A PANORAMIC VIEW OF THE CELL (Chap 7 pg. 112)

Every organism is composed of one of two structurally different types of cells: prokaryotic cells or eukaryotic cells. Only the bacteria and archaea have prokaryotic cells. Protists, plants, fungi, and animals all have eukaryotic cells.

## Prokaryotic and eukaryotic cells differ in size and complexity

All cells have several basic features in common: They are all bounded by a membrane, called a plasma membrane. Within the membrane is a semifluid substance, cytosol, in which organelles are found. All cells contain chromosomes, carrying genes in the form of DNA. And all cells have ribosomes, tiny organelles that make proteins according to instructions from the genes.

A major difference between prokaryotic and eukaryotic cells, indicated by their names, is that the chromosomes of a eukaryotic cell are located in a membrane-enclosed organelle called the nucleus. The word prokaryotic is from the Greek pro, before, and karyon, kernel, referring here to the nucleus. In a prokaryotic cell (FIGURE 7.4), the DNA is concentrated in a region called the nucleoid, but no membrane separates this region from the rest of the cell. In contrast, the eukaryotic cell (Greek eu, true, and karyon) has a true nucleus, bounded by a membranous nuclear envelope (see FIGURES 7.7 and 7.8, pp. 114-115). The entire region between the nucleus and the plasma membrane is called the cytoplasm, a term also used for the interior of a prokaryotic cell. Within the cytoplasm of a eukaryotic cell, suspended in cytosol, are a variety of membrane-bounded organelles of specialized form and function. These are absent in prokaryotic cells. Thus, the presence or absence of a true nucleus is just one example of the disparity in structural complexity between the two types of cells.

Fig 7-4. A prokaryotic cell. Lacking a true nucleus and the other membrane-enclosed organelles of the eukaryotic cell, the prokaryotic cell is much simpler in structure. Only organisms of the domains Bacteria and Archaea have prokaryotic cells

Fig 7-7. Overview of an animal cell. This drawing of an animal cell incorporates the most common structures of animal cells (no cell actually looks just like this). The cell has a variety of organelles ("little organs"), many of which are bounded by membranes. The most prominent organelle in an animal cell is usually the nucleus. Most of the cell's metabolic activities occur in the cytoplasm, the entire region between the nucleus and the plasma membrane. The cytoplasm contains many organelles suspended in a semifluid medium, the cytosol. Pervading much of the cytoplasm is a labyrinth of membranes called the endoplasmic reticulum (ER).

Fig 7-8. Overview of a plant cell. This drawing of a generalized plant cell reveals the similarities and differences between an animal cell and a plant cell. In addition to most of the features seen in an animal cell, a plant cell has membrane-enclosed organelles called plastids. The most important type of plastid is the chloroplast, which carries out photosynthesis. Many plant cells have a large central vacuole. Outside a plant cell's plasma membrane is a thick cell wall, perforated by channels called plasmodesmata.

Eukaryotic cells are generally much bigger than prokaryotic cells (see FIGURE 7.1). Size is a general aspect of cell structure that relates to function. The logistics of carrying out metabolism set limits on cell size. At the lower limit, the smallest cells known are bacteria called mycoplasmas, which have diameters between 0.1 and 1.0 mm. These are perhaps the smallest packages with enough DNA to program metabolism and enough enzymes and other cellular equipment to carry out the activities necessary for a cell to sustain itself and reproduce. Most bacteria are 1-10 mm in diameter, a dimension about ten times bigger than that of mycoplasmas. Eukaryotic cells are typically 10-100 mm in diameter, ten times bigger than bacteria.

Metabolic requirements also impose upper limits on the size that is practical for a single cell. As an object of a particular shape increases in size, its volume grows proportionately more than its surface area. (Area is proportional to a linear dimension squared, whereas volume is proportional to the linear dimension cubed.) Thus, the smaller the object, the greater its ratio of surface area to volume (FIGURE 7.5).

Fig 7-5. Geometric relationships explain why most cells are microscopic. In this diagram, cells are represented as boxes. Using arbitrary units of length, we can calculate the cell's surface area (in square units), volume (in cubic units), and surface-to-volume ratio. A high surface-to-volume ratio facilitates the exchange of materials between a cell and its environment.

At the boundary of every cell, the plasma membrane functions as a selective barrier that allows sufficient passage of oxygen, nutrients, and wastes to service the entire volume of the cell (FIGURE 7.6). For each square micrometer of membrane, only so much of a particular substance can cross per second. Rates of chemical exchange with the extracellular environment might be inadequate to maintain a cell with a very large cytoplasm. The need for a surface sufficiently large to accommodate the volume helps explain the microscopic size of most cells. Larger organisms do not generally have larger cells than smaller organisms--simply more cells.

Fig 7-6. The plasma membrane. The plasma membrane and the membranes of organelles consist of a double layer (bilayer) of phospholipids with various proteins attached to or embedded in it. The phospholipid tails in the interior of a membrane are hydrophobic; the phospholipid heads, the exterior proteins and parts of proteins, and any carbohydrate side chains are hydrophilic and in contact with the aqueous solution on either side of the membrane. Carbohydrate side chains are found only on the outer surface of the plasma membrane. The specific functions of a membrane depend on the kinds of phospholipids and proteins present.

Prokaryotic cells will be described in detail in Chapters 18 and 27, and the possible evolutionary relationships between prokaryotic and eukaryotic cells will be discussed in Chapter 28. Most of the discussion of cell structure that follows in this chapter applies to eukaryotic cells.

## Internal membranes compartmentalize the functions of a eukaryotic cell

In addition to the plasma membrane at its outer surface, a eukaryotic cell has extensive and elaborately arranged internal membranes, which partition the cell into compartments--the membranous organelles mentioned earlier. These membranes also participate directly in the cell's metabolism; many enzymes are built right into the membranes. Because the cell's compartments provide different local environments that facilitate specific metabolic functions, incompatible processes can go on simultaneously inside the same cell.

Membranes of various kinds are fundamental to the organization of the cell. In general, biological membranes consist of a double layer of phospholipids and other lipids. Embedded in this lipid bilayer or attached to its surfaces are diverse proteins (see FIGURE 7.6). However, each type of membrane has a unique composition of lipids and proteins suited to that membrane's specific functions. For example, enzymes embedded in the membranes of the organelles called mitochondria function in cellular respiration.

Before continuing with this chapter, examine the overviews of eukaryotic cells in FIGURES 7.7 and 7.8 on these two pages. These figures introduce the various organelles and provide a map of the cell for the detailed tour upon which we will now embark. FIGURES 7.7 and 7.8 also contrast animal and plant cells. As eukaryotic cells, they have much more in common than either has with any prokaryote. As you will see, however, there are important differences between plant and animal cells.

## THE NUCLEUS AND RIBOSOMES

At the first stop of our detailed tour of the cell are two organelles involved in the genetic control of the cell: the nucleus, which houses most of the cell's DNA, and the ribosomes, which use information from the DNA to make proteins.

## The nucleus contains a eukaryotic cell's genetic library

The nucleus contains most of the genes in the eukaryotic cell (some genes are located in mitochondria and chloroplasts). It is generally the most conspicuous organelle in a eukaryotic cell, averaging about 5 mm in diameter. The nuclear envelope encloses the nucleus (FIGURE 7.9), separating its contents from the cytoplasm.

Fig 7-9. The nucleus and its envelope. Within the nucleus is chromatin, consisting of DNA and proteins. When a cell prepares to divide, individual chromosomes become visible as the chromatin condenses. The nucleolus functions in ribosome synthesis. The nuclear envelope, which consists of two membranes separated by a narrow space, is perforated with pores and lined by a nuclear lamina.

The nuclear envelope is a double membrane. The two membranes, each a lipid bilayer with associated proteins, are separated by a space of about 20-40 nm. The envelope is perforated by pores that are about 100 nm in diameter. At the lip of each pore, the inner and outer membranes of the nuclear envelope are fused. An intricate protein structure called a pore complex lines each pore and regulates the entry and exit of certain large macromolecules and particles. Except at the pores, the nuclear side of the envelope is lined by the nuclear lamina, a netlike array of protein filaments (intermediate filaments) that maintains the shape of the nucleus. There is also much evidence for a nuclear matrix, a framework of fibers extending throughout the nuclear interior. (We will examine possible functions of the nuclear lamina and matrix in Chapter 19.)

Within the nucleus, the DNA is organized along with proteins into a fibrous material called chromatin. Stained chromatin usually appears through both light microscopes and electron microscopes as a diffuse mass. As a cell prepares to divide (reproduce), however, the thin chromatin fibers coil up (condense), becoming thick enough to be discerned as separate structures called chromosomes. Each eukaryotic species has a characteristic number of chromosomes. A typical human cell, for example, has 46 chromosomes in its nucleus; the exceptions are the sex cells (eggs and sperm), which have only 23 chromosomes in humans.

A prominent structure within the nondividing nucleus is the nucleolus, which appears through the electron microscope as a mass of densely stained granules and fibers adjoining part of the chromatin. Here a special type of RNA called ribosomal RNA is synthesized and assembled with proteins imported from the cytoplasm into the main components of ribosomes, called ribosomal subunits. These subunits then pass through the nuclear pores to the cytoplasm, where they can combine to form ribosomes. Sometimes there are two or more nucleoli; the number depends on the species and the stage in the cell's reproductive cycle.

As we saw in FIGURE 5.28, the nucleus directs protein synthesis by synthesizing messenger RNA (mRNA) and sending it to the cytoplasm via the nuclear pores. The mRNA is made according to instructions provided by the DNA (as is ribosomal RNA). Once an mRNA molecule reaches the cytoplasm, ribosomes translate its genetic message into the primary structure of a specific polypeptide. This process of translating genetic information is described in detail in Chapter 17.

## Ribosomes build a cell's proteins

Ribosomes, particles made of ribosomal RNA and protein, are the organelles that carry out protein synthesis; each is composed of two subunits (FIGURE 7.10). Cells that have high rates of protein synthesis have a particularly large number of ribosomes. For example, a human pancreas cell has a few million ribosomes. Not surprisingly, cells active in protein synthesis also have prominent nucleoli. (Keep in mind that both nucleoli and ribosomes, unlike most other organelles, are not enclosed in membrane.)

Fig 7-10. Ribosomes. (a) This electron micrograph of part of a pancreas cell shows many ribosomes, both free (in the cytosol) and bound (to the endoplasmic reticulum). The bound ribosomes of pancreas cells make a number of secretory proteins, including the hormone insulin and digestive enzymes. Bound ribosomes also make proteins destined for insertion into membranes or the interiors of other organelles. Free ribosomes mainly make proteins that remain dissolved in the cytosol. Bound and free ribosomes are identical and can alternate between these two roles. (b) This simplified diagram of a ribosome shows its two subunits.

Ribosomes build proteins in two cytoplasmic locales (see FIGURE 7.10). Free ribosomes are suspended in the cytosol, while bound ribosomes are attached to the outside of the endoplasmic reticulum or nuclear envelope. Most of the proteins made by free ribosomes will function within the cytosol; examples are enzymes that catalyze the first steps of sugar breakdown. Bound ribosomes generally make proteins that are destined either for insertion into membranes, for packaging within certain organelles such as lysosomes, or for export from the cell (secretion). Cells that specialize in protein secretion--for instance, the cells of the pancreas and other glands that secrete digestive enzymes--frequently have a high proportion of bound ribosomes. Bound and free ribosomes are structurally identical and can alternate between the two roles; the cell adjusts the relative numbers of each as its metabolism changes. You will learn more about ribosome structure and function in Chapter 17.

## THE ENDOMEMBRANE SYSTEM

Many of the different membranes of the eukaryotic cell are part of an endomembrane system. These membranes are related either through direct physical continuity or by the transfer of membrane segments as tiny vesicles (sacs made of membrane). Despite these relationships, the various membranes are not identical in structure and function. Moreover, the thickness, molecular composition, and metabolic behavior of a membrane are not fixed, but may be modified several times during the membrane's life. The endomembrane system includes the nuclear envelope, endoplasmic reticulum, Golgi apparatus, lysosomes, various kinds of vacuoles, and the plasma membrane (not actually an endomembrane in physical location, but nevertheless related to the endoplasmic reticulum and other internal membranes). We have already discussed the nuclear envelope and will now focus on the endoplasmic reticulum and the other endomembranes to which it gives rise.

## The endoplasmic reticulum manufactures membranes and performs many other biosynthetic functions

The endoplasmic reticulum (ER) is a membranous labyrinth so extensive that it accounts for more than half the total membrane in many eukaryotic cells. (The word endoplasmic means "within the cytoplasm," and reticulum is Latin for "little net.") The ER consists of a network of membranous tubules and sacs called cisternae (Latin, cisterna, a reservoir for a liquid). The ER membrane separates the internal compartment of the ER, called the cisternal space, from the cytosol. And because the ER membrane is continuous with the nuclear envelope, the space between the two membranes of the envelope is continuous with the cisternal space of the ER (FIGURE 7.11).

Fig 7-11. Endoplasmic reticulum (ER). A membranous system of interconnected tubules and flattened sacs called cisternae, the ER is also continuous with the nuclear envelope. (The drawing is a cutaway view.) The membrane of the ER encloses a compartment called the cisternal space. Rough ER, which is studded on its outer surface with ribosomes, can be distinguished from smooth ER in the electron micrograph (TEM).

There are two distinct, though connected, regions of ER that differ in structure and function: smooth ER and rough ER. Smooth ER is so named because its cytoplasmic surface lacks ribosomes. Rough ER appears rough through the electron microscope because ribosomes stud the cytoplasmic surface of the membrane. As already mentioned, ribosomes are also attached to the cytoplasmic side of the nuclear envelope's outer membrane, which is confluent with rough ER.

## Functions of Smooth ER

The smooth ER of various cell types functions in diverse metabolic processes, including synthesis of lipids, metabolism of carbohydrates, and detoxification of drugs and poisons.

Enzymes of the smooth ER are important to the synthesis of lipids, including oils, phospholipids, and steroids. Among the steroids produced by the smooth ER in animal cells are the sex hormones of vertebrates and the various steroid hormones secreted by the adrenal glands. The cells that actually synthesize and secrete these hormones--in the testes and ovaries, for example--are rich in smooth ER, a structural feature that fits the function of these cells.

Liver cells provide one example of the role of smooth ER in carbohydrate metabolism. Liver cells store carbohydrate in the form of glycogen, a polysaccharide. The hydrolysis of glycogen leads to the release of glucose from the liver cells, which is important in the regulation of sugar concentration in the blood. However, the first product of glycogen hydrolysis is glucose phosphate, an ionic form of the sugar that cannot exit the cell and enter the blood. An enzyme embedded in the membrane of the liver cell's smooth ER removes the phosphate from the glucose, which can then leave the cell.

Enzymes of the smooth ER help detoxify drugs and poisons, especially in liver cells. Detoxification usually involves adding hydroxyl groups to drugs, making them more soluble and easier to flush from the body. The sedative phenobarbital and other barbiturates are examples of drugs metabolized in this manner by smooth ER in liver cells. In fact, barbiturates, alcohol, and many other drugs induce the proliferation of smooth ER and its associated detoxification enzymes. This, in turn, increases tolerance to the drugs, meaning that higher doses are required to achieve a particular effect, such as sedation. Also, because some of the detoxification enzymes have relatively broad action, the proliferation of smooth ER in response to one drug can increase tolerance to other drugs as well. Barbiturate abuse, for example, may decrease the effectiveness of certain antibiotics and other useful drugs.

Muscle cells exhibit still another specialized function of smooth ER. The ER membrane pumps calcium ions from the cytosol into the cisternal space. When a muscle cell is stimulated by a nerve impulse, calcium rushes back across the ER membrane into the cytosol and triggers contraction of the muscle cell.

## Rough ER and the Synthesis of Secretory Proteins

Many types of specialized cells secrete proteins produced by ribosomes attached to rough ER. For example, certain cells in the pancreas secrete the protein insulin, a hormone, into the bloodstream (see FIGURE 7.10a). As a polypeptide chain grows from a bound ribosome, it is threaded into the cisternal space through a pore formed by a protein in the ER membrane. As it enters the cisternal space, the new protein folds into its native conformation. Most secretory proteins are glycoproteins, proteins that are covalently bonded to carbohydrates. The carbohydrate is attached to the protein in the ER by specialized molecules built into the ER membrane. The carbohydrate appendage of a glycoprotein is an oligosaccharide, the term for a relatively small polymer of sugar units.

Once secretory proteins are formed, the ER membrane keeps them separate from the proteins, produced by free ribosomes, that will remain in the cytosol. Secretory proteins depart from the ER wrapped in the membranes of vesicles that bud like bubbles from a specialized region called transitional ER. Such vesicles in transit from one part of the cell to another are called transport vesicles, and we will soon learn their fate.

## Rough ER and Membrane Production

In addition to making secretory proteins, rough ER is a membrane factory that grows in place by adding proteins and phospholipids. As polypeptides destined to be membrane proteins grow from the ribosomes, they are inserted into the ER membrane itself and are anchored there by their hydrophobic portions. The rough ER also makes its own membrane phospholipids; enzymes built into the ER membrane assemble phospholipids from precursors in the cytosol. The ER membrane expands and can be transferred in the form of transport vesicles to other components of the endomembrane system.

## The Golgi apparatus finishes, sorts, and ships cell products

After leaving the ER, many transport vesicles travel to the Golgi apparatus. We can think of the Golgi as a center of manufacturing, warehousing, sorting, and shipping. Here, products of the ER are modified and stored and then sent to other destinations. Not surprisingly, the Golgi apparatus is especially extensive in cells specialized for secretion.

The Golgi apparatus consists of flattened membranous sacs--cisternae--looking like a stack of pita bread (FIGURE 7.12). A cell may have several or even hundreds of these stacks. The membrane of each cisterna in a stack separates its internal space from the cytosol. Vesicles concentrated in the vicinity of the Golgi apparatus are engaged in the transfer of material between the Golgi and other structures.

Fig 7-12. The Golgi apparatus. The Golgi apparatus consists of stacks of flattened sacs, or cisternae, which, unlike ER cisternae, are not physically connected. (The drawing is a cutaway view.) A Golgi stack receives and dispatches transport vesicles and the products they contain. Materials received from the ER are modified and stored in the Golgi and eventually shipped to the cell surface or other destinations. Note the vesicles joining and leaving the cisternae. A Golgi stack has a structural and functional polarity, with a cis face that receives vesicles containing ER products and a trans face that dispatches vesicles (at right, TEM).

A Golgi stack has a distinct polarity, with the membranes of cisternae at opposite ends of the stack differing in thickness and molecular composition. The two poles of a Golgi stack are referred to as the cis face and the trans face; these act, respectively, as the receiving and shipping departments of the Golgi apparatus. The cis face is usually located near the ER. Transport vesicles move material from the ER to the Golgi. A vesicle that buds from the ER will add its membrane and the contents of its lumen (cavity) to the cis face by fusing with a Golgi membrane. The trans face gives rise to vesicles, which pinch off and travel to other sites.

Products of the ER are usually modified during their transit from the cis pole to the trans pole of the Golgi. Proteins and phospholipids of membranes may be altered. For example, various Golgi enzymes modify the oligosaccharide portions of glycoproteins. Oligosaccharides are first added to proteins in the rough ER, often during the process of polypeptide synthesis. The resulting glycoprotein is then modified as it passes through the rest of the ER and the Golgi. The Golgi removes some sugar monomers and substitutes others, producing a large variety of oligosaccharides.

In addition to its finishing work, the Golgi apparatus manufactures certain macromolecules by itself. Many polysaccharides secreted by cells are Golgi products, including pectins and certain other noncellulose polysaccharides made by plant cells and incorporated along with cellulose into their cell walls. (Cellulose is made by enzymes located within the plasma membrane, which directly deposit this polysaccharide on the outside surface.) Golgi products that will be secreted depart from the trans face of the Golgi inside transport vesicles that eventually fuse with the plasma membrane.

The Golgi manufactures and refines its products in stages, with different cisternae between the cis and trans ends containing unique teams of enzymes. Products in various stages of processing seem to be transferred from one cisterna to the next by vesicles.

Before a Golgi stack dispatches its products by budding vesicles from the trans face, it sorts these products and targets them for various parts of the cell. Molecular identification tags, such as phosphate groups that have been added to the Golgi products, aid in sorting. Finally, transport vesicles budded from the Golgi may have external molecules on their membranes that recognize "docking sites" on the surface of specific organelles or on the plasma membrane.

## Lysosomes are digestive compartments

A lysosome is a membrane-bounded sac of hydrolytic enzymes that the cell uses to digest macromolecules (FIGURE 7.13). There are lysosomal enzymes that can hydrolyze proteins, polysaccharides, fats, and nucleic acids--all the major classes of macromolecules. These enzymes work best in an acidic environment, at about pH 5. The lysosomal membrane maintains this low internal pH by pumping hydrogen ions from the cytosol into the lumen of the lysosome. If a lysosome breaks open or leaks its contents, the released enzymes are not very active, because the cytosol has a neutral pH. However, excessive leakage from a large number of lysosomes can destroy a cell by autodigestion. From this example we can see once again how important compartmental organization is to the functions of the cell: The lysosome provides a space where the cell can digest macromolecules safely, without the general destruction that would occur if hydrolytic enzymes roamed at large.

Fig 7-13. Lysosomes. (a) In this white blood cell from a rat, the lysosomes are very dark because of a specific stain that reacts with one of the products of digestion within the lysosome. This type of white blood cell ingests bacteria and viruses and destroys them in the lysosomes (TEM). (b) In the cytoplasm of this rat liver cell, an autophagic lysosome has engulfed two disabled organelles, a mitochondrion and a peroxisome (TEM).

Hydrolytic enzymes and lysosomal membrane are made by rough ER and then transferred to the Golgi apparatus for further processing. At least some lysosomes probably arise by budding from the trans face of the Golgi apparatus (FIGURE 7.14, p. 122). Proteins of the inner surface of the lysosomal membrane and the digestive enzymes themselves are probably spared from destruction by having three-dimensional conformations that protect vulnerable bonds from enzymatic attack.

Fig 7-14. The formation and functions of lysosomes. The ER and Golgi apparatus generally cooperate in the production of lysosomes containing active hydrolytic enzymes. Lysosomes digest (hydrolyze) materials taken into the cell and recycle materials from intracellular refuse. This figure shows one lysosome fusing with a food vacuole and another engulfing a damaged mitochondrion.

Lysosomes carry out intracellular digestion in a variety of circumstances. Amoebas and many other protists eat by engulfing smaller organisms or other food particles, a process called phagocytosis (from the Greek phagein, to eat, and kytos, vessel, referring here to the cell). The food vacuole formed in this way then fuses with a lysosome, whose enzymes digest the food (see FIGURE 7.14). Digestion products, including simple sugars, amino acids, and other monomers, pass into the cytosol and become nutrients for the cell. Some human cells also carry out phagocytosis. Among them are macrophages, cells that help defend the body by destroying bacteria and other invaders (see FIGURE 7.13a).

Lysosomes also use their hydrolytic enzymes to recycle the cell's own organic material, a process called autophagy.This occurs when a lysosome engulfs another organelle or a small amount of cytosol (see FIGURE 7.13b). The lysosomal enzymes dismantle the ingested material, and the organic monomers are returned to the cytosol for reuse. With the help of lysosomes, the cell continually renews itself. A human liver cell, for example, recycles half of its macromolecules each week.

Programmed destruction of cells by their own lysosomal enzymes is important in the development of many multicellular organisms. During the transforming of a tadpole into a frog, for instance, lysosomes in the cells of the tail destroy these cells. And the hands of human embryos are webbed until lysosomes digest the tissue between the fingers.

A variety of inherited disorders called lysosomal storage diseases affect lysosomal metabolism. A person afflicted with a storage disease lacks a functioning version of a hydrolytic enzyme normally present in lysosomes. The lysosomes become engorged with indigestible substrates, which begin to interfere with other cellular activities. In Pompe's disease, for example, the liver is damaged by an accumulation of glycogen due to the absence of a lysosomal enzyme needed to break down that polysaccharide. In Tay-Sachs disease, a lipid-digesting enzyme is missing or inactive, and the brain becomes impaired by an accumulation of lipids in the cells. Fortunately, storage diseases are rare in the general population. In the future, it may be possible to cure such a disorder by inserting genes (DNA) for the missing enzyme into the appropriate cells (see Chapter 20).

## Vacuoles have diverse functions in cell maintenance

Vacuoles and vesicles are both membrane-bounded sacs within the cell, but vacuoles are larger than vesicles. Vacuoles have various functions. Food vacuoles, formed by phagocytosis, have already been mentioned (see FIGURE 7.14). Many freshwater protists have contractile vacuoles that pump excess water out of the cell (see FIGURE 8.13). Mature plant cells generally contain a large central vacuole (FIGURE 7.15) enclosed by a membrane called the tonoplast, which is part of their endomembrane system. The central vacuole develops by the coalescence of smaller vacuoles, themselves derived from the endoplasmic reticulum and Golgi apparatus. The vacuole is in this way an integral part of the endomembrane system. Like all cellular membranes, the tonoplast is selective in transporting solutes; therefore, the solution inside the vacuole, called cell sap, differs in composition from the cytosol.

Fig 7-15. The plant cell vacuole. The central vacuole is usually the largest compartment in a plant cell, comprising 80% or more of a mature cell. The rest of the cytoplasm is generally confined to a narrow zone between the vacuolar membrane (tonoplast) and the plasma membrane. Functions of the vacuole include storage, waste disposal, protection, and growth (TEM).

The plant cell's central vacuole is a versatile compartment. It can hold reserves of important organic compounds, such as the proteins stockpiled in the vacuoles of storage cells in seeds. It is also the plant cell's main repository of inorganic ions, such as potassium and chloride. Many plant cells use their vacuoles as disposal sites for metabolic by-products that would endanger the cell if they accumulated in the cytosol. Some vacuoles contain pigments that color the cells, such as the red and blue pigments of petals that help attract pollinating insects to flowers. Vacuoles may also help protect the plant against predators by containing compounds that are poisonous or unpalatable to animals. The vacuole has a major role in the growth of plant cells, which elongate as their vacuoles absorb water, enabling the cell to become larger with a minimal investment in new cytoplasm. And because the cytosol often occupies only a thin layer between the plasma membrane and the tonoplast, the ratio of membrane surface to cytosolic volume is great, even for a large plant cell.

FIGURE 7.16 reviews the endomembrane system, showing the flow of membranes through the various organelles. As the membrane moves from the ER to the Golgi and then elsewhere, its molecular composition and metabolic functions are modified. The endomembrane system is a complex and dynamic player in the cell's compartmental organization.

Fig 7-16. Review: relationships among organelles of the endomembrane system. The red arrows show some of the pathways of membrane migration. The nuclear envelope is connected to the rough ER, which is also confluent with smooth ER. Membrane produced by the ER flows in the form of transport vesicles to the Golgi, which in turn pinches off vesicles that give rise to lysosomes and vacuoles. Even the plasma membrane expands by the fusion of vesicles born in the ER and Golgi. Coalescence of vesicles with the plasma membrane also releases secretory proteins and other products to the outside of the cell.

We'll continue our tour of the cell with some membranous organelles that are not closely related to the endomembrane system.

## OTHER MEMBRANOUS ORGANELLES

## Mitochondria and chloroplasts are the main energy transformers of cells

Organisms are open systems that transform the energy they acquire from their surroundings. In eukaryotic cells, mitochondria and chloroplasts are the organelles that convert energy to forms that cells can use for work. Mitochondria (singular, mitochondrion) are the sites of cellular respiration, the catabolic process that generates ATP by extracting energy from sugars, fats, and other fuels with the help of oxygen. Chloroplasts, found only in plants and algae, are the sites of photosynthesis. They convert solar energy to chemical energy by absorbing sunlight and using it to drive the synthesis of organic compounds from carbon dioxide and water.

Although mitochondria and chloroplasts are enclosed by membranes, they are not part of the endomembrane system. Their membrane proteins are made not by the ER, but by free ribosomes in the cytosol and by ribosomes contained within the mitochondria and chloroplasts themselves. Not only do these organelles have ribosomes, but they also contain a small amount of DNA. It is this DNA that programs the synthesis of the proteins made on the organelle's own ribosomes. (Proteins imported from the cytosol--constituting most of the organelle's proteins--are programmed by nuclear DNA.) Mitochondria and chloroplasts are semiautonomous organelles that grow and reproduce within the cell. In Chapters 9 and 10, we will focus on how mitochondria and chloroplasts function. We will consider the evolution of these organelles in Chapter 28. Here we are concerned mainly with the structure of these energy transformers.

## Mitochondria

Mitochondria are found in nearly all eukaryotic cells, including those of plants, animals, fungi, and protists. Some cells have a single large mitochondrion, but more often a cell has hundreds or even thousands of mitochondria; the number is correlated with the cell's level of metabolic activity. Mitochondria are about 1-10 mm long. Time-lapse films of living cells reveal mitochondria moving around, changing their shapes, and dividing in two, unlike the static cylinders seen in electron micrographs of dead cells.

The mitochondrion is enclosed by two membranes, each a phospholipid bilayer with a unique collection of embedded proteins (FIGURE 7.17). The outer membrane is smooth, but the inner membrane is convoluted, with infoldings called cristae. The inner membrane divides the mitochondrion into two internal compartments. The first is the intermembrane space, the narrow region between the inner and outer membranes. The second compartment, the mitochondrial matrix, is enclosed by the inner membrane. The matrix contains many different enzymes as well as the mitochondrial DNA and ribosomes. Some of the metabolic steps of cellular respiration are catalyzed by enzymes in the matrix. Other proteins that function in respiration, including the enzyme that makes ATP, are built into the inner membrane. The cristae give the inner mitochondrial membrane a large surface area that enhances the productivity of cellular respiration, another example of structure fitting function.

Fig 7-17. The mitochondrion, site of cellular respiration. The two membranes of the mitochondrion are evident in the drawing and micrograph (TEM). The cristae are infoldings of the inner membrane. The cutaway drawing shows the two compartments bounded by the membranes: the intermembrane space and the mitochondrial matrix.

## Chloroplasts

The chloroplast is a specialized member of a family of closely related plant organelles called plastids. Amyloplasts are colorless plastids that store starch (amylose), particularly in roots and tubers. Chromoplasts have pigments that give fruits and flowers their orange and yellow hues. Chloroplasts contain the green pigment chlorophyll, along with enzymes and other molecules that function in the photosynthetic production of sugar. These lens-shaped organelles, measuring about 2 mm by 5 mm, are found in leaves and other green organs of plants and in algae (FIGURE 7.18).

Fig 7-18. The chloroplast, site of photosynthesis. Chloroplasts are enclosed by two membranes separated by a narrow intermembrane space that constitutes an outer compartment. The inner membrane encloses a second compartment, containing a fluid called stroma. The stroma surrounds a third compartment, the thylakoid space, delineated by the thylakoid membrane. Thylakoid sacs (thylakoids) are stacked to form structures called grana, which are connected by thin tubules between individual thylakoids (TEM).

The contents of a chloroplast are partitioned from the cytosol by an envelope consisting of two membranes separated by a very narrow intermembrane space. Inside the chloroplast is another membranous system in the form of flattened sacs called thylakoids. In some regions, thylakoids are stacked like poker chips; each stack is called a granum (plural, grana). The fluid outside the thylakoids is the stroma, which contains the chloroplast DNA and ribosomes as well as many enzymes. Note that the thylakoid membrane divides the interior of the chloroplast into two compartments: the thylakoid space and the stroma. In Chapter 10, you will learn how this compartmental organization enables the chloroplast to convert light energy to chemical energy during photosynthesis.

As with mitochondria, the static and rigid appearance of chloroplasts in micrographs is not true to their dynamic behavior in the living cell. Their shapes are plastic, and they grow and occasionally pinch in two, reproducing themselves. They are mobile and move around the cell with mitochondria and other organelles along tracks of the cytoskeleton.

## The mitotic phase alternates with interphase in the cell cycle: an overview (pg. 217-223)

Mitosis is just one part of the cell cycle (FIGURE 12.4). In fact, the mitotic (M) phase, which includes both mitosis and cytokinesis, is usually the shortest part of the cell cycle. Mitotic cell division alternates with a much longer interphase, which often accounts for about 90% of the cycle. It is during interphase that the cell grows and copies its chromosomes in preparation for cell division. Interphase can be divided into subphases: the G1phase ("first gap"), the S phase, and the G2phase ("second gap"). During all three subphases, the cell grows by producing proteins and cytoplasmic organelles. However, chromosomes are duplicated only during the S phase (S stands for synthesis of DNA). Thus, a cell grows (G1), continues to grow as it copies its chromosomes (S), grows more as it completes preparations for cell division (G2), and divides (M). The daughter cells may then repeat the cycle.

Fig 12-4. The cell cycle. In a dividing cell, the mitotic (M) phase alternates with interphase, a growth period. The first part of interphase, called G1, is followed by the S phase, when the chromosomes replicate; the last part of interphase is called G2. In the M phase, mitosis divides the nucleus and distributes its chromosomes to the daughter nuclei, and cytokinessis divides the cytoplasm, producing two daughter cells.

Time-lapse films of living, dividing cells reveal the dynamics of mitosis as a continuum of changes. For purposes of description, however, mitosis is conventionally broken down into five subphases: prophase, prometaphase, metaphase, anaphase, and telophase. FIGURE 12.5, on pages 218-219, describes these stages in an animal cell. Be sure to study this figure thoroughly before progressing to the next section, which examines mitosis more closely.

Fig 12-5. The stages of mitotic cell division in an animal cell. The light micrographs show dividing lung cells from a newt, which has 22 chromosomes in its somatic cells. The chromosomes appear blue and the microtubules green. (The red fibers are intermediate filaments.) The schematic drawings show details not visible in the micrographs. For the sake of simplicity, only four chromosomes are drawn. (In plant cells, centrioles are lacking and cytokinesis occurs differently.

## The mitotic spindle distributes chromosomes to daughter cells: a closer look

Many of the events of mitosis depend on the mitotic spindle, which begins to form in the cytoplasm during prophase. This structure consists of fibers made of microtubules and associated proteins. While the mitotic spindle assembles, the microtubules of the cytoskeleton partially disassemble, probably providing the material used to construct the spindle. The spindle microtubules elongate by incorporating more subunits of the protein tubulin (see TABLE 7.2).

The assembly of spindle microtubules starts in the centrosome, a nonmembranous organelle that functions throughout the cell cycle to organize the cell's microtubules (it is also called the microtubule-organizing center). In animal cells, a pair of centrioles is located at the center of the centrosome, but the centrioles are not essential for cell division. In fact, the centrosomes of most plants lack centrioles, and if a researcher destroys the centrioles of an animal cell with a laser micro beam, a spindle nevertheless forms during mitosis.

During interphase, the single centrosome replicates to form two centrosomes (see FIGURE 12.5). As mitosis starts, the two centrosomes are located near the nucleus; they then move apart from each other during prophase and prometaphase, as spindle microtubules grow out from them. By the end of prometaphase, the two centrosomes, referred to as spindle poles in this context, are at opposite poles of the cell.

Each of the two joined chromatids of a chromosome has a kinetochore, a structure of proteins and specific sections of chromosomal DNA at the centromere. The chromosome's two kinetochores face in opposite directions. During prometaphase, some of the spindle microtubules attach to the kinetochores. When one of a chromosome's kinetochores is "captured" by microtubules, the chromosome begins to move toward the pole from which those microtubules come. However, this movement is checked as soon as microtubules from the opposite pole attach to the other kinetochore. What happens next is like a tug-of-war that ends in a draw. The chromosome moves first in one direction, then the other, back and forth, finally settling midway between the two poles of the cell (FIGURE 12.6). Meanwhile, microtubules that do not attach to kinetochores interact with nonkinetochore microtubules from the opposite pole of the cell. At metaphase, these microtubules overlap, and the centromeres of all the duplicated chromosomes are on a plane midway between the two poles. This plane is called the metaphase plate of the cell. The spindle is now complete.

Fig 12-6. The mitotic spindle at metaphase.

Let's now see how the structure of the completed spindle correlates with its function during anaphase. Anaphase commences suddenly when proteins holding together the sister chromatids of each chromosome are inactivated. Now that the chromatids are separate, full-fledged chromosomes, they move toward opposite poles of the cell. How do the kinetochore microtubules function in this poleward movement of chromosomes? Experimental evidence supports the hypothesis that kinetochores are equipped with motor proteins that "walk" a chromosome along the attached microtubules toward the nearest pole. Meanwhile, the microtubules shorten by depolymerizing at their kinetochore ends (FIGURE 12.7). (To review how motor proteins move an object along a microtubule, see FIGURE 7.21b.)

Fig 12-7. Testing a hypothesis for chromosome migration during anaphase.

What is the function of the nonkinetochore microtubules? In a dividing animal cell, these microtubules are responsible for elongating the whole cell during anaphase (see FIGURE 12.5). Nonkinetochore microtubules interdigitate across the metaphase plate, and during anaphase ones originating from opposite spindle poles move past each other toward their poles. The mechanism seems to be similar to the one that slides neighboring microtubules in a flagellum: Motor proteins attached to the nonkinetochore microtubules drive them past one another, using energy from ATP (see FIGURE 7.21a). At the same time, the nonkinetochore microtubules lengthen by the addition of tubulin subunits to their ends.

At the end of anaphase, duplicate sets of chromosomes have arrived at opposite poles of the elongated parent cell. Nuclei re-form during telophase. Cytokinesis generally begins during this last stage of mitosis.

## Cytokinesis divides the cytoplasm: a closer look

In animal cells, cytokinesis occurs by a process known as cleavage. The first sign of cleavage is the appearance of a cleavage furrow, which begins as a shallow groove in the cell surface near the old metaphase plate (FIGURE 12.8a). On the cytoplasmic side of the furrow is a contractile ring of actin microfilaments associated with molecules of the protein myosin. Actin and myosin are the same proteins responsible for muscle contraction, as well as many other kinds of cell movement. The contraction of the dividing cell's ring of microfilaments is like the pulling of drawstrings. The cleavage furrow deepens until the parent cell is pinched in two, producing two completely separated cells.

Fig 12-8. Cytokinesis in animal and plant cells.

Cytokinesis in plant cells, which have walls, is markedly different. There is no cleavage furrow. Instead, during telophase, vesicles derived from the Golgi apparatus move along microtubules to the middle of the cell, where they coalesce, producing a cell plate (FIGURE 12.8b). Cell wall materials carried in the vesicles collect in the cell plate as it grows. The cell plate enlarges until its surrounding membrane fuses with the plasma membrane along the perimeter of the cell. Two daughter cells result, each with its own plasma membrane. Meanwhile, a new cell wall arising from the contents of the cell plate has formed between the daughter cells.

FIGURE 12.9 is a series of micrographs of a dividing plant cell. Examining this figure will help you review mitosis and cytokinesis.

Fig 12-9. Mitosis in a plant cell. These light micrographs show mitosis in cells of an onion root.

## Mitosis in eukaryotes may have evolved from binary fission in bacteria

The complex cellular choreography of mitotic cell division solves the problem of correctly distributing copies of eukaryotic genomes to daughter cells. How did mitosis evolve? Given that prokaryotes preceded eukaryotes on Earth by billions of years, we might hypothesize that mitosis had its origins in simpler bacterial mechanisms of cell reproduction.

Prokaryotes (bacteria) reproduce by a type of cell division called binary fission, meaning literally "division in half." Most bacterial genes are carried on a single bacterial chromosome that consists of a circular DNA molecule and associated proteins. Although bacteria are smaller and simpler than eukaryotic cells, the problem of replicating their genomes in an orderly fashion and distributing the copies equally to two daughter cells is still formidable. The chromosome of the bacterium Escherichia coli, for example, when it is fully stretched out, is about 500 times longer than the length of the cell. Clearly, such a long chromosome must be highly coiled and folded within the cell--and it is.

Prokaryotes do not have mitotic spindles, so what brings about the separation of the two daughter chromosomes in a dividing bacterial cell? A hypothesis proposed in the 1960s suggested that separation of bacterial chromosomes results simply from the growth of new plasma membrane between two sites on the membrane where the chromosome copies are attached. Recent research, however, has challenged this model (FIGURE 12.10, p. 224). Rather than being a passive process, separation of daughter bacterial chromosomes involves active chromosomal movement. Once the DNA of the chromosome begins to replicate, the copies of the first replicated region--called the origin of replication--move apart rapidly. Using the techniques of modern DNA technology to tag the origins of replication with molecules that glow green in fluorescence microscopy (see TABLE 7.1), researchers have directly observed the movement of bacterial chromosomes. This movement is reminiscent of the poleward movements of the centromere regions of eukaryotic chromosomes during anaphase of mitosis, even though bacteria don't have mitotic spindles or even microtubules. How bacterial chromosomes move is still a mystery. The idea that prokaryotes might have molecules in any way like the microtubules and motor proteins used in eukaryotic mitosis is surprising and intriguing.

Fig 12-10. Bacterial cell division (binary fission). The single, circular bacterial chromosome replicates, and the two copies move apart by an unknown mechanism. Meanwhile, the cell grows in size. When chromosomal replication is complete, the plasma membrane grows inward to divide the cell in two as a new cell wall is deposited between the daughter cells. The example shown here is the bacterium Escherichia coli.

While the bacterial chromosome is replicating, the cell is growing. When replication is complete and the bacterium has reached about twice its initial size, its plasma membrane grows inward, dividing the parent cell into two daughter cells. Each cell inherits a complete genome.

As eukaryotes evolved, along with their larger genomes and nuclear envelopes, the ancestral process of binary fission somehow gave rise to mitosis. FIGURE 12.11 traces a hypothesis for the stepwise evolution of mitosis. Possible intermediate stages are represented by two unusual types of nuclear division found in certain modern unicellular algae. In both types, the nuclear envelope remains intact. In dinoflagellates, replicated chromosomes are attached to the nuclear envelope and separate as it elongates prior to cell division. In diatoms, a spindle within the nucleus separates the chromosomes.

Fig 12-11. A hypothesis for the evolution of mitosis. Researchers interested in the evolution of eukaryotic cell division have observed in modern organisms what they believe are mechanisms of division intermediate between the binary fission of bacteria and mitosis as it occurs in most eukaryotes. These schematic diagrams of a proposed evolutionary sequence do not show cell walls.

## Meiosis reduces chromosome number from diploid to haploid: a closer look (pgs 239-245)

Many of the steps of meiosis closely resemble corresponding steps in mitosis. Meiosis, like mitosis, is preceded by the replication of chromosomes. However, this single replication is followed by two consecutive cell divisions, called meiosis I and meiosis II. These divisions result in four daughter cells (rather than the two daughter cells of mitosis), each with only half as many chromosomes as the parent cell. Study the overview of meiosis in FIGURE 13.6, and be sure you understand the difference between homologous chromosomes and sister chromatids. The two chromosomes of a homologous pair are individual chromosomes that were inherited from different parents. Homologues appear alike in the microscope, but they have different versions of genes at some of their corresponding loci (for example, a gene for freckles on one chromosome and a gene for the absence of freckles at the same locus on the homologue).

Fig 13-6. Overview of meiosis: how meiosis reduces chromosome number. After the chromosomes replicate once, the diploid cell divides twice, yielding four haploid daughter cells. This overview tracks just one pair of homologous chromosomes, which for the sake of simplicity are drawn in the condensed state throughout (they would not normally be condensed during interphase). Homologues in this and later figures are colored red and blue to remind you that they carry different versions of some genes.

FIGURE 13.7 on pages 240-241 describes in some detail the two divisions of meiosis for an animal cell whose diploid number is 4. Study FIGURE 13.7 thoroughly before going on to the next section.

Fig 13-7. The stages of meiotic cell division. These diagrams show meiotic cell division for an animal cell with a diploid number of 4 (2n = 4). The behavior of the chromosomes is emphasized by the use of red and blue to differentiate the members of each homologous pair. For a discussion of the spindle and other features common to mitosis and meiosis, see Chapter 12.

## Mitosis and Meiosis Compared

Now that you have followed chromosomes through meiosis in FIGURE 13.7, let's summarize the key differences between meiosis and mitosis. The chromosome number is reduced by half in meiosis but not in mitosis. The genetic consequences of this difference are important. Whereas mitosis produces daughter cells genetically identical to their parent cell and to each other, meiosis produces cells that differ genetically from their parent cell and from each other.

FIGURE 13.8 compares the key steps of mitosis and meiosis. Although meiosis involves two cell divisions, three events that are unique to meiosis all occur during the first of the divisions, meiosis I:

Fig 13-8. A comparison of mitosis and meiosis. (In this chapter, we consider prophase to include prometaphase.)

During prophase I of meiosis, the duplicated chromosomes pair with their homologues, a process called synapsis. For part of prophase I, a protein "zipper"--the synaptonemal complex--holds the homologous chromosomes tightly together all along their lengths. When the synaptonemal complex disappears in late prophase, the four closely associated chromatids of a homologous pair are visible in the light microscope as a tetrad. Also visible in the light microscope are X-shaped regions called chiasmata (singular, chiasma). They represent a crossing of nonsister chromatids, which are two chromatids belonging to separate but homologous chromosomes. Chiasmata are the physical manifestations of a genetic rearrangement called crossing over, discussed in the next section. Neither synapsis nor chiasma formation occurs during mitosis.

At metaphase I of meiosis, homologous pairs of chromosomes, rather than individual chromosomes, align on the metaphase plate.

At anaphase I of meiosis, sister chromatids do not separate, as they do in mitosis. Rather, the two sister chromatids of each chromosome remain attached and go to the same pole of the cell. Meiosis I separates homologous pairs of chromosomes, not sister chromatids of individual chromosomes.

The second meiotic division, meiosis II, separates sister chromatids and is virtually identical in mechanism to mitosis. However, since the chromosomes do not replicate between meiosis I and meiosis II, the final outcome of meiosis is a halving of the number of chromosomes per cell--a reduction from two haploid sets to one haploid set in each cell.

## ORIGINS OF GENETIC VARIATION

How do we account for the genetic variation we observed in FIGURE 13.2? We are now ready to address this question.

## Sexual life cycles produce genetic variation among offspring

In species that reproduce sexually, the behavior of chromosomes during meiosis and fertilization is responsible for most of the variation that arises each generation. Let's examine three mechanisms that contribute to the genetic variation arising from sexual reproduction: independent assortment of chromosomes, crossing over, and random fertilization.

## Independent Assortment of Chromosomes

One way sexual reproduction generates genetic variation is shown in FIGURE 13.9, which features meiosis of a diploid cell with two homologous pairs of chromosomes. The red and blue colors distinguishing the maternal and paternal chromosomes of each homologous pair allow us to track individual chromosomes as meiosis proceeds and they are packaged in gametes. At metaphase I, the homologous pairs of chromosomes, each consisting of one maternal and one paternal chromosome, are situated on the metaphase plate. The orientations of the homologous pairs relative to the poles of the cell are random; there are two alternative possibilities for each pair. Thus, there is a fifty-fifty chance that a particular daughter cell of meiosis I will get the maternal chromosome of a certain homologous pair and a fifty-fifty chance that it will receive the paternal chromosome.

Fig 13-9. The results of alternative arrangements of two homologous chromosome pairs on the metaphase plate in meiosis I. In this figure we consider the consequences of meiosis in a hypothetical organism with a diploid chromosome number of 4 (2n = 4). The parental origins of the chromosomes are designated with different colors, blue for chromosomes inherited from one parent, red for chromosomes from the other parent. The positioning of each homologous pair of chromosomes at metaphase I is a matter of chance and determines which chromosomes will be packaged together in the haploid daughter cells.

Because each homologous pair of chromosomes is positioned independently of the other pairs at metaphase I--its orientation is as random as the flip of a coin--the first meiotic division results in independent assortment of maternal and paternal chromosomes into daughter cells. Each gamete represents one outcome of all possible combinations of maternal and paternal chromosomes. The number of combinations possible for gametes formed by meiosis starting with two homologous pairs of chromosomes (2n = 4, n = 2) is four, as shown in FIGURE 13.9. (Only two of the four combinations of gametes shown in the figure would result from meiosis of a single diploid cell, but starting with a large number of diploid cells, gametes of all four types would be produced in approximately equal numbers.) In the case of n = 3, eight combinations of chromosomes are possible for gametes. More generally, the number of combinations possible when chromosomes assort independently into gametes during meiosis is 2n, where n is the haploid number of the organism.

In the case of humans, the haploid number (n) in the formula is 23. Thus, the number of possible combinations of maternal and paternal chromosomes in the resulting gametes is 223, or about 8 million. The variety of gametes is analogous to the different combinations of heads and tails possible for the simultaneous tossing of 23 coins. Thus, each gamete that a human produces contains one of roughly 8 million possible assortments of chromosomes inherited from that individual's mother and father.

## Crossing Over

As a consequence of the independent assortment of chromosomes during meiosis, each of us produces a collection of gametes differing greatly in their combinations of the chromosomes we inherited from our two parents. But from what you have learned so far, it would seem that each individual chromosome in a gamete would be exclusively maternal or paternal in origin; that is, it would consist of DNA derived from our mother or father, but not from both. In fact, this is not the case. The process called crossing over produces recombinant chromosomes, which combine genes inherited from our two parents (FIGURE 13.10).

Fig 13-10. The results of crossing over during meiosis. During prophase of meiosis I, nonsister chromatids of homologous chromosomes exchange corresponding segments. Following these chromosomes through meiosis, we can see that crossing over gives rise to recombinant chromosomes, individual chromosomes that have some combination of DNA originally derived from two different parents.

Recent research has revealed that crossing over begins very early in prophase I, as homologous chromosomes pair loosely along their lengths and before the synaptonemal complex forms between them. The pairing is precise, the homologues aligning with each other gene by gene. In crossing over, homologous portions of two nonsister chromatids trade places. (For humans, an average of two or three such crossover events occur per chromosome pair.) After the synaptonemal complex disappears, the locations where these genetic exchanges have occurred are visible as chiasmata.

At metaphase II, chromosomes that contain one or more recombinant chromatids can be oriented in two alternative, nonequivalent ways with respect to other chromosomes, because their sister chromatids are no longer identical twins. The independent assortment of these nonidentical sister chromatids during meiosis II increases still more the number of genetic types of gametes that can result from meiosis.

You will learn more about crossing over in Chapter 15. The important point for now is that crossing over, by combining DNA inherited from two parents into a single chromosome, is an important source of genetic variation in sexual life cycles.

## Random Fertilization

The random nature of fertilization adds to the genetic variation arising from meiosis. Consider a zygote resulting from a mating between a woman and a man. A human ovum, representing one of approximately 8 million possible chromosome combinations, is fertilized by a single sperm cell, which represents one of 8 million different possibilities. Thus, even without considering crossing over, any two parents will produce a zygote with any of about 64 trillion (8 million X 8 million) diploid combinations. (If you calculate 223 X 223 exactly, you will find that the total is actually over 70 trillion.) Adding in the variation brought about by crossing over, the number of possibilities is truly astronomical. No wonder brothers and sisters can be so different. You really are unique.

So far, we have seen that there are three sources of genetic variability in a sexually reproducing population of organisms:

Independent assortment of homologous chromosomes during meiosis I (and of nonidentical sister chromatids during meiosis II)

Crossing over between homologous chromosomes during prophase of meiosis I

Random fertilization of an ovum by a sperm

All three mechanisms reshuffle the various genes carried by the individual members of a population. However, as you will learn in subsequent chapters, mutations are what ultimately create a population's diversity of genes.

## Evolutionary adaptation depends on a population's genetic variation

Having considered how sexual reproduction contributes to genetic variation in a population, we can relate these concepts to evolution, biology's core theme. Darwin recognized the importance of genetic variation in the evolutionary mechanism he called natural selection. Recall from Chapter 1 that a population evolves through the differential reproductive success of its variant members. On average, those individuals best suited to the local environment leave the most offspring, transmitting their genes in the process. This natural selection results in adaptation, the accumulation of the genetic variations favored by the environment. As the environment changes or a population moves, the population may survive if, in each generation, at least some of its members can cope effectively with the new conditions. Different genetic variations may work better than those that prevailed in the old time or place. Sex and mutations are the two sources of this variation, and we have considered the sexual contribution in this chapter.

Although Darwin realized that heritable variation is what makes evolution possible, he could not explain why offspring resemble--but are not identical to--their parents. Ironically, Gregor Mendel, a contemporary of Darwin, published a theory of inheritance that helps explain genetic variation, but his discoveries had no impact on biologists until 1900, more than 15 years after Darwin (1809-1882) and Mendel (1822-1884) had died. In the next chapter, you will learn how Mendel discovered the basic rules governing the inheritance of specific traits.

## DNA REPLICATION AND REPAIR (pgs 293-301)

The relationship between structure and function, one of the themes of biology, is manifest in the double helix. The idea that there is specific pairing of nitrogenous bases in DNA was the flash of inspiration that led Watson and Crick to the correct double helix. At the same time, they saw the functional significance of the base-pairing rules. They ended their classic paper with this wry statement: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." In the next section, you will learn about this basic mechanism of DNA replication. Some important details of the process will be presented in the section that follows.

## During DNA replication, base pairing enables existing DNA strands to serve as templates for new complementary strands

In a second paper that followed their announcement of the double helix, Watson and Crick stated their hypothesis for how DNA replicates:

Now our model for deoxyribonucleic acid is, in effect, a pair of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken, and the two chains unwind and separate. Each chain then acts as a template for the formation onto itself of a new companion chain, so that eventually we shall have two pairs of chains, where we only had one before. Moreover, the sequence of the pairs of bases will have been duplicated exactly.\*

FIGURE 16.7 illustrates Watson and Crick's basic idea. To make it easier to follow, the diagram shows only a short section of double helix, in untwisted form. Notice that if you cover one of the two DNA strands of FIGURE 16.7a, you can still determine its linear sequence of bases by referring to the unmasked strand and applying the base-pairing rules. The two strands are complementary; each stores the information necessary to reconstruct the other. When a cell copies a DNA molecule, each strand serves as a template (mold) for ordering nucleotides into a new complementary strand. One at a time, nucleotides line up along the template strand according to the base-pairing rules. The nucleotides are linked to form the new strands. Where there was one double-stranded DNA molecule at the beginning of the process, there are now two, each an exact replica of the "parent" molecule. The copying mechanism is analogous to using a photographic negative to make a positive image, which can in turn be used to make another negative, and so on. (See FIGURE 5.30 for a helical version of FIGURE 16.7.)

Fig 16-7. A model for DNA replication: the basic concept. In this simplification, a short segment of DNA has been untwisted into a structure that resembles a ladder. The rails of the ladder are the sugar-phosphate backbones of the two DNA strands; the rungs are the pairs of nitrogenous bases. Simple shapes symbolize the four kinds of bases. Dark blue represents DNA strands originally present in the parent cell; light blue represents newly synthesized DNA.

This model of gene replication remained untested for several years following publication of the DNA structure. The requisite experiments were simple in concept but difficult to perform. Watson and Crick's model predicts that when a double helix replicates, each of the two daughter molecules will have one old strand, derived from the parent molecule, and one newly made strand. This semiconservative model can be distinguished from a conservative model of replication, in which the parent molecule somehow emerges from the replication process intact (that is, it is conserved). In yet a third model, called the dispersive model, all four strands of DNA following replication have a mixture of old and new DNA (FIGURE 16.8, p. 294). Although mechanisms for conservative or dispersive DNA replication are not easy to devise, these models remained possibilities until they could be ruled out. Finally, in the late 1950s, Matthew Meselson and Franklin Stahl devised experiments that tested the three hypotheses. Their experiments supported the semiconservative model, as predicted by Watson and Crick (FIGURE 16.9, p. 294).

Fig 16-8. Three alternative models of DNA replication. The short segments of double helix here symbolize the DNA within a cell. Beginning with a parent cell, we follow the DNA for two generations of cells--two rounds of DNA replication. Newly made DNA is lighter blue.

Fig 16-9. The Meselson-Stahl experiment tested three models of DNA replication. Meselson and Stahl cultured E. coli bacteria for several generations on a medium containing a heavy isotope of nitrogen, 15N. The bacteria incorporated the heavy nitrogen into their nucleotides and then into their DNA. The scientists then transferred the bacteria to a medium containing 14N, the lighter, more common isotope of nitrogen. Any new DNA that the bacteria synthesized would be lighter than the "old" DNA made in the 15N medium. Meselson and Stahl could distinguish DNA of different densities by centrifuging DNA extracted from the bacteria. The centrifuge tubes in this drawing represent the results predicted by each of the three models in FIGURE 16.8. The first replication in the 14N medium produced a band of hybrid (15N-14N) DNA. This result eliminated the conservative model. A second replication produced both light and hybrid DNA, a result that eliminated the dispersive model and supported the semiconservative model.

The basic principle of DNA replication is elegantly simple. However, the actual process involves complex biochemical gymnastics, as we will now see.

## A large team of enzymes and other proteins carries out DNA replication

The bacterium E. coli has a single chromosome of about 5 million base pairs. In a favorable environment, an E. coli cell can copy all this DNA and divide to form two genetically identical daughter cells in less than an hour. Each of your cells has 46 DNA molecules in its nucleus, one giant molecule per chromosome. In all, that represents about 6 billion base pairs, or over a thousand times more DNA than is found in a bacterial cell. If we were to print the one-letter symbols for these bases (A, G, C, and T) the size of the letters you are now reading, the 6 billion bases of a single human cell would fill about 900 books as thick as this text. Yet it takes a cell just a few hours to copy all this DNA. This replication of an enormous amount of genetic information is achieved with very few errors--only one per billion nucleotides. The copying of DNA is remarkable in its speed and accuracy.

More than a dozen enzymes and other proteins participate in DNA replication. Much more is known about how this "replication machine" works in bacteria than in eukaryotes. However, most of the process seems to be fundamentally similar for prokaryotes and eukaryotes. In this section, we take a closer look at the basic steps.

## Getting Started: Origins of Replication

The replication of a DNA molecule begins at special sites called origins of replication. The bacterial chromosome, which is circular, has a single origin, a stretch of DNA having a specific sequence of nucleotides. Proteins that initiate DNA replication recognize this sequence and attach to the DNA, separating the two strands and opening up a replication "bubble." Replication of DNA then proceeds in both directions, until the entire molecule is copied (see FIGURE 18.11). In contrast to the bacterial chromosome, a eukaryotic chromosome may have hundreds or even thousands of replication origins. Multiple replication bubbles form and eventually fuse, thus speeding up the copying of the very long DNA molecules (FIGURE 16.10). As in bacteria, DNA replication proceeds in both directions from each origin. At each end of a replication bubble is a replication fork, a Y-shaped region where the new strands of DNA are elongating.

Fig 16-10. Origins of replication in eukaryotes.

## Elongating a New DNA Strand

Elongation of new DNA at a replication fork is catalyzed by enzymes called DNA polymerases. As nucleotides align with complementary bases along a template strand of DNA, they are added by polymerase, one by one, to the growing end of the new DNA strand. The rate of elongation is about 500 nucleotides per second in bacteria and 50 per second in human cells.

What is the source of energy that drives the polymerization of nucleotides to form new DNA strands? The nucleotides that serve as substrates for DNA polymerase are actually nucleo side triphosphates, which are nucleotides with three phosphate groups (FIGURE 16.11, p. 296). You have already encountered such a molecule--ATP. The only difference between the ATP of energy metabolism and the nucleoside triphosphate that supplies adenine to DNA is the sugar component, which is deoxyribose in the building block of DNA, but ribose in ATP. (As you might guess, ribose-containing ATP is a substrate for RNA synthesis.) Like ATP, the triphosphate monomers used for DNA synthesis are chemically reactive, partly because their triphosphate tails have an unstable cluster of negative charge. As each monomer joins the growing end of a DNA strand, it loses two phosphate groups as a molecule of pyrophosphate P - Pi). Subsequent hydrolysis of the pyrophosphate to two molecules of inorganic phosphate (Pi) is the exergonic reaction that drives the polymerization reaction.

Fig 16-11. Incorporation of a nucleotide into a DNA strand. When a nucleoside triphosphate links to the sugar-phosphate backbone of a growing DNA strand, it loses two of its phosphates as a pyrophosphate molecule. The enzyme catalyzing the reaction is a DNA polymerase, and hydrolysis of the bonds between the phosphate groups of the pyrophosphate provides the energy for the reaction.

## The Antiparallel Arrangement of the DNA Strands

There is more to the scenario of DNA synthesis at the replication fork. Until now, we have ignored an important feature of the double helix: The two DNA strands are antiparallel; that is, their sugar-phosphate backbones run in opposite directions. In FIGURE 16.12, the five carbons of one deoxyribose sugar of each DNA strand are numbered from 1' to 5'. (The prime sign is used to distinguish the carbon atoms of the sugar from the carbon and nitrogen atoms of the nitrogenous bases.) Notice in FIGURE 16.12 that a nucleotide's phosphate group is attached to the 5' carbon of deoxyribose. Notice also that the phosphate group of one nucleotide is joined to the 3' carbon of the adjacent nucleotide. The result is a DNA strand of distinct polarity. At one end, denoted the 3' end, a hydroxyl group is attached to the 3' carbon of the terminal deoxyribose. At the opposite end, the 5' end, the sugar-phosphate backbone terminates with the phosphate group attached to the 5' carbon of the last nucleotide. In the double helix, the two sugar-phosphate backbones are essentially upside down (antiparallel) relative to each other.

Fig 16-12. The two strands of DNA are antiparallel. The 5' 3' direction of one strand runs counter to the 5' 3' direction of the other strand. The numbers assigned to the carbon atoms of the deoxyribose units are shown for two of them.

How does the antiparallel structure of the double helix affect replication? DNA polymerases add nucleotides only to the free 3' end of a growing DNA strand, never to the 5' end. Thus, a new DNA strand can elongate only in the 5' 3' direction. With this in mind, let's examine a replication fork (FIGURE 16.13). Along one template strand, DNA polymerase can synthesize a continuous complementary strand by elongating the new DNA in the mandatory 5' 3' direction. The polymerase simply nestles in the replication fork on that template strand and continuously adds nucleotides to a complementary strand as the fork progresses. The DNA strand made by this mechanism is called the leading strand.

Fig 16-13. Synthesis of leading and lagging strands during DNA replication.

To elongate the other new strand of DNA, polymerase must work along the other template strand in the direction away from the replication fork. The DNA synthesized in this direction is called the lagging strand. The process is analogous to a sewing method called backstitching. As a replication bubble opens, a polymerase molecule can work its way away from a replication fork and synthesize a short segment of DNA. As the bubble grows, another short segment of the lagging strand can be made in a similar way. In contrast to the leading strand, which elongates continuously, the lagging strand is first synthesized as a series of segments. These pieces are called Okazaki fragments, after the Japanese scientist who discovered them. The fragments are about 100 to 200 nucleotides long in eukaryotes. Another enzyme, DNA ligase, joins (ligates) the sugar-phosphate backbones of the Okazaki fragments to create a single DNA strand.

## Priming DNA Synthesis

There is another important restriction for DNA polymerases. None of these enzymes can actually initiate synthesis of a polynucleotide; they can only add nucleotides to the end of an already existing chain that is base-paired with the template strand (see FIGURE 16.11). In the replication of cellular DNA, the start of a new chain, its primer, is not DNA, but a short stretch of RNA, the other class of nucleic acid. An enzyme called primase joins RNA nucleotides to make the primer, which is about 10 nucleotides long in eukaryotes (FIGURE 16.14). (Like all RNA-synthesizing enzymes, primase can start an RNA chain from scratch.) Another DNA polymerase later replaces the RNA nucleotides of the primers with DNA versions. Only one primer is required for a DNA polymerase to begin synthesizing the leading strand of new DNA. For the lagging strand, each Okazaki fragment must be primed; the primers are converted to DNA before ligase joins the fragments together.

Fig 16-14. Priming DNA synthesis with RNA. DNA polymerase cannot initiate a polynucleotide strand; it can only add to the 3' end of an already-started strand. The primer is a short segment of RNA synthesized by the enzyme primase. Each primer is eventually replaced by DNA.

## Other Proteins Assisting DNA Replication

You have learned about three kinds of proteins that function in DNA synthesis: DNA polymerase, ligase, and primase. Other kinds of proteins also participate; two of these are helicase and single-strand binding protein. A helicase is an enzyme that untwists the double helix at the replication fork, separating the two old strands. Molecules of single-strand binding protein then line up along the unpaired DNA strands, holding them apart while they serve as templates for the synthesis of new complementary strands.

FIGURE 16.15 summarizes the functions of the main proteins that cooperate in DNA replication. FIGURE 16.16 is a visual summary of DNA replication.

Fig 16-15. The main proteins of DNA replication and their functions.

Fig 16-16. A summary of DNA replication. The detailed diagram shows one replication fork, but as indicated in the overview diagram, replication usually occurs simultaneously at two forks, one at either end of a replication bubble. Notice in the overview diagram that a leading strand is initiated by an RNA primer (magenta), as is each Okazaki fragment in a lagging strand. Viewing each daughter strand in its entirety, you can see that half of it is made continuously as a leading strand, while the other half (on the other side of the origin) is synthesized in fragments as a lagging strand.

## The DNA Replication Machine as a Stationary Complex

It is traditional--and convenient--to represent DNA polymerase molecules as locomotives moving along a DNA "railroad track," but such a model is inaccurate in two important ways. First, the various proteins that participate in DNA replication actually form a single large complex, a DNA replication "machine." Second, this machine is probably stationary during the replication process. In eukaryotic cells, the multiple copies of the machine, perhaps grouped into "factories," may anchor to the nuclear matrix, a framework of fibers extending through the interior of the nucleus. Recent studies support a model in which DNA polymerase molecules "reel in" the parental DNA and "extrude" newly made daughter DNA molecules.

## Enzymes proofread DNA during its replication and repair damage in existing DNA

We cannot attribute the accuracy of DNA replication solely to the specificity of base pairing. Although errors in the completed DNA molecule amount to only one in a billion nucleotides, initial pairing errors between incoming nucleotides and those in the template strand are 100,000 times more common--an error rate of one in 10,000 base pairs. During DNA replication, DNA polymerase itself proofreads each nucleotide against its template as soon as it is added to the growing strand. Upon finding an incorrectly paired nucleotide, the polymerase removes the nucleotide and then resumes synthesis. (This action resembles correcting a word-processing error by using the "delete" key and then entering the correction.)

Mismatched nucleotides sometimes evade proofreading by DNA polymerase or arise after DNA synthesis is completed--by damage to a nucleotide base, for instance. In mismatch repair, cells use special enzymes to fix incorrectly paired nucleotides. Researchers spotlighted the importance of such proteins when they found that a hereditary defect in one of them is associated with a form of colon cancer. Apparently, this defect allows cancer-causing errors to accumulate in the DNA.

Maintenance of the genetic information encoded in DNA requires frequent repair of various kinds of damage to existing DNA. DNA molecules are constantly subjected to potentially harmful chemical and physical agents. Reactive chemicals (in the environment and occurring naturally in cells), radioactive emissions, X-rays, and ultraviolet light can change nucleotides in ways that can affect encoded genetic information, usually adversely. In addition, DNA bases often undergo spontaneous chemical changes under normal cellular conditions. Fortunately, changes in DNA are usually corrected before they become self-perpetuating mutations. Each cell continuously monitors and repairs its genetic material. Because repair of damaged DNA is so important to the survival of an organism, it is no surprise that many different DNA repair enzymes have evolved. Almost 100 are known in E. coli, and 130 have been identified so far in humans.

Most mechanisms for repairing DNA damage take advantage of the base-paired structure of DNA. Usually, a segment of the strand containing the damage is cut out (excised) by a DNA-cutting enzyme--a nuclease--and the resulting gap is filled in with nucleotides properly paired with the nucleotides in the undamaged strand. The enzymes involved in filling the gap are a DNA polymerase and ligase. DNA repair of this type is called nucleotide excision repair (FIGURE 16.17).

Fig 16-17. Nucleotide excision repair of DNA damage. A team of enzymes detects and repairs damaged DNA. This figure shows DNA containing a thymine dimer, a type of damage often caused by ultra-violet radiation. Repair enzymes can excise the damaged region from the DNA and replace it with a normal DNA segment.

One function of the DNA repair enzymes in our skin cells is to repair genetic damage caused by the ultraviolet rays of sunlight. One type of damage, the type shown in FIGURE 16.17, is the covalent linking of thymine bases that are adjacent on a DNA strand. Such thymine dimers cause the DNA to buckle and interfere with DNA replication. The importance of repairing this kind of damage is underscored by the disorder xeroderma pigmentosum, which in most cases is caused by an inherited defect in a nucleotide excision repair enzyme. Individuals with this disorder are hypersensitive to sunlight; mutations in their skin cells caused by ultraviolet light are left uncorrected and cause skin cancer.

## The ends of DNA molecules are replicated by a special mechanism

Most DNA repair processes involve DNA polymerases, but these enzymes are helpless to fix a "defect" that results from their own limitations. For linear DNA, such as the DNA of eukaryotic chromosomes, the fact that a DNA polymerase can only add nu cleotides to the 3' end of a preexisting polynucleotide leads to a potential problem. The usual replication machinery provides no way to complete the 5' ends of daughter DNA strands; as a result, repeated rounds of replication produce shorter and shorter DNA molecules (FIGURE 16.18, p. 300). If a cell divided enough times, essential genes would be deleted. Clearly, if this trend continued over generations, we would not be here today!

Fig 16-18. The end-replication problem. When a linear DNA molecule replicates, a gap is left at the 5' end of each new strand (light blue) because DNA polymerase can only add nucleotides to a 3' end. As a result, with each round of replication, the DNA molecules get slightly shorter. For simplicity we show only one end of a linear DNA molecule.

Prokaryotes avoid this problem by having circular DNA molecules (which have no ends), but what about eukaryotes? Eukaryotic chromosomal DNA molecules have special nucleotide sequences called telomeres at their ends (FIGURE 16.19). Telomeres do not contain genes; instead, the DNA consists of multiple repetitions of one short nucleotide sequence. The repeated unit in human telomeres, which is typical, is the six-nucleotide sequence TTAGGG. The number of repetitions in a telomere varies between about 100 and 1,000. Telomeric DNA protects the organism's genes from being eroded through successive rounds of DNA replication. In addition, telomeric DNA and special proteins associated with it somehow prevent the ends from activating the cell's systems for monitoring DNA damage. (The end of a DNA molecule that is "seen" as a double-strand break may otherwise trigger signal-transduction pathways leading to cell cycle arrest or cell death.)

Fig 16-19. Telomeres and telomerase. Eukaryotes deal with the end-replication issue by having expendable, noncoding sequences called telomeres at the ends of their DNA and the enzyme telomerase in some of their cells.

In the long term, over the course of generations, eukaryotic organisms need a way of restoring their shortened telomeres. This is provided by telomerase, a special enzyme that catalyzes the lengthening of telomeres. But how does telomerase synthesize DNA where the DNA template has been lost? Telomerase is unusual in having a short molecule of RNA along with its protein. The RNA contains a nucleotide sequence that serves as the template for new telomere segments at the 3' end of the telo-mere. FIGURE 16.19 shows how telomerase and DNA polymerase work together to lengthen telomeres.

Telomerase is not present in most cells of multicellular organisms like ourselves, and the DNA of dividing somatic cells does tend to be shorter in older individuals and in cultured cells that have divided many times. Thus, it is possible that telomeres are a limiting factor in the life span of certain tissues and even the organism as a whole. In any case, telomerase is present in germ-line cells, those that give rise to gametes. The enzyme produces long telomeres in these cells and hence in the newborn.

Intriguingly, researchers have also found telomerase in somatic cells that are cancerous. Cells from large tumors often have unusually short telomeres, as one would expect for cells that have undergone many cell divisions. Progressive shortening would presumably lead eventually to self-destruction of the cancer unless telomerase became available to stabilize telomere length. This is exactly what seems to happen in cancer cells and also in immortal strains of cultured cells (see Chapter 12). If telomerase is indeed an important factor in many cancers, it may provide a useful target for both cancer diagnosis and chemotherapy.

DNA replication provides the copies of genes that parents pass to offspring via gametes. However, it is not enough that genes be copied and transmitted; they must also be expressed. How do genes manifest themselves in phenotypic characters such as eye color? In the next chapter, we will examine the molecular basis of gene expression--how the cell translates genetic information encoded in DNA.

## Transcription and translation are the two main processes linking gene to protein: an overview (pgs 304-311

Genes provide the instructions for making specific proteins. But a gene does not build a protein directly. The bridge between DNA and protein synthesis is RNA. You learned in Chapter 5 that RNA is chemically similar to DNA, except that it contains ribose instead of deoxyribose as its sugar and has the nitrogenous base uracil rather than thymine (see FIGURE 5.29). Thus, each nucleotide along a DNA strand has deoxyribose as its sugar and A, G, C, or T as its base; each nucleotide along an RNA strand has ribose as its sugar and A, G, C, or U as its base. An RNA molecule almost always consists of a single strand.

It is customary to describe the flow of information from gene to protein in linguistic terms because both nucleic acids and proteins are polymers with specific sequences of monomers that convey information, much as specific sequences of letters communicate information in a language like English. In DNA or RNA, the monomers are the four types of nucleotides, which differ in their nitrogenous bases. Genes are typically hundreds or thousands of nucleotides long, each gene having a specific sequence of bases. Each polypeptide of a protein also has monomers arranged in a particular linear order (the protein's primary structure), but its monomers are the 20 amino acids. Thus, nucleic acids and proteins contain information written in two different chemical languages. To get from DNA, written in one language, to protein, written in the other, requires two major stages, transcription and translation.

Transcription is the synthesis of RNA under the direction of DNA. Both nucleic acids use the same language, and the information is simply transcribed, or copied, from one molecule to the other. Just as a DNA strand provides a template for the synthesis of a new complementary strand during DNA replication, it provides a template for assembling a sequence of RNA nucleotides. The resulting RNA molecule is a faithful transcript of the gene's protein-building instructions. This type of RNA molecule is called messenger RNA (mRNA), because it carries a genetic message from the DNA to the protein-synthesizing machinery of the cell (FIGURE 17.2a). (Transcription is the general term for the synthesis of any kind of RNA on a DNA template. Later in this chapter, you will learn about other types of RNA produced by transcription.)

Fig 17-2. Overview: the roles of transcription and translation in the flow of genetic information. In a cell, inherited information flows from DNA to RNA to protein. The two main stages of information flow are transcription and translation. In transcription, a gene provides the instructions for synthesizing a messenger RNA (mRNA) molecule. In translation, the information encoded in mRNA determines the order of amino acids that are joined to form a specific polypeptide. The sites of translation are ribosomes. A miniature version of part b (or sometimes part a) accompanies several figures later in the Chapter as an orientation diagram to help you see where a particular figure fits into the overall scheme.

Translation is the actual synthesis of a polypeptide, which occurs under the direction of mRNA. During this stage, there is a change in language: The cell must translate the base sequence of an mRNA molecule into the amino acid sequence of a polypeptide. The sites of translation are ribosomes, complex particles that facilitate the orderly linking of amino acids into polypeptide chains.

Although the basic mechanics of transcription and translation are similar for prokaryotes and eukaryotes, there is an important difference in the flow of genetic information within the cells. Because bacteria lack nuclei, their DNA is not segregated from ribosomes and the other protein-synthesizing equipment. Transcription and translation are coupled, with ribosomes attaching to the leading end of an mRNA molecule while transcription is still in progress (see FIGURE 17.22). In a eukaryotic cell, by contrast, the nuclear envelope separates transcription from translation in space and time (FIGURE 17.2b). Transcription occurs in the nucleus, and mRNA is dispatched to the cytoplasm, where translation occurs. But before they can leave the nucleus, eukaryotic RNA transcripts are modified in various ways to produce the final, functional mRNA. Thus, in a two-step process, the transcription of a eukaryotic gene results in pre-mRNA, and RNA processing yields the finished mRNA. A more general term for an initial RNA transcript is primary transcript.

Let's summarize the main point of our overview of protein synthesis: Genes program protein synthesis via genetic messages in the form of messenger RNA. Put another way, cells are governed by a molecular chain of command: DNA RNA protein. The next section discusses how the instructions for assembling amino acids into a specific order are encoded in nucleic acids.

## In the genetic code, nucleotide triplets specify amino acids

When biologists began to suspect that the instructions for protein synthesis were encoded in DNA, they recognized a problem: There are only four nucleotides to specify 20 amino acids. Thus, the genetic code cannot be a language like Chinese, where each written symbol corresponds to a single word. If each nucleotide base were translated into an amino acid, only 4 of the 20 amino acids could be specified. Would a language of two-letter code words suffice? The base sequence AG, for example, could specify one amino acid, and GT could specify another. Since there are four bases, this would give us 16 (that is, 42) possible arrangements--still not enough to code for all 20 amino acids.

Triplets of nucleotide bases are the smallest units of uniform length that can code for all the amino acids. If each arrangement of three consecutive bases specifies an amino acid, there can be 64 (that is, 43) possible code words--more than enough to specify all the amino acids. Experiments have verified that the flow of information from gene to protein is based on a triplet code: The genetic instructions for a polypeptide chain are written in the DNA as a series of three-nucleotide words. For example, the base triplet AGT at a particular position along a DNA strand results in the placement of the amino acid serine at the corresponding position of the polypeptide to be produced.

As you know, a cell does not directly translate a gene into amino acids. The intermediate step is transcription, during which the gene determines the sequence of base triplets along the length of an mRNA molecule. For each gene, only one of the two DNA strands is transcribed (FIGURE 17.3). This strand is called the template strand, because it provides the template for ordering the sequence of nucleotides in an RNA transcript. A given DNA strand can be the template strand in some regions of a DNA molecule, while in other regions along the double helix it is the complementary strand that functions as the template for RNA synthesis.

Fig 17-3. The triplet code. For each gene, one DNA strand functions as a template for transcription--the synthesis of a complementary mRNA molecule. The base-pairing rules for DNA synthesis also guide transcription, but uracil (U) takes the place of thymine (T) in RNA. During translation, the mRNA is read as a sequence of base triplets, called codons. Each codon specifies an amino acid to be added to the growing polypeptide chain. The mRNA is read in the 5' 3' direction.

An mRNA molecule is complementary rather than identical to its DNA template because RNA bases are assembled on the template according to base-pairing rules. The pairs are similar to those that form during DNA replication, except that U, the RNA substitute for T, pairs with A. Thus, when a DNA strand is transcribed, the base triplet ACC in DNA provides a template for UGG in the mRNA molecule. The mRNA base triplets are called codons. For example, UGG is the codon for the amino acid tryptophan (abbreviated Trp). (The term codon is also sometimes used for the complementary DNA base triplet. For example, the DNA codon corresponding to the RNA codon UGG is ACC.)

During translation, the sequence of codons along an mRNA molecule is decoded, or translated, into a sequence of amino acids making up a polypeptide chain. The codons are read in the 5' 3' direction along the mRNA. (To review what is meant by the 5' and 3' ends of a nucleic acid chain, see FIGURE 16.12.) Each codon specifies which one of the 20 amino acids will be incorporated at the corresponding position along a polypeptide. Because codons are base triplets, the number of nucleotides making up a genetic message must be three times the number of amino acids making up the protein product. For example, it takes 300 nucleotides along an RNA strand to code for a polypeptide that is 100 amino acids long.

## Cracking the Genetic Code

Molecular biologists cracked the code of life in the early 1960s, when a series of elegant experiments disclosed the amino acid translations of each of the RNA codons. The first codon was deciphered in 1961 by Marshall Nirenberg, of the National Institutes of Health, and his colleagues. Nirenberg synthesized an artificial mRNA by linking identical RNA nucleotides containing uracil as their base. No matter where this message started or stopped, it could contain only one codon in repetition: UUU. Nirenberg added this "poly(U)" to a test-tube mixture containing amino acids, ribosomes, and the other components required for protein synthesis. His artificial system translated the poly(U) into a polypeptide containing a single amino acid, phenylalanine (Phe), strung together as a long polyphenylalanine chain. Thus, Nirenberg determined that the mRNA codon UUU specifies the amino acid phenylalanine. Soon, the amino acids specified by the codons AAA, GGG, and CCC were also determined.

Although more elaborate techniques were required to decode mixed triplets such as AUA and CGA, all 64 codons were deciphered by the mid-1960s. As FIGURE 17.4 shows, 61 of the 64 triplets code for amino acids. Notice that the codon AUG has a dual function: It not only codes for the amino acid methionine (Met), but also functions as a "start" signal, or initiation codon. Genetic messages begin with the mRNA codon AUG, which signals the protein-synthesizing machinery to begin translating the mRNA at that location. (Because AUG also stands for methionine, polypeptide chains begin with methionine when they are synthesized. However, an enzyme may subsequently remove this starter amino acid from a chain.) The remaining three codons do not designate amino acids. Instead, they are "stop" signals, or termination codons, marking the end of translation.

Fig 17-4. The dictionary of the genetic code. The three bases of an mRNA codon are designated here as the first, second, and third bases, reading in the 5' 3' direction along the mRNA. (Practice using this dictionary by finding the codons in FIGURE 17.3.) The codon AUG not only stands for the amino acid methionine (Met) but also functions as a "start" signal for ribosomes to begin translating the mRNA at that point. Three of the 64 codons function as "stop" signals. Any one of these termination codons marks the end of a genetic message.

Notice in FIGURE 17.4 that there is redundancy in the genetic code, but no ambiguity. For example, although codons GAA and GAG both specify glutamic acid (redundancy), neither of them ever specifies any other amino acid (no ambiguity). The redundancy in the code is not altogether random. In many cases, codons that are synonyms for a particular amino acid differ only in the third base of the triplet. We will consider a possible benefit for this redundancy later in the chapter.

Our ability to extract the intended message from a written language depends on reading the symbols in the correct groupings--that is, in the correct reading frame. Consider this statement: "The red dog ate the cat." Group the letters incorrectly by starting at the wrong point, and the result will probably be gibberish: for example, "her edd oga tet hec at." The reading frame is also important in the molecular language of cells. The short stretch of polypeptide shown in FIGURE 17.3, for instance, will only be made correctly if the mRNA nucleotides are read from left to right (5' 3') in the groups of three shown in the figure: UGG UUU GGC UCA. Although a genetic message is written with no spaces between the codons, the cell's protein-synthesizing machinery reads the message as a series of nonoverlapping three-letter words. The message is not read as a series of overlapping words--UGG UUU, and so on--which would convey a very different message.

Let's summarize what we have just covered. Genetic information is encoded as a sequence of nonoverlapping base triplets, or codons, each of which is translated into a specific amino acid during protein synthesis.

## The genetic code must have evolved very early in the history of life

The genetic code is nearly universal, shared by organisms from the simplest bacteria to the most complex plants and animals. The RNA codon CCG, for instance, is translated as the amino acid proline in all organisms whose genetic code has been examined. In laboratory experiments, genes can be transcribed and translated after they are transplanted from one species to another (FIGURE 17.5). One important application is that bacteria can be programmed by the insertion of human genes to synthesize certain human proteins that have important medical uses. Such applications have produced many exciting developments in biotechnology, which you will learn about in Chapter 20.

Fig 17-5. A tobacco plant expressing a firefly gene. Because diverse forms of life share a common genetic code, it is possible to program one species to produce proteins characteristic of another species by transplanting DNA. In this experiment, researchers were able to incorporate a gene from a firefly into the DNA of a tobacco plant. The gene codes for the firefly enzyme that catalyzes the chemical reaction that releases energy in the form of light.

Exceptions to the universality of the genetic code are translation systems where a few codons differ from the standard ones. The main examples are found in certain single-celled eukaryotes, such as Paramecium, an organism you may know from the lab. Other examples are found in certain mitochondria and chloroplasts, which transcribe and translate the genes carried by their small amount of DNA. However, the evolutionary significance of the code's near universality is clear. A language shared by all living things must have been operating very early in the history of life--early enough to be present in the common ancestors of all modern organisms. A shared genetic vocabulary is a reminder of the kinship that bonds all life on Earth.

Now that we have considered the linguistic logic and evolutionary significance of the genetic code, we are ready to reexamine transcription, translation, and related topics in more detail.

## THE SYNTHESIS AND PROCESSING OF RNA

## Transcription is the DNA-directed synthesis of RNA: a closer look

Messenger RNA, the carrier of information from DNA to the cell's protein-synthesizing machinery, is transcribed from the template strand of a gene. An enzyme called an RNA polymerase pries the two strands of DNA apart and hooks together the RNA nucleotides as they base-pair along the DNA template (FIGURE 17.6). Like the DNA polymerases that function in DNA replication, RNA polymerases can add nucleotides only to the 3' end of the growing polymer. Thus, an RNA molecule elongates in its 5' 3' direction.

Fig 17-6. The stages of transcription: initiation, elongation, and termination. RNA polymerase moves along a gene from the promoter (green) to just beyond the terminator (red), assembling an RNA molecule (transcript) complementary to the gene's template strand. In a prokaryote, the RNA transcript of a protein-coding gene is immediately usable as mRNA; in a eukaryote, it must first undergo processing, as described on pp. 311-312.

Specific sequences of nucleotides along the DNA mark where transcription of a gene begins and ends. The DNA sequence where RNA polymerase attaches and initiates transcription is known as the promoter; the sequence that signals the end of transcription is called the terminator. Molecular biologists refer to the direction of transcription as "downstream" and the other direction as "upstream." These terms are also used to describe the positions of nucleotide sequences within the DNA or RNA. Thus, the promoter sequence in DNA is said to be upstream from the terminator. The stretch of DNA that is transcribed into an RNA molecule is called a transcription unit.

Bacteria have a single type of RNA polymerase that synthesizes not only mRNA but also other types of RNA that function in protein synthesis. In contrast, eukaryotes have three types of RNA polymerase in their nuclei, numbered I, II, and III. The one used for mRNA synthesis is RNA polymerase II. In the discussion of transcription that follows, we start with the features of mRNA synthesis common to both prokaryotes and eukaryotes and then describe some key differences.

The three stages of transcription, as shown in FIGURE 17.6 and described next, are initiation, elongation, and termination of the RNA chain. Study FIGURE 17.6 to familiarize yourself with the stages of transcription and the terms used to describe them.

## RNA Polymerase Binding and Initiation of Transcription

The promoter of a gene includes within it the transcription start point (the nucleotide where RNA synthesis actually begins) and typically extends several dozen nucleotide pairs "upstream" from the start point. In addition to serving as a binding site for RNA polymerase and determining where transcription starts, the promoter determines which of the two strands of the DNA helix is used as the template.

Certain sections of a promoter are especially important for binding RNA polymerase. In prokaryotes, the RNA polymerase itself specifically recognizes and binds to the promoter. In eukaryotes, a collection of proteins called transcription factors mediate the binding of RNA polymerase and the initiation of transcription. Only after certain transcription factors are attached to the promoter does the RNA polymerase bind to it. The completed assembly of transcription factors and RNA polymerase bound to the promoter is called a transcription initiation complex. FIGURE 17.7 shows the role of transcription factors and a crucial promoter DNA sequence called a TATA box in forming the initiation complex.

Fig 17-7. The initiation of transcription at a eukaryotic promoter. In eukaryotic cells, proteins called transcription factors mediate the initiation of transcription by RNA polymerase. The enzyme that transcribes protein-coding genes in eukaryotic cells is called RNA polymerase II.

The interaction between eukaryotic RNA polymerase and transcription factors is an example of the special importance of protein-protein interactions in controlling eukaryotic transcription (as we will discuss further in Chapter 19). Once the polymerase is firmly attached to the promoter DNA, the two DNA strands unwind there, and the enzyme starts transcribing the template strand.

## Elongation of the RNA Strand

As RNA polymerase moves along the DNA, it continues to untwist the double helix, exposing about 10 to 20 DNA bases at a time for pairing with RNA nucleotides (see FIGURE 17.6). The enzyme adds nucleotides to the 3' end of the growing RNA molecule as it continues along the double helix. In the wake of this advancing wave of RNA synthesis, the DNA double helix re-forms and the new RNA molecule peels away from its DNA template. Transcription progresses at a rate of about 60 nucleotides per second in eukaryotes.

A single gene can be transcribed simultaneously by several molecules of RNA polymerase following each other like trucks in a convoy. A growing strand of RNA trails off from each polymerase, with the length of each new strand reflecting how far along the template the enzyme has traveled from the start point (see FIGURE 17.22). The congregation of many polymerase molecules simultaneously transcribing a single gene increases the amount of mRNA transcribed from it, which helps the cell make the encoded protein in large amounts.

## Termination of Transcription

Transcription proceeds until after the RNA polymerase transcribes a terminator sequence in the DNA. The transcribed terminator--an RNA sequence--functions as the actual termination signal. There are several different mechanisms of transcription termination, the details of which are still somewhat murky. In the prokaryotic cell, transcription usually stops right at the end of the termination signal; when the polymerase reaches that point, it releases both the RNA and the DNA. By contrast, in the eukaryotic cell, the polymerase continues for hundreds of nucleotides past the termination signal, which is an AAUAAA sequence in the pre-mRNA (see FIGURE 17.8). But then, at a point about 10 to 35 nucleotides past the AAUAAA, the pre-mRNA is cut free from the enzyme. The cleavage site on the RNA is also the site for the addition of a poly(A) tail--one step of RNA processing, our next topic.

Fig 17-8. RNA processing: addition of the 5' cap and poly(A) tail. Enzymes modify the two ends of a eukaryotic pre-mRNA molecule. The modified ends help protect the RNA from degradation, and the poly(A) tail may promote the export of mRNA from the nucleus. When the mRNA reaches the cytoplasm, the modified ends, in conjunction with certain cytoplasmic proteins, facilitate ribosome attachment. The leader and trailer are not translated, nor is the poly(A) tail.

## Building a Polypeptide (pgs 317-319)

We can divide translation, the synthesis of a polypeptide chain, into three stages (analogous to those of transcription): initiation, elongation, and termination. All three stages require protein "factors" that aid mRNA, tRNA, and ribosomes in the translation process. For chain initiation and elongation, energy is also required. It is provided by the hydrolysis of GTP (guanosine triphosphate), a molecule closely related to ATP.

Initiation. The initiation stage of translation brings together mRNA, a tRNA bearing the first amino acid of the polypeptide, and the two subunits of a ribosome (FIGURE 17.17). First, a small ribosomal subunit binds to both mRNA and a special initiator tRNA. The small ribosomal subunit attaches to the leader segment at the 5' (upstream) end of the mRNA. In bacteria, rRNA of the small subunit base-pairs with a specific sequence of nucleotides within the mRNA leader; in eukaryotes, the 5' cap first tells the small subunit to attach to the 5' end of the mRNA. Downstream on the mRNA is the initiation codon, AUG, which signals the start of translation. The initiator tRNA, which carries the amino acid methionine, attaches to the initiation codon.

Fig 17-17. The initiation of translation.

The union of mRNA, initiator tRNA, and a small ribosomal subunit is followed by the attachment of a large ribosomal subunit, completing a translation initiation complex. Proteins called initiation factors are required to bring all these components together. The cell also spends energy in the form of a GTP molecule to form the initiation complex. At the completion of the initiation process, the initiator tRNA sits in the P site of the ribosome, and the vacant A site is ready for the next aminoacyl tRNA. The synthesis of a polypeptide is initiated at its amino end (see FIGURE 5.16b).

Elongation. In the elongation stage of translation, amino acids are added one by one to the preceding amino acid. Each addition involves the participation of several proteins called elongation factors and occurs in a three-step cycle (FIGURE 17.18):

Fig 17-18. The elongation cycle of translation. Not shown in this diagram are the proteins called elongation factors. The hydrolysis of GTP drives the elongation process.

Codon recognition. The mRNA codon in the A site of the ribosome forms hydrogen bonds with the anticodon of an incoming molecule of tRNA carrying its appropriate amino acid. An elongation factor ushers the tRNA into the A site. This step requires the hydrolysis of two molecules of GTP.

Peptide bond formation. An rRNA molecule of the large ribosomal subunit, functioning as a ribozyme, catalyzes the formation of a peptide bond that joins the polypeptide extending from the P site to the newly arrived amino acid in the A site. In this step, the polypeptide separates from the tRNA to which it was attached, and the amino acid at its carboxyl end bonds to the amino acid carried by the tRNA in the A site.

Translocation. The ribosome now translocates (moves) the tRNA in the A site, with its attached polypeptide, to the P site. As the tRNA moves, its anticodon remains hydrogen-bonded to the mRNA codon; the mRNA moves along with it and brings the next codon to be translated into the A site. Meanwhile, the tRNA that was in the P site is moved to the E (exit) site and from there leaves the ribosome. The translocation step requires energy, which is provided by hydrolysis of a GTP molecule. The mRNA is moved through the ribosome in one direction only, 5' end first; this is equivalent to the ribosome moving 5' 3'on the mRNA. The important point is that the ribosome and the mRNA move relative to each other, unidirectionally, codon by codon. The elongation cycle takes less than a tenth of a second and is repeated as each amino acid is added to the chain until the polypeptide is completed.

Termination. The final stage of translation is termination (FIGURE 17.19). Elongation continues until a stop codon in the mRNA reaches the A site of the ribosome. The special base triplets UAA, UAG, and UGA do not code for amino acids but instead act as signals to stop translation. A protein called a release factor binds directly to the stop codon in the A site. The release factor causes the addition of a water molecule instead of an amino acid to the polypeptide chain. This reaction hydrolyzes the completed polypeptide from the tRNA that is in the P site, freeing the polypeptide from the ribosome. The remainder of the translation assembly then comes apart.

Fig 17-19. The termination of translation.

## Polyribosomes

A single ribosome can make an average-sized polypeptide in less than a minute. Typically, however, a single mRNA is used to make many copies of a polypeptide simultaneously, because a number of ribosomes work on translating the message at the same time. Once a ribosome moves past the initiation codon, a second ribosome can attach to the mRNA, and thus, multiple ribosomes may trail along the same mRNA. Such strings of ribosomes, called polyribosomes, can be seen with the electron microscope (FIGURE 17.20). Polyribosomes are found in both prokaryotic and eukaryotic cells.

They help a cell to make many copes of a polypeptide very quickly.

Fig 17-20. Polyribosomes.

## From Polypeptide to Functional Protein

During and after its synthesis, a polypeptide chain begins to coil and fold spontaneously, forming a functional protein of specific conformation: a three-dimensional molecule with secondary and tertiary structure (see FIGURE 5.24). A gene determines primary structure, and primary structure in turn determines conformation. In many cases, a chaperone protein helps the polypeptide fold correctly (see FIGURE 5.26).

Additional steps--posttranslational modifications--may be required before the protein can begin doing its particular job in the cell. Certain amino acids may be chemically modified by the attachment of sugars, lipids, phosphate groups, or other additions. Enzymes may remove one or more amino acids from the leading (amino) end of the polypeptide chain. In some cases, a single polypeptide chain may be enzymatically cleaved into two or more pieces. For example, the protein insulin is first synthesized as a single polypeptide chain but becomes active only after an enzyme cuts out a central part of the chain, leaving a protein made up of two polypeptide chains connected by disulfide bridges (see FIGURE 5.22). In other cases, two or more polypeptides that are synthesized separately may join to become the subunits of a protein that has quaternary structure.