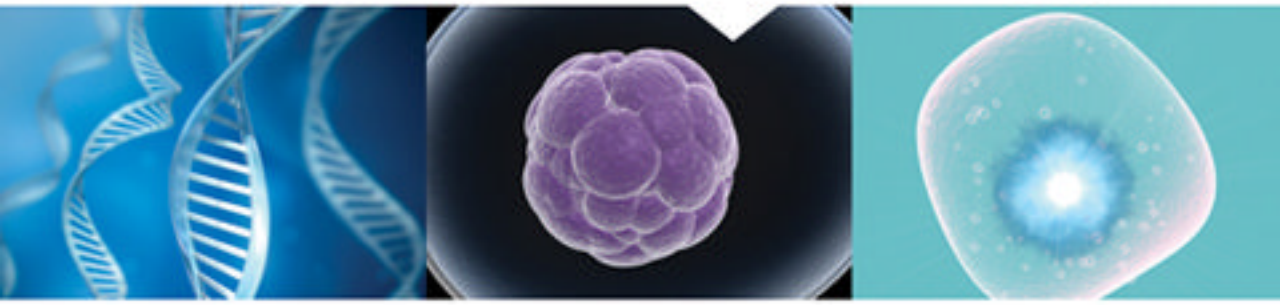
THE new biology



THE CELL

Nature's First Life-form

REVISED EDITION

JOSEPH PANNO, PH.D.

THE new biology

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Revised Edition

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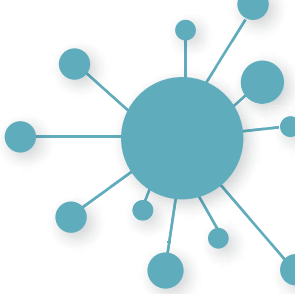


Preface

When the first edition of this set was being written, the new biology was just beginning to come into its potential and to experience some of its first failures. Dolly the sheep was alive and well and had just celebrated her fifth birthday. Stem cell researchers, working 12-hour days, were giddy with the prospect of curing every disease known to humankind, but were frustrated by inconsistent results and the limited availability of human embryonic stem cells. Gene therapists, still reeling from the disastrous Gelsinger trial of 1998, were busy trying to figure out what had gone wrong and how to improve the safety of a procedure that many believed would revolutionize medical science. And cancer researchers, while experiencing many successes, hit their own speed bump when a major survey showed only modest improvements in the prognosis for all of the deadliest cancers.

During the 1970s, when the new biology was born, recombinant technology served to reenergize the sagging discipline that biology had become. This same level of excitement reappeared in the 1990s with the emergence of gene therapy, the cloning of Dolly the sheep, and the successful cultivation of stem cells. Recently, great excitement has come with the completion of the human genome project and the genome sequencing of more than 100 animal and plant species. Careful analysis of these genomes has spawned a new branch of biological research known as comparative genomics. The information that scientists can now extract from animal genomes is expected to improve all other branches of biological science. Not to be outdone, stem cell researchers have found a way to produce embryo-like stem cells from ordinary skin cells. This achievement not only marks the end of the great stem cell debate, but it also provides an immensely powerful procedure, known as cellular dedifferentiation, for studying and manipulating the very essence of a cell. This procedure will become a crucial weapon in the fight against cancer and many other diseases.

The new biology, like our expanding universe, has been growing and spreading at an astonishing rate. The amount of information that is now available on these topics is of astronomical proportions. Thus, the problem of deciding what to leave out has become as difficult as the decision of what to include. The guiding principle in writing this set has always been to provide a thorough overview of the topics without overwhelming the reader with a mountain of facts and figures. To be sure, this set contains many facts and figures, but these have been carefully chosen to illustrate only the essential principles.

This edition, in keeping with the expansion of the biological disciplines, has grown to accommodate new material and new areas of research. Four new books have been added that focus on areas of biological research that are reaping the benefits of genome science

and modern research technologies. Thus, the New Biology set now consists of the following 10 volumes:

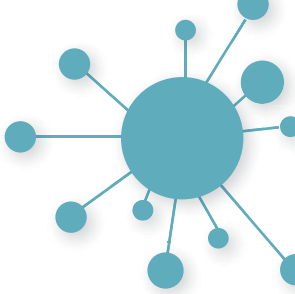
1. *Aging*, Revised Edition
2. *Animal Cloning*, Revised Edition
3. *Cancer*, Revised Edition
4. *The Cell*, Revised Edition
5. *Gene Therapy*, Revised Edition
6. *Stem Cell Research*, Revised Edition
7. *Genome Research*
8. *The Immune System*
9. *Modern Medicine*
10. *Viruses*

Many new chapters have been added to each of the original six volumes, and the remaining chapters have been extensively revised and updated. The number of figures and photos in each book has increased significantly, and all are now rendered in full color. The new volumes, following the same format as the originals, greatly expand the scope of the New Biology set and serve to emphasize the fact that these technologies are not just about finding cures for diseases but are helping scientists understand a wide range of biological processes. Even a partial list of these revelations is impressive: detailed information on every gene and every protein that is needed to build a human being; eventual identification of all cancer genes, stem cell–specific genes, and longevity genes; mapping of safe chromosomal insertion sites for gene therapy; and the identification of genes that control the growth of the human brain, the development of speech, and the maintenance of mental stability. In a stunning achievement, genome researchers have been able to trace the exact route our human ancestors used to emigrate from Africa nearly

65,000 years ago and even to estimate the number of individuals who made up the original group.

In addition to the accelerating pace of discovery, the new biology has made great strides in resolving past mistakes and failures. The Gelsinger trial was a dismal failure that killed a young man in the prime of his life, but gene therapy trials in the next 10 years will be astonishing, both for their success and for their safety. For the past 50 years, cancer researchers have been caught in a desperate struggle as they tried to control the growth and spread of deadly tumors, but many scientists are now confident that cancer will be eliminated by 2020. Viruses, such as HIV or the flu, are resourceful and often deadly adversaries, but genome researchers are about to put the fight on more rational grounds as detailed information is obtained about viral genes, viral life cycles, and viruses' uncanny ability to evade or cripple the human immune system.

These struggles and more are covered in this edition of the New Biology set. I hope the discourse will serve to illustrate both the power of science and the near superhuman effort that has gone into the creation and validation of these technologies.



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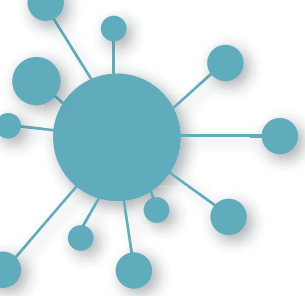


Introduction

Life began in the oceans of ancient Earth more than 3 billion years ago. At that time, the planet was a wild and stormy place with an atmosphere that was not fit to breathe. Although storms were violent, life could not have appeared without them. Lightning provided the energy to make certain molecules that all living things need, and wind churned up the surface of the soupy seas like a diligent cook stirring a pot. Storms had to stir that pot for a billion years before it finally happened: a tiny bubble, too small to see with the naked eye, gave birth to the first cell, and in the wink of a cosmic eye the Earth was teeming with life.

The first cells were little more than microscopic bags of chemicals that were capable of reproduction. They lived solitary lives, but eventually, after learning how to communicate with each other, they began to form small colonies consisting of three or four cells each. As time passed, cell-to-cell communication and cooperation

became so elaborate that the first simple colonies were transformed into complex multicellular plants and animals. The first cell colonies were produced almost 3 billion years ago by prokaryotes, a simple kind of cell more commonly known as bacteria. Super-cells called eukaryotes, appearing about 2 billion years ago, created much more elaborate colonies that eventually gave rise to true multicellular organisms. Eukaryotes are the direct descendants of the prokaryotes, but they are larger, more complex, and more adept at cell-to-cell communication. Plants and animals are all made from eukaryotes. The human body, for example, is made from more than 100 billion eukaryotes, a population consisting of more than 200 different cell types that are organized into organs and tissues. Our brains alone are constructed from 10 billion eukaryotes called neurons that are linked together in a network of enormous complexity. Some neurons in our brain are capable of communicating simultaneously with 100,000 other neurons. It is this level of complexity that produces our intellect and gives us the powers of speech and vision. In some sense, the human brain is the ultimate colony, the most intricate cellular community ever to appear on Earth.

Our understanding of the cell has increased tremendously since the 1970s when recombinant DNA technology was first introduced. This technology made it possible to study the structure and function of a cell in minute detail. Prior to the 1970s, biologists had only a basic understanding of the cell; they knew the DNA was located in the nucleus, that the cell was surrounded by what appeared to be a featureless membrane, and that the cell interior was full of structures called organelles, their functions largely unknown. Today, scientists have sequenced the entire human genome, as well as the genomes of many other organisms. They have determined the function of virtually every cellular organelle and have shown that the cell membrane, far from being featureless, contains a molecular forest that gives the cell its eyes, its ears, and the equipment it needs to capture food and to communicate with other cells.

By studying the cell, we improve our understanding of the living world and, in particular, our understanding of plant and animal physiology, genetics, and biochemistry. This wealth of information has revolutionized the biological and medical sciences. For human society, this knowledge translates into a dramatic reduction in mortalities due to infectious diseases and medical disorders. The war on cancer, launched almost 40 years ago, is finally approaching a stage where all cancers will be curable. Improved treatment and prognosis are now possible for many other disorders, such as cardiovascular disease, diabetes, and cystic fibrosis. Improved knowledge of the cell made it possible for researchers to isolate and culture stem cells, a very talented cell that may be used to treat spinal cord trauma and degenerative neurological diseases such as Alzheimer's disease and Parkinson's disease. In 2007, Japanese scientists discovered a way to make stem cells from ordinary skin cells by a process known as cellular reprogramming. This accomplishment may turn out to be the most important scientific advance of the 21st century.

This revised edition of *The Cell,* one volume in the New Biology set, contains updated and revised material throughout, accompanied by many photographs and line drawings, all of which are now in color. New material in the first two chapters covers the recent controversy over the teaching of creationism in public schools, life on other planets, bacterial populations, and the potential threat of certain pathogenic species. Chapter 3 has been expanded to provide a more detailed discussion of eukaryote structure and function, including protein synthesis, the glycocalyx, intercellular traffic, and cell-to-cell communication (or cell signaling). A cell's ability to communicate with other cells was essential for the development of multicellular creatures. Moreover, the corruption of that ability is central to many pathological conditions such as cancer and Alzheimer's disease. Chapter 6 has been extensively revised to better focus on the structural elements that were necessary for the transition to multicellular organisms. Two new chapters have been

added: chapter 7, which provides expanded coverage of the many kinds of cells that make up the human body, and chapter 9, which covers plant biology and the crucial role that photosynthesis played during the formation of Earth's biosphere. The final chapter, as before, provides extensive background material on recombinant DNA technology, DNA sequencing, and other topics that are relevant to cell biology. This resource material has been updated, particularly with regards to recent advances in DNA sequencing technology.

This is an exciting time in the field of biology. The completion of the genome project and the groundbreaking research of the 20th century are now beginning to bear fruit. At the dawn of a new century we face a future in which our understanding of the cell, and the world in which it lives, transforms every aspect of our lives, from vanquishing disease and illness to restoring our damaged biosphere. It is hoped that this new edition will help the reader stay abreast of this ever changing and very stimulating field.

The Origin of Life

Life began so long ago that many people believe it is impossible to reconstruct the events that led to the appearance of the first cell. The skepticism is understandable since there are no fossils from that period to study and our knowledge of the Earth's formative years is still rudimentary. Nevertheless, some progress has been made by studying the most primitive cells on Earth today and by conducting laboratory experiments that attempt to reconstruct, in a test tube, the conditions of ancient Earth.

The results suggest that life arose on Earth through purely natural means, driven by the force of natural selection as proposed by the theory of evolution. A renewed debate involving the theory of evolution and the origin of life on Earth began in 2005 when board members at several public high schools in the United States discussed the possibility of teaching creationism as an alternative to scientific theories. Creationism holds that God created life and that

the original life-forms have remained unchanged down through time. A modernized version of this belief, known as intelligent design, accepts the notion of evolutionary change but proposes that evolution is guided by a supernatural being. Public reaction to this debate has shown that the majority of Americans favor teaching creationism along with the theory of evolution. Later that year, however, a court in Pennsylvania ruled that a high school in Dover could not teach intelligent design or creationism because the constitution of the United States guarantees a separation between church and state. Subsequent attempts to introduce creationism in the public schools have also failed.

Proponents of intelligent design claim that proof for their theory is inherent in the complexity of life itself, which they maintain could not have occurred through natural means. Scientists agree that living systems are exceedingly complex but that it is unreasonable to judge their origins based on complexity alone. The theory of evolution suggests that organisms change over time to meet the demands of their environment. Thus, it is likely the complexity that is evident now was built up one step at a time from much simpler systems that existed millions of years ago.

The controversy surrounding creationism and intelligent design depends to a great extent on whether the Bible is taken as the literal word of God or whether it was simply inspired by a belief in God. Some Christian groups in the United States, who believe the Bible is the literal word of God, have gone to great lengths to discredit the theory of evolution. One such effort is the Creation Museum in northern Kentucky, which opened in 2007. In its displays and lectures, the museum attempts to show that the Earth is only 6,000 years old and that all the fossils and geological strata that scientific data have shown to be millions of years old were laid down in 2348 b.c.e., the year of the great flood and the launching of Noah's ark. In order to explain how the few species that were taken aboard the ark could have diversified into the great range of creatures that live on

Earth today, the museum states that God provided organisms with the power to change rapidly. Thus, the museum admits that evolution has occurred, but the mechanism they propose is nothing more than wishful thinking. Such extreme views polarize the debate and strengthen the resolve of scientists and many other people who believe that such ideas should not be taught in public schools.

In this chapter, the discussion will focus on the scientific evidence for the origin of life on Earth. Creationism and intelligent design will not be covered simply because these ideas cannot be studied or tested scientifically.

THE BIG BANG

Fifteen billion years ago, everything in the universe was a fluid substance compressed into an area about the size of a small marble. There was no matter of the kind that exists now: no iron, no copper, no carbon, and no oxygen. Just subatomic particles (bosons, leptons, and gluons) brought together by a crushing force of gravity. No one knows how long the universe remained in this state or even if time, as now known, existed. Scientists do know that it was extremely hot, with temperatures exceeding 10 billion degrees, 1,000 times hotter than the center of the Sun. Eventually something happened (no one knows exactly what), and that marble of unimaginable heat and density suddenly exploded. Within seconds, the temperature dropped enough for atomic nuclei to form, and after 400,000 years the temperature was low enough for the first elements to appear. The first of these was hydrogen, the simplest of all elements and the one that gave rise to all the rest. Although the universe was cooling down, it was still hot enough to fuse hydrogen atoms to produce helium. Enough hydrogen and helium were formed in this way to produce all of the stars and galaxies. Heat within the stars was sufficient to fuse hydrogen and helium atoms to form all the other elements that are now find in nature, such as carbon, iron, copper, and nitrogen.

THE IMPORTANCE OF VIOLENT STORMS

Ten billion years after the big bang, Earth was created as a molten ball of metal and stone thrown off by the Sun during the formation of the solar system. Additional material was added to our planet as it collided with asteroids and meteors. The high surface temperature liberated an enormous amount of water vapor from the nearly molten rocks. The vapor rose into the atmosphere, forming a heavy cloud layer that completely enshrouded the planet, effectively blocking the Sun's rays. During the subsequent half billion years, the Earth cooled down and the rains began to fall. This was no brief summer shower, but a pelting rain that lasted thousands of years and led to the formation of the oceans, which covered most of the Earth's surface just as they do today. The land was barren and wracked with volcanic eruptions that spewed noxious gases such as methane (CH_4) and ammonia (NH_3) into the atmosphere. The air contained very little, if any, free oxygen.

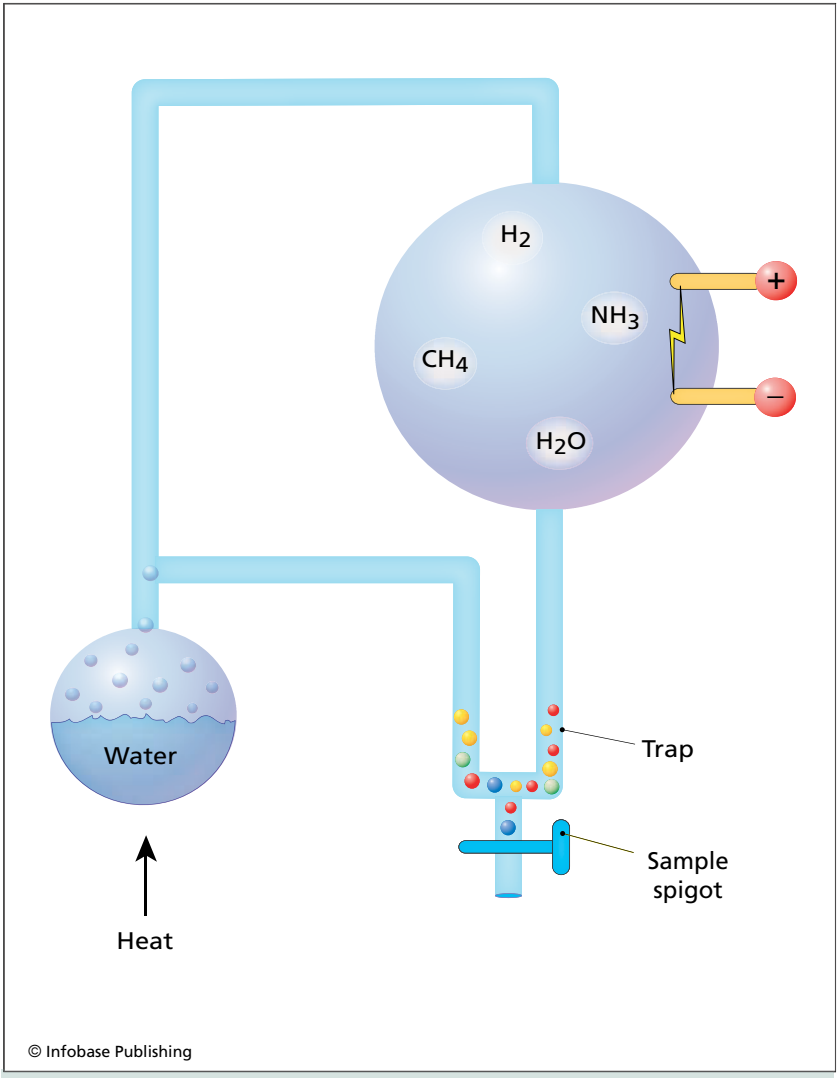
An artist's conception of prebiotic Earth showing volcanoes and stormy environment *(Steve Munsinger/Photo Researchers, Inc.)*

A planet with an atmosphere of methane and ammonia does not, at first glance, appear a likely candidate for the origin of life. Modern cells need oxygen to breathe and require four kinds of organic molecules: amino acids (building blocks for proteins), nucleic acids (building blocks for DNA and RNA), fats, and sugars. This is a short list but a long way from methane and ammonia. In the 1920s, the biochemists Aleksandr Oparin and J. B. S. Haldane suggested that the fierce electric storms of ancient Earth provided the necessary energy for the synthesis of organic compounds from methane and ammonia.

In 1953, Harold Urey, a professor at the University of Chicago, and his graduate student Stanley Miller decided to test the Oparin-Haldane theory. To conduct the experiment, Miller constructed a simple test-tube apparatus consisting of two round flasks connected by glass tubing. One of the flasks, containing water, simulated the ocean, and a second flask, filled with hydrogen, methane, and ammonia gases, served as the atmosphere. They passed an electric discharge through the flask containing the atmosphere to simulate lightning and heated the water in the flask to produce the high temperature of the young Earth. After a week, Urey and Miller tested the contents of the flask and to their great surprise found that the water contained large amounts of amino acids. By varying the conditions of their experiment, they were able to produce a wide variety of organic compounds, including nucleic acids, sugars, and fats.

ESSENTIAL MOLECULES FORMED SPONTANEOUSLY

The Urey-Miller experiment made it clear that the basic building blocks for life could have been made in the harsh, prebiotic (before life) Earth environment. Given the conditions of that period, it now seems almost inevitable that such molecules would be synthesized. These results, published in the journal *Science* in 1953, generated a great deal of excitement, both in the science community and among the general public. Many scientists believed they were close to



Urey-Miller experiment to simulate conditions on prebiotic Earth. Water is heated in a closed system containing methane (CH_4), ammonia (NH_3), and hydrogen (H_2) gases. An electric discharge is passed through the vaporized mixture to simulate lightning in the atmosphere. Synthesized compounds collect in the trap and are sampled by opening the spigot. The original experiment was run for a week or more before samples were collected.

understanding the origin of life itself, a feat that had seemed impossible just a few years earlier. The Urey-Miller experiment suggested that the conditions on Earth 4.5 billion years ago led to the formation of certain key organic molecules, which assembled themselves into larger molecules that eventually went on to form the first cell. This was an exciting experiment, but much of the optimism it generated began to fade under the cloud of an impenetrable paradox.

Modern cells depend heavily on an interaction between proteins and the nucleic acids deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The proteins are used to construct the cell, and a special group of them, called enzymes, control the many chemical reactions that are necessary for cells to live. DNA is a collection of blueprints, or genes, that stores the information to make the proteins. One kind of RNA, called messenger RNA (mRNA), serves as an intermediary between the genes and the cell's machinery for synthesizing proteins. Although it is possible for nucleic acids and proteins to self-assemble, it is extremely unlikely that the modern relationship between the three developed spontaneously. It comes down to the age-old question of which came first, the chicken or the egg: the nucleic acids or the proteins? Could DNA, or RNA, have self-assembled and then orchestrated the synthesis of the proteins? Or did the proteins self-assemble and then make their own blueprints using DNA, while ignoring RNA altogether? Before an attempt is made to resolve this paradox, it is necessary to discuss in a little more detail the kinds of molecules that cells need to survive.

Cells are biochemical entities that synthesize many thousands of molecules. Studying these chemicals and the biochemistry of the cell would be extremely difficult were it not for the fact that most of the chemical variation is based on six types of molecules that are assembled into just five types of macromolecules. The six basic molecules are amino acids, phosphate, glycerol, sugars, fatty acids, and nucleotides. The five macromolecules are proteins, DNA, RNA, phospholipids, and sugar polymers called polysaccharides.

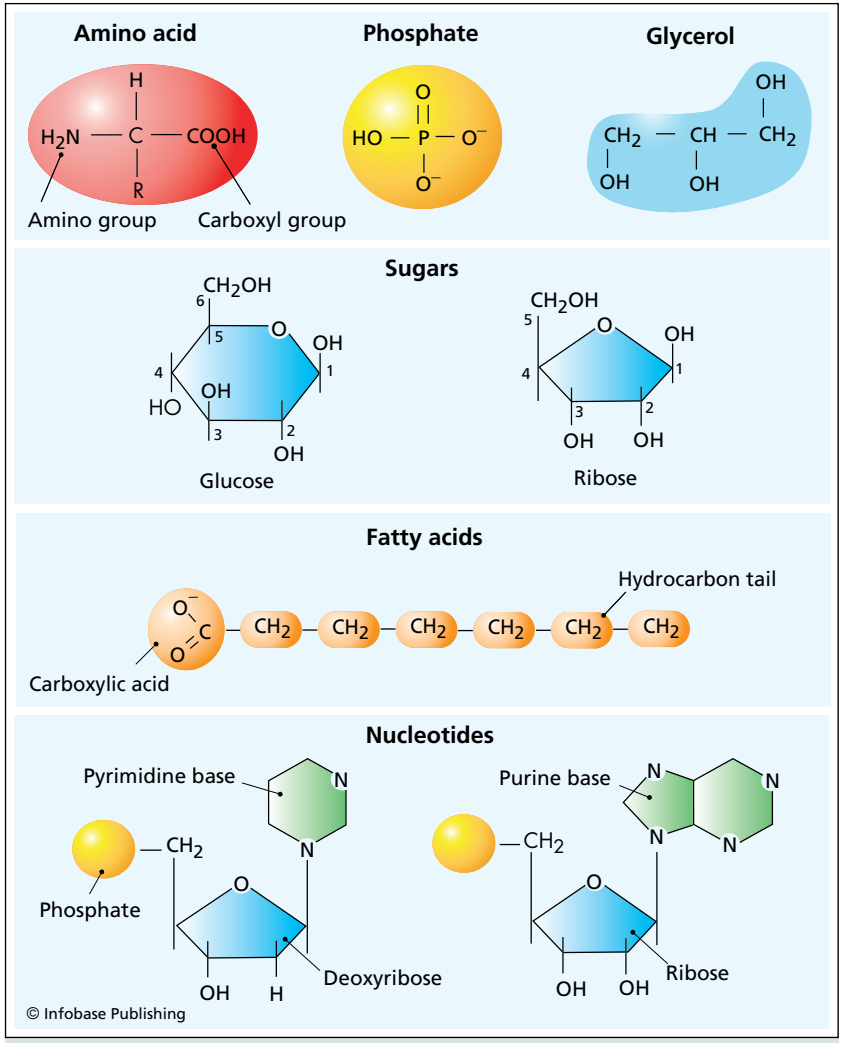
Molecules of the Cell

Amino acids have a simple core structure consisting of an amino group, a carboxyl group, and a variable R group attached to a carbon atom. There are 20 different kinds of amino acids, each with a unique R group. The simplest and most ancient amino acid is glycine, with an R group that consists only of hydrogen. The chemistry of the various amino acids varies considerably: Some carry a positive electric charge, while others are negatively charged or electrically neutral; some are water soluble (hydrophilic), while others are hydrophobic.

Phosphates are extremely important molecules that are used in the construction or modification of many other molecules. They are also used to store chemical-bond energy in the form of adenosine triphosphate (ATP). The production of phosphate-to-phosphate chemical bonds for use as an energy source is an ancient cellular process, dating back at least 2 billion years.

Glycerol is a simple three-carbon alcohol that is an important component of cell membranes and fat reservoirs. This molecule may have stabilized the membranes of prebiotic bubbles. Interestingly, it is often used today as an ingredient in a solution for making long-lasting soap bubbles.

Sugars are versatile molecules, belonging to a general class of compounds known as carbohydrates, which serve a structural role as well as providing energy for the cell. Glucose, a six-carbon sugar, is the primary energy source for most cells and the principal sugar used to glycosylate the proteins and lipids that form the outer coat of all cells. Plants have exploited the structural potential of sugars in their production of cellulose; wood, bark, grasses, and reeds are all polymers of glucose and other monosaccharides. Ribose, a five-carbon sugar, is a component of nucleic acids as well as the cell's main energy depot, ATP. The numbering convention for sugar carbon atoms is shown in the figure on page 9. Ribose carbons are numbered as 1′ (1 prime), 2′, and so on. Consequently, references to nucleic acids, which include ribose, often refer to the 3′ or 5′ carbon.



Molecules of the cell. Amino acids are the building blocks for proteins. Phosphate is an important component of many other molecules and is added to proteins to modify their behavior. Glycerol is a three-carbon alcohol that is an important ingredient in cell membranes and fat. Sugars, such as glucose, are a primary energy source for most cells and also have many structural functions. Fatty acids are involved in the production of cell membranes and storage of fat. Nucleotides are the building blocks for DNA and RNA. P: Phosphate, C: Carbon, H: Hydrogen, O: Oxygen, N: Nitrogen, R: Variable group.

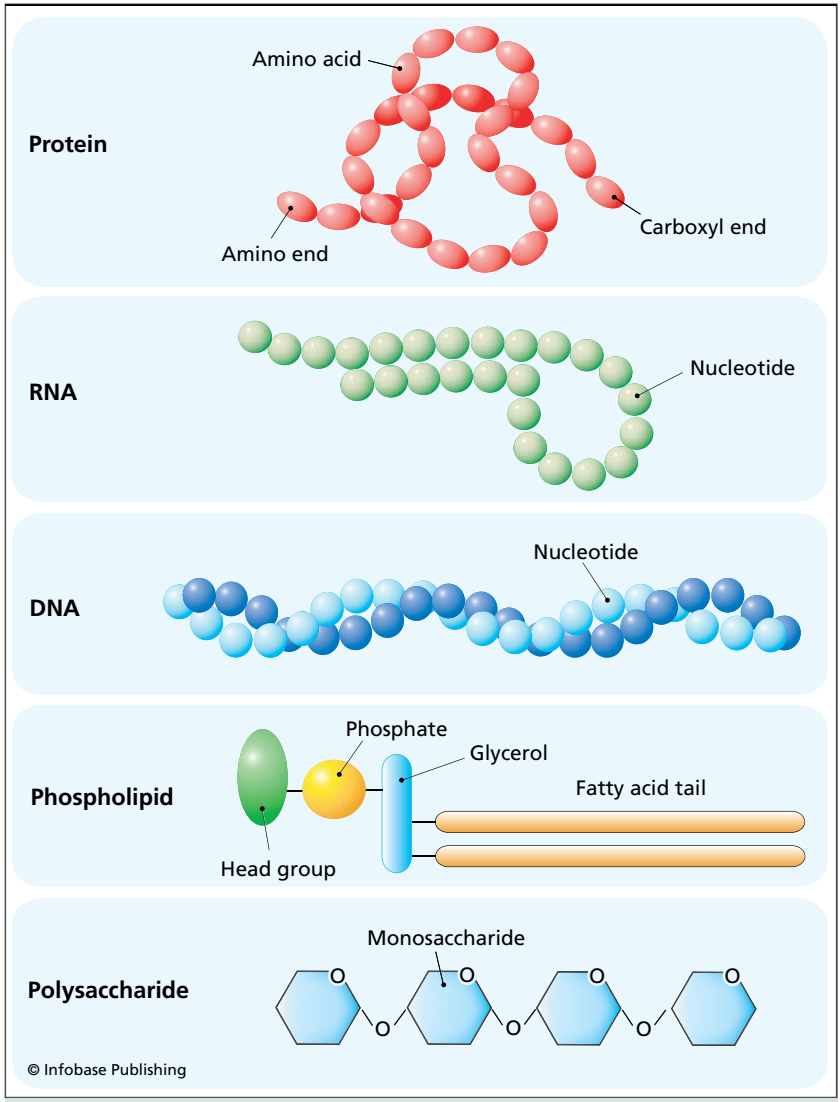
Fatty acids consist of a carboxyl group (the hydrated form is called carboxylic acid) linked to a hydrophobic hydrocarbon tail. These molecules are used in the construction of cell membranes and fat. The hydrophobic nature of fatty acids is critically important to the normal function of the cell membrane since it prevents the passive entry of water and water-soluble molecules.

Nucleotides are building blocks for DNA and RNA. These molecules consist of three components: a phosphate, a ribose sugar, and a nitrogenous (nitrogen-containing) ring compound that behaves as a base in solution (a base is a substance that can accept a proton in solution). Nucleotide bases appear in two forms: A single-ring nitrogenous base, called a pyrimidine, and a double-ringed base, called a purine. There are two kinds of purines (adenine and guanine) and three pyrimidines (uracil, cytosine, and thymine). Uracil is specific to RNA, substituting for thymine. In addition, RNA nucleotides contain ribose, whereas DNA nucleotides contain deoxyribose (hence the origin of their names). Ribose has a hydroxyl (OH) group attached to both the 2′ and 3′ carbons, whereas deoxyribose is missing the 2′ hydroxyl group.

Macromolecules of the Cell

The six basic molecules are used by all cells to construct five essential macromolecules: proteins, RNA, DNA, phospholipids, and polysaccharides. Macromolecules have primary, secondary, and tertiary structural levels. The primary structural level refers to the chain that is formed by linking the building blocks together. The secondary structure involves the bending of the linear chain to form a three-dimensional object. Tertiary structural elements involve the formation of chemical bonds between some of the building blocks in the chain to stabilize the secondary structure. A quaternary structure can also occur when two identical molecules interact to form a dimer or double molecule.

Proteins are long chains or polymers of amino acids. The primary structure is held together by peptide bonds that link the



Macromolecules of the cell. Protein is made from amino acids linked together to form a long chain that can fold up into a three-dimensional structure. RNA and DNA are long chains of nucleotides. RNA is generally single stranded but can form localized double-stranded regions. DNA is a double-stranded helix, with one strand coiling around the other. A phospholipid is composed of a hydrophilic head-group, a phosphate, a glycerol molecule, and two hydrophobic fatty acid tails. Polysaccharides are sugar polymers.

carboxyl end of one amino acid to the amino end of a second amino acid. Thus, once constructed, every protein has an amino end and a carboxyl end. An average protein consists of about 400 amino acids. There are 21 naturally occurring amino acids; with this number the cell can produce an almost infinite variety of proteins. Evolution and natural selection, however, have weeded out most of these, so that eukaryote cells function well with 10,000 to 30,000 different proteins. In addition, this select group of proteins has been conserved over the past 2 billion years (i.e., most of the proteins found in yeast can also be found, in modified form, in humans and other higher organisms). The secondary structure of a protein depends on the amino acid sequence and can be quite complicated, often producing three-dimensional structures possessing multiple functions.

RNA is a polymer of the ribonucleotides adenine, uracil, cytosine, and guanine. RNA is generally single stranded, but it can form localized double-stranded regions by a process known as complementary base pairing, whereby adenine forms a bond with uracil and cytosine pairs with guanine. RNA is involved in the synthesis of proteins and is a structural and enzymatic component of ribosomes.

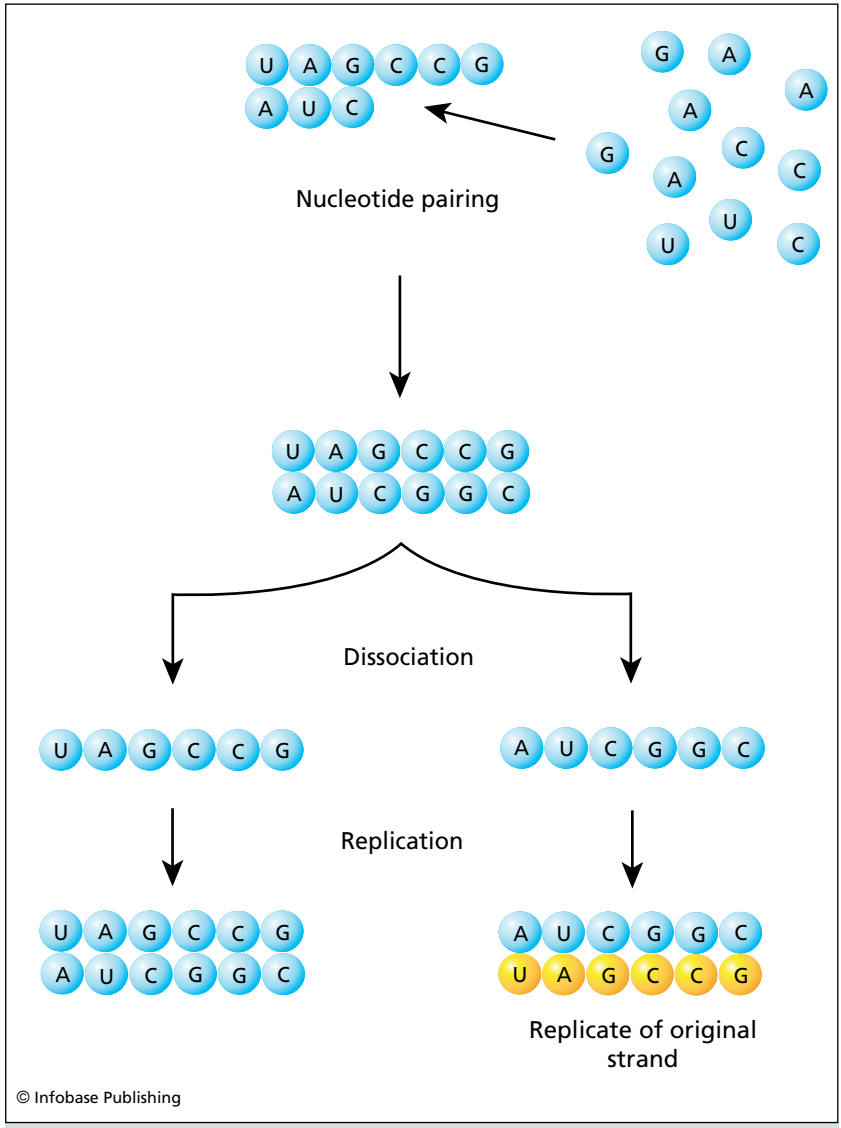
DNA is a double-stranded nucleic acid. This macromolecule encodes cellular genes and is constructed from adenine, thymine, cytosine, and guanine deoxyribonucleotides. The two DNA strands coil around each other like strands in a piece of rope, creating a double helix. The two strands are complementary throughout the length of the molecule: adenine pairs with thymine and cytosine pairs with guanine. Thus, if the sequence of one strand is known to be ATCGTC, the sequence of the other strand must be TAGCAG.

Phospholipids are the main component in cell membranes. These macromolecules are composed of a polar head group (usually an alcohol), a phosphate, glycerol, and two hydrophobic fatty acid tails. Fat that is stored in the body as an energy reserve has a structure similar to a phospholipid, being composed of three fatty acid

chains attached to a molecule of glycerol. The third fatty acid takes the place of the phosphate and head group of a phospholipid.

Polysaccharides are sugar polymers consisting of two or more monosaccharides: disaccharides (two monosaccharides) and oligosaccharides (three to 12 monosaccharides) where each are attached to proteins and lipids destined for the cell surface or the extracellular matrix. Polysaccharides, such as glycogen and starch, may contain several hundred monosaccharides and are stored in cells as an energy reserve.

All of the molecules shown in the figure on page 9 are assumed to have formed in the prebiotic oceans, and this was followed by auto-assembly of the macromolecules shown in the figure on page 11. Auto-assembly of the nucleic acids could have produced polymers that were 60 to 100 nucleotides long. With one DNA or RNA strand made, a second strand would have formed automatically through base pairing: the formation of chemical bounds between the nitrogenous bases. The chemistry of these bases is such that cytosine always pairs with guanine, while adenine always pairs with thymine. In the case of RNA, adenine pairs with uracil, an RNA-specific base not found in DNA that substitutes for thymine. Consequently, pairing is always between a purine and a pyrimidine, and the association between the two can form spontaneously. Base pairing, also known as hybridization, can form between two DNA molecules or between a DNA and an RNA molecule. Self-hybridization, involving a single DNA or RNA molecule, can also occur. Because the early oceans were hot, double-stranded DNA or RNA came apart through dissociation of the two chains. That is, the prevailing heat broke (or melted) the chemical bonds holding each nucleotide pair together without disrupting the two chains or strands. When the strands separate, the cycle repeats with another round of base pairing leading to the production of two more double-stranded molecules, one of which contains the original strand and the other contains its exact copy.



Self-replication of RNA through a process of base pairing and dissociation. Soon after replication, the two double strands separate into four single-stranded molecules, one of which (gold) is identical to the original strand. DNA can self-replicate through the same mechanisms.

By exploiting the properties of nucleotide base-pairing, coupled with the high temperatures of primitive Earth, short pieces of DNA and RNA can replicate without the aid of any other molecules. In modern cells, DNA remains double-stranded, and in prebiotic Earth, with much higher temperatures than exist today, it may still have been slower to dissociate than RNA. Thus, RNA replication would have proceeded much more quickly, producing a larger, more diverse population of molecules.

LIFE BEGAN IN AN RNA WORLD

The molecule that led to the first living cell would have to be able to replicate itself as well as function as an enzyme. DNA fulfills the first condition but it has no known enzymatic activity, and, at the time of the Urey-Miller experiment, this was thought to be the case for RNA as well. Both were believed to be incapable of regulating chemical reactions, so that neither could build protein molecules by themselves. Proteins, on the other hand, make efficient enzymes but cannot replicate themselves. This paradox was about to bring an end to origin-of-life studies when, in 1983, Thomas Cech at the University of Colorado and Sidney Altman at Yale University discovered ribozymes, RNA molecules capable of enzymatic activity. Cech isolated his ribozyme from the protozoan, *Tetrahymena thermophila,* whereas Altman discovered a similar ribozyme in *Escherichia coli.* For their discoveries, Cech and Altman received the 1989 Nobel Prize in chemistry. The discovery of ribozymes led almost immediately to the suggestion that the first cells came to life in an RNA world.

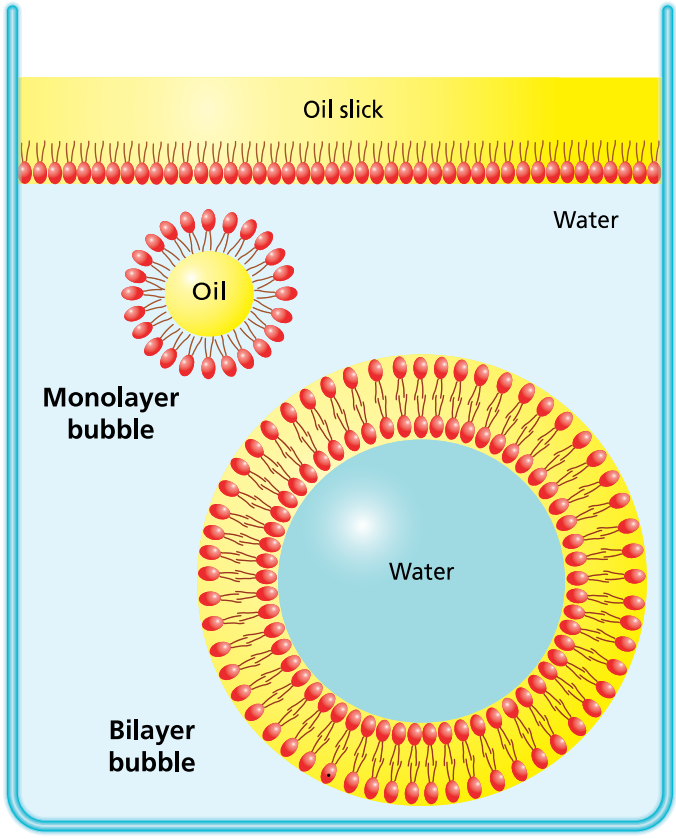
Ribozymes, assembled in the prebiotic oceans, could not only replicate themselves but could have catalyzed the formation of specific proteins, which in turn could have functioned as structural proteins or enzymes. Eventually, a protein enzyme appeared that could copy RNA into DNA (such an enzyme, called reverse

transcriptase, does exist), and when that happened, the cell's machinery approached a modern level of organization: DNA serving as the blueprint, and RNA acting as an intermediary in the process of protein synthesis. Shifting to a DNA-based genome meant that cells could become much more complex, since DNA, as a double-stranded molecule, is more stable than RNA and thus capable of storing information for many more genes.

OCEAN WAVES HELPED CREATE THE FIRST CELLS

The concept of the RNA world is very compelling, yet in itself it cannot explain the appearance of the first cell. The auto-assembly of ribozymes and proteins is of little use if they are not confined in some way. But how was this to happen? Organic molecules, newly synthesized by the raging storms, were swept along and dispersed by the wind and currents. If a ribozyme appeared that could make an especially useful protein, the association between the two would have been quickly lost. However, winds sweeping across the ocean have a way of driving things into shore, so it is possible that organic molecules collected and were concentrated along the seashore much like driftwood collecting on a beach. The water near the shore, being shallower than in the open oceans, would also tend to be warmer, concentrating the organic molecules even further through evaporation. Shorelines have another important property that is of interest here. Anyone who has stood on a beach and watched a wave break has witnessed one of the most important mechanisms for the formation of life on this planet: The foam that rolls onto shore after the wave breaks is composed of billions of bubbles.

In the prebiotic coastal waters, each bubble that formed collected a different sample of the water and, therefore, represented a unique individual, a separate experiment that could be acted upon by the forces of natural selection. But as one stands on a beach watching the waves break on the shore, one notices that the foam

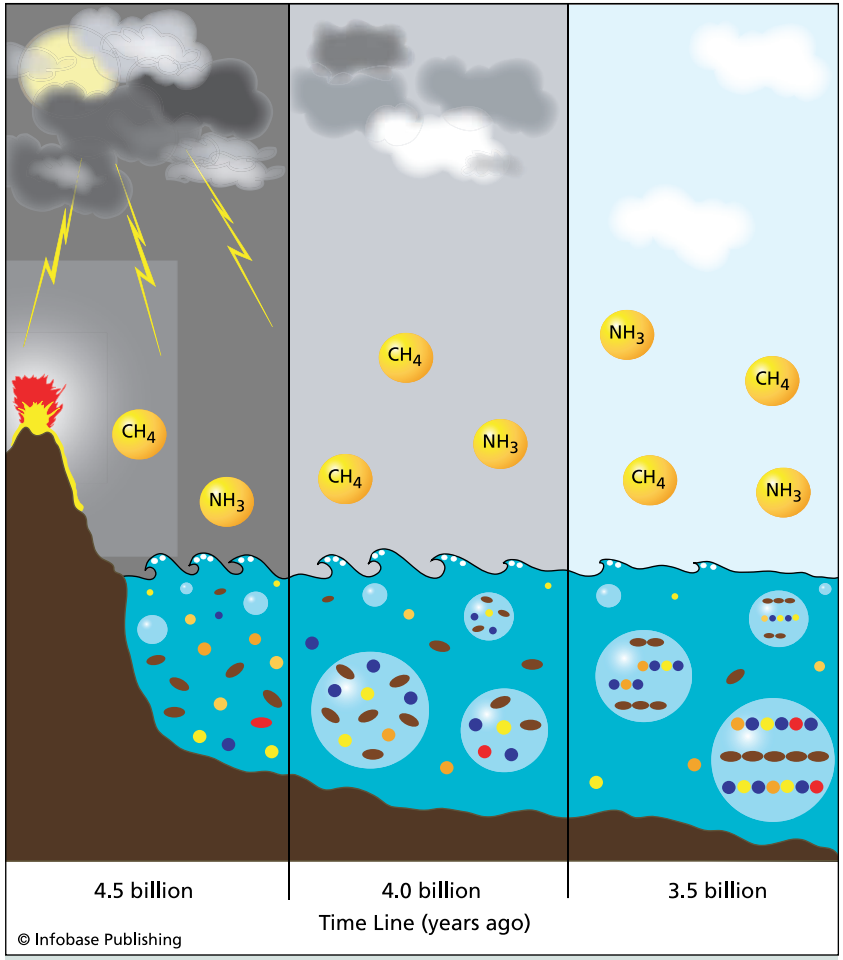


Phospholipid bubbles. Phospholipid molecules have a hydrophilic head end (red ovals) and two hydrophobic tails that do not mix with water and will avoid being surrounded by it. In an oil slick, the hydrophobic tails mix with the oil while the heads stay close to the water. In turbulence, phospholipids form two kinds of bubbles: a monolayer that can only capture a drop of oil and a bilayer that can capture a drop of water. The bilayer allows the hydrophobic tails to associate with themselves, while the heads associate with water on both the inside and outside surfaces of the bubble.

in the surf disappears very quickly. There is nothing to hold the bubbles together; that is, unless there happens to be a layer of oil on the surface of the water. Bubbles made from oil tend to have a much longer life span. Coincidentally, among the organic molecules synthesized in the prebiotic oceans was the oily compound, phospholipid. These molecules may be drawn as a bead, representing the hydrophilic head group, linked to the hydrophobic fatty acid tails. Phospholipids have the unusual property of being hydrophilic (able to mix with water) at the beaded end, but hydrophobic (unable to mix with water) at the tail end. This is curious behavior, but extremely important for the origin of life. Biologists believe that phospholipids were produced by the storms of ancient Earth, forming Earth's first oil slick very close to shore in relatively calm bays and lagoons. When the water was stirred up by driving wind and rolling surf, the phospholipids produced billions of tiny, stable bubbles.

Phospholipids have another curious, but very important, property: They can form bubbles out of a monolayer (single layer) of molecules or out of a bilayer (two layers) of molecules. In a monolayer, the external surface of the bubble is always the hydrophilic end of the molecule, whereas the inside of the bubble contains the hydrophobic portion. This type of bubble can only trap oils, not water, and therefore could never lead to the production of a cell. On the other hand, a bubble formed from a bilayer has a hydrophilic surface on both the exterior and interior surfaces. Such a bubble can trap water and water-soluble molecules like ribozymes, sugars, and proteins.

The lipid bilayer is a simple structure and at first glance may seem as though it is of little consequence, but life could not have arisen without it. Lipid-bilayer bubbles, forming in the seas of ancient Earth, could remain intact long enough to experiment with the molecules and macromolecules they captured when they were formed. If a bubble happened to pick up, or assemble, a protein that stabilized the walls of the bubble, then that bubble had an advantage over the others and would have extra time to experiment with the



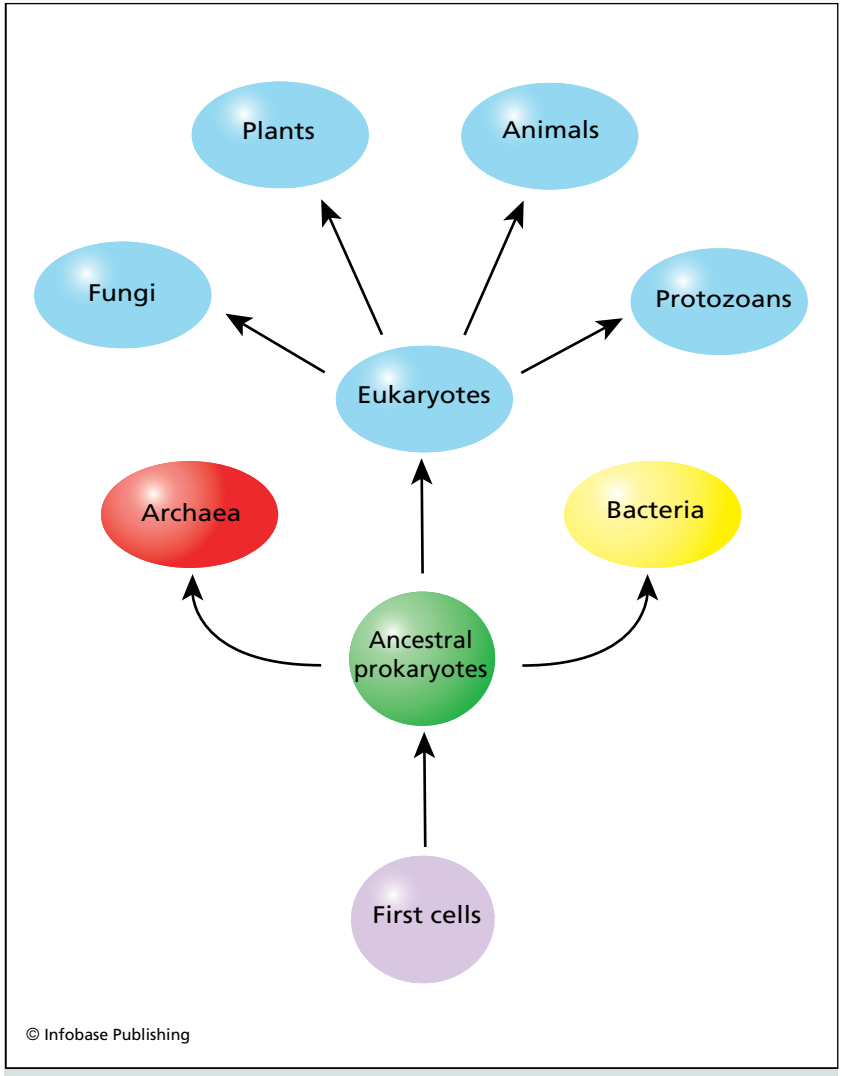
The origin of the f rst cells. Organic molecules essential for life were synthesized spontaneously 4.5 billion years ago when Earth was hot, stormy, and wracked with constant volcanic eruptions. Some of the organic molecules were captured by lipid bubbles (light blue spheres) formed by ocean turbulence near a shoreline, and by 3.5 million years ago the first cells learned how to assemble the molecules into a variety of polymers. Nucleic acids, amino acids, fats, and sugars were among the organic molecules produced in the prebiotic oceans; only the nucleic acids (colored circles) and amino acids (brown ovals) are shown. Major gases in the atmosphere included methane (CH_4) and ammonia (NH_3).

synthesis of novel ribozymes and proteins. When the bubbles burst, they released the results of their experiments into the water. When new bubbles formed, they may have captured some, or all, of those molecules and thus were given a head start through the inheritance of a simple gene pool. This simple form of genetic inheritance, acted upon by natural selection, may have transformed the prebiotic bubbles into the first cells.

Modern cells all have a membrane constructed from a phospholipid bilayer. From the very beginning, cells used the lipid bilayer to regulate their internal environment. The bilayer blocked, or impeded, the passive flow of most molecules into the cell, thus protecting the cell from the external environment. Cells exploited this property by embedding proteins in their membranes that would allow only certain molecules to gain entry. In this way, the cell could fine-tune the selection of what got in and what did not. Other proteins embedded in the membrane acted like sensory antennae, making it possible for cells to gain information about their immediate environment. Some of these proteins were used to detect the presence of food molecules, while others became specialized as transmitters and receivers, allowing the cells to communicate with each other. Cell-to-cell communication led to the next stage in the development of life on our planet. Single cells began to form colonies of increasing complexity, eventually transforming themselves into the multicellular creatures that now inhabit the Earth.

THE CLASSIFICATION OF CELLS

The first cells, appearing 3.5 billion years ago, quickly evolved into ancestral prokaryotes and, about 2 billion years ago, gave rise to archaea, bacteria, and eukaryotes, the three major divisions of life in the world. Eukaryotes, in turn, gave rise to plants, animals, protozoans, and fungi. Each of these groups represents a distinct phylogenetic kingdom. The archaea and bacteria represent a fifth



Cell classification. The first cells evolved into the ancestral prokaryotes, which gave rise to the archaea, bacteria, and eukaryotes, the three major divisions of life in the world. The archaea and bacteria are very similar anatomically but differ biochemically. Eukaryotes, anatomically and biochemically distinct from both the archaea and bacteria, gave rise to plants, animals, protozoans, and fungi.

kingdom, known as the monera or prokaryotes. The archaea are prokaryotes that are physically similar to bacteria (both lack a nucleus and internal organelles), but they have retained a primitive biochemistry and physiology that would have been commonplace 2 billion years ago. Most archaea are anaerobic and can live in extreme conditions of high temperature (sometimes hot enough to cook an egg) and high salt concentrations. All of these conditions were common on Earth 3 billion years ago and make the archaea seem like living fossils. For the archaea, oxygen is a toxic substance, and for this reason they are always found living underground or in deep thermal vents where the concentration of oxygen is very low. There are some bacteria that are also anaerobic, but they can tolerate higher concentrations of oxygen than can the archaea. Eukaryotes (meaning true nucleus) are much more complex than the prokaryotes, having many membrane-bounded organelles and a large genome. These cells are the primary focus of this book, and the overall New Biology set.

LIFE ON OTHER PLANETS

In 2004, the National Aeronautics and Space Administration (NASA) landed *Spirit* and *Opportunity,* two mobile robots, on opposite sides of the planet Mars. The main goal of this mission was to search for signs of water and to determine whether life exists on Mars or may have existed there sometime in the past. *Opportunity,* landing close to a thin outcrop of rock, found evidence to suggest that a shallow salty lake or sea once covered the immediate area. NASA scientists concluded from the data that the Martian environment of the past could have been favorable for the appearance of life. Over a five-year period, both landers provided abundant evidence for the existence of water on the red planet. Indeed, it is possible that Mars was once a watery world with vast oceans and inland seas.

Based on what is known about the origin of life on Earth, it is interesting to consider the possibility that life exists on Mars. On

Earth, the crucial ingredients for the emergence of life were water, heat, electrical storms, and the presence of methane and ammonia in the atmosphere. The presence of many extinct volcanoes on Mars suggests that the planet was once hot and geologically active, much like Earth in its early days. Mars, on the other hand, is 49 million miles farther away from the Sun than is the Earth. Thus, the surface temperature of Mars may never have been as high as it was on the young Earth. This could be a critical difference in that it may have reduced the amount of organic material produced during the prebiotic stage. The amount of prebiotic organic material and the length of time that Mars had oceans or lakes are extremely important because they determine not only whether life can appear but how long the first life-forms have before the prebiotic nutrients are consumed.

On Earth, it took about 500 million years for photosynthetic organisms to evolve from the first cells. Given that the young Earth had a large, active biosphere, it may be assumed that this is a reasonably good estimate of how long it would take for autotrophic cells to appear on any planet similar to Earth. It also gives us a rough estimate of the amount of prebiotic soup that an Earthlike planet can produce, that is, enough to last for at least 500 million years. But, in addition to being farther away from the Sun than Earth, Mars is also a smaller planet, about half the size of Earth. These two facts may have been crucial in determining the fate of life on Mars. If life did appear on Mars, given that planet's small size and lower surface temperatures, it is unlikely that the first Martian cells had enough prebiotic nutrients to get them through the heterotrophic-autotrophic transition. And without that transition, the young biosphere was doomed. Mars may have had microbes in its seas, but plants never evolved to colonize the land, and as a consequence the planet is now cold and barren.

A similar analysis may be applied to the possibility that life exists on other planets or moons in our solar system. In March 2006,

NASA scientists announced that the *Cassini* spacecraft, launched in 1997 to explore Saturn and its moons, had discovered liquid water reservoirs erupting in geysers, like those found in Yellowstone National Park, on Saturn's moon Enceladus. In 2005, the spacecraft made a similar discovery on Titan, Saturn's largest moon. Recent studies have shown that the geysers on Enceladus contain large quantities of sodium salts, thus supporting the view that the water, possibly an ocean of it, resides in rocky caverns just below the surface of the moon. These exciting discoveries suggest that Enceladus and Titan are geologically active and may offer an environment that could support life. Thermal vents not only provide heat, they also provide the regular, wavelike turbulence necessary for the production of prebiotic bubbles. If enough organic material was produced on Titan or Enceladus during their formative years, it is possible that microbes could have arisen on both moons. But as with Mars, the likelihood of life prospering in such an environment is poor at best.

NASA had hoped to answer the question of whether life ever arose on Mars by sending the spacecraft *Phoenix* to the planet in 2007. *Phoenix* landed in the northern polar region of Mars on May 25, 2008, and almost immediately began having problems with the robotic arm and shovel that was designed to collect soil samples for analysis. The samples were to be heated in an oven in order to produce the gases needed to identify any carbon compounds, the main signature of life, which may reside in the soil. But the team members spent a third of the mission (one month) trying in vain to get a soil sample into one of the lander's two prime analytical instruments (called a Thermal and Evolved-Gas Analyzer, or TEGA). Apparently, the operation failed owing to the temperature differential between the soil and the lander. When the relatively warm scoop, on the end of the robotic arm, collected a sample of the frozen soil, the soil thawed briefly before refreezing, effectively sticking the sample to the scoop.

However, in fall 2008, when hope was beginning to fade, NASA engineers managed to get a soil sample into an oven for analysis. The results were intriguing: The soil on Mars is very similar to soil from Antarctica, particularly from the upper dry valleys. Martian soil is salty and alkaline and contains traces of magnesium, sodium, potassium, and chloride. Although organic compounds, such as amino acids or phospholipids, were not detected, the presence of the salts is additional evidence for water on the planet. Sometime in the past, Mars had oceans that could have supported life. Shortly after obtaining these results, the *Phoenix* lander was retired from service. Thus, the answer to the question of whether life ever arose on Mars will have to await future missions.

Prokaryotes: Laying the Foundations

The first cells appeared on Earth more than 3 billion years ago and quickly evolved into prokaryotes, more commonly known as bacteria. For more than a billion years, prokaryotes were the only things alive on the face of the Earth. During those billion years, bacteria were likely confined to tide pools and shallow seas, but eventually they came to inhabit virtually every niche available in the water, on the land, and in the air.

OVERVIEW

All bacteria are extremely small and invisible to the naked eye. More than a thousand of these cells would fit within a period on this page. No one knew that bacteria, or any other cell, existed until a Dutch lens grinder named Antoni van Leeuwenhoek made the

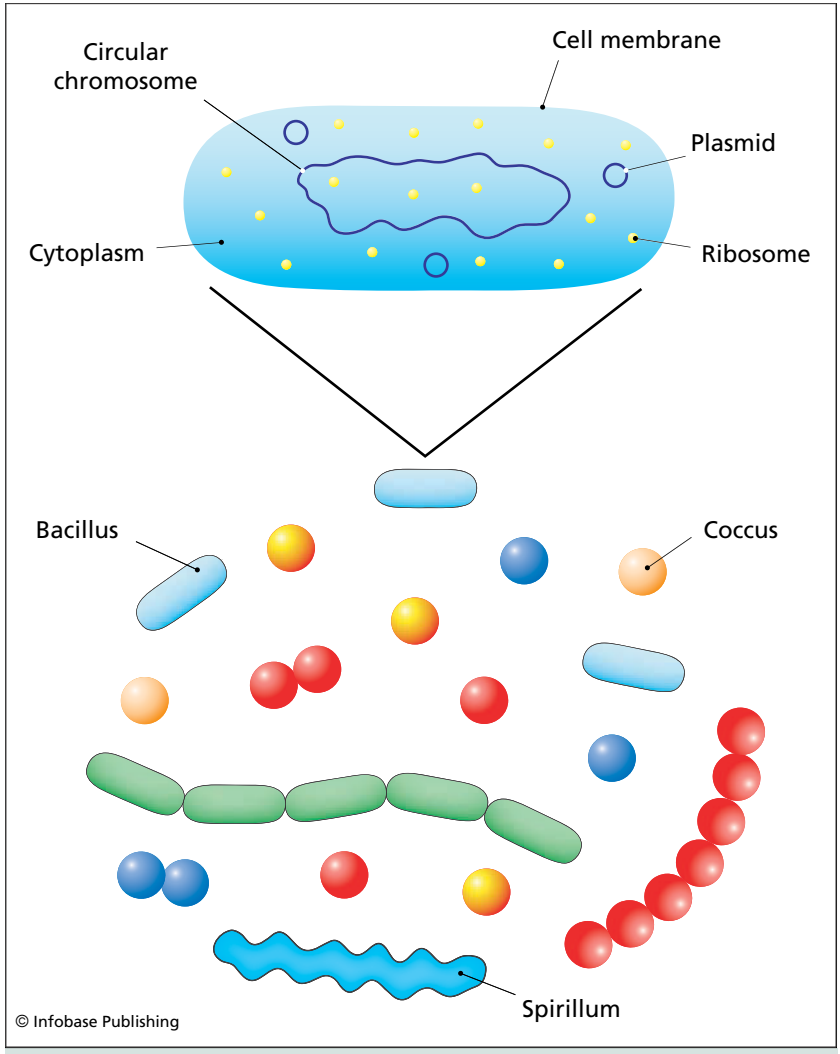
first high-resolution microscope in 1660. Leeuwenhoek's microscope consisted of a single lens mounted in a small brass frame, to which he attached a slender arm for holding a specimen. One September evening in 1683 he looked at a sample of dental plaque taken from his own teeth and the next morning wrote an excited letter to the Royal Society of London describing the many "animalcules" that he had discovered.

Two hundred years later, microscopes and the field of biology had developed to such an extent that more than 1,500 bacterial species had been discovered and described in detail (as described in chapter 10). This wealth of information not only set the stage for the new biology that was to come but also gave us the theoretical framework to understand many diseases that had plagued mankind since the origin of our species.

All bacteria have the same simple anatomy that consists primarily of the following three parts:

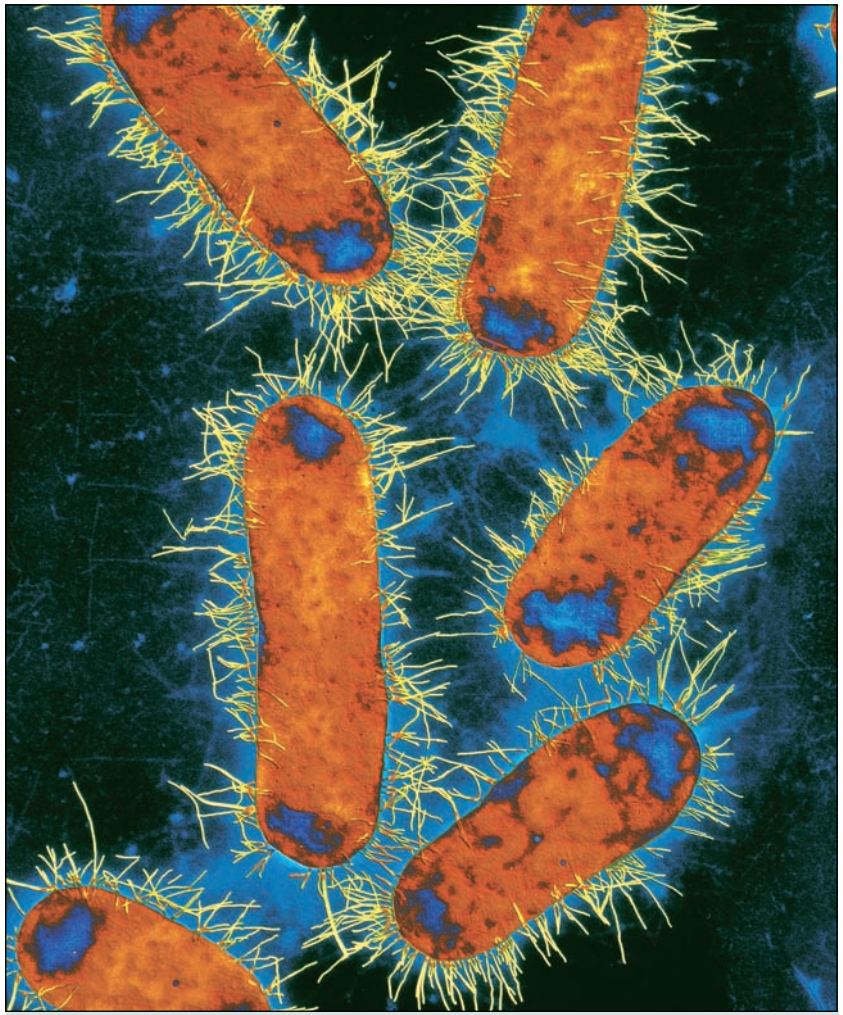
- a cell membrane
- protoplasm (or cytoplasm), and
- a chromosome.

The cell membrane, often surrounded by a cell wall, is a phospholipid bilayer, identical in kind to that which formed around the prebiotic bubbles. The cytoplasm is an aqueous gel that contains a wide assortment of enzymes and molecules and millions of spherical bodies called ribosomes that are involved in protein synthesis. Prokaryote ribosomes are complex structures consisting of more than 50 different proteins and three RNA molecules. Although the proteins outnumber the RNA, two-thirds of the ribosomes' mass is due to the RNA. Before ribozymes were discovered, it was assumed the proteins existed in the ribosome as enzymes. Scientists now know, however, that the RNA molecules

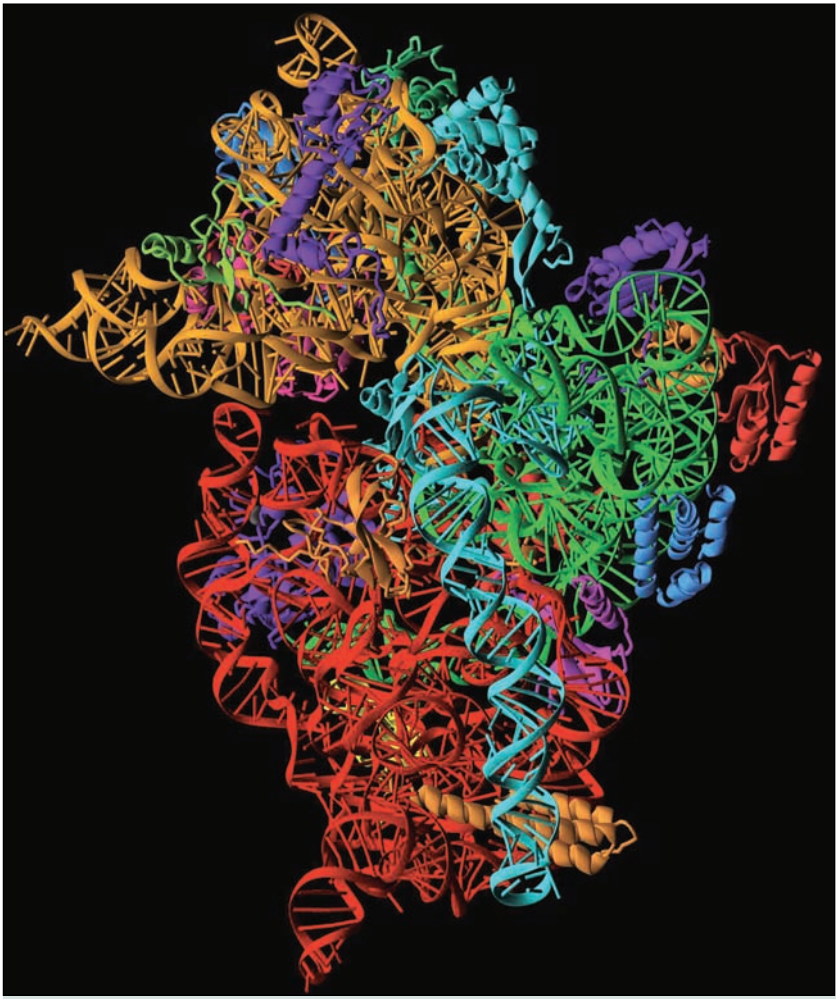


Prokaryotes. All prokaryotes have the same basic anatomy consisting of a cell membrane, cytoplasm, and a circular DNA chromosome. Some bacteria have a second, smaller chromosome called a plasmid, which may be present in multiple copies. The cytoplasm contains a wide assortment of enzymes and molecules, as well as ribosomes, protein-RNA complexes that are involved in protein synthesis. The cells may be spherical (coccus), rod shaped (bacillus), or wavy corkscrews (spirillum), appearing singly, in pairs, or linked together into short chains.

catalyze the formation of new proteins, while the ribosomal proteins serve a structural role, perhaps acting as a scaffold to hold the amino acids in position before they are linked together.



Colored scanning electron micrograph (SEM) of the rod-shaped, ciliated bacteria, *Escherichia coli*, commonly known as *E. coli*. These bacteria are a normal part of the intestinal flora but certain strains may cause gastroenteritis. *E. coli* is also commonly used in genetic studies. Magnification: 25,000×. *(Eye of Science/Photo Researchers, Inc.)*



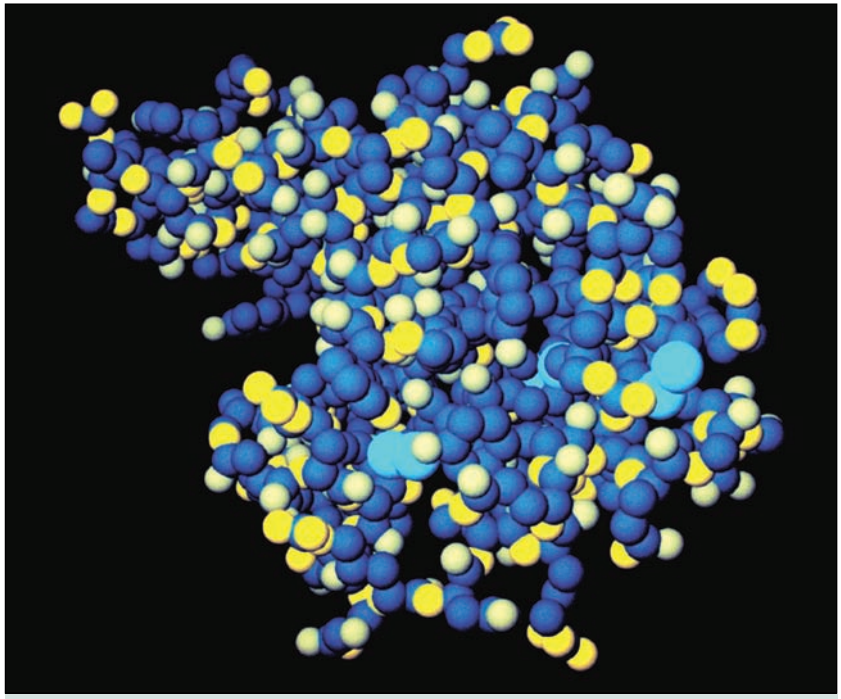
Molecule model of the 30S ribosomal subunit, which consists of protein (corkscrew structures) and RNA (coiled ladders). The overall shape of the molecule is determined by the RNA, which is also responsible for the catalytic function of the ribosome. *(V. Ramakrishnan, MRC Laboratory of Molecular Biology, Cambridge)*

The bacterial cytoplasm also contains the cell's chromosome: a single circular piece of DNA that holds all of the genes, collectively referred to as the genome. Molecular biologists, using recombinant

DNA technology (described in chapter 10), have found that a typical prokaryote has 2,000 to 4,500 genes, with each gene coding for a single protein. Many bacteria have a second, smaller chromosome called a plasmid. Like the main chromosome, the plasmid is circular, but it only carries two or three genes. Plasmid genes have been sequenced and are known to code for proteins that can neutralize antibiotics, such as penicillin or streptomycin. Placing antibiotic-resistant genes on an auxiliary chromosome is a brilliant maneuver. The cell can only have one copy of the main chromosome, but it can have many copies of the plasmid. Consequently, bacteria that have plasmids can produce a large amount of antibiotic-resistant proteins in a very short time. Plasmids make the control of pathogenic bacteria very difficult, but their existence was crucial for the development of the new biology.

Because bacteria have such a simple structure, it is often impossible, even under a high-powered microscope, to tell one species from another. In a few cases, there are clear structural differences: Some bacteria are spherical, whereas others are shaped like short rods. Both types of bacteria can appear singly or linked together into chains of varying lengths. Many rod-shaped bacteria are covered with hairlike cilia or have a single tail-like flagellum to propel the cell through the water. This type of bacterium is said to be motile. Biologists have measured the speed of these cells and have found that if they were the size of a rowboat, they could travel through the water at 56 miles per hour (90 km/hr). Spherical bacteria are never ciliated and therefore cannot propel themselves through the water. This type of bacterium is said to be nonmotile.

Despite the simple morphology of prokaryotes, their biochemistry is surprisingly complex. In addition to the ribosome, described above, prokaryotes synthesize thousands of complex macromolecules that are used to meet their physiological needs. Earth's environment, whether in water, soil, or air, contains an enormous variety of organic molecules, and for each molecule there is a bacterium that is able to use it as a source of food.

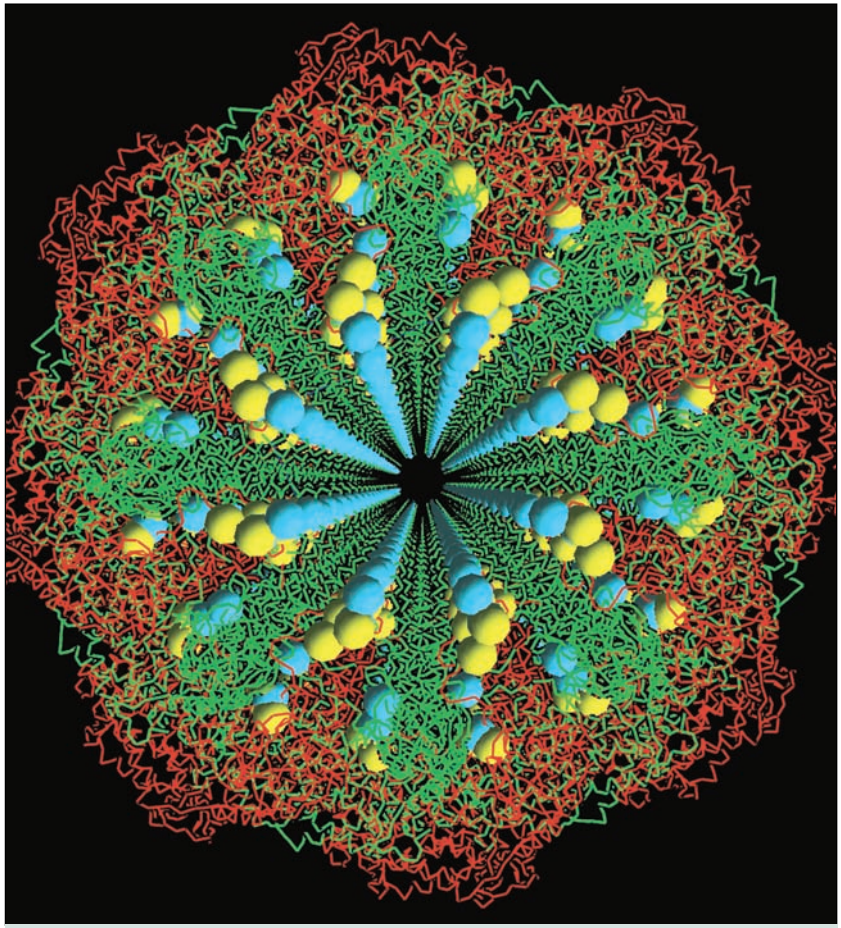


Computer generated model of lysozyme. This is an enzyme found in tears and mucus that protects against bacterial infection by literally dissolving the bacteria. *(Kenneth Eward/BioGrafx/Photo Researchers, Inc.)*

THE QUEST FOR POWER

When one strikes a match to wood in a fireplace, one gathers round to enjoy the warmth of the fire, produced by the release of energy stored in the molecular structure of the wood. By striking the match, one is triggering a chain reaction that liberates the energy in one roaring step. In their quest for power, cells learned to avoid roaring steps because they generate too much heat, so that most of the energy is lost.

Acquiring energy is one of the most important problems that prokaryotes had to solve. The first cells lived in an ocean filled with



Heat shock protein from the bacterium *Thermoplasma acidophilum*. This is a chaperonin heat shock protein, one that protects the bacterium from damage by high temperatures. The protein is made up of two chains (red and green). Eight pairs of these chains form a ring, and several rings are stacked to form a tubelike structure with a striking symmetry. *(Science Source/Photo Researchers, Inc.)*

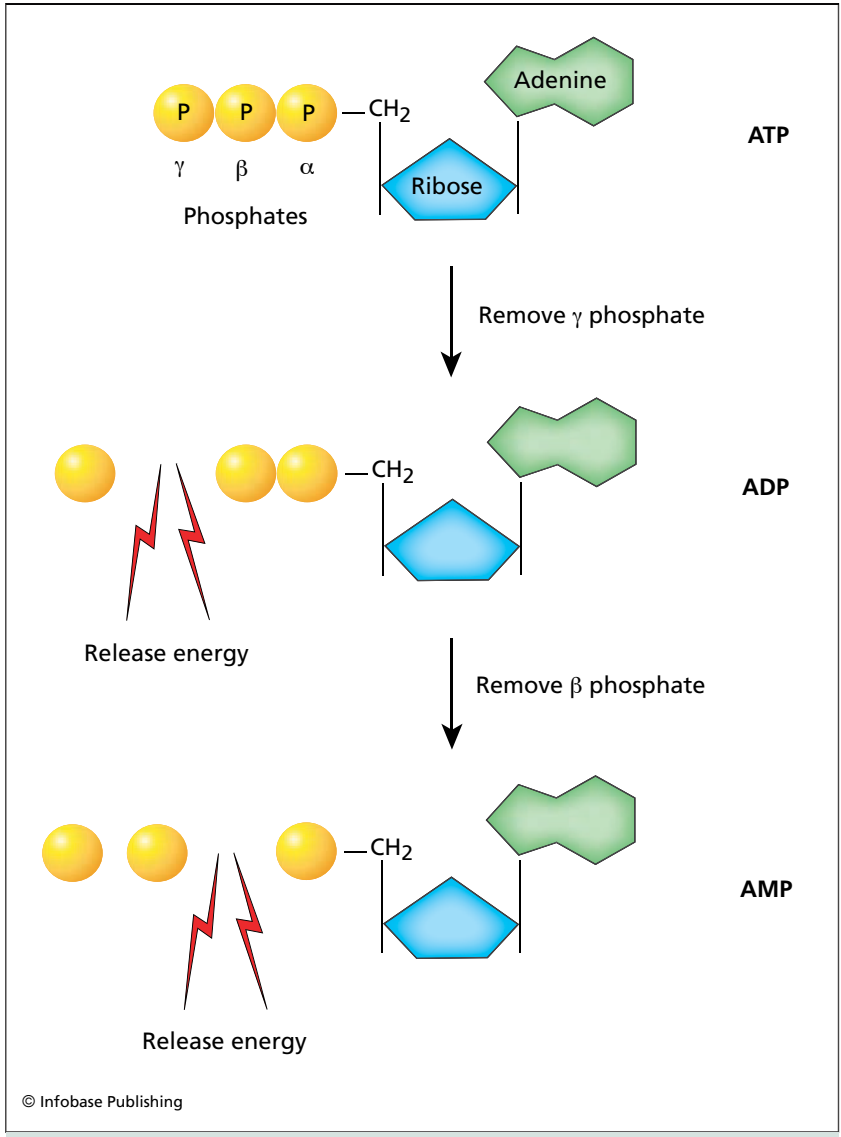
small molecules, produced in the prebiotic environment. Each of these molecules was like a small piece of wood that the cell could use as a source of energy. The trick was to extract the energy very

carefully, so as not to lose most of it in a burst of heat and light. Extracting energy in this way is the job of an enzyme. Enzymes can pick a molecule apart piece by piece, supplying small amounts of energy at each stage. The energy so released can be used directly to perform a task or it can be stored for later use. Extracting energy by breaking a molecule down is called catabolism. Anabolism is just the reverse; it uses energy to build molecules. Cellular metabolism is the combination of catabolic and anabolic activity.

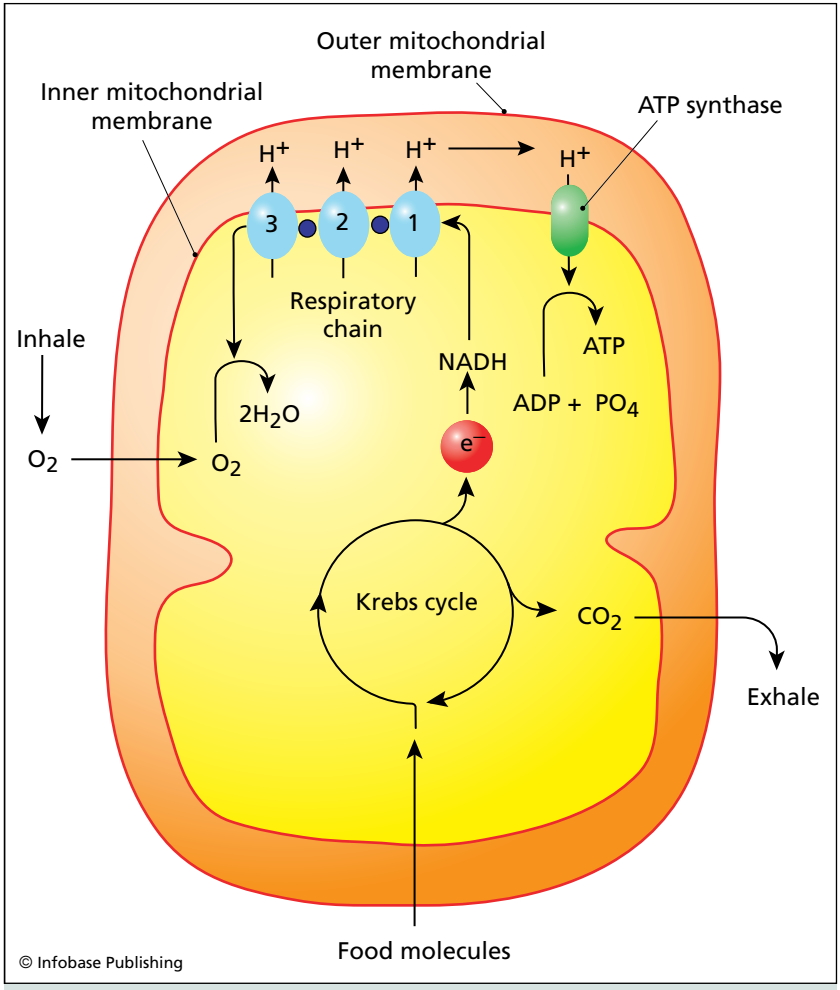
Of all the molecules created in the prebiotic oceans, glucose was the most important as a source of food. Cells prefer glucose to this day, and, for some, like the neurons in the human brain, it is the only molecule they will use. Prokaryotes can use glucose directly, or they can send it through an anabolic pathway to form a polymer called glycogen (a long chain of glucose molecules) that is stored in the cell for later use.

Prokaryotes also developed a method for storing some of the energy that is released when glucose is broken down. This procedure is called glycolysis, a very ancient catabolic pathway that produces two molecules of adenosine triphosphate (ATP) for every molecule of glucose that is broken down. ATP stores energy in phosphate bonds, and this energy is released when these bonds are broken. If an enzyme requires energy for a job that it has to do, it can either break (or hydrolyze) a phosphate bond on ATP or, if it lacks that ability, the cell recruits another enzyme that has ATPase activity (that is, the enzyme is able to hydrolyze the phosphate bond). Prokaryotes often recruit many enzymes to perform a given task. The complete catabolism of a molecule of glucose by the glycolytic pathway requires 10 different enzymes. Because it does not require oxygen, it is referred to as an anaerobic metabolic pathway.

A half-million years after the first prokaryotes appeared, some of them learned to build organic molecules by using energy collected from the Sun. A by-product of this photosynthetic pathway is the release of oxygen. Eventually, other cells, unable to perform photosynthesis, learned to use the oxygen to extract energy from



Adenosine triphosphate (ATP), the cell's energy depot. Energy is stored in the covalent bonds linking the phosphates together. Breaking the gamma (γ) phosphate bond releases energy, converting ATP to adenosine diphosphate (ADP). Additional energy may be released by breaking the beta (β) bond, converting ADP to adenosine monophosphate (AMP). AMP is converted back to ATP by mitochondria.



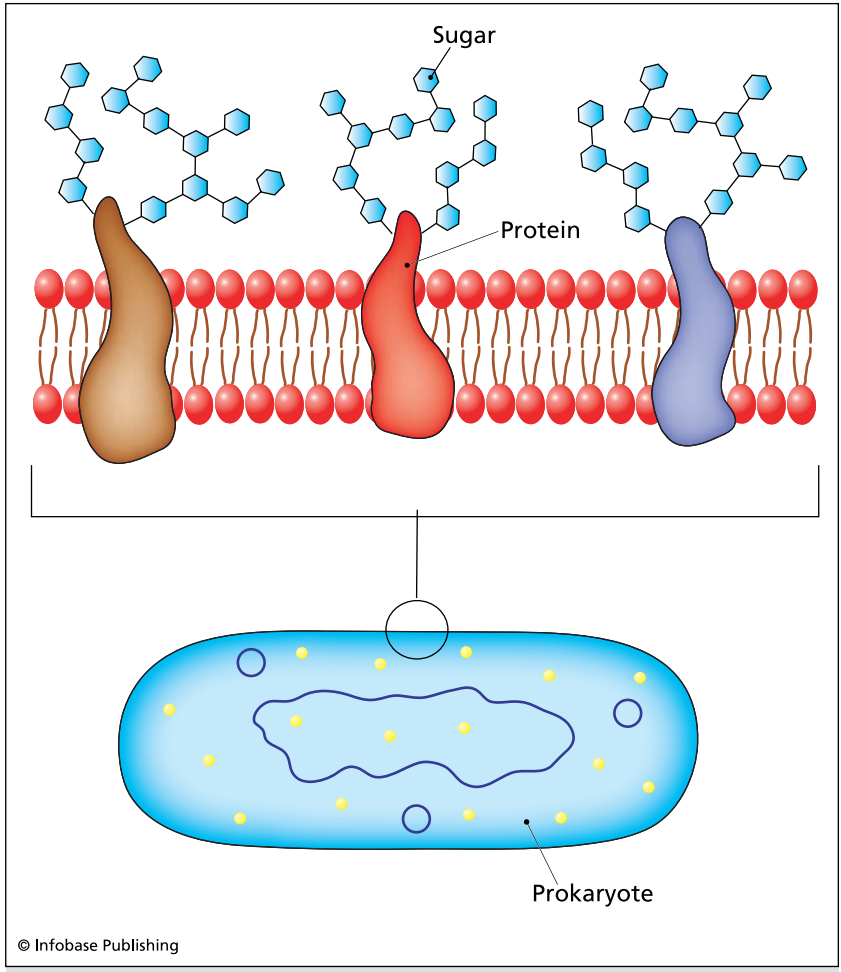
Production of ATP by mitochondria. Food molecules are processed through the Krebs cycle to produce electrons (e^-) that are carried by a molecule called NADH. The respiratory chain consists of three major components: NADH dehydrogenase (1), cytochrome b (2), and cytochrome oxidase (3). The first component in the chain captures the stored electrons by separating NADH into NAD and H^+ (not shown). The electrons travel through the chain powering a pumping function of each component resulting in a proton (H^+) concentration gradient across the inner membrane and are eventually transferred to oxygen (O_2) leading to the production of water. The protons, moving down their concentration gradient, power the synthesis of ATP by the synthetase. The only exhaust from this power plant is water, that the cell uses, and CO_2, a gas that is exhaled by the lungs.

an even wider variety of molecules than was possible with glycolysis. Two aerobic (requiring oxygen) metabolic pathways were developed: One of these is called the citric acid cycle (or the Krebs cycle, after the biochemist who discovered it), and the other is the electron transport chain (also called the respiratory chain). These two pathways work in tandem to extract energy from fats, simple sugars, polysaccharides, and amino acids.

Unlike glycolysis, the Krebs cycle stores most of the energy that it liberates in electrons that are carried by special molecules through the respiratory chain where their energy is used to make ATP. The by-products of these two pathways are water and carbon dioxide (CO_2). The coordinated activity of Krebs cycle and the respiratory chain is analogous to the way electricity is generated to run our factories and to make our homes comfortable. A power generator, usually at a hydroelectric dam, plays the role of the citric acid cycle, and the copper wires that carry the current are analogous to the respiratory chain. The electricity produced by the power plants is used to turn on lights, heaters, and motors. The cell uses the electricity that it generates for one thing: to make ATP.

Glycolysis, the Krebs cycle, and the respiratory chain are all run and assembled by protein enzymes. These metabolic pathways are used by all prokaryotes that are alive today. The glycolytic pathway and Krebs cycle are located in the protoplasm, while the respiratory chain is located in the cell membrane. Additional proteins necessary for collecting glucose and other sugars are also located in the cell membrane. These proteins, called glucose transporters, or carriers, are specially designed for bringing glucose into the cell. Glucose and other simple sugars can diffuse passively across the cell membrane, but it is a much slower process. Transporters provide a channel that allows the cell to take up glucose 100 times faster than by simple diffusion.

The ability to make glucose carriers that are embedded in the cell membrane was probably the most important event leading to the transition from the first cells to the ancestral prokaryotes. Once cells learned this trick, they expanded on it very quickly. Proteins were embedded in the membrane that could detect and import other



The prokaryote glycocalyx. The glycocalyx is a molecular "forest" consisting of glycoproteins that covers the outer surface of the cell membrane. In this forest, the "tree trunks" are protein, the "leaves" are sugar molecules, and the "branches" are chemical bonds. Glycoproteins have many jobs, including the transport and detection of food molecules.

sugars, such as maltose or lactose. They even learned how to make sugar receptors, embedded also in the membrane, which could signal the cell when a high concentration of glucose or maltose was encountered so the activity of the transporters could be stepped up

accordingly. The electron transport chain may have evolved from proteins that were originally embedded in the membrane to process or detect sugar molecules.

The sugar carriers, receptors, and components of the respiratory chain are all glycoproteins; that is, sugar molecules are attached to the proteins to enhance or modulate their behavior. Glycoproteins are like molecular trees, with the protein portion being the trunk and the sugar molecules forming the leaves and branches. It is almost as though the prokaryotes were building a forest with which to cover themselves, much in the way higher plants covered the surface of the Earth so many millions of years later. The molecular forest of a prokaryote is called the glycocalyx, and its importance to the cell cannot be overstated. This forest gives the cell its eyes, ears, and a sense of touch, in addition to energy-processing machinery. It is through the glycocalyx that cells learned how to communicate with one another, paving the way for the appearance of multicellular creatures.

CELLS ARE SELF-SUFFICIENT

Acquiring energy is not the only problem that prokaryotes had to deal with—they also had to keep their house in order and be able to make or repair every part of their structure. This was no small feat. By analogy, if a house is damaged the homeowner goes to the hardware store to buy lumber for the repairs, but for a cell there is no such store available. Instead, they have to *make* all of the materials needed before they can begin to repair themselves.

Prokaryotes have learned to build and repair themselves with the same molecules that they use for food. As mentioned earlier in this chapter, glucose can be converted into glycogen as a food reserve; after being cross-linked with a few amino acids, it is also used to make the cell wall. Higher plants use a similar trick: Wood is nothing more than an elaborate polymer of glucose. The trees that grow on the Earth and the lumber that is used to build our homes are all sugar compounds.

The prokaryotes use many other molecules both for food and for building materials: Fats can be used as a source of food or converted

to phospholipids and used to repair the cell membrane. Many proteins have enzymatic activity and are kept busy running the cell. However, there are also many nonenzymatic proteins that are synthesized as building materials and used to construct cilia, flagella, microtubules, the cytoskeleton, and all of the cellular organelles.

A NEW GENETIC ORDER

Cells came to life in an RNA world, where RNA functioned both as a molecule for storing genetic information and as an enzyme. But RNA is a relatively small and simple molecule. Like an old-fashioned computer with limited memory, it can only store small amounts of genetic information and its talents as an enzyme are severely limited. This was no great disadvantage to the first cells, since the molecules they were using as a source of energy were also very simple and did not require enzymes more elaborate than a ribozyme. But that world began to change as the small molecules were used up and replaced with more complex molecules, produced by the cells themselves and liberated into the water when they died. Now the cells were faced with a new challenge: Find a way to utilize molecules of increasing complexity.

Cells met the challenge, but there may have been a time when their survival was in jeopardy, for it meant they had to change the way they had been doing things for millions of years. They had to abandon the RNA world in favor of DNA and protein. Protein enzymes, being made from 20 different amino acids, can be much more complex than a ribozyme and therefore have a greater chance of being able to catabolize complex molecules. In addition, as the population of complex food molecules increased, cells were forced to keep pace by coming up with new enzymes to deal with them. RNA could not store the genetic information for all of the new protein enzymes that cells needed in order to survive. It is for this reason that DNA came to be the molecule of choice for the storage of genetic information.

In the RNA world, the genetic flow was simple and direct: Ribozymes were both genes and enzymes that could synthesize proteins directly. But the prokaryotes changed all that. DNA became the molecule of choice for storing the genes, and RNA was left in control of the protein-synthesis machinery. This three-step, unidirectional organization of DNA to RNA to protein is now used by all cells and has many advantages over the system that it replaced. DNA, being a much longer macromolecule than RNA, can store information for thousands of genes. In addition, because it is a double-stranded molecule, DNA is much more stable than RNA, and the presence of two strands provides a way of repairing the genes if they are ever damaged. That is, because of nucleotide pairing, the damaged strand can be repaired using the complementary strand as a guide.

In the new genetic order, the molecule carrying the gene itself is not being used to synthesize the protein, so there is no chance that it will be damaged during the translation process. There is a real-world analogy to this organization: Human architects and engineers make blueprints to guide them in the production of houses, cars, boats, and many other structures. Those blueprints are always kept in a safe place so nothing will ever happen to them, and the only time they are taken out of the filing cabinet is to make a copy, or a working blueprint, that is given to the carpenters to build a new home. The carpenters may spill coffee on it, step on it, or crumple it up if they want to, because they always have the original to refer back to. In the cell, the working blueprint is a molecule called messenger RNA (mRNA), so named because it is, in effect, carrying a genetic message from the chromosomes to the ribosomes. Copying a gene into mRNA occurs by a process known as transcription. Once the ribosomes receive the mRNA, the ribosomal RNA (rRNA) molecules use it to synthesize the protein in a process known as translation. Although RNA lost its job as a genetic reservoir, it remains in control of protein synthesis.

No one knows how DNA came to be the cell's gene bank, but it is possible the enzymatic proteins orchestrated the change themselves. There is a protein enzyme called reverse transcriptase that can copy RNA into DNA, and the reverse transcription process that it performs may have been the way the DNA genome originally appeared. In addition, protein enzymes are capable of surprisingly complex behavior, almost as though they are living entities unto themselves. In prokaryotes, and in all modern cells, there are proteins that take care of the DNA much like shepherds tending their flock. Some of these molecular shepherds constantly scan the DNA for damage and, if they find any, repair it. Several protein enzymes are in charge of replicating DNA in preparation for cell division, and still others are needed to copy a gene into messenger RNA in preparation for protein synthesis.

The interaction between the proteins and the DNA is of mutual benefit and important for the survival of the cell. All proteins have a very short life span, sometimes no more than a day or two, so it is to their advantage to take care of the genes. In a sense, this relationship between DNA and protein is a form of molecular symbiosis, in which both parties benefit. The cell as a whole is the ultimate beneficiary, since a good working relationship between the enzymes and the DNA makes it more adaptable and better able to survive in a changing environment.

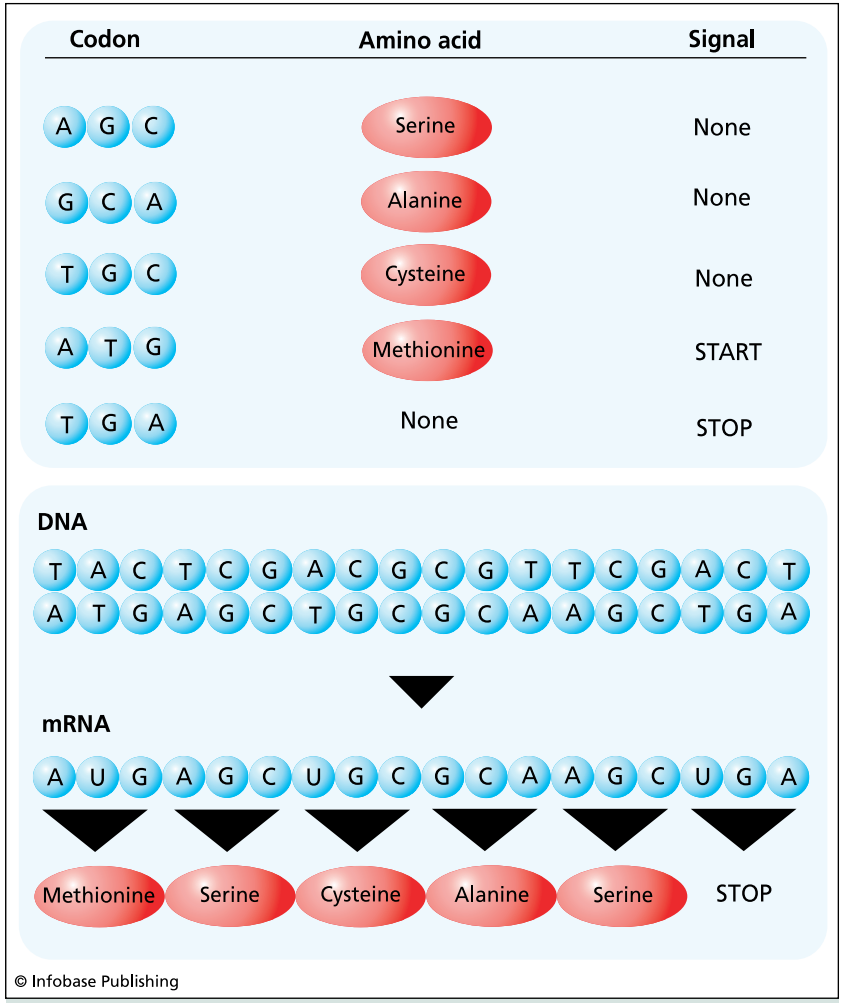
In building their new genetic order, the prokaryotes had to develop a method for interpreting the sequence information stored in the DNA molecule. DNA is a linear sequence of four different kinds of nucleotides, so the simplest approach would be to have each nucleotide specify a different amino acid: adenine would code for the amino acid glycine, cytosine for the amino acid lysine, and so on. This simple code may have been useful to the earliest cells, but it is limited to the construction of proteins consisting of only four different kinds of amino acids.

Prokaryotes undoubtedly experimented with a variety of coding methods before adopting the current system, in which a com-

bination of three out of the four possible DNA nucleotides, called a codon, specifies a single amino acid. With this scheme, it is possible to have a unique code for each of the 20 naturally occurring amino acids. For example, the codon AGC specifies the amino acid serine, whereas TGC specifies the amino acid cysteine. Codons are linked together to form a long continuous sequence that is called a gene. Not all of the codons code for amino acids. The sequence TGA signals the end of the gene, and a special codon, ATG, signals the start site, in addition to specifying the amino acid methionine. Thus, all proteins begin with this amino acid, although it is sometimes removed once construction of the protein is complete. An average protein may consist of 300 to 400 amino acids; since the codon consists of three nucleotides for each amino acid, a typical gene may be 900 to 1,200 nucleotides long. The codon system, developed by the prokaryotes, is also used by the eukaryotes, and for this reason is referred to as the universal genetic code.

Messenger RNA is the working copy of the gene, but it has the nucleotide uracil in place of thymine, which is found in DNA. Consequently, the DNA codon for methionine is ATG, but it is AUG on the mRNA. The ribosomal RNAs are programmed to recognize the codon as it appears on the mRNA. Once the protein is made, mRNA is broken down and the nucleotides recycled. Thus, mRNA has a very short life span, sometimes referred to as its half-life (the time it takes for half the population to disappear), which can range from a few seconds to a few hours. The short half-life of mRNA makes the cell very responsive to changing conditions in the environment and within the cell itself.

The complete genetic code used by all living things on Earth consists of 64 codons that specify 20 amino acids and the start and stop sites. The large number of codons is due to redundancy in the code; that is, several codons may specify the same amino acid. Human carpenters, as mentioned previously, use a two-dimensional blueprint to build a three-dimensional house, but prokaryotes do



Transcription and the genetic code. Five codons are shown, four specifying amino acids (protein subunits) and two that serve as start and stop signals. The codons, including the start and stop signals, are linked together to form a gene on the bottom, or coding, DNA strand. The coding strand is copied into messenger RNA (mRNA), which is used to synthesize the protein. Nucleotides appear as round beads: Adenine (A), Thymine (T), Cytosine (C), and Guanine (G). Amino acids appear as labeled elliptical beads. Note that in mRNA uracil (U) replaces the thymine (T) found in DNA.

THE UNIVERSAL GENETIC CODE

CODON	AMINO ACID	SIGNAL
GCA GCC GCG GCU	Alanine	–
UGC UGU	Cysteine	–
GAC GAU	Aspartic acid	–
GAA GAG	Glutamic acid	–
UUC UUU	Phenylalanine	–
GGA GGC GGG GGU	Glycine	–
CAC CAU	Histidine	–
AUA AUC AUU	Isoleucine	–
AAA AAG	Lysine	–
UUA UUG CUA CUC CUG CUU	Leucine	–
AUG	Methionine	Start
AAC AAU	Asparagine	–
CCA CCC CCG CCU	Proline	–
CAA CAG	Glutamine	–
AGA AGG CGA CGC CGG CGU	Arginine	–
AGC AGU UCA UCC UCG UCU	Serine	–
ACA ACC ACG ACU	Threonine	–
GUA GUC GUG GUU	Valine	–
UGG	Tryptophan	–
UAC UAU	Tyrosine	–
UAA UGA UAG	–	Stop

Note: Codons are written using the standard abbreviation for each nucleotide on the messenger RNA: adenine (A), uracil (U), cytosine (C), and guanine (G). All but two of the amino acids (methionine and tryptophan) have more than one codon. Note that in mRNA uracil replaces the thymine found in DNA.

better. They build three-dimensional objects (proteins), but they do it with a one-dimensional blueprint. Moreover, the invention of the codon, and the use of it to produce identical copies of the same

protein over and over again, is nature's first and most important cloning experiment.

BACTERIAL POPULATIONS

The classification of bacteria is based on the cell's morphology, its motility, the way they grow on a culture plate, the kind of sugars and other molecules that they are able to metabolize, and whether their metabolism is anaerobic or aerobic. In 1844, the Danish bacteriologist Hans Christian Gram introduced a simple staining procedure that provides a convenient way of distinguishing different types of bacteria that otherwise have the same physical appearance. The Gram stain exploits the chemical properties of the cell walls in different species of bacteria. Those bacteria that retain the stain, which is blue, are referred to as being gram positive and those that do not are gram negative. For example, *Myxobacteria, Staphylococcus,* and *Streptococcus* are Gram positive, whereas *Salmonella, Pseudomonas,* and *Cyanobacteria* are Gram negative. DNA sequence analysis of ribosomal RNA has also been used to assign cells to various taxonomic groups.

Bacteria are usually thought of as disease organisms, and many of them, the pathogenic bacteria, do cause very serious disorders. But most bacteria are nonpathogenic and can be found living in the water, in the soil, on our skin, and in our intestinal tracts. There are even bacteria living among the clouds. Because trees and elephants are so big, people tend to think that they, along with other plants and animals, contribute the most to the biomass of the Earth, but this is not the case. Bacteria not only outnumber us, they outweigh us by a wide margin.

Bacteria are always busy making a living, just as they have done for millions of years, and the thing that occupies most of their time is the extraction of energy from organic molecules. A by-product of the extraction process is the recycling of nutrients, an activity that is crucial to the maintenance of Earth's ecosystem. Carbon and nitrogen compounds, essential for the synthesis of proteins and nucleic

CHARACTERISTICS OF SOME NONPATHOGENIC BACTERIA

Cyanobacteria	Gram-negative, aerobic, nonmotile short rods that occur singly, in long filaments, and in irregular colonies. Cells are capable of photosynthesis and live in freshwater and marine habitats. They recycle organic matter.
Myxobacteria	Gram-negative, aerobic, motile rods that occur singly but can aggregate to form fruiting bodies and spores. Cells live in the soil and have a complex life cycle. They recycle terrestrial organic matter and build soil.
Pseudomonas	Gram-negative, aerobic, motile (with several flagella) rods that occur singly or in pairs. Cells live in soil and water and are able to decompose virtually any organic molecule, including crude oil. Important as recyclers and soil builders.
Spirillum	Gram-negative, aerobic, motile (with a single flagella), long spiral-shaped rod that always occurs singly. Cells live in freshwater and marine habitats. They recycle organic matter.
Lactobacillus	Gram-positive, aerobic, non-spore-forming rods that occur singly. Important in the food industry for the production of cheese, yogurt, sour cream, and other dairy products.

acids, would be lost from the system if not recycled by bacteria. In this regard, the cyanobacteria (sometimes called blue-green algae) are perhaps the most important. This is an extremely ancient group of filamentous bacteria.

Fossilized remains of cyanobacteria date back nearly 3 billion years and are the oldest fossils known. The discovery of fossilized cyanobacteria was crucial to our understanding of the origin of life and the conditions prevalent on the primitive Earth. Cyanobacteria are capable of photosynthesis and are believed to be the evolutionary forerunners of modern-day plant and algal chloroplasts. They

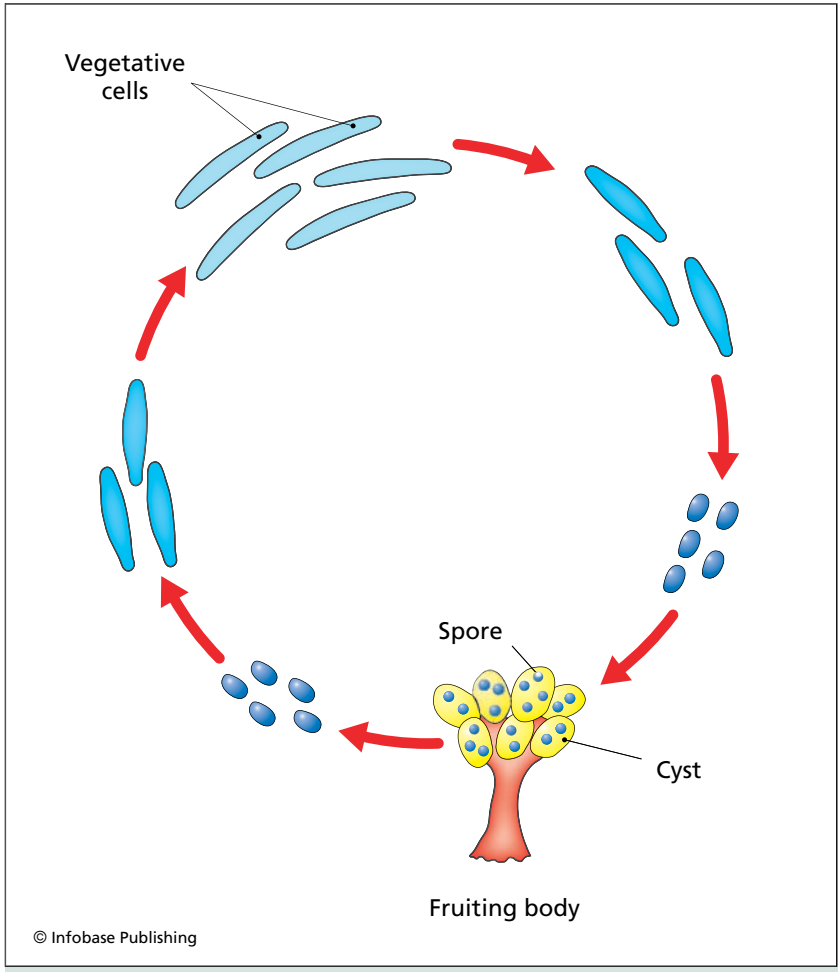
CHARACTERISTICS OF SOME PATHOGENIC BACTERIA

Salmonella	Gram-negative, aerobic, motile rods that occur singly. Two species, *gallainarum* and *pullorum,* cause food poisoning.
Streptococcus	Gram-positive, aerobic cocci (spheres) that occur singly or in short chains. Several species cause throat infections, tonsillitis, and food poisoning.
Staphylococcus	Gram-positive, aerobic cocci that occur singly or in grapelike clusters. *S. aureus* is a major cause of food poisoning. The symptoms are produced by a toxin.
Bacillus anthracis	Gram-positive, aerobic, nonmotile rods that occur singly. These are spore-forming bacteria that germinate when inhaled by people or cattle. The cells attack the lungs and the disease is usually fatal.
Vibrio cholerae	Gram-negative, aerobic, curved rods that occur singly. Ecology similar to pseudomonads. Causative agent of cholera, which is usually fatal if not treated promptly.
Clostridium botulinum	Gram-positive, anaerobic, spore-forming rod that produces a very potent neurotoxin. The spores are heat resistant and can survive in food that is not cooked properly. Infections are fatal if not treated promptly.

are also important members of the phytoplankton in the oceans and freshwater lakes, accounting for more than 25 percent of the biomass produced in aquatic ecosystems.

Terrestrial counterparts to the cyanobacteria are the myxobacteria and the pseudomonads, both of which are important for their ability to recycle organic compounds and for making soil. The myxobacteria are especially interesting because of their complex social behavior, which is unique among all of the prokaryotes. Myxobacteria live out most of their lives as vegetative cells that browse among the soil and leaf litter. If the food supply runs out,

the cells aggregate at the top of the leaf litter where they form a fruiting body that looks like a tiny cluster of grapes. Some of the cells form the stalk and storage capsules, called cysts, while the rest



Life cycle of myxobacteria. Vegetative cells feed on organic material in the soil. When food runs low, the cells are stimulated to form a fruiting body consisting of many cysts, which are full of spores. When conditions are favorable, the cysts break open and the spores germinate into vegetative cells.

form the spores that become sealed inside the cysts (the grapelike structures). Being on top of the leaf litter exposes the fruiting body to the wind, which may carry some of the cysts to other areas where, hopefully, there is more food available. When the conditions are right, the cysts rupture, releasing the spores, which germinate into vegetative cells. This remarkable life cycle is an example of the kind of colonial behavior, adopted by primitive cells, that eventually led to the appearance of multicellular creatures.

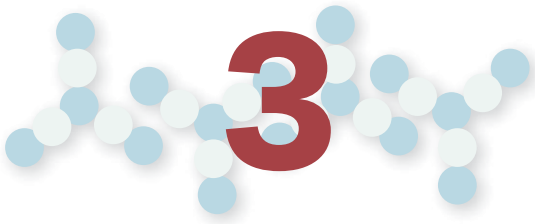
Not all bacteria live in the soil, air, or water: Many live on the skin or inside the bodies of animals. Those that live inside an animal's body are restricted to the oral cavity, the throat, and the digestive tract. For the most part, these bacteria are harmless, sometimes even beneficial. But there are a few that can make people and animals seriously ill, and in some cases the disease can be fatal. Some of the pathogenic bacteria that infect humans are species from the *Salmonella, Streptococcus,* and *Staphylococcus* genera, which cause food poisoning and, in the case of *Streptococcus,* serious throat infections. Although these bacteria can make people very sick, the diseases they produce are mild compared to those produced by especially virulent bacteria such as *Bacillus anthracis* and *Clostridium botulinum.*

The most notorious among this latter group is *Bacillus anthracis,* a spore-forming bacterium responsible for anthrax, a disease affecting cattle and humans. When an animal inhales *B. anthracis* spores it is like offering the bacteria a large plate of food. The spores germinate very quickly into vegetative cells and begin consuming the victim's lung tissue. Death usually follows within a few days. Antibiotics are available for this disease but are usually ineffective unless given to the patient on the day the spores are inhaled.

B. anthracis has been used by bioterrorists, who have exploited the deadliness of the disease and the hardiness of the anthrax spores, which can remain viable for years. The spores, which to the naked eye look like a fine powder, were used in 2001 to contaminate letters

that were sent to various destinations in the United States. Several people died after being exposed to these letters. Later that year, George W. Bush authorized the formation of the National Biodefense Analysis and Countermeasures Center (NBACC), a top-secret research facility that was originally located at the U.S. Army's aging biodefense campus at Fort Detrick in Frederick, Maryland. This site is one of only two facilities in the country officially permitted to handle the most dangerous biological agents on Earth (the other is at the Centers for Disease Control in Atlanta). In 2008, construction was completed on a new facility for NBACC (pronounced en-back) at Fort Detrick. The eight-story building houses NBACC's two major divisions: a forensic testing center to identify possible culprits in future biological attacks and the Biothreat Characterization Center (BTCC), which is charged with the task of predicting the nature of such attacks. Although well intentioned, the top-secret nature of the facility and the fact that extremely dangerous microbes will be cultured there have drawn extensive criticism from observers and the general public. Critics maintain that NBACC does not comply with the Biological and Toxin Weapons Convention, a 1972 international treaty outlawing the production and storage of biological weapons. Currently, the Obama administration, conferring with the previous administration, maintains that NBACC research is of a defensive nature and thus does not contravene the intent of the treaty. Facilities similar to NBACC have been built or are being planned in Europe, India, China, and Cuba.

The real threat of bioterrorism and the construction of facilities like NBACC call for the ratification of a new treaty that will address the conflicting issues of secrecy, to keep the information away from bioterrorists, and transparency, so that the public and government officials can make intelligent decisions about the implementation of biodefense strategies and policies.



Eukaryotes: Dawn of a New Era

The only thing in nature more complicated than a eukaryote is a bunch of eukaryotes working together; the heart is one example, the human brain is another. The complexity of these cells came about as a result of necessity and the struggle for survival. Life began in coastal waters that were filled with nutrients, produced by the heat and storms of prebiotic Earth. As the first cells consumed those nutrients, selection pressures led to the appearance of phototrophic prokaryotes, known as autotrophs, which could obtain their energy through photosynthesis. But many prokaryotes, known as heterotrophs, lacked this ability and in order to survive began hunting other prokaryotes for food. Scientists believe the eukaryotes evolved from this latter group of hunting heterotrophs. The new adaptations not only helped the eukaryotes hunt down their prey but also made it possible for those cells to communicate

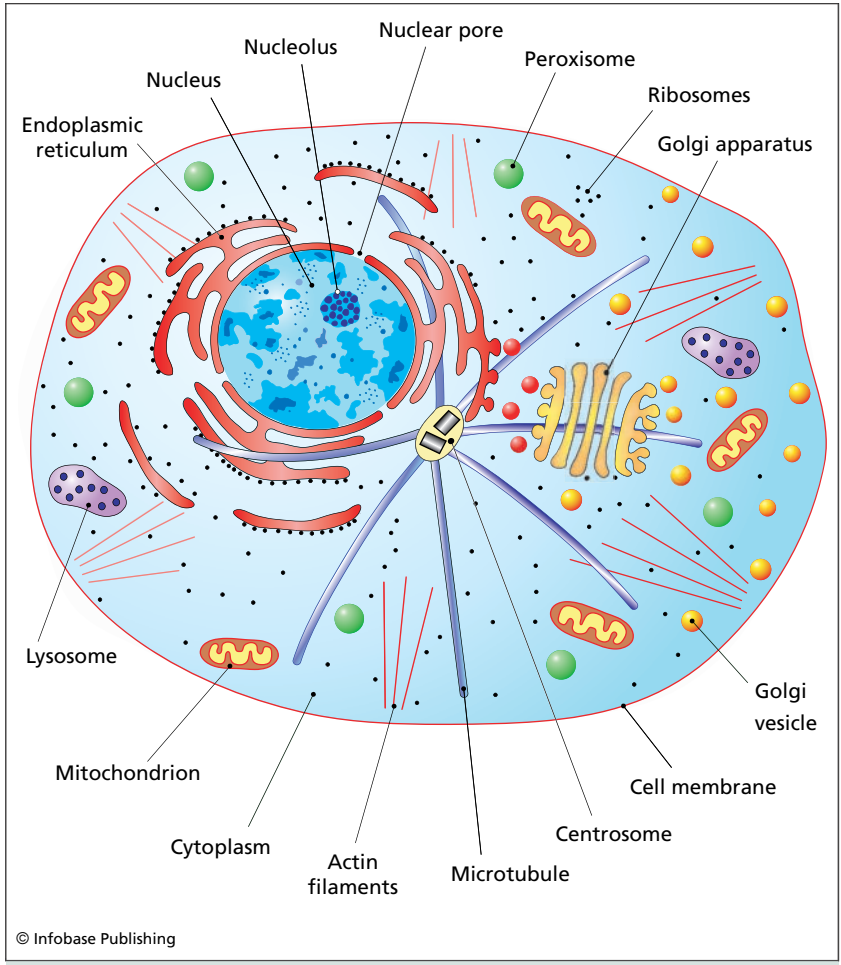
with one another and eventually to form colonies and multicellular creatures.

The emergence of the eukaryotes was an important event not only because a new life-form had emerged but also for the survival of the entire ecosystem. Eukaryotes went on to produce enormous populations of photosynthetic organisms both in the water and on the land. Decomposing land plants and animal excretions produced soil, which in turn provides a habitat for billions of prokaryotes and other creatures. These habitats could not have been produced by prokaryotes alone. Thus it is possible that without the eukaryotes life may have died out on this planet sometime after the first cells consumed the prebiotic nutrients. The eukaryotes that took the evolution of life to a new level and the adaptations that made this possible and distinguish them from the prokaryotes are the subject of this chapter.

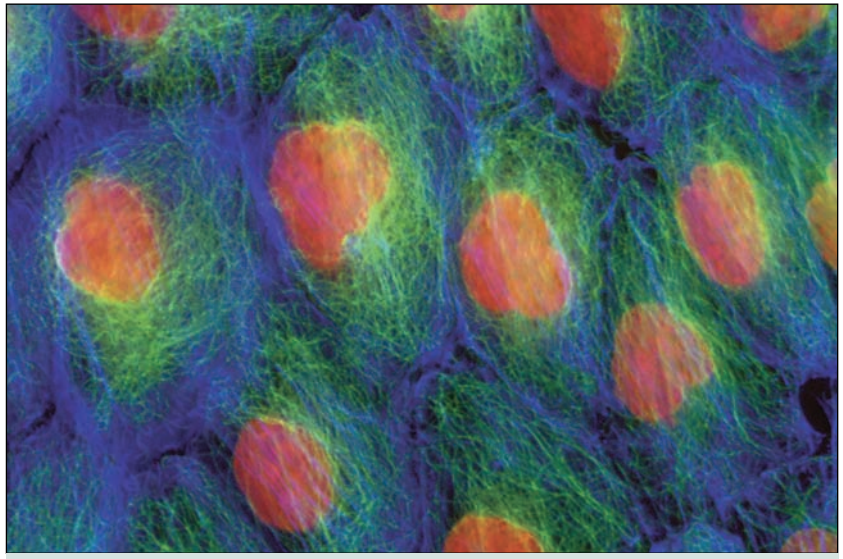
OVERVIEW

Eukaryotes (meaning "true nucleus") are much more complex than prokaryotes. In prokaryotes, genes are replicated, transcribed, and translated all in one compartment, the cell's protoplasm. Eukaryotes changed all of this by setting up special membrane-bounded compartments, or organelles, for each job. While the cytoplasm of a prokaryote is plain and homogeneous, the interior of a eukaryote is a maze of organelles, which includes the nucleus, nucleolus, endoplasmic reticulum (ER), Golgi complex, mitochondria, lysosomes, peroxisomes, and a weblike skeleton of protein fibers.

The eukaryote nucleus, bounded by a double phospholipid membrane, contains a DNA (deoxyribonucleic acid) genome on two or more linear chromosomes, each of which may contain thousands of genes. The nucleus also contains an assembly plant for ribosomal subunits called the nucleolus. The ER and the Golgi complex work together to glycosylate proteins and lipids (they attach sugar molecules to the proteins and lipids producing glycoproteins and

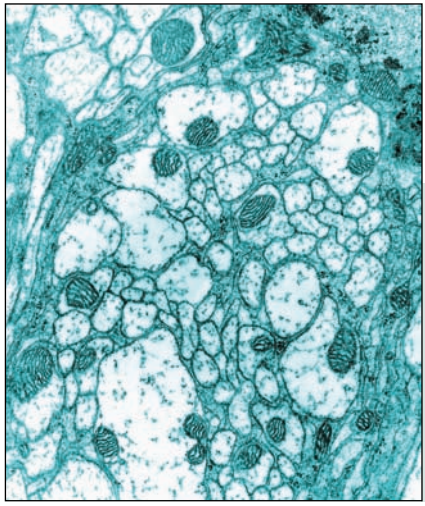


The eukaryote cell. The structural components shown here are present in organisms as diverse as protozoans, plants, and animals. The nucleus contains the DNA genome and an assembly plant for ribosomal subunits (the nucleolus). The endoplasmic reticulum (ER) and the Golgi work together to modify proteins, most of which are destined for the cell membrane. These proteins are sent to the membrane in Golgi vesicles. Mitochondria provide the cell with energy in the form of ATP. Ribosomes, some of which are attached to the ER, synthesize proteins. Lysosomes and peroxisomes recycle cellular material and molecules. The microtubules and centrosome form the spindle apparatus for moving chromosomes to the daughter cells during cell division. Actin filaments and a weblike structure consisting of intermediate filaments (not shown) form the cytoskeleton.

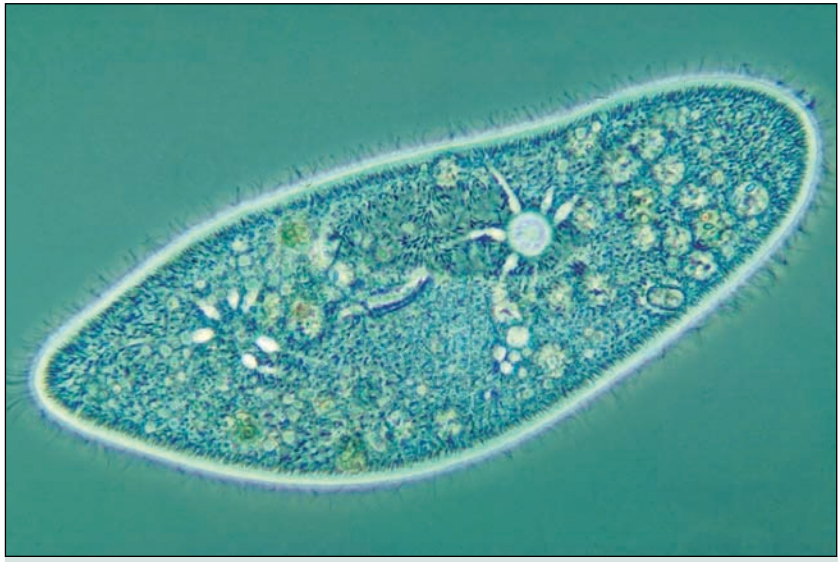


Color-enhanced micrograph of epithelial cells. The cells are isolated from the pancreas showing the distribution of DNA (red), cytoskeleton microtubules (green), and actin (blue). Magnification 2,520×. *(Jennifer Waters Shuler/Photo Researchers, Inc.)*

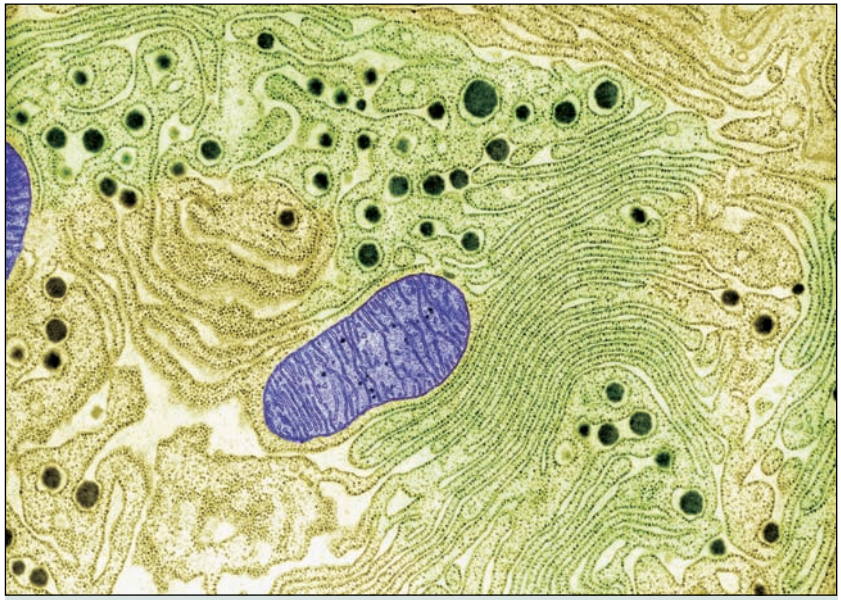
glycolipids), most of which are destined for the cell membrane to form a molecular forest known as the glycocalyx. The glycoproteins and glycolipids travel from the ER to the Golgi, and from the Golgi to their final destinations, in membrane-bounded vesicles (bubbles) that form by budding off the organelle by exocytosis. Thus, the cytoplasm contains many transport vesicles that originate from the ER and Golgi. The Golgi vesicles bud off the outer chamber or the one farthest from the ER. Mitochondria, once free-living prokaryotes, and the only other organelles with a double membrane, provide the cell with energy in the form of adenosine triphosphate (ATP). The production of ATP is carried out by an assembly of metal-containing proteins called the electron transport chain, located in the mitochondrion inner membrane. Ribosomes, some of which are attached to the ER, synthesize proteins. Those portions of the ER containing ribosomes are known as rough ER (RER). Lysosomes and peroxisomes recycle cellular material and molecules.



Color-enhanced transmission electron micrograph (TEM) of a section of a housefly's brain. This TEM shows the large number of mitochondria that is typical of extremely active tissue. Magnification 19,250×. *(courtesy of the author)*



Micrograph of the protozoan, *Paramecium candatum*. These cells have a characteristic shape of a slipper and their outer surface is covered in cilia. The cell interior is filled with circular food vacuoles. The bright star-shaped structures are contractile vacuoles, and the macronucleus can be seen lying below the one to the right. Magnification 160×. *(M. I. Walker/Photo Researchers, Inc.)*



Color-enhanced TEM of a bat pancreas cell, showing mitochondria and endoplasmic reticulum. Magnification 36,300×. *(Omikron/Photo Researchers, Inc.)*

The microtubules and centrosome form the spindle apparatus for moving chromosomes to the daughter cells during cell division. Actin filaments and a weblike structure consisting of intermediate filaments form the cytoskeleton, which gives the cell its strength, resiliency, and characteristic shape.

The functional organization of a eukaryote is similar to a carpentry shop. Imagine such a place where carpenters use a copy of a master blueprint, stored in the shop's office, to build wooden chairs. This shop builds many different kinds of chairs, and it keeps a blueprint for each one. All of the machines and tools that the carpenters use to build the chairs are located on the shop floor. Every morning, someone in the office takes a blueprint out of the filing cabinet, makes a photocopy of it, and then gives it to one of the carpenters. This blueprint may represent a standing order, or the shop may have

received an order that morning to produce a special chair. After the chairs are made, some of the carpenters take them into a finishing room, where the frames are painted, after which they go to a shipping room where they are packaged and sent out to the customers. The energy to power the tools and to heat and illuminate the shop comes from electricity produced at a hydroelectric dam. The shop communicates with its customers and with other shops by using the telephone or by sending a letter or an e-mail.

In a eukaryote cell, the shop floor is the cytoplasm and the shop office is the nucleus. The blueprints for everything that the cell makes are kept in the nucleus, but instead of being pictures on a piece of paper they are made out of DNA. The cell makes proteins of various kinds, and it keeps a blueprint for each one. The blueprints are genes that are arranged, end-to-end, on a very long molecule of DNA. That molecule is the chromosome, and eukaryotes always have more than one.

A eukaryote makes copies of some of its genes every day. Each copy is called a messenger RNA (mRNA), and after being delivered to the cytoplasm it is used to guide the production of a protein. The cell's carpenters are enzymes that use translation machinery in the cytoplasm to build the proteins. Some of the proteins remain in the cytoplasm to help run the cell, but most are sent to the ER, analogous to the finishing room, where they are glycosylated (painted with sugar), and then to the Golgi complex, where they mature before being packaged for export. These structures, the ER and the Golgi complex, have no counterparts among the prokaryotes. The sugar-coated (glycosylated) proteins from the Golgi are sent to the cell surface, where they form the glycocalyx. As described in chapter 2, the trunks of the trees in this forest are made from protein and the leaves are made from sugar molecules (the "glyco" part). The glycocalyx is part of the cell's communication hardware, allowing it to send and receive messages. Cell-surface glycoproteins also form the transporters and ion channels that serve as gateways into the cell. Prokaryotes have a

glycocalyx, but its form and function are very simple by comparison. Eukaryotes have refined this hardware to such an extent that a neuron in the human brain can send a signal to the big toe in a fraction of a second. Eukaryotes, like carpentry shops, consume a great deal of energy every day. Carpentry shops get their energy from a hydroelectric dam, whereas eukaryotes get their power from the mitochondria. A hydroelectric dam supplies power in the form of electrons, but mitochondria supply it in the form of ATP.

Although organized like a carpentry shop, the internal structure of a eukaryote is more like that of a coral reef. The cell's "reef" consists of the ER and the Golgi complex—the ER being the larger of the two, fanning out from the nucleus, like the rings of Saturn. A real coral reef is a very busy place, with a multitude of colorful fish going about their daily business. Traffic in and around the Golgi complex and the ER is equally hectic, with thousands of multicolored proteins, RNAs, and smaller molecules speeding around as they take care of a thousand chores each day. Traffic between the Golgi complex and the cell membrane alone consists of a constant stream of millions of transport bubbles.

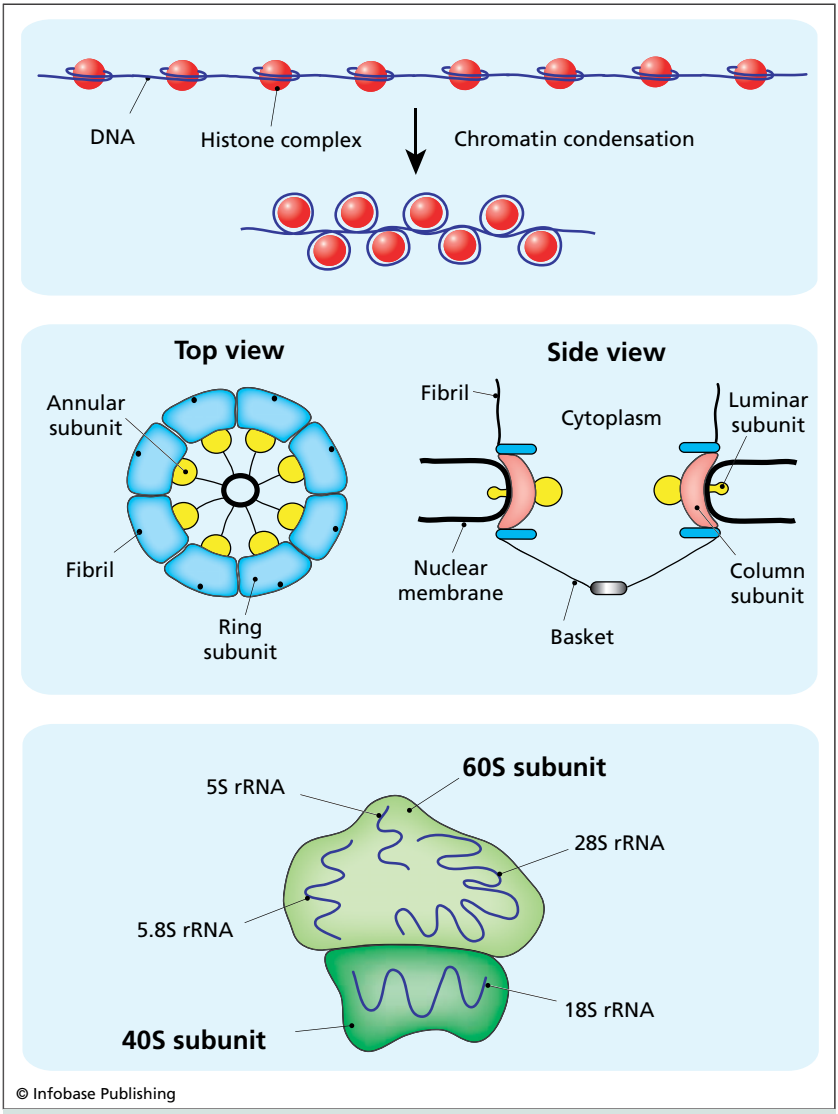
The behavior of eukaryote enzymes, particularly in the nucleus, is akin to that of shepherds tending their flock. Chromosomes, for example, are the most passive macromolecules in the cell, and they are tended by a special group of enzymes that act very much like shepherds. The enzymes move the chromosomes around in preparation for cell division, regulate the duplication (replication) of each chromosome and the copying of each gene (transcription), and are constantly inspecting the chromosomes for damage, repairing them when necessary. Why do these molecular shepherds spend so much time and energy caring for the chromosomes? The relationship between DNA and enzymes is an example of the most ancient and most successful cloning experiment. Enzymes have short lives, but a new and exact copy can always be produced as long as the genes are cared for and kept in good shape.

THE NUCLEUS

The nucleus houses the chromosomes and all of the enzymes necessary to replicate, transcribe, and repair the genes. The complexity of a eukaryote, compared with a prokaryote, suggests a larger genome, and this has been confirmed with the completion of the human genome project and the sequencing of several prokaryote genomes (discussed further in chapter 5). Bacteria usually have 2,000 to 4,500 genes, compared with 30,000 in the human genome. This one difference is at the heart of all the other differences between the prokaryotes and the eukaryotes.

Prokaryotes pack all of their genes onto a single, ring-shaped chromosome, but a structure such as this could not be used to store 30,000 genes. Eukaryotes solved this problem by splitting their genome into several linear chromosomes. Human cells, for example, have 46 chromosomes, 23 originating from the mother and 23 from the father. Each chromosome is a single, extremely long DNA molecule. This type of chromosome solves the problem of storing a large number of genes, but it becomes a problem when the cell tries to divide. Imagine unwinding and untangling 46 pieces of thread, each of which is several miles long, so they can be laid out, side-by-side, in nice, even rows. The chromosomes have to be aligned in this way for the successful completion of the cell cycle.

Moving long genetic threads around is a major problem, but one that eukaryotes solved by coating their chromosomes with special proteins, called histones, that can be manipulated with phosphokinases (enzymes that phosphorylate or add phosphate groups to proteins). Consequently, the chromosome of a eukaryote is not naked DNA, as is that of a prokaryote. There are several kinds of histones, most of which form a spherical structure called a nucleosome that makes the relaxed chromosome look like a string of beads. The complex of DNA and histones is called chromatin. Phosphorylating the nucleosomes is like releasing a stretched rubber band: The chromosome contracts to form a compact structure that is 10,000 times shorter than the bare piece of DNA. Just as a suitcase makes it possible for us to take our clothes on a trip, histones and the chromatin



Components of a eukaryote cell. Eukaryote chromosomes (top panel) are a complex of DNA and histones, called chromatin, which exists in extended and condensed states. The nuclear pore (middle panel) is an octagonal structure built from many protein subunits that provide a channel through the nuclear membrane. The ribosome (bottom panel) consists of two protein subunits (40S, 60S) that are associated with different ribosomal RNAs (rRNA). Translation is initiated when the two subunits bind to messenger RNA (mRNA).

structure they produce make it possible for the cell to package its genes in preparation for cell division.

Chromatin compaction, or condensation, is also used during interphase (the period between cell divisions) to help manage the chromosomes and as one mechanism for controlling gene expression. The packing ratio of interphase chromatin is about 1:1,000 over all, but there are regions where it can be much lower (the density increases as the ratio decreases). This variation in the density of the chromatin accounts for the blotchy appearance of most interphase nuclei. Areas of the nucleus that are very dark represent highly compacted chromatin, whereas the lighter regions contain chromatin in a more relaxed state. At the molecular level, chromatin condensation is an extremely dynamic process that is used to close down single genes or whole neighborhoods consisting of hundreds of genes. The mechanism by which this occurs is fairly straightforward: Highly condensed chromatin blocks the transcription machinery so it cannot get access to the gene. Details of this mechanism and others that control gene expression will be discussed in chapter 5.

Keeping the genes in one compartment and the protein-manufacturing machinery in another compartment requires a constant flow of traffic across the nuclear envelope. This traffic is not just the mRNA moving out to the cytoplasm; it also includes the proteins that are synthesized in the cytoplasm but stationed inside the nucleus. Histones, for example, must be imported at a rate of 1 million every three minutes. DNA replication and repair enzymes, and the protein components of the ribosome, which are assembled in the nucleolus, are also part of the traffic.

Eukaryotes evolved an adjustable nuclear pore with an exquisite octagonal geometry to cope with all the traffic moving in and out of the nucleus. The pore is constructed from four subunits, each of which is composed of more than one kind of protein and a basket-like assembly on the nuclear side. Altogether, nearly 50 different kinds of protein are used to construct this pore, and a typical cell

has 3,000 to 4,000 of them, evenly spaced over the surface of the nuclear envelope. At certain magnifications, the pores make the nuclear envelope look like the crater-marked surface of the Moon, although, unlike moon craters, nuclear "craters" are all the same size. See the figure on page 61.

The nuclear pore blocks the passage by simple diffusion of any molecule larger than a billionth of an inch (nine nanometers or 10^{-9} m) in diameter. Nuclear proteins that are larger than this gain access by a specific interaction with the cytosolic fibrils that causes the pore to open, somewhat like the iris diaphragm of a camera, just enough to let the molecule in. Scientists know the interaction between the protein and the fibril involves a nuclear localization sequence (NLS) on the protein itself, and, without it, the pore will not allow the protein to pass. The NLS is like a password or a passport written in the language of an amino acid sequence, usually located at the very end of the protein. A common password for a nuclear protein is lysine-lysine-lysine-arginine-lysine. If a nuclear protein presented itself to a nuclear pore with a mutated password, such as lysine-threonine-lysine-arginine-lysine, it would not get in. Scientists have been able to trick the nuclear pore by adding the correct password to a protein that does not normally belong in the nucleus. Such experiments have made it clear that the pore checks the password only and does not care about the identity of the protein. The mechanism by which the pore is able to recognize the correct password and to vary the diameter of its channel accordingly is still a mystery.

PROTEIN SYNTHESIS

Eukaryotes synthesize their proteins using ribosomes and the genetic code, just as prokaryotes do. But (as explained in chapter 2) ribosomes are constructed from a mixture of protein and RNA, and this fact created a problem for the eukaryotes. The RNA is made in the nucleus, but the protein is made in the cytoplasm: where should the ribosome be made? The RNA could be synthesized and shipped

out to the cytoplasm, where the ribosome could be assembled, or the protein components could be shipped into the nucleus for final assembly. But given that mRNA is also synthesized there, assembly of the ribosome in the nucleus could lead to a real disaster. If the ribosomes encountered the mRNA, the cell would end up synthesizing proteins in both the nucleus and the cytoplasm, which is exactly what the two compartments were designed to prevent. What a quandary! One solution would be to remake the ribosome, so it is all protein or all RNA. However, this would be a formidable task because the ribosome is a very complex structure that took many millions of years to evolve.

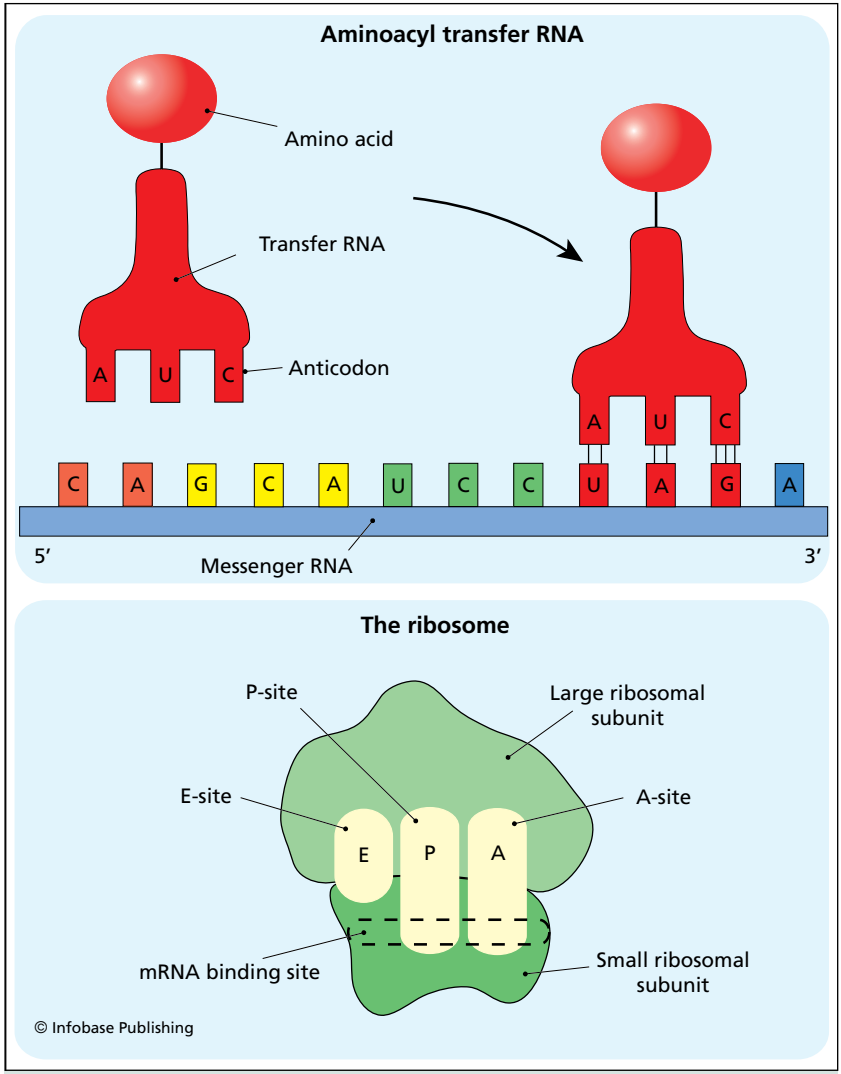
To appreciate the complexity of the ribosome, let us examine its components. The eukaryote ribosome consists of two major complexes, called the 40S and 60S subunits (the *S* refers to their rate of sedimentation in a centrifuge; the larger the S value, the larger the rRNA). The 40S subunit consists of 33 proteins and a single 18S (having 1,900 nucleotides) ribosomal RNA molecule (rRNA). The 60S subunit has 50 proteins and three different rRNA molecules that range in size from 120 nucleotides (5S) to 4,700 nucleotides (28S). A functional ribosome is produced when the 40S and 60S subunits bind to an mRNA. See the figure on page 61.

Trying to recreate such a complex structure is out of the question. Instead, the eukaryotes settled on a compromise strategy to keep the ribosome as it is (although they did add a few extra proteins and one extra RNA molecule) and to assemble the subunits in the nucleus but not join them together there. Subunit assembly occurs in the nucleolus, where the rRNAs are synthesized and where ribosomal proteins collect after entering the nucleus from the cytoplasm. Once assembled, the inactive subunits can be safely transported to a pore. Biologists believe the final activation of each subunit occurs as they pass through separate nuclear pores. Once they are in the cytoplasm they are free to associate with mRNA to initiate translation.

A crucial component of the translation machinery is transfer RNA (tRNA). This RNA acts like an adapter that matches a particular amino acid with its corresponding codon, as defined by the genetic code. When a tRNA is linked to an amino acid it is known as aminoacyl-tRNA. There is a separate tRNA for each amino acid that carries a three base-pair sequence, known as the anticodon, that is complementary to the amino acid's codon. The anticodon hybridizes with the codon by forming double or triple chemical bonds, with adenine (A) always pairing with uracil (U) and cytosine (C) always pairing with guanine (G). James Watson and Francis Crick discovered these pairing rules in the 1950s as they worked out a model for DNA structure. DNA nucleotide pairing rules were subsequently shown to apply to RNA base pairing as well. The translation of mRNA into protein requires extensive interaction between the ribosome and tRNAs. Consequently, the ribosome has three binding sites for these RNA molecules: the aminoacyl-tRNA site (the A-site), the peptidyl-tRNA site (the P-site), and the exit site (the E-site). The binding site for the messenger RNA is located on the small ribosomal subunit.

Translation occurs in the following three steps:

1. An aminoacyl tRNA enters the A-site where it hybridizes with the codon.
2. The ribosome joins the growing amino acid chain (also called a peptide or protein chain) to the new incoming amino acid and breaks the acyl bond holding the peptide chain to the tRNA docked in the P-site. (If the incoming aminoacyl tRNA is the first, the ribosome simply proceeds to the next step.)
3. The ribosome moves along the mRNA, in the 3′ direction, ejecting the tRNA in the P-site (if one is there), replacing it with the new peptidyl tRNA, while at the same time bringing the next codon into the A-site.



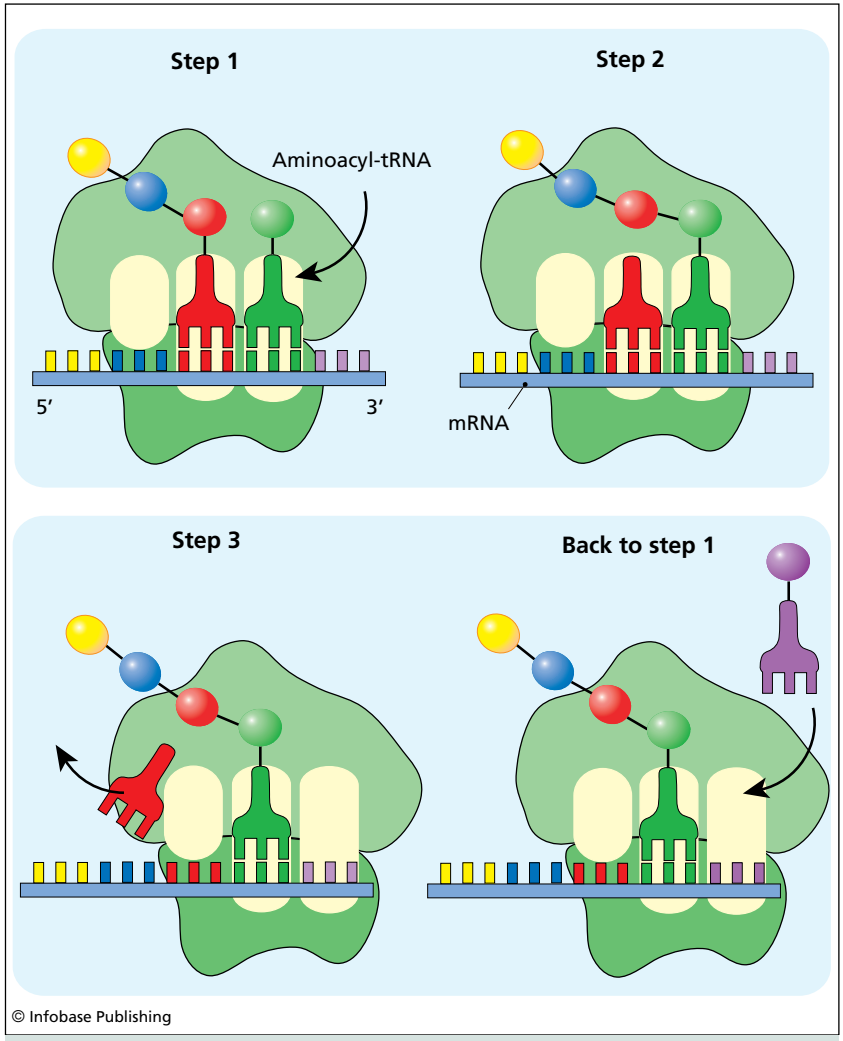
Translation machinery. Transfer RNA (tRNA) serves a crucial role in matching an amino acid to a specific codon. When tRNA is bound to an amino acid it is called aminoacyl tRNA. There is a separate tRNA, with an appropriate anticodon, for each amino acid. The anticodon forms double or triple bonds with the codon, with adenine (A) always pairing with uracil (U), and cytosine (C) always pairing with guanine (G). The ribosome (bottom) contains three tRNA binding sites: the aminoacyl-tRNA site (A), the peptidyl-tRNA site (P), and the exit site (E). The small subunit contains the binding site for the messenger RNA (mRNA).

The first step repeats when a new aminoacyl tRNA enters the A-site. The three steps repeat until a stop codon, such as UAG, appears in the A-site, thus terminating translation. The ribosome always translates the mRNA in the 5′ to the 3′ direction. As previously described, amino acids are joined together by a peptide bond that links the carboxyl end (COOH) of one amino acid to the amino end (NH2) of a second amino acid. Consequently, the codon for the first amino acid in the chain (the amino end of the protein) is always at the 5′ end of the mRNA. Likewise, the codon for the last amino acid in the chain (the carboxyl end of the protein) is at the 3′ end of the mRNA. The amino terminus of the translated protein in the figure is indicated by the yellow amino acid. (See figure on page 68.)

The translation machinery is helped along by two elongation factors, known as EF-1 and EF-2. These factors accelerate the process by supplying energy to the ribosome for the many manipulations it has to perform. EF-1 also serves to improve the accuracy of translation by escorting the incoming aminoacyl tRNA to the A-site where it inhibits the formation of the peptide bond until after the codon-anticodon pairing has been verified. Verification is the job of the ribosome; if the pairing is verified, EF-1 dissociates, thus allowing the formation of the bond and elongation of the amino acid chain. If the pairing is not verified, the incoming aminoacyl tRNA is ejected. Ribosomes can be forced to translate mRNA in a test tube without the elongation factors and, when this is done, the ribosome's accuracy is only about 60 percent. In the presence of EF-1 and EF-2, the accuracy of the ribosome is nearly 100 percent.

DESIGNING A MOLECULAR FOREST

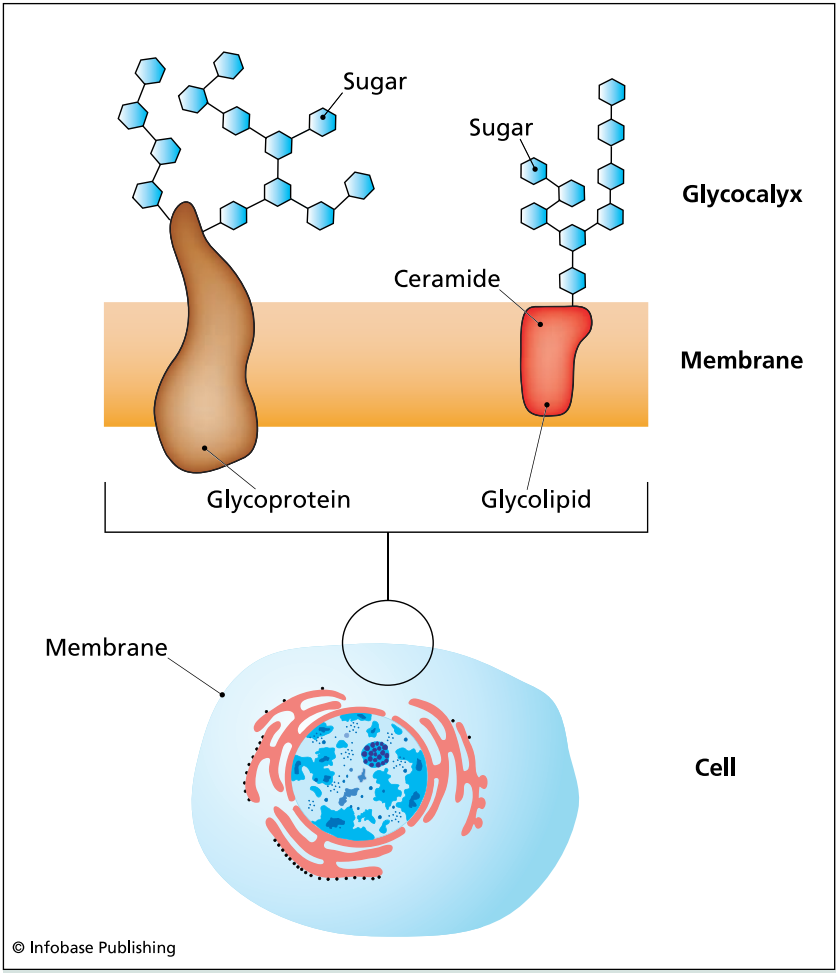
The eukaryote glycocalyx is more complex than the one produced by prokaryotes. Prokaryotes use the glycocalyx to collect and import food molecules, whereas the eukaryotes use it both for food collection and for cell-to-cell communication. Consequently, the eukaryote glycocalyx has a much more diverse collection of glycoproteins embedded in the membrane, and there is a higher proportion of glycolipids, particularly in nerve cells. Although it gets less



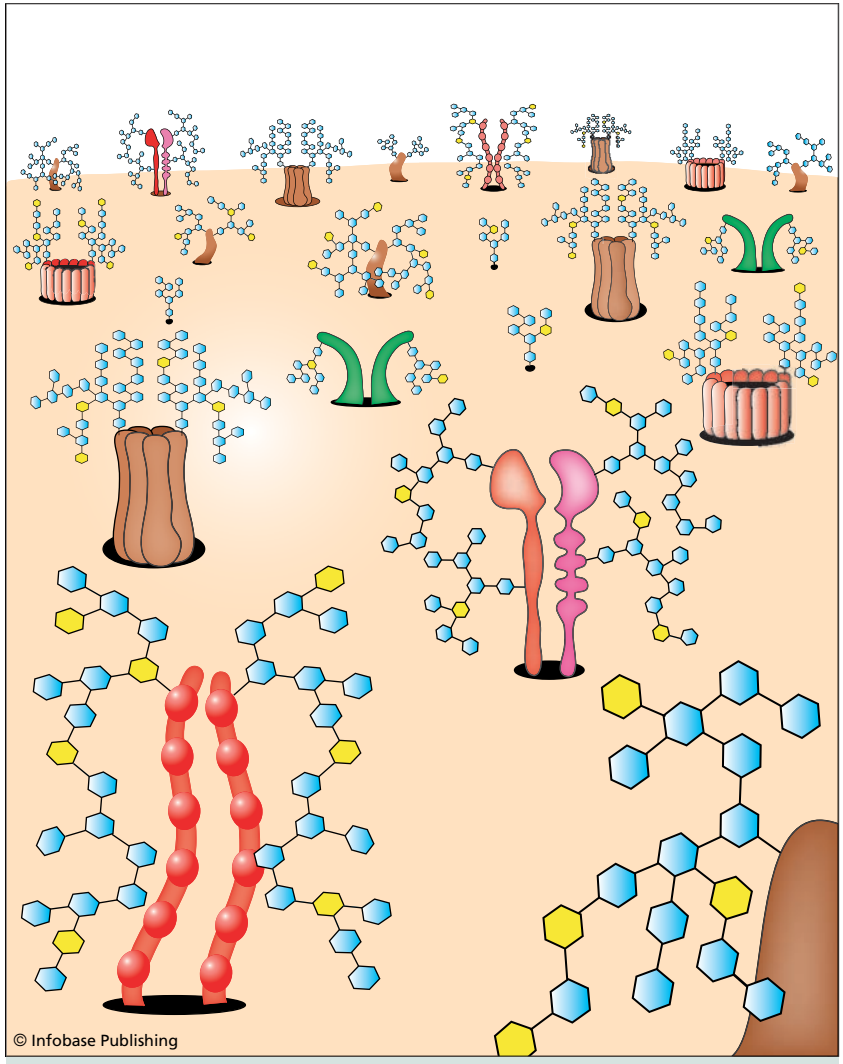
Translation. An aminoacyl-tRNA (green), with the appropriate anticodon, enters the A-site where it hybridizes with the codon (step 1). The ribosome joins the growing amino acid chain to the new incoming amino acid and breaks the acyl bond on the red aminoacyl tRNA (step 2). The ribosome moves to the right, in the 3′ direction, ejecting the red tRNA and bringing the next codon (violet) into the A-site (step 3). Step 1 repeats when the violet-colored aminoacyl-tRNA binds to the codon in the A-site. The yellow amino acid marks the amino terminus of the protein, which always corresponds to the 5′ end of the mRNA.

attention than the nucleus and DNA, the glycocalyx is arguably the most important structure the cell has. To illustrate, imagine an artist's studio, with paintbrushes, tubes of paint, easels, and canvases lying around. What is the most important thing in that studio? Surely the paintbrushes are the most important for without them the picture could not be produced. Someone else might say, "No, the paints are the most important, for you can always get along without brushes." But neither of these is as important as the painting itself. Everything in the studio is there for that one purpose: to paint the picture. No matter how beautiful or well-crafted the brushes and the easel are, they are merely the tools of the artist's trade. It is a similar situation in the cell. There are many fine structures, all carefully crafted, but they are there for one purpose: to make the glycocalyx, for without it the cell would have no eyes, no ears, no way to collect food, and no way to communicate with other cells and the outside world. Indeed, the two greatest structures in the cell, the ER and the Golgi complex are devoted to the production of glycoproteins and glycolipids, most of which are used to build and maintain the glycocalyx.

Proteins destined for the cell membrane carry a marker sequence much in the way nuclear proteins carry an NLS. When the ribosome detects the marker sequence, it moves to the surface of the ER where it threads the protein through a pore as it is being synthesized. Ribosomes, located on the surface of the ER, can be clearly seen in electron micrographs; those areas are referred to as the rough ER. Once the protein is inside the ER, it is glycosylated by several different enzymes that add the sugar molecules sequentially. This is analogous to a team of painters working on the same canvas. One painter might lay in the sky and ground, after which another paints the clouds and rocks. The glycosylating enzymes seem to follow a set of rules, because the same kinds of glycoproteins are produced over and over again, but the nature of those rules is still unclear. When the enzymes in the ER are finished, the glycoprotein is loaded into a transport vesicle (bubble) and sent to the Golgi complex.



The eukaryote glycocalyx. The eukaryote's molecular forest consists of glycoproteins and glycolipids. Two examples are shown at the top, a glycoprotein on the left and a glycolipid on the right. The glycoprotein trees have "trunks" made of protein and "leaves" made of sugar molecules. Glycolipids also have "leaves" made of sugar molecules, but the "trunks" are a fatty compound called ceramide that is completely submerged within the plane of the membrane. The glycocalyx has many jobs including cell-to-cell communication and the transport and detection of food molecules. It also provides recognition markers so the immune system can detect foreign cells.



A panoramic view of the glycocalyx. The glycoproteins in the cell's forest come in many different shapes and sizes, and they dominate the surface of most cells. The glycolipids all have the same ceramide trunks, but the molecular foliage varies considerably. All but three of the structures in this image are glycoproteins, but in nerve cells they are much more common.

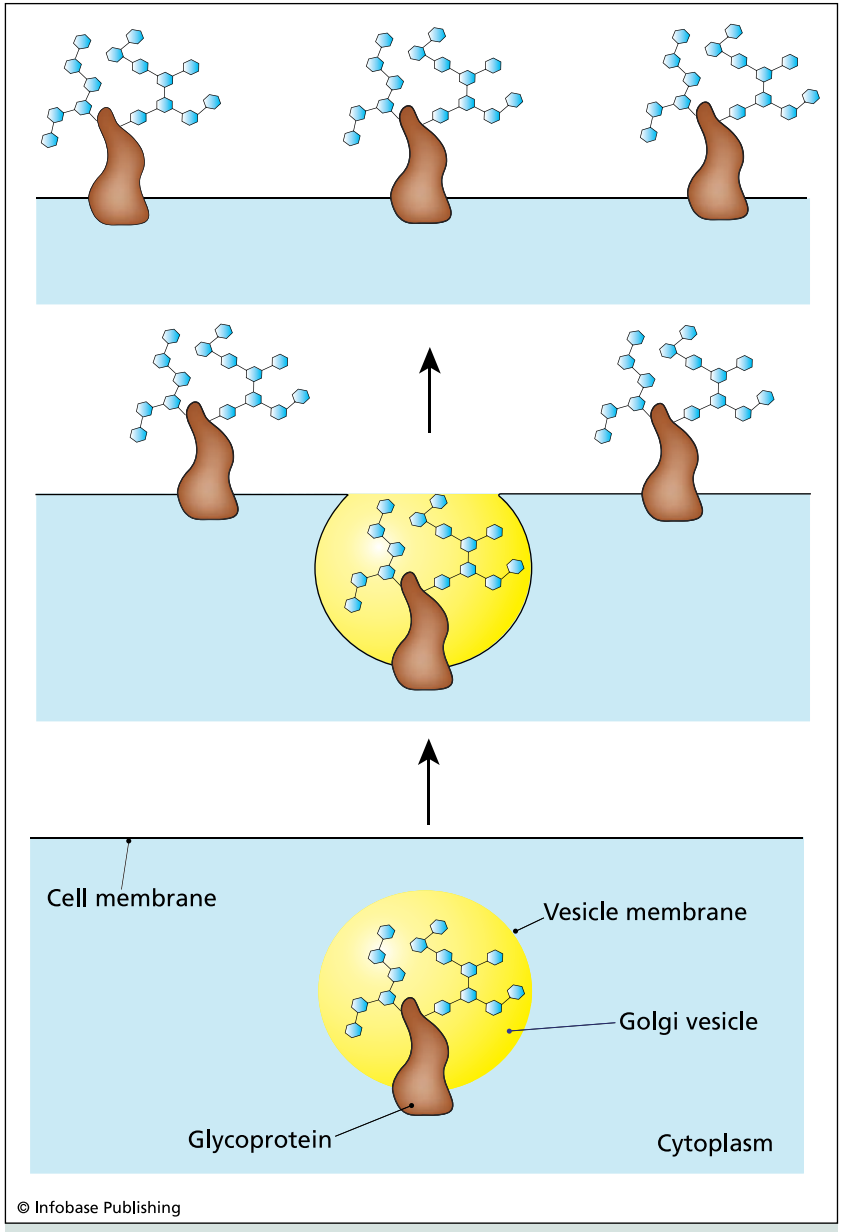
The Golgi complex has its own group of enzymes that specialize in refining the process begun in the ER. They begin by adding more sugar molecules. As successive enzymes modify the glycoprotein (now looking very much like a molecular tree), it moves from one Golgi chamber to the next, each time being transported by a Golgi vesicle. In each chamber, enzymes add, trim, or prune the molecular leaves on the trees. Again, as with ER glycosylation, the decision-making process guiding these tasks is poorly understood. When the enzymes are satisfied with the shape and appearance of the glycoprotein, it is loaded into a Golgi vesicle and sent out from the trans Golgi network (the outermost chamber) to the cell membrane. Fusion of the vesicle membrane with the cell membrane automatically plants the molecular tree.

Not all of the glycoproteins that pass through the ER or Golgi are destined for the cell membrane. Many are intended for the lysosomes, and some are secreted. Indeed, Golgi vesicles carrying lysosomal glycoproteins become that organelle, as will be described later in this chapter.

SYMBIOSIS AND THE QUEST FOR POWER

Prokaryotes developed two aerobic systems for extracting energy from food molecules: the Krebs cycle, which stores most of the energy as electrons, and the electron transport chain, which uses the energy to make ATP. In eukaryotes, both processes occur in an organelle called the mitochondrion. Consequently, these organelles are responsible for providing the cell with the ATP it needs to power all of its biochemical reactions (although a small amount of ATP is provided by glycolysis, which is carried out in the cell's cytoplasm).

For many years, cell biologists believed the mitochondria originated with the eukaryotes, but in 1910 the Russian biologist Konstantin Mereschovsky suggested that these organelles were once free-living prokaryotes. The earliest evidence in support of this notion, presented by the American biologist Lynn Margulis in 1970 focused on the fact that mitochondria have their own genome



Planting a molecular forest. Vesicles from the Golgi complex carry glycoproteins to the cell surface. Fusion of the vesicle membrane with the cell membrane automatically plants the molecular tree.

and are morphologically similar to bacteria. Since then, Margulis and others have described several interesting similarities between mitochondria and prokaryotes:

- Mitochondria divide by simple binary fission, as do bacteria.
- Synthesis of ATP occurs through the same pathway used by bacteria.
- Mitochondria and bacteria both have a single, circular DNA chromosome.
- The mitochondrial genome is very similar in structure and organization to that of many bacteria.

Today, most scientists accept the symbiogenesis theory, which states that the evolution of eukaryotes was associated with the acquisition of symbionts, particularly mitochondria (in animals) and chloroplasts (in plants), about 2 billion years ago. Consequently, mitochondria have been handed down from one generation to the next ever since that time. In addition, for all the animals in the world, inheritance of mitochondria is through the maternal line. They get their mitochondria from their mothers. This fact has been applied by scientists at the University of California, Berkeley, who traced the origins of the human species to a single "mitochondrial Eve" who apparently lived in Africa 100,000 to 200,000 years ago.

RECYCLING AND DEFENSE

The lysosome is a cell organelle that is responsible for recycling worn-out cell parts, such as mitochondria, membranes, and various molecules. Lysosomes are vesicles that are filled with powerful enzymes that can break down virtually any structure or component of the cell. Lysosomal enzymes are glycoproteins that are sent through the ER and Golgi complex, but the Golgi vesicles containing these

enzymes stay in the cytoplasm (rather than fusing with the cell membrane), where they mature into functional lysosomes.

Lysosomes are also very important for maintaining the cell membrane and other membrane-bounded structures. Traffic through the ER and the Golgi complex results in the fusion of ER vesicles with the Golgi and the fusion of Golgi vesicles with the cell membrane. If steps were not taken to recycle parts of both membrane systems they would grow very large, very quickly. In the case of the cell membrane, regular rounds of endocytosis serve to keep the membrane at a constant size. The endocytotic vesicle is fused with a lysosome where the parts are broken down, with some being returned to the Golgi and ER.

Cellular defense among animals is usually left to the immune system. But each cell does have built-in defense capabilities, left over from the time when cells were free living. This defense mechanism involves a coordination between the lysosomes and endocytosis. One form of endocytosis, called phagocytosis, makes it possible for the cell to engulf an invading bacterium. The phagocytic vesicle containing the bacterium fuses with a lysosome and breaks down the microbe to individual molecules, which are released into the cytoplasm or expelled from the cell. All eukaryotes have this defensive capability, but it is used most effectively by macrophages, an important cellular member of an animal's immune system.

THE CELLULAR RAILWAY

Vesicles that are released from the ER and the Golgi complex are not simply set loose to float freely through the cytoplasm. Each vesicle is equipped with special membrane-bound glycoproteins that are used to anchor it to a railway constructed of microtubules. Indeed, the ER and the Golgi are both anchored to this railway, which crisscrosses the cytoplasm in many different directions. The railway is part of the cytoskeleton.

The attachment of a vesicle to a microtubule is mediated by several proteins that associate with the anchor proteins. One of these proteins, called Dynein, is responsible for binding the entire complex to the railway. Dynein is also a motor protein that can "walk" along the microtubule, dragging the vesicle along with it. Dynein obtains its energy for its walking movements from ATP. Golgi vesicles travel along the microtubule railway until they get to within a micrometer (μm) of the cell membrane, and there they sit until they receive a "launch" signal. This signal may be a hormone or an increase in the amount of intercellular calcium. When the signal is received, the dynein complex releases the vesicle allowing it to float the remaining distance to the membrane. This is not a free-float but rather an electrical attraction between the vesicle, which carries a net positive charge, and the inner surface of the cell membrane, which carries a net negative charge. This charge difference is established by another set of glycoproteins embedded in the vesicle membrane and various molecules located in the cell membrane. Microtubules and motor proteins will be encountered again in the next chapter when the movement of chromosomes during cell cycle is discussed.

SUMMARY

The emergence of the eukaryotes came with the appearance of a super cell, about 2 billion years ago that was bigger, faster, and much more complex than an average prokaryote. Eukaryotes do everything on a grand scale: They are 10 times larger than a typical prokaryote, and they have 10 times the number of genes. Eukaryote genes are controlled by a team of transcription factors and are tended by a second team of gene monitors. The internal structure of a eukaryote, in contrast to prokaryotes, is composed of many membrane-bounded organelles. The main priority of a eukaryote is the construction of the forestlike glycocalyx; most of the internal structures, like the ER and the Golgi complex, are dedicated to this single purpose. An elaborate intercellular railway is devoted to the

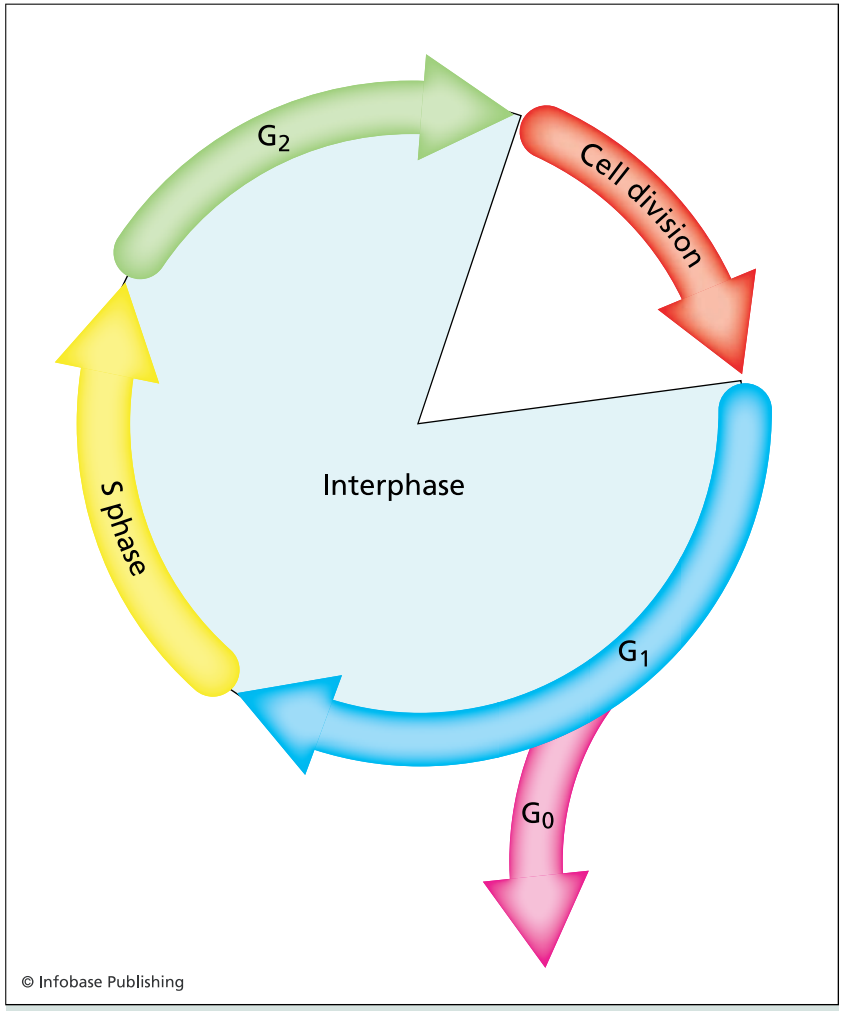
transportation of glycoproteins and glycolipids to and from the cell surface. The eukaryote glycocalyx has many important functions. It improves the efficiency of food gathering, particularly for single-cell eukaryotes with a predator lifestyle. It serves as communication hardware, making it possible for eukaryotes to communicate with each other, and thus paved the way for cell colonies and, eventually, true multicellular organisms. It provides cell-to-cell anchorage points and is a crucial component of connective tissue, the tissue that gives every animal its shape, texture, and dimensions.

The Cell Cycle

Cells inherited the power of reproduction from the prebiotic bubbles that split in half at regular intervals under the influence of their turbulent environment. This pattern of turbulent fragmentation followed by a brief period of calm is now stuck in the molecular psyche, or behavior pattern, of every cell. Even today, after 3 billion years, many bacteria still divide every 20 minutes. Though eukaryotes take longer to divide, their regularity is just as profound.

The regular alternation between division and calm has come to be known as the cell cycle. In studying this cycle, scientists have recognized different states of calm and different ways in which a cell can divide. The calm state of the cell cycle, referred to as interphase, is divided into three subphases called gap 1 (G_1), S phase (a period of DNA synthesis), and gap 2 (G_2). The conclusion of interphase, and with it the termination of G_2, occurs with division of the cell and a

return to G_1. Cells may leave the cycle by entering a special phase called G_0. Some cells, such as postmitotic neurons in an animal's brain, remain in G_0 for the life of the organism.



The cell cycle. Most cells spend their time cycling between a state of calm (interphase) and cell division. Interphase is further divided into three subphases: gap 1 (G_1), S phase (DNA synthesis), and gap 2 (G_2). Cells may exit the cycle by entering a special phase called G_0.

Although interphase is a period of relative calm, the cell grows continuously during this period, working hard to prepare for the next round of division. Two notable events are the duplication of the spindle (the centrosome and associated microtubules), a structure that is crucial for the movement of the chromosomes during cell division, and the appearance of an enzyme called maturation promoting factor (MPF) at the end of G_2. MPF is the enzyme that phosphorylates the histones in order to compact the chromosomes in preparation for cell division. MPF is also responsible for the breakdown of the nuclear membrane. When cell division is complete, MPF disappears, allowing the chromosomes to decondense and the nuclear envelope to reform.

Many of the events that occur during interphase are poorly understood, but scientists know the intention of all the labor is to ensure the production of two identical daughter cells. When the prebiotic bubbles divided, the two new bubbles were not necessarily identical to each other, as there were no mechanisms in place to ensure an equal distribution of the parent bubble's contents. The dissimilarity of the daughter bubbles was an advantage at that stage of development because it promoted diversity, but true cells had too much to lose to permit haphazard divisions. Thus, the appearance of true cells required mechanisms that guaranteed identical daughter cells.

Natural selection is the driving force behind the appearance of novel cellular mechanisms. If a cell happened onto a useful biochemical reaction, there would have been a strong selection pressure to preserve that innovation by providing a way for that cell to hand it down to succeeding generations. The acquisition of new traits and abilities is always linked to the appearance of enzymes with new talents. The earliest cells had already begun storing genetic information for their enzymes, first as an RNA genome and later as DNA. The problem they faced initially was the need to duplicate their genome accurately and to coordinate that process with cell division in order to ensure the production of identical daughter cells.

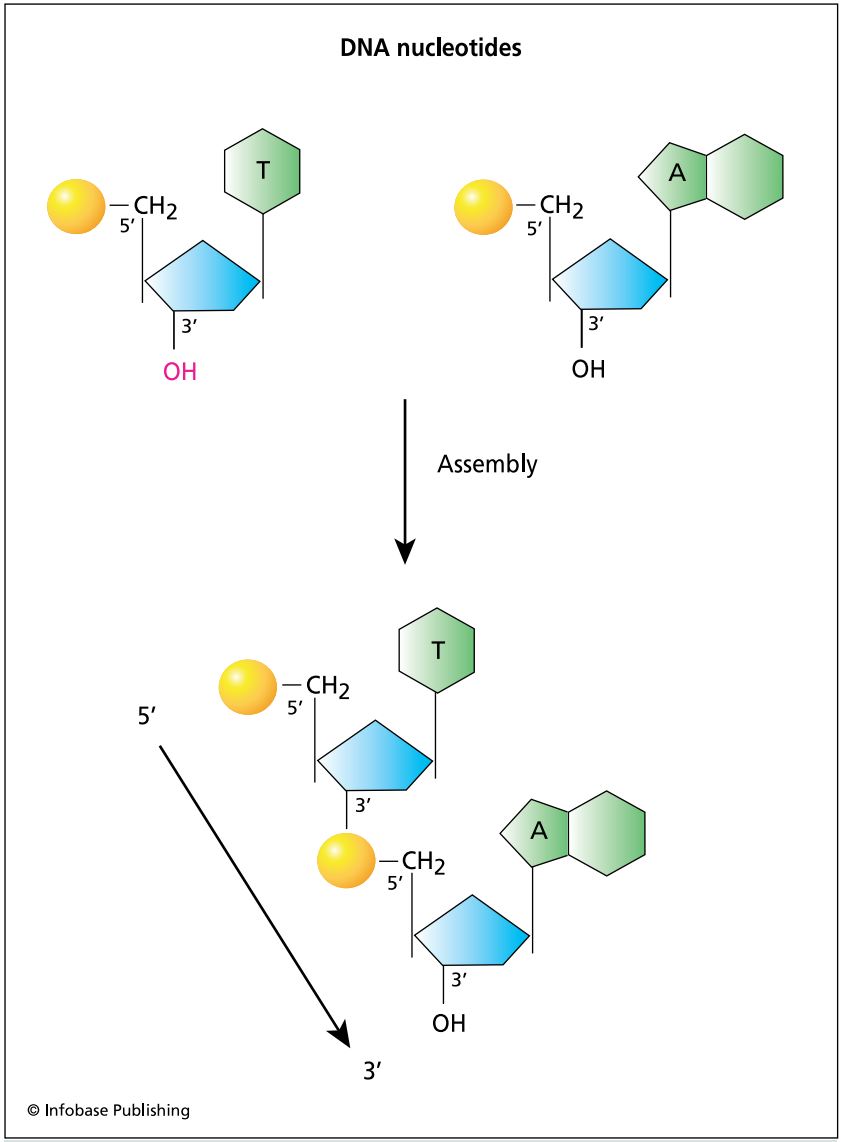
DNA REPLICATION

To understand how a cell duplicates its genome during S phase, it is necessary to understand something about the structure of a nucleotide and the way in which several nucleotides are linked together to form a single-stranded DNA molecule. Nucleotides, as seen in the figure on page 82, are linked together to form single-stranded DNA by the attachment of the phosphate group of one nucleotide to the 3′ carbon of a second nucleotide. Since the phosphate of the first nucleotide is attached to its 5′ carbon, the DNA chain is said to grow in the 5′ to 3′ direction. The internal OH group is lost when the two nucleotides are joined together.

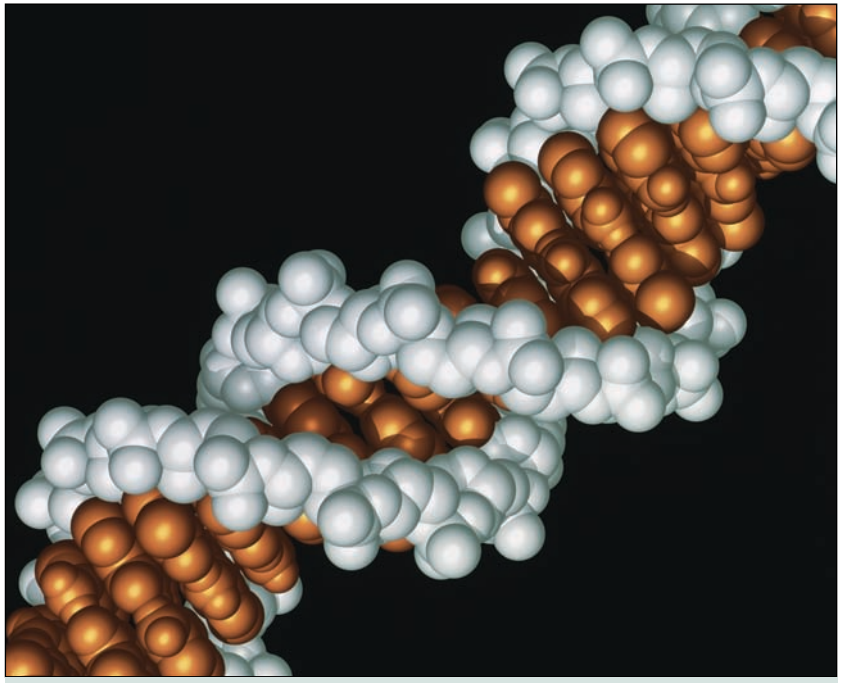
Double-stranded DNA forms when two chains of nucleotides interact through the formation of chemical bonds between complementary base pairs. Triple bonds are formed between cytosine and guanine, and double bonds are formed between adenine and thymine. The geometry of each DNA strand is such that they can only form a double-stranded molecule by being antiparallel, that is, the orientation of one strand is in the 5′ to 3′ direction, while the complementary strand runs 3′ to 5′.

In prokaryotes and eukaryotes, duplication of the genome occurs at replication bubbles, each of which contains two replication forks. The bubbles are regions of the DNA that have dissociated so that daughter strands can be synthesized. A prokaryote chromosome has a single replication bubble, whereas a eukaryote chromosome has many. Consequently, DNA replication in eukaryotes does not begin at one end of the chromosome and continue on until it reaches the other end; replication begins at many places simultaneously and progresses until all of the bubbles have fused, at which point duplication of the chromosome is complete.

DNA replication requires the coordinated effort of a team of enzymes, led by DNA helicase and primase. The helicase is a remarkable enzyme that is responsible for initiating the formation of the bubble and for separating the two DNA strands. This enzyme moves



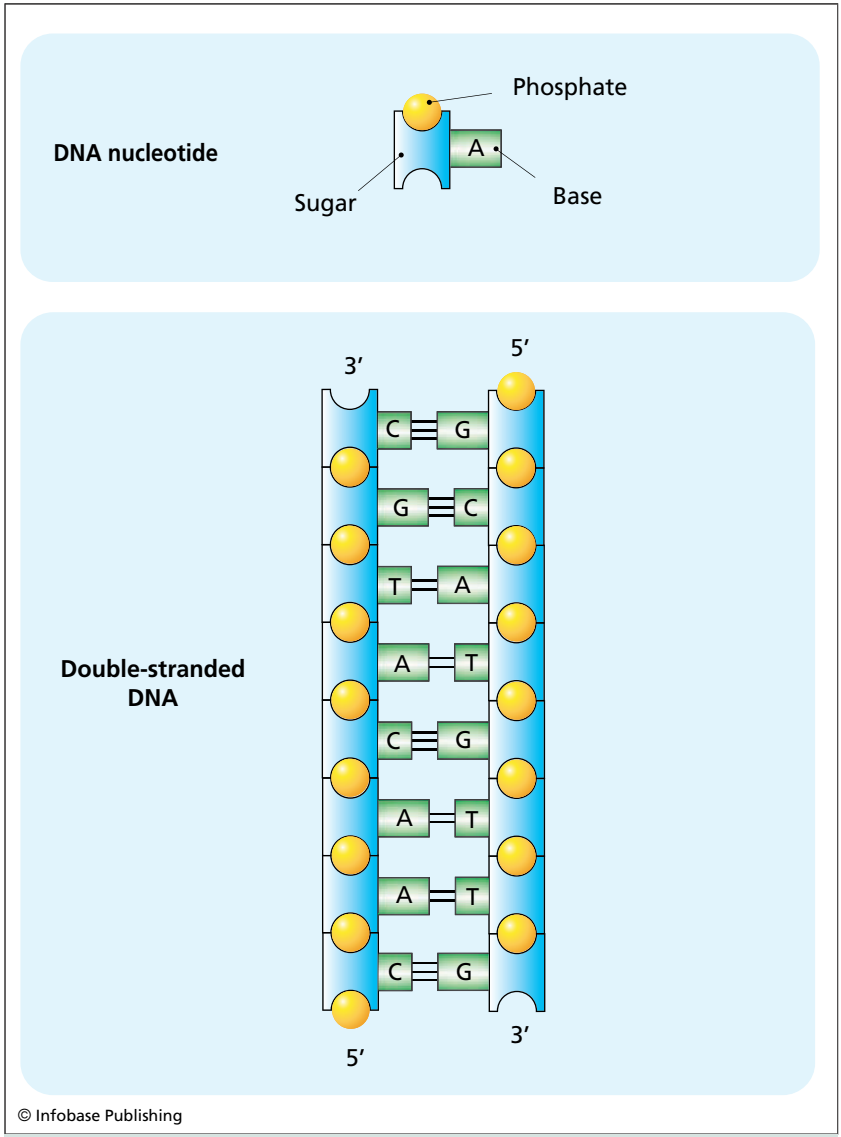
Nucleotide linkage. Nucleotides are linked together in a 5′ to 3′ direction by linking the phosphate of the adenine (A) nucleotide to the 3′ carbon of the thymine (T) nucleotide. The OH group on the T nucleotide (shown in blue) is lost in the process.



Computer model of DNA. The two strands coil around each other to form a helix that, when looking down on it from above, coils to the right. The spherical structures in this image represent the various atoms in the sugars and bases (bronze) and phosphates (light gray). *(K. Seddon & T. Evans/Photo Researchers, Inc.)*

at an astonishing rate, separating 1,000 nucleotides every second. Helicase gets its name from the fact that DNA is a helix, wherein the two strands coil around each other like two strands in a piece of rope. Consequently, helicase is not just separating the two strands but also unwinding them as the replication fork progresses.

The enzyme that is directly responsible for reading the template strand and for constructing the new daughter strand is called DNA polymerase. This enzyme reads the parental DNA in the 3′ to 5′ direction and creates a daughter strand that grows 5′ to 3′. DNA

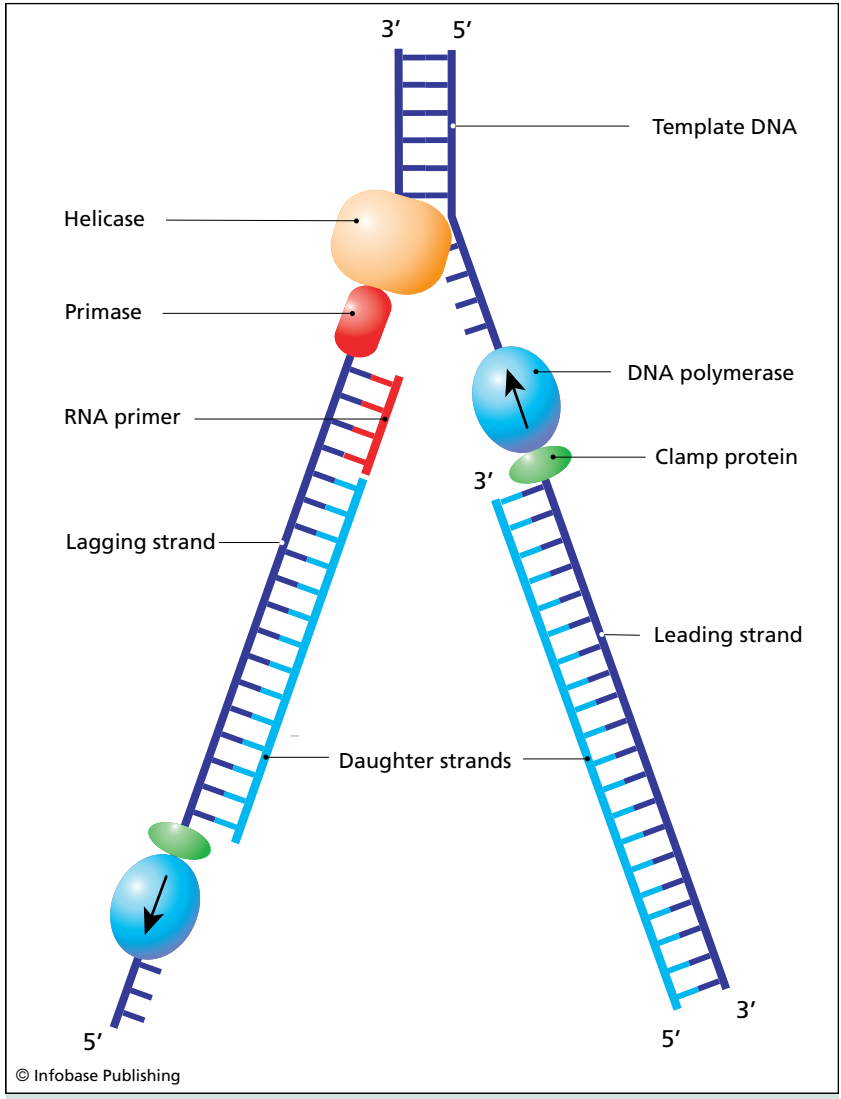


DNA structure. Nucleotides are linked together to form a double-stranded molecule. The bases are held together with double or triple bonds, and the two strands are antiparallel.

polymerase also has an editorial function: It checks the preceding nucleotide to make sure it is correct before it will add a nucleotide to the growing chain. The editor function of this enzyme introduces an interesting problem: How can the polymerase add the very first nucleotide, given that it has to check a preceding nucleotide before adding a new one? A special enzyme called primase, which is attached to the helicase, solves this problem. Primase synthesizes short pieces of RNA that form a DNA-RNA double-stranded region. The RNA becomes a temporary part of the daughter strand, thus priming the DNA polymerase by providing the crucial first nucleotide in the new strand. Once the chromosome is duplicated, DNA repair enzymes remove the RNA primers and replace them with DNA nucleotides.

For every replication fork, there is a leading and lagging strand. The leading strand reads 3′ to 5′ in the direction of the fork, whereas the lagging strand reads 5′ to 3′. Since DNA polymerase reads the template 3′ to 5′, the leading strand only requires a single primer, which is laid down as soon as the replication bubble forms. The lagging strand, however, has to have many primers, and these are laid down as the fork progresses. This is why the helicase and the primase, as shown in the figure on page 86, are located on the lagging strand. DNA polymerase can duplicate the leading strand continuously, in one long piece, whereas the lagging strand has to be duplicated with many discontinuous pieces, from one primer to the next.

This may seem like an awkward solution, but the alternative is to place a single primer at the 3′ tips of each strand and then replicate them both continuously. This would mean that one helicase and one polymerase would have to travel the entire length of each strand. Even with the helicase moving as fast as it does, it would be a very slow way of replicating the genome. Using multiple replication forks per chromosome is analogous to parallel processing, which can dramatically reduce the time it takes to complete a task.



DNA replication. The helicase separates the two strands so the DNA polymerase can synthesize new strands. The primase provides replication signals for the polymerase, in the form of RNA primers, and the clamp protein keeps the polymerase from falling off the DNA. The leading strand requires only a single primer (not shown). The lagging strand requires many primers, and the daughter strand is synthesized as a series of DNA fragments that are later joined into one continuous strand.

BINARY FISSION

Prokaryotes all divide by a simple process called binary fission. These cells grow continuously during interphase, and the growth is coordinated with the duplication of the chromosome. Soon after the replication bubble forms, one strand of the chromosome is attached to the membrane at one end of the cell and the other strand is attached to the opposite end. Growth of the cell and the completion of DNA replication are coordinated with the formation of a septum, or invagination of the cell membrane, which eventually divides the cell into two. The formation of the septum is initiated by a protein called FtsZ, but the details of the process are still unclear.

MITOSIS

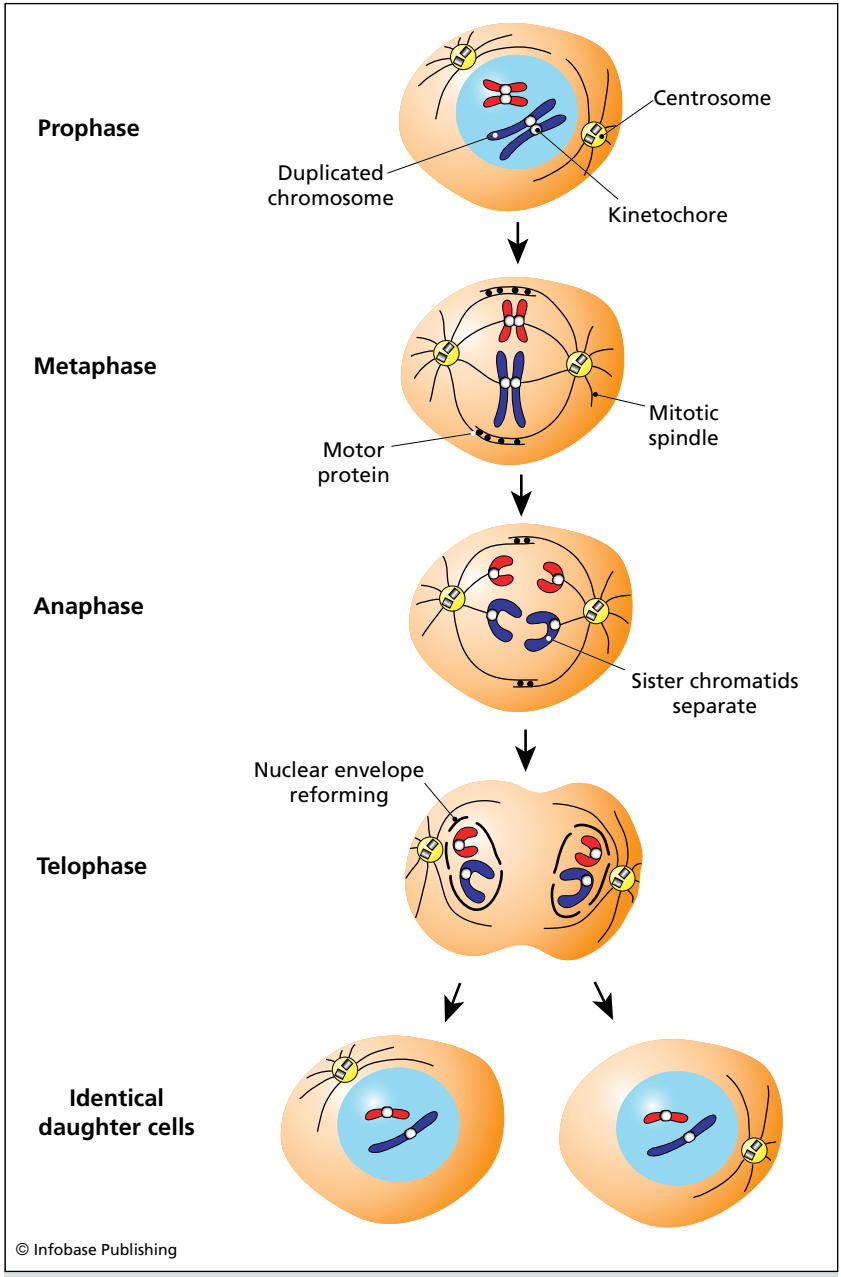
Cell division among the eukaryotes is more complicated than it is in prokaryotes. The biggest problem eukaryotes have to deal with, compared with prokaryotes, is their enormous genome, spread out over many chromosomes, which must be replicated, packaged, arranged, and sorted before the cell can divide. Division is by a process known as mitosis, which is divided into four stages: prophase, metaphase, anaphase, and telophase. All of these stages are marked out in accordance with the behavior of the nucleus and the chromosomes.

Prophase

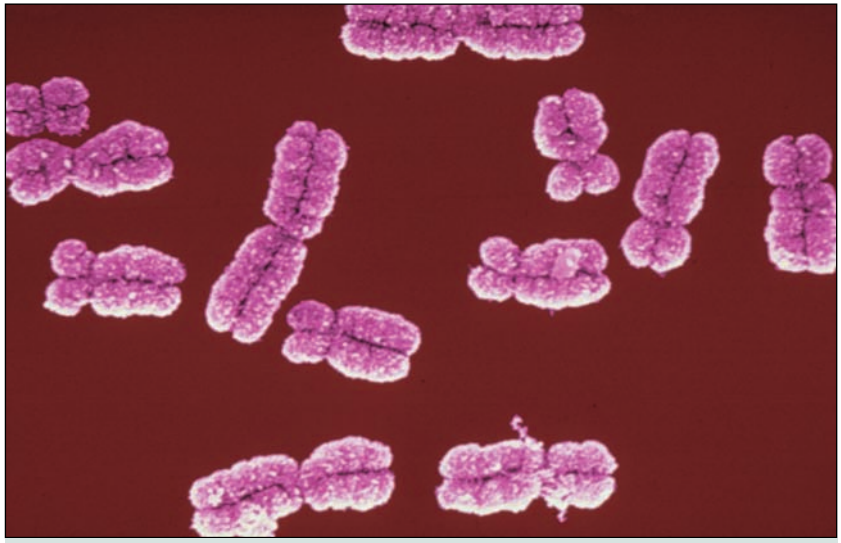
The duplicated chromosomes begin condensation, and the two centrosomes begin moving to opposite poles of the cell. Under the microscope, the chromosomes become visible as X-shaped structures, which are the two duplicated chromosomes, often called sister chromatids. A special region of each chromosome, called a centromere, holds the chromatids together. Proteins bind to the centromere to form a structure called the kinetochore.

Metaphase

The chromosomes are sorted out and aligned between the two centrosomes. By this time, the nuclear membrane has completely



Mitosis. Principal stage dealing with the movement and partitioning of the chromosomes between the future daughter cells. For clarity, only two chromosomes are shown.



Scanning electron micrograph of human chromosomes in metaphase. *(Biophoto Associates/Photo Researchers, Inc.)*

broken down. The two centrosomes and the microtubules fanning out between them form the mitotic spindle. The area in between the spindles, where the chromosomes are aligned, is often referred to as the metaphase plate. Some of the microtubules make contact with the kinetochores, while others overlap, with motor proteins situated in between. (For clarity, only two chromosomes are shown in the figure on page 88.) Eukaryotes are normally diploid, so a cell would have two copies of each chromosome, one from the mother and one from the father.

Anaphase

The duplicated chromosomes move to opposite poles of the cell. The first step is the release of an enzyme that breaks the bonds holding the kinetochores together, thus allowing the sister chromatids to separate from each other while remaining bound to their respective microtubules. Motor proteins then move along the microtubule dragging the chromosomes to opposite ends of the cell. Using energy

supplied by ATP, the motor proteins break the microtubule down as it drags the chromosome along, so that the microtubule is gone by the time the chromosome reaches the spindle pole. Throughout this process, the motor proteins and the chromosome manage to stay one step ahead of the disintegrating microtubule. The overlapping microtubules aid movement of the chromosomes toward the poles as another type of motor protein pushes the microtubules in opposite directions, effectively forcing the centrosomes toward the poles. This accounts for the greater overlap of microtubules in metaphase as compared with anaphase.

Telophase

The daughter chromosomes arrive at the spindle poles and decondense to form the relaxed chromatin characteristic of interphase nuclei. The nuclear envelope begins forming around the chromosomes, marking the end of mitosis. During the same period, a contractile ring, made of the proteins Myosin and Actin, begins pinching the parental cell in two. This stage, separate from mitosis, is called cytokinesis and leads to the formation of two daughter cells, each with one nucleus.

MEIOSIS

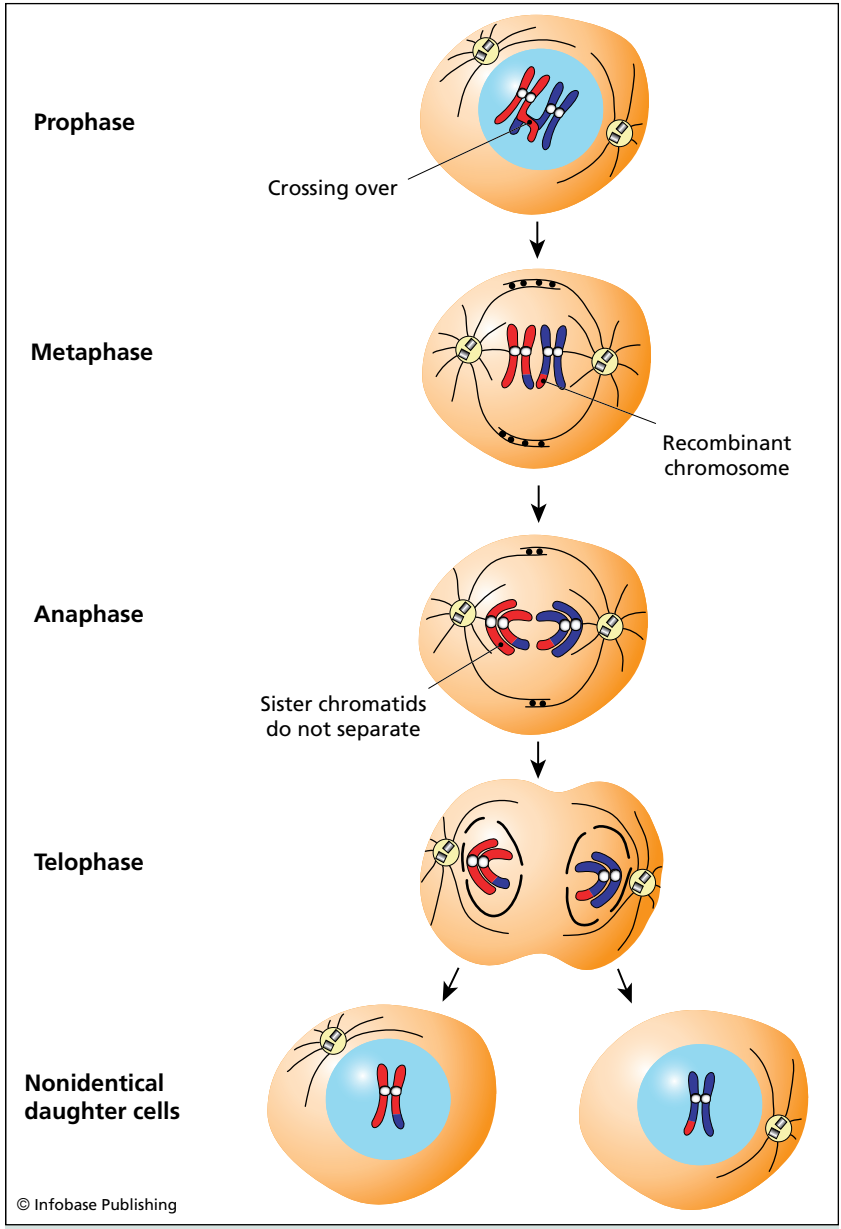
Unlike mitosis, which leads to the growth of an organism, meiosis is intended for sexual reproduction and occurs exclusively in ovaries and testes. Eukaryotes, being diploid, receive chromosomes from both parents; if gametes were produced using mitosis, a catastrophic growth in the number of chromosomes would occur each time a sperm fertilized an egg. Meiosis is a special form of cell division that produces haploid gametes (eggs and sperm), each possessing half as many chromosomes as the diploid cell. When haploid gametes fuse, they produce an embryo with the correct number of chromosomes.

The existence of meiosis was first suggested 100 years ago when microbiologists counted the number of chromosomes in somatic

and germ cells. The roundworm, for example, was found to have four chromosomes in its somatic cells but only two in its gametes. Many other studies also compared the amount of DNA in nuclei from somatic cells and gonads, always with the same result: The amount of DNA in somatic cells is exactly double the amount in fully mature gametes.

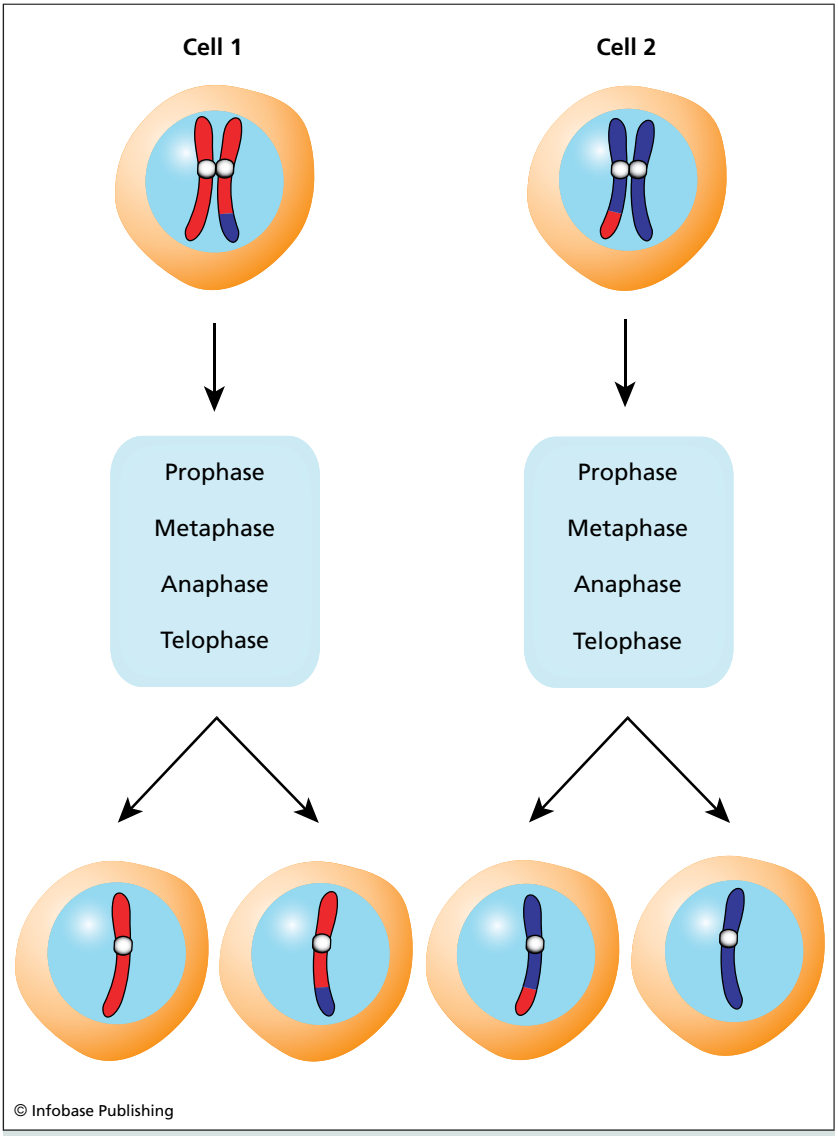
To understand how this could be, scientists studied cell division in the gonads and were able to show that meiosis occurs as two rounds of cell division with only one round of DNA synthesis. The two rounds of division were called meiosis I and meiosis II, and scientists observed that both could be divided into the same four stages known to occur in mitosis. Indeed, meiosis II is virtually identical to a mitotic division. Meiosis I resembles mitosis, but close examination shows three important differences: gene swapping occurs between homologous chromosomes in prophase; homologs (i.e., two homologous chromosomes) remain paired at metaphase, instead of lining up at the plate as is done in mitosis; and the kinetochores do not separate at anaphase.

Homologous chromosomes are two identical chromosomes that come from different parents. For example, humans have 23 chromosomes from the father and the same 23 from the mother. Each individual has a maternal chromosome 1 and a paternal chromosome 1 that carry the same genes but specify slightly different traits. Chromosome 1 may carry the gene for eye color, but the maternal version, or allele, may specify blue eyes, whereas the paternal allele specifies brown. During prophase, homologous pairs exchange large numbers of genes by swapping whole pieces of chromosome. Thus one of the maternal chromatids (colored red in the figure on page 92) ends up with a piece of paternal chromosome, and a paternal chromatid (dark blue) receives the corresponding piece of maternal chromosome. Mixing genetic material in this way is unique to meiosis, and it is one of the reasons sexual reproduction has been such a powerful evolutionary force.



Meiosis I. The most notable features include genetic recombination (crossing over) between the homologous chromosomes during prophase, comigration of the sister chromatids during anaphase, and the production of nonidentical daughter cells. Only one homologous pair is shown.

During anaphase of meiosis I, the kinetochores do not separate as they do in mitosis. The effect of this is to separate the maternal and paternal chromosomes by sending them to different daughter



Meiosis II. Cells 1 and 2 were produced in meiosis I. These cells divide mitotically but without replication of the chromosomes to produce four genetically unique haploid gametes.

cells, although the segregation is random. That is, the daughter cells receive a random assortment of maternal and paternal chromosomes, rather than one daughter cell receiving all paternal chromosomes and the other all maternal chromosomes. Random segregation, along with genetic recombination, accounts for the fact that while children resemble their parents, they do not look or act exactly like them. These two mechanisms are responsible for the remarkable adaptability of all eukaryotes.

Meiosis II begins immediately after the completion of meiosis I, which produces two daughter cells each containing a duplicated parental chromosome and a recombinant chromosome consisting of both paternal and maternal DNA. These two cells divide mitotically to produce four haploid cells, each of which is genetically unique, containing unaltered or recombinant maternal and paternal chromosomes. The example seen in the figure on page 93 follows the fate of a single homologous pair. In reality, the four haploid cells, if human, would contain 23 chromosomes each, some from the mother, some from the father, some remixed (recombinant), others unaltered.

Meiosis produces haploid cells by passing through two rounds of cell division with only one round of DNA synthesis. The process, however, is not just concerned with reducing the number of chromosomes but is also involved in stirring up the genetic pot in order to produce unique gametes that may someday give rise to an equally unique individual.

Genes

Genes are a precious biological commodity. They hold the key to a cell's survival and are the summation of countless natural experiments over millions of years of evolution. Nature has poked, prodded, modified, and tweaked each gene, almost to exhaustion. Those that fail the test or do not live up to expectations are eliminated. The genes that are around today, within humankind and within other organisms, are the survivors. The elaborate codes they carry and the proteins or RNA molecules they specify produce organisms that are also survivors: hardy creatures that are resourceful, diligent, and well adapted to their environment.

The attention nature pays to a gene is analogous to the labors of an artist who spends many hours carefully shaping, carving, and polishing a sculpture. Work on the sculpture may last for weeks or years and often goes through many revisions, each of which brings the object closer to the artist's vision. The difference between the

artist and nature is that nature works on its sculptures indirectly by polishing or altering genes, removing a base here, adding one there, and seeing what happens. In many cases, nature is never satisfied with the outcome, and so the gene and its product continue changing over time. Sometimes, however, nature seems to hit it right, and the gene remains unchanged for millions of years.

These and many other insights into the nature of the gene have been hard won over many years by thousands of researchers studying different species of plant and animal life. But the pace of this kind of work increased dramatically in the late 1990s with the initiation of the human genome project. The final draft of the human genome was completed in 2003 and provides a wealth of information regarding the structure, organization, and evolution of our genes. The recent trend is to extend this information by sequencing the genomes of many individuals in order to study the natural genetic variation within the human population.

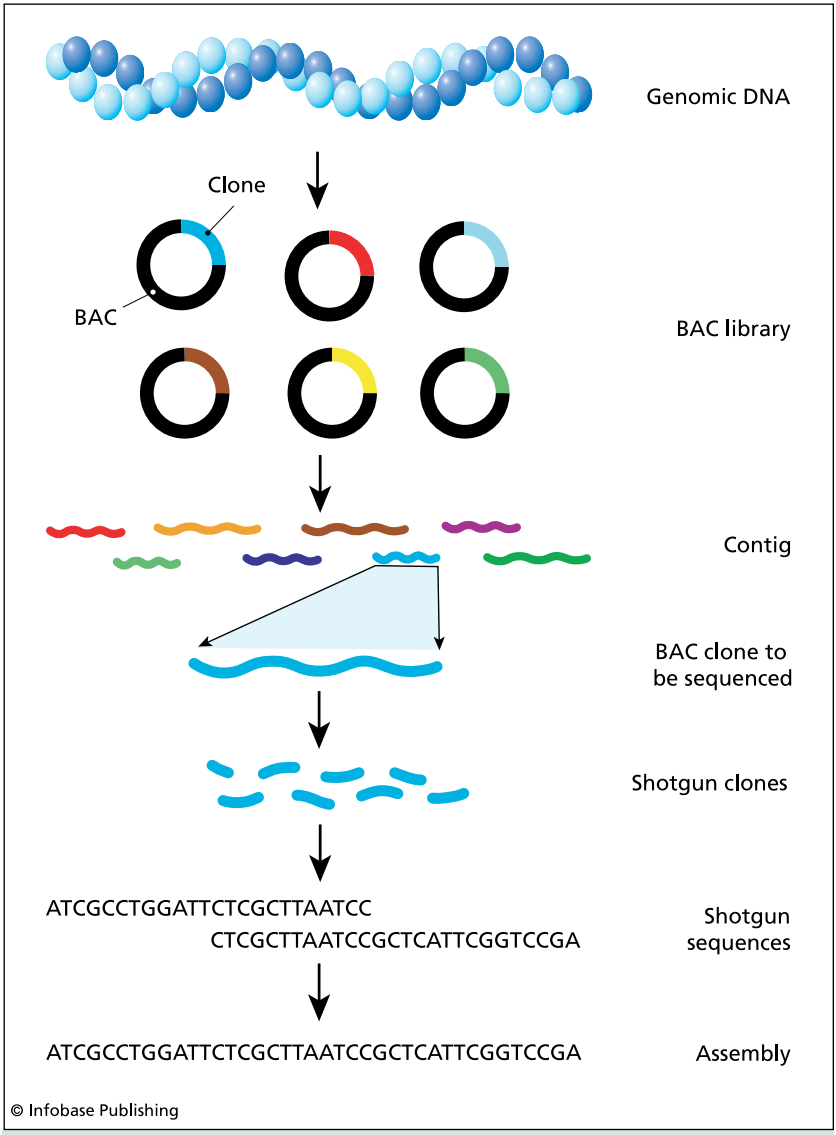
THE HUMAN GENOME PROJECT

Sequencing the entire human genome is an idea that grew over a period of 20 years, beginning in the early 1980s. At that time, the DNA-sequencing method invented by the British biochemist Fred Sanger, then at Cambridge, was but a few years old and had only been used to sequence viral or mitochondrial genomes. (See chapter 10 for a description of sequencing methods.) Indeed, one of the first genomes to be sequenced was that of bacteriophage G4, a virus that infects the bacterium *Escherichia coli.* The G4 genome consists of 5,577 nucleotide pairs (or base pairs, abbreviated bp) and was sequenced in Dr. Sanger's laboratory in 1979. By 1982, the Sanger protocol was used by others to sequence the genome of the animal virus SV40 (5,224 bp), the human mitochondrion (16,569 bp), and bacteriophage lambda (48,502 bp). Besides providing invaluable data, these projects demonstrated the feasibility of sequencing very large genomes.

The possibility of sequencing the entire human genome was first discussed at scientific meetings organized by the U.S. Department of Energy (DOE) between 1984 and 1986. A committee appointed by the U.S. National Research Council endorsed the idea in 1988 but recommended a broader program to include the sequencing of the genes of humans, bacteria, yeast, worms, flies, and mice. They also called for the establishment of research programs devoted to the ethical, legal, and social issues raised by human genome research. The program was formally launched in late 1990 as a consortium consisting of coordinated sequencing projects in the United States, Britain, France, Germany, Japan, and China. At about the same time, the Human Genome Organization (HUGO) was founded to provide a forum for international coordination of genomic research.

By 1995, the consortium had established a strategy, called hierarchical shotgun sequencing, which they applied to the human genome as well as to the other organisms mentioned. With this strategy, genomic DNA is cut into one-megabase (Mb) fragments (i.e., each fragment consists of 1 million bases) that are cloned into bacterial artificial chromosomes (BACs) to form a library of DNA fragments. The BAC fragments are partially characterized, then organized into an overlapping assembly called a contig. Clones are selected from the contigs for shotgun sequencing. That is, each shotgun clone is digested into small 1,000 bp fragments, sequenced, and then assembled into the final sequence with the aid of computers. Organizing the initial BAC fragments into contigs greatly simplifies the final assembly stage.

Sequencing of the human genome was divided into two stages. The first stage, completed in 2001, was a rough draft that covered about 80 percent of the genome with an estimated size of more than 3 billion bases (also expressed as 3 gigabases, or 3 Gb). The final draft, completed in April 2003, covers the entire genome, refines the data for areas of the genome that were difficult to sequence, and fills in many gaps that occurred in the rough draft. The final draft of



Hierarchical shotgun sequencing. Total genomic DNA is cut with a restriction enzyme into one megabase fragments (i.e., 1 million base pairs per fragment) that are cloned into bacterial artificial chromosomes (BACs) to form a library. The BAC fragments are partially characterized in order to organize them into an overlapping assembly called a contig. Clones are selected from the contigs for shotgun sequencing and final assembly.

the human genome gives us a great deal of information that may be divided into three categories: gene content, gene origins, and gene organization.

Gene Content

Analysis of the final draft has shown that the human genome consists of 3.2 Gb of DNA that encodes about 30,000 genes (estimates range between 30,000 to 40,000). The estimated number of genes is surprisingly low; many scientists had believed the human genome contained 100,000 genes. By comparison, the fruit fly has 13,338 genes and the simple roundworm, *Caenorhabditis elegans,* has 18,266. The genome data suggests that human complexity, as compared to the fruit fly or the worm, is not simply due to the absolute

FUNCTIONAL PROFILE OF KNOWN CELLULAR PROTEINS

CELLULAR PROCESS	NUMBER OF PROTEINS
Energy metabolism	5,200
DNA replication/repair	900
Transcription/translation	3,200
Signaling (intra- and extracellular)	3,100
Protein modifiers	850
Transport	1,200
Multifunctional proteins	400
Structural	900
Defense	1,050
Total	**16,800**

Note: The total shown is about half of the estimated 32,000 protein-encoding genes in the human genome. The discrepancy is due to the fact that many genes have an unknown function.

number of genes but involves the complexity of the proteins that are encoded by those genes. In general, human proteins tend to be much more complex than those of lower organisms. Data from the final draft and other sources provide a detailed overview of the functional profile of human cellular proteins.

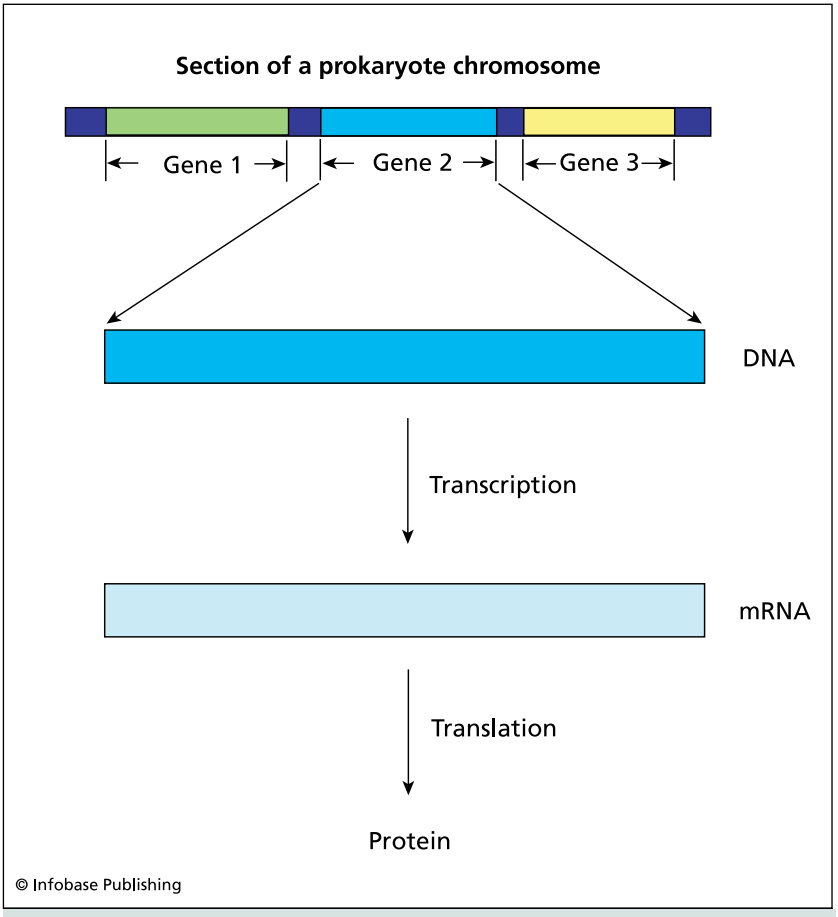
Gene Origins

Fully one-half of human genes originated as mobile genetic elements, also known as jumping genes (these will be discussed at length in a following section). Equally surprising is the fact that 220 of human genes were obtained by lateral gene transfer (LGT) from bacteria, rather than ancestral, or vertical, inheritance. In other words, humans and many other eukaryote organisms have obtained genes directly from bacteria, probably during episodes of infection, in a kind of natural gene therapy, or gene swapping. Scientists know this to be the case because while these genes occur in bacteria they are not present in yeast, fruit flies, or any other eukaryotes that have been tested. The extent of LGT can be truly astonishing. Recently, an American team at the Institute for Genomic Research in Rockville, Maryland, found that *Wolbachia pipientis,* a prokaryote that parasitizes the fly *Drosophila ananassae,* had transferred its entire genome, consisting of 45 genes, to one of the host's chromosomes. Successive generations of these flies were shown to have retained the parasite's genes whether or not Wolbachia infected them.

The function of most of the laterally transferred genes is unclear, although a few may code for basic metabolic enzymes. A notable exception is a gene that codes for an enzyme called monoamine oxidase (MAO). Monoamines are neurotransmitters, such as dopamine, norepinephrine, and serotonin, which are needed for neural signaling in the human central nervous system. MAO plays a crucial role in the turnover of these neurotransmitters. How MAO, obtained from bacteria, could have developed such an important role in human physiology is a great mystery. (Neurons and neurotransmitters are discussed in chapter 8.)

Gene Organization

In prokaryotes, genes are simply arranged in tandem along the chromosome, with little if any DNA separating one gene from the other. Each gene is transcribed into messenger RNA (mRNA), which is translated into protein. Indeed, in prokaryotes, which have no

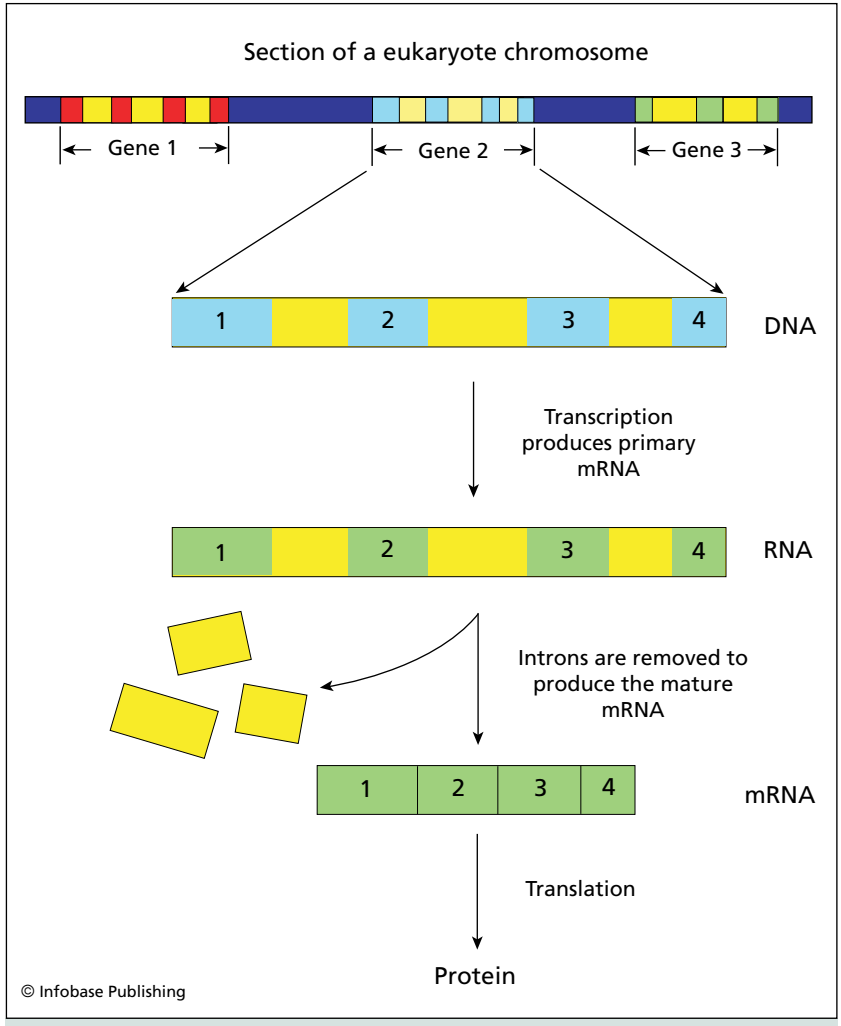


Prokaryote genes. The genes are arranged in tandem along the chromosome, with little if any DNA separating one gene from the other. The genes may code for protein, as shown at the top for gene 2, or ribosomal RNA (rRNA).

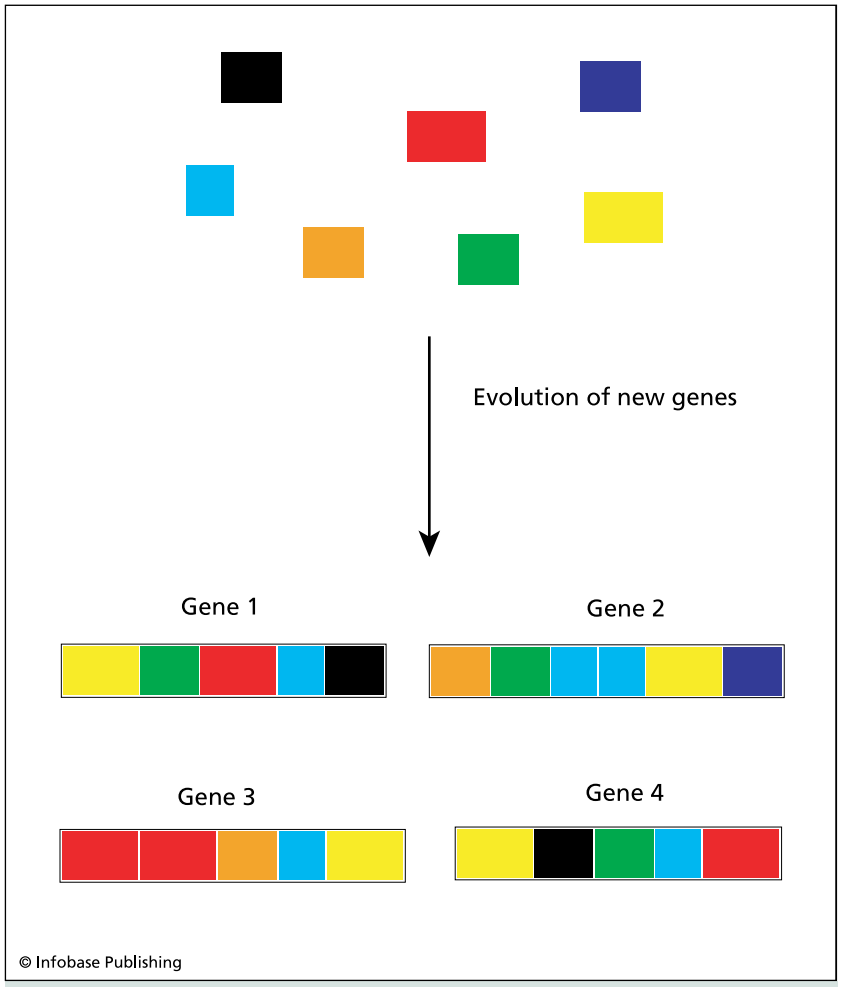
nucleus, translation often begins even before transcription is complete. In eukaryotes, gene organization is more complex. Data from the genome project shows clearly that eukaryote genes are split into subunits, called exons, and that each exon is separated by a length of DNA, called an intron. A gene, consisting of introns and exons, is separated from other genes by long stretches of noncoding DNA called intervening sequences. Eukaryote genes are transcribed into a primary RNA molecule that includes exon and intron sequences. The primary transcript never leaves the nucleus and is never translated into protein. Nuclear enzymes remove the introns from the primary transcript, after which the exons are joined together to form the mature mRNA. Thus, only the exons carry the necessary code to produce a protein.

Why do eukaryotes, all of which have split genes, go through a laborious procedure when the simple and direct method used by prokaryotes works so well and seems so eloquent? The answer lies with the need to maximize protein evolution and to protect the genome from insertional mutagenesis. The rate at which proteins evolve can be maximized by a process known as exon shuf ing. Any given protein usually has more than one job that it is good at. One end of a protein may be good at binding to a specific region of the genome, while the other end specializes in phosphorylating other proteins. The middle region of the protein may give it a special corkscrew shape. If each of these regions is encoded by three different exons, then it is easy to see how new proteins could emerge very quickly by recombining, or shuf ing, pre-existing exons.

Insertional mutagenesis occurs because our world is full of viruses that can infect eukaryote cells. The life cycle of many of these viruses is such that they are able to insert their genome into a chromosome of the host cell. If eukaryotes had a simple prokaryote-like genome, then no matter where the virus inserted it would likely destroy, damage, or mutate a gene. On the other hand, the eukaryote genome contains large regions of noncoding DNA (introns and intervening sequences) so that, in all probability, insertion of



Eukaryote genes. The genes are arranged in separate subunits called exons (dark gray) and introns (light gray). Each gene, which consists of introns and exons, is separated from other genes by long stretches of noncoding DNA called intervening sequences (white areas). The genes are transcribed into a primary RNA molecule that includes exon and intron sequences. Nuclear enzymes remove the introns from the primary transcript, and then the exons are joined together to form the mature mRNA, which is translated into protein. Transcription of ribosomal genes is similar, except that the exons become the individual rRNAs.



Exon shuf ing. New eukaryote genes can evolve without the appearance of new mutations simply by recombining, or shuffling, preexisting exons. In the figure, seven exons were used to produce four novel genes, each of which would code for a novel protein. Exon shuffling is not possible in a prokaryote genome.

a virus will not damage any of the genes. The only way a virus can damage any of our genes is if it happens to insert at an exon-intron boundary. If this happens, special DNA sequences located at the

boundary are corrupted, leading to the production of defective messenger RNA.

The fact is, human genes and the genes of most eukaryotes are hidden in a sea of noncoding DNA. As already mentioned, the human genome consists of 3.2 Gb of DNA and contains about 30,000 genes. With an average gene size of 1,200 bp (based on exons only), this amounts to a total of 36 Mb of coding DNA, or roughly 1 percent of the total DNA content. The vast majority of our DNA codes for nothing but has a crucial role in protecting our genes from marauding pieces of DNA.

THE FUTURE OF GENOMIC RESEARCH

The many insights into the structure, evolution, and organization of our genes that the genome project has provided are expected to revolutionize the study of many medical disorders. Various research communities, as diverse as nursing, psychiatry, and gene therapy, have already written summary articles outlining the many advances they expect to make with the available sequence data. Cancer research provides the most striking example of the impact this data has already had. Scientists at the Sanger Institute in Cambridge, England, have established a research program to use human sequence data to identify all cancer-causing genes in the human genome. Within a few months, they were able to isolate and fully characterize a gene that causes more than 70 percent of all malignant melanomas. Their analysis is so complete and so illuminating that they believe a cure for this deadly form of cancer will be available within a few years, and they expect to complete the identification of all other cancer genes by 2015.

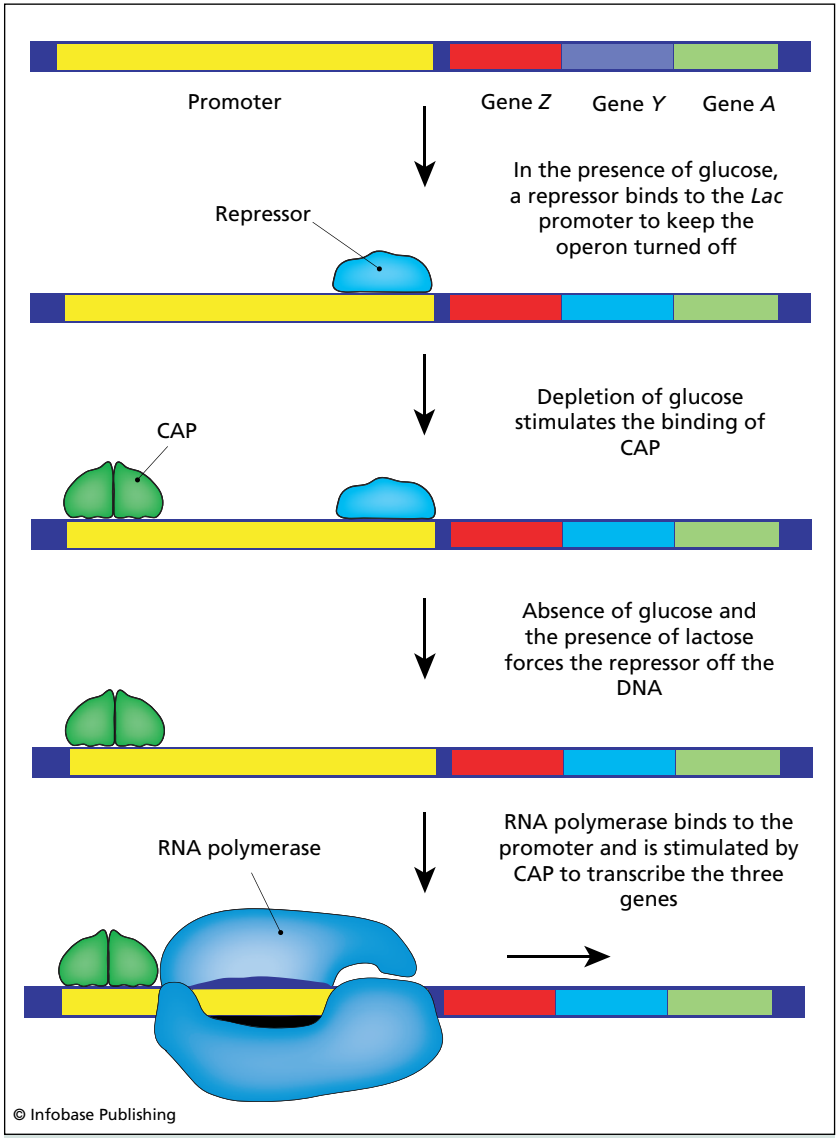
A similar research program has been established in the United States at the National Cancer Institute (NCI), a part of the National Institutes of Health (NIH). In 2006, NCI expanded its search for cancer genes by launching the Cancer Genetic Markers of Susceptibility (CGEMS) study to identify genetic alterations that make people susceptible to common forms of cancer, such as prostate and

breast cancer. CGEMS is a three-year initiative costing $14 million that will scan the entire human genome, isolated from each type of cancer, to identify relevant mutations. The initiative will begin with the scanning of a total of 2,500 samples from men who have been diagnosed with prostate cancer and men who have not. This program was greatly expanded in 2009 with the formation of the International Cancer Genome Consortium (ICGC). This consortium has set itself the goal of sequencing the genome of individual cancer patients. To maximize the quality of the data, each genome will be sequenced 20 times for each of 50 different types of cancer. Using ultrafast sequencers, the consortium hopes to complete its task in five years.

Cancer is only one of many prominent genetic diseases. Similar efforts are expected in the discovery and characterization of genes that cause neurological disorders, cardiovascular disease, diabetes, metabolic disorders, and aging.

TURNING GENES ON AND OFF

All genes come equipped with a controlling region, called the promoter, which serves the same function as a light switch and provides the binding site for the RNA polymerase that transcribes the gene into RNA. The switching function of the promoter is regulated by other proteins, known generally as transcription factors, that bind to the promoter in a way that either blocks the polymerase (the "off" position) or activates it (the "on" position). In some cases, two or more genes are controlled by the same promoter, so when the promoter switches on, all the genes are activated simultaneously (i.e., transcribed into RNA). This arrangement, in which a single promoter is linked to two or more genes, is known as an operon, and it is very common among prokaryotes. Indeed, it was the study of the bacterial *Lac* operon that gave geneticists their first insights into the control of gene expression. The *Lac* operon has three structural genes that code for proteins needed for the import and processing of the sugar lactose, from which this operon gets its name. For historical



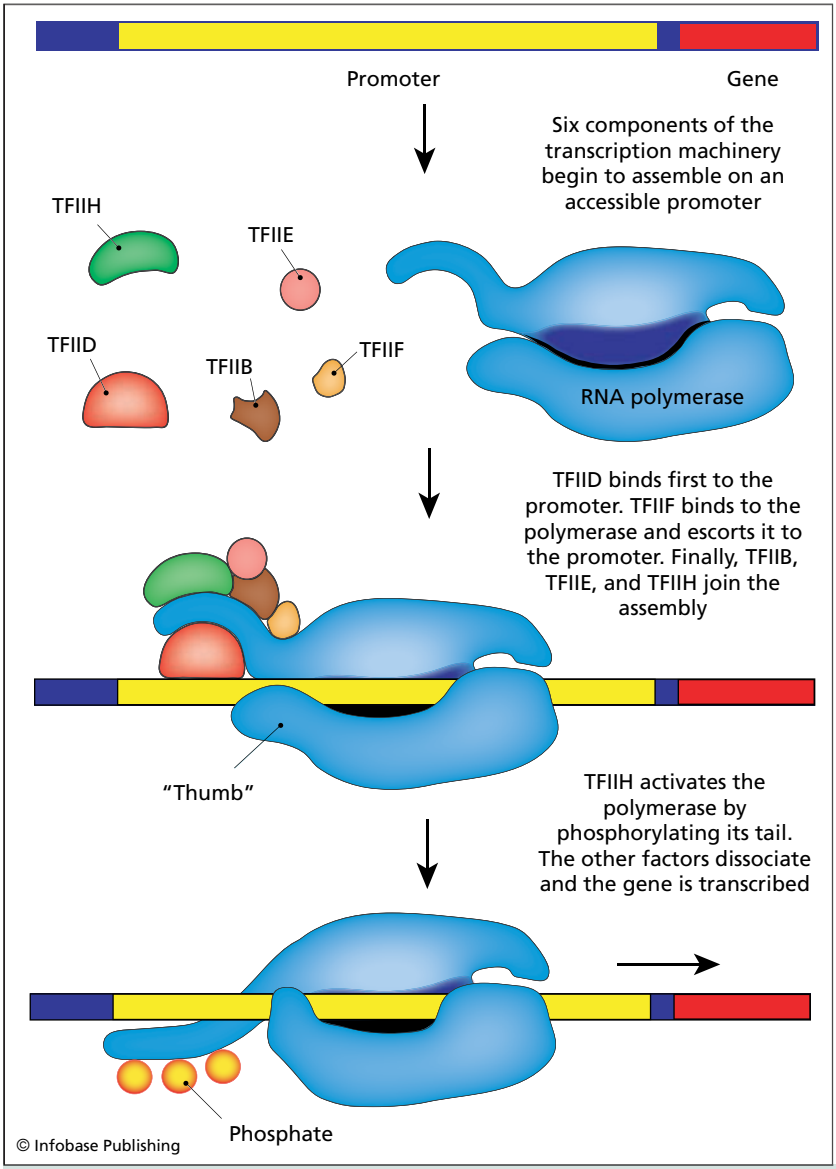
The *Lac* operon. This operon consists of three structural genes (*Z, Y,* and *A*) under the control of a single promoter. The repressor binds to a portion of the promoter called the operator. The catabolite activator protein (CAP) binds at the opposite end of the promoter, in a region known as the regulator, or CAP site. The *Lac* operon is designed to stay off as long as glucose is present.

reasons, these genes are known as *z, y,* and *a*. The *z* gene codes for β-galactosidase, an enzyme that hydrolyzes lactose to galactose and glucose. The *y* gene codes for a permease that facilitates the entry of lactose into the bacteria, and the *a* gene codes for a transacetylase that metabolizes lactose-like compounds.

A simplified scheme for controlling the *Lac* operon is shown in the figure on page 107. A repressor protein binds to the promoter in a region called the operator, thus preventing the binding of the polymerase. This happens whenever glucose, the preferred sugar, is available. As long as the repressor is bound to the operator, the operon is turned off. Depletion of glucose stimulates the binding of a protein called catabolite activator protein (CAP), to another area of the promoter, called the regulator, or CAP site. If lactose happens to be present when the glucose is depleted, the repressor is forced off the promoter, allowing the binding of the polymerase. Once bound, CAP activates the polymerase and the *Lac* operon is transcribed.

The control of gene expression in eukaryotes is more complex, but the basic logic remains the same. In this case, control occurs in three steps. First, special proteins expose the gene's promoter; second, transcription factors assemble on the promoter; and third, regulatory proteins activate the transcriptional machinery. Eukaryote regulatory proteins belong to three families represented by helix-loop-helix, zinc finger, and leucine zipper proteins. The various members of these families are able to recognize specific DNA sequences that are associated with the promoter of every gene. The interaction between the regulatory protein and the DNA is not passive. Rather than simply binding to the DNA, the protein changes the conformation of the local neighborhood by twisting the DNA into a more opened helical structure. The conformational change that the DNA undergoes exposes the promoter to the transcription machinery. This process is complex and not fully understood but is known to involve a relaxation of the DNA helix as well as alterations to the local chromatin conformation.

After the promoter is exposed, six components of the transcription machinery, including RNA polymerase and five transcription



The control of eukaryote gene expression. RNA polymerase and five transcription factors assemble on an exposed promoter. Once these components are in place, transcription factor IIH (TFIIH) is activated by a eukaryote-specific enhancer, analogous to the prokaryote CAP. In this case, the enhancer is located some distance, sometimes thousands of base pairs, upstream (i.e., to the left) from the polymerase. Only a portion of the gene is shown.

factors, quickly assemble. Once the transcription machinery is assembled, the regulatory protein activates a transcription factor, called IIH, which in turn activates the polymerase to permit transcription of the gene.

For a prokaryote, the decision to turn a particular gene on or off is relatively straightforward and usually involves signals that it receives directly from the environment. If one could read the mind of a bacterium, its thought processes might go something like the following:

> Is there any glucose around? If not, is there any lactose? If there is plenty of glucose on hand, keep the *Lac* operon off, because there is no point in running it otherwise. If we run out of glucose but detect lactose, turn the glucose operon off and the *Lac* operon on. If we run out of glucose and lactose, activate auxiliary operons that are capable of processing other sugars, such as maltose or arabinose. If there are no sugars around, activate the genes responsible for processing proteins, fats, and anything else that carries calories.

The control of gene expression in eukaryotes generally adheres to the logic established by the prokaryotes. However, in eukaryotes, the details of the process are more involved, requiring a greater number of steps and molecular participants. Eukaryotes are much more careful about controlling spurious and inappropriate gene expression. This is particularly true for multicellular eukaryotes, for which sloppy control of gene expression can lead to cancerous growths. The desire for precise control can be seen in the number of factors that are needed just to establish a transcription crew in eukaryotes as opposed to prokaryotes. A process that has many components automatically provides many pathways for controlling that process. This is critical for multicellular eukaryotes, all of which are absolutely dependent on careful regulation of gene expression.

GENE SILENCING

Scientists have recently discovered a whole new class of RNA molecules, called micro RNA (miRNA). These RNA molecules are about 22 nucleotides long, are noncoding (i.e., do not form mRNA), and can silence a gene by binding to, thus inactivating or destroying, the gene's mRNA. Thus, this type of genetic control is distinct from the mechanisms discussed in the preceding section. Gene silencing inhibits the expression of a gene without turning it off.

Since their discovery, miRNAs have been the subject of intensive research and have revolutionized our understanding of gene expression while greatly expanding the role of RNA in basic functions of the cell. MiRNAs have been found in virtually every plant and animal species thus far examined, from simple fungi to roundworms *(C. elegans),* mice, and humans (so far, they have not been identified among prokaryotes). The extent to which miRNAs are involved in animal development and physiology is truly impressive. The best-studied examples are as follows:

Neural development: A deletion of the gene encoding a specific miRNA called *miR-9* in fruit flies can cause duplication of sensory neurons, and the loss of other miRNAs is known to disrupt the normal development of the neuronal synapse.

Development of the immune system: It seems that miRNAs act as triggers that direct bone marrow progenitors along the B cell differentiation pathway. In one experiment, inactivation of a bone marrow–specific miRNA led to the development of fewer B cells and an excess of T cells.

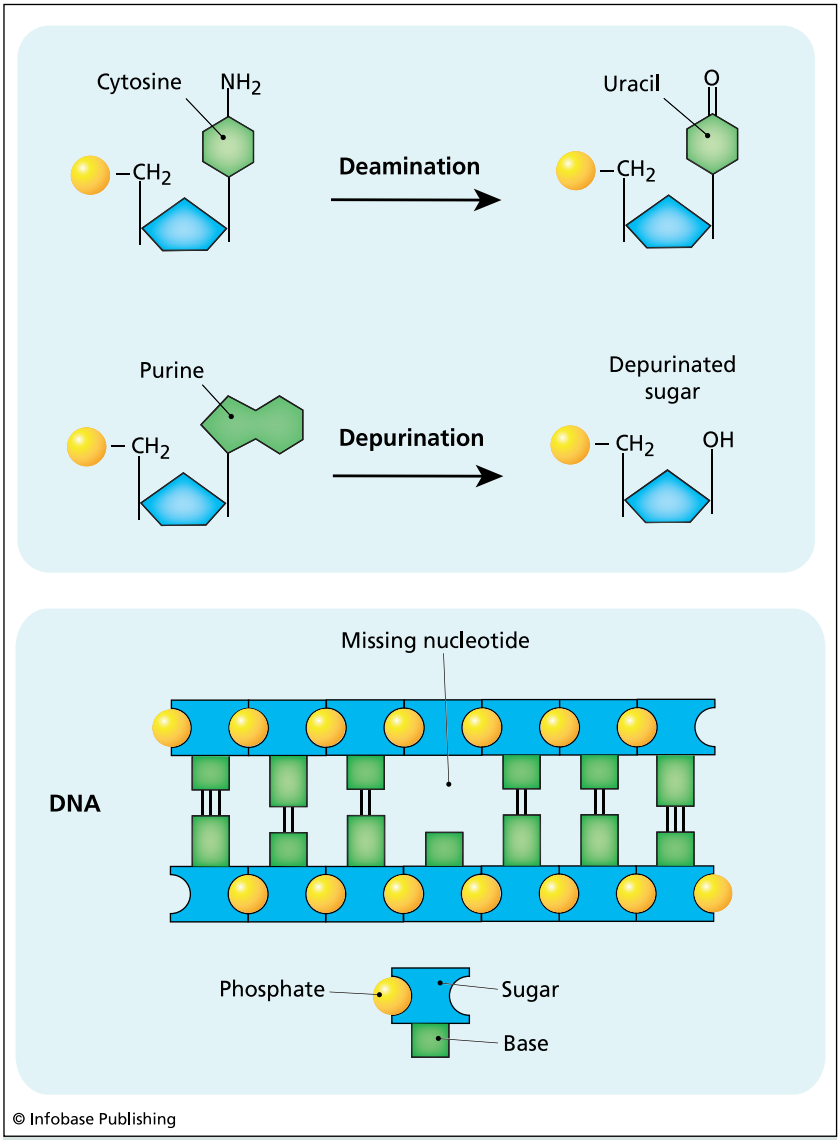
Maintenance of cells in an undifferentiated state: Embryonic stem cells express a special type of miRNA and, if these are inactivated, the cells begin to differentiate into a variety of adult cell types.

- **Immune response:** Certain miRNAs, found in the mouse and human, are known to bind to and inactivate invasive nucleic acids, such as viral genomes and transposons (see the section "Mobile Genetic Elements" below). Indeed, many scientists believe that miRNAs evolved as a defense mechanism against such invasions.
- **Cancer:** Overexpression of *miR-21* is associated with the occurrence of many types of cancer, including gliomas (brain tumors), breast cancer, and cancers of the lung and cervix.
- **Diseases of the heart:** Disruption of *miR-133* is associated with cardiac hypertrophy in mice and humans.
- **Hyper-muscularity:** Certain miRNAs act by silencing a gene coding for a skeletal muscle-inhibitory protein. When the expression of these miRNAs is disrupted, the musculature becomes overdeveloped.

Eukaryote cells use miRNAs to fine-tune gene expression. By studying these RNAs, scientists not only enhance their understanding of cellular functions but gain an immensely powerful strategy for treating diseases. If gliomas are produced by overexpression of *miR-21,* then interfering with this miRNA or its gene could prove to be an effective therapy. Other scientists are using the concept of gene silencing and RNA inhibition to study a wide range of genes and their products. The design of such experiments is relatively straightforward: Produce a miRNA that will silence the gene of interest and then simply monitor the effects that the interference has on specific cells or on the animal as a whole.

GENE MONITORS AND REPAIR CREWS

DNA is a very stable molecule, but it is not immutable. Every day, in a typical human cell, spontaneous chemical events, environmental pollutants, and radiation damage thousands of nucleotides. In



DNA damage from deamination and depurination. Deamination, or loss of an amino group (NH_2), converts cytosine to uracil. Depurination results in the loss of a purine nucleotide (top panel), leaving a gap, or missing tooth, in the DNA molecule (bottom panel). Both types of damage can lead to a catastrophic buildup of mutations if not repaired.

many cases, it takes only a single defective nucleotide within the coding region of a gene to produce an inactive, mutant protein. The most common forms of DNA damage are depurination and deamination. Depurination is the loss of a purine base (guanine or adenine) resulting in a gap in the DNA sequence, referred to as a missing tooth. Deamination converts cytosine to uracil, a base that is normally found only in RNA.

It has been estimated that about 5,000 purines are lost from each human cell every day and that over the same time period 100 cytosines are deaminated per cell. Depurination and deamination produce a great deal of damage and, in either case, the daughter strand ends up with a missing nucleotide and possibly a mutated gene, as the DNA-replication machinery simply bypasses the uracil or the missing tooth. If left unrepaired, the mutated genes will be passed on to all daughter cells, with catastrophic consequences for the organism as a whole.

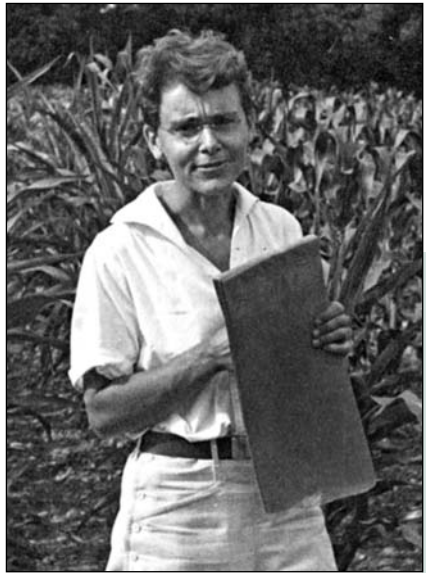
DNA damage caused by depurination is repaired by special nuclear proteins that detect the missing tooth, excise about 10 nucleotides on either side of the damage, and then, using the complementary strand as a guide, reconstruct the strand correctly. Deamination is dealt with by a remarkable group of DNA repair enzymes known as base-flippers. These enzymes monitor the DNA one nucleotide at a time. After binding to a nucleotide, a base-flipper breaks the hydrogen bonds holding the nucleotide to its complementary partner. It then performs the maneuver for which it gets its name. Holding onto the nucleotide, it rotates the base a full 180 degrees, inspects it carefully, and, if it detects any damage, cuts the base out and discards it. In this case, the base-flipper leaves the final repair to the missing-tooth crew that detects and repairs the gap as described previously. If the nucleotide is normal, the base-flipper rotates it back into place and reseals the hydrogen bonds. Base-flippers and the missing-tooth crew work 24 hours a day, seven days a week. Scientists have estimated that these crews

inspect and repair the entire genome of a typical human cell in less than 24 hours.

MOBILE GENETIC ELEMENTS

When first proposed by the pioneering American geneticist Barbara McClintock in 1951, the idea that genes could move from one location in the genome to some other location was greeted with disbelief and disdain. For more than 20 years this idea languished in a kind of scientific limbo until the advent of recombinant technology made it possible to prove the existence of these wandering genes, also known as transposons, transposable elements, and jumping genes. By the 1980s, McClintock's work was finally given the recognition it deserved, and in 1983, at the age of 81, she was awarded the Nobel Prize in physiology or medicine. She died on September 2, 1992.

McClintock's work provided several insights into the organization and evolution of the eukaryote genome. First, the position of some genes, within the genome, is flexible; second, the roles of



Barbara McClintock (1902–92) in a cornfield at Cold Spring Harbor Laboratories in the 1950s. Dr. McClintock received a Nobel Prize in 1983 for her discovery of transposable elements. *(Cold Spring Harbor Library and Archives)*

transposable elements, introns, and intervening sequences are interconnected; and third, viruses are direct descendents of jumping genes. With the completion of the genome project, it is now known that many of our genes were once transposable elements, including the 220 genes obtained by horizontal transfer from bacteria.

Insertional mutagenesis, discussed earlier in the chapter, is the connection between a jumping gene and an intervening sequence. As mentioned, only 1 percent of the human genome contains genes, with the rest of the DNA consisting of intervening sequences and introns. Our genome has evolved into a form that accommodates mobile genes. In such a genome, the odds of a transposable element damaging an existing gene are extremely small. When a jumping gene moves, it will, in all likelihood, reinsert into an intervening sequence, a region that lacks genes, where it may stay for thousands or millions of years. After having been duplicated, most transposons move again, thus producing many copies of the same gene, sprinkled around the genome. These copies of the original gene are then free to mutate into other genes that may eventually become useful to the organism. Thus, copies of the original transposon are the source of many of the genes now present in the human genome.

The flexibility of a transposable genome is perhaps the single most important characteristic that led to the explosive adaptability of eukaryotes and the many life-forms, especially the multicellular creatures, they produced. Such a genome is also important to modern medical therapies, such as gene therapy, that attempt to cure a disease by introducing a normal gene into the patient's genome. If our genome were organized like that of the prokaryotes, such a therapy would be nearly impossible.

Transposable elements have given us a flexible and extremely powerful genomic organization, but they have also given us the viruses and all the illnesses they produce, such as AIDS, polio, and the common cold. Somehow, millions of years ago, a jumping gene learned how to jump right out of the cell. It acquired this ability in

small steps as it moved from one place in the genome to another. A jumping gene that moves reinserts into an intervening sequence, devoid of other genes; occasionally, a transposon reinserts next to a gene, possibly one that codes for a protein that could serve as a capsid, a protein that protects a virus's genome, and forms the overall structure of the viral particle. The next time such a transposon moved it would take a copy of the potential capsid gene with it. Eventually, by moving from place to place, the transposon would have collected a large number of genes that not only made it possible for it to escape from the cell but also gave it the power to reinfect other cells. When that happened, a simple mobile genetic element went from being a molecular curiosity to a living thing, equipped with a life cycle and the power of reproduction.

From Cells to Bodies

Eukaryotes seem to have been destined for multicellularity. Not that they fared poorly as single cells. To the contrary, the phylogenetic class protista is one of the most successful and diverse groups of organisms around. But becoming multicellular requires a complex communication network backed up by far more genes and proteins than a prokaryote can muster. Prokaryotes did, however, lay the groundwork and formed the first, though temporary, association of cells in the form of fruiting bodies containing the spores of many individual cells.

THE ROAD TO MULTICELLULAR ORGANISMS

Myxobacteria, discussed in chapter 2, were the first cells to form brief multicellular associations for the purpose of reproduction. These prokaryotes spend most of their time as free-living vegetative cells. Starvation is the trigger that leads to the aggregation of

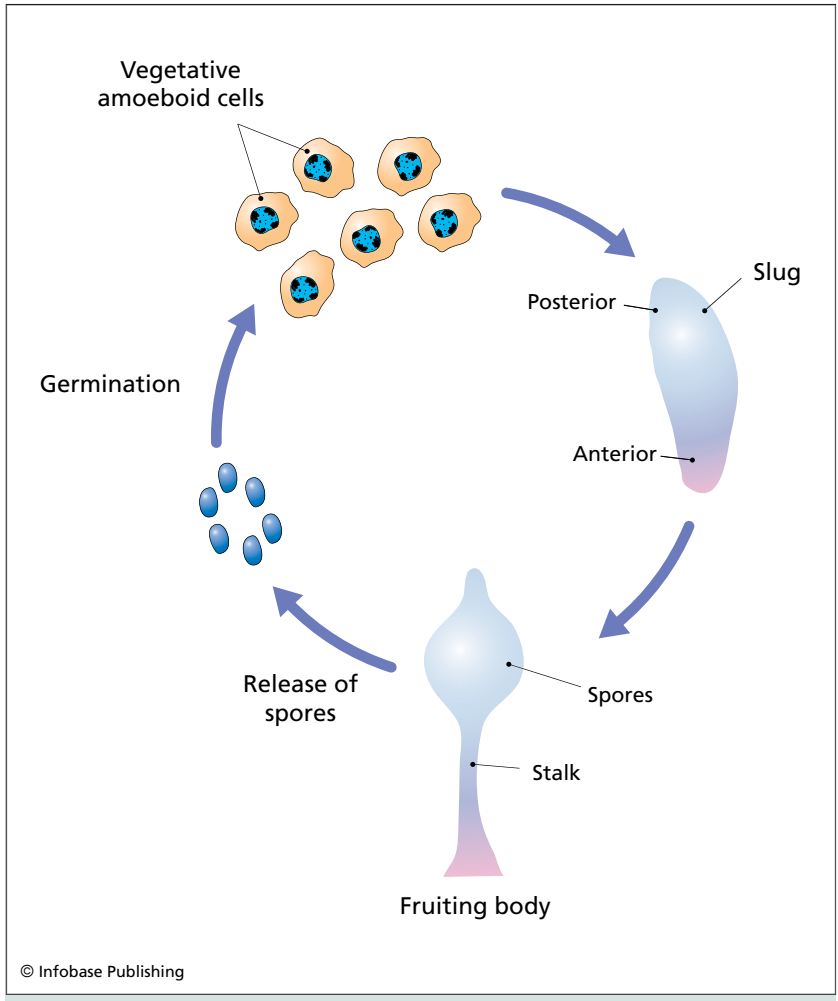
these cells into a sporulating fruiting body. As spores, the cells can hibernate until conditions improve; in addition, wind currents may carry them to other, more favorable locations, where they can germinate with the hope of finding food. This type of multicellular colonial behavior was refined by eukaryotes such as the amoeba, *Dictyostelium discoideum,* and the green algae, *Volvox*. Colonial forms eventually gave rise to true multicellular organisms such as sea sponges and sea cucumbers, followed by higher organisms, both aquatic and terrestrial.

Dictyostelium, a social amoeba that feeds on bacteria among the leaf litter of forest floors, is a eukaryote that has a life cycle similar to that of the myxobacteria. In this case, the formation of the fruiting body is preceded by the formation of a migrating slug, consisting of thousands of individual *Dictyostelium* cells. It is the slug phase that distinguishes this life cycle from that of the myxobacteria. The slug behaves like a single entity with a common purpose. It moves among the leaf litter as though it were a real slug or worm, testing the environment for a suitable place to form a fruiting body. Usually, the slug tries to migrate as far above the leaf litter as possible. In this way, the fruiting body gets maximum exposure to wind currents, improving the chance that the spores will be carried to a new location.

As with myxobacteria, the main stimulus for slug formation is starvation. When the individual cells run out of food they release a chemical called cyclic adenosine monophosphate (cAMP) that serves as a call for all of the *Dictyostelium* cells in the immediate neighborhood to aggregate. The hungriest cells answer the call first, forming the anterior of the slug. The less-hungry cells also answer the call, but they take their time in getting there. As a consequence, arriving late, they form the posterior of the slug. When the slug finds a suitable location, the anterior portion anchors itself to the substratum, and all of the cells in this region form the stalk, while the cells at the posterior form the spores. The cells forming the stalk

PROGRESSIVE STAGES OF MULTICELLULARITY

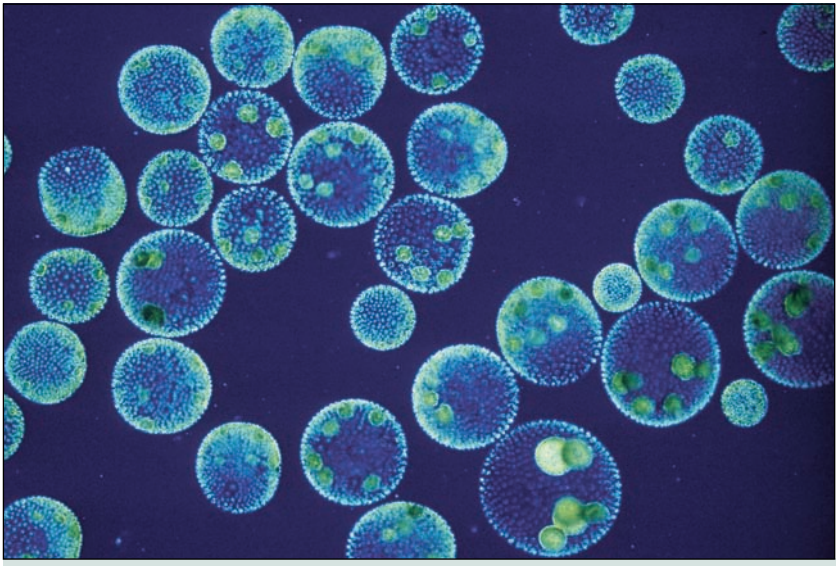
STAGE	DESCRIPTION
Cellular slugs	Temporary associations of identical cells, cooperating for a common purpose. The only known example among eukaryotes is *Dictyostelium*.
Cellular colonies	Permanent associations among identical cells. The colonies are capable of producing gametes (sperm and eggs). Two examples are *Gonium* and *Volvox*.
Sponges	Aquatic creatures constructed from different types of cells, including gametes, that differentiate during development. The cells are not organized into tissues, and there is no nervous system.
Cnidarians	Aquatic creatures that include the jellyfish, sea anemones, hydroids, and corals. The first appearance of tissues, a rudimentary nervous system, a mouth, and digestive tract. These animals are constructed from only eight cell types: epithelial, muscle, neural, glandular, interstitial, gonadal, mesenchyme, and the cnidoblast.
Mollusks	Organisms that include the clams, octopods, and snails, some of which are terrestrial. These animals have a well-developed mouth and digestive tract. In addition, some, like the octopus, have a highly developed central nervous system.
Echinoderms	Aquatic animals that include the sea stars, sea urchins, and sea cucumbers. All have well-developed tissues, including a digestive tract and nervous system.
Cartilaginous fish	Fish having a full set of organs and tissues but lacking a true skeleton. This group includes the sharks and rays.
Vertebrates	A subphylum of animals that are found in the water, on land, and in the air. They include the bony fishes, amphibians, reptiles, birds, and mammals. Organs and tissue systems are refined to an extraordinary degree, allowing reptiles, birds, and mammals to colonize the land.



Life cycle of *Dictyostelium*. Vegetative amoeboid cells feed on bacteria in the soil. When food runs low, the cells aggregate to form a migrating slug, which consists of hungry cells at the anterior and well-fed cells at the posterior. The slug eventually anchors itself to the substratum at its anterior end, which gives rise to the stalk, while the posterior differentiates into a fruiting body containing spores. Air currents spread the spores to new locations, and if conditions are favorable the spores germinate into vegetative cells.

are doomed; they die soon after the fruiting body is formed. There is an interesting logic associated with this arrangement. Stalk cells are the hungry ones, therefore they do not have the reserves necessary to make viable spores. The hungry cells, in effect, are being sacrificed for the good of the community as a whole. It is also interesting to note that this early form of multicellularity is associated with such complex behavior and the principle of cooperation for the good of the community.

The green algae, represented by *Gonium* and *Volvox,* produced the first permanent cellular colonies. Gonium colonies are small, concave discs, consisting of about 32 cells, whereas *Volvox,* resembling a green glass ball, has up to 50,000 cells. *Volvox* colonies have



***Volvox,* a colonial form of green algae.** A single colony may consist of more than 500 cells, each with a pair of whiplike tails (flagella). All the cells beat their flagella in unison, propelling the colony through the water. Very large colonies can exceed one mm in diameter and are easily visible to the naked eye. Many will be found to contain daughter and granddaughter cells in various stages of development. Magnification 16×. *(M. I. Walker/Photo Researchers, Inc.)*

Colorful red f nger sponge and brown tube sponges on Belize reef. *(Dennis Sabo/Shutterstock)*

cytoplasmic bridges that connect the cells together, allowing them to communicate with each other and to share nutrients. Each *Volvox* cell has a single flagellum, the beating of which is coordinated to propel the entire colony through the water like a rolling ball. The various cells within the colony seem to have specialized functions, as they cannot survive if the colony is disrupted. A small number of the cells are specialized for reproduction and serve as precursors for new colonies.

Although *Volvox* cells appear to be specialized, they are all essentially the same kind of cell, looking very much like *chlamydomonas,* the unicellular green algae that are believed to have given rise to the colonial forms. The next stage in the development of multicellularity came with the appearance of the sea sponges, animals that are constructed from several distinct cell types. These simple animals produce gametes that fuse to form an embryo. Development of the embryo is associated with cellular

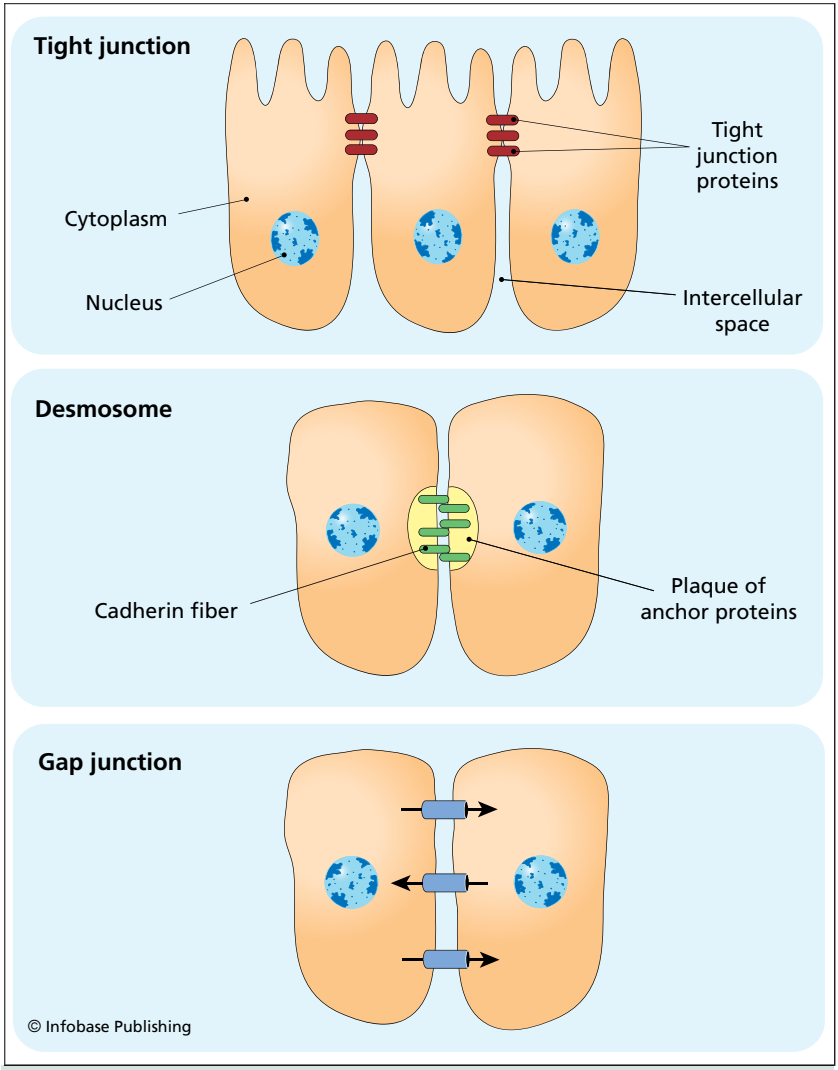
Crystal jellyf sh, *Aequorea victoria.* *(Dwight Smith/* Shutterstock)

differentiation by mechanisms that have been retained by higher animals. Sponges, though multicellular, do not have tissues or a nervous system.

A true nervous system made its first appearance with the evolution of the cnidarians (e.g., jellyfish and corals), animals that are made from only eight cell types but which possess a mouth, digestive tract, and tissues, such as epithelia and muscles. These tissues and organ systems were refined by the mollusks (snails and octopods) and echinoderms (sea stars and cucumbers), reaching an exquisite level of sophistication with the appearance of the vertebrates during the Cambrian period about 500 million years ago.

CELL JUNCTIONS

A *Volvox* colony is held together with simple cytoplasmic bridges. True multicellular creatures have evolved more sophisticated structures, called cell junctions, for holding the tissues and organs together. Three of the most important of these are tight junctions, desmosomes, and gap junctions.



Cell junctions. Tight junctions are constructed from membrane proteins that stitch the two cells together very tightly. Desmosomes, constructed from plaques and protein fibers called cadherins, hold the cells together but allow material to pass through the intercellular space. The gap junction is a hollow tube formed from six identical proteins called connexins (not shown). These junctions hold the cells together while allowing small molecules to pass freely between the cells, as indicated by the arrows. The "gap" refers to the large intercellular space. Note that the size of each cell junction, relative to the cell, has been exaggerated for clarity.

Tight Junctions

These junctions, found primarily in the epithelial lining of an animal's digestive tract, stitch two cells together so tightly that the intercellular space at the site of the junction is obliterated. This junction not only keeps the cells together but also performs a very important secondary function of blocking the movement of bacteria and small molecules from the lumen (interior) of the gut into the intercellular space. In other words, tight junctions make the gut leak-proof. Nutrients obtained from food that is eaten must pass through the gut's epithelial lining, where they are screened before being allowed to enter general circulation. Without the tight junctions, unwanted chemicals, possibly of a toxic nature, would be absorbed. In addition, the millions of bacteria that inhabit an animal's intestinal tract would quickly spread throughout the body, leading to a fatal infection.

Desmosomes

Most of the cells in an animal's body are held together by desmosomes, junctions that are like tiny organic rivets. A protein plaque, analogous to a rivet head, is located beneath the membranes of adjacent cells. Another protein, called Cadherin, projects from each plaque into the intercellular space where they form chemical bonds with each other, thus holding the two cells together. This junction is more relaxed than a tight junction, and molecules are free to diffuse throughout the intercellular space.

Gap Junctions

Cells need to be anchored to each other. In many cases, they also need to exchange fluids. The cytoplasmic bridges found in *Volvox* serve both of these functions. Gap junctions, consisting primarily of hollow protein tubes, provide both an anchor and a fluid conduit in higher organisms. The sharing of cytoplasm is especially important in cardiac muscle, where the contraction of each myocyte is coordinated by the movement of ions through gap junctions.

THE EXTRACELLULAR MATRIX

Cell junctions are not the only way for cells and tissues to hold together. Indeed, most of the "glue" that holds an animal together and gives it shape and volume is due to the extracellular matrix (ECM). The ECM is a collection of glycosylated proteins that are secreted by all cells to a certain extent but primarily by a special type of cell called a fibroblast. Once the ECM is produced, cells can attach to it, thus holding the tissue together without direct contact between the neighboring cells.

Cellular proteins mediating connection with the ECM are adhesion proteins found in the glycocalyx. The most important of these adhesion proteins are the integrins and the cadherins (see the panoramic view of the glycocalyx in chapter 3), both of which make direct contact with ECM proteins. To fortify the connection, the Integrins and the Cadherins are tightly bound to the cytoskeleton, in particular to actin filaments located just beneath the cell membrane. The ECM is also known as connective tissue and will be discussed further in the next chapter.

CELL COMMUNICATION

Eukaryotes expend a tremendous amount of time and energy building and maintaining the glycocalyx, a molecular forest that would, if people were small enough to walk through it, rival the beauty of any forest on the surface of the Earth. Despite this, eukaryotes build the glycocalyx not because they want to look good, but because they need to communicate with their environment, and especially with other cells. The glycoproteins in the cell's forest come in many different shapes and sizes, but functionally they all fall within one of three groups: transporters, ion channels, and receptors.

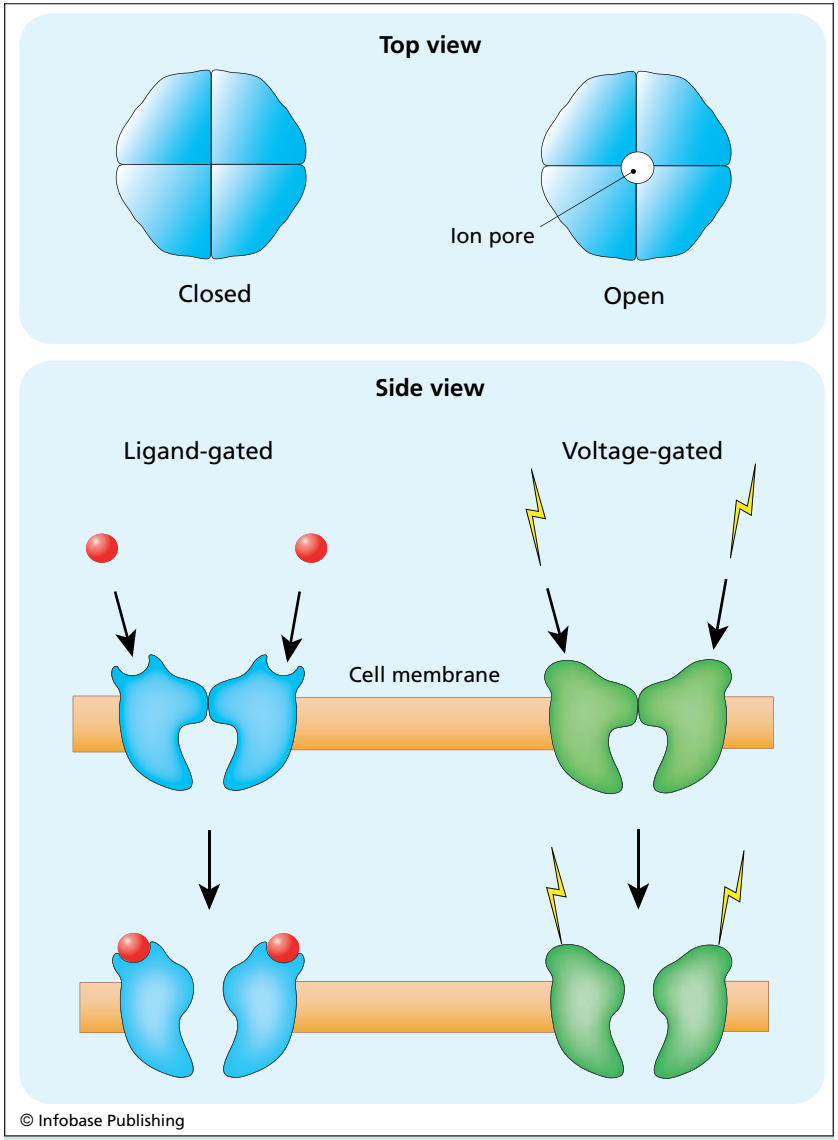
Transporters are designed to carry specific molecules (usually food of some kind) across the cell membrane. Each type of molecule, such as glucose, lactose, and amino acids, has its own transporter. The intestinal tract, which absorbs the nutrients from the food one

eats, has an enormous population of each kind of transporter. Virtually all of the nutrients are taken into intestinal cells by transporters and then released into the blood by transporters working in reverse, that is, moving molecules from inside the cell to the outside (into the circulatory system). Other tissues of the body also have transporters. Most animal cells use glucose as their primary source of energy; this is particularly true of the brain. Consequently, all of the tissues in an animal's body have a great number of glucose transporters. (Other sugars, brought in by the intestinal cells, are converted to glucose before being released into general circulation, so other kinds of sugar transporters are not needed outside the intestinal tract.)

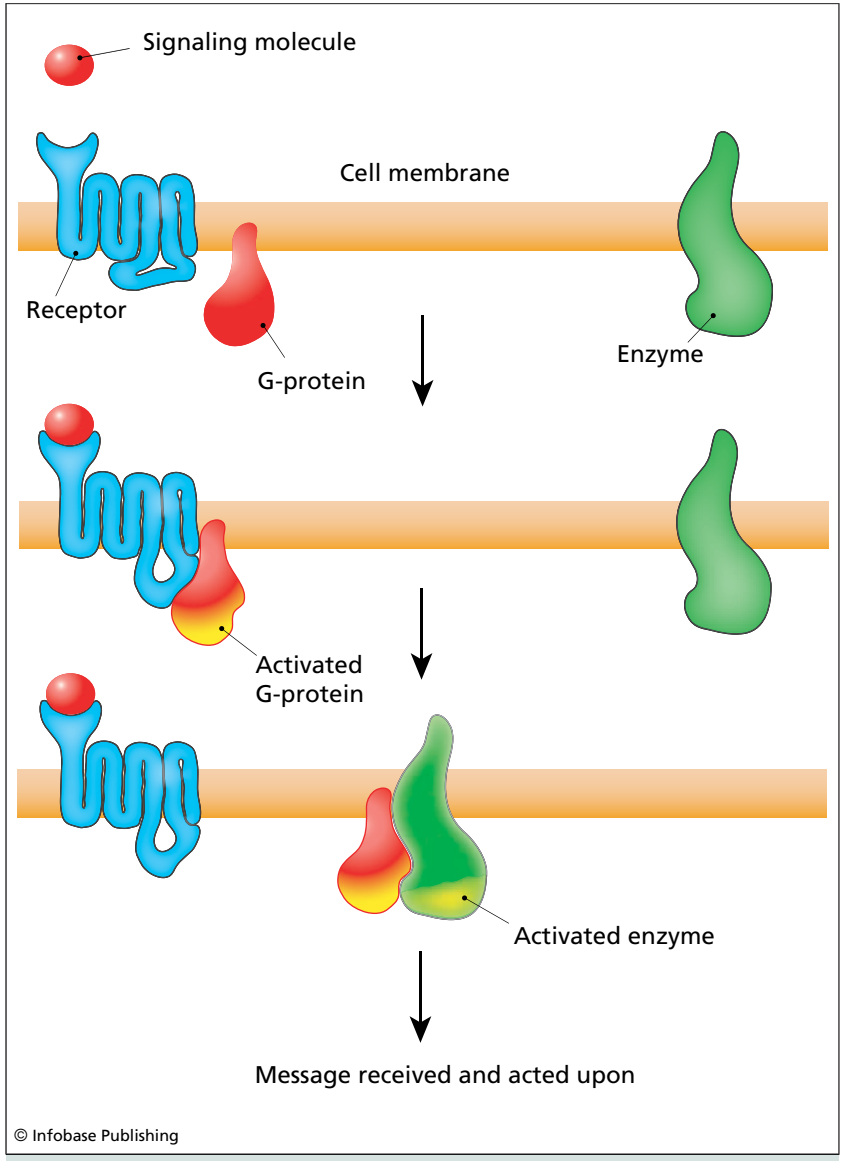
The second group of glycoproteins, the ion channels, represents a very powerful messaging system that uses the flow of ions into the cell as a means of communication; much in the way the flow of electrons down a phone line is used to speak with someone at a great distance. Ion channels are gated, that is, they can be opened and closed. Ligand-gated ion channels open when a signaling molecule binds to the channel. Voltage-gated channels open when they sense, or are jolted by, an electric field across the membrane.

Receptors, the third and most diverse group of cell-surface glycoproteins, are at the heart of the cell's ability to communicate with other cells, a distinction they share with ion channels. Each receptor is designed to respond to a specific signaling molecule. When a signaling molecule binds to its receptor, it sets off a chain of biochemical events in the recipient or target cell. The signal may cause the cell to increase or decrease the output of a product that is required by other cells in the body, or it may order the cell to grow, to stop growing, or even to commit suicide.

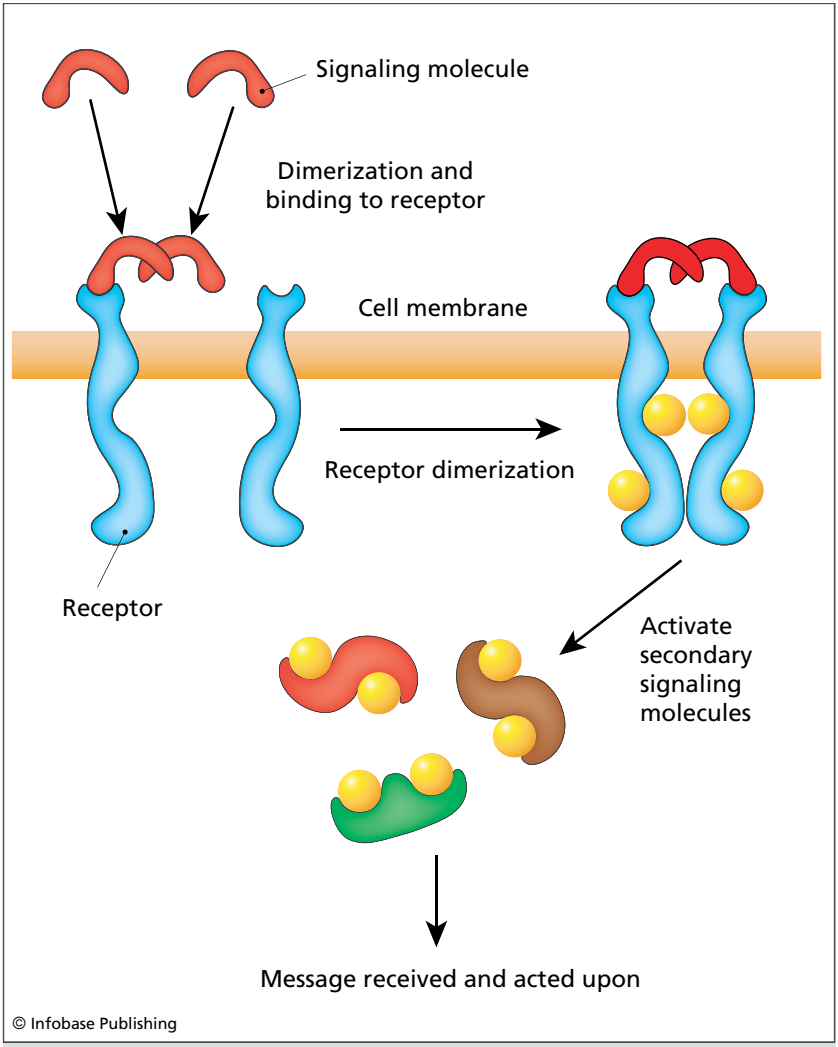
The most common type of receptor that cells use for communication is the G-protein linked receptor. A G-protein is an enzyme that gets its power from an energy reservoir called guanosine



Ion channels. Ligand-gated channels open when bound to a signaling molecule, and voltage-gated channels open when they receive an electrical jolt. Ion channels are glycoproteins that are an important part of the glycocalyx.



G-protein-linked receptor. The signaling molecule binds to the receptor, leading to the activation of the G-protein, which in turn activates an enzyme. The enzyme activates secondary messengers, which effect some change in the cell.



Enzyme-linked receptor. The primary signaling molecule forms a dimer (two identical molecules bound together) and binds to the receptor, stimulating dimerization of the receptor. This stimulates phosphokinase activity of the receptor, which phosphorylates itself and several secondary signaling molecules. The activated signaling molecules effect some change in the cell.

triphosphate (GTP), a close relative of the cell's main energy reservoir, adenosine triphosphate (ATP). Binding of a signaling molecule to one of these receptors leads to the activation of the G-protein, which in turn activates a transduction, or signal conversion, enzyme. This enzyme, in turn, produces or activates secondary messengers that cause the cell to carry out the appropriate response. This chain of events, from the moment the signaling molecule binds to its receptor, is called a signal transduction pathway. Alternatively, some signaling pathways use an enzyme-linked receptor, effectively cutting out the G-protein middleman. In a pathway such as this, the receptor itself is also a transduction enzyme, which is activated by the binding of a signaling molecule. The activated receptor phosphorylates, and thereby activates, secondary messengers that lead to a change in the activity or behavior of the target cell.

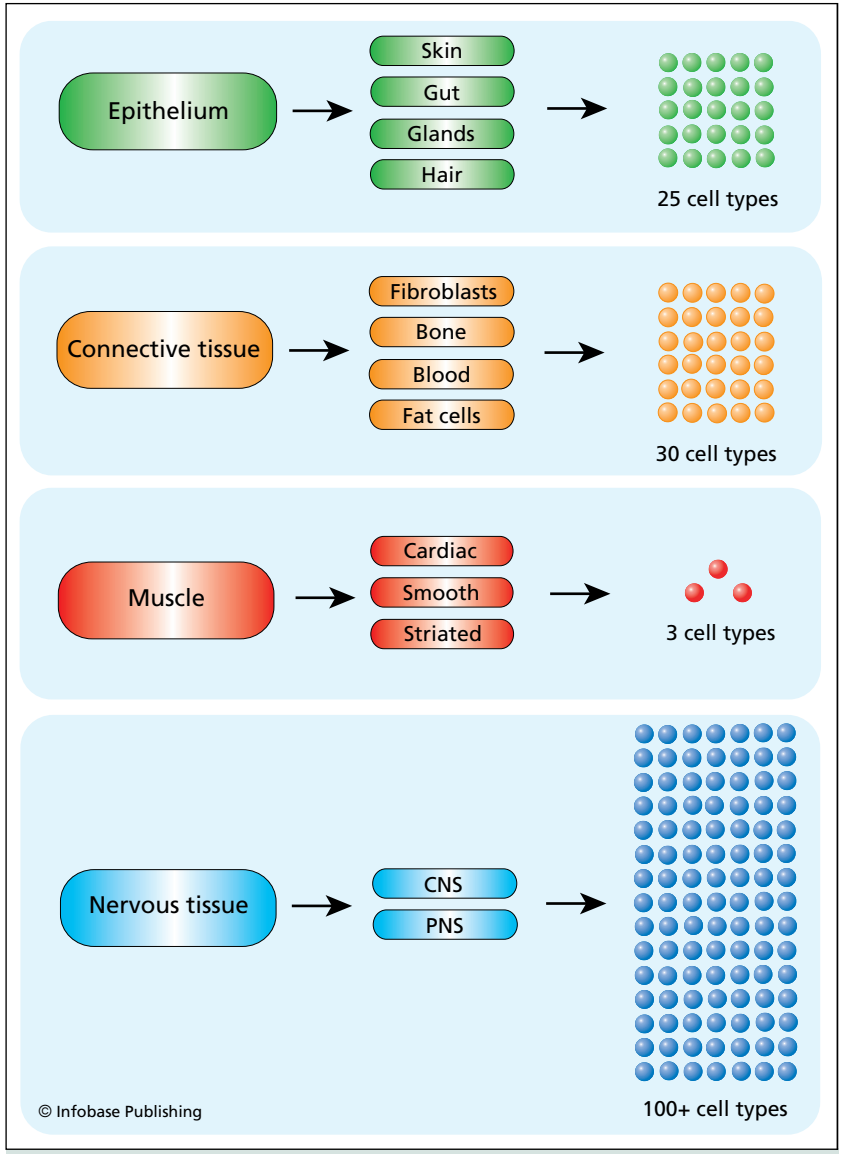
In a multicellular organism, such as a housefly or a human, communication is taken care of by the nervous system, using a combination of ligand-gated and voltage-gated ion channels, or by the endocrine system, which releases hormones as signals to other cells. These two systems coordinate virtually everything that goes on in an animal's body. The exquisite regulation of ion channels in neurons gives us our intellect, vision, hearing, and all other sensory perceptions. The endocrine system regulates our growth, sexual maturation, general energy levels, and even our mood from one day to the next. (The function of neurons and cell communication in general will be described in greater detail in chapter 8.) The regulation and coordination of ion channels for the purpose of cell-to-cell communication has no counterpart among the prokaryotes.

7

Cells in the Human Body

The evolution of multicellular creatures was accompanied by the diversification of cell types. The simplest multicellular animals, such as the sponges and cnidarians, are made from fewer than a dozen cell types, whereas humans are constructed from more than 200 different kinds of cells. These cells represent four primary tissues: epithelium, connective tissue, muscle, and nervous tissue. The primary tissues give rise to all the organs and fluids of the body. For example, epithelium produces skin, gut, glands, and hair, whereas connective tissue gives rise to fibroblasts, bone, blood, and fat cells. Each of the primary tissues is constructed from many different cell types. Based on cell variety alone, the most complex primary tissue is neural, consisting of more than 100 different kinds of neurons.

The many kinds of cells that make up an animal's body differ both in structure and function. This is not to say that a muscle cell, for example, contains different organelles than a neuron or liver



Cells of the human body. There are four primary tissues in the body: epithelium, connective tissue, muscle tissue, and neural tissue. These tissues, representing more than 200 cell types, give rise to the body's structures, blood, and other fluids. The greatest diversity of cell types is found among the tissues of the central nervous system (CNS) and peripheral nervous system (PNS).

cell; these cells are all eukaryotes and therefore have the same basic composition as shown previously. However, the external shape, the number of organelles, and even the DNA content vary widely among the differing cell types. To illustrate the extraordinary variety of cells that make up the human body, this chapter will describe examples of epithelium, connective tissue, and muscle, while nervous tissue (neurons) is covered in the following chapter. But the discussion begins with a brief overview of embryonic development, a process that determines the fate of every cell and the origin of every tissue.

EMBRYONIC DEVELOPMENT

All plants and animals begin life as a single cell, known as an egg or an oocyte. Fertilization of the egg triggers a developmental program that produces an organism made up of many millions, sometimes billions, of cells. But the creation of such a creature is not just a matter of the egg dividing many times to produce a huge number of cells. Instead, embryogenesis is a combination of cellular division and differentiation. Cell division increases the number of cells, while differentiation transforms those cells into many different kinds. Consequently, by the time a human infant is born, the child's body not only contains several billion cells, but those cells have differentiated into more than 200 distinct types that form all of the tissues and organs of the body.

Embryonic development is similar in most animal species and is divided into three major stages. The first stage is the formation of a blastula, a hollow ball or disk of embryonic cells known as blastomeres. The second stage occurs when the blastula invaginates and reorganizes itself to produce a gastrula, which defines the body axis and sets the stage for the appearance of the three basic germ layers known as ectoderm, mesoderm, and endoderm. These three germ layers give rise to all the cells and tissues of the body. Immediately after the formation of the three germ layers, the ectoderm begins to form the nervous system—this defines the third embryonic stage

known as neurulation. The ectoderm also gives rise to epithelium (skin), eyes, ears, the linings of blood vessels, and certain internal organs, such as the lungs and kidneys. The mesoderm originates with a population of amoeboid-like cells known as mesenchyme that migrate throughout the embryo's body, taking up positions between the ectoderm and the endoderm. Mesenchyme, and the mesoderm it produces, gives rise to blood cells, the skeleton, muscle, and blood vessels. Some of the mesenchyme cells persist in an undifferentiated state throughout the life of the individual, where they serve to replace damaged or worn-out mesodermal tissues. At this stage they are known as stem cells. In the late-stage embryo, the endoderm becomes surrounded by the mesoderm and the overlying ectoderm. This tissue is destined to form most of the internal organs of the body, such as the gut, the respiratory tract, the liver, and the pancreas.

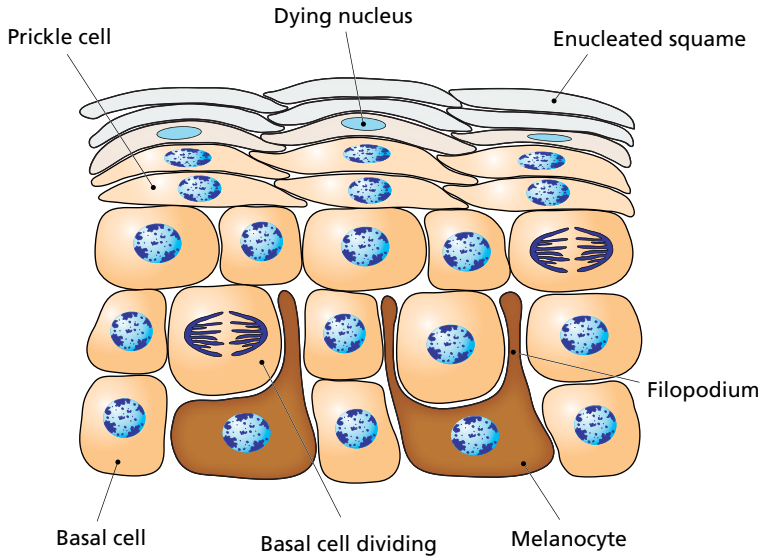
EPITHELIUM

Epithelium is the tissue that covers the body and forms the surface layer of many organs such as the lungs, stomach, intestines, and the inner and outer linings of blood vessels. Some organs, such as the lungs and kidneys, are composed almost entirely of epithelium. The most notable characteristic of epithelium is that the cells are tightly connected to each other to form a continuous uninterrupted sheet or organ. This close association between the cells is especially important in the skin, which serves as a barrier to the outside world, and in the gastrointestinal tract, which serves as a selective barrier to the gut contents. In this sense, the epithelium, unlike the other tissues, plays a vital role in the conservation of body fluids as well as shielding the body against infectious or noxious agents from the environment. The diverse roles of the epithelium are reflected in the various physical forms that these cells assume, which are generally of three types: squamous, columnar, and cuboidal. Squamous epithelia have a flattened profile and are typical of the outer skin

layers. Columnar epithelia have an elongated profile and are found most often in the digestive tract. Cuboidal cells often synthesize and secrete hormones and thus are typically found in glandular tissue, such as the liver or the pituitary gland. They are also located in the deeper layers of the skin.

Skin

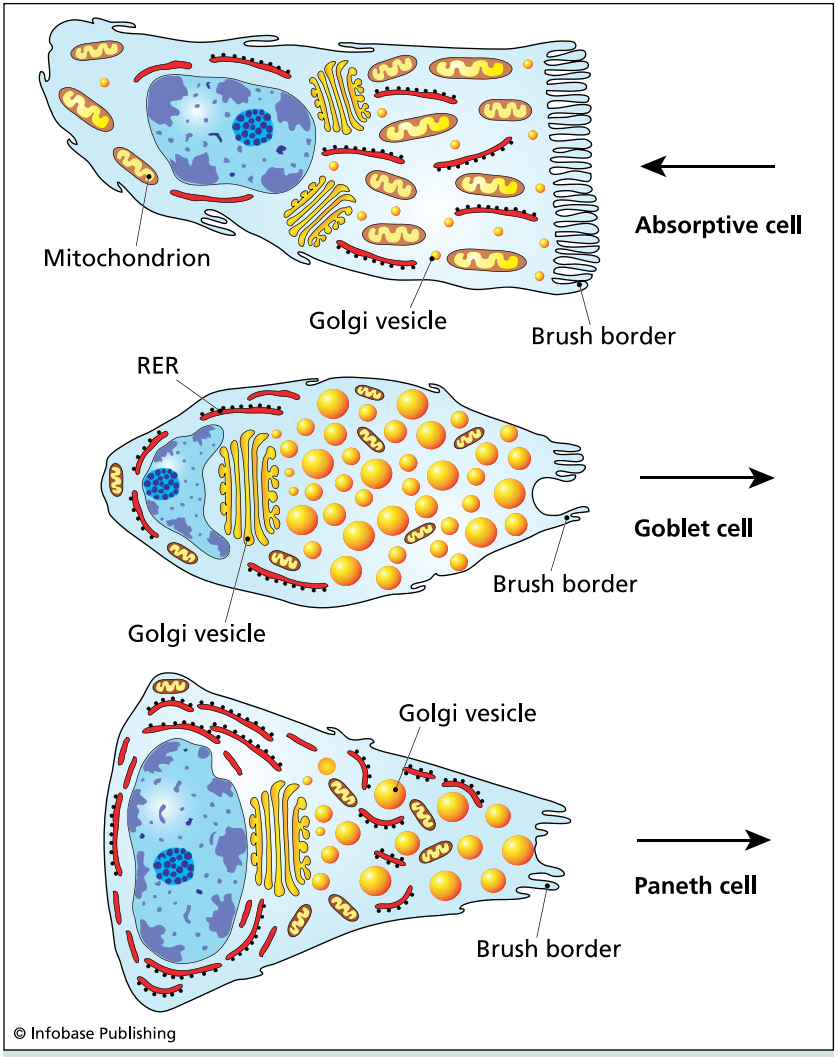
Human skin, made from epithelial cells, protects us from dehydration and microbial infections. It also contains sweat glands to help cool the body, sebaceous glands that oil the skin, and hair follicles. The barrier against microbes consists of a relatively dry physical barrier that is difficult for microbes to penetrate. The skin also has a large population of ion pumps, located in the membranes of every epithelial cell, that deposit hydrogen ions on the surface of the skin, producing an acid mantle capable of inhibiting the growth of most bacteria. Human epidermis is a stratified epithelium that consists of two populations of squamous cells: the outermost enucleated squames and the nucleated prickle cells. Just beneath the squamous cells are the cuboidal basal cells, and at the deepest layers are pigment-containing melanocytes that give the skin color and protect the inner cell layers from the damaging effects of ultraviolet radiation. The melanocytes and many of the basal cells are attached to the underlying connective tissue (described below). Melanocytes have long, thin fingerlike projections, called filopodia, that work their way in between the basal cells. The basal and squamous cell layers are in a constant state of change. Division of a basal cell is followed by a process called keratinization by which the daughter cells are transformed into prickle cells. Keratin is a tough protein that makes the outer cell layer resistant to abrasion. In the final stage of keratinization, the prickle cell loses its nucleus and the now dead and fully keratinized squame eventually flakes off from the surface. Keratinized squames from the scalp are called dandruff.



Structure of the epidermis. The epidermis is a stratified epithelium that forms the outer layer of the skin. It consists of three cell populations: squamous cells (the enucleated squames and the prickle cells), the cuboidal basal cells, and, at the deepest layers, pigment-containing melanocytes. The basal and squamous cell layers are in a constant state of change. Division of a basal cell is followed by keratinization, a process by which the daughter cells are transformed into prickle cells. Keratin is a tough protein that makes the outer cell layer resistant to abrasion. In the final stage of keratinization, the prickle cell loses its nucleus. The now dead and fully keratinized squame eventually flakes off from the surface. Keratinized squames from the scalp are called dandruff.

The Digestive Tract (The Gut)

The gut is made of different kinds of columnar epithelial cells that are specialized for absorbing nutrients from the food that is eaten and for secreting large quantities of proteins, lipoproteins, and glycoproteins needed both for digestion and to help control the



Epithelium of the digestive tract. Essential molecules are extracted from the food we eat by absorptive cells, equipped with many mitochondria to convert the molecules to ATP. Goblet cells secrete mucus into the intestinal tract for lubrication, and paneth cells secrete an antibacterial protein. All of these cells have brush borders to maximize the surface area of the membrane, either for secretion or absorption (indicated by the arrows). Goblet and paneth cells, both specialized for secretion, have a single large Golgi complex aimed directly at the brush border, that releases enormous vesicles.

enormous population of intestinal bacteria. These cells have evolved a shape that reflects the directionality of their activity. That is, they either absorb material from the intestinal tract or secrete material into the lumen of the tract. Consequently, these cells have an elongated shape, with one surface helping to form the lumen of the gut. This surface is the only one involved in absorption or secretion. The absorptive, goblet, and paneth cells of the small intestine are striking examples of epithelium.

The absorptive cells (also known as brush-border cells) absorb nutrients from the intestine along one surface, covered in microvilli (the brush border), that projects into the lumen of the tract. The cytoplasm of these cells is highly polar, with a large population of mitochondria and extensive rough endoplasmic reticulum (ER). The mitochondria contain the enzymatic machinery needed to process the incoming nutrients for the final production of ATP. (For more on this process, see chapters 2 and 3.) These cells, being specialized for absorption, do not have an extensive Golgi complex. Goblet cells are devoted to the secretion of mucus into the gut, which serves to lubricate the walls of the intestine, improving absorption and the movement of the gut contents. The cytoplasm of these cells has an extensive RER and a single large Golgi complex, aimed directly at the brush border, that releases enormous vesicles full of mucus (a special kind of glycoprotein). Paneth cells are part of the innate immune system (discussed later in this chapter), which helps control bacterial populations in the lumen of the gut by secreting proteins called cryptdins that kill bacteria. Like the goblet cells, paneth cells have a highly developed rough ER and Golgi complex. Release of the enormous secretory vesicles is polarized, directing all vesicles to the secretory surface.

Endocrine Glands

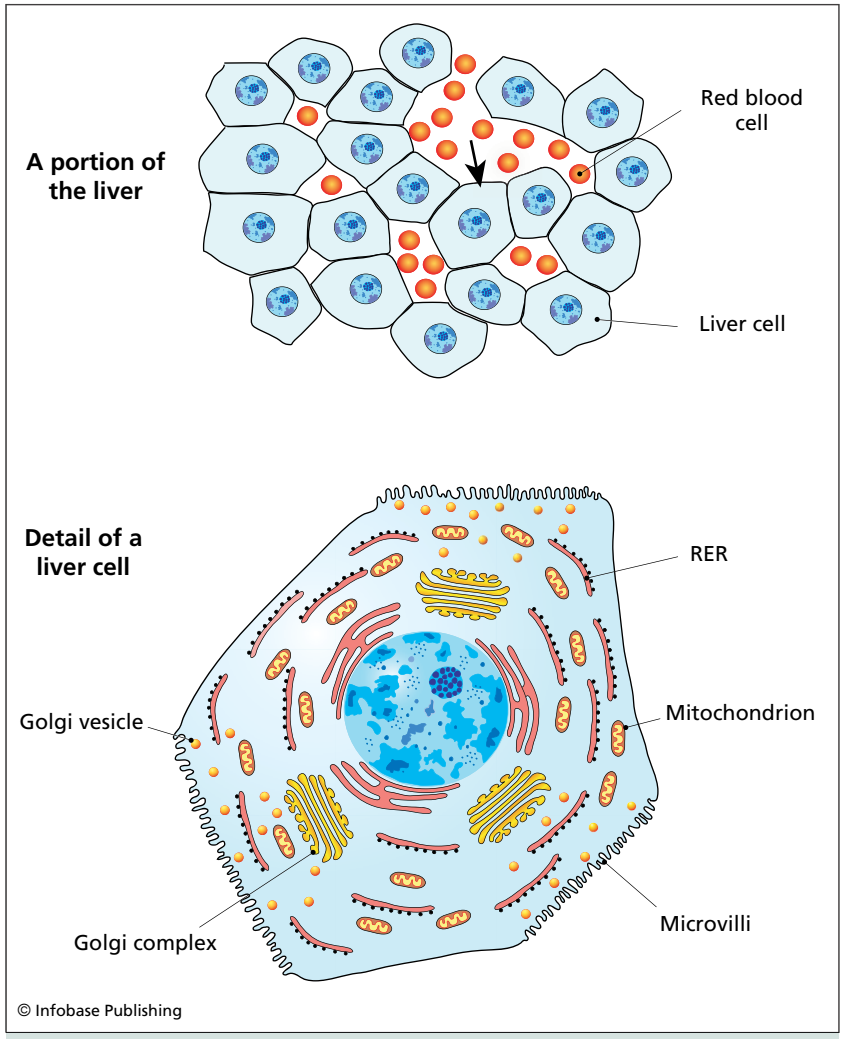
The human body has many endocrine glands that serve to coordinate digestion, reproduction, the fight-or-flight response, and the

uptake and utilization of food molecules from the blood. In this section, two of the most important glands: the liver and the pituitary gland will be discussed.

Liver

The liver, weighing almost three pounds, is the heaviest gland in the body and is constructed primarily of cuboidal epithelium (the liver cells). The five- or six-sided cells are loosely arranged around an intricate system of blood vessels and liver canals. The canals, called the portal system, deliver a substance called bile to the intestinal tract for the digestion of fatty compounds. Liver cells are extremely active metabolically, being involved in detoxifying the blood as well as manufacturing bile. Consequently, these cells have a large number of mitochondria, a very extensive endoplasmic reticulum, and several Golgi complexes. The cell membranes lining blood vessels, or the portal system, have many microvilli to facilitate transport into and out of the cell.

To maximize their biosynthetic capabilities, liver cells in humans and many other vertebrates have polyploid nuclei. That is, the DNA content has been duplicated fourfold to eightfold, compared to a haploid nucleus (i.e., a sperm or an egg nucleus). Consequently, liver cells may be found that are diploid, tetraploid, or octoploid (abbreviated as 2n, 4n, and 8n). Duplicating the genome provides additional copies of crucial genes, all of which may be transcribed simultaneously in order to increase the amount of protein being produced over a given period of time. This is a very clever strategy, making a single octoploid cell the functional equivalent of four diploid cells. Thus, a liver consisting of octoploid cells can be smaller than a diploid liver and still achieve the same output. The practice of maximizing cellular output by duplicating the entire genome is an ancient strategy commonly employed by insects. Insect cells have been found containing a quantity of DNA that is 100,000 times greater than that present in the animal's sperm or eggs.



Organization of the liver and structure of liver cells. The five- or six-sided cells, shown at the top, are loosely arranged around an intricate system of blood vessels and a system of liver canals, called the portal system, that contain bile secretions. A typical liver cell (bottom) has an extensive RER, a large number of mitochondria, and multiple Golgi complexes in addition to a large polyploid nucleus. The cell membranes lining blood vessels, or the portal system, have many microvilli to facilitate transport into and out of the cell. The magnified cell is from the image at the top. Its position is marked with an arrow.

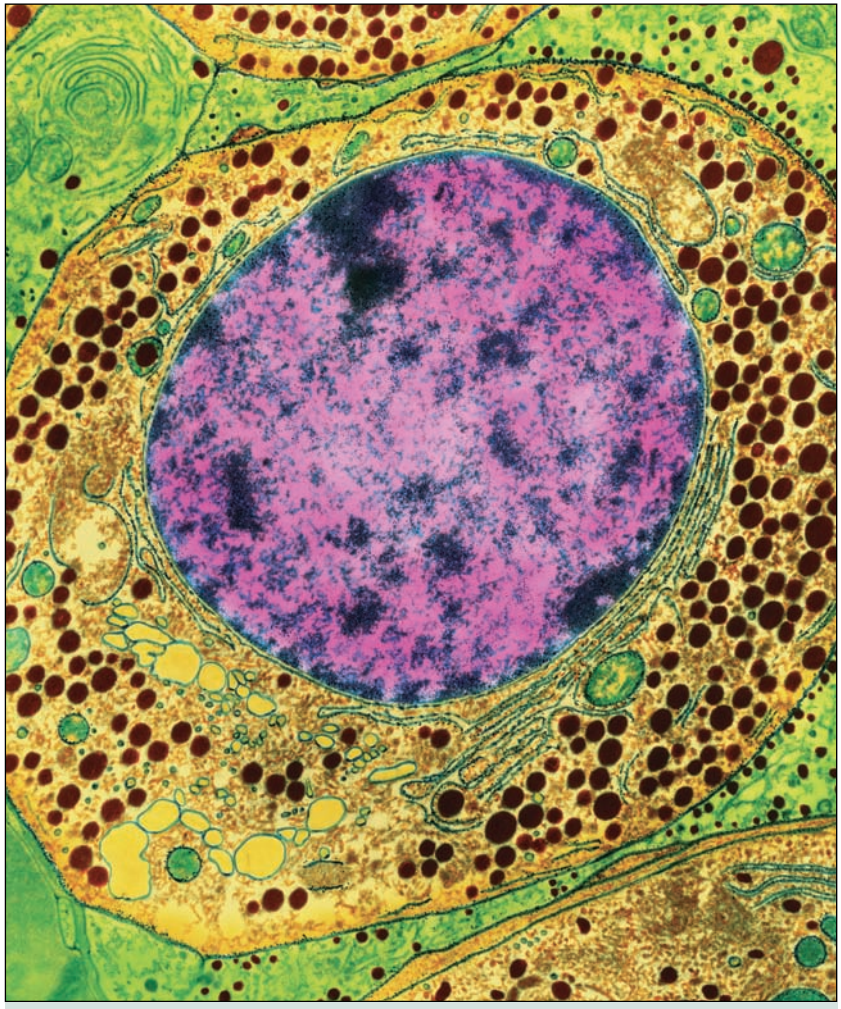
The Pituitary Gland

Another very important glandular epithelium is the pituitary gland, the master gland of the vertebrate body. Located at the base of the brain, it is about the size of a cashew nut and, despite its small size, is in charge of producing all of the hormones that coordinate the many physiological processes occurring in all vertebrates. This gland, constructed almost entirely of cuboidal epithelium, consists of 10 different cell types, each of which specializes in the synthesis and release of a different hormone. Each cell type is named after the hormone it produces. Thus, growth hormone–producing cells are known as GH cells or somatotrophs, and thyroid hormone–producing cells are known as thyrotrophs.

Hormones that stimulate and regulate the gonads (ovaries and testis) are produced by two kinds of gonadotrophs: one produces follicle-stimulating hormone and the other produces luteinizing hormone. Other pituitary cells synthesize hormones that stimulate the adrenal glands (adrenocorticotrophs or corticotrophs), regulate lactation (lactotrophs), and control the production of melanin in the skin (melanotrophs). The melanotrophs produce melanocyte-stimulating hormone (MSH), which is very important in lower vertebrates, such as lizards and amphibians, where its release can stimulate a rapid change in skin color. The two remaining cell types are special neurons known as neurosecretory cells that secrete oxytocin (oxytrophs) and vasopressin (vasotrophs). The cell bodies of the vasotrophs and the oxytrophs are located in the hypothalamus, but their axons are located in the posterior portion of the pituitary gland (neural structure is described in chapter 8). Oxytocin causes milk ejection from the breasts and contraction of the uterus during birth. Vasopressin regulates salt and water balance by stimulating the kidneys to retain water.

CONNECTIVE TISSUE

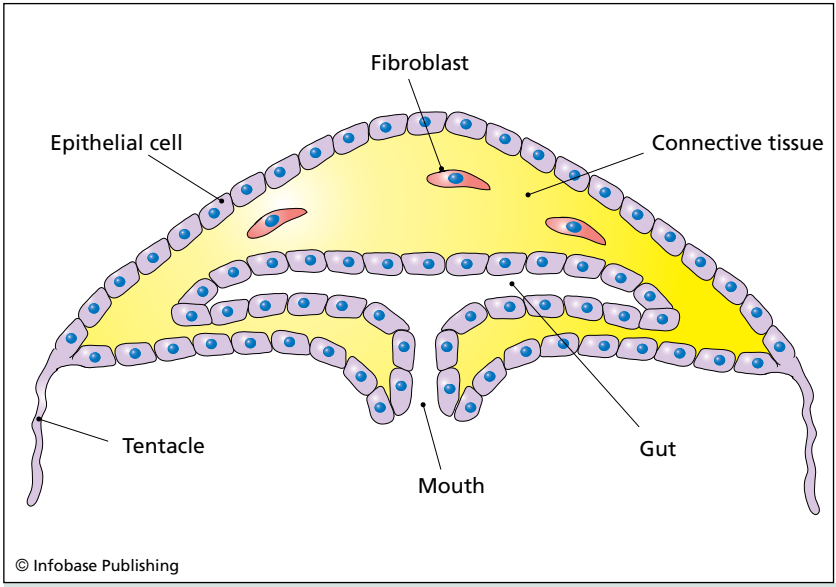
This tissue, derived from mesoderm, gives the body shape and volume and also provides mechanical support and a soft cushion



Colored transmission electron micrograph of a growth hormone-producing cell (somatotroph) from the pituitary gland. The pituitary gland is located at the base of the brain. The growth hormone can be seen in the numerous granules (brown) within the cell cytoplasm (yellow). Visible cell organelles include mitochondria (round, green) and the nucleus (purple and pink). There are large amounts of rough endoplasmic reticulum (thin green) with the protein-synthesizing ribosomes (black dots). Magnification 1,000×. *(Quest/Photo Researchers, Inc.)*

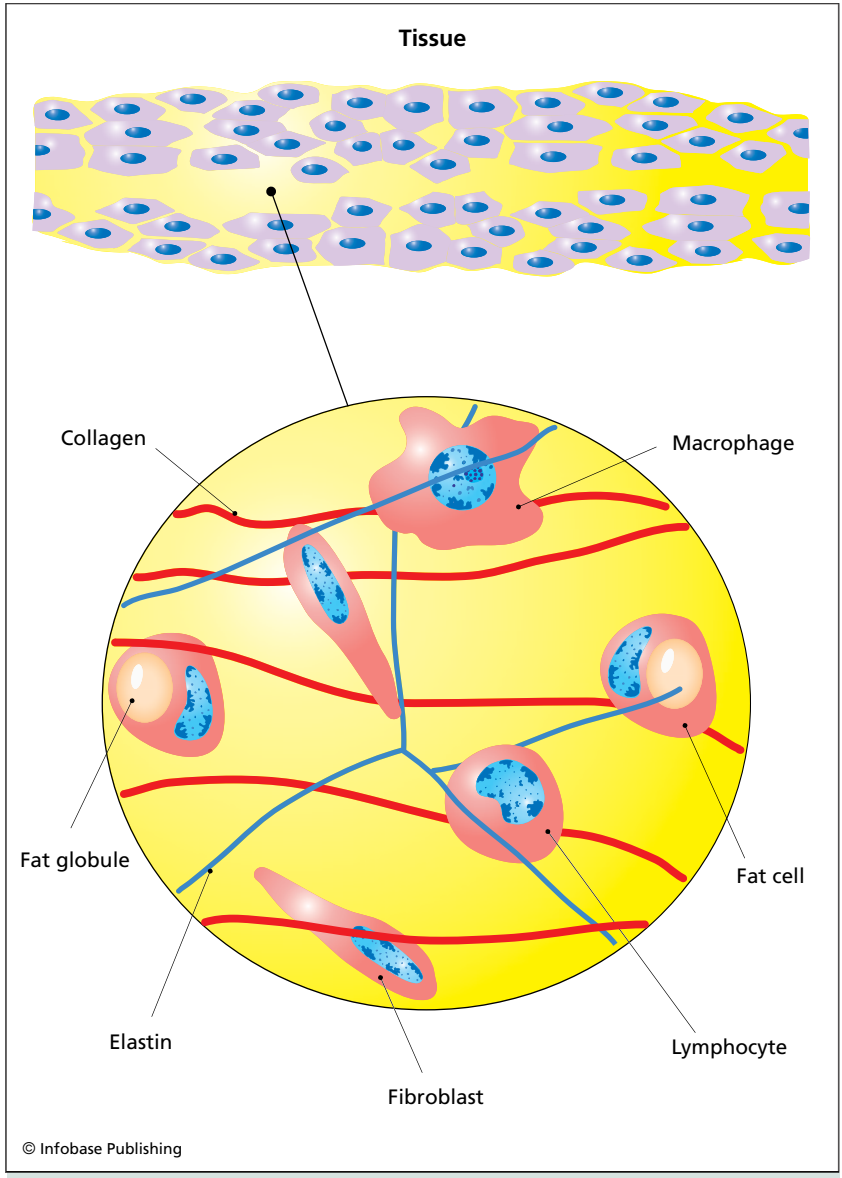
for internal organs. This type of tissue is produced by fibroblasts, bone-forming cells, and by blood cells. All of these cells originate from the embryonic mesenchyme and are renewed by the resident stem cell population. Connective tissue is distinct from epithelium in that the cells do not make direct contact with each other. Instead, they produce and secrete an enormous volume of extracellular matrix known as the ground substance. In soft tissues, the ground substance is produced by fibroblasts and consists primarily of collagen, elastin, and glycoproteins. The glycoproteins, called proteoglycans and glycosaminoglycans (GAGs), are highly glycosylated, so much so that they look like molecular versions of hairy caterpillars. The GAGs and proteoglycans attract and hold water, forming a jellylike substance that receives its strength from the embedded collagen fibers and its elasticity from elastin. Metal-reinforced cement and fiberglass (resin saturated plastic fibers) are analogous to this type of structure.

The best way to appreciate the importance of connective tissue is to consider the jellyfish. The shape of this animal is determined almost entirely by the connective tissue, which is produced by fibroblast-like cells. This tissue gives the creature volume and a cushioned support matrix for the internal organs. The epithelial cells (epithelium or skin) are attached to the connective tissue through interactions between the cell-surface glycoproteins and the glycoproteins of the connective tissue. Dehydration of the connective tissue is believed to be responsible for the thinning and wrinkling of the skin in aging humans and other mammals. In bone, the ground substance is also a mixture of collagen, elastin, and glycoproteins with the addition of minerals, such as calcium, to make the structure hard. In this case, bone-forming cells, known as chondroblasts, produce the ground substance. In blood, the ground substance is a liquid, known as plasma, within which the blood cells are suspended. Blood-forming cells located in the bone marrow produce the plasma.



Connective tissue in a jellyf sh. The importance of connective tissue is easy to see in a simple animal such as this. The shape of the creature is determined almost entirely by the jellylike connective tissue, which is produced and secreted by the fibroblasts. The internal organs, such as the gut and gonads (not shown), are embedded in the connective tissue. These properties of the connective tissue hold true for all animals, including humans. Note that the size of the epithelial cells, relative to the whole organism, is exaggerated for clarity.

Connective tissue also serves as the initial battlefield in the war against invading microbes, viruses, and other pathogens. Although many invading pathogens enter the body through the circulatory system, most gain entry through the connective tissue, and it is there they encounter the full fury of the body's immune system. The connective tissue provides millions of passageways that cells of the immune system can navigate, both during an attack and during day-to-day surveillance. The recruitment of large numbers of immune cells to fight an infection would be much more difficult if all the tissues of the body were tightly stitched together, leaving very little space



Connective tissue. All of the body's cells are embedded in connective tissue (colored yellow at the top in a sample of soft, loose tissue). A portion of the connective tissue is shown magnified at the bottom. This tissue consists of collagen fibers, elastin, and three principal cell types: fibroblasts, fat cells, and cells of the immune system (macrophages and lymphocytes).

between the cells. Thus, even in tightly connected tissue, such as the skin, there is still an extracellular matrix between the adjacent cells. Immune cells can protect such tissue, but it is a slower process.

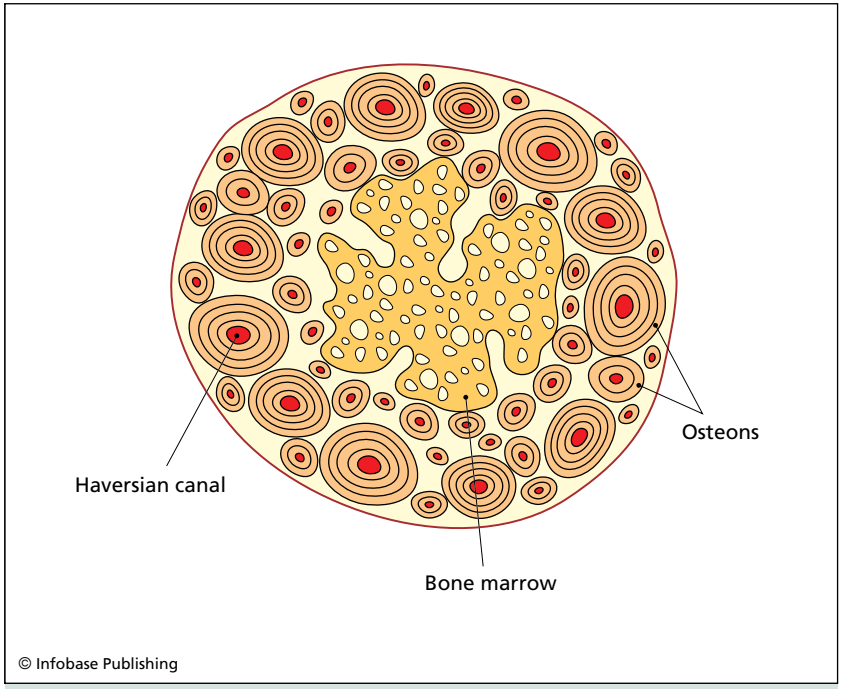
Finally, the connective tissue serves as a convenient storage place for fat reserves. Fat is stored within fat cells, and these take up residence within the connective tissue usually at specific sites within the body. Of course, the amount of fat stored varies with the diet and activity level of the individual, but females generally have a higher proportion of subcutaneous fat (i.e., stored in the connective tissue just beneath the skin) than do males. The storage of fat in this way ensures that all of the muscles of the body have a local energy reserve that can be quickly mobilized.

Fibroblasts

Fibroblasts produce most of the connective tissue in an animal's body and are one member of the connective-tissue cell family that includes osteoblasts, cartilage cells, fat cells, and smooth muscle cells. These cells are all derived from embryonic mesenchyme. Fibroblasts are large flat cells with a fusiform profile (shaped like a submarine). The nucleus is oval or elongated, and the cytoplasm is filled with an extensive endoplasmic reticulum, necessary for the production and secretion of collagen. After forming the connective tissue, fibroblasts remain embedded within the tissue where they are involved in maintenance and repair. An important part of their job is to repair wounded skin. Their activities are responsible for the formation of scar tissue, which consists primarily of collagen secreted by the fibroblasts.

Bone

The bones in our bodies are produced by osteoblasts that synthesize the structure as a collection of calcified collagen rods, known as osteons, each of which consists of concentric rings, much like the rings of a tree. Blood vessels, located in the Haversian canals at the center of each osteon, supply the tissue with oxygen and nutrients.

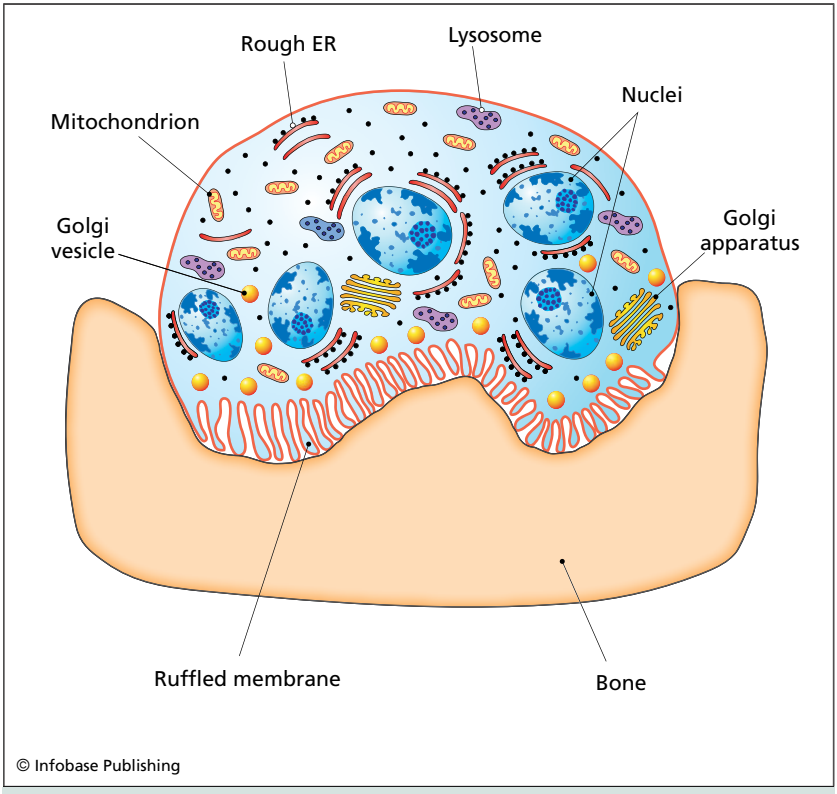


Bone structure. Compact or dense bone is constructed from long tubular structures called osteons that are built up in concentric rings. The center of each osteon, called the Haversian canal, contains blood vessels that supply the bone with oxygen and nourishment. The central portion of the bone is porous and is called trabecular bone or, more commonly, bone marrow.

Every year, as an infant grows to an adult, new osteons are added and elongated. This process is so regular that, barring traumas or starvation, anthropologists have been able to estimate the age of an individual at the time of death by determining the number of osteons in the bones. Osteoblasts are large cells with a variable shape that ranges from cuboidal to pyramidal. They have a large nucleus with a single prominent nucleolus and, like the fibroblasts, have a very extensive endoplasmic reticulum.

Bone is in a constant state of repair and remodeling, required when a bone is broken or fractured and to strengthen the bone

as result of physical exercise and repetitive use. In the latter case, remodeling can strengthen specific areas of a given bone that is subjected to repetitive stress. Estimates of bone replacement range from 5 percent to nearly 20 percent per year. Although osteoblasts are charged with the job of building the bone, remodeling is left to a different kind of cell known as an osteoclast. These are giant cells (more than 50 micrometers in diameter) with a remarkable anatomy. Osteoclasts have several nuclei, a cytoplasm that is stuffed



Osteoclast. The repair and remodeling of bone begins when osteoclasts dissolve away an area of bone by secreting acids and hydrolases, all along their ruffled membrane. Osteoclasts can bore deep into the bone, often forming long tunnels. Once the bone has been removed, osteoblasts move in to repair the area by secreting a new bone matrix.

with organelles, and a cell membrane that is highly ruf ed over half of the cell's surface. The ruf ed membrane is brought into contact with the bone where it secretes acids and hydrolases to dissolve away the bone matrix, after which the cavity is repaired by the osteoblasts. This repair cycle takes about 100 days. Osteoclasts function very much like their close relatives, the phagocytic cells of the immune system (macrophages), which remove dead or dying cells, as well as invading microbes, from the tissues. The steroid hormones, estrogen and progesterone, control bone renewal and remodeling. Age-related changes in the control of these hormones and the way osteoblasts and osteoclasts respond to it are believed to be the cause of osteoporosis, a bone disease that is very common among the elderly.

Blood

Human blood consists of two distinct populations of cells: red blood cells, which carry oxygen to all of the tissues, and white blood cells, which form the immune system.

Red Blood Cells

Red blood cells (RBC), also known as erythrocytes, form in the bone marrow from precursor cells known as erythroblasts, which in turn are produced by hemopoietic stem cells. These stem cells are descended from the same mesenchyme that gives rise to all the other connective tissue cells. Erythroblasts go through several stages of development marked by a gradual reduction in size and extreme biosynthetic activity focused primarily on the production of hemoglobin, the iron-containing protein that binds oxygen. In its final stage of development, the nucleus is extruded from the cell, leaving a mature erythrocyte consisting almost entirely of a cytoplasm filled with hemoglobin. Without a nucleus, the RBC cannot repair itself, and consequently these cells have a very short life span of about 100 days. The disadvantage of a short life span is more than offset by the extra hemoglobin and

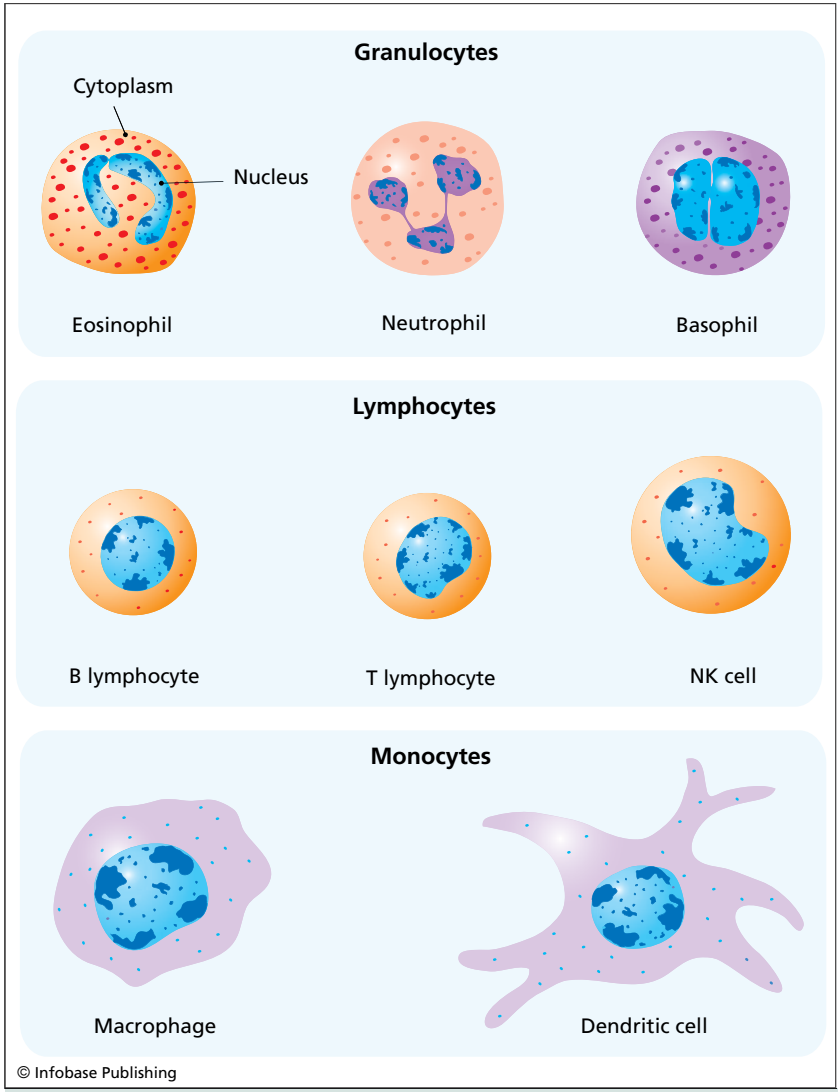
therefore additional oxygen that these cells are able to carry without the nucleus.

White Blood Cells

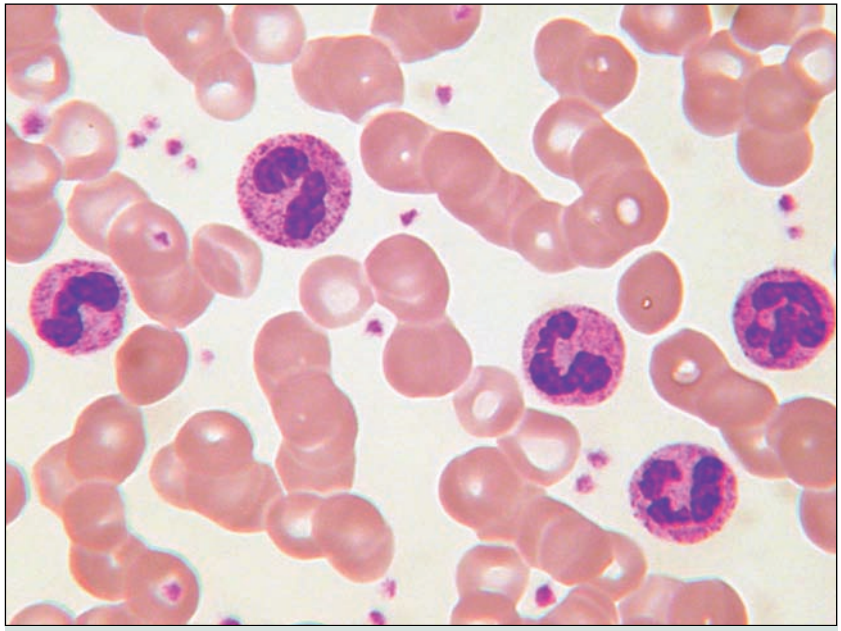
The human immune system is composed of a diverse group of white blood cells that are divided into three major categories, all of which are produced in the bone marrow or the thymus gland: granulocytes, monocytes, and lymphocytes. Granulocytes have a distinctive, lobular nucleus and are phagocytic (can eat cells and viruses). Monocytes are large phagocytic cells with an irregularly shaped nucleus. The largest monocytes, the macrophages, can engulf whole bacteria as well as damaged and senescent body cells. Lymphocytes have a smooth morphology and a large round nucleus. T-lymphocytes (also called T cells) and natural killer (NK) cells deal primarily with coordinating the immune response and with killing already infected body cells. B-lymphocytes (B cells) are nonphagocytic; they deal with an invading microbe by releasing antibodies.

Phagocytosis of an invading microbe by granulocytes and monocytes represents a first-line defense, called the innate response. All animals are capable of mounting this kind of defense. Activation of the lymphocytes leads to a more powerful, second line of defense called the adaptive response, which is found only in higher vertebrates. The adaptive response is initiated by monocytes, specifically, dendritic and Langerhans cells. These cells, after engulfing a virus or bacteria, literally tear the microbe apart and then embed the pieces, now called antigens, in their membrane. The antigens are presented to lymphocytes, which become activated when their receptors bind to the microbial antigens. Activated B-lymphocytes secrete antibodies specifically designed for that particular microbe. Activated T-lymphocytes and NK cells attack the microbe directly but are primarily concerned with locating and killing infected body cells.

The adaptive system can remember a pathogen long after it has been removed from the body. This why a specific bacteria or virus cannot make us sick twice. Once infected, an individual develops a



White blood cells. These cells are divided into three major categories: granulocytes, lymphocytes, and monocytes. Granulocytes have a distinctive lobular nucleus, granulated cytoplasm, and all are phagocytic (eat cells, viruses, and debris). Lymphocytes have a smooth morphology with a large round or kidney-shaped nucleus. B-lymphocytes are nonphagocytic but produce antibodies. T-lymphocytes and natural killer (NK) cells coordinate the immune response and can force infected cells to commit suicide. Monocytes are large phagocytic cells that can engulf bacteria and damaged or infected body cells.

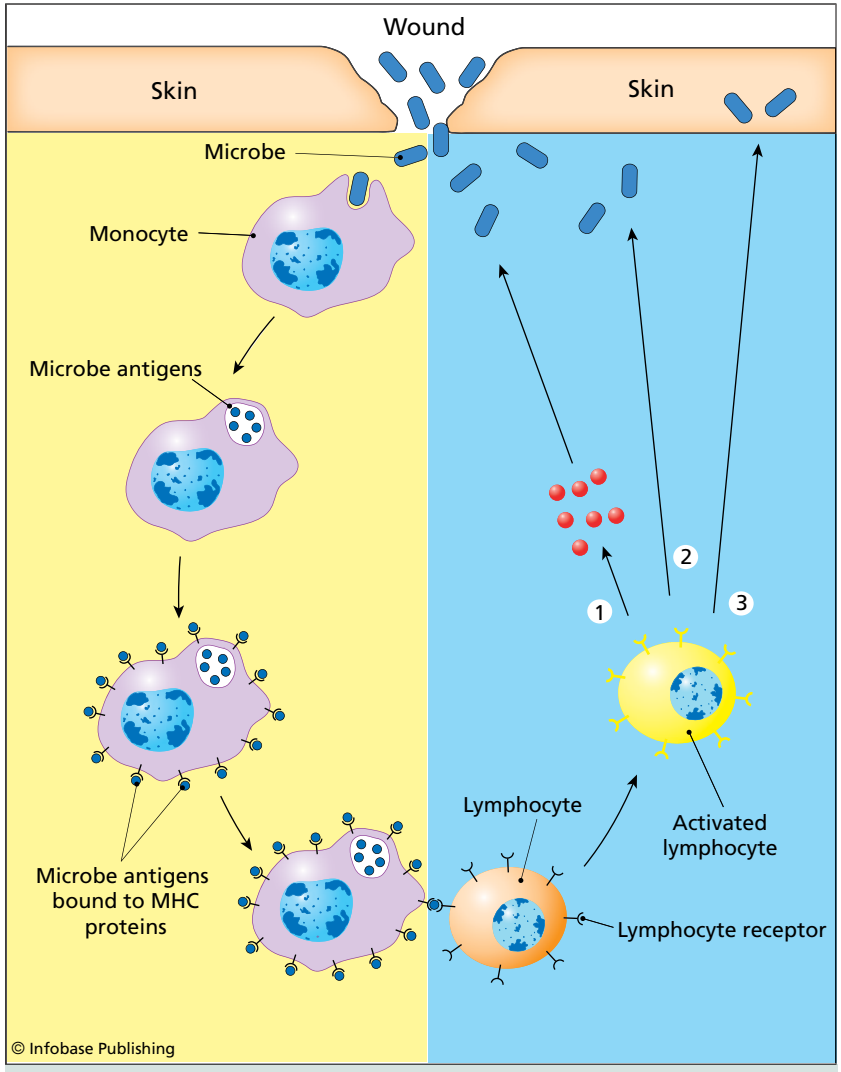


White blood cells. Magnification 1,000×. *(Dr. Fred Hossler/Visuals Unlimited)*

natural, lifelong immunity. We can also immunize ourselves against many diseases by injecting a crippled version of the pathogen or specific antigens from a pathogen into our bloodstream. This concoction of bits and pieces from a pathogen, called an immunizing serum, activates the adaptive response, leading to a lasting (though not always lifelong) immunity against the disease.

MUSCLE

One of the four primary tissues, muscle gives rise to only three cell types: skeletal, cardiac, and smooth muscle. Cardiac muscle forms the heart; smooth muscle is found in the digestive tract, blood vessels, and the uterine lining; and skeletal muscle moves the limbs, tongue, and eyes. Skeletal muscle is formed during embryonic development, when myoblasts fuse to form a multinucleated muscle cell (also known as a myofibril). Dozens of muscle cells join together

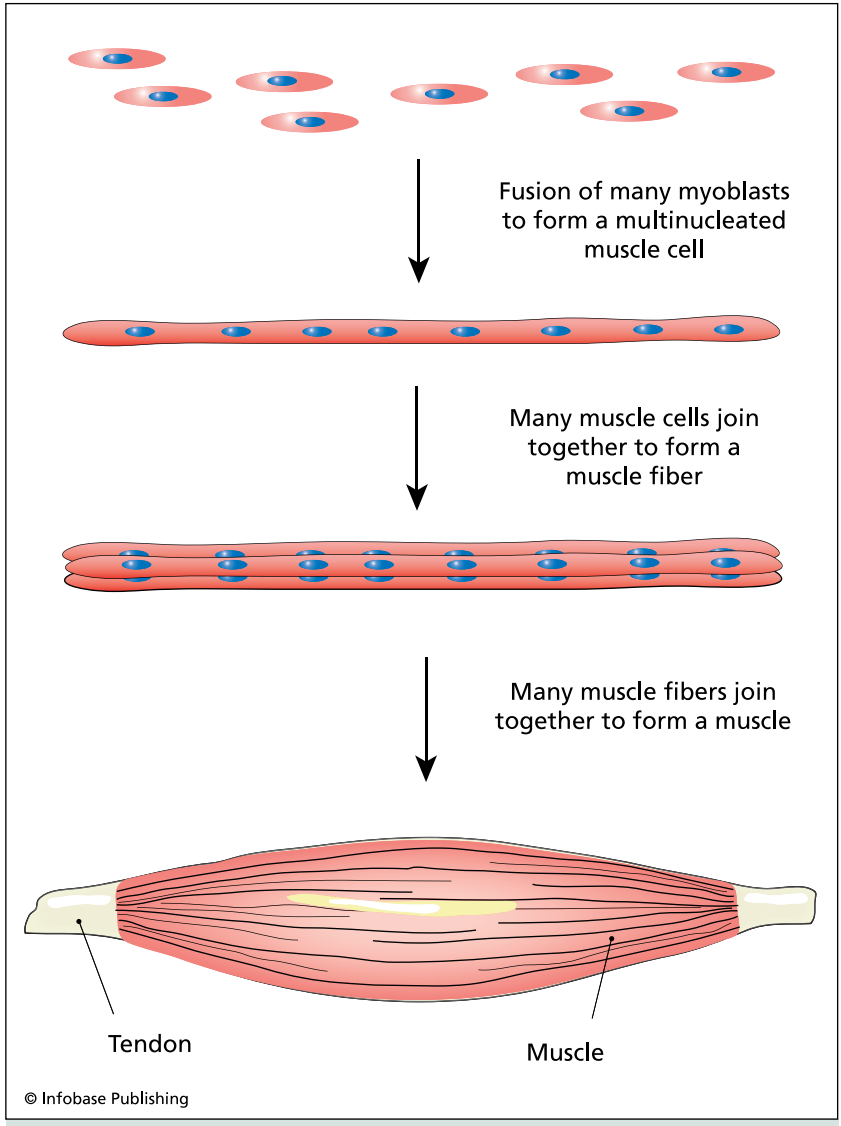


Innate and adaptive immune response. Phagocytosis of invading microbes is called the innate response (yellow zone). In higher vertebrates, microbe antigens, bound to special monocyte surface proteins called the major histocompatibility complex (MHC), are then presented to lymphocytes. Contact between the lymphocyte receptor and the antigen activates the lymphocyte and the adaptive response (blue zone), consisting of a three-pronged attack: 1) release of antibodies, which kill the microbes; 2) direct attack on the microbes; 3) destruction of infected cells.

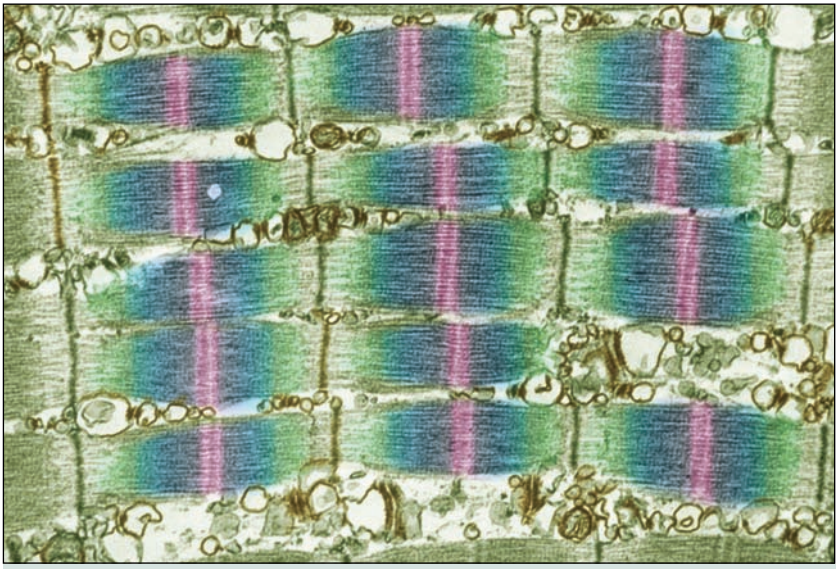
to form a muscle fiber, after which the fibers are joined together to make a muscle.

The contractile unit of a muscle cell is called a sarcomere. These units are kept perfectly aligned during the formation of the muscle fiber and, finally, the muscle itself. In this way, the muscle, composed of thousands of individual sarcomeres all aligned in register, contracts as though it were a single unit. Sarcomeres are constructed from four kinds of protein: Actin, Myosin, CapZ, and Alpha-actinin. Additional, accessory proteins are present, which stabilize muscle structure and help activate contraction. The precise and regularly repeating arrangement of the sarcomeres is visible in microscopic images of skeletal muscle as cross-striations. Consequently, skeletal muscle is also called striated muscle. The sarcomere contracts when the Myosin heads bind to the Actin and pull the Z discs toward the center of the sarcomere, which is called the midline. This description of muscle contraction, called the sliding-filament model (so named because the Actin "filament" slides along the Myosin), was first proposed more than 50 years ago by the British physiologist Sir Andrew Huxley (half brother of Aldous Huxley, the author of *Brave New World*). Huxley based his model on the biochemical analysis of contracting muscles, as well as microscopic images of relaxed and contracting skeletal muscle.

Muscle, like neurons, is an excitable tissue, meaning that it responds to electrochemical signals. Neurons pass the signal along to other neurons in a complex communication network, whereas muscles use the signal to activate contraction. Moving an arm, a leg, or a finger is possible because the brain sends a signal to a neuromuscular junction, a place where the nerve fiber contacts the muscle. The neural stimulus initiates the release of calcium from an internal storage depot, which activates simultaneous contraction of every sarcomere in the muscle. The length of time the calcium remains free depends on the length of the stimulus. If one picks up a heavy object and holds it in the air for a while, the muscles receive



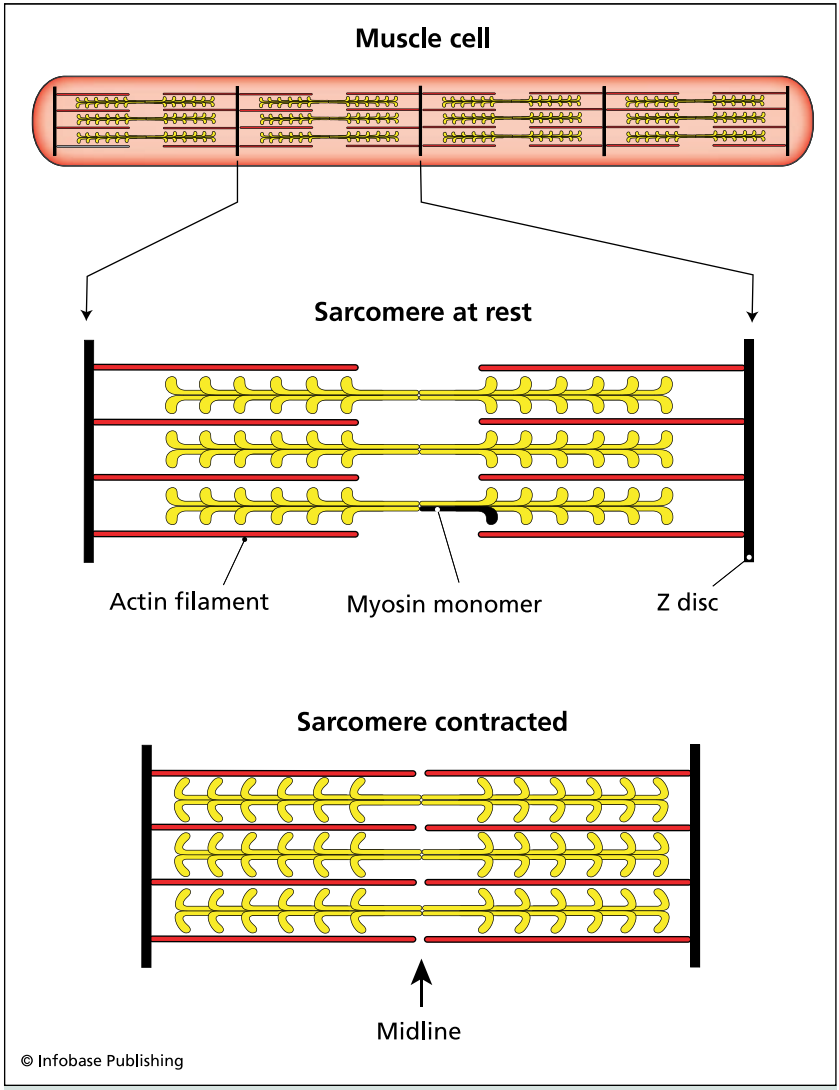
Skeletal muscle. During embryonic development, myoblasts fuse to form long, multinucleated muscle cells. The filamentous muscle cells, also called myofibrils, bundle together to form muscle fibers. Finally, many muscle fibers are joined together to form a muscle, anchored at both ends by tendons.



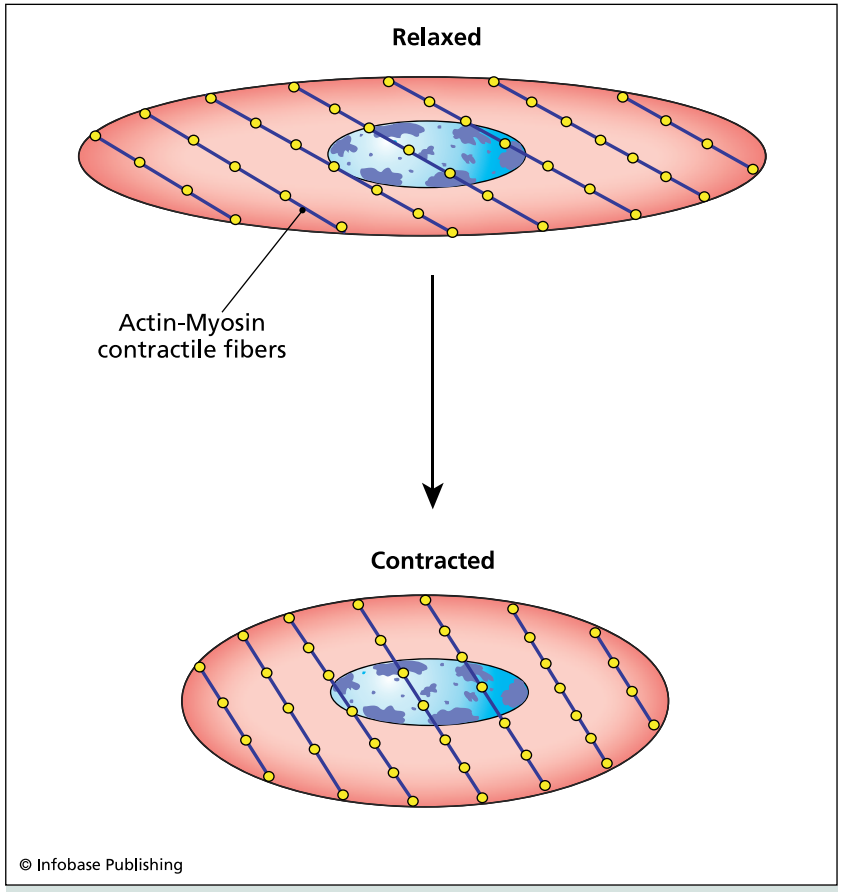
Colored transmission electron micrograph (TEM) of a longitudinal section through striated skeletal muscle. The striated banding pattern of the muscle fibrils is seen. The fibrils run in parallel (from left to right) and between them runs sarcoplasmic reticulum (SR) that transmits nerve impulses to the fibrils. Here, the SR contains many mitochondria. Within each fibril are contractile units called sarcomeres separated by lines. A sarcomere has protein filaments of myosin and actin that slide over each other, thereby causing the whole muscle to contract. Skeletal muscle is responsible for voluntary muscle movement in the body. *(Biology Media/Photo Researchers, Inc.)*

a steady stream of neural stimulation, keeping the concentration of free calcium at high levels. When the object is put down, the neural stimulation stops and the calcium is immediately returned to its storage depot so the muscle can relax.

Cardiac muscle has the same sarcomere-based contraction machinery as that found in skeletal muscle and thus is another example of striated muscle. The main difference between cardiac muscle and skeletal muscle is that the cardiac muscle has large numbers of gap junctions between the muscle cells, permitting a neural signal to



The sarcomere. A skeletal muscle cell (top) contains many repeats of the basic contraction unit, called a sarcomere. Each sarcomere (middle) is constructed from actin filaments, which are attached to a Z disc, and myosin monomers, the shape of a golf club (black). The Z discs are constructed from the proteins capZ and alpha-actinin. The sarcomere contracts (bottom) when the clubs, or heads, of the myosin monomers bind to the actin and pull the Z discs toward the midline (arrow). Actual contact between the myosin heads and the actin is not shown.



Smooth muscle. Fibers containing Actin and Myosin span the cell diagonally with respect to the long axis. Contraction of these fibers pulls the ends of the cell toward the center, greatly reducing its overall length.

pass freely through every cell in the organ. Consequently, a single impulse will stimulate all of the cells simultaneously, allowing the heart to beat as a single unit.

Smooth muscle gets its name from the fact that the contraction machinery is not organized into a sarcomere, and therefore these

muscles have a smooth, rather than striated appearance. Contraction is based on a simple and probably ancient arrangement of actin-myosin filaments. These filaments are arranged in a diagonal pattern from one end of the cell to the other and, as they contract, they pull the ends of the cell toward the center, greatly reducing its overall length. Smooth muscle is not capable of the speed or force of contraction that is typical for striated muscles, but they are ideal for tissues, such as intestine or uterine lining, that require slow, rhythmic contractions.

Neurons: Pushing Back the Night

A world without neurons is a dark world, populated by cells, plants, and simple creatures that never see the light of day. That world is a place devoid of vision, where there are no fish in the sea and no animals on the land; there is no music, no laughter, no emotion, and no intellect. The first cells appeared on Earth 3.5 billion years ago, but life as it is known today really began with the appearance of the neuron, about 2 billion years ago.

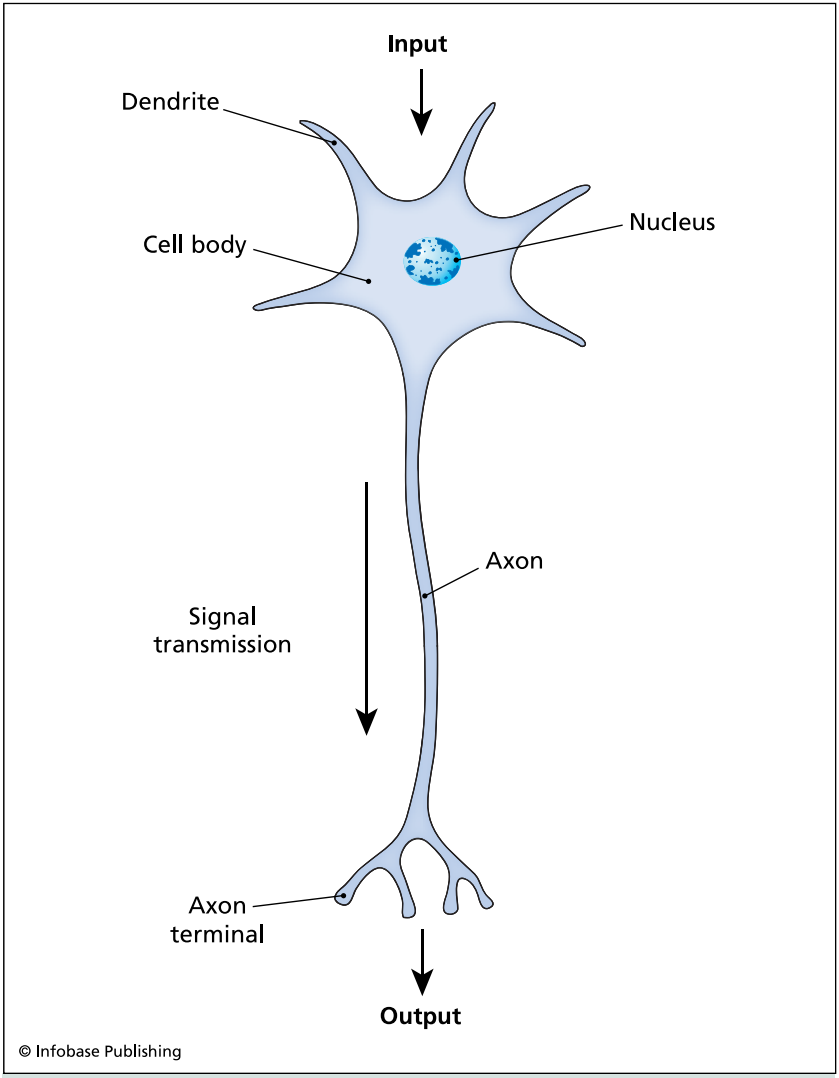
Neurons, and the things they do, defy the imagination. They make it possible for us to instantly recognize friends who walk into a room. One can tell at a glance if they are happy or sad and whether they are wearing the same clothes they wore the day before. One can talk to them, for hours on end sometimes, or we can get up and dance around the room. Neurons make all of this possible. They do it by forming intricate communication networks, usually consisting of billions of cells. By sending signals, fast and furiously, through

that network, neurons let us see the world, shape our intellect, and give us our personality.

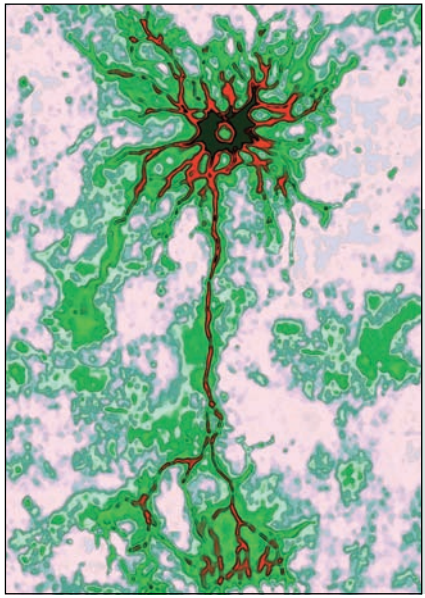
Scientists have only a vague notion of how neural networks accomplish so much. Considering a human personality and the entity that psychiatrists call a persona, or self-image, it is difficult to see how these intangible, possibly spiritual, aspects of our brain are generated by neurons simply talking to one another. Equally difficult to understand is the manner by which these networks store and access memories. The amount of memory in a personal computer is usually known; it is advertised with every machine, but the memory capacity of the human brain is still a mystery. Research in this area is still highly speculative, but many scientists believe the memory capacity of the human brain is infinite. This estimate is based on the observation that on any given day people always have room for new memories. Students never seem to reach that point in time when they have to stop and say, "Sorry, teacher, I can't learn any more, my brain is full." Skeptics would argue that our brains simply erase old memories to make room for the new, just as data on a computer is sometimes erased to make room for more files. And yet, psychologists insist that under hypnosis, people are able to remember things they once thought were lost. The memories are there, but retrieving them may be difficult.

NEURAL ANATOMY

Neurons are cells that are specially designed for communication and, like computers or other communication hardware, have a polarized anatomy that contains structures for receiving input and other structures for processing the output. A signal, in the form of an electrochemical current, enters a neuron at fingerlike projections, called dendrites, and is passed along to another neuron through the axon, a single projection, much longer than a dendrite. Signal reception, propagation through the cell, and transmission to another neuron take less than a millisecond and travel at a rate of 270 miles per hour (120 m/sec). Every axon forms a communication junction,



Neural anatomy. A neuron receives signals at fingerlike projections from the cell body, called dendrites, and passes them on to other neurons through an elongated process called an axon. The tip of an axon, which often branches into several terminals, makes contact with the dendrites of other neurons.



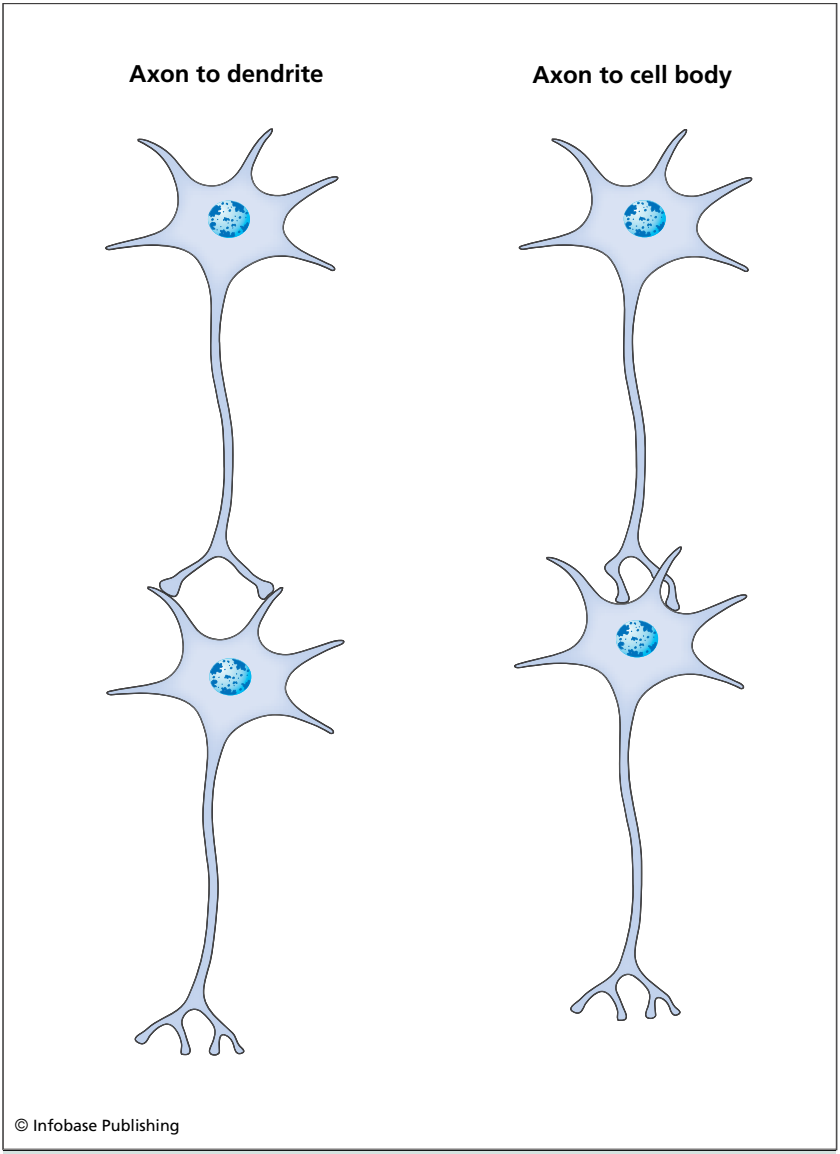
Human neuron. This consists of a cellular body (nucleus and Nissl substance, comprised of granular endoplasmic reticulum and ribosomes), secondary prolongations (dendrites), an axial prolongation (axon), and contact points between neurons and muscle fibers. *(James Cavallini/Photo Researchers, Inc.)*

called a synapse, with a dendrite or the cell body (soma) of another neuron. This simple scheme, repeated millions of times, makes possible the immensely complex neural circuitry of an animal's brain.

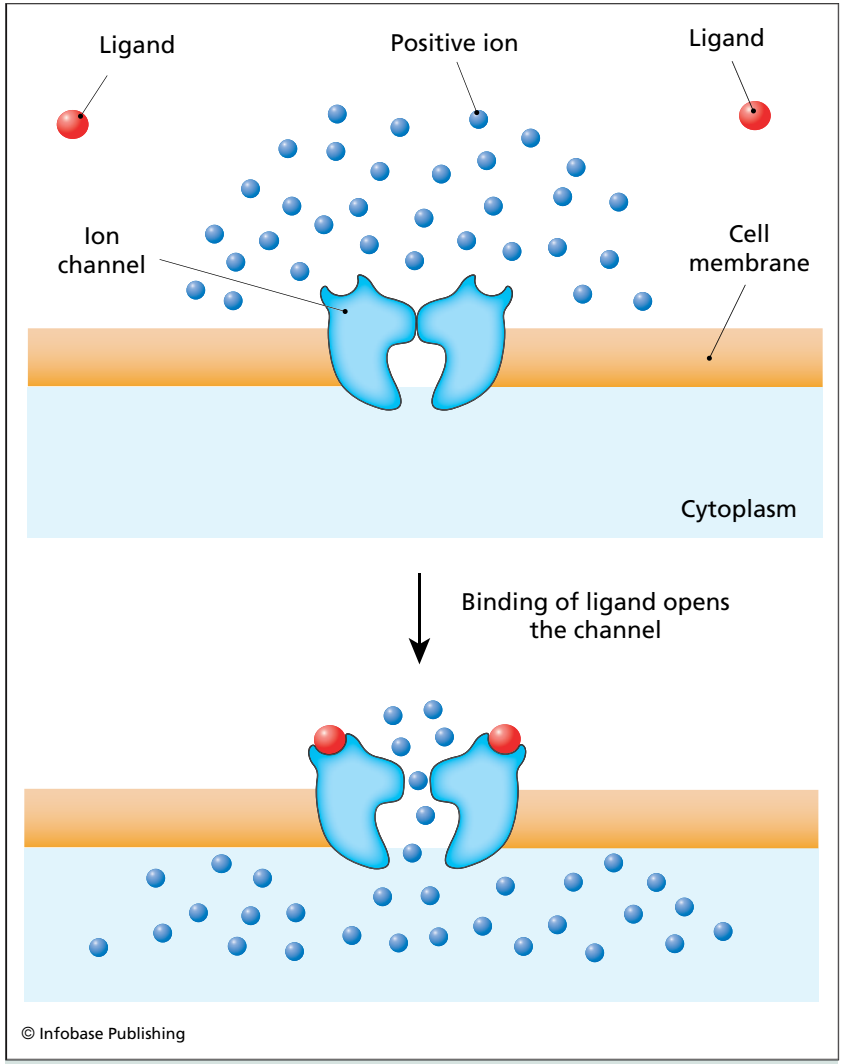
SIGNAL TRANSDUCTION

Propagation of a signal from one neuron to another depends on the coordinated operation of ion channels, located in the cell's membrane. Ion channels vary according to the type of ion that can pass through them, most commonly sodium (Na^+) and calcium (Ca^{++}), and the mechanism by which they are activated. Ligand-gated (Lg) ion channels are activated, or opened, when a signaling molecule, called a ligand, binds to the channel. Lg Na^+ channels are the most common type and serve to initiate neural signaling. Voltage-gated (Vg) channels are opened by an electrical stimulus. The most common channels of this kind permit the entry of Na^+ or Ca^{++} ions.

When Na^+ channels are closed, the ion concentration is greater on the exterior surface of the neuronal membrane than it is on the inside of the cell. Thus, the exterior is positively charged relative



Synaptic junctions. The synapse is usually formed between the axon and one or more dendrites, but axons may also form a synapse with the cell body.

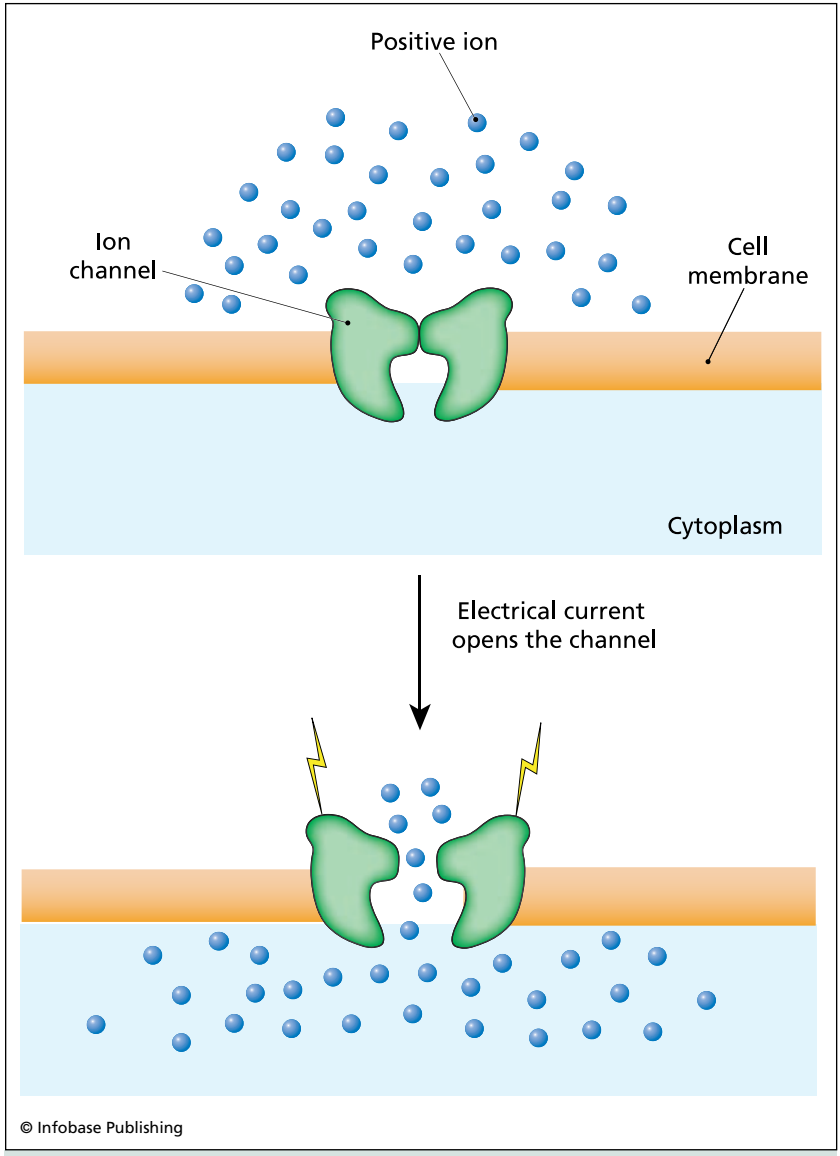


Ligand-gated (Lg) ion channel. The closed channel keeps positively charged ions outside the cell and, in this state, the membrane is said to be polarized (i.e., positive on the outside, relative to the inside). The channel opens when bound to a signaling (ligand) molecule, allowing the ions to rush inside, thus depolarizing the membrane and initiating an electrical current.

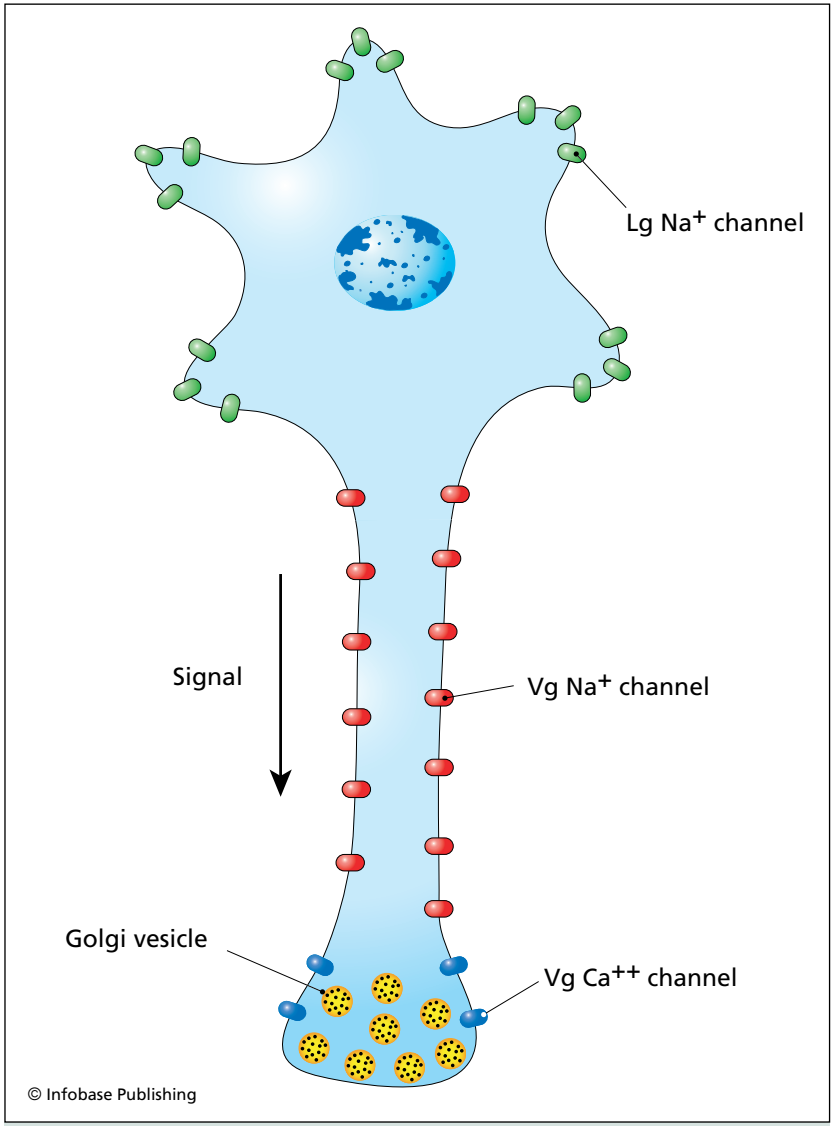
to the interior. In this resting state, the membrane is said to be polarized, and the potential difference across the membrane, known as the voltage, is relatively high. When Lg ion channels open, the Na+ rushes inside the cell, and in so doing, establishes an electrical current.

The same applies to a Vg Na^+ channel or a Vg Ca^{++} channel. Neurons receive, propagate, and transmit signals by coordinating the activation of Lg Na^+ channels, Vg Na^+ channels, and finally Vg Ca^{++} channels. The Lg Na^+ channels are always located in dendritic membranes (the input end of the cell). A special ligand, known as a neurotransmitter, sent by a neuron, binds to, and activates, Lg Na^+ channels. The opening of these channels initiates an electrical stimulus, through membrane depolarization. This signal spreads electrotonically or passively through the cytoplasm and decays rapidly with distance, but usually has enough strength to activate the first Vg Na^+ channels, located in the axonal membrane close to the cell body. The signal propagates along the axon toward the axonal terminus by sequential activation of Vg Na^+ channels. The strength of this signal is boosted each time a Vg channel opens, so it does not decay over the full length of the axon. The signal through the axon is called a spike potential, because the opening of each channel results in a renewed burst (spike) of the electrical current. Finally, the Vg Na^+ channels activate Vg Ca^{++} channels, located at the axonal terminus. The influx of calcium stimulates exocytosis of Golgi vesicles (fusion of the vesicles with the cell membrane), containing neurotransmitters that send the signal on to other neurons.

Signal propagation along the axon is unidirectional because the Na^+ channels, just behind the advancing signal, are deactivated. Special Na^+ pumps, driven by the hydrolysis of ATP, return the ion to the extra cellular space, thus repolarizing the membrane. This also serves to reactivate the channels in preparation for the next signal. The Vg Na^+ channels are capable of processing 500 signals



Voltage-gated (Vg) Ion channel. The electrical current that opens these channels originates with the opening of ligand-gated channels, after which Vg channels are opened by the current generated by other Vg channels.



Signal transmission. A ligand (neurotransmitter) binds to the Lg sodium (Na^+) channels, depolarizing the membrane and initiating an electrical current that opens the first Vg Na^+ channels in the axon. The signal propagates along the axon by sequential activation of the Vg Na^+ channels. Finally, the axonal current activates the Vg calcium (Ca^{++}) channels. Influx of calcium stimulates exocytosis of the Golgi vesicles, containing neurotransmitters that send the signal on to another neuron.

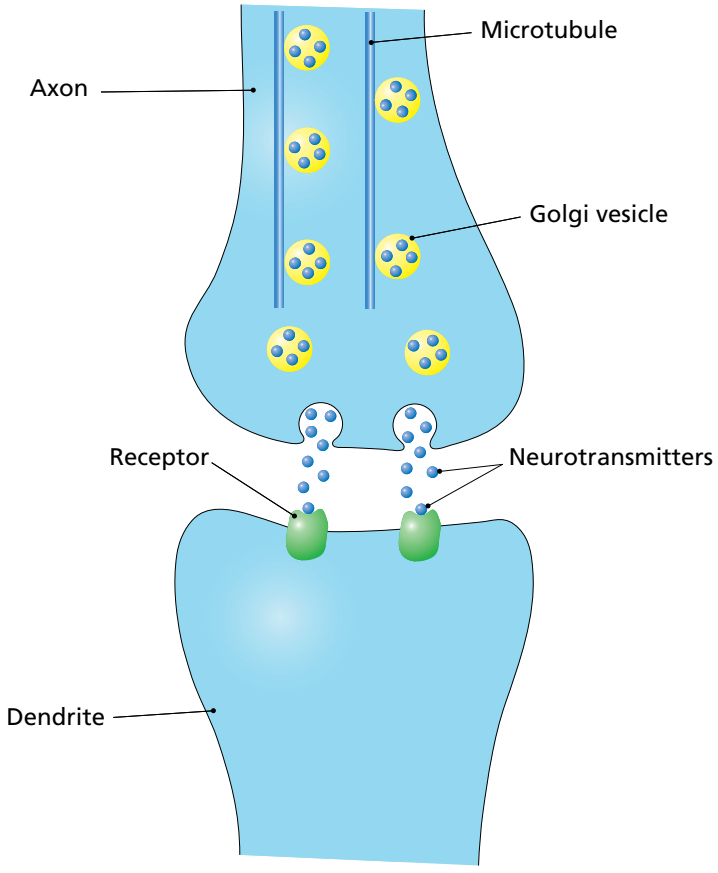
per second, and depolarization of the membrane involves the passage of up to 10 million Na^+ per second.

THE SYNAPTIC JUNCTION

The connection between an axon and a dendrite is called a synapse. Although neurons communicate through the synapse, they do not actually touch one another. Close inspection of a synapse shows a small gap separating the axon from the dendrite. A signal is transmitted across the gap by the release of neurotransmitters that are stored at the axon terminus in Golgi-derived vesicles. The vesicles travel to the axon terminus on a railroadlike transport network constructed of microtubules. When a neuron receives a signal, the Golgi vesicles, at the terminus, are released from the microtubules and fuse with the axonal membrane, dumping their cargo into the synaptic gap. The neurotransmitters quickly diffuse across the gap and bind to receptors on the dendritic membrane, triggering an electrochemical impulse in the target neuron, thus completing transmission of the signal. This may seem like an awkward way for neurons to signal each other, but the synaptic gap and the use of neurotransmitters are crucial for maintaining the strength of the signal over a network that consists of 100 billion cells. Each neuron in such a circuit, boosts the signal to its original strength, in a manner analogous to the signal boost that each Vg Na^+ channel provides as the signal travels along each axon.

MYELINATION

A neural circuit, much like an ordinary household electrical circuit, works better if the axons are insulated. Electrical wire is insulated with a plastic coating, but neurons are insulated by special cells that form a coating, called myelin, around each axon. Myelin is constructed from cells called oligodendrocytes and Schwann cells that wrap around axons to form a protective multilayered sheath. Oligodendrocytes, located in the central nervous system (CNS, consisting of the brain and spinal cord), can myelinate more than one axon at a time. Schwann cells, located in the peripheral nervous system (i.e.,



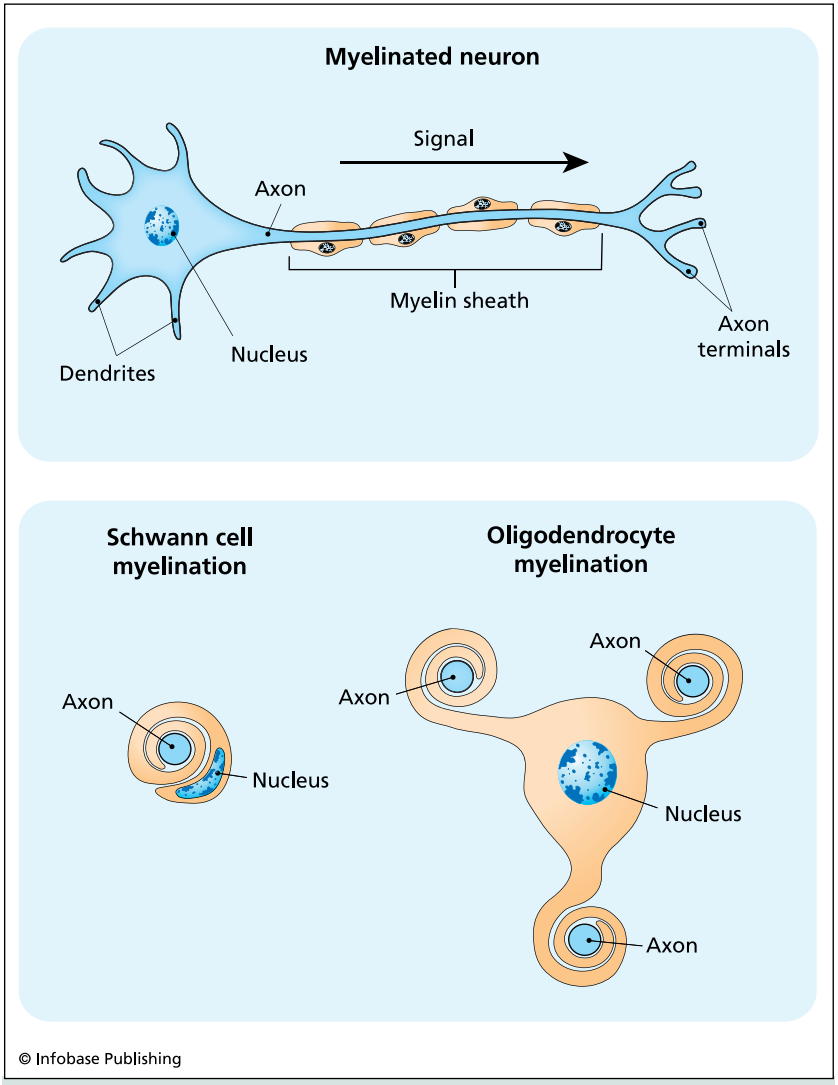
Synaptic junction. Axons and dendrites do not touch each other but are separated by a small gap called the synapse or synaptic junction. A signal is transmitted by the release of small molecules called neurotransmitters that are stored at the axon terminus in Golgi vesicles. Binding of the neurotransmitter to the receptor on the dendrite membrane completes the transmission. The Golgi vesicles travel to the axon terminus on a transportation network constructed from microtubules.

any nerves outside the CNS, such as those in the toes and fingers), wrap around a single axon. In humans, myelination is not complete at birth but it develops progressively as the individual grows and matures. The process is sensitive to the individual's environment and activity level. Neglected children, for example, have less myelination in certain brain regions than do other children. Mastering an activity such as playing the piano stimulates myelination, whereas a decrease in myelin content is known to be associated with certain mental disorders, such as schizophrenia and bipolar disorder. The existence of the myelin sheath greatly complicates medical procedures aimed at repairing a severed spinal cord or neural damage caused by Alzheimer's disease or Parkinson's disease.

NEUROTRANSMITTERS

Neurons synthesize the ligand, called a neurotransmitter, which is released at the axonal terminus to relay a signal to another neuron. Neurotransmitters are divided into two major groups. The first are fast, direct neurotransmitters that include acetylcholine (synthesized from acetyl-CoA and choline) and several amino acids (glutamate, aspartate, gamma-amino butyric acid, and glycine). The second group is slow, indirect neurotransmitters that include the monoamines (derived from various amino acids), such as epinephrine, dopamine, and serotonin. This group also includes a large number of neuropeptides. Fast, direct neurotransmitters bind to receptors that are ion channels. By contrast, the slow, indirect neurotransmitters bind to receptors that are linked to a signaling pathway and thus modulate the behavior of the target neuron through a series of biochemical steps, rather than generating an electrical current.

Acetylcholine (ACh) is one of the most ubiquitous neurotransmitters, being used by most vertebrate species in motor neurons (neurons that directly stimulate muscle) and various neurons in the CNS. Neurons that use ACh are referred to as being cholinergic.



Neural signaling and myelination. Communication between neurons is much more ef cient when the axons are insulated with a myelin sheath. Myelin is made by Schwann cells or oligodendrocytes wrapping around the axon. Oligodendrocytes can insulate more than one axon at a time.

GABA and glycine are both inhibitory neurotransmitters released by neurons in the CNS and peripheral nervous system (PNS) in vertebrates, crustaceans, and worms. Glutamine is an excitatory neurotransmitter found in the CNS of vertebrates, insects, and crustaceans. Glutamate receptors participate in modifying synaptic behavior and organization during long-term potentiation (learning and memory).

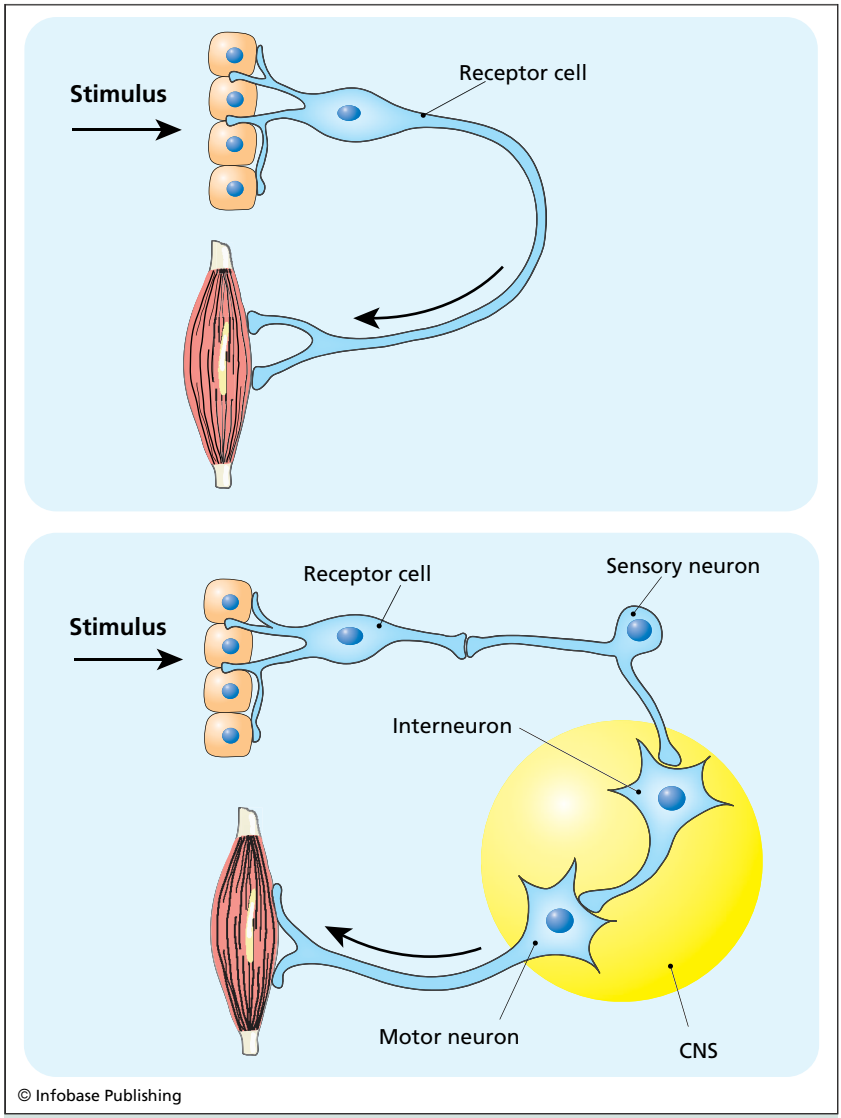
Norepinephrine (NE), synthesized from the amino acid phenylalanine, is released by various neurons in the CNS, as well as cells in the adrenal gland. Neurons releasing NE are said to be adrenergic. Psychoactive drugs, such as mescaline (from the peyote cactus) interfere with NE signal transmission in the CNS. Amphetamines mimic the function of NE, and cocaine interferes with NE inactivation, thus prolonging the stimulation of an adrenergic network. Dopamine, synthesized from the amino acid tyrosine, occurs throughout the CNS and acts through a signaling pathway. Dopamine is primarily a neuromodulator, being able to dampen the excitability of the target neuron. Parkinson's disease is a neurological disorder characterized by the degeneration and death of dopaminergic neurons, in an area of the brain known as the substantia nigra. Serotonin, synthesized from the amino acid tryptophan, also occurs throughout the CNS. Some of the receptors are ion channels, while others are linked to signaling pathways. Neuropeptides, consisting of about 30 amino acids, are widely distributed throughout the CNS and, like serotonin, bind to receptors that are ion channels or are linked to signaling pathways. One group of neuropeptides called the endogenous opioids are involved in regulating neural circuits controlling many physiological processes, including behavior, appetite, pain tolerance, stress, and shock. They are also involved in regulating the neuroendocrine system, primarily through their ability to reduce the excitability of neurons and neuroendocrine cells.

NEURAL CIRCUITS

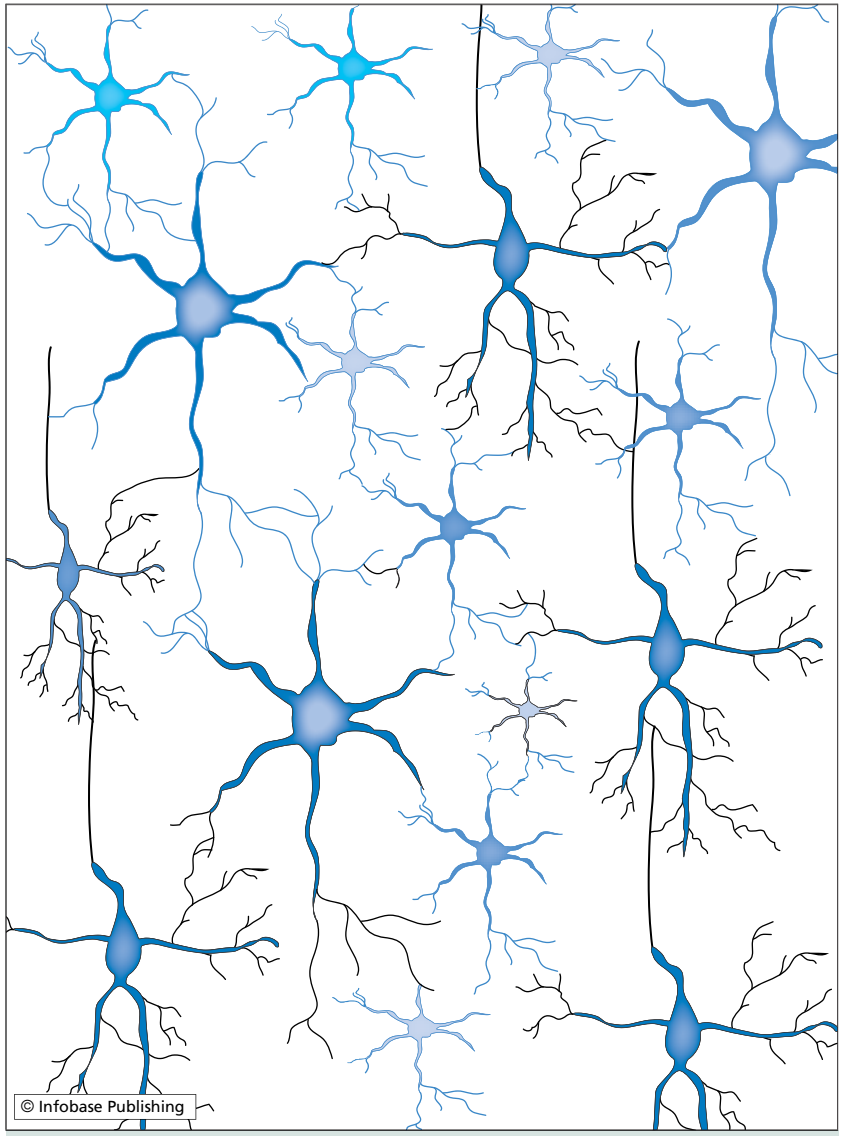
Neural circuits probably began with a single neuron acting as both a receptor cell and as a motor neuron capable of stimulating muscle or other tissue. This simple scheme evolved into a more elaborate system with the addition of specialized neurons, situated between the receptor and the target tissue. The additional circuitry became the central nervous system, capable of processing and evaluating the original stimulus before any action is taken.

Neural circuits in the human brain are enormously complex, consisting of more than 100 billion neurons, each forming thousands of synaptic junctions with other neurons. In total, it has been estimated that the human brain has more than a trillion synaptic junctions. One type of neuron in the cerebellum, called the Purkinje cell, has thousands of dendrites, receiving inputs from more than 200,000 other neurons. Circuits involving these neurons are part of the brain's machinery for controlling complex movements.

The complexity of the brain circuitry increases in humans and other mammals soon after birth. The increased complexity is not due to an increase in the number of neurons but rather is due to an increase in the number of synaptic junctions. The addition of new synapses increases the integrative and analytic capabilities of the circuit and may be necessary for the storage of new memories. Experiments with rats have shown that animals reared in a boring environment with minimal visual stimulation have fewer synapses than rats reared in stimulating environments or subjected to long-term training exercises. Extensive training and learning exercises also modify preexisting synapses in several ways: synaptic contacts increase in size, the number of Golgi vesicles stored at the axonal terminus increases, and there is an increase in the number of Ca^{++} channels in the pre-synaptic membrane. All of these changes are part of a complex process, known as long-term potentiation, that remodels the circuitry for enhanced functionality and memory storage.



Evolution of neural circuits. Circuits evolved from single-cell circuits consisting of a neuron that had the duel function of a receptor cell and a motor neuron, capable of stimulating a muscle or other tissue. More advanced circuits include sensory neurons and a central nervous system consisting of many different kinds of neurons that process and evaluate the stimulus before activating a motor neuron or other output neurons.



A neural circuit in the mammalian brain. Circuits in the brain consist of billions of neurons, each forming thousands of synaptic junctions with other neurons. These complex, highly integrated circuits give us our intellect, our emotions, our ability to see the world, and much more.

VISION

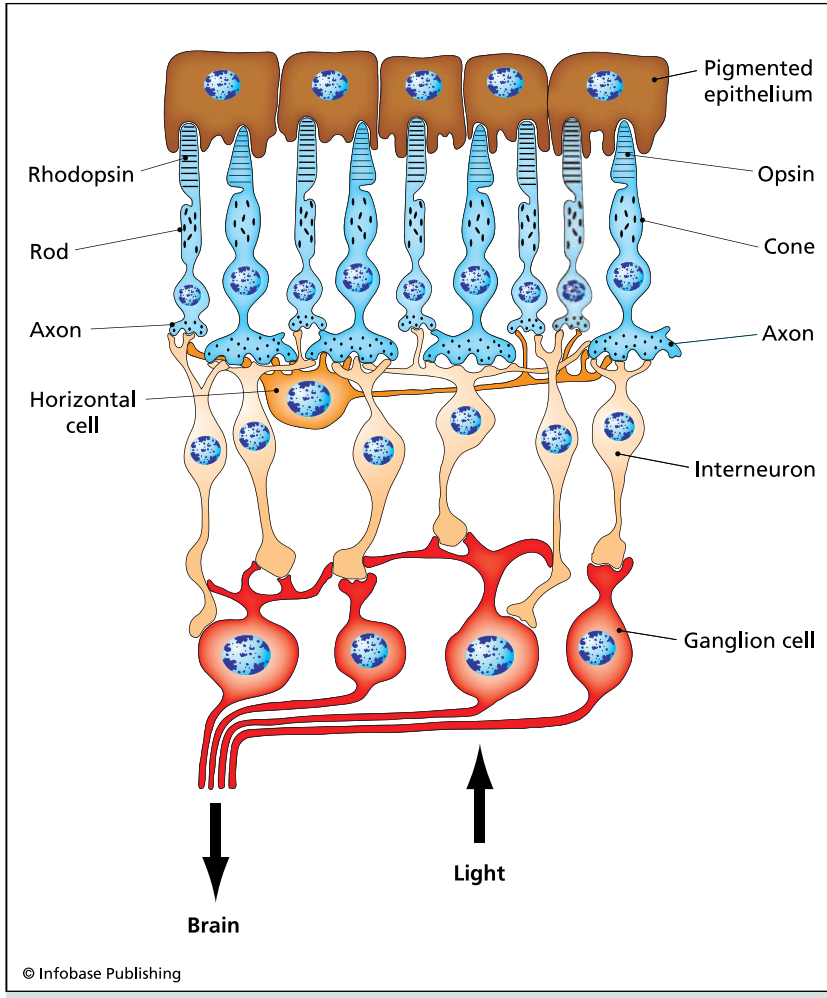
How do neurons make it possible for us to see the world? And not only to see it, but to see it in color, and to map changes quickly enough so there is no lag and no stutter in the image such as one gets while trying to watch a video on a cell phone or with a slow Internet connection. Our visual system consists of a front end (the eye and retina) that captures the image and a back end (the visual cortex) that interprets the signals from the eye and reconstructs a three-dimensional representation of the scene. The following discussion will be limited to the intricacies of the eye and the retina.

The Eye

The eye is like a camera that focuses an image onto a piece of film. It has an aperture (the pupil) that is regulated by the iris to vary the amount of light entering the eye, and it has a flexible lens that, with the aid of attached muscles, can alter its focal length so objects can be seen clearly whether they are inches or yards away. The interior of the eyeball is filled with a clear, viscous fluid called vitreous humor, which has the same refractive index as the lens. This minimizes the amount of scattered light to ensure the image is brought to a sharp focus on the retina, located at the back of the eye. An area near the center of the retina, called the fovea, is the place where the image is in the sharpest focus.

The Retina

The retina is a thin layer of nervous tissue, about 0.02 inches (0.5 mm) thick, located at the back of the eye. The diameter of the retina is about 0.9 inches (22 mm), whereas the diameter of the fovea is only about 0.2 inches (6 mm). The retina is composed of essentially four kinds of neurons: photoreceptors, interneurons, ganglion cells, and horizontal cells. Photoreceptors contain a light-absorbing visual pigment located in an area analogous to the dendrite. It is this pigment that initiates the neural reconstruction of the focused



The retina. The photoreceptors (rods and cones) are embedded in pigmented epithelium at the back of the retina. Interneurons connect the photoreceptors to ganglion cells that send their axons to the visual cortex of the brain. Horizontal cells provide a lateral circuit that is used to enhance the contrast of the image before it is transmitted to the brain. The RPE65 gene is expressed only in the cones.

image. Photoreceptors appear in two forms: the rods, specialized for the reception of black-and-white images, and the cones, which can detect color. Signals from the photoreceptors are conveyed to

Much of what is known about vision has been learned by studying the retina of cats. *(Eleanor Panno)*

the brain via the interneurons and ganglion cells. Axons from the ganglion cells are combined to form the optic nerve. The horizontal cells are used to process the image before it is sent on to the brain. Much of what is known about the retina was learned by studying the visual system of cats.

Rods

These photoreceptors have a visual pigment called rhodopsin. Each rod has 100 billion copies of this molecule, which allows the cell to detect a single photon. Rods cannot detect color, but their exquisite sensitivity makes them ideal for seeing at night or under low-light conditions. Rods are not found in the fovea, so the image they produce has a low resolution. This is one of the reasons why it is difficult to read small print in dim light. More than 80 percent of the photoreceptors in the human eye are rods.

Cones

These photoreceptors have a special visual pigment called cone opsin that is adapted for color sensitivity and day vision. There are three different forms of this pigment, each adapted for the detection of a different color, which in the human eye is blue, green, and yellow. Cones are concentrated in the fovea and produce the sharpest image. It is for this reason that one must look straight at an object

to get the clearest picture. The orientation of the cones and rods in the retina is in the opposite direction of the light pathway. This allows the retina to pull both of these photoreceptors deep into the pigmented epithelium at the back of the retina in order to guard against excessively bright light. This can occur, for example, when one leaves a movie theater on a bright sunny day. The initial fast response is a reduction in the pupil diameter to reduce the amount of light entering the eye. Within about 30 minutes, the retina will have pulled the photoreceptors into the pigmented epithelium, at which point the iris can reopen to its normal diameter.

Retinal Processing

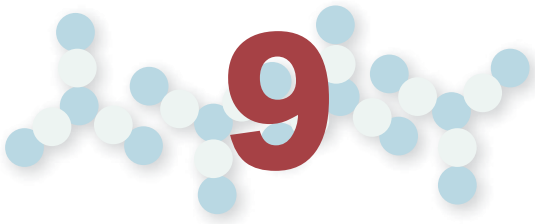
The retina does not simply relay the image to the visual cortex. Such an image would be badly degraded by the time it reached the brain and no amount of processing would be able to restore the quality of the original scene. Consequently, the retina is wired to process the image and, in particular, to enhance its contrast. This processing is enabled by horizontal neurons that provide a lateral retinal circuit. The lateral circuit allows the retina to sense the activity level of all of the photoreceptors simultaneously. Signals from photoreceptors that appear to be on the bright areas of the image are enhanced while signals from dark areas of the image are dampened down even further. This process, occurring throughout the retina, maximizes the contrast of the image, ensuring that what the brain receives is the clearest representation of the external scene.

A high-resolution retinal image is estimated to have 10,000 points or pixels (each photoreceptor is equivalent to a pixel). The retina has to be able to process this information fast enough to give the brain a sensation of fluid motion. Film animators know that for realistic motion they must prepare 24 frames for every second of the movie. Fewer frames will make the movie stutter and the characters will have unnatural, jerky movements. The retina has to work within this same time frame. That is, it must process the equivalent

of 24 frames per second in order to give us a smooth, realistic impression of the world. Given that 10,000 pixels constitute a single retinal frame, the retina needs to process 10,000 pixels × 24 frames per second, or 240,000 pixels per second. Scientists have estimated the speed of retinal processing at 600,000 pixels per second; more than enough to meet the demand.

SUMMARY

If neurons had never evolved on our planet, there would still be luxurious plant communities covering the surface of the Earth, microbes would be everywhere, and the seas would contain simple creatures like coral and sponges. All of these organisms are constructed from cells that communicate with each other, but they do so through a slow process involving the exchange of molecules that bind to cell-surface receptors. Neurons, by exploiting the properties of ion channels, introduced a lightning fast form of communication, without which the progression from single cells to complex multicellular bodies would not have been possible.



Plants

When the first cells appeared on Earth they were immersed in a nutrient broth that had everything they needed: nucleic acids, fats, carbohydrates, and minerals galore. But after half a billion years the prebiotic soup began to run thin, forcing the cell populations to come up with ever more clever ways of locating, ingesting, and processing whatever food they could find. Initially, this process led to a refinement in the biochemical pathways that cells used to extract the energy from available molecules, leading to the appearance of Krebs cycle and glycolysis, pathways that extract energy from food molecules, storing it for later use as molecules of ATP.

Even this level of efficiency and sophistication was not enough. Eventually there came a time when there was not enough food to go around and the young biosphere faced certain collapse. But then something strange happened: a group of prokaryotes found a way to extract energy directly from the Sun. Exactly how this happened

no one knows, but those seemingly magical cells marked the beginning of a new era that would transform the surface of the Earth. Today, that magic of 3 billion years ago is now a well-understood biological process called photosynthesis.

Some of the photosynthetic prokaryotes, now called chloroplasts, were taken up by eukaryotes in the same way that the animal lineage of eukaryotes took up mitochondria. Eukaryotes, equipped with chloroplasts, became a formidable new life-form. They went on to produce all of the algae and simple plants of the sea, as well as all of the vascular plants on land. These organisms, constituting the plant kingdom, transformed Earth's ecosystem from a barren landscape to a lush green paradise and in doing so made possible the emergence of the less endowed animal kingdom who, lacking the power of photosynthesis, had to forage for a living.

THE PLANT CELL

Plant cells are eukaryotes and thus have all of the basic structural features typical of animal cells, including a glycocalyx, Golgi complex, endoplasmic reticulum, and a nucleus. The most striking difference between an animal and a plant cell is that the latter possesses a cell wall and chloroplasts that are capable of photosynthesis.

The Cell Wall

Plant cells have a cell wall in addition to the cell membrane that is analogous to the extracellular matrix that surrounds most animal cells. The cell wall forms the woody part of many plants, giving them strength and resiliency, and with that the ability to grow to often-colossal sizes. Oak trees, hickory, maples, pine, and the towering redwoods of California owe their size and strength to the cell wall.

Plant cell walls, and the wood they produce, have played an important part in human history, going back at least 40,000 years: It was one of the earliest materials used for weapons and tools and for building shelters, campfires, canoes, and sailing ships. Balsa logs

and special reeds were used almost 1,000 years ago by seafaring pioneers who discovered the Hawaiian Islands and many other islands around the world. Canoes, dug out of fir logs or made from birch bark, were produced hundreds of years ago by Native Americans for fishing and for navigating oceans, lakes, and rivers. In 900 C.E., Vikings discovered North America by navigating the waters between Iceland and Labrador in enormous wooden canoes equipped with a single sail. Cross-Atlantic expeditions flourished throughout the 17th and 18th centuries with the construction of grand oak sailing ships. During this same period, oak, maple, and other hardwoods were the building material of choice for villages, homes, tools, and horse-drawn wagons. Even today, nearly all homes that are built in North America and Europe are framed with wood and filled with wooden furniture. Our civilization is practically built on a foundation made of wood. Humans are so close to wood that its beauty and utility are often taken for granted.

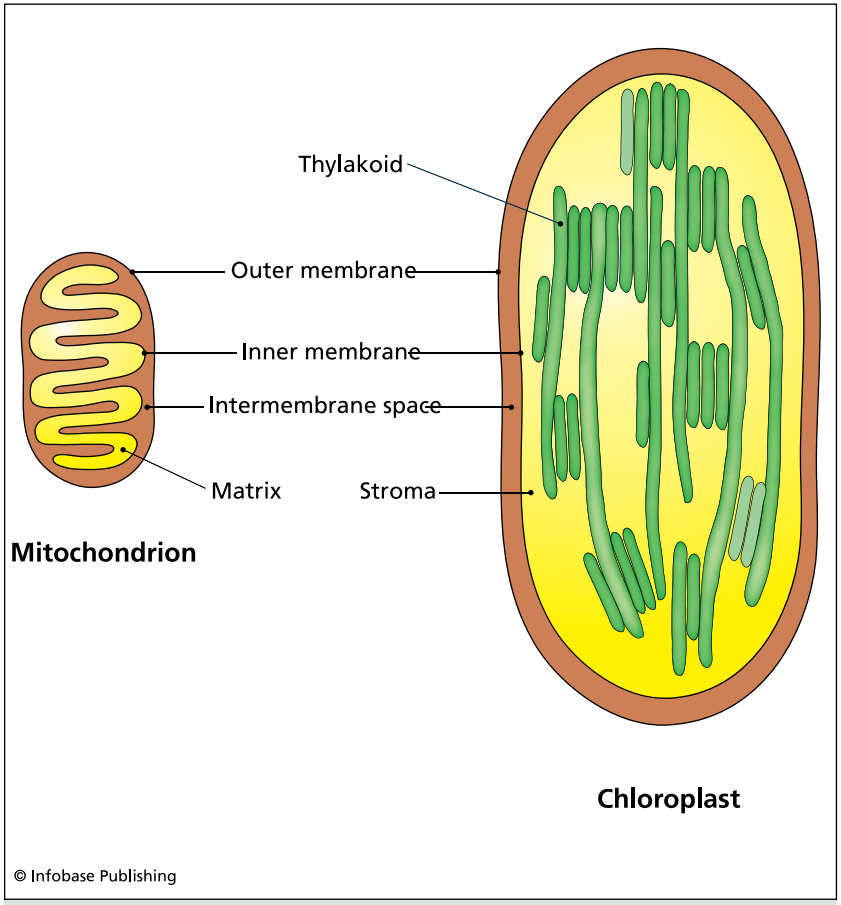
But what is this ubiquitous material, secreted by plant cells, that is so versatile? Simply stated, it is nothing more than polymerized glucose, a sugar that is also an important source of energy for both plants and animals. A bowl of glucose looks very much like a bowl of sucrose, the common table sugar, and nothing at all like a wooden board. Plants begin this remarkable transformation by forming and extruding long polymers of glucose into the intercellular space. These glucose polymers are called cellulose. Intermolecular hydrogen bonds hold adjacent cellulose polymers together to form crystalline aggregates, called cellulose microfibrils, which have the tensile strength of steel. The microfibrils are crosslinked to each other by different types of sugar polymers called glycans, which are made from glucose, xylose, and mannose. The ground substance for the cell wall is a very hydrated polysaccharide known as pectin, which serves the same function as the proteoglycans and glycosaminoglycans in the extracellular matrix of animal cells. The cell wall also contains a small amount

of protein similar to animal collagen that may serve to increase the flexibility of the structure. These sugar and protein polymers, when added together, form the subtle beauty, color, and texture of the material that is known as wood.

Photosynthesis

Plants synthesize all of the food they need by harvesting the energy of sunlight. All of the photosynthetic reactions are located within the chloroplasts, a plant cell organelle that was once a free-living photosynthetic prokaryote very similar to modern-day cyanobacteria. The acquisition of chloroplasts or mitochondria represents an ancient phylogenetic branch point that gave rise to the plant and animal kingdoms. Cells equipped with chloroplasts could obtain most of the nutrients they needed simply by basking in sunlight. Consequently, the unicellular and multicellular plants evolved a passive or sedentary lifestyle, floating along with the ocean currents as is typical of unicellular phytoplankton or rooted in the ground as is typical of seaweed and terrestrial plants.

Chloroplasts, like mitochondria, provide energy for the cell in the form of ATP and NADH, but there are many structural and functional differences between these two types of organelles. To begin with, a chloroplast is much larger than a mitochondrion and has a more complex inner structure. A mitochondrion has an outer cell membrane and an inner membrane that forms the cristae, the membrane structure that houses the electron transport chain and ATP synthase. The inner membrane surrounds the matrix, which is equivalent to cellular protoplasm (called cytoplasm in eukaryotes) in that it houses the DNA genome, ribosomes, and all of the enzymes for glycolysis and Krebs cycle. The chloroplast also has an inner and outer membrane, but in addition there is a third set of membranes that form disclike structures known as thylakoids. All of the photosynthetic machinery, known as the photosystem, is embedded in the thylakoid membranes. The protoplasmic space in



Comparison between a mitochondrion and a chloroplast. A chloroplast is nearly three times larger than a mitochondrion and in addition to the outer and inner membranes it has a third set of membranes that form the thylakoids. The chlorophyll and the photosystem proteins are located in the thylakoid membrane. Both of these organelles have their own DNA genome (not shown) located in the matrix or the stroma.

a mitochondrion is called the matrix, but in chloroplasts it is known as the stroma. The stroma, like the matrix, houses the DNA genome and the ribosomes. It is also the location of the Calvin cycle (also known as the carbon-fixation cycle) where nutrients are synthesized

using the ATP provided by the photosynthesis. The Calvin cycle, like photosynthesis, is unique to plants.

The photosystem consists of more than 25 different transmembrane proteins and many copies of a light-sensitive molecule called chlorophyll. The system has two components: an antenna complex and a photochemical reaction center. Light striking the antenna complex produces high-energy electrons in the chlorophyll, which are quickly passed to the reaction center. The reaction center, functioning much like the electron transport chain in mitochondria, uses the electrons to establish a hydrogen ion gradient across the thylakoid membrane, which drives the synthesis of ATP by ATP synthase. At the end of the chain, the high-energy electrons are transferred to phosphorylated NAD to produce NADPH. This compound is analogous to NADH, the electron carrier in mitochondria. The ATP and NADPH are sent to the stroma where they supply the energy required by the Calvin cycle to synthesize sugars, fatty acids, and amino acids. The Calvin cycle synthesizes carbon compounds by converting CO_2 and water into carbohydrate, the initial form of which is glucose. The simplified equation for this process is the following: $H_2O + CO_2 \Rightarrow CH_2O + O_2$. Thus, plants consume water and CO_2 and produce carbohydrates and O_2. This is complementary to the oxidative phosphorylation that occurs in the mitochondria of animal cells where O_2 and carbohydrates are consumed and CO_2 is produced. This simple equation is at the heart of the interdependence of the plant and animal kingdoms.

UNICELLULAR PLANTS

Unicellular plants, also known as algae, are aquatic and can be found in the sea and in freshwater lakes and rivers. Unicellular algae are free-floating organisms that remain close to the surface of the water to maximize their exposure to light. The free-floating lifestyle of the unicellular algae is called planktonic, and because these cells are capable of photosynthesis they are usually referred to as phytoplankton. This distinguishes them from benthic algae

that are attached to rocks or the ocean floor. The photosynthetic activity of phytoplankton is known as primary production, for the organic compounds these plants produce form the basis for the aquatic food chain that all aquatic animals depend on. Moreover, the total biomass and photosynthetic activity of phytoplankton are much greater than all land plants combined. Thus, even animals on the land depend on the productivity of marine phytoplankton.

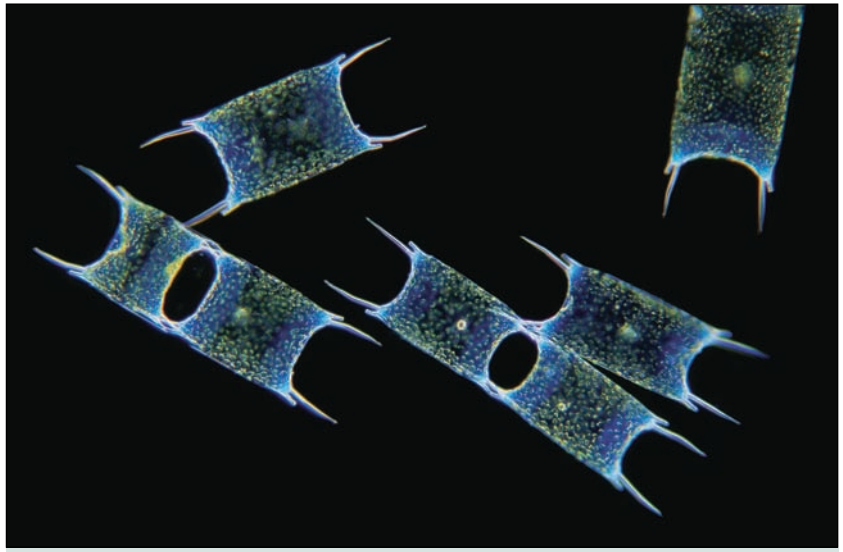
There are two major groups of marine phytoplankton, known as Chrysophyta and Pyrrophyta. The dominant chrysophytes are the diatoms and coccolithophores, while the dominant pyrrophytes are the dinoflagellates.

Diatoms

Diatoms, like most plants, have a cell wall, but in this case the wall is impregnated with silica (SiO_2) to produce a glasslike exoskeleton, or frustule, that has a beautiful "snowflake" architecture. The frustule consists of two closely fitting halves, the epitheca and a slightly smaller hypotheca, which when viewed from the side resemble a microscopic petri dish or round pillbox. Diatom frustules, viewed from the top, are either circular or pennate (shaped like a submarine) and are covered in fine meticulously arranged pores that allow material to pass into and out of the cell. The frustule also has many spinelike projections that some scientists believe act as a deterrent to diatom predators. Diatoms are very large cells, often reaching 500 μm in diameter or length. The internal structure consists of basic eukaryote organelles, in addition to a large number of chloroplasts and storage vacuoles that contain fats, sugars, and starch.

Coccolithophores

The coccolithophores are distinguished from the diatoms by their generally spherical shape and the fact that their cell wall is impregnated with small calcareous (containing calcium carbonate) plates called coccoliths. These organisms may be up to 0.002 inches (50 μm) in



Marine planktonic diatoms *(Odontella sinensis).* Diatoms are unicellular algae or protists that have beautiful glass shells consisting of two parts or valves that fit together like a tiny pillbox. Diatoms have yellow-brown photosynthetic pigments. Darkfield light micrograph, magnification 10x. *(Wim van Egmond/Visuals Unlimited)*

diameter and are very common in warm and temperate seas. In some areas, such as the Sargasso Sea, a single species, *Coccolithus huxleyi*, seems to be responsible for most of the primary productivity.

Diatoms and coccolithophores have been so numerous throughout Earth's history that their skeletons form much of the coastal seabeds and adjacent lands around the world. The white cliffs of Dover, for example, consist almost entirely of diatom and coccolithophore skeletons, a substance that is commonly referred to as diatomaceous earth.

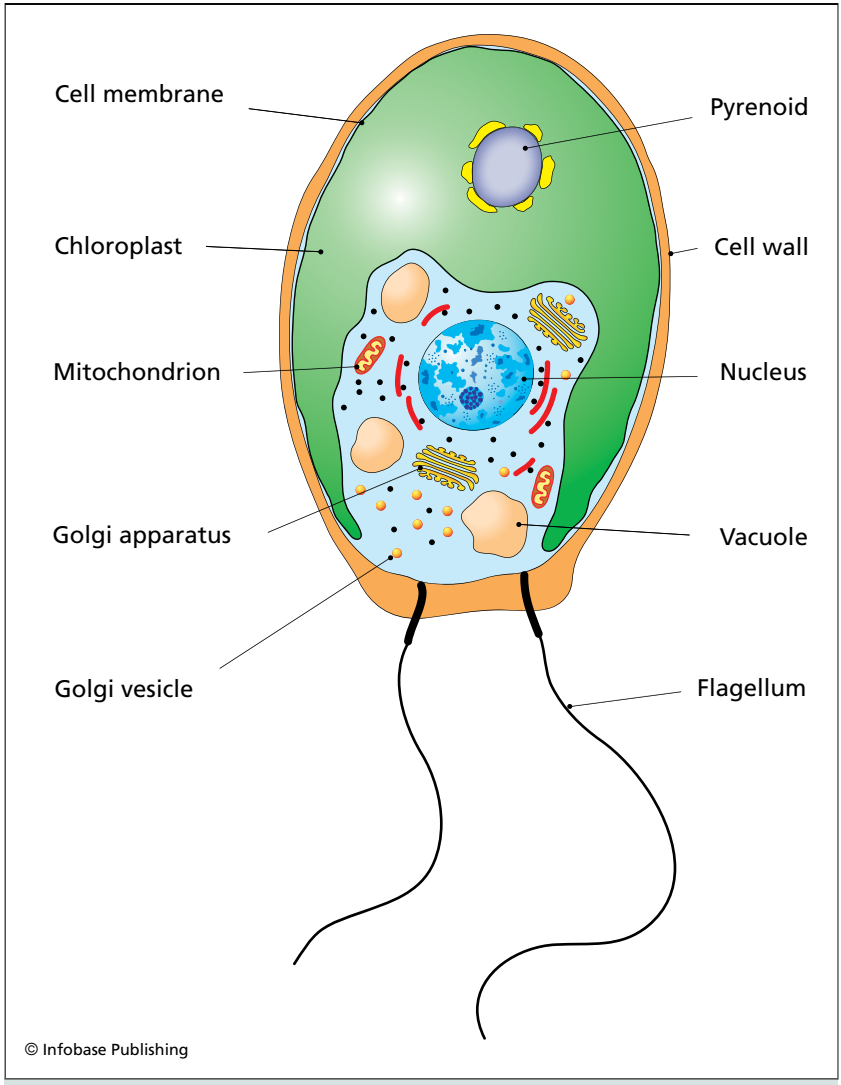
Dinoflagellates

These organisms are quite variable in size, ranging from 0.001 to 0.02 inches (25 to 500 µm). They tend to have an egglike or distinctly

conical shape and are equipped with two flagella that propel them through the water. The internal structure is dominated by the chloroplast, large nucleus, and storage vacuoles. Some species also have accessory pigments that give the cell a red or yellow color. During bloom conditions, when certain dinoflagellate populations increase dramatically, the color of the water turns red, producing the phenomenon known as a red tide. Several species of dinoflagellates, belonging to the genus *Gonyaulax,* are known to produce nerve toxins and during bloom conditions may cause extensive mortality in other marine organisms. The toxin produced by *Gonyaulax,* called saxitoxin, is 50 times more lethal than curare or strychnine. This toxin is often concentrated in the tissues of butter clams and other shellfish that feed on dinoflagellates. Human consumption of toxin-contaminated shellfish can be fatal. It is for this reason that health officials warn people not to eat shellfish during algal blooms or red tides.

The photosynthetic activity of phytoplankton is so important that scientists have spent a great deal of time measuring it in many places around the world. The method used is known as the light and dark bottle technique. With this procedure, measured changes in O_2 consumption and production are used to estimate photosynthetic rates. Seawater samples, containing phytoplankton, are collected and transferred to three different bottles: a light bottle (LB) made of clear transparent glass, a dark bottle (DB) covered with foil to exclude light, and an initial bottle (IB).

The O_2 concentration of the water in the initial bottles is determined immediately to establish the initial conditions in the light and dark bottles. The light and dark bottles are then lowered back to the depth at which they were collected and allowed to incubate for a few hours. At the end of the incubation period, the bottles are retrieved and the O_2 concentrations are determined as quickly as possible. The cells in the dark bottle cannot photosynthesize and so the amount of O_2 consumed (IB – DB) is a measure of cell respiration. The dark bottle also serves as a control for the experiment.



Chlamydomonas. These dinoflagellates range in size from 25 to 500 µm. They tend to have an egglike or conical shape and are equipped with two flagella that propel them through the water. The internal structure is dominated by the chloroplast, nucleus, and vacuoles, some of which are used to store food reserves. The pyrenoid is a special part of the chloroplast, unique to unicellular algae, that enhances the uptake of carbon dioxide.

The O_2 produced by the photosynthesizing cells in the light bottle (LB – IB) provides an estimate of net primary production. Photosynthesis can also be estimated by adding the carbon isotope C^{14} to LB and DB and then determining the amount of the isotope incorporated in LB during the incubation period. Methods such as these have been used to estimate annual primary production for all plant life at 100 billion metric tons of fixed carbon. Roughly 70 percent of this is due to the photosynthetic activity of marine phytoplankton.

THE SEAWEEDS

Seaweed, also known as benthic algae, are multicellular organisms that are normally attached to the rocks or sand in intertidal zones close to shore. Being attached to the ocean floor restricts the habitat of these algae to a narrow fringe along the periphery of the marine environment. This is in sharp contrast to the free-floating, far-ranging habitat of the phytoplankton. Seaweeds are represented by three divisions: Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae).

Anyone who has strolled along a beach knows that seaweeds are abundant and often form thick algae forests (known as kelp beds) just offshore. Very large kelp beds modify the local habitat for other marine organisms. Like a terrestrial forest, kelp beds provide food and habitat for a wide range of marine organisms. Small fish and even sea otters are known to take refuge among the kelp to escape a predator. Sea otters also use the kelp beds as convenient rafts that they can relax on, either to take nap or to eat their lunch.

Seaweeds are not as complex as land plants. They lack roots, flowers, seeds, and true leaves. Despite this, seaweeds have evolved an astonishing variety of shapes and sizes, all of which consist of a blade, stipe, and holdfast. Very large seaweeds include a fourth structural element, the pneumatocyst, which is a gas-filled ball that serves as a flotation device.

A piece of green seaweed that has washed up on shore. *(Amy Walters/Shutterstock)*

The Blade

The flattened, broad leafy structures of seaweeds are called blades. These range in complexity from a single filament to a complex level of branching that resembles land plants. The blades contain the chloroplasts and are the main sites for photosynthesis, although some photosynthesis occurs in the stipes and holdfast as well. The blades normally float on the surface of the water where they are tossed around by the surf and tides. Consequently, there are no top and bottom surfaces on seaweed blades as there are for leaves on land plants. Both sides are equally capable of photosynthesis. Since nearly all of the cells making up the blade are capable of photosynthesis, there are no veins for the conduction of nutrients as there are in higher plants.

The Stipe

A flexible, hollow tube or stipe forms the trunk of the seaweed, which often branches into several smaller stipes that give rise to the

blades. The stipe has to be strong enough to withstand the pounding of the surf but flexible enough to avoid breaking. Stipes often attain great lengths, sometimes as much as 30 meters (~ 33 yards), and possess conductive elements similar to those in the stems of terrestrial plants. Radioactive tracer studies have shown that these tissues do conduct nutrients from the blades to other parts of the plant.

The Holdfast

Although resembling roots, the holdfast does not absorb nutrients as higher plant roots do but is concerned only with attaching the plant to the seabed. Seaweeds that attach to rocks have holdfasts consisting of many short, sturdy rootlike fibers, whereas seaweeds anchored to sand or muddy bottoms have long thick fibers that penetrate deep into the ground.

TERRESTRIAL PLANTS

In moving from a coastal aquatic lifestyle to a life on dry land, plants had to overcome several obstacles, the most important of which was dehydration and the availability of water. The young Earth was a very hot place with surface temperatures that likely reached 104°F (40°C) on a daily basis. Any plant without easy access to water would quickly wilt and die in such an environment. And yet some did not die, and, by examining the morphology of simple seaweed and comparing it to modern-day land plants, scientists can explain why that was so.

Imagine a population of seaweeds living in a coastal habitat 1.5 billion yeas ago. At low tide, many of the immature seaweeds would have been pulled loose from the substratum and blown inland by the strong winds. Most of those unfortunate plants would have died of dehydration soon after reaching their terrestrial destination. But among that population were seaweeds that already had holdfasts adapted to burrowing into the ground. They might land on dry ground, but just beneath the surface, if they could only reach it, they

could find enough water and minerals to sustain them. Being out on the land, fully exposed to the Sun, would even maximize their photosynthetic capabilities. Thus it is reasonable to assume that the colonization of land required the evolution of holdfasts into true roots: structures that not only anchored the plant to the ground but also served to conduct water and minerals to other parts of the plant. A second and very important adaptation was the acquisition of a waxy cuticle covering the entire plant to reduce the amount of water lost through evaporation.

Descendents of those hardy pioneers likely stayed close to the ground, flopping over as they would have done at low tide back on the beach. But as the population density increased, the forces of natural selection would have altered the structure of the stipe, slowly transforming it into a woody trunk that allowed the plant to stand erect for unobstructed access to the light. Standing erect would have exposed the plant to hot, dry winds, thus increasing the threat of dehydration, but this problem was countered by the development of a thicker cuticle (bark), which reduced water loss to a minimum. These adaptations have a clear advantage, but they come at a price: the trunk, unlike a stipe, could no longer photosynthesize and must depend on receiving all of its nourishment from the blades. Seaweed blades and stipes have a limited capacity for circulating nourishment throughout the plant. This capacity was greatly extended and refined by land plants, which evolved a vascular system consisting of two kinds of tissue: the xylem, which carries water from the roots to the rest of the plant, and the phloem, which carries nourishment from the photosynthesizing leaves to the rest of the plant.

The remaining adaptations, which led to the appearance of true terrestrial plants, involved the transformation of the blade into a leaf and the production of seeds and flowers to enable reproduction on land. A seaweed blade is a nonvascularized, homogeneous structure that lacks a clearly defined top and bottom. This is not the case with a leaf, where all of the chloroplasts are restricted to cells

A flowering deciduous tree. *(courtesy of the author)*

located on the top surface. In addition, a leaf is a highly vascularized structure, which enables the distribution of nutrients to the rest of the plant. Seaweed reproduction is relatively straightforward: The gametes are released into the water where they combine to form new plants. Land plants, lacking ready access to bodies of water, evolved special structures (flowers) wherein the gametes fuse to produce embryos, which are released into the environment as seeds or seed pods. Seeds are sturdy structures that protect the embryos from desiccation until they encounter a favorable site for germination and growth.

PLANT COMMUNITIES

The colonization of the land by plants transformed the face of the Earth. What was once a barren, desolate landscape became a green oasis covered in trees, grasses, ferns, and moss. The evolution of plant habitats has produced five distinct plant communities or landscapes: rain forest, savanna, woods, desert, and tundra.

A tropical rain forest, common in South and Central America, is the richest, most diverse habitat (biome) on Earth, possessing at least two-thirds of all of Earth's plant and animal species. African savannas, dominated by grasses and the acacia tree, are home to enormous herds of gazelles, buffalo, elephants, zebra, and wildebeest. A savanna of similar proportions (the plains) once existed in North America, which supported the largest herds of buffalo and elk ever known to exist. Those herds supported Native American hunters for more than 10,000 years. Woodlands, such as the deciduous forests of eastern North America, are dominated by trees that lose their leaves in the winter: maple, birch, hickory, oak, and ash, all of which provide a rich habitat for a variety of herbivores, such as deer, elk, and moose. Tundra, although cold for much of the year, is home to a great variety of grasses, which support large herds of caribou. Even deserts, dry as they are, provide a habitat for many kinds of plants and animals. The Sonoran desert in Arizona, for example, provides a habitat for a large population of the giant saguaro cactus and many species of desert rodents, lizards, and snakes. A hillside, located on the eastern edge of this desert, is home to the longest-lived organism known to exist in the world: a pine tree named Methuselah that is 4,600 years old.

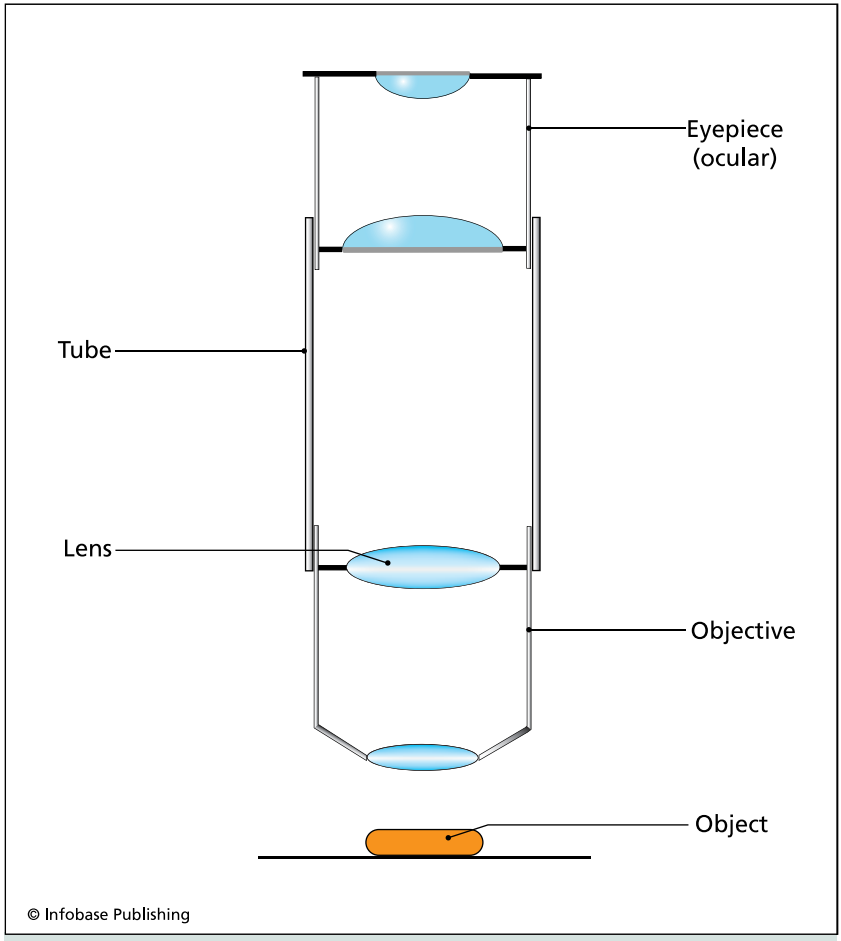
10

Resource Center

Studying the cell requires a great deal of hardware, technology, and experimental procedures. This chapter provides additional information that covers light microscopy, histology and histochemistry, and recombinant DNA technology.

LIGHT MICROSCOPY

Microscopes are among the oldest of scientific instruments and yet are just as important today as they were 120 years ago when microbiologists used them to study bacteria and the many diseases they cause. The earliest microscopes, invented in the 1600s by Antoni van Leeuwenhoek, were single lenses mounted in a brass frame that were held in the hand or attached to a table. These simple magnifying glasses could resolve bacteria and other microbes but were extremely difficult and tiring to use.



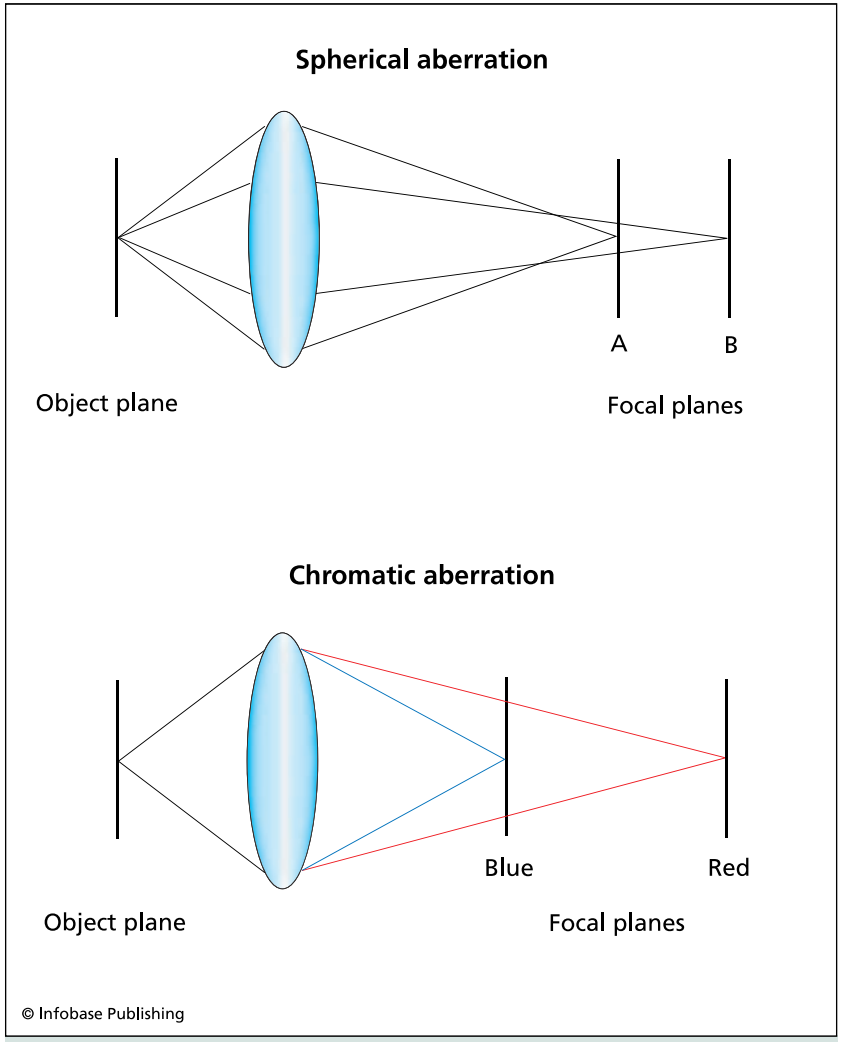
An 18th-century light microscope. The image is magnified by the objective and brought to focus at the eye by the eyepiece. The eyepiece and objective are compound lens systems that give high magnification but poor resolution and image quality.

By the 1700s, compound microscopes, using two or more lenses, were being made that offered higher magnifications than Leeuwenhoek's and were much easier to use, but the image quality

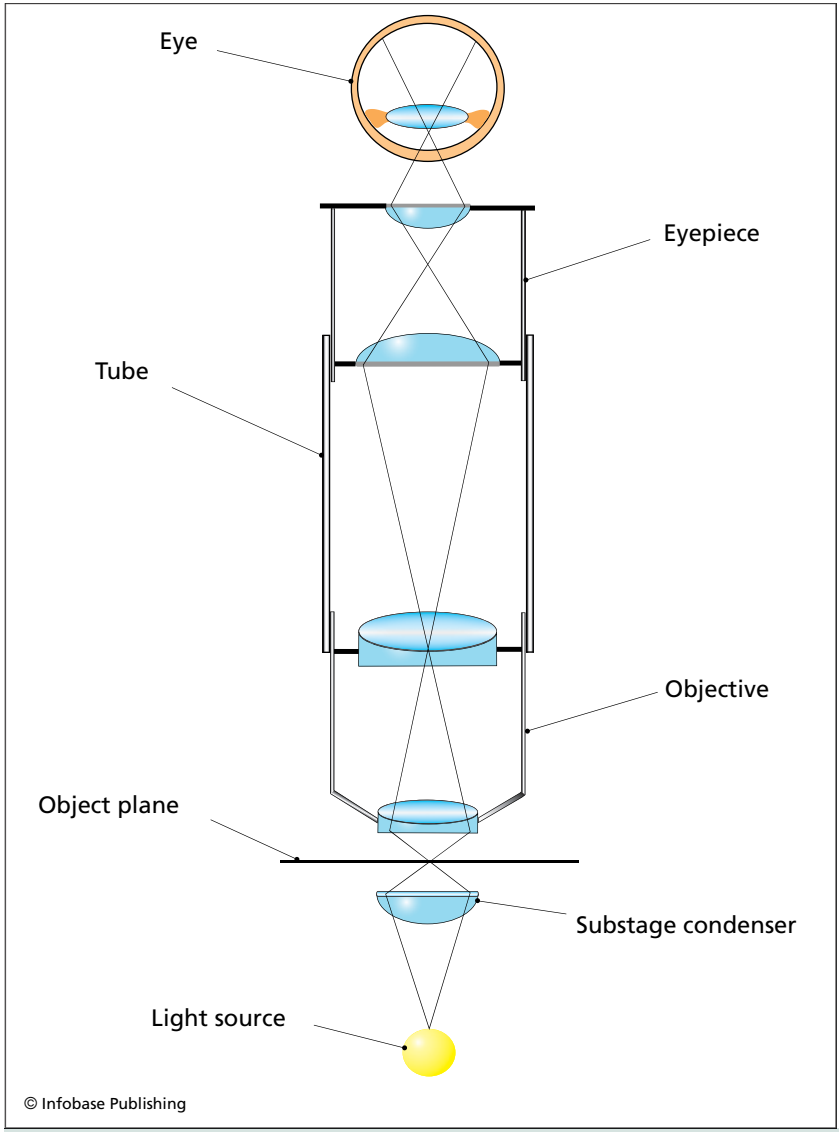
was not as good. These microscopes consisted essentially of an eyepiece, a metal tube, and an objective that formed the initial magnified image of the object. Adding two or more lenses together increased the overall power of a microscope but, at the same time, introduced optical artifacts that seriously degraded the image. The two most important of these artifacts are spherical and chromatic aberration.

Spherical and chromatic aberration arise as a natural consequence of the way light behaves when it passes through a glass lens, which has a higher density than the air around it. There are two simple rules of geometric optics that govern this behavior. First, light travels in a straight path, and second, the path bends (refracts) at an interface between two transparent media (i.e., the air and the lens). Lenses are designed with curved surfaces to magnify an object and to bring the image of that object to a single focal point. Spherical aberration occurs when light rays from an object are refracted by different parts of the lens and therefore do not come to a single focal point, producing an image that is fuzzy and out of focus. Chromatic aberration is a consequence of the multi-wavelength composition of white light, ranging from red (long wavelength) to blue (short wavelength). The extent to which light is refracted at an interphase depends on its wavelength, being high for blue light and low for red light. Therefore, the blue and red components of white light are refracted by a lens to separate focal points, producing a fuzzy image that has a red-and-blue halo.

In 1868, the German physicist Ernst Abbe discovered a way to construct objectives containing lens pairs and triplets that corrected chromatic and spherical aberration simultaneously. These lens systems, called apochromatic objectives, provide clear and undistorted images at the highest magnification possible (1,000X), while retaining a high resolution. The measure for resolution is the smallest distance at which two points can still be discerned. A resolution of one micrometer (μm), for instance, indicates that two



Spherical and chromatic lens aberrations. Spherical aberration is caused by variations in the amount of refraction that occurs over the surface of the lens. Consequently, light passing through peripheral areas of the lens comes to a different focal point (A) than light passing through central regions (B). Chromatic aberration occurs because the amount of refraction is greater for blue light than it is for red light. Thus, these two wavelengths and all wavelengths in between come to different focal points.

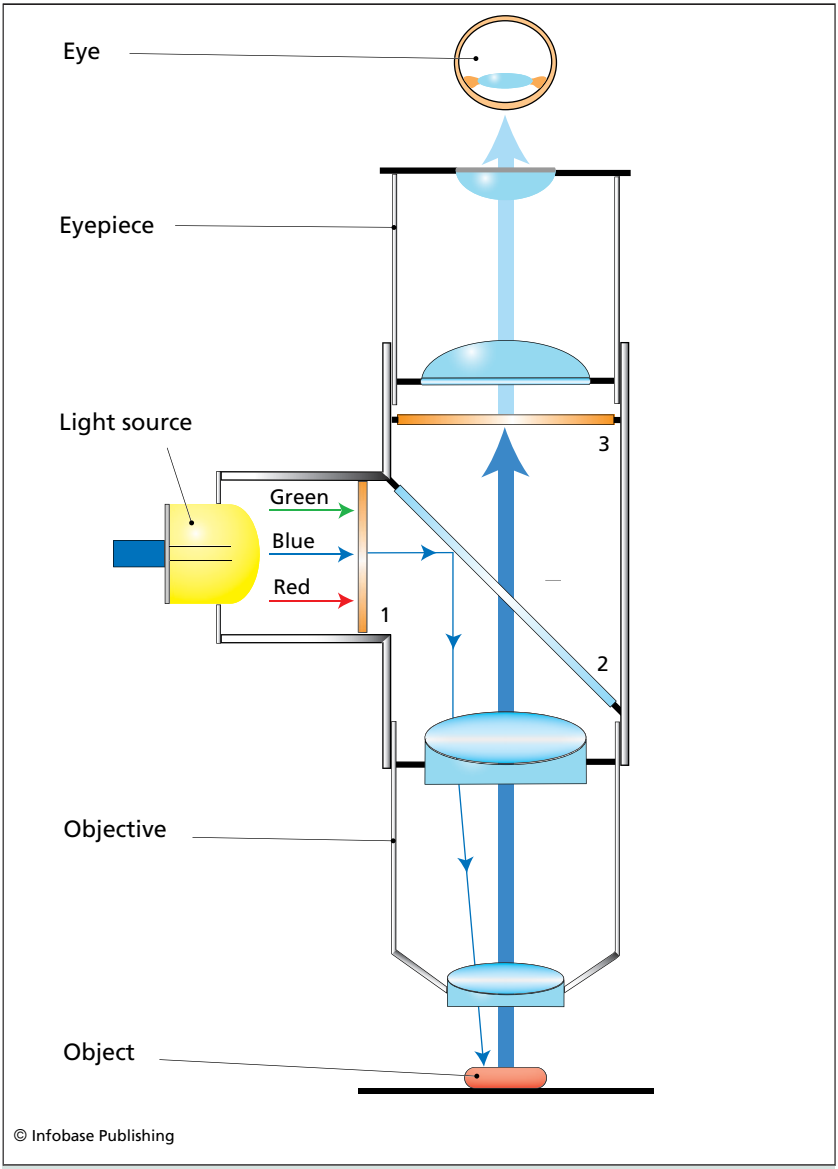


A light microscope corrected for chromatic and spherical aberration. Light is focused on the object by a condenser to enhance image quality and contrast. Chromatic and spherical aberration are corrected at the objective with precisely ground lens-pairs or triplets (two or three lenses fitted together). High-power objectives (100X) may have up to 10 separate lenses to correct for aberrations. The example shown above is a 10X objective, consisting of four lenses.

objects one µm apart can still be seen as separate objects, whereas if the objects were 0.5 µm apart they would appear to be a single object. Apochromatic objectives provide a resolution of 0.4 µm, very close to the theoretical limit for light microscopes of 0.2 µm (a distance of 0.2 µm is about 1/500 the diameter of a period on this page). The high resolution and clarity of the image provided by apochromatic objectives made it possible for scientists to study the structure and function of individual cells in a way that was never before possible. The quality of Abbe's lenses has never been surpassed, and apochromatic objectives are still standard on light microscopes today.

With the lens system perfected, microscopes quickly evolved from a simple monocular construction to binocular compound microscopes that are equipped with rotating turrets, holding a variety of objectives, camera attachments, video equipment, and special filtering systems for observing fluorescent images. In addition, microscopes with special optical systems, known as phase contrast and Nomarski's interference, were invented in the 1950s that made

A 19th-century monocular microscope. These instruments were capable of high resolution but dif cult to use for extended periods of time. *(courtesy of the author)*



A fluorescence microscope. A light source is filtered by a barrier filter (1) that blocks passage of all wavelengths, except for blue light, which is deflected by a beam-splitting mirror (2), directing it through the objective, where it stimulates fluorescent molecules in the object. Fluorescent light from the object passes freely through the beam splitter and is filtered of spurious fluorescence by a second barrier filter (3), before reaching the eye.

possible the observation of living cells without having to stain them with dyes. An important advance in the field of microscopy came with the introduction of the fluorescent microscope, an instrument that is used to observe cells and tissue sections that have been stained with fluorescent dyes. This microscope has standard objectives, but it includes special optical filters and a beam splitter for observing cells and tissues emitting fluorescent light. A fluorescent dye is a molecule that absorbs light of one wavelength, emitting it at another, longer wavelength. An example is the molecule fluorescein, which emits green light, at 520 nanometers (nm), when stimulated with blue light at 450 nm.

Monocular microscopes used at the turn of the 20th century could be carried around in a container not much bigger than a shoebox. Modern, fully equipped research microscopes are not only more powerful but also much bigger, weighing more than 100 pounds (45 kg) and easily filling the top of a lab bench. Indeed, some of these microscopes are so large and so elaborate they often need their own room.

ELECTRON MICROSCOPY

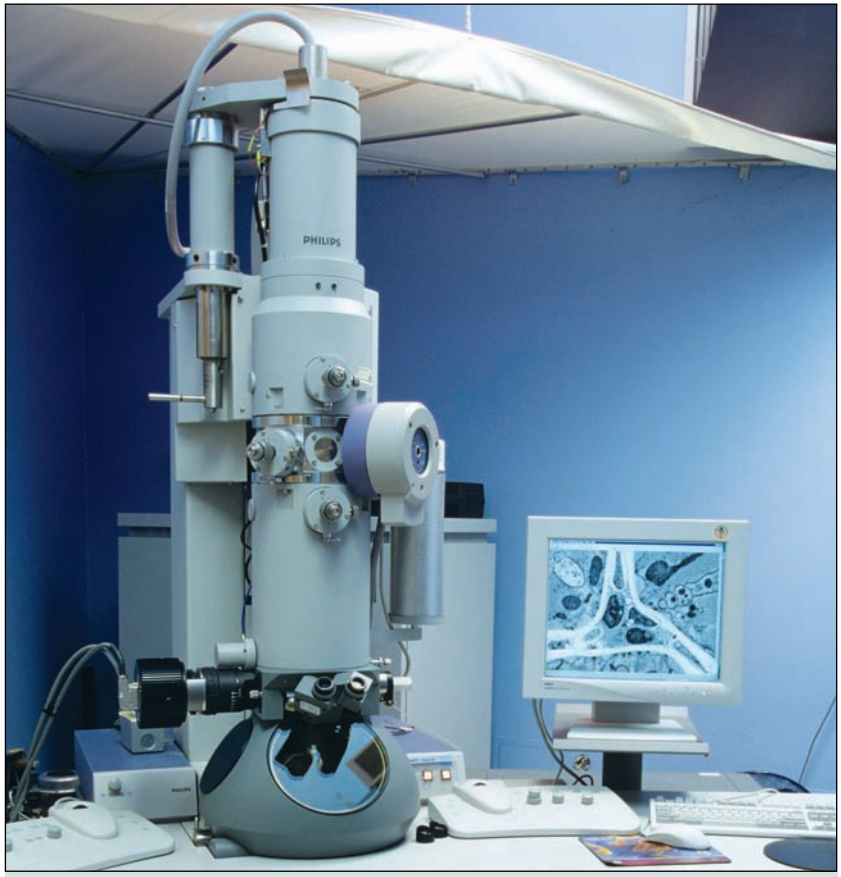
Electron microscopes obtain high-resolution images of cells and small organisms by using a focused beam of electrons rather than a beam of light. The main advantage is that an electron has a much shorter wavelength than does a beam of light, so the final resolution is much higher. The maximum resolution possible with a light microscope is 0.2 μm. With an electron microscope, the resolution is about 100 times better. This means that it is possible to see things in a cell, like ribosomes and nucleosomes, that are invisible under a light microscope.

The electron microscope focuses the beam of electrons by using magnetic lenses, and these are arranged in a way similar to that shown for the lenses in a light microscope. The electrons are accelerated through an electric field generated by 100,000 to 3 million volts. Because the beam of electrons are easily scattered by air molecules, the entire instrument has to be evacuated. It is

A modern binocular microscope that is used in cancer research. This particular instrument is connected to a computer, which displays the image field on the monitor and is equipped with software for analyzing and enhancing the image. *(Jean-Paul Chassenet/Photo Researchers, Inc.)*

for this reason that live specimens can never be studied with an instrument such as this.

There are two kinds of electron microscopes: transmission and scanning. The transmission electron microscope (TEM) is designed for studying tissue sections for the analysis of minute structures (molecules and organelles) inside a cell. Molecules can also be prepared and viewed separately. TEMs can magnify objects 50,000 times while displaying stunning detail. The scanning electron microscope (SEM) is designed for studying three-dimensional objects, cell organelles (chromosomes and ribosomes), and small insects. Useful magnifications with this instrument range from about 5,000 to 10,000 times.



Transmission electron microscope (TEM). *(Inga Spence/Visuals Unlimited)*

HISTOLOGY AND HISTOCHEMISTRY

Histology and histochemistry represent an overlapping collection of techniques that are used to study the structure and chemical properties of intact tissues. Histology means literally the study of tissue, whereas histochemistry refers to the use of specific stains to enhance the detection of microscopic detail based on known chemical or physical properties of the tissue. Thus, progress in histochemistry improved histology.

The microscopic characterization of human tissues, organs, and more than 200 human cell types, some of which are described in chapters 7 and 8, were originally obtained with a combination of histological and histochemical techniques. Studies involving these procedures are generally conducted on tissue sections or tissue smears that have been preserved with a chemical fixative and then stained with colored dyes. Histological preparations of tissue sections form the basis for our understanding of organ structure at the cellular level and are indispensable for the study of physiology. Histochemical analysis is usually focused more at the cellular level and was crucial for localizing DNA to the cell nucleus and for tracking the movement of molecules between the various subcellular compartments. Prominent examples included the movement of RNA from the nucleus to the cytoplasm, the movement of certain proteins from the cytoplasm back to the nucleus, and the transport of other proteins through the endoplasmic reticulum and Golgi complex.

Histology

Tissues are usually fixed, or preserved, before they are stained with a dye. Two fixatives that are commonly used are formalin (10 percent formaldehyde solution) and a mixture of alcohol and glacial acetic acid (3:1) called Clark's solution. Formalin is preferred for most applications, but Clark's solution is ideal for blood or chromosome smears. Staining tissues with various dyes to enhance the contrast of their microscopic image is a standard histological procedure. This approach takes advantage of biochemical differences between the various cellular compartments. The nucleus, for example, consists primarily of the negatively charged DNA, whereas the cytoplasm carries a net positive charge. Thus a positively charged dye, such as hemotoxylin, can be used to stain the nucleus, while a negatively charged dye, such as eosin, will stain the cytoplasm. These two dyes are normally used together; hemotoxylin stains the nucleus blue, while eosin counterstains the cytoplasm pink.

Histochemistry

Fixed tissues can be stained for specific cellular compounds such as DNA, RNA, lipids, and specific proteins. Of these procedures, the most important is the Feulgen reaction, which is used for detecting DNA. The Feulgen reaction involves hydrolyzing DNA nucleotides in a strong acid solution to expose aldehyde groups. The aldehydes, in turn, react with a special dye solution, called Schiff's reagent, resulting in a deep purple color. The Feulgen reaction is specific for DNA and does not stain other molecules in the nucleus or the cytoplasm. This method has been used to determine the amount of DNA in individual cell nuclei and to assess the degree of chromatin condensation.

BIOTECHNOLOGY

Biotechnology (also known as recombinant DNA technology) consists of several procedures that are used to study the structure and function of genes and their products. Central to this technology is the ability to clone specific pieces of DNA and to construct libraries of these DNA fragments that represent the genetic repertoire of an entire organism or a specific cell type. With these libraries at hand, scientists have been able to study the cell and whole organisms in unprecedented detail. The information so gained has revolutionized biology as well as many other disciplines, including medical science, pharmacology, psychiatry, and anthropology, to name but a few.

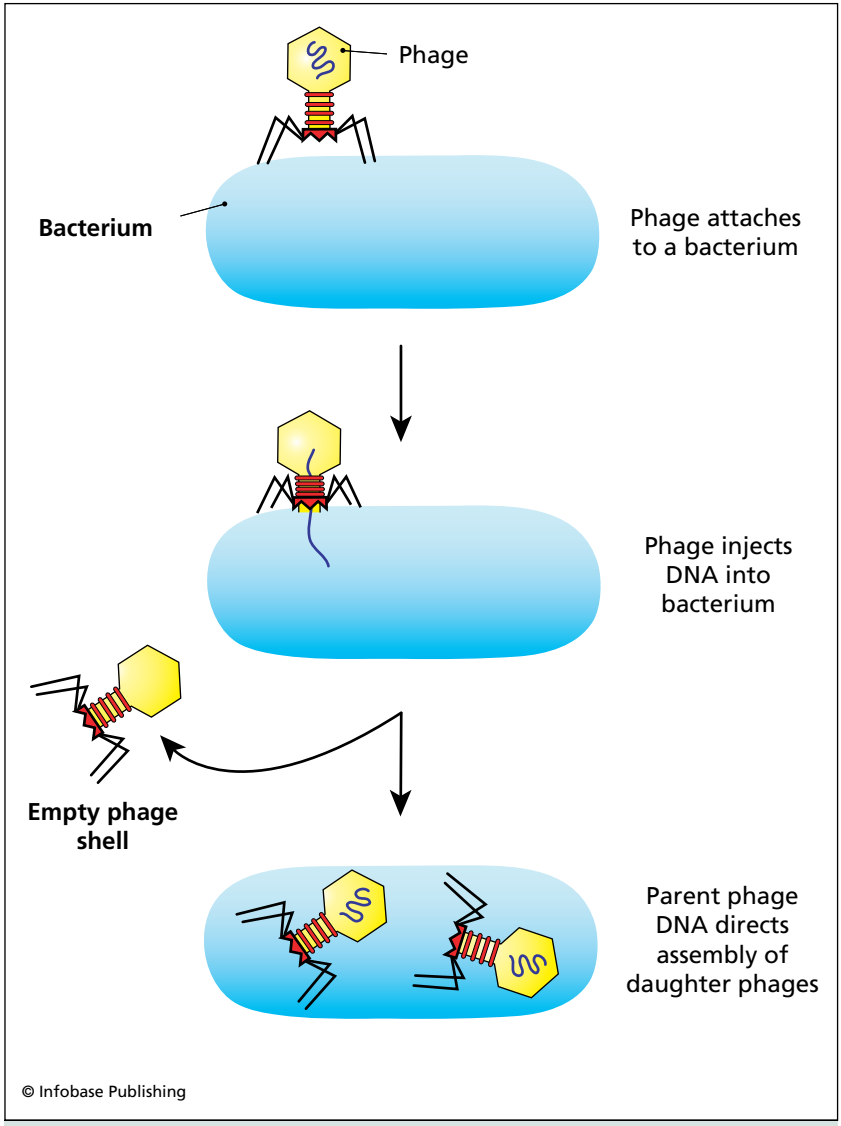
Historical Background

The historical roots of biotechnology can be traced back to the late 1800s. At that time, biologists were on a roll: Equipped with excellent, high-resolution compound microscopes and a few histochemical techniques, they were having tremendous success with the identification and treatment of many diseases that were caused by bacteria. Robert Koch, a German country doctor, proved that

anthrax, a fatal disease of cattle and humans, is caused by a bacterium, which he named *Bacillus anthracis*. Shortly after Koch's discovery, the great French chemist Louis Pasteur developed a vaccine, the first ever produced to treat anthrax. He quickly followed it up with another vaccine to treat rabies, a disease that was very common in Europe at the time. Paul Ehrlich, who worked in Koch's laboratory, produced a dramatically effective drug, which he called a magic bullet, to treat syphilis. The combined efforts of Koch, Pasteur, and Ehrlich led to treatments for tuberculosis, diphtheria, typhoid fever, and cholera. The knowledge they gained and the techniques they developed transformed the field of medicine from a confused, superstitious muddle into a highly efficient discipline for fighting and controlling infectious diseases.

But knowing how to identify bacteria and how to control them when they become infectious are still a long way from understanding how cells do what they do. Basic questions about the cell's genes, molecules, or metabolic pathways drew a complete blank. Not until the 1930s did biochemists begin unraveling some of the details of cellular behavior but knew nothing about the genes that encoded the enzymes they were discovering. They could not even agree on which type of molecule, DNA or protein, was the genetic material. Many scientists believed such questions could never be answered and that our knowledge of the cell would always remain superficial. By 1940, the euphoria of the late 1800s had given way to disappointment, confusion, and a sense of defeat.

The outlook began to brighten in 1952 when Martha Chase and Alfred Hershey proved that DNA, and not protein, is the genetic material of a cell. Their experiment depended on the fact that bacteria, like people, are subject to viral infections. A virus that infects a bacterium is called bacteriophage, or phage for short. Using the newly developed electron microscopes, other scientists had been able to observe a phage attaching to a bacterial cell, after

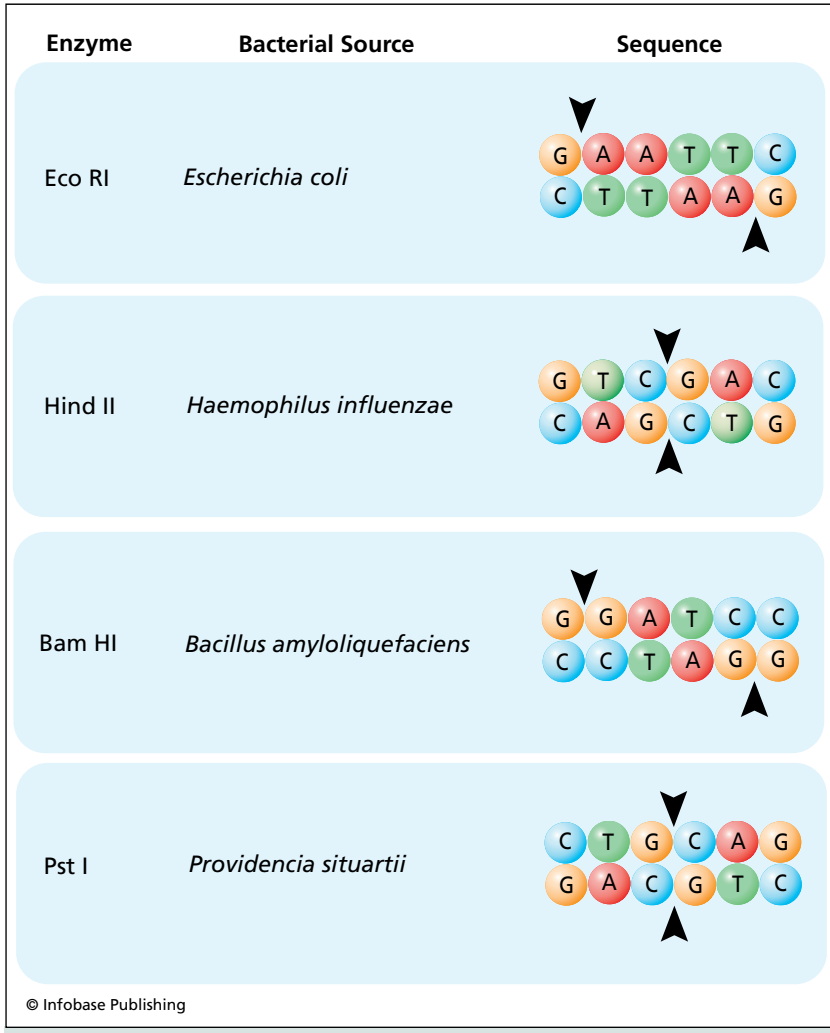


Identifying DNA as genetic material. By labeling the phages' DNA with an isotope of phosphorus and its protein with an isotope of sulfur, Martha Chase and Alfred Hershey were able to show that the phage always injects DNA into the host bacterium.

which the virus, acting like a tiny syringe, injected a long molecule into the bacterium. Within a few hours, phage particles could be seen forming inside the bacteria, after which the cell lysed, or burst open, releasing the newly made daughter phage to infect other bacteria. What did the parental phage inject into the bacteria: protein, DNA, or both? This is the question everyone wanted to answer. In a beautifully elegant experiment, Chase and Hershey showed that the phage always injects DNA into the bacterium, not protein.

With the identity of the cell's genetic material firmly established, many scientists turned their attention to learning more about DNA, and in 1954 James Watson and Francis Crick published a model for the structure of this macromolecule. DNA was shown to be a double helix, consisting of a linear sequence of four different nucleotides encoding the genetic information. In their paper, published in the journal *Nature,* Watson and Crick pointed out that the process of complementary nucleotide pairing provides a simple mechanism for gene duplication prior to cell division. Within 10 years, other scientists worked out the complete genetic code used by all living cells.

Understanding the molecular nature of the gene reinvigorated the biology community, giving biologists renewed hope that they could answer some of the following questions: How many genes does a prokaryote or a eukaryote have and how are they controlled? Which genes are necessary for the day-to-day running of the cell (the housekeeping genes) and which are needed for embryonic development? Is there a gene for intelligence the way there are genes for eye and hair color? Do cells become cancerous because a gene is not functioning properly, and, if so, which one is responsible? The questions are endless, and, despite the renewed enthusiasm, most biologists realized that it would take a new technology to provide the answers, a technology capable of isolating and amplifying specific genes so their sequence could be determined and their behavior studied in detail.



Restriction enzymes. These enzymes cut DNA at specific sites, as indicated by the arrows, and are named after the bacteria from which they were isolated.

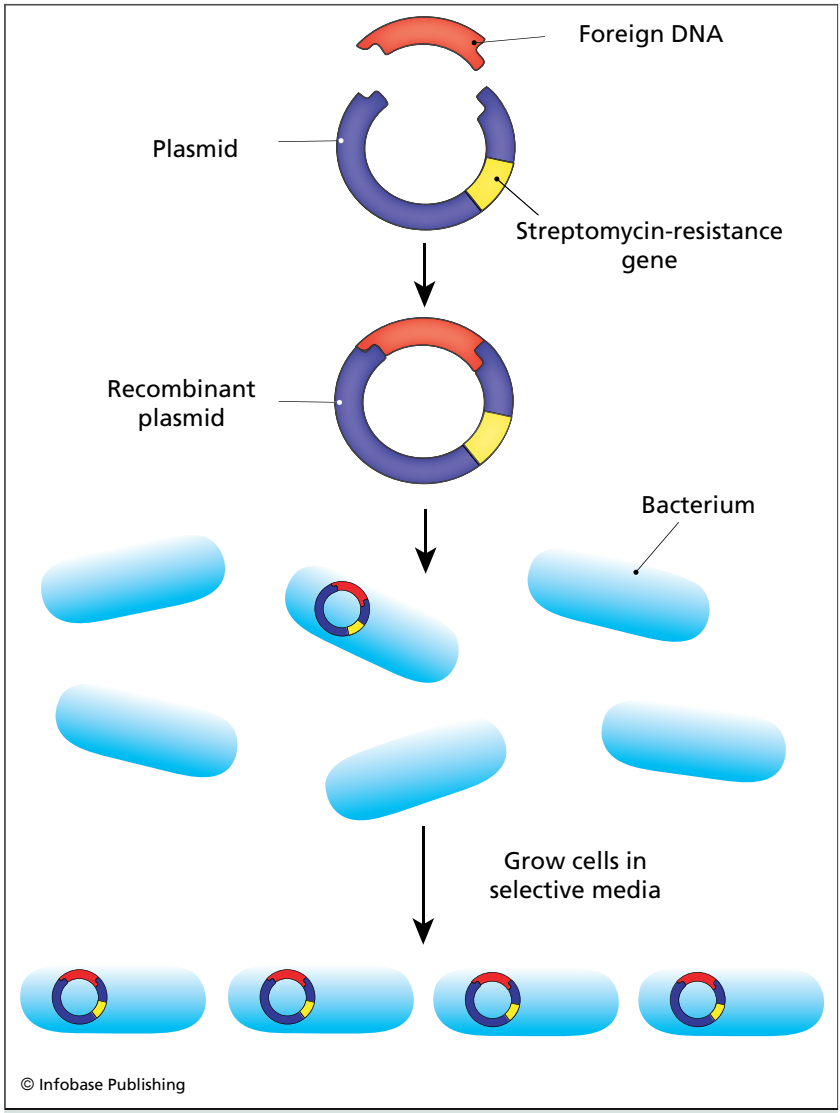
But throughout the 1960s there was no way to study a gene in detail. DNA encoded the genes in a sequence of nucleotides, but there was no way to determine the exact sequence, nor was it

possible to study a gene's activity or expression profile; that is, when it is turned on or off. This began to change in the 1970s with the isolation and purification of protein enzymes that could cut DNA at specific sites and other enzymes that could join two DNA fragments together. The first such protein to be discovered was DNA ligase, an enzyme that can join two pieces of DNA together and was later shown to be part of the cell's DNA replication and repair machinery. Other DNA-modifying enzymes, called restriction enzymes, cut DNA at sequence-specific sites, with different members of the family cutting at different sites. Restriction enzymes are isolated from bacteria, which use them to destroy or restrict the foreign DNA molecules they sometimes absorb from their environment. Since the discovery of restriction enzymes in 1970, more than 90 such enzymes have been isolated from more than 230 bacterial strains. The discovery of these enzymes led directly to the first DNA cloning experiments.

DNA Cloning

In 1973, scientists discovered that restriction enzymes, DNA ligase, and bacterial plasmids could be used to clone DNA molecules. Plasmids are small (about 3,000 base pairs) circular minichromosomes that occur naturally in bacteria and are often exchanged between cells by passive diffusion. A bacterium is said to be transfected when it acquires a new plasmid. For bacteria, the main advantage to swapping plasmids is that they often carry antibiotic resistance genes (a marker), so that a cell sensitive to ampicillin can become resistant simply by acquiring the right plasmid. For scientists, plasmid swapping provided an ideal method for amplifying or cloning a specific piece of DNA.

The first cloning experiment used a plasmid from the bacterium *Escherichia coli* that was cut with the restriction enzyme *Eco*RI. The plasmid had a single *Eco*RI site so the restriction enzyme simply opened the circular molecule. Foreign DNA, cut with the same



Cloning DNA in a plasmid. The foreign DNA and the plasmid are cut with the same restriction enzyme, allowed to fuse, and then sealed with DNA ligase. The recombinant plasmid is mixed with bacterial cells, some of which pick up the plasmid, allowing them to grow in a culture medium containing streptomycin. The bacteria's main chromosome is not shown.

restriction enzyme, was incubated with the plasmid. Because the plasmid and foreign DNA were both cut with *Eco*RI, the DNA could insert itself into the plasmid to form a hybrid, or recombinant plasmid, after which DNA ligase sealed the two together. The reaction mixture was added to a small volume of *E. coli* so that some of the cells could take up the recombinant plasmid before being transferred to a nutrient broth containing streptomycin. Only those cells carrying the recombinant plasmid, which contained an anti-streptomycin gene, could grow in the presence of this antibiotic. Each time the cells divided, the plasmid DNA was duplicated along with the main chromosome. After the cells had grown overnight, the foreign DNA had been amplified billions of times and was easily isolated for sequencing or expression studies. In this procedure, the plasmid is known as a cloning vector because it serves to transfer the foreign DNA into a cell.

DNA Libraries

The basic cloning procedure described above not only provides a way to amplify a specific piece of DNA but can also be used to construct DNA libraries. In this case, however, the cloning vector is a bacteriophage called lambda. The lambda genome is double-stranded DNA of about 40,000 base pairs (bp), much of which can be replaced by foreign DNA without sacrificing the ability of the virus to infect bacteria. This is the great advantage of lambda over a plasmid. Lambda can accommodate very long pieces of DNA, often long enough to contain an entire gene, whereas a plasmid cannot accommodate foreign DNA that is larger than 2,000 base pairs. Moreover, a bacteriophage has the natural ability to infect bacteria, so that the efficiency of transfection is 100 times greater than it is for plasmids.

The construction of a DNA library begins with the isolation of genomic DNA and its digestion with a restriction enzyme to produce fragments of 1,000 to 10,000 bp. These fragments are ligated

into lambda genomes, which are subjected to a packaging reaction to produce mature viral particles, most of which carry a different piece of the genomic DNA. This collection of viruses is called a genomic library and is used to study the structure and organization of specific genes. Clones from a library such as this contain the coding sequences, in addition to noncoding sequences such as introns, intervening sequences, promoters, and enhancers. An alternative form of a DNA library can be constructed by isolating messenger RNA (mRNA) from a specific cell type. This RNA is converted to the complimentary DNA (cDNA) using an RNA-dependent DNA polymerase called reverse transcriptase. The cDNA is ligated to lambda genomes and packaged as for the genomic library. This collection of recombinant viruses is known as a cDNA library and contains genes that were being expressed by the cells when the mRNA was extracted. It does not include introns or controlling elements as these are lost during transcription and the processing that occurs in the cell to make mature mRNA. Thus, a cDNA library is intended for the purpose of studying gene expression and the structure of the coding region only.

Labeling Cloned DNA

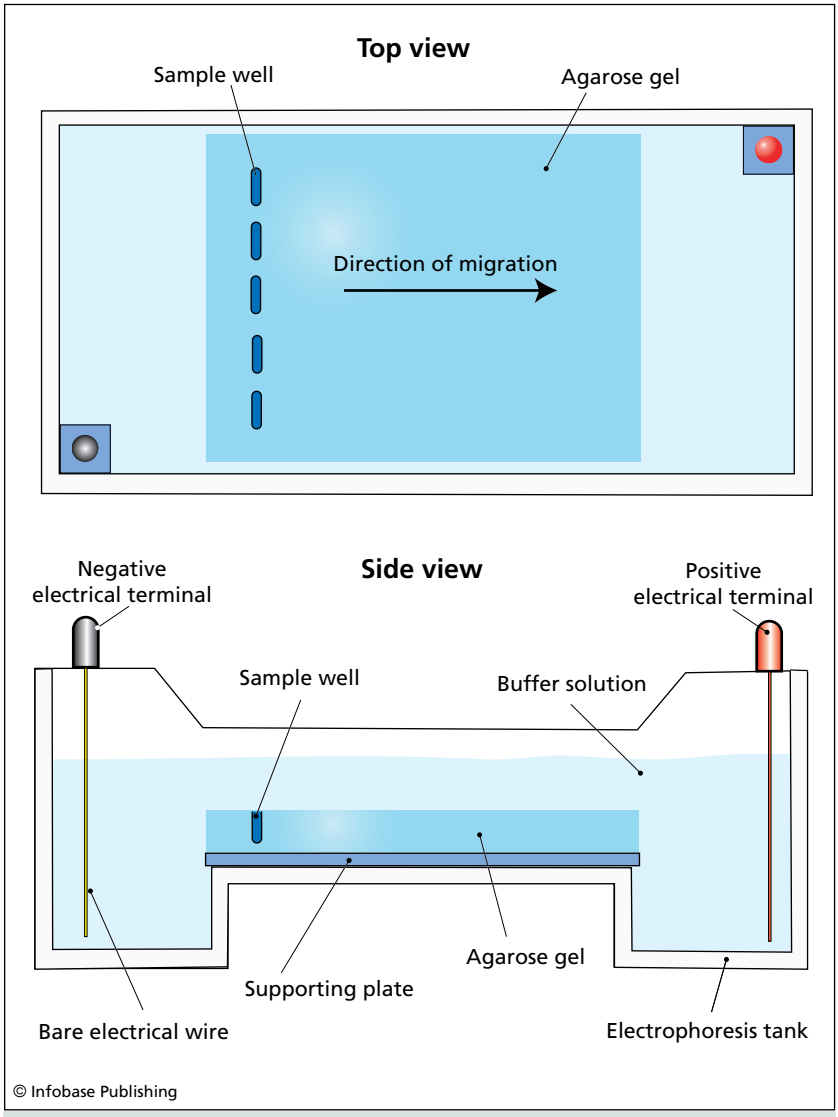
Many of the procedures used in biotechnology were inspired by the events that occur during DNA replication (described in chapter 4). This includes DNA sequencing, PCR (described below), and the labeling of cloned DNA for use as probes in expression studies. DNA replication involves duplicating one of the strands (the parent, or template strand) by linking nucleotides in an order specified by the template and depends on a large number of enzymes, the most important of which is DNA polymerase. This enzyme, guided by the template strand, constructs a daughter strand by linking nucleotides together. One such nucleotide is deoxyadenine triphosphate (dATP). Deoxyribonucleotides have a single hydroxyl group located at the 3′ carbon of the sugar group

while the triphosphate is attached to the 5′ carbon. The procedure for labeling DNA probes, developed in 1983, introduces radioactive nucleotides into a DNA molecule. This method supplies DNA polymerase with a single-stranded DNA template, a primer, and the four nucleotides, in a buffered solution to induce *in vitro* replication. The daughter strand, which becomes the labeled probe, is made radioactive by including a ^{32}P-labeled nucleotide in the reaction mix. The radioactive nucleotide is usually deoxy-cytosine triphosphate (dCTP) or dATP. The ^{32}P is always part of the α (alpha) phosphate (the phosphate closest to the 5′ carbon), as this is the one used by the polymerase to form the phosphodiester bond between nucleotides. Nucleotides can also be labeled with a fluorescent dye molecule.

Single-stranded DNA hexamers (six bases long) are used as primers, and these are produced in such a way that they contain all possible permutations of four bases taken six at a time. Randomizing the base sequence for the primers in this way ensures that there will be at least one primer site in a template that is only 50 bp long. Templates used in labeling reactions such as this are generally 100–800 bp long. This strategy of labeling DNA is known as random primer labeling.

Gel Electrophoresis

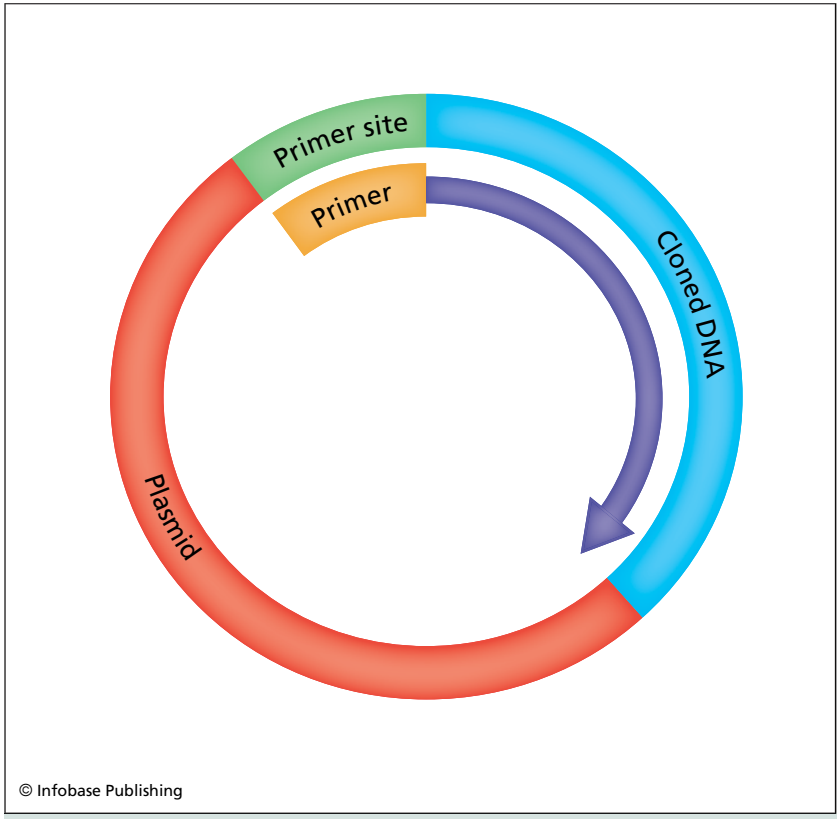
This procedure is used to separate DNA and RNA fragments by size in a slab of agarose (highly refined agar) or polyacrylamide subjected to an electric field. Nucleic acids carry a negative charge and thus will migrate toward a positively charged electrode. The gel acts as a sieving medium that impedes the movement of the molecules. Thus, the rate at which the fragments migrate is a function of their size; small fragments migrate more rapidly than large fragments. The gel, containing the samples, is run submerged in a special pH-regulated solution, or buffer. Agarose gels are run in a horizontal tank as shown in the figure: DNA sequencing gels, made of polyacrylamide, are much bigger and are run in a vertical tank.



Agarose gel electrophoresis. An agarose gel is placed in an electrophoresis tank, and submerged in a buffer solution. The electrical terminals are connected to power source, with the sample wells positioned near the negative terminal. When the current is turned on, the negatively charged nucleic acids migrate toward the positive terminal. The migration rate is an inverse function of molecular size (large molecules travel slower than small ones).

DNA Sequencing

A sequencing reaction developed by the British biochemist Dr. Fred Sanger in 1976 is a technique that takes its inspiration from the natural process of DNA replication. DNA polymerase requires a primer with a free 3′ hydroxyl group. The polymerase adds the first nucleotide to this group, and all subsequent bases are added to the 3′ hydroxyl of the previous base. Sequencing by the Sanger method

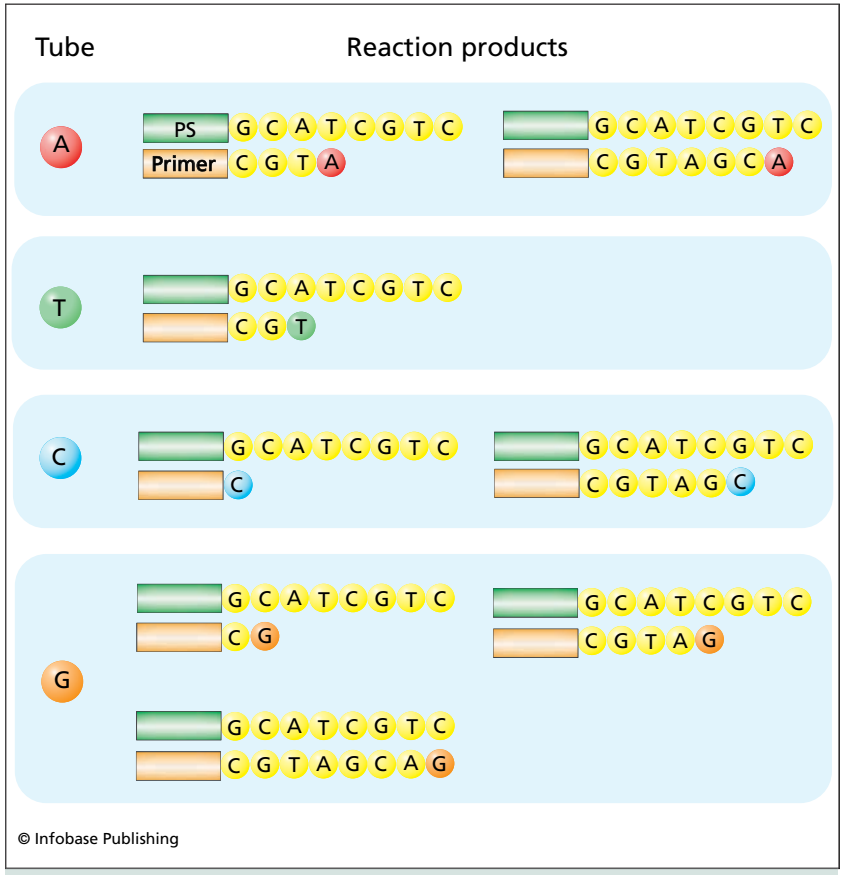


Plasmid primer site for DNA sequencing. The cloned DNA is inserted into the plasmid near an engineered primer site. Once the primer binds to the primer site, the cloned DNA may be replicated, as part of a sequencing reaction, in the direction indicated by the arrow. Only one strand of the double-stranded plasmid and cloned DNA is shown.

is usually performed with the DNA cloned into a special sequencing plasmid. This simplifies the choice of the primers since their sequence can be derived from the known plasmid sequence.

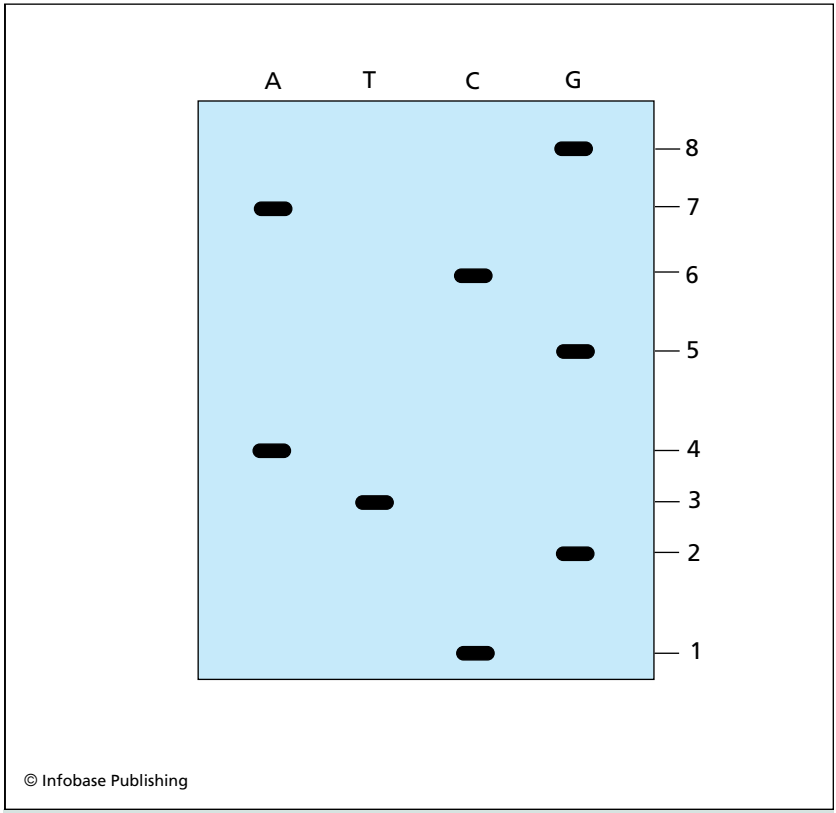
Once the primer binds to the primer site, the cloned DNA may be replicated. Sanger's method involves special nucleotide analogues lacking the 3′ hydroxyl group that are added to the reaction mix. These analogues, also known as dideoxynucleotides (ddATP, ddCTP, ddGTP, and ddTTP), terminate the growth of the daughter strand at the point of insertion. This can be used to determine the distance between each base on the daughter strand and the primer. These distances can be visualized by separating the Sanger reaction products on a polyacrylamide gel and then exposing the gel to X-ray film to produce an autoradiograph. The DNA sequence is read directly from this film beginning with the smallest fragment at the bottom of the gel (the nucleotide closest to the primer) and ending with the largest fragment at the top. A hypothetical sequencing reaction and subsequent autoradiograph are shown in the figures on pages 224 and 225. An autoradiograph of an actual sequencing gel is shown in the figure on page 226.

Sanger sequencing, as described above, is very accurate, but slow and costly. Refinements to the procedure were introduced in 2000 to meet the needs of the Human Genome Project. Semi-automated sequencing machines were built that were able to sequence DNA at the rate of about 67,000 bases per hour. This is roughly a 100-fold increase in speed over manual sequencing. Automated sequencers still rely on the Sanger reaction but with the following improvements: The dideoxynucleotides are color-coded by labeling them with four different fluorescent dyes (red, blue, green, and yellow) rather than a single monochromatic radioisotope. The sequencing reaction is set up in a single tube and is loaded into a single lane of a polyacrylamide gel. As the sample migrates through the gel, a detector in the machine records the passage of each nucleotide and identifies it by its fluorescent color. A computer records this information; thus the sequence of the template is determined by the time the gel has finished



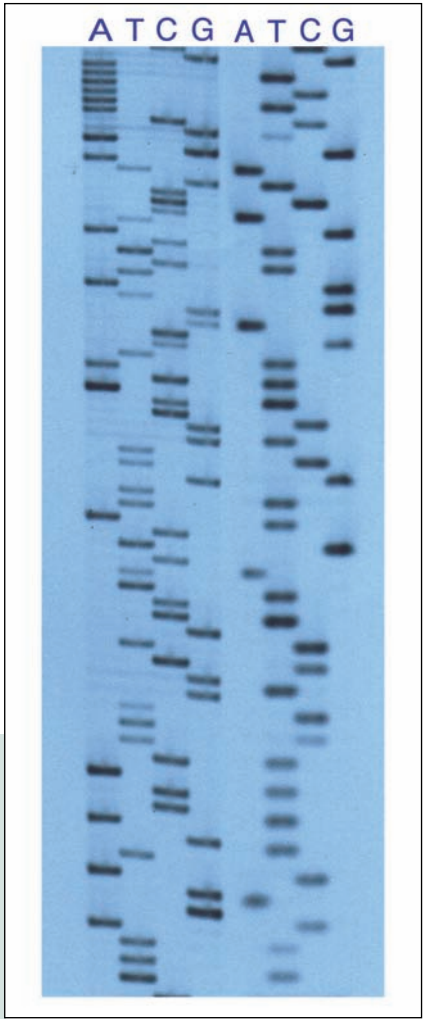
Example of a sequencing reaction. The reaction products are shown for each tube containing ddATP, ddTTP, ddCTP, and ddGTP (color coded as A, T, C, and G). The template, continuous with the primer site (PS) on the plasmid, is GCATCGTC. Replication of the template begins after the primer binds to the primer site. Note that the primer becomes part of the daughter strand. Incorporation of the di-deoxy nucleotide terminates the growth of the daughter strand.

running. Consequently, there is no need to expose the finished gel to X-ray film (which can take two days) or to spend many tedious hours reading the sequence manually from the film.



Hypothetical sequencing autoradiogram. The reaction products (shown in the figure on page 224) run from the top to the bottom, with the smallest fragment migrating at the highest rate. The sequence is read beginning with the smallest fragment on the gel (band # 1, in the "C" lane) and ending with the largest fragment at the top (band # 8, in the "G" lane). The sequence is CGTAGCAG. The complementary sequence is GCATCGTC. This is the template strand indicated in the figure on page 224.

Even with Sanger sequencing machines, it took hundreds of scientists nearly five years to sequence the 3 billion bases in the human genome, at an estimated cost of $800 million. Recently, scientists at 454 Life Sciences, a biotechnology company, have produced a system that they claim can sequence DNA at the astonishing rate



An autoradiogram of a portion of a DNA sequencing gel. A partial sequence (the first 20 bases) of the left set, beginning at the bottom of the "T" lane, is TTTAGGATGACCACTTTGGC. *(courtesy of the author)*

of 5 million bases per hour. At this rate, the human genome could be sequenced in just a few months at a cost of about $1 million. This sequencer does not use the Sanger reaction nor is it necessary to clone the template into a sequencing plasmid (great time-savers in themselves). The template is produced by digesting genomic DNA into small 300 to 800 base pair pieces, each of which are attached to tiny beads and amplified using the polymerase chain reaction (PCR) described below. The beads, now containing many copies of a single

template, are placed into microscopic wells on a fiber-optic plate. Each well is supplied with DNA polymerase, sequencing primer, and a buffering solution suitable for replication. The four nucleotides are then added to the system sequentially. The chemistry of the reaction solutions is such that a beam of light is released whenever the polymerase incorporates a nucleotide into the daughter strand. The machine, being equipped with a sensitive photometer, detects the light and a computer attached to the sequencer records the position of the well, thus keeping track of the sequence for each daughter strand. After all four of the nucleotides have passed through the system several times, the computer will have recorded a 100 base sequence for every template on the plate.

A single run of this machine takes about four hours to read the sequences in 200,000 wells. Thus the total sequence obtained in a single run is 20 million bases, or the 5 million bases per hour cited above. However, post-run software analysis of the data, which includes elimination of overlapping sequences, can reduce the total by several thousand bases. A major drawback to the 454 system is the fact that the computer must assemble the complete sequence from 200,000 templates, which limits the size of the genome that can be sequenced to 50 million bases.

Subsequent work has overcome this limitation, and in the spring of 2008 the 454 system was used to sequence the genome of Dr. James Watson in just four months at a cost of $1.5 million. With future refinements, it may be possible to sequence the entire human genome in one month for less than $10,000 (the stated target is $1,000). When this is accomplished, physicians will be able to call for patient-specific genome sequencing, which could have a dramatic impact on medical diagnostics and therapies.

Gene Expression

The production of a genomic or cDNA library, followed by the sequencing of isolated clones, is a very powerful method for characterizing genes and the genomes from which they came. In addition,

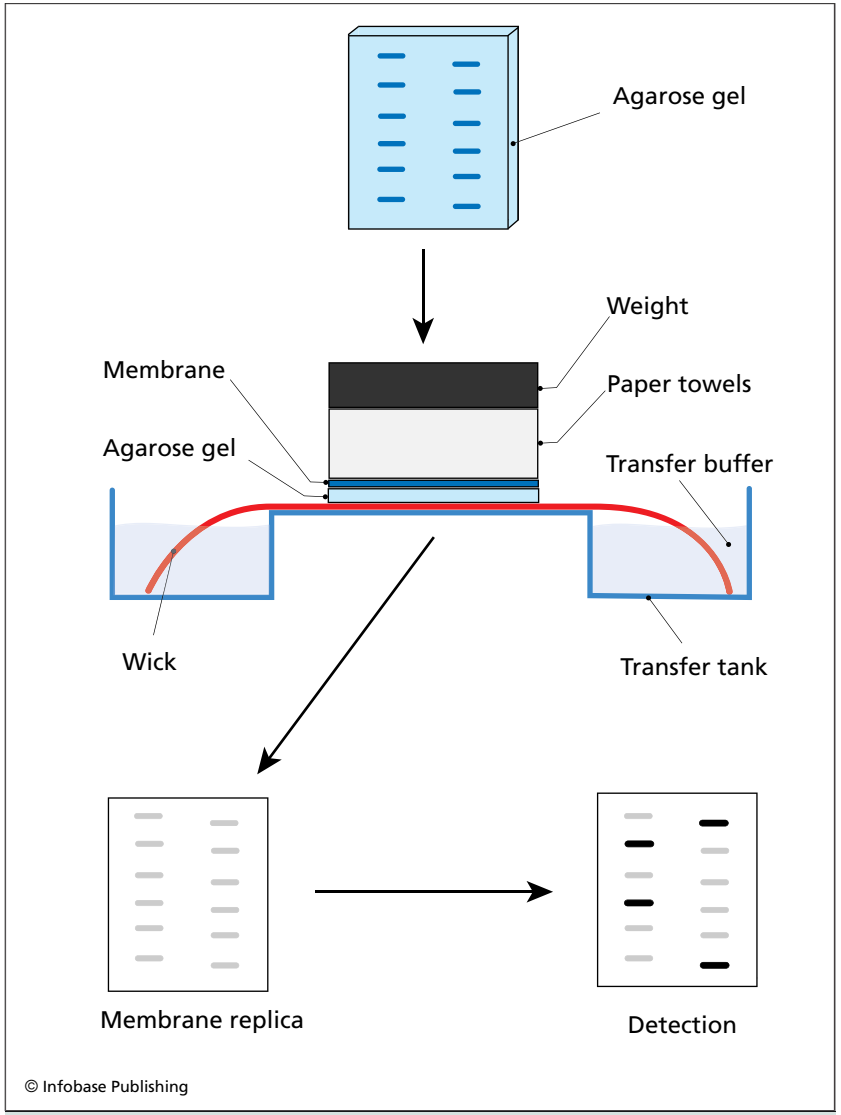
these libraries provide a way to determine the expression profile for a gene, that is, to determine which cells express the gene and exactly when the gene is turned on and off. Typical experiments may wish to determine the expression of specific genes in normal versus cancerous tissue or tissues obtained from groups of different ages. There are essentially three methods for doing this: RNA blotting, fluorescent in situ hybridization (FISH), and the polymerase chain reaction.

RNA Blotting

This procedure consists of the following steps:

1. Extract mRNA from the cells or tissue of interest.
2. Fractionate (separate by size) the mRNA sample using gel electrophoresis.
3. Transfer the fractionated sample to a nylon membrane (the blotting step).
4. Incubate the membrane with a gene fragment (usually a cDNA clone) that has been labeled with a radioisotope.
5. Expose the membrane to X-ray film to visualize the signal.

The RNA is transferred from the gel to a nylon membrane using a vacuum apparatus or a simple dish containing a transfer buffer topped by a large stack of ordinary paper towels and a weight. The paper towels pull the transfer buffer through the gel, eluting the RNA from the gel and trapping it on the membrane. The location of specific mRNAs can be determined by hybridizing the membrane to a radiolabeled cDNA or genomic clone. The hybridization procedure involves placing the membrane in a buffer solution containing a labeled probe. During a long incubation period, the probe binds to the target sequence immobilized on the membrane. A-T and G-C base pairing (also known as hybridization) mediate the binding between the probe and target. The double-stranded molecule thus formed is a hybrid between the RNA target, on the membrane, and



RNA transfer and membrane hybridization. RNA is fractionated on an agarose gel and then placed facedown on a paper wick in a transfer tank. The gel is overlain with a piece of nylon membrane, paper towels, and weight. The paper towels draw the buffer through the gel and the membrane. The flow of buffer elutes the RNA from the gel, transferring it to the membrane. A radiolabeled cDNA probe is hybridized to the membrane to detect specific mRNA transcripts. Note that the thickness of the membrane is exaggerated for clarity.

the DNA probe (thus the phrase "hybridizing the membrane"). The radiolabeled signals are visualized by exposing the membrane to X-ray film.

Fluorescent In Situ Hybridization

Studying gene expression does not always depend on RNA blots and membrane hybridization. In the 1980s, scientists found that cDNA probes could be hybridized to DNA or RNA in situ, that is, while located within cells or tissue sections fixed on a microscope slide. In this case, the probe is labeled with a fluorescent dye molecule rather than a radioactive isotope. The samples are then examined and photographed under a fluorescent microscope. FISH is an extremely powerful variation on RNA blotting. This procedure gives precise information regarding the identity of a cell that expresses a specific gene, information that usually cannot be obtained with membrane hybridization. Organs and tissues are generally composed of many different kinds of cells, which cannot be separated from each other using standard biochemical extraction procedures. Histological sections, however, show clearly the various cell types and, when subjected to FISH analysis, provide clear information as to which cells express specific genes. FISH is also used in clinical laboratories for the diagnosis of genetic abnormalities.

Polymerase Chain Reaction

PCR is simply repetitive DNA replication over a limited, primer-defined region of a suitable template. It provides a way of amplifying a short segment of DNA without going through the cloning procedures described above. The region defined by the primers is amplified to such an extent that it can be easily isolated for further study. The reaction exploits the fact that a DNA duplex, in a low-salt buffer, will melt (i.e., separate into two single strands) at 167 °F (75 °C), but will re-anneal (rehybridize) at 98.6°F (37°C).

The reaction is initiated by melting the template, in the presence of primers and polymerase in a suitable buffer, cooling quickly to 98.6°F (37°C), and allowing sufficient time for the polymerase to replicate both strands of the template. The temperature is then increased to 167°F (75°C) to melt the newly formed duplexes and after cooled to 98.6°F (37°C). At the lower temperature, more primer will anneal to initiate another round of replication. The heating-cooling cycle is repeated 20 to 30 times, after which the reaction products are fractionated on an agarose gel, and the region containing the amplified fragment is cut out of the gel and purified for further study. The DNA polymerase used in these reactions is isolated from thermophilic bacteria that can withstand temperatures of 158°F (70°C) to 176°F (80°C). PCR applications are nearly limitless. It is used to amplify DNA from samples containing, at times, no more than a few cells. It is being used in the development of ultrafast DNA sequencers (described above), identification of tissue samples in criminal investigations, amplification of ancient DNA obtained from fossils, and the identification of genes that are turned on or off during embryonic development, or during cellular transformation (cancer formation).

GENE AND PROTEIN NOMENCLATURE

Scientists who were, in effect, probing around in the dark have discovered many genes and their encoded proteins. Once discovered, the new genes or proteins had to be named. Usually the "name" is nothing more than a lab-book code or an acronym suggested by the system under study at the time. Sometimes it turns out, after further study, that the function observed in the original study is a minor aspect of the gene's role in the cell. It is for this reason that gene and protein names sometimes seem absurd and poorly chosen.

The International Committee on Standardized Genetic Nomenclature agreed to unify the rules and guidelines for gene and protein names for the mouse and rat. Similar committees have attempted to

standardize gene-naming conventions for human, frog, zebrafish, and yeast genes. In general, the gene name is expected to be brief and to begin with a lower case letter unless it is a person's name. The gene symbols are acronyms taken from the gene name and are expected to be three to five characters long and not more than 10. The symbols must be written with Roman letters and Arabic numbers. The same symbol is used for orthologs (i.e., the same gene) among different species, such as human, mouse, or rat. Thus, the gene sonic hedgehog is symbolized as shh and the gene myelocytomatosis is symbolized as myc.

Unfortunately, the various committees were unable to agree on a common presentation for the gene and protein symbols. A human gene symbol, for example, is italicized, uppercase letters and the protein is uppercase and not italicized. A frog gene symbol is lower case and the protein is uppercase, while neither is italicized. Thus the myc gene and its protein, for example, are written as *MYC* and MYC in humans, myc and MYC in frogs, and *Myc* and Myc in mice and rats. The later convention, *Myc* and Myc, is used throughout the New Biology set, regardless of the species.

WEIGHTS AND MEASURES

The following table presents some common weights, measures, and conversions that appear in this book.

QUANTITY	EQUIVALENT
Length	1 meter (m) = 100 centimeters (cm) = 1.094 yards = 39.37 inches 1 kilometer (km) = 1,000 m = 0.62 miles 1 foot = 30.48 cm 1 inch = 1/12 foot = 2.54 cm 1 cm = 0.394 inch = 10^{-2} (or 0.01) m 1 millimeter (mm) = 10^{-3} m 1 micrometer (μm) = 10^{-6} m 1 nanometer (nm) = 10^{-9} m 1 Ångström (Å) = 10^{-10} m

QUANTITY	EQUIVALENT
Mass	1 gram (g) = 0.0035 ounce 1 pound = 16 ounces = 453.6 grams 1 kilogram (kg) = 2.2 pounds (lb) 1 milligram (mg) = 10^{-3} g 1 microgram (µg) = 10^{-6} g
Volume	1 liter (l) = 1.06 quarts (US) = 0.264 gallon (US) 1 quart (US) = 32 fluid ounces = 0.95 liter 1 milliliter (ml) = 10^{-3} liter = 1 cubic centimeter (cc)
Temperature	°C = 5/9 (°F - 32) °F = (9/5 × °C) + 32
Energy	Calorie = the amount of heat needed to raise the temperature of 1 gram of water by 1°C. Kilocalorie = 1,000 calories. Used to describe the energy content of foods.

Glossary

acetyl A chemical group derived from acetic acid. Important in energy metabolism and for the modification of proteins.

acetyl-CoA A water-soluble molecule, coenzyme A (CoA) that carries acetyl groups in cells.

acetylcholine A neurotransmitter released at axonal terminals by cholinergic neurons. Found in the central and peripheral nervous system, and is released at the vertebrate neuromuscular junction.

acid A substance that releases protons when dissolved in water. Carries a net negative charge.

actin filament A protein filament formed by the polymerization of globular actin molecules. Forms the cytoskeleton of all eucaryotes and part of the contractile apparatus of skeletal muscle.

action potential A self-propagating electrical impulse that occurs in the membranes of neurons, muscles, photoreceptors, and hair cells of the inner ear.

active transport Movement of molecules across the cell membrane, utilizing the energy stored in ATP.

adenylate cyclase A membrane-bound enzyme that catalyzes the conversion of ATP to cyclic AMP. An important component of cell signaling pathways.

adherens junction A cell junction in which the cytoplasmic face of the membrane is attached to actin filaments

adipocyte A fat cell.

adrenaline (epinephrine) A hormone released by chromaffin cells in the adrenal gland. Prepares an animal for extreme activity, increases the heart rate and blood sugar levels.

adult stem cells Stem cells isolated from adult tissues, such as bone marrow or epithelium.

aerobic Refers to a process that either requires oxygen or occurs in its presence.

agar A polysaccharide isolated from sea weed that forms a gel when boiled in water and cooled to room temperature. Used by microbiologists as a solid culture medium for the isolation and growth of bacteria and fungi.

agarose A purified form of agar that is used to fractionate (separate by size) biomolecules.

allele An alternate form of a gene. Diploid organisms have two alleles for each gene, located at the same locus (position) on homologous chromosomes.

alpha helix A common folding pattern of proteins in which a linear sequence of amino acids twists into a right-handed helix stabilized by hydrogen bonds.

allogeneic transplant A cell, tissue, or organ transplant from an unrelated individual.

amino acid An organic molecule containing amino and carboxyl groups that is a building block of protein.

aminoacyl-tRNA synthetase An enzyme that attaches the correct amino acid to a tRNA.

amino terminus The end of a protein or polypeptide chain that carries a free amino group.

aminoacyl tRNA An amino acid linked by its carboxyl group to a hydroxyl group on tRNA.

amphipathic Having both hydrophilic and hydrophobic regions, as in a phospholipid.

anabolism A collection of metabolic reactions in a cell whereby large molecules are made from smaller ones.

anaerobic A cellular metabolism that does not depend on molecular oxygen.

anaphase A mitotic stage in which the two sets of chromosomes move away from each other towards opposite spindle poles.

anchoring junction A cell junction that attaches cells to each other.

angiogenesis Sprouting of new blood vessels from preexisting ones.

angstrom A unit of length, equal to 10^{-10} meter or 0.1 nanometer (nM), that is used to measure molecules and atoms.

anterior A position close to or at the head end of the body.

antibiotic A substance made by bacteria, fungi, and plants that is toxic to microorganisms. Common examples are penicillin and streptomycin.

antibody A protein made by B cells of the immune system in response to invading microbes.

anticodon A sequence of three nucleotides in tRNA that is complementary to a messenger RNA codon.

antigen A molecule that stimulates an immune response, leading to the formation of antibodies.

antigen-presenting cell A cell of the immune system, such as a monocyte, that presents pieces of an invading microbe (the antigen) to lymphocytes.

antiparallel The relative orientation of the two strands in a DNA double helix; the polarity of one strand is oriented in the opposite direction to the other.

antiporter A membrane carrier protein that transports two different molecules across a membrane in opposite directions.

apoptosis Regulated or programmed form of cell death that may be activated by the cell itself or by the immune system to force cells to commit suicide when they become infected with a virus or bacterium.

archaea The archaea are prokaryotes that are physically similar to bacteria (both lack a nucleus and internal organelles), but they have retained a primitive biochemistry and physiology that would have been commonplace 2 billion years ago.

asexual reproduction The process of forming new individuals without gametes or the fertilization of an egg by a sperm. Individuals produced this way are identical to the parent and referred to as a clone.

aster The star-shaped arrangement of microtubules that is characteristic of a mitotic or meiotic spindle.

ATP (adenosine triphosphate) A nucleoside consisting of adenine, ribose, and three phosphate groups that is the main carrier of chemical energy in the cell.

ATP synthase A protein located in the inner membrane of the mitochondrion that catalyzes the formation of ATP from ADP and inorganic phosphate using the energy supplied by the electron transport chain.

ATPase Any enzyme that catalyzes a biochemical reaction by extracting the necessary energy from ATP.

autogeneic transplant A patient receives a transplant of his or her own tissue.

autologous Refers to tissues or cells derived from the patient's own body.

autosome Any chromosome other than a sex chromosome.

autoradiograph (autoradiogram) X-ray film that has been exposed to X-rays or to a source of radioactivity. Used to vissualize internal structures of the body and radioactive signals from sequencing gels and DNA or RNA blots.

axon A long extension of a neuron's cell body that transmits an electrical signal to other neurons.

axonal transport The transport of organelles, such as Golgi vesicles, along an axon to the axonal terminus. Transport also flows from the terminus to the cell body.

B cell (B lymphocyte) A white blood cell that makes antibodies and is part of the adaptive immune response.

bacteria One of the most ancient forms of cellular life (the other is the archaea). Bacteria are procaryotes and some are known to cause disease.

bacterial artificial chromosome (BAC) A cloning vector that accommodates DNA inserts of up to 1 million base pairs.

bacteriophage A virus that infects bacteria. Bacteriophages were used to prove that DNA is the cell's genetic material and are now used as cloning vectors.

base A substance that can accept a proton in solution. The purines and pyrimidines in DNA and RNA are organic bases and are often referred to simply as bases.

base pair Two nucleotides in RNA or DNA that are held together by hydrogen bonds. Adenine bound to thymine or guanine bound to cytosine are examples of base pairs.

benign Tumors that grow to a limited size, and do not spread to other parts of the body.

beta sheet Common structural motif in proteins in which different strands of the protein run alongside each other and are held together by hydrogen bonds.

biopsy The removal of cells or tissues for examination under a microscope. When only a sample of tissue is removed, the procedure is called an incisional biopsy or core biopsy. When an entire lump or suspicious area is removed, the procedure is called an excisional biopsy. When a sample of tissue or fluid is removed with a needle, the procedure is called a needle biopsy or fine-needle aspiration.

biosphere The world of living organisms.

biotechnology A set of procedures that are used to study and manipulate genes and their products.

blastomere A cell formed by the cleavage of a fertilized egg. Blastomeres are the totipotent cells of the early embryo.

blotting A technique for transferring DNA (Southern blotting), RNA (northern blotting), or proteins (western blotting) from an agarose or polyacrylamide gel to a nylon membrane.

BRCA1 (breast cancer gene 1) A gene on chromosome 17 that may be involved in regulating the cell cycle. A person who inherits an altered version of the BRCA1 gene has a higher risk of getting breast, ovarian, or prostate cancer.

BRCA2 (breast cancer gene 2) A gene on chromosome 13 that, when mutated, increases the risk of getting breast, ovarian, or prostate cancer.

budding yeast The common name for the baker's yeast *Saccharomyces cerevisiae,* a popular experimental organism that reproduces by budding off a parental cell.

buffer A pH-regulated solution with a known electrolyte (salt) content. Used in the isolation, manipulation, and storage of biomolecules and medicinal products.

cadherin Belongs to a family of proteins that mediates cell-cell adhesion in animal tissues.

calorie A unit of heat. One calorie is the amount of heat needed to raise the temperature of 1 gram of water by 1°C. Kilocalories (1,000 calories) are used to describe the energy content of foods.

capsid The protein coat of a virus, formed by autoassembly of one or more proteins into a geometrically symmetrical structure.

carbohydrate A general class of compounds that includes sugars, containing carbon, hydrogen, and oxygen.

carboxyl group A carbon atom attached to an oxygen and a hydroxyl group.

carboxyl terminus The end of a protein containing a carboxyl group.

carcinogen A compound or form of radiation that can cause cancer.

carcinogenesis The formation of a cancer.

carcinoma Cancer of the epithelium, representing the majority of human cancers.

cardiac muscle Muscle of the heart. Composed of myocytes that are linked together in a communication network based on free passage of small molecules through gap junctions.

caspase A protease involved in the initiation of apoptosis.

catabolism Enzyme regulated breakdown of large molecules for the extraction of chemical-bond energy. Intermediate products are called catabolites.

catalyst A substance that lowers the activation energy of a reaction.

CD28 Cell-surface protein located in T cell membranes, necessary for the activation of T cells by foreign antigens.

cDNA (complementary DNA) DNA that is synthesized from mRNA, thus containing the complementary sequence. cDNA contains coding sequence, but not the regulatory sequences that are present in the genome. Labeled probes are made from cDNA for the study of gene expression.

cell adhesion molecule (CAM) A cell surface protein that is used to connect cells to each other.

cell body The main part of a cell containing the nucleus, Golgi complex, and endoplasmic reticulum. Used in reference to neurons that have long processes (dendrites and axons) extending some distance from the nucleus and cytoplasmic machinery.

cell coat (see **glycocalyx**)

cell fate The final differentiated state that a pluripotent embryonic cell is expected to attain.

cell-cycle control system A team of regulatory proteins that governs progression through the cell cycle.

cell-division-cycle gene (*cdc* gene) A gene that controls a specific step in the cell cycle.

cell-medicated immune response Activation of specific cells to launch an immune response against an invading microbe.

cell nuclear transfer Animal cloning technique whereby a somatic cell nucleus is transferred to an enucleated oocyte. Synonymous with somatic cell nuclear transfer.

celsius A measure of temperature. This scale is defined such that 0°C is the temperature at which water freezes, and 100°C is the temperature at which water boils.

central nervous system (CNS) That part of a nervous system that analyzes signals from the body and the environment. In animals, the CNS includes the brain and spinal cord.

centriole A cylindrical array of microtubules that is found at the center of a centrosome in animal cells.

centromere A region of a mitotic chromosome that holds sister chromatids together. Microtubules of the spindle fiber connect to an area of the centromere called the kinetochore.

centrosome Organizes the mitotic spindle and the spindle poles. In most animal cells it contains a pair of centrioles.

chiasma (plural chiasmata) An X-shaped connection between homologous chromosomes that occurs during meiosis I, representing a site of crossing-over, or genetic exchange between the two chromosomes.

chromatid A duplicate chromosome that is still connected to the original at the centromere. The identical pair are called sister chromatids.

chromatin A complex of DNA and proteins (histones and nonhistones) that forms each chromosome, and is found in the nucleus of all eucaryotes. Decondensed and thread-like during interphase.

chromatin condensation Compaction of different regions of interphase chromosomes that is mediated by the histones.

chromosome One long molecule of DNA that contains the organism's genes. In procaryotes the chromosome is circular and naked; in eucaryotes it is linear and complexed with histone and nonhistone proteins.

chromosome condensation Compaction of entire chromosomes in preparation for cell division.

clinical breast exam An exam of the breast performed by a physician to check for lumps or other changes.

cnidoblast A stinging cell found in the Cnidarians (jellyfish).

cyclic adenosine monophosphate (cAMP) A second messenger in a cell-signaling pathway that is produced from ATP by the enzyme adenylate cyclase.

cyclin A protein that activates protein kinases (cyclin-dependent protein kinases, or Cdk) that control progression from one stage of the cell cycle to another.

cytochemistry The study of the intracellular distribution of chemicals.

cytochrome Colored, iron-containing protein that is part of the electron transport chain.

cytotoxic T-cell A T-lymphocyte that kills infected body cells.

dendrite An extension of a nerve cell that receives signals from other neurons.

dexrazoxane A drug used to protect the heart from the toxic effects of anthracycline drugs such as doxorubicin. It belongs to the family of drugs called chemoprotective agents.

dideoxynucleotide A nucleotide lacking the 2′ and 3′ hydroxyl groups.

dideoxy sequencing A method for sequencing DNA that employs dideoxyribose nucleotides. Also known as the Sanger sequencing method, after Fred Sanger, a chemist who invented the procedure in 1976.

diploid A genetic term meaning two sets of homologous chromosomes, one set from the mother and the other from the father. Thus, diploid organisms have two versions (alleles) of each gene in the genome.

DNA (deoxyribonucleic acid) A long polymer formed by linking four different kinds of nucleotides together likes beads on a string. The sequence of nucleotides is used to encode an organism's genes.

DNA helicase An enzyme that separates and unwinds the two DNA strands in preparation for replication or transcription.

DNA library A collection of DNA fragments that are cloned into plasmids or viral genomes.

DNA ligase An enzyme that joins two DNA strands together to make a continuous DNA molecule.

DNA microarray A technique for studying the simultaneous expression of a very large number of genes.

DNA polymerase An enzyme that synthesizes DNA using one strand as a template.

DNA primase An enzyme that synthesizes a short strand of RNA that serves as a primer for DNA replication.

dorsal The backside of an animal. Also refers to the upper surface of anatomical structures, such as arms or wings.

dorsalventral The body axis running from the backside to the frontside or the upperside to the underside of a structure.

double helix The three-dimensional structure of DNA in which the two strands twist around each other to form a spiral.

doxorubicin An anticancer drug that belongs to a family of antitumor antibiotics.

Drosophila melanogaster Small species of fly, commonly called a fruit fly that is used as an experimental organism in genetics, embryology, and gerontology.

ductal carcinoma in situ (DCIS) Abnormal cells that involve only the lining of a breast duct. The cells have not spread outside the duct to other tissues in the breast. Also called intraductal carcinoma.

dynein A motor protein that is involved in chromosome movements during cell division.

dysplasia Disordered growth of cells in a tissue or organ, often leading to the development of cancer.

ectoderm An embryonic tissue that is the precursor of the epidermis and the nervous system.

electrochemical gradient A differential concentration of an ion or molecule across the cell membrane that serves as a source of potential energy and may polarize the cell electrically.

electron microscope A microscope that uses electrons to produce a high resolution image of the cell.

electrophoresis The movement of a molecule, such as protein, DNA or RNA, through an electric field. In practice, the molecules migrate through a slab of agarose or polyacrylamide that is immersed in a special solution and subjected to an electric field.

elution To remove one substance from another by washing it out with a buffer or solvent.

embryogenesis The development of an embryo from a fertilized egg.

embryonic stem cell (ES cell) A pluripotent cell derived from the inner cell mass (the cells that give rise to the embryo instead of the placenta) of a mammalian embryo.

endocrine cell A cell that is specialized for the production and release of hormones. Such cells make up hormone-producing tissue such as the pituitary gland or gonads.

endocytosis Cellular uptake of material from the environment by invagination of the cell membrane to form a vesicle called an endosome. The endosome's contents are made available to the cell after it fuses with a lysosome.

endoderm An embryonic tissue layer that gives rise to the gut.

endoplasmic reticulum (ER) Membrane-bounded chambers that are used to modify newly synthesized proteins with the addition of sugar molecules (glycosylation). When finished, the glycosylated proteins are sent to the Golgi apparatus in exocytotic vesicles.

enhancer A DNA regulatory sequence that provides a binding site for transcription factors capable of increasing the rate of transcription for a specific gene. Often located thousands of base pairs away from the gene it regulates.

enveloped virus A virus, containing a capsid that is surrounded by a lipid bilayer originally obtained from the membrane of a previously infected cell.

enzyme A protein or RNA that catalyzes a specific chemical reaction.

epidermis The epithelial layer, or skin, that covers the outer surface of the body.

ER marker sequence The amino terminal sequence that directs proteins to enter the endoplasmic reticulum (ER). This sequence is removed once the protein enters the ER.

erythrocyte A red blood cell that contains the oxygen-carrying pigment hemoglobin, used to deliver oxygen to cells in the body.

***Escherichia coli* (*E. coli*)** Rod shape, gram negative, bacterium that inhabits the intestinal tract of most animals and is used as an experimental organism by geneticists and biomedical researchers.

eukaryote (eucaryote) A cell containing a nucleus and many membrane-bounded organelles. All lifeforms, except bacteria and viruses, are composed of eucaryote cells.

euchromatin Lightly staining portion of interphase chromatin, in contrast to the darkly staining heterochromatin (condensed chromatin). Euchromatin contains most, if not all, of the active genes.

exocytosis The process by which molecules are secreted from a cell. Molecules to be secreted are located in Golgi-derived vesicles that fuse with the inner surface of the cell membrane, depositing the contents into the intercellular space.

exon Coding region of a eucaryote gene that is represented in messenger RNA, and thus directs the synthesis of a specific protein.

expression studies Examination of the type and quantity of mRNA or protein that is produced by cells, tissues, or organs.

fat A lipid material, consisting of triglycerides (fatty acids bound to glycerol), that is stored adipocytes as an energy reserve.

fatty acid A compound that has a carboxylic acid attached to a long hydrocarbon chain. A major source of cellular energy and a component of phospholipids.

fertilization The fusion of haploid male and female gametes to form a diploid zygote.

fibroblast The cell type that, by secreting an extracellular matrix, gives rise to the connective tissue of the body.

filopodium A finger-like projection of a cell's cytoplasmic membrane, commonly observed in amoeba and embryonic nerve cells.

filter hybridization The detection of specific DNA or RNA molecules, fixed on a nylon filter (or membrane), by incubating the filter with a labelled probe that hybridizes to the target sequence. Also known as membrane hybridization.

fixative A chemical that is used to preserve cells and tissues. Common examples are formaldehyde, methanol, and acetic acid.

flagellum Whip-like structure found in procaryotes and eucaryotes that are used to propel cells through water.

fluorescein Fluorescent dye that produces a green light when illuminated with ultraviolet or blue light.

fluorescent dye A dye that absorbs UV or blue light, and emits light of a longer wavelength, usually as green or red light.

fluorescent in situ hybridization (FISH) A procedure for detecting the expression of a specific gene in tissue sections or smears through the use of DNA probes labelled with a fluorescent dye.

fluorescent microscope A microscope that is equipped with special filters, and a beam splitter, for the examination of tissues and cells stained with a fluorescent dye.

follicle cell Cells that surround, and help feed, a developing oocyte.

G_0 G "zero" refers to a phase of the cell cycle. State of withdrawal from the cycle as the cell enters a resting or quiescent stage. Occurs in differentiated body cells, as well as developing oocytes.

G_1 Gap 1 refers to the phase of the cell cycle that occurs just after mitosis, and before the next round of DNA synthesis.

G_2 The Gap 2 phase of the cell cycle follows DNA replication and precedes mitosis.

gap junction A communication channel in the membranes of adjacent cells that allows free passage of ions and small molecules.

gel electrophoresis A procedure that is used to separate biomolecules by forcing them to migrate through a gel matrix (agarose or polyacrylamide) subjected to an electric field.

gene A region of the DNA that specifies a specific protein or RNA molecule that is handed down from one generation to the next. This region includes both the coding, noncoding and regulatory sequences.

gene regulatory protein Any protein that binds to DNA and thereby affects the expression of a specific gene.

gene repressor protein A protein that binds to DNA and blocks transcription of a specific gene.

gene therapy A method for treating disease whereby a defective gene, causing the disease, is either repaired, replaced or supplemented with a functional copy.

genetic code A set of rules that assigns a specific DNA or RNA triplet, consisting of a three base sequence, to a specific amino acid.

genome All of the genes that belong to a cell or an organism.

genomic library A collection of DNA fragments, obtained by digesting genomic DNA with a restriction enzyme, that are cloned into plasmid or viral vectors.

genomics The study of DNA sequences and their role in the function and structure of an organism.

genotype The genetic composition of a cell or organism.

germ cell Cells that develop into gametes, either sperm or oocytes.

glucose Six-carbon monoosaccharide (sugar) that is the principle source of energy for many cells and organisms. Stored as glycogen in animal cells and as starch in plants. Wood is an elaborate polymer of glucose and other sugars.

glycerol A three carbon alcohol that is an important component of phospholipids.

glycocalyx A molecular "forest", consisting of glycosylated proteins and lipids, that covers the surface of every cell. The glycoproteins and glycolipids, carried to the cell membrane by Golgi-derived vesicles, have many functions including the formation of ion channels, cell-signaling receptors, and transporters.

glycogen A polymer of glucose, used to store energy in an animal cell.

glycolysis The degradation of glucose with production of ATP.

glycoprotein Any protein that has a chain of glucose molecules (oligosaccharide) attached to some of the amino acid residues.

glycosylation The process of adding one or more sugar molecules to proteins or lipids.

glycosyl transferase An enzyme in the Golgi complex that adds glucose to proteins.

Golgi complex (Golgi apparatus) Membrane-bounded organelle in eucaryote cells that receives glycoproteins from the ER, which are modified and sorted before being sent to their final destination. The Golgi complex is also the source of glycolipids that are destined for the cell membrane. The glycoproteins and glycolipids leave the Golgi by exocytosis. This organelle is named after the Italian histologist, Camillo Golgi, who discovered it in 1898.

Gram stain A bacterial stain that detects different species of bacteria based on the composition of their cell wall. Bacteria that retain the Gram stain are colored blue (Gram positive), whereas those that do not are colored orange (Gram negative).

granulocyte A type of white blood cell that includes the neutrophils, basophils, and eosinophils.

growth factor A small protein (polypeptide) that can stimulate cells to grow and proliferate.

haploid Having only one set of chromosomes. A condition that is typical in gametes, such as sperm and eggs.

HeLa cell A tumor-derived cell line, originally isolated from a cancer patient in 1951. Currently used by many laboratories to study the cell biology of cancer and carcinogenesis.

helix-loop-helix A structural motif common to a group of gene regulatory proteins.

helper T cell A type of T lymphocyte that helps stimulate B cells to make antibody directed against a specific microbe or antigen.

hemoglobin An iron-containing protein complex, located in red blood cell, that picks up oxygen in the lungs and carries it to other tissues and cells of the body.

hemopoiesis Production of blood cells, occurring primarily in the bone marrow.

hematopoietic Refers to cells, derived form the bone marrow, that give rise to red and white blood cells.

hematopoietic stem cell transplantation (HSCT) The use of stem cells isolated from the bone marrow to treat leukemia and lymphoma.

hepatocyte A liver cell.

heterochromatin A region of a chromosome that is highly condensed and transcriptionally inactive.

histochemistry The study of chemical differentiation of tissues.

histology The study of tissues.

histone Small nuclear proteins, rich in the amino acids arginine and lysine, that form the nucleosome in eucaryote nuclei, a bead-like structure that is a major component of chromatin.

HIV The human immunodeficiency virus that is responsible for AIDS.

homolog One of two or more genes that have a similar sequence, and are descended from a common ancestor gene.

homologous Organs or molecules that are similar in structure because they have descended from a common ancestor. Used primarily in reference to DNA and protein sequences.

homologous chromosomes Two copies of the same chromosome, one inherited from the mother, and the other from the father.

hormone A signaling molecule, produced and secreted by endocrine glands. Usually released into general circulation for coordination of an animal's physiology.

housekeeping gene A gene that codes for a protein that is needed by all cells, irregardless of the cell's specialization. Genes encoding enzymes involved in glycolysis and Krebs cycle are common examples.

hybridization A term used in molecular biology (recombinant DNA technology), meaning the formation a double stranded nucleic acid through complementary base-pairing. A property that is exploited in filter hybridization, a procedure that is used to screen gene libraries, and to study gene structure and expression.

hydrolysis The breaking of a covalent chemical bond with the subsequent addition of a molecule of water.

hydrophilic A polar compound that mixes readily with water.

hydrophobic A non-polar molecule that dissolves in fat and lipid solutions, but not in water.

hydroxyl group (-OH) Chemical group consisting of oxygen and hydrogen that is a prominent part of alcohol.

image analysis A computerized method for extracting information from digitized microscopic images of cells or cell organelles.

immunofluorescence Detection of specific a cellular protein with the aid of a fluorescent dye that is coupled to an antibody.

immunoglobulin (Ig) An antibody made by B cells as part of the adaptive immune response.

incontinence Inability to control the flow of urine from the bladder (urinary incontinence) or the escape of stool from the rectum (fecal incontinence).

insertional mutagenesis Damage suffered by a gene when a virus or a jumping gene inserts itself into a chromosome.

***in situ* hybridization** A method for studying gene expression, whereby a labeled cDNA or RNA probe hybridizes to a specific mRNA in intact cells or tissues. The procedure is usually carried out on tissue sections or smears of individual cells.

in vitro Refers to cells growing in culture, or a biochemical reaction occurring in a test tube (Latin for "in glass").

in vivo A biochemical reaction, or a process, occurring in living cells or a living organism (Latin for "in life").

insulin Polypeptide hormone secreted by β (beta) cells in the vertebrate pancreas. Production of this hormone is regulated directly by the amount of glucose that is in the blood.

interleukin A small protein hormone, secreted by lymphocytes, to activate and coordinate the adaptive immune response.

interphase The period between each cell division, which includes the G_1, S, and G_2 phases of the cell cycle.

intron A section of a eucaryotic gene that is non-coding. It is transcribed, but does not appear in the mature mRNA.

ion channel A transmembrane channel that allows ions to diffuse across the membrane, down their electrochemical gradient.

ion An atom that has gained or lossed electrons, thus acquiring a charge. Common examples are Na^+ and Ca^{++} ions.

ischemia An inadequate supply of blood to a part of the body, caused by degenerative vascular disease.

Jak-STAT signaling pathway One of several cell signaling pathways that activates gene expression. The pathway is activated through cell surface receptors and cytoplasmic Janus kinases (Jaks), and signal transducers and activators of transcription (STATs).

karyotype A pictorial catalog of a cell's chromosomes, showing their number, size, shape, and overall banding pattern.

keratin Proteins produced by specialized epithelial cells called keratinocytes. Keratin is found in hair, fingernails, and feathers.

kilometer 1,000 meters, which is equal to 0.621 miles.

kinesin A motor protein that uses energy obtained from the hydrolysis of ATP to move along a microtubule.

kinetochore A complex of proteins that forms around the centromere of mitotic or meiotic chromosomes, providing an attachment site for microtubules. The other end of each microtubule is attached to a chromosome.

Krebs cycle (citric acid cycle) The central metabolic pathway in all eucaryotes and aerobic procaryotes. Discovered by the German chemist, Hans Krebs, in 1937. The cycle oxidizes acetyl groups derived from food molecules. The end products are CO_2, H_2O, and high-energy electrons, which pass via NADH and FADH2 to the respiratory chain. In eucaryotes, Krebs cycle is located in the mitochondria.

labeling reaction The addition of a radioactive atom or fluorescent dye to DNA or RNA for use as a probe in filter hybridization.

lagging strand One of the two newly synthesized DNA strands at a replication fork. The lagging strand is synthesized discontinuously,

and therefore, its completion lags behind the second, or leading, strand.

lambda bacteriophage A viral parasite that infects bacteria. Widely used as a DNA cloning vector.

leading strand One of the two newly synthesized DNA strands at a replication fork. The leading strand is made by continuous synthesis in the 5′ to 3′ direction.

leucine zipper A structural motif of DNA binding proteins, in which two identical proteins are joined together at regularly-spaced leucine residues, much like a zipper, to form a dimer.

leukemia Cancer of white blood cells.

lipid bilayer Two, closely aligned, sheets of phospholipids that forms the core structure of all cell membranes. The two layers are aligned such that the hydrophobic tails are interior, while the hydrophilic head groups are exterior on both surfaces.

liposome An artificial lipid bilayer vesicle used in membrane studies and as an artificial gene therapy vector.

locus A term from genetics that refers to the position of a gene along a chromosome. Different alleles of the same gene occupy the same locus.

long-term potentiation (LTP) A physical remodeling of synaptic junctions that receive continuous stimulation.

lumen A cavity completely surrounded by epithelial cells.

lymphocyte A type of white blood cell that is involved in the adaptive immune response. There are two kinds of lymphocytes: T lymphocytes and B lymphocytes. T lymphocytes (T cells) mature in the thymus, and attack invading microbes directly. B lymphocytes (B cells) mature in the bone marrow, and make antibodies that are designed to immobilize or destroy specific microbes or antigens.

lysis The rupture of the cell membrane followed by death of the cell.

lysosome Membrane-bounded organelle of eucaryotes that contains powerful digestive enzymes.

macromolecule A very large molecule that is built from smaller molecular subunits. Common examples are DNA, proteins, and polysaccharides.

magnetic resonance imaging (MRI) A procedure in which radio waves and a powerful magnet linked to a computer are used to cre-

ate detailed pictures of areas inside the body. These pictures can show the difference between normal and diseased tissue. MRI makes better images of organs and soft tissue than other scanning techniques, such as CT or X-ray. MRI is especially useful for imaging the brain, spine, the soft tissue of joints, and the inside of bones. Also called nuclear magnetic resonance imaging.

major histocompatibility complex Vertebrate genes that code for a large family of cell-surface glycoproteins that bind foreign antigens and present them to T cells to induce an immune response.

malignant Refers to the functional status of a cancer cell that grows aggressively and is able to metastasize, or colonize, other areas of the body.

mammography The use of X-rays to create a picture of the breast.

MAP-kinase (mitogen-activated protein kinase) A protein kinase that is part of a cell proliferation-inducing signaling pathway.

M-cyclin A eucaryote enzyme that regulates mitosis.

meiosis A special form of cell division by which haploid gametes are produced. This is accomplished with two rounds of cell division, but only one round of DNA replication.

melanocyte A skin cell that produces the pigment melanin.

membrane The lipid bilayer, and the associated glycocalyx, that surrounds and encloses all cells.

membrane channel A protein complex that forms a pore or channel through the membrane for the free passage of ions and small molecules.

membrane potential A build-up of charged ions on one side of the cell membrane establishes an electrochemical gradient that is measured in millivolts (mV). An important characteristic of neurons as it provides the electrical current, when ion channels open, that enable these cells to communicate with each other.

mesoderm An embryonic germ layer that gives rise to muscle, connective tissue, bones, and many internal organs.

messenger RNA (mRNA) An RNA transcribed from a gene that is used as the gene template by the ribosomes, and other components of the translation machinery, to synthesize a protein.

metabolism The sum total of the chemical processes that occur in living cells.

metaphase The stage of mitosis at which the chromosomes are attached to the spindle but have not begun to move apart.

metaphase plate Refers to the imaginary plane established by the chromosomes as they line up at right angles to the spindle poles.

metaplasia A change in the pattern of cellular behavior that often precedes the development of cancer.

metastasis Spread of cancer cells from the site of the original tumor to other parts of the body.

methyl group (-CH_3) Hydrophobic chemical group derived from methane. Occurs at the end of a fatty acid.

meter Basic unit in the metric system. Equal to 39.4 inches or 1.09 yards.

micrograph Photograph taken through a light, or electron, microscope.

micrometer (μm or micron) Equal to 10^{-6} meters.

microtubule A fine cylindrical tube made of the protein tubulin, forming a major component of the eucaryote cytoskeleton.

millimeter (mm) Equal to 10^{-3} meters.

mitochondrion (plural mitochondria) Eucaryote organelle, formerly free-living, that produces most of the cell's ATP

mitogen A hormone or signaling molecule that stimulates cells to grow and divide.

mitosis Division of a eucaryotic nucleus. From the Greek *mitos,* meaning a thread, in reference to the threadlike appearance of interphase chromosomes.

mitotic chromosome Highly condensed duplicated chromosomes held together by the centromere. Each member of the pair is referred to as a sister chromatid.

mitotic spindle Array of microtubules, fanning out from the polar centrioles, and connecting to each of the chromosomes.

molecule Two or more atoms linked together by covalent bonds.

monoclonal antibody An antibody produced from a B-cell derived clonal line. Since all of the cells are clones of the original B cell, the antibodies produced are identical.

monocyte A type of white blood cell that is involved in the immune response.

motif An element of structure or pattern that may be a recurring domain in a variety of proteins.

M phase The period of the cell cycle (mitosis or meiosis) when the chromosomes separate and migrate to the opposite poles of the spindle.

multipass transmembrane protein A membrane protein that passes back and forth across the lipid bilayer.

multipotency The property by which an undifferentiated animal cell can give rise to many of the body's cell types.

mutant A genetic variation within a population.

mutation A heritable change in the nucleotide sequence of a chromosome.

myelin sheath Insulation applied to the axons of neurons. The sheath is produced by oligodendrocytes in the central nervous system, and by Schwann cells in the peripheral nervous system.

myeloid cell White blood cells other than lymphocytes.

myoblast Muscle precursor cell. Many myoblasts fuse into a syncytium, containing many nuclei, to form a single muscle cell.

myocyte A muscle cell.

NAD (nicotine adenine dinucleotide) Accepts a hydride ion (H^-), produced by the Krebs cycle, forming NADH, the main carrier of electrons for oxidative phosphorylation.

NADH dehydrogenase Removes electrons from NADH and passes them down the electron transport chain.

nanometer (nm) Equal to 10^{-9} meters or 10^{-3} microns.

National Institutes of Health (NIH) A biomedical research center located in the United States. NIH consists of over 25 research institutes, including the National Institute of Aging (NIA) and the National Cancer Institute (NCI). All of the institutes are funded by the federal government.

natural killer cell (NK cell) A lymphocyte that kills virus-infected cells in the body. They also kill foreign cells associated with a tissue or organ transplant.

neuromuscular junction A special form of synapse between a motor neuron and a skeletal muscle cell.

neuromodulator A chemical released by neurons at a synapse that modifies the behavior of the targeted neuron(s).

neuron A cell specially adapted for communication that forms the nervous system of all animals.

neurotransmitter A chemical released by neurons at a synapse that activates the targeted neuron.

non–small cell lung cancer A group of lung cancers that includes squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. The small cells are endocrine cells.

northern blotting A technique for the study of gene expression. Messenger RNA (mRNA) is fractionated on an agarose gel and then transferred to a piece of nylon filter paper (or membrane). A specific mRNA is detected by hybridization with a labeled DNA or RNA probe. The original blotting technique invented by E. M. Southern inspired the name. Also known as RNA blotting.

nuclear envelope The double membrane (two lipid bilayers) enclosing the cell nucleus.

nuclear localization signal (NLS) A short amino acid sequence located on proteins that are destined for the cell nucleus, after they are translated in the cytoplasm.

nuclei acid DNA or RNA, a macromolecule consisting of a chain of nucleotides.

nucleolar organizer Region of a chromosome containing a cluster of ribosomal RNA genes that gives rise to the nucleolus.

nucleolus A structure in the nucleus where ribosomal RNA is transcribed and ribosomal subunits are assembled.

nucleoside A purine or pyrimidine linked to a ribose or deoxyribose sugar.

nucleosome A bead-like structure, consisting of histone proteins.

nucleotide A nucleoside containing one or more phosphate groups linked to the 5′ carbon of the ribose sugar. DNA and RNA are nucleotide polymers.

nucleus Eucaryote cell organelle that contains the DNA genome on one or more chromosomes.

oligodendrocyte A myelinating glia cell of the vertebrate central nervous system.

oligomer A short polymer, usually consisting of amino acids (oligopeptides), sugars (oligosaccharides), or nucleotides (oligonucleotides). Taken from the Greek word, *oligos,* meaning few or little.

oligo labeling A method for incorporating labeled nucleotides into a short piece of DNA or RNA. Also known as the random-primer labeling method.

oncogene A mutant form of a normal cellular gene, known as a proto-oncogene, that can transform a cell to a cancerous phenotype.

oocyte A female gamete or egg cell.

operator A region of a procaryote chromosome that controls the expression of adjacent genes.

operon Two or more procaryote genes that are transcribed into a single mRNA.

organelle A membrane bounded structure, occurring in eucaryote cells, that has a specialized function. Examples are the nucleus, Golgi complex, and endoplasmic reticulum.

osmosis The movement of solvent across a semi-permeable membrane that separates a solution with a high concentration of solutes from one with a low concentration of solutes. The membrane must be permeable to the solvent, but not to the solutes. In the context of cellular osmosis, the solvent is always water, the solutes are ions and molecules, and the membrane is the cell membrane.

osteoblast Cells that form bones.

ovulation Rupture of a mature follicle with subsequent release of a mature oocyte from the ovary.

oxidative phosphorylation Generation of high energy electrons from food molecules that are used to power the synthesis of ATP from ADP and inorganic phosphate. The electrons are eventually transferred to oxygen, to complete the process. Occurs in bacteria and mitochondria.

p53 A tumor suppressor gene that is mutated in about half of all human cancers. The normal function of the *p53* protein is to block passage through the cell cycle when DNA damage is detected.

parthenogenesis A natural form of animal cloning whereby an individual is produced without the formation of haploid gametes and the fertilization of an egg.

pathogen An organism that causes disease.

PCR (polymerase chain reaction) A method for amplifying specific regions of DNA by temperature cycling a reaction mixture containing the template, a heat-stable DNA polymerase, and replication primers.

peptide bond The chemical bond that links amino acids together to form a protein.

pH Measures the acidity of a solution as a negative logarithmic function (p) of H^+ concentration (H). Thus, a pH of 2.0 (10^{-2} molar H^+) is acidic, whereas a pH of 8.0 (10^{-8} molar H^+) is basic.

phagocyte A cell that engulfs other cells or debri by phagocytosis.

phagocytosis A process whereby cells engulf other cells or organic material by endocytosis. A common practice among protozoans, and cells of the vertebrate immune system. (From the Greek *phagein,* to eat.)

phenotype Physical characteristics of a cell or organism.

phospholipid The kind of lipid molecule used to construct cell membranes. Composed of a hydrophilic head-group, phosphate, glycerol, and two hydrophobic fatty acid tails.

phosphorylation A chemical reaction in which a phosphate is covalently bonded to another molecule.

phosphokinase An enzyme that adds phosphate to proteins.

photoreceptor A molecule or cell that responds to light.

photosynthesis A biochemical process in which plants, algae, and certain bacteria use energy obtained from sunlight to synthesize macromolecules from CO_2 and H_2O.

phylogeny The evolutionary history of a group of organisms, usually represented diagrammatically as a phylogenetic tree.

pinocytosis A form of endocytosis whereby fluid is brought into the cell from the environment.

pixel One element in a data array that represents an image or photograph.

placebo An inactive substance that looks the same, and is administered in the same way, as a drug in a clinical trial.

plasmid A minichromosome, often carrying antibiotic-resistant genes, that occurs naturally among procaryotes. Used extensively as a DNA cloning vector.

platelet A cell fragment, derived from megakaryocytes and lacking a nucleus, that is present in the bloodstream, and is involved in blood coagulation.

ploidy The total number of chromosomes (n) that a cell has. Ploidy is also measured as the amount of DNA (C) in a given cell, relative to a

haploid nucleus of the same organism. Most organisms are diploid, having two sets of chromosomes, one from each parent, but there is great variation among plants and animals. The silk gland of the moth *Bombyx mori,* for example, has cells that are extremely polyploid, reaching values of 100,000C, flowers are often highly polyploid, and vertebrate hepatocytes may be 16C.

pluripotency The property by which an undifferentiated animal cell can give rise to most of the body's cell types.

poikilotherm An animal incapable of regulating its body temperature independent of the external environment. It is for this reason that such animals are restricted to warm tropical climates.

point mutation A change in DNA, particularly in a region containing a gene, that alters a single nucleotide.

polarization A term used to describe the re-establishment of a sodium ion gradient across the membrane of a neuron. Polarization followed by depolarization is the fundamental mechanism by which neurons communicate with each other.

polyploid Possessing more than two sets of homologous chromosomes.

polyploidization DNA replication in the absence of cell division. Provides many copies of particular genes and thus occurs in cells that highly active metabolically (see ploidy).

polyacrylamide A tough polymer gel that is used to fractionate DNA and protein samples.

portal system A system of liver vessels that carries liver enzymes directly to the digestive tract.

post-mitotic Refers to a cell that has lost the ability to divide.

probe Usually a fragment of a cloned DNA molecule that is labeled with a radioisotope or fluorescent dye, and used to detect specific DNA or RNA molecules on Southern or Northern blots.

progenitor cell A cell that has developed from a stem cell, but can still give rise to a limited variety of cell types.

proliferation A process whereby cells grow and divide.

promoter A DNA sequence to which RNA polymerase binds to initiate gene transcription.

prophase The first stage of mitosis. The chromosomes are duplicated and are beginning to condense, but are attached to the spindle.

protein A major constituent of cells and organisms. Proteins, made by linking amino acids together, are used for structural purposes, and regulate many biochemical reactions in their alternative role as enzymes. Proteins range in size from just a few amino acids to over 200.

protein glycosylation The addition of sugar molecules to a protein.

proto-oncogene A normal gene that can be converted to a cancer-causing gene (oncogene) by a point mutation or through inappropriate expression.

protozoa Free living, single-cell eucaryotes that feed on bacteria and other microorganisms. Common examples are *Paramecium* and *Amoeba*. Parasitic forms are also known that inhabit the digestive and urogenital tract of many animals, including humans.

P-site The binding site on the ribosome for the growing protein (or peptide) chain.

purine A nitrogen-containing compound that is found in RNA and DNA. Two examples are adenine and guanine.

pyrimidine A nitrogen-containing compound found in RNA and DNA. Examples are cytosine, thymine, and uracil (RNA only).

radioactive isotope An atom with an unstable nucleus that emits radiation as it decays.

random primer labeling A method for incorporating labeled nucleotides into a short piece of DNA or RNA.

randomized clinical trial A study in which the participants are assigned by chance to separate groups that compare different treatments; neither the researchers nor the participants can choose which group. Using chance to assign people to groups means that the groups will be similar and that the treatments they receive can be compared objectively. At the time of the trial, it is not known which treatment is best.

reagent A chemical solution designed for a specific biochemical or histochemical procedure.

recombinant DNA A DNA molecule that has been formed by joining two or more fragments from different sources.

refractive index A measure of the ability of a substance to bend a beam of light expressed in reference to air which has, by definition, a refractive index of 1.0.

regulatory sequence A DNA sequence to which proteins bind that regulate the assembly of the transcriptional machinery.

replication bubble Local dissociation of the DNA double helix in preparation for replication. Each bubble contains two replication forks.

replication fork The Y-shape region of a replicating chromosome. Associated with replication bubbles.

replication origin (origin of replication, ORI) The location at which DNA replication begins.

respiratory chain (electron transport chain) A collection of iron- and copper-containing proteins, located in the inner mitochondrion membrane, that utilize the energy of electrons traveling down the chain to synthesize ATP.

restriction map The size and number of DNA fragments obtained after digesting with one or more restriction enzymes.

restriction enzyme An enzyme that cuts DNA at specific sites.

retrovirus A virus that converts its RNA genome to DNA once it has infected a cell.

reverse transcriptase An RNA-dependent DNA polymerase. This enzyme synthesizes DNA by using RNA as a template, the reverse of the usual flow of genetic information from DNA to RNA.

ribosomal RNA (rRNA) RNA that is part of the ribosome, and serves both a structural and functional role, possibly by catalyzing some of the steps involved in protein synthesis.

ribosome A complex of protein and RNA that catalyzes the synthesis of proteins.

rough endoplasmic reticulum (rough ER) Endoplasmic reticulum that has ribosomes bound to its outer surface.

Saccharomyces Genus of budding yeast that are frequently used in the study of eucaryote cell biology.

sarcoma Cancer of connective tissue.

Schwann cell Glia cell that produces myelin in the peripheral nervous system.

screening Checking for disease when there are no symptoms.

senescence (from the Latin word *senex,* meaning "old man" or "old age") Physical and biochemical changes that occur in cells and organisms with age.

signal transduction A process by which a signal is relayed to the interior of a cell where it elicits a response at the cytoplasmic or nuclear level.

smooth muscle cell Muscles lining the intestinal tract and arteries. Lacks the striations typical of cardiac and skeletal muscle, giving it a smooth appearance when viewed under a microscope.

somatic cell Any cell in a plant or animal except those that produce gametes (germ cells or germ cell precursors).

somatic cell nuclear transfer Animal cloning technique whereby a somatic cell nucleus is transferred to an enucleated oocyte. Synonymous with cell nuclear transfer or replacement.

Southern transfer The transfer of DNA fragments from an agarose gel to a piece of nylon filter paper. Specific fragments are identified by hybridizing the filter to a labeled probe. Invented by the Scottish scientist, E. M. Southern, in 1975. Also known as DNA blotting.

stem cell Pluripotent progenitor cell, found in embryos and various parts of the body, that can differentiate into a wide variety of cell types.

steroid A hydrophobic molecule with a characteristic four-ringed structure. Sex hormones, such as estrogen and testosterone, are steroids.

structural gene A gene that codes for a protein or an RNA. Distinguished from regions of the DNA that are involved in regulating gene expression, but are noncoding.

synapse A neural communication junction between an axon and a dendrite. Signal transmission occurs when neurotransmitters, released into the junction by the axon of one neuron, stimulate receptors on the dendrite of a second neuron.

syncytium A large multi-nucleated cell. Skeletal muscle cells are syncytiums produced by the fusion of many myoblasts.

syngeneic transplants A patient receives tissue or an organ from an identical twin.

tamoxifen A drug that is used to treat breast cancer. Tamoxifen blocks the effects of the hormone estrogen in the body. It belongs to the family of drugs called antiestrogens.

T cell (T lymphocyte) A white blood cell involved in activating and coordinating the immune response.

telomere The end of a chromosome. Replaced by the enzyme telomerase with each round of cell division to prevent shortening of the chromosomes.

telophase The final stage of mitosis in which the chromosomes decondense and the nuclear envelope reforms.

template A single strand of DNA or RNA whose sequence serves as a guide for the synthesis of a complementary, or daughter, strand.

therapeutic cloning The cloning of a human embryo for the purpose of harvesting the inner cell mass (embryonic stem cells).

topoisomerase An enzyme that makes reversible cuts in DNA to relieve strain or to undo knots.

totipotency The property by which an undifferentiated animal cell can give rise to all of the body's cell types. The fertilized egg and blastomeres from an early embryo are the only cells possessing this ability.

trans Golgi network The membrane surfaces where glycoproteins and glycolipids exit the Golgi complex in transport vesicles.

transcription The copying of a DNA sequence into RNA, catalyzed by RNA polymerase.

transcription factor A general term referring to a wide assortment of proteins needed to initiate or regulate transcription.

transfection Introduction of a foreign gene into a eukaryote or prokaryote cell.

transfer RNA (tRNA) A collection of small RNA molecules that transfer an amino acid to a growing polypeptide chain on a ribosome. There is a separate tRNA for amino acid.

transgenic organism A plant or animal that has been transfected with a foreign gene.

translation A ribosome-catalyzed process whereby the nucleotide sequence of a mRNA is used as a template to direct the synthesis of a protein.

transposable element (transposon) A segment of DNA that can move from one region of a genome to another.

ultrasound (ultrasonography) A procedure in which high-energy sound waves (ultrasound) are bounced off internal tissues or organs

producing echoes that are used to form a picture of body tissues (a sonogram).

umbilical cord blood stem cells Stem cells, produced by a human fetus and the placenta, that are found in the blood that passes from the placenta to the fetus.

vector A virus or plasmid used to carry a DNA fragment into a bacterial cell (for cloning) or into a eukaryote to produce a transgenic organism.

vesicle A membrane-bounded bubble found in eucaryote cells. Vesicles carry material from the ER to the Golgi and from the Golgi to the cell membrane.

virus A particle containing an RNA or DNA genome surrounded by a protein coat. Viruses are cellular parasites that cause many diseases.

western blotting The transfer of protein from a polyacrylamide gel too a piece of nylon filter paper. Specific proteins are detected with labeled antibodies. The name was inspired by the original blotting technique invented by the Scottish scientist E. M. Southern in 1975. Also known as protein blotting.

xenogeneic transplants (xenograft) A patient receives tissue or an organ from an animal of a different species.

yeast Common term for unicellular eucaryotes that are used to brew beer and make bread. Bakers yeast, *Saccharomyces cerevisiae,* are also widely used in studies on cell biology.

zygote A diploid cell produced by the fusion of a sperm and egg.

Further Resources

BOOKS

Alberts, Bruce, Dennis Bray, Karen Hopkins, and Alexander Johnson. *Essential Cell Biology.* Second ed. New York: Garland Publishing, 2003. A basic introduction to cellular structure and function that is suitable for high school students.

Alberts, Bruce, et al. *Molecular Biology of the Cell.* Fifth ed. New York: Garland Publishing, 2008. Advanced coverage of cell biology that is suitable for senior high school students and undergraduates.

Keller, Evelyn Fox. *A Feeling for the Organism: The Life and Work of Barbara McClintock.* New York: W. H. Freeman and Company, 1983. An enjoyable book that chronicles Dr. McClintock's work that led to her discovery of "jumping genes" and the chilly response she received from a skeptical scientific community.

Krause, W. J. *Krause's Essential Human Histology for Medical Students.* Boca Raton, Fla.: Universal Publishers, 2005. This book goes well with histology videos provided free on Google video.

De Kruif, Paul. *Microbe Hunters.* New York: Harcourt, 2002. First published in 1926, this book about pathogenic bacteria and viruses has been an inspiration to young biologists ever since.

Lentz, Thomas L. *Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure.* Philadelphia, Pa.: Saunders, 1971. Although hard to find, this book has some of the finest drawings of the cell ever produced.

Mader, Sylvia S. *Biology.* Ninth ed. Boston: McGraw-Hill Companies, 2007. A basic high school biology textbook.

Margulis, Lynn, and Dorion Sagan. *Dazzle Gradually: Reflections on the Nature of Nature.* White River Junction, Vt.: Chelsea Green Publishing, 2007. Examines many interesting aspects of cellular evolution.

Margulis, Lynn, and K. V. Schwartz. *Five Kingdoms: An Illustrated Guide to Phyla of Life on Earth.* New York: Freeman, 1998. Describes the evolution of bacteria, archaea, plants, animals, and fungi.

Panno, Joseph. *Animal Cloning: The Science of Nuclear Transfer.* Rev. ed. New York: Facts On File, 2010. Medical applications of cloning technology are discussed including therapeutic cloning.

———. *Cancer: The Role of Genes, Lifestyle, and Environment.* Rev. ed. New York: Facts On File, 2010. The basic nature of cancer written for the general public and young students.

———. *Gene Therapy: Treatments and Cures for Genetic Diseases.* Rev. ed. New York: Facts On File, 2010. Discusses not only the great potential of this therapy but also its dangers and its many failures.

———. *Human Aging: Modern Theories and Therapies.* Rev. ed. New York: Facts On File, 2010. Explains why and how people age and how the process may be reversed or modified.

———. *Stem Cell Research: Medical Applications & Ethical Controversies.* Rev. ed. New York: Facts On File, 2010. All about a special type of cell, the stem cell, and its use in medical therapies.

Spangenburg, Ray, and Diane Kit Moser. *Barbara McClintock: Pioneering Geneticist.* New York: Chelsea House, 2008. Good biographical volume on the life and work of the acclaimed scientist.

JOURNAL AND MAGAZINE ARTICLES

Barrick, Jeffery, and Ronald Breaker. "The Power of Riboswitches." *Scientific American* 296 (2007): 50–57. Discusses recently dis-

covered RNA molecules that appear to regulate many cellular processes.

Boyd, Scott. "Everything You Wanted to Know about Small RNA but Were Afraid to Ask." *Laboratory Investigation* 88 (2008): 569–578. A brief overview of the functions of micro RNAs.

Carroll, Sean, Benjamin Prud'homme, and Nicolas Gompel. "Regulating Evolution." *Scientific American* 298 (2008): 61–67. The role of gene expression in the evolution of life on Earth.

Church, George. "Genomes for All." *Scientific American* 294 (2006): 46–54. This article describes fast and cheap DNA sequencers that could make it possible for everyone to have their genome sequenced, giving new meaning to personalized medicine.

Collins, Francis, Michael Morgan, and Aristides Patrinos. "The Human Genome Project: Lessons from Large-Scale Biology." *Science* 300 (2003): 286–290. Provides an overview of the many organizational problems that had to be overcome in order to complete the project.

Hotopp, Julie, et al. "Widespread Lateral Gene Transfer from Intracellular Bacteria to Multicellular Eukaryotes." *Science* 317 (2007): 1,753–1,756. This article details the transmission of genes from the bacterium *Wolbachia pipientis* to four insect and four nematode species.

Miller, Jon, Eugenie Scott, and Shinji Okamoto. "Public Acceptance of Evolution." *Science* 313 (2006): 765–766. Evaluates the extent to which the American public is willing to accept the theory of evolution.

Mirsky, Steve. "Teach the Science." *Scientific American* 294 (February 2006): 36–38. This article profiles the work of Dr. Eugenie Scott, a scientist who opposes the teaching of creationism or intelligent design in public schools.

Sanderson, Katherine. "Penguins on Mars?" *Nature News* (June 27, 2008). The *Phoenix* lander has discovered that Martian soil is like Antarctica's.

Schmitt, Harrison. "From the Moon to Mars." *Scientific American* 301 (July 2009). A fascinating look at the problems astronauts will face while exploring Mars.

Song, Hongjun, Charles Stevens, and Fred Gage. "Astroglia Induce Neurogenesis from Adult Neural Stem Cells." *Nature* 417 (2002): 39–44. This research paper is directed at professional scientists, but is recommended to beginning students for the beautiful fluorescent images of neurons growing in culture.

Stein, William, Frank Mannolini, Linda VanAller Hernick, et al. "Giant Cladoxylopsid Trees Resolve the Enigma of the Earth's Earliest Forest Stumps at Gilboa." *Nature* 446 (2007): 904–907. This paper discusses the structure and evolution of Earth's earliest trees, which appeared in the Devonian age about 380 million years ago.

INTERNET ARTICLES

Ainsworth, Claire. "Cell Biology: Stretching the imagination." *Nature* 456 (2008): 696–699. Available online. URL: http://www.nature.com/news/2008/081210/full/456696a.html. Accessed January 10, 2009. This news article profiles Dr. Donald Ingber and his work, which shows how responsive cells are to mechanical stimulation.

Amos, Jonathan. "Ancient Sponges Leave Their Mark." BBC News (February 4, 2009). Available online. URL: http://news.bbc.co.uk/2/hi/science/nature/7871099.stm. Accessed February 4, 2009. An analyis of chemical residues on ancient rocks has shown that sponges appeared on Earth 635 million years ago.

———. "Misty Caverns on Enceladus Moon." BBC News (June 24, 2009). Available online. URL: http://news.bbc.co.uk/2/hi/science/nature/8115148.stm. Accessed July 3, 2009. This article discusses the possibility that Enceladus has abundant water located in caverns just below the surface.

BBC News. "Ancient Rocks 'Built by microbes'" (June 7, 2006). Available online. URL: http://news.bbc.co.uk/2/hi/science/

nature/5055604.stm. Accessed July 3, 2009. An analysis of ancient rocks in western Australia suggests that they were constructed by microbes over 3 billion years ago.

———. "New Minerals Point to Wetter Mars" (October 29, 2008). Available online. URL: http://news.bbc.co.uk/2/hi/science/nature/7696669.stm. Accessed July 3, 2009. NASA scientists have discovered hydrated silica (opal), which suggests that Mars had surface water for many millions of years.

Centers for Disease Control and Prevention. U.S. Department of Health and Human Resources. "Anthrax: What You Need to Know." Available online: URL: http://emergency.cdc.gov/agent/anthrax/needtoknow.asp. Accessed July 3, 2009. Discusses anthrax within the context of a bioterrorist attack.

Chang, Kenneth. "Gauging Age of Universe Becomes More Precise." *New York Times* (March 9, 2008). Available online. URL: http://www.nytimes.com/2008/03/09/science/space/09cosmos.html?scp=1&sq=Gaugin g%20Age%20of%20Universe%20Becomes%20More%20Precise&st=cse. Accessed July 3, 2009. According to NASA scientists, the universe is 13.73 billion years old.

———. "NASA Has Its Closest Look at Geysers on Saturn Moon." *New York Times* (August 15, 2008). Available online. URL: http://www.nytimes.com/2008/08/16/science/space/16cassini.html?scp=3&sq=Saturn %20Moon%20Has%20Geysers&st=cse. Accessed July 3, 2009. This article describes the work of NASA scientists who have been photographing and analyzing geysers of vapor and icy particles being produced on Enceladus, one of Saturn's smallest moons.

———. "Paleontology and Creationism Meet but Don't Mesh." *New York Times* (June 29, 2009). Available online. URL: http://www.nytimes.com/2009/06/30/science/30muse.html?ref=science. Accessed July 2, 2009. This article profiles the Creation Museum in Petersburg, Kentucky.

Dean, Cornelia. "Roving Defender of Evolution, and of Room for God." *New York Times* (April 29, 2008). Available online. URL: http://www.nytimes.com/2008/04/29/science/29prof.html?scp=43&sq=The%20Evoluti on%20Debate&st=cse. Accessed July 2, 2009. This article profiles the work of Dr. Francisco Ayala, a biologist who maintains that belief in evolution does not rule out belief in God.

Dreifus, Claudia. "Deep in the Sea, Imagining the Cradle of Life on Earth." *New York Times* (October 16, 2007). Available online. URL: http://www.nytimes.com/2007/10/16/science/16conv.html?scp=1&sq=Deep%20in%2 0the%20Sea,%20Imagining%20the%20Cradle%20of%20Life%20on%20Earth&st=cse. Accessed July 2, 2009. An article profiling the work of Dr. Cindy Lee Van Dover, a marine biologist who studies the ecology of the ocean floor.

Food and Drug Administration (U.S.). *The Bad Bug Book*. Available online. URL: http://vm.cfsan.fda.gov/~mow/intro.html. Accessed July 2, 2009. This handbook provides basic facts regarding pathogenic microorganisms and natural toxins. The information comes from the Food and Drug Administration, the Centers for Disease Control and Prevention, the USDA Food Safety Inspection Service, and the National Institutes of Health.

Gallup. "On Darwin's Birthday, Only 4 in 10 Believe in Evolution" (February 11, 2009). Available online. URL: http://www.gallup.com/poll/114544/Darwin-Birthday-Believe-Evolution.aspx. Accessed July 2, 2009. The full results for a Gallup poll that correlates education level and church attendance with a belief or disbelief in the theory of evolution.

Harmon, Amy. "A Teacher on the Front Line as Faith and Science Clash." *New York Times* (August 23, 2008). Available online. URL: http://www.nytimes.com/2008/08/24/education/24evolution.html?scp=1&sq=A%20Te acher%20on%20the%20Front%20Line%20as%20Faith%20and%20Science%20Clash&st=cse. Accessed July 2, 2009. This article profiles David Campbell,

a high school biology teacher in Florida, who teaches evolution to students who believe in the literal word of the Bible.

Museum of Paleontology. University of California, Berkeley. "Bacteria: Life History and Ecology." Available online. URL: http://www.ucmp.berkeley.edu/bacteria/bacterialh.htm. Accessed July 2, 2009. A comprehensive coverage of bacteriology.

National Aeronautics and Space Administration. "NASA Orbiter Reveals Details of a Wetter Mars." Available online. URL: http://www.nasa.gov/mission_pages/MRO/main/index.html. Accessed July 2, 2009. Complete coverage of the Mars Reconnaissance Orbiter, including its latest and very dramatic findings regarding the existence of water on the red planet.

———. "Phoenix Mars Lander" Available online. URL: http://www.nasa.gov/mission_pages/phoenix/main/index.html. Accessed July 2, 2009. Complete NASA coverage of the lander's activity.

Nature Publishing Group. "Double Helix: 50 Years of DNA." Available online. URL: http://www.nature.com/nature/dna50/index.html. Accessed July 2, 2009. A special issue of many articles assembled by the journal *Nature* to commemorate the 50th anniversary of James Watson and Francis Crick's classic paper describing the structure of DNA.

Ocean World. "Coral reefs." Available online. URL: http://oceanworld.tamu.edu/students/coral/. Accessed July 2, 2009. A collection of articles about reef ecology produced by Texas A&M University.

Wade, Nicholas. "Atlas Squeaked: A Complete Map of the Brain of a Mouse." *New York Times* (September 26, 2006). Available online. URL: http://www.nytimes.com/2006/09/26/science/26brain.html?scp=1&sq=Atlas%20Squea ked:%20A%20Complete%20Map%20of%20the%20Brain%20of%20a%20Mouse&st =cse. Accessed July 2, 2009. This articles profiles a $41 million project to map gene expression in specific neurons throughout the brain of a mouse.

———. "How Human Cells Get Their Marching Orders." *New York Times* (August 15, 2006). Available online. URL: http://www.nytimes.com/2006/08/15/science/15skin.html?scp=1&sq=How%20Human %20Cells%20Get%20Their%20Marching%20Orders&st=cse. Accessed July 2, 2009. When old cells in the human body die, new ones form to take their place. This article describes research that is attempting to explain how new cells know where to go.

WEB SITES

Complete Work of Charles Darwin (UK). Available online. URL: http://darwin-online.org.uk/. Accessed July 2, 2009. This site contains all of Charles Darwin's publications and most of his private papers. All of this material, including audiobooks, may be downloaded free of charge.

Department of Energy Human Genome Project (U.S.). Available online. URL: http://genomics.energy.gov. Accessed July 2, 2009. Covers every aspect of the human genome project with extensive color illustrations.

Genetic Science Learning Center at the University of Utah. Available online. URL: http://learn.genetics.utah.edu/. Accessed July 2, 2009. An excellent resource for beginning students. This site contains information and illustrations covering basic cell biology, human genetics, animal cloning, stem cells and other new biology topics.

Google Video. Available online. URL: http://video.google.com/videosearch?q=histology+tissue&emb=0&aq =3&oq=histology#. Accessed July 2, 2009. This site contains many videos covering human histology and cell biology.

Institute of Molecular Biotechnology, Jena, Germany. Available online. URL: http://www.imb-jena.de/IMAGE.html. Accessed July 2, 2009. Image library of biological macromolecules.

National Aeronautics and Space Administration. Available online. URL: http://www.nasa.gov/home/index.html. Accessed July 2,

2009. Many fascinating articles describing NASA's recent missions to Mars and Saturn, and much more.

National Center for Biotechnology Information. Available online. URL: http://www.ncbi.nlm.nih.gov. Accessed July 2, 2009. This site, established by the National Institutes of Health, is an excellent resource for anyone interested in biology. The NCBI provides access to GenBank (DNA sequences), literature databases (Medline and others), molecular databases, and topics dealing with genomic biology. With the literature database, for example, anyone can access Medline's 11,000,000 biomedical journal citations to research biomedical questions. Many of these links provide free access to full-length research papers.

National Health Museum Resource Center. Washington, D.C. Available online. URL: www.accessexcellence.org/RC/. Accessed July 2, 2009. Covers many areas of biological research, supplemented with extensive graphics and animations.

National Human Genome Research Institute (U.S.). Available online. URL: http://www.genome.gov/. Accessed July 2, 2009. The institute supports genetic and genomic research, including the ethical, legal, and social implications of genetics research.

National Institutes of Health. (U.S.). Available online. URL: http://www.nih.gov. Accessed July 2, 2009. The NIH posts information on their Web site that covers a broad range of topics including general health information, cell biology, aging, cancer research, and much more.

Nature Publishing Group. Available online. URL: http://www.nature.com/nature/supplements/collections/humangenome/commentaries/ Accessed July 2, 2009. The journal *Nature* has provided a comprehensive guide to the human genome. This site provides links to the definitive historical record for the sequences and analyses of human chromosomes, All papers, which are free for downloading, are based on the final draft produced by the Human Genome Project.

Sanger Institute (U.K.). Available online. URL: http://www.sanger.ac.uk. Accessed July 2, 2009. DNA sequencing center, named after Fred Sanger, inventor of the most commonly used method for sequencing DNA. The institute is also involved in projects that apply human DNA sequence data to find cures for cancer and other medical disorders.

U.S. Food and Drug Administration. Available online. URL: http://www.fda.gov. Accessed July 2, 2009. Provides extensive coverage of general health issues and regulations.

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