rotates when the inner protein ring attached to the rod turns with respect to the outer ring fixed to the cell wall. The inner ring is an H^+ ion channel, a proton pump that uses the flow of protons into the cell to power the movement of the inner ring past the outer one. The membrane wall anchor of the flagellum is called the basal body. ***b.*** Electron micrograph of bacterial flagellum.

thick. Pili are more important in adhesion than movement, and they also have a role in exchange of genetic information (discussed later).

Endospore formation

Some prokaryotes are able to form **endospores,** developing a thick wall around their genome and a small portion of the cytoplasm when they are exposed to environmental stress. These endospores are highly resistant to environmental stress, especially heat, and when environmental conditions improve, they can germinate and return to normal cell division to form new individuals after decades or even centuries.

The bacteria that cause tetanus, botulism, and anthrax are all capable of forming spores. With a puncture wound, tetanus endospores may be driven deep into the skin where conditions are favorable for them to germinate and cause disease, or even death.

The interior of prokaryotic cells is organized

The most fundamental characteristic of prokaryotic cells is their simple interior organization. Prokaryotic cells lack the extensive functional compartmentalization seen within eukaryotic cells, but they do have the following structures:

Internal membranes. Many prokaryotes possess invaginated regions of the plasma membrane that function in respiration or photosynthesis (figure 28.9).

Nucleoid region. Prokaryotes lack nuclei and generally do not possess linear chromosomes. Instead, their genes are encoded within a single double-stranded ring of DNA that is highly condensed to form a visible region of the cell known as the **nucleoid region.** Many prokaryotic cells also possess plasmids, which as described earlier are small, independently replicating circles of DNA. Plasmids contain only a few genes, and although these genes may confer a selective advantage, they are not essential for the cell's survival.

Ribosomes. Prokaryotic ribosomes are smaller than those of eukaryotes and differ in protein and RNA content. Antibiotics such as tetracycline and chloramphenicol can tell the difference, however—they bind to prokaryotic ribosomes and block protein synthesis, but they do not bind to eukaryotic ribosomes.

Figure 28.9 Prokaryotic cells often have complex internal membranes. *a.* This aerobic bacterium exhibits extensive respiratory membranes (long dark curves that hug the cell wall) within its cytoplasm not unlike those seen in mitochondria. *b.* This cyanobacterium has thylakoid-like membranes (ripple-like shapes along the edges and in the center) that provide a site for photosynthesis.

Learning Outcomes Review 28.3

The three basic shapes of prokaryotes are rod-shaped, spherical, and spiral-shaped. Bacteria have a cell wall containing peptidoglycan, which is the basis for the Gram stain. Gram-positive bacteria have a thick cell wall, relative to gram-negative species. Many also have an external capsule. Some bacteria have flagella and pili. Some can form heat-resistant endospores. Although prokaryotes do not have membrane-bounded organelles, the interior of the cell is organized and may include infolding of the plasma membrane. Prokaryotic DNA is localized in a nucleoid region.

- ***What would be the simplest method to determine whether two bacteria belong to the same species?***

28.4 Prokaryotic Genetics

Learning Outcomes

1. ***Contrast the mechanisms of DNA exchange in prokaryotes.***
2. ***Explain genetic mapping in E. coli.***
3. ***Describe how genetics explains the spread of antibiotic resistance.***

In sexually reproducing populations, traits are transferred vertically from parent to child. Prokaryotes do not reproduce sexually, but they can exchange DNA between different cells. This horizontal gene transfer occurs when genes move from one cell to another by **conjugation,** requiring cell-to-cell contact, or by means of viruses *(transduction).* Some species of bacteria can also pick up genetic material directly from the environment *(transformation).*

All of these processes have been observed in archaea, but the study of archaeal genetics is still in its infancy because of the difficulty in culturing most species. We concentrate here on bacterial systems, primarily *E. coli,* which has been studied extensively.

Conjugation depends on the presence of a conjugative plasmid

Plasmids may encode functions that can confer an advantage to the cell, such as antibiotic resistance, on which natural selection can operate—but they are not required for normal function. In some cases, plasmids can be transferred from one cell to another via conjugation. The best known plasmid capable of transfer is called the **F plasmid,** for fertility factor; cells containing F plasmids are termed $\mathbf{F^+}$ cells, and cells that lack the F plasmid

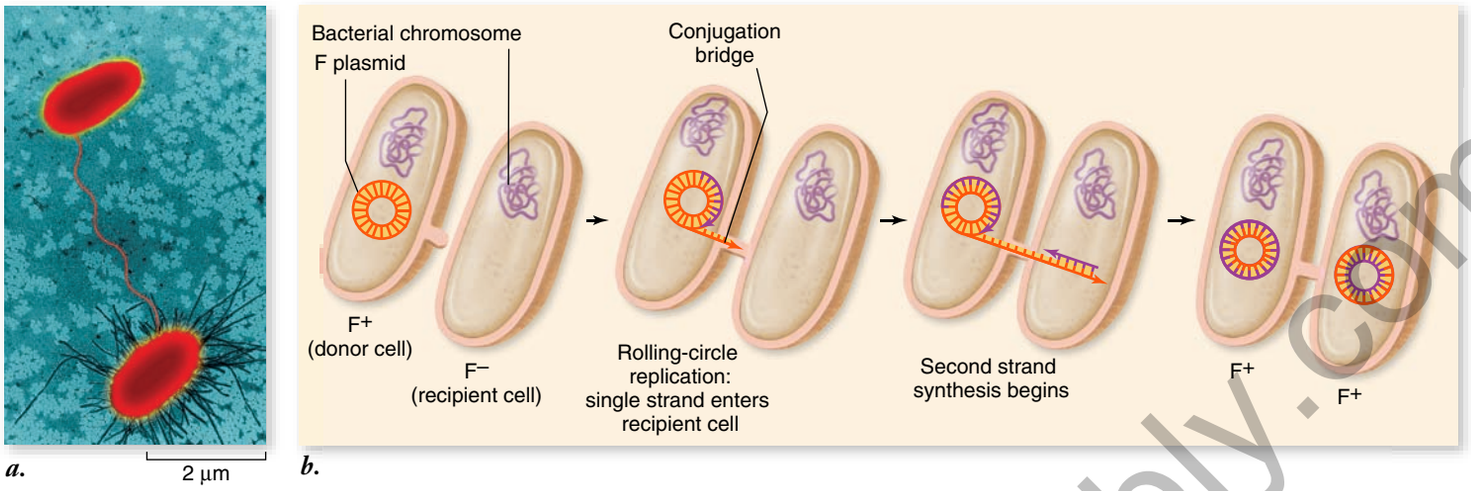


Figure 28.10 Conjugation bridge and transfer of F plasmid between F⁺ and F⁻ cell. ***a.*** The electron micrograph shows two *E. coli* cells caught in the act of conjugation. The connection between the cells is the extended F pilus. ***b.*** F⁻ cells are converted to F⁺ cells by the transfer of the F plasmid. The cells are joined by a conjugation bridge and the plasmid is replicated in the donor cell, displacing one parental strand. The displaced strand is transferred to the recipient cell then replicated. After successful transfer, the recipient cell becomes an F⁺ cell capable of expressing genes for the F pilus and acting as a donor.

are F⁻ cells. The F plasmid occurs in *E. coli* and, like all plasmids, acts as an independent genetic entity that nevertheless depends on the cell for replication. Studies involving the F plasmid were critical to our current understanding of bacterial genetics and the organization of the *E. coli* chromosome.

F plasmid transfer

The F plasmid contains a DNA replication origin and several genes that promote its transfer to other cells. These genes encode protein subunits that assemble on the surface of the bacterial cell, forming a hollow pilus that is necessary for the transfer process (figure 28.10*a*).

First, the F plasmid binds to a site on the interior of the F⁺ cell just beneath the pilus, now called a *conjugation bridge*. Then, by a process called *rolling-circle replication*, the F plasmid begins to copy its DNA at the binding point. As it is replicated, the displaced single strand of the plasmid passes into the other cell. There, a complementary strand is added, creating a new, stable F plasmid (figure 28.10*b*).

Recombination between the F plasmid and host chromosome

The F plasmid can integrate into the host chromosome by recombining with it (see chapter 13). The molecular events in this process are similar to events during meiosis in eukaryotes when crossing over (recombination) exchanges material between chromosomes. This process is also called homologous recombination. In the case of the F plasmid and the *E. coli* chromosome, a single recombination event between two circles produces a larger circle, consisting of the chromosome and the integrated plasmid. This integration is actually mediated by host-encoded proteins, but it takes advantage of regions in the F plasmid called insertion sequences (IS) that also exist in the *E. coli* chromosome. These IS elements are actually transposable elements that probably moved from the chromosome to the F plasmid.

When the F plasmid is integrated into the chromosome, the cell is called an **Hfr cell** for high frequency of recombination (figure 28.11), because now transfer by the F plasmid will include chromosomal DNA. The site on the F plasmid where transfer initiates is located in the middle of the integrated plasmid, so that the entire chromosome would have to be transferred to also

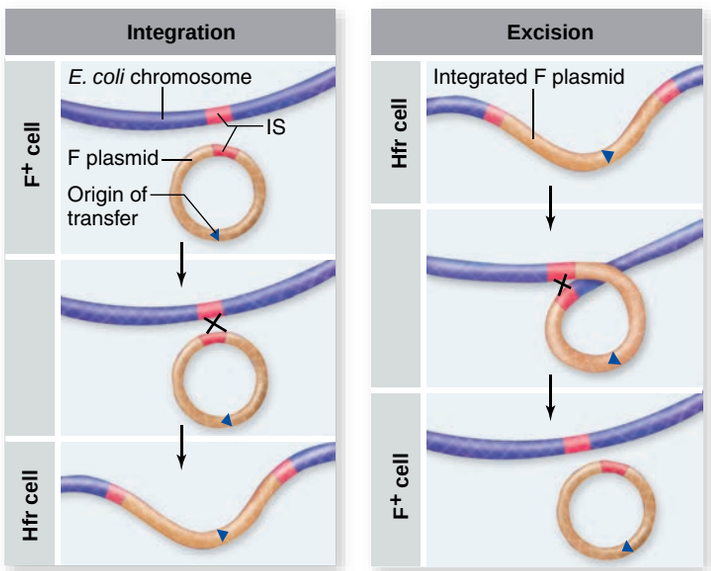


Figure 28.11 Integration and excision of F plasmid. The F plasmid contains short insertion sequences (IS) that also exist in the chromosome. This allows the plasmid to pair with the chromosome, and a single recombination event between two circles leads to a larger circle. This integrates the plasmid into the chromosome, creating an Hfr cell, as shown on the left. The process is reversible because the IS sequences in the integrated plasmid can pair, and now a recombination event will return the two circles and convert the Hfr back to an F⁺ cell as shown on the right.

transfer all of the integrated plasmid. The transfer of the entire chromosome takes around 100 minutes, and the conjugation bridge is usually broken before that time. This leads to transfer of portions of donor chromosome that can then replace regions of the recipient chromosome by homologous recombination. This occurs by *two* recombination events between the linear piece and the circular chromosome, similar to a double crossover in eukaryotic meiosis.

Geneticists have taken advantage of this process to map the order of genes in the *E. coli* chromosome. Genes close to the origin of transfer are transferred early in the process, and those far from the origin are transferred later. If the process of mating is experimentally interrupted at different times, then gene order can be mapped based on time of entry of each gene (figure 28.12). The entry of genes can be detected by using a donor with wild-type alleles that can replace mutant alleles in the recipient by homologous recombination as described. These experiments have shown that the *E. coli* chromosome is indeed circular, and the genetic map is therefore circular. The units of the map are minutes, and the entire map is 100 minutes long.

The F plasmid can also excise itself by reversing the integration process. In this case, the IS elements bounding the integrated plasmid pair and now a single recombination event will restore the two circles (see figure 28.11). If excision is inaccurate, the F plasmid can pick up some chromosomal DNA in the process. This creates what is called an F plasmid that can then be transferred rapidly and in its entirety to another cell. In this case, the cell already has the same genetic material in its chromosome as that carried by the F′. This makes the cell a **partial diploid,** sometimes called a **merodiploid.** Merodiploids can be used to determine if new isolated mutations are alleles of known genes. This is done by using wild types of alleles of known genes of the F′ plasmid to provide normal function heterozygous to unknown mutant alleles in the chromosome.

Viruses transfer DNA by transduction

Horizontal transfer of DNA can also be mediated by bacteriophage. In **generalized transduction,** virtually any gene can be transferred between cells; in **specialized transduction,** only a few genes are transferred.

Generalized transduction

Generalized transduction can be thought of as an accident of the biology of some types of lytic phage (see chapter 27). In these viruses, after the viral genome is replicated and the phage head is constructed, the phage packaging machinery stuffs DNA into the phage head until no more fits, so-called headfull packaging. Sometimes the phage begins with bacterial DNA instead of phage DNA and packages this DNA into a phage head (figure 28.13). When this viral particle goes on to infect another cell, it injects the bacterial DNA into the infected cell instead of viral DNA. This DNA can then be incorporated into the recipient chromosome by homologous recombination. Similar to transfer by Hfr cells described earlier, two recombination events are necessary to integrate the linear piece of DNA into the circular chromosome (see figure 28.13).

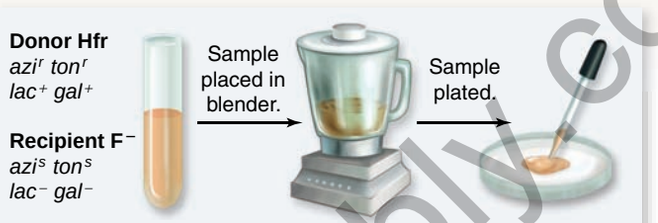
Generalized transduction has also been used for mapping purposes in *E. coli*, although the logic is different from that in conjugation. In transduction, the closer together two genes are, the more likely it is that they will be transferred in a single transduction event. This can be expressed mathematically as the *cotransduction frequency*. Correlation of maps from the two

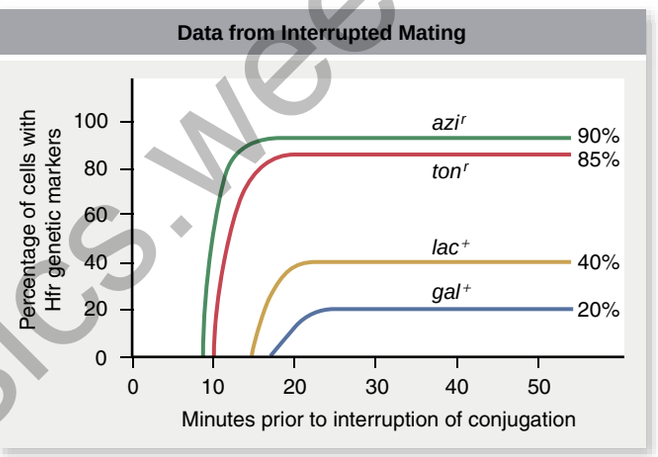
SCIENTIFIC THINKING

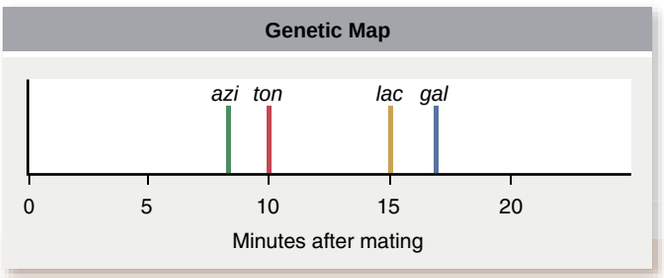
Hypothesis: *Conjugation using Hfr strains involves the linear transfer of information from donor to recipient cell.*

Prediction: *If there is a linear transfer of information, then different markers should appear in a time sequence.*

Test: *Mating strains are agitated at time points to break the conjugation bridge, then plated to determine genotype.*







Result: *The different genes from the donor strain appear in a linear time sequence.*

Conclusion: *The transfer of genetic information is linear. This sequence can be used to construct a genetic map ordering the genes on the chromosomes.*

Further Experiments: *Can other methods of DNA exchange also be used for genetic mapping?*

Figure 28.12 Interrupted mating experiment allows construction of genetic map.

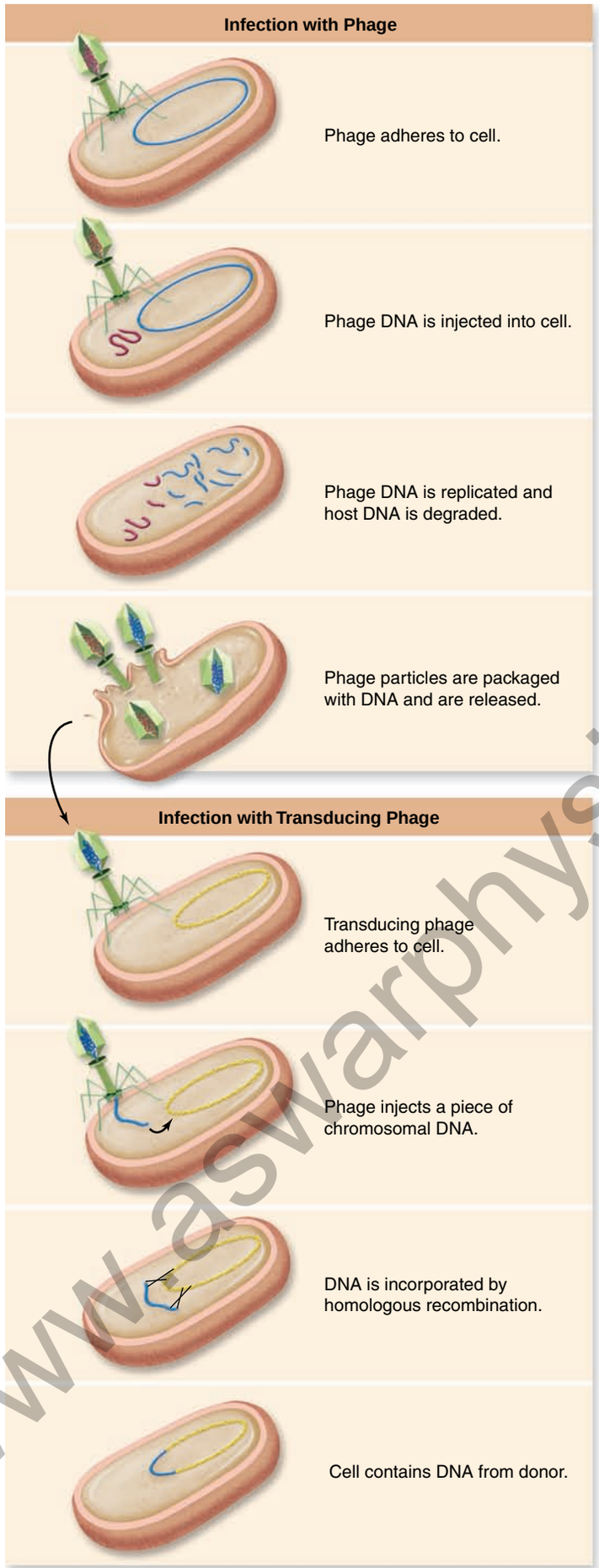


Figure 28.13 Transduction by generalized transducing phage. When some phage infect cells, they degrade the host DNA into pieces. When the phage package their DNA, they can package host DNA in place of phage DNA to produce a transducing phage as shown on the top. When a transducing phage infects a cell, it injects host DNA that can then be integrated into the host genome by homologous recombination. With a linear piece of DNA, it requires two recombination events, which replace the chromosomal DNA with the transducing DNA as shown on the bottom. If the new allele is different from the old, the cell's phenotype will change.

methods allows an empirical conversion between cotransduction frequency and minutes in the genetic map.

Specialized transduction

Specialized transduction is limited to phage that exhibit a lysogenic life cycle (see chapter 27). The prototype for this is phage λ from *E. coli*. When λ infects a cell and its genome integrates into the host chromosome, it does not destroy the cell but is passed on by cell division. This integration event is similar to the integration of the F plasmid, except that in the case of λ the recombination is a site-specific event mediated by phage-encoded proteins.

In this lysogenic state, the phage is called a prophage and it is dormant. The prophage encodes the functions necessary to eventually excise itself and undergo lytic growth, leading to the death of the cell. If this excision event is imprecise, it may take some chromosomal DNA with it, in the process making a specialized transducing phage. These phage carry both phage genes and chromosomal genes, unlike generalized transducing phage that carry only chromosomal DNA.

Because the phage head can carry only as much DNA as is found in the phage genome, imprecise excision results in deletion of phage genes. Thus specialized transducing phage may be defective if genes necessary for phage growth are lost in the process.

Specialized transducing phage particles can then integrate into the chromosome, just like wild-type phage, also making the cell diploid for the genes carried by the phage. Phage particles that can integrate as prophages may become trapped in the host genome if the genes necessary for excision become defective by mutation or are lost. The *E. coli* genome contains a number of such cryptic prophage, some of which encode functions important to the cell and must now be considered part of the host genome.

Transformation is the uptake of DNA directly from the environment

Transformation is a naturally occurring process in some species, such as the bacteria that were studied by Frederick Griffith (see chapter 14). Griffith discovered the process despite not knowing what chemical component was transferred. Transformation occurs when one bacterial cell has died and ruptured,

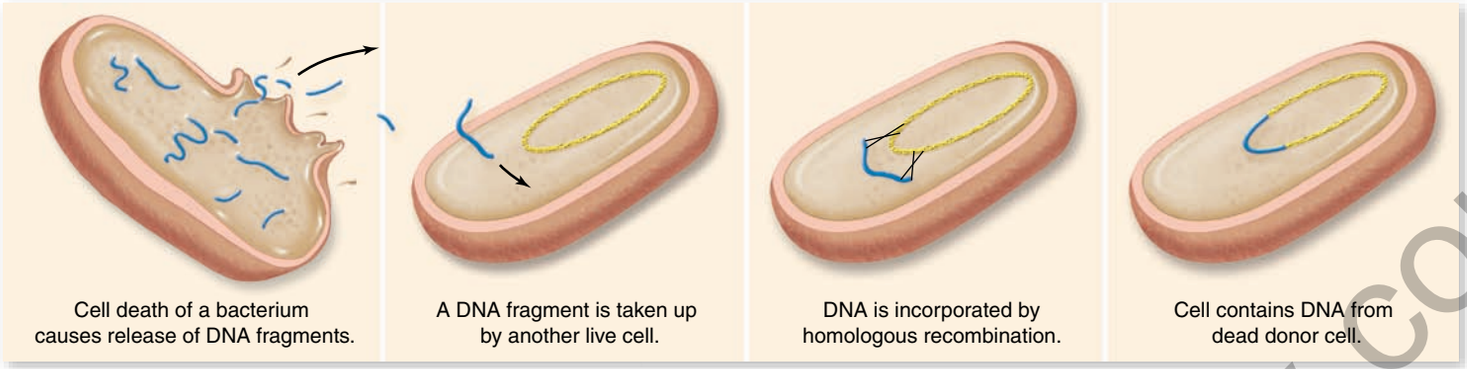


Figure 28.14 Natural transformation. Natural transformation occurs when one cell dies and releases its contents to the surrounding environment. The DNA is usually fragmented, and small pieces can be taken up by other, living cells. The DNA taken up can replace chromosomal DNA by homologous recombination as in conjugation and transduction. If the new DNA contains different alleles from the chromosome, the phenotype of the cell changes, possibly providing a selective advantage.

spilling its fragmented DNA into the surrounding environment. This DNA can be taken up by another cell and incorporated into its genome, thereby transforming it (figure 28.14). When the uptake occurs under natural conditions, it is termed natural transformation. Some species of both gram-positive and gram-negative bacteria exhibit natural transformation, although the mechanisms seem to differ between the groups.

The proteins involved in the process of natural transformation are all encoded by the bacterial chromosome. The implication is that natural transformation may be the only one of the mechanisms of DNA exchange that evolved as part of normal cellular machinery. The transfer of chromosomal DNA by either conjugation or transduction can be thought of as accidents of plasmid or phage biology, respectively.

Transformation is also important in molecular cloning, but *E. coli* does not exhibit natural transformation. When transformation is accomplished in the laboratory it is called artificial transformation. Artificial transformation is useful for cloning and DNA manipulation (see chapter 17).

Antibiotic resistance can be transferred by resistance plasmids

Some conjugative plasmids pick up antibiotic resistance genes, becoming resistance plasmids, or **R plasmids.** The rapid transfer of newly acquired, antibiotic resistance genes by plasmids has been an important factor in the appearance of the resistant strains of the pathogen *Staphylococcus aureus* discussed in the next section.

The means by which resistance plasmids acquire antibiotic resistance genes is often through transposable elements, which were described in chapter 18. These elements can move from chromosome to chromosome or from plasmid to chromosome and back again, and they can transfer antibiotic resistance genes in the process. If a conjugative plasmid picks up these genes, then the bacterium carrying it has a selective advantage in the presence of those antibiotics.

An important example in terms of human health involves the Enterobacteriaceae, the family of bacteria to which the common intestinal bacterium *E. coli* belongs. This family contains many pathogenic bacteria, including the organisms that cause dysentery, typhoid, and other major diseases. At times, some of the genetic material from these pathogenic species is exchanged with or transferred to *E. coli* by transmissible plasmids or bacteriophage. Because of its abundance in the human digestive tract, *E. coli* poses a special threat if it acquires harmful traits, as seen by the outbreaks of the food-borne O157:H7 strain of *E. coli*. Infection with this strain of *E. coli* can lead to serious illness. This is a new strain of *E. coli* that evolved by acquiring genes for pathogenic traits. Evidence suggests this occurred by both transduction and the acquisition of a large virulence plasmid by conjugation.

Variation can also arise by mutation

Just as with any organism, mutations can arise spontaneously in bacteria. Certain factors, especially those that damage DNA, such as radiation, ultraviolet light, and various chemicals, increase the likelihood of mutation.

A typical bacterium such as *E. coli* contains about 5000 genes. The probability of mutation occurring by chance is about in one out of every million copies of a gene. With 5000 genes in a bacterium, we can predict that approximately 1 out of every 200 bacteria will have a mutation. With adequate food and nutrients, a population of *E. coli* can double in 20 minutes. Because bacteria multiply so rapidly, mutations can spread rapidly in a population and can change the characteristics of that population in a relatively short time.

In the laboratory, bacteria are grown on different substrates, called *growth media*, that reflect their nutritional needs. For a particular species, the medium that contains only those nutrients required for wild-type growth is termed a *minimal medium*. A mutant that can no longer survive on minimal medium and needs particular nutritional supplements, such as an amino acid, is called an **auxotroph. Replica plating** allows identification of bacterial auxotrophs from a master plate of rich growth media by isolating individual colonies and observing their growth (or failure to grow) on different supplemented media. The technique is somewhat like using a rubber stamp—an impression of colonies growing in a Petri plate is made on a velvet surface, and then this surface is pressed onto different

media in other plates. The impression contains many thousands if not millions of cells from each colony—and each colony has grown from a single cell. In this way, a bacterium with a highly specific mutation can be isolated, identified, and grown.

The ability of prokaryotes to change rapidly in response to new challenges often has adverse effects on humans. A number of antibiotic-resistant strains of the bacterium *Staphylococcus aureus* (termed methicillin-resistant *Staphylococcus aureus*, or MRSA) had been known in hospital settings for some time. More recently these have been observed in infections out of the hospital setting, so-called community acquired MRSA.

Of most concern among these strains is **vancomycin-resistant *Staphylococcus aureus*** (VRSA). This appears to have arisen rapidly by mutation and is alarming because vancomycin is the drug of last resort, making these strains and the infections they cause very difficult to stop. *Staphylococcus* infections, or "staph" infections for short, provide an excellent example of the way in which mutation and intensive selection can bring about rapid change in bacterial populations.

Learning Outcomes Review 28.4

Prokaryotic DNA exchange is horizontal, from donor cell to recipient cell. DNA can be exchanged by conjugation via plasmids, by transduction via viruses, and by transformation through the direct uptake of DNA from the environment. These forms of DNA exchange can be used experimentally to map genes. Variation in prokaryotes also arises by mutation. Extensive use of antibiotics has lead to selection for resistant organisms. Resistance genes can be transferred, rapidly spreading resistance.

- ***How does transfer of genetic information in bacteria differ from eukaryotic sex?***

28.5 Prokaryotic Metabolism

Learning Outcomes

1. ***Describe the different ways that prokaryotes acquire energy and carbon.***
2. ***Explain how bacterial proteins can cause disease in humans.***

The variation seen in prokaryotes manifests itself most noticeably in biochemical rather than morphological diversity. Wide variation has been found in the types of metabolism prokaryotes exhibit, especially in the means by which they acquire energy and carbon.

Prokaryotes acquire carbon and energy in four basic ways

Prokaryotes have evolved many mechanisms to acquire the energy and carbon they need for growth and reproduction. Many are *autotrophs* that obtain their carbon from inorganic CO_2. Other prokaryotes are *heterotrophs* that obtain at least some of their carbon from organic molecules, such as glucose. Depending on the method by which they acquire energy, autotrophs and heterotrophs are categorized as follows:

Photoautotrophs. Many bacteria carry out photosynthesis, using the energy of sunlight to build organic molecules from carbon dioxide. The **cyanobacteria** use chlorophyll *a* as the key light-capturing pigment and H_2O as an electron donor, releasing oxygen gas as a by-product. They are therefore oxygenic, and their method of photosynthesis is very similar to that found in algae and plants.

Other bacteria use bacteriochlorophyll as their light-capturing pigment and H_2S as an electron donor, leaving elemental sulfur as the by-product. These bacteria do not produce oxygen (anoxygenic) and have a simpler method of photosynthesis. These are the purple and green sulfur bacteria.

Archaeal species also carry out photosynthesis, the simplest form known. This involves a single protein, bacteriorhodopsin, that uses energy from light to translocate protons across a membrane. This then provides a proton motive force for ATP synthesis. Recent surveys of microbial diversity in marine ecosystems using DNA sequencing have found a new relative of the rhodopsin family called proteorhodopsin. First found in a bacterial species, these proteorhodopsins are quite widespread, found in bacterial, archaeal, and even algal species. This raises the possibility that photosynthesis in marine systems may be more widespread and complex than previously thought.

Chemolithoautotrophs. Some prokaryotes obtain energy by oxidizing inorganic substances. Nitrifiers, for example, oxidize ammonia or nitrite to obtain energy, producing the nitrate that is taken up by plants. This process is called **nitrification,** and it is essential in terrestrial ecosystems because plants primarily absorb nitrogen in the form of nitrate.

Other chemolithoautotrophs oxidize sulfur, hydrogen gas, and other inorganic molecules. On the dark ocean floor at depths of 2500 m, entire ecosystems subsist on prokaryotes that oxidize hydrogen sulfide as it escapes from thermal vents.

Photoheterotrophs. The so-called purple and green nonsulfur bacteria use light as their source of energy but obtain carbon from organic molecules, such as carbohydrates or alcohols that have been produced by other organisms.

Chemoheterotrophs. The majority of prokaryotes obtain both carbon atoms and energy from organic molecules. These include decomposers and most pathogens. Human beings and all nonphotosynthetic eukaryotes are chemoheterotrophs as well.

Some bacteria can attack other cells directly

Invading pathogens of the genera *Yersinia* can introduce proteins directly into host cells by a specialized form of secretion. (*Yersinia pestis* is the bacterial species responsible for bubonic plague.) Most proteins secreted by gram-negative

bacteria have special signal sequences that allow them to pass through the bacterium's double membrane. The proteins secreted by *Yersinia* lacked a key signal sequence that two known secretion mechanisms require for transport. The proteins must therefore have been secreted by means of a third type of system, which researchers called the *type III system*. This kind of system acts like a kind of molecular syringe allowing the pathogen to inject proteins directly into the cytoplasm of host cells.

As more bacterial species are studied, the genes coding for the type III system are turning up in other gram-negative animal pathogens, and even in more distantly related plant pathogens. The genes seem more closely related to one another than are the bacteria. Furthermore, the genes are similar to those that code for bacterial flagella.

These proteins are used to transfer other virulence proteins, such as toxins, into nearby eukaryotic cells. Given the similarity of the type III genes to the genes that code for flagella, the transfer proteins may form a flagellum-like structure that shoots virulence proteins into the host cells. Once in the eukaryotic cells, the virulence proteins affect the host's response to the pathogen.

In *Yersinia*, proteins secreted by the type III system are injected into macrophages; the proteins disrupt signals that tell the macrophages to engulf bacteria. *Salmonella* and *Shigella* use their type III proteins to enter the cytoplasm of eukaryotic cells, and thus they are protected from the immune system of their host. The proteins secreted by certain strains of *E. coli* alter the cytoskeleton of nearby intestinal eukaryotic cells, resulting in a bulge onto which the bacterial cells can tightly bind.

Bacteria are costly plant pathogens

Although the majority of commercially relevant plant pathogens are fungi, many diseases of plants are associated with particular heterotrophic bacteria. Almost every kind of plant is susceptible to one or more kinds of bacterial disease, including blights, soft rots, and wilts. Fire blight, which destroys pear and apple trees and related plants, is a well-known example of bacterial disease.

The early symptoms of these plant diseases vary, but they are commonly manifested as spots of various sizes on the stems, leaves, flowers, or fruits. Most bacteria that cause plant diseases are members of the group of rod-shaped gram-negative bacteria known as pseudomonads.

Learning Outcomes Review 28.5

Prokaryotes exhibit amazing metabolic diversity with both autotrophic and heterotrophic species. Photoautotrophs use light as an energy source; chemolithoautotrophs oxidize inorganic compounds. Photoheterotrophs use light as an energy source and organic compounds as carbon sources. Chemoheterotrophs use organic compounds for both energy and carbon. Bacterial animal pathogens attack host cells with toxic proteins that disrupt the host's immune response, among other effects.

- ***Why is metabolism a better way than morphology to characterize prokaryotes?***

28.6 Human Bacterial Disease

Learning Outcomes

1. ***Describe common human bacterial pathogens.***
2. ***Explain how bacteria can cause ulcers.***
3. ***Identify sexually transmitted diseases caused by bacteria.***

In the early 20th century, before the discovery and widespread use of antibiotics, infectious diseases killed nearly 20% of all U.S. children before they reached the age of five. Sanitation and antibiotics considerably improved the situation. In recent years, however, we have seen the appearance or reappearance of many bacterial diseases, including cholera, leprosy, tetanus, bacterial pneumonia, whooping cough, diphtheria, and Lyme disease (table 28.1). Members of the genus *Streptococcus* are associated with scarlet fever, rheumatic fever, pneumonia, "flesh-eating disease," and other infections. Tuberculosis, another bacterial disease, is still a leading cause of death in humans worldwide.

Bacteria have many different methods to spread through a susceptible population. Tuberculosis and many other bacterial diseases of the respiratory tract are mostly spread through the air in droplets of mucus or saliva. Diseases such as typhoid fever, paratyphoid fever, and bacillary dysentery are spread by fecal contamination of food or water. Lyme disease and Rocky Mountain spotted fever are spread to humans by tick vectors.

Tuberculosis has infected humans for all of recorded history

Tuberculosis (TB) has been a scourge to humanity for thousands of years. There is evidence that peoples from ancient Egypt and pre-Columbian South America died from TB; the TB bacillus *(Mycobacterium tuberculosis)* has been identified in prehistoric mummies. TB afflicts the respiratory system, thwarts the immune system, and is easily transmitted from person to person through the air.

The spread of tuberculosis

Currently, about one-third of all people worldwide are regularly exposed to *Mycobacterium tuberculosis*. An estimated 9.27 million new cases were diagnosed, and 1.8 million deaths occurred in 2007. In 2006, the World Health Organization reported the incidence of TB falling in five of six WHO regions, but the numbers continue to rise in Africa driven by the spread of HIV.

Since the mid-1980s, the United States has experienced a resurgence of TB. This peaked in the mid-1990s and has been declining since, although the rate of decline is leveling off. The latest statistics from the CDC indicate 13,300 TB cases in 2007, down from 13,754 cases in 2006.

Tuberculosis treatment

Most TB patients are placed on multiple, expensive antibiotics for six to twelve months. Alarming outbreaks of **multidrug-resistant**

TABLE 28.1 Important Human Bacterial Diseases

Disease	Pathogen	Vector/Reservoir	Epidemiology
Anthrax	*Bacillus antbracis*	Animals, including processed skins	Bacterial infection that can be transmitted through contact or ingestion. Rare except in sporadic outbreaks. May be fatal.
Botulism	*Clostridium botulinum*	Improperly prepared food	Contracted through ingestion or contact with wound. Produces acute toxic poison; can be fatal.
Chlamydia	*Chlamydia trachomatis*	Humans, sexually transmitted disease (STD)	Urogenital infections with possible spread to eyes and respiratory tract. Increasingly common over past 20 years.
Cholera	*Vibrio cholerae*	Human feces, plankton	Causes severe diarrhea that can lead to death by dehydration; 50% peak mortality rate if untreated. A major killer in times of crowding and poor sanitation; over 100,000 died in Rwanda in 1994 outbreak.
Dental caries	*Streptococcus mutans, Streptococcus sabrinus*	Humans	A dense collection of these bacteria on the surface of teeth leads to secretion of acids that destroy minerals in tooth enamel; sugar alone does not cause caries.
Diphtheria	*Corynebacterium diphtheriae*	Humans	Acute inflammation and lesions of respiratory mucous membranes. Spread through respiratory droplets. Vaccine available.
Gonorrhea	*Neisseria gonorrhoeae*	Humans only	STD, on the increase worldwide. Usually not fatal.
Hansen disease (leprosy)	*Mycobacterium leprae*	Humans, feral armadillos	Chronic infection of the skin; worldwide incidence about 10–12 million, especially in southeast Asia. Spread through contact with infected individuals.
Lyme disease	*Borrelia burgdorferi*	Ticks, deer, small rodents	Spread through bite of infected tick. Lesion followed by malaise, fever, fatigue, pain, stiff neck, and headache.
Peptic ulcers	*Helicobacter pylori*	Humans	Originally thought to be caused by stress or diet, most peptic ulcers now appear to be caused by this bacterium; good news for ulcer sufferers because it can be treated with antibiotics.
Plague	*Yersinia pestis*	Fleas of wild rodents: rats and squirrels	Killed one-fourth of the population of Europe in the 14th century; endemic in wild rodent populations of the western United States today.
Pneumonia	*Streptococcus, Mycoplasma, Chlamydia, Haemophilus*	Humans	Acute infection of the lungs; often fatal without treatment. Vaccine for streptococcal pneumonia available.
Tuberculosis	*Mycobacterium tuberculosis*	Humans	An acute bacterial infection of the lungs, lymph, and meninges. Its incidence is on the rise, complicated by the development of new strains of the bacterium that are resistant to antibiotics.
Typhoid fever	*Salmonella typhi*	Humans	A systemic bacterial disease of worldwide incidence. Fewer than 500 cases a year are reported in the United States. Spread through contaminated water or foods (such as improperly washed fruits and vegetables). Vaccines are available for travelers.
Typhus	*Rickettsia typhi*	Lice, rat fleas, humans	Historically a major killer in times of crowding and poor sanitation; transmitted from human to human through the bite of infected lice and fleas. Peak untreated mortality rate of 70%.

(MDR) strains of TB have occurred, however, in the United States and worldwide. These MDR strains are resistant to most of the best available anti-TB medications. MDR TB is of particular concern because it requires much more time and is more expensive to treat. Also, it is more likely to prove fatal.

This spread of MDR TB is likely due to the extremely long course of antibiotics required to treat the disease. Patients often quit taking the antibiotics before completing the course, setting up conditions in their bodies to allow drug-resistant bacteria to thrive.

The basic principles of TB treatment and control are to make sure all patients complete a full course of medication, so that all of the bacteria causing the infection are killed and drug-resistant strains do not develop. Great efforts are being made to ensure that high-risk individuals who are infected but not yet sick receive preventive therapy under observation. Such programs are approximately 90% effective in reducing the likelihood of developing active TB and spreading it to others. These efforts are having an effect, since the disease is on the decline in the United States, decreasing by 3.3% from 2006 to 2007.

Bacterial biofilms are involved in tooth decay

Bacteria and other organisms may form mixed cultures on certain surfaces that are extremely difficult to treat. On teeth, this biofilm, or plaque, consists largely of bacterial cells surrounded by a polysaccharide matrix. Most of the bacteria in plaque are filaments of rod-shaped cells classified as various species of *Actinomyces*, which extend out perpendicular to the surface of the tooth. Many other bacterial species are also present in plaque.

Tooth decay, or dental caries, is caused by the bacteria present in the plaque, which persist especially in places that are difficult to reach with a toothbrush. Diets that are high in simple sugars are especially harmful to teeth because certain bacteria, notably *Streptococcus sobrinus* and *S. mutans*, ferment the sugars to lactic acid. This acid production reduces the pH in the area around the plaque, breaking down the structure of the hydroxyapatite that makes tooth enamel hard. As the enamel degenerates, the remaining soft matrix of the tooth becomes vulnerable to bacterial attack.

Bacteria can cause ulcers

Bacteria can also be the cause of disease states that on the surface appear to have no infectious basis. Peptic ulcer disease is due to craterlike lesions in the gastrointestinal tract that are exposed to peptic acid. Ulcers can be caused by drugs, such as nonsteroidal anti-inflammatory drugs, and also by some tumors of the pancreas that cause an oversecretion of peptic acid. In 1982, a bacterium named *Campylobacter pylori* (now named *Helicobacter pylori*) was isolated from gastric juices. Over the years evidence has accumulated that this bacterium is actually the causative agent in the majority of cases of peptic ulcer disease.

Antibiotic therapy can now eliminate *H. pylori*, treating the cause of the disease, and not just the symptoms. The discovery of the action of this bacterial species illustrates how even disease states that appear to be unrelated to infectious disease may actually be caused by cryptic (unknown) infection.

Many sexually transmitted diseases are bacterial

A number of bacteria cause sexually transmitted diseases (STDs), three particularly important examples of which are gonorrhea, syphilis, and chlamydia (figure 28.15).

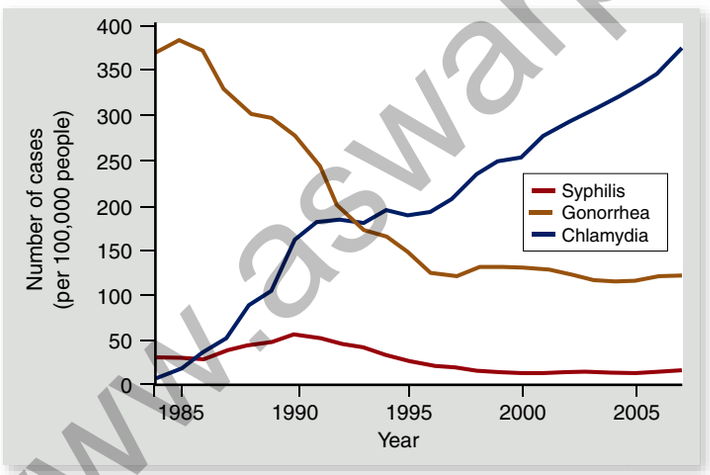


Figure 28.15 **Trends in sexually transmitted diseases in the United States.**

Inquiry question

How is it possible for the incidence of one STD (chlamydia) to rise as another (gonorrhea) falls?

Gonorrhea

Gonorrhea is one of the most prevalent communicable diseases in North America. Caused by the bacterium *Neisseria gonorrhoeae*, gonorrhea can be transmitted through sexual intercourse or any other sexual contact in which body fluids are exchanged, such as oral or anal intercourse. It can also pass from mother to baby during delivery through the birth canal.

The incidence of gonorrhea has been on the decline in the United States, but it remains a serious threat worldwide. Of particular concern is the appearance of antibiotic-resistant strains of *N. gonorrhoeae*.

Syphilis

Syphilis, a very destructive STD, was once prevalent and deadly but is now less common due to the advent of blood-screening procedures and antibiotics. Syphilis is caused by a spirochete bacterium, *Treponema pallidum*, transmitted during sexual intercourse or through direct contact with an open syphilis chancre sore. The bacterium can also be transmitted from a mother to her fetus, often causing damage to the heart, eyes, and nervous system of the baby.

Once inside the body, the disease progresses in four distinct stages. The first, or primary stage, is characterized by the appearance of a small, painless, often unnoticed sore called a *chancre*. The chancre resembles a blister and occurs at the location where the bacterium entered the body about three weeks following exposure. This stage of the disease is highly infectious, and an infected person may unwittingly transmit the disease to others. This sore heals without treatment in approximately four weeks, deceptively indicating a "cure" of the disease, although the bacterium remains in the body.

The second stage of syphilis, or secondary syphilis, is marked by a rash, a sore throat, and sores in the mouth. The bacteria can be transmitted at this stage through kissing or contact with an open sore. Commonly at this point, the disease enters the third stage, a latent period. This latent stage of syphilis is symptomless and may last for several years. At this point, the person is no longer infectious, but the bacteria are still present in the body, attacking the internal organs.

The final stage of syphilis is the most debilitating, as the damage done by the bacteria in the third stage becomes evident. Sufferers at this stage of syphilis experience heart disease, mental deficiency, and nerve damage, which may include loss of motor functions or blindness.

Chlamydia

Chlamydia is caused by an unusual bacterium. *Chlamydia trachomatis* is genetically a bacterium but is an obligate intracellular parasite, much like a virus in this respect. It is susceptible to antibiotics but it depends on its host to replicate its genetic material. The bacterium is transmitted through vaginal, anal, or oral intercourse with an infected person.

Chlamydia is called the "silent STD" because women usually experience no symptoms until after the infection has become established. In part because of this symptomless nature, the incidence of chlamydia has skyrocketed, increasing from 142 cases per 100,000 population in 1988 to 544 cases per 100,000 population in 2007.

The effects of an established chlamydia infection on the female body are extremely serious. Chlamydia can cause pelvic inflammatory disease (PID), which can lead to sterility and sometimes death.

It has recently been established that infection of the male or female reproductive tract by chlamydia can cause heart disease. Chlamydiae produce a peptide similar to one produced by cardiac muscle. As the body's immune system tries to fight off the infection, it recognizes and reacts to this peptide. The similarity between the bacterial and cardiac peptides confuses the immune system, and T cells attack cardiac muscle fibers, inadvertently causing inflammation of the heart and other problems.

Within the last few years, two types of tests for chlamydia have been developed. The treatment for the disease is antibiotics, usually tetracycline, which can penetrate the eukaryotic plasma membrane to attack the bacterium. Any woman who experiences the symptoms associated with this STD or who is at risk of developing an STD should be tested for the presence of the chlamydia bacterium; otherwise, her fertility may be at risk.

Learning Outcomes Review 28.6

Many human diseases are due to bacterial infection, including tuberculosis, streptococcal and staphylococcal infection, and sexually transmitted diseases. The causative agent of most peptic ulcers is *Helicobacter pylori,* an inhabitant of the digestive tract. Bacteria are responsible for many STDs, including gonorrhea, syphilis, and chlamydia. In many cases symptoms of infection disappear although the disease is still present, and all can have serious consequences if untreated, especially for women.

- ***Why is infection by most pathogens not fatal?***

28.7 Beneficial Prokaryotes

Learning Outcomes

1. ***Recognize the role of prokaryotes in the global cycling of elements.***
2. ***Describe examples of bacterial/eukaryote symbiosis.***
3. ***Explain how bacteria can be used for bioremediation.***

Prokaryotes were largely responsible for creating the current properties of the atmosphere and the soil through billions of years of their activity. Today, they still affect the Earth and human life in many important ways.

Prokaryotes are involved in cycling important elements

Life on Earth is critically dependent on the cycling of chemical elements between organisms and the physical environments in which they live—that is, between the living and nonliving elements of ecosystems. Prokaryotes, algae, and fungi play many key roles in this chemical cycling, a process discussed in detail in chapter 58.

Decomposition

The carbon, nitrogen, phosphorus, sulfur, and other atoms of biological systems all have come from the physical environment, and when organisms die and decay, these elements all return to it. The prokaryotes and fungi that carry out the decomposition portion of chemical cycles, releasing a dead organism's atoms to the environment, are called *decomposers.*

Fixation

Other prokaryotes play important roles in fixation, the other half of chemical cycles, helping to return elements from inorganic forms to organic forms that heterotrophic organisms can use.

Carbon. The role of photosynthetic prokaryotes in fixing carbon is obvious. The organic compounds that plants, algae, and photosynthetic prokaryotes produce from CO_2 pass up through food chains to form the bodies of all the ecosystem's heterotrophs. Ancient cyanobacteria are thought to have added oxygen to the Earth's atmosphere as a by-product of their photosynthesis. Modern photosynthetic prokaryotes continue to contribute to the production of oxygen.

Nitrogen. Less obvious, but no less critical to life, is the role of prokaryotes in recycling nitrogen. The nitrogen in the Earth's atmosphere is in the form of N_2 gas. A triple covalent bond links the two nitrogen atoms and is not easy to break. Among the Earth's organisms, only a very few species of prokaryotes are able to accomplish this feat, reducing N_2 to ammonia (NH_3), which is used to build amino acids and other nitrogen-containing biological molecules. When the organisms that contain these molecules die, decomposers return nitrogen to the soil as ammonia. This is then converted to nitrate (NO^{3-}) by nitrifying bacteria, making nitrogen available for plants. The nitrate can also be converted back into molecular nitrogen by *denitrifiers* that return the nitrogen to the atmosphere, completing the cycle.

To fix atmospheric nitrogen, prokaryotes employ an enzyme complex called nitrogenase, encoded by a set of genes called *nif* ("nitrogen fixation") genes. The nitrogenase complex is extremely sensitive to oxygen and is found in a wide range of free-living prokaryotes.

In aquatic environments, nitrogen fixation is carried out largely by cyanobacteria such as *Anabaena*, which forms long chains of cells. Because the nitrogen fixation process is strictly anaerobic, individual cyanobacteria cells may develop into *heterocysts*, specialized nitrogen-fixing cells impermeable to oxygen.

In soil, nitrogen fixation occurs in the roots of plants that harbor symbiotic colonies of nitrogen-fixing bacteria. These associations include *Rhizobium* (a genus of proteobacteria; see figure 28.5) with legumes, *Frankia* (an actinomycete) with many woody shrubs, and *Anabaena* with water ferns.

Prokaryotes may live in symbiotic associations with eukaryotes

Many prokaryotes live in symbiotic association with eukaryotes. **Symbiosis** refers to the ecological relationship between different species that live in direct contact with each other. The symbiotic

association of nitrogen-fixing bacteria with plant roots is an example of *mutualism*, a form of symbiosis in which both parties benefit. The bacteria supply the plant with useful nitrogen, and the plant supplies the bacteria with sugars and other organic nutrients (see chapter 39).

Many bacteria live symbiotically within the digestive tracts of animals, providing nutrients to their hosts. Cattle and other grazing mammals are unable to digest cellulose in the grass and plants they eat because they lack the required cellulase enzyme. Colonies of cellulase-producing bacteria inhabiting the gut allow cattle to digest their food (see chapter 48 for a fuller account). Similarly, humans maintain large colonies of bacteria in the large intestine that produce vitamins—particularly B_{12} and K—that the body cannot make.

Many bacteria inhabit the outer surfaces of animals and plants without doing damage. These associations are examples of *commensalism*, in which one organism (the bacterium) receives benefits while the animal or plant is neither benefited nor harmed.

Parasitism is a form of symbiosis in which one member (in this case, the bacterium) benefits, and the other (the infected animal or plant) is harmed. Infection might be considered a form of parasitism.

Bacteria are used in genetic engineering

Because the genetic code is universal, a gene from a human can be inserted into a bacterial cell, and the bacterium produces a human protein. The use of bacteria in genetic engineering was discussed in chapter 17, and it is a large part of modern molecular biology.

In addition to the production of pharmaceutical agents such as insulin, discussed in chapter 17, applying genetic engineering methods to produce improved strains of bacteria for commercial use holds promise for the future. Bacteria are now widely used as "biofactories" in the commercial production of a variety of enzymes, vitamins, and antibiotics. Immense cultures of bacteria, often genetically modified to enhance performance, are used to produce commercial acetone and other industrially important compounds.

Bacteria can be used for bioremediation

The use of organisms to remove pollutants from water, air, and soil is called *bioremediation*. The normal functioning of sewage treatment plants depends on the activity of microorganisms. In sewage treatment plants, the solid matter from raw sewage is broken down by bacteria and archaea naturally present in the sewage. The end product, methane gas (CH_4) is often used as an energy source to heat the treatment plant.

Biostimulation, that is, the addition of nutrients such as nitrogen and phosphorus sources, has been used to encourage the growth of naturally occurring microbes that can degrade crude oil spills. This approach was used successfully to clean up the Alaskan shoreline after the crude oil spill of the Exxon Valdez in 1998. Similarly, biostimulation has been used to encourage the growth of naturally occurring microbial flora in contaminated groundwater. Current efforts include those concentrated on the use of endogenous microbes such as *Geobacter* (see figure 28.5) to eliminate radioactive uranium from groundwater contaminated during the cold war.

Chlorinated compounds released into the environment by a variety of sources are another serious pollutant. Some bacteria can actually use these compounds for energy by performing reductive dehalogenation that is linked to electron transport, a process termed *halorespiration*. Although still at the development stage, the use of such bacteria to remove halogenated compounds from toxic waste holds great promise.

Learning Outcomes Review 28.7

Prokaryotes are vital to ecosystems for both recycling elements and fixation, or making elements available in organic form. Bacteria are involved in fixation of both carbon and nitrogen and are the only organisms that can fix nitrogen. These nitrogen-fixing bacteria may live in symbiotic association with plants. Bacteria are a key component of waste treatment, and they are also being used in bioremediation to remove toxic compounds introduced into the environment.

- ***Does the information about nitrogen fixation shed any light on the practice of crop rotation?***

Chapter Review

28.1 The First Cells

Microfossils indicate that the first cells were probably prokaryotic.
The oldest microfossils are 3.5 billion years old. Stromatolites, a combination of sedimentary deposits and precipitated materials, are as old as 2.7 billion years.

Isotopic data indicate that carbon fixation is an ancient process.
Relatively higher levels of carbon-12 in fossils compared with neighboring rocks indicate the action of ancient carbon fixation.

Some hydrocarbons found in ancient rocks may have biological origins.
Biomarkers such as lipids indicate that cyanobacteria are at least 2.7 billion years old.

28.2 Prokaryotic Diversity

Prokaryotes are fundamentally different from eukaryotes.
Prokaryotic features include unicellularity, small circular DNA, division by binary fission, lack of internal compartmentalization, a singular flagellum, and metabolic diversity.

Despite similarities, bacteria and archaea differ fundamentally.

Bacteria and archaea differ in four key areas: plasma membranes, cell walls, DNA replication, and gene expression.

Archaeal lipids have ether instead of ester linkages and can form tetraether monolayers. The cell walls of bacteria contain peptidoglycans, but those of archaea do not.

Both bacteria and archaea DNA have a single replication origin, but the origin and the replication proteins are different. Archaeal initiation of DNA replication and RNA polymerases are more like those of eukaryotes.

Most prokaryotes have not been characterized.

Nine clades of prokaryotes have been found so far, but many bacteria have not been studied (see figure 28.5).

28.3 Prokaryotic Cell Structure

Prokaryotes have three basic forms: rods, cocci, and spirals.

Prokaryotes have a tough cell wall and other external structures.

Bacteria are classified as gram-positive or gram-negative based on the Gram stain (see figure 28.6). Gram-positive bacteria have a thick peptidoglycan layer in the cell wall that contains teichoic acid (see figure 28.7). Gram-negative bacteria have a thin peptidoglycan layer and an outer membrane containing lipopolysaccharides in their cell wall (see figure 28.7).

Some bacteria have a gelatinous layer, the capsule, enabling the bacterium to adhere to surfaces and evade an immune response.

Many bacteria have a slender, rigid, helical flagellum composed of flagellin, which can rotate to drive movement (see figure 28.8). Some bacteria have hairlike pili that have roles in adhesion and exchange of genetic information.

Some bacteria form highly resistant endospores in response to environmental stress.

The interior of prokaryotic cells is organized.

In prokaryotes, invaginated regions of the plasma membrane function in respiration and photosynthesis. The nucleoid region contains a compacted circular DNA with no bounding membrane.

Prokaryotic ribosomes are smaller than those of eukaryotes and some antibiotics work by binding to these ribosomes, blocking protein synthesis.

28.4 Prokaryotic Genetics

Conjugation depends on the presence of a conjugative plasmid.

DNA can be exchanged by conjugation (see figure 28.10), which depends on the presence of conjugative plasmids like the F plasmid in *E coli*. The F^+ donor cell transfers the F plasmid to the F^- recipient cell.

The F plasmid can also integrate into the bacterial genome. Excision may be imprecise, so that the F plasmid carries genetic information from the host.

Viruses transfer DNA by transduction (see figure 28.13).

Generalized transduction occurs when viruses package host DNA and transfer it on subsequent infection. Specialized transduction is limited to lysogenic phage.

Transformation is the uptake of DNA directly from the environment (see figure 28.14).

Transformation occurs when cells take up DNA from the surrounding medium. It can be induced artificially in the laboratory.

Antibiotic resistance can be transferred by resistance plasmids.

R plasmids have played a significant role in the appearance of strains resistant to antibiodies, such as *S. aureus* and *E. coli* O157:H7.

Variation can also arise by mutation.

Mutations can occur spontaneously in bacteria due to radiation, UV, and various chemicals.

28.5 Prokaryotic Metabolism

Prokaryotes acquire carbon and energy in four basic ways.

Photoautotrophs carry out photosynthesis and obtain carbon from carbon dioxide. Chemolithoautotrophs obtain energy by oxidizing inorganic substances. Photoheterotrophs use light for energy but obtain carbon from organic molecules. Chemoheterotrophs, the largest group, obtain carbon and energy from organic molecules.

Some bacteria can attack other cells directly.

Some bacteria release proteins through their cell walls, and these proteins may transfer other, virulent proteins into eukaryotic cells.

Bacteria are costly plant pathogens.

Gram-negative bacteria known as pseudomonads are responsible for most plant diseases.

28.6 Human Bacterial Disease (see table 28.1)

Bacterial diseases are spread through mucus or saliva droplets, contaminated food and water, and insect vectors.

Tuberculosis has infected humans for all of recorded history.

Tuberculosis continues to be a major public health problem. Treatment requires a long course of antibiotics.

Bacterial biofilms are involved in tooth decay.

Bacteria can cause ulcers.

Most stomach ulcers are caused by infection with *Helicobacter pylori*.

Many sexually transmitted diseases are bacterial.

The potentially dangerous sexually transmitted diseases gonorrhea, syphilis, and chlamydia are caused by bacteria.

28.7 Beneficial Prokaryotes

Prokaryotes are involved in cycling important elements.

Prokaryotes are involved in the recycling of carbon and nitrogen; only bacteria can fix nitrogen.

Prokaryotes may live in symbiotic associations with eukaryotes.

Bacteria are used in genetic engineering.

Genetically engineered prokaryotes can be used to produce human pharmaceutical agents and other useful products.

Bacteria can be used for bioremediation.

Review Questions

UNDERSTAND

1. Which of the following would be an example of a biomarker?
   a. A microfossil found in a meteorite
   b. A hydrocarbon found in an ancient rock layer
   c. An area that is high in carbon-12 concentration in a rock layer
   d. A newly discovered formation of stromatolites
2. A cell that can use energy from the sun, and CO_2 as a carbon source is a
   a. photoautotroph.
   b. chemoautotroph.
   c. photoheterotroph.
   d. chemoheterotroph.
3. Gram-positive (+) and gram-negative (–) bacteria are characterized by differences in
   a. the cell wall: gram+ have peptidoglycan, gram– have pseudo-peptidoglycan.
   b. the plasma membrane: gram+ have ester-linked lipids, gram– have ether-linked lipids.
   c. the cell wall: gram+ have a thick layer of peptidoglycan and gram– have an outer membrane.
   d. chromosomal structure: gram+ have circular chromosomes, gram– have linear chromosomes.
4. Which of the following characteristics is unique to the archaea?
   a. A fluid mosaic model of plasma membrane structure
   b. The use of an RNA polymerase during gene expression
   c. Ether-linked phospholipids
   d. A single origin of DNA replication
5. The horizontal transfer of DNA using a plasmid is an example of
   a. generalized transduction.
   b. binary fission.
   c. transformation.
   d. conjugation.
6. The disease tuberculosis is
   a. caused by a bacterial pathogen.
   b. an emerging disease that is now worldwide.
   c. caused by a viral pathogen.
   d. not treatable with antibiotics.
7. Prokaryotes participate in the global cycling of
   a. proteins and nucleic acids.
   b. carbon and nitrogen.
   c. carbohydrates and lipids.
   d. all of the above.

APPLY

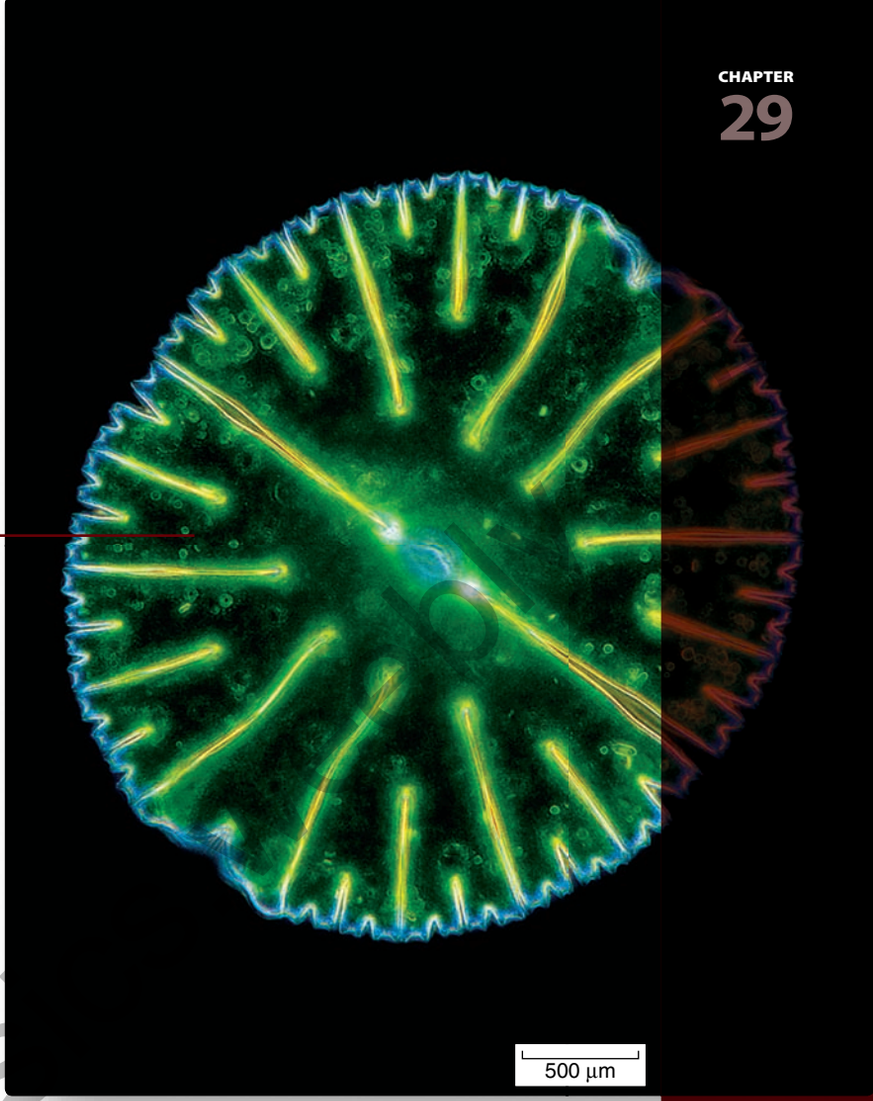
1. Which of the following is typically not associated with a prokaryote?
   a. Horizontal transfer of genetic information
   b. A lack of internal compartmentalization
   c. Multiple, linear chromosomes
   d. A cell size of 1 μm
2. The mechanisms of DNA exchange in prokaryotes share the feature of
   a. vertical transmission of information.
   b. horizontal transfer of information.
   c. requiring cell contact.
   d. the presence of a plasmid in one cell.
3. The cell wall in both gram-positive and gram-negative cells is
   a. composed of phospholipids.
   b. a target for antibiotics that affect peptidoglycan synthesis.
   c. composed of peptidoglycan.
   d. surrounded by a membrane.
4. The three domains of life
   a. represent variations of the same basic cell type.
   b. include two different basic cell types.
   c. consist of three different basic cell types.
   d. describe current cells but say nothing about their history.
5. Ulcers and tooth decay do not appear related, but in fact both
   a. are due to eating particular kinds of foods.
   b. are caused by viral infection.
   c. are caused by environmental factors.
   d. can be due to bacterial infection.
6. Bacteria lack independent internal membrane systems, but are able to perform photosynthesis and respiration, both of which use membranes. They are able to perform these functions because
   a. they actually have internal membranes, but only for these functions.
   b. invaginations of the plasma membrane can provide an internal membrane surface.
   c. they take place outside of the cell between the membrane and the cell wall.
   d. they use protein-based structures to take the place of internal membraes.
7. Plants cannot fix nitrogen, yet some plants do not need nitrogen from the soil. This is because
   a. of a symbiotic association with a bacterium that can fix nitrogen.
   b. these plants are the exceptions that can fix nitrogen.
   c. they have been infected by a parasitic virus that can fix nitrogen.
   d. they are able to obtain nitrogen from the air.

SYNTHESIZE

1. If a new form of carbon fixation was discovered that was not biased toward carbon-12, would this affect our analysis of the earliest evidence for life?
2. Frederick Griffith's experiments (see chapter 14) played an important role in showing that DNA is the genetic material. Griffith showed that dead virulent bacteria mixed with live nonvirulent bacteria could cause pneumonia in mice. Live rough bacteria could also be cultured from the infected mice. The difference between the two strains is a polysaccharide capsule found in the smooth strain. Given what you have learned in this chapter, how would you explain these observations?
3. In the 1960s, it was common practice to prescribe multiple antibiotics to fight bacterial infections. It is also often the case that patients do not always take the entire "course" of their antibiotics. Antibiotic resistance genes are often found on conjugative plasmids. How do these factors affect the evolution of antibiotic resistance and of resistance to multiple antibiotics in particular?
4. Soil-based nitrogen-fixing bacteria appear to be highly vulnerable to exposure to UV radiation. Suppose that the ozone level continues to be depleted, what are the long-term effects on the planet?

Chapter 29

Protists



Chapter Outline

Introduction

For more than half of the long history of life on Earth, all life was microscopic. The biggest organisms that existed for over 2 billion years were single-celled bacteria fewer than 6 µm thick. These prokaryotes lacked internal membranes, except for invaginations of surface membranes in photosynthetic bacteria.

The first evidence of a different kind of organism is found in tiny fossils in rock 1.5 billion years old. These fossil cells are much larger than bacteria (up to 10 times larger) and contain internal membranes and what appear to be small, membrane-bounded structures. The complexity and diversity of form among these single cells is astonishing. The step from relatively simple to quite complex cells marks one of the most important events in the evolution of life, the appearance of a new kind of organism, the eukaryote. Eukaryotes that are clearly not animals, plants, or fungi have been lumped together and called protists.

29.1 Eukaryotic Origins and Endosymbiosis

Learning Outcomes

1. *List the defining features of eukaryotes.*
2. *Define endosymbiosis and explain how it relates to the evolution of mitochondria and chloroplasts.*
3. *Explain why mitosis is not believed to have evolved all at once.*

Eukaryotic cells are distinguished from prokaryotes by the presence of a cytoskeleton and compartmentalization that includes a nuclear envelope and organelles. The exact sequence of events that led to large, complex eukaryotic cells is unknown, but several key events are agreed upon. Loss of a rigid cell wall allowed membranes to fold inward, increasing surface area. Membrane flexibility also made it possible for one cell to engulf another.

Fossil evidence dates the origins of eukaryotes

Indirect chemical traces hint that eukaryotes may go as far back as 2.7 billion years, but no fossils as yet support such an early appearance. In rocks about 1.5 billion years old, we begin to see the first microfossils that are noticeably different in appearance from the earlier, simpler forms, none of which were more than 6 μm in diameter (figure 29.1). These cells are much larger than those of prokaryotes and have internal membranes and thicker walls.

These early fossils mark a major event in the evolution of life: A new kind of organism had appeared. These new cells are called eukaryotes, from the Greek words meaning "true nucleus," because they possess an internal structure called a nucleus. All organisms other than prokaryotes are eukaryotes.

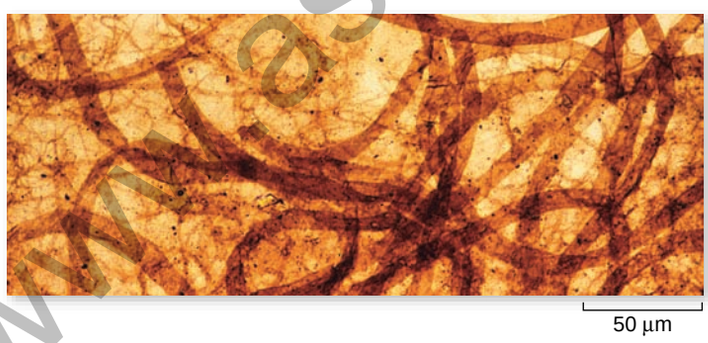


Figure 29.1 Early eukaryotic fossil. Fossil algae that lived in Siberia 1 BYA.

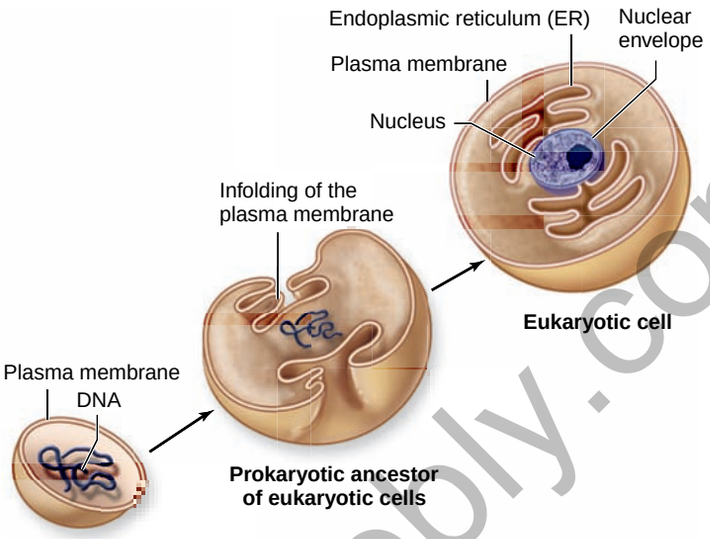


Figure 29.2 Origin of the nucleus and endoplasmic reticulum. Many prokaryotes today have infoldings of the plasma membrane (see also figure 27.6). The eukaryotic internal membrane system, called the endoplasmic reticulum (ER), and the nuclear envelope may have evolved from such infoldings of the plasma membrane, encasing the DNA of prokaryotic cells that gave rise to eukaryotic cells.

In the sections that follow, the origins of eukaryotic internal structure are considered. Keep in mind that, as discussed in chapter 24, horizontal gene transfer occurred frequently while eukaryotic cells were evolving. Eukaryotic cells evolved not only through horizontal gene transfer, but through infolding of membranes and engulfing other cells. Today's eukaryotic cell is the result of cutting and pasting of DNA and organelles from different species.

The nucleus and ER arose from membrane infoldings

Many prokaryotes have infoldings of their outer membranes extending into the cytoplasm that serve as passageways to the surface. The network of internal membranes in eukaryotes is called the endoplasmic reticulum (ER), and the nuclear envelope, an extension of the ER network that isolates and protects the nucleus, is thought to have evolved from such infoldings (figure 29.2).

Mitochondria evolved from engulfed aerobic bacteria

Bacteria that live within other cells and perform specific functions for their host cells are called *endosymbiotic bacteria*. Their widespread presence in nature led biologist Lynn Margulis in the early 1970s to champion the theory of endosymbiosis, which was first proposed by Konstantin

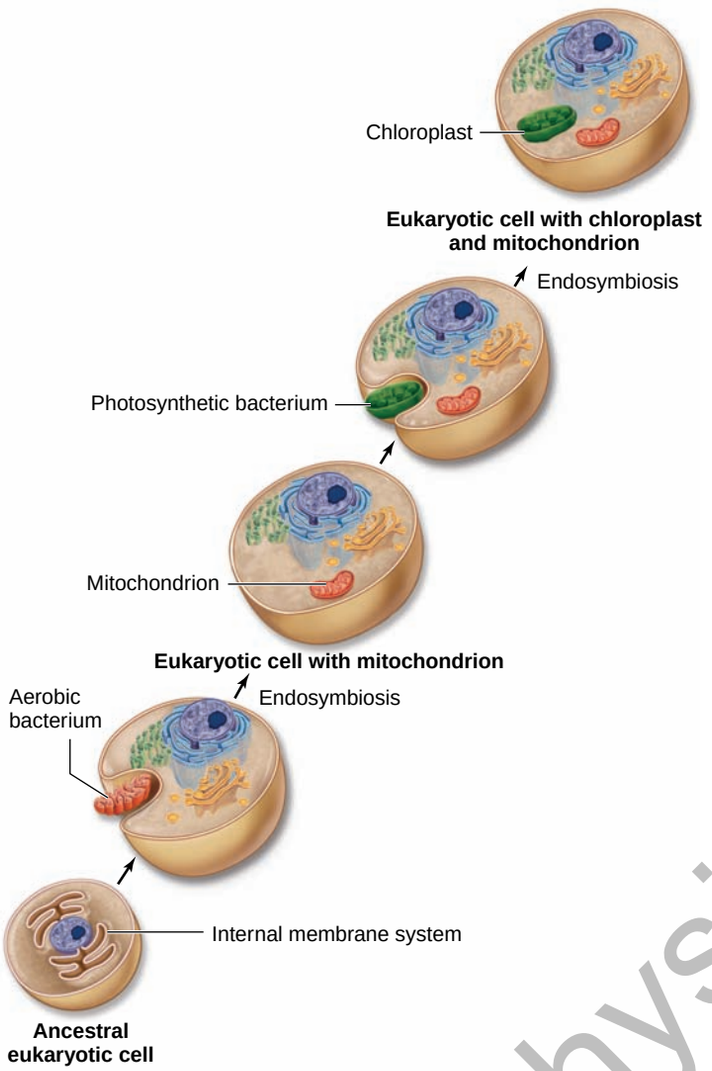


Figure 29.3 The theory of endosymbiosis. Scientists propose that ancestral eukaryotic cells, which already had an internal system of membranes, engulfed aerobic bacteria, which then became mitochondria in the eukaryotic cell. Chloroplasts also originated this way, with eukaryotic cells engulfing photosynthetic bacteria.

Mereschkowsky in 1905. Endosymbiosis means living together in close association.

Endosymbiosis, a concept that is now widely accepted, suggests that a critical stage in the evolution of eukaryotic cells involved endosymbiotic relationships with prokaryotic organisms. According to this theory, energy-producing bacteria may have come to reside within larger bacteria, eventually evolving into what we now know as mitochondria (figure 29.3). Possibly the original host cell was anaerobic with hydrogen-dependent metabolic pathways. The symbiont had a form of respiration that produced H_2. The host depended on the symbiont for H_2 under anaerobic conditions and was able later to adapt to an O_2-rich atmosphere using the symbiont's respiratory pathways.

Chloroplasts evolved from engulfed photosynthetic bacteria

Photosynthetic bacteria may have come to live within other larger bacteria, leading to the evolution of chloroplasts, the photosynthetic organelles of plants and algae (see figure 29.3). The history of chloroplast evolution is an example of the care that must be taken in phylogenetic studies. All chloroplasts are likely derived from a single line of cyanobacteria, but the organisms that host these chloroplasts are not monophyletic. This apparent paradox is resolved by considering the possibility of secondary, and even tertiary endosymbiosis. Figure 26.8 explains how red and green algae both obtained their chloroplasts by engulfing photosynthetic cyanobacteria. The brown algae most likely obtained their chloroplasts by engulfing one or more red algae, a process called **secondary endosymbiosis** (figure 29.4). (As mentioned, green algae are considered in the following chapter even though they are protists.)

A phylogenetic tree based only on chloroplast gene sequences from red and green algae reveals an incredibly close evolutionary relationship. This tree is misleading, however, because it is not possible to tell just from these data how

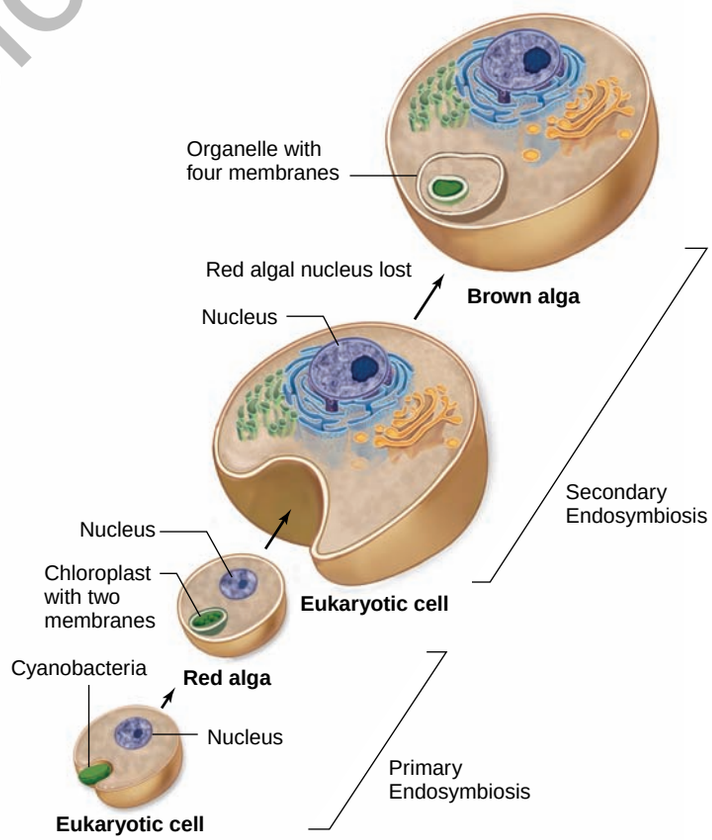


Figure 29.4 Endosymbiotic origins of chloroplasts in red and brown algae.

much the two algal lines had diverged at the time they engulfed the same line of cyanobacteria. Morphological and chemical traits are more helpful than chloroplast gene sequences in sorting out red and green algal relations. More data and analyses are still needed to confirm the position of red algae in figure 29.5.

Endosymbiosis is supported by a range of evidence

The fact that we now witness so many symbiotic relationships lends general support to the endosymbiotic theory. Even stronger support comes from the observation that present-day organelles such as mitochondria and chloroplasts contain their own DNA, which is remarkably similar to the DNA of bacteria in size and character. During the billion and a half years in which mitochondria have existed as endosymbionts within eukaryotic cells, most of their genes have been transferred to the chromosomes of the host cells—but not all. Each mitochondrion still has its own genome, a circular, closed molecule of DNA similar to that found in bacteria, on which are located genes encoding the essential proteins of oxidative metabolism. These genes are transcribed within the mitochondrion, using mitochondrial ribosomes that are smaller than those of eukaryotic cells, very much like bacterial ribosomes in size and structure. Many antibiotics that inhibit protein synthesis in bacteria also inhibit protein synthesis in mitochondria and chloroplasts, but not in the cytoplasm. Chloroplasts and mitochondria replicate via binary fission, not mitosis, further supporting bacterial origins.

Mitosis evolved in eukaryotes

The mechanisms of mitosis and cytokinesis, now so common among eukaryotes, did not evolve all at once. Traces of very different, and possibly intermediate, mechanisms survive today in some of the eukaryotes. In fungi and in some groups of protists, for example, the nuclear membrane does not dissolve, as it does in plants, animals, and most other protists, and mitosis is confined to the nucleus. When mitosis is complete in these organisms, the nucleus divides into two daughter nuclei, and only then does the rest of the cell divide. We do not know whether mitosis without nuclear membrane dissolution represents an intermediate step on the evolutionary journey, or simply a different way of solving the same problem. We cannot see the interiors of dividing cells well enough in fossils to be able to trace the history of mitosis.

Learning Outcomes Review 29.1

Eukaryotes are organisms that contain a nucleus and other membrane-bounded organelles. Endoplasmic reticulum and the nuclear membrane are believed to have evolved from infoldings of the outer membranes. According to the endosymbiont theory, mitochondria and chloroplasts evolved from engulfed bacteria that remained intact. Mitochondria, chloroplasts, and centrioles have their own DNA, which is similar to that of prokaryotes. Mitosis did not evolve all at once; different mechanisms persist in different organisms.

- ***What evidence supports the endosymbiont theory?***

Inquiry question

How could you distinguish between primary and secondary endosymbiosis by looking at micrographs of cells with chloroplasts?

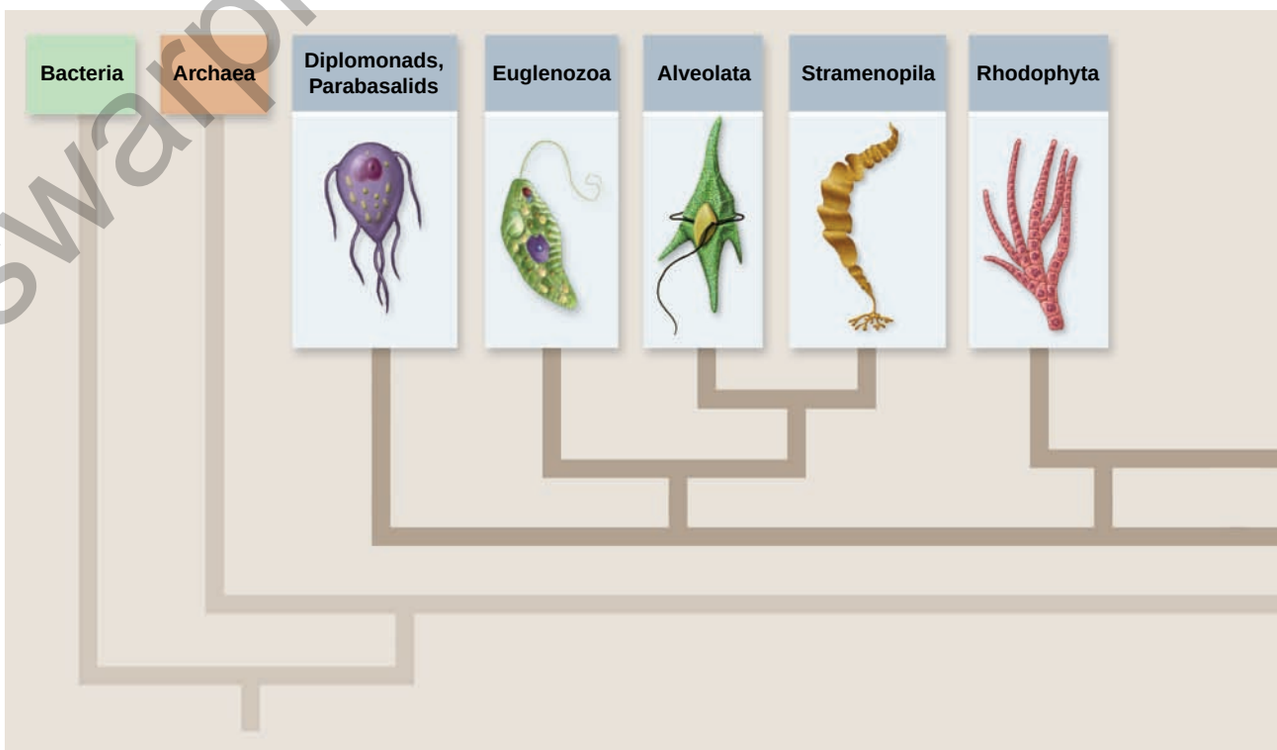


Figure 29.5 The challenge of protistan classification. Our understanding of the evolutionary relationships among protists is currently in flux. The most recent data support seven major, monophyletic groups within the protists. Consider this a working model, not fact. The green algae (Chlorophyta) are not truly monophyletic in that another branch, Streptophyta, gave rise to the land plants. Protist lineages are shaded in blue.

29.2 Defining Protists

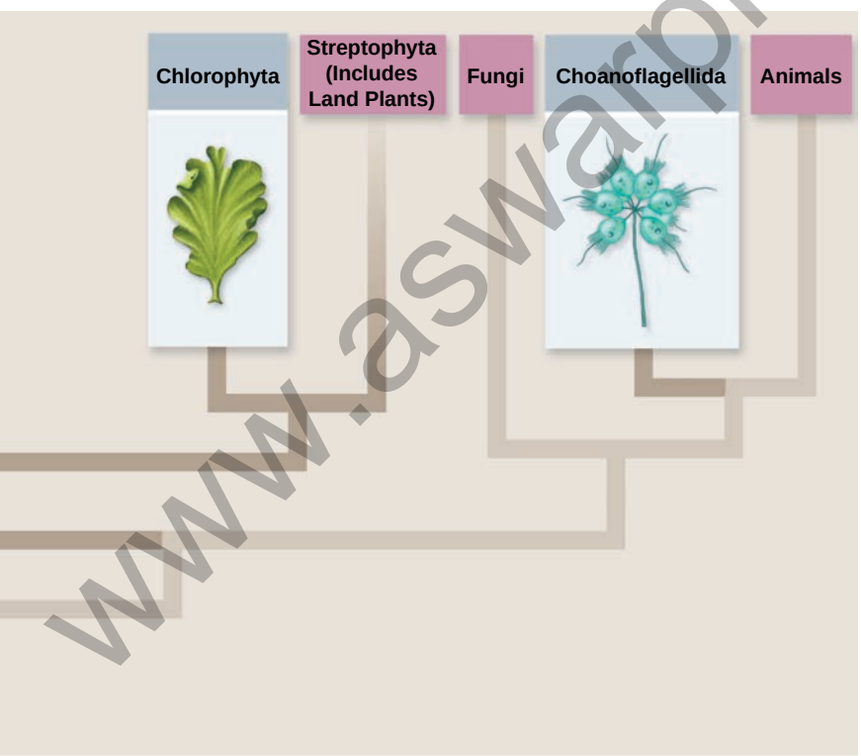
Learning Outcomes

1. *Describe the feature that distinguishes protists from other eukaryotes.*
2. *Define monophyletic.*
3. *Describe the various kinds of protist cell surfaces.*
4. *List the two main means of locomotion used by protists.*
5. *Distinguish between phototrophs, phagotrophs, and osmotrophs.*

Protists are the most diverse of the four kingdoms in the domain Eukarya. Protists are united on the basis of a single negative characteristic: They are eukaryotes that are not fungi, plants, or animals. In all other respects, they vary considerably, with no uniting features. Many are unicellular, but numerous colonial and multicellular groups also exist. Most are microscopic, but some are as large as trees. They represent all symmetries and exhibit all types of nutrition. The origin of eukaryotes, which began with ancestral protists, is among the most significant events in the evolution of life.

Protista is not monophyletic

One of the most important statements we can make about the kingdom Protista is that it is paraphyletic and not a kingdom at all; as a matter of convenience, single-celled eukaryotic organisms have typically been grouped together and called protists. This lumps 200,000 different and only distantly related forms together. The "single-kingdom" classification of the Protista is artificial and not representative of any evolutionary relationships. You may be wondering why biologists continue to refer to the protista as a kingdom and why we have a chapter devoted to the protists. While we wait for the evolutionary relationships among protists to be sorted out, lumping the protists together allows us to explore the biology of a number of fascinating eukaryotes that might otherwise disappear from the pages of your textbook.



Monophyletic clades have been identified among the protists

Applications of a variety of molecular methods are providing insights into the relationships among protists. Many questions about how to classify the protists are being addressed with these techniques. Are protists best considered as several different kingdoms, each of equal rank with animals, plants, and fungi? Are some of the protists actually members of other kingdoms? While these questions continue to be debated, new information is becoming available concerning which organisms among the protists are most likely to be monophyletic.

In this chapter, we group the 15 major protist phyla into seven major monophyletic groups, based on our current understanding of phylogeny (figure 29.5). Although these lineages may change, this approach allows us to examine groups with many shared traits. Keep in mind that about 60 of the protist lineages cannot yet be placed on the tree of life with any confidence! Protists exemplify the challenges and excitement of the revolutionary changes in taxonomy and phylogeny we explored in chapter 26. Understanding the evolution of protists is key to understanding the origins of plants, fungi, and animals.

Because green algae and land plants form a monophyletic clade, the green algae are explored in more detail in the following chapter on plant diversity. The characteristics of green algae and land plants are best understood when considered in concert because of their shared evolutionary history. The remaining six monophyletic clades that are loosely called protists are examined in this chapter.

Protist cell surfaces vary widely

Protists possess a varied array of cell surfaces. Some protists, such as amoebas, are surrounded only by their plasma membrane. All other protists have a plasma membrane with an extracellular matrix (ECM) deposited on the outside of the membrane. Some ECMs form strong cell walls; for instance, diatoms and foraminifera secrete glassy shells of silica.

Many protists with delicate surfaces are capable of surviving unfavorable environmental conditions. How do they manage to survive so well? They form cysts, which are dormant forms with resistant outer coverings in which cell metabolism is more or less completely shut down. Not all cysts are so sturdy, however. Vertebrate parasitic amoebas, for example, form cysts that are quite resistant to gastric acidity, but will not tolerate desiccation or high temperature.

Protists have several means of locomotion

Movement in protists is also accomplished by diverse mechanisms. Protists move chiefly by either flagellar rotation or pseudopodial movement. Many protists wave one or more flagella to propel themselves through the water, and others use banks of short, flagella-like structures called cilia to create water currents for their feeding or propulsion. Pseudopods (Greek, meaning "false feet") are the chief means of locomotion among amoebas, whose pseudopods are large, blunt extensions of the cell body called lobopodia. Other related protists extend thin, branching protrusions called filopodia. Still other protists extend long, thin pseudopods called axopodia supported by axial rods of microtubules. Axopodia can be extended or retracted. Because the tips can adhere to adjacent surfaces, the cell can move by a rolling motion, shortening the axopodia in front and extending those in the rear.

Protists have a range of nutritional strategies

Protists can be heterotrophic or autotrophic. Some autotrophic protists are photosynthetic and are called **phototrophs.** Others are heterotrophs that obtain energy from organic molecules synthesized by other organisms.

Among the heterotrophic protists are some called *phagotrophs*, which ingest visible particles of food by pulling them into intracellular vesicles called food vacuoles or phagosomes. Lysosomes fuse with the food vacuoles, introducing enzymes that digest the food particles within. Digested molecules are absorbed across the vacuolar membrane.

Protists that ingest food in soluble form are called *osmotrophs*. Another example of the protists' tremendous nutritional flexibility is seen in *mixotrophs*, protists that are both phototrophic and heterotrophic.

Protists reproduce asexually and sexually

Protists typically reproduce asexually, although some have an obligate sexual reproductive phase and others undergo sexual reproduction at times of stress, including food shortages.

Asexual reproduction

Asexual reproduction involves mitosis, but the process often differs from the mitosis in multicellular animals. For example, the nuclear membrane often persists throughout mitosis, with the microtubular spindle forming within it.

In some species, a cell simply splits into nearly equal halves after mitosis. Sometimes the daughter cell is considerably smaller than its parent and then grows to adult size—a type of cell division called **budding.** In *schizogony*, common among some protists, cell division is preceded by several nuclear divisions. This allows cytokinesis to produce several individuals almost simultaneously.

Sexual reproduction

Most eukaryotic cells also possess the ability to reproduce sexually, something prokaryotes cannot do at all. Meiosis (see chapter 11) is a major evolutionary innovation that arose in ancestral protists and allows for the production of haploid cells from diploid cells. Sexual reproduction is the process of producing offspring by fertilization, the union of two haploid cells. The great advantage of sexual reproduction is that it allows for frequent genetic recombination, which generates the variation that is the starting point of evolution. Not all eukaryotes reproduce sexually, but most have the capacity to do so. The evolution of meiosis and sexual reproduction contributed to the tremendous explosion of diversity among the eukaryotes.

Protists are the bridge to multicellularity

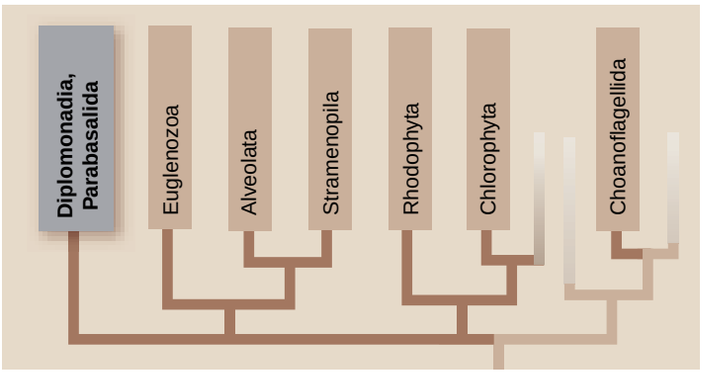
Diversity was also promoted by the development of *multicellularity*. Some single eukaryotic cells began living in association with others, in colonies. Eventually, individual members of the colony began to assume different duties, and the colony began to take on the characteristics of a single individual. Multicellularity has arisen many times among the eukaryotes. Practically every organism big enough to be seen with the unaided eye, including all animals and plants, is multicellular. The great advantage of multicellularity is that it fosters specialization; some cells devote all of their energies to one task, other cells to another. Few innovations have had as great an influence on the history of life as the specialization made possible by multicellularity.

Learning Outcomes Review 29.2

A monophyletic group is one in which all members have a single common ancestor. Protista is paraphyletic, however, so it is not really a kingdom. The major protist phyla have been grouped into seven major monophyletic groups. All protists have plasma membranes, but other cell-surface components, such as deposited extracellular material (ECM), are highly variable. Protists mainly use flagella or pseudopodial movement to propel themselves. Phototrophic protists carry out photosynthesis; phagotrophs ingest food particles; and osmotrophs ingest dissolved nutrients. Sexual reproduction is common, but asexual reproduction also occurs in many groups. Multicellular organisms likely arose from colonial protists.

- ***Why is Kingdom Protista considered to be a paraphyletic group?***
- ***What would be the advantage of movement by pseudopodia?***

29.3 *Diplomonads and Parabasalids: Flagellated Protists Lacking Mitochondria*



Learning Outcomes

1. *List the main features of diplomonads and parabasalids.*
2. *Give examples of diplomonads and parabasalids.*

What was the first eukaryote like? We cannot be sure, but the *diplomonads* and the *parabasalids* likely had early eukaryotic ancestors. Although these groups have similar features, their differences put them into separate clades.

Diplomonads have two nuclei

Diplomonads are unicellular and move with flagella. This group lacks mitochondria, but has two nuclei. *Giardia intestinalis* is an example of a diplomonad (figure 29.6). *Giardia* is a parasite that can pass from human to human via contaminated water and cause diarrhea. Mitochondrial genes are found in their nuclei, leading to the conclusion that *Giardia* evolved from aerobes. Electron micrographs of *Giardia* cells stained with mitochondrial-specific antibodies reveal degenerate mitochondria. Thus, *Giardia* is unlikely to represent an early protist.

Parabasalids have undulating membranes

Parabasalids contain an intriguing array of species. Some live in the gut of termites and digest cellulose, the main component of the termite's wood-based diet. The symbiotic relationship is one layer more complex because these parabasalids have a symbiotic relationship with bacteria that also aid in the digestion of cellulose. The persistent activity of these three symbiotic organisms from three different kingdoms can lead to the collapse of a home built of wood or recycle tons of fallen trees in a forest. Another parabasalid, *Trichomonas vaginalis*, causes a sexually transmitted disease in humans.

Parabasalids have undulating membranes that assist in locomotion (figure 29.7). Like diplomonads, parabasalids also use flagella to move and lack mitochondria. The lack of mitochondria in both groups is now believed to be a derived rather than an ancestral trait.

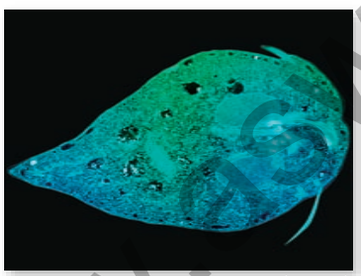


Figure 29.6 ***Giardia intestinalis.*** This parasitic diplomonad lacks a mitochondrion.

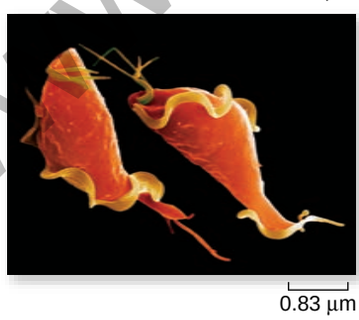


Figure 29.7 **Undulating membrane characteristic of parabasalids.** Vaginitis can be caused by this parasite species, *Trichomonas vaginalis.*

Learning Outcomes Review 29.3

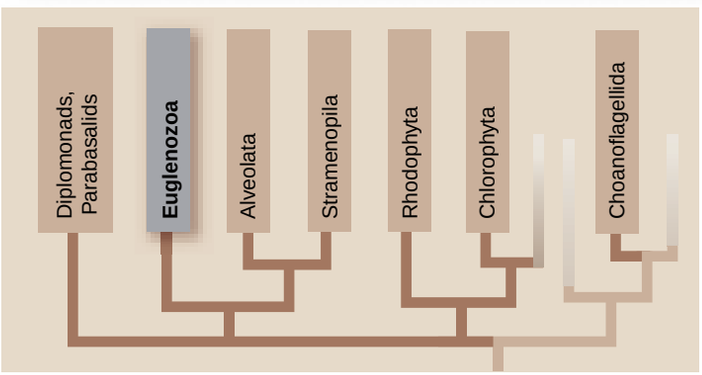
The ancestors of diplomonads and parabasalids are likely to be among the earliest eukaryotes. Diplomonads lack mitochondria but may contain mitochondrial genes. They are unicellular, have two nuclei, and move with flagella; an example is *Giardia.* Parabasalids also lack mitochondria and use flagella and undulating membranes for locomotion; an example is *Trichomonas.*

- *In what type of habitat would it be useful to use undulating membranes for locomotion?*

29.4 Euglenozoa: A Diverse Group in Which Some Members Have Chloroplasts

Learning Outcomes

1. *Explain why Euglenozoa cannot be classified as either plants or animals.*
2. *Describe the distinguishing feature of kinetoplastids.*



Among their distinguishing features, a number of the *Euglenozoa* have acquired chloroplasts through endosymbiosis. None of the algae are closely related to Euglenozoa, a reminder that endosymbiosis is widespread.

Euglenoids are free-living eukaryotes with anterior flagella

Euglenoids diverged early and were among the earliest free-living eukaryotes to possess mitochondria. Euglenoids clearly illustrate the impossibility of distinguishing "plants" from "animals" among the protists. About one-third of the approximately 40 genera of euglenoids have chloroplasts and are fully autotrophic; the others lack chloroplasts, ingest their food, and are heterotrophic.

Some euglenoids with chloroplasts may become heterotrophic in the dark; the chloroplasts become small and nonfunctional. If they are put back in the light, they may become green within a few hours. Photosynthetic euglenoids may sometimes feed on dissolved or particulate food.

Individual euglenoids range from 10 to 500 μm long and vary greatly in form. Interlocking proteinaceous strips arranged in a helical pattern form a flexible structure called the *pellicle*, which lies within the plasma membrane of the euglenoids. Because its pellicle is flexible, a euglenoid is able to change its shape.

Reproduction in this phylum occurs by mitotic cell division. The nuclear envelope remains intact throughout the process of mitosis. No sexual reproduction is known to occur in this group.

Euglena, the best known euglenoid

In *Euglena* (figure 29.8), the genus for which the phylum is named, two flagella are attached at the base of a flask-shaped opening called the *reservoir*, which is located at the anterior end of the cell. One of the flagella is long and has a row of very fine, short, hairlike projections along one side. A second, shorter flagellum is located within the reservoir but does not emerge from it. Contractile vacuoles collect excess water from all parts of the organism and empty it into the reservoir, which apparently helps regulate the osmotic pressure within the organism. The stigma, which also occurs in the green algae (phylum Chlorophyta), helps these photosynthetic organisms move toward light.

Cells of *Euglena* contain numerous small chloroplasts. These chloroplasts, like those of the green algae and plants, contain chlorophylls *a* and *b*, together with carotenoids. Although the chloroplasts of euglenoids differ somewhat in structure from those of green algae, they probably had a common origin. *Euglena's* photosynthetic pigments are light-sensitive (figure 29.9). It seems likely that euglenoid chloroplasts ultimately evolved from a symbiotic relationship through ingestion of green algae. Recent phylogenetic evidence indicates that *Euglena* had multiple origins within the Euglenoids, and the concept of a single *Euglena* genus is now being debated.

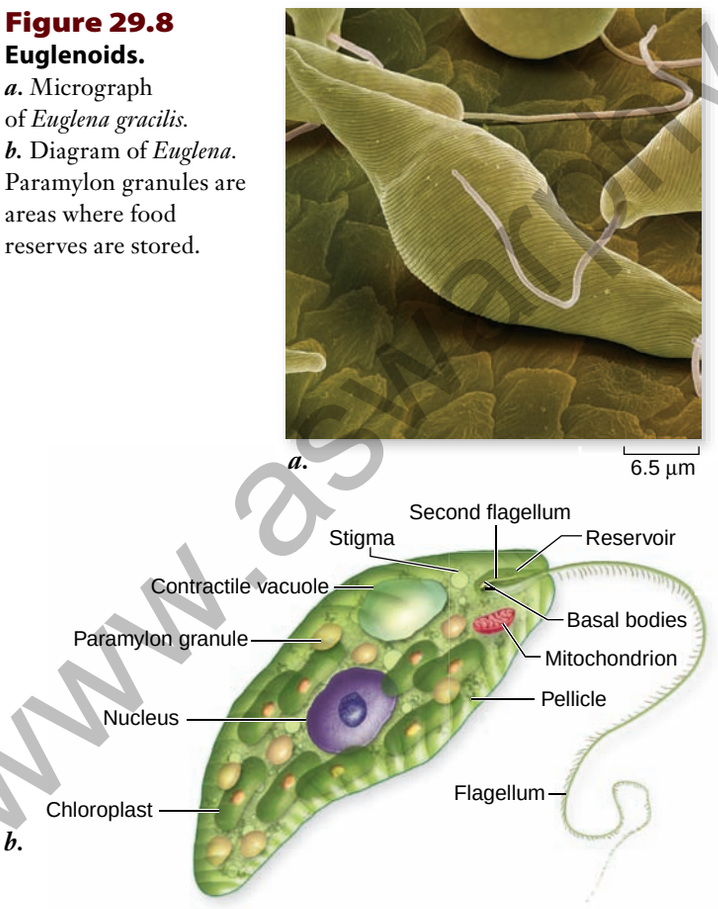


Figure 29.8
Euglenoids.
a. Micrograph of *Euglena gracilis.* ***b.*** Diagram of *Euglena.* Paramylon granules are areas where food reserves are stored.

Kinetoplastids are parasitic

A second major group within the Euglenozoa is the *kinetoplastids.* The name kinetoplastid refers to a unique, single mitochondrion in each cell. The mitochondria have two types of DNA: minicircles and maxicircles. (Remember that prokaryotes have circular DNA, and mitochondria had prokaryotic origins.) This mitochondrial DNA is responsible for very rapid glycolysis and also for an unusual kind of editing of the RNA by guide RNAs encoded in the minicircles.

Trypanosomes: Disease-causing kinetoplastids

Parasitism has evolved multiple times within the kinetoplastids. Trypanosomes are a group of kinetoplastids that cause many serious human diseases, the most familiar being trypanosomiasis, also known as African sleeping sickness, which causes extreme lethargy and fatigue (figure 29.10).

Leishmaniasis, which is transmitted by sand flies, is a trypanosomic disease that causes skin sores and in some cases can affect internal organs, leading to death. About 1.5 million new cases are reported each year. The rise in leishmaniasis in South America correlates with the move of infected individuals from rural to urban environments, where there is a greater chance of spreading the parasite.

Chagas disease is caused by *Trypanosoma cruzi.* At least 90 million people, from the southern United States to Argentina, are at risk of contracting *T. cruzi* from small wild mammals that carry the parasite and can spread it to other mammals and humans through skin contact with urine and feces. Blood transfusions have also increased the spread of the infection. Chagas disease can lead to severe cardiac and digestive problems in humans and domestic animals, but it appears to be tolerated in the wild mammals.

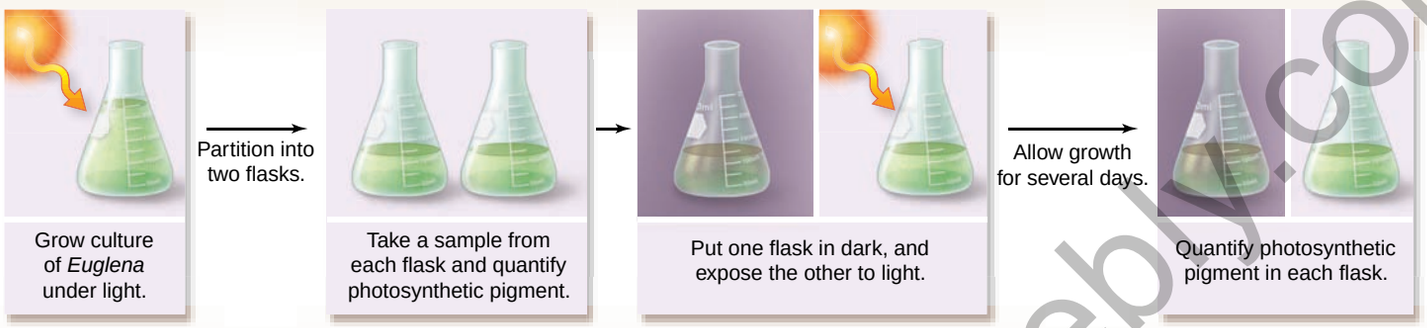
Control is especially difficult because of the unique attributes of these organisms. For example, tsetse fly-transmitted trypanosomes have evolved an elaborate genetic mechanism for repeatedly changing the antigenic nature of their protective glycoprotein coat, thus dodging the antibodies their hosts produce against them (see chapter 52). Only a single one out of some 1000 variable-surface glycoprotein (VSG) genes is expressed at a time. A VSG gene is usually duplicated and moved to 1 of about 20 expression sites near the telomere where it is transcribed. Only one expression site is transcribed at a time.

SCIENTIFIC THINKING

Hypothesis: Euglena *cells do not retain photosynthetic pigments in a dark environment.*

Prediction: *Photosynthetic pigments will be degraded when light-grown* Euglena *cells are transferred to the dark and new pigment will not be produced.*

Test: *Grow* Euglena *under normal light conditions. Transfer the culture to two flasks. Take a sample from each flask and measure the amount of photosynthetic pigments in each. Maintain one flask in the light and transfer the other to the dark. After several days, extract the photosynthetic pigments from each flask, and compare amounts with each other and with initial levels.*



Result: *Photosynthetic pigment levels are lower in the dark-grown flask than in the light-grown one. Pigment levels in the dark-grown flask are lower than at the beginning of the experiment. Pigment levels in the light-grown flask are unchanged.*

Conclusion: *The hypothesis is supported. Maintenance of* Euglena *in the dark resulted in a loss of photosynthetic pigment. Pigments were degraded in the dark-grown flask.*

Further Experiments: *Transfer dark-grown flasks back to the light and measure changes in pigment levels over time. Are original pigment levels restored after growth in light?*

Figure 29.9 **Effect of light on *Euglena* photosynthetic pigments.**

In the guts of the flies that spread them, trypanosomes are noninfective. When they are ready to transfer to the skin or bloodstream of their host, trypanosomes migrate to the salivary glands and acquire the thick coat of glycoprotein antigens that protect them from the host's antibodies. Later, when they are taken up by a tsetse fly, the trypanosomes again shed their coats.

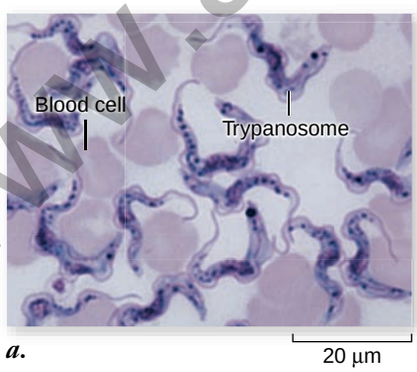
The production of vaccines against such a system is complex, but tests are under way. Releasing sterilized flies to impede the reproduction of populations is another technique being tried to control the fly population. Traps made of dark cloth and scented like cows, but poisoned with insecticides, have likewise proved effective.

The recent sequencing of the genomes of the three kinetoplastids described earlier revealed a core of common genes in all three, as described in chapter 24. The devastating toll of all three on human life could be alleviated by the development of a single drug targeted at one or more of the core proteins shared by the three parasites.

Learning Outcomes Review 29.4

The Euglenozoa were among the earliest protists to contain mitochondria. This group contains phototrophs and heterotrophs. Some members have chloroplasts that remain nonfunctional unless light is present, and some phototrophs may feed if food particles are present. The kinetoplastids contain a single mitochondrion with two types of DNA and the ability to edit RNA with RNA guides. Trypanosomes are disease-causing kinetoplastids.

- ***How does a contractile vacuole regulate osmotic pressure in a Euglena cell?***



a.

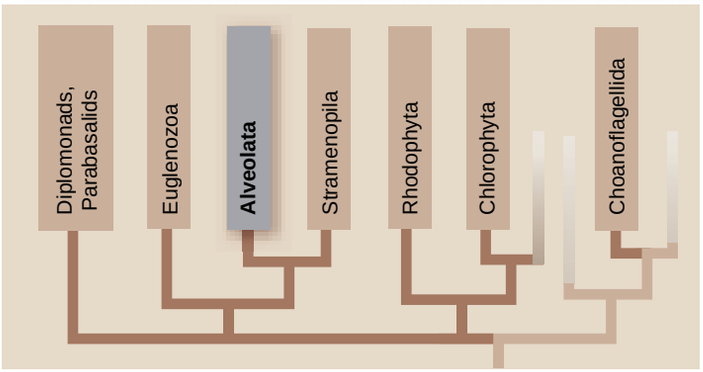
b.

Figure 29.10 **A kinetoplastid.**
a. *Trypanosoma* among red blood cells. The nuclei (dark-staining bodies), anterior flagella, and undulating, changeable shape of the trypanosomes are visible in this photomicrograph. ***b.*** The tsetse fly, shown here sucking blood from a human arm, can carry trypanosomes.

29.5 Alveolata: Protists with Submembrane Vesicles

Learning Outcomes

1. *Identify the distinguishing feature of the members of Alveolata.*
2. *Describe the swimming motion of a dinoflagellate.*
3. *Explain the function of the apical complex in Apicomplexans.*



Members of the **Alveolata** include the *dinoflagellates*, *apicomplexans*, and *ciliates*, all of which have a common lineage but diverse modes of locomotion. One common trait is the presence of flattened vesicles called alveoli (hence the name alveolata) stacked in a continuous layer below their plasma membranes (figure 29.11). The alveoli may function in membrane transport, similar to Golgi bodies.

Dinoflagellates are photosynthesizers with distinctive features

Most dinoflagellates are photosynthetic unicells with two flagella. Dinoflagellates live in both marine and freshwater environments. Some dinoflagellates are luminous and contribute to the twinkling or flashing effects seen in the sea at night, especially in the tropics.

The flagella, protective coats, and biochemistry of dinoflagellates are distinctive, and the dinoflagellates do not appear to be directly related to any other phylum. Plates made of a cellulose-like material, often encrusted with silica, encase the dinoflagellate cells (figure 29.12). Grooves at the junctures of these plates usually house the flagella, one encircling the cell like a belt, and the other perpendicular to it. By beating in their grooves, these flagella cause the dinoflagellate to spin as it moves.

Most dinoflagellates have chlorophylls *a* and *c*, in addition to carotenoids, so that in the biochemistry of their chloroplasts, they resemble the diatoms and the brown algae. Possibly this lineage acquired such chloroplasts by forming endosymbiotic relationships with members of those groups.

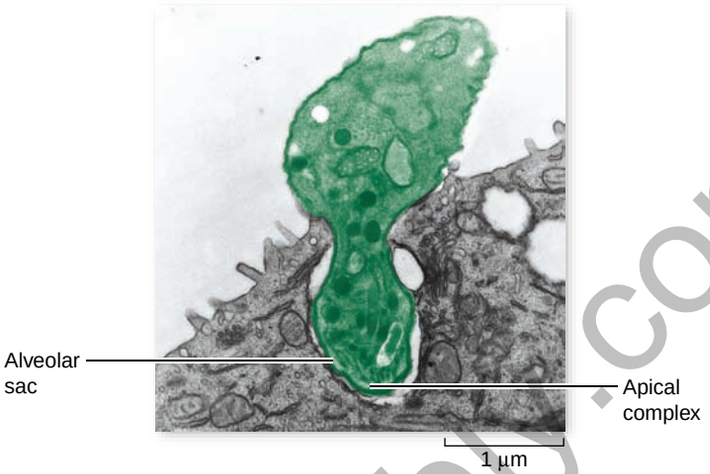


Figure 29.11 Alveoli are a continuum of vesicles just below the plasma membrane of dinoflagellates, apicomplexans, and ciliates. The apical complex of apicomplexans forces the parasite into host cells.

Red tide: Overgrowth of dinoflagellates

The poisonous and destructive "red tides" that occur frequently in coastal areas are often associated with great population explosions, or "blooms," of dinoflagellates, whose pigments color the water (figure 29.13). Red tides have a profound, detrimental effect on the fishing industry worldwide. Some 20 species of dinoflagellates produce powerful toxins that inhibit the diaphragm and cause respiratory failure in many vertebrates. When the toxic dinoflagellates are abundant, many fishes, birds, and marine mammals may die.

Although sexual reproduction does occur under starvation conditions, dinoflagellates reproduce primarily by asexual cell division. Asexual cell division relies on a unique form of mitosis in which the permanently condensed chromosomes divide within a permanent nuclear envelope. After the numerous chromosomes duplicate, the nucleus divides into two daughter nuclei.

Also, the dinoflagellate chromosome is unique among eukaryotes in that the DNA is not generally complexed with

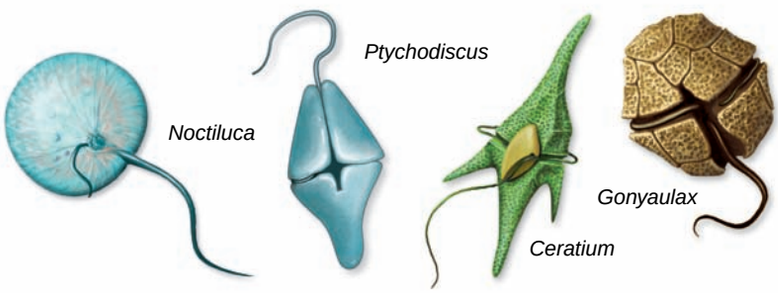


Figure 29.12 Some dinoflagellates. *Noctiluca*, which lacks the heavy cellulose armor characteristic of most dinoflagellates, is one of the bioluminescent organisms that cause the waves to sparkle in warm seas. In the other three genera, the shorter, encircling flagellum is seen in its groove, with the longer one projecting away from the body of the dinoflagellate. (Not drawn to scale.)

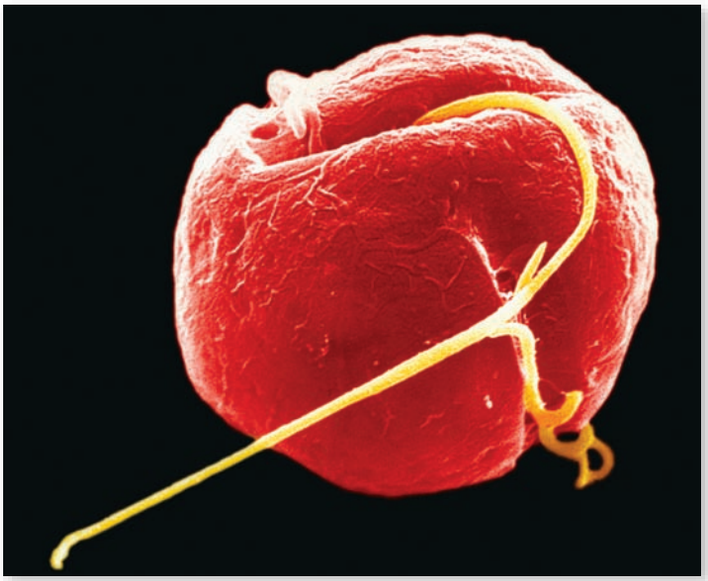


Figure 29.13 Red tide. Although small in size, huge populations of dinoflagellates, including this *Gymnopodium* species, can color the sea red and release toxins into the water.

histone proteins. In all other eukaryotes, the chromosomal DNA is complexed with histones to form nucleosomes, structures that represent the first order of DNA packaging in the nucleus (chapter 10). How dinoflagellates maintain distinct chromosomes with a small amount of histones remains a mystery.

Apicomplexans include the malaria parasite

Apicomplexans are spore-forming parasites of animals. They are called apicomplexans because of a unique arrangement of fibrils, microtubules, vacuoles, and other cell organelles at one end of the cell, termed an *apical complex* (see figure 29.11). The apical complex is a cytoskeletal and secretory complex that enables the apicomplexan to invade its host. The best known apicomplexan is the malarial parasite *Plasmodium*. (The use of the genome sequence of the parasite and the mosquito that carries it is discussed in chapter 24.)

Plasmodium and malaria

Plasmodium glides inside the red blood cells of its host with amoeboid-like contractility. Like other apicomplexans, *Plasmodium* has a complex life cycle involving sexual and asexual phases and alternation between different hosts, in this case mosquitoes *(Anopheles gambiae)* and humans (figure 29.14). Even though *Plasmodium* has mitochondria, it grows best in a low-O_2, high-CO_2 environment.

Efforts to eradicate malaria have focused on (1) eliminating the mosquito vectors; (2) developing drugs to poison the parasites that have entered the human body; and (3) developing vaccines. From the 1940s to the 1960s, wide-scale applications of dichlorodiphenyltrichloroethane (DDT) killed mosquitoes in the United States, Italy, Greece, and certain areas of Latin America. For a time, the worldwide elimination of malaria appeared possible. But this hope was soon crushed by the development of DDT-resistant mosquitoes in many regions. Furthermore, the use of DDT has had serious

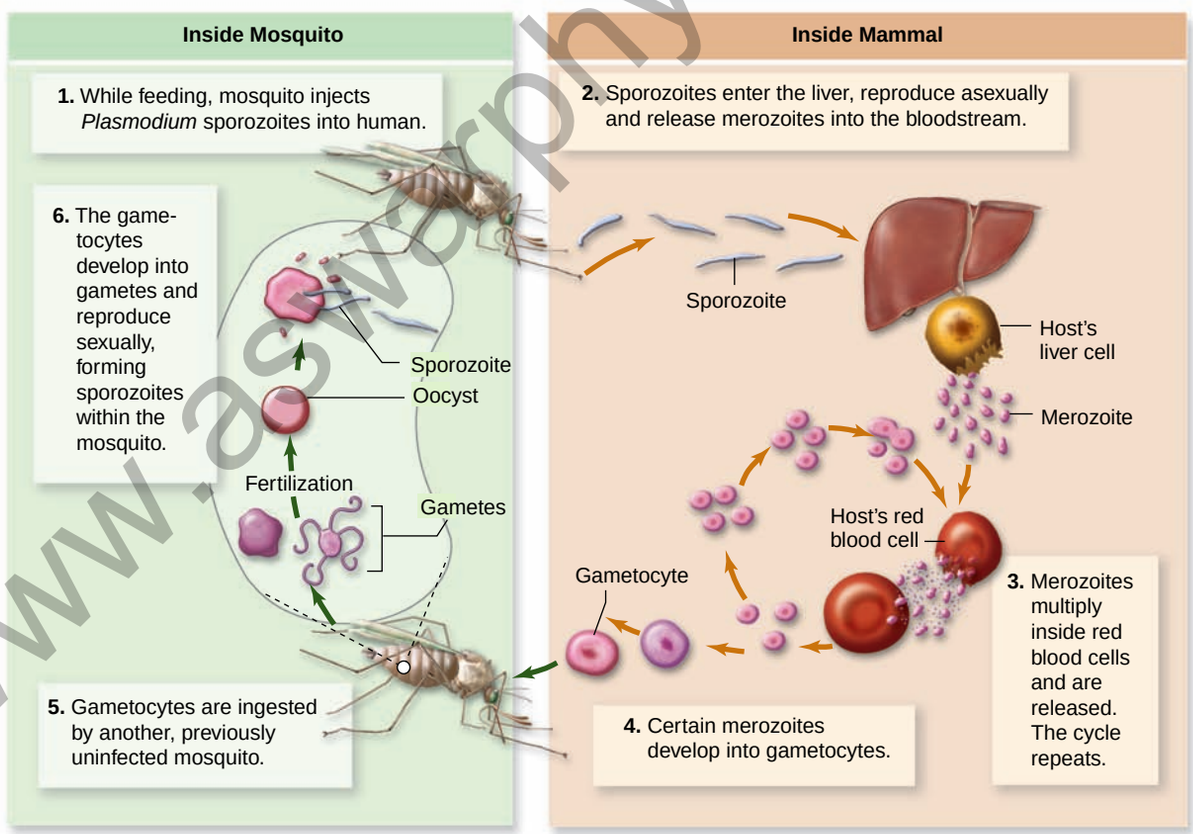


Figure 29.14 The life cycle of *Plasmodium*. *Plasmodium*, the apicomplexan that causes malaria, has a complex life cycle that alternates between mosquitoes and mammals.

Figure 29.15
Gregarine entering a cell.

environmental consequences. In addition to the problems with resistant strains of mosquitoes, strains of *Plasmodium* have appeared that are resistant to the drugs historically used to kill them, including quinine.

An experimental vaccine containing a surface protein of one malaria-causing parasite, *P. falciparum*, seems to induce the immune system to defend against future infections. In tests, six out of seven vaccinated people did not get malaria after being bitten by mosquitoes that carried *P. falciparum*. Many are hopeful that this new vaccine may be able to fight malaria. (Chapter 24 contains a discussion of the genome sequences of both *Plasmodium* and its mosquito vector.)

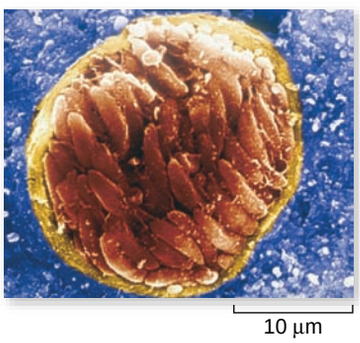
Gregarines

Gregarines are another group of apicomplexans that use their distinctive apical complex to attach themselves in the intestinal epithelium of arthropods, annelids, and mollusks. Most of the gregarine body, aside from the apical complex, is in the intestinal cavity, and nutrients appear to be obtained through the apicomplex attachment to the cell (figure 29.15).

Toxoplasma

Using its apical complex, *Toxoplasma gondii* invades the epithelial cells of the human gut. Most individuals infected with the parasite mount an immune response, preventing any permanent damage. In the absence of a fully functional immune system, however, *Toxoplasma* can damage brain (figure 29.16), heart, and skeletal tissues, in addition to gut and lymph tissue, during extended infections. Individuals with AIDS are particularly susceptible to *Toxoplasma* infection. If a pregnant women touches a cat litter box, *Toxoplasma* parasites from the cat can, if ingested, cross the placental barrier and harm the developing fetus with an immature immune system.

Figure 29.16
Micrograph of a cyst filled with *Toxoplasma*.
Toxoplasma can enter the brain and form cysts filled with slowly replicating parasites.



Ciliates are characterized by their mode of locomotion

As the name indicates, most ciliates feature large numbers of cilia (tiny beating hairs). These heterotrophic, unicellular protists are 10 to 3000 µm long. Their cilia are usually arranged either in longitudinal rows or in spirals around the cell. Cilia are anchored to microtubules beneath the plasma membrane (see chapter 5), and they beat in a coordinated fashion. In some groups, the cilia have specialized functions, becoming fused into sheets, spikes, and rods that may then function as mouths, paddles, teeth, or feet.

The ciliates have a pellicle, a tough but flexible outer covering, that enables them to squeeze through or move around obstacles.

Micronucleus and macronucleus

All known ciliates have two different types of nuclei within their cells: a small **micronucleus** and a larger **macronucleus** (figure 29.17). Macronuclei divide by mitosis and are essential for the physiological function of the well-known ciliate *Paramecium*. The micronucleus of some individuals of *Tetrahymena pyriformis*, a common laboratory species, was experimentally removed in the 1930s, and their descendants continue to reproduce asexually to this day! *Paramecium*, however, is not immortal. The cells divide asexually for about 700 generations and then die if sexual reproduction has not occurred. The micronucleus in ciliates is evidently needed only for sexual reproduction.

Vacuoles

Ciliates form vacuoles for ingesting food and regulating water balance. Food first enters the gullet, which in *Paramecium* is lined with cilia fused into a membrane (see figure 29.17). From the gullet, the food passes into food vacuoles, where enzymes and hydrochloric acid aid in its digestion. Afterward, the vacuole empties its waste contents through a special pore in the pellicle called the *cytoproct*, which is essentially an exocytotic

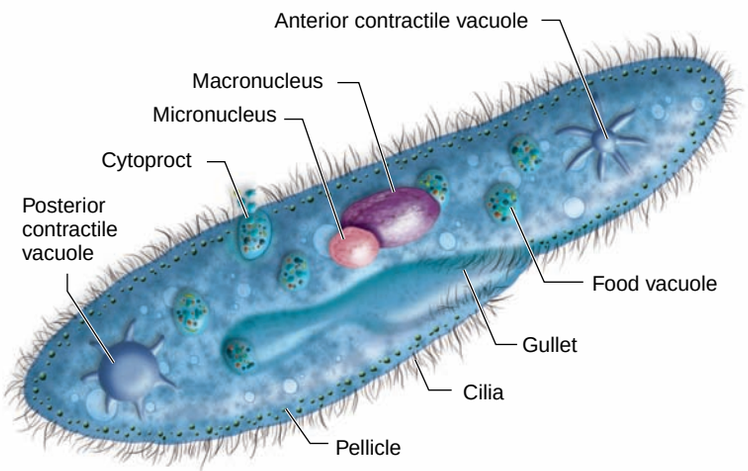


Figure 29.17 ***Paramecium.*** The main features of this ciliate include cilia, two nuclei, and numerous specialized organelles.

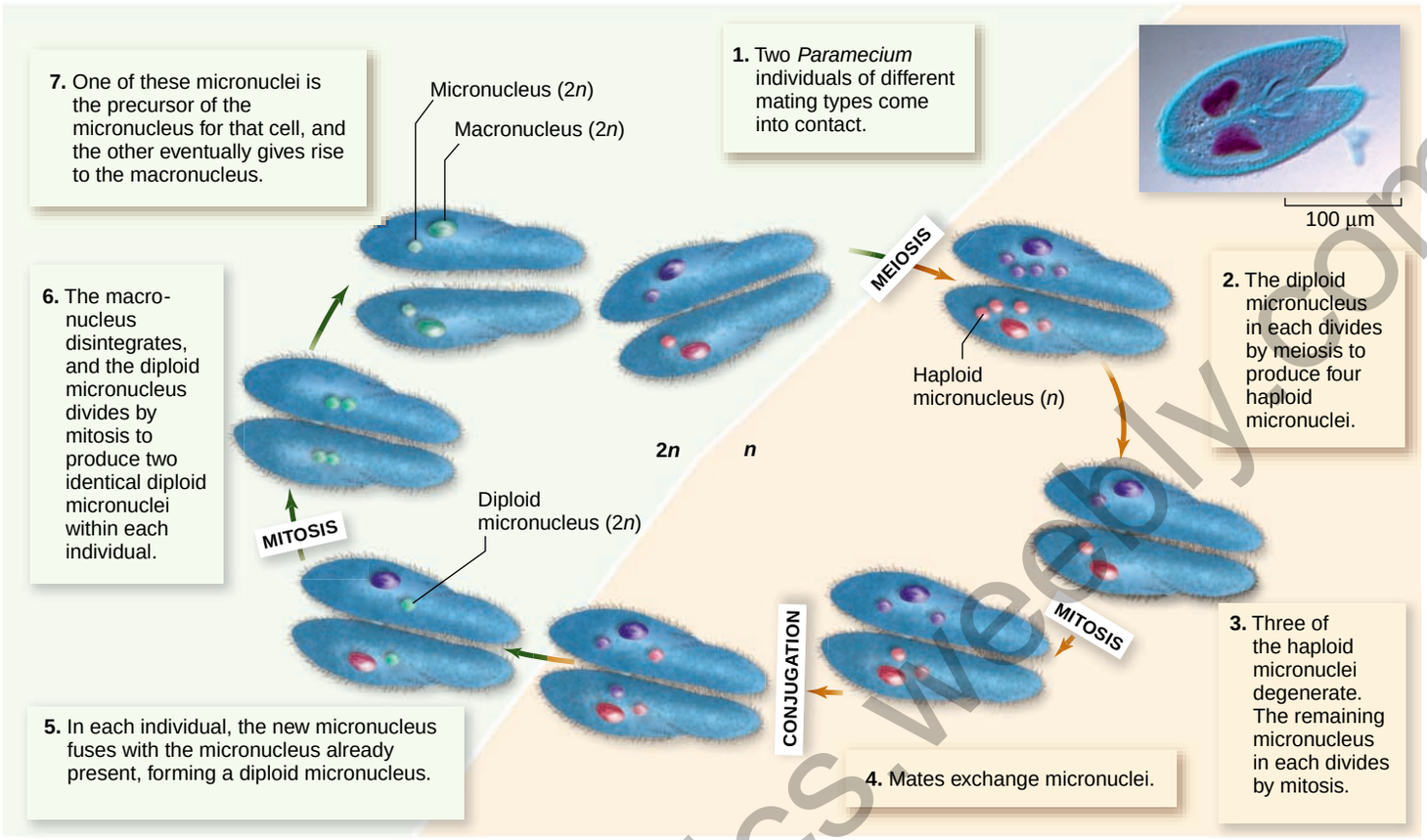


Figure 29.18 Life cycle of *Paramecium*. In sexual reproduction, two mature cells fuse in a process called conjugation.

vesicle that appears periodically when solid particles are ready to be expelled.

The contractile vacuoles, which regulate water balance, periodically expand and contract as they empty their contents to the outside of the organism.

Conjugation: Exchange of micronuclei

Like most ciliates, *Paramecium* undergoes a sexual process called conjugation, in which two individual cells remain attached to each other for up to several hours (figure 29.18).

Paramecia have multiple mating types. Only cells of two different genetically determined mating types can conjugate. Meiosis in the micronuclei produces several haploid micronuclei, and the two partners exchange a pair of their micronuclei through a cytoplasmic bridge between them.

In each conjugating individual, the new micronucleus fuses with one of the micronuclei already present in that individual, resulting in the production of a new diploid micronucleus. After conjugation, the macronucleus in each cell disintegrates, and the new diploid micronucleus undergoes mitosis, thus giving rise to two new identical diploid micronuclei in each individual.

One of these micronuclei becomes the precursor of the future micronuclei of that cell, while the other micronucleus undergoes multiple rounds of DNA replication, becoming the new macronucleus. This complete segregation of the genetic material is unique to the ciliates and makes them ideal organisms for the study of certain aspects of genetics.

"Killer" strains

Paramecium strains that kill other, sensitive strains of *Paramecium* long puzzled researchers. Initially, killer strains were believed to have genes coding for a substance toxic to sensitive strains. The true source of the toxin turned out to be an endosymbiotic bacterium in the "killer" strains. If this bacterium is engulfed by a "nonkiller" strain, the toxin is released, and the sensitive *Paramecium* dies.

Learning Outcomes Review 29.5

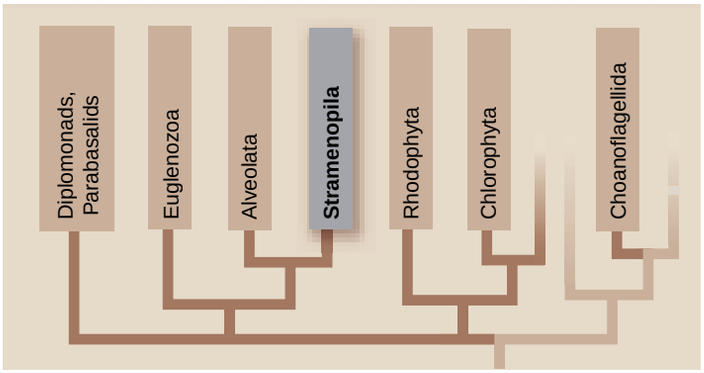
All members of the Alveolata contain flattened vesicles called alveoli. Dinoflagellates have pairs of flagella arranged perpendicular to each other, which causes them to swim with a spinning motion. Blooms of dinoflagellates cause red tides. Apicomplexans are animal parasites that produce a structure called an apical complex, which is composed of cytoskeleton and secretory structures and aids in penetrating their host. The ciliates are unicellular, heterotrophic protists with cilia used for feeding and propulsion.

- ***What would be a major difficulty in finding a poison to fight the malaria-causing protist Plasmodium?***

29.6 Stramenopila: Protists with Fine Hairs

Learning Outcomes

1. *Describe the characteristic features of the Stramenopila.*
2. *Describe the composition of the unique shells of diatoms.*
3. *Explain how the oomycetes are distinguished from other protists.*



Stramenopiles include *brown algae*, *diatoms*, and the *oomycetes* (water molds). The name *stramenopila* refers to unique, fine hairs (figure 29.19) found on the flagella of members of this group, although a few species have lost their hairs during evolution.

Brown algae include large seaweeds

Brown algae are the most conspicuous seaweeds in many northern regions (figure 29.20). The life cycle of the brown algae is marked by an alternation of generations between a multicellular sporophyte (diploid) and a multicellular gametophyte (haploid) (figure 29.21). Some sporophyte cells go through meiosis and produce spores. These spores germinate and undergo mitosis to produce the large individuals we recognize, such as the kelps. The gametophytes are often much smaller, filamentous individuals, perhaps a few centimeters in width.

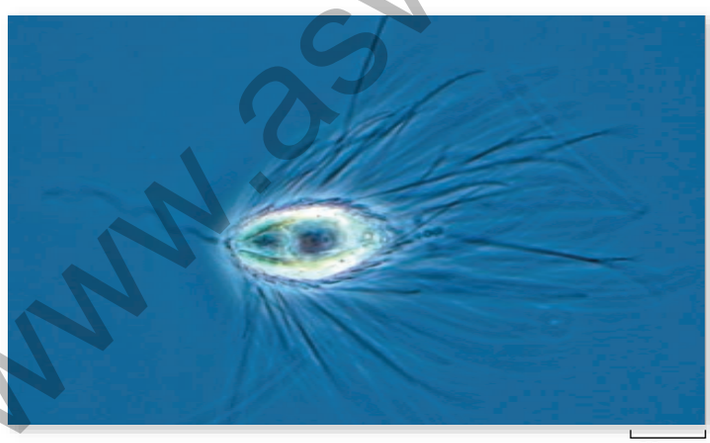


Figure 29.19 Stramenopiles have very fine hairs on their flagella.

Figure 29.20 Brown alga. The giant kelp, *Macrocystis pyrifera*, grows in relatively shallow water along the coasts throughout the world and provides food and shelter for many different kinds of organisms.

Even in an aquatic environment, transport can be a challenge for the very large brown algal species. Distinctive transport cells that stack one upon the other enhance transport within some species (see figure 23.10). However, even though the large kelp look like plants, it is important to realize that they do not contain the complex tissues such as xylem that are found in plants.

Diatoms are unicellular organisms with double shells

Diatoms, members of the phylum Chrysophyta, are photosynthetic, unicellular organisms with unique double shells made of opaline silica, which are often strikingly marked (figure 29.22). The shells of diatoms are like small boxes with lids, one half of the shell fitting inside the other. Their chloroplasts, containing chlorophylls *a* and *c*, as well as carotenoids, resemble those of the brown algae and dinoflagellates. Diatoms produce a unique carbohydrate called chrysolaminarin.

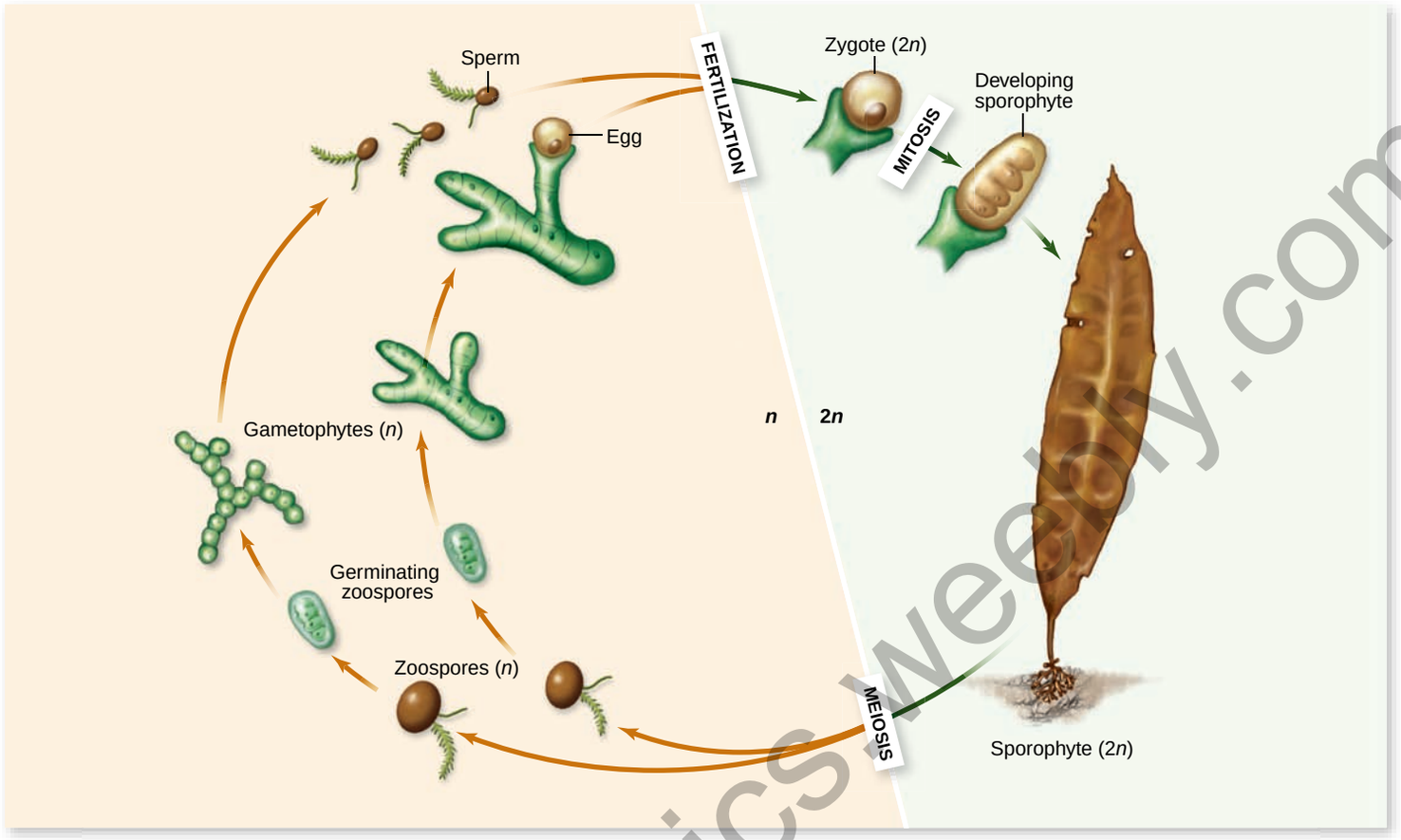


Figure 29.21 Life cycle of *Laminaria*, a brown alga. Multicellular haploid and diploid stages are found in this life cycle, although the male and female gametophytes are quite small.

Some diatoms move by using two long grooves, called *raphes*, which are lined with vibrating fibrils (figure 29.23). The exact mechanism is still being unraveled and may involve the ejection of mucopolysaccharide streams from the raphe that propel the diatom. Pencil-shaped diatoms can slide back and forth over each other, creating an ever-changing shape.

Oomycetes, the "water molds," have some pathogenic members

All oomycetes are either parasites or saprobes (organisms that live by feeding on dead organic matter). At one time, these organisms were considered fungi, which is the origin of the term *water mold* and why their name contains *-mycetes*.

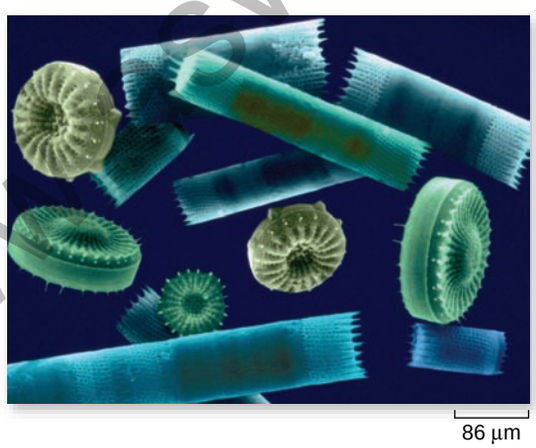


Figure 29.22 Diatoms. These different radially symmetrical diatoms have unique silica, two-part shells.

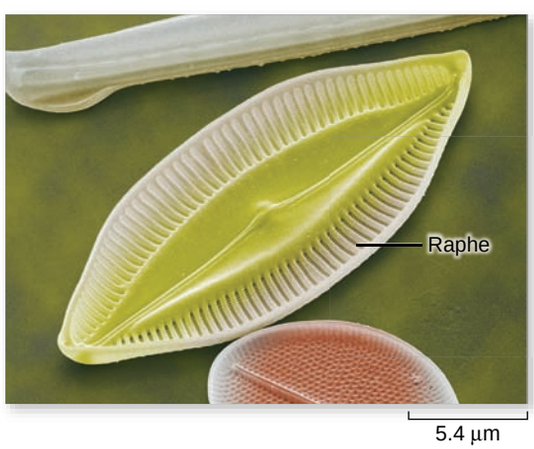


Figure 29.23 Diatom raphe are lined with fibrils that aid in locomotion.

They are distinguished from other protists by the structure of their motile spores, or zoospores, which bear two unequal flagella, one pointed forward and the other backward. Zoospores are produced asexually in a sporangium. Sexual reproduction involves the formation of male and female reproductive organs that produce gametes. Most oomycetes are found in water, but their terrestrial relatives are plant pathogens.

Phytophthora infestans, which causes late blight of potatoes, was responsible for the Irish potato famine of 1845 and 1847. During the famine, about 400,000 people starved to death or died of diseases complicated by starvation and about 2 million Irish immigrated to the United States and elsewhere.

Another oomycete, *Saprolegnia*, is a fish pathogen that can cause serious losses in fish hatcheries. When these fish are released into lakes, the pathogen can infect amphibians and kill millions of amphibian eggs at a time at certain locations. This pathogen is thought to contribute to the phenomenon of amphibian decline.

Learning Outcomes Review 29.6

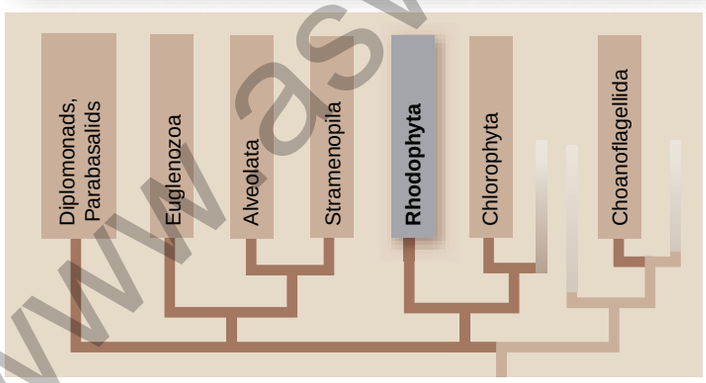
Most members of the Stramenopila have fine hairs on their flagella. Brown algae are large seaweeds that provide food and habitat for marine organisms. They undergo an alternation of generations. Diatoms are unicellular with silica in their cell walls, which forms a shell with two halves. Some can propel themselves. Oomycetes are unique in the production of zoospores that bear two unequal flagella.

- ***How could you distinguish between the sporophyte and the gametophyte of a brown alga?***

29.7 *Rhodophyta: Red Algae*

Learning Outcomes

1. ***List the major characteristics of red algae.***
2. ***Describe how humans use red algae.***



Rhodophyta, the red algae, range in size from microscopic organisms to *Schizymenia borealis* with blades as long as 2 m (figure 29.24). Sushi rolls are wrapped in nori, a red alga. Red algal polysaccharides are used commercially to thicken ice cream and cosmetics.

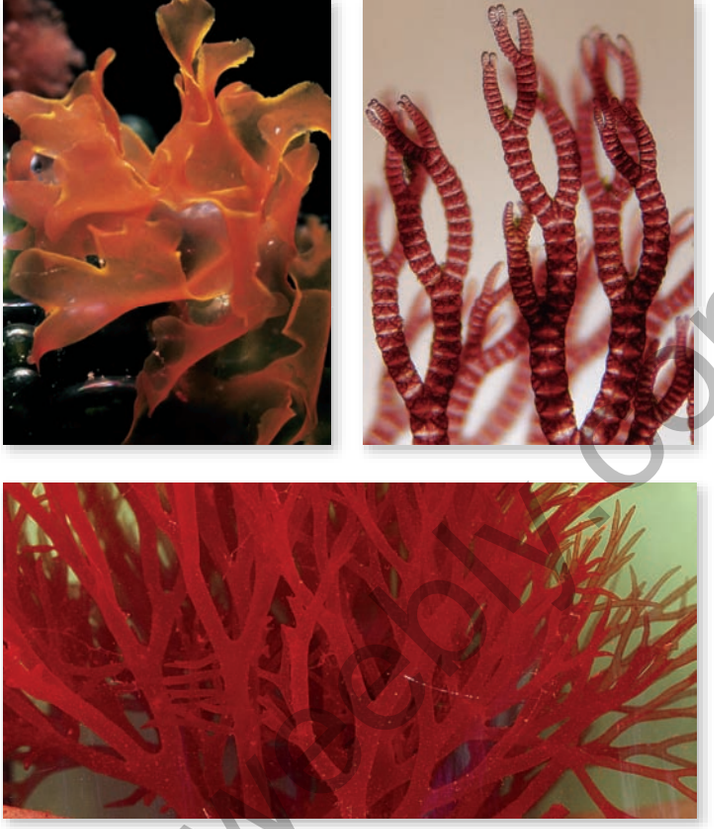


Figure 29.24 **Red algae come in many forms and sizes.**

This lineage lacks flagella and centrioles, and has the accessory photosynthetic pigments phycoerythrin, phycocyanin, and allophycocyanin, which are arranged within structures called *phycobilisomes*. They reproduce using alternation of generations.

The origin of the over 7000 species of Rhodophyta has been a source of controversy. Evidence supporting very early eukaryotic origins and a common ancestry with green algae has been considered. Molecular comparisons of the chloroplasts in red and green algae support a single endosymbiotic origin for both.

Comparisons of the nuclear DNA coding for the large subunit of RNA polymerase II from two red algae, a green alga, and another protist support the conclusion that the Rhodophyta emerged before the evolutionary lineage that led to plants, animals, and fungi.

How can we reconcile the data from plastid and nuclear DNA? The host cells and the cyanobacterial symbionts probably did not follow congruent evolutionary pathways. The host cell that gave rise to red algae may have been distinct from the one that gave rise to plants. One possibility is that different host cells engulfed the same bacterial symbiont. Tentatively, we will treat Rhodophyta and Chlorophyta (the green algae; see chapter 30) as sister clades based on the substantial amount of chloroplast data.

Learning Outcomes Review 29.7

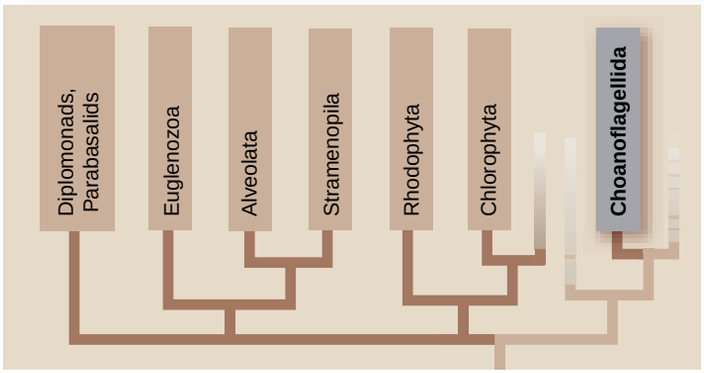
Red algae vary greatly in size and produce accessory pigments that may give them a red color. They lack centrioles and flagella, and they reproduce using an alternation of generations. Humans use red algae as a food and a thickening agent. The evolutionary origin of red algae is a subject of controversy.

- ***Why would you expect to get different results from analysis of nuclear DNA versus plastid DNA?***

29.8 Choanoflagellida: Possible Animal Ancestors

Learning Outcome

1. *Describe the evolutionary significance of the choanoflagellates.*



Choanoflagellates are most like the common ancestor of the sponges and, indeed, all animals. Choanoflagellates have a single emergent flagellum surrounded by a funnel-shaped, contractile collar composed of closely placed filaments, a structure that is exactly matched in the sponges, which are animals. These protists feed on bacteria strained out of the water by their collar. Colonial forms resemble freshwater sponges (figure 29.25).

The close relationship of choanoflagellates to animals was further demonstrated by the strong homology between a surface receptor (a tyrosine kinase receptor) found in choanoflagellates and sponges. This surface receptor initiates a signaling pathway involving phosphorylation (see chapter 9).

Learning Outcome Review 29.8

The choanoflagellates are believed to be the closest relatives of animals. Colonial forms are similar to freshwater sponges, and both organisms have a homologous cell surface receptor.

- *What other types of studies might connect choanoflagellates with sponges?*

Figure 29.25
Colonial choanoflagellates resemble their close animal relatives, the sponges.

29.9 Protists Without a Clade

Learning Outcomes

1. *Explain how amoebas move.*
2. *Distinguish between the shells of most foraminifera and those of diatoms.*
3. *Distinguish between cellular and plasmodial slime molds.*

Many protists remain to be placed on the tree of life. The following examples are of particular importance to human health and the environment.

Amoebas are paraphyletic

So far, we have organized the protists based on their closest relatives. Some lineages vary tremendously if you consider just a single trait. For example, the stramenopiles include autotrophic, marine algae, and terrestrial plant pathogens. As seen in chapter 25, it is also possible for unrelated organisms to acquire similar traits. That is the case with amoebas, which have similar cell morphology, but are not monophyletic.

Rhizopoda: True amoebas

Amoebas move from place to place by means of their pseudopods. Pseudopods are flowing projections of cytoplasm that extend and pull the amoeba forward or engulf food particles. An amoeba puts a pseudopod forward and then flows into it (figure 29.26). Microfilaments of actin and myosin similar to those found in muscles are associated with these movements. The pseudopods can form at any point on the cell body so that it can move in any direction.

Actinopoda: Radiolarians

The pseudopods of amoeboid cells give them truly amorphous bodies. One group, however, has more distinct structures. Members of the phylum Actinopoda, often called *radiolarians*, secrete glassy exoskeletons made of silica. These skeletons give the unicellular organisms a distinct shape, exhibiting either bilateral or radial symmetry. The shells of different species form many elaborate and beautiful shapes, with pseudopods extruding outward

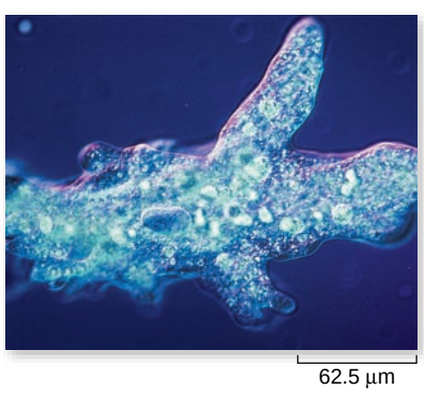


Figure 29.26
Amoeba proteus.
The projections are pseudopods; an amoeba moves by flowing into them.

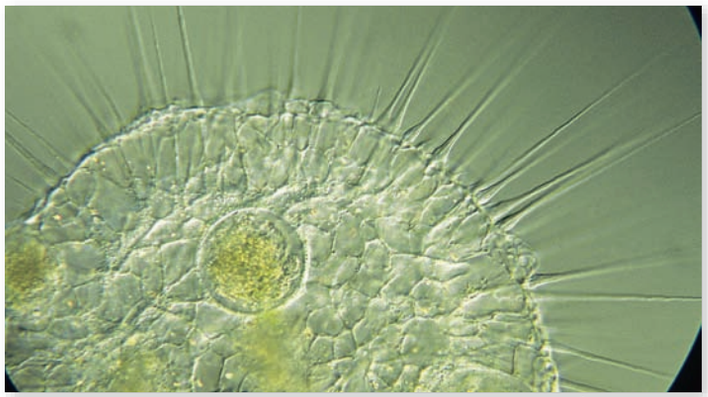


Figure 29.27 ***Actinosphaerium*** **with needle-like pseudopods.**

along spiky projections of the skeleton (figure 29.27). Microtubules support these cytoplasmic projections.

Foraminifera fossils created huge limestone deposits

Members of the phylum Foraminifera are heterotrophic marine protists. They range in diameter from about 20 µm to several centimeters. They resemble tiny snails and can form 3-m-deep layers in marine sediments. Characteristic of the group are pore-studded shells (called *tests*) composed of organic materials usually reinforced with grains of calcium carbonate, sand, or even plates from shells of echinoderms or spicules (minute needles of calcium carbonate) from sponge skeletons.

Depending on the building materials they use, foraminifera may have shells of very different appearance. Some of them are brilliantly colored red, salmon, or yellow-brown.

Most foraminifera live in sand or are attached to other organisms, but two families consist of free-floating planktonic organisms. Their tests may be single-chambered, but are more often multichambered, and they sometimes have a spiral shape resembling that of a tiny snail. Thin cytoplasmic projections called *podia* emerge through openings in the tests (figure 29.28).

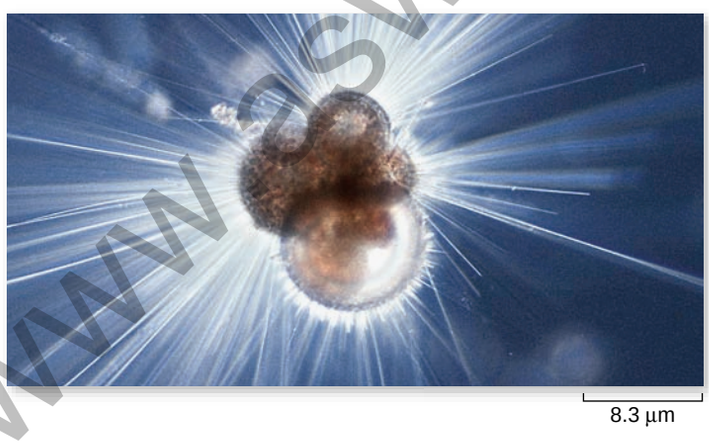


Figure 29.28 **A representative of the foraminifera.** Podia, thin cytoplasmic projections, extend through pores in the calcareous test, or shell, of this living foram.

Podia are used for swimming, gathering materials for the tests, and feeding. Foraminifera eat a wide variety of small organisms.

The life cycles of foraminifera are extremely complex, involving alternation between haploid and diploid generations. Foraminifera have contributed massive accumulations of their tests to the fossil record for more than 200 million years. Because of the excellent preservation of their tests and the striking differences among them, forams are very important as geological markers. The pattern of occurrence of different forams is often used as a guide in searching for oil-bearing strata. Limestones all over the world, including the famous White Cliffs of Dover in southern England, are often rich in forams (figure 29.29).

Slime molds exhibit "group behavior"

Slime molds originated at least three distinct times, and the three lineages are very distantly related. Like water molds, these organisms were once considered fungi. We will explore two lineages: the plasmodial slime molds, which are huge, single-celled, multinucleate, oozing masses, and the cellular slime molds, in which single cells combine and differentiate, creating an early model of multicellularity.

Plasmodial slime molds

Plasmodial slime molds stream along as a **plasmodium,** a nonwalled, multinucleate mass of cytoplasm that resembles a moving mass of slime (figure 29.30). This form is called the *feeding phase*, and the plasmodia may be orange, yellow, or another color.

Plasmodia show a back-and-forth streaming of cytoplasm that is very conspicuous, especially under a microscope. They are able to pass through the mesh in cloth or simply to flow around or through other obstacles. As they move, they engulf and digest bacteria, yeasts, and other small particles of organic matter.

A multinucleated *Plasmodium* cell undergoes mitosis synchronously, with the nuclear envelope breaking down, but only at late anaphase or telophase. Centrioles are absent.

Figure 29.29 **White Cliffs of Dover.** The limestone that forms these cliffs is composed almost entirely of fossil shells of protists, including foraminifera.

Figure 29.30 A plasmodial protist. This multinucleate pretzel slime mold, *Hemitrichia serpula*, moves about in search of the bacteria and other organic particles that it ingests.

Figure 29.31 Sporangia of a plasmodial slime mold. These *Arcyria* sporangia are found in the phylum Myxomycota.

When either food or moisture is in short supply, the plasmodium migrates relatively rapidly to a new area. Here it stops moving and either forms a mass in which spores differentiate or divides into a large number of small mounds, each of which produces a single, mature **sporangium,** the structure in which spores are produced. These sporangia are often beautiful and extremely complex in form (figure 29.31). The spores are highly resistant to unfavorable environmental influences and may last for years if kept dry.

Cellular slime molds

The cellular slime molds have become an important group for the study of cell differentiation because of their relatively simple developmental systems (figure 29.32). The individual organisms behave as separate amoebas, moving through the soil and ingesting bacteria. When food becomes scarce, the individuals aggregate to form a moving "slug." Cyclic adenosine monophosphate (cAMP) is sent out in pulses by some of the cells, and other cells move in the direction of the cAMP to form the slug. In the cellular slime mold *Dictyostelium discoideum*, this slug goes through morphogenesis to make stalk and spore cells. The spores then go on to form a new amoeba if they land in a moist habitat.

Learning Outcomes Review 29.9

Amoebas such as Rhizopodia and Actinopoda are polyphyletic. They move with the aid of pseudopods. Many members of the foraminifera occupy marine habitats. Most of them have calcium carbonate shells responsible for large fossil deposits of limestone, whereas most diatoms have silica shells. At least three lineages of slime mold exist. Cellular slime molds are multicellular, and plasmodial slime molds consist of large multinucleate single cells.

- ***Would you say that cellular slime molds are closely related to plasmodial slime molds?***

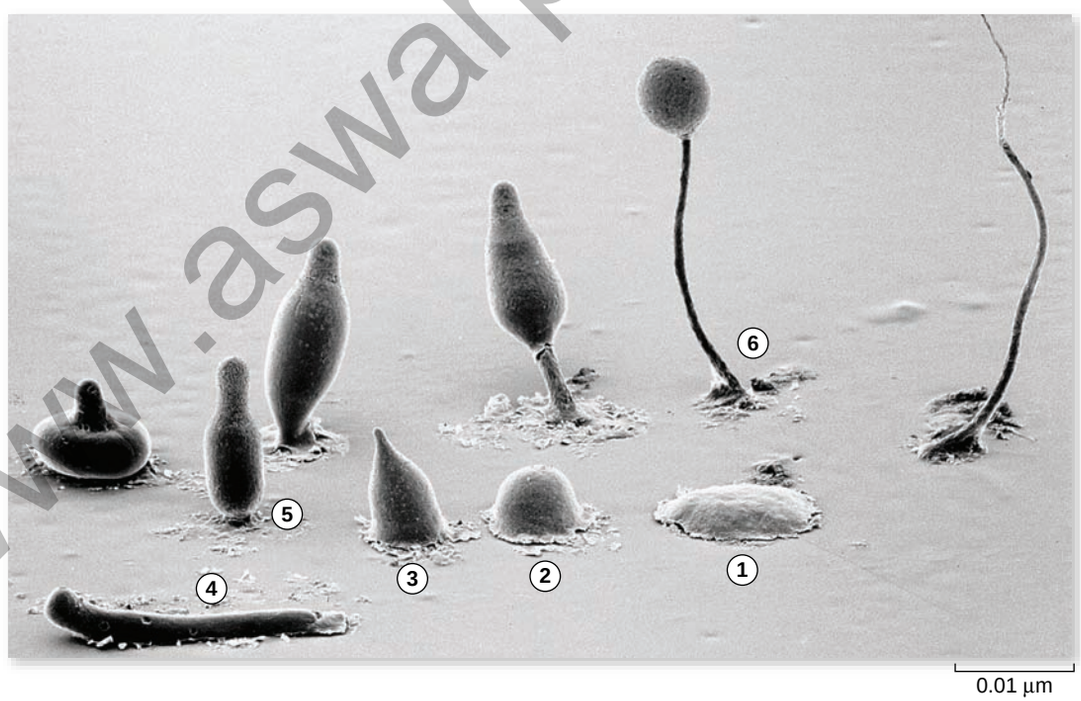
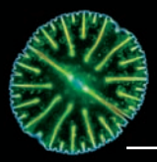


Figure 29.32 Development in *Dictyostelium discoideum,* a cellular slime mold. 1. First, a spore germinates, forming an amoeba that feeds and reproduces until the food runs out. At that point, amoebas aggregate and move toward a fixed center. 2. The aggregated amoebas begin to form a mound. 3. The mound produces a tip and begins to fall sideways. 4. Next, the aggregate forms a multicellular "slug," 2–3 mm long, that migrates toward light. 5. The slug stops moving and a process called culmination begins. Cells differentiate into stalk and spore cells. 6. In the mature fruiting body, amoebas become encysted as spores.



Chapter Review

29.1 Eukaryotic Origins and Endosymbiosis

Protista is not monophyletic.

Protists are the most diverse of the four kingdoms in the domain Eukarya; however, Kingdom Protista is polyphyletic.

Monophyletic clades have been identified among the protists.

The correct classification of protists is under debate. Seven major monophyletic groups are considered in this chapter.

Fossil evidence dates the origins of eukaryotes.

Although eukaryotes may have arisen earlier, the fossil evidence of their appearance dates back to 1.5 BYA.

The nucleus and ER arose from membrane infoldings.

Mitochondria evolved from engulfed aerobic bacteria.

According to the theory of endosymbiosis, ancestral eukaryotic cells engulfed aerobic bacteria, which then became mitochondria.

Chloroplasts evolved from engulfed photosynthetic bacteria.

Chloroplasts are believed to have arisen when ancestral eukaryotic cells engulfed photosynthetic bacteria.

Endosymbiosis is supported by a range of evidence.

Centrioles may have also arisen by endosymbiosis. In support of endosymbiosis, several organelles are found to contain their own DNA, which closely resembles that of prokaryotes.

Genes have migrated from endosymbiotic organelles.

Many genes in the mitochondria have moved into the eukaryotic nucleus over time.

Mitosis evolved in eukaryotes.

Mechanisms of mitosis vary among organisms, suggesting that the process did not evolve all at once.

29.2 Defining Protists

Protista is not monophyletic.

Monophyletic clades have been identified among the protists.

Protist cell surfaces vary widely.

Extracellular material (ECM) may cover the plasma membrane.

Protists have several means of locomotion.

Protists mainly use flagella or pseudopods for locomotion, although many other means of propulsion are found.

Protists have a range of nutritional strategies.

Protists include phototrophs, heterotrophs, (phagotrophs or osmotrophs), and mixotrophs capable of both modes.

Protists reproduce asexually and sexually.

Protists can reproduce asexually by mitosis, budding, or schizogony. They may also carry out sexual reproduction.

Protists are the bridge to multicellularity.

Colonial protists may be the precursors of multicellular organisms.

29.3 Diplomonads and Parabasalids: Flagellated Protists Lacking Mitochondria

Diplomonads have two nuclei.

Diplomonads are unicellular, move with flagella, and have two nuclei.

Parabasalids have undulating membranes.

Parabasalids use flagella and undulating membranes for locomotion.

29.4 Euglenozoa: A Diverse Group in Which Some Members Have Chloroplasts

Euglenoids are free-living eukaryotes with anterior flagella.

Euglenoids can produce chloroplasts to carry out photosynthesis in the light. They contain a pellicle and move via anterior flagella.

Kinetoplastids are parasitic.

Kinetoplastids are parasitic and are distinctive in having a single, unique mitochondrion with two types of circular DNA.

29.5 Alveolata: Protists with Submembrane Vesicles

Dinoflagellates are photosynthesizers with distinctive features.

Dinoflagellates have pairs of flagella arranged so that they swim with a spinning motion. Blooms of dinoflagellates cause red tides.

Apicomplexans include the malaria parasite.

Apicomplexans are spore-forming animal parasites. They have a unique arrangement of organelles at one end of the cell, called the apical complex, which is used to invade the host.

Ciliates are characterized by their mode of locomotion.

Ciliates are unicellular, heterotrophic protists that use numerous cilia for feeding and propulsion. Each cell has a macronucleus and a micronucleus. Micronuclei are exchanged during conjugation.

29.6 Stramenopila: Protists with Fine Hairs

Brown algae include large seaweeds.

Brown algae are typically large seaweeds that undergo an alternation of generations, producing gametophyte and sporophyte stages.

Diatoms are unicellular organisms with double shells.

Diatoms have silica in their cell walls. Each diatom produces two overlapping glassy shells that fit like a box and lid.

Oomycetes, the "water molds," have some pathogenic members.

Oomycetes are parasitic and are unique in the production of asexual spores (zoospores) that bear two unequal flagella.

29.7 Rhodophyta: Red Algae

Red algae produce accessory pigments that may give them a red color. They lack centrioles and flagella, and they reproduce using an alternation of generations.

29.8 Choanoflagellida: Possible Animal Ancestors

Colonial choanoflagellates are structurally similar to freshwater sponges, and molecular similarities have been found.

29.9 Protists Without a Clade

Amoebas are paraphyletic.

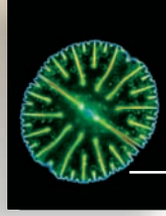
Amoebas have formed through several lineages. Two main groups are Rhizopoda and Actinopoda (radiolarians).

Foraminifera fossils created huge limestone deposits.

The Foraminifera are heterotrophic marine protists with pore-studded shells primarily formed by deposit of calcium carbonate.

Slime molds exhibit "group behavior."

Two of the three lineages of slime molds are the plasmodial slime molds and the cellular slime molds. All can aggregate to form a moving "slug" that produces spores.



Review Questions

UNDERSTAND

1. Fossil evidence of eukaryote dates back to *Hemitrichia serpula*
   a. 2.5 BYA.
   b. 1.5 BYA.
   c. 2.5 MYA.
   d. 1.5 MYA.
2. DNA is not found in this organelle.
   a. Endoplasmic reticulum
   b. Nucleus
   c. Chloroplast
   d. Centriole
   e. Mitochondrion
3. The products of budding are
   a. two cells of equal size.
   b. two cells, one of which is smaller than the other.
   c. many cells of equal size.
   d. many cells of variable size.
4. Both diplomonads and parabasalids
   a. contain chloroplasts.
   b. have multinucleate cells.
   c. lack mitochondria.
   d. have silica in their cell walls.
5. Trypanosomes are examples of
   a. euglenoids.
   b. diplomonads.
   c. parabasilids.
   d. kinetoplastids.
6. The function of the apical complex in Apicomplexans is to
   a. propel the cell through water.
   b. penetrate host tissue.
   c. absorb food.
   d. detect light.
7. If a cell contains a pellicle, it
   a. can change shape readily.
   b. is shaped like a sphere.
   c. is shaped like a torpedo.
   d. must have a contractile vacuole.
8. Stramenopila are
   a. tiny flagella.
   b. large cilia.
   c. small hairs on flagella.
   d. pairs of large flagella.
9. Choose all of the following that exhibit an alternation of multicellular generations.
   a. Dinoflagellates
   b. Brown algae
   c. Red algae
   d. Diatoms
10. Choose all of the following that are photosynthetic.
    a. Diatoms
    b. Ciliates
    c. Apicomplexans
    d. Dinoflagellates
11. Which is most likely the ancestor of animals?
    a. Trypanosomes
    b. Diplomonads
    c. Ciliates
    d. Choanoflagellates
12. When food is scarce, cells of this organism communicate with each other to form a multicellular slug.
    a. Cellular slime molds
    b. True amoebas
    c. Foraminifera
    d. Diatoms

APPLY

1. Analyze the following statements and choose the one that most accurately supports the endosymbiotic theory.
   a. Mitochondria rely on mitosis for replication.
   b. Chloroplasts contain DNA but translation does not occur in chloroplasts.
   c. Vacuoles have double membranes.
   d. Antibiotics that inhibit protein synthesis in bacteria can have the same effect on mitochondria.
2. Determine which feature of the choanoflagellates was likely the most significant for the evolution of animals?
   a. Flagellum with a funnel-shaped, contractile collar also found in sponges
   b. A tyrosine kinase receptor on the surface of choanoflagellates that has strong homology to fungi
   c. A colonial form that resembles some fungi
   d. Eyespots that are similar to ribbonworms
3. Examine the life cycle of cellular slime molds, and determine which feature affords the greatest advantage for surviving food shortages.
   a. Cellular slime molds produce spores when starved.
   b. Cellular slime molds are saprobes.
   c. A diet of bacteria ensures there will never be a shortage of food.
   d. Cellular slime molds use cAMP to guide each other to food sources.

SYNTHESIZE

1. Modern taxonomic treatments rely heavily on phylogenetic data to classify organisms. In the past, taxonomists often used a morphological species concept, in which species were defined based on similarities in growth form. Give an example to show how a morphological species concept would group a set of protists differently than a phylogenetic species concept would.
2. Three methods have been used to try to eradicate malaria. One is to eliminate the mosquito vectors of the parasite, a second is to kill the parasites after they entered the human body, and the third is to develop a vaccine against the parasite, allowing the human immune system to provide protection from the disease. Which do you suppose is the most promising in the long run? Why? Think about both the biology of the disease and the efficacy of carrying out each of the methods on a large scale.
3. Design an experiment to demonstrate that cells of cellular slime molds are attracted to cyclic-AMP. Then, design a follow-up experiment to determine whether they are always attracted to cAMP or only when resources are scarce.

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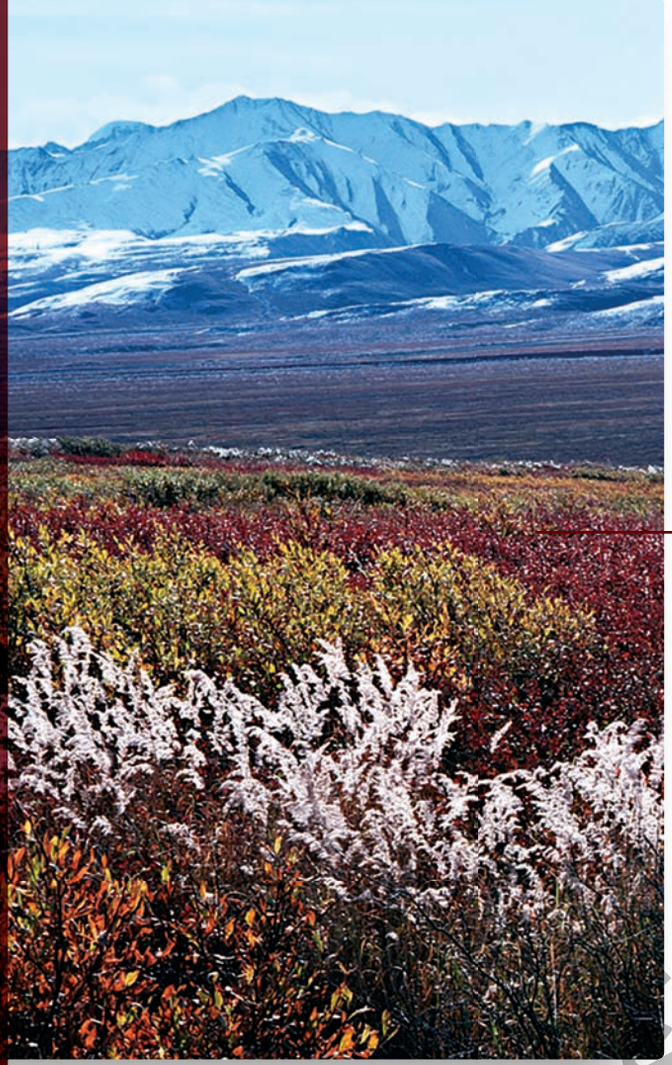


Chapter 30

Green Plants

Chapter Outline

Introduction

Colonization of land by plants fundamentally altered the history of life on Earth. A terrestrial environment offers abundant CO_2 and solar radiation for photosynthesis. But for at least 500 million years, the lack of water and higher ultraviolet (UV) radiation on land confined green algal ancestors to an aquatic environment. Evolutionary innovations for reproduction, structural support, and prevention of water-loss are key in the story of plant adaptation to land. The evolutionary shift on land to life cycles dominated by a diploid generation masks recessive mutations arising from higher UV exposure. As a result, larger numbers of alleles persist in the gene pool, creating greater genetic diversity. Numerous evolutionary solutions to terrestrial challenges have resulted in over 300,000 species of plants dominating all terrestrial communities today, from forests to alpine tundra and from agricultural fields to deserts. Plants affect almost every aspect of our lives, from improving environmental quality to providing pharmaceuticals, food, fuels, building materials, and clothing. This chapter explores the evolutionary history and strategies of the green plants.

30.1 Defining Plants

Learning Outcomes

1. *Explain the relationship between the different algae clades and plants.*
2. *Describe the haplodiplontic life cycle.*
3. *Distinguish between a sporophyte and a gametophyte.*
4. *Identify two major environmental challenges for land plants and associated adaptations.*

As you saw in chapter 26, the phylogenetic revolution has completely altered our definition of a plant. We now know that all green algae and the land plants shared a common ancestor a little over 1 BYA, and the two groups are now recognized as a kingdom or crown group referred to as the Viridiplantae, or simply, the green plants. DNA sequence data are consistent with the claim that a single individual gave rise to all plants. Thus, plants are all members of the Viridiplantae, extending an older definition of plants to include the green algae.

Plants are photoautotrophic, but not all photoautotrophs are members of the Viridiplantae. The definition of a plant is broad, but it excludes the red and brown algae. All

algae—red, brown, and green—shared a primary endosymbiotic event 1.5 BYA. But sharing an ancestral chloroplast lineage is not the same as being monophyletic. Red and green algae last shared a common ancestor about 1.4 BYA. Brown algae became photosynthetic through endosymbiosis with a eukaryotic red alga that had itself already acquired a photosynthetic cyanobacterium, as described in the preceding chapter.

Plants are also not fungi, which are more closely related to metazoan animals (see chapter 32). Fungi, however, were essential to the colonization of land by plants, enhancing plants' nutrient uptake from the soil.

Land plants evolved from freshwater algae

Some saltwater algae evolved to thrive in a freshwater environment. Just a single species of freshwater green algae gave rise to the entire terrestrial plant lineage, from mosses through the flowering plants (angiosperms). Given the incredibly harsh conditions of life on land, it is not surprising that all land plants share a single common ancestor. Exactly what this ancestral alga was is still a mystery, but close relatives, members of the charophytes, exist in freshwater lakes today.

The green algae split into two major clades: the chlorophytes, which never made it to land, and the charophytes, which are sister to all the land plants (figure 30.1). Land plants, although diverse, have certain characteristics in common. Unlike the charophytes, land plants have multicellular haploid and diploid stages. Diploid embryos are also land plant innovations. Over time, the trend has been toward more embryo protection and a smaller haploid stage in the life cycle.

Land plants have adapted to terrestrial life

Unlike their freshwater ancestors, most land plants have only limited amounts of water available. As an adaptation to living on land, most plants are protected from desication—the tendency of organisms to lose water to the air—by a waxy surface material called the cuticle that is secreted onto their exposed surfaces. The cuticle is relatively impermeable, preventing water loss. This solution, however, limits the gas exchange essential for respiration and photosynthesis. Gas diffusion into and out of a plant occurs through tiny mouth-shaped openings called **stomata** (singular, *stoma*), which allows water to diffuse out at the same time. Chapter 36 describes how stomata can be closed at times to limit water loss.

Moving water within plants is a challenge that increases with plant size. Members of the land plants can be distinguished based on the presence or absence of **tracheids,** specialized cells that facilitate the transport of water and minerals (see chapter 36). Tracheophytes have specialized transport cells called tracheids and have evolved highly efficient transport systems: water-conducting xylem and food-conducting phloem strands of tissues in their stems, roots, and leaves. Some plants that grow in aquatic environments, including water lilies, have tracheids. Aquatic tracheophytes had terrestrial ancestors that adapted back to a watery environment.

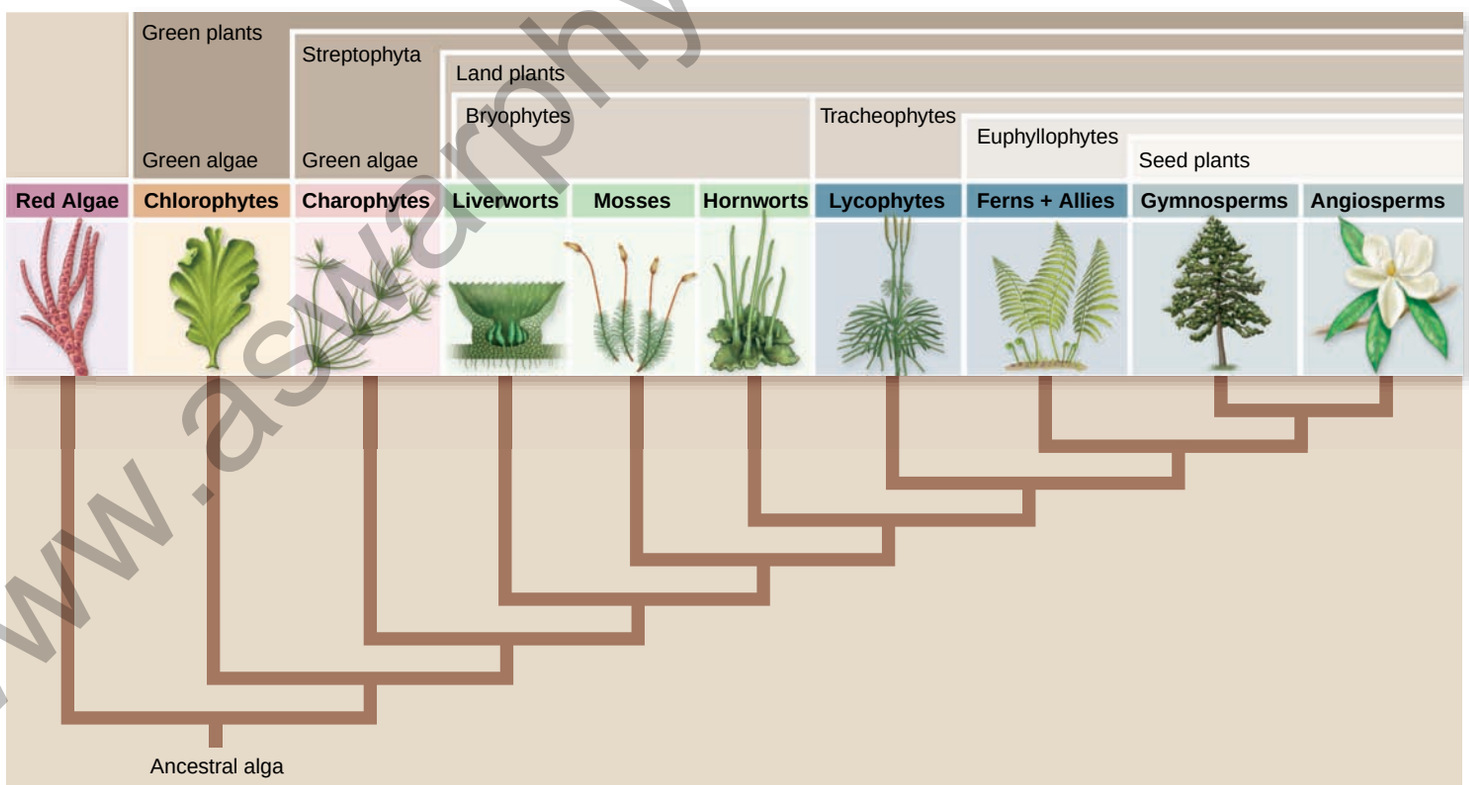


Figure 30.1 **Green plant phylogeny.**

Terrestrial plants are exposed to higher intensities of UV irradiation than aquatic algae, increasing the chance of mutation. Diploid genomes mask the effect of a single, deleterious allele. All land plants have both haploid and diploid generations, and the evolutionary shift toward a dominant diploid generation allows for greater genetic variability to persist in terrestrial plants.

Most multicellular Viridiplantae have haplodiplontic life cycles. Many multicellular green algae and all land plants have haplodiplontic life cycles and undergo mitosis after both gamete fusion and meiosis. The result is a multicellular haploid individual and a multicellular diploid individual—unlike in the human life cycle, in which gamete fusion directly follows meiosis. Humans have a **diplontic** life cycle, meaning that only the diploid stage is multicellular; by contrast, the land plant life cycle is **haplodiplontic,** having multicellular haploid and diploid stages.

The haplodiplontic cycle produces alternation of generations

The basic haplodiplontic cycle is summarized in figure 30.2. Many brown, red, and green algae are also haplodiplontic. Humans produce gametes via meiosis, but land plants actually produce gametes by *mitosis* in a multicellular, haploid individual. The diploid generation, or **sporophyte,** alternates with the haploid generation, or **gametophyte.** Sporophyte means "spore plant," and gametophyte means "gamete plant." These terms indicate the kinds of reproductive cells the respective generations produce.

The diploid sporophyte produces haploid spores (not gametes) by meiosis. Meiosis takes place in structures called sporangia, where diploid **spore mother cells (sporocytes)** undergo meiosis, each producing four haploid **spores.** Spores are the first cells of the gametophyte generation. Spores divide by mitosis, producing a multicellular, haploid gametophyte.

The haploid gametophyte is the source of gametes. When the gametes fuse, the zygote they form is diploid and is the first cell of the next sporophyte generation. The zygote grows into a diploid sporophyte by mitosis and produces sporangia in which meiosis ultimately occurs.

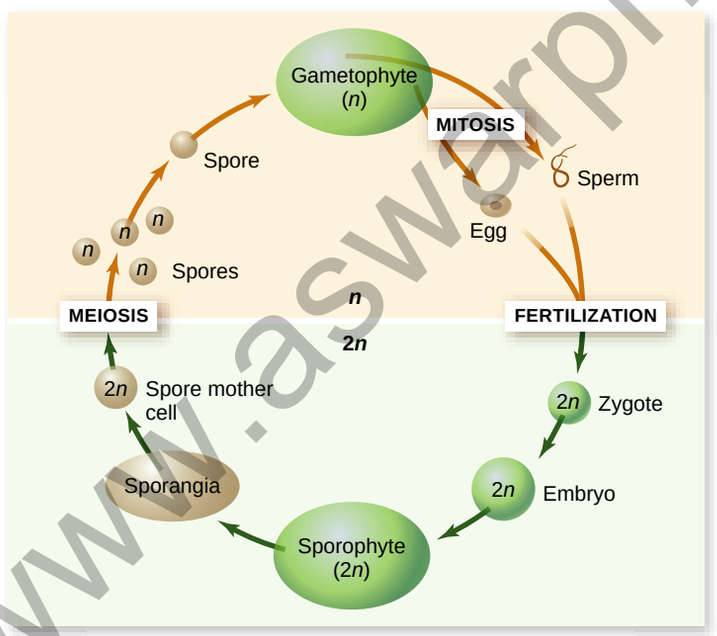


Figure 30.2 A generalized multicellular plant life cycle. Note that both haploid and diploid individuals can be multicellular. Also, spores are produced by meiosis, while gametes are produced by mitosis.

The relative sizes of haploid and diploid generations vary

All land plants are haplodiplontic; however, the haploid generation consumes a much larger portion of the life cycle in mosses and ferns than it does in gymnosperms and angiosperms. In mosses, liverworts, and ferns, the gametophyte is photosynthetic and free-living. When you look at mosses, what you see is largely gametophyte tissue; the sporophytes are usually smaller, brownish or yellowish structures attached to the tissues of the gametophyte. In other plants, the gametophyte is usually nutritionally dependent on the sporophyte. When you look at a gymnosperm or angiosperm, such as most trees, the largest, most visible portion is a sporophyte.

Although the sporophyte generation can get very large, the size of the gametophyte is limited in all plants. The gametophyte generation of mosses produces gametes at its tips. The egg is stationary, and sperm lands near the egg in a droplet of water. If the moss were the height of a sequoia, not only would vascular tissue be needed for conduction and support, but the sperm would have to swim up the tree! In contrast, the small gametophyte of the fern develops on the forest floor where gametes can meet. Tree ferns are especially abundant in Australia; the haploid spores the sporophyte trees produce fall to the ground and develop into gametophytes.

Having completed an overview of plant life cycles, we next consider the major plant groups within Viridiplantae. As we proceed, you will see a reduction of the gametophyte from group to group, a loss of multicellular **gametangia** (structures in which gametes are produced), and increasing specialization for life on land, including the remarkable structural adaptations of the flowering plants, which are the dominant plants today.

Learning Outcomes Review 30.1

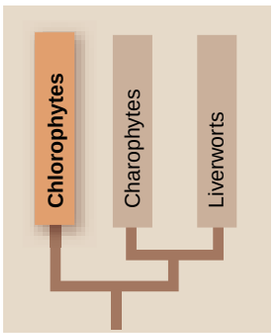
All algae acquired chloroplasts necessary for photosynthesis, but green algae diverged from red algae after that event. A single freshwater green alga successfully invaded land; its descendants eventually developed reproductive strategies, conducting systems, stomata, and cuticles as adaptations. Green plants (Viridiplantae) include all green algae and the land plants. Most plants have a haplodiplontic life cycle, a haploid form alternates with a diploid form in a single organism. Diploid sporophytes produce haploid spores by meiosis. Each spore can develop into a haploid gametophyte by mitosis; the gametophyte form produces haploid gametes, again by mitosis. When the gametes fuse, the diploid sporophyte is formed once more.

- ***How would you distinguish a small aquatic tracheophyte from a freshwater alga?***
- ***What distinguishes gamete formation in plants from gamete formation in humans?***

30.2 Chlorophytes and Charophytes: Green Algae

Learning Outcomes

1. *Explain why chlorophytes are considered close relatives of land plants.*
2. *Explain why charophytes are considered the closest relatives of land plants.*



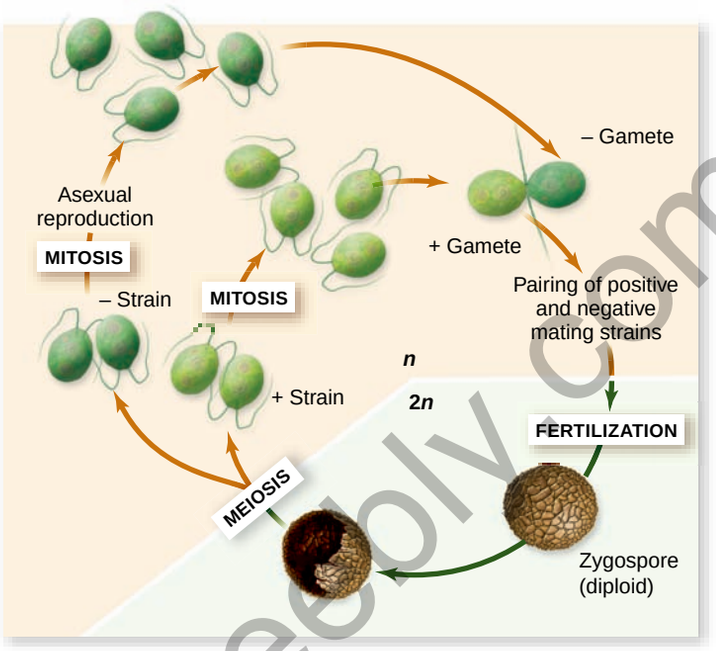
Green algae have two distinct lineages: the chlorophytes, discussed here, and another lineage, the **streptophytes,** that gave rise to the land plants (see figure 30.1). The chlorophytes are of special interest here because of their unusual diversity and lines of specialization. The chlorophytes have an extensive fossil record dating back 900 million years. Modern chlorophytes closely resemble land plants, especially in their chloroplasts, which are biochemically similar to those of the plants. They contain chlorophylls *a* and *b*, as well as carotenoids.

Chlorophytes can be unicellular

Early green algae probably resembled *Chlamydomonas reinhardtii*, diverging from land plants over 1 BYA (figure 30.3). Individuals are microscopic (usually less than 25 µm long), green, and rounded, and they have two flagella at the anterior end. They are soil dwellers that move rapidly in water by beating their flagella in opposite directions. Most individuals of *Chlamydomonas* are haploid. *Chlamydomonas* reproduces asexually as well as sexually, but because it is always unicellular the life cycle is not haplodiplontic (see figure 30.3).

Several lines of evolutionary specialization have been derived from organisms such as *Chlamydomonas*, *including* the evolution of nonmotile, unicellular green algae. *Chlamydomonas* is capable of retracting its flagella and settling down as an immobile unicellular organism if the pond in which it lives dries out. Some common algae found in soil and bark, such as *Chlorella*, are essentially like *Chlamydomonas* in this trait, but they do not have the ability to form flagella.

Genome-sequencing projects are providing new insights into the evolution of the Viridiplantae. A comparison of the 6968 protein families predicted by the *Chlamydomonas* genome were compared with a red algal genome and two streptophyte genomes (moss and *Arabidopsis*). Of these proteins, 172 are found only in the Viridiplantae. Comparing these conserved proteins in the green plants will provide new information about the evolution of the green plants.



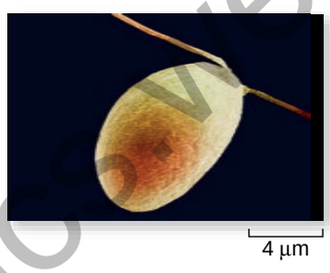


Figure 30.3
***Chlamydomonas* life cycle.** This single-celled chlorophyte has both asexual and sexual reproduction. Unlike multicellular green plants, gamete fusion is not followed by mitosis. © Dr. Richard Kessel & Dr. Gene Shih/Visuals Unlimited

Colonial chlorophytes have some cell specialization

Multicellularity arose many times in the eukaryotes. Colonial chlorophytes provide examples of cellular specialization, an aspect of multicellularity. A line of specialization from cells like those of *Chlamydomonas* concerns the formation of motile, colonial organisms. In these genera of green algae, the *Chlamydomonas*-like cells retain some of their individuality.

The most elaborate of these organisms is *Volvox* (figure 30.4), a hollow sphere made up of a single layer of

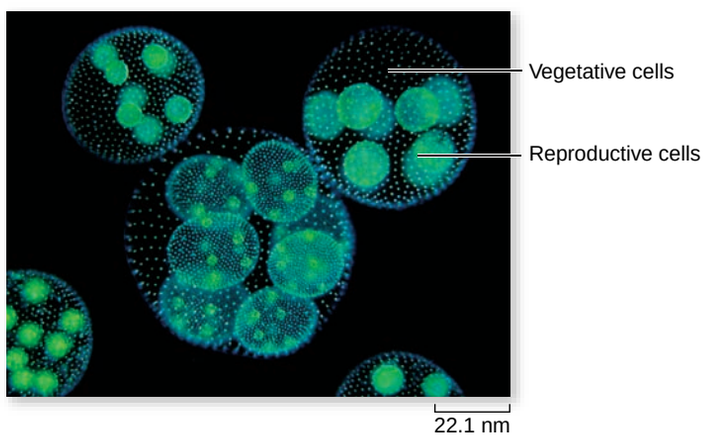
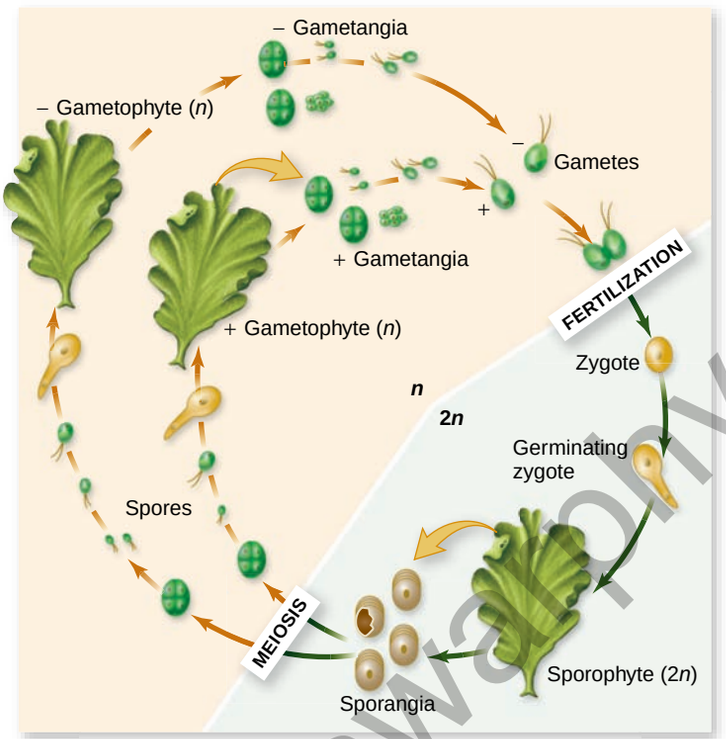


Figure 30.4 ***Volvox.*** This chlorophyte forms a colony where some cells specialize for reproduction.

500 to 60,000 individual cells, each cell having two flagella. Only a small number of the cells are reproductive. Some reproductive cells may divide asexually, bulge inward, and give rise to new colonies that initially remain within the parent colony. Others produce gametes.

Multicellular chlorophytes can have haplodiplontic life cycles

Haplodiplontic life cycles are found in some chlorophytes and the streptophytes, which include both charophytes and land plants. *Ulva*, a multicellular chlorophyte, has identical gametophyte and sporophyte generations that consist of flattened sheets two cells thick (figure 30.5). Unlike the charophytes, none of the ancestral chlorophytes gave rise to land plants.



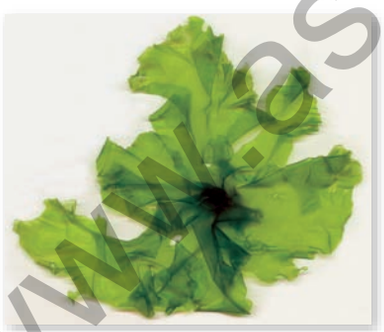


Figure 30.5 Life cycle of *Ulva*. This chlorophyte alga has a haplodiplontic life cycle. The gametophyte and sporophyte are multicellular and identical in appearance.

Inquiry question

Are *Ulva* gametes formed by meiosis? Explain your response.

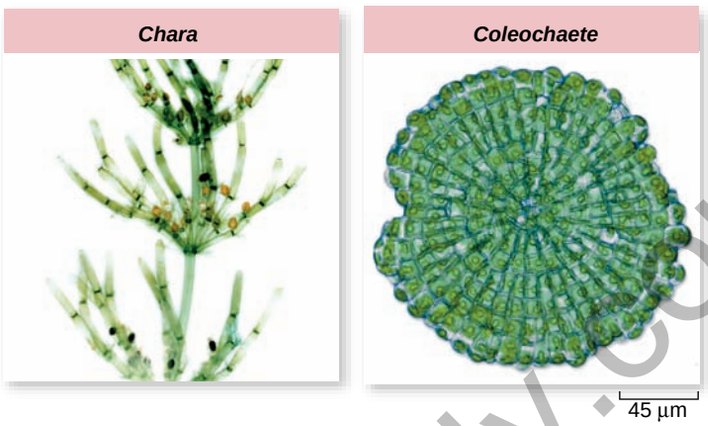
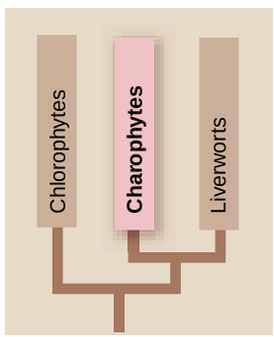


Figure 30.6 *Chara*, a member of the Charales, and *Coleochaete*, a member of the Coleochaetales, represent the two clades most closely related to land plants.

Charophytes are the closest relatives to land plants



Charophytes, a clade of streptophytes, are also green algae, and they are distinguished from chlorophytes by their close phylogenetic relationship to the land plants. Charophytes have haplontic life cycles, indicating that the evolution of a diplontic embryo and haplodiplontic life cycle occurred after the move onto land.

Identifying which of the charophyte clades is sister (most closely related) to the land plants puzzled biologists for a long time. The charophyte algae fossil record is scarce. Currently, the molecular evidence from rRNA and DNA sequences favors the charophytes as the green algal clade in the streptophytes.

The two candidate Charophyta clades have been the Charales, with about 300 species, and the Coleochaetales, with about 30 species (figure 30.6). Both lineages are primarily freshwater algae, but the Charales are huge, relative to the microscopic Coleochaetales. Both clades have similarities to land plants. *Coleochaete* and its relatives have cytoplasmic linkages between cells called *plasmodesmata*, which are found in land plants. The species *Chara* in the Charales undergoes mitosis and cytokinesis like land plant cells. Sexual reproduction in both relies on a large, nonmotile egg and flagellated sperm. These gametes are more similar to those of land plants than many charophyte relatives. Both charophyte clades form green mats around the edges of freshwater ponds and marshes. One species must have successfully inched its way onto land through adaptations to drying.

Learning Outcomes Review 30.2

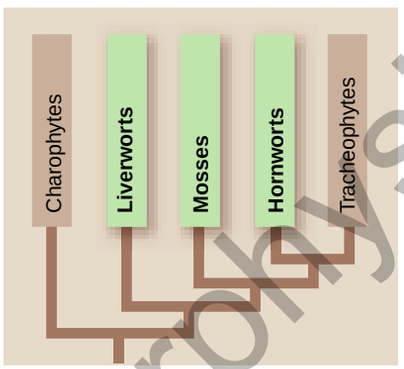
The chlorophytes have chloroplasts very similar to those of land plants. Specializations in this group include the evolution of nonmotile, unicellular species that can tolerate drying and formation of colonial organisms that exhibit a degree of cell specialization. Charophytes are the green algae that are most likely sister to the land plants based on molecular evidence, morphology, and reproduction.

- ***What major barrier must be overcome for sexual reproduction of land-based organisms?***

30.3 Bryophytes: Dominant Gametophyte Generation

Learning Outcome

1. ***Describe adaptations of bryophytes for terrestrial environments.***



Bryophytes are the closest living descendants of the first land plants. Plants in this group are also called nontracheophytes because they lack the derived transport cell called a *tracheid.*

Fossil evidence and molecular systematics can be used to reconstruct early terrestrial plant life. Water and gas availability were limiting factors. These plants likely had little ability to regulate internal water levels and likely tolerated desication, traits found in most extant mosses, although some are aquatic.

Algae, including the Charales, lack roots. Fungi and early land plants cohabitated, and the fungi formed close associations with the plants that enhanced water uptake. The tight symbiotic relationship between fungi and plants, called **mycorrhizal associations,** are also found in many existing bryophytes. More information on mycorrhizal fungi is found in chapter 31.

Bryophytes are unspecialized but successful in many environments

The approximately 24,700 species of bryophytes are simple but highly adapted to a diversity of terrestrial environments, even deserts. Most bryophytes are small; few exceed 7 cm in height. Bryophytes have conducting cells other than tracheids for water and nutrients. The tracheid is a derived trait that characterizes the tracheophytes, all land plants but the bryophytes. Bryophytes are sometimes called nonvascular plants, but *nontracheophyte* is a more accurate term because they do have conducting cells of different types.

Scientists now agree that bryophytes consist of three quite distinct clades of relatively unspecialized plants: liverworts, mosses, and hornworts. Their gametophytes are photosynthetic and are more conspicuous than the sporophytes. Sporophytes are attached to the gametophytes and depend on them nutritionally in varying degrees. Some of the sporophytes are completely enclosed within gametophyte tissue; others are not and usually turn brownish or straw-colored at maturity. Like ferns and certain other vascular (tracheophyte) plants, bryophytes require water (such as rainwater) to reproduce sexually, tracing back to their aquatic origins. It is not surprising that they are especially common in moist places, both in the tropics and temperate regions.

Liverworts are an ancient phylum

The Old English word *wyrt* means "plant" or "herb." Some common liverworts (phylum Hepaticophyta) have flattened gametophytes with lobes resembling those of liver—hence the name "liverwort." Although the lobed liverworts are the best known representatives of this phylum, they constitute only about 20% of the species (figure 30.7). The other 80% are leafy and superficially resemble mosses. The gametophytes are prostrate instead of erect, and the rhizoids are one-celled.

Some liverworts have air chambers containing upright, branching rows of photosynthetic cells, each chamber having a pore at the top to facilitate gas exchange. Unlike stomata, the pores are fixed open and cannot close.

Sexual reproduction in liverworts is similar to that in mosses. Lobed liverworts may form gametangia in umbrella-like structures. Asexual reproduction occurs when lens-shaped pieces of tissue that are released from the gametophyte grow to form new gametophytes.

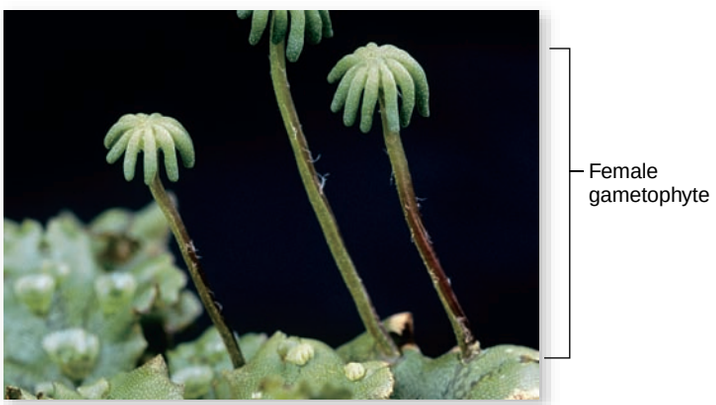


Figure 30.7 A common liverwort, *Marchantia* (phylum Hepaticophyta). The microscopic sporophytes are formed by fertilization within the tissues of the umbrella-shaped structures that arise from the surface of the flat, green, creeping gametophyte.

Mosses have rhizoids and water-conducting tissue

Unlike other bryophytes, the gametophytes of mosses typically consist of small, leaflike structures (not true leaves, which contain vascular tissue) arranged spirally or alternately around a stemlike axis (figure 30.8); the axis is anchored to its substrate by means of rhizoids. Each rhizoid consists of several cells that absorb water, but not nearly the volume of water that is absorbed by a vascular plant root.

Moss leaflike structures have little in common with leaves of vascular plants, except for the superficial appearance of the green, flattened blade and slightly thickened midrib that runs lengthwise down the middle. Only one cell layer thick (except at the midrib), they lack vascular strands and stomata, and all the cells are haploid.

Water may rise up a strand of specialized cells in the center of a moss gametophyte axis. Some mosses also have specialized food-conducting cells surrounding those that conduct water.

Moss reproduction

Multicellular gametangia are formed at the tips of the leafy gametophytes (figure 30.9). Female gametangia **(archegonia)** may develop either on the same gametophyte as the male gametangia **(antheridia)** or on separate plants. A single egg is produced in the swollen lower part of an archegonium, whereas numerous sperm are produced in an antheridium.

When sperm are released from an antheridium, they swim with the aid of flagella through a film of dew or rainwater to the archegonia. One sperm (which is haploid) unites with an egg (also haploid), forming a diploid zygote. The zygote divides by mitosis and develops into the sporophyte, a slender, basal stalk with a swollen capsule, the *sporangium*, at its tip. As the sporophyte develops, its base is embedded in gametophyte tissue, its nutritional source.

Figure 30.8 A hair-cup moss, *Polytrichum* (phylum Bryophyta). The leaflike structures belong to the gametophyte. Each of the yellowish-brown stalks with a capsule, or sporangium, at its summit is a sporophyte.

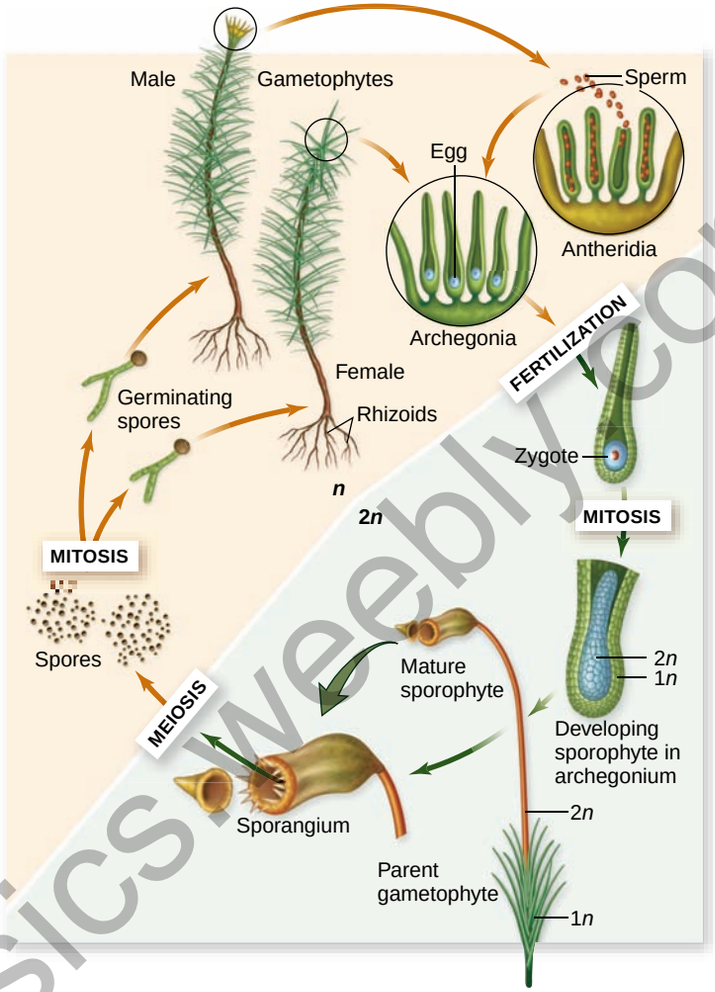


Figure 30.9 Life cycle of a typical moss. The majority of the life cycle of a moss is in the haploid state. The leafy gametophyte is photosynthetic, but the smaller sporophyte is not and is nutritionally dependent on the gametophyte. Water is required to carry sperm to the egg.

The sporangium is often cylindrical or club-shaped. Spore mother cells within the sporangium undergo meiosis, each producing four haploid spores. In many mosses at maturity, the top of the sporangium pops off, and the spores are released. A spore that lands in a suitable damp location may germinate and grow, using mitosis, into a threadlike structure, which branches to form rhizoids and "buds" that grow upright. Each bud develops into a new gametophyte plant consisting of a leafy axis.

Moss distribution

In the Arctic and the Antarctic, mosses are the most abundant plants. The greatest diversity of moss species, however, is found in the tropics. Many mosses are able to withstand prolonged periods of drought, although mosses are not common in deserts.

Most mosses are highly sensitive to air pollution and are rarely found in abundance in or near cities or other areas with high levels of air pollution. Some mosses, such as the peat mosses *(Sphagnum)*, can absorb up to 25 times their weight in

water and are valuable commercially as a soil conditioner or as a fuel when dry.

The moss genome

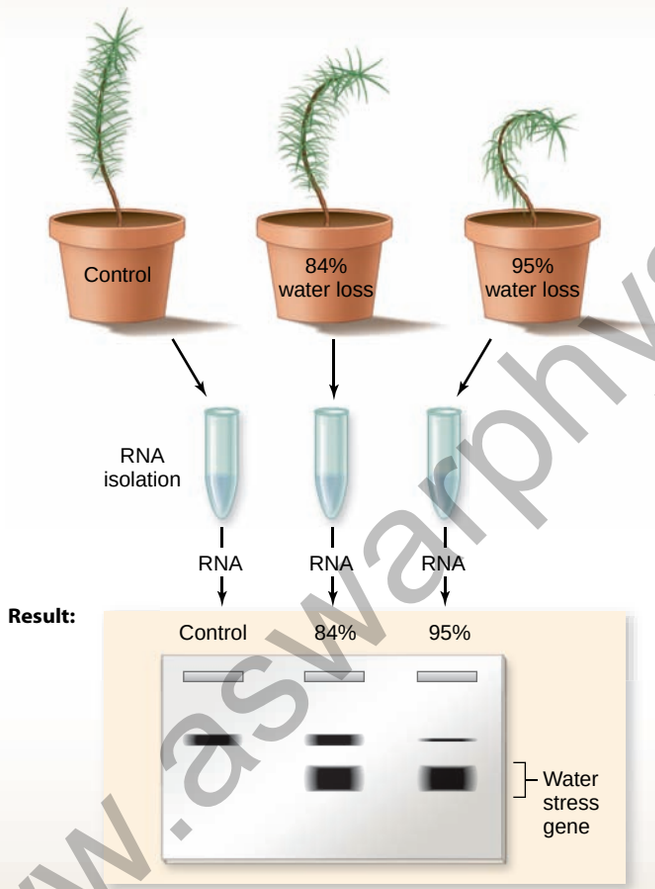
Moss plants can survive extreme water loss—an adaptive trait in the early colonization of land that has been lost from vegetative tissues of tracheophytes (figure 30.10). Desication tolerance and phylogenetic position were among the traits that led researchers to sequence the genome of the moss *Physcomitrella patens* as being the first streptophyte that is not a seed plant. Although the moss genome is a single genome bracketed by *Chlamydomonas* and the flowering plant *Arabidopsis*, many evolutionary hints are hidden within it. Evidence indicates the loss of genes associated with a watery life, including flagellar arms, which have completely vanished in the flowering plants. Genes associated with tolerance of terrestrial stresses, including temperature and water availability, are absent in *Chlamydomonas* and present in moss. The genome data add rich sets of traits to be used in phylogenetic analyses.

SCIENTIFIC THINKING

Hypothesis: *Desiccation tolerance genes in moss and flowering plants first appeared in a common ancestor.*

Prediction: *The late embryogenesis abundant (LEA) protein gene, a desiccation tolerance gene, from flowering plants will be expressed in moss plants when they experience severe water loss.*

Test: *Isolate RNA from moss plants that have not been water stressed (control), have been dehydrated to 84% water loss, and have been dehydrated to 95% water loss. Load a gel with equal amounts of RNA from each treatment. Probe the gel with a cDNA sequence for the LEA gene that is labeled.*



Conclusion: *Moss and flowering plants share a gene that is expressed under water stress conditions.*

Further Experiments: *Are other stress genes shared by bryophytes and flowering plants? Repeat the experiment with other stress-induced genes.*

Figure 30.10 Moss and flowering plants share desication tolerance genes.

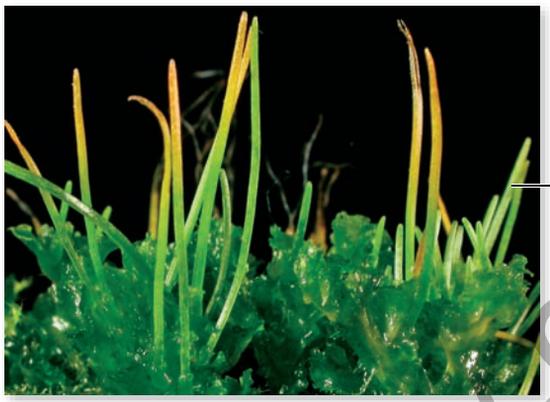


Figure 30.11 Hornworts (phylum Anthocerotophyta). Hornwort sporophytes are seen in this photo. Unlike the sporophytes of other bryophytes, most hornwort sporophytes are photosynthetic.

Hornworts developed stomata

The origin of hornworts (phylum Anthocerotophyta) is a puzzle. They are most likely among the earliest land plants, yet the earliest hornwort fossil spores date from the Cretaceous period (65 to 145 MYA), when angiosperms were emerging.

The small hornwort sporophytes resemble tiny green broom handles or horns, rising from filmy gametophytes usually less than 2 cm in diameter (figure 30.11). The sporophyte base is embedded in gametophyte tissue, from which it derives some of its nutrition. However, the sporophyte has stomata to regulate gas exchange, is photosynthetic, and provides much of the energy needed for growth and reproduction. Hornwort cells usually have a single large chloroplast.

Learning Outcome Review 30.3

The bryophytes exhibit adaptations to terrestrial life. Moss adaptations include rhizoids to anchor the moss body and to absorb water, and water-conducting tissues. Mosses are found in a variety of habitats, and some can survive droughts. Hornworts developed stomata that can open and close to regulate gas exchange.

- ***What might account for the abundance of mosses in the Arctic and Antarctic?***

30.4 Tracheophyte Plants: Roots, Stems, and Leaves

Learning Outcomes

1. *Explain the evolutionary significance of tracheids.*
2. *Analyze the claim that roots, stems, and leaves are evolutionary innovations unique to tracheophytes.*

The first tracheophytes with a relatively complete record belonged to the phylum Rhyniophyta. We are not certain what the earliest of these vascular plants looked like, but fossils of *Cooksonia* provide some insight into their characteristics (figure 30.12).

Cooksonia, the first known vascular land plant, appeared in the late Silurian period about 420 MYA, but is now extinct. It was successful partly because it encountered little competition as it spread out over vast tracts of land. The plants were only a few centimeters tall and had no roots or leaves. They consisted of little more than a branching axis, the branches forking evenly and expanding slightly toward the tips. They were **homosporous** (producing only one type of spore). Sporangia formed at branch tips. Other ancient vascular plants that followed evolved more complex arrangements of sporangia.

Vascular tissue allows for distribution of nutrients

Cooksonia and the other early plants that followed it became successful colonizers of the land by developing efficient water- and food-conducting systems called *vascular tissues*. These tissues consist of strands of specialized cylindrical or elongated cells that form a network throughout a plant, extending from near the tips of the roots, through the stems, and into true leaves, defined by the presence of vascular tissue in the blade. One type of vascular tissue, **xylem,** conducts water and dissolved minerals upward from the roots; another type of tissue, **phloem,** conducts sucrose and hormones throughout the plant. Vascular tissue enables enhanced height and size in the tracheophytes. It develops in the sporophyte, but (with a few exceptions) not in the gametophyte. (Vascular tissue structure is discussed more fully in chapter 38.) A cuticle and stomata are also characteristic of vascular plants.

Figure 30.12 ***Cooksonia*, the first known vascular land plant.** This fossil represents a plant that lived some 420 MYA. *Cooksonia* belongs to phylum Rhyniophyta, consisting entirely of extinct plants. Its upright, branched stems, which were no more than a few centimeters tall, terminated in sporangia, as seen here. It probably lived in moist environments such as mudflats, had a resistant cuticle, and produced spores typical of vascular plants.

Inquiry question

Explain why tracheophytes may have had a selective advantage during the evolution of land plants.

Tracheophytes include seven extant phyla grouped in three clades

Three clades of vascular plants exist today: (1) lycophytes (club mosses), (2) pterophytes (ferns and their relatives), and (3) seed plants. Advances in molecular systematics have changed the way we view the evolutionary history of vascular plants. Whisk ferns and horsetails were long believed to be distinct phyla that were transitional between bryophytes and vascular plants. Phylogenetic evidence now shows they are the closest living relatives to ferns, and they are grouped as pterophytes.

Tracheophytes dominate terrestrial habitats everywhere, except for the highest mountains and the tundra. The haplodiplontic life cycle persists, but the gametophyte has been reduced in size relative to the sporophyte during the evolution of tracheophytes. A similar reduction in multicellular gametangia has occurred as well.

Stems evolved prior to roots

Fossils of early vascular plants reveal stems, but no roots or leaves. The earliest vascular plants, including *Cooksonia*, had transport cells in their stems, but the lack of roots limited the size of these plants.

Roots provide structural support and transport capability

True roots are found only in the tracheophytes. Other, somewhat similar structures enhance either transport or support in non-tracheophytes, but only roots have a dual function—providing both transport and support. Lycophytes diverged from other tracheophytes before roots appeared, based on fossil evidence. It appears that roots evolved at least two separate times.

Leaves evolved more than once

Leaves increase surface area of the sporophyte, enhancing photosynthetic capacity. Lycophytes have single vascular strands supporting relatively small leaves called lycophylls. True leaves, called euphylls, are found only in ferns and seed plants, having distinct origins from lycophylls (figure 30.13). Lycophylls may have resulted from vascular tissue penetrating small, leafy protuberances on stems. Euphylls most likely arose from branching stems that became webbed with leaf tissue.

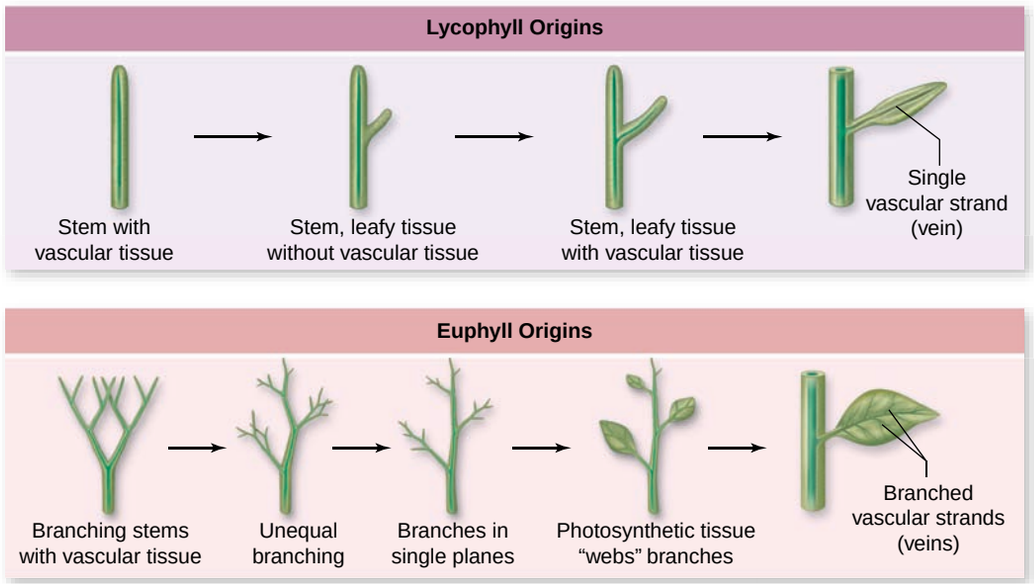


Figure 30.13 Evolution of leaves.

About 400 million years separates the appearance of vascular tissue and the wide euphyll leaf—a curiously large amount of time. The current hypothesis is that a 90% drop in atmospheric CO_2 360 MYA allowed for the increase in leaf size because of an increase in the number of stomata on a leaf. Large, horizontal leaves capture 200% more radiation than thin, axial leaves. Although beneficial for photosynthesis, larger leaves correspondingly increase leaf temperature, which can be lethal. Stomatal openings in the leaf enhance the movement of water out of the leaf, thereby cooling it. The density of stomata on leaf surfaces correlates with CO_2 concentration, as the stomatal openings are essential for gas exchange. As the atmospheric CO_2 levels dropped, plants could not obtain sufficient CO_2 for photosynthesis. In the low-CO_2 atmosphere, natural selection favored plants with higher stomatal densities. Higher stomatal densities favored larger leaves with a photosynthetic advantage that did not overheat. Leaves up to 120 mm wide and 160 mm long have been identified in the fossil record from that time period.

Seeds are another innovation in some phyla

Seeds are highly resistant structures well suited to protecting a plant embryo from drought and to some extent from predators. In addition, almost all seeds contain a supply of food for the young plant. Lycophytes and pterophytes do not have seeds.

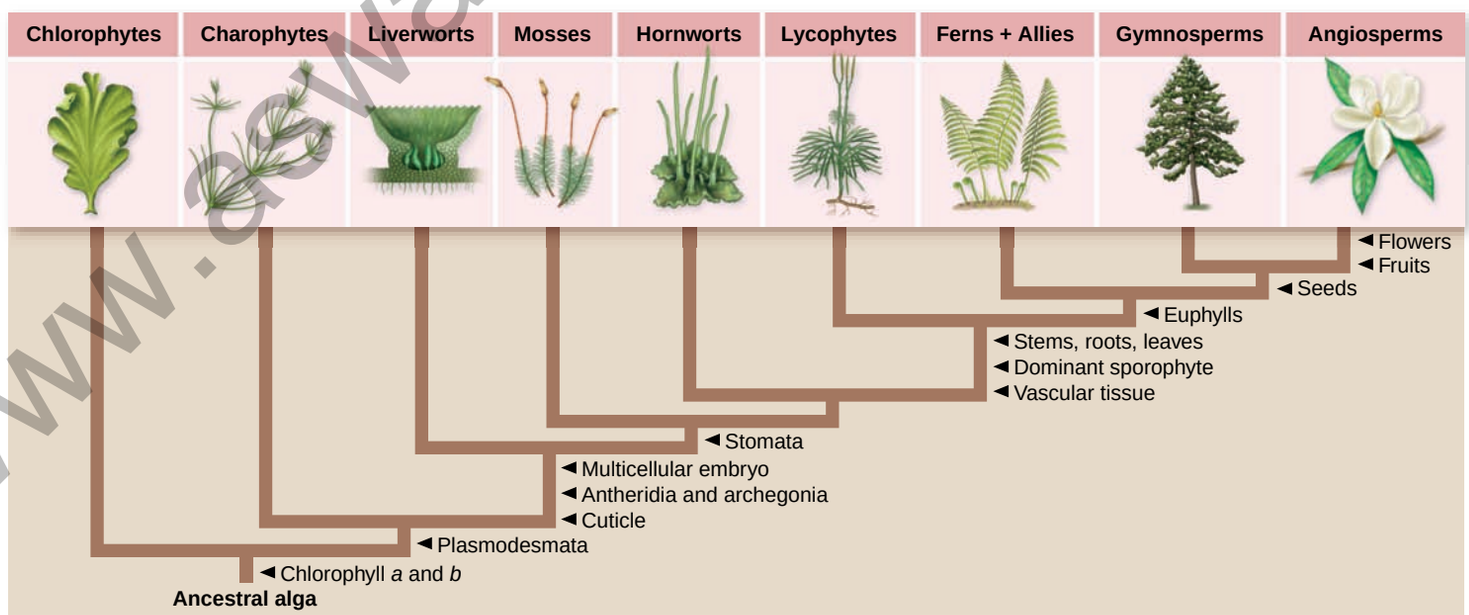
Fruits in the flowering plants (angiosperms) add a layer of protection to seeds and attract animals that assist in seed dispersal, expanding the potential range of the species. Flowers allow plants to secure the benefits of wide outcrossing in promoting genetic diversity. Before moving on to the specifics of lycophytes and pterophytes, review the evolutionary history of terrestrial innovations in the land plants illustrated in figure 30.14. The advantages conferred by seeds have led to the current dominance of seed plants in terrestrial environments.

Learning Outcomes Review 30.4

Most tracheophytes have well-developed vascular tissues, including tracheids, that enable efficient delivery of water and nutrients throughout the organism. They also exhibit specialized roots, stems, leaves, cuticles, and stomata. Many produce seeds, which protect and nourish embryos.

- ***Why would vascular tissue be prevalent in the sporophyte, but not the gametophyte, generation?***

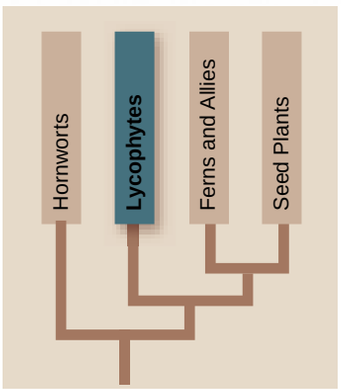
Figure 30.14 Land plant innovations.



30.5 Lycophytes: Dominant Sporophyte Generation and Vascular Tissue

Learning Outcome

1. *Explain features that differentiate lycophytes from bryophytes.*



The earliest vascular plants lacked seeds. Members of four phyla of living vascular plants also lack seeds, as do at least three other phyla known only from fossils. As we explore the adaptations of the vascular plants, we focus on both reproductive strategies and the advantages of increasingly complex transport systems.

The lycophytes (club mosses) are relic species of an ancient past when vascular plants first evolved (figure 30.15). They are the sister group to all vascular plants. Several genera of club mosses, some of them treelike, became extinct about 270 MYA. Today, club mosses are worldwide in distribution but are most abundant in the tropics and moist temperate regions.

Members of the 12 to 13 genera and about 1150 living species of club mosses superficially resemble true mosses, but once their internal vascular structure and reproductive processes became known, it was clear that they are unrelated to mosses. The sporophyte stage is the dominant (obvious) stage; sporophytes have leafy stems that are seldom more than 30 cm long.

Figure 30.15 A club moss. *Selaginella moellendorffii's sporophyte generation* grows on moist forest floors.

Selaginella moellendorffii is a lycophyte whose genome is now being analyzed. Comparisons with the moss genome will help us understand more about genes that are important in a dominant sporophyte generation and in the evolution of vascular tissue. Are the genes new or were they co-opted from the gametophyte generation?

Learning Outcome Review 30.5

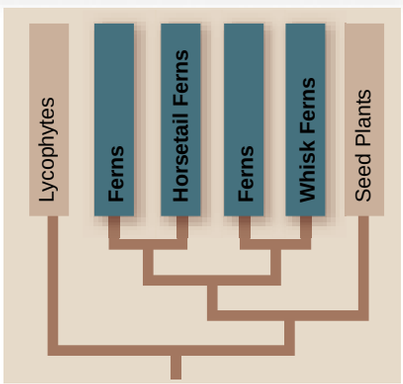
Lycophytes are basal to all other vascular plants. Although they superficially resemble bryophytes, they contain tracheid-based vascular tissues, and their reproductive cycle is like that of other vascular plants; however, they lack vascularized leaves.

- *What events might have contributed to the extinction of large club mosses 270 MYA?*

30.6 Pterophytes: Ferns and Their Relatives

Learning Outcomes

1. *List the features exhibited by pterophytes.*
2. *Contrast pterophyte and moss sporophytes.*



The phylogenetic relationships among ferns and their near relations are still being sorted out. A common ancestor gave rise to two clades: One clade diverged to produce a line of ferns and horsetails; the other diverged to yield another line of ferns and whisk ferns—ancient-looking plants.

Whisk ferns and horsetails are close relatives of ferns. Like lycophytes and bryophytes, they all form antheridia and archegonia. Free water is required for the process of fertilization, during which the sperm, which have flagella, swim to and unite with the eggs. In contrast, most seed plants have nonflagellated sperm.

Whisk ferns lost their roots and leaves secondarily

In whisk ferns, which occur in the tropics and subtropics, the sporophytic generation consist merely of evenly forking green stems without roots (figure 30.16). The two or three species of

Figure 30.16
A whisk fern. Whisk ferns have no roots or leaves. The green, photosynthetic stems have yellow sporangia attached.

Figure 30.17
A horsetail, *Equisetum telmateia*. This species forms two kinds of erect stems; one is green and photosynthetic, and the other, which terminates in a spore-producing "cone," is mostly light brown.

the genus *Psilotum* do, however, have tiny, green, spirally arranged flaps of tissue lacking veins and stomata. Another genus, *Tmesipteris*, has more leaflike appendages. Currently, systematists believe that whisk ferns lost leaves and roots when they diverged from others in the fern lineage.

Given the simple structure of whisk ferns, it was particularly surprising to discover that they are monophyletic with ferns. The gametophytes of whisk ferns are essentially colorless and are less than 2 mm in diameter, but they can be up to 18 mm long. They form symbiotic associations with fungi, which furnish their nutrients. Some develop elements of vascular tissue and have the distinction of being the only gametophytes known to do so.

Horsetails have jointed stems with brushlike leaves

The 15 living species of horsetails are all homosporous. They constitute a single genus, *Equisetum*. Fossil forms of *Equisetum* extend back 300 million years to an era when some of their relatives were treelike. Today, they are widely scattered around the world, mostly in damp places. Some that grow among the coastal redwoods of California may reach a height of 3 m, but most are less than a meter tall (figure 30.17).

Horsetail sporophytes consist of ribbed, jointed, photosynthetic stems that arise from branching underground *rhizomes* with roots at their nodes. A whorl of nonphotosynthetic, scalelike leaves emerges at each node. The hollow stems have silica deposits in the epidermal cells of the ribs, and the interior parts of the stems have two sets of vertical, tubular canals. The larger outer canals, which alternate with the ribs, contain air, while the smaller inner canals opposite the ribs contain water. Horsetails are also called scouring rushes because pioneers of the American West used them to scrub pans.

Ferns have fronds that bear sori

Ferns are the most abundant group of seedless vascular plants, with about 11,000 living species. Recent research indicates that they may be the closest relatives to the seed plants.

The fossil record indicates that ferns originated during the Devonian period about 350 MYA and became abundant and varied in form during the next 50 million years. Their apparent ancestors were established on land as much as 375 MYA. Rainforests and swamps of lycopsid and fern trees growing in the Eastern United States and Europe over 300 MYA formed the coal currently being mined. Today, ferns flourish in a wide range of habitats throughout the world; however, about 75% of the species occur in the tropics.

The conspicuous sporophytes may be less than a centimeter in diameter (as in small aquatic ferns such as *Azolla*), or more than 24 m tall, with leaves up to 5 m or longer in the tree ferns (figure 30.18). The sporophytes and the much smaller

Figure 30.18
A tree fern (phylum Pterophyta) in the forests of Malaysia. The ferns are by far the largest group of seedless vascular plants.