CHAPTER 1

Introduction to Cell Biology

CONTENTS

WE ARE CELLS

We are made of cells. Cells make up our skin, our organs, and our muscles. The brain, the seat of our thoughts and desires, is made of cells. Our blood vessels teem with cells. Fertilization is no more or less than a joining of two separate cells to produce a single new cell, which then multiplies to produce the embryo. When we grow from a tiny embryo into a large adult, we do so by adding more and more cells. When we get sick, it is often because our cells have run amok. And when we grow old, it is because our cells gradually give up the ghost. After we die and are buried, soon the only remnants of our existence are bones, teeth, and hair, structures that were sculpted in life by the ceaseless activity of cells. Many medicines work by changing how cells behave, and in recent years cells themselves are being used as medicines to cure sick people. Because all living things are made of one or more cells, the origin of life corresponds to the origin of cells. Starting with this chapter, we will explore what cells are and how they work, themes that will be expanded throughout this book.

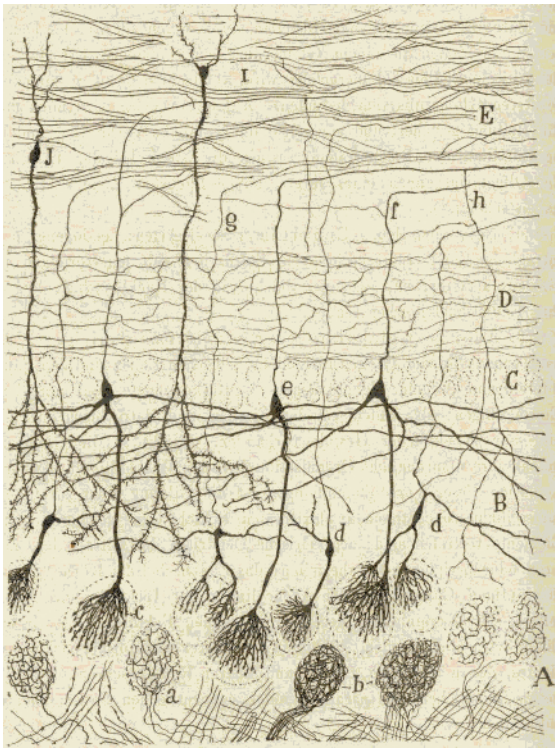


Diagram of nerve cells from the cat brain, hand-drawn by Santiago Ramón y Cajal. Ramón y Cajal was the first to recognize that the brain is made up of huge numbers of individual cells, rather than a continuous connected network as proposed by his competitor, Camillo Golgi. Ramón y Cajal and Golgi fought a protracted battle over this point, but eventually the meticulous detail of Ramón y Cajal's work convinced the world that the brain is indeed a collective of individual cells.

SOURCE: Histology of the Nervous System of Man and Vertebrates by Cajal (1995) Fig. "Neurons in the Cat Brain." by Permission of Oxford University Press.

1.1 The Discovery of Cells

Cells, and the structures they comprise, are too small to be directly seen, heard, or touched. In spite of this tremendous handicap, cells are the subject of hundreds of thousands of publications each year, with virtually every aspect of their minuscule structure coming under scrutiny. In many ways, the study of cell and molecular biology stands as a tribute to human curiosity for seeking to discover and to human creative intelligence for devising the complex instruments and elaborate techniques by which these discoveries can be made. This is not to imply that cell and molecular biologists have a monopoly on these noble traits. At one end of the scientific spectrum, astronomers are utilizing an orbiting telescope to capture images of primordial galaxies that are so far from Earth they appear to us today as they existed more than 13 billion years ago, only a few hundred million years after the Big Bang. At the other end of the spectrum, nuclear physicists have recently forced protons to collide with one another at velocities approaching the speed of light, confirming the existence of a hypothesized particle—the Higgs boson—that is proposed to endow all other subatomic particles with mass. Clearly, our universe consists of worlds within worlds, all aspects of which make for fascinating study.

As will be apparent throughout this book, cell and molecular biology is *reductionist*; that is, it is based on the view that knowledge of the parts of the whole can explain the character of the whole. When viewed in this way, our feeling for the wonder and mystery of life may be replaced by the need to explain everything in terms of the workings of the "machinery" of the living system. To the degree to which this occurs, it is hoped that this loss can be replaced by an equally strong appreciation for the beauty and complexity of the mechanisms underlying cellular activity.

Microscopy

Because of their small size, cells can only be observed with the aid of a **microscope**, an instrument that provides a magnified image of a tiny object. We do not know when humans first discovered the remarkable ability of curved-glass surfaces to bend light and form images. Spectacles were first made in Europe in the thirteenth century, and the first compound (double-lens) light microscopes were constructed by the end of the sixteenth century. By the mid-1600s, a handful of pioneering scientists had used their handmade microscopes to uncover a world that would never have been revealed to the naked eye. The discovery of cells (**FIGURE 1.1a**) is generally credited to Robert Hooke, an English microscopist who, at age 27, was awarded the position of curator of the Royal Society of London, England's foremost scientific academy. One of the many questions Hooke attempted to answer was why stoppers made of cork (part of the bark of trees) were so well suited to holding air in a bottle. As he wrote in 1665: "I took a good clear piece of cork, and with a Pen-knife sharpen'd as keen as a Razor, I cut a piece of it off, and . . . then examining it with a *Microscope*, me thought I could perceive it to appear a little porous . . . much like a Honeycomb." Hooke called the pores *cells* because they reminded him of the cells inhabited by monks living in a monastery. In actual fact, Hooke had observed the empty cell walls of dead plant tissue, walls that had originally been produced by the living cells they surrounded.

(a)

(b)

FIGURE 1.1 The discovery of cells. (*a*) One of Robert Hooke's more ornate compound (double-lens) microscopes. (Inset) Hooke's drawing of a thin slice of cork, showing the honeycomb-like network of "cells." (*b*) Single-lens microscope used by Antonie van Leeuwenhoek to observe bacteria and other microorganisms. The biconvex lens, which was capable of magnifying an object approximately 270 times and providing a resolution of approximately 1.35 µm, was held between two metal plates.

SOURCE: (a) The Granger Collection, New York; inset Biophoto Associates/Getty Images, Inc.; (b) © Bettmann/Corbis

Meanwhile, Antonie van Leeuwenhoek, a Dutchman who earned a living selling clothes and buttons, was spending his spare time grinding lenses and constructing simple microscopes of remarkable quality (Figure 1.1*b*). For 50 years, Leeuwenhoek sent letters to the Royal Society of London describing his microscopic observations—along with a rambling discourse on his daily habits and the state of his health. Leeuwenhoek was the first to examine a drop of pond water under the microscope and, to his amazement, observe the teeming microscopic "animalcules" that darted back and forth before his eyes. He was also the first to describe various forms of bacteria, which he obtained from water in which pepper had been soaked and from scrapings from his teeth. His initial letters to the Royal Society describing this previously unseen world were met with such skepticism that the society dispatched its curator, Robert Hooke, to confirm the observations. Hooke did just that, and Leeuwenhoek was soon a worldwide celebrity, receiving visits in

Holland from Peter the Great of Russia and the queen of England.

Cell Theory

It wasn't until the 1830s that the widespread importance of cells was realized. In 1838, Matthias Schleiden, a German lawyer turned botanist, concluded that, despite differences in the structure of various tissues, plants were made of cells and that the plant embryo arose from a single cell. In 1839, Theodor Schwann, a German zoologist and colleague of Schleiden's, published a comprehensive report on the cellular basis of animal life. Schwann concluded that the cells of plants and animals are similar structures and proposed these two tenets of the **cell theory**:

- All organisms are composed of one or more cells.
- The cell is the structural unit of life.

Schleiden and Schwann's ideas on the *origin* of cells proved to be less insightful; both agreed that cells could arise from noncellular materials. Given the prominence that these two scientists held in the scientific world, it took a number of years before observations by other biologists were accepted as demonstrating that cells did not arise in this manner any more than organisms arose by spontaneous generation. By 1855, Rudolf Virchow, a German pathologist, had made a convincing case for the third tenet of the cell theory:

- Cells can arise only by division from a preexisting cell.

1.2 Basic Properties of Cells

Just as plants and animals are alive, so too are cells. Life, in fact, is the most basic property of cells, and cells are the smallest units to exhibit this property. Unlike the parts of a cell, which simply deteriorate if isolated, whole cells can be removed from a plant or animal and cultured in a laboratory where they will grow and reproduce for extended periods of time. If mistreated, they may die. Death can also be considered one of the most basic properties of life, because only a living entity faces this prospect. Remarkably, cells within the body generally die "by their own hand"—the victims of an internal program that causes cells that are no longer needed or cells that pose a risk of becoming cancerous to eliminate themselves.

The first culture of human cells was begun by George and Martha Gey of Johns Hopkins University in 1951. The cells were obtained from a malignant tumor and named HeLa cells after the donor, Henrietta Lacks. HeLa cells—descended by cell division from this first cell sample—are still being grown in laboratories around the world today (**FIGURE 1.2**). Because they are so much simpler to study than cells situated within the body, cells grown **in vitro** (i.e., in culture, outside the body) have become an essential tool of cell and molecular biologists. In fact, much of the information that will be discussed in this book has been obtained using cells grown in laboratory cultures.

We will begin our exploration of cells by examining a few of their most fundamental properties.

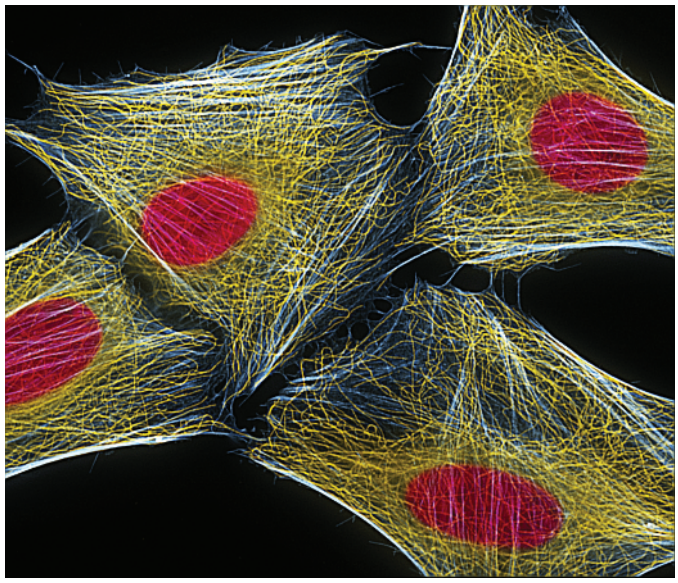


FIGURE 1.2 **HeLa cells**, such as the ones pictured here, were the first human cells to be kept in culture for long periods of time and are still in use today. Unlike normal cells, which have a finite lifetime in culture, these cancerous HeLa cells can be cultured indefinitely as long as conditions are favorable to support cell growth and division.

SOURCE: Torsten Wittmann/Photo Researchers, Inc.

Cells Are Highly Complex and Organized

Complexity is a property that is evident when encountered, but difficult to describe. For the present, we can think of complexity in terms of order and consistency. The more complex a structure, the greater the number of parts that must be in their proper place, the less tolerance for errors in the nature and interactions of the parts, and the more regulation or control that must be exerted to maintain the system. Cellular activities can be remarkably precise. DNA duplication, for example, occurs with an error rate of less than one mistake every ten million nucleotides incorporated—and most of these are quickly corrected by an elaborate repair mechanism that recognizes the defect.

During the course of this book, we will have occasion to consider the complexity of life at several different levels. We will discuss the organization of atoms into small-sized molecules; the organization of these molecules into giant polymers; and the organization of different types of polymeric molecules into complexes, which in turn are organized into subcellular organelles and finally into cells. As will be apparent, there is a great deal of consistency at every level. Each type of cell has a consistent appearance when viewed under a high-powered electron microscope; that is, its organelles have a particular shape and location, from one individual of a species to another. Similarly, each type of organelle has a consistent composition of macromolecules, which are arranged in a predictable pattern. Consider the cells lining your intestine that are responsible for removing nutrients from your digestive tract. **FIGURE 1.3** illustrates the many different levels of organization present in such a tissue.

The epithelial cells that line the intestine are tightly connected to each other like bricks in a wall (Figure 1.3 inset 1).

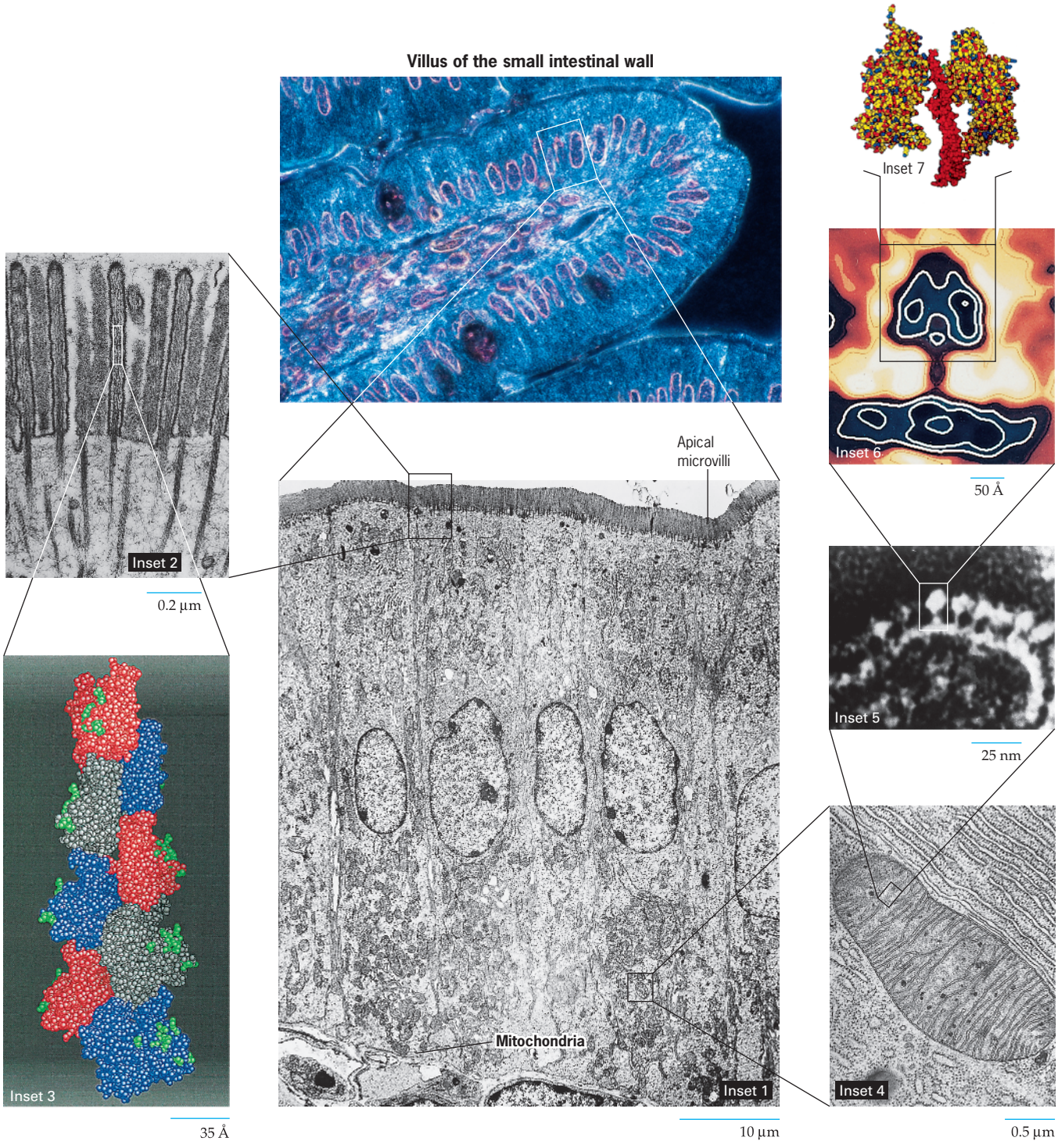


FIGURE 1.3 **Levels of cellular and molecular organization.** The brightly colored photograph of a stained section shows the microscopic structure of a villus of the wall of the small intestine, as seen through the light microscope. Inset 1 shows an electron micrograph of the epithelial layer of cells that lines the inner intestinal wall. The apical surface of each cell, which faces the channel of the intestine, contains a large number of microvilli involved in nutrient absorption. The basal region of each cell contains large numbers of mitochondria, where energy is made available to the cell. Inset 2 shows the apical microvilli; each microvillus contains a bundle of actin filaments. Inset 3 shows the actin protein subunits that make up each filament. Inset 4 shows an individual mitochondrion similar to those found in the basal region of the epithelial cells. Inset 5 shows a portion of an inner membrane of a mitochondrion including the stalked particles that project from the membrane and correspond to the sites where ATP is synthesized. Insets 6 and 7 show molecular models of the ATP-synthesizing machinery, which is discussed at length in Chapter 9.

SOURCE: Light micrograph Cecil Fox/Photo Researchers; inset 1 courtesy of Shakti P. Kapur, Georgetown University Medical Center; inset 2 from Mark S. Mooseker and Lewis G. Tilney, *J. Cell Biol.* 67:729, 1975, reproduced with permission of the Rockefeller University Press; inset 3 courtesy of Kenneth C. Holmes; inset 4 Keith R. Porter/Photo Researchers; inset 5 courtesy of Humberto Fernandez-Moran; inset 6 courtesy of Roderick A. Capaldi; inset 7 courtesy of Wolfgang Junge, Holger Lill, and Siegfried Engelbrecht, University of Osnabrück, Germany.

The apical ends of these cells, which face the intestinal channel, have long processes (*microvilli*) that facilitate absorption of nutrients (inset 2). The microvilli are able to project outward from the apical cell surface because they contain an internal skeleton made of filaments, which in turn are composed of protein (*actin*) monomers polymerized in a characteristic array (inset 3). At their basal ends, intestinal cells have large numbers of mitochondria (inset 4) that provide the energy required to fuel various membrane transport processes. Each mitochondrion is composed of a defined pattern of internal membranes, which in turn are composed of a consistent array of proteins, including an electrically powered ATP-synthesizing machine that projects from the inner membrane like a ball on a stick (insets 5–7).

One of the truly fascinating aspects of cells is that they achieve organization at many different scales using physical processes that are essentially random. Even though living cells are highly complex and ordered, it has become increasingly evident in recent years that random (*stochastic*) events play a critical role in all cellular activities. Many of the molecules within living cells are in a constant state of random movement, propelled by thermal energy they acquire from their environment. Cells have evolved the capacity to utilize this movement in highly directed ways. We can consider one example of this phenomenon, keeping in mind that many other cases could be described. Proteins are complex molecules often consisting of hundreds of amino acid building blocks and attaining molecular masses over a hundred thousand Daltons. Despite their huge size, proteins consist of a polypeptide chain that has to fold into a precisely defined three-dimensional (native) structure. If it fails to fold properly, the protein will lack meaningful function. In 1969, Cyrus Levinthal of Columbia University identified certain features of this folding process that became known as Levinthal's paradox. For one part of the paradox, Levinthal noted that, if protein folding depended solely on random molecular movements, it would require a period of time greater than the age of the universe for a protein to fold into its native structure. According to this scenario, the time it would take for a protein to fold properly might be compared to the period required for a monkey sitting at a piano to compose one of Beethoven's concertos. The paradox inherent in protein folding becomes evident knowing that, despite their enormous complexity, proteins actually acquire their native structures within fractions of a second. How is the paradox resolved? Even though folding of a protein is driven by random thermal motion, the process occurs in stepwise fashion so that the protein folds along pathways in which less structured intermediates guide the formation of better formed subsequent intermediates. In other words, the folding pathway allows proteins to rapidly "jump" from one step to the next until the native structure is reached. To carry over the solution of the protein folding paradox to the monkey at the piano, it would be as if every time the monkey tapped an appropriate key, that note would be recorded, allowing the monkey to move toward the next note in the concerto. As long as the monkey was an active player, the composition of the concerto could be accomplished quite rapidly. It can be said that these types of events are "biased." They depend upon random activities, but they lead to directed outcomes because they select for intermediate stages that lie on the path leading to the desired outcome.

Fortunately for cell and molecular biologists, evolution has moved rather slowly at the levels of biological organization with which they are concerned. Whereas a human and a cat, for example, have very different anatomical features, the cells that make up their tissues, and the organelles that make up their cells, are very similar. The actin filament portrayed in Figure 1.3, inset 3, and the ATP-synthesizing enzyme of inset 6 are virtually identical to similar structures found in such diverse organisms as humans, snails, yeast, and redwood trees. Information obtained by studying cells from one type of organism often has direct application to other forms of life. Many of the most basic processes, such as the synthesis of proteins, the conservation of chemical energy, or the construction of a membrane, are remarkably similar in all living rganisms.

Cells Possess a Genetic Program and the Means to Use It

Organisms are built according to information encoded in a collection of genes, which are constructed of DNA. The human genetic program contains enough information, if converted to words, to fill millions of pages of text. Remarkably, this vast amount of information is packaged into a set of chromosomes that occupies the space of a cell nucleus—hundreds of times smaller than the dot on this *i*.

Genes are more than storage lockers for information: They constitute the recipes for constructing cellular structures, the directions for running cellular activities, and the program for making more of themselves. The molecular structure of genes allows for changes in genetic information (mutations) that lead to variation among individuals, which forms the basis of biological evolution. Discovering the mechanisms by which cells use and transmit their genetic information has been one of the greatest achievements of science in recent decades.

Cells Are Capable of Producing More of Themselves

Just as individual organisms are generated by reproduction, so too are individual cells. Cells reproduce by division, a process in which the contents of a "mother" cell are distributed into two "daughter" cells. Prior to division, the genetic material is faithfully duplicated, and each daughter cell receives a complete and equal share of genetic information. In most cases, the two daughter cells have approximately equal volume. In some cases, however, as occurs when a human oocyte undergoes division, one of the cells can retain nearly all of the cytoplasm, even though it receives only half of the genetic material (**FIGURE 1.4**).

Cells Acquire and Utilize Energy

Every biological process requires the input of energy. Virtually all of the energy utilized by life on the Earth's surface arrives in the form of electromagnetic radiation from the sun. The energy of light is trapped by light-absorbing pigments present in the membranes of photosynthetic cells (**FIGURE 1.5**). Light energy is converted by photosynthesis into chemical energy that is stored in energy-rich carbohydrates, such as sucrose or starch. For most animal cells, energy arrives prepackaged, often in the form of

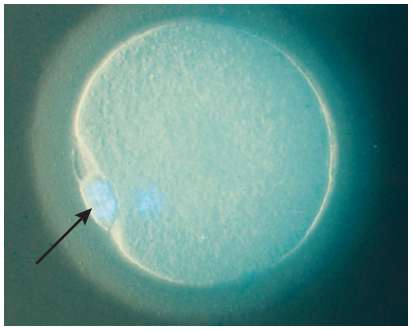


FIGURE 1.4 **Cell reproduction.** This mammalian oocyte has recently undergone a highly unequal cell division in which most of the cytoplasm has been retained within the large oocyte, which has the potential to be fertilized and develop into an embryo. The other cell is a nonfunctional remnant that consists almost totally of nuclear material (indicated by the blue-staining chromosomes, arrow).

SOURCE: Courtesy of Jonathan van Blerkom.

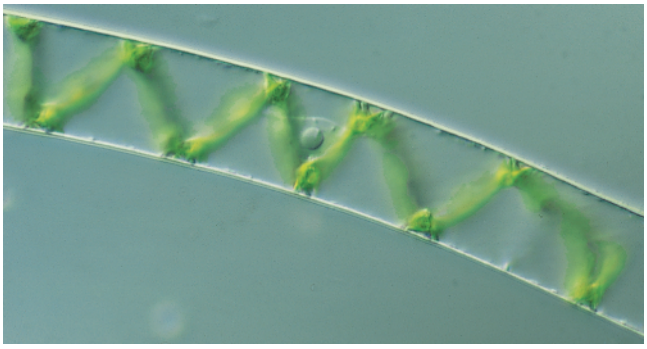


FIGURE 1.5 **Acquiring energy.** A living cell of the filamentous alga *Spirogyra*. The ribbon-like chloroplast, which is seen to zigzag through the cell, is the site where energy from sunlight is captured and converted to chemical energy during photosynthesis.

SOURCE: M. I. Walker/Photo Researchers, Inc.

the sugar glucose. In humans, glucose is released by the liver into the blood where it circulates through the body delivering chemical energy to all the cells. Once in a cell, the glucose is disassembled in such a way that its energy content can be stored in a readily available form (usually as ATP) that is later put to use in running all of the cell's myriad energy-requiring activities. Cells expend an enormous amount of energy simply breaking down and rebuilding the macromolecules and organelles of which they are made. This continual "turnover," as it is called, maintains the integrity of cell components in the face of inevitable wear and tear and enables the cell to respond rapidly to changing conditions.

Cells Carry Out a Variety of Chemical Reactions

Cells function like miniaturized chemical plants. Even the simplest bacterial cell is capable of hundreds of different chemical transformations, none of which occurs at any significant rate in the inanimate world. Virtually all chemical changes that take place in cells require *enzymes*—molecules that greatly increase the rate at which a chemical reaction occurs. The sum total of the chemical reactions in a cell represents that cell's **metabolism**.

Cells Engage in Mechanical Activities

Cells are sites of bustling activity. Materials are transported from place to place, structures are assembled and then rapidly disassembled, and, in many cases, the entire cell moves itself from one site to another. These types of activities are based on dynamic, mechanical changes within cells, many of which are initiated by changes in the shape of "motor" proteins. Motor proteins are just one of many types of molecular "machines" employed by cells to carry out mechanical activities.

Cells Are Able to Respond to Stimuli

Some cells respond to stimuli in obvious ways; a single-celled protist, for example, moves away from an object in its path or moves toward a source of nutrients. Cells within a multicellular plant or animal respond to stimuli less obviously. Most cells are covered with *receptors* that interact with substances in the environment in highly specific ways. Cells possess receptors to hormones, growth factors, and extracellular materials, as well as to substances on the surfaces of other cells. A cell's receptors provide pathways through which external stimuli can evoke specific responses in target cells. Cells may respond to specific stimuli by altering their metabolic activities, moving from one place to another, or even committing suicide.

Cells Are Capable of Self-Regulation

In recent years, a new term has been used to describe cells: *robustness*. Cells are robust, that is, hearty or durable, because they are protected from dangerous fluctuations in composition and behavior. Should such fluctuations occur, specific feedback circuits are activated that serve to return the cell to the appropriate state. In addition to requiring energy, maintaining a complex, ordered state requires constant regulation. The importance of a cell's regulatory mechanisms becomes most evident when they break down. For example, failure of a cell to correct a mistake when it duplicates its DNA may result in a debilitating mutation, or a breakdown in a cell's growth-control safeguards can transform the cell into a cancer cell with the capability of destroying the entire organism. We are gradually learning how a cell controls its activities, but much more is left to discover.

Consider the following experiment conducted in 1891 by Hans Driesch, a German embryologist. Driesch found that he could completely separate the first two or four cells of a sea urchin embryo and each of the isolated cells would proceed to develop into a normal embryo (**FIGURE 1.6**). How can a cell that is normally destined to form only part of an embryo regulate its own activities and form an entire embryo? How does the isolated cell recognize the absence of its neighbors, and how does this recognition redirect the entire course of the cell's development? How can a part of an embryo have a sense of the whole? We are not able to answer these questions much better today than we were more than a hundred years ago when the experiment was performed.

Throughout this book we will be discussing processes that require a series of ordered steps, much like the assembly-line

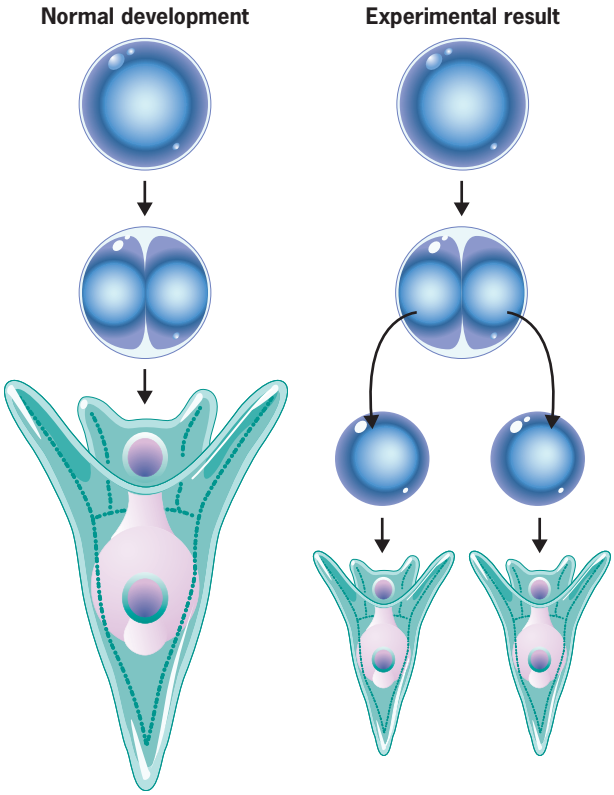
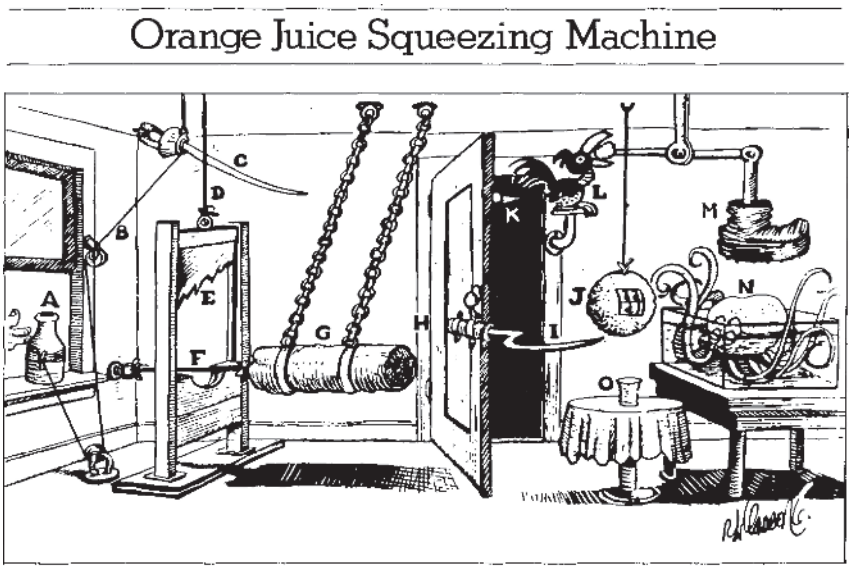


FIGURE 1.6 **Self-regulation.** The left panel depicts the normal development of a sea urchin in which a fertilized egg gives rise to a single embryo. The right panel depicts an experiment in which the cells of an early embryo are separated from one another after the first division, and each cell is allowed to develop in isolation. Rather than developing into half of an embryo, as it would if left undisturbed, each isolated cell recognizes the absence of its neighbor, regulating its development to form a complete (although smaller) embryo.

construction of an automobile in which workers add, remove, or make specific adjustments as the car moves along. In the cell, the information for product design resides in the nucleic acids, and the construction workers are primarily proteins. It is the presence of these two types of macromolecules that, more than any other factor, sets the chemistry of the cell apart from that of the nonliving world. In the cell, the workers must act without the benefit of conscious direction. Each step of a process must occur spontaneously in such a way that the next step is automatically triggered. In many ways, cells operate in a manner analogous to the orange-squeezing contraption discovered by "The Professor" and shown in **FIGURE 1.7**. Each type of cellular activity requires a unique set of highly complex molecular tools and machines—the products of eons of natural selection and biological evolution. A primary goal of biologists is to understand the molecular structure and role of each component involved in a particular activity, the means by which these components interact, and the mechanisms by which these interactions are regulated.

Cells Evolve

How did cells arise? Of all the major questions posed by biologists, this question may be the least likely ever to be answered. It is presumed that cells evolved from some type of precellular life form, which in turn evolved from nonliving organic materials that were present in the primordial seas. Whereas the origin of cells is shrouded in near-total mystery, the evolution of cells can be studied by examining organisms that are alive today. If you were to observe the features of a bacterial cell living in the human intestinal tract (see **FIGURE 1.18a**) and a cell that is part of the lining of that tract (**FIGURE 1.3**), you would be struck by



Professor Butts steps into an open elevator shaft and when he lands at the bottom he finds a simple orange squeezing machine. Milkman takes empty milk bottle (**A**), pulling string (**B**) which causes sword (**C**) to sever cord (**D**) and allow guillotine blade (**E**) to drop and cut rope (**F**) which releases battering ram (**G**). Ram bumps against open door (**H**), causing it to close. Grass sickle (**I**) cuts a slice off end of orange (**J**)–at the same time spike (**K**) stabs "prune hawk" (**L**) he opens his mouth to yell in agony, thereby releasing prune and allowing diver's boot (**M**) to drop and step on sleeping octopus (**N**). Octopus awakens in a rage and, seeing diver's face which is painted on orange, attacks it and crushes it with tentacles, thereby causing all the juice in the orange to run into glass (**O**).

Later on you can use the log to build a log cabin where you can raise your son to be President like Abraham Lincoln.

FIGURE 1.7 **Cellular activities** are often analogous to this "Rube Goldberg machine" in which one event "automatically" triggers the next event in a reaction sequence.

SOURCE: Rube Goldberg is the ® and © of Rube Goldberg, Inc.

the differences between the two cells. Yet both of these cells, as well as all other cells that are present in living organisms, share many features, including a common genetic code, a plasma membrane, and ribosomes. According to one of the tenets of modern biology, all living organisms have evolved from a single, common ancestral cell that lived more than three billion years ago. Because it gave rise to all the living organisms that we know of, this ancient cell is often referred to as the *last universal common ancestor* (or *LUCA*). We will examine some of the events that occurred during the evolution of cells in the Experimental Pathway at the end of the chapter. Future chapters will explore biochemical aspects of the origin of life. Keep in mind that evolution is not simply an event of the past, but an ongoing process that continues to modify the properties of cells that will be present in organisms that have yet to appear. For example, evolution of drug resistance in bacteria is a major health concern and will be discussed in Human Prespective in Chapter 3.

1.3 Two Fundamentally Different Classes of Cells

Once the electron microscope became widely available, biologists were able to examine the internal structure of a wide variety of cells. It became apparent from these studies that there were two basic classes of cells—prokaryotic and eukaryotic—distinguished by their size and the types of internal structures, or **organelles**, they contain (**FIGURE 1.8**). The existence of two distinct classes of cells, without any known intermediates, represents one of the most fundamental evolutionary divisions in the biological world. The structurally simpler **prokaryotic** cells include bacteria, whereas the structurally more complex **eukaryotic** cells include protists, fungi, plants, and animals.[1]

[1]Those interested in examining a proposal to do away with the concept of prokaryotic versus eukaryotic organisms can read a brief essay by N. R. Pace in *Nature* 441:289, 2006.

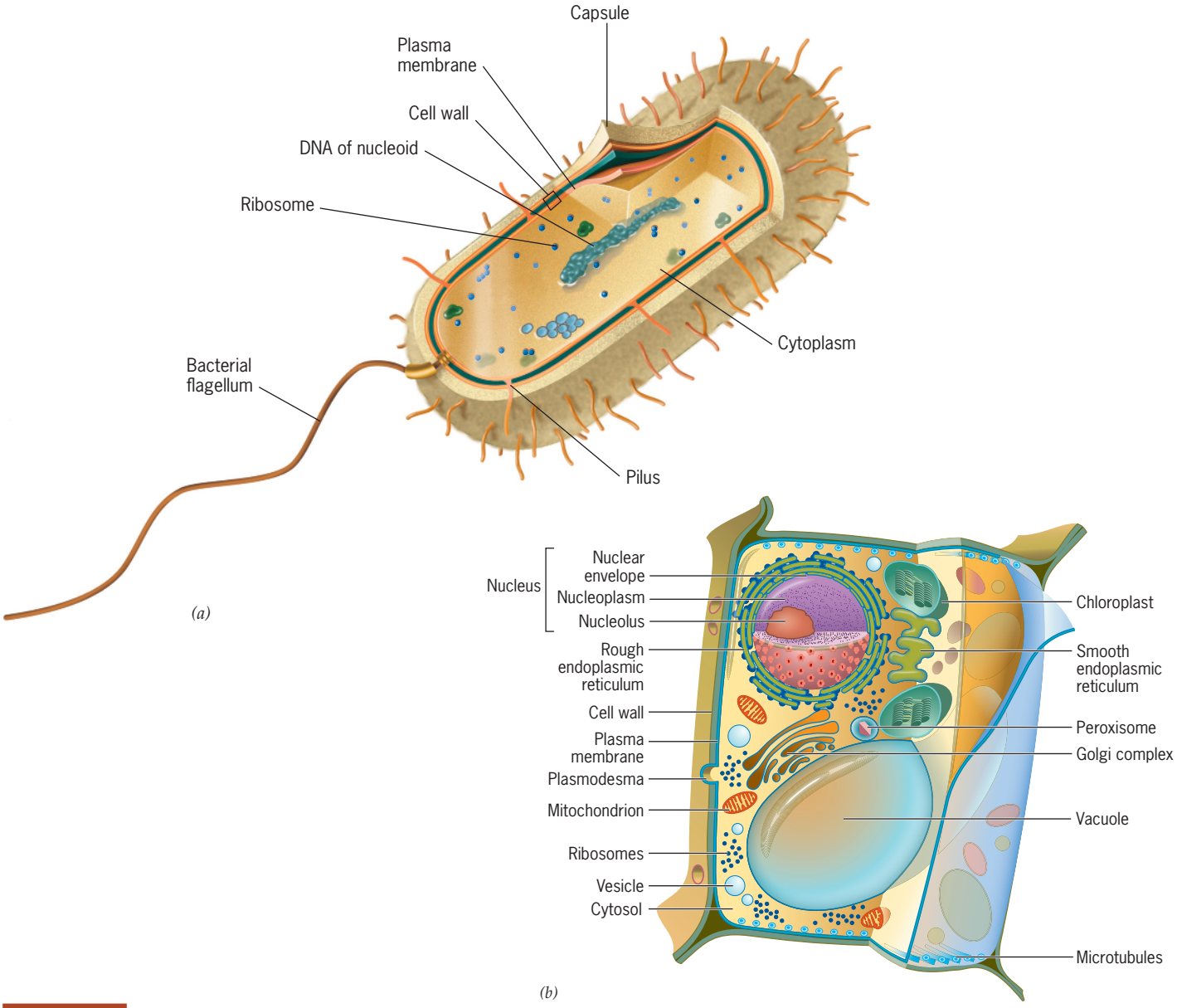


FIGURE 1.8 **The structure of cells.** Schematic diagrams of a "generalized" bacterial (*a*), plant (*b*), and animal (*c*) cell. Note: Organelles are not drawn to scale.

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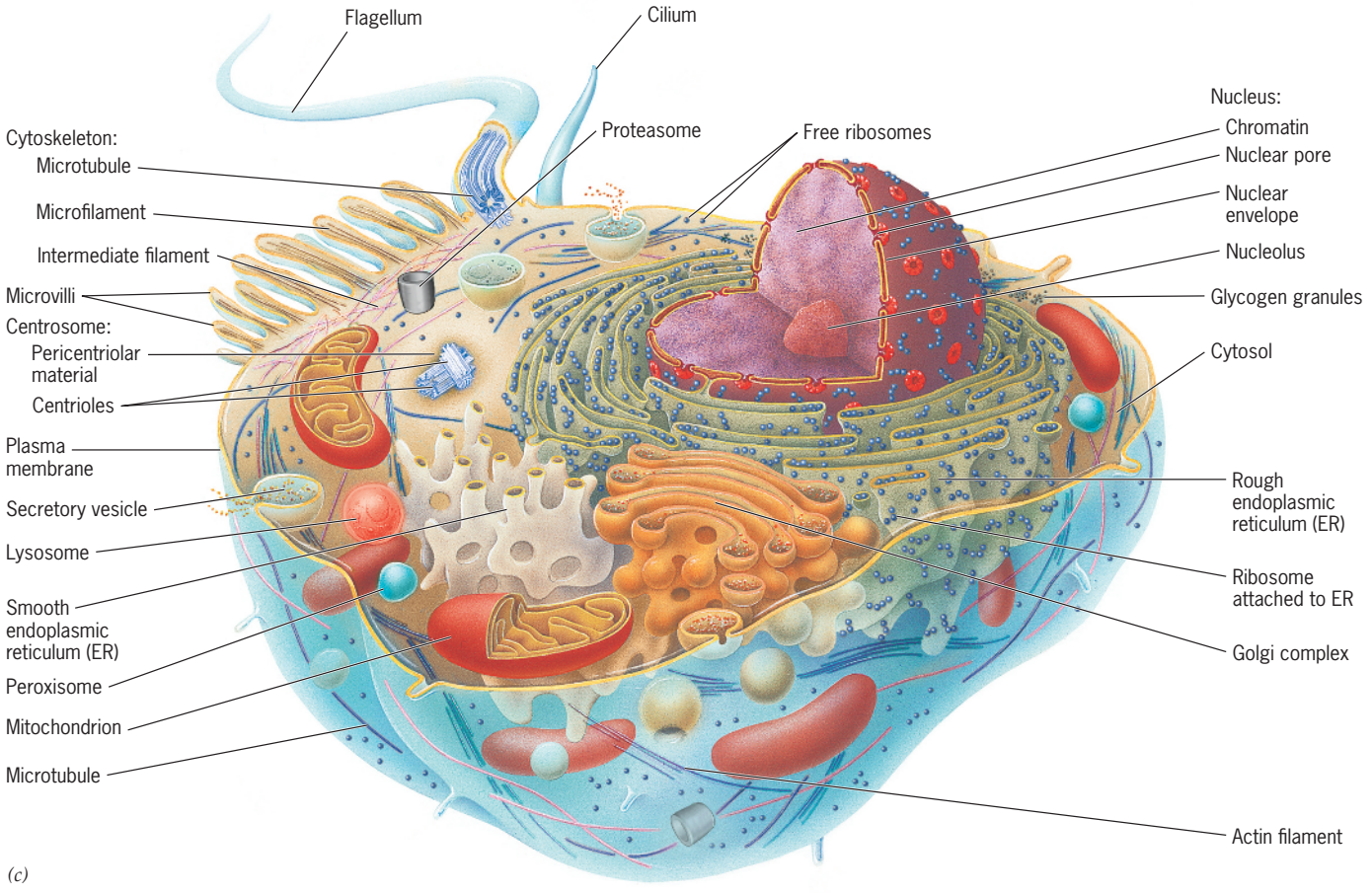


FIGURE 1.8 (*Continued*):

We are not sure when prokaryotic cells first appeared on Earth. Evidence of prokaryotic life has been obtained from rocks approximately 2.7 billion years of age. Not only do these rocks contain what appear to be fossilized microbes, they contain complex organic molecules that are characteristic of particular types of prokaryotic organisms, including cyanobacteria. It is unlikely that such molecules could have been synthesized abiotically, that is, without the involvement of living cells. Cyanobacteria almost certainly appeared by 2.4 billion years ago, because that is when the atmosphere became infused with molecular oxygen (O_2), which is a by-product of the photosynthetic activity of these prokaryotes. The dawn of the age of eukaryotic cells is also shrouded in uncertainty. Complex multicellular animals appear rather suddenly in the fossil record approximately 600 million years ago, but there is considerable evidence that simpler eukaryotic organisms were present on Earth more than one billion years earlier. The estimated time of appearance on Earth of several major groups of organisms is depicted in **FIGURE 1.9**. Even a superficial examination of Figure 1.9 reveals how "quickly" life arose following the formation of Earth and cooling of its surface and how long it took for the subsequent evolution of complex animals and plants.

The following brief comparison between prokaryotic and eukaryotic cells reveals many basic differences between the two types, as well as many similarities (see Figure 1.8). The similarities and differences between the two types of cells are listed in Table 1.1. The shared properties reflect the fact that eukaryotic cells almost

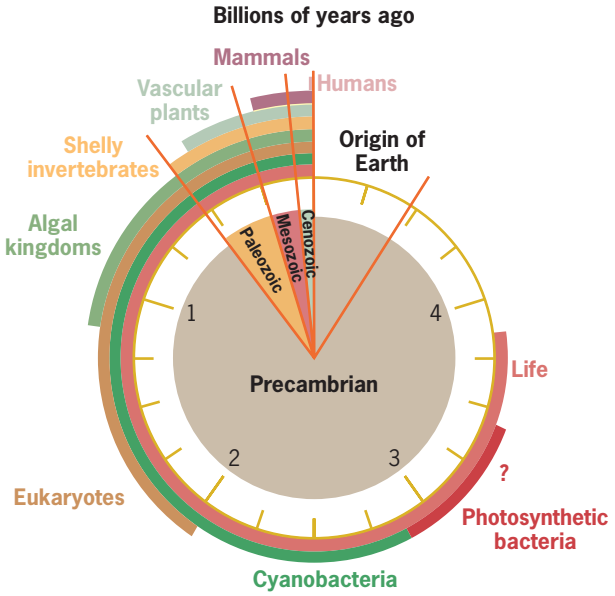


FIGURE 1.9 **Earth's biogeologic clock.** A portrait of the past five billion years of Earth's history showing a proposed time of appearance of major groups of organisms. Complex animals (shelly invertebrates) and vascular plants are relatively recent arrivals. The time indicated for the origin of life is speculative. In addition, photosynthetic bacteria may have arisen much earlier, hence the question mark. The geologic eras are indicated in the center of the illustration.

SOURCE: From D. J. Des Marais, *Science* 289:1704, 2001. Copyright © 2001. Reprinted with permission from AAAS.

TABLE 1.1 A Comparison of Prokaryotic and Eukaryotic Cells

Features held in common by the two types of cells:

- Plasma membrane of similar construction
- Genetic information encoded in DNA using identical genetic code
- Similar mechanisms for transcription and translation of genetic information, including similar ribosomes
- Shared metabolic pathways (e.g., glycolysis and TCA cycle)
- Similar apparatus for conservation of chemical energy as ATP (located in the plasma membrane of prokaryotes and the mitochondrial membrane of eukaryotes)
- Similar mechanism of photosynthesis (between cyanobacteria and green plants)
- Similar mechanism for synthesizing and inserting membrane proteins
- Proteasomes (protein digesting structures) of similar construction (between archaebacteria and eukaryotes)
- Cytoskeletal filaments built of proteins similar to actin and tubulin

Features of eukaryotic cells not found in prokaryotes:

- Division of cells into nucleus and cytoplasm, separated by a nuclear envelope containing complex pore structures
- Complex chromosomes composed of DNA and associated proteins that are capable of compacting into mitotic structures
- Complex membranous cytoplasmic organelles (includes endoplasmic reticulum, Golgi complex, lysosomes, endosomes, peroxisomes, and glyoxisomes)
- Specialized cytoplasmic organelles for aerobic respiration (mitochondria) and photosynthesis (chloroplasts)
- Complex cytoskeletal system (including actin filaments, intermediate filaments, and microtubules) and associated motor proteins
- Complex flagella and cilia
- Ability to ingest particulate material by enclosure within plasma membrane vesicles (phagocytosis)
- Cellulose-containing cell walls (in plants)
- Cell division using a microtubule-containing mitotic spindle that separates chromosomes
- Presence of two copies of genes per cell (diploidy), one from each parent
- Presence of three different RNA synthesizing enzymes (RNA polymerases)
- Sexual reproduction requiring meiosis and fertilization

certainly evolved from prokaryotic ancestors. Because of their common ancestry, both types of cells share an identical genetic language, a common set of metabolic pathways, and many common structural features. For example, both types of cells are bounded by plasma membranes of similar construction that serve as a selectively permeable barrier between the living and nonliving worlds. Both types of cells may be surrounded by a rigid, nonliving *cell wall* that protects the delicate life form within. Although the cell walls of prokaryotes and eukaryotes may have similar functions, their chemical composition is very different.

Internally, eukaryotic cells are much more complex—both structurally and functionally—than prokaryotic cells (Figure 1.8). The difference in structural complexity is evident in the electron micrographs of a bacterial and an animal cell shown in Figures 1.18*a* and 1.10, respectively. Both contain a nuclear region that houses the cell's genetic material, surrounded by cytoplasm. The genetic material of a prokaryotic cell is present in a **nucleoid**: a poorly demarcated region of the cell that lacks a boundary membrane to separate it from the surrounding cytoplasm. In contrast, eukaryotic cells possess a **nucleus**: a region bounded by a complex membranous structure called the *nuclear envelope*. This difference in nuclear structure is the basis for the terms *prokaryotic* (*pro* = before, *karyon* = nucleus) and *eukaryotic* (*eu* = true, *karyon* = nucleus). Prokaryotic cells contain relatively small amounts of DNA; the DNA content of bacteria ranges from about 600,000 base pairs to nearly 8 million base pairs and encodes between about 500 and several thousand proteins.[2] Although a "simple" baker's yeast cell has only slightly more DNA (12 million base pairs encoding about 6200 proteins) than the most complex prokaryotes, most eukaryotic cells contain considerably more genetic information. Both prokaryotic and eukaryotic cells have DNA-containing chromosomes. Eukaryotic cells possess a number of separate chromosomes, each containing a single linear molecule of DNA. In contrast, nearly all prokaryotes that have been studied contain a single, circular chromosome. More importantly, the chromosomal DNA of eukaryotes, unlike that of prokaryotes, is tightly associated with proteins to form a complex nucleoprotein material known as **chromatin**.

The cytoplasm of the two types of cells is also very different. The cytoplasm of a eukaryotic cell is filled with a great diversity of structures, as is readily apparent by examining an electron micrograph of nearly any plant or animal cell (**FIGURE 1.10**). Even yeast, the simplest eukaryote, is much more complex structurally than an average bacterium, even though these two organisms have a similar number of genes. Eukaryotic cells contain an array of membrane-bound organelles. Eukaryotic organelles include mitochondria, where chemical energy is made available to fuel cellular activities; an endoplasmic reticulum, where many of a cell's proteins and lipids are manufactured; Golgi complexes, where materials are sorted, modified, and transported to specific cellular destinations; and a variety of simple membrane-bound vesicles of varying dimension. Plant cells contain additional membranous organelles, including chloroplasts, which are the sites of photosynthesis, and often a single large vacuole that can occupy most of the volume of the cell. Taken as a group, the membranes of the eukaryotic cell serve to divide the cytoplasm into compartments within which specialized activities can take place. In contrast, the cytoplasm of prokaryotic cells is essentially devoid of membranous structures. The complex photosynthetic membranes of the cyanobacteria are a major exception to this generalization (see Figure 1.15).

The cytoplasmic membranes of eukaryotic cells form a system of interconnecting channels and vesicles that function in the transport of substances from one part of a cell to another, as well as between the inside of the cell and its environment. Because of their small size, directed intracytoplasmic communication is less important in prokaryotic cells, where the necessary movement of materials can be accomplished by simple diffusion.

Eukaryotic cells also contain numerous structures lacking a surrounding membrane. Included in this group are the elongated tubules and filaments of the cytoskeleton, which participate in cell contractility, movement, and support. It was thought for many years that prokaryotic cells lacked any trace of a cytoskeleton, but primitive cytoskeletal filaments have been found in bacteria (see Chapter 13). It is still fair to say that the prokaryotic cytoskeleton is much simpler, both structurally and functionally, than that of eukaryotes. Both eukaryotic and prokaryotic cells possess ribosomes, which are nonmembranous particles that function as "workbenches" on which the proteins of the cell are manufactured. Even though ribosomes of prokaryotic and eukaryotic cells have

[2]Eight million base pairs is equivalent to a DNA molecule nearly 3 mm long.

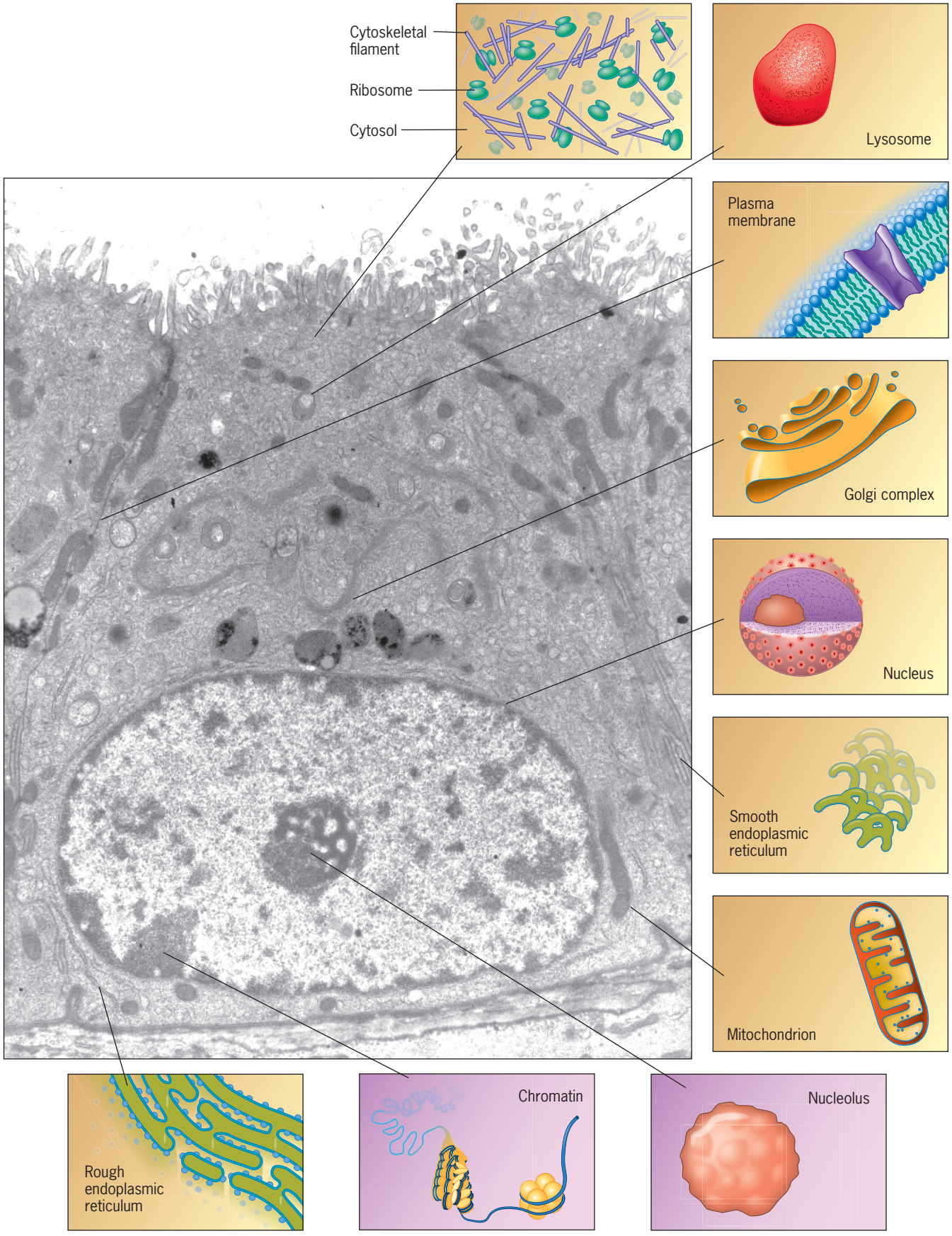


FIGURE 1.10 **The structure of a eukaryotic cell.** This epithelial cell lines the male reproductive tract in the rat. A number of different organelles are indicated and depicted in schematic diagrams around the border of the figure.

SOURCE: David M. Phillips/Photo Researchers, Inc.

considerably different dimensions (those of prokaryotes are smaller and contain fewer components), these structures participate in the assembly of proteins by a similar mechanism in both types of cells. **FIGURE 1.11** is a colorized electron micrograph of a portion of the cytoplasm near the thin edge of a single-celled eukaryotic organism. This is a region of the cell where membrane-bound organelles tend to be absent. The micrograph shows individual filaments of the cytoskeleton (orange) and other large macromolecular complexes of the cytoplasm (turquoise). Most of these complexes are ribosomes. It is evident from this type of image that the cytoplasm of a eukaryotic cell is extremely crowded, leaving very little space for the soluble phase of the cytoplasm, which is called the **cytosol**.

Other major differences between eukaryotic and prokaryotic cells can be noted. Eukaryotic cells divide by a complex process of mitosis in which duplicated chromosomes condense into compact structures that are segregated by an elaborate microtubule-containing apparatus (**FIGURE 1.12**). This apparatus, which is called a *mitotic spindle*, allows each daughter cell to receive an equivalent array of genetic material. In prokaryotes, there is no mitotic spindle to separate the genome copies after replication. It was once thought that the two copies are separated by attaching the DNA to the cell surface allowing the growth of the cell membrane to pull them apart. However, live cell imaging showed that the DNA separates faster than the cell grows, and the precise mechanism by which prokaryotes segregate their genomes remains an open question. Some current models are based on regulated compaction or folding of the DNA so that the two copies would fold into two separate masses, thus separating them.

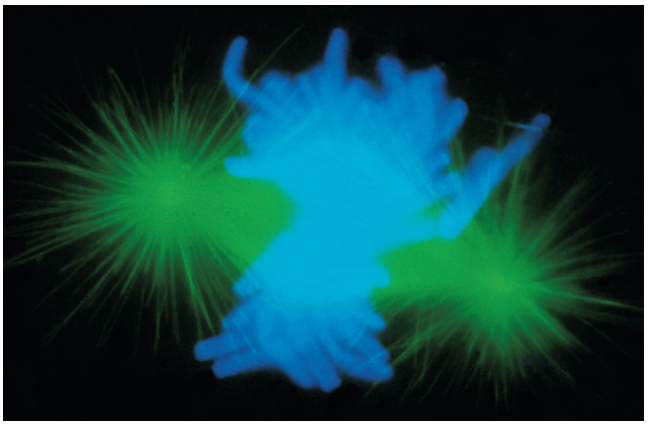


FIGURE 1.12 Cell division in eukaryotes requires the assembly of an elaborate chromosome-separating apparatus called the mitotic spindle, which is constructed primarily of microtubules. The microtubules in this micrograph appear green because they are bound by an antibody that is linked to a green fluorescent dye. The chromosomes, which were about to be separated into two daughter cells when this cell was fixed, are stained blue.

SOURCE: Courtesy of Conly L. Rieder.

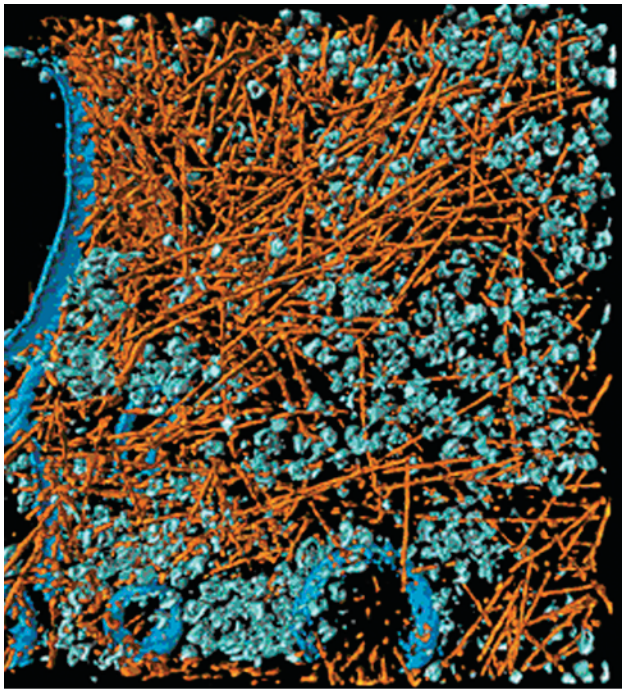


FIGURE 1.11 The cytoplasm of a eukaryotic cell is a crowded compartment. This colorized electron micrographic image shows a small region near the edge of a single-celled eukaryotic organism that had been quickly frozen prior to microscopic examination. The three-dimensional appearance is made possible by capturing two-dimensional digital images of the specimen at different angles and merging the individual frames using a computer. Cytoskeletal filaments are shown in orange, macromolecular complexes (primarily ribosomes) are turquoise, and portions of cell membranes are blue.

SOURCE: From Ohad Medalia et al., *Science* 298:1211, 2002, Figure 3*a*. © 2002, reprinted with permission from AAAS. Photo provided courtesy of Wolfgang Baumeister.

For the most part, prokaryotes are nonsexual organisms. They contain only one copy of their single chromosome and have no processes comparable to meiosis, gamete formation, or true fertilization. Even though true sexual reproduction is lacking among prokaryotes, some are capable of *conjugation*, in which a piece of DNA is passed from one cell to another (**FIGURE 1.13**). However, the recipient almost never receives a whole chromosome from the donor, and the condition in which the recipient cell contains both its own and its partner's DNA is fleeting. The cell soon reverts back to possession of a single chromosome. Although prokaryotes may not be as efficient as eukaryotes in exchanging DNA with other members of their own species, they are more adept than eukaryotes at picking up and incorporating foreign DNA from their environment, which has had considerable impact on microbial evolution (page 30).

Eukaryotic cells possess a variety of complex locomotor mechanisms, whereas those of prokaryotes are relatively simple. The movement of a prokaryotic cell may be accomplished by a thin protein filament, called a *flagellum*, which protrudes from the cell and rotates (**FIGURE 1.14a**). The rotations of the flagellum, which can exceed 1000 times per second, exert pressure against the surrounding fluid, propelling the cell through the medium. Certain eukaryotic cells, including many protists and sperm cells, also possess flagella, but the eukaryotic versions are much more complex than the simple protein filaments of bacteria (Figure 1.14*b*), and they generate movement by a different mechanism.

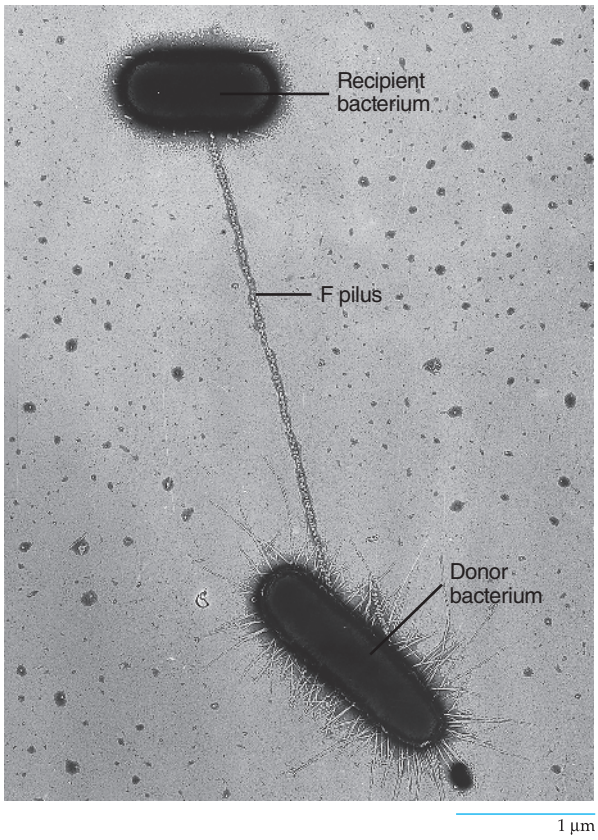
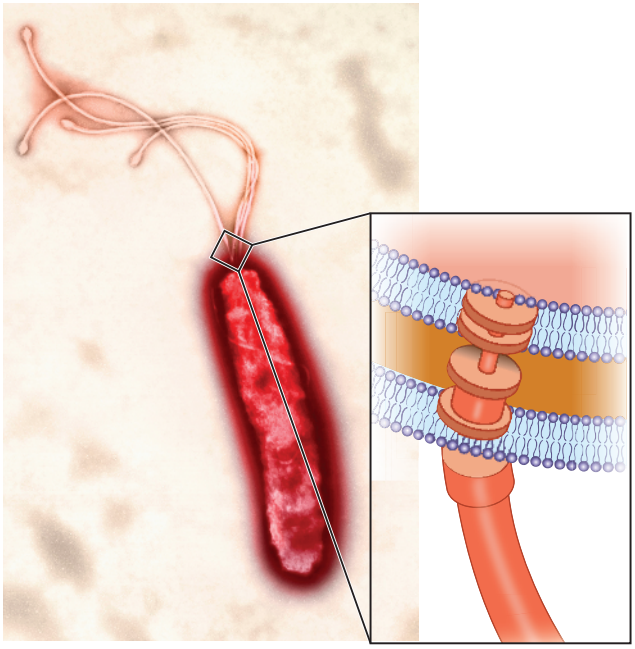


FIGURE 1.13 **Bacterial conjugation.** Electron micrograph showing a conjugating pair of bacteria joined by a structure of the donor cell, termed the F pilus, through which DNA is thought to be passed.
SOURCE: Courtesy of Charles C. Brinton, Jr., and Judith Carnahan.

In the preceding paragraphs, many of the most important differences between the prokaryotic and eukaryotic levels of cellular organization were mentioned. We will elaborate on many of these points in later chapters. Before you dismiss prokaryotes as inferior, keep in mind that these organisms have remained on Earth for more than three billion years, and at this very moment, trillions of them are clinging to the outer surface of your body and feasting on the nutrients within your digestive tract. We think of these organisms as individual, solitary creatures, but recent insights have shown that they live in complex, multispecies communities called *biofilms*. The layer of plaque that grows on our teeth is an example of a biofilm. Different cells in a biofilm may carry out different specialized activities, not unlike the cells in a plant or an animal. Consider also that, metabolically, prokaryotes are very sophisticated, highly evolved organisms. For example, a bacterium, such as *Escherichia coli*, a common inhabitant of both the human digestive tract and the laboratory culture dish, has the ability to live and prosper in a medium containing one or two low-molecular-weight organic compounds and a few inorganic ions. Other bacteria are able to live on a diet consisting solely of inorganic substances. One species of bacteria has been found in wells more than a thousand meters below the Earth's surface living on basalt rock and molecular hydrogen (H_2) produced by inorganic reactions. In contrast, even the most metabolically talented cells in your body require a variety of organic compounds, including a number of vitamins and other essential substances they cannot make on their own. In fact, many of these essential dietary ingredients are produced by the bacteria that normally live in the large intestine.



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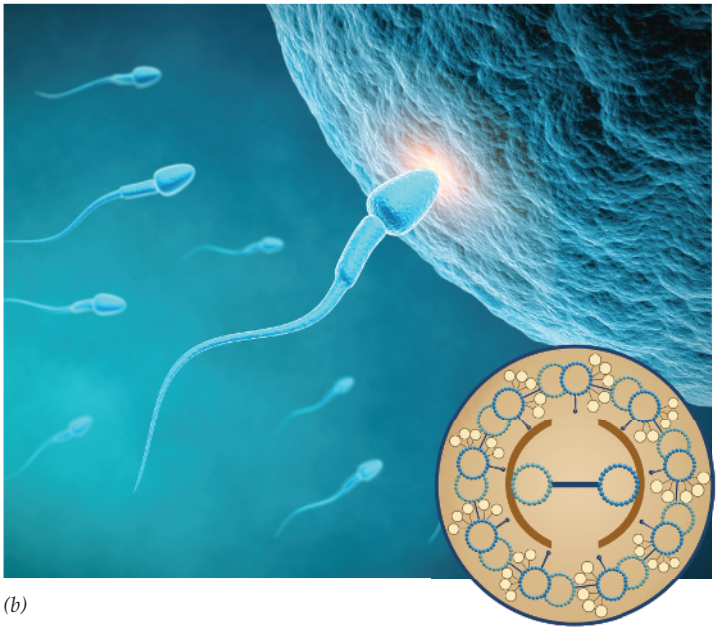


(b)

FIGURE 1.14 **The difference between prokaryotic and eukaryotic flagella.** (*a*) The bacterium *Helicobacter* with its numerous flagella. Inset shows a portion of a single bacterial flagellum, which consists largely of a single protein called flagellin. (*b*) Each of these human sperm cells is powered by the undulatory movements of a single flagellum. The inset shows a cross section of the central core of a mammalian sperm flagellum. The flagella of eukaryotic cells are so similar that this cross section could just as well have been taken of a flagellum from a protist or green alga.
SOURCE: (a) Heather Davies/Science Photo Library/Corbis (b) Sashkin/Shutterstock.

1.4 Types of Prokaryotic Cells

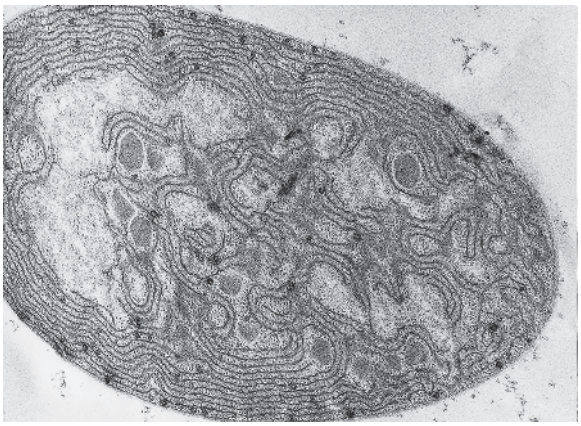
The distinction between prokaryotic and eukaryotic cells is based on structural complexity (as detailed in Table 1.1) and not on phylogenetic relationship. Prokaryotes are divided into two major taxonomic groups, or domains: the Archaea (or archaebacteria) and the Bacteria (or eubacteria). Members of the Archaea are more closely related to eukaryotes than they are to the other group of prokaryotes (the Bacteria). The experiments that led to the discovery that life is represented by three distinct branches are discussed in the Experimental Pathways at the end of the chapter.

Domain Archaea and Domain Bacteria

The domain Archaea includes several groups of organisms whose evolutionary ties to one another are revealed by similarities in the nucleotide sequences of their nucleic acids. The best known Archaea are species that live in extremely inhospitable environments; they are often referred to as "extremophiles." Included among the Archaea are the methanogens [prokaryotes capable of converting CO_2 and H_2 gases into methane (CH_4) gas]; the halophiles (prokaryotes that live in extremely salty environments, such as the Dead Sea or certain deep sea brine pools that possess a salinity equivalent to 5M $MgCl_2$); acidophiles (acid-loving prokaryotes that thrive at a pH as low as 0, such as that found in the drainage fluids of abandoned mine shafts); and thermophiles (prokaryotes that live at very high temperatures). Included in this last-named group are hyperthermophiles, which live in the hydrothermal vents of the ocean floor. The latest record holder among this group has been named "strain 121" because it is able to grow and divide in superheated water at a temperature of 121°C, which just happens to be the temperature used to sterilize surgical instruments in an autoclave. Recent analyses of soil and ocean microbes indicate that many members of the Archaea are also at home in habitats of normal temperature, pH, and salinity.

All other prokaryotes are classified in the domain Bacteria. This domain includes the smallest known cells, the mycoplasma (0.2 µm diameter), which are the only known prokaryotes to lack a cell wall and to contain a genome with fewer than 500 genes. Bacteria are present in every conceivable habitat on Earth, from the permanent ice shelf of the Antarctic to the driest African deserts, to the internal confines of plants and animals. Bacteria have even been found living in rock layers situated several kilometers beneath the Earth's surface. Some of these bacterial communities are thought to have been cut off from life on the surface for more than one hundred million years. The most complex prokaryotes are the cyanobacteria. Cyanobacteria contain elaborate arrays of cytoplasmic membranes, which serve as sites of photosynthesis (**FIGURE 1.15a**). The membranes of cyanobacteria are very similar to the photosynthetic membranes present within the chloroplasts of plant cells. As in eukaryotic plants, photosynthesis in cyanobacteria is accomplished by splitting water molecules, which releases molecular oxygen.

Many cyanobacteria are capable not only of photosynthesis, but also of **nitrogen fixation**, the conversion of nitrogen (N_2) gas into reduced forms of nitrogen (such as ammonia, NH_3) that can be used by cells in the synthesis of nitrogen-containing organic compounds, including amino acids and nucleotides. Those species capable of both photosynthesis and nitrogen fixation can survive on the barest of resources—light, N_2, CO_2, and H_2O. It is not surprising, therefore, that cyanobacteria are usually the first organisms to colonize the bare rocks rendered lifeless by a scorching volcanic eruption. Another unusual habitat occupied by cyanobacteria is illustrated in Figure 1.15*b*.



(a)

(b)

FIGURE 1.15 Cyanobacteria. (*a*) Electron micrograph of a cyanobacterium showing the cytoplasmic membranes that carry out photosynthesis. These concentric membranes are very similar to the thylakoid membranes present within the chloroplasts of plant cells, a reminder that chloroplasts evolved from a symbiotic cyanobacterium. (*b*) Cyanobacteria living inside the hairs of these polar bears are responsible for the unusual greenish color of their coats.

SOURCE: (a) Courtesy of Norma J. Lang. (b) Courtesy Zoological Society of San Diego.

Prokaryotic Diversity

For the most part, microbiologists are familiar only with those microorganisms they are able to grow in a culture medium. When a patient suffering from a respiratory or urinary tract infection sees his or her physician, one of the first steps often taken is to culture the pathogen. Once it has been cultured, the organism can be identified and the proper treatment prescribed. It has proven relatively easy to culture *most* disease-causing prokaryotes, but the same is not true for those living free in nature. The problem is compounded by the fact that prokaryotes are barely visible in a light microscope and their morphology is often not very distinctive. To date, roughly 6000 species of

prokaryotes have been identified by traditional techniques, which is less than one-tenth of 1 percent of the millions of prokaryotic species thought to exist on Earth! Our appreciation for the diversity of prokaryotic communities has increased dramatically in recent years with the use of molecular techniques that do not require the isolation of a particular organism.

Suppose one wanted to learn about the diversity of prokaryotes that live in the upper layers of the Pacific Ocean off the coast of California. Rather than trying to culture such organisms, which would prove largely futile, a researcher could concentrate the cells from a sample of ocean water, extract the DNA, and analyze certain DNA sequences present in the preparation. All organisms share certain genes, such as those that code for the RNAs present in ribosomes or the enzymes of certain metabolic pathways. Even though all organisms may share such genes, the sequences of the nucleotides that make up the genes vary considerably from one species to another. This is the basis of biological evolution. By using techniques that reveal the variety of DNA sequences of a particular gene in a particular habitat, one learns directly about the diversity of species that live in that habitat. Recent sequencing techniques have become so rapid and cost-efficient that virtually all of the genes present in the microbes of a given habitat can be sequenced, generating a collective genome, or *metagenome*. This approach can provide information about the types of proteins these organisms manufacture and thus about many of the metabolic activities in which they engage.

These same molecular strategies are being used to explore the remarkable diversity among the trillions of "unseen passengers" that live on or within our own bodies, in habitats such as the intestinal tract, mouth, vagina, and skin. This collection of microbes, which is known as the human *microbiome*, is the subject of several international research efforts aimed at identifying and characterizing these organisms in people of different age, diet, geography, and state of health. It has already been demonstrated, for example, that obese and lean humans have markedly different populations of bacteria in their digestive tracts. As obese individuals lose weight, their bacterial profile shifts toward that of the leaner individuals. One recent study of fecal samples taken from 124 people of varying weight revealed the presence within the collective population of more than 1000 different species of bacteria. Taken together, these microbes contained more than 3 million distinct genes—approximately 150 times as many as the number present in the human genome. Among the functions of proteins encoded by these microbial genomes are the synthesis of vitamins, the breakdown of complex plant sugars, and the prevention of growth of pathogenic organisms.

By using sequence-based molecular techniques, biologists have found that most habitats on Earth are teeming with previously unrecognized prokaryotic life. One estimate of the sheer numbers of prokaryotes in the major habitats of the Earth is given in Table 1.2. It is noteworthy that more than 90 percent of these organisms are now thought to live in the subsurface sediments well beneath the oceans and upper soil layers. Nutrients can be so scarce in some of these deep sediments that microbes living there are thought to divide only once every several hundred years! Table 1.2 also provides an estimate of the amount of carbon that is sequestered in the world's prokaryotic cells. To put this number into more familiar terms, it is roughly comparable to the total amount of carbon present in all of the world's plant life.

TABLE 1.2 Number and Biomass of Prokaryotes in the World

Environment	No. of prokaryotic cells, × 10^{28}	Pg of C in prokaryotes*
Aquatic habitats	12	2.2
Oceanic subsurface	355	303
Soil	26	26
Terrestrial subsurface	25–250	22–215
Total	415–640	353–546

*1 petagram (Pg) = 10^{15}g.
Source: W. B. Whitman et al., *Proc. Nat'l. Acad. Sci. U.S.A.* 95:6581, 1998.

1.5 Types of Eukaryotic Cells

In many regards, the most complex eukaryotic cells are not found inside of plants or animals, but rather among the single-celled (*unicellular*) protists, such as those pictured in **FIGURE 1.16**. All of the machinery required for the complex activities in which this organism engages—sensing the environment, trapping food, expelling excess fluid, evading predators—is housed within the confines of a single cell.

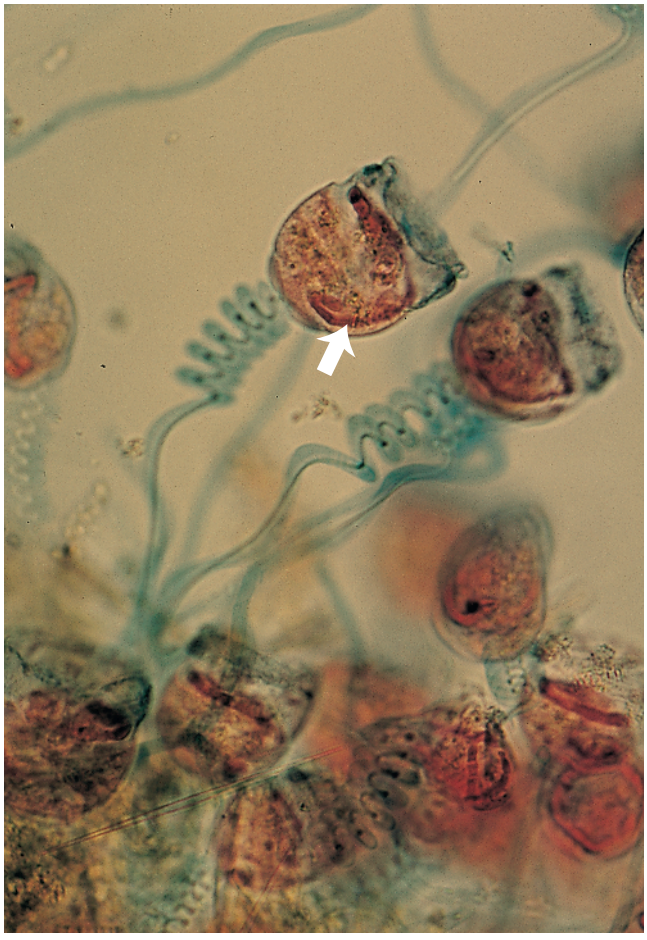


FIGURE 1.16 Vorticella, a complex ciliated protist. A number of these unicellular organisms are seen here; most have withdrawn their "heads" due to shortening of the blue-stained contractile ribbon in the stalk. Each cell has a single large nucleus, called a macronucleus (arrow), which contains many copies of the genes.
SOURCE: Carolina Biological Supply Co./Phototake.

Cell Differentiation

Complex unicellular organisms represent one evolutionary pathway. An alternate pathway has led to the evolution of multicellular organisms in which different activities are conducted by different types of specialized cells. Specialized cells are formed by a process called **differentiation**. A fertilized human egg, for example, will progress through a course of embryonic development that leads to the formation of approximately 250 distinct types of differentiated cells. Some cells become part of a particular digestive gland, others part of a large skeletal muscle, others part of a bone, and so forth (**FIGURE 1.17**). The pathway of differentiation followed by each embryonic cell depends primarily on the signals it receives from the surrounding environment; these signals in turn depend on the position of that cell within the embryo. As discussed in the accompanying Human Perspective, researchers are learning how to control the process of differentiation in the culture dish and applying this knowledge to the treatment of complex human diseases.

As a result of differentiation, different types of cells acquire a distinctive appearance and contain unique materials. Skeletal muscle cells contain a network of precisely aligned filaments composed of unique contractile proteins; cartilage cells become surrounded by a characteristic matrix containing polysaccharides and the protein collagen, which together provide mechanical support; red blood cells become disk-shaped sacks filled with a single protein, hemoglobin, which transports oxygen; and so forth. Despite their many differences, the various cells of a multicellular plant or animal are composed of similar organelles. Mitochondria, for example, are found in essentially all types of cells. In one type, however, they may have a rounded shape, whereas in another they may be highly elongated and thread-like. In each case, the number, appearance, and location of the various organelles can be correlated with the activities of the particular cell type. An analogy might be made to a variety of orchestral pieces: all are composed of the same notes, but varying arrangement gives each its unique character and beauty.

Model Organisms

Living organisms are highly diverse, and the results obtained from a particular experimental analysis may depend on the particular organism being studied. As a result, cell and molecular

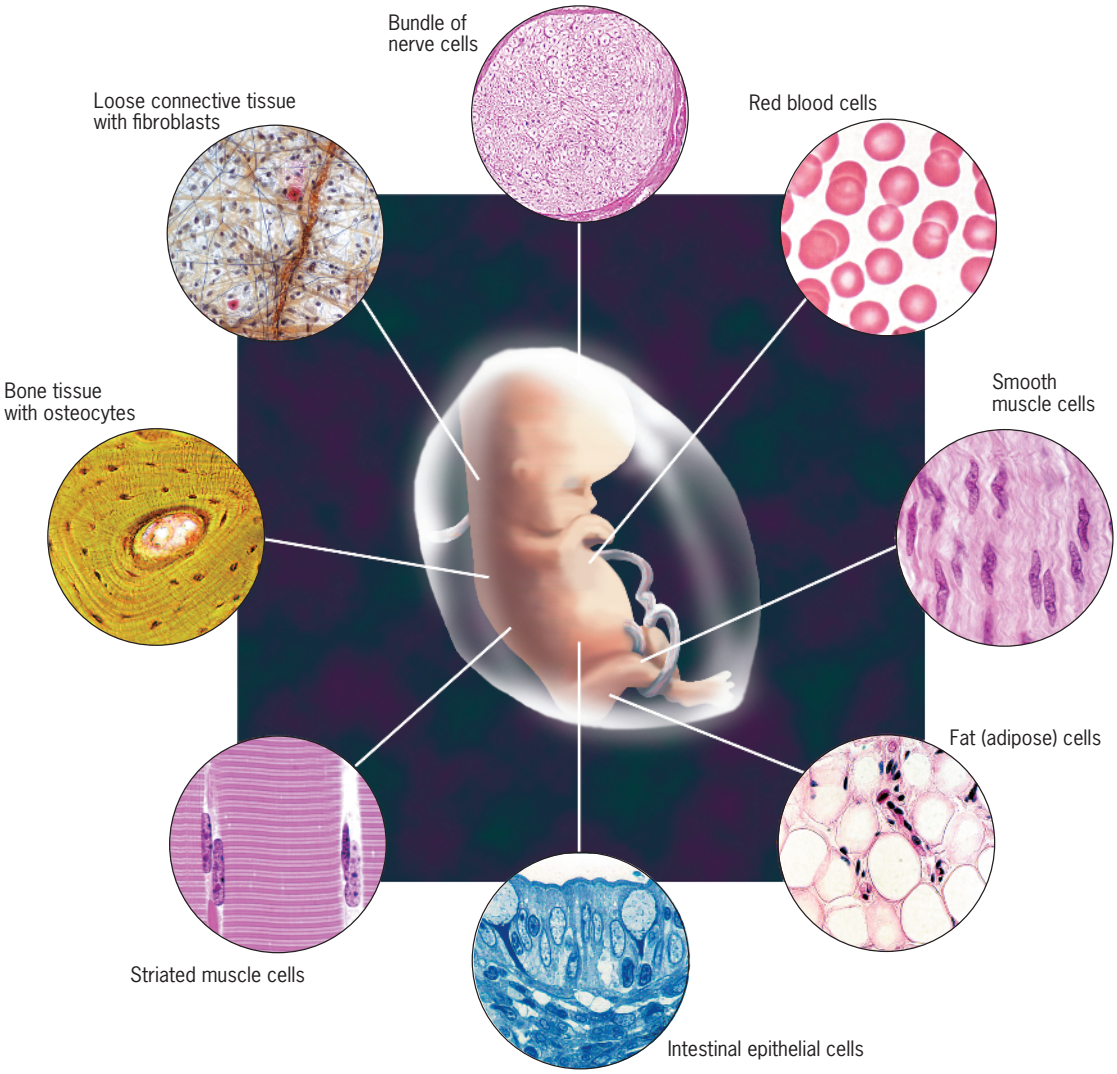
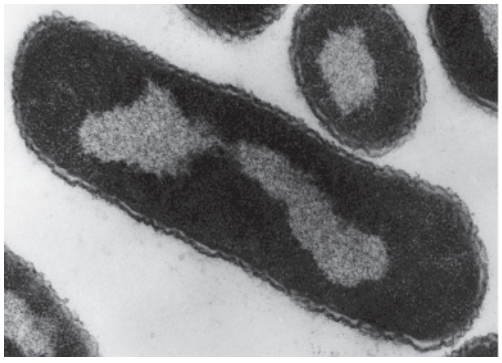


FIGURE 1.17 **Pathways of cell differentiation.** A few of the types of differentiated cells present in a human fetus.
SOURCE: Micrographs Courtesy of Michael Ross, University of Florida.

biologists have focused considerable research activities on a small number of "representative" or **model organisms**. It is hoped that a comprehensive body of knowledge built on these studies will provide a framework to understand those basic processes that are shared by most organisms, especially humans. This is not to suggest that many other organisms are not widely used in the study of cell and molecular biology. Nevertheless, six model organisms—one prokaryote and five eukaryotes—have captured much of the attention: a bacterium, *E. coli*; a budding yeast, *Saccharomyces cerevisiae*; a flowering plant, *Arabidopsis thaliana*; a nematode, *Caenorhabditis elegans*; a fruit fly, *Drosophila melanogaster*; and a mouse, *Mus musculus*. Each of these organisms has specific advantages that make it particularly useful as a research subject for answering certain types of questions. Each of these organisms is pictured in **FIGURE 1.18**, and a few of their advantages as research systems



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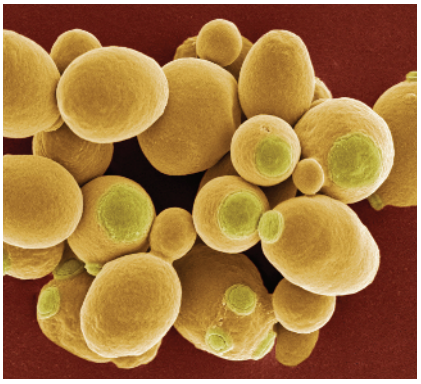
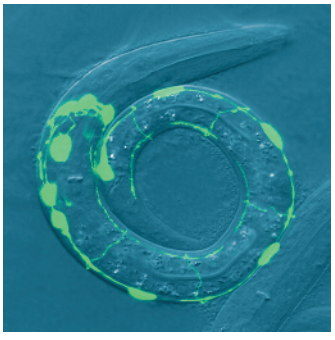
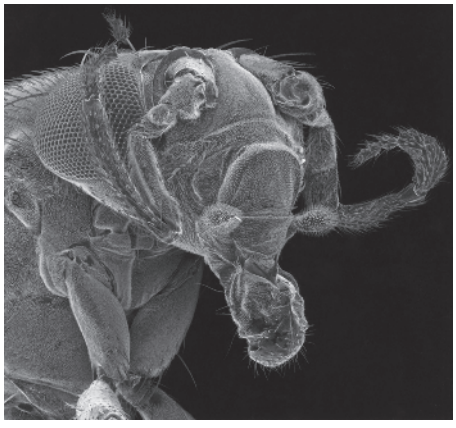


(b)

(c)



(d)



(e)

(f)

FIGURE 1.18 Six model organisms. (*a*) *Escherichia coli* is a rod-shaped bacterium that lives in the digestive tract of humans and other mammals. Much of what we will discuss about the basic molecular biology of the cell, including the mechanisms of replication, transcription, and translation, was originally worked out on this one prokaryotic organism. The relatively simple organization of a prokaryotic cell is illustrated in this electron micrograph. (*b*) *Saccharomyces cerevisiae*, more commonly known as baker's yeast or brewer's yeast. It is the least complex of the eukaryotes commonly studied, yet it contains a surprising number of proteins that are homologous to proteins in human cells. Such proteins typically have a conserved function in the two organisms. The species has a small genome encoding about 6200 proteins; it can be grown in a haploid state (one copy of each gene per cell rather than two as in most eukaryotic cells); and it can be grown under either aerobic (O_2-containing) or anaerobic (O_2-lacking) conditions. It is ideal for the identification of genes through the use of mutants. (*c*) *Arabidopsis thaliana*, a weed (called the thale cress) that is related to mustard and cabbage, which has an unusually small genome (120 million base pairs) for a flowering plant, a rapid generation time, and large seed production, and it grows to a height of only a few inches. (*d*) *Caenorhabditis elegans*, a microscopic-sized nematode, consists of a defined number of cells (roughly 1000), each of which develops according to a precise pattern of cell divisions. The animal is easily cultured, can be kept alive in a frozen state, has a transparent body wall, a short generation time, and facility for genetic analysis. This micrograph shows the larval nervous system, which has been labeled with the green fluorescent protein (GFP). The 2002 Nobel Prize was awarded to the researchers who pioneered its study. (*e*) *Drosophila melanogaster*, the fruit fly, is a small but complex eukaryote that is readily cultured in the lab, where it grows from an egg to an adult in a matter of days. *Drosophila* has been a favored animal for the study of genetics, the molecular biology of development, and the neurological basis of simple behavior. Certain larval cells have giant chromosomes, whose individual genes can be identified for studies of evolution and gene expression. In the mutant fly shown here, a leg has developed where an antenna would be located in a normal (wild type) fly. (*f*) *Mus musculus*, the common house mouse, is easily kept and bred in the laboratory. Thousands of different genetic strains have been developed, many of which are stored simply as frozen embryos due to lack of space to house the adult animals. The "nude mouse" pictured here develops without a thymus gland and, therefore, is able to accept human tissue grafts that are not rejected.

SOURCE: (a) Biophoto Associates/Photo Researchers; (b) Biophoto Associates/Photo Researchers; (c) Courtesy of Erik Jorgensen, University of Utah. From *Trends Genetics*, Vol. 14, Cover#12, 1998, with permission from Elsevier; (d) Courtesy of Erik Jorgensen, University of Utah. From *Trends Genetics*, Vol. 14, Cover#12, 1998, with permission from Elseviere: David Scharf/Photo Researchers, Inc. f: Ted Spiegel/© Corbis Images.

are described in the accompanying legend. We will concentrate in this text on results obtained from studies on mammalian systems—mostly on the mouse and on cultured mammalian cells—because these findings are most applicable to humans. But a large portion of what we know about mammalian cells was first discovered by experiments in other model organisms that are easier to work with. Thus, we will have many occasions to describe research carried out on the cells of other species. You may be surprised to discover how similar you are at the cell and molecular level to these much smaller and simpler organisms.

1.6 The Sizes of Cells and Their Components

FIGURE 1.19 shows the relative size of a number of structures of interest in cell biology. Two units of linear measure are most commonly used to describe structures within a cell: the **micrometer** (μm) and the **nanometer** (nm). One μm is equal to 10^{-6} meters, and one nm is equal to 10^{-9} meters. The **angstrom** (Å), which is equal to one-tenth of a nm, is commonly employed by molecular biologists for atomic dimensions. One angstrom is roughly equivalent to the diameter of a hydrogen atom. Large biological molecules (i.e., macromolecules) are described in either angstroms or nanometers. Myoglobin, a typical globular protein, is approximately 4.5 nm × 3.5 nm × 2.5 nm; highly elongated proteins (such as collagen or myosin) are over 100 nm in length; and DNA is approximately 2.0 nm in width. Complexes of macromolecules, such as ribosomes, microtubules, and microfilaments, are between 5 and 25 nm in diameter. Despite their tiny dimensions, these macromolecular complexes constitute remarkably sophisticated "nanomachines" capable of performing a diverse array of mechanical, chemical, and electrical activities.

Cells and their organelles are more easily defined in micrometers. Nuclei, for example, are approximately 5–10 μm in diameter, and mitochondria are approximately 2 μm in length. Prokaryotic cells typically range in length from about 1 to 5 μm, eukaryotic cells from about 10 to 30 μm. There are a number of reasons most cells are so small. Consider the following.

- Most eukaryotic cells possess a single nucleus that contains only two copies of most genes. Because genes serve as templates for the production of information-carrying messenger RNAs, a cell can only produce a limited number of these messenger RNAs in a given amount of time. The greater a cell's cytoplasmic volume, the longer it will take to synthesize the number of messages required by that cell.
- As a cell increases in size, the surface area/volume ratio decreases.[3] The ability of a cell to exchange substances with its environment is proportional to its surface area. If a cell were to grow beyond a certain size, its surface would not be sufficient to take up the substances (e.g., oxygen, nutrients) needed to support its metabolic activities. Cells that are specialized for absorption of solutes, such as those of the intestinal epithelium, typically possess microvilli, which greatly increase the surface area available for exchange (see Figure 1.3). The interior of a large plant cell is typically filled by a large, fluid-filled vacuole rather than metabolically active cytoplasm (see Figure 12.36*b*).
- A cell depends to a large degree on the random movement of molecules (*diffusion*). Oxygen, for example, must diffuse from the cell's surface through the cytoplasm to the interior of its mitochondria. The time required for diffusion is proportional to the square of the distance to be traversed. For example, O_2 requires only 100 microseconds to diffuse a distance of 1 μm, but requires 10^6 times as long to diffuse a distance of 1 mm. As a cell becomes larger and the distance from the surface to the interior becomes greater, the time required for diffusion to move substances in and out of a metabolically active cell becomes prohibitively long.

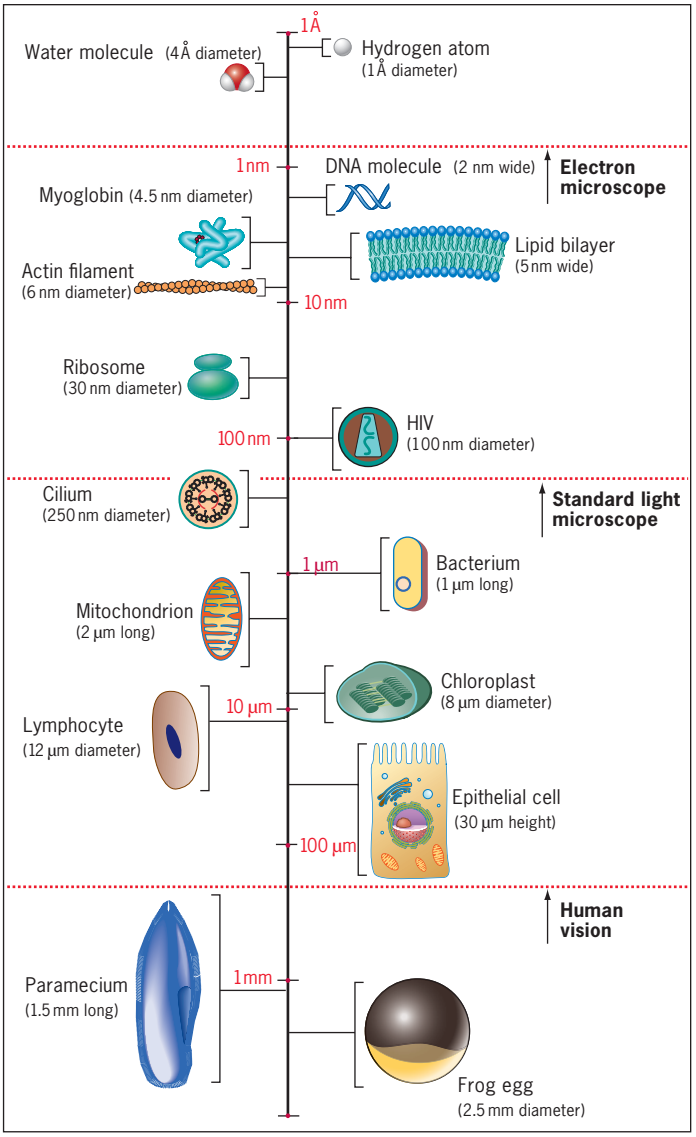


FIGURE 1.19 **Relative sizes of cells and cell components.** These structures differ in size by more than seven orders of magnitude.

[3]You can verify this statement by calculating the surface area and volume of a cube whose sides are 1 cm in length versus a cube whose sides are 10 cm in length. The surface area/volume ratio of the smaller cube is considerably greater than that of the larger cube.

But despite these constraints, some eukaryotic cells can be extremely large. The free-living single celled organism *Stentor coeruleus*, which lives in freshwater ponds, grows to be more than a millimeter long, and the giant single-celled green alga *Acetabularia* is more than 10 cm long. The gargantuan single-celled green alga *Caulerpa* can grow to a length of several meters and contains millions of nuclei in a common cytoplasm. Examples of large cell size are not restricted to such strange organisms, however. Indeed, we have some examples in our own bodies. Neurons send out extremely long processes; motor neurons in the human spinal cord, for example, send out axons that can be a meter long.

The fact that cells are collections of nanomachines has inspired a research field known as *synthetic biology*, whose ultimate goal is to create some minimal type of living cell in the laboratory out of the same types of component parts found in real cell. Synthetic biology uses the molecules, molecular complexes, and organelles of a cell as buiding blocks, as suggested by the cartoon in **FIGURE 1.20**. One motivation of these researchers is simply to accomplish the feat and, in the process, demonstrate that life at the cellular level emerges spontaneously when the proper constituents are brought together from chemically synthesized materials. At this point in time, biologists have only begun the first steps in this direction. Such work holds the potential to illuminate the possible origins of life and to launch an entirely new approach to biotechnology. However, creating life may raise interesting moral and even religious questions. A more modest goal of synthetic biology is to develop novel life forms, using existing organisms as a starting point, that have a unique value in medicine and industry, or in cleaning up the environment.

If, as most biologists would argue, the properties and activities of a cell spring from the genetic blueprint of that cell, then it should be possible to create a new type of cell by introducing a new genetic blueprint into the cytoplasm of an existing cell. This feat was accomplished by J. Craig Venter and colleagues in 2007, when they replaced the genome of one bacterium with a genome isolated from a closely related species, effectively transforming one species into the other. By 2010, after overcoming a number of stubborn technical roadblocks, the team was able to accomplish a similar feat using a copy of a bacterial genome that had been assembled (inside of a yeast cell) from fragments of DNA that had been chemically synthesized in the laboratory. The synthetic copy of the donor genome, which totaled approximately 1.1 million base pairs of DNA, contained a number of modifications introduced by the researchers. The modified copy of the genome (from *M. mycoides*) was transplanted into a cell of a closely related bacterial species (*M. capricolum*), where it replaced the host's original genome. Following genome transplantation, the recipient cell rapidly took on the characteristics of the species from which the donor DNA has been derived. In effect, these researchers have produced cells containing a "genetic skeleton" to which they can add combinations of new genes taken from other organisms.

Researchers around the world are attempting to genetically engineer organisms to possess metabolic pathways capable of producing pharmaceuticals, hydrocarbon-based fuel molecules, and other useful chemicals from cheap, simple precursors. Several companies are growing genetically engineered cyanobacteria capable of producing diesel fuel from sunlight, water, and CO_2. Researchers at another company have genetically engineered the common lab bacterium *E. coli* to ferment the complex polysaccharides present in seaweed into the biofuel ethanol. This feat required the introduction into *E. coli* of a combination of genes derived from three other bacterial species. Work has also begun on "rewriting" the yeast genome, signifying that eukaryotic cells have also become part of the effort to design genetically engineered biological manufacturing plants.

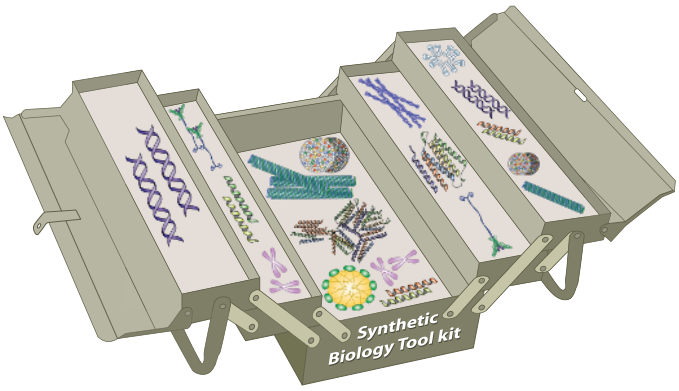


FIGURE 1.20 **The synthetic biologist's toolkit of the future?** Such a toolkit would presumably contain nucleic acids, proteins, lipids, and many other types of biomolecules.

In principle, the work described in the Human Perspective, in which one type of cell is directed into the formation of an entirely different type of cell, is also a form of synthetic biology. As a result of these many efforts, biologists are no longer restricted to studying cells that are available in Nature, but can also turn their attention to cells that can become available through experimental manipulation.

1.7 Viruses

By the end of the nineteenth century, the work of Louis Pasteur and others had convinced the scientific world that infectious diseases of plants and animals were due to bacteria. But studies of tobacco mosaic disease in tobacco plants and hoof-and-mouth disease in cattle pointed to the existence of another type of infectious agent. It was found, for example, that sap from a diseased tobacco plant could transmit mosaic disease to a healthy plant, even when the sap showed no evidence of bacteria in the light microscope. To gain further insight into the size and nature of the infectious agent, Dmitri Ivanovsky, a Russian biologist, forced the sap from a diseased plant through filters whose pores were so small that they retarded the passage of the smallest known bacterium. The filtrate was still infective, causing Ivanovsky to conclude in 1892 that certain diseases were caused by pathogens that were even smaller, and presumably simpler,

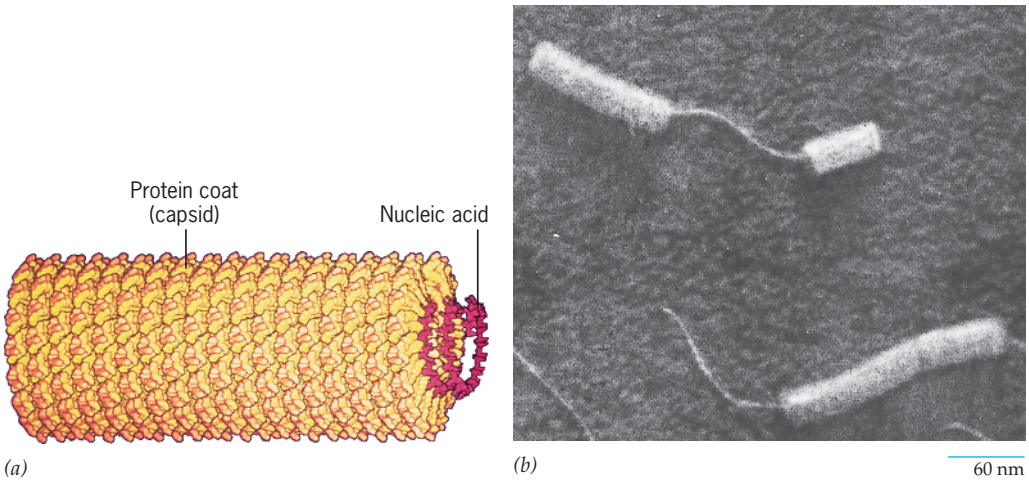


FIGURE 1.21 Tobacco mosaic virus (TMV). (*a*) Model of a portion of a TMV particle. The protein subunits, which are identical along the entire rod-shaped particle, enclose a single helical RNA molecule (red). (*b*) Electron micrograph of TMV particles after phenol has removed the protein subunits from the middle part of the upper particle and the ends of the lower particle. Intact rods are approximately 300 nm long and 18 nm in diameter.

SOURCE: (a) Courtesy of Gerald Stubbs, Keuchi Namba, and Donald Caspar; (b) Courtesy M.K. Corbett.

than the smallest known bacteria. These pathogens became known as **viruses**.

In 1935, Wendell Stanley of the Rockefeller Institute reported that the virus responsible for tobacco mosaic disease could be crystallized and that the crystals were infective. Substances that form crystals have a highly ordered, well-defined structure and are vastly less complex than the simplest cells. Stanley mistakenly concluded that tobacco mosaic virus (TMV) was a protein. In fact, TMV is a rod-shaped particle consisting of a single molecule of RNA surrounded by a helical shell composed of protein subunits (**FIGURE 1.21**).

Viruses are responsible for dozens of human diseases, including AIDS, polio, influenza, ebola, measles, and a few types of cancer. Viruses occur in a wide variety of very different shapes, sizes, and constructions, but all of them share certain common properties. All viruses are obligatory intracellular parasites; that is, they cannot reproduce unless present within a host cell. Depending on the specific virus, the host may be a plant, animal, or bacterial cell. Outside of a living cell, the virus exists as a particle, or **virion**, which is little more than a macromolecular package. The virion contains a small amount of genetic material that, depending on the virus, can be single-stranded or double-stranded, RNA or DNA. Remarkably, some viruses have as few as three or four different genes, but others may have as many as several hundred. The genetic material of the virion is surrounded by a protein capsule, or *capsid*. Virions are macromolecular aggregates, inanimate particles that by themselves are unable to reproduce, metabolize, or carry on any of the other activities associated with life. For this reason, viruses are not considered to be organisms and are not described as being alive.

Viral capsids are generally made up of a specific number of subunits. There are numerous advantages to construction by subunit, one of the most apparent being an economy of genetic information. If a viral coat is made of many copies of a single protein, as is that of TMV, or a few proteins, as are the coats of many other viruses, the virus needs only one or a few genes to code for its protein container. Many viruses have a capsid whose subunits are organized into a polyhedron, that is, a structure having planar faces. A particularly common polyhedral shape of viruses is the 20-sided *icosahedron*. For example, adenovirus, which causes respiratory infections in mammals, has an icosahedral capsid (**FIGURE 1.22a**). In many animal viruses, including the *human immunodeficiency virus* (*HIV*) responsible for AIDS, the protein capsid is surrounded by a lipid-containing outer envelope that is derived from the modified plasma membrane of the host cell as the virus buds from the host-cell surface (Figure 1.22*b*). Bacterial viruses, or *bacteriophages*, are among the most complex viruses (Figure 1.22*c*). They are also the most abundant biological entities on Earth. The T bacteriophages (which were used in key experiments that revealed the structure and properties of the genetic material) consist of a polyhedral head containing DNA, a cylindrical stalk through which the DNA is injected into the bacterial cell, and tail fibers, which together cause the particle to resemble a landing module for the moon.

Each virus has on its surface a protein that is able to bind to a particular surface component of its host cell. For example, the protein that projects from the surface of the HIV particle (labeled gp120 in Figure 1.22*b*, which stands for glycoprotein of molecular mass 120,000 daltons[4]) interacts with a specific protein (called CD4) on the surface of certain white blood cells, facilitating entry of the virus into its host cell. The interaction between viral and host proteins determines the specificity of the virus, that is, the types of host cells that the virus can enter and infect. Some viruses have a wide *host range*, being able to infect cells from a variety of different organs or host species. The virus that causes rabies, for example, is able to infect many different types of mammalian hosts, including dogs, bats, and humans. Most viruses, however, have a relatively narrow host range. This is true, for example, of human cold and influenza viruses, which are generally able to infect only the respiratory epithelial cells of human hosts.

A change in host-cell specificity can have striking consequences. This point is dramatically illustrated by the 1918 influenza pandemic, which killed more than 30 million people worldwide. The virus was especially lethal in young adults, who

[4]One dalton is equivalent to one unit of atomic mass, the mass of a single hydrogen (^{1}H) atom.

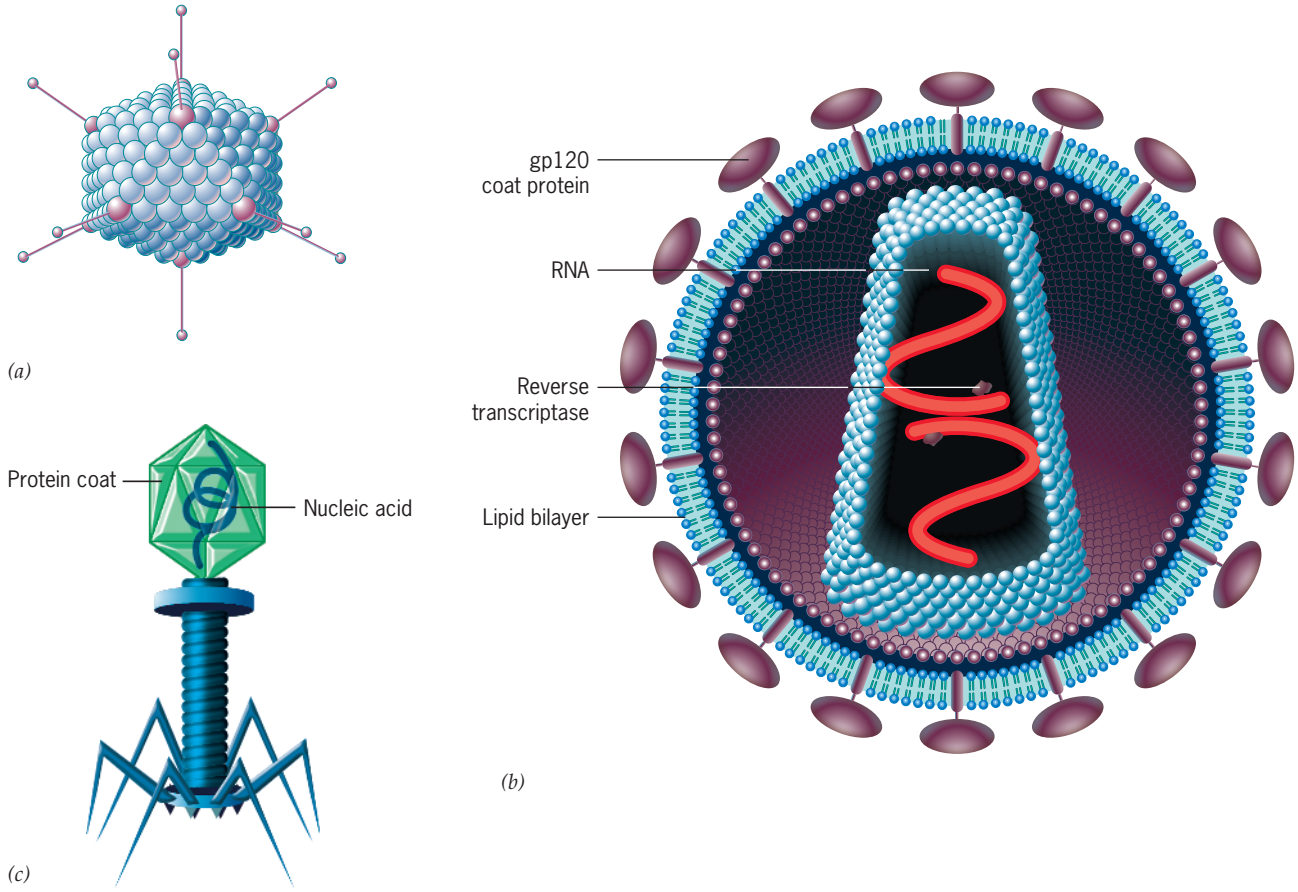


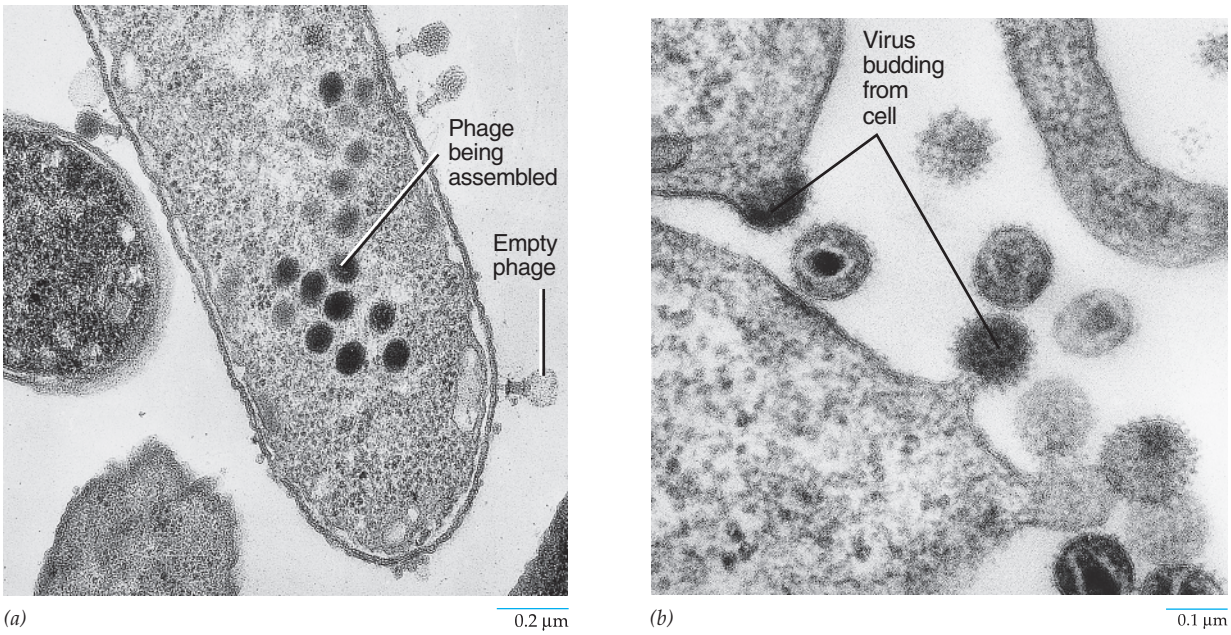
FIGURE 1.22 **Virus diversity.** The structures of (*a*) an adenovirus, (*b*) a human immunodeficiency virus (HIV), and (*c*) a T-even bacteriophage. *Note:* These viruses are not drawn to the same scale.

do not normally fall victim to influenza. In fact, the 675,000 deaths from this virus in the United States temporarily lowered average life expectancy by several years. In one of the most acclaimed—and controversial—feats of the past few years, researchers have been able to determine the genomic sequence of the virus responsible for this pandemic and to reconstitute the virus in its full virulent state. This was accomplished by isolating the viral genes (which are part of a genome consisting of eight separate RNA molecules encoding 11 different proteins) from the preserved tissues of victims who had died from the infection 90 years earlier. The best preserved samples were obtained from a Native American woman who had been buried in the Alaskan permafrost. The sequence of the "1918 virus" suggested that the pathogen had jumped from birds to humans. Although the virus had accumulated a considerable number of mutations, which adapted it to a mammalian host, it had never exchanged genetic material with that of a human influenza virus as had been thought likely.

Analysis of the sequence of the 1918 virus has provided some clues to explain why it was so deadly and how it spread so efficiently from one human to another. Using the genomic sequence, researchers reconstituted the 1918 virus into infectious particles, which were found to be exceptionally virulent in laboratory tests. Whereas laboratory mice normally survive infection by modern human influenza viruses, the reconstituted 1918 strain killed 100 percent of infected mice and produced enormous numbers of viral particles in the animals' lungs. Because of the potential risk to public health, publication of the full sequence of the 1918 virus and its reconstitution went forward only after approval by governmental safety panels and the demonstration that existing influenza vaccines and drugs protect mice from the reconstituted virus.

There are two basic types of viral infection. (1) In most cases, the virus arrests the normal synthetic activities of the host and redirects the cell to use its available materials to manufacture viral nucleic acids and proteins, which assemble into new virions. Viruses, in other words, do not grow like cells; they are assembled from components directly into the mature-sized virions. Ultimately, the infected cell ruptures (*lyses*) and releases a new generation of viral particles capable of infecting neighboring cells. An example of this type of *lytic* infection is shown in **FIGURE 1.23a**. (2) In other cases, the infecting virus does not lead to the death of the host cell, but instead inserts (*integrates*) its DNA into the DNA of the host cell's chromosomes. The integrated viral DNA is called a **provirus**. An integrated provirus can have different effects depending on the type of virus and host cell. For example,

- Bacterial cells containing a provirus behave normally until exposed to a stimulus, such as ultraviolet radiation, that activates the dormant viral DNA, leading to the lysis of the cell and release of viral progeny.



(a) (b)

FIGURE 1.23 **A virus infection.** (*a*) Micrograph showing a late stage in the infection of a bacterial cell by a bacteriophage. Virus particles are being assembled within the cell, and empty phage coats are still present on the cell surface. (*b*) Micrograph showing HIV particles budding from an infected human lymphocyte.

SOURCE: (a) Courtesy of Jonathan King and Erika Hartwig; (b) Courtesy of Hans Gelderblom.

- Some animal cells containing a provirus produce new viral progeny that bud at the cell surface without lysing the infected cell. Human immunodeficiency virus (HIV) acts in this way; an infected cell may remain alive for a period, acting as a factory for the production of new virions (Figure 1.23*b*).
- Some animal cells containing a provirus lose control over their own growth and division and become malignant. This phenomenon is readily studied in the laboratory by infecting cultured cells with the appropriate tumor virus.

Viruses are not without their virtues. Because the activities of viral genes mimic those of host genes, investigators have used viruses for decades as a research tool to study the mechanism of DNA replication and gene expression in their much more complex hosts. In addition, viruses are now being used as a means to introduce foreign genes into human cells, a technique that will likely serve as the basis for the treatment of human diseases by gene therapy. Last, insect- and bacteria-killing viruses may play an increasing role in the war against insect pests and bacterial pathogens. Bacteriophages have been used for decades to treat bacterial infections in eastern Europe and Russia, while physicians in the West have relied on antibiotics. Given the rise in antibiotic-resistant bacteria, bacteriophages may be making a comeback on the heels of promising studies on infected mice. Several biotechnology companies are now producing bacteriophages intended to combat bacterial infections and to protect certain foods from bacterial contamination.

Viroids

It came as a surprise in 1971 to discover that viruses are not the simplest types of infectious agents. In that year, T. O. Diener of the U.S. Department of Agriculture reported that potato spindle-tuber disease, which causes potatoes to become gnarled and cracked, is caused by an infectious agent consisting of a small circular RNA molecule that totally lacks a protein coat. Diener named the pathogen a **viroid**. The RNAs of viroids range in size from about 240 to 600 nucleotides, one-tenth the size of the smaller viruses. No evidence has been found that the naked viroid RNA encodes any proteins. Rather, any biochemical activities in which viroids engage take place using host-cell proteins. For example, duplication of the viroid RNA within an infected cell utilizes the host's RNA polymerase II, an enzyme that normally transcribes the host's DNA into messenger RNAs. Viroids are thought to cause disease by interfering with the cell's normal path of gene expression. The effect on crops can be serious: A viroid disease called cadang-cadang has devastated the coconut palm groves of the Philippines, and another viroid has wreaked havoc on the chrysanthemum industry in the United States. The discovery of a different type of infectious agent even simpler than a viroid is described in the Human Perspective in Chapter 2.

THE HUMAN PERSPECTIVE

The Prospect of Cell Replacement Therapy

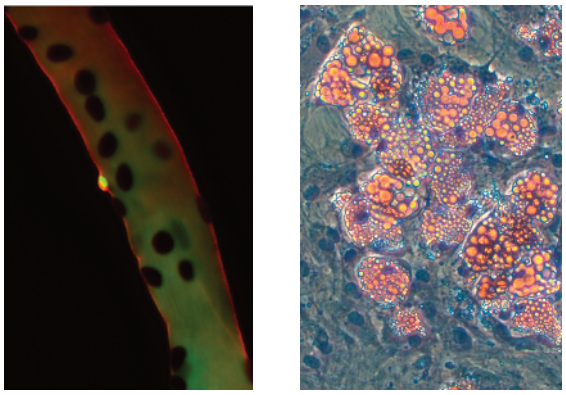
Many human diseases result from the deaths of specific types of cells. Type 1 diabetes, for example, results from the destruction of beta cells in the pancreas; Parkinson's disease occurs with the loss of dopamine-producing neurons in the brain; and heart failure can be traced to the death of cadiac muscle cells (cardiomyocytes) in the heart. Imagine the possibilities if we could isolate cells from a patient, convert them into the cells that are needed by that patient, and then infuse them back into the patient to restore the body's lost function. Recent studies have given researchers hope that one day this type of therapy will be common place. To better understand the concept of cell replacement therapy, we can consider a procedure used widely in current practice known as *bone marrow transplantation* in which cells are extracted from the pelvic bones of a donor and infused into the body of a recipient.

Bone marrow transplantation is used most often to treat lymphomas and leukemias, which are cancers that affect the nature and number of white blood cells. To carry out the procedure, the patient is exposed to a high level of radiation and/or toxic chemicals, which kills the cancer cells, but also kills all of the cells involved in the formation of red and white blood cells. This treatment has this effect because blood-forming cells are particularly sensitive to radiation and toxic chemicals. Once a person's blood-forming cells have been destroyed, they are replaced by bone marrow cells transplanted from a healthy donor. Bone marrow can regenerate the blood tissue of the transplant recipient because it contains a small percentage of cells that can proliferate and restock the patient's blood-forming bone marrow tissue.[1] These blood-forming cells in the bone marrow are termed **hematopoietic stem cells** (or **HSCs**), and they were discovered in the early 1960s by Ernest McCulloch and James Till at the University of Toronto. HSCs are responsible for replacing the millions of red and white blood cells that age and die every minute in our bodies (see Figure 17.6). Amazingly, a *single* HSC is capable of reconstituting the entire hematopoietic (blood-forming) system of an irradiated mouse. An increasing number of parents are saving the blood from the umbilical cord of their newborn baby as a type of "stem-cell insurance policy" in case that child should ever develop a disease that might be treated by administration of HSCs. Now that we have described one type of cell replacement therapy, we can consider several other types that have a much wider therapeutic potential. We will divide these potential therapies into four types.

Adult Stem Cells

Hematopoietic stem cells in the bone marrow are an example of an *adult* stem cell. **Stem cells** are defined as undifferentiated cells that (1) are capable of self-renewal, that is, production of more cells like themselves, and (2) are multipotent, that is, are capable of differentiating into two or more mature cell types. HSCs of the bone marrow are only one type of adult stem cell. Most, if not all, of the organs in a human adult contain stem cells that are capable of replacing the particular cells of the tissue in which they are found. Even the adult brain, which is not known for its ability to regenerate, contains stem cells that can generate new neurons and glial cells (the supportive cells of the brain). **FIGURE 1a** shows an isolated stem cell present in adult skeletal muscle; these "satellite cells," as they are called, are thought to divide and differentiate as needed for the repair of injured muscle tissue. Figure 1*b* shows a culture of adipose (fat) cells that have differentiated in vitro from adult stem cells that are present within fat tissue.

The adult human heart contains stem cells that are capable of differentiating into the cells that form both the muscle tissue of the heart (the cardiomyocytes of the myocardium) and the heart's blood vessels. It had been hoped that these cardiac stem cells might have the potential to regenerate healthy heart tissue in a patient who had experienced a serious heart attack. This hope has apparently been realized based on the appearance of two landmark reports in late 2011 on the results from clinical trials of patients that had suffered significant heart-tissue damage following heart attacks. Stem cells were harvested from each of the patients during heart surgeries, expanded in number through in vitro culture, and then infused back into each patient's heart. Over the next few months, a majority of treated patients experienced significant replacement (e.g., 50 percent) of the damaged heart muscle by healthy tissue derived from the infused stem cells. This regeneration of heart tissue was accompanied by a clear improvement in quality of life compared to patients in the placebo group that did not receive stem cells. Adult stem cells are an ideal system for cell replacement therapies because they represent an *autologous* treatment; that is, the cells are taken from the same



(a) *(b)*

FIGURE 1 **An adult muscle stem cell.** (*a*) A portion of a muscle fiber, with its many nuclei stained blue. A single stem cell (yellow) is seen to be lodged between the outer surface of the muscle fiber and an extracellular layer (or basement membrane), which is stained red. The undifferentiated stem cell exhibits this yellow color because it expresses a protein that is not present in the differentiated muscle fiber. (*b*) Adult stem cells undergoing differentiation into adipose (fat) cells in culture. Stem cells capable of this process are present in adult fat tissue and also bone marrow.

SOURCE: (a) From Charlotte A. Collins; et al., Cell 122:291, 2005; by permission of Elsevier; (b) Courtesy of Thermo Fisher Scientific, fom Nature 451:855, 2008.

continued

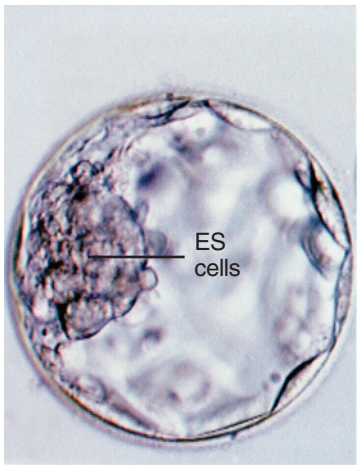
patient in which they are used. Consequently, these stem cells do not face the prospect of immune rejection. These dramatic results with cardiac stem cells rekindled interest in adult stem cells, which had waned after a number of failed attempts to direct stem cells isolated from bone marrow to regenerate diseased tissues. The great majority of adult stem cell therapies under development use a type of adult stem cell known as a mesenchymal stem cell (MSC). These can be obtained from bone marrow but they are different from the HSCs discussed above in that they do not produce blood cells but rather a variety of other cell types found in various tissues and organs. MSCs can also be obtained from fat tissue obtained during liposuction procedures. Currently there are well over 100 controlled clinical trials underway for treating a wide range of diseases with MSC-derived cells, including heart disease, diabetes, and immune diseases such as Lupus and Crohn's disease. An MSC-based therapy called "Prochymal" became the first FDA approved stem cell therapy. It is used to treat Crohn's disease as well as to treat immune reactions that can occur in patients who receive bone marrow transplants.

Embryonic Stem Cells

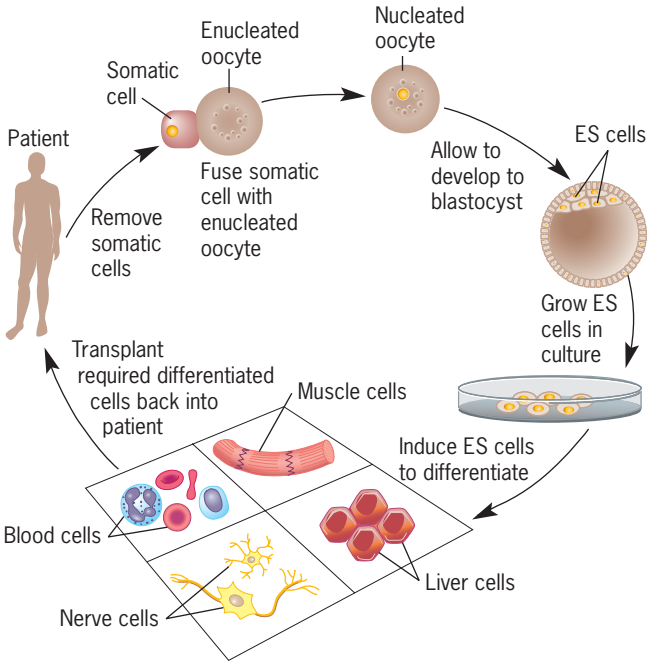
Much of the excitement that has been generated in the field over the past decade or two has come from studies on **embryonic stem (ES) cells**, which are a type of stem cell isolated from very young mammalian embryos (**FIGURE 2a**). These are the cells in the early embryo that give rise to all of the various structures of the mammalian fetus. Unlike adult stem cells, ES cells are *pluripotent*; that is, they are capable of differentiating into every type of cell in the body. In most cases, human ES cells have been isolated from embryos provided by in vitro fertilization clinics. Worldwide, dozens of genetically distinct human ES cell lines, each derived from a single embryo, are available for experimental investigation.

The long-range goal of clinical researchers is to learn how to coax ES cells to differentiate in culture into each of the many cell types that might be used for cell replacement therapy. Considerable progress has been made in this pursuit, and numerous studies have shown that transplants of differentiated, ES-derived cells can improve the condition of animals with diseased or damaged organs. The first trial in humans was begun in 2009 on patients who had experienced debilitating spinal cord injuries. The trial to treat spinal cord injuries utilized cells, called oligodendrocytes, that produce the myelin sheaths that become wrapped around nerve cells (see Figure 8.5). The oligodendrocytes transplanted into these patients were differentiated from human ES cells that were cultured in a medium containing insulin, thyroid hormone, and a combination of certain growth factors. This particular culture protocol had been found to direct the differentiation of ES cells into oligodendrocytes rather than any other cell type. Unfortunately, no significant improvement was reported in the treated patients, and the company conducting the trial decided to cease further involvement in the effort.

Embryonic stem cell therapy is currently under intense study as a treatment for retinal degeneration diseases such as macular degeneration. At the time of this writing there are eight government-approved clinical trials using ES cells induced to differentiate into retinal pigmented epithelial cells, a key cell type within the retina, in an attempt to cure different forms of retinal degeneration.



(a)



(b)

FIGURE 2 Embryonic stem cells; their isolation and potential use. (*a*) Micrograph of a mammalian blastocyst, an early stage during embryonic development, showing the inner cell mass, which is composed of pluripotent ES cells. Once isolated, such cells are readily grown in culture. (*b*) A potential procedure for obtaining differentiated cells for use in cell replacement therapy. A small piece of tissue is taken from the patient, and one of the somatic cells is fused with a donor oocyte whose own nucleus had been previously removed. The resulting oocyte (egg), with the patient's cell nucleus, is allowed to develop into an early embryo, and the ES cells are harvested and grown in culture. A population of ES cells are induced to differentiate into the required cells, which are subsequently transplanted into the patient to restore organ function. (At the present time, it has not been possible to obtain blastocyst stage embryos, that is, ones with ES cells, from any primate species by the procedure shown here, although it has been accomplished using an oocyte from which the nucleus is not first removed. The ES cells that are generated in such experiments are triploid; that is, they have three copies of each chromosome—one from the oocyte and two from the donor nucleus—rather than two, as would normally be the case. Regardless, these triploid ES cells are pluripotent and capable of transplantation.)

SOURCE: © Phanie/SuperStock

The primary risk with the therapeutic use of ES cells is the unnoticed presence of undifferentiated ES cells among the differentiated cell population. Undifferentiated ES cells are capable of forming a type of benign tumor, called a teratoma, which may contain a bizarre mass of various differentiated tissues, including hair and teeth. The formation of a teratoma within the central nervous system could have severe consequences. In addition, the culture of ES cells at the present time involves the use of nonhuman biological materials, which also poses potential risks.

The ES cells used in these early trials were derived from cell lines that had been isolated from human embryos unrelated to the patients who are being treated. Such cells face the prospect of immunologic rejection by the transplant recipient. It may be possible, however, to "customize" ES cells so that they possess the same genetic makeup of the individual who is being treated. This may be accomplished one day by a roundabout procedure called *somatic cell nuclear transfer* (*SCNT*), shown in Figure 2*b*, that begins with an unfertilized egg—a cell that is obtained from the ovaries of an unrelated woman donor. In this approach, the nucleus of the unfertilized egg would be replaced by the nucleus of a cell from the patient to be treated, which would cause the egg to have the same chromosome composition as that of the patient. The egg would then be allowed to develop to an early embryonic stage, and the ES cells would be removed, cultured, and induced to differentiate into the type of cells needed by the patient. Because this procedure involves the formation of a human embryo that is used only as a source of ES cells, there are major ethical questions that must be settled before it could be routinely practiced. In addition, the process of SCNT is so expensive and technically demanding that it is highly improbable that it could ever be practiced as part of any routine medical treatment. It is more likely that, if ES cell-based therapy is ever practiced, it would depend on the use of a bank of hundreds or thousands of different ES cells. Such a bank could contain cells that are close enough as a tissue match to be suitable for use in the majority of patients.

Induced Pluripotent Stem Cells

It had long been thought that the process of cell differentiation in mammals was irreversible; once a cell had become a fibroblast, or white blood cell, or cartilage cell, it could never again revert to any other cell type. This concept was shattered in 2006 when Shinya Yamanaka and co-workers of Kyoto University announced a stunning discovery; his lab had succeeded in reprogramming a fully differentiated mouse cell—in this case a type of connective tissue fibroblast—into a pluripotent stem cell. They accomplished the feat by introducing into the mouse fibroblast the genes that encoded four key proteins that are characteristic of ES cells. These genes (*Oct4, Sox2, Klf4,* and *Myc*, known collectively as OSKM) are thought to play a key role in maintaining the cells in an undifferentiated state and allowing them to continue to self-renew. The genes were introduced into cultured fibroblasts using gene-carrying viruses, and those rare cells that became reprogrammed were selected from the others in the culture by specialized techniques. They called this new type of cells *induced pluripotent cells* (*iPS cells*) and demonstrated that they were indeed pluripotent by injecting them into a mouse blastocyst and finding that they participated in the differentiation of all the cells of the body, including eggs and sperm. Within the next year or so the same reprogramming feat had been accomplished in several labs with human cells. What this means is that researchers now have available to them an unlimited supply of pluripotent cells that can be directed to differentiate into various types of body cells using similar experimental protocols to those already developed for ES cells.

Indeed, iPS cells have already been used to correct certain disease conditions in experimental animals, including sickle cell anemia in mice as depicted in **FIGURE 3**. Based on the promising results of animal experiments, attempts to use iPS cells in patients are beginning. The first clinical trial of an iPS cell based therapy was begun in 2014. Similar to ongoing embryonic stem cell trials mentioned above, this trial is testing the use of iPS cell derived retinal pigmented epithelial cells to treat macular degeneration. Results from this trial were not available at the time of this writing.

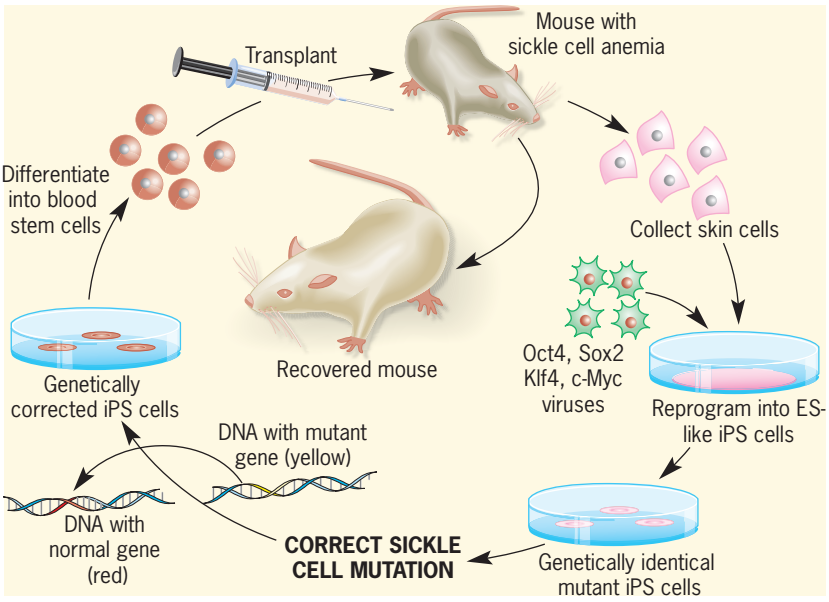


FIGURE 3 Steps taken to generate induced pluripotent stem (iPS) cells for use in correcting the inherited disease sickle cell anemia in mice. Skin cells are collected from the diseased animal, reprogrammed in culture by introducing the four required genes that are ferried into the cells by viruses, and allowed to develop into undifferentiated pluripotent iPS cells. The iPS cells are then treated so as to replace the defective (globin) gene with a normal copy, and the corrected iPS cells are caused to differentiate into normal blood stem cells in culture. These blood stem cells are then injected back into the diseased mouse, where they proliferate and differentiate into normal blood cells, thereby curing the disorder.

SOURCE: Reprinted from an illustration by Rudolf Jaenisch, *Cell* 132:5, 2008, with permission from Elsevier.

continued

The utility of iPS cells may extend far beyond cell replacement therapy. iPS cells have also been prepared from adult cells taken from patients with a multitude of genetic disorders. Researchers are then able to follow the differentiation of these iPS cells in culture into the specialized cell types that are affected by the particular disease. It is hoped that such studies will reveal the mechanisms of disease formation as it unfolds in a culture dish just as it would normally occur in an unobservable way deep within the body. These "diseased iPS cells" have been referred to as "patients in a Petri dish." The clinical relevance of these cells can be illustrated by an example. iPS cells derived from patients with a heart disorder called long QT syndrome differentiate into cardiac muscle cells that exhibit irregular contractions ("beats") in culture. This disease-specific phenotype seen in culture can be corrected by several medicines normally prescribed to treat this disorder. Moreover, when these cardiomyocytes that had differentiated from the diseased iPS cells were exposed to the drug cisapride, the irregularity of their contractions increased. Cisapride is a drug that was used to treat heartburn before it was pulled from the market in the United States after it was shown to cause heart arrhythmias in certain patients. Results of this type suggest that differentiated cells derived from diseased iPS cells will serve as valuable targets for screening potential drugs for their effectiveness in halting disease progression.

Unlike ES cells, the generation of iPS cells does not require the use of an embryo. This feature removes all of the ethical reservations that accompany work with ES cells and also makes it much easier to generate these cells in the lab. However, as research on iPS cells has increased, the therapeutic potential for these cells has become less clear. For the first several years of study, it was thought that iPS cells and ES cells were essentially indistinguishable. Recent studies, however, have shown that iPS cells lack the "high quality" characteristic of ES cells and that not all iPS cells are the same. For example, iPS cells exhibit certain genomic abnormalities that are not present in ES cells, including the presence of mutations and extra copies of random segments of the genome. In addition, the DNA-containing chromatin of iPS cells retains certain traces of the original cells from which they were derived, which means that they are not completely reprogrammed into ES-like, pluripotent cells. This residual memory of their origin makes it is easier to direct iPS cells toward differentiation back into the cells from which they were derived than into other types of cells. It may be that these apparent deficiencies in iPS cells will not be a serious impediment in the use of these cells to treat diseases that affect adult tissues, but it has raised important questions. There are other issues with iPS cells as well. It will be important to develop efficient cell reprogramming techniques that do not use genome-integrating viruses because such cells carry the potential of developing into cancers. Progress has been made in this regard, but the efficiency of iPS cell formation typically drops when other procedures are used to introduce genes.

Like ES cells, undifferentiated iPS cells also give rise to teratomas, so it is essential that only fully differentiated cells are transplanted into human subjects. Also like ES cells, the iPS cells in current use have the same tissue antigens as the donors who originally provided them, so they would stimulate an immune attack if they were to be transplanted into other human recipients. Unlike the formation of ES cells, however, it will be much easier to generate personalized, tissue-compatible iPS cells, because they can be derived from a simple skin biopsy from each patient. Still, it does take considerable time, expense, and technical expertise to generate a population of iPS cells from a specific donor. Consequently, if iPS cells are ever developed for widespread therapeutic use, they would likely come from a large cell bank that could provide cells that are close tissue matches to most potential recipients. It may also be possible to remove all of the genes from iPS cells that normally prevent them from being transplanted into random recipients.

Direct Cell Reprogramming

In 2008 the field of cellular reprogramming took another unexpected turn with the announcement that one type of differentiated cell had been converted directly into another type of differentiated cell, a case of "transdifferentiation." In this report, the acinar cells of the pancreas, which produce enzymes responsible for digestion of food in the intestine, were transformed into pancreatic beta cells, which synthesize and secrete the hormone insulin. The reprogramming process occurred directly, in a matter of a few days, without the cells passing through an intermediate stem cell state—and it occurred while the cells remained in their normal residence within the pancreas of a live mouse. This feat was accomplished by injection into the animals of viruses that carried three genes known to be important in differentiation of beta cells in the embryo. In this case, the recipients of the injection were diabetic mice, and the transdifferentiation of a significant number of acinar cells into beta cells allowed the animals to regulate their blood sugar levels with much lower doses of insulin. It is also noteworthy that the adenoviruses used to deliver the genes in this experiment do not become a permanent part of the recipient cell, which removes some of the concerns about the use of viruses as gene carriers in humans. Since this initial report, a number of laboratories have developed in vitro techniques to directly convert one type of differentiated cell (typically a fibroblast) into another type of cell, such as a neuron, cardiomyocyte, or blood-cell precursor, in culture, without passing through a pluripotent intermediate. In all of these cases, transdifferentiation occurs when the original cells are forced to express certain genes that play a role in the normal embryonic differentiation of the other cell type. It is too early to know whether this type of direct reprogramming strategy has therapeutic potential, but it certainly raises the prospect that diseased cells that need to be replaced might be formed directly from other types of cells within the same organ.

Reference

1. Bone marrow transplantation can be contrasted to a simple blood transfusion where the recipient receives differentiated blood cells (especially red blood cells and platelets) present in the circulation.

EXPERIMENTAL PATHWAYS

The Origin of Eukaryotic Cells

We have seen in this chapter that cells can be divided into two groups: prokaryotic cells and eukaryotic cells. Almost from the time this division of cellular life was proposed, biologists have been fascinated by the question: What is the origin of the eukaryotic cell? It is generally agreed that prokaryotic cells (1) arose before eukaryotic cells and (2) gave rise to eukaryotic cells. The first point can be verified directly from the fossil record, which shows that prokaryotic cells were present in rocks approximately 2.7 billion years old (page 9), which is roughly one billion years before any evidence is seen of eukaryotes. The second point follows from the fact that the two types of cells have to be related to one another because they share many complex traits (e.g., very similar genetic codes, enzymes, metabolic pathways, and plasma membranes) that could not have evolved independently in different organisms.

Until about 1970, it was generally believed that eukaryotic cells evolved from prokaryotic cells by a process of gradual evolution in which the organelles of the eukaryotic cell became progressively more complex. Acceptance of this concept changed dramatically about that time largely through the work of Lynn Margulis, then at Boston University. Margulis resurrected an idea that had been proposed earlier, and dismissed, that certain organelles of a eukaryotic cell—most notably the mitochondria and chloroplasts—had evolved from smaller prokaryotic cells that had taken up residence in the cytoplasm of a larger host cell.[1] This hypothesis is referred to as the **endosymbiont theory** because it describes how a single "composite" cell of greater complexity could evolve from two or more separate, simpler cells living in a symbiotic relationship with one another.

Our earliest prokaryotic ancestors were presumed to have been anaerobic heterotrophic cells: *anaerobic* meaning they derived their energy from food matter without employing molecular oxygen (O_2) and *heterotrophic* meaning they were unable to synthesize organic compounds from inorganic precursors (such as CO_2 and water), but instead had to obtain preformed organic compounds from their environment. These prokaryotic ancestors are then thought to have acquired the ability to form internal membrane compartments, allowing formation of a nucleus by containing the DNA within an internal membrane. This development of internal membranes produced the first organism that would be considered eukaryote-like in terms of having a nucleus or other internal compartments (**FIGURE 1**). Because this is the first organism that subsequently gave rise to all eukaryotes, it is known as the *first eukaryotic common ancester* (FECA). Although the presence of internal membranes was once though to be an exclusively eukaryotic trait, it is now known that some bacteria can in fact form extensive complex internal membrane systems. The most dramatic example known to date is the bacterium *Gemmata obscuriglobus*, which forms a variety of complex internal membranes (**FIGURE 2**). However, careful three-dimensional reconstructions of *G. obscuriglobus* structure show that these membranes do not form closed compartments like eukaryotic organelles.[2] It thus appears that the key step in producing the FECA was not formation of internal membranes per se,

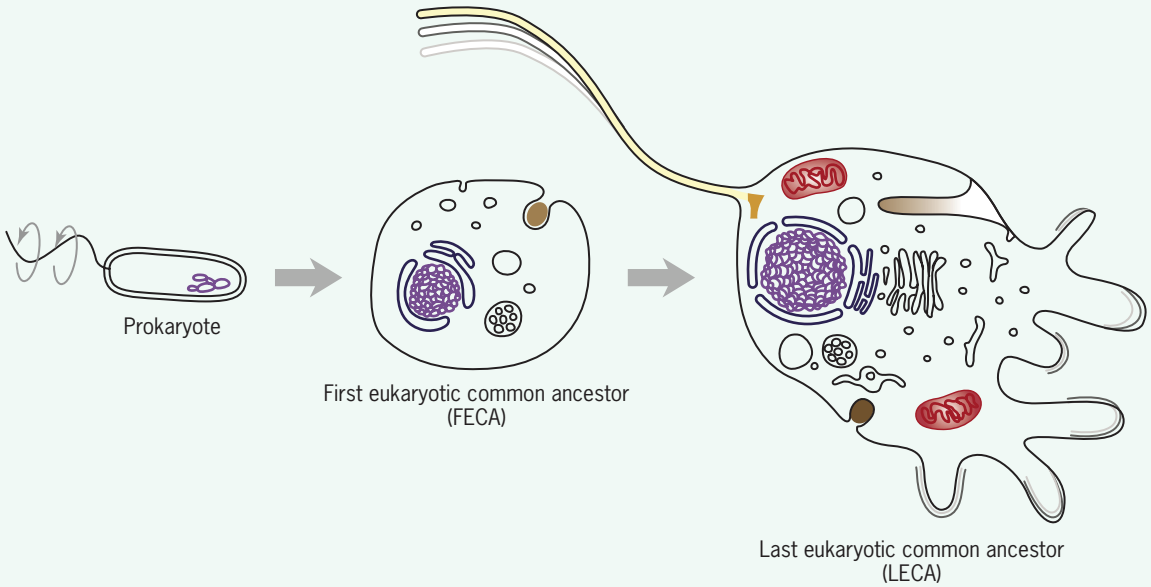


FIGURE 1 **A model depicting stages in the evolution of eukaryotes.** Starting from a prokaryotic ancestor, internal compartments began to develop, leading to an organism with internal membrane compartments such as a nucleus. Such an organism is known as the First Eukaryotic Common Ancestor (FECA). The molecular machinery for making internal membranes then allowed the FECA to engulf and maintain endosymbiotic organisms, allowing acquisition of mitochondria. Additional evolutionary innovations gave rise to cellular features common to all eukaryotic lineages, including cilia, intron splicing, and meiosis. The organism that had all these traits, and therefore gave rise to all existing eukaryotic lineages, is known as the Last Eukaryotic Common Ancestor (LECA). After the LECA arose, further evolutionary steps, such as endosymbiosis of photosynthetic bacteria to produce chloroplasts, gave rise to different classes of eukaryotic cells.

SOURCE: From F.D. Mast et al., *Trends Cell Biol.* 24:435–442, 2014.

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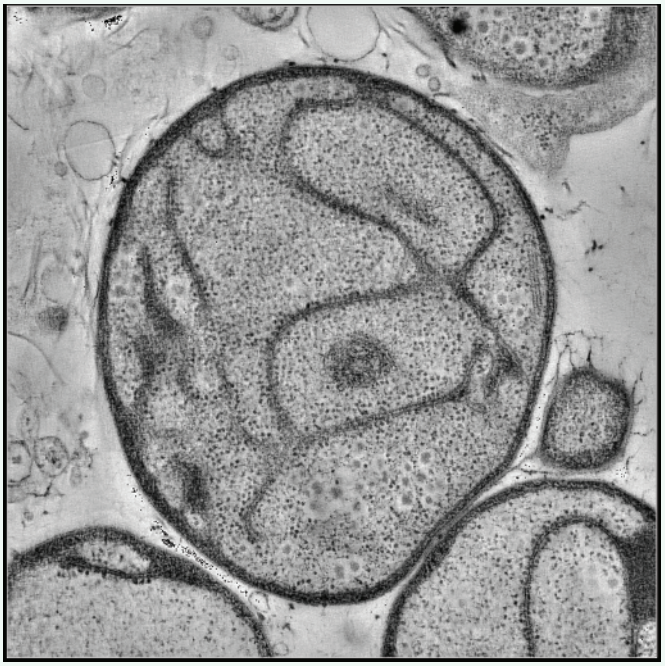

FIGURE 2 Prokaryotes with complex internal membrane systems. Electron micrograph of *Gemmata obscuriglobus*, a bacterium with a complex set of internal membranes. Although these membranes do not form closed organelles as they would in eukaryotes, they show that a potential for membrane organization exists even in prokaryotes.

SOURCE: From R. Santarella-Mellwig et al., *Plos Biol*. 11:E1001565, 2013.

but the further development of these membranes into closed internal compartments, particularly a compartment surrounding the DNA to produce a nucleus.

According to the endosymbiont theory, the next step in the evolution of modern eukaryotes was when a descendent of the FECA cell ingested a small, aerobic prokaryote which somehow resisted digestion within the cytoplasm, taking up residence as a permanent endosymbiont. As the host cell reproduced, so did the endosymbiont, so that a colony of these composite cells was soon produced. Over many generations, endosymbionts lost many of the traits that were no longer required for survival, and the once-independent oxygen-respiring microbes evolved into precursors of modern-day mitochondria. A cell whose ancestors had formed through the sequence of symbiotic events just described could have given rise to a line of cells that evolved other basic characteristics of eukaryotic cells, including additional internal organelles (endoplasmic reticulum, Golgi complex, lysosomes), a complex cytoskeleton including cilia, intron splicing, and both mitotic and meiotic cell division. These characteristics, which are shared among all existing eukaryotic lineages, are proposed to have arisen by a gradual process of evolution, rather than in a single step as might occur through acquisition of an endosymbiont. All eukaryotes alive today descended from the cell that acquired these traits, and it is therefore known as the *last eukaryotic common ancester* (LECA). Current research on evolutionary cell biology is focused on reconstructing the molecular, structural, and functional features of the FECA and LECA by comparing features of existing eukaryotic and prokaryotic lineages. The oldest fossils thought to be the remains of eukaryotes date back about 1.8 billion years.

Margulis proposed that the acquisition of another endosymbiont, specifically a cyanobacterium, converted an early heterotrophic eukaryote into an ancestor of photosynthetic eukaryotes: the green algae and plants.[3] The acquisition of chloroplasts (roughly one billion years ago) must have been one of the last steps in the sequence of endosymbioses because these organelles are only present in plants and algae. In contrast, all known groups of eukaryotes either (1) possess mitochondria or (2) show definitive evidence they have evolved from organisms that possessed these organelles.[a] The concept that mitochondria and chloroplasts arose via evolution from symbiotic organisms is now supported by an overwhelming body of evidence, some of which will be described in numerous chapters of this text.

The division of all living organisms into two categories, prokaryotes and eukaryotes, reflects a basic dichotomy in the structures of cells, but it is not necessarily an accurate phylogenetic distinction, that is, one that reflects the evolutionary relationships among living organisms. How does one determine evolutionary relationships among organisms that have been separated in time for billions of years, such as prokaryotes and eukaryotes? Modern taxonomic schemes that attempt to classify organisms are based on comparisons of the DNA sequences of living organisms.[4] Differences between organisms in the sequence of nucleotides that make up a nucleic acid are the result of mutations in DNA that have been transmitted to offspring. Mutations can accumulate in a given gene at a relatively constant rate over long periods of time. Consequently, comparisons of nucleotide sequences can be used to determine how closely organisms are related to one another. For example, two organisms that are closely related, that is, have diverged only recently from a common ancestor, should have fewer sequence differences in a particular gene than two organisms that are distantly related, that is, do not have a recent common ancestor. Using this type of sequence information as an "evolutionary clock," researchers can construct phylogenetic trees showing proposed pathways by which different groups of living organisms may have diverged from one another during the course of evolution.

Beginning in the mid-1970s, Carl Woese and his colleagues at the University of Illinois began a series of studies that compared the nucleotide sequence in different organisms of the RNA molecule that resides in the small subunit of the ribosome. This RNA—which is called the 16S rRNA in prokaryotes or the 18S rRNA in eukaryotes—was chosen because it is present in large quantities in all cells, it is easy to purify, and it tends to change only slowly over long periods of evolutionary time, which means that it could be used to study relationships of very distantly related organisms. In one of their first studies, Woese and his colleagues analyzed the rRNA present in the ribosomes of chloroplasts from the photosynthetic protist *Euglena*.[5] They found that the sequence of this chloroplast rRNA molecule was much more similar to that of the 16S rRNA found in ribosomes of cyanobacteria than it was to its 18S counterpart in the ribosomes from eukaryotic

[a]There are a number of anaerobic unicellular eukaryotes (e.g., the intestinal parasite *Giardia*) that lack mitochondria. For years, these organisms formed the basis for a proposal that mitochondrial endosymbiosis was a late event that took place after the evolution of these mitochondria-lacking groups. However, recent analysis of the nuclear DNA of these organisms indicates the presence of genes that were likely transferred to the nucleus from mitochondria, suggesting that the ancestors of these organisms lost their mitochondria during the course of evolution.

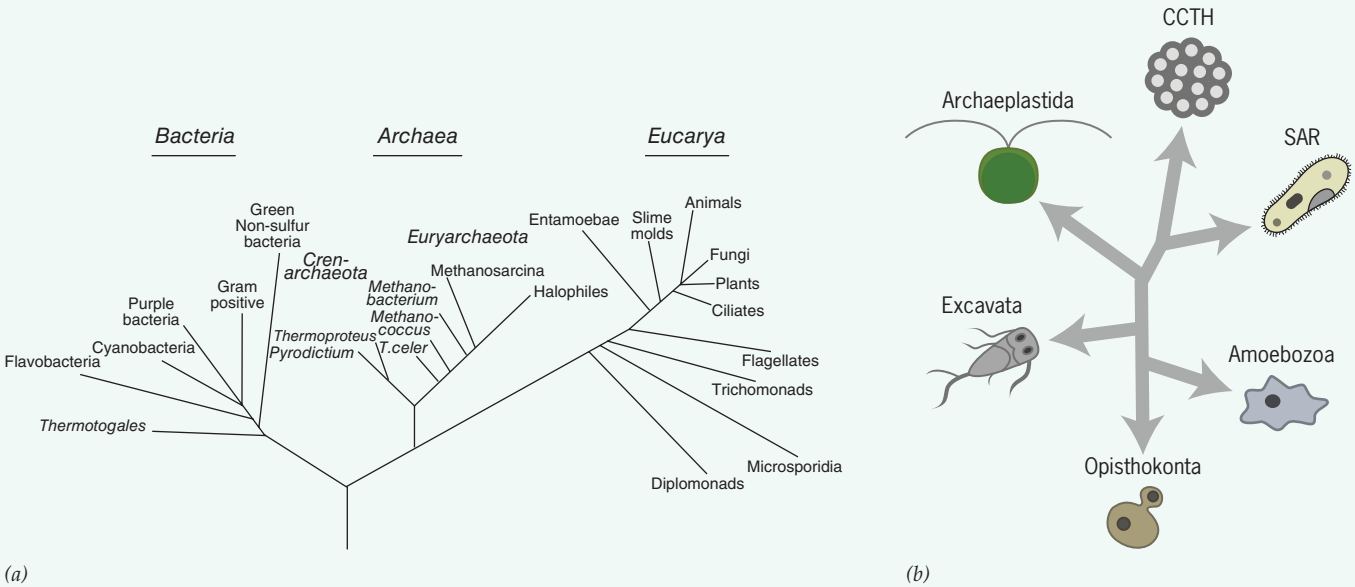

FIGURE 3 **Domains of life.** (*a*) A phylogenetic tree based on rRNA sequence comparisons showing the three domains of life. The Archaea are divided into two subgroups as indicated. (*b*) Phylogenetic relation between existing eukaryotic lineages. Although initial analysis of rRNA such as in Panel A suggested a series of early and late branching events to produce different lineages of eukaryoties, more careful analysis of genes and genomes now suggests that six major lineages all diverged from the LECA to produce distinct classes of eukaryotes known as "supergroups." Animals and fungi, while looking different, are highly similar at the molecular level and together form a single group, the Opisthokonta. Plants and green algae, again while looking different, are closely related by molecular phylgeny and form a group called Archaeplastida. A wide range of other eukaryotes including many different species formerly lumped together as "protists" are now clearly divided into four distinct groups with unfamiliar names Excavata (which includes the parasites Giardia and Naegleria), Amoebozoa, SAR (which includes ciliates like Paramecium as well as diatoms and brown algae such as giant kelp), and CCTH (composed entirely of obscure and unfamiliar single-celled organisms whose biology is poorly understood).

SOURCE: (a) From C. R. Woese et al., *Proc. Nat'l. Acad. Sci. U.S.A.* 87:4578, 1990; (b) From F.D. Mast et al., *Tends Cell Biol.* 24:435–442, 2014.

cytoplasm. This finding provided strong evidence for the symbiotic origin of chloroplasts from cyanobacteria.

In 1977, Woese and George Fox published a landmark paper in the study of molecular evolution.[6] They compared the nucleotide sequences of small-subunit rRNAs that had been purified from 13 different prokaryotic and eukaryotic species. They found that the sequences clustered into three distinct groups, such that the rRNAs within each group are much more similar to one another than they are to rRNAs of the other two groups. The first of the groups contained only eukaryotes; the second group contained the "typical" bacteria (gram-positive, gram-negative, and cyanobacteria); and the third group contained several species of methanogenic (methane-producing) "bacteria." Woese and Fox concluded, to their surprise, that the methanogenic organisms "appear to be no more related to typical bacteria than they are to eukaryotic cytoplasms." These results suggested that the members of these three groups represent three distinct evolutionary lines that branched apart from one another at a very early stage in the evolution of cellular organisms. Consequently, they assigned these organisms to three different kingdoms, which they named the Urkaryotes, Eubacteria, and Archaebacteria, a terminology that divided the prokaryotes into two fundamentally distinct groups.

Subsequent research provided support for the concept that prokaryotes could be divided into two distantly related lineages, and it expanded the ranks of the archaebacteria to include at least two other groups, the thermophiles, which live in hot springs and ocean vents, and the halophiles, which live in very salty lakes and seas. In 1989, two published reports rooted the tree of life and suggested that the archaebacteria were actually more closely related to eukaryotes than they were to eubacteria.[7,8] Both groups of researchers compared the amino acid sequences of several proteins that were present in a wide variety of different prokaryotes, eukaryotes, mitochondria, and chloroplasts. A phylogenetic tree constructed from sequences of ribosomal RNAs, which comes to the same conclusion, is shown in **FIGURE 3a.**[9] In this latter paper, Woese and colleagues proposed a revised taxonomic scheme, which has been widely accepted. In this scheme, the archaebacteria, eubacteria, and eukaryotes are assigned to separate domains, which are named Archaea, Bacteria, and Eucarya, respectively.[b] Similar DNA sequence analysis studies have shown that eukaryotes then split into six distinct lineages (Figure 3*b*), of which animals including humans fall into a group known as "opisthokonts." According to the model in Figure 3*a*, the first major split in the tree of life produced two separate lineages, one leading to the Bacteria and the other leading to both the Archaea and the Eucarya. If this view is correct, it was an archaebacterium, not a eubacterium, that took in a symbiont and gave rise to the lineage that led to the first eukaryotic cells. Although the host prokaryote was presumably an archaebacterium, the symbionts that evolved into

[b]Many biologists dislike the terms *archaebacteria* and *eubacteria*. Although these terms have gradually faded from the literature, being replaced simply by *archaea* and *bacteria*, many researchers in this field continue to use the former terms in published articles. Given that this is an introductory chapter in an introductory text, we have continued to refer to these organisms as archaebacteria and eubacteria to avoid possible confusion over the meaning of the term *bacterial*.

continued

mitochondria and chloroplasts were almost certainly eubacteria, as indicated by their close relationship with modern members of this group.

Until 1995, phylogenetic trees of the type shown in Figure 3*a* were based primarily on the analysis of the gene encoding the 16S–18S rRNA. By then, phylogenetic comparisons of a number of other genes were suggesting that the scheme depicted in Figure 3*a* might be oversimplified. Questions about the origin of prokaryotic and eukaryotic cells came into sharp focus between 1995 and 1997 with the publication of the entire sequences of a number of prokaryotic genomes, both archaebacterial and eubacterial, and the genome of a eukaryote, the yeast *Saccharomyces cerevisiae*. Researchers could now compare the sequences of hundreds of genes simultaneously, and this analysis raised a number of puzzling questions and blurred the lines of distinction between the three domains.[10] For example, the genomes of several archaebacteria showed the presence of a significant number of eubacterial genes. For the most part, those genes in archaebacteria whose products are involved with informational processes (chromosome structure, transcription, translation, and replication) were very different from their counterparts in eubacterial cells and, in fact, resembled the corresponding genes in eukaryotic cells. This observation fit nicely with the scheme in Figure 3*a*. In contrast, many of the genes in archaebacteria that encode the enzymes of metabolism exhibited an unmistakable eubacterial character.[11,12] The genomes of eubacterial species also showed evidence of a mixed origin, often containing a significant number of genes that bore an archaebacterial character.[13]

Most investigators who study the origin of ancient organisms have held on to the basic outline of the phylogenetic tree as demarcated in Figure 3*a* and argue that the presence of eubacteria-like genes in archaebacteria, and vice versa, is the result of the transfer of genes from one species to another, a phenomenon referred to as *horizontal gene transfer* (*HGT*), sometimes also called *lateral gene transfer.*[14] According to the original premise that led to the phylogenetic tree of Figure 3*a*, genes are inherited from one's parents, not from one's neighbors. This is the premise that allows an investigator to conclude that two species are closely related when they both possess a gene (e.g., the rRNA gene) of similar nucleotide sequence. If, however, cells can pick up genes from other species in their environment, then two species that are actually unrelated may possess genes of very similar sequence. An early measure of the importance of horizontal gene transfer in the evolution of prokaryotes came from a study that compared the genomes of two related eubacteria, *Escherichia* and *Salmonella*. It was found that 755 genes or nearly 20 percent of the *E. coli* genome is derived from "foreign" genes transferred into the *E. coli* genome over the past 100 million years, which is the time when the two eubacteria diverged. These 755 genes were acquired as the result of at least 234 separate lateral transfers from many different sources.[15] (The effect of horizontal gene transfer on antibiotic resistance in pathogenic bacteria is discussed in the Human Perspective of Chapter 3.)

If genomes are a mosaic composed of genes from diverse sources, how does one choose which genes to use in determining phylogenetic relationships? According to one viewpoint, genes that are involved in informational activities (transcription, translation, replication) make the best subjects for determining phylogenetic relationships, because such genes are less likely to be transferred laterally than genes involved in metabolic reactions.[16] These authors argue that the products of informational genes (e.g., rRNAs) are parts of large complexes whose components must interact with many other molecules. It is unlikely that a foreign gene product could become integrated into the existing machinery. When "informational genes" are used as the subjects of comparison, archaebacteria and eubacteria tend to separate into distinctly different groups, whereas archaebacteria and eukaryotes tend to group together as evolutionary relatives, just as they do in Figure 3.[See reference 17 for further discussion.]

Analysis of eukaryotic genomes has produced similar evidence of a mixed heritage. Studies of the yeast genome show unmistakable presence of genes derived from both archaebacteria and eubacteria. The "informational genes" tend to have an archaeal character and the "metabolic genes" a eubacterial character.[18] There are several possible explanations for the mixed character of the eukaryotic genome. Eukaryotic cells may have evolved from archaebacterial ancestors and then picked up genes from eubacteria with which they shared environments. In addition, some of the genes in the nucleus of a eukaryotic cell are clearly derived from eubacterial genes that have been transferred from the genome of the symbionts that evolved into mitochondria and chloroplasts.[19] A number of researchers have taken a more radical position and proposed that the eukaryote genome was originally derived from the fusion of an archaebacterial and a eubacterial cell followed by the integration of their two genomes.[e.g.,20] Given these various routes of gene acquisition, it is evident that no simple phylogenetic tree, such as that depicted in Figure 3*a*, can represent the evolutionary history of the entire genome of an organism.[Reviewed in 21–23] Instead, each gene or group of genes of a particular genome may have its own unique evolutionary tree, which can be a disconcerting thought to scientists seeking to determine the origin of our earliest eukaryotic ancestors.

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Synopsis

The cell theory has three tenets. (1) All organisms are composed of one or more cells; (2) the cell is the basic organizational unit of life; and (3) all cells arise from preexisting cells. (p. 2)

The properties of life, as exhibited by cells, can be described by a collection of properties. Cells are very complex and their substructure is highly organized and predictable. The information to build a cell is encoded in its genes. Cells reproduce by cell division; their activities are fueled by chemical energy; they carry out enzymatically controlled chemical reactions; they engage in numerous mechanical activities; they respond to stimuli; and they are capable of a remarkable level of self-regulation. (p. 3)

Cells are either prokaryotic or eukaryotic. Prokaryotic cells are found only among archaebacteria and eubacteria, whereas all other types of organisms—protists, fungi, plants, and animals—are composed of eukaryotic cells. Prokaryotic and eukaryotic cells share many common features, including a similar cellular membrane, a common system for storing and using genetic information, and similar metabolic pathways. Prokaryotic cells are the simpler type, lacking the complex membranous organelles (e.g., endoplasmic reticulum, Golgi complex, mitochondria, and chloroplasts), chromosomes, and cytoskeleton characteristic of the cells of eukaryotes. The two cell types can also be distinguished by their mechanism of cell division, their locomotor structures, and the type of cell wall they produce (if a cell wall is present). Complex plants and animals contain many different types of cells, each specialized for particular activities. (p. 8)

Cells are almost always microscopic in size. Bacterial cells are typically 1 to 5 μm in length, whereas eukaryotic cells are typically 10 to 30 μm. Cells are microscopic in size for a number of reasons: their nuclei possess a limited number of copies of each gene; the surface area (which serves as the cell's exchange surface) becomes limiting as a cell increases in size; and the distance between the cell surface and interior becomes too great for the cell's needs to be met by simple diffusion. (p. 18)

Viruses are noncellular pathogens that can only reproduce when present within a living cell. Outside of the cell, the virus exists as a macromolecular package, or virion. Virions occur in a variety of shapes and sizes, but all of them consist of viral nucleic acid enclosed in a wrapper containing viral proteins. Viral infections may lead to either (1) the destruction of the host cell with accompanying production of viral progeny, or (2) the integration of viral nucleic acid into the DNA of the host cell, which often alters the activities of that cell. Viruses are not considered to be living organisms. (p. 19)

Conceptual Questions

1.1 Discovery of Cells

1. When Robert Hooke first described cells, what was he actually looking at?
2. What are the three components of cell theory?

1.2 Basic Properties of Cells

3. List the fundamental properties shared by all cells. Describe the importance of each of these properties.
4. Describe the features of cells that suggest that all living organisms are derived from a common ancestor.
5. What is the source of energy that supports life on Earth? How is this energy passed from one organism to the next?

1.3 Two Fundamentally Different Classes of Cells

6. Compare a prokaryotic and eukaryotic cell on the basis of structural, functional, and metabolic differences.

1.4 Types of Prokaryotic Cells

7. Which group of prokaryotes is best known for containing many extremophiles?

1.5 Types of Eukaryotic Cells

8. What is the importance of cell differentiation?

1.6 The Sizes of Cells and Their Components

9. Why are cells almost always microscopic?
10. If a mitochondrion were 2 μm in length, how many angstroms would it be? How many nanometers? How many millimeters?

1.7 Viruses

11. What properties distinguish a virus from a bacterium?
12. What types of infections are viruses able to cause?
13. Compare and contrast: nucleoid and nucleus; the flagellum of a bacterium and a sperm; an archaebacterium and a cyanobacterium; nitrogen fixation and photosynthesis; bacteriophages and tobacco mosaic virus; a provirus and a virion.

Analytic Questions

1. Consider some question about cell structure or function that you would be interested in answering. Would the data required to answer the question be easier to collect by working on an entire plant or animal or on a population of cultured cells? What might be the advantages and disadvantages of working on a whole organism versus a cell culture?
2. Figure 1.3 shows an intestinal epithelial cell with large numbers of microvilli. What is the advantage to the organism of having these microvilli? What do you expect would happen to an individual that lacked such microvilli as the result of an inherited mutation?
3. The first human cells to be successfully cultured were derived from a malignant tumor. Do you think this simply reflects the availability of cancer cells, or might such cells be better subjects for cell culture? Why?
4. The drawings of plant and animal cells in Figure 1.8*b,c* include certain structures that are present in plant cells but absent in animal cells. How do you think each of these structures affects the life of the plant?
5. It was noted that cells possess receptors on their surface that allow them to respond to specific stimuli. Many cells in the human body possess receptors that allow them to bind specific hormones that circulate in the blood. Why do you think these hormone receptors are important? What would be the effect on the physiological activities of the body if cells lacked these receptors, or if all cells had the same receptors?
6. If you were to argue that viruses are living organisms, what features of viral structure and function might you use in your argument?
7. If we presume that activities within cells do occur in a manner analogous to that shown in the Rube Goldberg cartoon of Figure 1.7, how would this differ from a human activity, such as building a car on an assembly line or shooting a free throw in a basketball game?
8. Unlike bacterial cells, the nucleus of a eukaryotic cell is bounded by a double-layered membrane studded by complex pores. How do you think this might affect traffic between the DNA and cytoplasm of a eukaryotic cell compared to that of a prokaryotic cell?
9. Examine the photograph of the ciliated protist in Figure 1.16 and consider some of the activities in which this cell engages that a muscle or nerve cell in your body does not.
10. Which type of cell would you expect to achieve the largest volume: a highly flattened cell or a spherical cell? Why?
11. Suppose you were a scientist living in the 1890s and were studying a disease of tobacco crops that stunted the growth of the plants and mottled their leaves. You find that the sap from a diseased plant, when added to a healthy plant, is capable of transmitting the disease to that plant. You examine the sap in the best light microscopes of the period and see no evidence of bacteria. You force the sap through filters whose pores are so small that they retard the passage of the smallest known bacteria, yet the fluid that passes through the filters is still able to transmit the disease. Like Dimitri Ivanovsky, who conducted these experiments more than a hundred years ago, you would probably conclude that the infectious agent was an unknown type of unusually small bacterium. What kinds of experiments might you perform today to test this hypothesis?
12. Most evolutionary biologists believe that all mitochondria have evolved from a single ancestral mitochondrion and all chloroplasts have evolved from a single ancestral chloroplast. In other words, the symbiotic event that gave rise to each of these organelles occurred only once. If this is the case, where on the phylogenetic tree of Figure 3*a* of the Experimental Pathways section, page 29, would you place the acquisition of each of these organelles?
13. Publication of the complete sequence of the 1918 flu virus and reconstitution of active viral particles was met with great controversy. Those who favored publication of the work argued that this type of information can help to better understand the virulence of influenza viruses and help develop better therapeutics against them. Those opposed to its publication argued that the virus could be reconstituted by bioterrorists or that another pandemic could be created by the accidental release of the virus by a careless investigator. What is your opinion on the merits of conducting this type of work?