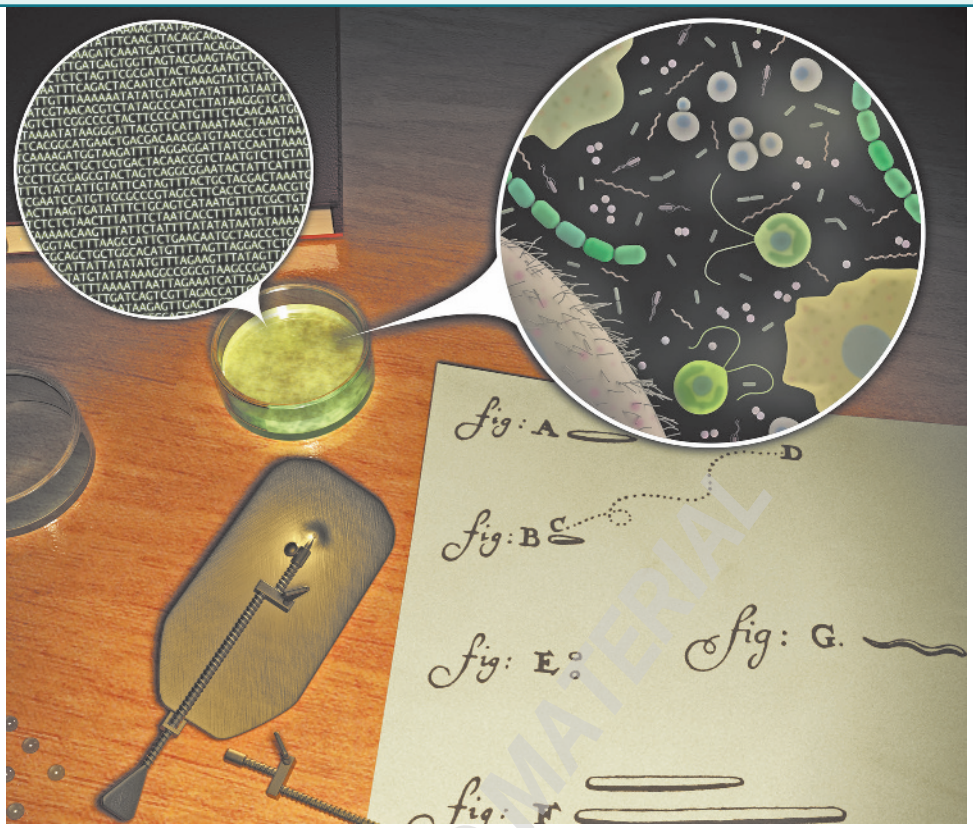


Copyright © 2013 John Wiley & Sons, Inc. All rights reserved.

The Microbial World

COPYRIGHTED MATERIAL

Anton van Leeuwenhoek was a successful textile merchant in the city of Delft, in the Netherlands, in the late seventeenth century. He used magnifying lenses in his trade to examine cloth, but in 1665, after reading Robert Hooke's book *Micrographia*, van Leeuwenhoek became fascinated with using microscopes to explore the natural world. Hooke, an Englishman of about the same age as van Leeuwenhoek, had laboriously constructed microscopes that magnified objects roughly 30 times and used them to examine the fine structure of materials both living and dead. His greatest contribution to biology was the discovery of cells, which he first observed in cork slices, as the units from which living organisms are assembled. Hooke's writings inspired van Leeuwenhoek, who enjoyed blowing glass and grinding tiny lenses, to fabricate simple but remarkably powerful microscopes. Some of the 400 or so microscopes that van Leeuwenhoek built magnified images almost 300-fold, and could be used to observe objects one-tenth the size that Hooke had seen. If we consider that the best modern light microscopes of today are limited to around 1000-fold magnification, van Leeuwenhoek's accomplishments are even more astounding!

With his extraordinary lenses, van Leeuwenhoek pushed the frontiers of human knowledge to ever-smaller dimensions. No one had imagined living creatures so small they could not be seen by the human eye, yet van Leeuwenhoek saw them all around us, on us, even inside us. In a letter to the Royal Society of London in 1684, he related that:

> The number of these Animals in the scurf of a man's Teeth, are so many, that I believe they exceed the number of Men in a kingdom. For upon the examination of a small parcel of it, no thicker than a Horse-hair, I found too many living Anima's therein, that I guess there might have been 1000 in a quantity of matter no bigger then the 1/100 part of a sand.

In another letter, he confided with amazement that:

> I then most always saw, with great wonder, that in the said matter there were many very little living animalcules, very prettily a-moving.

Thus, this modest Dutch merchant revealed a whole new "microscopic" world to humanity. Van Leeuwenhoek discovered microorganisms.

CHAPTER NAVIGATOR

As you study the key topics, make sure you review the following elements:

Microbiology involves the study of bacteria, archaea, eukaryal microorganisms, and viruses.

- Table 1.1: Macromolecules in microbial cells
- Animation: Classification systems
- Mini-Paper: The Three Domains of Life
- Toolbox 1.1: Polymerase Chain Reaction Amplification of rRNA Genes

Studies of microbes provide insight into their evolution, physiology, and genetics.

- Perspective 1.1: Creating Life in the Laboratory: The Miller–Urey Experiment
- Animation: Endosymbiosis
- Figure 1.18: Glycolysis, fermentation, and aerobic respiration
- Figure 1.22: Effects of mutations

Microbes interact with the world around them in myriad ways.

- Figure 1.24: Role of microbes in the global nitrogen cycle
- Figure 1.25: Recombinant DNA techniques

Microbes remain important causes of disease throughout the world.

- Microbes in Focus 1.1: *Bacillus anthracis*: Linking a Microbe to a Disease
- Figure 1.30: Infectious disease deaths in the United States during the twentieth century
- Figure 1.32: Impact of malaria in sub-Saharan Africa

Connections FOR THIS CHAPTER:

Development of antimicrobial and antiviral drugs (Section 24.2)

Evolution of eukaryal cells through an endosymbiotic process (Section 3.4)

Oxygenic and anoxygenic photosynthesis (Section 6.7)

Epidemiology: The study of how infectious diseases spread within populations (Section 18.3)

Introduction

Roughly three-and-a half centuries ago, Anton van Leeuwenhoek described microbial life with wonder in his voice. Today, many of us are talking about microbes with fear in our voices. In a few short months, SARS-CoV-2, the virus that causes COVID-19, spread throughout the world, infecting millions of people, overwhelming healthcare systems, and crippling the global economy. However, despite the tragedies associated with this virus, we know more about it than van Leeuwenhoek ever could have imagined. Today's electron microscopes can magnify objects 500,000-fold, a far cry from the 300-fold magnification achieved by van Leeuwenhoek. With improved sequencing techniques and bioinformatics tools, we can analyze and compare the genetic material of microbes. Carefully planned experiments allow us to understand how microbes replicate, and evaluate the effectiveness of antimicrobial drugs. Advancements in the field of microbiology truly have been remarkable.

From all of this information, we now know that microbes are enormously abundant and diverse, that they appeared billions of years before humans, and that the health of the entire biosphere depends on its tiniest microbial inhabitants. We also know that microbes interact with each other and with multicellular organisms, including humans, in many ways. Because of our increased understanding of microbes, we now can use them to help us in many agricultural and industrial settings. Additionally, we now better understand how our own bodies work. We also have learned to fear microbes; some of them, like SARS-CoV-2, cause diseases that have resulted in the suffering and death of untold millions of people through the ages.

Throughout this book, we will explore all of these aspects of microbiology. While we will use specific examples to illustrate our points, we will focus on the general principles. We will emphasize the relationships between microbes and the evolutionary history of biological processes. We will also learn how the study of microbes relates to various other disciplines, like genetics, chemistry, and environmental science. Finally, we will see that microbiology itself is a dynamic, evolving science. Our knowledge of microbiology is predicated on thoughtful, interesting, and exciting experiments. Much more still awaits our discovery. As we note throughout this book, we do not know all the answers. We probably do not even know all the questions! The field of microbiology is ever changing. Today's basic research will lead to tomorrow's revelations.

So, let's start our exploration of this dynamic field. In this chapter and in the book as a whole, we first will learn about the microbes. Then, we will examine the physiology and genetics of microbes and think about how we can cultivate them. Next, we will look at the how microbes interact with their environment and how we can utilize them. Finally, we

will explore the role of microbes in disease. We will frame our initial discussion around these questions:

- What is microbiology? (1.1)
- What do we know about the physiology, genetics, and cultivation of microbes? (1.2)
- How do microbes interact with the world around them? (1.3)
- How are microbes associated with disease? (1.4)

1.1 The Microbes

- What is microbiology?

Microorganisms are microscopic forms of life—organisms that are too small to see with the unaided eye. They usually consist of a single cell and include bacteria, archaea, fungi, protozoa, and algae. We will include viruses in many of our discussions as well. Viruses are not living, but they are microscopic; they use biological molecules and cellular machinery (borrowed from their host) to replicate, and they can cause infectious diseases like some microorganisms. Although viruses are not microorganisms, we can refer to them as **microbes**, a more general term that includes microorganisms and viruses. **Microbiology**, then, is the study of microbes.

Our relationship with the microbial world is complex and dynamic. On one hand, harmful bacteria, viruses, fungi, and protozoa kill millions of people each year and sicken billions. On the other hand, beneficial microbes associated with our bodies help us digest food and protect us from potentially harmful microbial invaders **(Figure 1.1)**. Some microbes cause crops to fail, while others provide essential nitrogen to plant roots through symbiotic relationships. Some microbes cause food to rot, but others carry out fermentations that produce yogurt, wine, beer, and other foods and beverages **(Figure 1.2)**.

In the past few decades, we have learned so much about the molecular machinery of life through the study of microbes, like the bacterium *Escherichia coli*. Indeed, scientists now routinely alter microbial cells to produce high-value, lifesaving

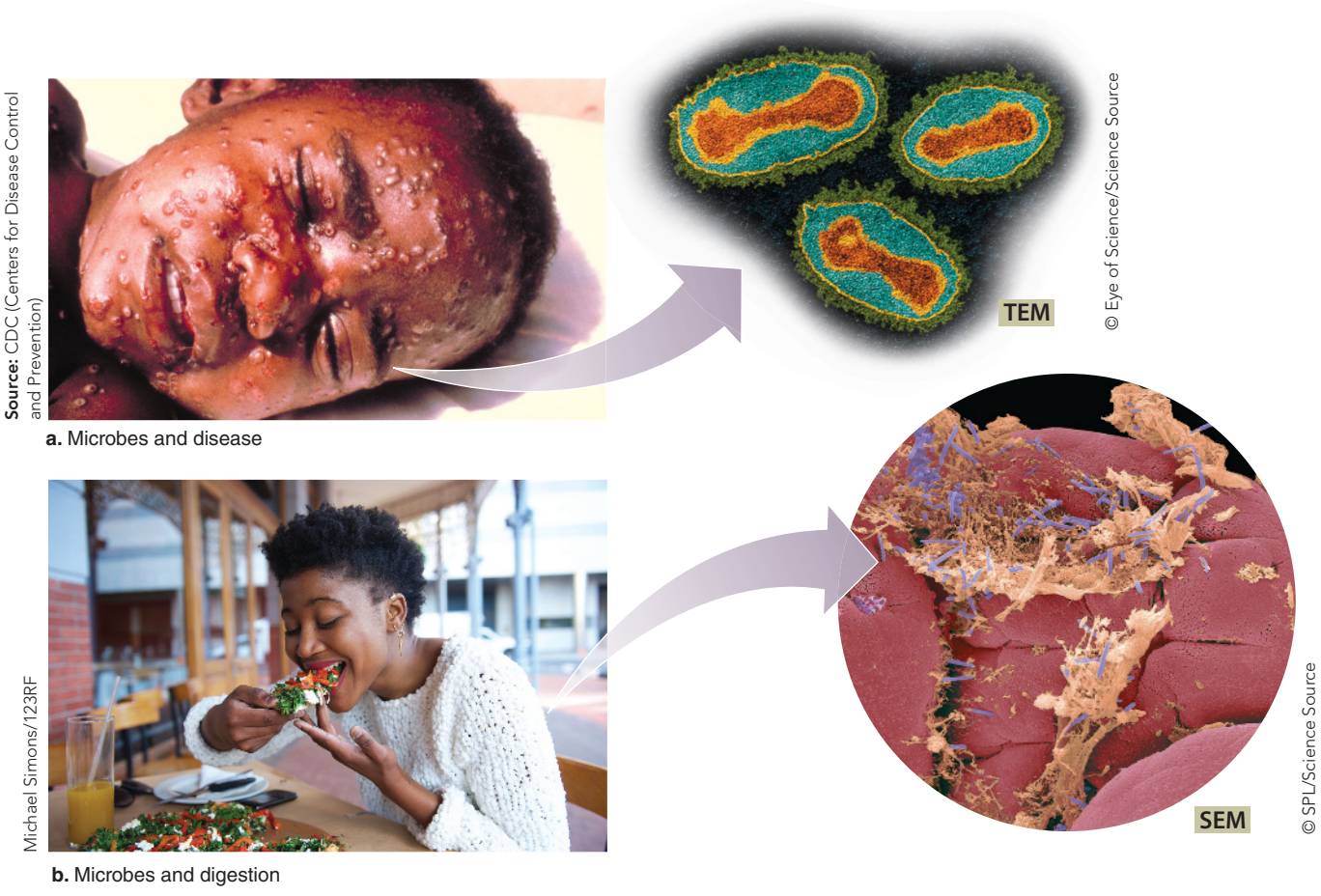


FIGURE 1.1 **Microbes and humans** **a.** Some microbes cause horrific infectious diseases, like smallpox. Man with smallpox (*left*); color-enhanced smallpox viruses (*right*). **b.** Other microbes, particularly those that reside in our gut, do not usually cause disease and help us digest the food that we eat *(left)*. Food debris (yellow) and bacteria (purple) in the small intestine (*right*).



© Nigel Cattlin/Alamy Stock Photo

a. Some microbes infect important agricultural plants.



© Inga Spence/Science Source

b. Other microbes provide nutrients to plants.



© Courtesy of Dave Wessner

c. Many microbes cause food to spoil.



© flubydust/E+/Getty Images

d. Other microbes aid in food and beverage preparation.

FIGURE 1.2 **Microbes and food** **a.** Soybean rust, a disease caused by a fungus, causes significant crop losses every year. **b.** Nitrogen-fixing bacteria interact with the roots of certain plants, forming nodules. The bacteria provide essential nutrients to plants, thereby aiding in their growth. **c.** These rotting tomatoes show growth of fungi. **d.** For centuries, humans have used microbes to help produce cheese, yogurt, wine, and beer.

medical products **(Figure 1.3)**. Whether helpful or harmful, the microbial world is deeply intertwined with our lives and with the very fabric of life on Earth. Let's begin our exploration of microbiology, then, by asking a very fundamental question. What is life?

The Basis of Life

So, what is life? This question has fascinated humans for millennia—perhaps since our ancestors first developed conscious, introspective thought. As biologists, we will focus on a practical definition of "life" that distinguishes living organisms from non-living objects.

First, living organisms are composed of **cells**, the smallest units of life as we know it. Second, living organisms are capable of:

- **Metabolism**: A controlled set of chemical reactions that extract energy and nutrients from the environment and transform them into new biological materials.
- **Growth**: An increase in the mass of biological material.
- **Reproduction**: The production of new copies of the organism.

To accomplish these tasks, organisms contain a biological instruction set to guide their actions. These instructions need to be reproduced as the organism itself reproduces. Other features that living organisms share include:

- Genetic variation, allowing the possibility of **evolution**, or inherited change within a population, through natural selection over the course of multiple generations.
- Response to external stimuli and adaptation to the local environment (within genetic and physiological constraints).
- **Homeostasis**: Active regulation of their internal environment to maintain relative constancy.

Does this list represent a complete description of what it means to be alive? Probably not. It's easy to come up with

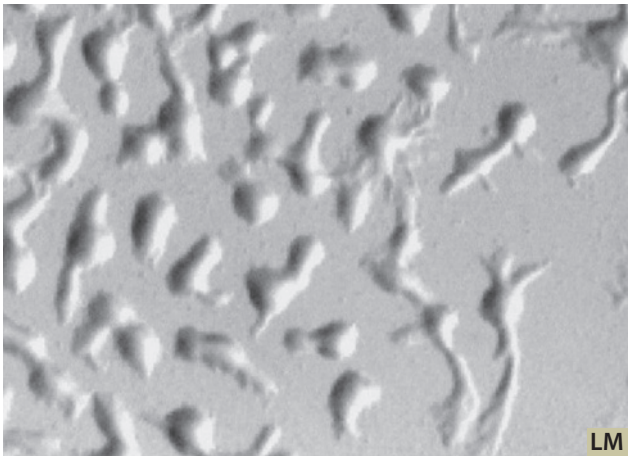


Maximilian Stock Ltd/Getty Images

FIGURE 1.3 Microbes and medicine Using recombinant DNA techniques, researchers can alter genes of microbes such that the microbes produce large quantities of medically important compounds. As we will see later in this chapter, human insulin today is produced by the bacterium *Escherichia coli*. Here, researchers monitor conditions in a large-scale bioreactor used to grow recombinant bacteria.

situations that challenge these criteria. Consider the curious case of bacterial endospores, specialized, metabolically inert cells produced by some bacterial species under highly stressful conditions. After shutting down metabolism, growth, and reproduction, endospores can remain dormant for long periods of time, even thousands of years, awaiting a favorable environment to germinate. Is an endospore alive during this state of suspended animation? These spores have all the components of living cells and, when conditions are appropriate, they will again develop into cells that meet the criteria listed above. What about *Nanoarchaeum equitans*, an archaeon that we will explore in Chapter 4? Apparently, it can survive only when in contact with a host cell. As we will see later in this section, viruses—subcellular microbes—represent an even more interesting anomaly to the standard definition of life. These microbes replicate and evolve. We could argue that they respond to external stimuli. Does the presence of these properties make them alive? Most likely, our definition of life should be applied holistically; an organism may not exhibit all of these traits at all times.

Most microorganisms live and function as single, autonomous cells. A free-living unicellular, or single-celled, organism can carry out all the necessary functions of metabolism, growth, and reproduction without physical connection to any other cells. In contrast, multicellular organisms are composed of many physically connected and genetically identical cells. The constituent cells that contribute to a multicellular organism can have distinct, specialized functions. A complex organism like a human can have hundreds of cell types organized into tissues and organs. Although the distinction between unicellular and multicellular organisms seems obvious, you might rethink this issue later as we learn more about the microbial world. Some unicellular organisms, for instance, can survive only in close association with cells of another species. Other unicellular microorganisms can communicate, behave socially, form three-dimensional structures containing millions of cells with different functions, and enter into dependent relationships with other cells. These behaviors blur the boundaries between unicellular and multicellular lifestyles. The slime mold *Dictyostelium discoideum*, for instance, exists as a rather typical unicellular organism when food is readily available. However, individual cells aggregate and form a complex structure during periods of nutrient depletion, with cells differentiating to assume specialized tasks **(Figure 1.4)**. Before we investigate these more unusual arrangements, though, let's learn more about the chemical makeup of cells.



© Courtesy Richard A. Firtel

a. Unicellular form



© Carolina Biological/Medical Images.com

b. Aggregation and differentiation

FIGURE 1.4 Developmental stages of *Dictyostelium discoideum* **a.** The slime mold *Dictyostelium discoideum* exists as a unicellular organism when food is plentiful. **b.** When its food supply becomes limited, cells aggregate in response to a cellular signal, forming a multicellular slug. Cells then begin to differentiate, eventually forming a stalk and fruiting body.

Chemical Makeup of Cells

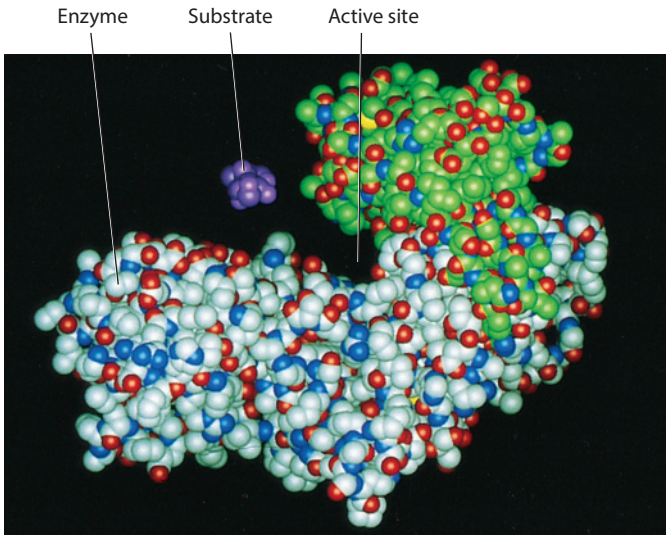
As we shall see in this section, all cells share some basic features. Notably, all cells are built from **macromolecules**, large, complex molecules composed of simpler subunits **(Table 1.1)**. Typically, macromolecules make up over 90 percent of a cell's dry weight, or the weight obtained after the removal of all water. In this section, we will explore the four major types of macromolecules found in cells: polypeptides, nucleic acids, lipids, and polysaccharides. For each, we will look briefly at their structure and functions.

Polypeptides, polymers of amino acids, constitute the most abundant class of macromolecules. Polypeptides, also often referred to as proteins, fold into elaborate structures and can execute a vast array of important jobs. Some proteins function as **enzymes**, macromolecules that catalyze chemical reactions within the cell **(Figure 1.5)**. Other proteins may facilitate the movement of material into or out of the cell. Still other proteins comprise critical structures such as microfilaments that facilitate cell movement **(Table 1.2)**.

Nucleic acids, polymers of nucleotides, make up most of the remainder of the macromolecules within a cell. This category includes deoxyribonucleic acid (DNA), a polymer of deoxyribonucleotides, and ribonucleic acid (RNA), a polymer of ribonucleotides. Individual nucleotides are composed of a sugar molecule (deoxyribose in DNA, ribose in RNA), a phosphate moiety, and one of four nitrogen-containing bases (abbreviated A, T, C, and G in DNA; A, U, C, and G in RNA). In all cells, DNA constitutes the main informational molecule, containing instructions for the production of RNA molecules. These RNA molecules fulfill numerous functions within the cell, most of which are associated with protein production.

Lipids, hydrophobic hydrocarbon molecules, represent another important class of macromolecules. The primary role of lipids in most cells is to form the foundation of the plasma membrane, a barrier surrounding the cell that, quite simply, separates inside from outside. This membrane restricts the movement of materials into and out of the cell, thereby allowing the cell to capture and concentrate nutrients for metabolism and growth, and prevent the products of metabolism from escaping **(Figure 1.6)**.

Polysaccharides are polymers of monosaccharides, or sugars. These molecules are composed entirely of carbon, hydrogen, and oxygen, with the general formula of $C_m(H_2O)_n$. Some polysaccharides serve as energy storage molecules. Starch and glycogen, for instance, are both polymers of the monosaccharide glucose ($C_6H_{12}O_6$). Other polysaccharides serve as structural molecules. Cellulose, the primary



© Courtesy T. A. Steitz, Yale University

FIGURE 1.5 Structure and function of enzymes In cells, many polypeptides function as enzymes, macromolecules that can catalyze chemical reactions. The function of the enzyme depends on its structure. The three-dimensional shape creates an active site with which the substrate interacts.

TABLE 1.1 Macromolecules in microbial cells

Macromolecule	Subunits	Functions	Dry weight of cell (%)
Polypeptides	Amino acids	Enzymes catalyze the vast majority of biochemical reactions in the cell. Other proteins are structural components of cells.	50–55
Nucleic acids	Deoxyribonucleotides	Informational: DNA provides the instructions for assembly and reproduction of the cell.	2–5
	Ribonucleotides	Many functions, most of which are involved in the production of polypeptides. Some serve structural or catalytic functions.	15–20
Lipids	Diverse structures	Structural: Make up cellular membranes that form physical boundaries between the inside of a cell and its surroundings and membranes of internal organelles.	10
Polysaccharides	Sugars	Structural (such as cellulose and chitin) and energy storage (such as glycogen and starch).	6–7

TABLE 1.2 Functions of selected polypeptides

Polypeptide	Location	Function
RNA polymerase	Cytoplasm of bacteria and archaea, nucleus of eukarya	Produces RNA molecules from DNA template
Glycogen phosphorylase	Cytoplasm	Conversion of glycogen into glucose monomers
K^+ channel	Plasma membrane	Passive transport of K^+ across the membrane, from an area of high concentration to an area of low concentration
Na^+/K^+ ATPase	Plasma membrane	Active transport of Na^+ and K^+ across the membrane, from areas of low concentration to areas of high concentration
Flagellin	Bacterial flagellum	Monomers polymerize to form flagellum, which aids in bacterial motility
FtsZ	Associated with plasma membrane of bacteria	Key component of cell division machinery

structural component of plant cell walls, also is a polymer of glucose monomers. Chitin, the primary structural component of fungal cell walls, consists of a derivative of glucose: *N*-acetylglucosamine. Many bacterial and archaeal cells use other polysaccharides for their cell walls.

The Domains of Life

Although polypeptides, nucleic acids, lipids, and polysaccharides exist in all living organisms, major groups of organisms also differ in substantial ways. Today, we categorize all living organisms and, by extension, their cells, into three domains: *Bacteria, Archaea*, and *Eukarya*. Until the late 1900s, however, biologists divided cells into only two types: **prokaryotes** and **eukaryotes (Figure 1.7)**. The term *eukaryote* is derived from Greek roots meaning "true kernel," in contrast to the term *prokaryote*, which translates as "before kernel." The "kernel" refers to the membrane-enclosed nucleus of eukaryal cells. The nucleus contains the genetic material of the eukaryal cell during most of the cell cycle and was clearly visible to microscopists in the 1800s. Its function as the organizer of the hereditary material was not understood until well into the 1900s.

Prokaryotes and eukaryotes also differ strikingly in the organization of their genetic material. Prokaryotes usually contain a single circular chromosomal DNA molecule. In contrast, eukaryotes usually contain multiple linear DNA molecules. At some point in their life cycle, most eukaryal organisms have two copies, or a 2*n* complement, of their genetic material. Most prokaryotes, in contrast, possess a single copy of their genetic material.

Conventional wisdom through the better part of the twentieth century stated that prokaryotes represented a fairly uniform group, until scientists started looking in more detail at the molecular machinery for the most ancient, important, and conserved processes in cells—the synthesis of DNA, RNA, and polypeptides. In the 1970s, microbiologists studying some prokaryotes noted that their molecular machinery resembled that of eukaryotes more than it did

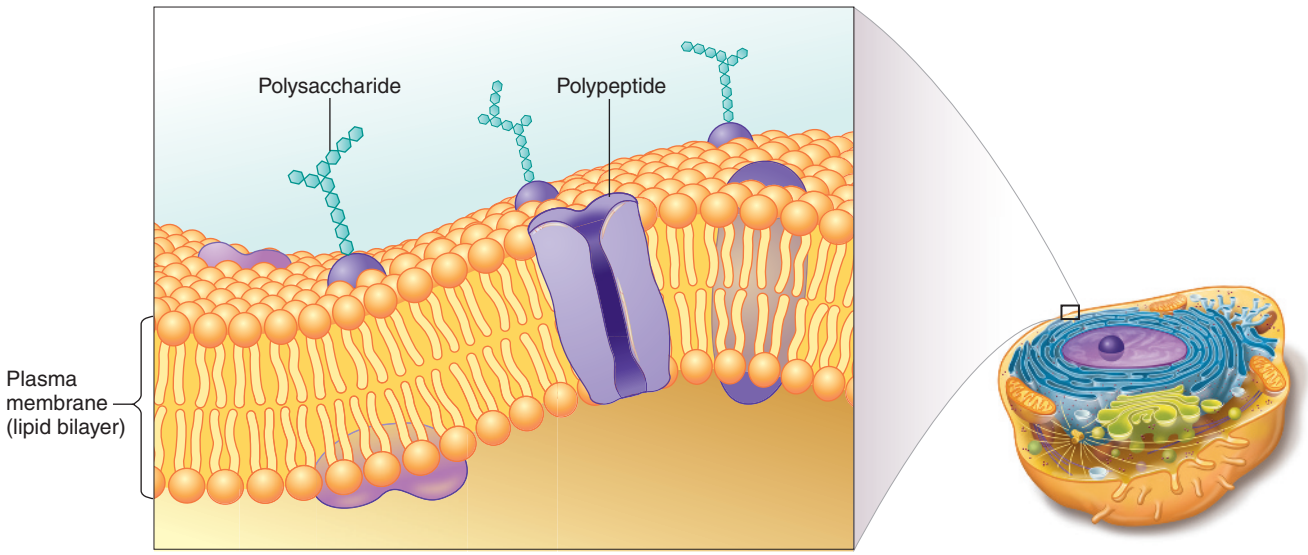
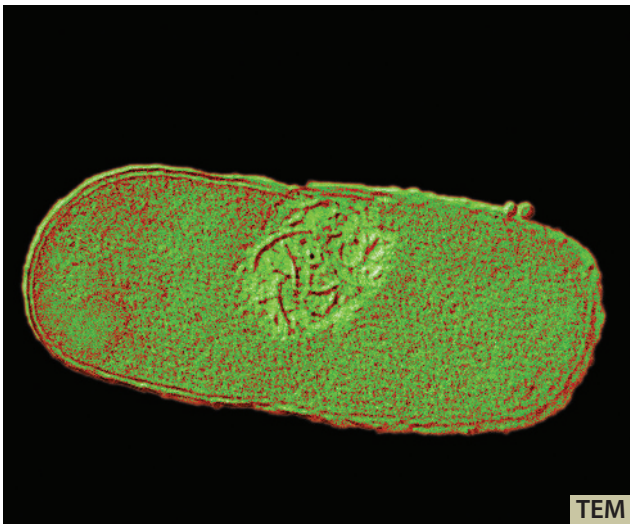
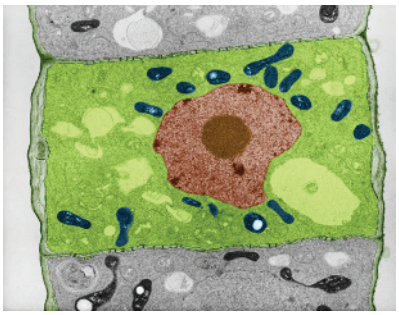


FIGURE 1.6 Plasma membrane All cells are enclosed within a lipid-based membrane that compartmentalizes the cell, allowing the composition of the inside and outside to differ. In most organisms, the membrane consists of a lipid bilayer. However, this membrane is not impervious. Various polypeptides and polysaccharides are associated with the membrane. These macromolecules help the cell control the movement of materials into and out of the cell.



© Dr. Klaus Boller/Science Source



© David M. Phillips/Science Source

a. Prokaryotic cell **b.** Eukaryotic cell

FIGURE 1.7 Prokaryotic and eukaryotic cells **a.** Prokaryotic cells, as shown in this colorized micrograph of *Escherichia coli*, lack a membrane-bound nucleus. They include organisms in the domains *Bacteria* and *Archaea*. **b.** Eukaryotic cells contain a membrane-bound nucleus, as shown in purple in this artist's rendition of a plant cell. Until the latter decades of the twentieth century, biologists divided all cells into these two main types: prokaryotes and eukaryotes. Today, we recognize that all living organisms really should be divided into three categories, or domains: *Bacteria*, *Archaea*, and *Eukarya*. Eukarya contain nuclei. Bacteria and archaea do not.

other prokaryotes. Leading the way in these studies was Dr. Carl Woese of the University of Illinois. Woese focused his attention on the structure and sequence of one of the RNA molecules that serves as a scaffold for assembly of the ribosome—the small subunit (SSU) ribosomal RNA. This molecule is a critical component of the ribosome in all living organisms and interacts with the messenger RNA during translation (see Section 8.4). His work paved the way for a revolution in thinking about the **phylogeny**, or evolutionary history, of organisms **(Figure 1.8)**. His studies also led to a major revision in the taxonomy, or the classification, of living organisms. Because of Woese's work, we now categorize all living organisms into one of three domains: *Bacteria*, *Archaea*, or *Eukarya* **(Mini-Paper)**.

Animation: Classification Systems

However, even this classification scheme may soon change. Today, some microbiologists argue that there are two domains of life—*Bacteria* and *Archaea*. Eukarya, they postulate, exist within the archaeal domain (see Section 4.3).

Thanks largely to the development of the **polymerase chain reaction (PCR)**, a technique that allows researchers to quickly amplify specific pieces of DNA **(Toolbox 1.1)** and the development of rapid, cost-effective DNA sequencing techniques, we now have a richer and more accurate phylogenetic tree. This tree is consistent with the idea that the archaeal and eukaryal domains shared a common ancestor after they split from the bacterial domain. It probably is impossible, though, to determine when the divergence of these lineages actually occurred; microorganisms do not fossilize well. That said, fossilized stromatolites, mineralized mats built up by layer upon layer of photosynthetic bacteria and other microbes in shallow marine habitats, have been observed in rock formations nearly 3.5 billion years old (see Figure 1.13). If such elaborate microbial communities, including bacteria capable of photosynthesis (see Section 2.4), existed 3.5 billion years ago, then the split between *Bacteria* and the *Archaea*/*Eukarya* domains probably occurred much earlier.

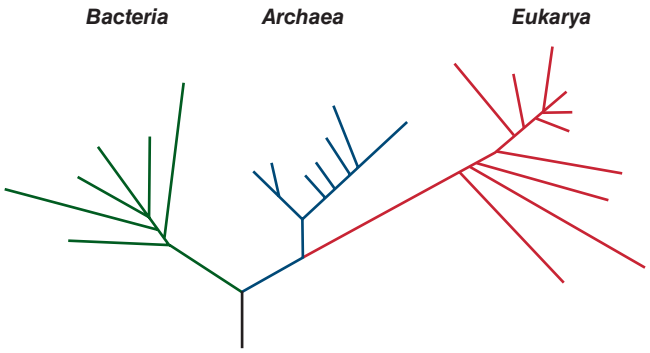


FIGURE 1.8 Phylogenetic tree of life By comparing the sequences of small subunit (SSU) ribosomal RNA gene sequences, researchers now classify all living organisms into one of three domains: *Bacteria*, *Archaea*, or *Eukarya*. In this phylogenetic tree, bacteria are shown in green, archaea are shown in blue, and eukarya are shown in red. The linear distance between the endpoints of any two lines is proportional to the sequence similarity of the SSU rRNA gene sequences from the organisms corresponding to the endpoints. Sequence similarity reflects evolutionary distance.

Mini-Paper: A Focus on the Research

The Three Domains of Life

Source: Based on C. R. Woese, O. Kandler, and M. L. Wheelis. 1990. Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87: 4576–4579.

Context

Aristotle categorized life into just two fundamental groups, animals and plants, and this categorization persisted until the dawn of microbiology as a science. In 1868, two centuries after van Leeuwenhoek's discovery of microbial life, German biologist Ernst Haeckel proposed a third fundamental group, or kingdom, *Protista*, for microscopic life-forms. In 1938, Herbert Copeland suggested that microorganisms should actually be divided into two kingdoms, *Protista* and *Bacteria*, thereby recognizing the difference between eukaryotic and prokaryotic cells. Twenty years later, Robert Whittaker advocated further separation of eukaryotic microorganisms into kingdoms of *Fungi* and *Protista* but kept prokaryotic cells in a single kingdom called *Monera*. This five-kingdom taxonomic system—*Animalia*, *Plantae*, *Fungi*, *Protista*, and *Monera*—became the accepted standard for the next few decades until DNA and protein sequences became widely accessible.

Carl Woese, a microbiologist at the University of Illinois, was fascinated by a group of prokaryotes known at the time as "archaebacteria." Initially, these strange microorganisms were found primarily in marginal environments such as anoxic sediments, hypersaline ponds, and hot springs. Other than their curious ability to colonize extreme habitats, the feature of archaebacteria that generated the most interest among the wider biological community was the ability of some of these organisms to produce methane. These microorganisms remain the only organisms known to produce this gas.

To understand the phylogeny, or evolutionary history, of these methane-producing microorganisms, Woese followed the lead of Zuckerkandl and Pauling, who had first demonstrated in the 1960s that comparisons of protein sequences could reveal evolutionary relationships. If two organisms were closely related, these researchers reasoned, then the amino acid sequence of a common protein in the organisms should be very similar. Conversely, if two organisms were distantly related, then the amino acid sequence of a common protein should be more divergent. Woese and colleagues began to focus not on protein sequences, but on RNA sequences. Woese reasoned that the ribosomal RNAs (rRNAs), because of their universal presence in all cells, could be excellent molecules to compare. In bacteria, the 16S rRNA molecule is part of the small subunit of the ribosome. In eukaryotes, the equivalent ribosomal RNA is the 18S rRNA. These molecules, collectively referred to as "small subunit (SSU) rRNA" molecules, are critical in the ribosome, helping to bring together the ribosomal structure and interacting with messenger RNA. Not only are ribosomal RNAs universally distributed, but they also have the same function in all cells.

The SSU rRNA gene sequence has been referred to as a "molecular chronometer," a slowly ticking clock that measures evolutionary time. The sequence of this molecule changes very slowly because of the functional constraints on the molecule. Random mutations that occur within the gene encoding the small subunit rRNA often have serious negative consequences, so relatively few changes are passed on to subsequent generations. Nevertheless, there are enough differences in the roughly 1,600 nucleotide sequence to differentiate between species to map patterns of similarity. If one assumes that overall mutation rates are similar between species (which seems to be true with respect to rRNA genes), then one can quantify sequence differences between SSU rRNA genes in multiple species to infer relationships. Ultimately, Woese discovered that the methane-producing microorganisms were no more closely related to other bacteria than they were to the eukaryotes. They were not bacteria at all! Let's examine the scientific work that led to this conclusion.

Experiments

The 1990 Woese *et al*. paper actually presented no new experimental data. Its importance was in articulating a new view of the phylogeny of life. To understand the genesis of this idea, we should step back and examine the data. The biggest challenge Woese faced in the 1970s in developing ribosomal RNA sequences as a tool for phylogenetic analysis was the difficulty in determining such sequences. Woese and colleagues developed a laborious method to infer the sequence of 16S rRNA molecules, which they described in a 1977 article, "Comparative cataloging of 16S ribosomal ribonucleic acid: A molecular approach to procaryotic systematics," *Journal of Bacteriology*, vol. 27, pp. 44–57. First, RNA was extracted from cells. The isolated rRNA then was cut into small chunks using a ribonuclease enzyme that yields short fragments of nucleic acid usually 5–20 bases long. The sequence of each oligonucleotide was determined by further chemical and enzymatic analysis. In its original incarnation, this method did not actually yield a complete rRNA sequence, but rather a catalog of short oligonucleotide sequences present in the rRNA. Catalogs from different species then were compared.

The underlying assumption of molecular sequence comparisons is that the number of nucleotide differences between two sequences is proportional to the time since the two species diverged from a common ancestor. Species that share a more recent common ancestor will have fewer differences than species that have been separated for longer periods of time. Exactly how long ago two species separated depends on the rate at which mutations accumulate, which can be very difficult, if not impossible, to know. Fortunately, to determine phylogenetic relationships, we do not need to know *exact* times of divergence. We are just interested in *relative* times: if organisms A and B shared a common ancestor *after* they shared an ancestor with organism C, then A and B would have fewer sequence differences with each other than either would have with C. The Woese method essentially took each 16S rRNA sequence and compared it against all of the other species. A method for quantifying the similarity between sequences was developed to yield an association coefficient between 0 and 1. A perfect match between sequences would result in an association coefficient of 1, whereas no matches would give a score of 0. From these scores, a computer algorithm was used to plot the most likely phylogenetic tree.

Starting with papers in 1977 and continuing through the 1980s, Woese and colleagues built a "universal phylogenetic tree" through comparison of SSU rRNA sequences from diverse organisms, including members of each of the five kingdoms of life, as defined at that time. The great strength of this universal tree is that it compares all organisms using a common standard, a molecule they all possess. Woese noted that this universal tree supports *three* primary branches of life, not the five kingdoms previously

accepted. In the 1990 paper, Woese and his coauthors propose that these very ancient branches—*Bacteria*, *Eukarya* (or *Eucarya*, as it is spelled in the 1990 paper), and *Archaea*—be referred to as domains (see Figure 1.8).

The domain *Archaea* is composed of species previously known as archaebacteria. Though archaea lack a nucleus, they turned out to be no more similar to bacteria than they are to eukaryotic organisms, or eukarya. In fact, Woese's tree suggests that the domains *Archaea* and *Eukarya* share a more recent common ancestor than either does with the domain *Bacteria*. As we will see in Chapter 4, archaea are similar in size and shape to bacteria. Many of the enzymes used by archaea for DNA replication, transcription, and translation, however, more closely resemble the corresponding enzymes found in eukarya. Perhaps most interestingly, the plasma membrane of archaea differs chemically from the plasma membranes of bacteria or eukarya. The exact evolutionary history of these organisms is yet to be determined.

In this three-domain phylogenetic tree, *Monera* and *Protista* disappear as kingdoms. In fact, if kingdoms are to be defined by equivalent depth of branching—which implies roughly equivalent evolutionary times since divergence—rRNA gene sequence comparisons support many more kingdoms than were previously known, most of which are populated by microorganisms. As we will see in Chapter 4, Woese and coauthors proposed two kingdoms within the domain *Archaea*: *Crenarchaeota* and *Euryarchaeota*. The authors also noted the presence of heat-loving microorganisms in several other branches of life. It is quite possible, then, that the organism at the root of the tree (the last common ancestor of all life on Earth) was thermophilic, or heat-loving. We will return to this point in Section 1.2.

Impact

For the first time, biologists could create a natural taxonomic system in which all organisms are compared by the same criteria. The realization that the prokaryotes could not be united as a phylogenetic group raised many questions regarding the validity of the five-kingdom system. Not surprisingly, there was initial resistance. Many scientists challenged the validity of this approach and the computational methods on which it was based. Since Woese first conducted his experiments, there have been methodological improvements, specifically in the ability to amplify entire rRNA genes using the polymerase chain reaction (PCR; see Toolbox 1.1), followed by rapid and straightforward DNA sequencing. The basic concept of using rRNA gene sequence comparisons to derive phylogenetic relationships is now accepted as an essential method of phylogenetic analysis.

Sequence-based phylogenies have had more impact on microbiology than on any other branch of science. Since this paper was published in 1990, databases containing ribosomal RNA gene sequences have grown explosively. The universal phylogenetic tree is now much richer and more complex. Using PCR, microbiologists can characterize organisms using rRNA gene sequences even if the microorganisms cannot be grown in culture. Because the majority of microbes apparently will not grow in laboratory culture (see Section 7.2), this approach is enormously important for understanding the true diversity of life on Earth and its evolutionary history. Proposals for new bacterial and archaeal kingdoms, based on rRNA gene analysis of uncultured organisms, have appeared regularly since 1990. Classification of eukaryal microorganisms also is affected by rRNA-based phylogenetics.

Ribosomal RNA sequences do not tell the entire evolutionary story of an organism. In the last decade, the DNA sequences of hundreds of entire genomes have been determined, most of them from microorganisms. It is clear that microbes are rampant sharers of genes, which we will discuss more in Chapters 10, 11, and 21. Although it is likely that rRNA genes are rarely shared and do accurately reflect the evolutionary history of the "core" genome, large fractions of genetic material in many organisms may have distinct histories, a finding that Carl Woese could scarcely have imagined when he began his revolutionary efforts to clarify microbial taxonomy.

Questions for Discussion

1. **What features make SSU rRNA gene sequences ideal for phylogenetic studies?**
2. **What drawbacks do you see with the use of rRNA for these studies?**
3. **If we discover forms of life on another planet, would studies of rRNA gene sequences be useful for categorizing these life-forms? Explain.**

Although all cells share many features, studies have demonstrated clearly that bacteria, eukarya, and archaea are evolutionarily distinct. Some of their differences are listed in **Table 1.3**. We will discuss each of these types of cells in much more detail in Chapters 2–4.

Viruses

Are viruses alive? They are not cellular. As we noted earlier, though, they certainly replicate and evolve. Viruses, however, require host cells for replication. Outside of a host cell, virus particles are essentially inert. An isolated virus has no metabolism—it takes up no nutrients and extracts no energy from its environment. Viruses also lack most of the basic machinery needed for the synthesis of macromolecules. Viruses do not respond to stimuli, except perhaps when they bind to receptors on a new host cell, and they do not maintain internal homeostasis. When a virus enters a host cell, it does not grow and reproduce in the same sense that cellular organisms do. Virus particles are more or less completely disassembled in the host cell, and new virus particles are only assembled after the genetic material has been replicated and the host cell has synthesized new viral proteins. Cellular organisms have no comparable state of disassembly during their growth and reproduction.

Connections **Viruses infect all cellular forms of life. They replicate in various ways, but all depend on using host cell machinery for their replication. This makes them obligate intracellular parasites. We will examine viral replication in** Section 9.3.

Toolbox 1.1

Polymerase Chain Reaction Amplification of rRNA Genes

The method of ribosomal RNA sequencing developed by Carl Woese was extremely laborious. Fortunately, techniques just being developed in the 1970s and 1980s, as Woese and colleagues were initially developing the universal phylogenetic tree, made nucleic acid sequencing much simpler. The most important of these techniques was the polymerase chain reaction (PCR). With this technique, researchers can create millions of copies of a specific piece of DNA. **Animation: Polymerase chain reaction**

Kary Mullis, then a scientist at Cetus Corporation, a biotechnology company in Emeryville, California, invented PCR in 1983 and was awarded the Nobel Prize in Chemistry in 1993 for this discovery. The technique basically mirrors the process of DNA replication utilized by all cells. However, rather than replicating an entire DNA molecule, PCR results in the repeated replication, or amplification, of a small, defined segment of a larger DNA molecule. The reaction requires only a few basic reagents:

- DNA containing the sequence to be amplified
- Deoxyribonucleotides (dATP, dCTP, dTTP, and dGTP)
- DNA polymerase
- Oligonucleotide primers

The process begins with the denaturation of double-stranded DNA, making it single-stranded. This step is achieved by heating the DNA to around 95°C for a short period of time. The primers, generally 15–30 nucleotide-long pieces of single-stranded DNA synthesized in the laboratory, then bind to complementary regions on this newly denatured DNA. The primers are designed such that one primer binds to one strand of the denatured DNA while the other primer binds to the other strand of the DNA. Additionally, the two primers bind to regions of the DNA flanking the sequence to be amplified **(Figure B1.1)**.

After the primers bind, the DNA polymerase begins generating new DNA, using the denatured DNA as a template. We will see in Section 8.2 that DNA polymerases generate new DNA by attaching deoxyribonucleotides to primers bound to a template strand. Because PCR usually employs only two primers that bind on either side of a specific region of DNA, these primers delineate which segment of the original DNA molecule will be replicated. The whole process, then, involves three steps:

- Denaturation, or melting, of the DNA
- Attachment, or annealing, of the primers
- Generation of new DNA by DNA polymerase

These steps are repeated multiple times, resulting in the exponential amplification of the region bounded by the two primers. After 10 cycles, the number of these amplified DNA molecules increases over 1000-fold. After 20 cycles, the increase is over 1 million-fold, and 30 cycles will generate over 1 billion new copies!

The crucial development that made PCR widely usable was the discovery of thermostable DNA polymerases that could withstand the high temperature (> 90°C) used to separate DNA strands. The first thermostable polymerase used for PCR was Taq DNA polymerase, which came originally from the bacterium *Thermus aquaticus*, isolated from hot springs in Yellowstone National Park in the United States. Today, a variety of thermostable DNA polymerases are commercially available, several of which have been isolated from heat-loving archaea. We will discuss these fascinating microorganisms more throughout Chapter 4.

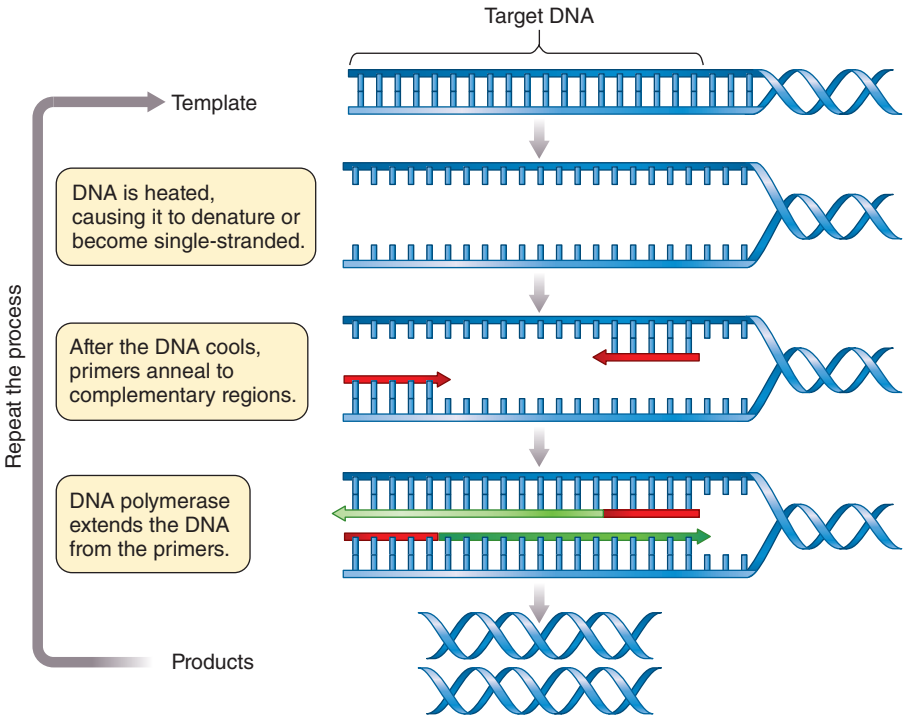


FIGURE B1.1 The polymerase chain reaction (PCR) After heating the DNA to denature, or separate, the two strands, the mixture is cooled, allowing the short, single-stranded DNA primers to anneal to their complementary regions. The DNA polymerase then extends these primers, using the opposite strand as a template. This process is repeated multiple times, resulting in the generation of many copies of the segment of DNA bounded by the primers. In this fashion, a small amount of input DNA can be amplified sufficiently to perform routine chemical analyses.

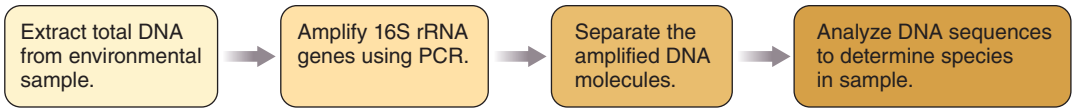


FIGURE B1.2 Use of PCR to identify microorganisms With PCR, microbial species can be identified simply by isolating a little of their DNA. As shown in this schematic, DNA can be isolated from an environmental source without isolating and growing pure cultures of specific bacterial species. By doing PCR with primers specific for the 16S rRNA gene, this region of the genome can be amplified and subsequently sequenced, thereby providing the investigator with enough information to determine which species are present.

PCR has revolutionized SSU rRNA gene sequence analysis and species identification. Today, just a bit of chromosomal DNA, extracted from an environmental sample, can be used as a template for PCR **(Figure B1.2)**. The PCR-amplified DNA products then can be separated and sequenced. By analyzing the sequences, researchers can accurately identify the species present in the original sample without isolating and growing pure cultures. The techniques available today certainly represent big improvements over Woese's method of sequencing fragments of rRNA molecules.

The uses of PCR in microbiology extend much further. PCR-based tests have been developed to detect the human pathogen *Chlamydophila pneumoniae*, a bacterium that typically is difficult to identify. As we will see in Perspective 5.2, a form of PCR routinely is used to monitor the viral load, or amount of virus present, in people with HIV disease. PCR also allows us to learn more about microbes that currently cannot be grown in the laboratory, a topic we will explore in Section 7.2. Virtually all areas of microbiology have been affected by the conceptually simple polymerase chain reaction.

Test Your Understanding

Explain how PCR amplification would differ if a standard DNA polymerase instead of a thermostable DNA polymerase such as Taq were used. What would be the result and why?

Although viruses are not cellular, they are still very important biological entities to study **(Figure 1.9)**. Viruses are molecular parasites that probably have been around since shortly after the first cells evolved. Microbiologists are interested in viruses not only because they cause many important infectious diseases in humans, crop plants, and livestock, but also because they are fascinating biological systems in their own right. Viruses have taught us a great deal about how cellular organisms function. As parasites, viruses must adapt to their host organism. To be taken up by host cells, most viruses have evolved to bind to host cell surface molecules and often enter cells by hijacking host systems ordinarily used for taking up non-viral molecules. Many viruses rely on host enzymes for the production of mRNA, and all viruses use host cell ribosomes for the production of proteins. By studying how viruses use the machinery of their host cells, scientists have gained insight into many critical processes in eukaryal, bacterial, and archaeal cells.

Microbes as Research Models

Basic research on the structure and function of microbes has laid a solid foundation for understanding the biology of all cells, including our own. Unicellular microorganisms generally possess the same genetic code and many of the same biochemical pathways as multicellular organisms. Additionally, microbes have many advantages for use in research:

- Many are easily cultivated in the lab using inexpensive equipment; they grow rapidly to high cell density on cheap nutrient sources.
- They facilitate the production of enzymes, other proteins, and various biomolecules for industrial and medical uses.
- Most have relatively small numbers of genes to analyze. Even the largest bacterial and archaeal genomes

TABLE 1.3 Selected characteristics of the three domains

	Bacteria	*Archaea*	*Eukarya*
Nuclear membrane	No	No	Yes
Membrane-bound organelles	Rare, a few types found in a few species	Rare, a few types found in a few species	Multiple distinct types, found in all species
Plasma membrane	Similar to *Eukarya*	Different from *Bacteria* and *Eukarya*	Similar to *Bacteria*
Cell wall	Found in nearly all species, constructed of peptidoglycan	Found in nearly all species, constructed of various materials	Found in some species, constructed of various materials
RNA polymerases	Single polymerase	Single polymerase, eukaryal-like RNA pol II	Three main polymerases (RNA pol I, II, and III)
Histones	Histone-like proteins	Yes	Yes

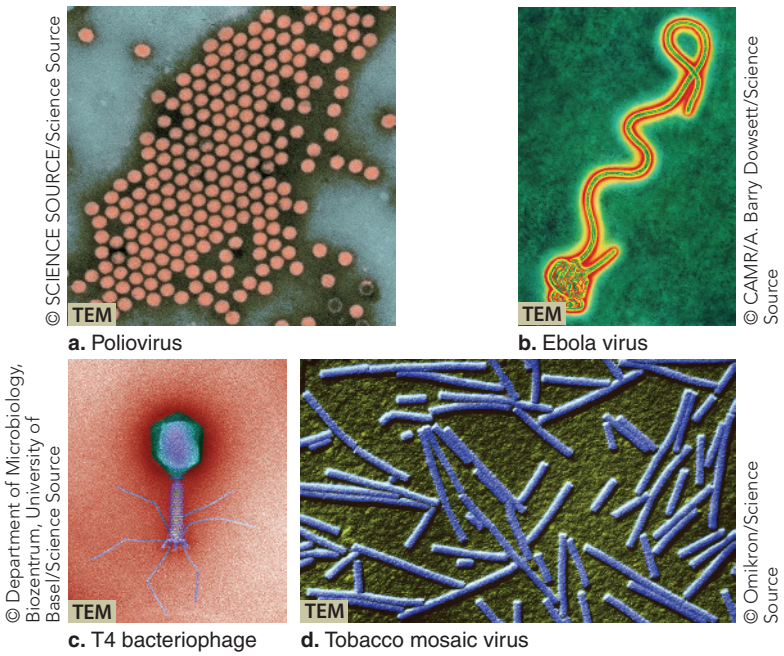


FIGURE 1.9 Viruses Different viruses have different shapes, and some can cause horrific infectious diseases. All images are artificially colored to enhance their appearance. **a.** Poliovirus, the cause of paralytic polio. **b.** Ebola virus, the cause of hemorrhagic fever, a rapidly progressing, highly fatal disease. **c.** T4 bacteriophage, a virus that infects bacteria and has been extensively used in research. **d.** Tobacco mosaic virus, a virus that infects plants, was the first virus to be discovered.

are smaller than the smallest eukaryal genomes, and eukaryal microorganisms have substantially fewer genes than complex multicellular eukarya.

- Many can be genetically manipulated much more easily than complex eukarya.

Popular microbial model systems for research include the intestinal bacterium *Escherichia coli* and the eukaryal yeast *Saccharomyces cerevisiae*, which is also known as baker's yeast or brewer's yeast because of its long historical use in food and beverage production **(Figure 1.10)**. These model microorganisms have been subjected to the vast experimental armaments of the fields of biochemistry, genetics, molecular biology, and cell biology. Our current understanding of the complexities of biochemical pathways; DNA replication and cell division; the nature of genes; the control of gene expression; and protein synthesis, folding, and function has arisen largely from studies of model microbes.

Research on the biology of microbial cells has virtually unlimited practical applications. For example, to understand how some antimicrobial drugs work against their microbial targets while sparing host cells, we need to understand differences in structure between bacterial and eukaryal cells, or perhaps between fungal and human cells. Paul Ehrlich, a towering figure in the history of medicine and immunology, was among the first to recognize that such differences had medical implications. From his experience in the field of histology, Ehrlich was familiar with dyes that differentially stained bacterial and human cells. Based on this observation, he speculated that molecular "magic bullets" that specifically target microbial invaders were feasible. He had little knowledge of the actual structures present on or in cells of any kind, but this concept that certain drugs may adversely affect specific types of cells, while sparing other types of cells, remains at the heart of our drug development initiatives today.

Members of Ehrlich's research group discovered an organic arsenic-containing compound, arsphenamine, which in 1910 became the first effective commercial drug for the treatment of *Treponema pallidum*, the bacterium that causes the sexually transmitted disease syphilis **(Figure 1.11)**. Because it also exhibited toxicity to host cells, arsphenamine, known by its trade name Salvarsan, was abandoned in the 1940s in favor of penicillin, the first widely used antibiotic capable of killing many different kinds of bacteria.

Salvarsan's historical importance was in establishing that lethal agents specifically targeted at microbial cells are indeed possible. In the century since Salvarsan came on the market, an enormous amount has been learned about the molecular differences between bacterial and eukaryal cells. Hundreds of

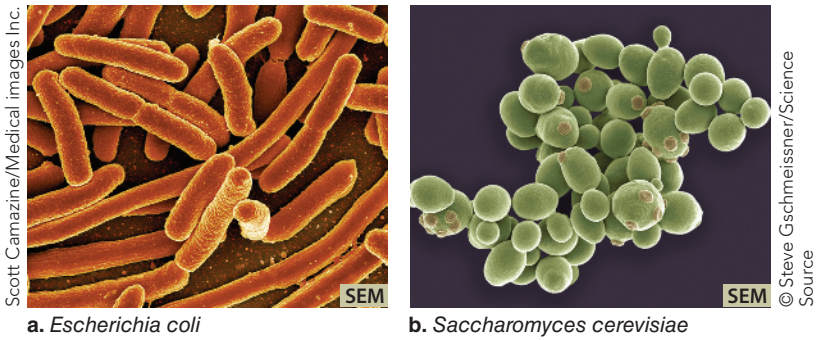
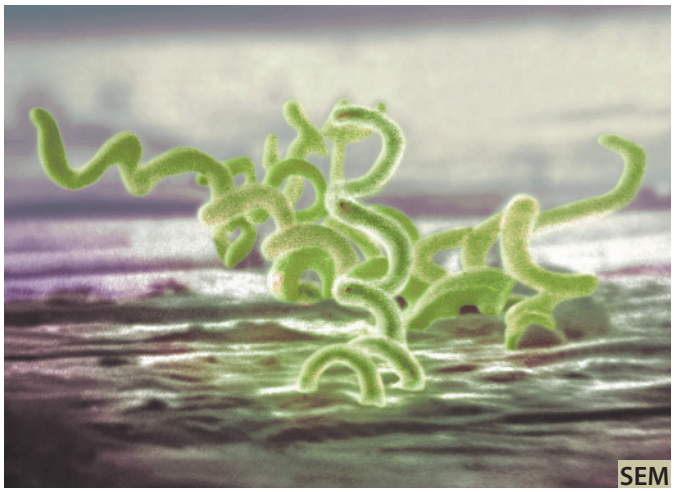


FIGURE 1.10 Microbes as model organisms Microbes have been used extensively in research. Because they replicate quickly, are cheap to grow, and have relatively simple structures, they have been used extensively to study basic cellular processes like DNA replication, transcription, and translation. Two of the most-studied microbial model organisms are **a.** the bacterium *Escherichia coli* and **b.** the eukarya *Saccharomyces cerevisiae*, a yeast.



© SCIENCE SOURCE/Science Source

FIGURE 1.11 The development of antimicrobial drugs The bacterium *Treponema pallidum* causes syphilis, a sexually transmitted disease. Salvarsan, the first commercially available drug to combat syphilis, was developed in 1910. Although Salvarsan prevented the replication of this bacterium, it also was toxic to human cells, and its use was curtailed after the development of penicillin.

new antimicrobial and antiviral drugs have been discovered, and hopefully there will be many more to come.

Connections **Basic research into the structure and replication of microbes has led to the development of numerous antimicrobial and antiviral drugs. Many of the currently approved drugs for the treatment of HIV, for instance, interfere with specific viral enzymes needed for the production of new virus particles. We will discuss how a particular class of these drugs—nucleoside analogs—works in** Section 24.2.

1.1 Fact Check

1. What are the key features of living organisms?
2. Describe the macromolecules found in cells.
3. What are the three domains of living organisms?
4. Explain why microbes are useful model systems in research and provide examples of microbial model systems.

1.2 Microbial Physiology, Genetics, and Cultivation

- What do we know about the physiology, genetics, and cultivation of microbes?

Although groups of microbes may be different from each other, they all share common information processes. Indeed, one of the most remarkable aspects of life as we know it is the constancy of these information processes. In all cells, the main informational molecule is double-stranded DNA. In all cells, a specific type of RNA, messenger RNA, or mRNA, serves as the conduit between the information in DNA and the actual production of proteins. In all cells, the code used to convert the information present in DNA to RNA to protein is the same. This conserved genetic code probably represents the most compelling evidence for evolution. All living organisms share a common informational pathway, suggesting that all living organisms share a common ancestor.

Because all living organisms, and the genetic processes of these organisms, are evolutionarily related, we will begin our exploration of microbial genetics by examining the origins of life. We then will look at how genetic processes occur in microbes and how microbiologists study these processes. We will end this section with a brief overview of how microbes acquire and use energy.

The Evolution of Life on Earth

Earth is home to a huge variety of microbes. To understand how this incredible diversity evolved, we need to consider the history of Earth and the origins of life itself. The geochemical changes that have occurred on Earth in the past 4 billion years—in the oceans, on land, and in the atmosphere—have been dramatic. These changes have profoundly affected, and were profoundly affected by, microorganisms. The vast majority of the living organisms we see today, at least without microscopes, are large multicellular eukarya that arose within the last few hundred million years, the last 10 percent of Earth's history. But most of the major evolutionary events that moved life toward today's world occurred in the distant past, when microbes alone ruled the planet. To get some perspective on this point, let's take a brief walk through the history of life.

Prebiotic Evolution

When Earth formed approximately 4.5 billion years ago (abbreviated ybp, for years before present), it was a hot and sterile place. Oceans of liquid water formed around 4 billion ybp, once the crust and atmosphere had cooled sufficiently for liquid water to condense **(Figure 1.12)**. These oceans may have been partially or completely converted to steam on multiple occasions by the energy of asteroid impacts, which were far more common in the early solar system. Depending on when the first life-forms evolved, such impacts could have resulted in mass extinctions, coupled with selection for life-forms that could live in this extreme environment. By 3.8 billion ybp, life clearly had gained a permanent foothold. The first microorganisms appeared as life transformed from a semiorganized set of chemicals and reactions to a true cellular form. By 3.5 billion ybp, microbial cells were abundant on Earth, as is evident from fossilized stromatolites containing cyanobacteria-like structures **(Figure 1.13)**.

Cyanobacteria, we should note, are photosynthetic bacteria. The evolution of these organisms, and their oxygen-releasing photosynthetic capabilities, led to the eventual oxygenating of Earth's atmosphere. Given that multicellular

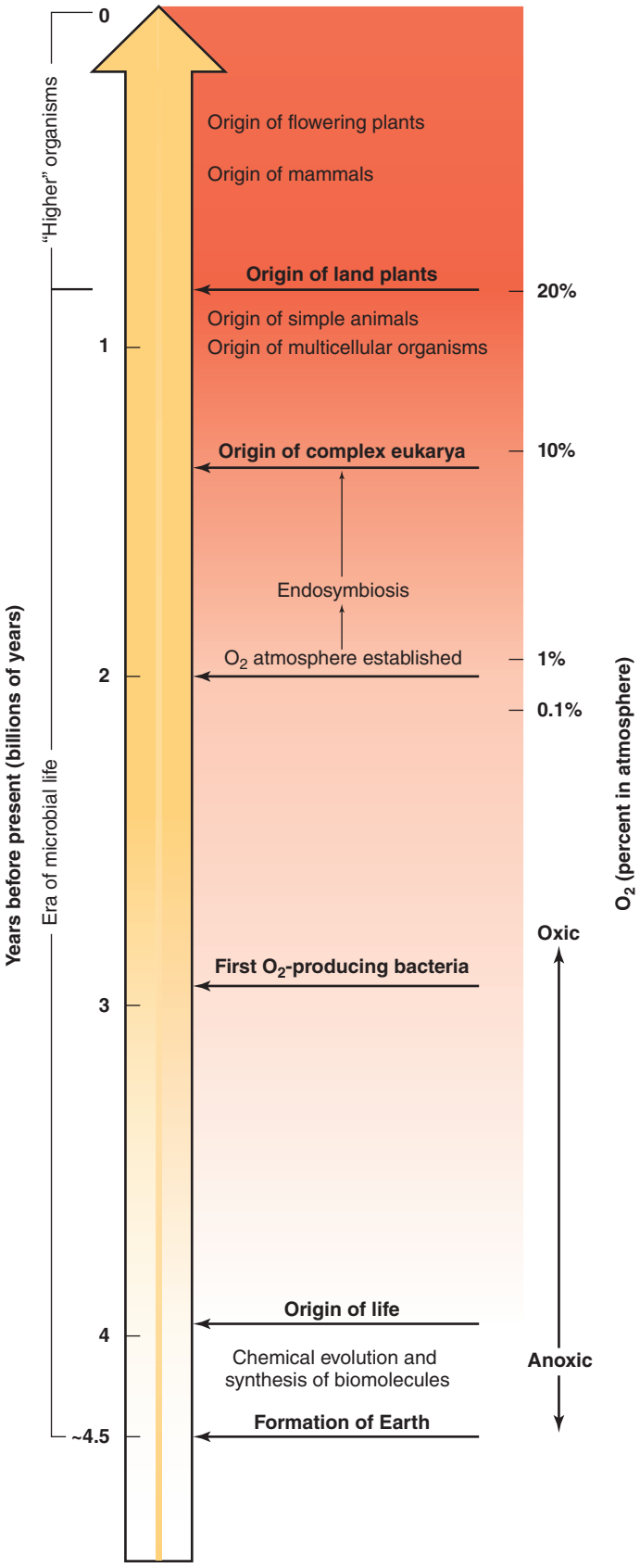


FIGURE 1.12 Evolutionary time line of life on Earth Current evidence suggests that Earth formed roughly 4.5 billion years ago. Life appeared around 3.8 billion ybp. Some early eukarya first appeared around 1.5 billion ybp. Mammals first appeared about 200 million ybp. Oxygen did not become a major constituent of the atmosphere until the advent of oxygen-producing photosynthesis.

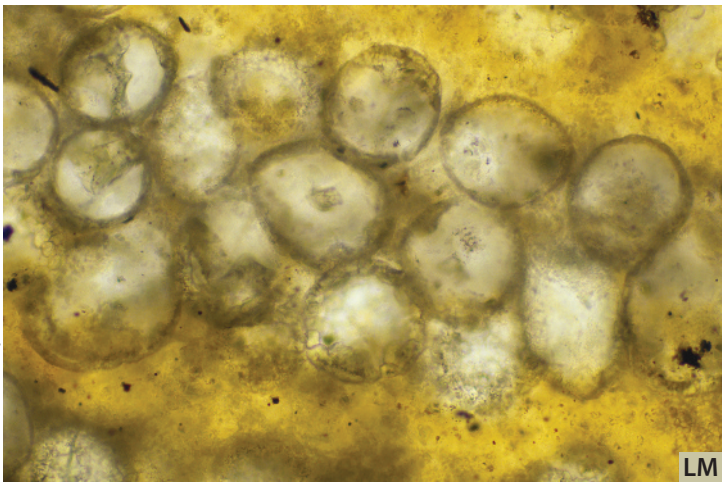
algae and marine invertebrates are not evident in the fossil record until 0.5 billion ybp, it appears that microbial life ruled Earth for over 3 billion years. Only during the last 500 million years has Earth seen the rise of plants and animals! Our planet has changed drastically since its violent birth, but with the exception of dramatic events like asteroid impacts and volcanic eruptions, changes have occurred gradually. Microbes had plenty of time to evolve an incredible array of talents, allowing them to exploit every possible habitat. Given the eons that have gone by, we can only imagine the diversity of microbial life that has existed since Earth's origins; we still do not fully comprehend the richness of microbial life on present-day Earth.

When life first appeared, Earth was a harsh place. The average temperature was quite hot, probably over 50°C. The composition of the atmosphere is not known for sure, but researchers hypothesize that it had a high concentration of CO_2, perhaps up to 30 percent. Other atmospheric gases may have included nitrogen (N_2) and hydrogen (H_2). Whether gases such as ammonia (NH_3), methane (CH_4), cyanide (HCN), and hydrogen sulfide (H_2S) were present in substantial concentrations is not known with certainty. It is clear, though, that there was little or no molecular oxygen (O_2). The oceans probably were fairly acidic due to the high concentration of dissolved CO_2. By comparison, today's atmosphere consists of about 0.03 percent CO_2 and 21 percent O_2, with a moderate average temperature of 13°C. What changed the O_2 and CO_2 concentrations so dramatically since life began? Microbial activities over the past 4 billion years are part of the answer.

The First Microbial Life

It is generally assumed that life-forms present on early Earth have not survived unaltered to modern times. Conditions on our planet have changed radically over the past 4 billion years, and it is reasonable to assume that evolutionary innovations incorporated into living systems during that time outcompeted and displaced primitive cells long ago. Nevertheless, the biochemical origins of life are of great interest. As we look outward for life elsewhere in our solar system and beyond, simple living systems—microorganisms—are far more likely to be discovered than are advanced civilizations in flying saucers. The better we understand the evolution of life on Earth, the better idea we have of what to look for elsewhere.

Many hypotheses have addressed the origin of life. The Miller–Urey experiment envisioned an early Earth where organic molecules accumulated in the oceans, creating a rich "prebiotic soup" from which organized cellular life eventually emerged **(Perspective 1.1)**. Perhaps the organic molecules would have required a surface on which to accumulate rather than simply floating in the open ocean. Günter Wächtershäuser has theorized that life evolved on iron-containing surfaces, such as iron pyrite (FeS_2), an insoluble, positively charged surface with affinity for organic compounds. Metabolic processes that occur in modern cells could have evolved from reactions among surface-bound organic compounds. Many modern proteins, such as cytochromes and hemes, bind iron and other metallic atoms; many enzymes, including DNA polymerases, require bound metals for activity. These interactions with metals may reflect a very ancient relationship.



© Michael Abbey/Science Source

a. Fossils of cyanobacteria



© Courtesy Josh D. Neufeld

b. Stromatolites

FIGURE 1.13 **Ancient fossils** **a.** Fossilized cyanobacteria. These fossils of cyanobacteria were found in Wyoming and date back about 50 million years. **b.** Stromatolites. Modern stromatolites, like these from Western Australia, can be observed in several locations. Fossilized stromatolites that are 3.5 billion years old have been identified, indicating that photosynthetic bacteria existed on Earth at least this long ago.

As we try to envision the origins of life, the formation of chains of ribonucleotides (RNA) and amino acids (polypeptides) clearly represents a necessary event. These polymers, as we noted, contain information and carry out cellular functions. Some researchers, including Carl Woese, have suggested that information was initially stored in RNA molecules rather than DNA, leading to the **RNA world** hypothesis for the origins of life. We now know that certain RNA molecules, **ribozymes**, can catalyze chemical reactions, much like the protein-based enzymes with which we are more familiar. Early in the history of life, then, RNA may have had dual functions, serving as the primary informational molecule and catalyzing important reactions.

Of course, the true stories of the origin of genetic molecules and origin of life certainly are more complex. Most scientific stories are more complex than they initially seem! Several researchers have faulted the RNA world hypothesis. They argue that RNA and rudimentary peptides must have evolved in concert. In 2019, chemists at the Scripps Research in California postulated that life originated from an even more complex mixture. The first genetic molecule, they conjecture, may have been composed of both RNA and DNA subunits. Only later did pure forms of these molecules arise.

While we think about the origins of life, let's ask another question. How did the first membranes form? We know that hydrocarbons coupled to charged groups, such as phosphates, form polar lipids, which can spontaneously organize into micelles and even bilayer membranes that close back upon themselves to form a sealed compartment **(Figure 1.14)**. Perhaps such primitive membranes initially formed around fragments of minerals, such as FeS_2, that also happened to serve as surfaces for the aggregation of organic molecules, as just discussed. If a membrane encapsulated an informational molecule and a catalytic molecule, like a ribozyme, then something like a cell would have been formed.

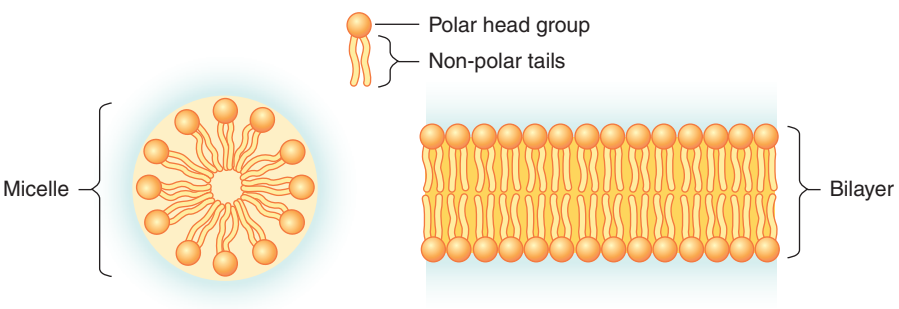


FIGURE 1.14 **Micelles and bilayer membranes** Phospholipids, consisting of non-polar fatty acid tails and polar phosphate heads, can spontaneously form micelles, spherical units with polar surfaces and non-polar cores. These molecules also can form bilayers, in which the non-polar regions exist between two polar surfaces.

Perspective 1.1

Creating Life in the Laboratory: the Miller–Urey Experiment

We cannot go back in time to the prebiotic world to watch how life actually evolved. We can imagine scenarios, formulate hypotheses, and test them in the laboratory to gain insight into potential pathways to life. In 1953, Stanley Miller described the first laboratory investigation intended to simulate prebiotic Earth. Miller, then a graduate student at the University of Chicago, designed a reactor with his mentor, Harold Urey, to test for abiotic production of biologically relevant molecules. The Miller–Urey experiment revolutionized the thinking of many scientists, and a fair number of non-scientists as well, about the origin of life on Earth.

The Miller–Urey experiment started with a water-filled flask, heated by a burner. The atmosphere in the apparatus was intended to simulate that of primitive Earth and consisted of a mixture of ammonia (NH_3), methane (CH_4), and hydrogen (H_2). The water was heated to boiling, at which time water vapor mixed with the various gases in the atmosphere and circulated through the apparatus. A separate chamber discharged electrical sparks through the atmosphere, after which the tubing was cooled so that water vapor would condense and drip back into the original flask **(Figure B1.3)**.

After a few days of continuous operation, Miller observed that the water in the flask was changing color. Within a week, the solution in the flask had turned a deep red and had become turbid. Miller examined the solution after a week and found that the solution contained organic molecules, or molecules containing carbon-hydrogen bonds. Most notably, the simple amino acids glycine and alanine were readily detectable, along with aspartic acid. These amino acids are among the 20 primary amino acids used by life on Earth for protein synthesis and presumably were abundant as life evolved. Miller's flask provided the first evidence that such molecules could be synthesized from inorganic precursors under conditions believed to mimic primordial Earth, where energy inputs could have included electrical discharge from lightning strikes as well as heat from both the sun and Earth's crust.

The Miller–Urey experiment stimulated other scientists to design experiments to simulate hypothetical atmospheric, oceanic, and geological conditions on prebiotic Earth. Experiments done in hydrogen-rich reducing atmospheres similar to Miller's showed that many amino acids, nitrogenous bases, and other organic compounds could be produced abiotically. Questions about these experiments arose, however, as the consensus of scientists shifted in the 1970s to favor an atmosphere on early Earth that was much richer in CO_2 and N_2. In a Miller-type apparatus, these atmospheric conditions yield few or no organic molecules such as amino acids. Models of early Earth conditions continued to evolve. By 2005, scenarios with much higher levels of H_2 in early atmospheres, co-existing with CO_2, were tested. Jeffrey Bada, a professor at Scripps Institution of Oceanography and a former graduate student of Miller's, further altered the composition and atmosphere in a Miller-type reactor and was able to reproduce Miller's yield of organic material.

Because we do not know the exact chemical and physical conditions on early Earth, controversies continue. Predicting the pathway by which life arose depends greatly on what conditions are assumed to have existed. To further complicate the situation, some evidence suggests that substantial amounts of organic molecules could have been delivered to Earth by comets and asteroids crashing into the planet during the tumultuous early days of the solar system. Hydrocarbons and complex nitrogen-containing organic molecules, including amino acids, have been detected on comets in space and in meteorites on Earth. The magnitude of the contribution of these impacts remains unclear.



Stanley Miller in laboratory in 1952

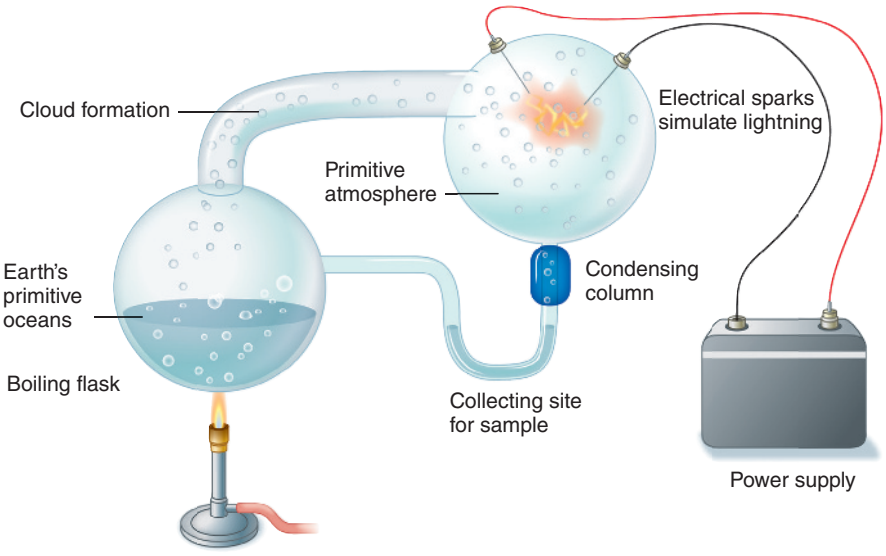


FIGURE B1.3 The Miller–Urey experiment To explore the origins of life, Miller (pictured here) and Urey created an early Earth atmosphere within a closed apparatus. After supplying heat and electricity to the apparatus (simulating energy sources on ancient Earth), they extracted samples for analysis. Their chemical analysis showed that organic molecules had formed, including amino acids. This experiment suggested that the building blocks for living systems could have formed on Earth from small inorganic precursors.

Although RNA may have been the major informational and catalytic molecule initially, double-stranded DNA eventually supplanted RNA as the major informational molecule and proteins arose as the major catalytic molecules, giving rise to the first living organism. Carl Woese refers to this organism as a **progenote**, a cell hypothesized to store information in genes not yet linked together on chromosomes **(Figure 1.15)**.

When a progenote replicated itself, each of the progeny may have had a different subset of the parental genes, and thus a number of variations could have arisen in one generation. Genetic variation and mutation were probably frequent, given that these primitive cells would have lacked the sophisticated genetic repair mechanisms seen in modern cells. Though they might not have looked much like modern life, progenotes would have been just as subject to Darwinian evolution. Darwinian evolution, we should note, depends on (1) genetic variation in a population, (2) the environment exerting selective pressure(s), and (3) differential reproductive success among genetic variants as a result of that selective pressure. Primitive cells that not only survived, but also reproduced more efficiently and passed gene sets to their progeny, would have spread most rapidly. Assuming that genetic variation was common in progenotes, the rate of evolutionary change could have been quite high.

The Appearance of Eukarya

How did complex cells arise? Most biologists now agree that mitochondria and chloroplasts, two of the most distinctive organelles in eukaryal cells **(Figure 1.16)**, are derived from bacterial cells through a process known as "endosymbiosis." These organelles have their own circular genomes, though these genomes are far smaller than the genomes of contemporary bacteria. The genes present in the chloroplast and mitochondrial genomes include a gene encoding 16S rRNA, the SSU rRNA molecule present in bacterial ribosomes. Sequence analysis of the mitochondrial and chloroplast 16S rRNA gene indicates that mitochondria and chloroplasts are related to specific groups of bacteria.

Animation: Endosymbiosis

Lynn Margulis, a biologist who worked at the University of Massachusetts–Amherst, long championed endosymbiosis as a mechanism for the origins of mitochondria and chloroplasts. Indeed, substantial molecular evidence indicates that these organelles became part of eukaryal cells through endosymbiotic relationships that became permanent. Mitochondria probably were added first to a developing eukaryal cell; they are present in most, but not all, modern eukarya. We can imagine that the endosymbiont provided the host with extra ATP. In return, the host provided the endosymbiont with nutrients and a safe place to live. The extra ATP ultimately allowed for an increase in cell size, and for multicellular arrangements.

Chloroplasts probably were added later, leading to the evolution of algae and plants. A bacterium capable of photosynthesis, the ability to harvest the energy present in sunlight to produce organic molecules, would provide a host with a new energy source and allow the host to expand into new habitats. During the evolution of eukarya, multiple endosymbiotic events leading to chloroplasts may have occurred. We now know that some differences exist between the chloroplasts present in different algal and plant groups. Regardless, photosynthesis brought solar power to eukaryal cells. Algae and plants, as a result, have been phenomenally successful, and now account for a major fraction of the biomass on land (plants) and sea (algae). Once these mitochondria and chloroplasts became permanently established in eukaryal hosts, their own genomes degenerated until only a few essential genes were left. By 500 million years ago, multicellular eukarya had begun to dominate the macroscopic landscape of life on Earth. Microbes had set the stage for them.

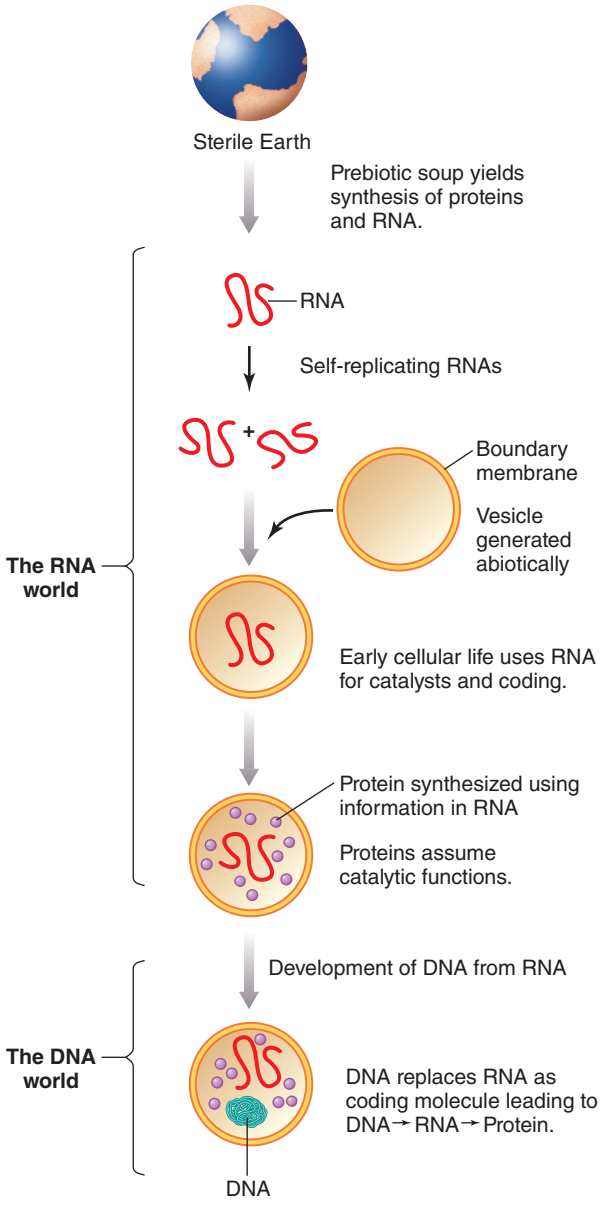
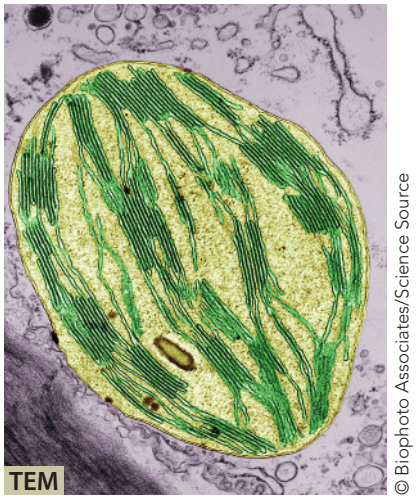


FIGURE 1.15 The origins of life We probably will never know for sure how life began, but we can make a reasonable hypothesis. Shortly after Earth was formed, natural processes yielded a variety of substances, including RNA. Self-replicating and catalytic RNA molecules became enclosed within a boundary membrane, leading to an early version of cellular life. Eventually, proteins became the major catalytic molecules and DNA supplanted RNA as the dominant informational molecule.



a. Mitochondrion



b. Chloroplast

FIGURE 1.16 Mitochondria and chloroplasts These two distinctive eukaryal organelles probably originated via endosymbiosis. **a.** Mitochondrion, which may have originated when a developing eukaryal cell engulfed a bacterial cell capable of undergoing efficient aerobic respiration. **b.** Chloroplast, which may have originated in a similar fashion when an early eukaryal cell engulfed a cyanobacterial-like bacterium capable of photosynthesis.

Connections **We will look more closely at the origins of eukaryal cells in** Section 3.4 **and explore the evidence supporting the endosymbiotic theory. In** Chapter 15, **we'll see that endosymbiotic relationships between microbes and host cells are actually very common in nature. In most cases, the host cannot survive without the essential functions supplied by the endosymbiont.**

The evolution of oxygenic photosynthesis in cyanobacteria led to the abundance of molecular oxygen we see in the atmosphere today. Highly efficient aerobic respiration became possible. Aerobically respiring bacteria then entered into the symbioses that led to mitochondria in eukaryal cells. Without mitochondria to power cellular metabolism efficiently, large multicellular eukaryal organisms would never have appeared.

Photosynthesis and Respiration

All microorganisms, indeed, all living organisms, must acquire energy and produce macromolecules. Most macroscopic eukarya exhibit relatively limited types of metabolism. Microorganisms, as we will see briefly in this section and more fully in Chapter 6, exhibit more diverse types of metabolism. This metabolic diversity allows microorganisms to inhabit a wide range of habitats. Because different microorganisms can utilize various nutrients, they can exist in environments that may be uninhabitable by other organisms.

In a very basic sense, all living organisms need organic molecules. **Heterotrophs**, or "other feeders," ingest organic molecules. These preformed molecules can be used for the biosynthesis of other macromolecules or as an energy source. **Autotrophs**, or "self feeders," can produce their own organic molecules from an inorganic carbon source. We typically think of plants when we think of autotrophs. These photosynthetic organisms harvest the energy present in sunlight to convert the carbon in CO_2 into organic molecules.

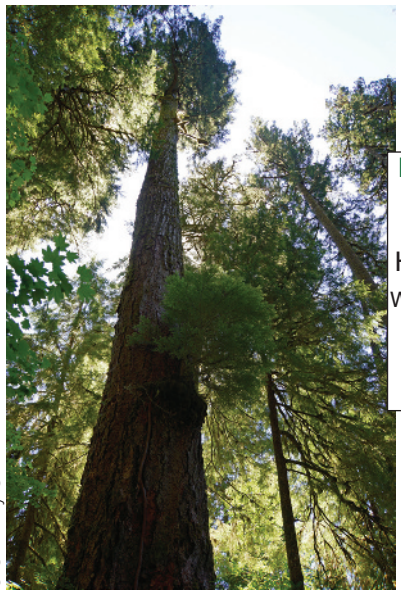
In cyanobacteria, the presumed descendants of the organisms that gave rise to chloroplasts, membrane-bound pigment molecules absorb the energy present in sunlight. This absorbed energy results in the transfer of an electron from the pigment molecule to a series of membrane-bound proteins. Ultimately, the movement of this electron powers the formation of ATP, the energy currency of the cell **(Figure 1.17)**. The cells can use this ATP to incorporate inorganic carbon from CO_2 into an organic molecule. To complete the process, the pigment molecule must regain an electron to replace the one that was lost. In cyanobacteria and plants, the pigment molecule regains this electron by removing electrons from water, resulting in the liberation of O_2:

$$2\,H_2O \rightarrow 4\,H^+ + 4\,e^- + O_2$$

This liberation of oxygen via photosynthesis ultimately led to the increased atmospheric O_2 concentration that we discussed previously.

Connections **Not all photosynthetic organisms produce O_2 as a by-product. Some photosynthetic organisms gain electrons from hydrogen sulfide (H_2S) instead of water. For these organisms, elemental sulfur (S^0), not O_2, is liberated. This anoxygenic photosynthesis will be discussed in more detail in** Section 6.7.

Regardless of how organisms acquire organic molecules, all living organisms also need a mechanism of oxidizing those molecules to generate ATP. One of the simplest means



© Courtesy Olivia Fukui

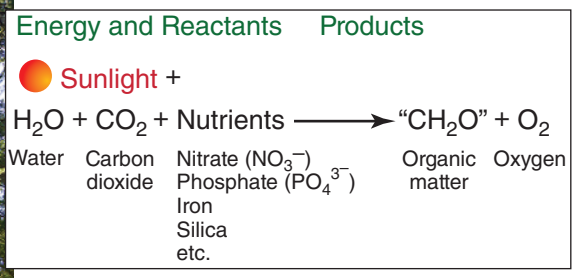


FIGURE 1.17 Overview of oxygenic photosynthesis Energy from sunlight is harvested by a membrane-bound pigment molecule, resulting in the loss of an electron. The harvested energy is used to form organic molecules from CO_2. The electron lost by the pigment molecule is replaced by the breakdown of water, resulting in the generation of O_2.

of acquiring energy from organic molecules is glycolysis, the reaction in which glucose is converted to pyruvate, with the subsequent generation of two ATP molecules:

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{Pi} + 2\ \text{NAD}^+ \rightarrow 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ \text{H}^+$$

In some cases, glycolysis is coupled with fermentation, a process in which the NADH produced by glycolysis is converted back to NAD^+ and the pyruvate molecules are converted to a waste product, such as ethanol or lactate. While this system of obtaining energy from glucose is fairly simple, it is not particularly efficient; much of the potential energy present in the original glucose molecule is not converted to ATP **(Figure 1.18)**.

A more effective means of obtaining ATP from glucose involves respiration, a set of metabolic processes whereby the glucose is more completely utilized, resulting in the production of larger quantities of ATP. Plants, animals, and many microorganisms undergo aerobic respiration, a form of respiration that involves the addition of electrons to O_2, resulting in the formation of H_2O. Some bacteria perform respiration in which a terminal electron acceptor other than oxygen is used. This type of respiration generally results in the formation of less ATP than respiration involving oxygen.

The presence of O_2 in the atmosphere had profound consequences for life on Earth. Respiration utilizing oxygen allows organisms to harvest a significant amount of energy from organic molecules. During this process though, oxygen is converted to a series of highly reactive molecules. These by-products can damage cells through their ability to oxidize other molecules and their ability to generate even more potent compounds through interactions with light. Until oxygenic photosynthesis evolved, *all* life, by necessity, was anaerobic. When O_2 began accumulating in the atmosphere, organisms either had to develop strategies for defending themselves against these dangerous by-products or retreat to habitats where O_2 remained less abundant or absent. This remains true today. Anaerobic microorganisms are still with us, living in specialized habitats free from molecular oxygen.

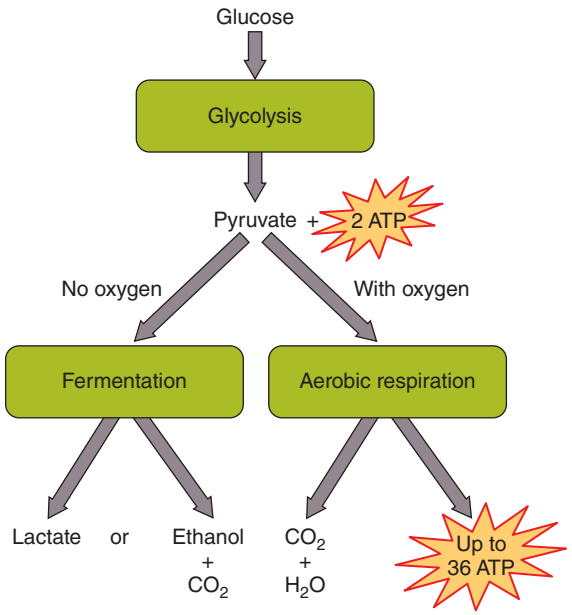


FIGURE 1.18 Glycolysis, fermentation, and aerobic respiration In almost all organisms, glucose can be converted to pyruvate, resulting in the production of some ATP. The process of aerobic respiration allows cells to generate much more ATP from pyruvate. In plants, animals, and many bacteria, respiration occurs in the presence of oxygen (aerobic respiration) and results in the generation of CO_2 and H_2O. Some bacteria undergo anaerobic respiration, utilizing a terminal electron acceptor other than oxygen. If respiration cannot occur, some organisms undergo fermentation reactions. Two well-studied types of fermentation result in the production of lactate or ethanol. These products of fermentation generally are toxic to the microorganism but may be helpful to humans.

Connections **During aerobic respiration, O_2 can be converted to hydrogen peroxide (H_2O_2). This reactive molecule can damage cells. As a result, most organisms that can survive in the presence of O_2 produce catalase, an enzyme that converts H_2O_2 to H_2O and O_2. We will learn more about toxic oxygen species in** Section 7.1.

The increase of O_2 in the atmosphere also created an ozone layer in the stratosphere, approximately 10–25 miles above Earth's surface. Ozone (O_3) is generated naturally from O_2 and strongly absorbs short wavelength ultraviolet (UV) light. UV light is dangerous to cells because it causes damaging chemical reactions in DNA. Because UV light does not penetrate water very well, aquatic life is fairly well protected from its harmful effects. Terrestrial organisms, however, are not so lucky. The accumulation of ozone in the atmosphere reduced the amount of UV radiation reaching Earth's surface. The rise of O_2 in the atmosphere, therefore, indirectly facilitated the colonization of land by microorganisms.

DNA and RNA: The Genetic Molecules

In all organisms today, genes are linked together in long molecules of double-stranded DNA. DNA is an elegant, albeit enormously long, molecule whose structure is beautifully suited for information storage and replication. Because each strand of a double-stranded DNA molecule is complementary to the other strand, one can easily imagine a mechanism of faithfully replicating the molecule. If the two strands denature, or come apart, then each strand contains the information necessary to re-form the other strand **(Figure 1.19)**. When a cell divides, identical copies of DNA can be produced so that each progeny cell will contain the exact same informational molecule.

The companion nucleic acid, RNA, also plays a critical role in the information flow in cells **(Figure 1.20)**. Messenger RNA (mRNA) is synthesized from a DNA template during the process of transcription and delivers instructions to the ribosome for the production of a specific chain of amino acids. Ribosomes are the protein synthesis factories of the cell. During translation (polypeptide synthesis), transfer RNAs (tRNAs) deliver amino acids to the ribosome. The ribosome itself is composed of proteins and ribosomal RNA (rRNA) molecules, which provide a structural framework. One of the rRNAs contributes catalytically to peptide bond formation. As we noted earlier, the functional versatility of RNA has suggested to scientists that it could have played a central role in the origin of living systems.

Connections **Even though most living organisms share a common genetic code, significant differences in some of the details of the code and various genetic processes exist between members of the domains *Eukarya*, *Archaea*, and *Bacteria*. We will examine translation more in** Section 8.4 **and explore some of these domain- and organism-specific differences.**

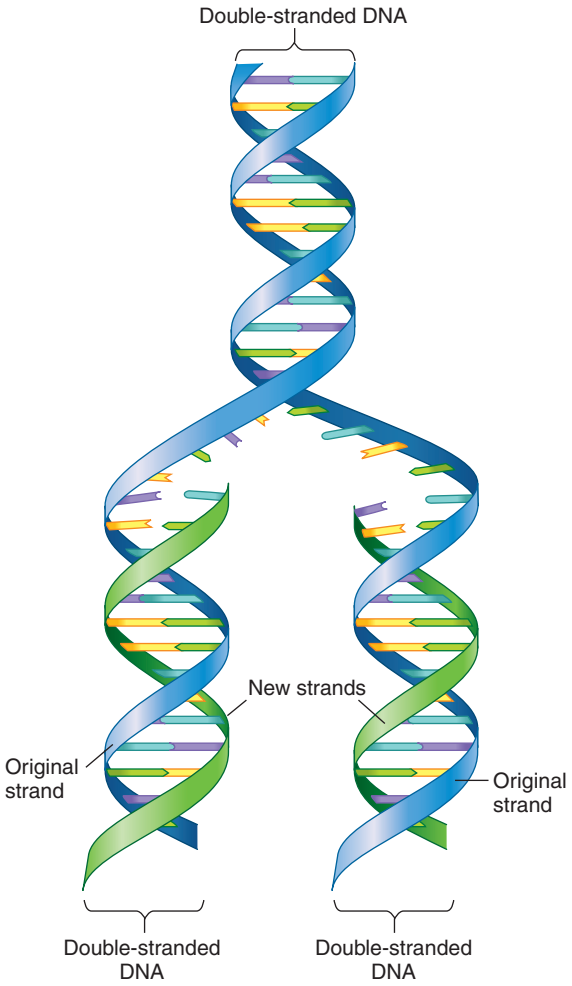


FIGURE 1.19 **Replication of DNA** Because of the double-stranded nature of DNA, and the fact that each strand within the double-stranded molecule is complementary to the other strand, a method of replication seems quite obvious. If the two strands are separated, then each strand can serve as a template for the formation of another new strand, resulting in the production of two identical double-stranded molecules.

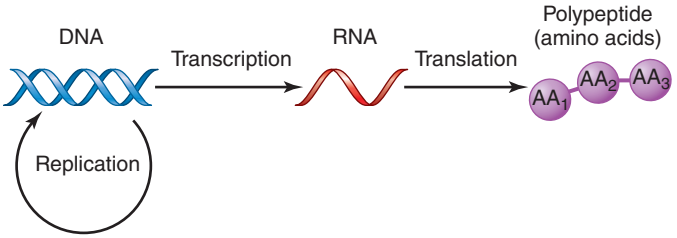
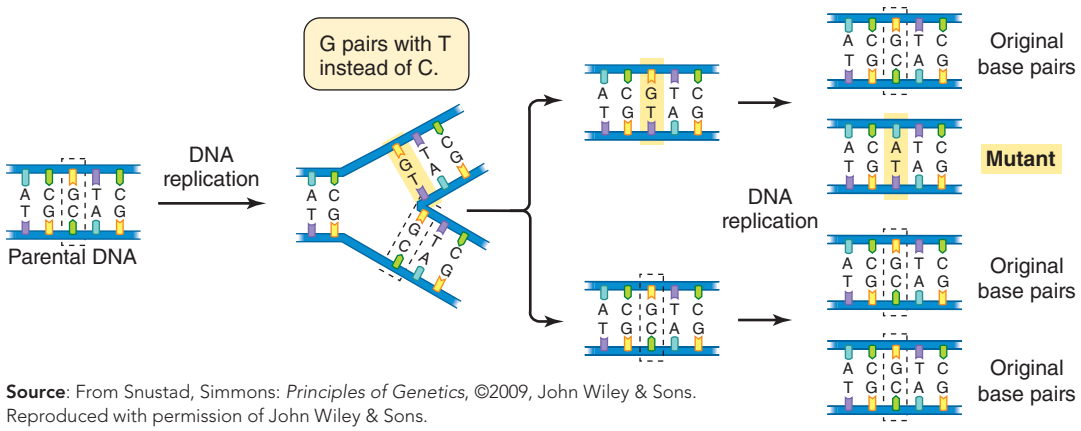


FIGURE 1.20 **The flow of information in cells** The main informational molecule, DNA, generally is replicated prior to cell division, ensuring that each daughter cell possesses the same genetic material. Information within DNA is copied into RNA during transcription. The information in RNA specifies a corresponding sequence of amino acids during translation of the RNA into a polypeptide.



Source: From Snustad, Simmons: *Principles of Genetics*, ©2009, John Wiley & Sons. Reproduced with permission of John Wiley & Sons.

FIGURE 1.21 Heritable changes in the DNA During DNA replication, errors may occur, such as in this example where G pairs with T instead of with C. When this molecule of DNA undergoes a second round of replication, one of the new molecules will contain the original CG base pair, while the other newly formed molecule will contain a mutant TA base pair at this location. The mutation, at this point, has become incorporated into this molecule of DNA and will appear in all progeny DNA.

Genetic Analysis

The ability to faithfully replicate the genome and convert the information contained within the genome into functional proteins represents only part of the story. As we noted earlier, living organisms must contain some genetic variation in order for evolution to occur. The ultimate source of this variation is **mutation**, a heritable change in the genome. As we will first see in Section 8.5, mutations can result from errors made during replication or from various physical or chemical insults to the DNA. Regardless of their source, these changes in the genotype, or genetic composition of an organism, can be passed on when the genome replicates and the cell divides **(Figure 1.21)**. More importantly, these mutations may alter the genetic information in such a way that the proteins produced by the cell differ **(Figure 1.22)**. These changes in the proteins, in turn, can alter the phenotype, or observable characteristics of the cell.

Although bacteria do not undergo sexual reproduction by the formation and uniting of gametes, they do exchange genes between cells, a process referred to as horizontal gene transfer. As we will see in Section 10.4, genetic material can be transferred between bacteria in several ways. Moreover, more and more evidence suggests that genetic material can be transferred not only between different bacterial types, but also between organisms in different domains. This exchange of genetic material muddies somewhat our ability to construct a perfect phylogenetic tree of all living organisms **(Figure 1.23)**.

Animation: Tree of life

In recent years, improved DNA-sequencing techniques have allowed researchers to determine the exact DNA sequences of entire genomes of organisms. Rather than just studying specific genes, we now can study entire genomes, a field of inquiry known as genomics. Along with these improved sequencing techniques, we also now have improved computing power. The computer tools available today allow us to analyze and compare genomes, which is the basis of the burgeoning field of bioinformatics. With these new tools at our disposal, we are learning more about the evolutionary history of life, the diversity of organisms, and the functioning of our cells. The flood of microbial genome sequences in recent years has clearly shown that horizontal gene transfer is ubiquitous in microorganisms. In reality, most microbial genomes are composites of DNA fragments with distinct evolutionary histories.

Connections **Genomics has revealed the profound impacts of gene transfer in regard to pathogen evolution. In** Section 21.4, **we'll explore several examples of how transfer events have led to the development of a pathogen.**

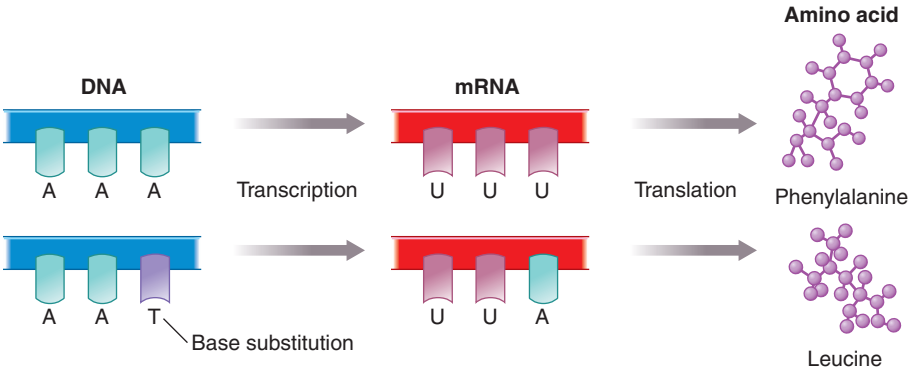


FIGURE 1.22 Effects of mutations Alterations in the DNA sequence can lead to changes in the mRNA and amino acids that constitute the resulting polypeptides. Here, a change in the DNA sequence results in a change in the amino acid present in the resulting polypeptide. Amino acid changes could alter the shape of the polypeptide, which, in turn, may affect the phenotype of the organism.

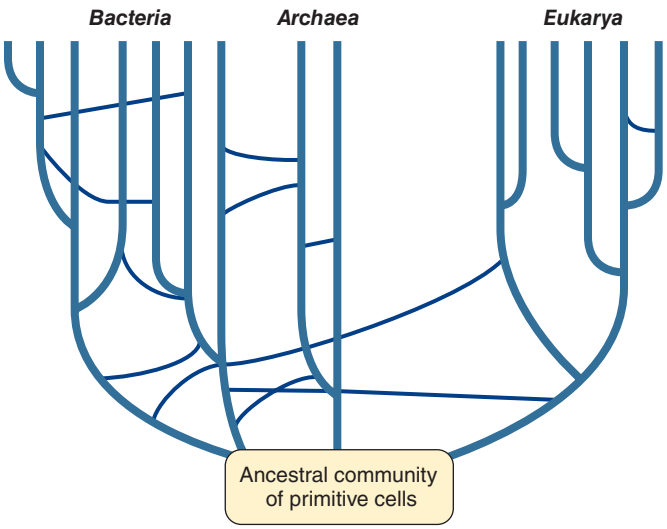


FIGURE 1.23 Genetic exchange occurs between species and between domains Gene transfer usually takes place between members of the same species by sexual reproduction or other transfer of DNA. However, now there is evidence that gene transfer also can occur among bacteria, archaea, and eukarya (indicated by thin lines). Although this genetic exchange does not disrupt the phylogenetic tree, it does mean that individual genomes may contain segments of DNA obtained recently from distantly related organisms.

1.2 Fact Check

1. Describe how double-stranded DNA, mRNA, tRNA, and rRNA are involved in the information flow in cells.
2. How might the first membranes have formed, and how could this event have resulted in something resembling a cell?
3. Describe how mitochondria may have arisen in eukaryal cells.
4. Differentiate between the terms *heterotroph* and *autotroph*.
5. Describe the roles of glycolysis, fermentation, and respiration in energy production in different environments.

1.3 Microbial Ecology and Biotechnology

- How do microbes interact with the world around them?

So far we have seen that microbes not only share many structural and functional features, but also exhibit a great deal of diversity. We also have seen, at least briefly, evolved, how microorganisms acquire and use energy, and how genetic information is transmitted and interpreted within microbes. As we continue our introduction to microbiology, let's ask two more important questions. First, how do microorganisms interact with their environment? Second, how can we utilize these various interactions? As we will see, these two questions are intricately linked.

As we mentioned in the previous section, the evolution of photosynthesis resulted in the increased level of atmospheric oxygen that makes our existence possible. In later chapters, we will see that microbes affect biogeochemical cycling of many other chemicals, from nitrogen to carbon to phosphorus. They interact not only with chemicals, but also with each other and with various macroscopic organisms. Moreover, we have learned how to harness some of their remarkable powers. From the use of microbes to produce foods and drinks to the use of microbes to produce life-saving medicines, we are intricately tied to our microbial partners!

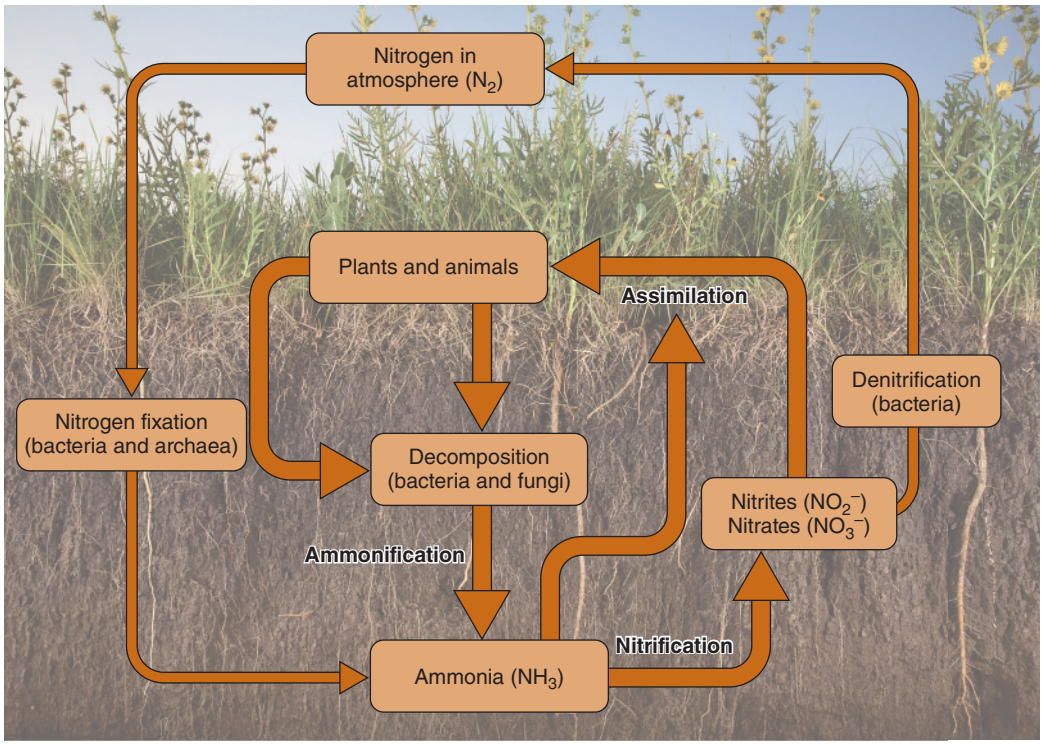
Microorganisms and Biogeochemical Cycling

Microbes have been intimately involved in modulating conditions within the **biosphere**, those regions of Earth that can support life. Not only did microorganisms, through photosynthesis, create the oxygen-rich atmosphere on which most life on Earth relies, but they are also involved in **biogeochemical cycling**, the transitioning of various chemicals between organic and inorganic forms. The amount of carbon contained within living bacteria on Earth, for instance, is estimated to be nearly as great as the amount of carbon in all the multicellular organisms combined. Photosynthetic cyanobacteria convert CO_2 from the atmosphere into organic molecules. Microbial metabolism ultimately converts much of this organic carbon back into CO_2.

As we saw in Section 1.1, nucleic acids and polypeptides, two important categories of cellular macromolecules, contain nitrogen. Only certain types of bacteria and archaea can convert nitrogen gas (N_2) from the atmosphere into forms that can be readily used by other organisms to form these molecules. This **nitrogen fixation** is accomplished by both free-living bacteria and bacteria living in symbiotic associations with plants **(Figure 1.24)**. Bacteria also convert nitrogen present in organic material back to N_2 gas through both denitrification and ammonia oxidation. In terrestrial habitats, denitrification and ammonia oxidation are of special concern to farmers, as they can decrease the fertility of agricultural soils. In the marine environment, these processes can limit ocean productivity.

Microbial Interactions

Not surprisingly, microbes do not exist in isolation. As we will investigate more thoroughly in Chapter 14, microbes exist in diverse communities of organisms that interact with each other and the environment. As we will see in Chapter 3, vast numbers of eukaryal microorganisms exist within the Rio



JIM RICHARDSON/National Geographic Image Collection

FIGURE 1.24 Role of microbes in the global nitrogen cycle Only certain bacteria and archaea can convert gaseous nitrogen (N_2) into forms that can be used by other living organisms. Nitrogen incorporated into ammonia (NH_3), nitrites (NO_2^-), and nitrates (NO_3^-) can be used to synthesize amino acids. These molecules also can be used in the synthesis of nucleic acids. Other microorganisms decompose nitrogen-containing molecules and act on the resulting products, converting various forms of nitrogen back into N2 and thereby completing the nitrogen cycle.

Tinto, a river in Spain with a pH of approximately 2. As we will see in Chapter 4, communities of organisms live in the waters surrounding deep sea thermal vents, in a large part dependent on archaea that thrive in the superheated water near these thermal vents. As we will see in Chapter 15, many microbes live with, in, or on various plants, invertebrates, and vertebrates.

Microbes, of course, also live in and on humans. Van Leeuwenhoek, remember, described microorganisms living on a person's teeth over 300 years ago. In fact, we can view the human body as a complex ecosystem. Microbes exist throughout our bodies, often in a symbiotic relationship with us. Microorganisms within our body produce vitamins for us and help us digest our food. Some microbes, as we will discuss in Chapter 23, even help us control the replication of other, unwanted microbes, thereby aiding in the prevention of disease.

Biotechnology and Industrial Microbiology

Scientists studying genetics and molecular biology revolutionized all of biology in the late 1970s with the development of methods for producing **recombinant DNA** molecules—DNA sequences linked together to form a single molecule that never existed previously in the natural world. This revolutionary technology has allowed genes from humans and many other organisms to be inserted into bacteria and other microbes. This technique has allowed microbes to be used as low-cost manufacturing plants for the production of valuable proteins such as human insulin and human growth hormone. The incredible range of applications for recombinant DNA methods could not have been realized without the availability of well-studied microorganisms such as *Escherichia coli*.

The process of creating recombinant DNA is surprisingly simple. From bacteria, researchers first can isolate plasmids, small circular DNA molecules that replicate independently of the chromosome. Using restriction endonucleases, bacterial enzymes that cleave DNA at specific locations, researchers can insert foreign DNA into a plasmid, thereby creating a new molecule. Finally, this recombinant DNA molecule can be added back to bacteria. As the bacteria replicate, the recombinant plasmid also replicates. More importantly, foreign genes inserted into the plasmid will be expressed **(Figure 1.25)**.

Following a related process, researchers at Genentech, a biotechnology company based in San Francisco, announced in 1978 that they had engineered *E. coli* to produce human insulin. This achievement marked the first time that a medical product was produced via recombinant DNA technology. Today, a vast majority of insulin used by people with diabetes is produced in an analogous fashion.

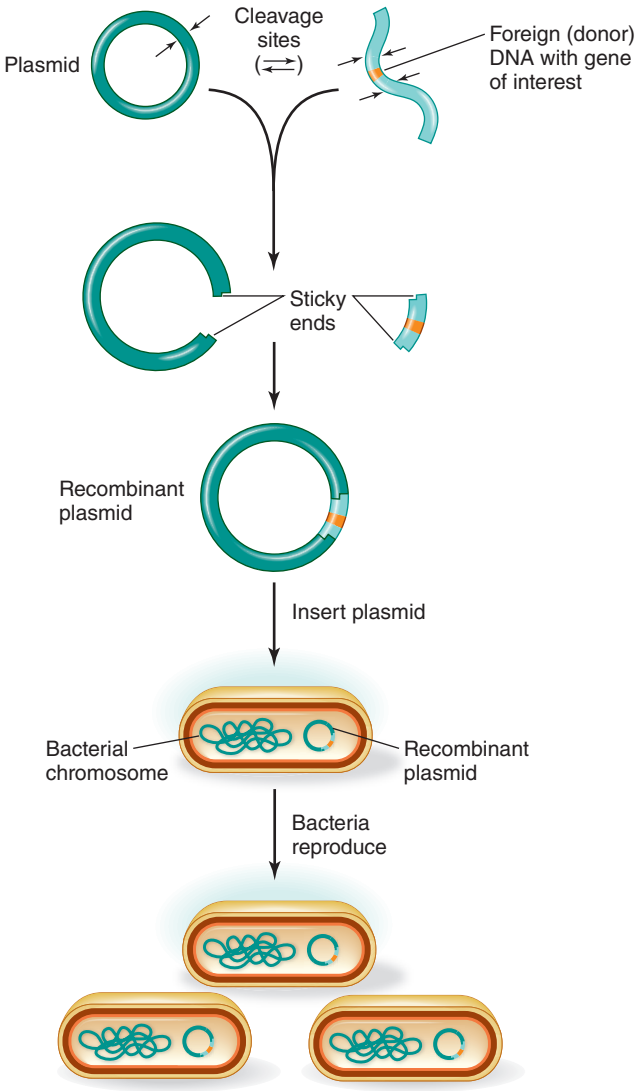


FIGURE 1.25 Recombinant DNA techniques Plasmid DNA and donor DNA can be cleaved with a restriction endonuclease, or restriction enzyme. When mixed together, the foreign DNA can become incorporated into the linearized plasmid, resulting in the formation of a recombinant plasmid. This plasmid can be introduced into bacteria. As the bacteria reproduce, the plasmid will be replicated, resulting in the replication of the foreign DNA. If the donor DNA contains a gene, then the bacteria can express that gene, resulting in the production of a foreign protein, coded by the donor (foreign) DNA.

Connections **As we will see in** Section 16.2, **researchers now can use techniques like site-directed mutagenesis to intentionally modify natural gene products. In** Section 16.4, **we will investigate how biotechnology may help to alleviate our dependence on fossil fuels.**

1.3 Fact Check

1. Explain how bacteria are involved in nitrogen cycling.
2. Describe how a recombinant DNA molecule can be constructed.

1.4 Microbes and Disease

- How are microbes associated with disease?

Although we now know that some microbes can cause disease, this concept was not common knowledge for most of human history. People thought that diseases had various causes—from angry gods to bad air. These views prevailed mostly because people could not see the bacteria associated with bacterial infections. Even after the development of the microscope, people still did not understand that microbes could be transmitted from person to person. Rather, many assumed that microbes arose from inanimate materials, a process known as spontaneous generation. This belief negated a need to even consider the transmission or prevention of microbial diseases. If microbes arose spontaneously, then there was no reason to investigate how a person became infected.

While many scientists worked to disprove the widely held belief in spontaneous generation, Louis Pasteur's experiments in the mid-1800s provided the most compelling evidence refuting this idea. As with so many classic experiments, his experiment was both simple and elegant. Pasteur added nutrient broths to swan-necked flasks and then boiled the broths to kill any contaminating microorganisms. He then observed the broths for signs of microbial growth **(Figure 1.26)**.

With this approach, Pasteur reasoned, outside air could enter the flask. Bacteria present in the outside air, though, would become trapped in the neck of the flask, never coming in contact with the sterile broth. No bacteria, he hypothesized, would grow. If the flask were tilted such that the broth traveled to the neck of the flask and then returned to the upright position, however, bacteria trapped in the neck would flow back into the nutrient broth and growth would occur. In other words, microbial life in the broth could result only from microbial life present in the neck of the flask; it did not arise spontaneously.

Connections **What signs of microbial growth did Pasteur observe? In** Section 7.1, **we will examine how bacteria grow under standard laboratory conditions. In** Section 7.4, **we will explore how to block this growth.**

The Identification of Infectious Agents

About 200 years after van Leeuwenhoek's first observations of microbes (and just 15 years after Pasteur showed that microorganisms do not arise by spontaneous generation), Robert Koch provided the first clear demonstration that a bacterium, *Bacillus anthracis* **(Microbes in Focus 1.1)**, was the cause of a specific disease, anthrax, in livestock.

Robert Koch was a German physician and scientist and a true pioneer in the field of microbiology. Aside from his anthrax work **(Figure 1.27)**, he was responsible not only for

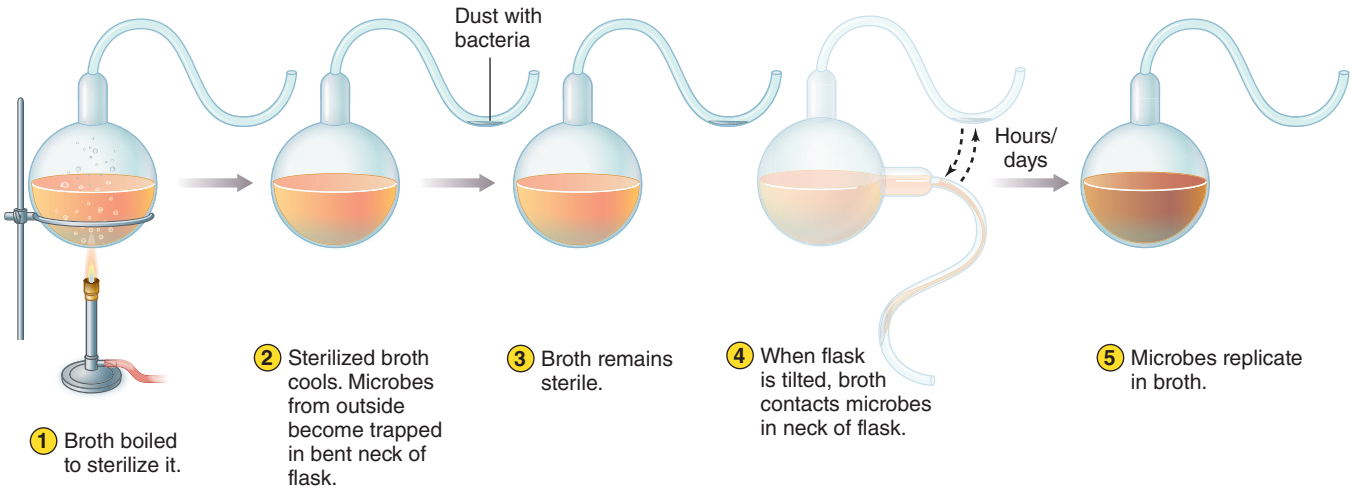


FIGURE 1.26 Process Diagram: Pasteur refutes spontaneous generation By showing that bacterial growth did not occur in sterile broth within swan-necked flasks but did occur when the sterile broth contacted outside contaminants, Pasteur demonstrated that microorganisms did not arise spontaneously.

developing important laboratory techniques—for example, the use of media solidified with agar for the isolation of bacteria in the laboratory—but also for developing clear criteria linking particular microorganisms to specific diseases. We will explore the details of these criteria, referred to as Koch's postulates, in Section 18.4.

Koch used these criteria to establish the cause of other important diseases, including tuberculosis, which is caused by the bacterium *Mycobacterium tuberculosis*. The identification of other infectious microbes quickly followed. The late 1800s, in fact, were a golden age of sorts, in which medical science finally began to achieve real insight into many infectious diseases.

Connections **As our understanding of infectious diseases and our experimental techniques have changed, so too have our interpretations of Koch's postulates. Molecular correlates of his basic postulates are explored in** Section 18.4.

The Effects of Infectious Diseases

As van Leeuwenhoek observed, many microbes exist in and on our bodies. Most of the time, these microbial partners are just that—partners. When the relationship between microbes and host becomes unbalanced, then disease can arise. Microbial diseases, as we all know, have profoundly affected human life. As we will see in the final section of this book, our understanding of how microbes cause disease—and how we can prevent these diseases or alleviate their effects—is constantly changing.

We all feel the effects of microbial infections through the course of our lives. A runny nose and a cough often are signs of a cold, which may be due to a rhinovirus infection of the upper respiratory tract. The onset of winter portends the flu season, when hundreds of millions of people each year suffer from fevers, headaches, coughs, and muscle aches caused by the influenza virus. The average person in the United States experiences several bouts of diarrhea per year, which could have any number of different causes, including viruses, bacteria, or protozoa,

Microbes in Focus 1.1

Bacillus anthracis: Linking a Microbe to a Disease

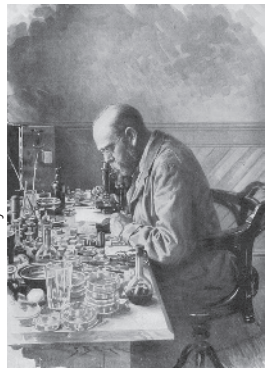
Habitat: Infects various mammals, including cattle, sheep, and horses. Also can infect humans. Spores can be found in soil.

Description: Gram-positive rod-shaped bacterium measuring approximately 1 μm in width and 3 μm in length

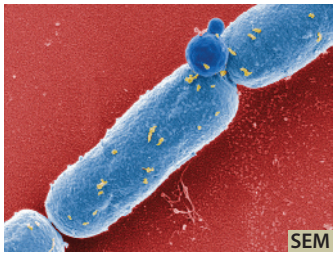
Key Features: When nutrients are lacking, *B. anthracis* can form endospores, metabolically inert structures that are largely resistant to harsh environmental conditions. The endospores can begin replicating again when conditions improve, even after years in the endospore state. Although naturally occurring human infections with *B. anthracis* are rare, this bacterium still remains actively studied by researchers, mainly because of its potential use as a bioweapon. We will explore this topic more in Chapter 7.



© Dr. James Feeley/CDC


© Chronicle/Alamy Stock Photo

a. Robert Koch



© Dr. Gary Gaugler/Science Source

b. *Bacillus anthracis*


AP Images/CHUCK BIGGER

c. Vaccination

FIGURE 1.27 Robert Koch proved that anthrax was caused by a microorganism Koch showed that a specific microbe, the bacterium *Bacillus anthracis*, caused anthrax in livestock. Koch also developed Koch's postulates, a series of criteria that needed to be met to prove that a specific microorganism caused a specific disease. **a.** Robert Koch. **b.** *B. anthracis* (color enhanced). **c.** Cow about to receive anthrax vaccination.

all of which can be acquired through food or drink. If you are studying this book in the United States, Canada, or Europe, it's unlikely (but not unheard of) that you will have to deal with the constant threat of infections with serious or even life-threatening consequences. If you live in one of the many regions of the world where affordable effective public health measures are lacking, infectious diseases may have a more prominent role in your life. AIDS, malaria, tuberculosis, and other diseases of microbial origin take millions of lives per year in these regions; we are far from a solution to this enormous problem.

History is full of examples of the powerful impact of infectious diseases. Modern medicine has greatly improved our ability to deal with these diseases. We now understand how infectious diseases spread, and the widespread availability of vaccines and antimicrobial drugs facilitates prevention and treatment. But we have not eliminated the threat of new infectious diseases. The human immunodeficiency virus (HIV), which was only discovered in the 1980s, has caused over 40 million deaths since then, and there is still no vaccine to prevent HIV infection. The influenza virus has caused devastating pandemics, or worldwide outbreaks, in centuries past, and now there is great concern that a new serotype could enter the human population, with potentially catastrophic consequences. The 2014–2016 outbreak of Ebola virus devastated parts of western Africa. Finally, the COVID-19 virus wreaked havoc on people throughout the world in 2020.

Connections **As we saw during the summer and fall of 2009, the H1N1 strain of influenza virus quickly spread throughout the world. What factors led to the rapid dissemination of this microbe? We will discuss epidemiology, or the study of how diseases spread in populations, in** Section 18.3.

An exceptionally devastating pandemic occurred when plague, popularly referred to as "The Black Death," killed a third or more of the population of Europe, Asia, and North Africa in a 60-year span from 1340 to 1400 **(Figure 1.28)**. Plague is caused by the bacterium *Yersinia pestis*, which infects rodents and humans and is transmitted between them by fleas **(Figure 1.29)**. This bacterium is commonly present in rodents such as mice, rats, and squirrels in many parts of the world. The plague pandemic of the 1300s is thought to have originated in central Asia or China and probably moved westward with trading caravans and/or Mongol armies. Historical records indicate that plague was absolutely devastating. Up to 60 million people, perhaps half of its population, may have died from plague in China in the 1300s. In Europe, 25–50 million people perished, largely between 1347 and 1351. Several million more people were killed by plague in the Middle East and northern Africa.

To put the global death toll due to the plague pandemic of the 1300s into perspective, imagine a modern pandemic that resulted in the death of *over 1 billion people* within a few years! Even the modern AIDS and influenza pandemics pale by comparison. In the Middle Ages, people did not understand the underlying cause of this horrifying disease. Supernatural explanations of disease were universally accepted; diseases were punishments from God or the results of curses, witchcraft, or "bad air." The true cause of plague, in fact, was not discovered until 1893, when Alexandre Yersin isolated the bacterium *Y. pestis* during a plague outbreak in Hong Kong. Today, plague is rare and treatable with antibiotics.

The massive social disruptions caused by epidemics such as plague in the Middle Ages are rivaled only by the effects of war, famine, and natural disasters. From the perspective of public health, these events are not unrelated. Until very recently, most people who died during wartime, both military and civilian, were victims of microbes. Before the advent of antibiotics during World War II, battlefield wounds were highly likely to become infected, the end result of which was often death. Troops in the field for prolonged periods often were affected by disease. Cholera, typhus, and dysentery were common due to the poor sanitation conditions. Diseases spread by carriers such as lice and fleas, which thrive in crowded conditions, also are a great risk to soldiers and displaced civilians (refugees) during wartime. Malnutrition resulting from war or famine (or both) blunts the effectiveness of the immune system, leaving soldiers and civilians more susceptible to disease.

Control of Infectious Diseases

As researchers learned more about the causes of infectious diseases in the late 1800s and early 1900s, the medical treatment of these diseases gained a scientific basis. Even before Koch linked specific microbes to specific diseases, the British physician Joseph Lister had discovered the value of cleanliness and disinfection measures in reducing mortality from post-surgical and post-childbirth infections. Doctors' offices and hospitals strove for the universal application of such disinfection

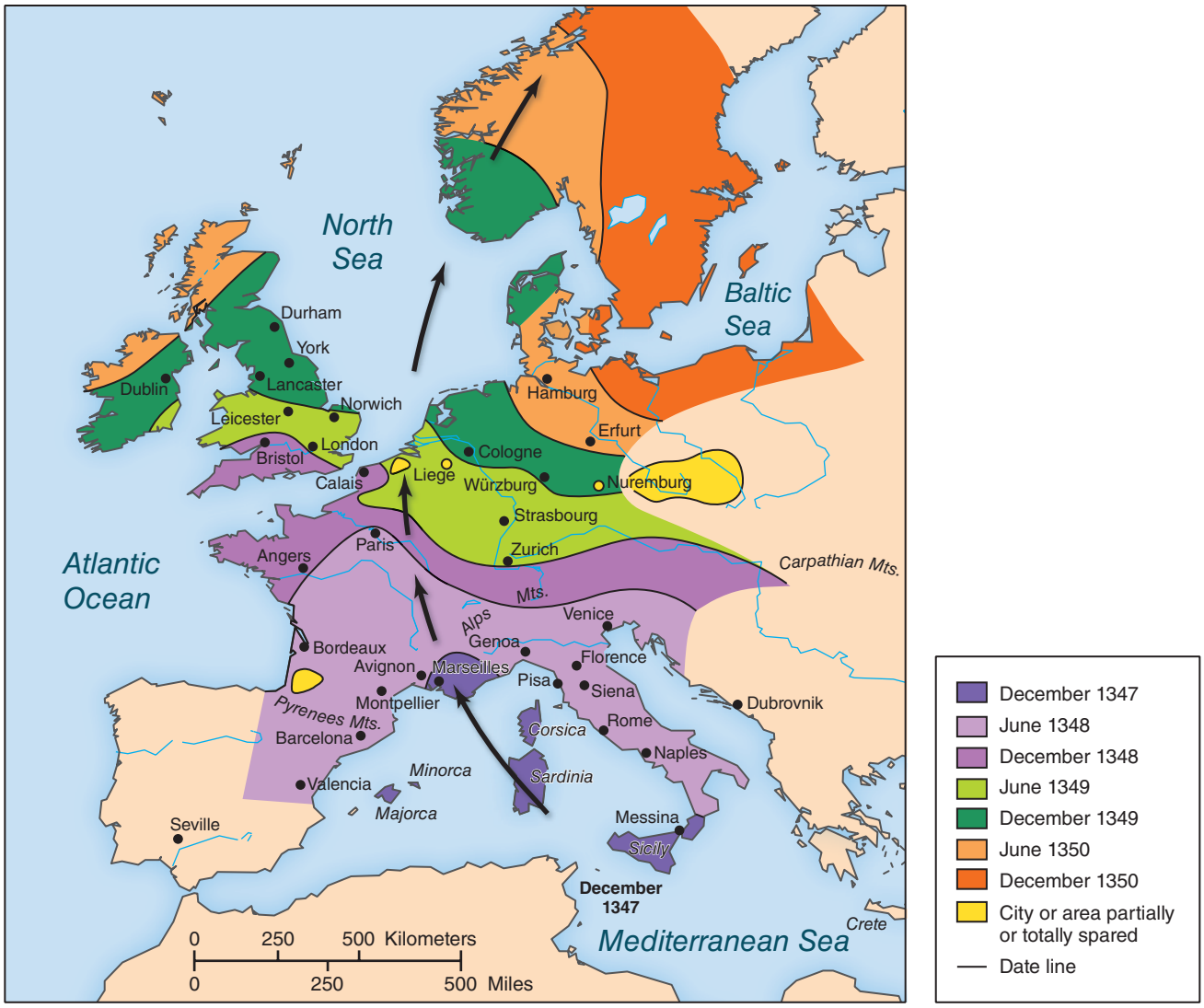


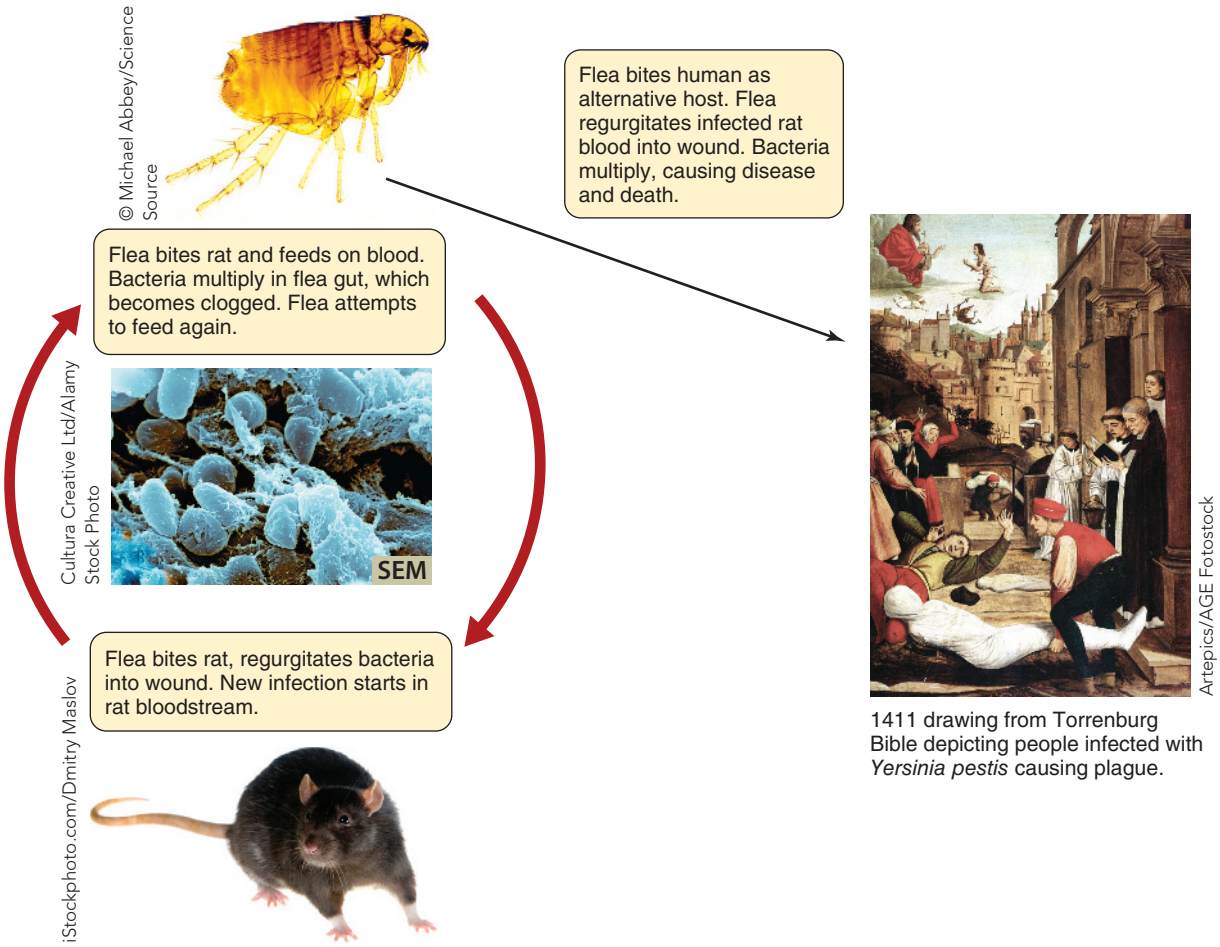
FIGURE 1.28 The Great Plague Also referred to as The Black Death, the plague spread rapidly throughout Europe in the 1300s. In a 60-year span, roughly a third of the European population died from this infectious disease. The dates on the map show how rapidly the disease moved through Europe in an era without the modern means of rapid mass transport. Poor sanitation and a lack of knowledge about the causes of infectious diseases contributed to its spread.

measures to decrease the incidence of disease. Although these methods of preventing infections were effective, methods for treating infectious diseases remained inadequate.

Even though Salvarsan was the first scientifically developed antimicrobial to be marketed commercially, it was not until penicillin and sulfa drugs came into use in the 1940s that antimicrobial drugs had a major impact on the treatment of infectious diseases. Indeed, people in developed countries saw a dramatic decrease in deaths due to infectious disease during the twentieth century because of improved sanitation and the development of antimicrobial drugs and vaccines **(Figure 1.30)**. A new problem, however, now faces us. Increasingly, infections result from antibiotic-resistant bacteria. Our existing antibiotics are not effective against these microorganisms, and our ability to treat some infectious diseases is declining.

Connections **Recall that bacteria are masters at sharing genes through horizontal gene transfer. This facilitates the passing of genes for antimicrobial resistance. History has shown that the more effective an antimicrobial drug is, the more it is prescribed and the faster resistance spreads, rendering the drug ineffective. See** Section 24.3 **for more on the acquisition of drug resistance.**

Vaccines also have had an enormous impact in reducing the sickness and death associated with infectious diseases. Vaccination involves exposing a person to an inactivated or weakened version of a microbe, or even just a part of the microbe, to create immunity to a disease. The past century has seen vaccines developed for many deadly diseases, including polio, diphtheria, and rabies. Historically, vaccination began roughly 2,000 years ago in China and India as a defense against smallpox. Smallpox,



1411 drawing from Torrenburg Bible depicting people infected with *Yersinia pestis* causing plague.

FIGURE 1.29 Transmission of plague The plague is caused by the bacterium *Yersinia pestis*, which typically infects rats and is transmitted by fleas. If an infected flea bites a human, then the bacterium can be transmitted to the human, resulting in disease and, potentially, death.

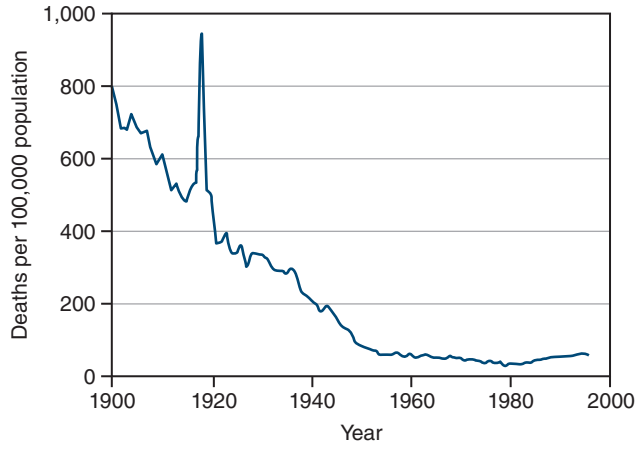


FIGURE 1.30 Infectious disease deaths in the United States during the twentieth century Deaths associated with infectious diseases decreased dramatically in the United States during the twentieth century. The spike in deaths due to infectious diseases around 1920 represents the increased deaths caused by the 1918 influenza pandemic. While the overall infectious disease burden in the United States has remained low, new infectious agents, like HIV, have emerged and existing infectious diseases, like measles, have shown an increased prevalence.

a viral disease, was common in Europe and Asia, in some areas being responsible for 20 percent of all deaths. It was fatal to one of every four people infected. Those who survived carried characteristic smallpox scars for the rest of their lives, but they also carried a lifelong protection from the disease.

Vaccination against smallpox was occasionally practiced in eighteenth-century Europe, but it was the famous experiment of English physician Edward Jenner in 1796 that popularized the procedure. Jenner used material from a cowpox infection of a milkmaid to inoculate a boy, who was later shown to be immune to smallpox **(Figure 1.31)**. We now know that cowpox virus is closely related to the smallpox virus, explaining why exposure to it generated immunity to smallpox. Thanks to the smallpox vaccine, in fact, no naturally occurring cases of smallpox have occurred since 1977. In one of the greatest achievements in medical history, we have eliminated this scourge from the face of Earth.

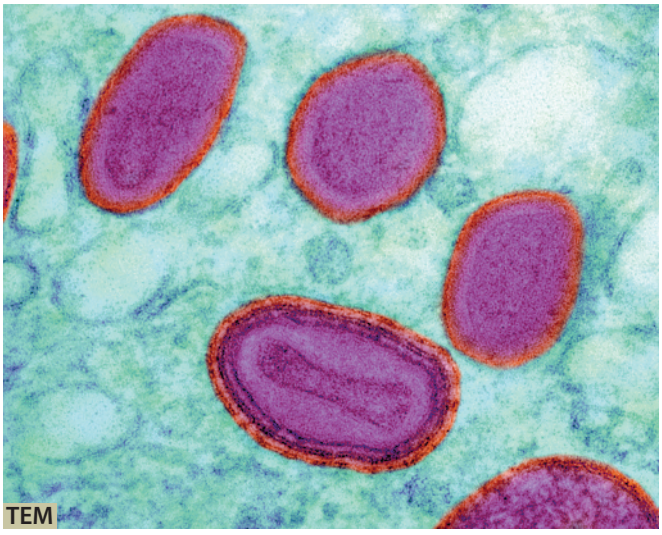
People in developing regions of the world, particularly in sub-Saharan Africa, continue to suffer from infectious diseases. These diseases include AIDS, tuberculosis, and malaria, which combined take nearly five million lives per year **(Figure 1.32)**. Vaccines are not yet available that can prevent HIV or malaria infection, and the current tuberculosis vaccine is far from ideal. Drugs to treat these three diseases are available, but they are


© SPL/Science Source

a. Drawing of cowpox lesions on a milkmaid's hand.


© Archives Charmet/Bridgeman Images

b. Painting depicting Jenner inoculating a boy against smallpox



© SPL/Science Source

c. Vaccinia virus

FIGURE 1.31 Edward Jenner and the development of vaccination Although the Chinese practiced forms of vaccination against smallpox for hundreds of years, vaccination was not practiced in Europe until the end of the eighteenth century. In 1796, Edward Jenner showed that he could inoculate a boy with material obtained from the pox marks on a woman infected with cowpox and provide the boy with immunity to smallpox. Global vaccinations have eliminated smallpox. **a.** 1798 illustration from Jenner of cowpox lesions on a milkmaid's hand. **b.** Painting of Jenner vaccinating a person against smallpox. **c.** Vaccinia virus, the virus used as the smallpox vaccine.

expensive, which means that many people do not have adequate access to the therapies. Additionally, it is not uncommon to find people in developing countries simultaneously affected by at least two of these diseases, lowering their life expectancy even further.

Other major historical factors that have reduced death from infectious diseases include improvements in personal hygiene, public sanitation, and food and water safety. Indoor plumbing, water treatment measures, and large-scale sewage disposal systems have led to a decrease in waterborne infectious diseases. **Pasteurization**, the process in which milk is heated briefly to kill most microorganisms, freezers, and refrigerators all have contributed to the increased safety of food. Many of us take these advances for granted. Unfortunately, these simple measures are not universally available. As a result, we now are faced with what Dr. Paul Farmer refers to as the "great epi (epidemiology) divide." People in developing countries and people in developed countries without access to adequate health care suffer a disproportionate infectious disease burden.

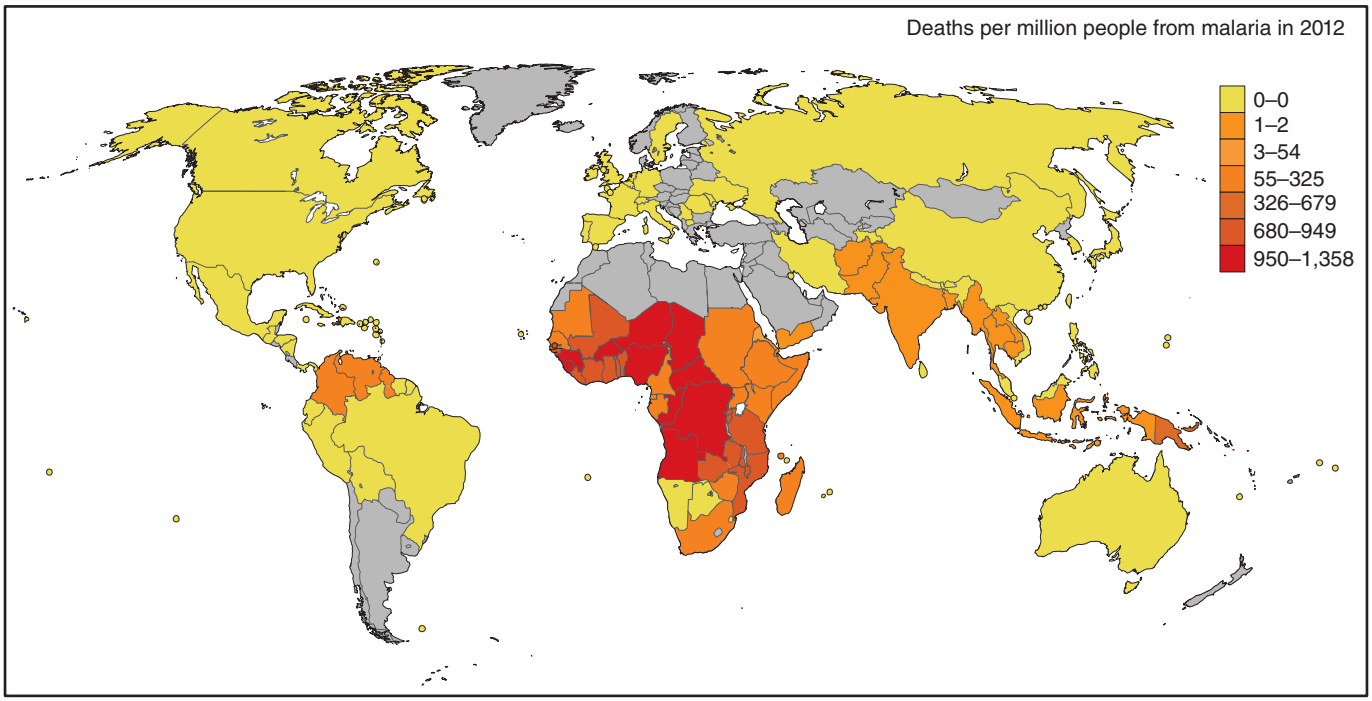
In this chapter, we have barely scratched the surface of microbiology. We have been introduced to the microbes. We have learned about the evolution of life and the genetics of microbes. We have begun to explore how microorganisms acquire energy. Finally, we have seen how microbes can cause diseases. In later chapters, we will further explore these topics. We will see how important microbes are in our world. As we investigate these topics, we will see that extensive experimental evidence supports our current understanding of the microbial world. We also will see that many questions and ambiguities still remain.

Throughout this book, we will see that microbiology is a dynamic science. Microbiologists continue to make new discoveries using an expanding selection of laboratory tools. They also continue to refine, modify, and, occasionally, reject existing hypotheses. As we saw in the Mini-Paper, inquisitive minds, continuous experimentation, and the development of new tools can combine to result in completely new ways of seeing the world around us. Such dramatic advances have occurred throughout the history of microbiology and certainly will continue to occur **(Table 1.4)**.

During our exploration, we will make connections between topics. We will see how intimately related microbes are to each other, to their environment, and to us. We will see how the genetics, physiology, and habitat of microbes are intertwined. We also will see how, as the evolutionary biologist Theodosius Dobzhansky wrote in 1973, "Nothing in biology makes sense except in the light of evolution." Most importantly, we will see that the microbial world presents as many surprises to us today as it did to van Leeuwenhoek over 300 years ago.

1.4 Fact Check

1. Why was the role of microbes in causing disease not accepted for so many years?
2. What is spontaneous generation, and how was it disproved?
3. Describe measures that have been effective in controlling and reducing deaths from infectious diseases.



Source: From Malaria world map-Deaths per million persons WHO 2012 in Malaria: An Ancient and Deadly Foe. Licensed under CC BY-SA 4.0. Public Domain

FIGURE 1.32 Impact of malaria in sub-Saharan Africa During the twentieth century, global deaths associated with malaria, an infectious disease primarily caused by the protozoan *Plasmodium falciparum*, decreased dramatically. However, malaria remains a major problem in sub-Saharan Africa. According to the World Health Organization, about 9 in 10 deaths due to malaria in 2012 occurred in this region. Many other infectious diseases also disproportionately affect people in developing countries and people without clean water or access to adequate health care.

TABLE 1.4 Selected advances in microbiology

Year	Scientist	Advance
1600s	Anton van Leeuwenhoek	Uses microscope to see microorganisms
1860s	Louis Pasteur	Disproves idea of spontaneous generation
1860s	Joseph Lister	Practices infection control
1876	Robert Koch	Identifies *Bacillus anthracis* as cause of anthrax
1928	Alexander Fleming	Discovers penicillin
1950s	Jonas Salk and Albert Sabin	Develop poliovirus vaccines
1966	Lynn Margulis	Proposes endosymbiotic theory
1983	Kary Mullis	Invents PCR
1990	Carl Woese	Proposes three-domain classification of living organisms
1995	Craig Venter	Publishes first complete bacterial genome sequence
1998	Jo Handelsman	Coins the term *metagenomics*
2003	Rita Colwell	Shows that cholera infections can be prevented by simple filtration
2006	Harald zur Hausen	Discovers that human papillomaviruses cause cervical cancer, leading to development of the HPV vaccine
2012	Jennifer Doudna	Discovers CRISPR/Cas9 system for editing DNA

The Rest of the Story

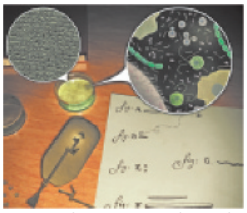
Van Leeuwenhoek noted that the number of microbes on a person's teeth may "exceed the number of Men in a kingdom." It most certainly does. Today, many researchers estimate that the human body consists of 10^{13} human cells. On us and in us, though, there may be an additional 10^{13} microbial cells. In other words, one could argue that we are equal parts microbe and human! The study of these microbes has moved well beyond the use of the microscope. In this chapter, we reviewed how the microbes present today have evolved to occupy a plethora of habitats using a vast array of metabolic processes, even within the human body. Growth conditions for many of these microbes are well understood, and they have been isolated and grown in the laboratory. However, a large subset still remains a mystery because their specific growth microenvironments have not been determined or cannot be reproduced experimentally.

In 2007, the Human Microbiome Project was launched with an aim to identify, analyze, and catalog the hundreds of microbial species residing in or on the human body (the human microbiota). As part of this project, researchers amplified and sequenced bacterial small subunit ribosomal RNA (SSU rRNA) genes, as described in Section 1.1, from various body sites on nearly 250 people. The initial results, published in June of 2012, were astounding. Every one of us is home to hundreds of different bacterial species, and the inhabitants of one person often differ from the inhabitants of another person. This "non-cultivation-based" approach ultimately will facilitate the genetic sequencing and more complete understanding of the microbes that have a major influence on human health and disease.

Image in Action

Anton van Leeuwenhoek is examining a sample of pond water using the microscope he built by hand. Today, microbiologists also can analyze DNA sequences (represented in the upper left) to study environmental samples.

1. What types of microorganisms (and from what domains of life) did Anton van Leeuwenhoek most likely observe? What microbes was van Leeuwenhoek unable to observe with his simple microscope? Explain.
2. Imagine that you were given access to the same drop of pond water observed by van Leeuwenhoek. How could you use PCR to identify or characterize some of the microbes he observed or even those he didn't observe?



Copyright © 2013 John Wiley & Sons, Inc. All rights reserved.

Summary

Section 1.1: What is microbiology?

Microbiology is the study of **microorganisms**, including bacteria, archaea, and eukaryal microorganisms, and viruses. Collectively, we can refer to all of them as **microbes**.

- All living organisms share certain features, including **metabolism**, **growth**, **reproduction**, genetic variation resulting in **evolution**, response to outside stimuli, and internal **homeostasis**.
- A **cell** is the simplest structure capable of carrying out all the processes of life.
- All cells contain various **macromolecules**, including **polypeptides**, **nucleic acids**, **lipids**, and **polysaccharides**. Many polypeptides function as **enzymes**.
- Historically, all living organisms were classified as **eukaryotes** or **prokaryotes**, depending on whether they did or did not have a nucleus. Today, the taxonomy of living organisms consists of three domains: *Bacteria*, *Archaea*, and *Eukarya*.
- This classification scheme reflects the **phylogeny** of all living organisms.
- Our ability to classify microorganisms has been aided greatly by the **polymerase chain reaction (PCR)**.
- Viruses are subcellular and can be classified as microbes.
- Because of their relatively simple structures, microbes have been useful research models.

Section 1.2: What do we know about the physiology, genetics, and cultivation of microbes?

All living organisms possess remarkably similar informational molecules and processes for converting this genetic information into functional molecules. This conservation of genetic processes provides compelling evidence that all living organisms are evolutionarily related.

- Life probably evolved on Earth around 3.8 billion years ago. Simple organic molecules, possibly associated with iron-containing surfaces, became enclosed within a lipid membrane.

- The identification of **ribozymes** lends support to the **RNA world** hypothesis of the origin of life. The precursors of life, the so-called **progenote**, may have used RNA as the major informational molecule.
- Eukarya arose through endosymbiosis, in which free-living bacteria became engulfed within a developing eukaryal cell, providing the host with the ability to harvest sunlight or undergo aerobic respiration.
- In all cells, the main informational molecule is double-stranded DNA, a molecule ideally suited for containing information and for faithful replication. This DNA-based information is converted into functional molecules through the processes of transcription and translation.
- **Mutation** in the DNA allows for genetic variation, a prerequisite for evolution.
- All living organisms must obtain organic molecules. **Heterotrophs** ingest them. **Autotrophs** produce their own organic molecules.

Section 1.3: How do microbes interact with the world around them?

- Microbial metabolism has affected the **biosphere**, and microbes are intimately involved in the **biogeochemical cycling** of many chemicals, including **nitrogen fixation**.
- Microbes interact with each other and other organisms in many complex ways.
- Our understanding of the basic genetic processes has led to the development of **recombinant DNA** technologies. We now can alter DNA molecules and produce human proteins in microbial hosts.
- Recent advances in DNA-sequencing technologies and increased computing power have led to the emerging fields of genomics and bioinformatics.

Section 1.4: How are microbes associated with disease?

The work of a number of microbiologists, including Louis Pasteur, led to the development and acceptance of the germ theory of disease.

- Koch's postulates, developed in the 1800s, provide a means of demonstrating that a particular microorganism causes a particular disease.
- Infectious diseases have had, and continue to have, a profound impact on humans.
- Today, we have an assortment of antibiotics, antivirals, and vaccines that treat or prevent many infectious diseases. The development of these therapies has depended in large part on our understanding of the structure of these microbes and their replication strategies. Other techniques, like **pasteurization**, also have led to a decrease in the incidence of certain infectious diseases.
- Unfortunately, infectious diseases remain horrific threats to people throughout the world.

Application Questions

1. A researcher is studying a newly discovered microbe and must first determine whether this microbe should be considered alive. What characteristics will she examine to make this determination?

2. Given what we learned about proteins in Section 1.1, what might be the effects of an antimicrobial drug that halts protein synthesis in cells?

3. Imagine a researcher is examining several cell samples in the laboratory and needs to categorize each sample as either bacterial or eukaryal. What features should the researcher examine and why?

4. Inspired by the Miller–Urey experiment described in Perspective 1.1, a researcher continues to explore the origins of life on Earth. The first task is to design a simulation of prebiotic Earth. What conditions should the researcher consider when creating this simulation?

5. According to the endosymbiotic theory, mitochondria and chloroplasts are derived from bacterial cells. What evidence exists to support this theory?

6. If a species of microbe were discovered that did not appear to mutate, scientists probably would hypothesize that its lack of mutation would be detrimental to its evolution. Explain why the scientists would make this prediction.

7. In Section 1.2, oxygenic photosynthesis and aerobic respiration were described. Based on our discussion of these two processes, explain why oxygenic photosynthesis must have evolved on Earth before aerobic respiration.

8. Oxygen is not always a "good thing." In fact, O_2 can be considered dangerous. Explain why oxygen can be harmful and what microbes must do to deal with the dangers of an oxygenated world.

9. In Section 1.4, we discuss the great epi (epidemiology) divide between developing and developed countries. List several policy and funding changes that could be implemented in developing countries to decrease the number of infectious disease cases. Explain each of these recommendations.

10. A research group has received funding to study the diversity and evolutionary relatedness of microbes in a marine ecosystem. The head of the laboratory has decided to sequence and analyze the SSU rRNA genes of the microorganisms in this ecosystem.

a. What are SSU rRNA genes?

b. Why are these gene sequences ideal for studying the evolutionary relationships of the microorganisms found there?

c. How is this process conducted?

d. After sequencing and analyzing three gene samples, the researchers conclude that two of the samples are from closely related microorganisms, while the third sample is very distantly related. Describe the sequencing results that would lead to this conclusion.

11. In Section 1.1, we discussed Paul Ehrlich's foundational research in which he noted differences between bacterial and human cells. Those observed differences became the basis for the development of antimicrobial drugs that "spare" eukaryal human cells. Based on these observed differences between bacterial and eukaryal cells:

a. Identify an aspect of bacteria that would not be a good target for a drug because it would not "spare" the host. Explain why this bacterial component is not a good choice for an antimicrobial drug.

b. Identify an aspect of bacteria that might be worth further exploration as a potential target for an antimicrobial drug that would not harm human cells. Explain.

Suggested Reading

Fleischmann, R. D., M. D. Adams, O. White, R. A. Clayton, E. F. Kirkness, A. R. Kerlavage, C. J. Bult, J. F. Tomb, B. A. Dougherty, J. M. Merrick, et al. 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae Rd*. Science 269:496–512.

Kruger, K., P. J. Grabowski, A. J. Zaug, J. Sands, D. E. Gottschling, and T. R. Cech. 1982. Self-splicing RNA: Autoexcision and autocyclization of the ribosomal RNA intervening sequence of *Tetrahymena*. Cell 31:147–157.

Miller, S. L., 1953. A production of amino acids under possible primitive Earth conditions. Science 117:528–529.

Miller, S. L., and H. C. Urey. 1959. Organic compound synthesis on the primitive earth. Science 130:245–251.

Turnbaugh, P. J., R. E. Ley, M. Hamady, C. M. Fraser-Liggett, R. Knight, and J. I. Gordon. 2007. The human microbiome project. Nature 449:804–810.

Woese, C. R., O. Kandler, and M. L. Wheelis. 1990. Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87:4576–4579.