CHAPTER **NINETEEN** 19

Sexual Reproduction and the Power of Genetics

Individual cells reproduce by replicating their DNA and dividing in two. This basic process of cell proliferation occurs in all living species—in the cells of multicellular organisms and in free-living cells such as bacteria and yeasts—and it allows each cell to pass on its genetic information to future generations.

Yet reproduction in a multicellular organism—in a fish or a fly, a person or a plant—is a much more complicated affair. It entails elaborate developmental cycles, in which all of the organism's cells, tissues, and organs must be generated afresh from a single cell. This starter cell is no ordinary cell. It has a very peculiar origin: for most animal and plant species, it is produced by the union of a pair of cells that hail from two completely separate individuals—a mother and a father. As a result of this cell fusion—a central event in *sexual reproduction*—two genomes merge to form the genome of a new individual. The mechanisms that govern genetic inheritance in sexually reproducing organisms are therefore different, and more complex, than those that operate in organisms that pass on their genetic information asexually—by a straighforward cell division or by budding off a brand new individual.

In this chapter, we explore the cell biology of sexual reproduction. We discuss what organisms gain from sex, and we describe how they do it. We examine the reproductive cells produced by males and females, and we explore the specialized form of cell division, called *meiosis*, that generates them. We discuss how Gregor Mendel, a nineteenth-century Austrian monk, deduced the basic logic of genetic inheritance by studying the progeny of pea plants. Finally, we describe how scientists can exploit the genetics of sexual reproduction to gain insights into human biology, human origins, and the molecular underpinnings of human disease.

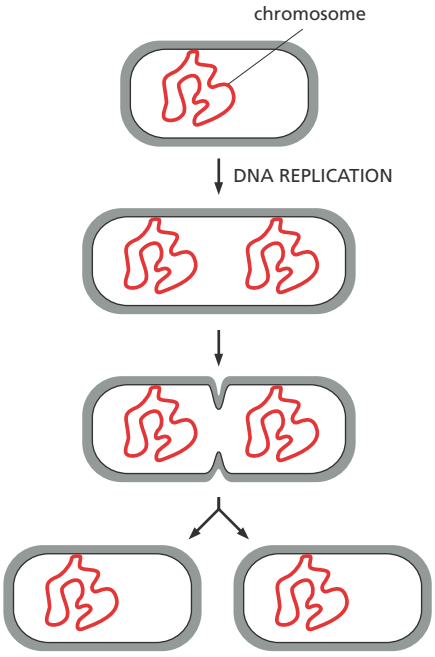


Figure 19–1 Bacteria reproduce by simple cell division. The division of one bacterium into two takes 20–25 minutes under ideal growth conditions.

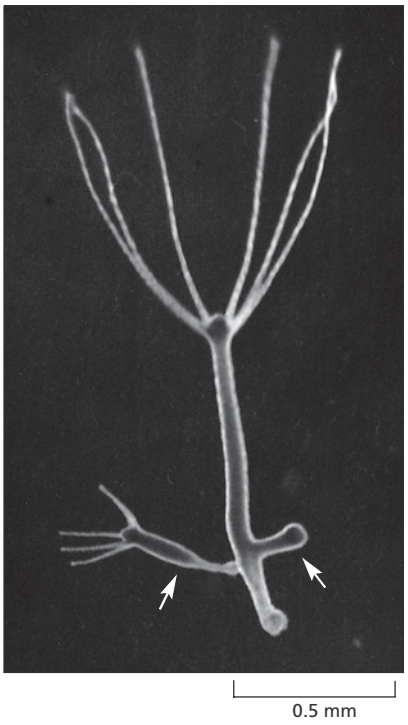


Figure 19–2 A hydra reproduces by budding. This form of asexual reproduction involves the production of buds (*arrows*), which pinch off to form progeny that are genetically identical to their parent. Eventually, these buds will detach from their parent and live independently. (Courtesy of Amata Hornbruch.)

THE BENEFITS OF SEX

Most of the creatures we see around us reproduce sexually. However, many organisms, especially those invisible to the naked eye, can produce offspring without resorting to sex. Most bacteria and other single-celled organisms multiply by simple cell division (**Figure 19–1**). Many plants also reproduce asexually, forming multicellular offshoots that later detach from the parent to make new independent plants. Even in the animal kingdom, there are species that can procreate without sex. Hydra produce young by budding (**Figure 19–2**). Certain worms, when split in two, can regenerate the "missing halves" to form two complete individuals. And in some species of insects, lizards, and even birds, the females can lay eggs that develop *parthenogenetically*—without the help of males, sperm, or fertilization—into healthy daughters that can also reproduce the same way.

But while such forms of **asexual reproduction** are simple and direct, they give rise to offspring that are genetically identical to the parent organism. **Sexual reproduction**, on the other hand, involves the mixing of DNA from two individuals to produce offspring that are genetically distinct from one another and from both their parents. This mode of reproduction apparently has great advantages, as the vast majority of plants and animals have adopted it.

Sexual Reproduction Involves Both Diploid and Haploid Cells

Organisms that reproduce sexually are generally *diploid*: each cell contains two sets of chromosomes—one inherited from each parent. Because the two parents are members of the same species, the *maternal* chromosome set and the *paternal* chromosome set are very similar. The most notable difference between them is the *sex chromosomes*, which, in some species, distinguish males from females. With the exception of these sex chromosomes, the maternal and paternal versions of every chromosome—called the maternal and paternal **homologs**—carry the same set of genes. Each diploid cell, therefore, carries two copies of every gene (except for those found on the sex chromosomes, which may be present in only one copy).

Unlike the majority of cells in a diploid organism, however, the specialized cells that perform the central process in sexual reproduction—the **germ cells**, or **gametes**—are *haploid*: they each contain only one set of chromosomes. For most organisms, the males and females produce different types of gametes. In animals, one is large and nonmotile and is referred to as the *egg*; the other is small and motile and is referred to as the *sperm* (**Figure 19–3**). These two dissimilar haploid gametes join together to regenerate a diploid cell, called the fertilized egg, or *zygote*, which has chromosomes from both the mother and father. The zygote thus produced develops into a new individual with a diploid set of chromosomes that is distinct from that of either parent (**Figure 19–4**).

For almost all multicellular animals, including vertebrates, practically the whole life cycle is spent in the diploid state. The haploid cells exist only briefly and are highly specialized for their function as genetic ambassadors. These haploid gametes are generated from diploid precursor cells by a specialized form of reductive division called *meiosis*, a process we discuss shortly. This precursor cell lineage, which is dedicated solely to the production of germ cells, is called the **germ line**. The cells forming the rest of the animal's body—the **somatic cells**—ultimately leave no

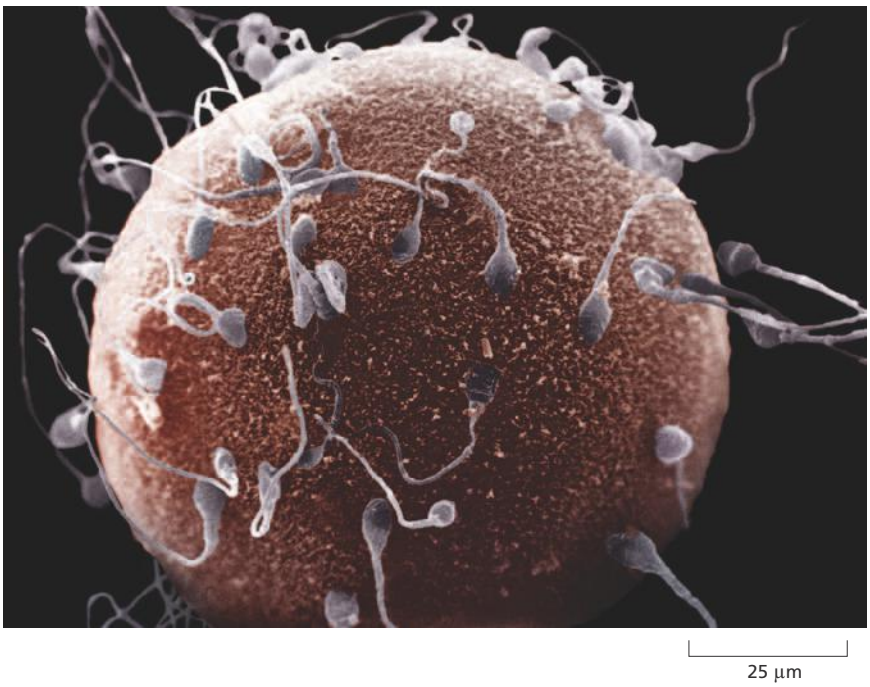


Figure 19–3 Despite their tremendous difference in size, sperm and egg contribute equally to the genetic character of the offspring. This difference in size between male and female gametes (in which eggs contain a large quantity of cytoplasm, whereas sperm contain almost none) is consistent with the fact that the cytoplasm is not the basis of inheritance. If it were, the female's contribution to the makeup of the offspring would be much greater than the male's. Shown here is a scanning electron micrograph of an egg with human sperm bound to its surface. Although many sperm are bound to the egg, only one will fertilize it. (Courtesy of David M. Phillips/Photo Researchers, Inc.)

progeny of their own (**Figure 19–5** and see Figure 9–3). They exist, in effect, only to help the cells of the germ line survive and propagate.

The sexual reproductive cycle thus involves an alternation of haploid cells, each carrying a single set of chromosomes, with generations of diploid cells, each carrying two sets of chromosomes. One benefit of this arrangement is that it allows sexually reproducing organisms to produce offspring that are genetically diverse, as we discuss next.

Sexual Reproduction Generates Genetic Diversity

Sexual reproduction produces novel chromosome combinations. During meiosis, the maternal and paternal chromosome sets present in diploid germ-line cells are partitioned out into the single chromosome sets of the gametes. Each gamete will receive a mixture of maternal homologs and paternal homologs; when the genomes of two gametes combine during fertilization, they produce a zygote with a unique chromosomal complement.

Of course, if the maternal and paternal homologs carry the same genes, why should such chromosomal assortment matter? One answer is that although the set of genes on each homolog is the same, the paternal and maternal version of each gene is not. Genes occur in variant versions, called **alleles**. For any given gene, many different alleles may be present in the "gene pool" of a species. The existence of these variant alleles means that the two copies of any given gene in a particular individual are likely to be somewhat different from each other—and from those carried by other individuals. What makes individuals within a species genetically unique is the inheritance of different combinations of alleles. And with its cycles of diploidy, meiosis, haploidy, and cell fusion, sex breaks up old combinations of alleles and generates new ones.

Sexual reproduction also generates genetic diversity through a second mechanism—genetic recombination. We discuss this process, which scrambles the genetic information on each chromosome during meiosis, a bit later.

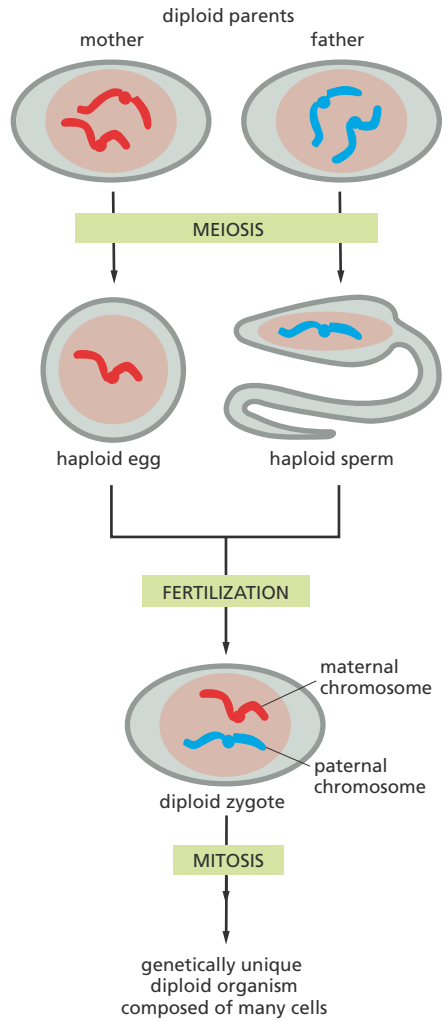


Figure 19–4 Sexual reproduction involves both haploid and diploid cells. Sperm and egg are produced by meiosis of diploid germ-line cells. During fertilization, a haploid egg and a haploid sperm fuse to form a diploid zygote. For simplicity, only one chromosome is shown for each gamete, and the sperm cell has been greatly enlarged. Human gametes have 23 chromosomes, and the egg is much larger than the sperm (see, for example, Figure 19–3).

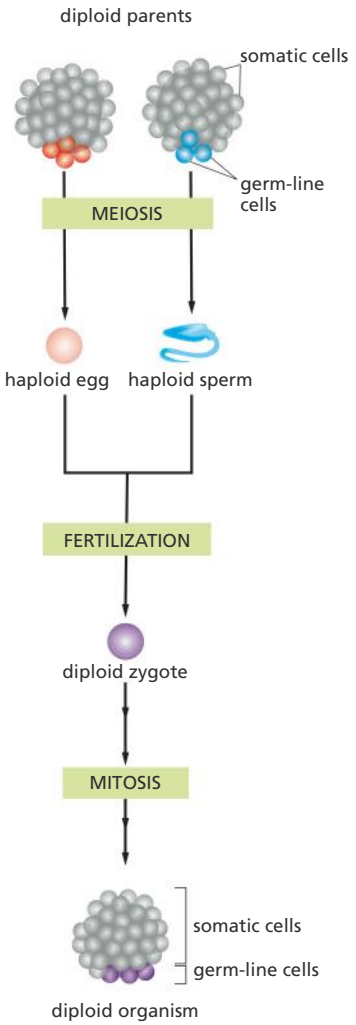


Figure 19–5 Germ-line cells and somatic cells carry out fundamentally different functions. In sexually reproducing animals, diploid germ-line cells, which are specified early in development, give rise to haploid gametes by meiosis. The gametes propagate genetic information into the next generation. Somatic cells (*gray*) form the body of the organism and are therefore necessary to support sexual reproduction, but they themselves leave no progeny.

Sexual Reproduction Gives Organisms a Competitive Advantage in a Changing Environment

The processes that generate genetic diversity during meiosis operate at random, as we will shortly discuss. That means that the alleles an individual receives from its parents are as likely to represent a change for the worse as they are a change for the better. Why, then, should the ability to try out new genetic combinations give organisms that reproduce sexually an evolutionary advantage over those that "breed true" through an asexual process? This question continues to perplex evolutionary geneticists, but one advantage seems to be that reshuffling genetic information through sexual reproduction can help a species survive in an unpredictably variable environment. If two parents produce many offspring with a wide variety of gene combinations, they increase the odds that at least one of their progeny will have a combination of features necessary for survival in a variety of environmental conditions. They are more likely, for example, to survive infections by bacteria, viruses, and parasites, which themselves continually change in a never-ending evolutionary battle. This genetic gamble may explain why even unicellular organisms, such as yeasts, intermittently indulge in a simple form of sexual reproduction. Typically, they switch on this behavior as an alternative to ordinary cell division when times are hard and starvation looms. Yeasts with a genetic defect that makes them unable to reproduce sexually show a reduced ability to evolve and adapt when they are subjected to harsh conditions.

Sexual reproduction may also be advantageous for another reason. In any population, new mutations continually occur, giving rise to new alleles—and many of these new mutations may be harmful. Sexual reproduction can speed up the elimination of these deleterious alleles and help to prevent them from accumulating in the population. By mating with only the fittest males, females select for good combinations of alleles and allow bad combinations to be lost from the population more efficiently than they would otherwise be. According to this theory, which is supported by some careful calculations of costs and benefits, sexual reproduction is favored because males can serve as a genetic filtering device: the males who succeed in mating allow the best, and only the best, collections of genes to be passed on, whereas males who fail to mate serve as a genetic "trash can"—a way of discarding bad collections of alleles from the population. Of course, for social organisms especially, it must be conceded that males may sometimes make themselves useful in other ways.

Whatever its advantages, sex has clearly been favored by evolution. In the following section, we review the central features of this popular form of reproduction, beginning with meiosis, the process by which gametes are formed.

MEIOSIS AND FERTILIZATION

Our modern understanding of the fundamental cycle of events involved in sexual reproduction grew out of discoveries reported in 1888, when Theodor Boveri noted that the fertilized egg of a parasitic roundworm contains four chromosomes, whereas the worm's gametes (sperm and egg) contain only two. This study was the first to demonstrate that gametes are **haploid**—they carry only a single set of chromosomes. All of the other cells of the body, including the germ-line cells that give rise to the gametes, are **diploid**—they carry two sets of chromosomes, one derived from the mother and the other from the father. Therefore, sperm and eggs must be produced by a special kind of "reductive" cell division in which the number of chromosomes is precisely halved (see Figure 19–4). The term **meiosis** was coined to describe this form of cell division; it comes from a Greek word meaning "diminution," or "lessening."

From Boveri's experiments on worms and other species, it became clear that the behavior of the chromosomes, which at that time were simply microscopic bodies of unknown function, matched the pattern of inheritance, in which the two parents make equal contributions to determining the character of the progeny despite the enormous difference in size between egg and sperm (see Figure 19–3). This was the first clue that chromosomes contain the material of heredity. The study of sexual reproduction and meiosis therefore has a central place in the history of cell biology.

In this section, we describe the cell biology of sexual reproduction from a modern point of view, focusing on the elaborate dance of chromosomes that occurs when a cell undertakes meiosis. We begin with an overview of how meiosis distributes chromosomes to the gametes. We then take a closer look at how chromosomes pair, recombine, and are segregated into germ cells during meiosis, thereby shuffling the maternal and paternal genes into novel combinations. We also discuss what happens when meiosis goes awry. Finally, we consider briefly the process of fertilization, through which gametes come together to form a new, genetically distinct individual.

Meiosis Involves One Round of DNA Replication Followed by Two Rounds of Cell Division

Before a diploid cell divides by mitosis, it duplicates its two sets of chromosomes. This duplication allows a full set of chromosomes—including a complete maternal set plus a complete paternal set—to be transmitted to each daughter cell (discussed in Chapter 18). Although meiosis ultimately halves this diploid chromosome complement—producing haploid gametes that carry only a single set of chromosomes—it, too, begins with a round of chromosome duplication. The subsequent reduction in chromosome number occurs because this single round of duplication is followed by two successive cell divisions without further DNA replication (**Figure 19–6**). One can imagine that meiosis might instead occur by a simple modification of mitotic cell division: if DNA replication (S phase) were omitted completely, a single round of cell division could produce two haploid cells directly. But, for reasons that are still unclear, this is not the way meiosis works.

Meiosis begins in specialized diploid germ-line cells that reside in the ovaries or testes. Like somatic cells, these germ-line cells are diploid; each contains two copies of every chromosome—a paternal homolog, inherited from the organism's father, and a maternal homolog, inherited from its mother. In the first step of meiosis, all of these chromosomes are duplicated, and the resulting copies remain closely attached to each other, as they would during an ordinary mitosis (see "Prophase" in Panel 18–1, pp. 622–623). The next phase of the process, however, is unique to meiosis. Each duplicated paternal chromosome first locates and then attaches itself to the corresponding duplicated maternal homolog, a process called *pairing*. Pairing ensures that the homologs will segregate properly during the two subsequent cell divisions and that each of the final gametes will receive a complete haploid set of chromosomes.

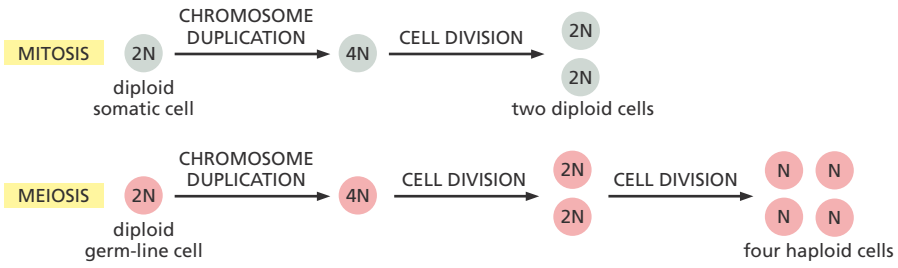


Figure 19–6 Mitosis and meiosis both begin with a round of chromosome duplication. In mitosis, this duplication is followed by a single round of cell division to yield two diploid cells. In meiosis, chromosome duplication in a diploid germ-line cell is followed by two rounds of cell division, without further DNA replication, to produce four haploid cells. *N* represents the number of chromosomes in the haploid cell.

Together, the two successive meiotic cell divisions, called *meiotic division I* (meiosis I) and *meiotic division II* (meiosis II), parcel out one complete set of chromosomes to each of the four haploid cells produced. Because the assignment of each homolog to the haploid daughter cells is random, each of the resulting gametes will receive a different mixture of maternal and paternal chromosomes.

Thus, meiosis produces four cells that are genetically dissimilar and that contain exactly half as many chromosomes as the original parent germ-line cell. Mitosis, in contrast, produces two genetically identical daughter cells. **Figure 19–7** summarizes the molecular events that distinguish

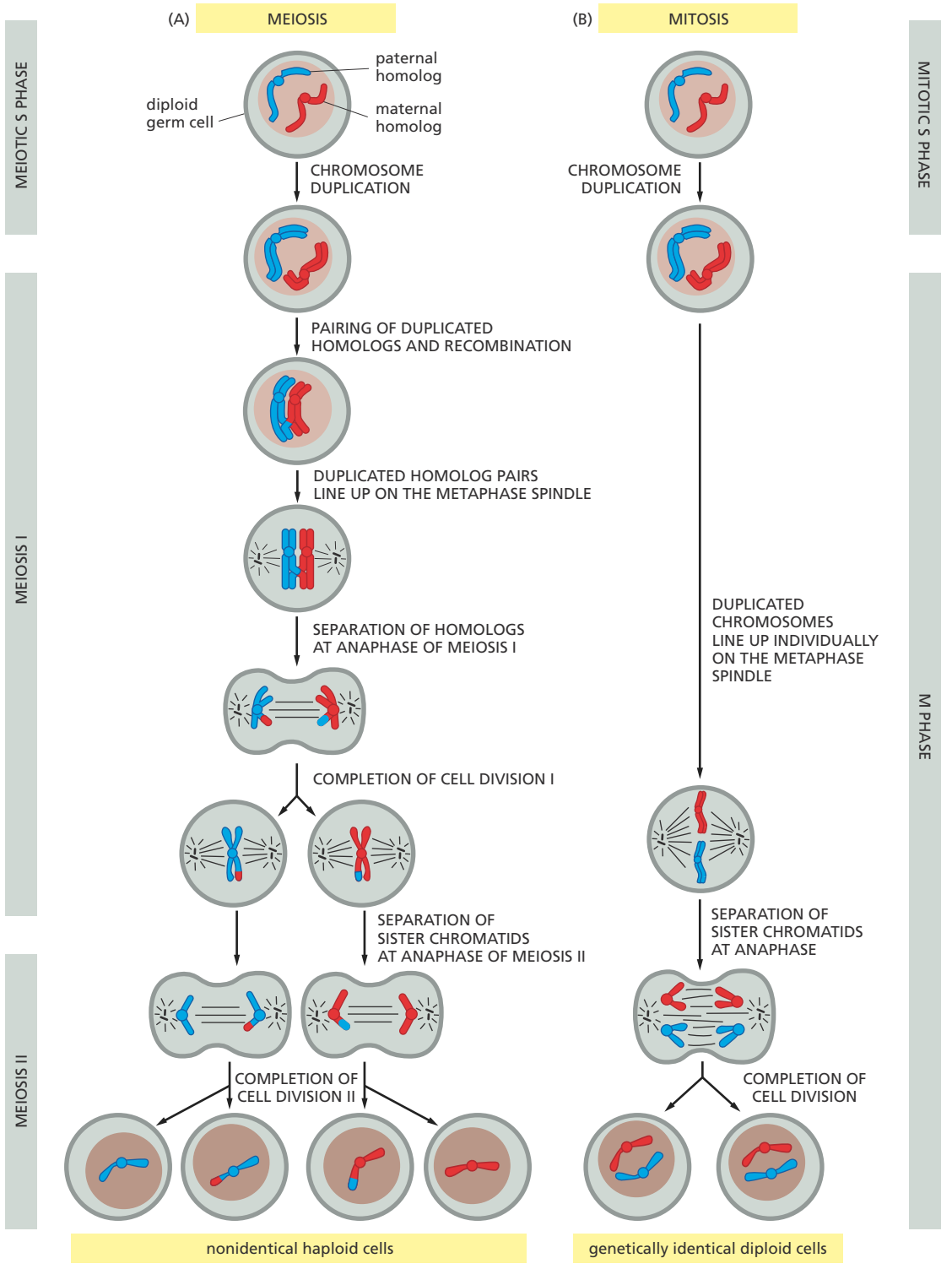


Figure 19–7 Meiosis generates four nonidentical haploid cells, whereas mitosis produces two identical diploid cells. As in Figure 19–4, only one pair of homologous chromosomes is shown. (A) In meiosis, two cell divisions are required after chromosome duplication to produce haploid cells. Each diploid cell that enters meiosis therefore produces four haploid cells, whereas (B) each diploid cell that divides by mitosis produces two diploid cells. Although mitosis and meiosis II are usually accomplished within hours, meiosis I can last days, months, or even years, because of the long time spent in prophase I.

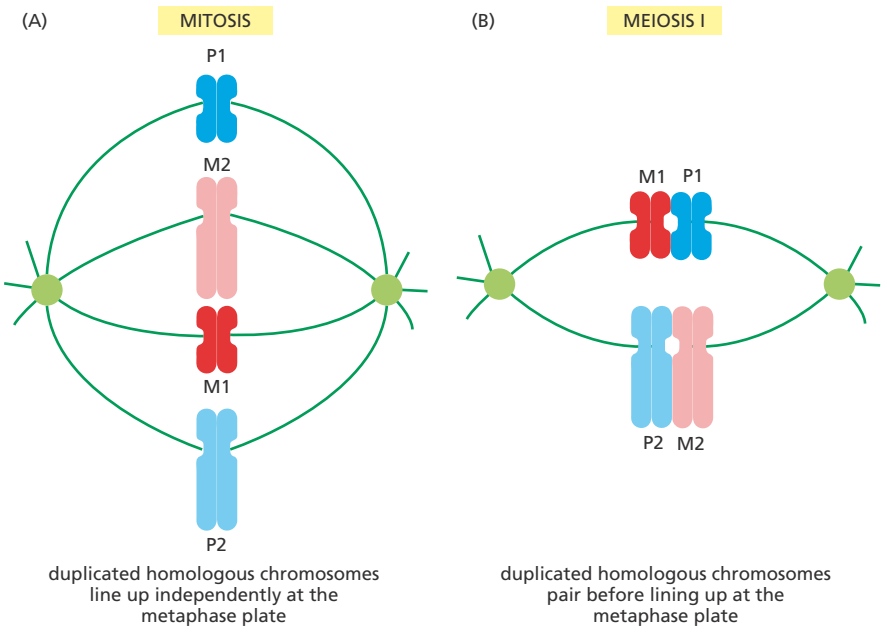


Figure 19–8 During meiosis, duplicated homologous chromosomes pair before lining up on the meiotic spindle. (A) In mitosis, the individual duplicated maternal (M) and paternal (P) chromosomes line up independently at the metaphase plate; each consists of a pair of sister chromatids, which will separate just before the cell divides. (B) By contrast, in division I of meiosis, duplicated maternal and paternal homologs pair long before lining up at the metaphase plate. The maternal and paternal homologs separate during the first meiotic division, and the sister chromatids separate during meiosis II. The mitotic and meiotic spindles are shown in *green*.

these two types of cell division—differences we now discuss in greater detail, beginning with the meiosis-specific pairing of maternal and paternal chromosomes.

Meiosis Requires the Pairing of Duplicated Homologous Chromosomes

As mentioned earlier, before a eukaryotic cell divides—by either meiosis or mitosis—it first duplicates all of its chromosomes. The twin copies of each duplicated chromosome, called **sister chromatids**, at first remain tightly linked along their length. The way these duplicated chromosomes are handled, however, differs between meiosis and mitosis. In mitosis, as we discuss in Chapter 18, the duplicated chromosomes line up, single file, at the metaphase plate (**Figure 19–8A**). As mitosis continues, the sister chromatids separate and are segregated into one or other of the two daughter cells.

In meiosis, however, the need to halve the number of chromosomes introduces an extra demand on the cell-division machinery. To ensure that each of the four haploid cells produced by meiosis will receive a single sister chromatid from each chromosome set, a germ-line cell must keep track of both the maternal and paternal **homologous chromosomes** (homologs). It does so by **pairing** the duplicated homologs before they line up at the metaphase plate (**Figure 19–8B**). Each pairing forms a structure called a **bivalent**, in which all four sister chromatids stick together until the cell is ready to divide (**Figure 19–9**). The maternal and paternal homologs will separate during meiotic division I, and the individual sister chromatids will separate during meiotic division II.

How the homologs (and the two sex chromosomes) recognize each other during pairing is still not fully understood. In many organisms, the initial association depends on an interaction between matching maternal and paternal DNA sequences at numerous sites that are widely dispersed along the homologous chromosomes. Once formed, bivalents are very stable: they remain associated throughout the long prophase of meiosis I, a stage that in some organisms can last for years.

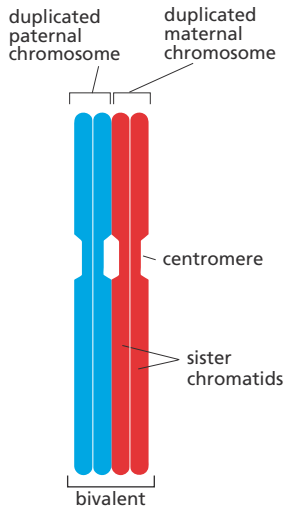


Figure 19–9 Duplicated maternal and paternal chromosomes pair during meiosis I to form bivalents. Each bivalent contains four sister chromatids and forms during prophase of meiosis I, well before attaching to the meiotic spindle.

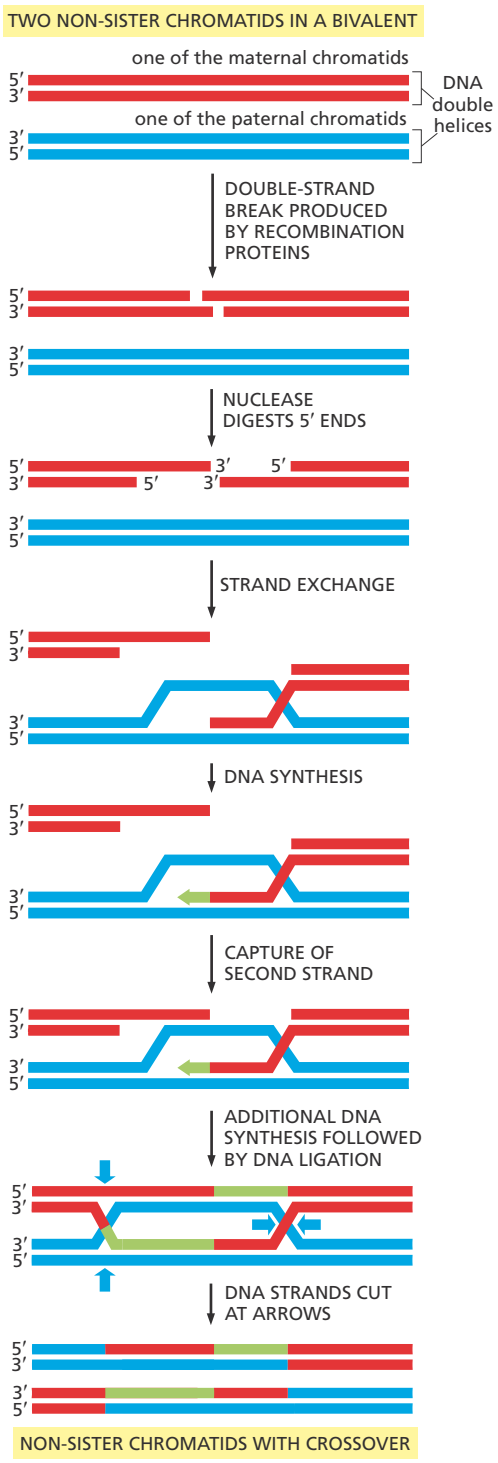


Figure 19–10 During meiosis I, non-sister chromatids in each bivalent swap segments of DNA. Here, only two of the four sister chromatids in the bivalent are shown, each drawn as a DNA double helix. During meiosis, the protein complexes that carry out this homologous recombination (not shown) first produce a double-strand break in the DNA of one of the chromatids (either the maternal or paternal chromatid) and then promote the formation of a cross-strand exchange with the other chromatid. When this exchange is resolved, each chromatid will contain a segment of DNA from the other. Many of the steps that produce chromosome crossovers during meiosis resemble those that guide the repair of DNA double-strand breaks in somatic cells (see Figure 6–30).

Crossing-Over Occurs Between the Duplicated Maternal and Paternal Chromosomes in Each Bivalent

The picture of meiotic division I we have just painted is greatly simplified, in that it leaves out a crucial feature. In sexually reproducing organisms, the pairing of the maternal and paternal chromosomes is accompanied by **homologous recombination**, a process in which two identical or very similar nucleotide sequences exchange genetic information. In Chapter 6, we discussed how homologous recombination is used to mend damaged chromosomes from which genetic information has been lost. This type of repair, uses information from an intact DNA double helix to restore the correct nucleotide sequence to a damaged, newly duplicated homolog (see Figure 6–30). A similar process takes place when homologous chromosomes pair during the long prophase of the first meiotic division. In meiosis, however, the recombination occurs between the non-sister chromatids in each bivalent, rather than between the identical sister chromatids within each duplicated chromosome. As a result, the maternal and paternal homologs end up physically swapping homologous chromosomal segments in a complex, multistep process called **crossing-over** (**Figure 19–10**).

Crossing-over is facilitated by the formation of a *synaptonemal complex*. As the duplicated homologs pair, this elaborate protein complex helps to hold the bivalent together and align the homologs so that strand exchange can readily occur between the non-sister chromatids. Each of the chromatids in a duplicated homolog (that is, each of these very long DNA double helices) can form a crossover with either (or both) of the chromatids from the other chromosome in the bivalent. The synaptonemal complex also helps space out the crossover events that take place along each chromosome.

By the time prophase I ends, the synaptonemal complex has disassembled, allowing the homologs to separate along most of their length. But each bivalent remains held together by at least one **chiasma** (plural **chiasmata**), a structure named after the Greek letter chi, χ, which is shaped like a cross. Each chiasma corresponds to a crossover between two non-sister chromatids (**Figure 19–11A**). Most bivalents contain more than one chiasma, indicating that multiple crossovers occur between homologous chromosomes (**Figure 19–11B and C**). In human oocytes—the cells that give rise to the egg—an average of two to three crossover events occur within each bivalent (**Figure 19–12**).

Crossovers that occur during meiosis are a major source of genetic variation in sexually reproducing species. By scrambling the genetic constitution of each of the chromosomes in the gamete, crossing-over helps to produce individuals with novel assortments of alleles. Crossing-over also has a second important role in meiosis. By holding homologous

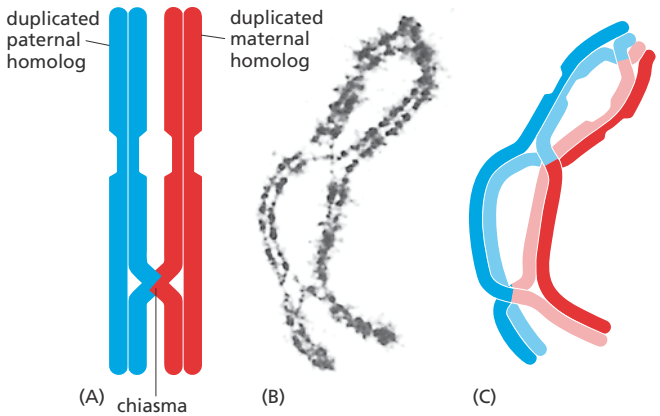


Figure 19–11 Crossover events create chiasmata between non-sister chromatids in each bivalent. (A) Schematic set of paired homologs in which one crossover event has occurred, creating a single chiasma. (B) Micrograph of a grasshopper bivalent with three chiasmata. (C) As the maternal and paternal homologs start to separate in meiosis I, chiasmata like those shown here help to hold the bivalent together. (B, courtesy of Bernard John.)

chromosomes together during prophase I, the chiasmata help ensure that the maternal and paternal homologs will segregate from one another correctly at the first meiotic division, as we discuss next.

Chromosome Pairing and Crossing-Over Ensure the Proper Segregation of Homologs

In most organisms, crossing-over during meiosis is required for the correct segregation of the two duplicated homologs into separate daughter nuclei. The chiasmata created by crossover events keep the maternal and paternal homologs bundled together until the spindle separates them during meiotic anaphase I. Before anaphase I, the two poles of the spindle pull on the duplicated homologs in opposite directions, and the chiasmata resist this pulling (**Figure 19–13A**). In so doing, the chiasmata help to position and stabilize bivalents at the metaphase plate.

In addition to the chiasmata, which hold the maternal and paternal homologs together, *cohesin* proteins (described in Chapter 18) keep the sister chromatids glued together along their entire length at meiosis I (see Figures 19–11B and 18–18). At the start of anaphase I, the cohesin proteins that hold the chromosome arms together are suddenly degraded. This release allows the arms to separate and the recombined homologs to be pulled apart (**Figure 19–13B**). This release is necessary because if the arms did not come apart, the duplicated maternal and paternal homologs would remain tethered to one another by the homologous DNA segments they had exchanged.

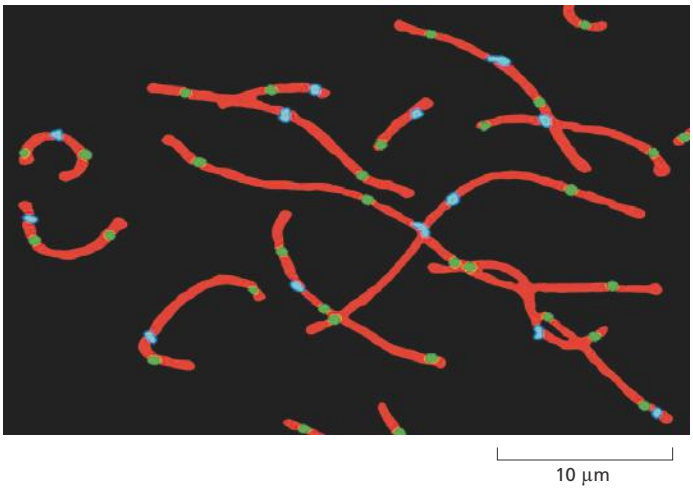


Figure 19–12 Multiple crossovers can occur between the duplicated homologous chromosomes in a bivalent. Fluorescence micrograph shows a spread of chromosomes from a human oocyte (egg-cell precursor) at the stage where all four chromatids—both maternal and paternal homologs—are still tightly associated: each single long thread (stained *red*) is a bivalent, containing four DNA double helices. Sites of crossing-over are marked by the presence of a protein (stained *green*) that is a key component of the meiotic recombination machinery. *Blue* staining marks the position of centromeres (see Figure 19–9). (From C. Tease et al., *Am. J. Hum. Genet.* 70:1469–1479, 2002. With permission from Elsevier.)

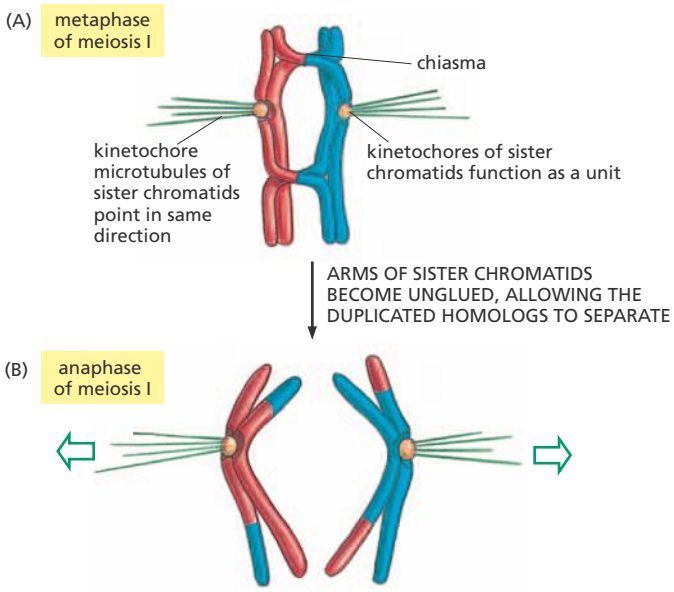


Figure 19–13 Chiasmata help ensure proper segregation of duplicated homologs during the first meiotic division. (A) In metaphase of meiosis I, chiasmata created by crossing-over hold the maternal and paternal homologs together. At this stage, cohesin proteins (not shown) keep the sister chromatids glued together along their entire length. The kinetochores of sister chromatids function as a single unit in meiosis I, and microtubules that attach to them point toward the same spindle pole. (B) At anaphase of meiosis I, the cohesins holding the arms of the sister chromatids together are suddenly degraded, allowing the homologs to be separated. Cohesins in the centromere continue to hold the sister chromatids together as the homologs are pulled apart.

The Second Meiotic Division Produces Haploid Daughter Cells

To separate the sister chromatids and produce cells with a haploid amount of DNA, a second round of division, meiosis II, follows soon after the first—without further DNA replication or any significant interphase period. A meiotic spindle forms, and the kinetochores on each pair of sister chromatids now attach to kinetochore microtubules that point in opposite directions, as they would in an ordinary mitotic division. At anaphase of meiosis II, the remaining, meiosis-specific cohesins—located in the centromere—are degraded, and the sister chromatids are drawn into different daughter cells (**Figure 19–14**). The entire process is shown in **Movie 19.1**.

Haploid Gametes Contain Reassorted Genetic Information

Even though they share the same parents, no two siblings are genetically the same (unless they are identical twins). These genetic differences are initiated long before sperm meets egg, when meiosis I produces two kinds of randomizing genetic reassortment.

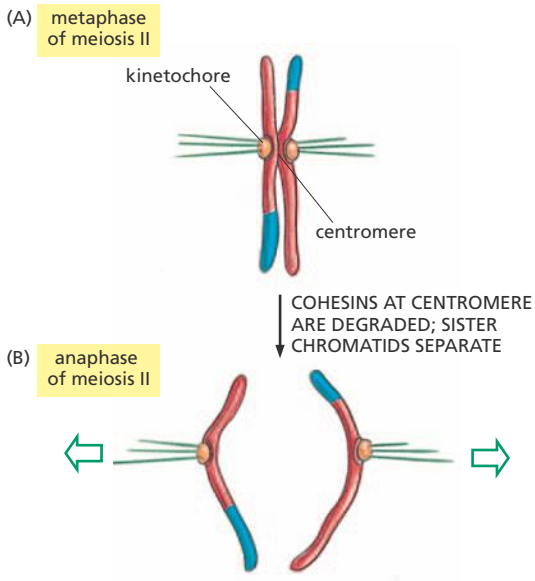


Figure 19–14 In meiosis II, as in mitosis, the kinetochores on each sister chromatid function independently, allowing the two sister chromatids to be pulled to opposite poles. (A) In metaphase of meiosis II, the kinetochores of the sister chromatids point in opposite directions. (B) At anaphase of meiosis II, the cohesins holding the sister chromatids together at the centromere are degraded, allowing kinetochore microtubules to pull the two sister chromatids to opposite poles.

First, as we have seen, the maternal and paternal chromosomes are shuffled and dealt out randomly during meiosis I. Although the chromosomes are carefully distributed so that each cell receives one and only one copy of each chromosome, the choice between the maternal or paternal homolog is made by chance, like the flip of a coin. Thus, each gamete contains the maternal versions of some chromosomes and the paternal versions of others (**Figure 19–15A**). This random assortment depends solely on the way each bivalent happens to be positioned when it lines up on the spindle during metaphase of meiosis I. Whether the maternal or paternal homolog is captured by the microtubules from one pole or the other depends on which way the bivalent is facing when the microtubules connect to its kinetochore (see Figure 19–13). Because the orientation of each bivalent at the moment of capture is completely random, the assortment of maternal and paternal chromosomes is random as well.

Thanks to this random reassortment of maternal and paternal homologs, an individual could in principle produce 2^n genetically different gametes, where n is the haploid number of chromosomes. With 23 chromosomes to choose from, each human, for example, could in theory produce 2^{23}—or 8.4×10^6—genetically distinct gametes. The actual number of different gametes each person can produce, however, is much greater than that, because the crossing-over that takes place during meiosis provides a second source of randomized genetic reassortment. Between two and three crossovers occur on average between each pair of human homologs, generating new chromosomes with novel assortments of maternal and paternal alleles. Because crossing-over occurs at more or less random sites along the length of a chromosome, each meiosis will produce four sets of entirely novel chromosomes (**Figure 19–15B**).

Taken together, the random reassortment of maternal and paternal chromosomes, coupled with the genetic mixing of crossing-over, provides a

QUESTION 19–1

Why do you think that organisms do not use the first steps of meiosis (up to and including meiotic cell division I) for the ordinary mitotic division of somatic cells?

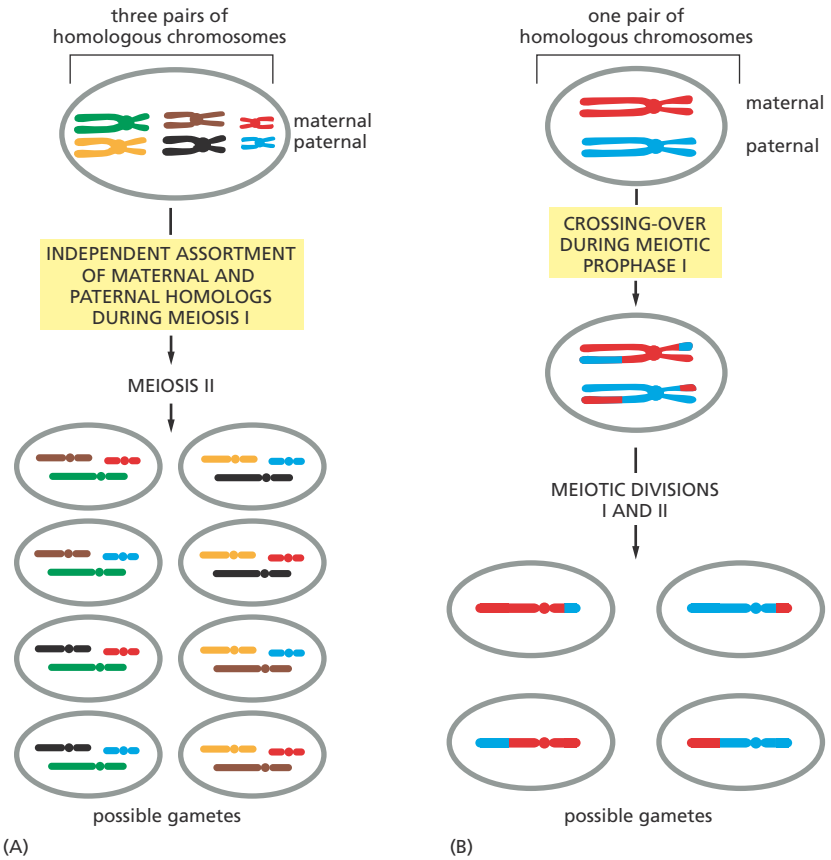


Figure 19–15 Two kinds of genetic reassortment generate new chromosome combinations during meiosis. (A) The independent assortment of the maternal and paternal homologs during meiosis produces 2^n different haploid gametes for an organism with n chromosomes. Here $n = 3$, and there are 2^3, or 8, different possible gametes. For simplicity, chromosome crossing-over is not shown here. (B) Crossing-over during meiotic prophase I exchanges segments of DNA between homologous chromosomes and thereby reassorts genes on each individual chromosome. For simplicity, only a single pair of homologous chromosomes is shown. Both independent chromosome assortment and crossing-over occur during every meiosis.

nearly limitless source of genetic variation in the gametes produced by a single individual. Considering that every person is formed by the fusion of such gametes, produced by two completely different individuals, the richness of human variation that we see around us, even within a single family, is not at all surprising.

Meiosis Is Not Flawless

The sorting of chromosomes that takes place during meiosis is a remarkable feat of molecular bookkeeping: in humans, each meiosis requires that the starting cell keep track of 92 chromosomes (23 pairs, each of which has duplicated), handing out one complete set to each gamete. Not surprisingly, mistakes can occur in the distribution of chromosomes during this elaborate process.

Occasionally, homologs fail to separate properly—a phenomenon known as *nondisjunction*. As a result, some of the haploid cells that are produced lack a particular chromosome, while others have more than one copy of it. Upon fertilization, such gametes form abnormal embryos, most of which die. Some survive, however. *Down syndrome*, for example—a disorder associated with cognitive disability and characteristic physical abnormalities—is caused by an extra copy of Chromosome 21. This error results from nondisjunction of a Chromosome 21 pair during meiosis I, giving rise to a gamete that contains two copies of that chromosome instead of one (**Figure 19–16**). When this abnormal gamete fuses with a normal gamete at fertilization, the resulting embryo contains three copies of Chromosome 21 instead of two. This chromosome imbalance produces an extra dose of the proteins encoded by Chromosome 21 and thereby interferes with the proper development of the embryo and normal functions in the adult.

The frequency of chromosome mis-segregation during the production of human gametes is remarkably high, particularly in females: nondisjunction occurs in about 10% of the meioses in human oocytes, giving rise to eggs that contain the wrong number of chromosomes (a condition called *aneuploidy*). Aneuploidy occurs less often in human sperm, perhaps because sperm development is subjected to more stringent quality

QUESTION 19–2

Ignoring the effects of chromosome crossovers, an individual human can in principle produce $2^{23} = 8.4 \times 10^6$ genetically different gametes. How many of these possibilities can be "sampled" in the average life of (A) a female and (B) a male, given that women produce one egg a month during their fertile years, whereas men can make hundreds of millions of sperm each day?

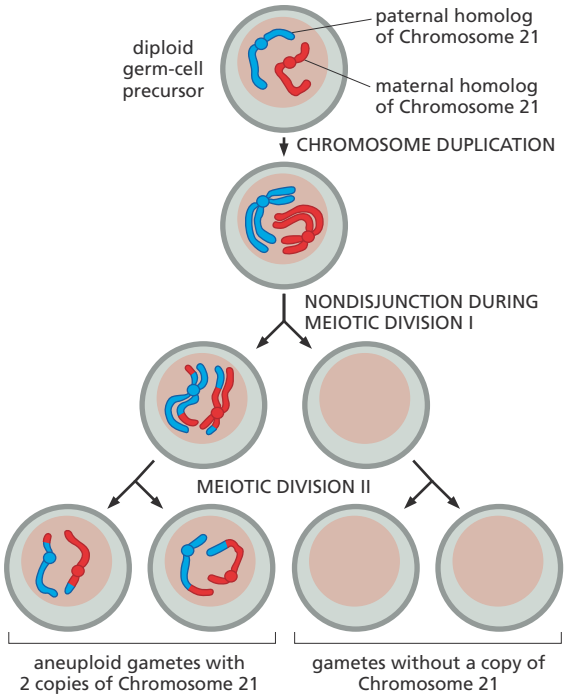


Figure 19–16 Errors in chromosome segregation during meiosis can result in gametes with incorrect numbers of chromosomes. In this example, the duplicated maternal and paternal copies of Chromosome 21 fail to separate normally during the first meiotic division. As a result, two of the gametes receive no copy of the chromosome, while the other two gametes receive two copies instead of the proper single copy. Gametes that receive an incorrect number of chromosomes are called *aneuploid* gametes. If one of them participates in the fertilization process, the resulting zygote will also have an abnormal number of chromosomes. A child that receives three copies of Chromosome 21 will have Down syndrome.

control than egg development. If meiosis goes wrong in male cells, a cell-cycle checkpoint mechanism is activated, arresting meiosis and leading to cell death by apoptosis. Regardless of whether the segregation error occurs in the sperm or the egg, nondisjunction is thought to be one reason for the high rate of miscarriages (spontaneous abortions) in early pregnancy in humans.

Fertilization Reconstitutes a Complete Diploid Genome

Having seen how chromosomes are parceled out during meiosis to form haploid germ cells, we now briefly consider how they are reunited in the process of **fertilization** to form a new zygote with a diploid set of chromosomes.

Of the 300 million human sperm ejaculated during coitus, only about 200 reach the site of fertilization in the oviduct. Sperm are attracted to an ovulated egg by chemical signals released by both the egg and the supporting cells that surround it. Once a sperm finds the egg, it must migrate through a protective layer of cells and then bind to, and tunnel through, the egg coat, called the *zona pellucida*. Finally, the sperm must bind to and fuse with the underlying egg plasma membrane (**Figure 19–17**). Although fertilization normally occurs by this process of sperm–egg fusion, it can also be achieved artificially by injecting the sperm directly into the egg cytoplasm; this is often done in infertility clinics when there is a problem with natural sperm–egg fusion.

Although many sperm may bind to an egg (see Figure 19–3), only one normally fuses with the egg plasma membrane and introduces its DNA into the egg cytoplasm. The control of this step is especially important because it ensures that the fertilized egg—also called a **zygote**—will contain two, and only two, sets of chromosomes. Several mechanisms prevent multiple sperm from entering an egg. In one mechanism, the first successful sperm triggers the release of a wave of Ca^{2+} ions in the egg cytoplasm. This flood of Ca^{2+} in turn triggers the secretion of enzymes that cause a "hardening" of the zona pellucida, which prevents "runner up" sperm from penetrating the egg. The Ca^{2+} wave also helps trigger the development of the egg. To watch a fertilization-induced calcium wave, see **Movie 19.2**.

The process of fertilization is not complete, however, until the two haploid nuclei (called *pronuclei*) come together and combine their chromosomes into a single diploid nucleus. Soon after the pronuclei fuse, the diploid cell begins to divide, forming a ball of cells that—through repeated rounds of cell division and differentiation—will give rise to an embryo and, eventually, an adult organism. Fertilization marks the beginning of one of the most remarkable phenomena in all of biology—the process by which a single-celled zygote initiates the developmental program that directs the formation of a new individual.

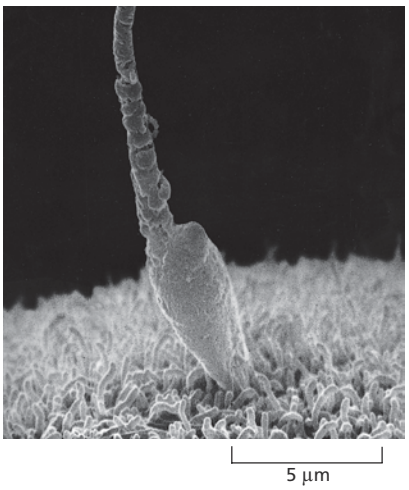


Figure 19–17 A sperm binds to the plasma membrane of an egg. Shown here is a scanning electron micrograph of a human sperm coming in contact with a hamster egg. The egg has been stripped of its zona pellucida, exposing the plasma membrane, which is covered in fingerlike microvilli. Such uncoated hamster eggs were sometimes used in infertility clinics to assess whether a man's sperm were capable of penetrating an egg. The zygotes resulting from this test do not develop. (Courtesy of David M. Phillips.)

Figure 19–18 One disproven theory of inheritance suggested that genetic traits are passed down solely from the father. In support of this particular theory of uniparental inheritance, some early microscopists fancied that they could detect a small, perfectly formed human crouched inside the head of each sperm.

MENDEL AND THE LAWS OF INHERITANCE

In organisms that reproduce without sex, the genetic material of the parent is transmitted faithfully to its progeny. The resulting offspring are thus genetically identical to a single parent. Before Mendel started working with peas, some biologists suspected that inheritance might work that way in humans (**Figure 19–18**).

Although children resemble their parents, they are not carbon copies of either the mother or the father. Thanks to the mechanisms of meiosis just described, sex breaks up existing collections of genetic information, shuffles alleles into new combinations, and produces offspring that tend

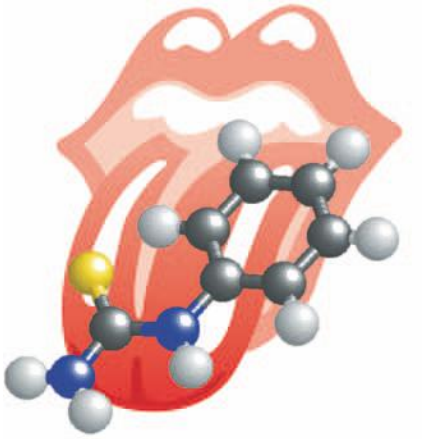


Figure 19–19 Some people taste it, some people don't. The ability to taste the chemical phenylthiocarbamide (PTC) is governed by a single gene. Although geneticists have known since the 1930s that the inability to taste PTC is inherited, it was not until 2003 that researchers identified the responsible gene, which encodes a bitter-taste receptor. Nontasters produce a PTC receptor protein that contains amino acid substitutions that are thought to reduce the receptor's activity.

to exhibit a mixture of traits derived from both parents, as well as novel ones. The ability to track characteristics that show some variation from one generation to the next enabled geneticists to begin to decipher the rules that govern heredity in sexually reproducing organisms.

The simplest traits to follow are those that are easy to see or to measure. In humans, these include the tendency to sneeze when exposed to bright sun, whether a person's earlobes are attached or pendulous, or the ability to detect certain odors or flavors (**Figure 19–19**). Of course, the laws of inheritance were not worked out by studying people with pendulous earlobes, but by following traits in organisms that are easy to breed and that produce large numbers of offspring. Gregor Mendel, the father of genetics, focused on peas. But similar breeding experiments can be performed in fruit flies, worms, dogs, cats, or any other plant or animal that possesses characteristics of interest, because the same basic laws of inheritance apply to all sexually reproducing organisms, from peas to people.

In this section, we describe the logic of genetic inheritance in sexually reproducing organisms. We see how the behavior of chromosomes during meiosis—their segregation into gametes that then unite at random to form genetically unique offspring—explains the experimentally derived laws of inheritance. But first, we discuss how Mendel, breeding peas in his monastery garden, discovered these laws more than 150 years ago.

Mendel Studied Traits That Are Inherited in a Discrete Fashion

Mendel chose to study pea plants because they are easy to cultivate in large numbers and could be raised in a small space—such as an abbey garden. He controlled which plants mated with which by removing sperm (pollen) from one plant and brushing it onto the female structures of another. This careful cross-pollination ensured that Mendel could be certain of the parentage of every pea plant he examined.

Perhaps more important for Mendel's purposes, pea plants were available in many varieties. For example, one variety has purple flowers, another has white. One variety produces seeds (peas) with smooth skin, another produces peas that are wrinkled. Mendel chose to follow seven traits—such as flower color and pea shape—that were distinct, easily observable, and, most importantly, inherited in a discrete fashion: for example, the plants have either purple flowers or white flowers—nothing in between (**Figure 19–20**).

Mendel Disproved the Alternative Theories of Inheritance

The breeding experiments that Mendel performed were straightforward. He started with stocks of genetically pure, "true-breeding" plants—those that produce offspring of the same variety when allowed to self-fertilize. If he followed pea color, for example, he used plants with yellow peas that always produced offspring with yellow peas, and plants with green peas that always produced offspring with green peas.

Mendel's predecessors had focused on organisms that varied in multiple traits. These investigators often wound up trying to characterize offspring whose appearance differed in such a complex way that they could not easily be compared with their parents. But Mendel took the unique approach of studying each trait one at a time. In a typical experiment, he would cross-pollinate two of his true-breeding varieties. He then recorded the inheritance of the chosen trait in the next generation. For example, Mendel crossed plants producing yellow peas with plants producing green peas and discovered that the resulting hybrid offspring,

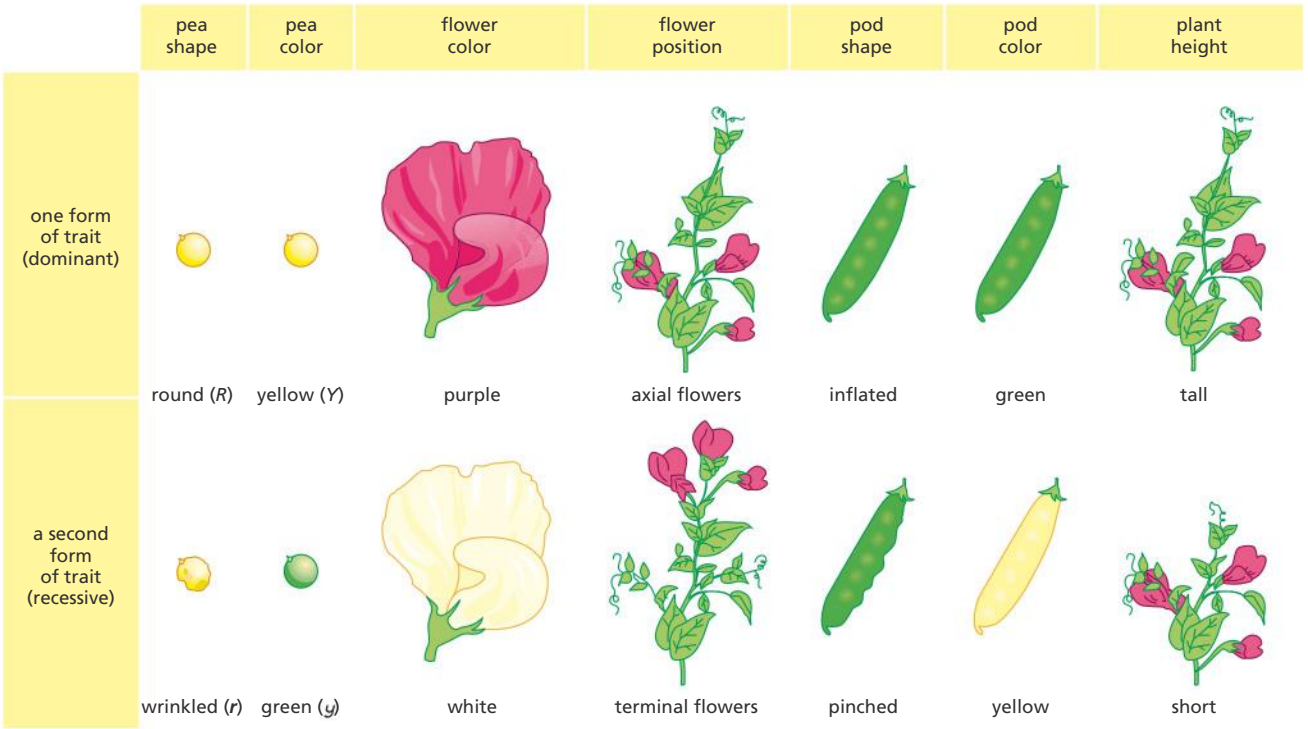


Figure 19–20 Mendel studied seven traits that are inherited in a discrete fashion. For each trait, the plants display either one variation or the other, with nothing in between. As we will see shortly, one form of each trait is dominant, whereas the other is recessive.

called the first filial, or F_1, generation, all had yellow peas (**Figure 19–21**). He obtained a similar result for every trait he followed: the F_1 hybrids all resembled only one of their two parents.

Had Mendel stopped there—observing only the F_1 generation—he might have developed some mistaken ideas about the nature of heredity: these results appear to support the theory of uniparental inheritance, which states that the appearance of the offspring will match one parent or the other (see, for example, Figure 19–18). Fortunately, Mendel took his breeding experiments to the next step: he crossed the F_1 plants with one another (or allowed them to self-fertilize) and examined the results.

Mendel's Experiments Revealed the Existence of Dominant and Recessive Alleles

One look at the offspring of Mendel's initial cross-fertilization experiments, such as those shown in Figure 19–21, raises an obvious question: what happened to the traits that disappeared in the F_1 generation? Did the plants bearing green peas, for example, fail to make a genetic contribution to their offspring? To find out, Mendel allowed the F_1 plants to self-fertilize. If the trait for green peas had been lost, then the F_1 plants would produce only plants with yellow peas in the next, F_2, generation. Instead, he found that the "disappearing trait" returned: although three-quarters of the offspring in the F_2 generation had yellow peas, one-quarter had green peas (**Figure 19–22**). Mendel saw the same type of behavior for each of the other six traits he examined.

To account for these observations, Mendel proposed that the inheritance of traits is governed by hereditary factors (which we now call genes) and that these factors come in alternative versions that account for the variations seen in inherited characteristics. The gene dictating pea color, for example, exists in two "flavors"—one that directs the production of yellow peas and one that directs production of green peas. Such alternative versions of a gene are now called *alleles*, and the whole collection of alleles possessed by an individual—its genetic makeup—is called its **genotype**.

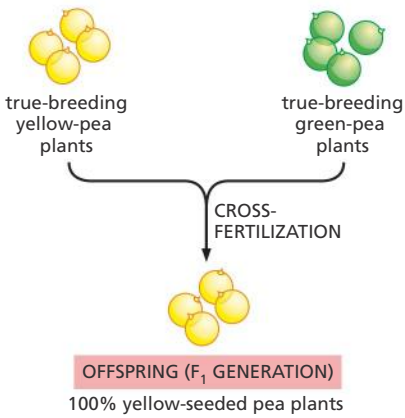


Figure 19–21 True-breeding varieties, when cross-fertilized with each other, produce hybrid offspring that resemble one parent. In this case, true-breeding green-pea plants, crossed with true-breeding yellow-pea plants, always produce offspring with yellow peas.

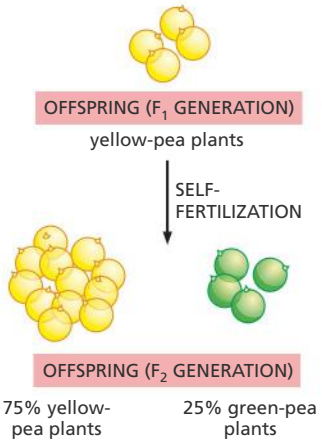


Figure 19–22 The appearance of the F_2 generation shows that an individual carries two alleles of each gene. When the F_1 plants in Figure 19–21 are allowed to self-fertilize (or are bred with each other), 25% of the progeny produce green peas.

Mendel's major conceptual breakthrough was to propose that for each characteristic, an organism must inherit two copies, or alleles, of each gene—one from its mother and one from its father. The true-breeding parental strains, he theorized, each possessed a pair of identical alleles—the yellow-pea plants possessed two alleles for yellow peas, the green-pea plant two alleles for green peas. An individual that possesses two identical alleles is said to be **homozygous** for that trait. The F_1 hybrid plants, on the other hand, had received two dissimilar alleles—one specifying yellow peas and the other green. These plants were **heterozygous** for the trait of interest.

The appearance, or **phenotype**, of the organism depends on which versions of each allele it inherits. To explain the disappearance of a trait in the F_1 generation—and its reappearance in the F_2 generation—Mendel supposed that for any pair of alleles, one allele is *dominant* and the other is *recessive*, or hidden. The dominant allele, whenever it is present, would dictate the plant's phenotype. In the case of pea color, the allele that specifies yellow peas is dominant; the green-pea allele is recessive.

One important consequence of heterozygosity, and of dominance and recessiveness, is that not all of the alleles that an individual carries can be detected in its phenotype. Humans have about 30,000 genes, and each of us is heterozygous for a very large number of these. Thus, we all carry a great deal of genetic information that remains hidden in our personal phenotype but that can turn up in future generations.

Each Gamete Carries a Single Allele for Each Character

Mendel's theory—that for every gene, an individual inherits one copy from its mother and one copy from its father—raised some logistical issues. If an organism has two copies of every gene, how does it pass only one copy of each to its progeny? And how do these gene sets come together again in the resulting offspring?

Mendel postulated that when sperm and eggs are formed, the two copies of each gene present in the parent separate so that each gamete receives only one allele for each trait. For his pea plants, each egg (ovum) and each sperm (pollen) receives only one allele for pea color (either yellow or green), one allele for pea shape (smooth or wrinkled), one allele for flower color (purple or white), and so on. During fertilization, sperm carrying one or other allele unites with an egg carrying one or other allele to produce a fertilized egg or zygote with two alleles. Which type of sperm unites with which type of egg at fertilization is entirely a matter of chance.

This principle of heredity is laid out in Mendel's first law, the **law of segregation**. It states that the two alleles for each trait separate (or segregate) during gamete formation and then unite at random—one from each parent—at fertilization. According to this law, the F_1 hybrid plants with yellow peas will produce two classes of gametes: half the gametes will get a yellow-pea allele and half will get a green-pea allele. When the hybrid plants self-pollinate, these two classes of gametes will unite at random. Thus, four different combinations of alleles can come together in the F_2 offspring (**Figure 19–23**). One-quarter of the F_2 plants will receive two alleles specifying green peas; these plants will obviously produce green peas. One-quarter of the plants will receive two yellow-pea alleles and will produce yellow peas. But one-half of the plants will inherit one allele for yellow peas and one allele for green. Because the yellow allele is dominant, these plants—like their heterozygous F_1 parents—will all produce yellow peas. All told, three-quarters of the offspring will have yellow peas and one-quarter will have green peas. Thus Mendel's law of segregation explains the 3:1 ratio that he observed in the F_2 generation.

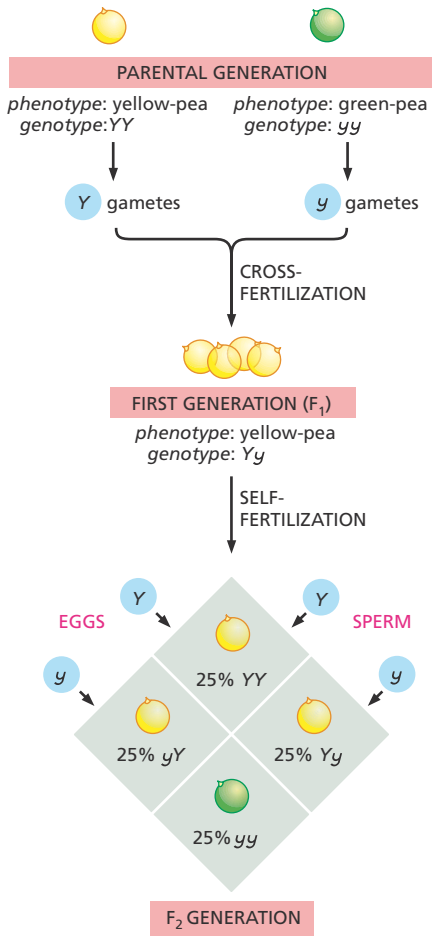


Figure 19–23 Parent plants produce gametes that each contain one allele for each trait; the phenotype of the offspring depends on which combination of alleles it receives. Here we see both the genotype and phenotype of the pea plants that were bred in the experiments illustrated in Figures 19–21 and 19–22. The true-breeding yellow-pea plants produce only *Y*-bearing gametes; the true-breeding green plants produce only *y* gametes. The F_1 offspring of a cross between these parents all produce yellow peas, and they have the genotype *Yy*. When these hybrid plants are bred with each other, 75% of the offspring have yellow peas, 25% have green. The gray box at the bottom, called a Punnett square after a British mathematician who was a follower of Mendel, allows one to track the segregation of alleles during gamete formation and to predict the outcomes of breeding experiments like the one outlined in Figure 19–22. According to the system invented by Mendel, capital letters indicate a dominant allele and lowercase letters a recessive allele.

Mendel's Law of Segregation Applies to All Sexually Reproducing Organisms

Mendel's law of segregation explained the data for every trait he examined in pea plants, and he replicated his basic findings with corn and beans. But his rules governing inheritance are not limited to plants: they apply to all sexually reproducing organisms (**Figure 19–24**).

Consider a phenotype in humans that reflects the action of a single gene. The major form of *albinism*—Type II albinism—is a rare condition that is inherited in a recessive manner in many animals, including humans. Like the pea plants that produce green seeds, albinos are homozygous recessive: their genotype is *aa*. The dominant allele of the gene (denoted *A*) encodes an enzyme involved in making melanin, the pigment responsible for most of the brown and black color present in hair, skin, and the retina of the eye. Because the recessive allele codes for a version of this enzyme that is only weakly active or completely inactive, albinos have white hair, white skin, and pupils that look pink because a lack of melanin in the eye allows the red color of the hemoglobin in blood vessels in the retina to be visible.

The trait for albinism is inherited in the same manner as any other recessive trait, including Mendel's green peas. If a Type II albino man (genotype *aa*) has children with a Type II albino woman (also *aa*), all of their children will be albino (*aa*). However, if a nonalbino man (*AA*) marries and has children with an albino woman (*aa*), their children will all be heterozygous (*Aa*) and normally pigmented (**Figure 19–25**). If two nonalbino individuals with an *Aa* genotype start a family, each of their children would have a 25% chance of being an albino (*aa*).

Of course, humans generally don't have families large enough to guarantee accurate Mendelian ratios. (Mendel arrived at his ratios by breeding

Figure 19–24 Mendel's law of segregation applies to all sexually reproducing organisms. Dogs are bred specifically to enhance certain phenotypic traits, including a diverse range of body size, coat color, head shape, snout length, ear position, and fur patterns. Scientists have been conducting genetic analyses on scores of dog breeds to search for the alleles that underlie these common canine characteristics. A single growth factor gene has been linked to body size, and three additional genes have been shown to account for coat length, curliness, and the presence or absence of "furnishings"—bushy eyebrows and beards—in almost all dog breeds. (Courtesy of Ester Inbar.)

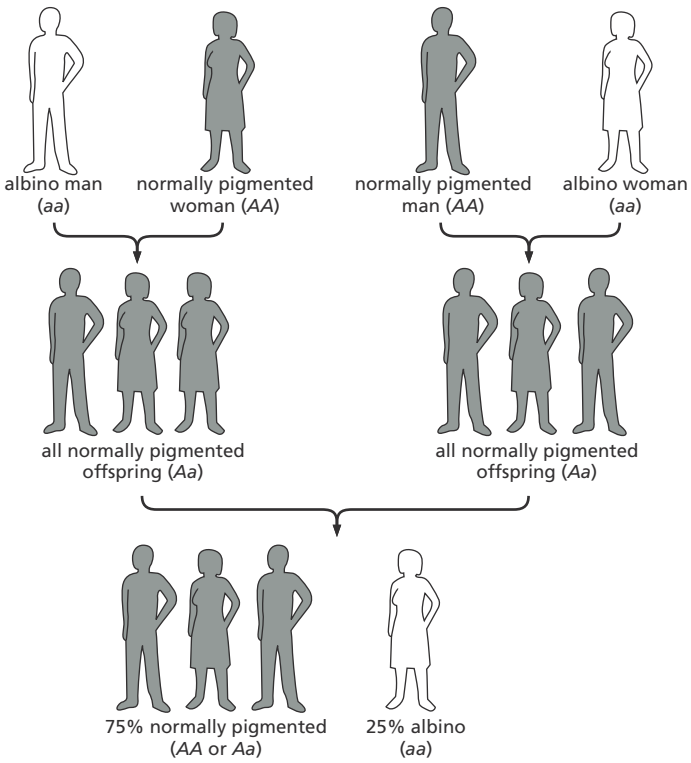


Figure 19–25 Recessive alleles all follow the same Mendelian laws of inheritance. Here, we trace the inheritance of Type II albinism, a recessive trait that is associated with a single gene in humans. Note that normally pigmented individuals can be either homozygous (*AA*) or heterozygous (*Aa*) for the dominant allele *A*.

and counting thousands of pea plants for most of his crosses.) Geneticists that follow the inheritance of specific traits in humans get around this problem by working with large numbers of families—or with several generations of a few large families—and preparing **pedigrees** that show the phenotype of each family member for the relevant trait. **Figure 19–26** shows the pedigree for a family that harbors a recessive allele for deafness. It also illustrates an important practical consequence of Mendel's laws: first-cousin marriages create a greatly increased risk of producing children that are homozygous for a deleterious recessive mutation.

Alleles for Different Traits Segregate Independently

Mendel deliberately simplified the problem of heredity by starting with breeding experiments that focused on the inheritance of one trait at a time, called *monohybrid crosses*. He then turned his attention to multihybrid crosses, examining the simultaneous inheritance of two or more apparently unrelated traits.

In the simplest situation, a *dihybrid cross*, Mendel followed the inheritance of two traits at once: for example, pea color and pea shape. In the case of pea color, we have already seen that yellow is dominant over

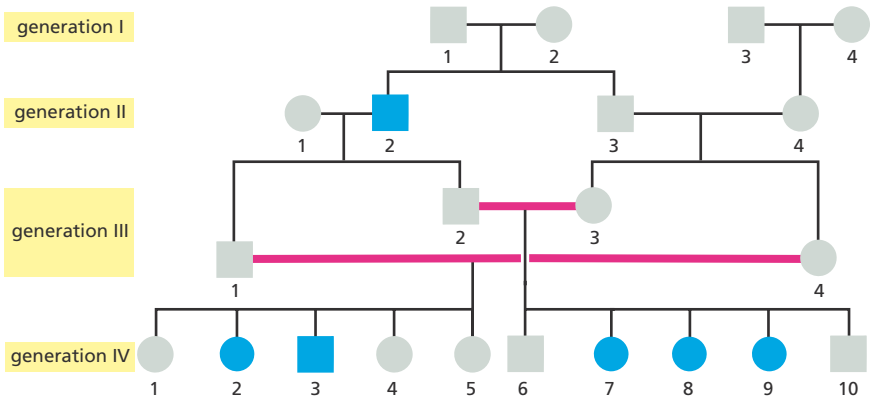


Figure 19–26 A pedigree shows the risks of first-cousin marriages. Shown here is an actual pedigree for a family that harbors a rare recessive mutation causing deafness. According to convention, *squares* represent males, *circles* are females. Here, family members that show the deaf phenotype are indicated by a *blue* symbol, those that do not by a *gray* symbol. A *black* horizontal line connecting a male and female represents a mating between unrelated individuals, and a *red* horizontal line represents a mating between blood relatives. The offspring of each mating are shown underneath, in order of their birth from left to right.

Individuals within each generation are labeled sequentially from left to right for purposes of identification. In the third generation in this pedigree, for example, individual 2, a man who is not deaf, marries his first cousin, individual 3, who is also not deaf. Three out of their five children (individuals 7, 8, and 9 in the fourth generation) are deaf. Meanwhile, individual 1, the brother of 2, also marries a first cousin, individual 4, the sister of 3. Two out of their five children are deaf. (Adapted from Z.M. Ahmed et al., *BMC Med. Genet.* 5:24, 2004. With permission from BMC Medical Genetics.)

green; for pea shape, round is dominant over wrinkled (see Figure 19–20). What would happen when plants that differ in both of these characters are crossed? Again, Mendel started with true-breeding parental strains: the dominant strain produced yellow round peas (its genotype is *YYRR*), the recessive strain produced green wrinkled peas (*yyrr*). One possibility is that the two characters, pea color and shape, would be transmitted from parents to offspring as a linked package. In other words, plants would always produce either yellow round peas or green wrinkled ones. The other possibility is that pea color and shape would be inherited independently, which means that at some point plants that produce a novel mix of traits—yellow wrinkled peas or green round peas—would arise.

The F_1 generation of plants all showed the expected phenotype: each produced peas that were yellow and round. But this result would occur whether or not the parental alleles were linked. When the F_1 plants were then allowed to self-fertilize, the results were clear: the two alleles for seed color segregated independently from the two alleles for seed shape, producing four different pea phenotypes: yellow-round, yellow-wrinkled, green-round, and green-wrinkled (**Figure 19–27**). Mendel tried his seven pea characters in various pairwise combinations and always observed a characteristic 9:3:3:1 phenotypic ratio in the F_2 generation. The independent segregation of each pair of alleles during gamete formation is Mendel's second law—the **law of independent assortment**.

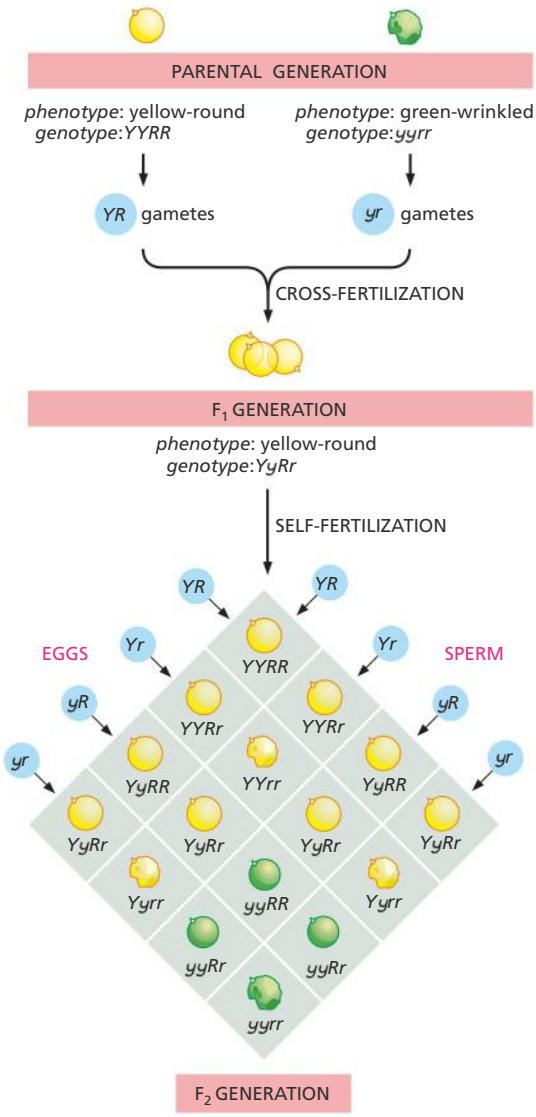


Figure 19–27 A dihybrid (two trait) cross demonstrates that alleles can segregate independently. Alleles that segregate independently are packaged into gametes in all possible combinations. So the *Y* allele is equally likely to be packaged with the *R* or *r* allele during gamete formation; and the same holds true for the *y* allele. Thus four classes of gametes are produced in roughly equal numbers: *YR, Yr, yR,* and *yr.* When these gametes are allowed to combine at random to produce the F_2 generation, the resulting pea phenotypes are yellow-round, yellow-wrinkled, green-round, and green-wrinkled in a ratio of 9:3:3:1.

The Behavior of Chromosomes During Meiosis Underlies Mendel's Laws of Inheritance

So far we have discussed alleles and genes as if they are disembodied entities. We now know that Mendel's "factors"—the things we call genes—are carried on chromosomes that are parceled out during the formation of gametes and then brought together in novel combinations in the zygote at fertilization. Chromosomes therefore provide the physical basis for Mendel's laws, and their behavior during meiosis and fertilization—which we discussed earlier—explains these laws perfectly.

During meiosis, the maternal and paternal homologs—and the genes that they contain—pair and then separate from each other as they are parceled out into gametes. These maternal and paternal chromosome copies will possess different variants—or alleles—of many of the genes they carry. Take, for example, a pea plant that is heterozygous for the yellow-pea gene (*Yy*). During meiosis, the chromosomes bearing the *Y* and *y* alleles will be separated, producing two types of haploid gametes—ones that contain a *Y* allele and others that contain a *y*. In a plant that self-fertilizes, these haploid gametes come together to produce the diploid individuals of the next generation—which may be *YY*, *Yy*, or *yy*. Together, the meiotic mechanisms that distribute the alleles into gametes and the combining of gametes at fertilization provide the physical foundation for Mendel's law of segregation.

But what about independent assortment of multiple traits? Because each pair of duplicated homologs attaches to the spindle and lines up at the metaphase plate independently during meiosis, each gamete will inherit a random mixture of paternal and maternal chromosomes (see Figure 19–15A). Thus the alleles of genes on different chromosomes will segregate independently.

Consider a pea plant that is heterozygous for both seed color (*Yy*) and seed shape (*Rr*). The homolog pair carrying the color alleles will attach to the meiotic spindle with a certain orientation: whether the *Y*-bearing homolog or its *y*-bearing counterpart is captured by the microtubules from one pole or the other depends on which way the bivalent happens to be facing at the moment of attachment (**Figure 19–28**). The same is true for the homolog pair carrying the alleles for seed shape. Thus, whether the final gamete receives the *YR*, *Yr*, *yR*, or *yr* allele combination depends entirely on which way the two homolog pairs were facing when they were captured by the meiotic spindle; each outcome has the same degree of randomness as the tossing of a coin.

Even Genes on the Same Chromosome Can Segregate Independently by Crossing-Over

Mendel studied seven traits, each of which is controlled by a separate gene. It turns out that most of these genes are located on different chromosomes, which readily explains the independent segregation he observed. But the independent segregation of different traits does not necessarily require that the responsible genes lie on different chromosomes. If two genes are far enough away from each other on the same chromosome, they will also sort independently, because of the crossing-over that occurs during meiosis. As we discussed earlier, when duplicated homologs pair to form bivalents, the maternal and paternal homologs always undergo crossing-over. This genetic exchange can separate alleles that were formerly together on the same chromosome, causing them to segregate into different gametes (**Figure 19–29**). We now know, for

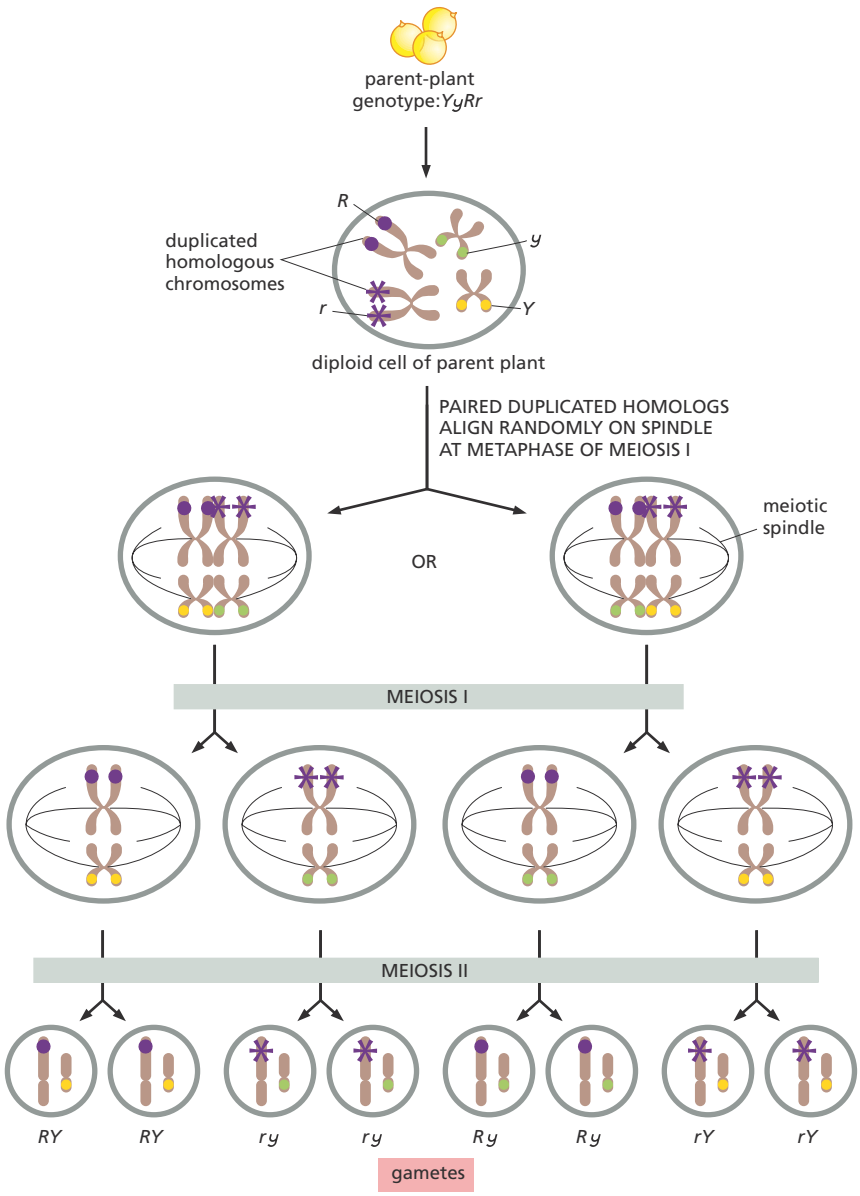


Figure 19–28 The separation of duplicated homologous chromosomes during meiosis explains Mendel's laws of segregation and independent assortment. Here we show independent assortment of the alleles for seed color, yellow (*Y*) and green (*y*), and for seed shape, round (*R*) and wrinkled (*r*), as an example of how two genes on different chromosomes segregate independently. Although crossovers are not shown, they would not affect the independent assortment of these traits, as the two genes lie on different chromosomes.

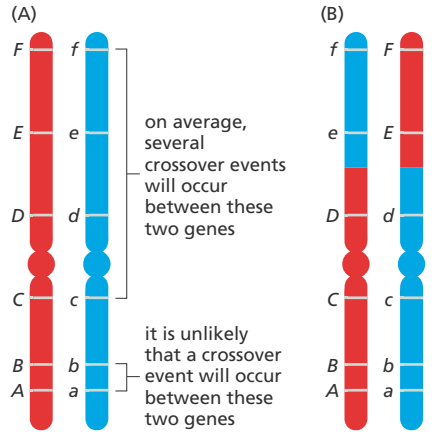


Figure 19–29 Genes that lie far enough apart on the same chromosome will segregate independently. (A) Because several crossover events occur randomly along each chromosome during prophase of meiosis I, two genes on the same chromosome will obey Mendel's law of independent assortment if they are far enough apart. Thus, for example, there is a high probability of crossovers occurring in the long region between *C*/*c* and *F*/*f*, meaning that a gamete carrying the *F* allele will wind up with the *c* allele as often as it will the *C* allele. In contrast, the *A*/*a* and *B*/*b* genes are close together, so there is only a small chance of crossing-over between them: thus the *A* allele is likely to be co-inherited with the *B* allele, and the *a* allele with the *b* allele. From the frequency of recombination, one can estimate the distances between the genes. (B) An example of a crossover that has separated the *C*/*c* and *F*/*f* alleles, but not the *A*/*a* and *B*/*b* alleles.

example, that the genes for pea shape and pod color that Mendel studied are located on the same chromosome, but because they are far apart they segregate independently.

Not all genes segregate independently as per Mendel's second law. If genes lie close together on a chromosome, they are likely to be inherited as a unit. For example, human genes associated with red–green color-blindness and hemophilia are typically inherited together for this reason. By measuring how frequently genes are co-inherited, geneticists can determine whether they reside on the same chromosome and, if so, how far apart they are. These measurements of *genetic linkage* have been used to map the relative positions of the genes on each chromosome of many organisms. Such **genetic maps** have been crucial for isolating and characterizing mutant genes responsible for human genetic diseases such as cystic fibrosis.

Mutations in Genes Can Cause a Loss of Function or a Gain of Function

Mutations produce heritable changes in DNA sequence. They can arise in various ways (discussed in Chapter 6) and can be classified by the effect

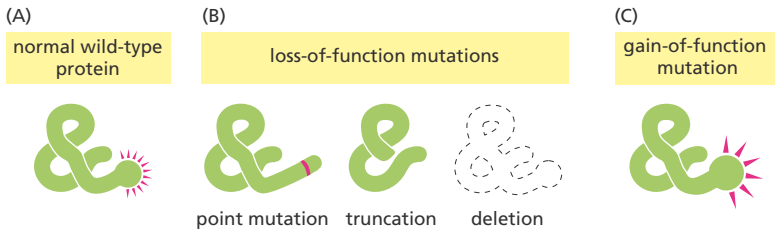


Figure 19–30 Mutations in protein-coding genes can affect the protein product in a variety of ways. (A) In this example, the normal or "wild-type" protein has a specific function denoted by the *red rays*. (B) Various loss-of-function mutations decrease or eliminate this activity. (C) Gain-of-function mutations boost this activity, as shown, or lead to an increase in the amount of the normal protein (not shown).

they have on gene function. Mutations that reduce or eliminate the activity of a gene are called **loss-of-function mutations** (Figure 19–30). An organism in which both alleles of a gene bear loss-of-function mutations will generally display an abnormal phenotype—one that differs from the most commonly occurring phenotype (although the difference may sometimes be subtle and hard to detect). By contrast, the heterozygote, which possesses one mutant allele and one normal, "wild-type" allele, generally makes enough active gene product to function normally and retain a normal phenotype. Thus loss-of-function mutations are usually recessive, because—for most genes—decreasing the normal amount of gene product by 50% has little impact.

In the case of Mendel's peas, the gene that dictates seed shape codes for an enzyme that helps convert sugars into branched starch molecules. The dominant, wild-type allele, *R*, produces an active enzyme; the recessive, mutant allele, *r*, does not. Because they lack this enzyme, plants that are homozygous for the *r* allele contain more sugar and less starch than plants that possess the dominant *R* allele, which gives their peas a wrinkled appearance (see Figure 19–20). The sweet peas available in the supermarket are often wrinkled mutants of the same type that Mendel studied.

Mutations that increase the activity of a gene or its product, or result in the gene being expressed in inappropriate circumstances, are called **gain-of-function mutations** (see Figure 19–30). Such mutations are usually dominant. For example, as we saw in Chapter 16, certain mutations in the *Ras* gene generate a form of the protein that is always active. Because the normal Ras protein is involved in controlling cell proliferation, the mutant protein drives cells to multiply inappropriately, even in the absence of signals that are normally required to stimulate cell division—thereby promoting the development of cancer. About 30% of all human cancers contain such dominant, gain-of-function mutations in the *Ras* gene.

Each of Us Carries Many Potentially Harmful Recessive Mutations

As we saw in Chapter 9, mutations provide the fodder for evolution. They can alter the fitness of an organism, making it either less or more likely for the individual to survive and leave progeny. Natural selection determines whether these mutations are preserved: those that confer a selective advantage on an organism tend to be perpetuated, while those that compromise an organism's fitness or ability to procreate tend to be lost.

The great majority of chance mutations are either neutral, with no effect on phenotype, or deleterious. A deleterious mutation that is dominant—one that exerts its negative effects when present even in a single copy—will be eliminated almost as soon as it arises. In an extreme case, if a mutant organism is unable to reproduce, the mutation that causes that failure will be lost from the population when the mutant individual dies. For deleterious mutations that are recessive, things are a little more complicated. When such a mutation first arises, it will generally

be present in only a single copy. The organism that caries the mutation can produce just as many progeny as other individuals; the majority of these progeny will inherit a single copy of the mutation, and they too will appear fit and healthy. But as they and their descendants begin to mate with one another, some individuals will inherit two copies of the mutant allele and display an abnormal phenotype.

If such a homozygous individual fails to reproduce, two copies of the mutant allele will be lost from the population. Eventually, an equilibrium is reached, where the rate at which new mutations occur in the gene balances the rate at which these mutant alleles are lost through matings that yield abnormal homozygous mutant individuals. As a consequence, many deleterious recessive mutations are present in heterozygous individuals at a surprisingly high frequency, even though homozygous individuals showing the deleterious phenotype are rare. Thus the most common form of hereditary deafness (due to mutations in a gene that encodes a gap-junction protein; see Figure 20–29) occurs in about one in 4000 births, but about one in 30 of us are carriers of a loss-of-function mutant allele of the gene.

QUESTION 19–3

Imagine that each chromosome undergoes one and only one crossover event on each chromatid during each meiosis. How would the co-inheritance of traits that are determined by genes at opposite ends of the same chromosome compare with the co-inheritance observed for genes on two different chromosomes? How does this compare with the actual situation?

GENETICS AS AN EXPERIMENTAL TOOL

Our understanding of how chromosomes shuttle genetic information from one generation to the next did more than demystify the basis of inheritance: it united the science of genetics with other life sciences, from cell biology and biochemistry to physiology and medicine. **Genetics** provides a powerful way to discover what specific genes do and how variations in those genes underlie the differences between one species and another or between individuals within a species. Such knowledge also has practical benefits, as understanding the genetic and biological basis of diseases can help us to better diagnose, treat, and prevent them.

In this section, we outline the *classical genetic approach* to identifying genes and determining how they influence the phenotype of an experimental organism such as yeast or flies. The process begins with the generation of a very large number of mutants and the identification of those rare individuals that show a phenotype of interest. By analyzing these rare mutant individuals and their progeny, we can track down the genes responsible and work out what these genes normally do—and how mutations that alter their activity affect how an organism looks and behaves.

Modern technologies—particularly new methods for obtaining and comparing genome sequences—have made it possible to analyze the genotypes of large numbers of individuals, including humans. In the final part of this section, we discuss how analyses of DNA collected from human families and populations all over the world are providing clues about our evolutionary history and about the genes that influence our susceptibility to disease.

The Classical Genetic Approach Begins with Random Mutagenesis

Before the advent of recombinant DNA technology (discussed in Chapter 10), most genes were identified and characterized by observing the processes disrupted when the gene was mutated. This type of analysis begins with the isolation of mutants that have an interesting or unusual phenotype: fruit flies that have white eyes or curly wings or that become paralyzed when exposed to high temperatures, for example. Working backward from the abnormal phenotype, one then determines the change

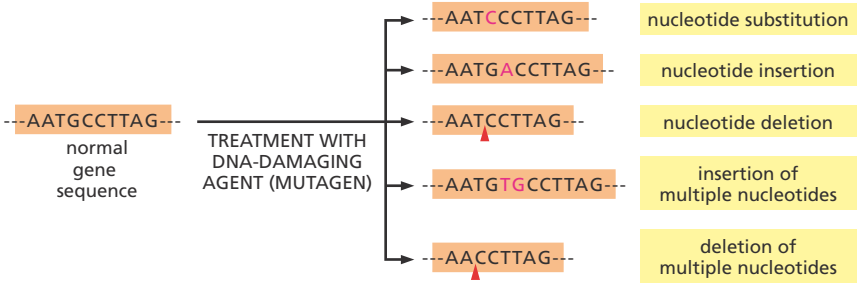


Figure 19–31 Mutations come in various forms. Different mutagens tend to produce different types of changes. Some common types of mutation are shown here. Other examples include changes in larger segments of DNA, including deletions, duplications, and chromosomal rearrangements (not shown).

in DNA that is responsible. This **classical genetic approach**—searching for mutant phenotypes and then isolating the responsible genes—is most easily performed in "model organisms" that reproduce rapidly and are amenable to genetic manipulation, such as bacteria, yeasts, nematode worms, zebrafish, and fruit flies. A brief review of this classical approach is presented in **Panel 19–1** (p. 669).

Although spontaneous mutants with interesting phenotypes can be found by combing through a collection of thousands or millions of organisms, the process can be made much more efficient by generating mutations artificially with agents that damage DNA, called *mutagens*. Different mutagens generate different types of DNA mutations (**Figure 19–31**). Not all mutations will lead to a noticeable change in phenotype. But by treating large numbers of organisms with mutagens, collections of mutants can be generated quickly, increasing the odds of finding an interesting phenotype, as we discuss next.

Genetic Screens Identify Mutants Deficient in Specific Cell Processes

A **genetic screen** typically involves examining many thousands of mutagenized individuals to find the few that show a specific altered phenotype of interest. To search for genes involved in cell metabolism, for example, one might screen mutagenized bacterial or yeast cells to pick out those that have lost the ability to grow in the absence of a particular amino acid or other nutrient.

Even genes involved in complex phenotypes, such as social behavior, can be identified by genetic screens in multicellular organisms. For example, by screening for worms that feed alone rather than in clusters as do wild-type individuals, scientists identified and isolated a gene that affects this "social behavior" (**Figure 19–32**).

Advances in modern technologies have made it possible to carry out genome-wide, high-throughput genetic screens on collections of

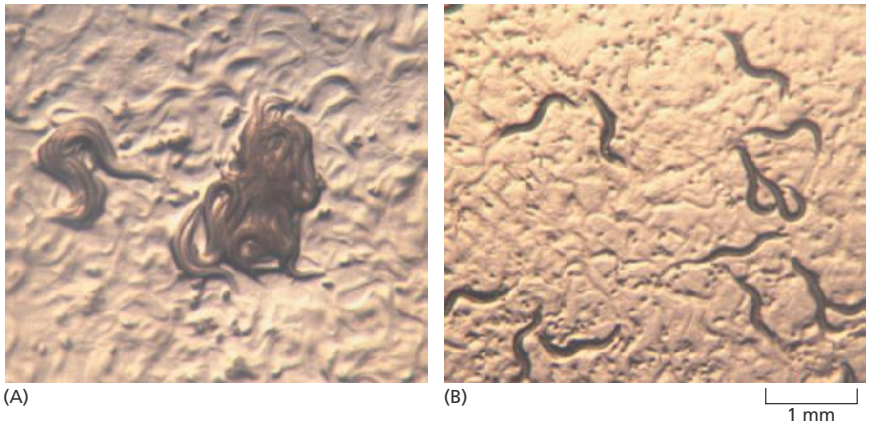


Figure 19–32 Genetic screens can be used to identify mutations that affect an animal's behavior. (A) Wild-type *C. elegans* engage in social feeding. The worms swim around until they encounter their neighbors and only then settle down to feed. (B) Mutant worms dine alone. (Courtesy of Cornelia Bargmann, cover of *Cell* 94, 1998. With permission from Elsevier.)

GENES AND PHENOTYPES

Gene: a functional unit of inheritance, corresponding to the segment of DNA coding for a protein or noncoding RNA molecule.

Genome: all of an organism's DNA sequences.

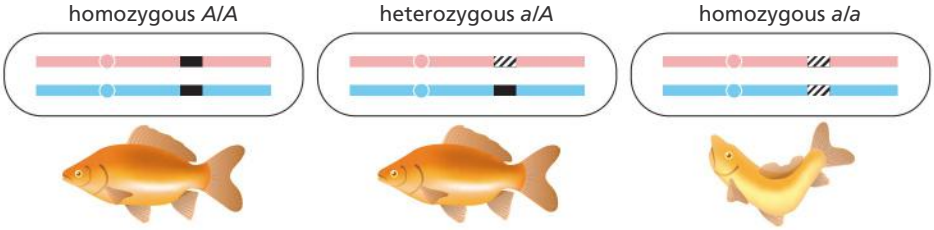
locus: the site of a gene in the genome

Wild type: the normal, naturally occurring type

Mutant: differing from the wild type because of a genetic change (a mutation)

GENOTYPE: the specific set of alleles forming the genome of an individual

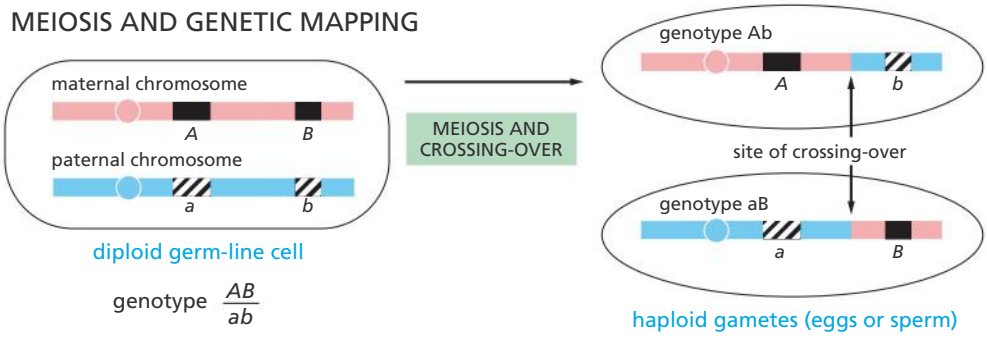
PHENOTYPE: the visible or functional characteristics of the individual



allele *A* is dominant (relative to *a*); allele *a* is recessive (relative to *A*)

In the example above, the phenotype of the heterozygote is the same as that of one of the homozygotes; in cases where it is different from both homozygotes, the two alleles are said to be co-dominant.

MEIOSIS AND GENETIC MAPPING



diploid germ-line cell

genotype $\frac{AB}{ab}$

haploid gametes (eggs or sperm)

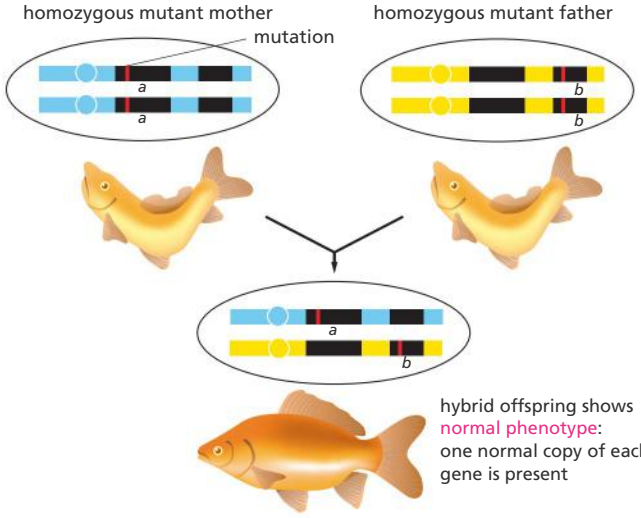
The greater the distance between two loci on a single chromosome, the greater is the chance that they will be separated by crossing-over occurring at a site between them. If two genes are thus reassorted in x% of gametes, they are said to be separated on a chromosome by a genetic map distance of x map units (or x centimorgans).

TWO GENES OR ONE?

Given two mutations that produce the same phenotype, how can we tell whether they are mutations in the same gene? If the mutations are recessive (as they most often are), the answer can be found by a complementation test.

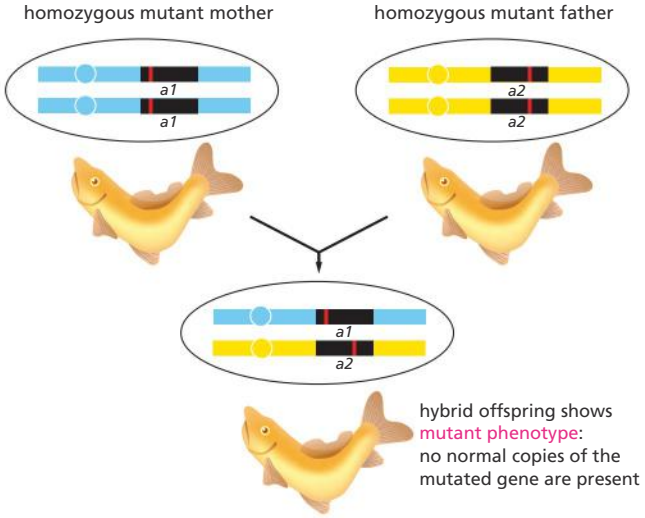
In the simplest type of complementation test, an individual who is homozygous for one mutation is mated with an individual who is homozygous for the other. The phenotype of the offspring gives the answer to the question.

COMPLEMENTATION:
MUTATIONS IN TWO DIFFERENT GENES



hybrid offspring shows normal phenotype: one normal copy of each gene is present

NONCOMPLEMENTATION:
TWO INDEPENDENT MUTATIONS IN THE SAME GENE



hybrid offspring shows mutant phenotype: no normal copies of the mutated gene are present

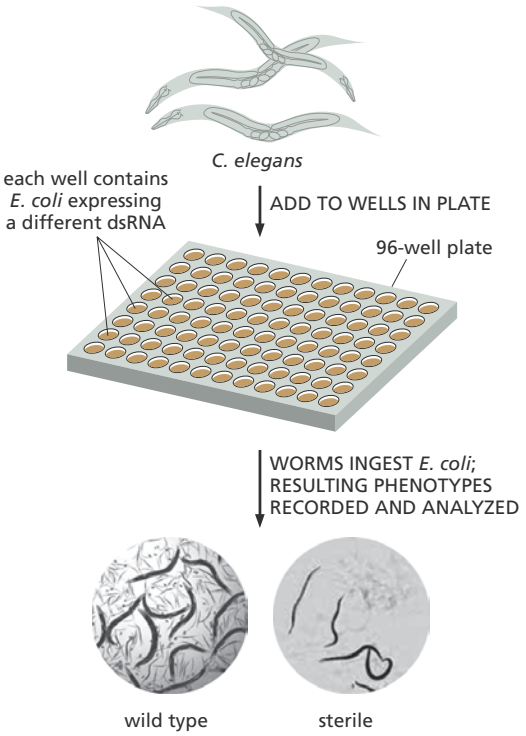


Figure 19–33 RNA interference provides a convenient method for conducting genome-wide genetic screens. In this experiment, each well in this 96-well plate is filled with *E. coli* that produce a different double-stranded (ds), interfering RNA. *E. coli* are a standard diet for *C. elegans* raised in the laboratory. Each interfering RNA matches the nucleotide sequence of a single *C. elegans* gene, thereby inactivating it. About 10 worms are added to each well, where they ingest the genetically modified bacteria. The plate is incubated for several days, which gives the RNAs time to inactivate their target genes—and the worms time to grow, mate, and produce offspring. The plate is then examined in a microscope, which can be controlled robotically, to screen for genes that affect the worms' ability to survive, reproduce, develop, and behave. Shown here are wild-type worms alongside a mutant that shows an impaired ability to reproduce. (From B. Lehner et al., *Nat. Genet.* 38:896–903, 2006. With permission from Macmillan Publishers Ltd.)

individuals in which nearly all of the protein-coding genes have been individually inactivated. Moreover, such mutant collections can often be screened using automated robots. For example, investigators have made use of RNA interference (explained in Figure 10–34) to generate nematode worms in which the activity of every protein-coding gene has been disrupted, with each worm being deficient in just one gene. These collections can be rapidly screened for dramatic changes in phenotype, such as stunted growth, uncoordinated movement, decreased fertility, or impaired embryonic development (**Figure 19–33**). Using this strategy, the genes needed for a particular characteristic can be identified.

Conditional Mutants Permit the Study of Lethal Mutations

Genetic screens are a powerful approach for isolating and characterizing mutations that are compatible with life—those that change the appearance or behavior of an organism without killing it. A problem arises, however, if we wish to study essential genes—those that are absolutely required for fundamental cell processes, such as RNA synthesis or cell division. Defects in these genes are usually lethal, which means that special strategies are needed to isolate and propagate such mutants: if the mutants cannot be bred, their genes cannot be studied.

If the organism is diploid—a mouse or a pea plant, say—and the mutant phenotype is recessive, there is a simple solution. Individuals that are heterozygous for the mutation will have a normal phenotype and can be propagated. When they are mated with one another, 25% of the progeny will be homozygous mutants and will show the lethal mutant phenotype; 50% will be heterozygous carriers of the mutation like their parents and can maintain the breeding stock.

But what if the organism is haploid, as is the case for many yeast and bacteria? One way to study lethal mutations in such organisms makes use of *conditional mutants*, in which the protein product of the mutant gene is only defective under certain conditions. For example, in mutants that are *temperature-sensitive*, the protein functions normally within a

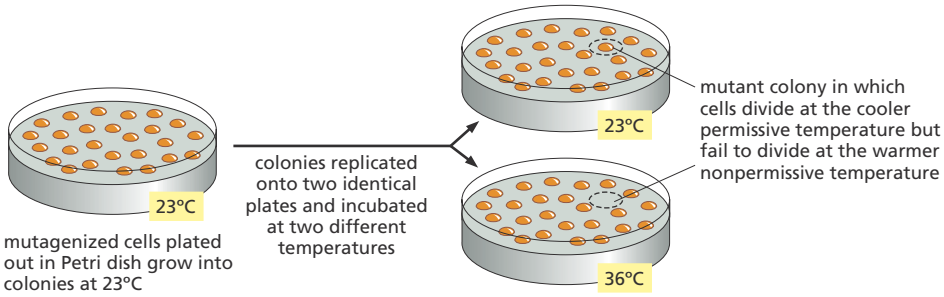


Figure 19–34 Temperature-sensitive mutants are valuable for identifying the genes and proteins involved in essential cell processes. In this example, yeast cells are treated with a mutagen, spread on a culture plate at a relatively cool temperature, and allowed to proliferate to form colonies. The colonies are then transferred to two identical Petri plates using a technique called replica plating. One of these plates is incubated at a cool temperature, the other at a warmer temperature. Those cells that contain a temperature-sensitive mutation in a gene essential for proliferation can be readily identified, because they divide at the cooler permissive temperature—but fail to divide at the warmer nonpermissive temperature.

certain range of temperatures (called the *permissive* temperature) but can be inactivated by a shift to a *nonpermissive temperature* outside this range. Thus the abnormal phenotype can be switched on and off simply by changing the temperature. A cell containing a temperature-sensitive mutation in an essential gene can be propagated at the permissive temperature and then be driven to display its mutant phenotype by a shift to a nonpermissive temperature (**Figure 19–34**).

Many temperature-sensitive bacterial mutants were isolated to identify the genes that encode the bacterial proteins required for DNA replication; investigators treated large populations of bacteria with mutagens and then screened for cells that stopped making DNA when they were warmed from 30°C to 42°C. Similarly, temperature-sensitive yeast mutants were used to identify many of the proteins involved in regulating the cell cycle (see How We Know, pp. 30–31) and in transporting proteins through the secretory pathway (see Figure 15–28).

A Complementation Test Reveals Whether Two Mutations Are in the Same Gene

A large-scale genetic screen can turn up many mutant organisms with the same phenotype. These mutations might affect the same gene or they might affect different genes that function in the same process. How can we distinguish between the two? If the mutations are recessive and cause a loss of function, a **complementation test** can reveal whether they affect the same or different genes.

In the simplest type of complementation test, an individual that is homozygous for one recessive mutation is mated with an individual that is homozygous for the other mutation. If the two mutations affect the same gene, the offspring will show the mutant phenotype, because they carry only defective copies of the gene in question. If, in contrast, the mutations affect different genes, the resulting offspring will show the normal, wild-type phenotype, because they will have one normal copy (and one mutant copy) of each gene (see Panel 19–1, p. 669).

Whenever the normal phenotype is restored in such a test, the alleles inherited from the two parents are said to complement each other (**Figure 19–35**). For example, complementation tests on mutants identified during genetic screens have revealed that 5 genes are required for yeast cells

Figure 19–35 A complementation test can reveal that mutations in two different genes are responsible for the same abnormal phenotype. When an albino (white) bird from one strain is bred with an albino from a different strain, the resulting offspring have normal coloration. This restoration of the wild-type plumage implies that the two white breeds lack color because of recessive mutations in different genes. (From W. Bateson, *Mendel's Principles of Heredity,* 1st ed. Cambridge, UK: Cambridge University Press, 1913. With permission from Cambridge University Press.)

to digest the sugar galactose, that 20 genes are needed for *E. coli* to build a functional flagellum, and many hundreds are essential for the normal development of an adult nematode worm from a fertilized egg.

Rapid and Cheap DNA Sequencing Has Revolutionized Human Genetic Studies

Genetic screens in model experimental organisms have been spectacularly successful in identifying genes and relating them to various phenotypes, including many that are conserved between these organisms and humans. But the same approach cannot be used in humans. Unlike flies, worms, yeast, and bacteria, humans do not reproduce rapidly, and intentional mutagenesis in humans is out of the question. Moreover, an individual with a serious defect in an essential process such as DNA replication would die long before birth—before we can assess the phenotype.

Nonetheless, humans are becoming increasingly attractive subjects for genetic studies. Because the human population is so large, spontaneous, nonlethal mutations have arisen in all human genes—many times over. A substantial proportion of these remain in the genomes of present-day humans. The most deleterious of these mutations are discovered when the mutant individuals call attention to themselves by seeking medical help—a uniquely human behavior.

With the recent advances that have enabled the sequencing of entire human genomes cheaply and quickly, we can now identify such mutations and study their evolution and inheritance in ways that were impossible even a few years ago. By comparing the sequences of thousands of human genomes from all around the world, we can now identify directly the DNA differences that distinguish one individual from another. These differences hold clues to our evolutionary origins and can be used to explore the roots of disease.

Linked Blocks of Polymorphisms Have Been Passed Down from Our Ancestors

When we compare the sequences of multiple human genomes, we find that any two individuals will differ in about 1 nucleotide pair in 1000. Most of these variations are common and relatively harmless. When two sequence variants coexist in the population and are both common, the variants are called **polymorphisms**. The majority of polymorphisms are due to the substitution of a single nucleotide, called **single-nucleotide polymorphisms** or **SNPs** (**Figure 19–36**). The rest are due largely to insertions or deletions—called *indels* when the change is small, or *copy number variants* (*CNVs*) when it is large.

Although these common variants can be found throughout the genome, they are not scattered randomly—or even independently. Instead, they tend to travel in groups called **haplotype blocks**—combinations of polymorphisms or other DNA markers that are inherited as a unit.

QUESTION 19–4

When two individuals from different isolated, inbred subpopulations of a species come together and mate, their offspring often show "hybrid vigor": that is, they appear more robust, healthy, and fertile than either parent. Can you suggest a possible explanation for this phenomenon?

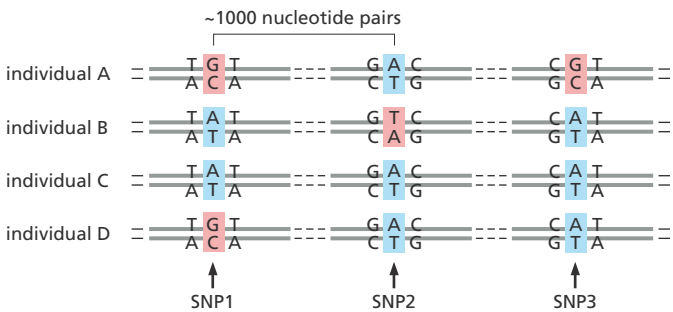


Figure 19–36 Single-nucleotide polymorphisms (SNPs) are sites in the genome where two or more alternative choices of a nucleotide are common in the population. Most such variations in the human genome occur at locations where they do not significantly affect a gene's function.

To understand why such haplotype blocks exist, we need to consider our evolutionary history. It is thought that modern humans expanded from a relatively small population—perhaps around 10,000 individuals—that existed in Africa about 60,000 years ago. Among that small group of our ancestors, some individuals will have carried one set of genetic variants, others a different set. The chromosomes of a present-day human represent a shuffled combination of chromosome segments from different members of this small ancestral group of people. Because only about two thousand generations separate us from them, large segments of these ancestral chromosomes have passed from parent to child, unbroken by the crossover events that occur during meiosis. (Remember, only a few crossovers occur between each set of homologous chromosomes (see Figure 19–12).)

As a result, certain sets of DNA sequences—and their associated polymorphisms—have been inherited in linked groups, with little genetic rearrangement across the generations. These are the haplotype blocks. Like genes that exist in different allelic forms, haplotype blocks also come in a limited number of variants that are common in the human population, each representing a combination of DNA polymorphisms passed down from a particular ancestor long ago.

Our Genome Sequences Provide Clues to our Evolutionary History

A detailed examination of haplotype blocks has provided intriguing insights into the history of human populations. New alleles of genes are continually being generated by mutation; many of these variants will be neutral, in that they will not affect the reproductive success of the individual. These have a chance of becoming common in the population. The more time that has elapsed since the origin of a relatively common allele like a SNP, the smaller should be the haplotype block that surrounds it: over the course of many generations, crossover events will have had many chances to separate an ancient allele from other polymorphisms nearby. Thus by comparing the sizes of haplotype blocks from different human populations, it is possible to estimate how many generations have elapsed since the origin of a specific neutral mutation. Combining such genetic comparisons with archaeological findings, scientists have traced our history from that small set of ancestors and deduce the most probable routes our ancestors took when they left Africa (**Figure 19–37**).

More recent studies, comparing the genome sequences of living humans with those of Neanderthals and another extinct relation from southern Siberia, suggest that our exit from Africa was a bit more convoluted. Some of us share a few percent of our nucleotide sequences with these

Figure 19–37 The human populations that are now dispersed around the world originated in Africa about 60,000 to 80,000 years ago. The map shows the routes of the earliest successful human migrations. Dotted lines indicate two alternative routes that our ancestors seem to have taken out of Africa. Studies of the size of haplotype blocks suggest that modern Europeans are descended from a small ancestral population that existed about 30,000 to 50,000 years ago. Haplotype blocks in a Nigerian population are significantly smaller, indicating that the Nigerian population was established before the European. These findings agree with archaeological findings, which suggest that the ancestors of modern native Australians (*solid red* arrows)—and of modern European and Middle Eastern populations (migration routes not shown)—reached their destinations about 45,000 years ago. (Modified from P. Forster and S. Matsumura, *Science* 308:965–966, 2005. With permission from AAAS.)

archaic humans, suggesting that a number of our ancestors interbred with their neighbors as they made their way across the globe.

Genome analyses can also be used to estimate when and where humans acquired mutations that have conferred an evolutionary benefit, such as resistance to infection. Such favorable mutations will rapidly accumulate in the population because individuals that carry it will be more likely to survive an epidemic and pass the mutation on to their offspring. A haplotype analysis can be used to "date" the appearance of such a favorable mutation. If it cropped up in the population relatively recently, there will have been fewer opportunities for recombination to break up the DNA sequence around it, so the surrounding haplotype block will be large.

Such is the case for two alleles that confer resistance to malaria. These alleles are widespread in Africa, where malaria is rife. They are embedded in unusually large haplotype blocks, suggesting that they arose recently in the African gene pool—probably about 2500 years ago for one of them and about 6500 years ago for the other. In this way, analyses of modern human genomes can highlight important events in ancient human history, including our initial exposures to specific infections.

Polymorphisms Can Aid the Search for Mutations Associated with Disease

The study of polymorphisms may also have more practical relevance to human health. CNVs, indels, and SNPs can be used as markers for building human genetic maps or for conducting searches for mutations that predispose individuals to a specific disease. Mutations that give rise, in a reproducible way, to rare but clearly defined abnormalities, such as albinism or congenital deafness, can often be identified by studies of affected families. Such single-gene, or monogenic, disorders are often referred to as *Mendelian* because their pattern of inheritance is as easy to track as the wrinkled peas and purple flowers that were studied by Mendel. But for many common diseases, the genetic roots are more complex. Instead of a single allele of a single gene, such disorders stem from a combination of contributions from multiple genes. For these *multigenic* conditions, such as diabetes or arthritis, population studies are often helpful in tracking down the genes that increase the risk of getting the disease.

In population studies, investigators collect DNA samples from a large number of people who have the disease and compare them to samples from a group of people who do not have the disease. They look for variants—SNPs, for example—that are more common among the people who have the disease. Because DNA sequences that are close together on a chromosome tend to be inherited together, the presence of such SNPs could indicate that an allele that increases the risk of the disease might lie nearby (**Figure 19–38**). Although, in principle, the disease could be caused by the SNP itself, the culprit is much more likely to be a change that is merely linked to the SNP.

Such *genome-wide association studies*—which initially focused on SNPs—have been used to search for genes that predispose individuals to common diseases, including diabetes, coronary artery disease, rheumatoid arthritis, and even depression. One such study is described in **How We Know** (pp. 676–677). For many of these conditions, environmental as well as genetic factors play an important part in determining susceptibility. Disappointingly, most of the DNA polymorphisms identified increase the risk of disease only slightly. But by providing insights into the molecular mechanisms underlying common disorders, these results—and newer, more powerful ways of analyzing the differences in human populations—should eventually lead to better approaches to disease treatment and prevention.

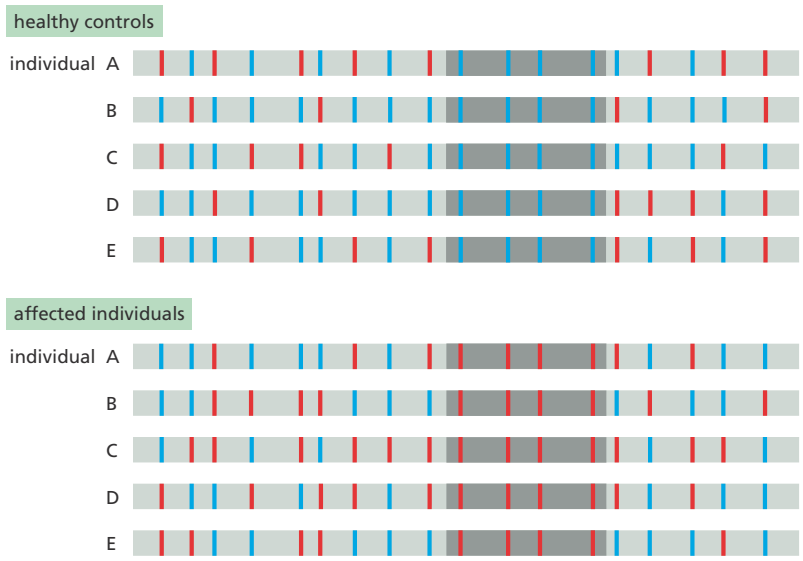


Figure 19–38 Genes that affect the risk of developing a common disease can often be tracked down through linkage to SNPs. Here, the patterns of SNPs are compared between two sets of individuals—a set of healthy controls and a set affected by a particular common disease. A segment of a typical chromosome is shown. For most polymorphic sites in this segment, it is a random matter whether an individual has one SNP variant (*red* vertical bars) or another (*blue* vertical bars); and the same randomness is seen both for the control group and for the affected individuals. However, in the part of the chromosome that is shaded in *darker gray*, a bias is seen, such that most normal individuals have the *blue* SNP variants, whereas most affected individuals have the *red* SNP variants. This suggests that this region contains, or is close to, a gene that is genetically linked to these red SNP variants and that predisposes to the disease. Using carefully selected controls and thousands of affected individuals, this approach can help track down disease-related genes, even when they confer only a slight increase in the risk of developing the disease.

Genomics Is Accelerating the Discovery of Rare Mutations that Predispose Us to Serious Disease

The genetic variants that have thus far allowed us to track our ancestors and identify some of the genes that increase our risk of disease are common ones. They arose long ago in our evolutionary past and are now present, in one form or another, in a substantial portion (1% or more) of the population. Such polymorphisms are thought to account for about 90% of the differences between one person's genome and another. But when we try to tie these common variants to differences in disease susceptibility or other heritable traits, such as height, we find that they do not have as much predictive power as we had anticipated: thus, for example, most confer relatively small increases—less than twofold—in the risk of developing a common disease.

In contrast to polymorphisms, rare DNA variants—those much less frequent in humans than SNPs—can have large effects on the risk of developing some common diseases. For example, a number of different loss-of-function mutations, each individually rare, have been found to increase greatly the predisposition to autism and schizophrenia. Many of these are *de novo* mutations, which arose spontaneously in the germ-line cells of one or other parent. The fact that these mutations arise spontaneously with some frequency could help explain why these common disorders—each observed in about 1% of the population—remain with us, even though the affected individuals leave few or no descendants. These rare mutations, which may arise in any one of hundreds of different genes, can greatly increase the risk of autism and schizophrenia—and could explain much of their clinical variability. Because they are kept rare by natural selection, most such variants with a large effect on risk would be missed by genome-wide association studies.

Now that the price of DNA sequencing has plummeted, the most efficient and cost-effective way to identify these rare, large-effect mutations is by sequencing all the exons (the *exome*)—or even the whole genomes—of affected individuals, along with those of their parents and siblings as controls. Although exome sequencing will miss the noncoding variations that affect gene regulation, the majority of rare, large-effect mutations have thus far been found to lie within exons; common, small-effect variations, by contrast, have been found mainly in noncoding sequences.

QUESTION 19–5

In a recent automated analysis, thousands of SNPs across the genome were analyzed in pooled DNA samples from humans who had been sorted into groups according to their age. For the vast majority of these sites, there was no change in the relative frequencies of different variants as these humans aged. Sometimes, albeit rarely, a particular variant at one position was found to decrease in frequency progressively for people over 50 years old. Which of the possible explanations seems most likely?

A. The nucleotide in that SNP at that position is unstable, and mutates with age.

B. Those people born more than 50 years ago came from a population that tended to lack the disappearing SNP variant.

C. The SNP variant alters an important gene product in a way that shortens the human life-span, or is linked to a neighboring allele that has this effect.

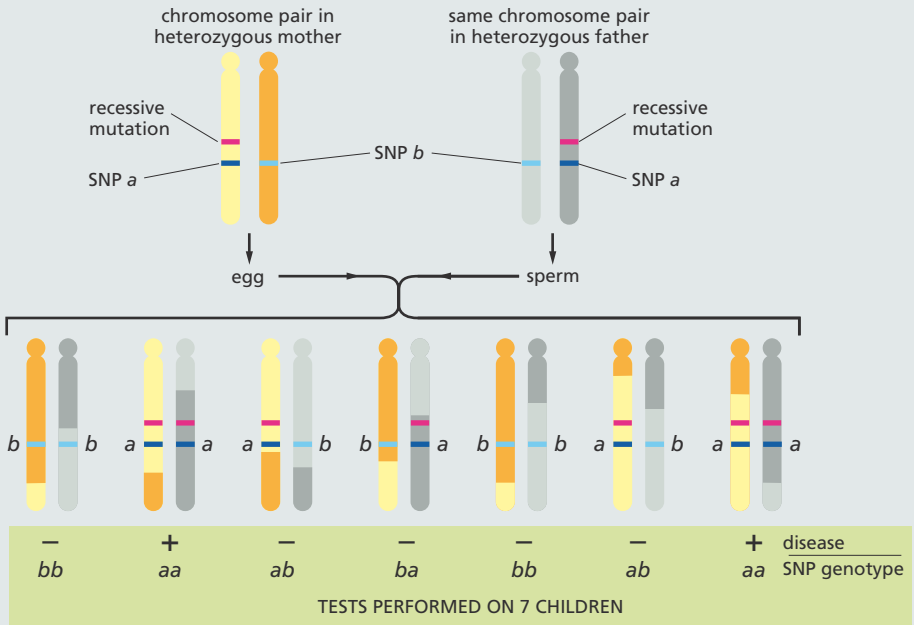
USING SNPs TO GET A HANDLE ON HUMAN DISEASE

For diseases that have their roots in genetics, finding the gene or genes responsible can be the first step toward improved diagnosis, treatment, and even prevention. The task is not simple, but having access to polymorphisms such as SNPs can help. In 1999, an international group of scientists set out to collect and catalog 300,000 SNPs—the single-nucleotide polymorphisms that are common in the human population (see Figure 19–36). Today, the database has grown to include a catalog of more than 17 million SNPs. These SNPs not only help to define the differences between one individual and another; for geneticists, they also serve as signposts that can point the way toward the genes involved in common human disorders, such as diabetes, obesity, asthma, arthritis, and even gallstones and restless leg syndrome.

Making a map

One way that SNPs have facilitated the search for alleles that predispose to disease is by providing the physical markers needed to construct detailed genetic linkage maps. A genetic linkage map displays the relative locations of a set of genes. Such maps are based on the frequency with which two alleles are co-inherited—something we can discover by seeing how often the phenotypic traits associated with those alleles show up together in an individual. Genes that lie close to one another on the same chromosome will be inherited together much more frequently than those that lie farther apart. By determining how often crossing-over separates two genes, the relative distance between them can be calculated (see Panel 19–1, p. 669).

The same sort of analysis can be used to discover linkage between a SNP and an allele. One simply looks for co-inheritance of the SNP with a certain phenotype, such as an inherited disease. Finding such a linkage indicates that the mutation responsible for the phenotype is either the SNP itself or, more likely, that lies close to the SNP (**Figure 19–39**). And because we know the exact location in the human genome sequence of every SNP we examine, the linkage tells us the neighborhood in which the causative mutation resides. A more detailed analysis of the DNA in that region—to look for deletions, insertions, or other functionally significant abnormalities in the DNA sequence of affected individuals—can then lead to a precise identification of the critical gene.



Disease is seen only in progeny with SNP genotype *aa*.
CONCLUSION: Recessive mutation causing the disease is co-inherited with SNP *a*. If this same correlation is observed in other families that have been examined, the mutation causing the disease must lie close to SNP *a*.

Figure 19–39 SNP analysis can pin down the location of a mutation that causes a genetic disease. In this approach, one studies the co-inheritance of a specific human phenotype (here a genetic disease) with a particular set of SNPs. The figure shows the logic for the common case of a family in which both parents are carriers of a recessive mutation. If individuals with the disease, and only such individuals, are homozygous for a particular SNP, then the SNP and the recessive mutation that causes the disease are likely to be close together on the same chromosome, as shown here. To prove that an apparent linkage is statistically significant, a few dozen individuals from such families may need to be examined. With more individuals and using more SNPs, it is possible to locate the mutation more precisely. These days it can be just as fast and cheap to use whole-genome sequencing to find the mutation.

Such linkage analyses are usually carried out in families that are particularly prone to a disorder—the larger the family, the better. And the method works best where there is a simple cause-and-effect relationship, such that a particular mutant gene directly and reliably causes the disorder—as is the case, for example, for the mutant gene that causes cystic fibrosis. But most common disorders are not like this. Instead, many factors affect the disease risk—some genetic, some environmental, some just a matter of chance. For such conditions, a different approach is needed to identify risk genes.

Making associations

Genome-wide association studies allow us to discover common genetic variants that affect the risk for a common disease, even if each variant alters susceptibility only slightly. Because mutations that destroy the activity of a key gene are likely to have a disastrous effect on the fitness of the mutant individual, they tend to be eliminated from the population by natural selection and so are rarely seen. Genetic variants that alter a gene's function only slightly, on the other hand, are much more common. By tracking down these common variants, or polymorphisms, we can sniff out some of the genes that contribute to the biology of common diseases.

Genome-wide association studies use genetic markers, such as SNPs, that are located throughout the genome to compare directly the DNA sequences of two populations: individuals who have a particular disease and those who do not. The approach identifies SNPs that are present in the people who have the disease more often than would be expected by chance.

Consider the case of *age-related macular degeneration* (*AMD*), a degenerative disorder of the retina that is a leading cause of blindness in the elderly. To search for genetic variations that are associated with AMD, researchers looked at a panel of just over 100,000 SNPs that spanned the genome. They determined the nucleotide sequence at each of these SNPs in 96 people who had AMD, and 50 who did not. Among the 100,000 SNPs, they discovered that one particular SNP was present significantly more often in the individuals who had the disease (**Figure 19–40**).

The SNP is located in a gene called *Cfh* (*complement factor H*). But it falls within one of the gene's introns and appears unlikely to have any effect on the protein product. This SNP itself, therefore, did not seem likely to be the cause of the increase in susceptibility to AMD. But it focused the researchers' attention on the Cfh gene. So they resequenced the region to look for additional polymorphisms that might also be inherited more often by people with AMD, along with the SNP that they had already identified. They discovered three variants that affect the amino acid sequence of the Cfh protein. One substitutes a histidine for a tyrosine at one particular place in the protein, and it was strongly associated with the disease (and almost always coupled with the original SNP that had put the researchers on the track of the *Cfh* gene). Individuals who carried two copies of this risky allele were five to seven times more likely to develop AMD than those who harbored a different allele of the *Cfh* gene.

Several other research teams, using a similar genetic association approach, have also pointed to *Cfh* variants as increasing the likelihood of developing AMD, making it almost certain that the *Cfh* gene has something to do with the biology of the disease. The Cfh protein is part of the complement system; it helps prevent the system from becoming overactive, a condition that can lead to inflammation and tissue damage. Interestingly, the environmental risk factors associated with the disease—smoking, obesity, and age—also affect inflammation and the activity of the complement system. Thus, whatever the detailed mechanism by which the *Cfh* gene influences the risk of AMD, the finding that complement is critical could lead to new tests for the early diagnosis of the disorder, as well as potential new avenues for treatment.

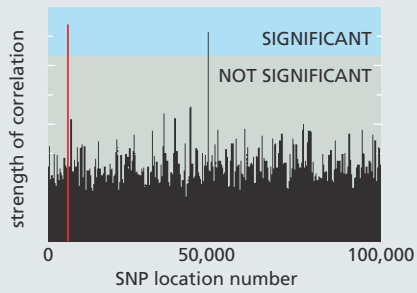


Figure 19–40 Genome-wide association studies identify DNA variations that are significantly more frequent in people with a given disease. In this study, scientists examined more than 100,000 SNPs in each of 146 people. The x-axis of the graph shows the relative position of each SNP in the genome, starting at the left with the SNPs on Chromosome 1. The y-axis shows the strength of each SNP's observed correlation with AMD. The *blue* region indicates a cutoff level for statistical significance, corresponding to a probability of less than 5% of finding that strength of correlation by pure chance anywhere among the whole set of 100,000 tested SNPs. The SNP marked in *red* is the one that led the way to the relevant gene, *Cfh*. The initial association of the other prominent SNP (*black*) with the disease disappeared when additional sequencing at that site was performed. (Adapted from R.J. Klein et al., *Science* 308:385–389, 2005. With permission from AAAS.)

Exome and genome sequencing efforts are turning up many previously unreported genetic variants—in both disease and apparently healthy populations. One recent study suggests that we all harbor about 100 loss-of-function mutations in protein-coding genes—20 of which eliminate the activity of both gene copies—indicating that humans do not actually need all of our genes to develop and function as "normal."

ESSENTIAL CONCEPTS

- Sexual reproduction involves the cyclic alternation of diploid and haploid states: diploid germ-line cells divide by meiosis to form haploid gametes, and the haploid gametes from two individuals fuse at fertilization to form a new diploid cell—the zygote.
- During meiosis, the maternal and paternal homologs are parceled out to gametes such that each gamete receives one copy of each chromosome. Because the segregation of these homologs occurs randomly, and crossing-over occurs between them, many genetically different gametes can be produced from a single individual.
- In addition to enhancing genetic mixing, crossing-over helps ensure the proper segregation of chromosomes during meiosis.
- Although most of the mechanical features of meiosis are similar to those of mitosis, the behavior of the chromosomes is different: meiosis produces four genetically distinct haploid cells by two consecutive cell divisions, whereas mitosis produces two genetically identical diploid cells by a single cell division.
- Mendel unraveled the laws of heredity by studying the inheritance of a handful of discrete traits in pea plants.
- Mendel's law of segregation states that the maternal and paternal alleles for each trait separate from one another during gamete formation and then reunite randomly during fertilization.
- Mendel's law of independent assortment states that, during gamete formation, different pairs of alleles segregate independently of one another.
- The behavior of chromosomes during meiosis explains both of Mendel's laws.
- If two genes are close to each other on a chromosome, they tend to be inherited as a unit; if they are far apart, they will typically be separated by crossing-over. The frequency with which two genes are separated by crossovers can be used to construct a genetic map that shows their order on a chromosome.
- Mutant alleles can be either dominant or recessive. If a single copy of the mutant allele alters the phenotype of an individual that also possesses a wild-type allele, the mutant allele is dominant; if not, it is recessive.
- Complementation tests reveal whether two mutations that produce the same phenotype affect the same gene or different genes.
- Mutant organisms can be generated by treating animals with mutagens, which damage DNA. Such mutants can then be screened to identify phenotypes of interest and, ultimately, to isolate the responsible genes.
- With the possible exception of identical twins, no two human genomes are alike. Each of us carries a unique set of polymorphisms—variations in nucleotide sequence that in some cases contribute to our individual phenotypes.
- Some of the common polymorphisms—including SNPs, indels, and CNVs—provide useful markers for genetic mapping.

- The human genome consists of large haplotype blocks—segments of nucleotide sequence that have been passed down intact from our distant ancestors and, in most individuals, have not yet been broken up by crossovers. The relative sizes of haplotype blocks can give us clues to our evolutionary history.
- DNA sequencing studies are identifying an increasing number of rare mutations that can greatly increase the risk of developing the most common human disorders.

KEY TERMS

allele
asexual reproduction
bivalent
chiasma (plural chiasmata)
classical genetic approach
complementation test
crossing-over
diploid
fertilization
gain-of-function mutation
gamete
genetic map
genetic screen
genetics
genotype
germ cell
germ line
haploid
haplotype block
heterozygous
homolog
homologous chromosome
homologous recombination
homozygous
law of independent assortment
law of segregation
loss-of-function mutation
meiosis
pairing
pedigree
phenotype
polymorphism
recombination
segregation
sexual reproduction
sister chromatid
SNP (single-nucleotide polymorphism)
somatic cell
zygote

QUESTIONS

QUESTION 19–6

It is easy to see how deleterious mutations in bacteria, which have a single copy of each gene, are eliminated by natural selection: the affected bacteria die and the mutation is thereby lost from the population. Eukaryotes, however, have two copies of most genes—that is, they are diploid. Often an individual with two normal copies of the gene (homozygous normal) is indistinguishable in phenotype from an individual with one normal copy and one defective copy of the gene (heterozygous). In such cases, natural selection can operate only against an individual with two copies of the defective gene (homozygous defective). Consider the situation in which a defective form of the gene is lethal when homozygous, but without effect when heterozygous. Can such a mutation ever be eliminated from the population by natural selection? Why or why not?

QUESTION 19–7

Which of the following statements are correct? Explain your answers.

A. The egg and sperm cells of animals contain haploid genomes.

B. During meiosis, chromosomes are allocated so that each germ cell obtains one and only one copy of each of the different chromosomes.

C. Mutations that arise during meiosis are not transmitted to the next generation.

QUESTION 19–8

What might cause chromosome nondisjunction, where two copies of the same chromosome end up in the same daughter cell? What could be the consequences of this event occurring (a) in mitosis and (b) in meiosis?

QUESTION 19–9

Why do sister chromatids have to remain paired in division I of meiosis? Does the answer suggest a strategy for washing your socks?

QUESTION 19–10

Distinguish between the following genetic terms:

A. Gene and allele.

B. Homozygous and heterozygous.

C. Genotype and phenotype.

D. Dominant and recessive.

QUESTION 19–11

You have been given three wrinkled peas, which we shall call A, B, and C, each of which you plant to produce a mature pea plant. Each of these three plants, once self-pollinated, produces only wrinkled peas.

A. Given that you know that the wrinkled-pea phenotype is recessive, as a result of a loss-of-function mutation, what can you say about the genotype of each plant?

B. Can you safely conclude that each of the three plants carries a mutation in the same gene?

C. If not, how could you rule out the possibility that each plant carries a mutation in a different gene, each of which gives the wrinkled-pea phenotype?

QUESTION 19–12

Susan's grandfather was deaf, and passed down a hereditary form of deafness within Susan's family as shown in **Figure Q19–12**.

A. Is this mutation most likely to be dominant or recessive?

B. Is it carried on an autosome or a sex chromosome? Why?

C. A complete SNP analysis has been done for all of the 11 grandchildren (4 affected, and 7 unaffected). In comparing these 11 SNP results, how long a haplotype block would you expect to find around the critical gene? How might you detect it?

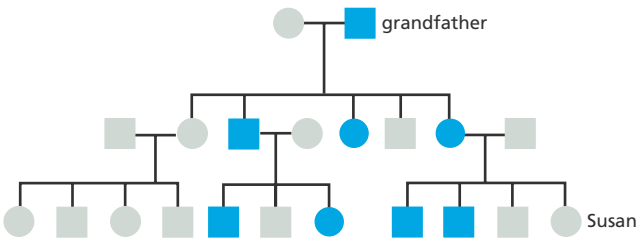


Figure Q19–12

QUESTION 19–13

Given that the mutation causing deafness in the family shown in Figure 19–26 is very rare, what is the most probable genotype of each of the four children in generation II?

QUESTION 19–14

In the pedigree shown in **Figure Q19–14**, the first born in each of three generations is the only person affected by a dominant genetically inherited disease, D. Your friend concludes that the first child born has a greater chance of inheriting the mutant *D* allele than do later children.

A. According to Mendel's laws, is this conclusion plausible?

B. What is the probability of obtaining this result by chance?

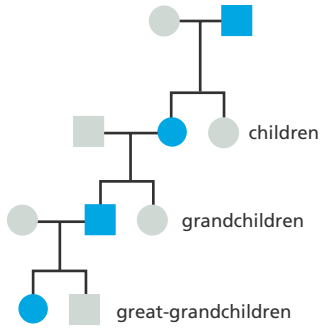


Figure Q19–14

C. What kind of additional data would be needed to test your friend's idea?

D. Is there any way in which your friend's hypothesis might turn out to be right?

QUESTION 19–15

Suppose one person in 100 is a carrier of a fatal recessive mutation, such that babies homozygous for the mutation die soon after birth. In a population where there are 1,000,000 births per year, how many babies per year will be born with the lethal homozygous condition?

QUESTION 19–16

Certain mutations are called *dominant-negative mutations.* What do you think this means and how do you suppose these mutations act? Explain the difference between a dominant-negative mutation and a gain-of-function mutation.

QUESTION 19–17

Discuss the following statement: "We would have no idea today of the importance of insulin as a regulatory hormone if its absence were not associated with the devastating human disease diabetes. It is the dramatic consequences of its absence that focused early efforts on the identification of insulin and the study of its normal role in physiology."

QUESTION 19–18

Early genetic studies in *Drosophila* laid the foundation for our current understanding of genes. *Drosophila* geneticists were able to generate mutant flies with a variety of easily observable phenotypic changes. Alterations from the fly's normal brick-red eye color have a venerable history because the very first mutant found by Thomas Hunt Morgan was a white-eyed fly (**Figure Q19–18**). Since that time, a large number of mutant flies with intermediate eye colors have been isolated and given names that challenge your color sense: garnet, ruby, vermilion, cherry, coral, apricot, buff, and carnation. The mutations responsible for these eye-color phenotypes are all recessive. To determine whether the mutations affected the same or different genes, homozygous flies for each mutation were bred to one another in pairs and the eye colors of their progeny were noted. In **Table Q19–18**, a + or a – indicates the phenotype of the progeny flies produced by mating the fly listed at the top of the column with the fly listed to the left of the row;

TABLE Q19–18 COMPLEMENTATION ANALYSIS OF *DROSOPHILA* EYE-COLOR MUTATIONS

Mutation	white	garnet	ruby	vermilion	cherry	coral	apricot	buff	carnation
white	–	+	+	+	–	–	–	–	+
garnet		–	+	+	+	+	+	+	+
ruby			–	+	+	+	+	+	+
vermilion				–	+	+	+	+	+
cherry					–	–	–	–	+
coral						–	–	–	+
apricot							–	–	+
buff								–	+
carnation									–

+ indicates that progeny of a cross between individuals showing the indicated eye colors are phenotypically normal; – indicates that the eye color of the progeny is abnormal.

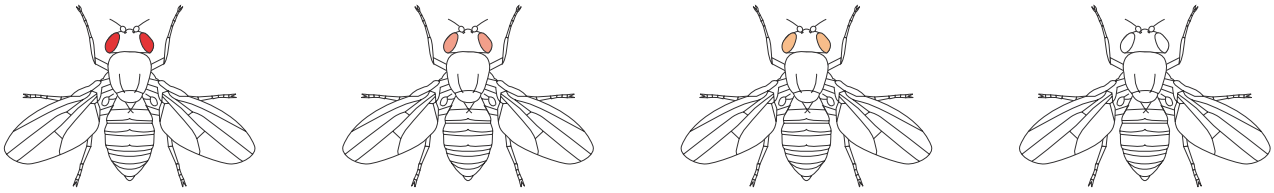


Figure Q19–18

brick-red wild-type eyes are shown as + and other colors are indicated as –.

A. How is it that flies with two different eye colors—ruby and white, for example—can give rise to progeny that all have brick-red eyes?

B. Which mutations are alleles of the same gene and which affect different genes?

C. How can different alleles of the same gene give different eye colors?

QUESTION 19–19

What are single-nucleotide polymorphisms (SNPs), and how can they be used to locate a mutant gene by linkage analysis?

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CHAPTER **TWENTY** 20

Cell Communities: Tissues, Stem Cells, and Cancer

Cells are the building blocks of multicellular organisms. This seems a simple statement, but it raises deep problems. Cells are not like bricks: they are small and squishy. How can they be used to construct a giraffe or a giant redwood tree? Each cell is enclosed in a flimsy membrane less than a hundred-thousandth of a millimeter thick, and it depends on the integrity of this membrane for its survival. How, then, can cells be joined together robustly to form muscles that can lift an elephant's weight? Most mysterious of all, if cells are the building blocks, where is the builder and where are the architect's plans? How are all the different cell types in a plant or an animal produced, with each in its proper place in an elaborate pattern (**Figure 20–1**)?

Most of the cells in multicellular organisms are organized into cooperative assemblies called **tissues**, such as the nervous, muscle, epithelial, and connective tissues found in vertebrates (**Figure 20–2**). In this chapter, we begin by discussing the architecture of tissues from a mechanical point of view. We will see that tissues are composed not only of cells, with their internal framework of cytoskeletal filaments (discussed in Chapter 17), but also of **extracellular matrix**, which cells secrete around themselves; it is this matrix that gives supportive tissues such as bone or wood their strength. The matrix provides one way to bind cells together, but cells can also attach to one another directly. Thus, we also discuss the *cell junctions* that link cells together in the flexible, mobile tissues of animals. These junctions transmit forces either from the cytoskeleton of one cell to that of the next, or from the cytoskeleton of a cell to the extracellular matrix.

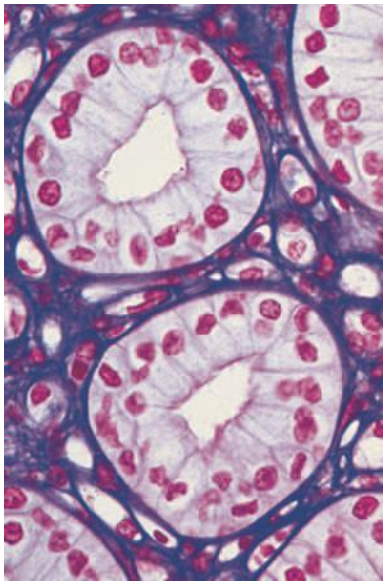


Figure 20–1 Multicellular organisms are built from organized collections of cells. This section of cells in the urine-collecting ducts of the kidney was stained with a combination of dyes, hematoxylin and eosin, commonly used in histology. Each duct is made of closely packed "principal" cells (with nuclei stained *red*), which form an epithelial tube, seen here in cross section as a ring. The ducts are embedded in an extracellular matrix, stained *purple* and populated by other types of cells. (From P.R. Wheater et al., Functional Histology, 2nd ed. London: Churchill Livingstone, 1987.)

But there is more to the organization of tissues than mechanics. Just as a building needs plumbing, telephone lines, and other fittings, so an animal tissue requires blood vessels, nerves, and other components formed from a variety of specialized cell types. All the tissue components have to be appropriately organized and coordinated, and many of them require continual maintenance and renewal. Cells die and have to be replaced with new cells of the right type, in the right places, and in the right numbers. In the third section of this chapter, we discuss how these processes are organized, as well as the crucial role that *stem cells*, self-renewing undifferentiated cells, play in the renewal and repair of some tissues.

Disorders of tissue renewal are a major medical concern, and those due to the misbehavior of mutant cells underlie the development of *cancer*. We discuss cancer in the final section of this chapter and of the book as a whole. Its study requires a synthesis of knowledge of cells and tissues at every level, from the molecular biology of DNA repair to the principles of natural selection and the social organization of cells in tissues. Many fundamental advances in cell biology have been driven by cancer research, and basic cell biology in return continues to deepen our understanding of the disease and provide us with renewed optimism about its treatment.

EXTRACELLULAR MATRIX AND CONNECTIVE TISSUES

Plants and animals have evolved their multicellular organization independently, and their tissues are constructed on different principles. Animals prey on other living things—and often are preyed on by other animals—and for this they must be strong and agile: they must possess tissues capable of rapid movement, and the cells that form those tissues must be able to generate and transmit forces and to change shape quickly. Plants, by contrast, are sedentary: their tissues are more or less rigid, although their cells are weak and fragile if isolated from the supporting matrix that surrounds them.

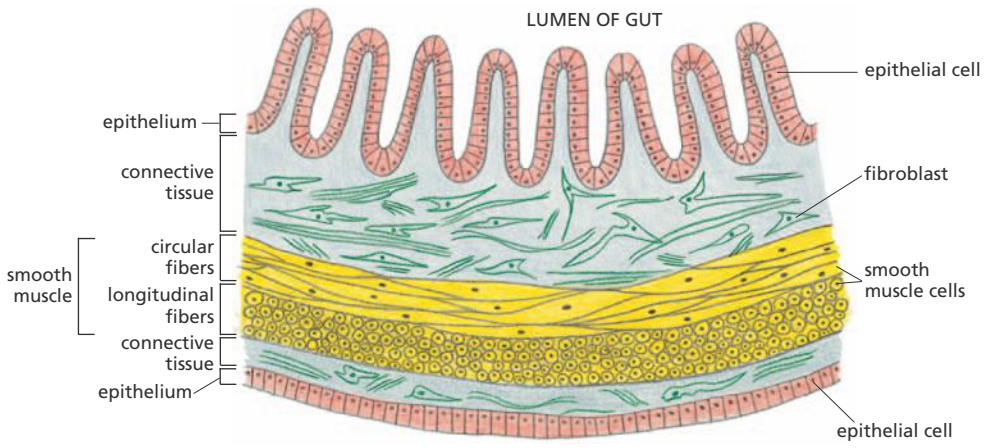


Figure 20–2 Cells are organized into tissues. Simplified drawing of a cross section through part of the wall of the intestine of a mammal. This long, tubelike organ is constructed from epithelial tissues (*red*), connective tissues (*green*), and muscle tissues (*yellow*). Each tissue is an organized assembly of cells, held together by cell–cell adhesions, extracellular matrix, or both.

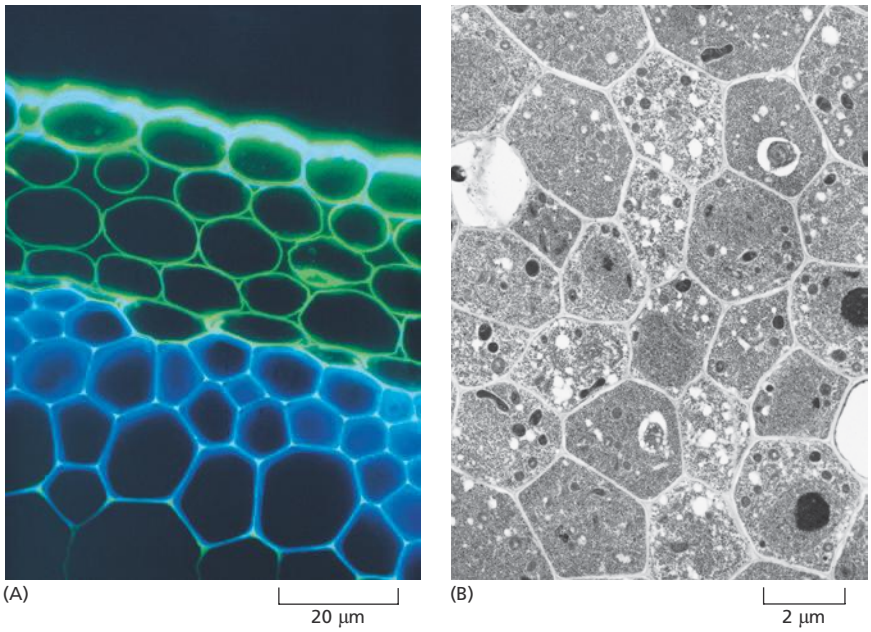


Figure 20–3 Plant tissues are strengthened by cell walls. (A) A cross section of part of the stem of the flowering plant *Arabidopsis* is shown, stained with fluorescent dyes that label two different cell wall polysaccharides—cellulose in *blue*, and pectin in *green*. The cells themselves are unstained and invisible in this preparation. Regions rich in both cellulose and pectin appear white. Pectin predominates in the outer layers of cells, which have only primary cell walls (deposited while the cell is still growing). Cellulose is more plentiful in the inner layers, which have thicker, more rigid secondary cell walls (deposited after cell growth has ceased). (B) Cells and their walls are clearly seen in this electron micrograph of the young cells in the root of the same plant. These cells are much smaller than those in the stem, as can be seen by the different scale bars in the two micrographs. (Courtesy of Paul Linstead.)

In plants, the supportive matrix is called the **cell wall**, a boxlike structure that encloses, protects, and constrains the shape of each of its cells (**Figure 20–3**). Plant cells themselves synthesize, secrete, and control the composition of this extracellular matrix: a cell wall can be thick and hard, as in wood, or thin and flexible, as in a leaf. But the principle of tissue construction is the same in either case: many tiny boxes are cemented together, with a delicate cell living inside each one. Indeed, as we noted in Chapter 1, it was the close-packed mass of microscopic chambers that Robert Hooke saw in a slice of cork three centuries ago that inspired the term "cell."

Animal tissues are more diverse. Like plant tissues, they consist of both cells and extracellular matrix, but these components are organized in many different ways. In some tissues, such as bone or tendon, extracellular matrix is plentiful and mechanically all-important; in others, such as muscle or epidermis, extracellular matrix is scanty, and the cytoskeletons of the cells themselves carry the mechanical load. We begin with a brief discussion of plant cells and tissues, before considering those of animals.

Plant Cells Have Tough External Walls

A naked plant cell, artificially stripped of its wall, is a delicate and vulnerable thing. With care, it can be kept alive in culture; but it is easily ruptured, and even a small decrease in the osmotic strength of the culture medium can cause the cell to swell and burst. Its cytoskeleton lacks the tension-bearing intermediate filaments found in animal cells, and as a result, it has virtually no tensile strength. An external wall, therefore, is essential.

The plant cell wall has to be tough, but it does not necessarily have to be rigid. Osmotic swelling of the cell, limited by the resistance of the cell wall, can keep the chamber distended, and a mass of such swollen chambers cemented together forms a semirigid tissue. Such is the state of a crisp lettuce leaf (**Figure 20–4**). If water is lacking so that the cells shrink, the leaf wilts.

Most newly formed cells in a multicellular plant initially make relatively thin *primary cell walls*, which can slowly expand to accommodate cell growth (see Figure 20–3B). The driving force for cell growth is the same

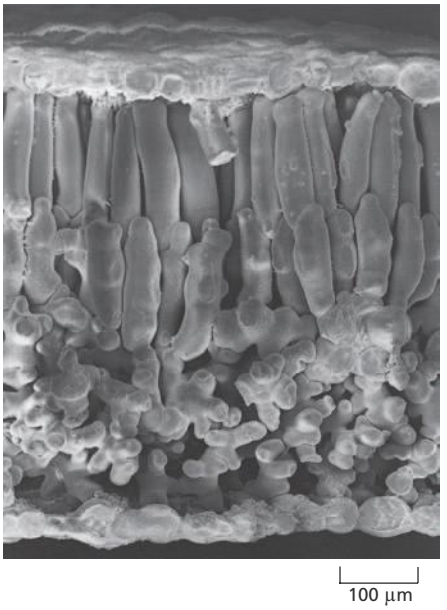


Figure 20–4 A scanning electron micrograph shows the cells in a crisp lettuce leaf. The cells, swollen by osmotic pressure, are stuck together via their walls. (Courtesy of Kim Findlay.)

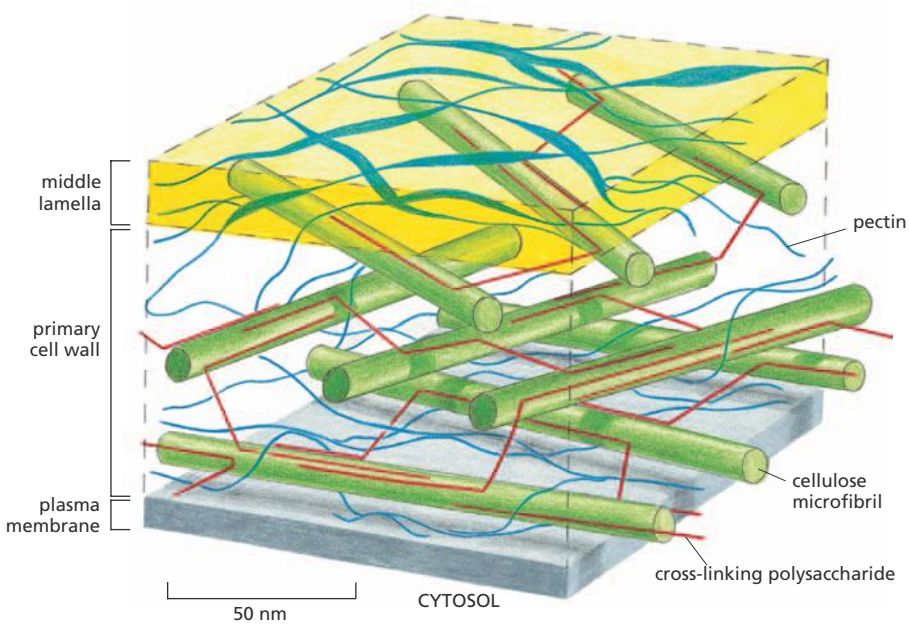


Figure 20–5 A scale model shows a portion of a primary plant cell wall. The *green* bars represent cellulose microfibrils, which provide tensile strength. Other polysaccharides (*red* strands) cross-link the cellulose microfibrils, while the polysaccharide pectin (*blue* strands) fills the spaces between the microfibrils, providing resistance to compression. The middle lamella (*yellow*) is rich in pectin and is the layer that cements one cell wall to another.

as that keeping the lettuce leaf crisp—a swelling pressure, called the *turgor pressure*, that develops as the result of an osmotic imbalance between the interior of the plant cell and its surroundings. Once cell growth stops and the wall no longer needs to expand, a more rigid *secondary cell wall* is often produced (see Figure 20–3A)—either by thickening of the primary wall or by deposition of new layers with a different composition underneath the old ones. When plant cells become specialized, they generally produce specially adapted types of walls: waxy, waterproof walls for the surface epidermal cells of a leaf; hard, thick, woody walls for the xylem cells of the stem; and so on.

Cellulose Microfibrils Give the Plant Cell Wall Its Tensile Strength

Like all extracellular matrices, plant cell walls derive their tensile strength from long fibers oriented along the lines of stress. In higher plants, the long fibers are generally made from the polysaccharide *cellulose*, the most abundant organic macromolecule on Earth. These **cellulose microfibrils** are interwoven with other polysaccharides and some structural proteins, all bonded together to form a complex structure that resists both compression and tension (**Figure 20–5**). In woody tissue, a highly cross-linked network of *lignin* (a complex polymer built from aromatic alcohol groups) is deposited within this matrix to make it more rigid and waterproof.

For a plant cell to grow or change its shape, the cell wall has to stretch or deform. Because the cellulose microfibrils resist stretching, their orientation governs the direction in which the growing cell enlarges: if, for example, they are arranged circumferentially as a corset, the cell will grow more readily in length than in girth (**Figure 20–6**). By controlling the

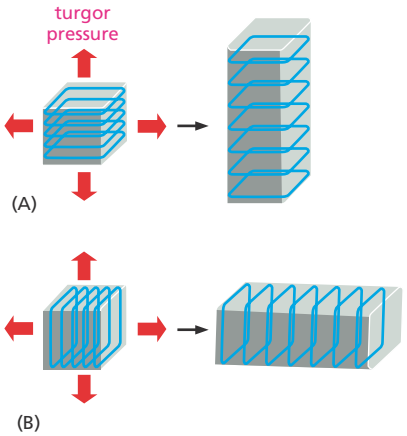


Figure 20–6 The orientation of cellulose microfibrils within the plant cell wall influences the direction in which the cell elongates. The cells in (A) and (B) start off with identical shapes (shown here as *cubes*) but with different orientations of cellulose microfibrils in their walls. Although turgor pressure is uniform in all directions, each cell tends to elongate in a direction perpendicular to the orientation of the microfibrils, which have great tensile strength. The final shape of an organ, such as a shoot, is determined by the direction in which its cells expand.

way that it lays down its wall, the plant cell consequently controls its own shape and thus the direction of growth of the tissue to which it belongs.

Cellulose is produced in a radically different way from most other extracellular macromolecules. Instead of being made inside the cell and then exported by exocytosis (discussed in Chapter 15), it is synthesized on the outer surface of the cell by enzyme complexes embedded in the plasma membrane. These complexes transport sugar monomers across the plasma membrane and incorporate them into a set of growing polymer chains at their points of membrane attachment. Each set of chains assembles to form a cellulose microfibril. The enzyme complexes move in the membrane, spinning out new polymers and laying down a trail of oriented cellulose microfibrils behind them (**Figure 20–7A**).

The paths followed by the enzyme complexes dictate the orientation in which cellulose is deposited in the cell wall; but what guides the enzyme complexes? Just underneath the plasma membrane, microtubules are aligned exactly with the cellulose microfibrils outside the cell (**Figure 20–7B**). These microtubules serve as tracks that help guide the movement of the enzyme complexes (**Figure 20–7C**). In this curiously indirect way, the cytoskeleton controls the shape of the plant cell and the modeling of the plant tissues. We will see that animal cells use their cytoskeleton to control tissue architecture in a much more direct manner.

QUESTION 20–1

Cells in the stem of a seedling that is grown in the dark orient their microtubules horizontally. How would you expect this to affect the growth of the plant?

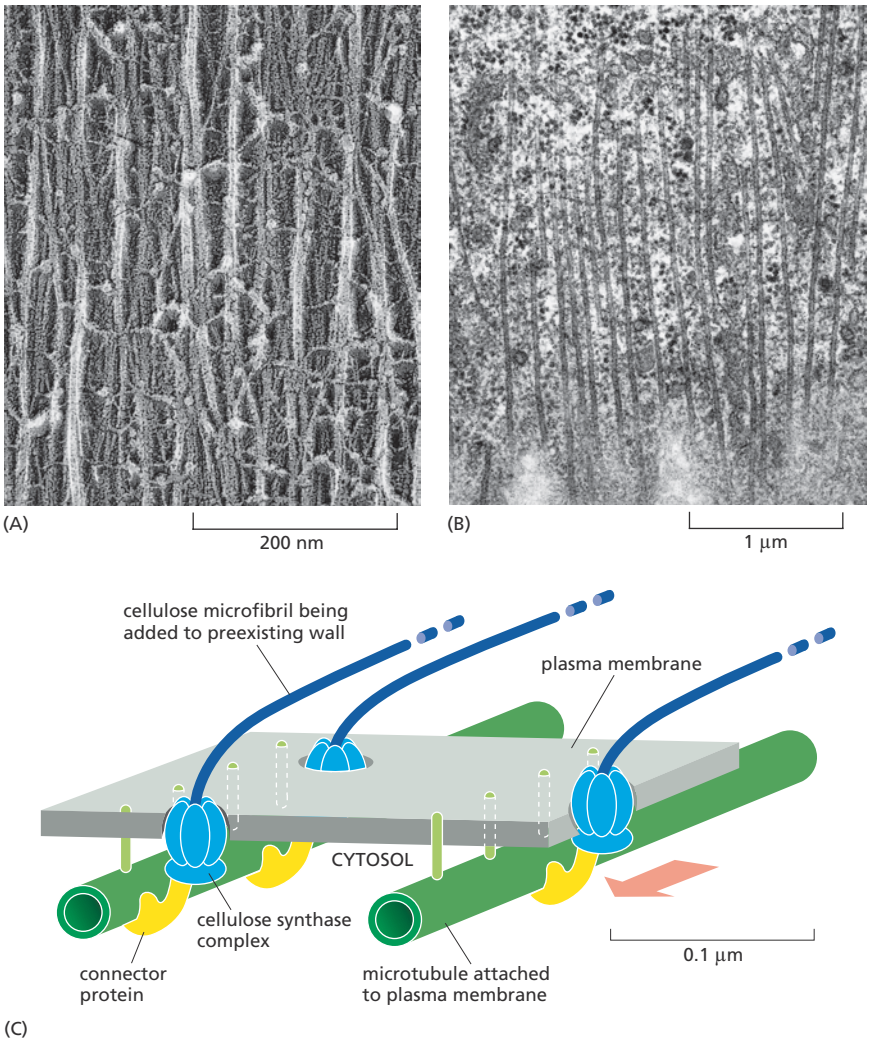


Figure 20–7 Microtubules help direct the deposition of cellulose in the plant cell wall. Electron micrographs show (A) oriented cellulose microfibrils in a plant cell wall and (B) microtubules just beneath a plant cell's plasma membrane. (C) The orientation of the newly deposited extracellular cellulose microfibrils (*dark blue*) is determined by the orientation of the underlying intracellular microtubules (*dark green*). The large *cellulose synthase* enzyme complexes are integral membrane proteins that continuously synthesize cellulose microfibrils on the outer face of the plasma membrane. The distal ends of the stiff microfibrils become integrated into the texture of the cell wall, and their elongation at the other end pushes the synthase complex along in the plane of the plasma membrane (*red* arrow). The cortical array of microtubules attached to the plasma membrane by transmembrane proteins (*green* vertical bars) helps determine the direction in which the microfibrils are laid down. (A, courtesy of Brian Wells and Keith Roberts; B, courtesy of Brian Gunning.)

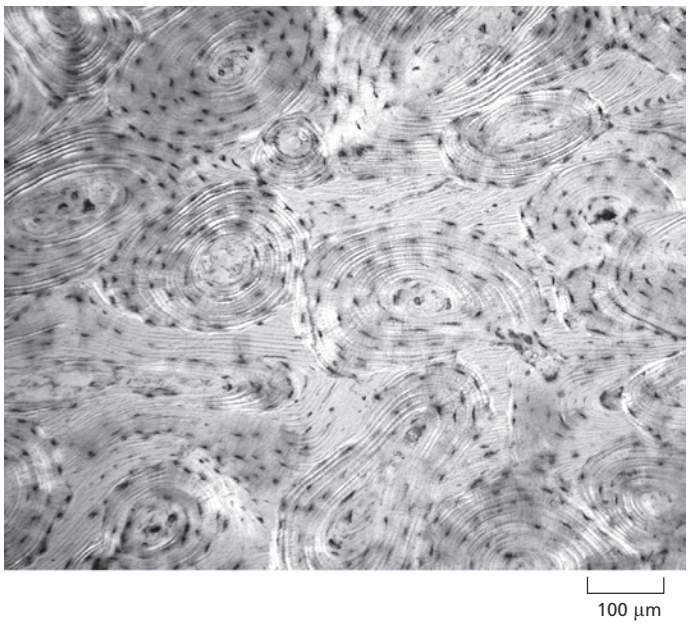


Figure 20–8 Extracellular matrix is plentiful in connective tissue such as bone. In this micrograph, cells in a cross section of bone appear as small, dark, antlike objects embedded in the bone matrix, which occupies most of the volume of the tissue and provides all its mechanical strength. The alternating light and dark bands are layers of matrix containing oriented collagen fibrils (made visible with the help of polarized light). Calcium phosphate crystals (not visible) filling the interstices between the collagen fibrils make bone matrix resistant to both compression and tension, like reinforced concrete.

Animal Connective Tissues Consist Largely of Extracellular Matrix

It is traditional to distinguish four major types of tissues in animals: connective, epithelial, nervous, and muscular. But the basic architectural distinction is between connective tissues and the rest. In **connective tissues**, extracellular matrix is plentiful and carries the mechanical load. In other tissues, such as epithelia, extracellular matrix is scanty, and the cells are directly joined to one another and carry the mechanical load themselves. We discuss connective tissues first.

Animal connective tissues are enormously varied. They can be tough and flexible like tendons or the dermis of the skin; hard and dense like bone; resilient and shock-absorbing like cartilage; or soft and transparent like the jelly that fills the interior of the eye. In all these examples, the bulk of the tissue is occupied by extracellular matrix, and the cells that produce the matrix are scattered within it like raisins in a pudding (**Figure 20–8**). In all of these tissues, the tensile strength—whether great or small—is chiefly provided not by a polysaccharide, as in plants, but by a fibrous protein: collagen. The various types of connective tissues owe their specific characters to the type of collagen that they contain, to its quantity, and, most importantly, to the other molecules that are interwoven with it in varying proportions. These include the rubbery protein *elastin*, which gives the walls of arteries their resilience as blood pulses through them, as well as a host of specialized polysaccharide molecules, which we discuss shortly.

Collagen Provides Tensile Strength in Animal Connective Tissues

Collagen is a protein found in all animals, and it comes in many varieties. Mammals have about 20 different collagen genes, coding for the variant forms of collagen required in different tissues. Collagens are the chief proteins in bone, tendon, and skin (leather is pickled collagen), and they constitute 25% of the total protein mass in a mammal—more than any other type of protein.

The characteristic feature of a typical collagen molecule is its long, stiff, triple-stranded helical structure, in which three collagen polypeptide

QUESTION 20–2

Mutations in the genes encoding collagens often have detrimental consequences, resulting in severely crippling diseases. Particularly devastating are mutations that change glycines, which are required at every third position in the collagen polypeptide chain so that it can assemble into the characteristic triple-helical rod (see Figure 20–9).

A. Would you expect collagen mutations to be detrimental if only one of the two copies of a collagen gene is defective?

B. A puzzling observation is that the change of a glycine residue into another amino acid is most detrimental if it occurs toward the amino terminus of the rod-forming domain. Suggest an explanation for this.

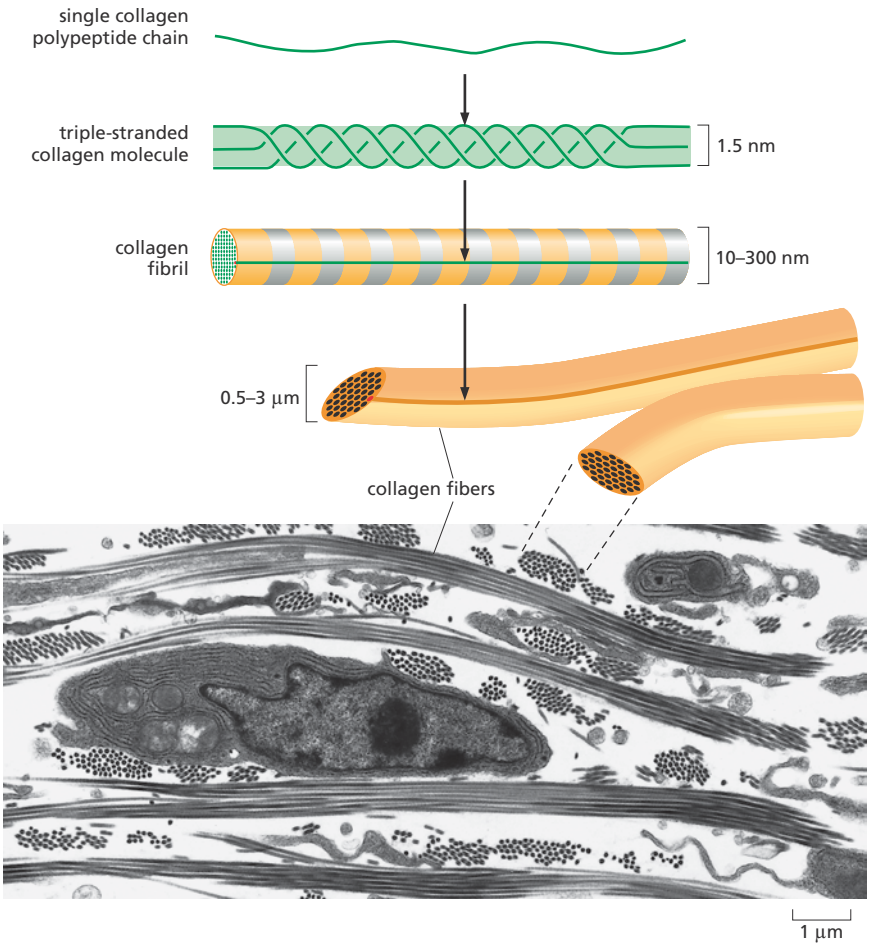


Figure 20–9 Collagen fibrils are organized into bundles. The drawings show the steps of collagen assembly, from individual polypeptide chains to triple-stranded collagen molecules, then to fibrils and, finally, fibers. The electron micrograph shows fully assembled collagen in the connective tissue of embryonic chick skin. The fibrils are organized into bundles (fibers), some running in the plane of the section, others approximately at right angles to it. The cell in the micrograph is a fibroblast, which secretes collagen and other extracellular matrix components. (Photograph from C. Ploetz et al., *J. Struct. Biol.* 106:73–81, 1991. With permission from Elsevier.)

chains are wound around one another in a ropelike superhelix (see Figure 4–29A). Some types of collagen molecules in turn assemble into ordered polymers called *collagen fibrils*, which are thin cables 10–300 nm in diameter and many micrometers long; these can pack together into still thicker *collagen fibers* (**Figure 20–9**). Other types of collagen molecules decorate the surface of collagen fibrils and link the fibrils to one another and to other components in the extracellular matrix.

The connective-tissue cells that manufacture and inhabit the extracellular matrix go by various names according to the tissue: in skin, tendon, and many other connective tissues, they are called **fibroblasts** (**Figure 20–10** and see Figure 20–9); in bone, they are called *osteoblasts*. They make both the collagen and the other macromolecules of the matrix. Almost all of these molecules are synthesized intracellularly and then secreted in the standard way, by exocytosis (discussed in Chapter 15). Outside the cell, they assemble into huge, cohesive aggregates. If assembly were to occur prematurely, before secretion, the cell would become choked with its own products. In the case of collagen, the cells avoid this catastrophe by secreting collagen molecules in a precursor form, called *procollagen*, with additional peptide extensions at each end that obstruct premature assembly into collagen fibrils. Extracellular enzymes—called procollagen proteinases—cut off these terminal extensions to allow assembly only after the molecules have emerged into the extracellular space (**Figure 20–11**).

Some people have a genetic defect in one of these proteinases, or in procollagen itself, so that their collagen fibrils do not assemble correctly.

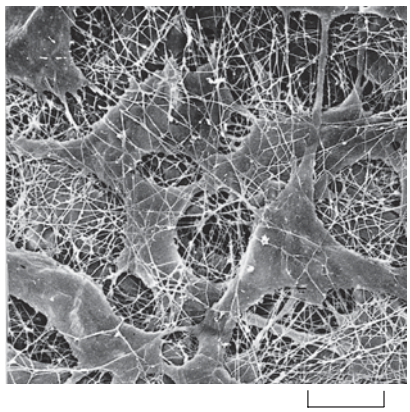


Figure 20–10 Fibroblasts produce the extracellular matrix of some connective tissues. A scanning electron micrograph showing fibroblasts and collagen fibers in connective tissue from the cornea of a rat. Other components that normally form a hydrated gel filling the spaces between the collagen fibrils and fibers have been removed by enzyme and acid treatment. (From T. Nishida et al., *Invest. Ophthalmol. Vis. Sci.* 29:1887–1890, 1988. With permission from ARVO.)

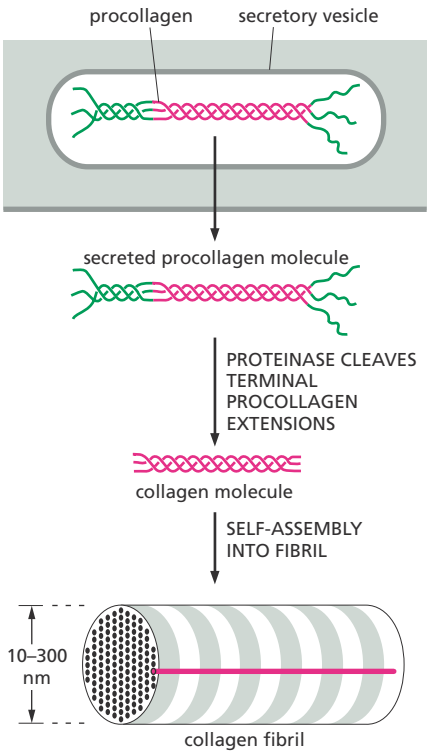


Figure 20–11 Procollagen precursors are cleaved to form mature collagen outside the cell. Collagen is synthesized as a procollagen molecule that has unstructured peptides at either end. These peptides prevent collagen from assembling into a fibril inside the fibroblast. When the procollagen is secreted, an extracellular enzyme removes its terminal peptides, producing mature collagen molecules. These molecules can then self-assemble into ordered collagen fibrils (see also Figure 20–9).

As a result, their connective tissues have a lower tensile strength and are extraordinarily stretchable (**Figure 20–12**).

Cells in tissues have to be able to degrade matrix as well as make it. This ability is essential for tissue growth, repair, and renewal; it is also important where migratory cells, such as macrophages, need to burrow through the thicket of collagen and other extracellular matrix polymers. Matrix proteases that cleave extracellular proteins play a part in many disease processes, ranging from arthritis, where they contribute to the breakdown of cartilage in affected joints, to cancer, where they help cancer cells invade normal tissue.

Cells Organize the Collagen That They Secrete

To do their job, collagen fibrils must be correctly aligned. In skin, for example, they are woven in a wickerwork pattern, or in alternating layers with different orientations so as to resist tensile stress in multiple directions (**Figure 20–13**). In tendons, which attach muscles to bone, they are aligned in parallel bundles along the major axis of tension.

The connective-tissue cells that produce collagen control this orientation, first by depositing the collagen in an oriented fashion and then by rearranging it. During development of the tissue, fibroblasts work on the collagen they have secreted, crawling over it and pulling on it—helping to compact it into sheets and draw it out into cables. This mechanical role of fibroblasts in shaping collagen matrices has been demonstrated dramatically in cell culture. When fibroblasts are mixed with a meshwork of randomly oriented collagen fibrils that form a gel in a culture dish, the fibroblasts tug on the meshwork, drawing in collagen from their surroundings and compacting it. If two small pieces of embryonic tissue containing fibroblasts are placed far apart on a collagen gel, the intervening collagen becomes organized into a dense band of aligned fibers that connect the two explants (**Figure 20–14**). The fibroblasts migrate out from the explants along the aligned collagen fibers. Thus, the fibroblasts influence the alignment of the collagen fibers, and the collagen fibers in turn affect the distribution of the fibroblasts. Fibroblasts presumably play a similar role in generating long-range order in the extracellular matrix inside the body—in helping to create tendons, for example, and the tough, dense layers of connective tissue that ensheathe and bind together most organs. Fibroblast migration is also important for healing wounds (**Movie 20.1**).

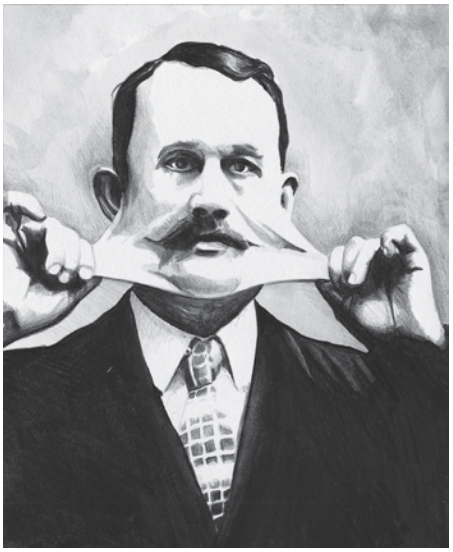


Figure 20–12 Incorrect collagen assembly can cause the skin to be hyperextensible. James Morris, "the elastic skin man," from a photograph taken in about 1890. Abnormally stretchable skin is part of a genetic syndrome that results from a defect in collagen assembly. In some individuals, this condition arises from a lack of an enzyme that converts procollagen to collagen.

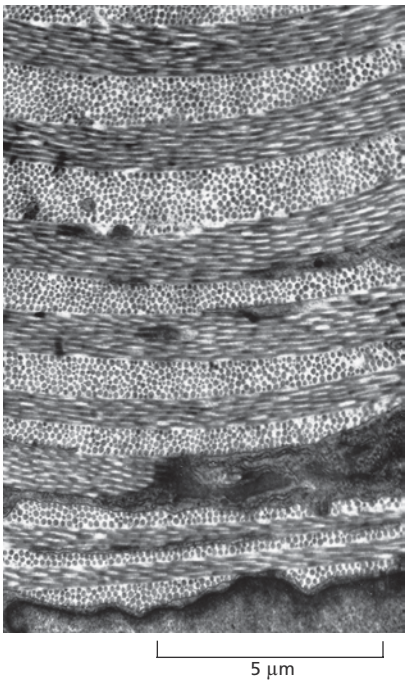


Figure 20–13 Collagen fibrils in skin are arranged in a plywoodlike pattern. The electron micrograph shows a cross section of tadpole skin. Successive layers of fibrils are laid down nearly at right angles to each other (see also Figure 20–9). This arrangement is also found in mature bone and in the cornea. (Courtesy of Jerome Gross.)

Integrins Couple the Matrix Outside a Cell to the Cytoskeleton Inside It

If cells are to pull on the matrix and crawl over it, they must be able to attach to it. Cells do not attach well to bare collagen. Another extracellular matrix protein, **fibronectin**, provides a linkage: part of the fibronectin molecule binds to collagen, while another part forms an attachment site for a cell (**Figure 20–15A and B**).

A cell attaches itself to fibronectin by means of a receptor protein, called an **integrin**, which spans the cell's plasma membrane. When the extracellular domain of the integrin binds to fibronectin, the intracellular domain binds (through a set of adaptor molecules) to an actin filament inside the cell (**Figure 20–15C**). Without this internal anchorage to the cytoskeleton, integrins would be ripped out of the flimsy lipid bilayer of the plasma membrane as the cell attempted to pull itself along the matrix.

It is the formation and breakage of the attachments on either end of an integrin molecule that allows a cell to crawl through a tissue, grabbing hold of the matrix at its front end and releasing its grip at the rear (see Figure 17–33). Integrins coordinate these "catch-and-release" manoeuvers by undergoing remarkable conformational changes. Binding to a molecule on one side of the plasma membrane causes the integrin molecule to stretch out into an extended, activated state so that it can then latch onto a different molecule on the opposite side—an effect that operates in either direction across the membrane (**Figure 20–16**). Thus, an intracellular signaling molecule can activate the integrin from the cytosolic side, causing it to reach out and grab hold of an extracellular structure. Similarly, binding to an external structure can switch on intracellular signaling pathways by activating protein kinases that associate with the intracellular end of the integrin. In this way, a cell's external attachments help regulate whether it lives or dies, and—if it does survive—whether it grows, divides, or differentiates.

Humans make at least 24 kinds of integrins, each of which recognizes distinct extracellular molecules and has distinct functions, depending on

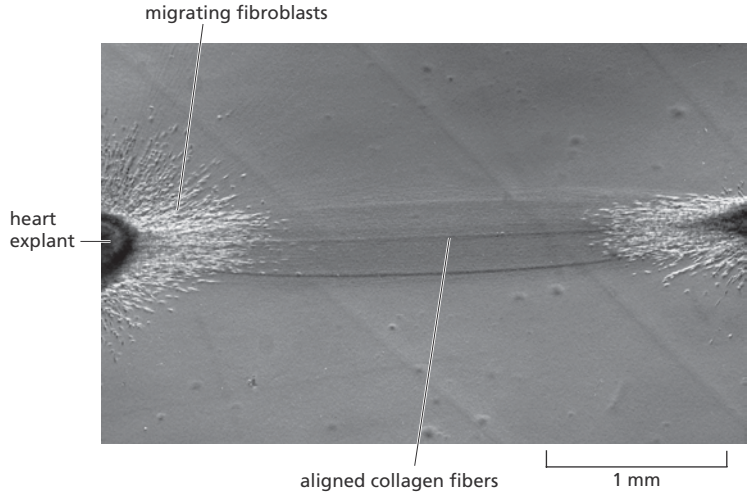


Figure 20–14 Fibroblasts influence the alignment of collagen fibers. This micrograph shows a region between two pieces of embryonic chick heart (rich in fibroblasts, as well as heart muscle cells) that have grown in culture on a collagen gel for four days. A dense tract of aligned collagen fibers has formed between the explants, presumably as a result of the fibroblasts, which have migrated out from the explants, tugging on the collagen. Elsewhere in the culture dish, the collagen remains disorganized and unaligned, so that it appears uniformly gray. (From D. Stopak and A.K. Harris, *Dev. Biol.* 90:383–398, 1982. With permission from Elsevier.)

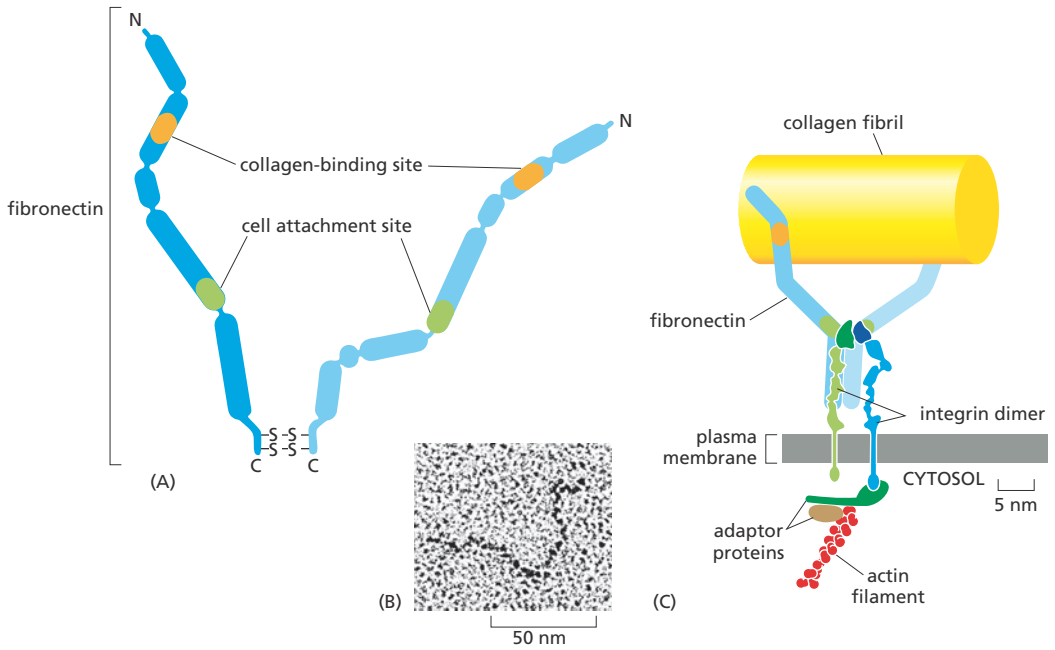


Figure 20–15 Fibronectin and integrin proteins help attach a cell to the extracellular matrix. Fibronectin molecules outside the cell bind to collagen fibrils. Integrins in the plasma membrane bind to the fibronectin and tether it to the cytoskeleton inside the cell. (A) Diagram and (B) electron micrograph of a molecule of fibronectin. (C) The transmembrane linkage mediated by an integrin protein (*blue* and *green* dimer). The integrin molecule transmits tension across the plasma membrane: it is anchored inside the cell via adaptor proteins to the actin cytoskeleton and externally via fibronectin to other extracellular matrix proteins, such as the collagen fibril shown. The integrin shown here links fibronectin to an actin filament inside the cell. Other integrins can connect different extracellular proteins to the cytoskeleton (usually to actin filaments, but sometimes to intermediate filaments). (B, from J. Engel et al., *J. Mol. Biol.* 150:97–120, 1981. With permission from Elsevier.)

the cell type in which it resides. For example, the integrins on white blood cells help the cells crawl out of blood vessels at sites of infection so as to deal with marauding microbes. People who lack this type of integrin develop a disease called *leucocyte adhesion deficiency* and suffer from repeated bacterial infections. A different form of integrin is found on blood platelets, and individuals who lack this integrin bleed excessively because their platelets cannot bind to the necessary clotting factor in the extracellular matrix.

Gels of Polysaccharides and Proteins Fill Spaces and Resist Compression

While collagen provides tensile strength to resist stretching, a completely different group of macromolecules in the extracellular matrix of

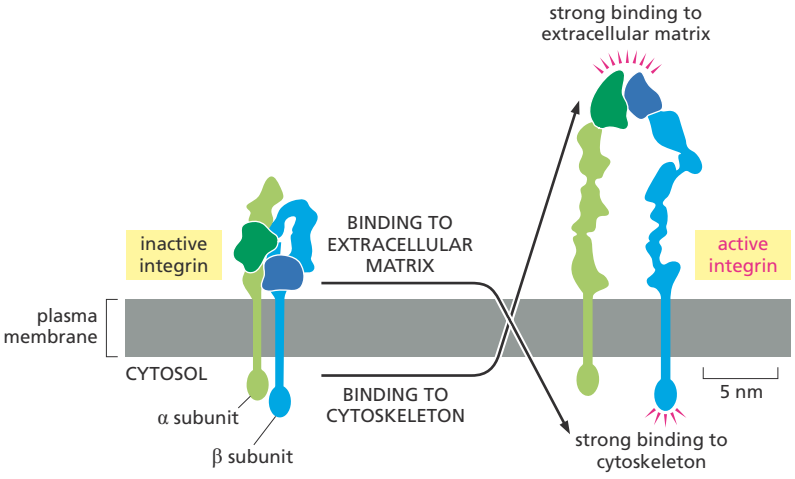


Figure 20–16 An integrin protein switches to an active conformation when it binds to molecules on either side of the plasma membrane. An integrin protein consists of two different subunits, α (*green*) and β (*blue*), which can switch between a folded, inactive form and an extended, active form. The switch to the activated state can be triggered by binding to an extracellular matrix molecule (such as fibronectin) or to intracellular adaptor proteins that then link the integrin to the cytoskeleton (see Figure 20–15). In both cases, the conformational change alters the integrin so that its opposite end rapidly forms a counterbalancing attachment to the appropriate structure. In this way, the integrin creates a mechanical linkage across the plasma membrane. (Based on T. Xiao at al., *Nature* 432:59–67, 2004. With permission from Macmillan Publishers Ltd.)

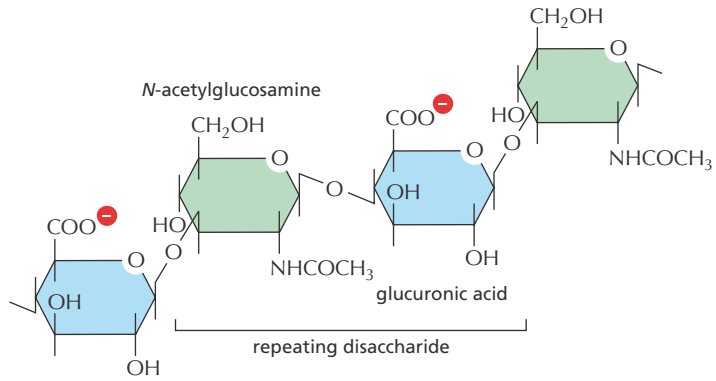
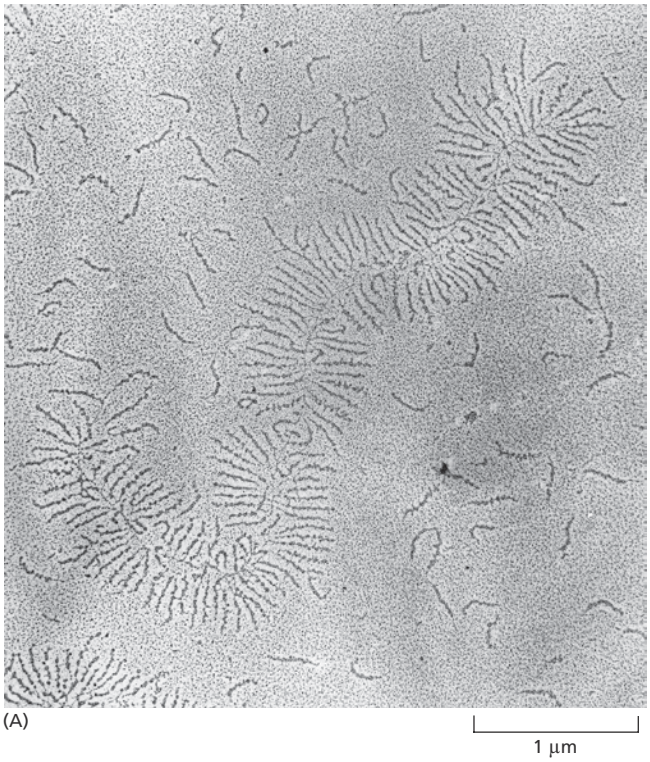


Figure 20–17 Glycosaminoglycans (GAGs) are built from repeating disaccharide units. Hyaluronan, a relatively simple GAG, is shown here. It consists of a single long chain of up to 25,000 repeated disaccharide units, each carrying a negative charge (*red*). As in other GAGs, one of the sugar monomers (*green*) in each disaccharide unit is an amino sugar. Many GAGs have additional negatively charges, often from sulfate groups (not shown).

animal tissues provides the complementary function, resisting compression. These are the **glycosaminoglycans** (**GAGs**), negatively charged polysaccharide chains made of repeating disaccharide units (**Figure 20–17**). GAGs are usually covalently linked to core proteins to form **proteoglycans**, which are extremely diverse in size, shape, and chemistry. Typically, many GAG chains are attached to a single core protein, which may in turn be linked at one end to another GAG, creating an enormous aggregate resembling a bottlebrush, with a molecular weight in the millions (**Figure 20–18**).

In dense, compact connective tissues such as tendon and bone, the proportion of GAGs is small, and the matrix consists almost entirely of collagen (or, in the case of bone, of collagen plus calcium phosphate crystals). At the other extreme, the jellylike substance in the interior of the eye consists almost entirely of one particular type of GAG, plus water,



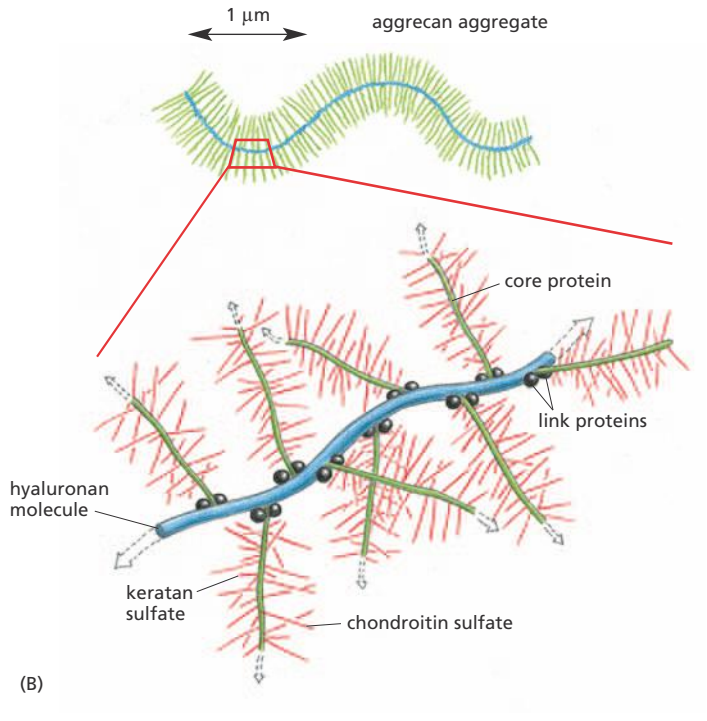


Figure 20–18 Proteoglycans and GAGs can form large aggregates. (A) Electron micrograph of an aggregate from cartilage, spread out on a flat surface. Many free subunits—themselves large proteoglycan molecules—can also be seen. (B) Schematic drawing of the giant aggregate illustrated in (A), showing how it is built up from GAGs (*red* and *blue*) and proteins (*green* and *black*). The mass of such a complex can be 10^8 daltons or more, and it occupies a volume equivalent to that of a bacterium, which is about 2×10^{-12} cm^3. (A, courtesy of Lawrence Rosenberg.)

with only a small amount of collagen. In general, GAGs are strongly hydrophilic and tend to adopt highly extended conformations, which occupy a huge volume relative to their mass (see Figure 20–18). Thus GAGs act as effective "space fillers" in the extracellular matrix of connective tissues.

Even at very low concentrations, GAGs form hydrophilic gels: their multiple negative charges attract a cloud of cations, such as Na^+, that are osmotically active, causing large amounts of water to be sucked into the matrix. This gives rise to a swelling pressure, which is balanced by tension in the collagen fibers interwoven with the proteoglycans. When the matrix is rich in collagen and large quantities of GAGs are trapped in its meshes, both the swelling pressure and the counterbalancing tension are enormous. Such a matrix is tough, resilient, and resistant to compression. The cartilage matrix that lines the knee joint, for example, has this character: it can support pressures of hundreds of kilograms per square centimeter.

Proteoglycans perform many sophisticated functions in addition to providing hydrated space around cells. They can form gels of varying pore size and charge density that act as filters to regulate the passage of molecules through the extracellular medium. They can bind secreted growth factors and other proteins that serve as extracellular signals for cells. They can block, encourage, or guide cell migration through the matrix. In all these ways, the matrix components influence the behavior of cells, often the same cells that make the matrix—a reciprocal interaction that has important effects on cell differentiation and the arrangement of cells in a tissue. Much remains to be learned about how cells weave the tapestry of matrix molecules and how the chemical messages they deposit in this intricate biochemical fabric are organized and act.

QUESTION 20–3

Proteoglycans are characterized by the abundance of negative charges on their sugar chains. How would the properties of these molecules differ if the negative charges were not as abundant?

EPITHELIAL SHEETS AND CELL JUNCTIONS

There are more than 200 visibly different cell types in the body of a vertebrate. The majority of these are organized into **epithelia** (singular **epithelium**)—multicellular sheets in which the cells are joined together, side to side. In some cases, the sheet is many cells thick, or *stratified*, as in the epidermis (the outer layer of the skin); in other cases, it is a *simple epithelium*, only one cell thick, as in the lining of the gut. Epithelial cells can take many forms. They can be tall and columnar, squat and cuboidal, or flat and squamous (**Figure 20–19**). Within a given sheet, the cells may be all the same type or a mixture of different types. Some epithelia, like the epidermis, act mainly just as a protective barrier; others have complex biochemical functions. Some secrete specialized products such as hormones, milk, or tears; others, such as the epithelium lining the gut, absorb nutrients; yet others detect signals, such as light, sensed by

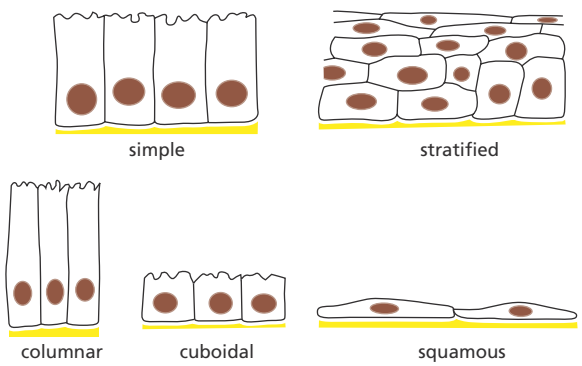


Figure 20–19 Cells can be packed together in different ways to form an epithelial sheet. Five basic types of epithelia are shown.

the layer of photoreceptors in the retina of the eye, or sound, sensed by the epithelium containing the auditory hair cells in the ear (see Figure 12–27). Despite these and many other variations, one can recognize a standard set of structural features that virtually all animal epithelia share. The arrangement of cells into epithelia is so commonplace that one easily takes it for granted; yet it requires a collection of specialized devices, as we will see, and these are common to a wide variety of epithelial cell types.

Epithelia cover the external surface of the body and line all its internal cavities, and they must have been an early feature in the evolution of animals. Cells joined together into an epithelial sheet create a barrier, which has the same significance for the multicellular organism that the plasma membrane has for a single cell. It keeps some molecules in, and others out; it takes up nutrients and exports wastes; it contains receptors for environmental signals; and it protects the interior of the organism from invading microorganisms and fluid loss.

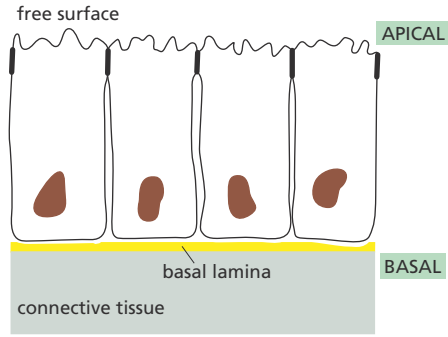


Figure 20–20 A sheet of epithelial cells has an apical and a basal surface. The basal surface sits on a specialized sheet of extracellular matrix called the basal lamina, while the apical surface is free.

Epithelial Sheets Are Polarized and Rest on a Basal Lamina

An epithelial sheet has two faces: the **apical** surface is free and exposed to the air or to a watery fluid; the **basal** surface is attached to a sheet of connective tissue called the basal lamina (**Figure 20–20**). The **basal lamina** consists of a thin, tough sheet of extracellular matrix, composed mainly of a specialized type of collagen (type IV collagen) and a protein called *laminin* (**Figure 20–21**). Laminin provides adhesive sites for integrin molecules in the basal plasma membranes of epithelial cells, and it thus serves a linking role like that of fibronectin in other connective tissues.

The apical and basal faces of an epithelium are chemically different, reflecting the polarized organization of the individual epithelial cells: each has a top and a bottom, with different properties and functions. This polarity is crucial for epithelial function. Consider, for example, the simple columnar epithelium that lines the small intestine of a mammal. It mainly consists of two intermingled cell types: absorptive cells, which

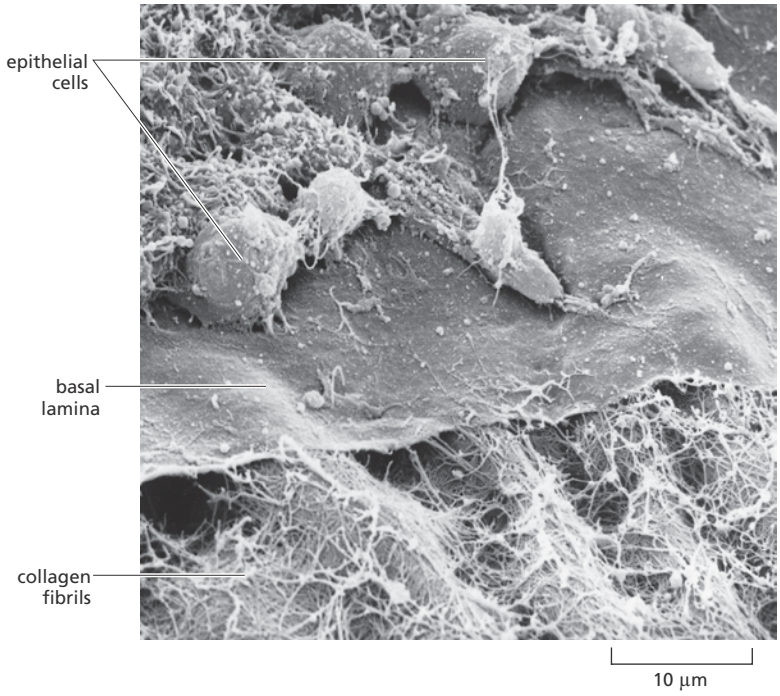


Figure 20–21 The basal lamina supports a sheet of epithelial cells. Scanning electron micrograph of a basal lamina in the cornea of a chick embryo. Some of the epithelial cells have been removed to expose the upper surface of the matlike basal lamina, which is woven from type IV collagen and laminin proteins. A network of other collagen fibrils in the underlying connective tissue interacts with the lower face of the lamina. (Courtesy of Robert Trelstad.)

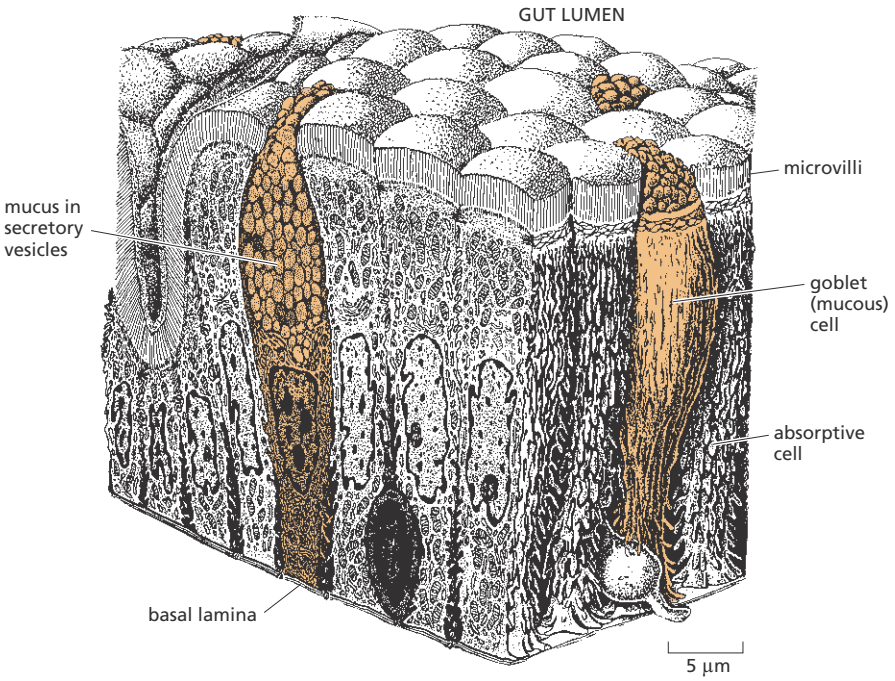


Figure 20–22 Functionally polarized cell types line the intestine. Absorptive cells, which take up nutrients from the intestine, are mingled in the gut epithelium with goblet cells (*brown*), which secrete mucus into the gut lumen. The absorptive cells are often called *brush-border cells,* because of the brushlike mass of microvilli on their apical surface; the microvilli serve to increase the area of apical plasma membrane for the transport of small molecules into the cell. The goblet cells owe their gobletlike shape to the mass of secretory vesicles that distends the cytoplasm in their apical region. (Adapted from R. Krstić, Human Microscopic Anatomy. Berlin: Springer, 1991. With permission from Springer-Verlag.)

take up nutrients, and goblet cells (so called because of their shape), which secrete the mucus that protects and lubricates the gut lining (**Figure 20–22**). Both cell types are polarized. The absorptive cells import food molecules from the gut lumen through their apical surface and export these molecules from their basal surface into the underlying tissues. To do this, absorptive cells require different sets of membrane transport proteins in their apical and basal plasma membranes (see Figure 12–14). The goblet cells also have to be polarized, but in a different way: they have to synthesize mucus and then discharge it from their apical end only (see Figure 20–22); their Golgi apparatus, secretory vesicles, and cytoskeleton are all polarized so as to bring this about. For both types of epithelial cells polarity depends on the junctions that the cells form with one another and with the basal lamina. These in turn control the arrangement of an elaborate system of membrane-associated intracellular proteins that create the polarized organization of the cytoplasm.

Tight Junctions Make an Epithelium Leak-proof and Separate Its Apical and Basal Surfaces

Epithelial **cell junctions** can be classified according to their function. Some provide a tight seal to prevent the leakage of molecules across the epithelium through the gaps between its cells; some provide strong mechanical attachments; and some provide for a special type of intimate chemical communication. In most epithelia, all these types of junctions are present. As we will see, each type of junction is characterized by its own class of membrane proteins.

The sealing function is served (in vertebrates) by **tight junctions**. These junctions seal neighboring cells together so that water-soluble molecules cannot easily leak between them. If a tracer molecule is added to one side of an epithelial cell sheet, it will usually not pass beyond the tight junction (**Figure 20–23A and B**). The tight junction is formed from proteins called *claudins* and *occludins*, which are arranged in strands along the lines of the junction to create the seal (**Figure 20–23C**). Without tight

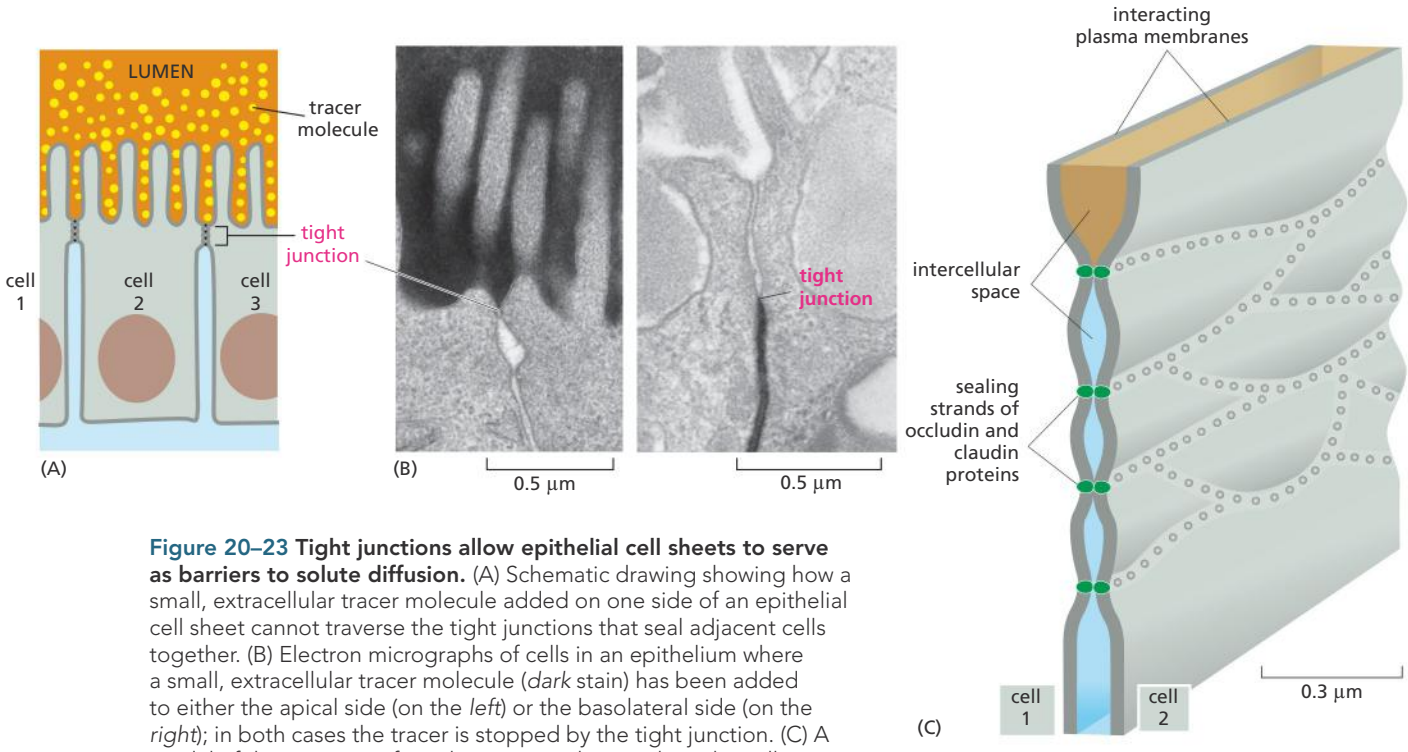


Figure 20–23 Tight junctions allow epithelial cell sheets to serve as barriers to solute diffusion. (A) Schematic drawing showing how a small, extracellular tracer molecule added on one side of an epithelial cell sheet cannot traverse the tight junctions that seal adjacent cells together. (B) Electron micrographs of cells in an epithelium where a small, extracellular tracer molecule (*dark* stain) has been added to either the apical side (on the *left*) or the basolateral side (on the *right*); in both cases the tracer is stopped by the tight junction. (C) A model of the structure of a tight junction, showing how the cells are sealed together by branching strands of transmembrane proteins, called claudins and occludins (*green*), in the plasma membranes of the interacting cells. Each type of protein binds to the same type in the apposed membrane (not shown). (B, courtesy of Daniel Friend.)

junctions to prevent leakage, the pumping activities of absorptive cells such as those in the gut would be futile, and the composition of the extracellular fluid would become the same on both sides of the epithelium. Tight junctions also play a key part in maintaining the polarity of the individual epithelial cells in two ways. First, the tight junctions around the apical region of each cell prevents diffusion of proteins within the plasma membrane and so keeps the apical domain of the plasma membrane different from the basal (or basolateral) domain (see Figure 11–32). Second, in many epithelia, the tight junctions are sites of assembly for the complexes of intracellular proteins that govern the apico-basal polarity of the cell interior.

Cytoskeleton-linked Junctions Bind Epithelial Cells Robustly to One Another and to the Basal Lamina

The cell junctions that hold an epithelium together by forming mechanical attachments are of three main types. *Adherens junctions* and *desmosomes* bind one epithelial cell to another, while *hemidesmosomes* bind epithelial cells to the basal lamina. All of these junctions provide mechanical strength by the same strategy: the proteins that form the cell adhesion span the plasma membrane and are linked inside the cell to cytoskeletal filaments. In this way, the cytoskeletal filaments are tied into a network that extends from cell to cell across the whole expanse of the epithelial sheet.

Adherens junctions and desmosomes are both built around transmembrane proteins that belong to the **cadherin** family: a cadherin molecule in the plasma membrane of one cell binds directly to an identical cadherin molecule in the plasma membrane of its neighbor (**Figure 20–24**).

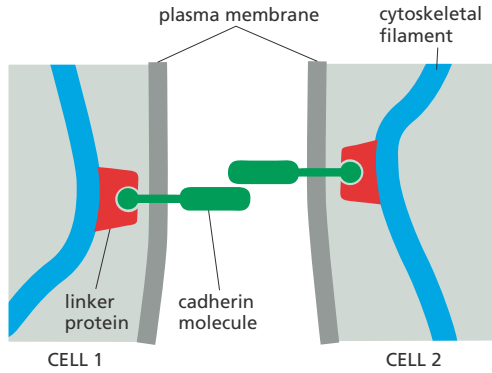


Figure 20–24 Cadherin molecules mediate mechanical attachment of one cell to another. Identical cadherin molecules in the plasma membranes of adjacent cells bind to each other extracellularly; inside the cell, they are attached, via linker proteins, to cytoskeletal filaments—either actin filaments or keratin intermediate filaments. As cells touch one another, their cadherins become concentrated at the point of attachment (Movie 20.2).

Such binding of like-to-like is called *homophilic* binding. In the case of cadherins, binding also requires that Ca^{2+} be present in the extracellular medium—hence the name.

At an **adherens junction**, each cadherin molecule is tethered inside its cell, via several linker proteins, to actin filaments. Often, the adherens junctions form a continuous adhesion belt around each of the interacting epithelial cells; this belt is located near the apical end of the cell, just below the tight junctions (**Figure 20–25**). Bundles of actin filaments are thus connected from cell to cell across the epithelium. This network of actin filaments can contract, giving the epithelial sheet the capacity to develop tension and to change its shape in remarkable ways. By shrinking the apical surface of an epithelial sheet along one axis, the sheet can roll itself up into a tube (**Figure 20–26A and B**). Alternatively, by shrinking its apical surface locally along all axes at once, the sheet can invaginate into a cup and eventually create a spherical vesicle by pinching off from the rest of the epithelium (**Figure 20–26C**). Epithelial movements such as these are important in embryonic development, where they create structures such as the neural tube (see Figure 20–26B), which gives rise to the central nervous system, and the lens vesicle, which develops into the lens of the eye (see Figure 20–26C).

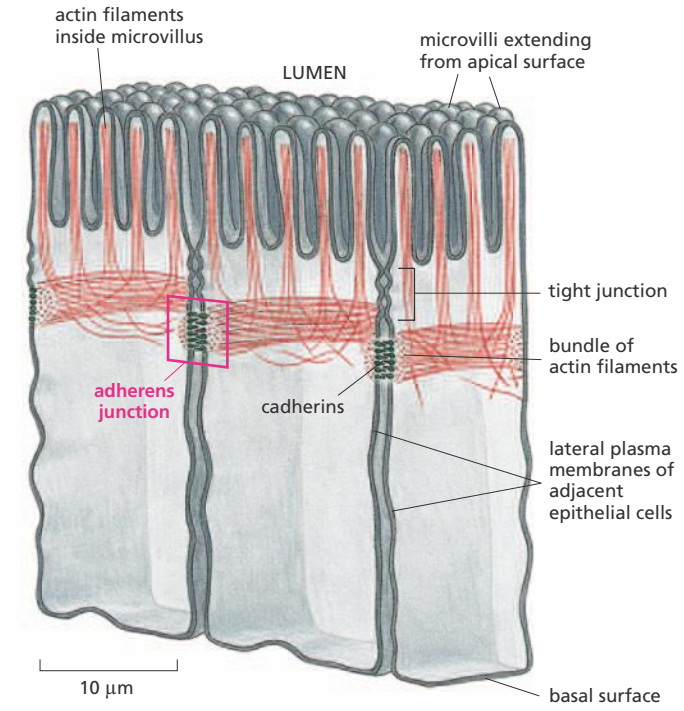
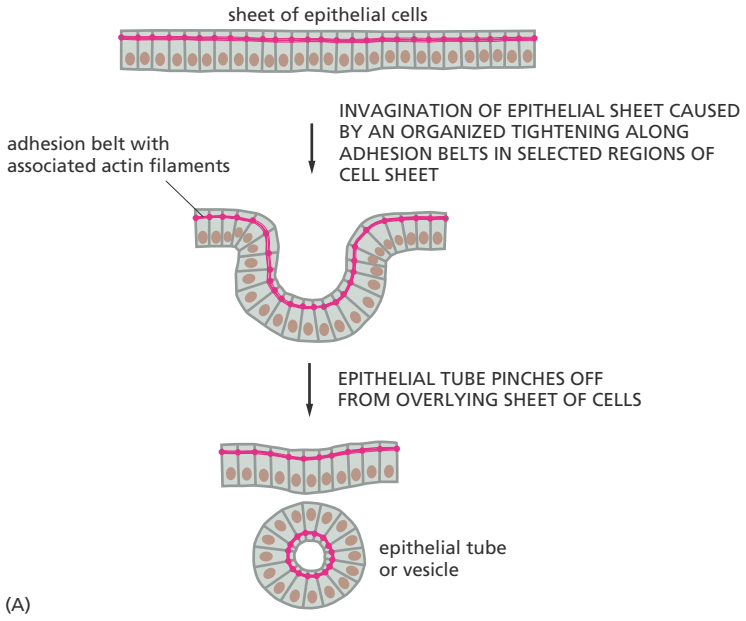
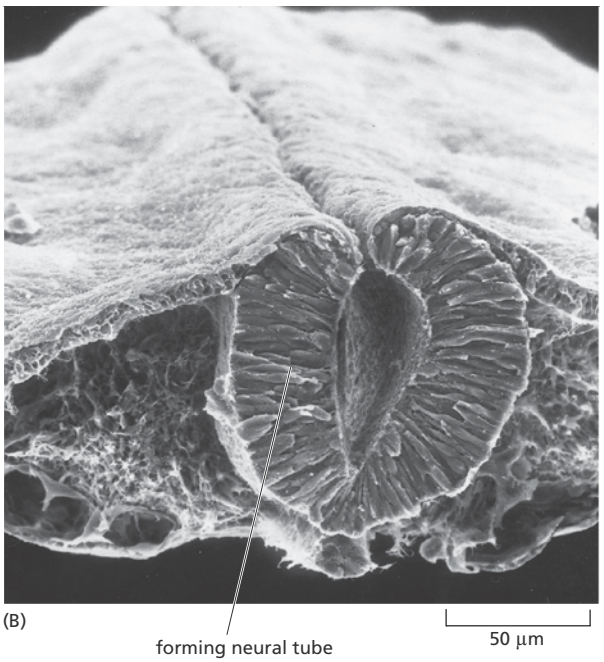


Figure 20–25 Adherens junctions form adhesion belts around epithelial cells in the small intestine. A contractile bundle of actin filaments runs along the cytoplasmic surface of the plasma membrane near the apex of each cell. These bundles are linked to those in adjacent cells via transmembrane cadherin molecules (see Figure 20–24).

At a **desmosome**, a different set of cadherin molecules connects to *keratin filaments*—the intermediate filaments found specifically in epithelial cells (see Figure 17–5). Bundles of ropelike keratin filaments criss-cross the cytoplasm and are "spot-welded" via desmosome junctions to the bundles of keratin filaments in adjacent cells (**Figure 20–27**). This arrangement confers great tensile strength on the epithelial sheet and is characteristic of tough, exposed epithelia such as the epidermis of the skin.

Blisters are a painful reminder that it is not enough for epidermal cells to be firmly attached to one another: they must also be anchored to the underlying connective tissue. As we noted earlier, the anchorage is mediated by integrins in the cells' basal plasma membranes. The extracellular domains of these integrins bind to laminin in the basal lamina; inside the cell, the integrin tails are linked to keratin filaments, creating a structure that looks superficially like half a desmosome. These attachments





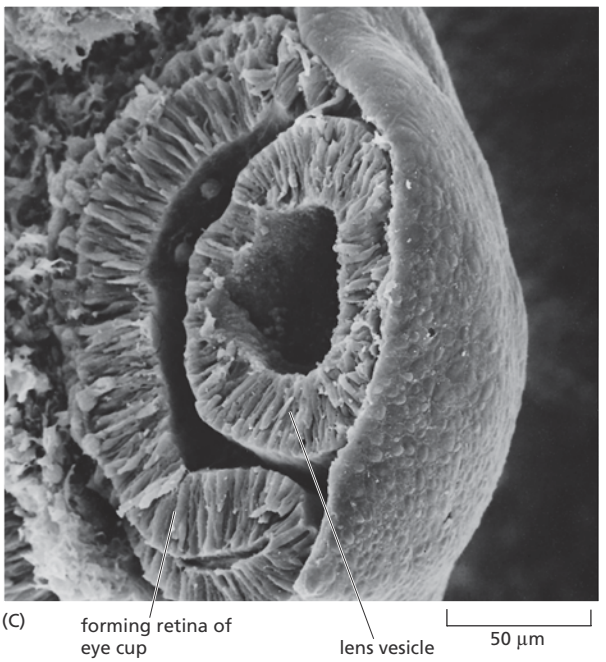


Figure 20–26 Epithelial sheets can bend to form a tube or a vesicle. Contraction of apical bundles of actin filaments linked from cell to cell via adherens junctions causes the epithelial cells to narrow at their apex. Depending on whether the contraction of the epithelial sheet is oriented along one axis, or is equal in all directions, the epithelium will either roll up into a tube or invaginate to form a vesicle. (A) Diagram showing how an apical contraction along one axis of an epithelial sheet can cause the sheet to form a tube. (B) Scanning electron micrograph of a cross section through the trunk of a two-day chick embryo, showing the formation of the neural tube by the process shown in (A). Part of the epithelial sheet that covers the surface of the embryo has thickened and rolled up by apical contraction; the opposing folds are about to fuse, after which the structure will pinch off to form the neural tube. (C) Scanning electron micrograph of a chick embryo showing the formation of the eye cup and lens. A patch of surface epithelium overlying the forming eye cup has become concave and has pinched off as a separate vesicle—the lens vesicle—within the eye cup. This process is driven by an apical narrowing of epithelial cells in all directions. (B, courtesy of Jean-Paul Revel; C, courtesy of K.W. Tosney.)

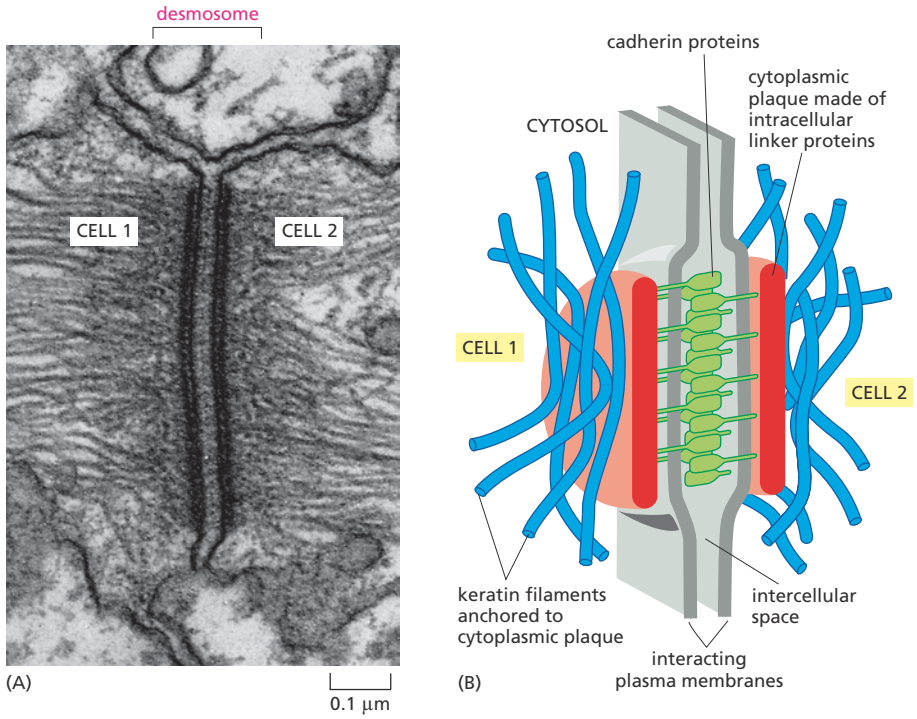


Figure 20–27 Desmosomes link the keratin intermediate filaments of one epithelial cell to those of another. (A) An electron micrograph of a desmosome joining two cells in the epidermis of newt skin, showing the attachment of keratin filaments. (B) Schematic drawing of a desmosome. On the cytoplasmic surface of each interacting plasma membrane is a dense plaque composed of a mixture of intracellular linker proteins. A bundle of keratin filaments is attached to the surface of each plaque. The cytoplasmic tails of transmembrane cadherin proteins bind to the outer face of each plaque; their extracellular domains interact to hold the cells together. (A, from D.E. Kelly, *J. Cell Biol.* 28:51–72, 1966. With permission from The Rockefeller University Press.)

of epithelial cells to basal lamina beneath them are therefore called **hemidesmosomes** (**Figure 20–28**).

QUESTION 20–4

Analogs of hemidesmosomes are the focal contact sites described in Chapter 17, which are also sites where the cell attaches to the extracellular matrix. These junctions are prevalent in fibroblasts but largely absent in epithelial cells. On the other hand, hemidesmosomes are prevalent in epithelial cells but absent in fibroblasts. In focal contact sites, intracellular connections are made to actin filaments, whereas in hemidesmosomes connections are made to intermediate filaments. Why do you suppose these two different cell types attach differently to the extracellular matrix?

Gap Junctions Allow Cytosolic Inorganic Ions and Small Molecules to Pass from Cell to Cell

The final type of epithelial cell junction, found in virtually all epithelia and in many other types of animal tissues, serves a totally different purpose. In the electron microscope, this **gap junction** appears as a region where the membranes of two cells lie close together and exactly parallel, with a very narrow gap of 2–4 nm between them. The gap, however, is not entirely empty; it is spanned by the protruding ends of many identical, transmembrane protein complexes that lie in the plasma membranes of the two apposed cells. These complexes, called *connexons*, are aligned end-to-end to form narrow, water-filled channels across the two plasma membranes (**Figure 20–29A**). The channels allow inorganic ions and small, water-soluble molecules (up to a molecular mass of about 1000 daltons) to move directly from the cytosol of one cell to the cytosol of the other. This creates an electrical and a metabolic coupling between the cells. Gap junctions between cardiac muscle cells, for example, provide

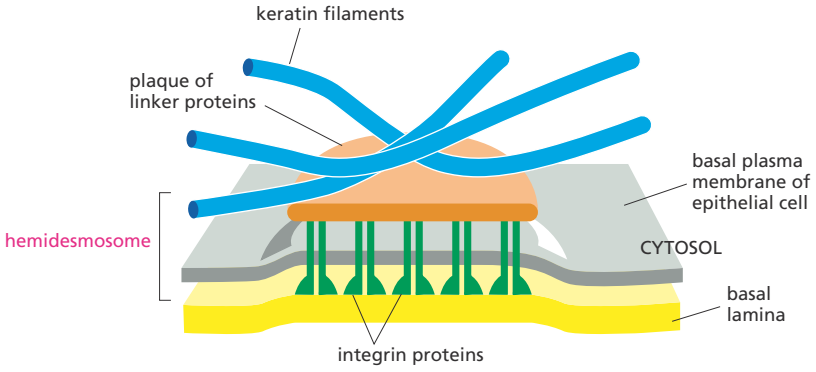


Figure 20–28 Hemidesmosomes anchor the keratin filaments in an epithelial cell to the basal lamina. The linkage is mediated by transmembrane integrins.

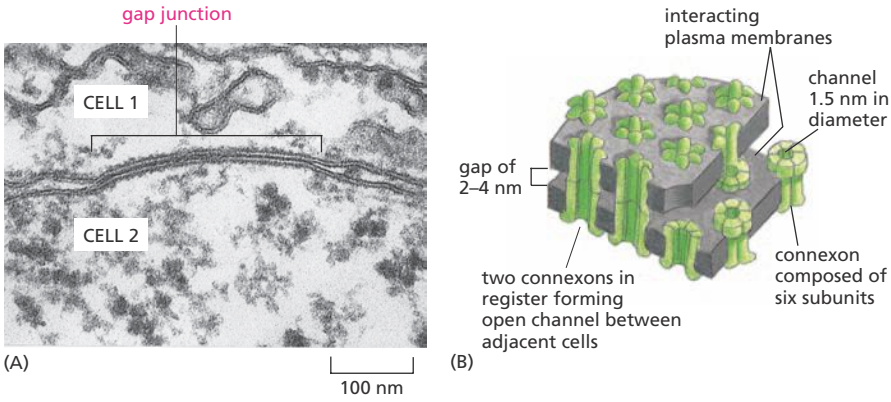


Figure 20–29 Gap junctions provide neighboring cells with a direct channel of communication. (A) Thin-section electron micrograph of a gap junction between two cells in culture. (B) A model of a gap junction. The drawing shows the interacting plasma membranes of two adjacent cells. The apposed membranes are penetrated by protein assemblies called *connexons* (*green*), each of which is formed from six identical protein subunits. Two connexons join across the intercellular gap to form an aqueous channel connecting the cytosols of the two cells. (A, from N.B. Gilula, in Cell Communication [R.P. Cox, ed.], pp. 1–29. New York: Wiley, 1974. With permission from John Wiley & Sons, Inc.)

the electrical coupling that allows electrical waves of excitation to spread synchronously through the heart, triggering the coordinated contraction of the cells that produces each heart beat.

Gap junctions in many tissues can be opened or closed in response to extracellular or intracellular signals. The neurotransmitter dopamine, for example, reduces gap-junction communication within a class of neurons in the retina in response to an increase in light intensity (**Figure 20–30**). This reduction in gap-junction permeability alters the pattern of electrical signaling and helps the retina switch from using rod photoreceptors, which are good detectors of low light, to cone photoreceptors, which detect color and fine detail in bright light. The function of gap junctions—and of the other junctions found in animal cells—are summarized in **Figure 20–31**.

Plant tissues lack all the types of cell junctions we have discussed so far, as their cells are held together by their cell walls. Curiously, however, they have a functional counterpart of the gap junction. The cytoplasms of adjacent plant cells are connected via minute communicating channels called **plasmodesmata**, which span the intervening cell walls. In contrast to gap-junction channels, plasmodesmata are cytoplasmic channels lined with plasma membrane (**Figure 20–32**). Thus in plants, the cytoplasm is, in principle, continuous from one cell to the next. Inorganic small molecules, and even macromolecules—including some proteins and regulatory RNAs—can pass through plasmodesmata. The controlled traffic of transcription regulators and regulatory RNAs from one cell to another is important in plant development.

QUESTION 20–5

Gap junctions are dynamic structures that, like conventional ion channels, are gated: they can close by a reversible conformational change in response to changes in the cell. The permeability of gap junctions decreases within seconds, for example, when the intracellular Ca^{2+} concentration is raised. Speculate why this form of regulation might be important for the health of a tissue.

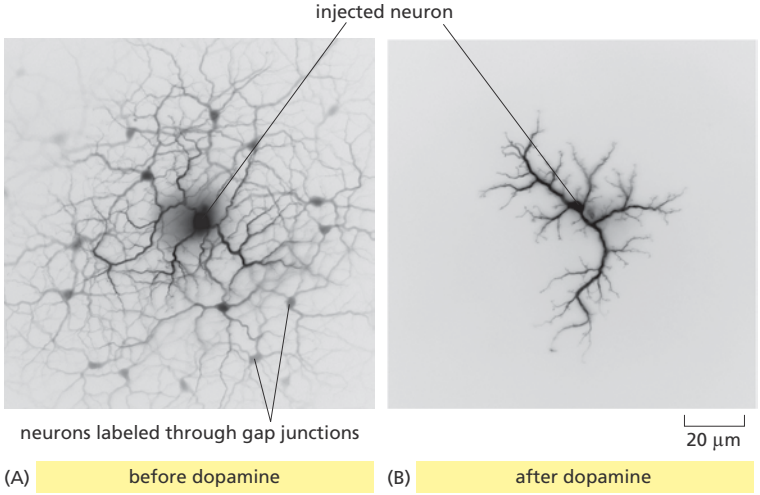
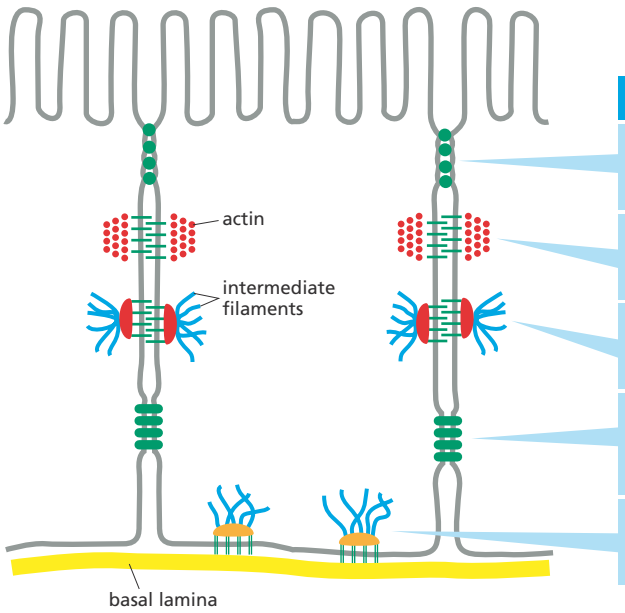


Figure 20–30 Extracellular signals can regulate the permeability of gap junctions. (A) A neuron in a rabbit retina (center) was injected with a dye that passes readily through gap junctions. The dye diffuses rapidly from the injected cell to label the surrounding neurons, which are connected by gap junctions. (B) Treatment of the retina with the neurotransmitter dopamine prior to dye injection decreases the permeability of the gap junctions and hampers the spread of the dye. (Courtesy of David Vaney.)



name	function
tight junction	seals neighboring cells together in an epithelial sheet to prevent leakage of extracellular molecules between them; helps polarize cells
adherens junction	joins an actin bundle in one cell to a similar bundle in a neighboring cell
desmosome	joins the intermediate filaments in one cell to those in a neighbor
gap junction	forms channels that allow small, intracellular, water-soluble molecules, including inorganic ions and metabolites, to pass from cell to cell
hemidesmosome	anchors intermediate filaments in a cell to the basal lamina

Figure 20–31 Several types of cell junctions are found in epithelia in animals. Whereas tight junctions are peculiar to epithelia, the other types also occur, in modified forms, in various nonepithelial tissues.

TISSUE MAINTENANCE AND RENEWAL

One cannot contemplate the organization of tissues without wondering how these astonishingly patterned structures come into being. This question raises an even more challenging one—a puzzle that is one of the most ancient and fundamental in all of biology: how is a complex multicellular organism generated from a single fertilized egg?

In the process of development, the fertilized egg cell divides repeatedly to give a clone of cells—about 10,000,000,000,000 for a human—essentially all containing the same genome but specialized in different ways. This clone has a structure. It may take the form of a daisy or an oak tree, a sea urchin, a whale, or a mouse (**Figure 20–33**). The structure is determined by the genome that the fertilized egg contains. The linear sequence of A, G, C, and T nucleotides in the DNA directs the production of a variety of distinct cell types, each expressing different sets of genes and arranged in a precise, intricate, three-dimensional pattern, which self-assembles during development.

Although the final structure of an animal's body may be enormously complex, it is generated by a limited repertoire of cell activities. Examples of all these activities have been discussed in earlier pages of this book. Cells grow, divide, migrate, and die. They form mechanical attachments and generate forces that bind epithelial sheets. They differentiate by switching on or off the production of specific sets of proteins and regulatory

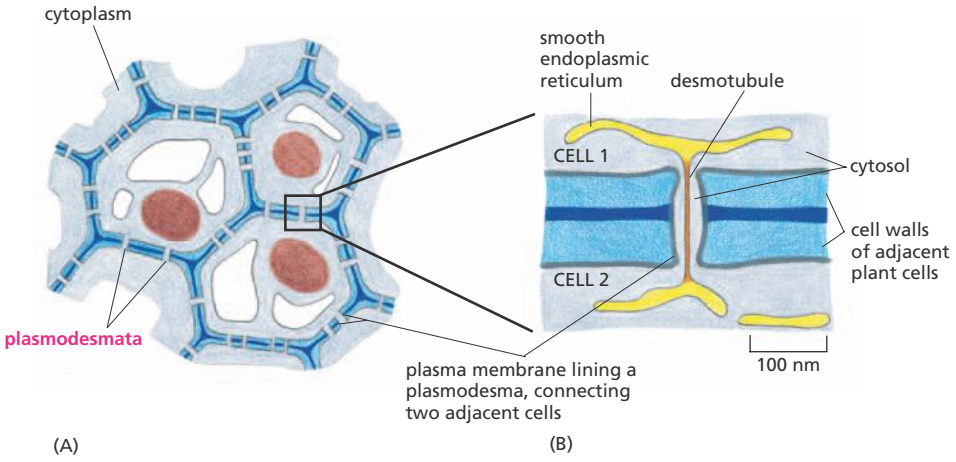


Figure 20–32 Plant cells are connected via plasmodesmata. (A) The cytoplasmic channels of plasmodesmata pierce the plant cell wall and connect the interiors of all cells in a plant. (B) Each plasmodesma is lined with plasma membrane common to the two connected cells. It usually also contains a fine tubular structure, the desmotubule, derived from smooth endoplasmic reticulum.

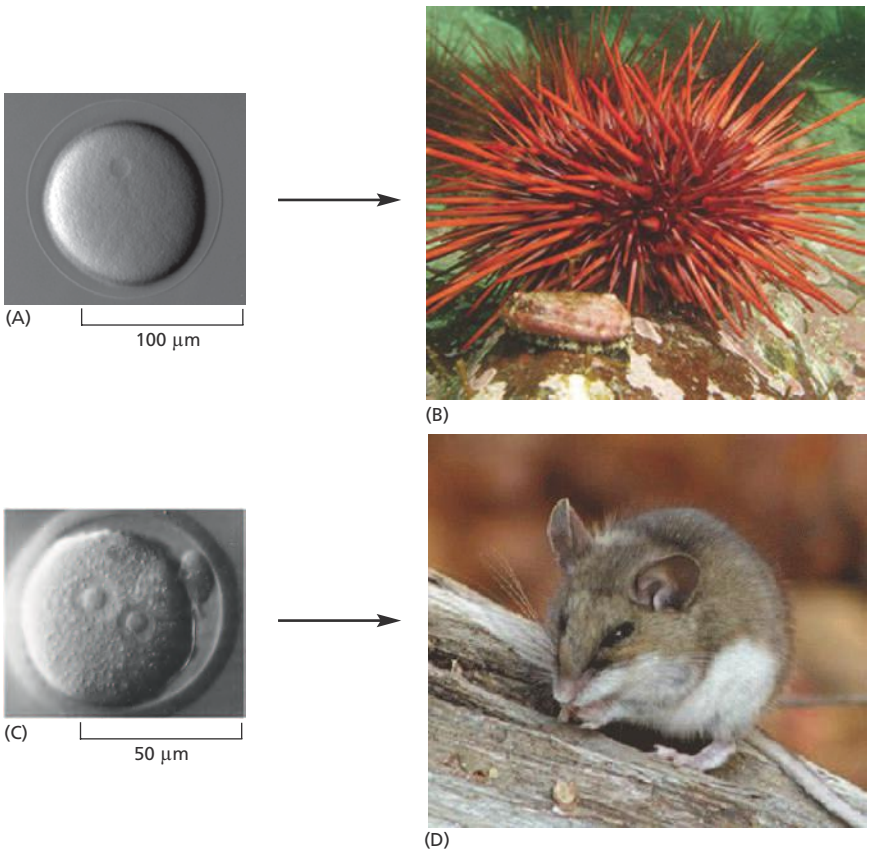


Figure 20–33 The genome of the fertilized egg determines the ultimate structure of the clone of cells that will develop from it. (A and B) A sea-urchin egg gives rise to a sea urchin; (C and D) a mouse egg gives rise to a mouse. (A, courtesy of David McClay; B, courtesy of Alaska Department of Fish and Game; C, courtesy of Patricia Calarco, from G. Martin, *Science* 209:768–776, 1980, with permission from AAAS; D, courtesy of US Department of Agriculture, Agricultural Research Service.)

RNAs. They produce molecular signals to influence neighboring cells, and they respond to signals that neighboring cells deliver to them. They remember the effects of previous signals they have received, and so progressively become more and more specialized in the characteristics they adopt. The genome, identical in virtually every cell, defines the rules by which these various possible cell activities are called into play. Through its operation in each cell individually, the genome guides the whole intricate process by which a multicellular organism is generated from a fertilized egg. **Movies 1.1**, **20.3**, and **20.4** offer some visual examples of how development unfurls for the embryos of a frog, a fruit fly, and a zebrafish, respectively.

For developmental biologists, the challenge is to explain how genes orchestrate the entire sequence of interlocking events that lead from the egg to the adult organism. We will not attempt to set out an answer to this problem here: we do not have space to do it justice, even though a great deal of the genetic and cell biological basis of development is now understood. But the same basic activities that combine to create the organism during development continue even in the adult body, where fresh cells are continually generated in precisely controlled patterns. It is this more limited topic that we discuss in this section, focusing on the organization and maintenance of the tissues of adult vertebrates.

Tissues Are Organized Mixtures of Many Cell Types

Although the specialized tissues in our body differ in many ways, they all have certain basic requirements, usually provided for by a mixture of cell types, as illustrated for the skin in **Figure 20–34**. As discussed earlier, all tissues need mechanical strength, which is often supplied by a supporting framework of connective tissue inhabited by fibroblasts. In this connective tissue, blood vessels lined with endothelial cells satisfy the need for oxygen, nutrients, and waste disposal. Likewise, most tissues

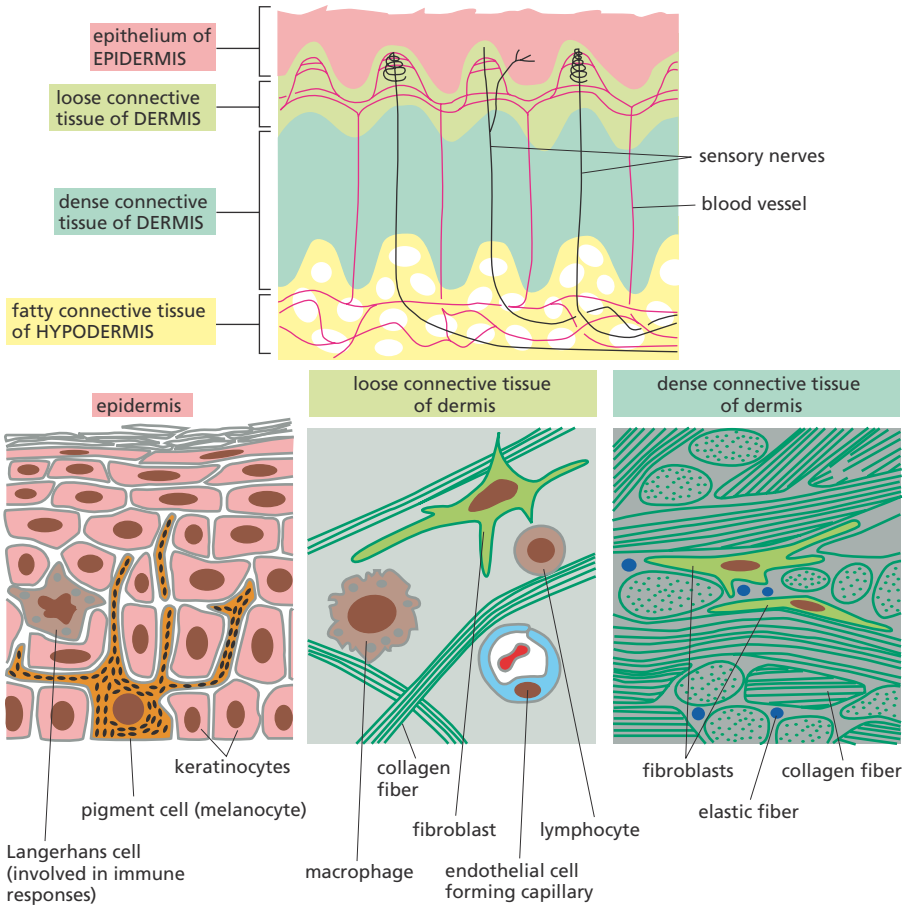


Figure 20–34 Mammalian skin is made of a mixture of cell types. Schematic diagrams showing the cellular architecture of the main layers of thick skin. Skin can be viewed as a large organ composed of two main tissues: epithelial tissue (the *epidermis*) on the outside, and connective tissue on the inside. The outermost layer of the epidermis consists of flat dead cells, whose intracellular organelles have disappeared (see Figure 20–37). The connective tissue consists of the tough *dermis* (from which leather is made) and the underlying fatty *hypodermis*. The dermis and hypodermis are richly supplied with blood vessels and nerves; some of the nerves extend into the epidermis, as shown.

are innervated by nerve-cell axons, which are ensheathed by Schwann cells that can wrap around the axons to provide electrical insulation. Macrophages dispose of dead and damaged cells and other unwanted debris, and lymphocytes and other white blood cells combat infection. Most of these cell types originate outside the tissue and invade it, either early in the course of its development (endothelial cells, nerve-cell axons, and Schwann cells) or continually during life (macrophages and other white blood cells).

A similar supporting apparatus is required to maintain the principal specialized cells of many tissues: the contractile cells of muscle, the secretory cells of glands, or the blood-forming cells of bone marrow, for example. Almost every tissue is therefore an intricate mixture of many cell types that must remain different from one another while coexisting in the same environment. Moreover, in almost all adult tissues, cells are continually dying and being replaced; throughout this hurly-burly of cell replacement and tissue renewal, the organization of the tissue must be preserved.

Three main factors contribute to this stability.

1. *Cell communication*: each type of specialized cell continually monitors its environment for signals from other cells and adjusts its behavior accordingly; in fact, the very survival of most cells depends on such social signals (discussed in Chapter 16). This communication ensures that new cells are produced and survive only when and where they are required.
2. *Selective cell adhesion*: because different cell types have different cadherins and other cell adhesion molecules in their plasma membrane, they tend to stick selectively, by homophilic binding, to other cells of the same type. They may also form selective attachments to certain other cell types and to specific extracellular matrix components. The selectivity of these cell adhesions prevents the different cell types in a tissue from becoming chaotically mixed.

3. *Cell memory*: as discussed in Chapter 8, specialized patterns of gene expression, evoked by signals that acted during embryonic development, are afterward stably maintained, so that cells autonomously preserve their distinctive character and pass it on to their progeny. A fibroblast divides to produce more fibroblasts, an endothelial cell divides to produce more endothelial cells, and so on.

Different Tissues Are Renewed at Different Rates

Tissues vary enormously in their rate and pattern of *cell turnover*. At one extreme is nervous tissue, in which most of the nerve cells last a lifetime without replacement. At the other extreme is the intestinal epithelium, in which cells are replaced every three to six days. Between these extremes there is a spectrum of different rates and styles of cell turnover and tissue renewal. Bone (see Figure 20–8) has a turnover time of about ten years in humans, involving renewal of the matrix as well as of cells: old bone matrix is slowly eaten away by a set of cells called *osteoclasts*, akin to macrophages, while new matrix is deposited by another set of cells, *osteoblasts*, akin to fibroblasts. New red blood cells in humans are generated continually by blood-forming precursor cells in the bone marrow; they are released into the bloodstream, where they recirculate continually for about 120 days before being removed and destroyed in the liver and spleen. In the skin, the outer layers of the epidermis are continually flaking off and being replaced from below, so that the epidermis is renewed with a turnover time of about two months. And so on.

Our life depends on these renewal processes. A large dose of ionizing radiation blocks cell division and thus halts renewal: within a few days, the lining of the intestine, for example, becomes denuded of cells, leading to the devastating diarrhea and water loss characteristic of acute radiation sickness.

Clearly, there have to be elaborate control mechanisms to keep cell production and cell loss in balance in the normal, healthy adult body. Cancers originate through violation of these controls, allowing cells in the self-renewing tissues to survive and proliferate to excess. To understand cancer, therefore, it is important to understand the normal social controls on cell turnover that cancer perverts.

Stem Cells Generate a Continuous Supply of Terminally Differentiated Cells

Most of the specialized, or **differentiated**, cells that need continual replacement are themselves unable to divide. Red blood cells, the epidermal cells on the skin surface, and the absorptive and goblet cells of the gut epithelium are all examples of this type. Such cells are referred to as *terminally differentiated*: they lie at the dead end of their developmental pathway.

The cells that replace the terminally differentiated cells that are lost are generated from a stock of proliferating *precursor cells*, which themselves usually derive from a much smaller number of self-renewing **stem cells**. Both stem cells and proliferating precursor cells are retained in the corresponding tissues along with the differentiated cells. Stem cells are not differentiated and can divide without limit (or at least for the lifetime of the animal). When a stem cell divides, though, each daughter has a choice: either it can remain a stem cell, or it can embark on a course leading to terminal differentiation, usually via a series of precursor cell divisions (**Figure 20–35**). The job of the stem cells and precursor cells, therefore, is not to carry out the specialized function of the differentiated cells, but

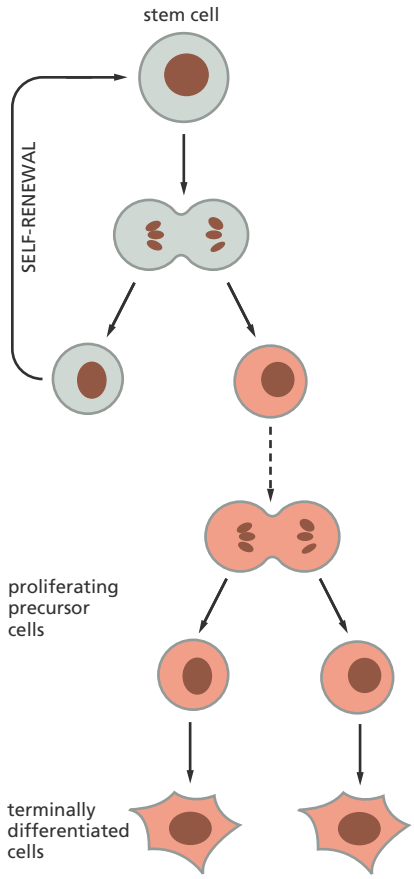


Figure 20–35 When a stem cell divides, each daughter can either remain a stem cell or go on to become terminally differentiated. The terminally differentiated cells usually develop from precursor cells that divide a limited number of times before they terminally differentiate. Stem-cell divisions can also produce two stem cells or two precursor cells, as long as the pool of stem cells is maintained.

QUESTION 20–6

Why does ionizing radiation stop cell division?

rather to produce cells that will. Stem cells are usually present in small numbers and often have a nondescript appearance, making them difficult to identify. Although stem cells and precursor cells are not differentiated, they are nonetheless developmentally restricted: under normal conditions, they stably express sets of transcription regulators that ensure that their differentiated progeny will be of the appropriate cell types.

The pattern of cell replacement varies from one stem-cell-based tissue to another. In the lining of the small intestine, for example, the absorptive and secretory cells are arranged as a single-layered, simple epithelium covering the surfaces of the fingerlike villi that project into the gut lumen. This epithelium is continuous with the epithelium lining the *crypts*, which descend into the underlying connective tissue (**Figure 20–36A**). The stem cells lie near the bottom of the crypts, where they give rise mostly to proliferating precursor cells, which move upward in the plane of the epithelial sheet. As they move upward, the precursor cells terminally differentiate into absorptive or secretory cells, which are shed into the gut lumen and die when they reach the tips of the villi (**Figure 20–36B**).

A contrasting example is the epidermis, a stratified epithelium. In the epidermis, proliferating stem cells and precursor cells are confined to the basal layer, adhering to the basal lamina. The differentiating cells travel

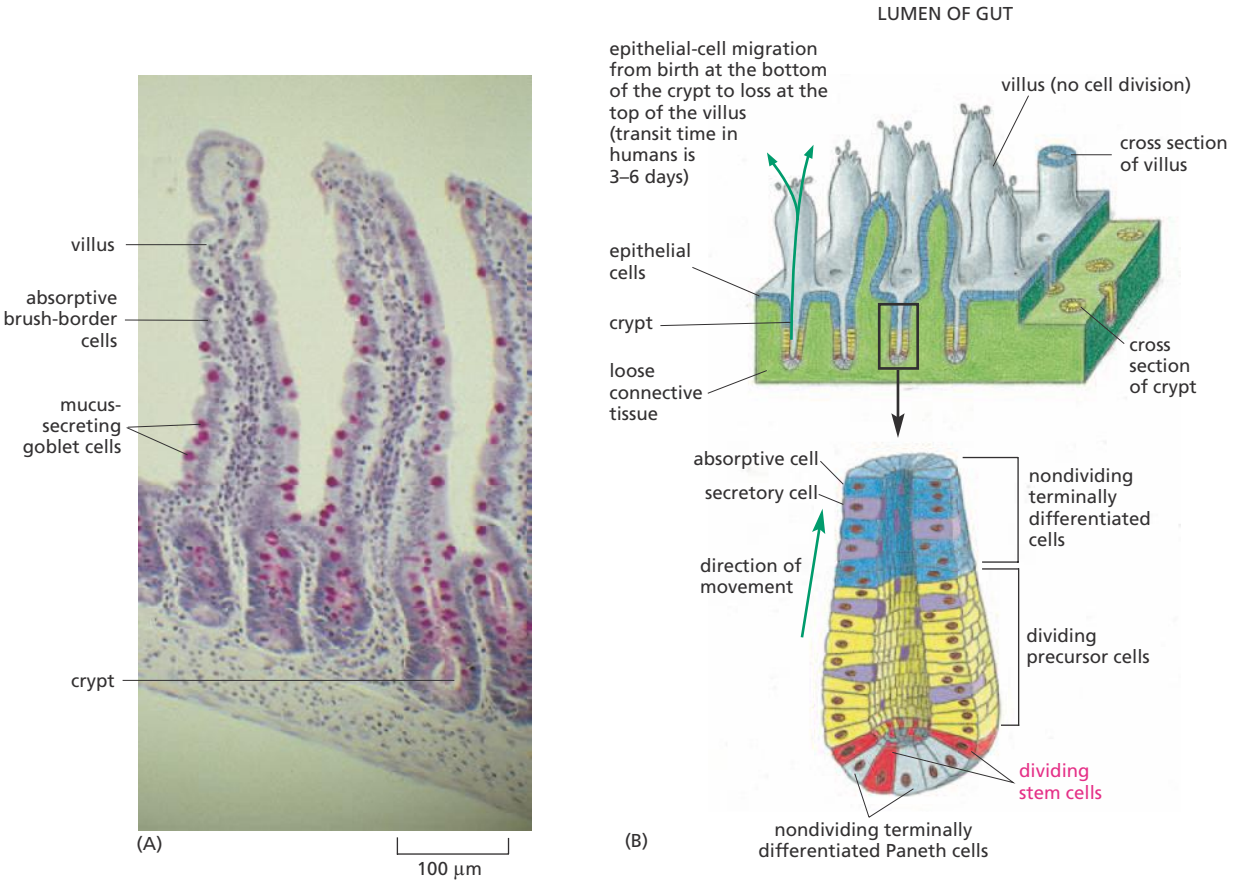


Figure 20–36 Renewal occurs continuously in the epithelial lining of the adult mammalian intestine. (A) Micrograph of a section of part of the lining of the small intestine, showing the villi and crypts. Mucus-secreting goblet cells (stained *purple*) are interspersed among the absorptive brush-border cells in the epithelium covering the villi. Smaller numbers of two other secretory cell types—enteroendocrine cells (not visible here), which secrete gut hormones, and Paneth cells, which secrete antibacterial proteins—are also present and derive from the same stem cells. (B) Cartoon showing the pattern of cell turnover and the proliferation of stem cells and precursor cells. The stem cells give rise mainly to proliferating precursor cells that slide continuously upward and terminally differentiate into secretory or absorptive cells, which are shed from the tip of the villus. The stem cells also give rise directly to terminally differentiated Paneth cells, which remain at the bottom of the crypt.

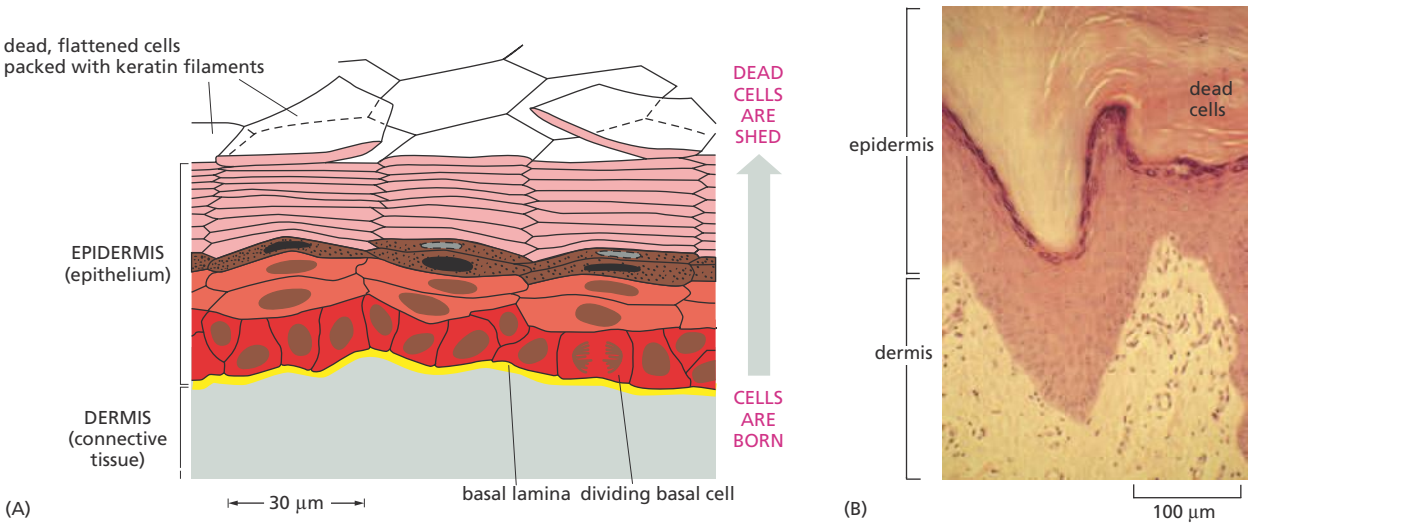


Figure 20–37 The epidermis of the skin is renewed from stem cells in its basal layer. (A) The basal layer contains a mixture of stem cells and dividing precursor cells that are produced from the stem cells. On emerging from the basal layer, the precursor cells stop dividing and move outward, differentiating as they go. Eventually, the cells undergo a special form of cell death: the nucleus and other organelles disintegrate, and the cell shrinks to the form of a flattened scale, packed with keratin filaments. The scales are ultimately shed from the skin surface. (B) Light micrograph of a cross section through the sole of a human foot, stained with hematoxylin and eosin.

outward from their site of origin in a direction perpendicular to the plane of the cell sheet; terminally differentiated cells and their corpses are eventually shed from the skin surface (**Figure 20–37**).

Often, a single type of stem cell gives rise to several types of differentiated progeny: the stem cells of the intestine, for example, produce absorptive cells, goblet cells, and several other secretory cell types. The process of blood-cell formation, or *hemopoiesis*, provides an extreme example of this phenomenon. All of the different cell types in the blood—both the red blood cells that carry oxygen and the many types of white blood cells that fight infection (**Figure 20–38**)—ultimately derive from a shared *hemopoietic stem cell* found in the bone marrow (**Figure 20–39**).

Specific Signals Maintain Stem-Cell Populations

Every stem-cell system requires control mechanisms to ensure that new cells are generated in the appropriate places and in the right numbers. The controls depend on extracellular signals exchanged between the stem cells, their progeny, and other cell types in the area. These signals, and the intracellular signaling pathways they activate, fall into a surprisingly small number of families, corresponding to half-a-dozen basic signaling mechanisms, some of which are discussed in Chapter 16. These few mechanisms are used again and again—in different combinations, evoking different responses in different contexts, in both the embryo and the adult.

Almost all these signaling mechanisms contribute to the task of maintaining the complex organization of a stem-cell system such as that of the intestine. Thus, a class of signal molecules known as the **Wnt proteins** serves to promote the proliferation of the stem cells and precursor cells at the base of each intestinal crypt (**Figure 20–40**). Cells in the crypt produce, in addition, other signals that act at longer range to prevent activation of the Wnt pathway outside the crypts. They also exchange yet other signals to control their diversification, so that some differentiate into secretory cells while others become absorptive cells.

QUESTION 20–7

Why do you suppose epithelial cells lining the gut are renewed frequently, whereas most neurons last for the lifetime of the organism?

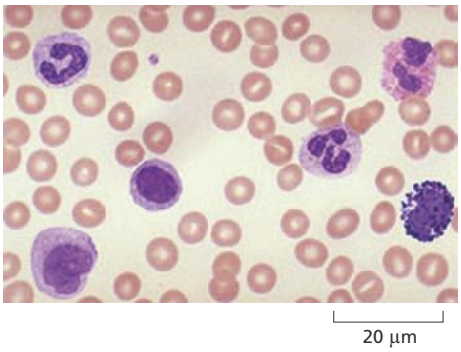


Figure 20–38 Blood contains many circulating cell types, all derived from a single type of stem cell. A sample of blood is smeared onto a glass coverslip, chemically fixed (see Panel 1–1, p.10), and stained with hematoxylin, which reacts with nucleic acids. Microscopic examination reveals numerous small erythrocytes (red blood cells), which lack DNA. Larger, purple-stained cells are different types of white blood cell: lymphocytes, eosinophils, basophils, neutrophils, and monocytes. Blood smears of this kind are routinely used as a clinical test in hospitals. (Courtesy of Peter Takizawa.)

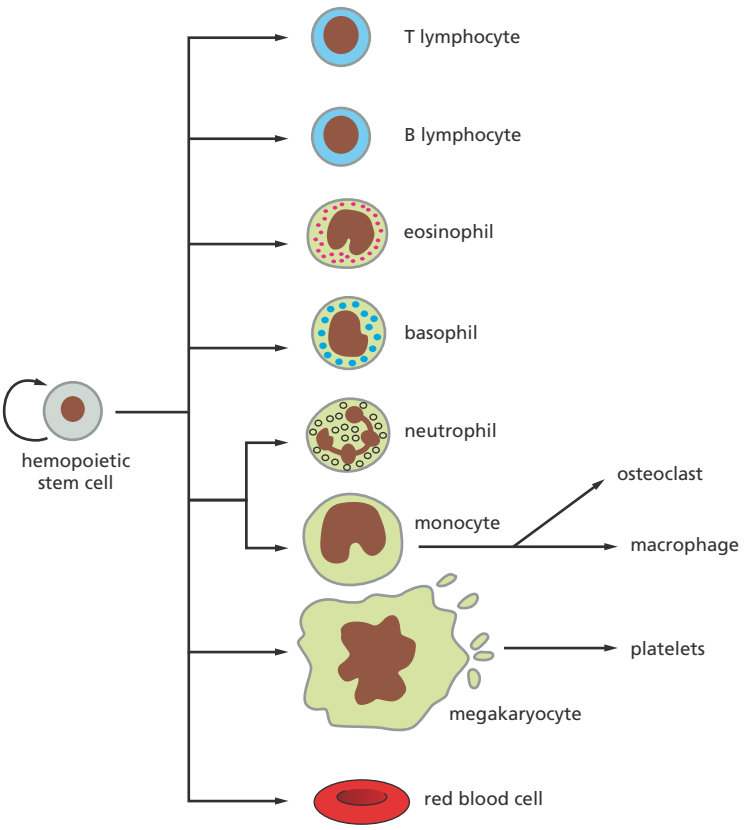


Figure 20–39 A hemopoietic stem cell divides to generate more stem cells, as well as precursor cells (not shown) that proliferate and differentiate into the mature blood cell types found in the circulation. The macrophages found in many tissues of the body and the osteoclasts that eat away bone matrix originate from the same precursor cells, as do a number of other cell types not shown in this scheme. Megakaryocytes give rise to blood platelets by shedding cell fragments (Movie 20.5). A large number of extracellular signal molecules are known to act at various points in this cell lineage to control the production of each cell type and to maintain appropriate numbers of precursor cells and stem cells.

Disorders of these signaling mechanisms disrupt the structure of the gut lining. In particular, as we see later, defects in the regulation of Wnt signaling underlie the commonest forms of human intestinal cancer.

Stem Cells Can Be Used to Repair Lost or Damaged Tissues

Because stem cells can proliferate indefinitely and produce progeny that differentiate, they provide for both continual renewal of normal tissue and repair of tissue lost through injury. For example, by transfusing a few hemopoietic stem cells into a mouse whose own blood stem cells have been destroyed by irradiation, it is possible to fully repopulate the animal with new blood cells and ultimately rescue it from death by anemia, infection, or both. A similar approach is used in the treatment of human leukemia with irradiation (or cytotoxic drugs) followed by bone marrow transplantation.

Although stem cells taken directly from adult tissues such as bone marrow have already proven their clinical value, another type of stem cell, first identified through experiments in mice, may have even greater

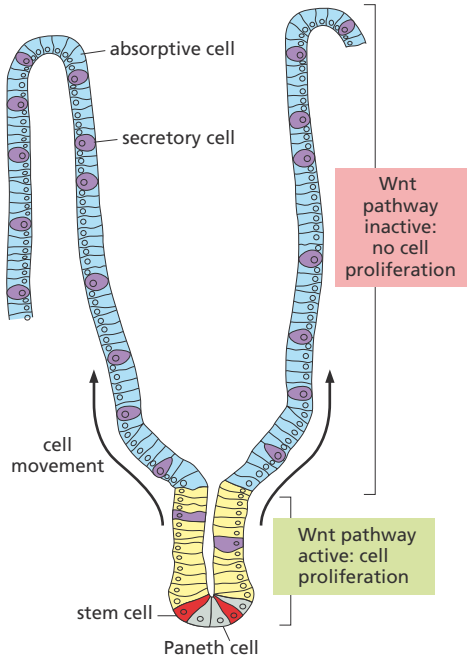


Figure 20–40 The Wnt signaling pathway helps to control the production of differentiated cells from stem cells in the intestinal crypt. Wnt signaling maintains proliferation in the crypt. The Wnt proteins are secreted by cells in and around the crypt base, especially the Paneth cells—a subclass of terminally differentiated secretory cells that are generated from the gut stem cells, but that move down to the crypt bottom instead of up to the tip of the villus. Paneth cells have a dual function: they secrete antimicrobial peptides to keep infection at bay, and at the same time they provide the signals to sustain the stem-cell population from which they themselves derive.

potential—both for treating and understanding human disease. It is possible, through cell culture, to derive from early mouse embryos an extraordinary class of stem cells called **embryonic stem cells**, or **ES cells**. Under appropriate conditions, these cells can be kept proliferating indefinitely in culture and yet retain unrestricted developmental potential and are thus said to be **pluripotent**: if the cells from the culture dish are put back into an early embryo, they can give rise to all the tissues and cell types in the body, including germ cells. Their descendants in the embryo are able to integrate perfectly into whatever site they come to occupy, adopting the character and behavior that normal cells would show at that site. These cells can also be induced to differentiate in culture into a large variety of cell types (**Figure 20–41**).

Cells with properties similar to those of mouse ES cells can now be derived from early human embryos, creating a potentially inexhaustible supply of cells that might be used for the replacement or repair of mature human tissues that are damaged. For example, experiments in mice suggest that it should be possible to use ES cells to replace the skeletal muscle fibers that degenerate in victims of muscular dystrophy, the nerve cells that die in patients with Parkinson's disease, the insulin-secreting cells that are destroyed in type 1 diabetics, and the cardiac muscle cells that die during a heart attack. Perhaps one day it might even become possible to grow entire organs from ES cells by a recapitulation of embryonic development (**Figure 20–42**).

There are, however, many hurdles to be cleared before such dreams can become reality. One major problem concerns immune rejection: if the transplanted cells are genetically different from the cells of the patient into whom they are grafted, they are likely to be rejected and destroyed by the immune system. Beyond the practical scientific difficulties, there have been ethical concerns about the use of human embryos and the purposes to which human ES cells might be put. One anxiety, for example, has centered on the possibility of using ES cells for human "cloning." But what exactly does this mean?

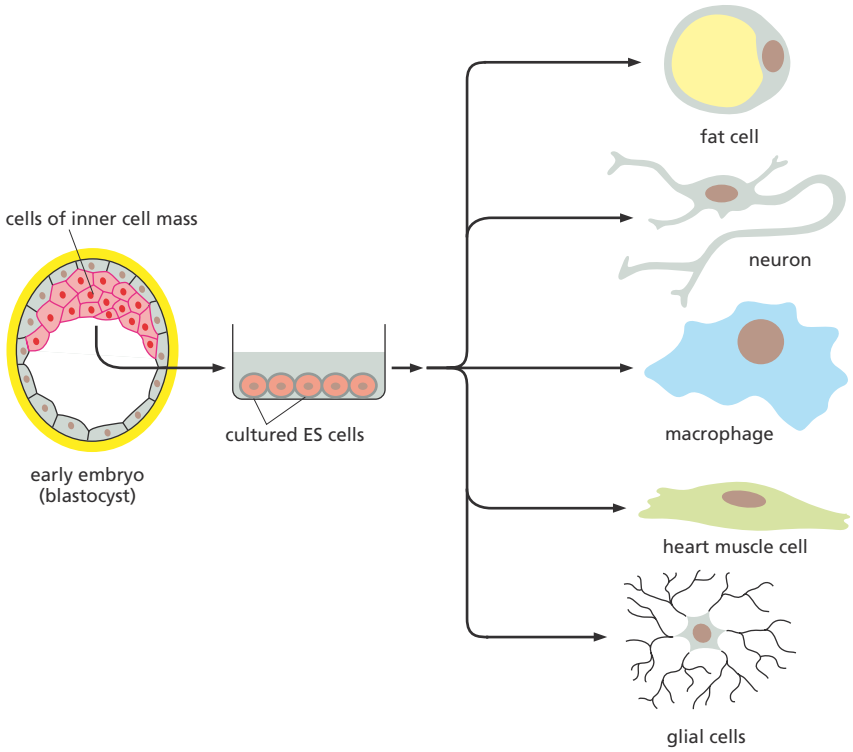


Figure 20–41 ES cells derived from an embryo can give rise to all of the tissues and cell types of the body. ES cells are harvested from the inner cell mass of an early embryo and can be maintained indefinitely as pluripotent stem cells in culture. If they are put back into an embryo, they will integrate perfectly and differentiate to suit whatever environment they are placed in. Alternatively, these cells can be induced to differentiate into specific cell types in culture when provided with the appropriate extracellular signal molecules (Movie 20.6). (Based on data from E. Fuchs and J.A. Segré, *Cell* 100:143–155, 2000. With permission from Elsevier.)

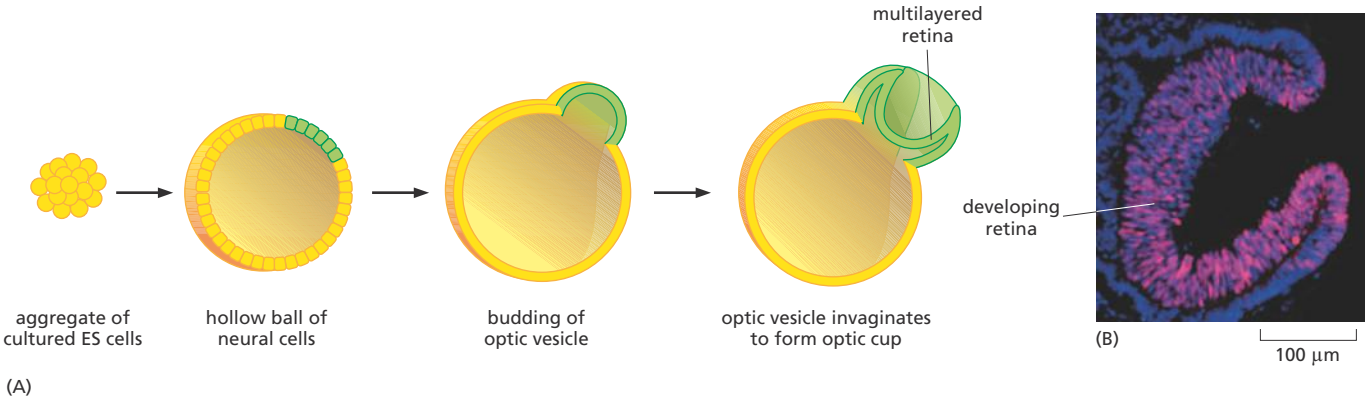


Figure 20–42 Cultured ES cells can give rise to a three-dimensional organ. (A) Remarkably, under appropriate conditions, mouse ES cells in culture can proliferate, differentiate, and interact to form a three-dimensional, eye-like structure, which includes a multilayered retina similar in organization to the one that forms *in vivo*. (B) Fluorescent micrograph of an optic cup formed by ES cells in culture. The structure includes a developing retina, containing multiple layers of neural cells, which produce a protein (*pink*) that serves as a marker for retinal tissue. (A, Adapted from M. Eiraku and Y. Sasai, *Curr. Opin. Neurobiol.* 22: 768–777, 2012; B, from M. Eiraku et al., *Nature* 472:51–56, 2011. With permission from Macmillan Publishers Ltd.)

Therapeutic Cloning and Reproductive Cloning Are Very Different Enterprises

The term "cloning" has been used in confusing ways as a shorthand term for several quite distinct types of procedure, particularly in public debates about the ethics of stem-cell research. It is important to understand the distinctions.

As biologists define the term, a clone is simply a set of cells or individuals that are essentially genetically identical, by virtue of their descent from a single ancestor cell. The simplest type of cloning is the cloning of cells in a culture dish. For instance, one can take a single epidermal stem cell from the skin and let it proliferate in culture to obtain a large clone of genetically identical epidermal cells. Such cells could be used to help reconstruct the skin of a badly burned patient. This kind of cloning is no more than an extension by artificial means of the processes of cell proliferation and differentiation that occur in a normal human body.

The cloning of entire multicellular animals, called **reproductive cloning**, is a very different enterprise, involving a far more radical departure from the ordinary course of nature. As we discuss in Chapter 19, each individual animal normally has both a mother and a father and is not genetically identical to either of them. In reproductive cloning, the need for two parents and sexual union is bypassed. For mammals, this difficult feat has been achieved in mice and sheep and a variety of other domestic animals by a technique called *nuclear transplantation*. The procedure begins with an unfertilized egg cell. The nucleus of this haploid gamete is sucked out or destroyed, and in its place a nucleus from a regular diploid cell is introduced. The diploid donor cell can, for example, be taken from a tissue of an adult individual. The hybrid cell, consisting of a diploid donor nucleus in a host egg cytoplasm, is allowed to develop for a few days in culture. In a small proportion of cases, this cell will give rise to an early embryo (a blastocyst) containing about 200 cells, which is then transferred into the uterus of a foster mother (**Figure 20–43**). If the experimenter is lucky, development continues as it would in a normal embryo, eventually giving rise to a whole new animal. An individual produced in this way, should be genetically identical to the adult individual who donated the diploid cell (except for the small amount of genetic information in mitochondria, which are inherited with the egg cytoplasm).

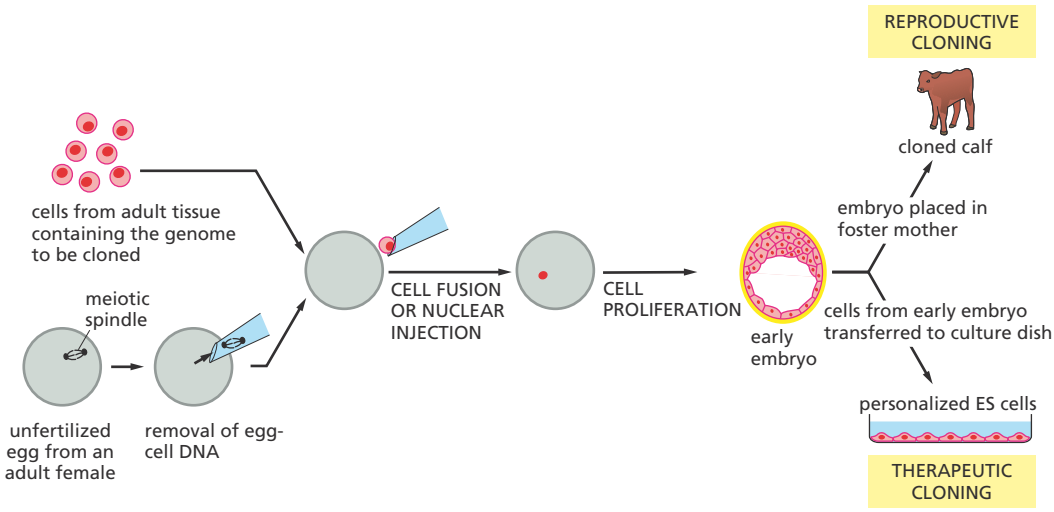


Figure 20–43 Nuclear transplantation can be used for "cloning" in two quite different senses of the word. In reproductive cloning, a whole new multicellular individual is generated; in therapeutic cloning, only cells (personalized ES cells) are produced. Both procedures begin with nuclear transplantation, in which a nucleus taken from an adult cell is transferred into the cytoplasm of an enucleated egg cell, so as to create a cell that has an embryonic character but carries the genes of the adult cell.

A different procedure, called **therapeutic cloning**, uses the technique of nuclear transplantation to produce cultured ES cells, rather than a cloned animal (see Figure 20–43). This approach is an elaborate method for generating *personalized ES cells*, with the aim of generating various cell types that can be used for tissue repair or to study disease mechanisms. Because the cells obtained are genetically almost identical to the original donor cell, they can be grafted back into the adult from whom the donor nucleus was taken, thereby minimizing immunological rejection. Nuclear transplantation, however, is technically very difficult, and it has only recently been possible to use it to produce personalized human ES cells. Moreover, the procedure requires a supply of human egg cells, which raises ethical issues. Indeed, nuclear transplantation into human egg cells is outlawed in some countries.

Induced Pluripotent Stem Cells Provide a Convenient Source of Human ES-like Cells

The problems associated with making personalized ES cells by nuclear transplantation can now be bypassed by an alternative approach, in which cells are taken from an adult tissue, grown in culture, and reprogrammed into an ES-like state by artificially driving the expression of a set of three transcription regulators called Oct3/4, Sox2, and Klf4. This treatment is sufficient to convert fibroblasts into cells with practically all the properties of ES cells, including the ability to proliferate indefinitely and differentiate in diverse ways and to contribute to any tissue (**Figure 20–44**). These ES-like cells are called **induced pluripotent stem cells** (**iPS cells**). The conversion rate is low, however—only a tiny proportion of the fibroblasts make the switch—and there are serious worries about the safety of implanting into humans derivatives of cells with such an abnormal developmental history. Much work remains to be done to allow this approach to be used to treat human diseases.

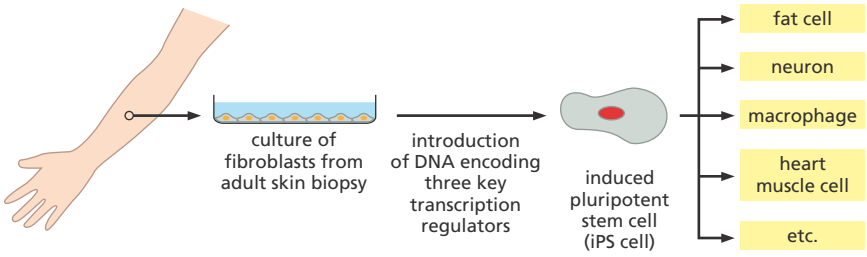


Figure 20–44 Induced pluripotent stem cells (iPS cells) can be generated by transformation of cultured cells isolated from adult tissues. In the example shown, genes that encode several transcription regulators normally expressed in ES cells are introduced into cultured fibroblasts using genetically manipulated viruses as vectors. After a few weeks in culture, a small proportion of the fibroblasts have transformed into cells that look and behave like ES cells and have the same ability as ES cells to differentiate into any of the cell types in the body.

Meanwhile, however, human ES cells and especially human iPS cells are proving to be valuable in other ways. They can be used to generate large, homogeneous populations of differentiated human cells of a specific type in culture; these can be used to test for potential toxic or beneficial effects of candidate drugs on specific human cell types. Moreover, it is possible to create iPS cells containing the genomes of patients who suffer from a genetic disease, and to use these patient-specific stem cells to study the disease mechanism and to search for drugs that might be useful in the treatment of that disease. An example is Timothy syndrome, a rare genetic disease caused by mutations in a gene that encodes a specific type of Ca^{2+} channel. The defective channel fails to close properly after opening, leading to abnormalities in heart rhythm and, in some individuals, to autism. The iPS cells produced from such individuals have been coaxed to differentiate in culture into neurons and heart muscle cells, which are now being used to study the physiological consequences of the Ca^{2+} channel abnormality and to hunt for drugs that can correct the defects.

In addition, experiments on the pluripotent stem cells themselves are providing insights into some of the many unsolved mysteries of developmental and stem-cell biology, including the mechanisms that make the specialized characters of most cells in adult tissues so remarkably stable under normal circumstances.

CANCER

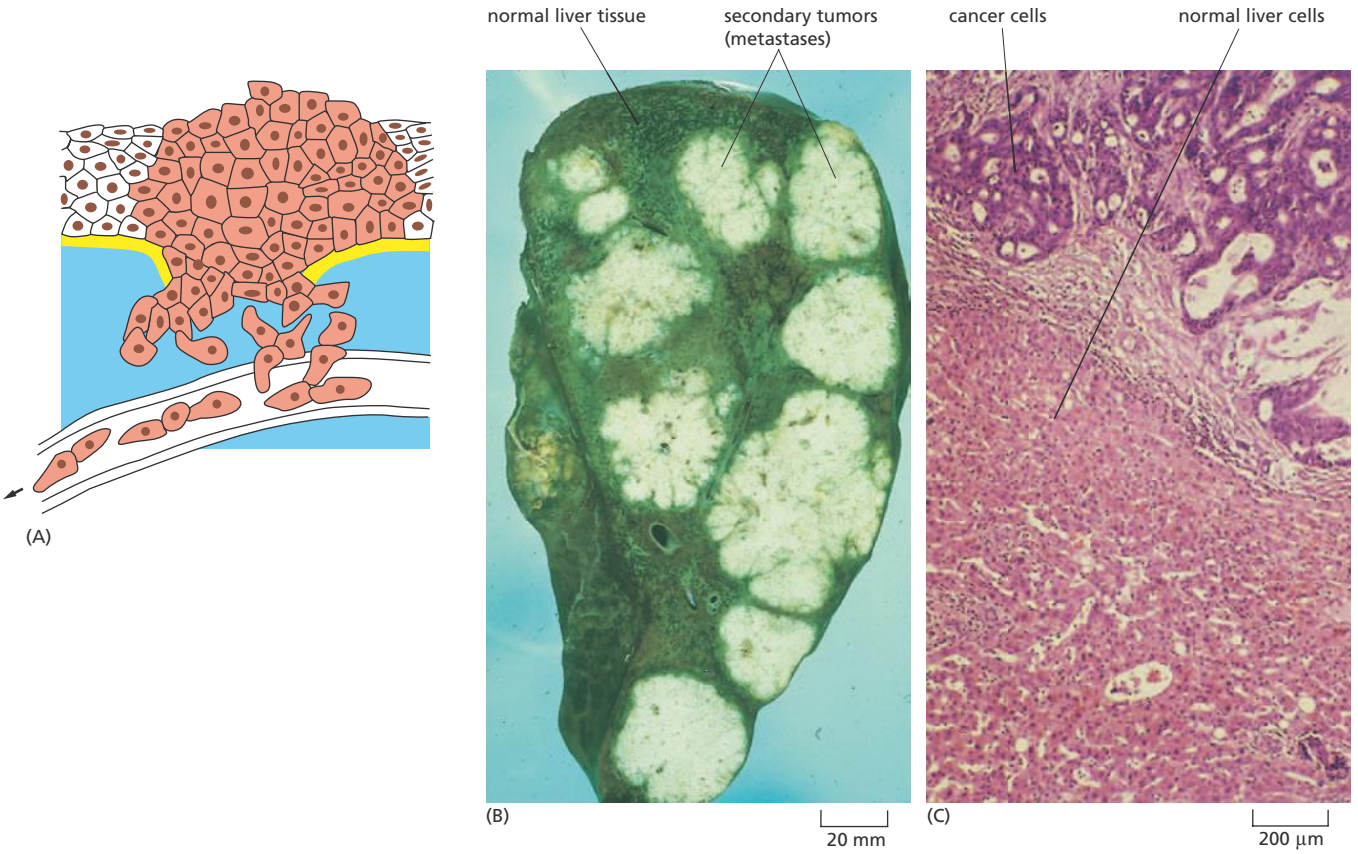
Humans pay a price for having tissues that can renew and repair themselves. The delicately adjusted mechanisms that control these processes can go wrong, leading to catastrophic disruption of tissue structure. Foremost among the diseases of tissue renewal is **cancer**, which stands alongside infectious illness, malnutrition, war, and heart disease as a major cause of death in human populations. In Europe and North America, for example, one in five of us will die of cancer.

Cancer arises from violations of the basic rules of social cell behavior. To make sense of the origins and progression of the disease, and to devise treatments, we have to draw upon almost every part of our knowledge of how cells work and interact in tissues. Conversely, much of what we know about cell and tissue biology has been discovered as a by-product of cancer research. In this section, we examine the causes and mechanisms of cancer, the types of cell misbehavior that contribute to its progress, and the ways in which we hope to use our understanding to defeat these misbehaving cells and, hence, the disease. Although there are many types of cancer, each with distinct properties, we will refer to them collectively by the umbrella term "cancer," as they are united by certain common principles.

Cancer Cells Proliferate, Invade, and Metastasize

As tissues grow and renew themselves, each individual cell must adjust its behavior according to the needs of the organism as a whole. The cell must divide only when new cells of that type are needed, and refrain from dividing when they are not; it must live as long as it is needed, and kill itself when it is not; it must maintain its specialized character; and it must occupy its proper place and not stray into inappropriate territories.

In a large organism, no significant harm is done if an occasional single cell misbehaves. But a potentially devastating breakdown of order occurs when a single cell suffers a genetic alteration that allows it to survive and divide when it should not, producing daughter cells that behave in the same antisocial way. Such a relentlessly expanding clone of abnormal



cells can disrupt the organization of the tissue, and eventually that of the body as a whole. It is this catastrophe that happens in cancer.

Cancer cells are defined by two heritable properties: they and their progeny (1) proliferate in defiance of the normal constraints and (2) invade and colonize territories normally reserved for other cells (**Movie 20.7**). It is the combination of these socially deviant features that creates the lethal danger. Cells that have the first property but not the second proliferate excessively but remain clustered together in a single mass, forming a tumor. But the tumor in this case is said to be *benign*, and it can usually be removed cleanly and completely by surgery. A tumor is cancerous only if its cells have the ability to invade surrounding tissue, in which case the tumor is said to be *malignant*. Malignant tumor cells with this invasive property often break loose from the primary tumor and enter the bloodstream or lymphatic vessels, where they form secondary tumors, or **metastases**, at other sites in the body (**Figure 20–45**). The more widely the cancer spreads, the harder it is to eradicate.

Figure 20–45 Cancers invade surrounding tissues and often metastasize to distant sites. (A) To give rise to a colony in a new site—called a secondary tumor or metastasis—the cells of a primary tumor in an epithelium must typically cross the basal lamina, migrate through connective tissue, and get into either blood or lymphatic vessels. They then have to exit from the bloodstream or lymph and settle, survive, and proliferate in a new location. (B) Secondary tumors in a human liver, originating from a primary tumor in the colon. (C) Higher-magnification view of one of the secondary tumors, stained differently to show the contrast between the normal liver cells and the cancer cells. (B and C, courtesy of Peter Isaacson.)

Epidemiological Studies Identify Preventable Causes of Cancer

Prevention is always better than cure, but to prevent cancer we need to know what causes it. Do factors in our environment or features of our way of life trigger the disease and help it to progress? If so, what are they? Answers to these questions come mainly from *epidemiology*—the statistical analysis of human populations, looking for factors that correlate with disease incidence. This approach has provided strong evidence that the environment plays an important part in the causation of most cases of cancer. The types of cancers that are common, for example, vary from country to country, and studies of migrants show that it is usually where people live, rather than where they were born, that governs their cancer risk.

Although it is still hard to discover which specific factors in the environment or lifestyle are significant, and many remain unknown, some have been precisely identified. For example, it was noted long ago that cervical cancer, which arises in the epithelium lining the cervix (neck) of the uterus, was much more common in women who were sexually experienced than in those who were not, suggesting a cause related to sexual activity. We now know, through modern epidemiological studies, that most cases of cervical cancer depend on infection of the cervical epithelium with certain subtypes of a common virus, called *human papillomavirus*. This virus is transmitted through sexual intercourse and can sometimes, if one is unlucky, provoke uncontrolled proliferation of the infected cells. Knowing this, we can attempt to prevent the cancer by preventing the infection—for example, by vaccination against papillomavirus. Such a vaccine is now available, conferring a high level of protection if given to young people before they become sexually active.

In the great majority of human cancers, however, viruses do not appear to play a part: as we will see, cancer is not an infectious disease. But epidemiology reveals that other factors increase the risk of cancer. Obesity is one such factor. Smoking tobacco is another: tobacco smoke is not only responsible for almost all cases of lung cancer, but it also raises the incidence of several other cancers, such as those of the bladder. By stopping the use of tobacco, we could prevent about 30% of all cancer deaths. No other single policy or treatment is known that would have such a dramatic impact on the cancer death rate.

As we will explain, although environmental factors affect the incidence of cancer and are critical for some forms of the disease, it would be wrong to conclude that they are the only cause of cancers. No matter how hard we try to prevent cancer by healthy living, we will never be able to eradicate this disease. To devise effective treatments, we need to derive a deep understanding of the biology of cancer cells and the mechanisms that underlie the growth and spread of tumors.

Cancers Develop by an Accumulation of Mutations

Cancer is fundamentally a genetic disease: it arises as a consequence of pathological changes in the information carried by DNA. It differs from other genetic diseases in that the mutations underlying cancer are mainly somatic mutations—those that occur in individual somatic cells of the body—as opposed to germ-line mutations, which are handed down via the germ cells from which the entire multicellular organism develops.

Most of the identified agents known to contribute to the causation of cancer, including ionizing radiation and most chemical carcinogens, are mutagens: they cause changes in the nucleotide sequence of DNA. But even in an environment that is free of tobacco smoke, radioactivity, and all the other external mutagens that worry us, mutations will occur spontaneously as a result of fundamental limitations on the accuracy of DNA replication and DNA repair (discussed in Chapter 6). In fact, environmental carcinogens other than tobacco smoke probably account for only a small fraction of the mutations responsible for cancer, and elimination of all these external risk factors would still leave us prone to the disease.

Although DNA is replicated and repaired with great accuracy, an average of one mistake slips by for every 10^9 or 10^{10} nucleotides copied, as we discuss in Chapter 6. This means that spontaneous mutations occur at an estimated rate of about 10^{-6} or 10^{-7} mutations per gene per cell division, even without encouragement by external mutagens. About 10^{16} cell divisions take place in a human body in the course of an average lifetime; thus, every single gene is likely to have acquired a mutation on more

than 10^9 separate occasions in any individual. From this point of view, the problem of cancer seems to be not why it occurs, but why it occurs so infrequently.

The explanation is that it takes more than a single mutation to turn a normal cell into a cancer cell. Precisely how many are required is still a matter of debate, but for most full blown cancers it could be at least 10—and, as we will see, they have to affect the right type of gene. These mutations do not all occur at once, but sequentially, usually over a period of many years.

Cancer, therefore, is most often a disease of old age, because it takes a long time for an individual clone of cells—those derived from a common founder—to accumulate a large number of mutations (see Figure 6–32). In fact, most human cancer cells not only contain many mutations, but they are also genetically unstable. This **genetic instability** results from mutations that interfere with the accurate replication and maintenance of the genome and thereby increase the mutation rate itself. Sometimes, the increased mutation rate may result from a defect in one of the many proteins needed to repair damaged DNA or to correct errors in DNA replication. Sometimes, there may be a defect in the cell-cycle checkpoint mechanisms that normally prevent a cell with damaged DNA from attempting to divide before it has completed the repair (discussed in Chapter 18). Sometimes, there may be a fault in the machinery of mitosis, which can lead to chromosomal damage, loss, or gain. These potential sources of genetic instability are summarized in **Table 20–1**.

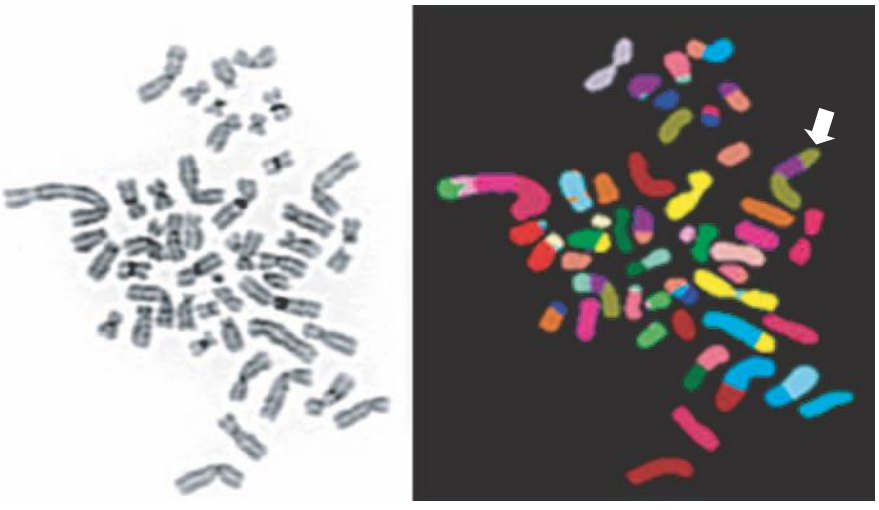
Genetic instability can generate extra chromosomes, as well as chromosome breaks and rearrangements—gross abnormalities that can be seen in a karyotype (**Figure 20–46**). It can also help drive the evolution of cancer, as we now discuss.

TABLE 20–1 A VARIETY OF FACTORS CAN CONTRIBUTE TO GENETIC INSTABILITY

Defects in DNA replication
Defects in DNA repair
Defects in cell-cycle checkpoint mechanisms
Mistakes in mitosis
Abnormal chromosome numbers

Cancer Cells Evolve, Giving Them an Increasingly Competitive Advantage

The mutations that lead to cancer do not cripple the mutant cells. On the contrary, they give these cells a competitive advantage over their neighbors. It is this advantage enjoyed by the mutant cells that leads to disaster for the organism as a whole. As an initial population of mutant cells grows, it slowly evolves: new chance mutations occur, some of which are favored by natural selection because they enhance cell proliferation and



(A) (B)

Figure 20–46 Cancer cells often have highly abnormal chromosomes, reflecting genetic instability. In the example shown here, chromosomes were prepared from a breast cancer cell in metaphase, spread on a glass slide, and stained with (A) a general DNA stain or (B) a combination of fluorescent stains that give a different color for each human chromosome. The staining (displayed in false color) shows multiple translocations, including one chromosome (*white* arrow) that has undergone two translocations, so that it is now made up of two pieces of chromosome 8 (*olive*) and a piece of chromosome 17 (*purple*). The karyotype also contains 48 chromosomes, instead of the normal 46. Such abnormalities in chromosome number can further cause chromosome-segregation errors when the cell divides, so that the degree of genetic disruption goes from bad to worse (see Table 20–1). (Courtesy of Joanne Davidson and Paul Edwards.)

QUESTION 20–8

About 10^{16} cell divisions take place in a human body during a lifetime, yet an adult human body consists of only about 10^{13} cells. Why are these two numbers so different?

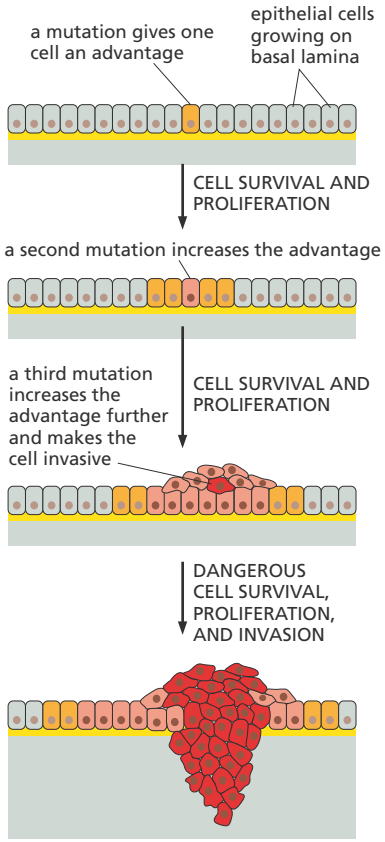


Figure 20–47 Tumors evolve by repeated rounds of mutation, proliferation, and natural selection. The final outcome is a fully malignant tumor. At each step, a single cell undergoes a mutation that enhances its ability to proliferate, or survive, or both, so that its progeny become a dominant clone in the tumor. Proliferation of this clone then hastens occurrence of the next step of tumor progression by increasing the size of the cell population at risk of undergoing an additional mutation. Some cancers contain multiple malignant clones, each with its own collection of mutations, in addition to a common set of mutations that reflect the tumor's origin from a founding mutant cell (not shown).

cell survival. This process of random mutation followed by selection culminates in the genesis of cancer cells that run riot within the population of cells that form the body, upsetting its regular structure (**Figure 20–47**).

Non-mutagenic environmental or lifestyle factors such as obesity may favor the development of cancer by altering the selection pressures that operate in tissues. A glut of circulating nutrients, or abnormal increases in hormones, mitogens, or growth factors, for example, may help cells with dangerous mutations survive, grow, and proliferate. Eventually, cells emerge that have all the abnormalities required for full-blown cancer.

To be successful, a cancer cell must acquire a whole range of abnormal properties—a collection of subversive behaviors. A proliferating precursor cell in the epithelial lining of the gut, for example, must undergo changes that permit it to carry on dividing when it would normally stop (see Figure 20–36). That cell and its progeny must also be able to avoid cell death, displace their normal neighbors, and attract a blood supply to nourish continued tumor growth. For the tumor cells to then become invasive, they must be able to detach from the epithelial sheet and digest their way through the basal lamina into the underlying connective tissue. To spread to other organs and form *metastases*, they must be able to get in, and then out, of blood or lymph vessels and settle, survive, and proliferate in new sites (see Figure 20–45).

Different cancers require different combinations of properties. Nevertheless, we can draw up a general list of characteristics that distinguish cancer cells from normal cells.

1. Cancer cells have a reduced dependence on signals from other cells for their survival, growth, and division. Often, this is because they contain mutations in components of the cell signaling pathways that normally respond to such stimuli. An activating mutation in a *Ras* gene (discussed in Chapter 16), for example, can cause an intracellular signal for proliferation even in the absence of the extracellular cue that would normally be needed to turn Ras on, like a faulty doorbell that rings even when nobody is pressing the button.
2. Cancer cells can survive levels of stress and internal derangement that would cause normal cells to kill themselves by apoptosis. This avoidance of cell suicide is often the result of mutations in genes that regulate the intracellular death program responsible for apoptosis (discussed in Chapter 18). For example, about 50% of all human cancers have an inactivating mutation in the *p53* gene. The p53 protein normally acts as part of a DNA damage response that causes cells with DNA damage to either cease dividing (see Figure 18–15) or die by apoptosis. Chromosome breakage, for example, if not repaired, will generally cause a cell to commit suicide; but if the cell is defective in p53, it may survive and divide, creating highly abnormal daughter cells that have the potential for further mischief.
3. Unlike most normal human cells, cancer cells can often proliferate indefinitely. Most normal human somatic cells will only divide a limited number of times in culture, after which they permanently stop; this is at least partly because they have lost the ability to produce the enzyme *telomerase*, so the telomeres at the ends of their chromosomes become progressively shorter with each cell division (see page 210). Cancer cells typically break through this proliferation barrier by reactivating production of telomerase, enabling them to maintain telomere length indefinitely.

4. Most cancer cells are genetically unstable, with a greatly increased mutation rate and an abnormal number of chromosomes.
5. Cancer cells are abnormally invasive, at least partly because they often lack certain cell adhesion molecules, such as cadherins, that help hold normal cells in their proper place.
6. Cancer cells have an abnormal metabolism that makes them avid for nutrients, which they use to fuel their biosynthesis and growth, rather than for energy generation by oxidative phosphorylation.
7. Cancer cells can survive and proliferate in abnormal locations, whereas most normal cells die when misplaced. This colonization of unfamiliar territory may result from the ability of cancer cells to produce their own extracellular survival signals and to suppress their apoptosis program (as described in #2, above).

To understand the molecular biology of cancer, we have to identify the mutations responsible for these abnormal properties.

Two Main Classes of Genes Are Critical for Cancer: Oncogenes and Tumor Suppressor Genes

Investigators have made use of a variety of approaches to track down the genes and mutations that are critical for cancer—from studying viruses that cause cancer in chickens to following families in which a particular cancer occurs unusually often. Though many of the most important of these genes have been identified, the hunt for others continues.

For many cancer-critical genes, the dangerous mutations are ones that render the encoded protein hyperactive. These *gain-of-function mutations* have a dominant effect: only one gene copy needs to be mutated to cause trouble. The resulting mutant gene is called an **oncogene**, and the corresponding normal form of the gene is called a **proto-oncogene** (**Figure 20–48A**). **Figure 20–49** shows a variety of ways in which a proto-oncogene can be converted into its corresponding oncogene.

For other genes, the danger lies in mutations that destroy their activity. These *loss-of-function mutations* are generally recessive: both copies of the gene must be lost or inactivated before an effect is seen; the normal

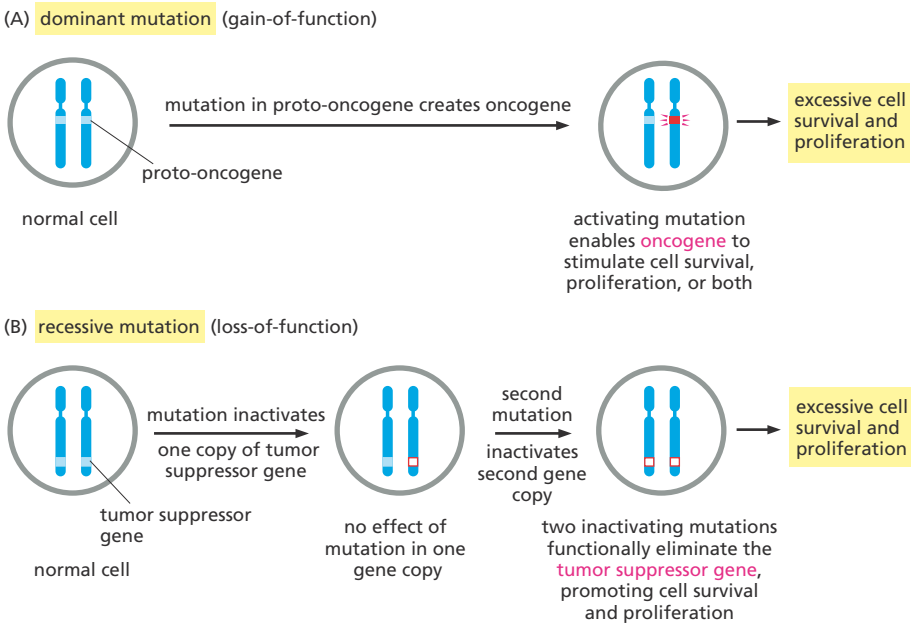


Figure 20–48 Genes that are critical for cancer are classified as proto-oncogenes or tumor suppressor genes, according to whether the dangerous mutations are dominant or recessive. (A) Oncogenes act in a dominant manner: a gain-of-function mutation in a single copy of the proto-oncogene can drive a cell toward cancer. (B) Loss-of-function mutations in tumor suppressor genes generally act in a recessive manner: the function of both copies of the gene must be lost to drive a cell toward cancer. In this diagram, normal genes are represented by *light blue* squares, activating mutations by *red* rays, and inactivating mutations by hollow *red* rectangles.

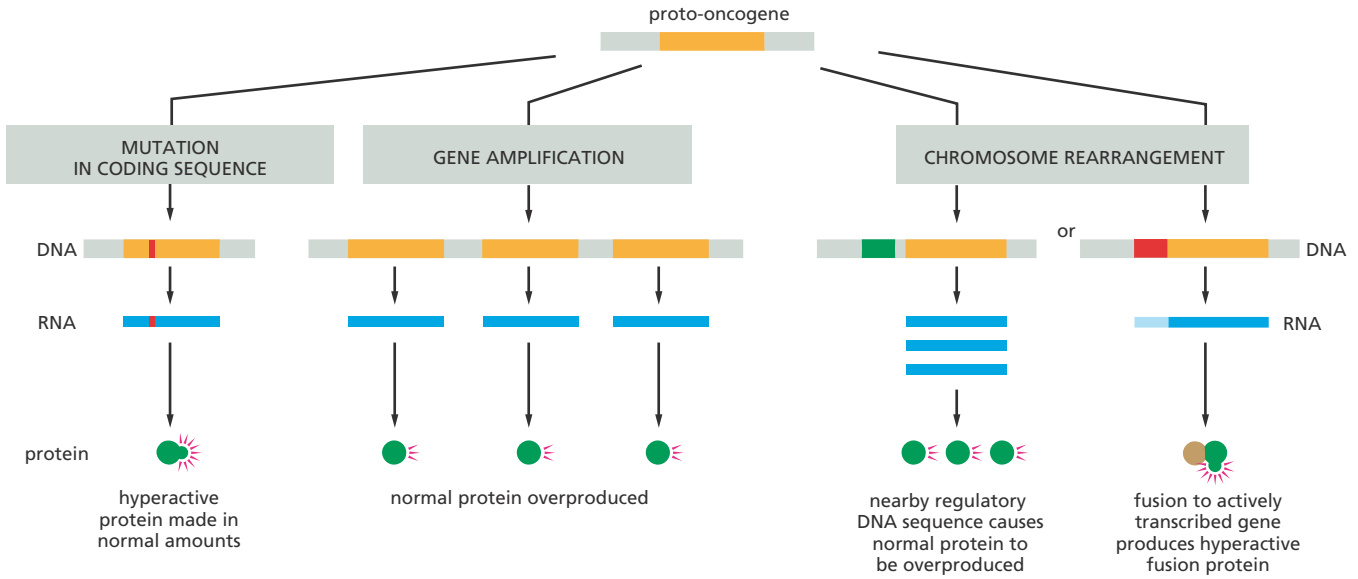


Figure 20–49 Several kinds of genetic change can convert a proto-oncogene into an oncogene. In each case, the change leads to an increase in the gene's function—that is, it is a gain-of-function mutation.

gene is called a **tumor suppressor gene** (Figure 20–48B). In addition to such genetic alterations, tumor suppressor genes can also be silenced by *epigenetic changes*, which alter gene expression without changing the gene's nucleotide sequence (as discussed in Chapter 8). Epigenetic changes are thought to silence some tumor suppressor genes in most human cancers. **Figure 20–50** highlights a few of the ways in which the activity of a tumor suppressor gene can be lost.

The variety of proto-oncogenes and tumor suppressor genes code for proteins of many different types, corresponding to the many kinds of misbehavior that cancer cells display. Some of these proteins are involved in signaling pathways that regulate cell survival, cell growth, or cell division. Others take part in DNA repair, mediate the DNA damage response, modify chromatin, or help regulate the cell cycle or apoptosis. Still others (such as cadherins) are involved in cell adhesion or other properties critical for metastasis, or have roles that we do not yet properly understand.

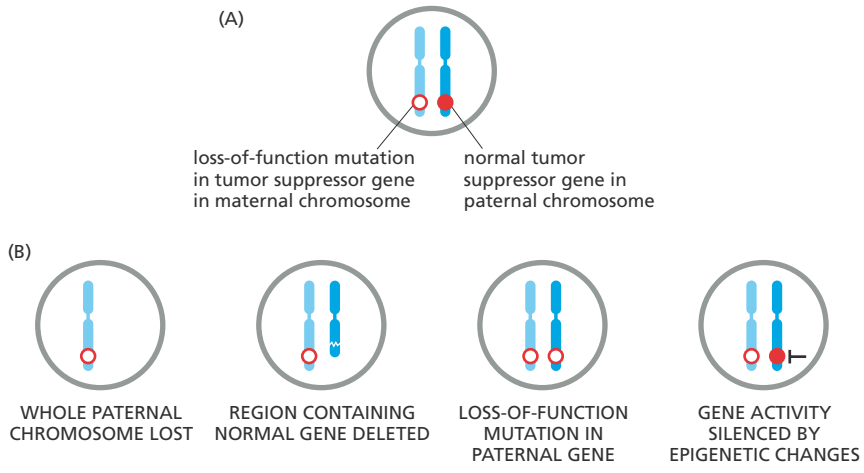


Figure 20–50 Several kinds of genetic events can eliminate the activity of a tumor suppressor gene. Note that both copies of such a gene must be lost to eliminate its function. (A) A cell in which the maternal copy of the suppressor gene is inactive because of loss-of-function mutation. (B) The same cell in which the paternal copy of the gene is inactivated in different ways, as shown.

Cancer-causing Mutations Cluster in a Few Fundamental Pathways

From the point of view of a cancer cell, oncogenes and tumor suppressor genes—and the mutations that affect them—are flip sides of the same coin. Activation of an oncogene and inactivation of a tumor suppressor gene can both promote the development of cancer. And both types of mutations are called into play in most cancers. In classifying cancer-critical genes, it seems that the type of mutation—gain-of-function or loss-of-function—matters less than the pathway in which it acts.

Rapid, low-cost DNA sequencing is now providing an unprecedented amount of information about the mutations that drive a variety of cancers. We can now compare the complete genome sequences of the cancer cells from a patient's tumor to the genome sequence of the noncancerous cells in the same individual—or of cancer cells that have spread to another location in the body. By putting together such data from many different patients, we can begin to draw up exhaustive lists of the genes that are critical for specific classes of cancer; and by analysis of data from a single patient, we can deduce the "family tree" of his or her cancer cells, showing how the progeny of the original founder cell have evolved and diversified as they multiplied and metastasized to different sites.

One remarkable finding has been that many of the genes mutated in individual tumors fall into a small number of key regulatory pathways: those that govern the initiation of cell proliferation, control cell growth, and regulate the cell's response to DNA damage and stress. For example, in almost every case of glioblastoma—the most common type of human brain tumor—mutations disrupt all three of these fundamental pathways, and the same pathways are subverted, in one way or another, in almost all human cancers (**Figure 20–51**). In any given patient, only a single gene tends to be mutated in each pathway, but not always the same gene: it is the under- or overactivity of the pathway that matters for cancer development, not the way in which this malfunction is achieved. Because the same three fundamental control systems are subverted in so wide a variety of cancers, it seems that their misregulation must be key to most cancers' success.

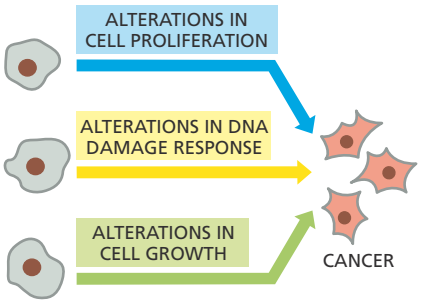


Figure 20–51 Three key regulatory pathways are perturbed in almost all human cancers. These pathways regulate cell proliferation, cell growth, and the cell's response to DNA damage or stress.

Colorectal Cancer Illustrates How Loss of a Tumor Suppressor Gene Can Lead to Cancer

Colorectal cancer provides one well-studied example of how a tumor suppressor can be identified and its role in tumor growth determined. Colorectal cancer arises from the epithelium lining the colon and rectum; most cases are seen in old people and do not have any discernible hereditary cause. A small proportion of cases, however, occur in families that are exceptionally prone to the disease and show an unusually early onset. In one set of such "predisposed" families, the affected individuals develop colorectal cancer in early adult life, and the onset of their disease is foreshadowed by the development of hundreds or thousands of little tumors, called polyps, in the epithelial lining of the colon and rectum.

By studying these families, investigators traced the development of the polyps to a deletion or inactivation of a tumor suppressor gene called *APC*—for *Adenomatous Polyposis Coli*. (Note that the protein encoded by this gene is different from the anaphase-promoting complex, also abbreviated APC, discussed in Chapter 18.) Affected individuals inherit one mutant copy of the gene and one normal copy. Although one normal gene copy is enough for normal cell behavior, all the cells of these individuals are only one mutational step away from total loss of the gene's function (as compared to two steps away for a person who inherits two

normal copies of the gene). The individual tumors arise from cells that have undergone a somatic mutation that inactivates the remaining good copy. Because the number of new mutations required is smaller, the disease strikes these individuals at an earlier age.

But what about the great majority of colorectal cancer patients, who have inherited two good copies of *APC* and do not have the hereditary condition or any significant family history of cancer? When their tumors are analyzed, it turns out that in more than 60% of cases, although both copies of *APC* are present in the adjacent normal tissue, the tumor cells themselves have lost or inactivated both copies of this gene, presumably through two independent somatic mutations.

All these findings clearly identify *APC* as a tumor suppressor gene and, knowing its sequence and mutant phenotype, one can begin to decipher how its loss helps to initiate the development of cancer. As explained in **How We Know** (pp. 722–723), the *APC* gene was found to encode an inhibitory protein that normally restricts the activation of the Wnt signaling pathway, which is involved in stimulating cell proliferation in the crypts of the gut lining, as described earlier (see Figure 20–40). When *APC* is lost, the pathway is hyperactive and epithelial cells proliferate to excess, generating a polyp (**Figure 20–52**). Within this growing mass of tissue, further mutations occur, sometimes resulting in invasive cancer (**Figure 20–53**).

An Understanding of Cancer Cell Biology Opens the Way to New Treatments

The better we understand the tricks that cancer cells use to survive, proliferate, and spread, the better are our chances of finding ways to defeat them. The task is made more challenging because cancer cells are highly mutable and, like weeds or parasites, rapidly evolve resistance to treatments used to exterminate them. Moreover, because mutations arise randomly, every case of cancer is likely to have its own unique combination of genes mutated. Even within an individual patient, tumor cells do not all contain the same genetic lesions. Thus, no single treatment is likely to work in every patient, or even for every cancer cell within the same patient. And the fact that cancers generally are not detected until the primary tumor has reached a diameter of 1 cm or more—by

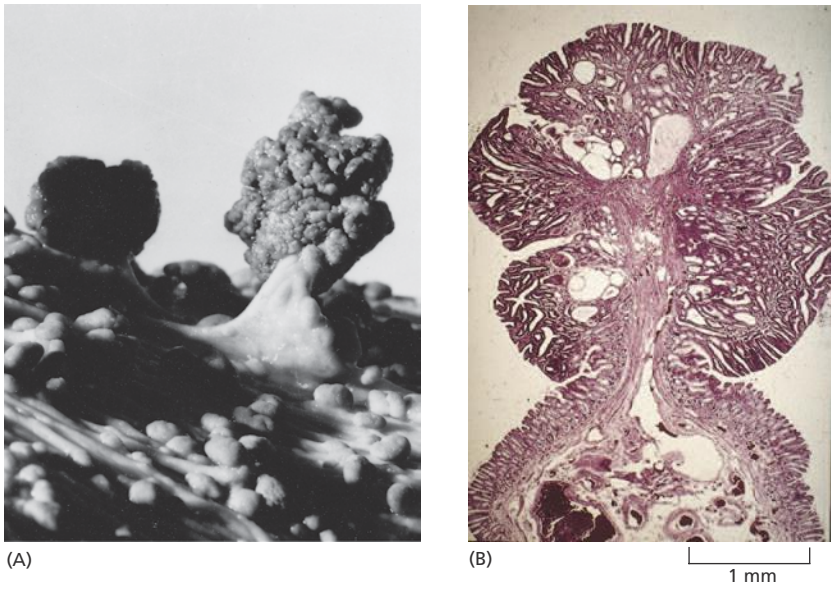


Figure 20–52 Colorectal cancer often begins with loss of the tumor suppressor gene *APC*, leading to growth of a polyp. (A) Thousands of small polyps, and a few much larger ones, are seen in the lining of the colon of a patient with an inherited *APC* mutation (whereas individuals without an *APC* mutation might have one or two polyps). Through further mutations, some of these polyps will progress to become invasive cancers, unless the tissue is removed surgically. (B) Cross section of one such polyp; note the excessive quantities of deeply infolded epithelium, corresponding to crypts full of abnormal, proliferating cells. (A, courtesy of John Northover and Cancer Research UK; B, courtesy of Anne Campbell.)

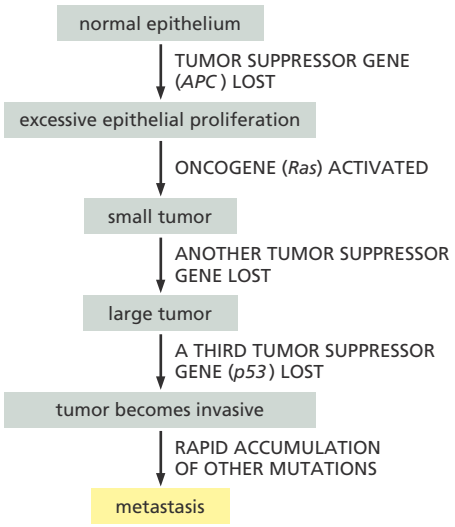


Figure 20–53 A polyp in the epithelial lining of the colon or rectum, caused by loss of the *APC* gene, can progress to cancer by accumulation of further mutations. The diagram shows a sequence of mutations that might underlie a typical case of colorectal cancer. After the initial mutation, all subsequent mutations occur randomly in a single cell that had already acquired the previous mutations. A sequence of events such as that shown here would usually be spread over 10 to 20 years or more. Though most colorectal cancers are thought to begin with loss of the *APC* tumor suppressor gene, the subsequent sequence of mutations is quite variable; indeed, many polyps never progress to cancer.

which time it consists of hundreds of millions of cells that are already genetically diverse and often have already begun to metastasize (**Figure 20–54**)—makes treatment even harder still.

Yet, in spite of these difficulties, an increasing number of cancers can be treated effectively. Surgery remains a highly effective tactic, and surgical techniques are continually improving: in many cases, if a cancer has not spread far, it can often be cured by simply cutting it out. Where surgery fails, therapies based on the intrinsic peculiarities of cancer cells can be used. Lack of normal cell-cycle control mechanisms, for example, may help make cancer cells particularly vulnerable to DNA damage: whereas a normal cell will halt its proliferation until such damage is repaired, a cancer cell may charge ahead regardless, producing daughter cells that may die because they inherit too many unrepaired breakages in their chromosomes. Presumably for this reason, cancer cells can often be killed by doses of radiotherapy or DNA-damaging chemotherapy that leave normal cells relatively unharmed.

Surgery, radiation, and chemotherapy are long-established treatments, but many novel approaches are also being enthusiastically pursued. In some cases, as with loss of a normal response to DNA damage, the very feature that helps to make the cancer cell dangerous also makes it vulnerable, enabling doctors to kill it with a properly targeted treatment. Some cancers of the breast and ovary, for example, owe their genetic instability to the lack of a protein (Brca1 or Brca2) needed for accurate repair of double-strand breaks in DNA (discussed in Chapter 6); the cancer cells survive by relying on alternative types of DNA repair mechanisms. Drugs that inhibit one of these alternative DNA repair mechanisms kill the cancer cells by raising their genetic instability to such a level that the cells die from chromosome fragmentation when they attempt to divide. Normal cells, which have an intact double-strand break repair mechanism, are relatively unaffected, and the drugs seem to have few side effects.

Another set of strategies aims to use the immune system to kill the tumor cells, taking advantage of tumor-specific cell-surface molecules to target the attack. Antibodies that recognize these tumor molecules can be produced *in vitro* and injected into the patient to mark the tumor cells for destruction. Other antibodies, aimed at the immune cells, can promote the elimination of cancer cells by neutralizing the inhibitory cell-surface molecules that keep the immune system's killer cells in check. The latter antibodies have been remarkably effective in clinical trials and, in principle, should be useful for treating a variety of different cancers.

In some cancers, it is becoming possible to target the products of specific oncogenes directly so as to block their action, causing the cancer cells to die. In chronic myeloid leukemia (CML), the misbehavior of the cancer cells depends on a mutant intracellular signaling protein (a tyrosine kinase) that causes the cells to proliferate when they should not. A small drug molecule, called imatinib (trade name Gleevec), blocks the activity

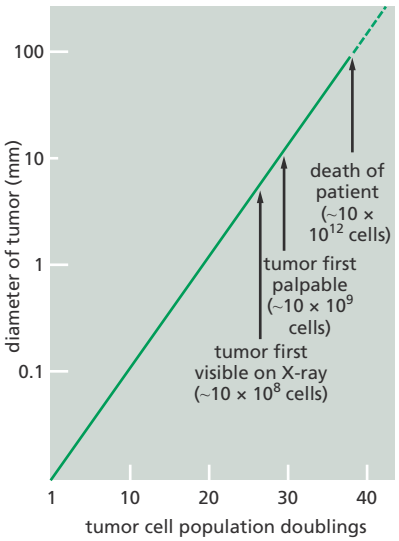


Figure 20–54 A tumor is generally not diagnosed until it has grown to contain hundreds of millions of cells. Here, the growth of a typical tumor is plotted on a logarithmic scale. Years may elapse before the tumor becomes noticeable. The doubling time of a typical breast tumor, for example, is about 100 days.

MAKING SENSE OF THE GENES THAT ARE CRITICAL FOR CANCER

The search for genes that are critical for cancer sometimes begins with a family that shows an inherited predisposition to a particular form of the disease. *APC*—a tumor suppressor gene that is frequently deleted or inactivated in colorectal cancer—was tracked down by searching for genetic defects in such families prone to the disease. But identifying the gene is only half the battle. The next step is determining what the normal gene does in a normal cell—and why alterations in the gene promote cancer.

Guilt by association

Determining what a gene—or its encoded product—does inside a cell is not a simple task. Imagine isolating an uncharacterized protein and being told that it acts as a protein kinase. That information does not reveal how the protein functions in the context of a living cell. What proteins does the kinase phosphorylate? In which tissues is it active? What role does it have in the growth, development, or physiology of the organism? A great deal of additional information is required to understand the biological context in which the kinase acts.

Most proteins do not function in isolation: they interact with other proteins in the cell. Thus one way to begin to decipher a protein's biological role is to identify its binding partners. If an uncharacterized protein interacts with a protein whose role in the cell is understood, the function of the unknown protein is likely to be in some way related. The simplest method for identifying proteins that bind to one another tightly is co-immunoprecipitation (see Panel 4–3, pp. 164–165). In this technique, an antibody is used to capture and precipitate a specific target protein from an extract prepared by breaking open cells; if this target protein is associated tightly with another protein, the partner protein will precipitate as well. This is the approach that was taken to characterize the Adenomatous Polyposis Coli gene product, APC.

Two groups of researchers used antibodies against APC to isolate the protein from extracts prepared from cultured human cells. The antibodies captured APC along with a second protein. When the researchers examined the amino acid sequence of this partner, they recognized the protein as β-catenin.

The discovery that APC interacts with β-catenin initially led to some wrong guesses about the role of APC in colorectal cancer. In mammals, β-catenin was known primarily for its role at adherens junctions, where it serves as a linker to connect membrane-spanning cadherin proteins to the intracellular actin cytoskeleton (see, for example, Figure 20–24). Thus, for some time, scientists thought that APC might be involved in cell adhesion. But within a few years, it emerged that β-catenin also has another completely different function. It is this unexpected function that turned out to be the one that is relevant for understanding APC's role in cancer.

Wingless flies

Not long before the discovery that APC binds to β-catenin, developmental biologists working on the fruit fly *Drosophila* had noticed that the human β-catenin protein is very similar in amino acid sequence to a *Drosophila* protein called Armadillo. Armadillo was known to be a key protein in a signaling pathway that has an important role in normal development in flies. The pathway is activated by the Wnt family of extracellular signal proteins, the founding member of which was called *Wingless*, after its mutant phenotype in flies. Wnt proteins bind to receptors on the surface of a cell, switching on an intracellular signaling pathway that ultimately leads to the activation of a set of genes that influence cell growth, division, and differentiation. Mutations in any of the proteins in this pathway lead to developmental errors that disrupt the basic body plan of the fly. The least devastating mutations cause flies to develop without wings; most mutations, however, result in the death of the embryo. In either case, the damage is done through effects on gene expression. This strongly suggested that Armadillo, and hence its vertebrate homolog β-catenin, were not just involved in cell adhesion, but somehow mediated the control of gene expression through the Wnt signaling pathway.

Although the Wnt pathway was discovered and studied intensively in fruit flies, it was later found to control many aspects of development in vertebrates, including mice and humans. Indeed, some of the proteins in the Wnt pathway function almost interchangeably in *Drosophila* and vertebrates. The direct link between β-catenin and gene expression became clear from work in mammalian cells. Just as APC could be used as "bait" to catch its partner β-catenin by immunoprecipitation, so β-catenin could be used as bait to catch the next protein in the signaling pathway. This was found to be a transcription regulator called LEF-1/TCF, or TCF for short. It too was found to have a *Drosophila* counterpart in the Wnt pathway, and a combination of *Drosophila* genetics and mammalian cell biology revealed how the gene control mechanism works.

Wnt transmits its signal by promoting the accumulation of "free" β-catenin (or, in flies, Armadillo)—that is, of β-catenin that is not locked up in cell junctions. This free protein migrates from the cytoplasm into the nucleus. There it binds to the TCF transcription regulator, creating a complex that activates transcription of various

Wnt-responsive genes, including genes whose products stimulate cell proliferation (**Figure 20–55**).

It turns out that APC regulates the activity of this pathway by facilitating degradation of β-catenin and thereby preventing it from activating TCF in cells where no Wnt signal has been received (see Figure 20–55A). Loss of APC allows the concentration of β-catenin to rise, so that TCF is activated and Wnt-responsive genes are turned on even in the absence of a Wnt signal. But how does this promote the development of colorectal cancer? To find out, researchers turned to mice that lack TCF4, a member of the TCF gene family that is specifically expressed in the gut epithelial lining.

Tales from the crypt

Although it may seem counterintuitive, one of the most direct ways of finding out what a gene normally does is to see what happens to the organism when that gene is missing. If one can pinpoint the processes that are disrupted or compromised, one can begin to decipher the gene's function.

With this in mind, researchers generated "knockout" mice in which the gene encoding TCF4 was disrupted. The mutation is lethal: mice lacking TCF4 die shortly after birth. But the animals showed an interesting abnormality in their intestines. The intestinal crypts, which contain the stem cells responsible for the renewal of the gut lining (see Figure 20–36), completely failed to develop. The researchers concluded that TCF4 is normally required for maintaining the pool of proliferating gut stem cells.

When APC is missing, we see the other side of the coin: without APC to promote its degradation, β-catenin accumulates in excessive quantities, binds to the TCF4 transcription regulator, and thereby overactivates the TCF4-responsive genes. This drives the formation of polyps by promoting the inappropriate proliferation of gut stem cells. Differentiated progeny cells continue to be produced and discarded into the gut lumen, but the crypt cell population grows too fast for this disposal mechanism to keep pace. The result is crypt enlargement and a steady increase in the number of crypts. The growing mass of tissue bulges out into the gut lumen as a polyp (see Figure 20–52 and **Movie 20.8**). A number of additional mutations are needed, however, to convert this primary tumor into an invasive cancer.

More than 60% of human colorectal tumors harbor mutations in the *APC* gene. Interestingly, among the minority class of tumors that retain functional APC, about a quarter have activating mutations in β-catenin instead. These mutations tend to make the β-catenin protein more resistant to degradation and thus produce the same effect as loss of APC. In fact, mutations that enhance the activity of β-catenin have been found in a wide variety of other tumor types, including melanomas, stomach cancers, and liver cancers. Thus, the genes that encode proteins that act in the Wnt signaling pathway provide multiple targets for mutations that can spur the development of cancer.

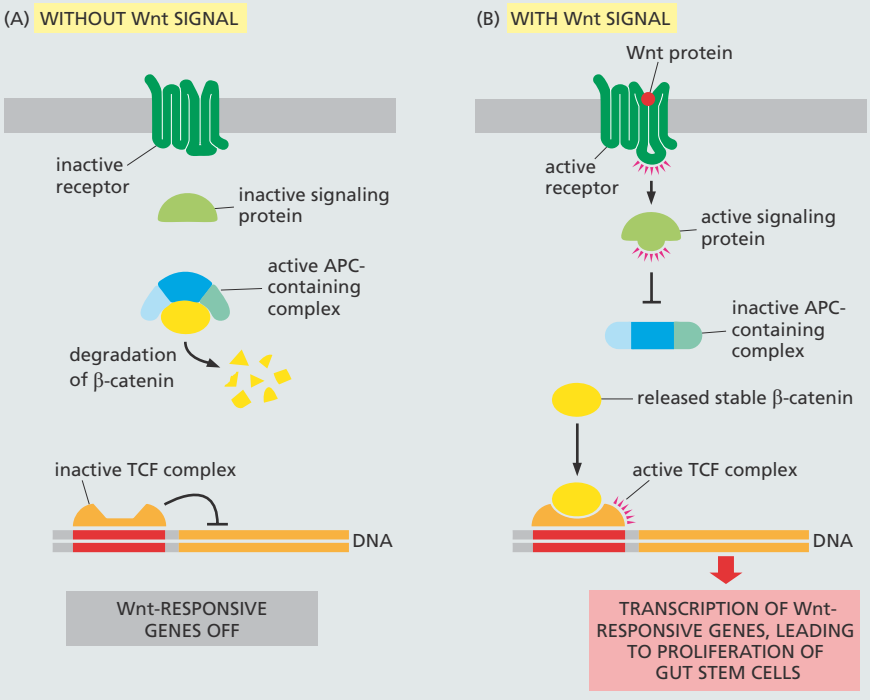


Figure 20–55 The APC protein keeps the Wnt signaling pathway inactive when the cell is not exposed to Wnt protein. It does this by promoting degradation of the signaling molecule β-catenin. In the presence of Wnt, or in the absence of active APC, free β-catenin becomes plentiful and combines with the transcription regulator TCF to drive transcription of Wnt-responsive genes and, ultimately, the proliferation of stem cells in the intestinal crypt (see Figure 20–40). In the colon, mutations that inactivate APC initiate tumors by causing excessive activation of the Wnt signaling pathway.

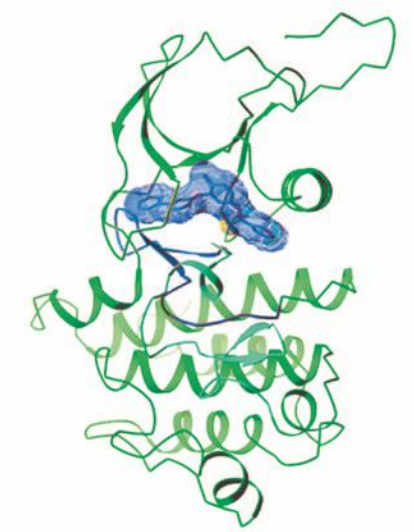


Figure 20–56 The drug Gleevec (imatinib) blocks the activity of a hyperactive oncogenic protein, thus inhibiting the growth of cancers that depend on that protein. The structure of a complex of Gleevec (solid *blue*) with the tyrosine kinase domain of the Abl protein (ribbon diagram), as determined by X-ray crystallography. (From T. Schindler et al., *Science* 289:1938–1942, 2000. With permission from AAAS.)

of this hyperactive mutant kinase (**Figure 20–56**). The results have been a dramatic success: in many patients, the abnormal proliferation and survival of the leukemic cells are strongly inhibited, providing many years of symptom-free survival. The same drug is also effective in some other cancers that depend on similar oncogenes.

With these examples before us, we can hope that our modern understanding of the molecular biology of cancer will soon allow us to devise effective rational treatments for even more forms of cancer. At the same time, cancer research has taught us many important lessons about basic cell biology. The applications of that knowledge go far beyond the treatment of cancer, giving us insight into the way the whole living world works.

ESSENTIAL CONCEPTS

- Tissues are composed of cells and extracellular matrix.
- In plants, each cell surrounds itself with extracellular matrix in the form of a cell wall, which is made chiefly of cellulose and other polysaccharides.
- An osmotic swelling pressure on plant cell walls keeps plant tissue turgid.
- Cellulose microfibrils in the plant cell wall confer tensile strength, while other polysaccharide components resist compression.
- The orientation in which the cellulose microfibrils are deposited controls the orientation of plant cell growth.
- Animal connective tissues provide mechanical support; these tissues consist mainly of extracellular matrix, which is secreted by a sparse scattering of embedded cells.
- In the extracellular matrix of animals, tensile strength is provided by the fibrous protein collagen, while glycosaminoglycans (GAGs), covalently linked to proteins to form proteoglycans, act as space-fillers and provide resistance to compression.
- Transmembrane integrin proteins link extracellular matrix proteins such as collagen and fibronectin to the intracellular cytoskeleton of cells that contact the matrix.
- Cells are connected via cell junctions in epithelial sheets that line all external and internal surfaces of the animal body.
- Proteins of the cadherin family span the epithelial cell plasma membrane and bind to identical cadherins in adjacent epithelial cells.
- At an adherens junction, the cadherins are linked intracellularly to actin filaments; at a desmosome junction, they are linked to keratin intermediate filaments.
- During development, the actin bundles at the adherens junctions that connect cells in an epithelial sheet can contract, causing the epithelium to bend and pinch off, forming an epithelial tube or vesicle.
- Hemidesmosomes attach the basal face of an epithelial cell to the basal lamina, a specialized sheet of extracellular matrix; the attachment is mediated by transmembrane integrin proteins, which are linked to intracellular keratin filaments.
- Tight junctions seal one epithelial cell to the next, barring the diffusion of water-soluble molecules across the epithelium.
- Gap junctions form channels that allow the direct passage of inorganic ions and small, hydrophilic molecules from cell to cell; plasmodesmata in plants form a different type of channel that allows both small and large molecules to pass from cell to cell.

- Most tissues in vertebrates are complex mixtures of cell types that are subject to continual turnover.
- The tissues of an adult animal are maintained and renewed by the same basic processes that generated them in the embryo: cell proliferation, cell movement, and cell differentiation. As in the embryo, these processes are controlled by intercellular communication, selective cell–cell adhesion, and cell memory.
- In many tissues, nondividing, terminally differentiated cells are generated from stem cells, usually via the proliferation of precursor cells.
- Embryonic stem cells (ES cells) can proliferate indefinitely in culture and remain capable of differentiating into any cell type in the body—that is, they are pluripotent.
- Induced pluripotent stem cells (iPS cells), which resemble ES cells, can be generated from cells of adult human tissues through the artificial expression of a small set of transcription regulators.
- Cancer cells fail to obey the social constraints that normally ensure that cells survive and proliferate only when and where they should, and do not invade regions where they do not belong.
- Cancers arise from the accumulation of many mutations in a single somatic cell lineage; they are genetically unstable, having increased mutation rates and, often, chromosomal abnormalities.
- Unlike most normal human cells, cancer cells typically express telomerase, enabling them to proliferate indefinitely without losing DNA at their chromosome ends.
- Most human cancer cells harbor mutations in the *p53* gene, allowing them to survive and divide even when their DNA is damaged.
- The mutations that promote cancer can do so either by converting proto-oncogenes into hyperactive oncogenes or by inactivating tumor suppressor genes.
- Sequencing of cancer genomes reveals that most cancers have mutations that subvert the same three key pathways, controlling cell proliferation, cell growth, and the response to DNA damage and stress. In different cases of cancer, these pathways are subverted in different ways.
- Knowing the molecular abnormalities that underlie a particular cancer, one can begin to design specifically targeted treatments.

KEY TERMS

adherens junction
apical
basal
basal lamina
cadherin
cancer
cell junction
cell wall
cellulose microfibril
collagen
connective tissue
desmosome
embryonic stem (ES) cell
epithelium (plural epithelia)
extracellular matrix
fibroblast
fibronectin
gap junction
genetic instability
glycosaminoglycan (GAG)
hemidesmosome
induced pluripotent stem (iPS) cell
integrin
metastasis
oncogene
plasmodesma
(plural plasmodesmata)
pluripotent
proteoglycan
proto-oncogene
reproductive cloning
stem cell
therapeutic cloning
tight junction
tissue
tumor suppressor gene
Wnt protein

QUESTIONS

QUESTION 20–9

Which of the following statements are correct? Explain your answers.

A. Gap junctions connect the cytoskeleton of one cell to that of a neighboring cell or to the extracellular matrix.

B. A wilted plant leaf can be likened to a deflated bicycle tire.

C. Because of their rigid structure, proteoglycans can withstand a large amount of compressive force.

D. The basal lamina is a specialized layer of extracellular matrix to which sheets of epithelial cells are attached.

E. Skin cells are continually shed and are renewed every few weeks; for a permanent tattoo, it is therefore necessary to deposit pigment below the epidermis.

F. Although stem cells are not differentiated, they are specialized and therefore give rise only to specific cell types.

QUESTION 20–10

Which of the following substances would you expect to spread from one cell to the next through (a) gap junctions and (b) plasmodesmata: glutamic acid, mRNA, cyclic AMP, Ca^{2+}, G proteins, and plasma membrane phospholipids?

QUESTION 20–11

Discuss the following statement: "If plant cells contained intermediate filaments to provide the cells with tensile strength, their cell walls would be dispensable."

QUESTION 20–12

Through the exchange of small metabolites and ions, gap junctions provide metabolic and electrical coupling between cells. Why, then, do you suppose that neurons communicate primarily through synapses rather than through gap junctions?

QUESTION 20–13

Gelatin is primarily composed of collagen, which is responsible for the remarkable tensile strength of connective tissue. It is the basic ingredient of jello; yet, as you probably experienced many times yourself while consuming the strawberry-flavored variety, jello has virtually no tensile strength. Why?

QUESTION 20–14

"The structure of an organism is determined by the genome that the egg contains." What is the evidence on which this statement is based? Indeed, a friend challenges you and suggests that you replace the DNA of a stork's egg with human DNA to see if a human baby results. How would you answer him?

QUESTION 20–15

Leukemias—that is, cancers arising through mutations that cause excessive production of white blood cells—have an earlier average age of onset than other cancers. Propose an explanation for why this might be the case.

QUESTION 20–16

Carefully consider the graph in **Figure Q20–16**, showing the number of cases of colon cancer diagnosed per 100,000 women per year as a function of age. Why is this graph so steep and curved, if mutations occur with a similar frequency throughout a person's life-span?

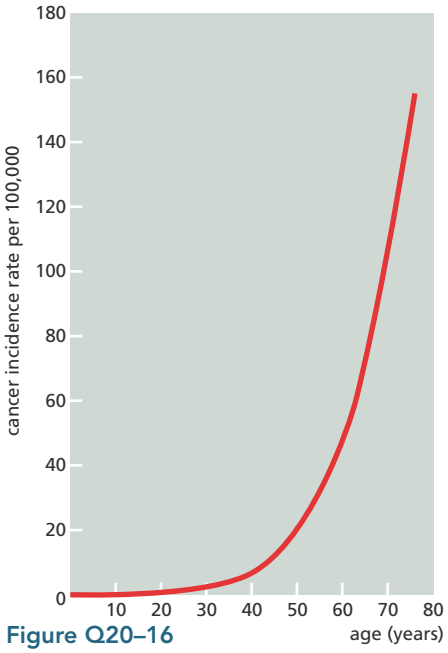


Figure Q20–16

QUESTION 20–17

Heavy smokers or industrial workers exposed for a limited time to a chemical carcinogen that induces mutations in DNA do not usually begin to develop cancers characteristic of their habit or occupation until 10, 20, or even more years after the exposure. Suggest an explanation for this long delay.

QUESTION 20–18

High levels of the female sex hormone estrogen increase the incidence of some forms of cancer. Thus, some early types of contraceptive pills containing high concentrations of estrogen were eventually withdrawn from use because this was found to increase the risk of cancer of the lining of the uterus. Male transsexuals who use estrogen preparations to give themselves a female appearance have an increased risk of breast cancer. High levels of androgens (male sex hormones) increase the risk of some other forms of cancer, such as cancer of the prostate. Can one infer that estrogens and androgens are mutagenic?

QUESTION 20–19

Is cancer hereditary?

Answers

Chapter 1

ANSWER 1–1 Trying to define life in terms of properties is an elusive business, as suggested by this scoring exercise (**Table A1–1**). Vacuum cleaners are highly organized objects, and take matter and energy from the environment and transform the energy into motion, responding to stimuli from the operator as they do so. On the other hand, they cannot reproduce themselves, or grow and develop—but then neither can old animals. Potatoes are not particularly responsive to stimuli, and so on. It is curious that standard definitions of life usually do not mention that living organisms on Earth are largely made of organic molecules, that life is carbon based. As we now know, the key types of "informational macromolecules"—DNA, RNA, and protein—are the same in every living species.

TABLE A1–1 PLAUSIBLE "LIFE" SCORES FOR A VACUUM CLEANER, A POTATO, AND A HUMAN

Characteristic	Vacuum cleaner	Potato	Human
1. Organization	Yes	Yes	Yes
2. Homeostasis	Yes	Yes	Yes
3. Reproduction	No	Yes	Yes
4. Development	No	Yes	Yes
5. Energy	Yes	Yes	Yes
6. Responsiveness	Yes	No	Yes
7. Adaptation	No	Yes	Yes

ANSWER 1–2 Most random changes to the shoe design would result in objectionable defects: shoes with multiple heels, with no soles, or with awkward sizes would obviously not sell and would therefore be selected against by market forces. Other changes would be neutral, such as minor variations in color or in size. A minority of changes, however, might result in more desirable shoes: deep scratches in a previously flat sole, for example, might create shoes that would perform better in wet conditions; the loss of high heels might produce shoes that are more comfortable. The example illustrates that random changes can lead to significant improvements if the number of trials is large enough and selective pressures are imposed.

ANSWER 1–3 It is extremely unlikely that you created a new organism in this experiment. Far more probably, a spore from the air landed in your broth, germinated, and gave rise to the cells you observed. In the middle of the nineteenth century, Louis Pasteur invented a clever apparatus to disprove the then widely accepted belief that life could arise spontaneously. He showed that sealed flasks never grew anything if properly heat-sterilized first. He overcame the objections of those who pointed out the lack of oxygen or who suggested that his heat sterilization killed the life-generating principle, by using a special flask with a slender "swan's neck," which was designed to prevent spores carried in the air from contaminating the culture (**Figure A1–3**). The cultures in these flasks never showed any signs of life; however, they were capable of supporting life, as could be demonstrated by washing some of the "dust" from the neck into the culture.

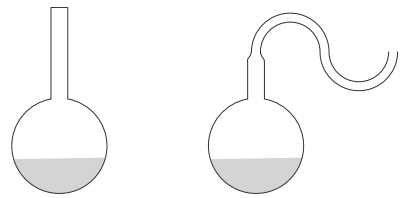


Figure A1–3

ANSWER 1–4 6×10^{39} (= 6×10^{27} g/10^{-12} g) bacteria would have the same mass as the Earth. And $6 \times 10^{39} = 2^{t/20}$, according to the equation describing exponential growth. Solving this equation for t results in $t = 2642$ minutes (or 44 hours). This represents only 132 generation times(!), whereas 5×10^{14} bacterial generation times have passed during the last 3.5 billion years. Obviously, the total mass of bacteria on this planet is nowhere close to the mass of the Earth. This illustrates that exponential growth can occur only for very few generations, i.e., for minuscule periods of time compared with evolution. In any realistic scenario, food supplies very quickly become limiting.

This simple calculation shows us that the ability to grow and divide quickly when food is ample is only one factor in the survival of a species. Food is generally scarce, and individuals of the same species have to compete with one another for the limited resources. Natural selection favors mutants that either win the competition or find ways to exploit food sources that their neighbors are unable to use.

ANSWER 1–5 By engulfing substances, such as food particles, eukaryotic cells can sequester them and feed

on them efficiently. Bacteria, in contrast, have no way of capturing lumps of food; they can export substances that help break down food substances in the environment, but the products of this labor must then be shared with other cells in the same neighborhood.

ANSWER 1–6 Conventional light microscopy is much easier to use and requires much simpler instruments. Objects that are 1 μm in size can easily be resolved; the lower limit of resolution is 0.2 μm, which is a theoretical limit imposed by the wavelength of visible light. Visible light is nondestructive and passes readily through water, making it possible to observe living cells. Electron microscopy, on the other hand, is much more complicated, both in the preparation of the sample (which needs to be extremely thinly sliced, stained with electron-dense heavy metal, and completely dehydrated) and in the nature of the instrument. Living cells cannot be observed in an electron microscope. The resolution of electron microscopy is much higher, however, and biological objects as small as 1 nm can be resolved. To see any structural detail, microtubules, mitochondria, and bacteria would need to be analyzed by electron microscopy. It is possible, however, to stain them with specific dyes and then determine their location by light microscopy; if the dye is fluorescent, the stained objects can be seen with high resolution in a fluorescence microscope.

ANSWER 1–7 Because the basic workings of cells are so similar, a great deal has been learned from studying model systems. Brewer's yeast is a good model system, because yeast cells are much simpler than human cancer cells. We can grow cells inexpensively and in vast quantities, and we can manipulate them genetically and biochemically much more easily than human cells. This allows us to use yeast to decipher the ground rules governing how cells divide and grow. Cancer cells divide when they should not (and therefore give rise to tumors), and a basic understanding of how cell division is normally controlled is therefore directly relevant to the cancer problem. Indeed, the National Cancer Institute, the American Cancer Society, and many other institutions that are devoted to finding a cure for cancer strongly support basic research on various aspects of cell division in different model systems, including yeast.

ANSWER 1–8 Check your answers using the Glossary and Panel 1–2 (p. 25).

ANSWER 1–9

A. False. The hereditary information is encoded in the cell's DNA, which in turn specifies its proteins (via RNA).
B. True. Bacteria do not have a nucleus.
C. False. Plants are composed of eukaryotic cells that contain chloroplasts as cytoplasmic organelles. The chloroplasts are thought to be evolutionarily derived from prokaryotic cells.
D. True. The number of chromosomes varies from one organism to another, but is constant in all cells (except germ cells) of the same organism.
E. False. The cytosol is the cytoplasm excluding all membrane-enclosed organelles.
F. True. The nuclear envelope is a double membrane, and mitochondria are surrounded by both an inner and an outer membrane.
G. False. Protozoans are single-celled organisms and therefore do not have different tissues or cell types. They have a complex structure, however, that has highly specialized parts.
H. Somewhat true. Peroxisomes and lysosomes contain enzymes that catalyze the breakdown of substances produced in the cytosol or taken up by the cell. One can argue, however, that many of these substances are degraded to generate food molecules, and as such are certainly not "unwanted."

ANSWER 1–10 One average brain cell weighs 10^{-9} g (= 1000 g/10^{12}). Because 1 g of water occupies 1 ml = 1 cm^3 (= 10^{-6} m^3), the volume of one cell is 10^{-15} m^3 (= 10^{-9} g × 10^{-6} m^3/g). Taking the cube root yields a side length of 10^{-5} m, or 10 μm (10^6 μm = 1 m) for each cell. The page of the book has a surface of 0.057 m^2 (= 21 cm × 27.5 cm), and each cell has a footprint of 10^{-10} m^2 (10^{-5} m × 10^{-5} m). Therefore, 57 × 10^7 (= 0.057 m^2/10^{-10} m^2) cells fit on this page when spread out as a single layer. Thus, 10^{12} cells would occupy 1750 pages (= 10^{12}/[57 × 10^7]).

ANSWER 1–11 In this plant cell, A is the nucleus, B is a vacuole, C is the cell wall, and D is a chloroplast. The scale bar is about 10 μm, the width of the nucleus.

ANSWER 1–12 The three major filaments are actin filaments, intermediate filaments, and microtubules. Actin filaments are involved in rapid cell movement, and are the most abundant filaments in a muscle cell; intermediate filaments provide mechanical stability and are the most abundant filaments in epidermal cells of the skin; and microtubules function as "railroad tracks" for intracellular movements, and are responsible for the separation of chromosomes during cell division. Other functions of all these filaments are discussed in Chapter 17.

ANSWER 1–13 It takes only 20 hours, i.e., less than a day, before mutant cells become more abundant in the culture. Using the equation provided in the question, we see that the number of the original ("wild-type") bacterial cells at time t minutes after the mutation occurred is $10^6 \times 2^{t/20}$. The number of mutant cells at time t is $1 \times 2^{t/15}$. To find out when the mutant cells "overtake" the wild-type cells, we simply have to make these two numbers equal to each other (i.e., $10^6 \times 2^{t/20} = 2^{t/15}$). Taking the logarithm to base 10 of both sides of this equation and solving it for t results in t = 1200 minutes (or 20 hours). At this time, the culture contains 2×10^{24} cells ($10^6 \times 2^{60} + 1 \times 2^{80}$). Incidentally, 2×10^{24} bacterial cells, each weighing 10^{-12} g, would weigh 2×10^{12} g (= 2×10^9 kg, or 2 million tons!). This can only have been a thought experiment.

ANSWER 1–14 Bacteria continually acquire mutations in their DNA. In the population of cells exposed to the poison, one or a few cells may harbor a mutation that makes them resistant to the action of the drug. Antibiotics that are poisonous to bacteria because they bind to certain bacterial proteins, for example, would not work if the proteins have a slightly changed surface so that binding occurs more weakly or not at all. These mutant bacteria would continue dividing rapidly while their cousins are slowed down. The antibiotic-resistant bacteria would soon become the predominant species in the culture.

ANSWER 1–15 $10^{13} = 2^{(t/1)}$. Therefore, it would take only 43 days [t = 13/log(2)]. This explains why some cancers can progress extremely rapidly. Many cancer cells divide

much more slowly, however, or die because of their internal abnormalities or because they do not have sufficient blood supply, and the actual progression of cancer is therefore usually slower.

ANSWER 1–16 Living cells evolved from nonliving matter, but grow and replicate. Like the material they originated from, they are governed by the laws of physics, thermodynamics, and chemistry. Thus, for example, they cannot create energy *de novo* or build ordered structures without the expenditure of free energy. We can understand virtually all cellular events, such as metabolism, catalysis, membrane assembly, and DNA replication, as complicated chemical reactions that can be experimentally reproduced, manipulated, and studied in test tubes.

Despite this fundamental reducibility, a living cell is more than the sum of its parts. We cannot randomly mix proteins, nucleic acids, and other chemicals together in a test tube, for example, and make a cell. The cell functions by virtue of its organized structure, and this is a product of its evolutionary history. Cells always come from preexisting cells, and the division of a mother cell passes both chemical constituents and structures to its daughters. The plasma membrane, for example, never has to form *de novo*, but grows by expansion of a preexisting membrane; there will always be a ribosome, in part made up of proteins whose function it is to make more proteins, including those that build more ribosomes.

ANSWER 1–17 In a multicellular organism, different cells take on specialized functions and cooperate with one another, so that any one cell type does not have to perform all activities for itself. Through such divisions of labor, multicellular organisms are able to exploit food sources that are inaccessible to single-celled organisms. A plant, for example, can reach the soil with its roots to take up water and nutrients, while at the same time, its leaves above ground can harvest light energy and CO_2 from the air. By protecting its reproductive cells with other specialized cells, the multicellular organism can develop new ways to survive in harsh environments or to fight off predators. When food runs out, it may be able to preserve its reproductive cells by allowing them to draw upon resources stored by their companions—or even to cannibalize relatives (a common process, in fact).

ANSWER 1–18 The volume and the surface area are 5.24×10^{-19} m^3 and 3.14×10^{-12} m^2 for the bacterial cell, and 1.77×10^{-15} m^3 and 7.07×10^{-10} m^2 for the animal cell, respectively. From these numbers, the surface-to-volume ratios are 6×10^6 m^{-1} and 4×10^5 m^{-1}, respectively. In other words, although the animal cell has a 3375-fold larger volume, its membrane surface is increased only 225-fold. If internal membranes are included in the calculation, however, the surface-to-volume ratios of both cells are about equal. Thus, because of their internal membranes, eukaryotic cells can grow bigger and still maintain a sufficiently large membrane area, which—as we shall discuss in more detail in later chapters—is required for many essential functions.

ANSWER 1–19 There are many lines of evidence for a common ancestor. Analyses of modern-day living cells show an amazing degree of similarity in the basic components that make up the inner workings of otherwise vastly different cells. Many metabolic pathways, for example, are conserved from one cell to another, and the compounds that make up nucleic acids and proteins are the same in all living cells, even though it is easy to imagine that a different choice of compounds (e.g., amino acids with different side chains) would have worked just as well. Similarly, it is not uncommon to find that important proteins have closely similar detailed structures in prokaryotic and eukaryotic cells. Theoretically, there would be many different ways to build proteins that could perform the same functions. The evidence overwhelmingly shows that most important processes were "invented" only once and then became fine-tuned during evolution to suit the particular needs of specialized cells and specific organisms.

It seems highly unlikely, however, that the first cell survived to become the primordial founder cell of today's living world. As evolution is not a directed process with a purposeful progression, it is more likely that there were a vast number of unsuccessful trial cells that replicated for a while and then became extinct because they could not adapt to changes in the environment or could not survive in competition with other types of cells. We can therefore speculate that the primordial ancestor cell was a "lucky" cell that ended up in a relatively stable environment in which it had a chance to replicate and evolve.

ANSWER 1–20 See **Figure A1–20**.

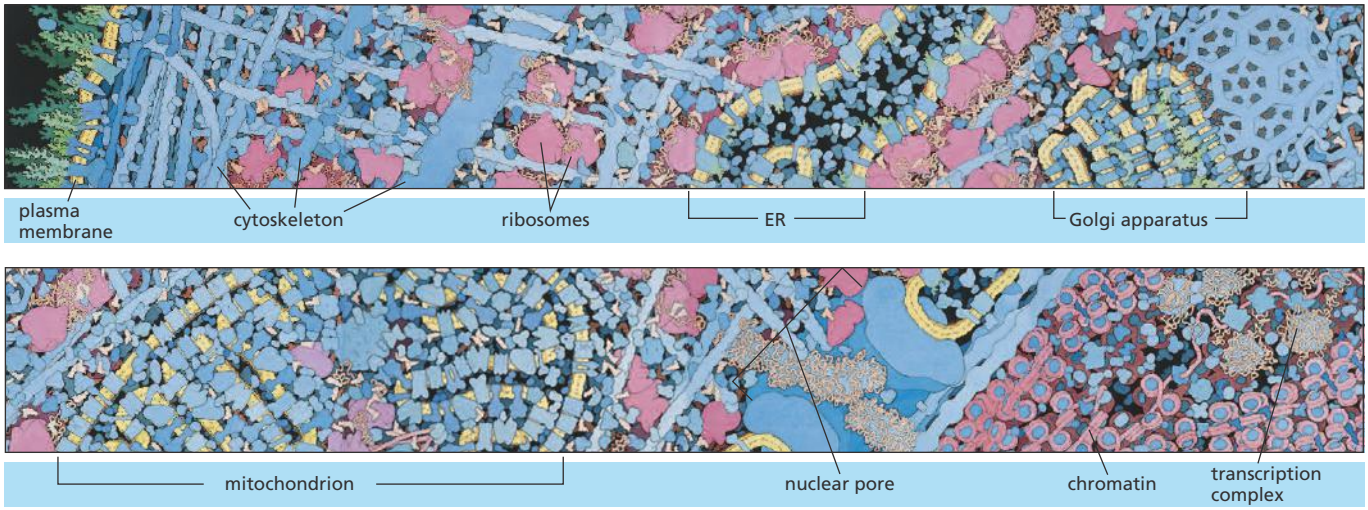


Figure A1–20 Courtesy of D. Goodsell.

ANSWER 1–21 A quick inspection might reveal beating cilia on the cell surface; their presence would tell you that the cell was eukaryotic. If you don't see them—and you are quite likely not to—you will have to look for other distinguishing features. If you are lucky, you might see the cell divide. Watch it then with the right optics, and you might be able to see condensed mitotic chromosomes, which again would tell you that it was a eukaryote. Fix the cell and stain it with a dye for DNA: if this is contained in a well-defined nucleus, the cell is a eukaryote; if you cannot see a well-defined nucleus, the cell may be a prokaryote. Alternatively, stain it with fluorescent antibodies that bind actin or tubulin (proteins that are highly conserved in eukaryotes but absent in bacteria). Embed it, section it, and look with an electron microscope: can you see organelles such as mitochondria inside your cell? Try staining it with the Gram stain, which is specific for molecules in the cell wall of some classes of bacteria. But all these tests might fail, leaving you still uncertain. For a definitive answer, you could attempt to analyze the sequences of the DNA and RNA molecules that it contains, using the sophisticated methods described later in this book. The sequences of highly conserved molecules, such as those that form the core components of the ribosome, provide a molecular signature that can tell you whether your cell is a eukaryote, a bacterium, or an archaeon. If you can't detect any RNA, you are probably looking not at a cell but at a piece of dirt.

Chapter 2

ANSWER 2–1 The chances are excellent because of the enormous size of Avogadro's number. The original cup contained one mole of water, or 6×10^{23} molecules, and the volume of the world's oceans, converted to cubic centimeters, is 1.5×10^{24} cm^3. After mixing, there should be on average 0.4 of an ancient water molecule per cm^3 ($6 \times 10^{23}/1.5 \times 10^{24}$), or 7.2 molecules in 18 g of Pacific Ocean.

ANSWER 2–2

A. The atomic number is 6; the atomic weight is 12 (= 6 protons + 6 neutrons).
B. The number of electrons is six (= the number of protons).
C. The first shell can accommodate two and the second shell eight electrons. Carbon therefore needs four additional electrons (or would have to give up four electrons) to obtain a full outermost shell. Carbon is most stable when it shares four additional electrons with other atoms (including other carbon atoms) by forming four covalent bonds.
D. Carbon 14 has two additional neutrons in its nucleus. Because the chemical properties of an atom are determined by its electrons, the chemical behavior of carbon 14 is identical to that of carbon 12.

ANSWER 2–3 The statement is correct. Both ionic and covalent bonds are based on the same principles: electrons can be shared equally between two interacting atoms, forming a nonpolar covalent bond; electrons can be shared unequally between two interacting atoms, forming a polar covalent bond; or electrons can be completely lost from one atom and gained by the other, forming an ionic bond. There are bonds of every conceivable intermediate state, and for borderline cases it becomes arbitrary whether a bond is described as a very polar covalent bond or an ionic bond.

ANSWER 2–4 The statement is correct. The hydrogen–oxygen bond in water molecules is polar, so that the oxygen atom carries a more negative charge than the hydrogen atoms. These partial negative charges are attracted to the positively charged sodium ions but are repelled from the negatively charged chloride ions.

ANSWER 2–5

A. Hydronium (H_3O^+) ions result from water dissociating into protons and hydroxyl ions, each proton binding to a water molecule to form a hydronium ion ($2H_2O \rightarrow H_2O + H^+ + OH^- \rightarrow H_3O^+ + OH^-$). At neutral pH, i.e., in the absence of an acid providing more H_3O^+ ions or a base providing more OH^- ions, the concentrations of H_3O^+ ions and OH^- ions are equal. We know that at neutrality the pH = 7.0, and therefore, the H^+ concentration is 10^{-7} M. The H^+ concentration equals the H_3O^+ concentration.
B. To calculate the ratio of H_3O^+ ions to H_2O molecules, we need to know the concentration of water molecules. The molecular weight of water is 18 (i.e., 18 g/mole), and 1 liter of water weighs 1 kg. Therefore, the concentration of water is 55.6 M (= 1000 [g/l]/[18 g/mole]), and the ratio of H_3O^+ ions to H_2O molecules is 1.8×10^{-9} (= $10^{-7}/55.6$); i.e., fewer than two water molecules in a billion are dissociated at neutral pH.

ANSWER 2–6 The synthesis of a macromolecule with a unique structure requires that in each amino acid position only one stereoisomer is used. Changing one amino acid from its L- to its D-form would result in a different protein. Thus, if for each amino acid a random mixture of the D- and L-forms were used to build a protein, its amino acid sequence could not specify a single structure, but many different structures (2^N different structures would be formed, where N is the number of amino acids in the protein).

Why L-amino acids were selected in evolution as the exclusive building blocks of proteins is a mystery; we could easily imagine a cell in which certain (or even all) amino acids were used in the D-forms to build proteins, as long as these particular stereoisomers were used exclusively.

ANSWER 2–7 The term "polarity" can be used in two ways. In one meaning, it refers to directional asymmetry—for example, in linear polymers such as polypeptides (which have an N-terminus and a C-terminus) or nucleic acids (which have a 3′ and a 5′ end). Because the covalent bonds that link the subunits together form only between the amino and the carboxyl groups of the amino acids in a polypeptide, and between the 3′ and the 5′ ends of nucleotides in a nucleic acid, polypeptides and nucleic acids always have two different ends, which give the chain a defined chemical polarity.

In the other meaning, polarity refers to a separation of electric charge in a bond or molecule. This kind of polarity promotes hydrogen-bonding to water molecules, and because the water solubility, or hydrophilicity, of a molecule depends upon its being polar in this sense, the term "polar" also indicates water solubility.

ANSWER 2–8 A major advantage of condensation reactions is that they are readily reversible by hydrolysis (and water is readily available in the cell). This allows cells to break down their macromolecules (or macromolecules of other organisms that were ingested as food) and to recover

the subunits intact so that they can be "recycled," i.e., used to build new macromolecules.

ANSWER 2–9 Many of the functions that macromolecules perform rely on their ability to associate with and dissociate from other molecules readily. This allows cells, for example, to remodel their interior when they move or divide, and to transport components from one organelle to another. Covalent bonds would be too stable for such a purpose, requiring a specific enzyme to break each kind of bond.

ANSWER 2–10

A. True. All nuclei are made of positively charged protons and uncharged neutrons; the only exception is the hydrogen nucleus, which consists of only one proton.
B. False. Atoms are electrically neutral. The number of positively charged protons is always balanced by an equal number of negatively charged electrons.
C. True—but only for the cell nucleus (see Chapter 1), and not for the atomic nucleus discussed in this chapter.
D. False. Elements can have different isotopes, which differ only in their number of neutrons.
E. True. In certain isotopes, the large number of neutrons destabilizes the nucleus, which decomposes in a process called radioactive decay.
F. True. Examples include granules of glycogen, a polymer of glucose, found in liver cells; and fat droplets, made of aggregated triacylglycerols, found in fat cells.
G. True. Individually, these bonds are weak and readily broken by thermal motion, but because interactions between two macromolecules involve a large number of such bonds, the overall binding can be quite strong, and because hydrogen bonds form only between correctly positioned groups on the interacting macromolecules, they are very specific.

ANSWER 2–11

A. One cellulose molecule has a molecular weight of $n \times (12[C] + 2 \times 1[H] + 16[O])$. We do not know n, but we can determine the ratio with which the individual elements contribute to the weight of cellulose. The contribution of carbon atoms is 40% [= 12/(12 + 2 + 16) × 100%]. Therefore, 2 g (40% of 5 g) of carbon atoms are contained in the cellulose that makes up this page. The atomic weight of carbon is 12 g/mole, and there are 6×10^{23} atoms or molecules in a mole. Therefore, 10^{23} carbon atoms [= (2 g/12 [g/mole]) × 6×10^{23} (molecules/mole)] make up this page.
B. The volume of the page is 4×10^{-6} m^3 (= 21.2 cm × 27.6 cm × 0.07 mm), which is the same as the volume of a cube with a side length of 1.6 cm (= $\sqrt[3]{4 \times 10^{-6}}$ m^2). Because we know from part A that the page contains 10^{23} carbon atoms, geometry tells us that there could be about 4.6×10^7 carbon atoms (= $\sqrt[3]{10^{23}}$) lined up along each side of this cube. Therefore, in cellulose, about 200,000 carbon atoms (= $4.6 \times 10^7 \times 0.07 \times 10^{-3}$ m/1.6×10^{-2} m) span the thickness of the page.
C. If tightly stacked, 350,000 carbon atoms with a 0.2-nm diameter would span the 0.07-mm thickness of the page.
D. There are two reasons for the 1.7-fold difference in the two calculations: (1) carbon is not the only atom in cellulose; and (2) paper is not an atomic lattice of precisely arranged cellulose molecules (as a diamond would be for precisely arranged carbon atoms), but a random meshwork of fibers.

ANSWER 2–12

A. The occupancies of the three electron shells, from the nucleus outward, are 2, 8, 8.
B.

helium	already has full level
oxygen	gain 2
carbon	gain 4 or lose 4
sodium	lose 1
chlorine	gain 1

C. Helium with its fully occupied electron shell is chemically unreactive. Sodium and chlorine, on the other hand, are extremely reactive and readily form Na^+ and Cl^- ions, which can form ionic bonds to produce NaCl (table salt).

ANSWER 2–13 Whether a substance is a liquid or gas at a given temperature depends on the attractive forces between its molecules. H_2S is a gas at room temperature and H_2O is a liquid because the hydrogen bonds that hold H_2O molecules together do not form between H_2S molecules. A sulfur atom is much larger than an oxygen atom, and because of its larger size, the outermost electrons are not as strongly attracted to the nucleus of the sulfur atom as they are in an oxygen atom. Consequently, the hydrogen–sulfur bond is much less polar than the hydrogen–oxygen bond. Because of the reduced polarity, the sulfur in a H_2S molecule is not strongly attracted to the hydrogen atoms in an adjacent H_2S molecule, and hydrogen bonds, which are so predominant in water, do not form.

ANSWER 2–14 The reactions are diagrammed in **Figure A2–14**, where R_1 and R_2 are amino acid side chains.

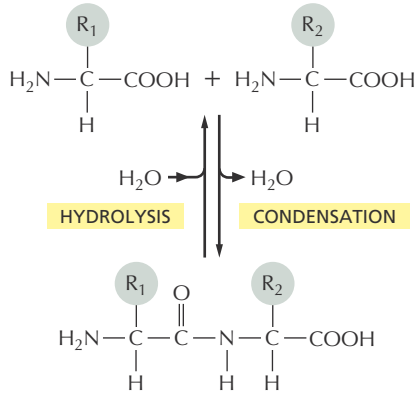


Figure A2–14

ANSWER 2–15

A. False. The properties of a protein depend on both the amino acids it contains and the order in which they are linked together. The diversity of proteins is due to the almost unlimited number of ways in which 20 different amino acids can be combined in a linear sequence.
B. False. Phospholipids assemble into bilayers in an aqueous environment by noncovalent forces. Lipid bilayers are therefore not macromolecules.
C. True. The backbone of nucleic acids is made up of alternating ribose (or deoxyribose in DNA) and phosphate groups. Ribose and deoxyribose are sugars.
D. True. About half of the 20 naturally occurring amino acids have hydrophobic side chains. In folded proteins, many of these side chains face toward the inside of a folded-up globular protein, because they are repelled from water.

E. True. Hydrophobic hydrocarbon tails contain only nonpolar bonds. Thus, they cannot participate in hydrogen-bonding and are repelled from water. We consider the underlying principles in more detail in Chapter 11.
F. False. RNA contains the four listed bases, but DNA contains T instead of U. T and U are very much alike, however, and differ only by a single methyl group.

ANSWER 2–16

A. (a) 400 (= 20^2); (b) 8000 (= 20^3); (c) 160,000 (= 20^4).
B. A protein with a molecular weight of 4800 daltons is made of about 40 amino acids; thus there are 1.1×10^{52} (= 20^{40}) different ways to make such a protein. Each individual protein molecule weighs 8×10^{-21} g (= $4800/6 \times 10^{23}$); thus a mixture of one molecule of each weighs 9×10^{31} g (= 8×10^{-21} g $\times$ 1.1×10^{52}), which is 15,000 times the total weight of the planet Earth, weighing 6×10^{24} kg. You would need a quite large container, indeed.
C. Given that most cell proteins are even larger than the one used in this example, it is clear that only a minuscule fraction of the total possible amino acid sequences are used in living cells.

ANSWER 2–17 Because all living cells are made up of chemicals and because all chemical reactions (whether in living cells or in test tubes) follow the same rules, an understanding of basic chemical principles is fundamentally important to the understanding of cell biology. For this reason, in later chapters, we will frequently refer back to these principles, on which all of the more complicated pathways and reactions that occur in cells are based.

ANSWER 2–18

A. Hydrogen bonds form between two specific chemical groups; one is always a hydrogen atom linked in a polar covalent bond to an oxygen or a nitrogen atom, and the other is usually a nitrogen or an oxygen atom. Van der Waals attractions are weaker and occur between any two atoms that are in close enough proximity. Both hydrogen bonds and van der Waals attractions are short-range interactions that come into play only when two molecules are already in close proximity. Both types of bonds can therefore be thought of as means of "fine-tuning" an interaction, i.e., helping to position two molecules correctly with respect to each other once they have been brought together by diffusion.
B. Van der Waals attractions would occur in all three examples. Hydrogen bonds would form only in (c).

ANSWER 2–19 Noncovalent bonds form between the covalently linked subunits of a macromolecule such as a polypeptide or RNA chain causing the chain to fold into a unique shape. These noncovalent bonds include hydrogen bonds, ionic interactions, van der Waals attractions, and hydrophobic interactions. Because these interactions are weak, they can be broken with relative ease; thus, most macromolecules can be unfolded by heating, which increases thermal motion.

ANSWER 2–20 Amphipathic molecules have both a hydrophilic and a hydrophobic end. Their hydrophilic end can hydrogen-bond to water, but their hydrophobic end is repelled from water because it interferes with the water structure. Consequently, the hydrophobic ends of amphipathic molecules tend to be exposed to air at air–water interfaces, or, in the interior of an aqueous solution, they will always cluster together to minimize their contact with water molecules. (See **Figure A2–20**.)

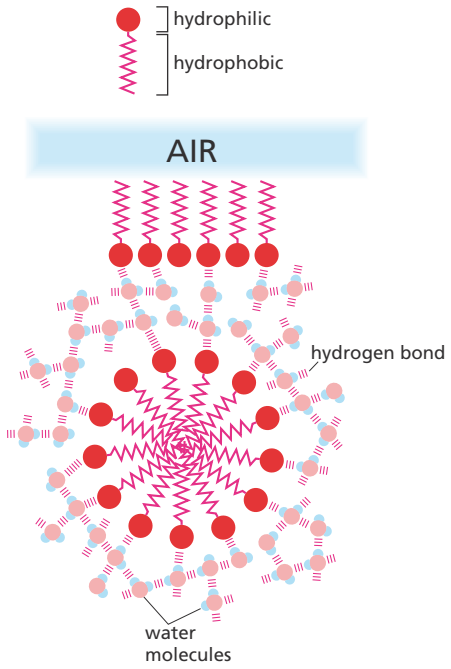


Figure A2–20

ANSWER 2–21

A,B. (A) and (B) are both correct formulas of the amino acid phenylalanine. In formula (B), phenylalanine is shown in the ionized form that exists in an aqueous solution, where the basic amino group is protonated and the acidic carboxylic group is deprotonated.
C. Incorrect. This structure of a peptide bond is missing a hydrogen atom bound to the nitrogen.
D. Incorrect. This formula of an adenine base features one double bond too many, creating a five-valent carbon atom and a four-valent nitrogen atom.
E. Incorrect. In this formula of a nucleoside triphosphate, there should be two additional oxygen atoms, one between each of the phosphorus atoms.
F. This is the correct formula of ethanol.
G. Incorrect. Water does not hydrogen-bond to hydrogens bonded to carbon. The lack of the capacity to hydrogen-bond makes hydrocarbon chains hydrophobic, i.e., water-hating.
H. Incorrect. Na and Cl form an ionic bond, Na^+Cl^-, but a covalent bond is drawn.
I. Incorrect. The oxygen atom attracts electrons more than the carbon atom; the polarity of the two bonds should therefore be reversed.
J. This structure of glucose is correct.
K. Almost correct. It is more accurate to show that only one hydrogen is lost from the $-NH_2$ group, and the –OH group is lost from the –COOH group.

Chapter 3

ANSWER 3–1 The equation represents the "bottom line" of photosynthesis, which occurs as a large set of individual reactions that are catalyzed by many individual enzymes. Because sugars are more complicated molecules than CO_2 and H_2O, the reaction generates a more ordered state inside the cell. As demanded by the second law of thermodynamics, this increase in order must be accompanied by a greater increase in disorder, which occurs because heat is generated at many steps on the long pathway leading to the products summarized in this equation.

ANSWER 3–2 Oxidation is defined as removal of electrons, and reduction represents a gain of electrons. Therefore, (A) is an oxidation, and (B) is a reduction. The *red* carbon atom in (C) remains largely unchanged; the neighboring carbon atom, however, loses a hydrogen atom (i.e., an electron and a proton) and hence becomes oxidized. The *red* carbon atom in (D) becomes oxidized because it loses a hydrogen atom, whereas the *red* carbon atom in (E) becomes reduced because it gains a hydrogen atom.

ANSWER 3–3

A. Both states of the coin, H and T, have an equal probability. There is therefore no driving force, i.e., no energy difference, that would favor H turning to T or vice versa. Therefore, $\Delta G° = 0$ for this reaction. However, a reaction proceeds if H and T coins are not present in the box in equal numbers. In this case, the concentration difference between H and T creates a driving force and $\Delta G \neq 0$; when the reaction reaches equilibrium—i.e., when there are equal numbers of H and T—$\Delta G = 0$.

B. The amount of shaking corresponds to the temperature, as it results in the "thermal" motion of the coins. The activation energy of the reaction is the energy that needs to be expended to flip the coin, i.e., to stand it on its rim, from where it can fall back facing either side up. Jigglase would speed up the flipping by lowering the energy required for this; it could, for example, be a magnet that is suspended above the box and helps lift the coins. Jigglase would not affect where the equilibrium lies (at an equal number of H and T), but it would speed up the process of reaching the equilibrium, because in the presence of jigglase more coins would flip back and forth.

ANSWER 3–4 See **Figure A3–4**. Note that $\Delta G°_{X \rightarrow Y}$ is positive, whereas $\Delta G°_{Y \rightarrow Z}$ and $\Delta G°_{X \rightarrow Z}$ are negative. The graph also shows that $\Delta G°_{X \rightarrow Z} = \Delta G°_{X \rightarrow Y} + \Delta G°_{Y \rightarrow Z}$. We do not know from the information given in Figure 3–12 how high the activation energy barriers are; they are therefore drawn to an arbitrary height (solid lines). The activation energies would be lowered by enzymes that catalyze these reactions, thereby speeding up the reaction rates (dotted lines), but the enzymes would not change the $\Delta G°$ values.

ANSWER 3–5 The reaction rates might be limited by (1) the concentration of the substrate, i.e., how often a molecule of CO_2 collides with the active site on the enzyme; (2) how many of these collisions are energetic enough to lead to a reaction; and (3) how fast the enzyme can release

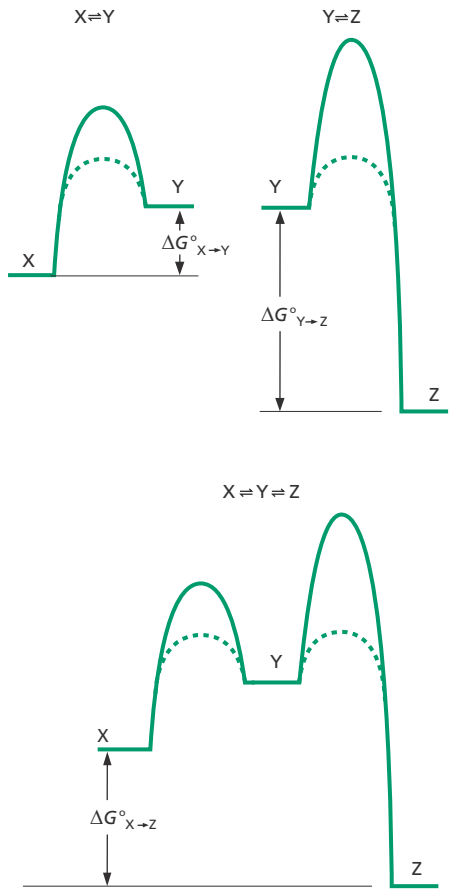


Figure A3–4

the products of the reaction and therefore be free to bind more CO_2. The diagram in **Figure A3–5** shows that the enzyme lowers the activation energy barrier, so that more CO_2 molecules have sufficient energy to undergo the reaction. The area under the curve from point A to infinite energy or from point B to infinite energy indicates the total number of molecules that will react without or with the enzyme, respectively. Although not drawn to scale, the ratio of these two areas should be 10^7.

ANSWER 3–6 All reactions are reversible. If the compound AB can dissociate to produce A and B, then it must also be possible for A and B to associate to form AB. Which of the two reactions predominates depends on the equilibrium constant of the reaction and the concentration of A, B, and AB (as discussed in Figure 3–19). Presumably, when this enzyme was isolated its activity was detected by

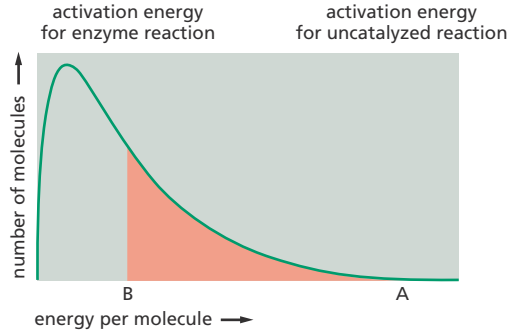


Figure A3–5

supplying A and B in relatively large amounts and measuring the amount of AB generated. But suppose, however, that in the cell there is a large concentration of AB, in which case the enzyme would actually catalyze AB → A + B. (This question is based on an actual example in which an enzyme was isolated and named according to the reaction in one direction, but was later shown to catalyze the reverse reaction in living cells.)

ANSWER 3–7

A. The rocks in Figure 3–30B provide the energy to lift the bucket of water. In the reaction $X + ATP \rightarrow Y + ADP + P_i$, ATP hydrolysis is driving the reaction; thus ATP corresponds to the rocks on top of the cliff. The broken debris in Figure 3–30B corresponds to ADP and P_i, the products of ATP hydrolysis. In the reaction, ATP hydrolysis is coupled to the conversion of X to Y. X, therefore, is the starting material, the bucket on the ground, which is converted to Y, the bucket at its highest point.

B. (i) The rock hitting the ground would be the futile hydrolysis of ATP—for example, in the absence of an enzyme that uses the energy released by the ATP hydrolysis to drive an otherwise unfavorable reaction; in this case, the energy stored in the phosphoanhydride bond of ATP would be lost as heat. (ii) The energy stored in Y could be used to drive another reaction. If Y represented the activated form of amino acid X, for example, it could undergo a condensation reaction to form a peptide bond during protein synthesis.

ANSWER 3–8 The free energy ΔG derived from ATP hydrolysis depends on both the $\Delta G°$ and the concentrations of the substrate and products. For example, for a particular set of concentrations, one might have

$$\Delta G = -12 \text{ kcal/mole} = -7.3 \text{ kcal/mole} + 0.616 \ln \frac{[ADP] \times [P_i]}{[ATP]}$$

ΔG is smaller than $\Delta G°$, largely because the ATP concentration in cells is high (in the millimolar range) and the ADP concentration is low (in the 10 μM range). The concentration term of this equation is therefore smaller than 1 and its logarithm is a negative number.

$\Delta G°$ is a constant for the reaction and will not vary with reaction conditions. ΔG, in contrast, depends on the concentrations of ATP, ADP, and phosphate, which can be somewhat different between cells.

ANSWER 3–9 Reactions B, D, and E all require coupling to other, energetically favorable reactions. In each case, higher-order structures are formed that are more complicated and have higher-energy bonds than the starting materials. In contrast, reaction A is a catabolic reaction that leads to compounds in a lower energy state and will occur spontaneously. The nucleoside triphosphates in reaction C contain enough energy to drive DNA synthesis (see Figure 3–41).

ANSWER 3–10

A. Nearly true, but strictly speaking, false. Because enzymes enhance the rate but do not change the equilibrium point of a reaction, a reaction will always occur in the absence of the enzyme, though often at a minuscule rate. Moreover, competing reactions may use up the substrate more quickly, thus further impeding the desired reaction. Thus, in practical terms, without an enzyme, some reactions may never occur to an appreciable extent.

B. False. High-energy electrons are more easily transferred, i.e., more loosely bound to the donor molecule. This does not mean that they move any faster.

C. True. Hydrolysis of an ATP molecule to form AMP also produces a pyrophosphate (PP_i) molecule, which in turn is hydrolyzed into two phosphate molecules. This second reaction releases almost the same amount of energy as the initial hydrolysis of ATP, thereby approximately doubling the energy total yield.

D. True. Oxidation is the removal of electrons, which reduces the diameter of the carbon atom.

E. True. ATP, for example, can donate both chemical-bond energy and a phosphate group.

F. False. Living cells have a particular kind of chemistry in which most oxidations are energy-releasing events; under different conditions, however, such as in a hydrogen-containing atmosphere, reductions would be energy-releasing events.

G. False. All cells, including those of cold- and warm-blooded animals, radiate comparable amounts of heat as a consequence of their metabolic reactions. For bacterial cells, for example, this becomes apparent when a compost pile heats up.

H. False. The equilibrium constant of the reaction $X \leftrightarrow Y$ remains unchanged. If Y is removed by a second reaction, more X is converted to Y so that the ratio of X to Y remains constant.

ANSWER 3–11 The free-energy difference ($\Delta G°$) between Y and X due to three hydrogen bonds is –3 kcal/mole. (Note that the free energy of Y is lower than that of X, because energy would need to be expended to break the bonds to convert Y to X. The value for $\Delta G°$ for the transition X → Y is therefore negative.) The equilibrium constant for the reaction is therefore about 100 (from Table 3–1, p. 98); i.e., there are about 100 times more molecules of Y than of X at equilibrium. An additional three hydrogen bonds would increase $\Delta G°$ to –6 kcal/mole and increase the equilibrium constant about another 100-fold to 10^4. Thus, relatively small differences in energy can have a major effect on equilibria.

ANSWER 3–12

A. The equilibrium constant is defined as $K = [AB]/([A] \times [B])$. The square brackets indicate the concentration. Thus, if A, B, and AB are each 1 μM (10^{-6} M), K will be 10^6 liters/mole [$= 10^{-6}/(10^{-6} \times 10^{-6})$].

B. Similarly, if A, B, and AB are each 1 nM (10^{-9} M), then K will be 10^9 liters/mole.

C. This example illustrates that interacting proteins that are present in cells in lower concentrations need to bind to each other with higher affinities so that a significant fraction of the molecules are bound at equilibrium. In this particular case, lowering the concentration by 1000-fold (from μM to nM) requires an increase in the equilibrium constant by 1000-fold to maintain the AB protein complex in the same proportion (corresponding to –4.3 kcal of free energy; see Table 3–1). This corresponds to about four or five extra hydrogen bonds.

ANSWER 3–13 The statement is correct. The criterion for whether a reaction proceeds spontaneously is ΔG, not $\Delta G°$,

and takes the concentrations of the reacting components into account. A reaction with a negative $\Delta G°$, for example, would not proceed spontaneously under conditions where there is a large enough excess of products, i.e., more than at equilibrium. Conversely, a reaction with a positive $\Delta G°$ might spontaneously go forward under conditions where there is a huge excess of substrate.

ANSWER 3–14

A. A maximum of 57 ATP molecules (= 686/12) corresponds to the total energy released by the complete oxidation of glucose to CO_2 and H_2O.
B. The overall efficiency of ATP production would be about 53%, calculated as the ratio of actually produced ATP molecules (30) divided by the number of ATP molecules that could be obtained if all the energy stored in a glucose molecule could be harvested as chemical energy in ATP (57).
C. During the oxidation of 1 mole of glucose, 322 kcal (the remaining 47% of the available 686 kcal in one mole of glucose that is not stored as chemical energy in ATP) would be released as heat. This amount of energy would heat your body by 4.3°C (= 322 kcal/75 kg). This is a significant amount of heat, considering that 4°C of elevated temperature would be a quite incapacitating fever and that 1 mole (180 g) of glucose is no more than two cups of sugar.
D. If the energy yield were only 20%, then instead of 47% in the example above, 80% of the available energy would be released as heat and would need to be dissipated by your body. The heat production would be more than 1.7-fold higher than normal, and your body would certainly overheat.
E. The chemical formula of ATP is $C_{10}H_{12}O_{13}N_5P_3$, and its molecular weight is therefore 503 g/mole. Your resting body therefore hydrolyzes about 80 moles (= 40 kg/0.503 kg/mole) of ATP in 24 hours (this corresponds to about 1000 kcal of liberated chemical energy). Because every mole of glucose yields 30 moles of ATP, this amount of energy could be produced by oxidation of 480 g glucose (= 180 g/mole × 80 moles/30).

ANSWER 3–15 This scientist is definitely a fake. The 57 ATP molecules would store 684 kcal (= 57 × 12 kcal) of chemical energy, which implies that the efficiency of ATP production from glucose would have been greater than 99%. This impossible degree of efficiency would leave virtually no energy to be released as heat, and this release is required according to the laws of thermodynamics.

ANSWER 3–16

A. From Table 3–1 (p. 98) we know that a free-energy difference of 4.3 kcal/mole corresponds to an equilibrium constant of 10^{-3}, i.e., $[A^*]/[A] = 10^{-3}$. The concentration of A* is therefore 1000-fold lower than that of A at equilibrium.
B. The ratio of A to A* would be unchanged. Lowering the activation energy barrier with an enzyme would accelerate the rate of the reaction, i.e., it would allow more molecules in a given time period to convert from A → A* and from A* → A, but it would not affect the ratio of A → A* at equilibrium.

ANSWER 3–17

A. The mutant mushroom would probably be safe to eat. ATP hydrolysis can provide approximately –12 kcal/mole of energy. This amount of energy shifts the equilibrium point of a reaction by an enormous factor: about 10^8-fold (from Table 3–1, p. 98, we see that –5.7 kcal/mole corresponds to an equilibrium constant of 10^4; thus, –12 kcal/mole corresponds to about 10^8. Note that, for coupled reactions, energies are additive, whereas equilibrium constants are multiplied). Therefore, if the energy of ATP hydrolysis cannot be utilized by the enzyme, 10^8-fold less poison is made. This example illustrates that coupling a reaction to the hydrolysis of an activated carrier molecule can shift the equilibrium point drastically.
B. It would be risky to consume this mutant mushroom. Slowing down the reaction rate would not affect its equilibrium point, and if the reaction were allowed to proceed for a long enough time, the mushroom would likely be loaded with poison. It is possible that the reaction would not reach equilibrium, but it would not be advisable to take a chance.

ANSWER 3–18 Enzyme A is beneficial. It allows the interconversion of two energy-carrier molecules, both of which are required as the triphosphate form for many metabolic reactions. Any ADP that is formed is quickly converted to ATP, and thus the cell maintains a high ATP/ADP ratio. Because of enzyme A, called nucleotide phosphokinase, some of the ATP is used to keep the GTP/GDP ratio similarly high.

Enzyme B would be highly detrimental to the cell. Cells use NAD^+ as an electron acceptor in catabolic reactions and must maintain high concentrations of this form of the carrier as it is used in reactions that break down glucose to make ATP. In contrast, NADPH is used as an electron donor in biosynthetic reactions and is kept at high concentration in the cells so as to allow the synthesis of nucleotides, fatty acids, and other essential molecules. Since enzyme B would deplete the cell's reserves of both NAD^+ and NADPH, it would decrease the rates of both catabolic and biosynthetic reactions.

ANSWER 3–19 Because enzymes are catalysts, enzyme reactions have to be thermodynamically feasible; the enzyme only lowers the activation energy barrier that otherwise slows the rate with which the reaction occurs. Heat confers more kinetic energy to substrates so that a higher fraction of them can surmount the normal activation energy barrier. Many substrates, however, have many different ways in which they could react, and all of these potential pathways will be enhanced by heat. An enzyme, by contrast, acts selectively to facilitate only one particular pathway that, in evolution, was selected to be useful for the cell. Heat, therefore, cannot substitute for enzyme function, and chicken soup must exert its claimed beneficial effects by other mechanisms, which remain to be discovered.

ANSWER 3–20

A. When $[S] << K_M$, the term $([S] + K_M)$ approaches K_M. Therefore, the equation is simplified to rate = $V_{max}[S]/K_M$. Therefore, the rate is proportional to [S].
B. When $[S] = K_M$, the term $[S]/([S] + K_M)$ equals ½. Therefore, the reaction rate is half of the maximal rate V_{max}.

C. If [S] >> K_M, the term ([S] + K_M) approaches [S]. Therefore, [S]/([S] + K_M) equals 1 and the reaction occurs at its maximal rate V_{max}.

ANSWER 3–21 The substrate concentration is 1 mM. This value can be obtained by substituting values in the equation, but it is simpler to note that the desired rate (50 μmole/sec) is exactly half of the maximum rate, V_{max}, where the substrate concentration is typically equal to the K_M. The two plots requested are shown in **Figure A3–21**. A plot of 1/rate versus 1/[S] is a straight line because rearranging the standard equation yields the equation listed in Question 3–23B.

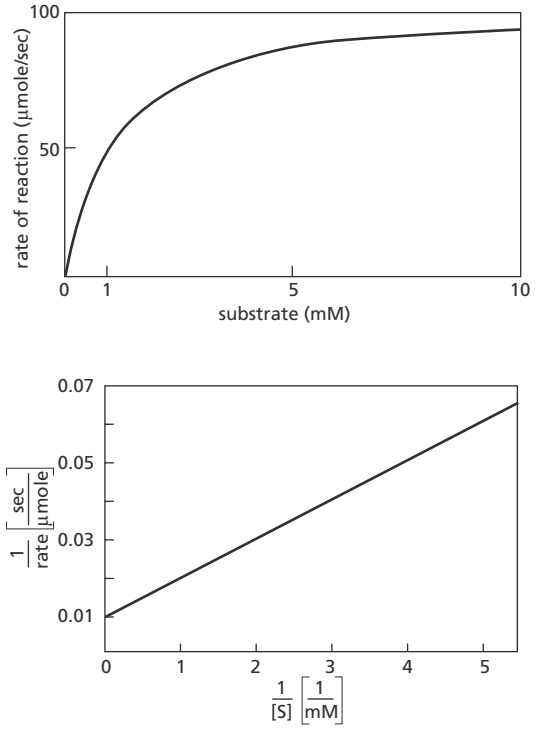


Figure A3–21

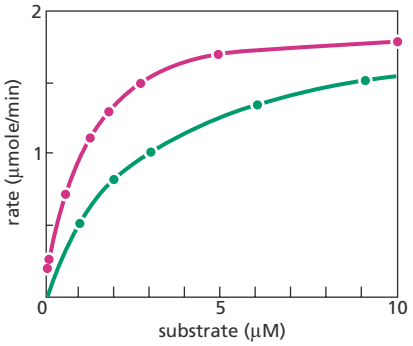
ANSWER 3–22 If [S] is very much smaller than K_M, the active site of the enzyme is mostly unoccupied. If [S] is very much greater than K_M, the reaction rate is limited by the enzyme concentration (because most of the catalytic sites are fully occupied).

ANSWER 3–23

A,B. The data in the boxes have been used to plot the red curve and red line in **Figure A3–23**. From the plotted data, the K_M is 1 μM and the V_{max} is 2 μmole/min. Note that the data are much easier to interpret in the linear plot, because the curve in (A) approaches, but never reaches, V_{max}.

C. It is important that only a small quantity of product is made, because otherwise the rate of reaction would decrease as the substrate was depleted and product accumulated. Thus the measured rates would be lower than they should be.

D. If the K_M increases, then the concentration of substrate needed to give a half-maximal rate is increased. As more substrate is needed to produce the same rate, the enzyme-catalyzed reaction has been inhibited by the phosphorylation. The expected data plots for the phosphorylated enzyme are the green curve and the green line in Figure A3–23.



DATA FOR A AND B

[S] (μM)	1/[S] [1/μM]	rate (μmole/min)	1/rate [min/μmole]
0.08	12.50	0.15	6.7
0.12	8.30	0.21	4.8
0.54	1.85	0.70	1.4
1.23	0.81	1.1	0.91
1.82	0.56	1.3	0.77
2.72	0.37	1.5	0.67
4.94	0.20	1.7	0.59
10.00	0.10	1.8	0.56

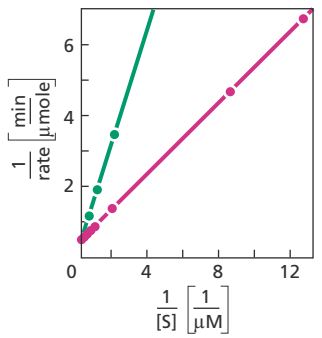


Figure A3–23

Chapter 4

ANSWER 4–1 Urea is a very small organic molecule that functions both as an efficient hydrogen-bond donor (through its $-NH_2$ groups) and as an efficient hydrogen-bond acceptor (through its –C=O group). As such, it can squeeze between hydrogen bonds that stabilize protein molecules and thus destabilize protein structures. In addition, the nonpolar side chains of a protein are held together in the interior of the folded structure because they would disrupt the structure of water if they were exposed. At high concentrations of urea, the hydrogen-bonded network of water molecules becomes disrupted so that these hydrophobic forces are significantly diminished. Proteins unfold in urea as a consequence of its effect on these two forces.

ANSWER 4–2 The amino acid sequence consists of alternating nonpolar and charged or polar amino acids. The resulting strand in a β sheet would therefore be polar on one side and hydrophobic on the other. Such a strand would probably be surrounded on either side by similar

strands that together form a β sheet with a hydrophobic face and a polar face. In a protein, such a β sheet (called "amphipathic," from the Greek *amphi,* "of both kinds," and *pathos*, "passion," because of its two surfaces with such different properties) would be positioned so that the hydrophobic side would face the protein's interior and the polar side would be on its surface, exposed to the water outside.

ANSWER 4–3 Mutations that are beneficial to an organism are selected in evolution because they confer a reproductive or survival advantage to the organism. Examples might be the better utilization of a food source, enhanced resistance to environmental insults, or an enhanced ability to attract a mate for sexual reproduction. In contrast, useless proteins are detrimental to organisms, as the metabolic energy required to make them is a wasted cost. If such mutant proteins were made in excess, the synthesis of normal proteins would suffer because the synthetic capacity of the cell is limited. In more severe cases, a mutant protein could interfere with the normal workings of the cell; a mutant enzyme that still binds an activated carrier molecule but does not catalyze a reaction, for example, may compete for a limited amount of this carrier and therefore inhibit normal processes. Natural selection therefore provides a strong driving force that eliminates both useless and harmful proteins.

ANSWER 4–4 Strong reducing agents that break all of the S–S bonds would cause all of the keratin filaments to separate. Individual hairs would be weakened and fragment. Indeed, strong reducing agents are used commercially in hair-removal creams sold by your local pharmacist. However, mild reducing agents are used in treatments that either straighten or curl hair, the latter requiring hair curlers. (See **Figure A4–4**.)

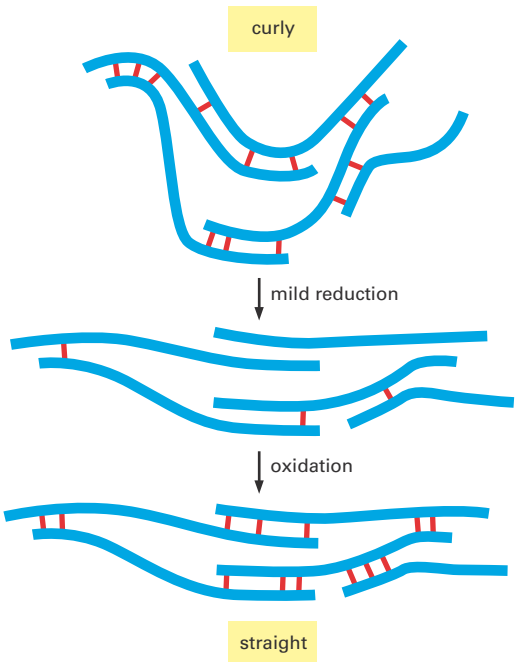


Figure A4–4

ANSWER 4–5 See **Figure A4–5**.

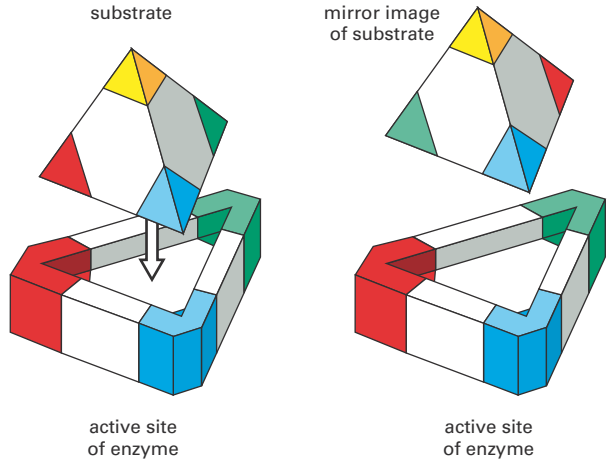


Figure A4–5

ANSWER 4–6

A. Feedback inhibition from Z that affects the reaction B → C would increase the flow through the B → X → Y → Z pathway, because the conversion of B to C is inhibited. Thus, the more Z there is, the more production of Z would be stimulated. This is likely to result in an uncontrolled "runaway" amplification of this pathway.
B. Feedback inhibition from Z affecting Y → Z would only inhibit the production of Z. In this scheme, however, X and Y would still be made at normal rates, even though both of these intermediates are no longer needed at this level. This pathway is therefore less efficient than the one shown in Figure 4–38.
C. If Z is a positive regulator of the step B → X, then the more Z there is, the more B will be converted to X and therefore shunted into the pathway producing more Z. This would result in a runaway amplification similar to that described for (A).
D. If Z is a positive regulator of the step B → C, then accumulation of Z leads to a redirection of the pathway to make more C. This is a second possible way, in addition to that shown in the figure, to balance the distribution of compounds into the two branches of the pathway.

ANSWER 4–7 Both nucleotide binding and phosphorylation can induce allosteric changes in proteins. These can have a multitude of consequences, such as altered enzyme activity, drastic shape changes, and changes in affinity for other proteins or small molecules. Both mechanisms are quite versatile. An advantage of nucleotide binding is the fast rate with which a small nucleotide can diffuse to the protein; the shape changes that accompany the function of motor proteins, for example, require quick nucleotide replenishment. If the different conformational states of a motor protein were controlled by phosphorylation, for example, a protein kinase would either need to diffuse into position at each step, a much slower process, or be associated permanently with each motor protein. One advantage of phosphorylation is that it requires only a single amino acid on the protein's surface, rather than a specific binding site. Phosphates can therefore

be added to many different side chains on the same protein (as long as protein kinases with the proper specificities exist), thereby vastly increasing the complexity of regulation that can be achieved for a single protein.

ANSWER 4–8 In working together in a complex, all three proteins contribute to the specificity (by binding to the safe and key directly). They help position one another correctly, and provide the mechanical bracing that allows them to perform a task that they could not perform individually (the key is grasped by two of the proteins, for example). Moreover, their functions are generally coordinated in time (for instance, the binding of ATP to one subunit is likely to require that ATP has already been hydrolyzed to ADP by another).

ANSWER 4–9 The α helix is right-handed. The three strands that form the large β sheet are antiparallel. There are no knots in the polypeptide chain, presumably because a knot would interfere with the folding of the protein into its three-dimensional conformation after protein synthesis.

ANSWER 4–10

A. True. Only a few amino acid side chains contribute to the active site. The rest of the protein is required to maintain the polypeptide chain in the correct conformation, provide additional binding sites for regulatory purposes, and localize the protein in the cell.
B. True. Some enzymes form covalent intermediates with their substrates (see middle panels of Figure 4–35); however, in all cases the enzyme is restored to its original structure after the reaction.
C. False. β sheets can, in principle, contain any number of strands because the two strands that form the rims of the sheet are available for hydrogen-bonding to other strands. (β sheets in known proteins contain from 2 to 16 strands.)
D. False. It is true that the specificity of an antibody molecule is exclusively contained in polypeptide loops on its surface; however, these loops are contributed by both the folded light and heavy chains (see Figure 4–33).
E. False. The possible linear arrangements of amino acids that lead to a stably folded protein domain are so few that most new proteins evolve by alteration of old ones.
F. True. Allosteric enzymes generally bind one or more molecules that function as regulators at sites that are distinct from the active site.
G. False. Although single noncovalent bonds are weak, many such bonds acting together are a major contributor to the three-dimensional structure of macromolecules.
H. False. Affinity chromatography separates specific macromolecules because of their interactions with specific ligands, not because of their charge.
I. False. The larger an organelle is, the more centrifugal force it experiences and the faster it sediments, despite an increased frictional resistance from the fluid through which it moves.

ANSWER 4–11 In an α helix and in the central strands of a β sheet, all of the N–H and C=O groups in the polypeptide backbone are engaged in hydrogen bonds. This gives considerable stability to these secondary structural elements, and it allows them to form in many different proteins.

ANSWER 4–12 No. It would not have the same or even a similar structure, because the peptide bond has a polarity. Looking at two sequential amino acids in a polypeptide chain, the amino acid that is closer to the N-terminal end contributes the carboxyl group and the other amino acid contributes the amino group to the peptide bond that links the two amino acids. Changing their order would put the side chains into different positions with respect to the peptide backbone and therefore change the way the polypeptide folds.

ANSWER 4–13 As it takes 3.6 amino acid residues to complete a turn of an α helix, this sequence of 14 amino acids would make close to 4 full turns. It is remarkable because its polar and hydrophobic amino acids are spaced so that all the polar ones are on one side of the α helix and all the hydrophobic residues are on the other. It is therefore likely that such an amphipathic α helix is exposed on the protein surface with its hydrophobic side facing the protein's interior. In addition, two such helices might wrap around each other as shown in Figure 4–16.

ANSWER 4–14

A. ES represents the enzyme–substrate complex.
B. Enzyme and substrate are in equilibrium between their free and bound states; once bound to the enzyme, a substrate molecule may either dissociate again (hence the bidirectional arrows) or be converted to product. As the substrate is converted to product (with the concomitant release of free energy), however, a reaction usually proceeds strongly in the forward direction, as indicated by the unidirectional arrow.
C. The enzyme is a catalyst and is therefore liberated in an unchanged form after the reaction; thus, E appears at both ends of the equation.
D. Often, the product of a reaction resembles the substrate sufficiently that it can also bind to the enzyme. Any enzyme molecules that are bound to the product (i.e., are part of the EP complex) are unavailable for catalysis; excess P therefore inhibits the reaction by lowering the concentration of free E.
E. Compound X would act as an inhibitor of the reaction and work similarly by forming an EX complex. However, since P has to be made before it can inhibit the reaction, it takes longer to act than X, which is present from the beginning of the reaction.

ANSWER 4–15 The polar amino acids Ser, Ser-P, Lys, Gln, His, and Glu are more likely to be found on a protein's surface, and the hydrophobic amino acids Leu, Phe, Val, Ile, and Met are more likely to be found in its interior. The oxidation of two cysteine residues to form a disulfide bond eliminates their potential to form hydrogen bonds and therefore makes them even more hydrophobic; thus disulfide bonds are usually found in the interior of proteins. Irrespective of the nature of their side chains, the most N-terminal amino acid and the most C-terminal amino acid each contain a charged group (the amino and carboxyl groups, respectively, that mark the ends of the polypeptide chain) and hence are usually found on the protein's surface.

ANSWER 4–16 Many secondary structural elements are not stable in isolation but are stabilized by other parts of the polypeptide chain. Hydrophobic regions of fragments, which would normally be hidden in the inside of a folded protein,

would be exposed to water in an aqueous solution; such fragments would tend to aggregate nonspecifically, and not have a defined structure, and they would be inactive for ligand binding, even if they contained all of the amino acids that would normally contribute to the ligand-binding site. A protein domain, in contrast, is considered a folding unit, and fragments of a polypeptide chain that correspond to intact domains are often able to fold correctly. Thus, separated protein domains often retain their activities, such as ligand binding, if the binding site is contained entirely within the domain. Thus the most likely place in which the polypeptide chain of the protein in Figure 4–19 could be severed to give rise to stable fragments is at the boundary between the two domains (i.e., at the loop between the two α helices at the bottom right of the structure shown).

ANSWER 4–17 Because of the lack of secondary structure, the C-terminal region of neurofilament proteins undergoes continual Brownian motion. The high density of negatively charged phosphate groups means that the C-terminals also experience repulsive interactions, which cause them to stand out from the surface of the neurofilament like the bristles of a brush. In electron micrographs of a cross section of an axon, the region occupied by the extended C-terminals appears as a clear zone around each neurofilament, from which organelles and other neurofilaments are excluded.

ANSWER 4–18 The heat-inactivation of the enzyme suggests that the mutation causes the enzyme to have a less stable structure. For example, a hydrogen bond that is normally formed between two amino acid side chains might no longer be formed because the mutation replaces one of these amino acids with a different one that cannot participate in the bond. Lacking such a bond that normally helps to keep the polypeptide chain folded properly, the protein partially or completely unfolds at a temperature at which it would normally be stable. Polypeptide chains that denature when the temperature is raised often aggregate, and they rarely refold into active proteins when the temperature is decreased.

ANSWER 4–19 The motor protein in the illustration can move just as easily to the left as to the right and so will not move steadily in one direction. However, if just one of the steps is coupled to ATP hydrolysis (for example, by making detachment of one foot dependent on binding of ATP and coupling the reattachment to hydrolysis of the bound ATP), then the protein will show unidirectional movement that requires the continued consumption of ATP. Note that, in principle, it does not matter which step is coupled to ATP hydrolysis (**Figure A4–19**).

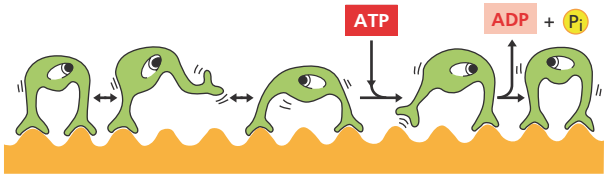


Figure A4–19

ANSWER 4–20 The slower migration of small molecules through a gel-filtration column occurs because smaller molecules have access to many more spaces in the porous beads that are packed into the column than do larger molecules. However, it is important that the flow rate through the column is slow enough to give the smaller molecules sufficient time to diffuse into the spaces inside the beads. At very rapid flow rates, all molecules will move rapidly around the beads, so that large and small molecules will now tend to exit together from the column.

ANSWER 4–21 The α helix in the figure is right-handed, whereas the coiled-coil is left-handed. The reversal occurs because of the staggered positions of hydrophobic side chains in the α helix.

ANSWER 4–22 The atoms at the binding sites of proteins must be precisely located to fit the molecules that they bind. Their location in turn requires the precise positioning of many of the amino acids and their side chains in the core of the protein, distant from the binding site itself. Thus, even a small change in this core can disrupt protein function by altering the conformation at a binding site far away.

Chapter 5

ANSWER 5–1

A. False. The polarity of a DNA strand commonly refers to the orientation of its sugar–phosphate backbone, one end of which contains a phosphate group and the other a hydroxyl group.
B. True. G-C base pairs are held together by three hydrogen bonds, whereas A-T base pairs are held together by only two.

ANSWER 5–2 Histone octamers occupy about 9% of the volume of the nucleus. The volume of the nucleus is

$$V = 4/3 \times 3.14 \times (3 \times 10^3 \text{ nm})^3$$

$$V = 1.13 \times 10^{11} \text{ nm}^3$$

The volume of the histone octamers is

$$V = 3.14 \times (4.5 \text{ nm})^2 \times (5 \text{ nm}) \times (32 \times 10^6)$$

$$V = 1.02 \times 10^{10} \text{ nm}^3$$

The ratio of the volume of histone octamers to the nuclear volume is 0.09; thus, histone octamers occupy about 9% of the nuclear volume. Because the DNA also occupies about 9% of the nuclear volume, together they occupy about 18% of the volume of the nucleus.

ANSWER 5–3 In contrast to most proteins, which accumulate amino acid changes over evolutionary time, the functions of histone proteins must involve nearly all of their amino acids, so that a change in any position would be deleterious to the cell.

ANSWER 5–4 Men have only one copy of the X chromosome in their cells; a defective gene carried on it therefore has no backup copy. Women, on the other hand, have two copies of the X chromosome in their cells, one inherited from each parent, so a defective copy of the gene on one X chromosome can generally be compensated for by a normal copy on the other chromosome. This is the case with regard to the gene that causes color blindness. However, during female development, one X chromosome in each cell is inactivated by compaction into

heterochromatin, shutting down gene expression from that chromosome (see Figure 5–30). This occurs at random in each cell to one or the other of the two X chromosomes, and therefore some cells of the woman will express the mutant copy of the gene, whereas others will express the normal copy. This results in a retina in which on average only every other cone cell is color sensitive, and women carrying the mutant gene on one X chromosome therefore see colored objects with reduced resolution.

A woman who is color-blind must have two defective copies of this gene, one inherited from each parent. Her father must therefore carry the mutation on his X chromosome; because this is his only copy of the gene, he would be color-blind. Her mother could carry the defective gene on either or both of her X chromosomes: if she carried it on both, she would be color-blind; if she carried it on one, she would have color vision but reduced resolution, as described above. Several different types of inherited color blindness are found in the human population; this question applies to only one type.

ANSWER 5–5

A. The complementary strand reads 5′-TGATTGTGGACAAAAATCC-3′. Paired DNA strands have opposite polarity, and the convention is to write a single-stranded DNA sequence in the 5′-to-3′ direction.

B. The DNA is made of four nucleotides (100% = 13% A + *x*% T + *y*% G + *z*% C). Because A pairs with T, the two nucleotides are represented in equimolar proportions in DNA. Therefore, the bacterial DNA in question contains 13% thymidine. This leaves 74% [= 100% – (13% + 13%)] for G and C, which also form base pairs and hence are equimolar. Thus $y = z = 74/2 = 37$.

C. A single-stranded DNA molecule that is *N* nucleotides long can have any one of 4^N possible sequences, but the number of possible double-stranded DNA molecules is more difficult to calculate. Many of the 4^N single-stranded sequences will be the complement of another possible sequence in the list; for example, 5′-AGTCC-3′ and 5′-GGACT-3′ form the same double-stranded DNA molecule and therefore count as a single, double-stranded possibility. If *N* is an odd number, then every single-stranded sequence will complement another sequence in the list so that the number of double-stranded sequences will be 0.5×4^N. If *N* is an even number, then there will be slightly more than this, since some sequences will be self-complementary (such as 5′-ACTAGT-3′) and the actual value can be calculated to be $0.5 \times 4^N + 0.5 \times 4^{N/2}$.

D. To specify a unique sequence which is *N* nucleotides long, 4^N has to be larger than 3×10^6. Thus, $4^N > 3 \times 10^6$, solved for *N*, gives $N > \ln(3 \times 10^6)/\ln(4) = 10.7$. Thus, on average, a sequence of only 11 nucleotides in length is unique in the genome. Performing the same calculation for the genome size of an animal cell yields a minimal stretch of 16 nucleotides. This shows that a relatively short sequence can mark a unique position in the genome and is sufficient, for example, to serve as an identity tag for one specific gene.

ANSWER 5–6 If the wrong bases were frequently incorporated during DNA replication, genetic information could not be inherited accurately. Life, as we know it, could not exist. Although the bases can form hydrogen-bonded pairs as indicated, these do not fit into the structure of the double helix. The angle with which the A residue is attached to the sugar–phosphate backbone is vastly different in the A-C pair, and the spacing between the two sugar–phosphate strands is considerably increased in the A-G pair, where two large purine rings interact. Consequently, it is energetically unfavorable to incorporate a wrong base in DNA, and such errors occur only very rarely.

ANSWER 5–7

A. The bases V, W, X, and Y can form a DNA-like double-helical molecule with virtually identical properties to those of bona fide DNA. V would always pair with X, and W with Y. Therefore, the macromolecules could be derived from a living organism that uses the same principles to replicate its genome as those used by organisms on Earth. In principle, different bases, such as V, W, X, and Y, could have been selected during evolution on Earth as building blocks for DNA. (Similarly, there are many more conceivable amino acid side chains than the set of 20 selected in evolution that make up all proteins.)

B. None of the bases V, W, X, or Y can replace A, T, G, or C. To preserve the distance between the two sugar–phosphate strands in a double helix, a pyrimidine always has to pair with a purine (see, for example, Figure 5–6). Thus, the eight possible combinations would be V-A, V-G, W-A, W-G, X-C, X-T, Y-C, and Y-T. Because of the positions of hydrogen-bond acceptors and hydrogen-bond donor groups, however, no stable base pairs would form in any of these combinations, as shown for the pairing of V and A in **Figure A5–7**, where only a single hydrogen bond could form.

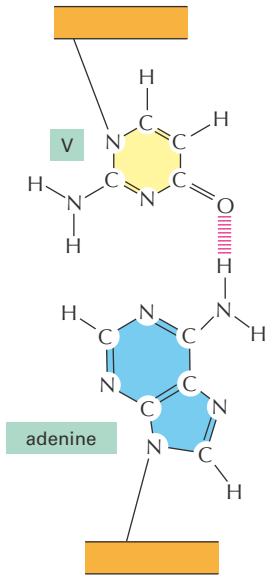


Figure A5–7

ANSWER 5–8 As the two strands are held together by hydrogen bonds between the bases, the stability of a DNA double helix is largely dependent on the number of hydrogen bonds that can be formed. Thus two parameters determine the stability: the number of nucleotide pairs and the number of hydrogen bonds that each nucleotide pair

contributes. As shown in Figure 5–6, an A-T pair contributes two hydrogen bonds, whereas a G-C pair contributes three hydrogen bonds. Therefore, helix C (containing a total of 34 hydrogen bonds) would melt at the lowest temperature, helix B (containing a total of 65 hydrogen bonds) would melt next, and helix A (containing a total of 78 hydrogen bonds) would melt last. Helix A is the most stable, largely owing to its high GC content. Indeed, the DNA of organisms that grow in extreme temperature environments, such as certain prokaryotes that grow in geothermal vents, has an unusually high GC content.

ANSWER 5–9 The DNA would be enlarged by a factor of 2.5×10^6 ($= 5 \times 10^{-3}/2 \times 10^{-9}$ m). Thus the extension cord would be 2500 km long. This is approximately the distance from London to Istanbul, San Francisco to Kansas City, Tokyo to the southern tip of Taiwan, and Melbourne to Cairns. Adjacent nucleotides would be about 0.85 mm apart (which is only about the thickness of a stack of 12 pages of this book). A gene that is 1000 nucleotide pairs long would be about 85 cm in length.

ANSWER 5–10

A. It takes two bits to specify each nucleotide pair (for example, 00, 01, 10, and 11 would be the binary codes for the four different nucleotides, each paired with its appropriate partner).
B. The entire human genome (3×10^9 nucleotide pairs) could be stored on two CDs ($3 \times 10^9 \times 2$ bits/4.8×10^9 bits).

ANSWER 5–11

A. True.
B. False. Nucleosome core particles are approximately 11 nm in diameter.

ANSWER 5–12 The definitions of the terms can be found in the Glossary. DNA assembles with specialized proteins to form *chromatin*. At a first level of packing, *histones* form the core of *nucleosomes*. In a nucleosome, the DNA is wrapped almost twice around this core. Between nuclear divisions—that is, in interphase—the *chromatin* of the *interphase chromosomes* is in a relatively extended form in the nucleus, although some regions of it, the *heterochromatin*, remain densely packed and are transcriptionally inactive. During nuclear division—that is, in mitosis—replicated chromosomes become condensed into *mitotic chromosomes*, which are transcriptionally inactive and are designed to be readily distributed between the two daughter cells.

ANSWER 5–13 Colonies are clumps of cells that originate from a single founder cell and grow outward as the cells divide again and again. In the lower colony of Figure Q5–13, the *Ade2* gene is inactivated when placed near a telomere, but apparently it can become spontaneously activated in a few cells, which then turn white. Once activated in a cell, the *Ade2* gene continues to be active in the descendants of that cell, resulting in clumps of white cells (the white sectors) in the colony. This result shows both that the inactivation of a gene positioned close to a telomere can be reversed and that this change is passed on to further generations. This change in *Ade2* expression probably results from a spontaneous decondensation of the chromatin structure around the gene.

ANSWER 5–14 In the electron micrographs, one can detect chromatin regions of two different densities; the densely stained regions correspond to heterochromatin, while less condensed chromatin is more lightly stained. The chromatin in A is mostly in the form of condensed, transcriptionally inactive heterochromatin, whereas most of the chromatin in B is decondensed and therefore potentially transcriptionally active. The nucleus in A is from a reticulocyte, a red blood cell precursor, which is largely devoted to making a single protein, hemoglobin. The nucleus in B is from a lymphocyte, which is active in transcribing many different genes.

ANSWER 5–15 Helix A is right-handed. Helix C is left-handed. Helix B has one right-handed strand and one left-handed strand. There are several ways to tell the handedness of a helix. For a vertically oriented helix, like the ones in Figure Q5–15, if the strands in front point up to the right, the helix is right-handed; if they point up to the left, the helix is left-handed. Once you are comfortable identifying the handedness of a helix, you will be amused to note that nearly 50% of the "DNA" helices shown in advertisements are left-handed, as are a surprisingly high number of the ones shown in books. Amazingly, a version of Helix B was used in advertisements for a prominent international conference, celebrating the 30-year anniversary of the discovery of the DNA helix.

ANSWER 5–16 The packing ratio within a nucleosome core is 4.5 [(147 bp × 0.34 nm/bp)/(11 nm) = 4.5]. If there is an additional 54 bp of linker DNA, then the packing ratio for "beads-on-a-string" DNA is 2.3 [(201 bp × 0.34 nm/bp)/(11 nm + {54 bp × 0.34 nm/bp}) = 2.3]. This first level of packing represents only 0.023% (2.3/10,000) of the total condensation that occurs at mitosis.

Chapter 6

ANSWER 6–1

A. The distance between replication forks 4 and 5 is about 280 nm, corresponding to 824 nucleotides (= 280/0.34). These two replication forks would collide in about 8 seconds. Forks 7 and 8 move away from each other and would therefore never collide.
B. The total length of DNA shown in the electron micrograph is about 1.5 μm, corresponding to 4400 nucleotides. This is only about 0.002% [= (4400/1.8×10^8) × 100%] of the total DNA in a fly cell.

ANSWER 6–2 Although the process may seem wasteful, it is not possible to proofread during the initial stages of primer synthesis. To start a new primer on a piece of single-stranded DNA, one nucleotide needs to be put in place and then linked to a second and then to a third, and so on. Even if these first nucleotides were perfectly matched to the template strand, they would bind with very low affinity, and it would consequently be difficult to distinguish the correct from incorrect bases by a hypothetical primase with proofreading activity; the enzyme would therefore stall. The task of the primase is to "just polymerize nucleotides that bind reasonably well to the template without worrying too much about accuracy." Later, these sequences are removed and replaced by DNA polymerase, which uses newly synthesized (and therefore proofread) DNA as its primer.

ANSWER 6–3

A. Without DNA polymerase, no replication can take place at all. RNA primers will be laid down at the origin of replication.
B. DNA ligase links the DNA fragments that are produced on the lagging strand. In the absence of ligase, the newly replicated DNA strands will remain as fragments, but no nucleotides will be missing.
C. Without the sliding clamp, the DNA polymerase will frequently fall off the DNA template. In principle, it can rebind and continue, but the continual falling off and rebinding will be time-consuming and will greatly slow down DNA replication.
D. In the absence of RNA-excision enzymes, the RNA fragments will remain covalently attached to the newly replicated DNA fragments. No ligation will take place, because the DNA ligase will not link DNA to RNA. The lagging strand will therefore consist of fragments composed of both RNA and DNA.
E. Without DNA helicase, the DNA polymerase will stall because it cannot separate the strands of the template DNA ahead of it. Little or no new DNA will be synthesized.
F. In the absence of primase, RNA primers cannot begin on either the leading or the lagging strand. DNA replication therefore cannot begin.

ANSWER 6–4 DNA damage by deamination and depurination reactions occurs spontaneously. This type of damage is not the result of replication errors and is therefore equally likely to occur on either strand. If DNA repair enzymes recognized such damage only on newly synthesized DNA strands, half of the defects would go uncorrected. The statement is therefore incorrect.

ANSWER 6–5 If the old strand were "repaired" using the new strand that contains a replication error as the template, then the error would become a permanent mutation in the genome. The old information would be erased in the process. Therefore, if repair enzymes did not distinguish between the two strands, there would be only a 50% chance that any given replication error would be corrected.

ANSWER 6–6 The argument is severely flawed. You cannot transform one species into another simply by introducing random changes into the DNA. It is exceedingly unlikely that the 5000 mutations that would accumulate every day in the absence of the DNA repair enzyme would be in the very positions where human and chimpanzee DNA sequences are different. It is very likely that, at such a high mutation frequency, many essential genes would be inactivated, leading to cell death. Furthermore, your body is made up of about 10^{13} cells. For you to turn into an ape, not just one but many of these cells would need to be changed. And even then, many of these changes would have to occur during development to effect changes in your body plan (making your arms longer than your legs, for example).

ANSWER 6–7

A. False. Identical DNA polymerase molecules catalyze DNA synthesis on the leading and lagging strands of a bacterial replication fork. The replication fork is asymmetrical because the lagging strand is synthesized in pieces that are then stitched together.
B. False. Only the RNA primers are removed by an RNA nuclease; Okazaki fragments are pieces of newly synthesized DNA on the lagging strand that are eventually joined together by DNA ligase.
C. True. With proofreading, DNA polymerase has an error rate of one mistake in 10^7 nucleotides polymerized; 99% of its errors are corrected by DNA mismatch repair enzymes, bringing the final error rate to one in 10^9.
D. True. Mutations would accumulate rapidly, inactivating many genes.
E. True. If a damaged nucleotide also occurred naturally in DNA, the repair enzyme would have no way of identifying the damage. It would therefore have only a 50% chance of fixing the right strand.
F. True. Usually, multiple mutations of specific types need to accumulate in a somatic cell lineage to produce a cancer. A mutation in a gene that codes for a DNA repair enzyme can make a cell more liable to accumulate further mutations, thereby accelerating the onset of cancer.

ANSWER 6–8 With a single origin of replication, which launches two DNA polymerases in opposite directions on the DNA, each moving at 100 nucleotides per second, the number of nucleotides replicated in 24 hours will be 1.73×10^7 ($= 2 \times 100 \times 24 \times 60 \times 60$). To replicate all the 6×10^9 nucleotides of DNA in the cell in this time, therefore, will require at least 348 ($= 6 \times 10^9 / 1.73 \times 10^7$) origins of replication. The estimated 10,000 origins of replication in the human genome are therefore more than enough to satisfy this minimum requirement.

ANSWER 6–9

A. Dideoxycytosine triphosphate (ddCTP) is identical to dCTP, except it lacks the 3′-hydroxyl group on the sugar ring. ddCTP is recognized by DNA polymerase as dCTP and becomes incorporated into DNA; because it lacks the crucial 3′-hydroxyl group, however, its addition to a growing DNA strand creates a dead end to which no further nucleotides can be added. Thus, if ddCTP is added in large excess, new DNA strands will be synthesized until the first G (the nucleotide complementary to C) is encountered on the template strand. ddCTP will then be incorporated instead of C, and the extension of this strand will be terminated.
B. If ddCTP is added at about 10% of the concentration of the available dCTP, there is a 1 in 10 chance of its being incorporated whenever a G is encountered on the template strand. Thus a population of DNA fragments will be synthesized, and from their lengths one can deduce where the G residues are located on the template strand. This strategy forms the basis of methods used to determine the sequence of nucleotides in a stretch of DNA (discussed in Chapter 10).

   The same chemical phenomenon is exploited by a drug, 3′-azido-3′-deoxythymidine (AZT), that is now commonly used in HIV-infected patients to treat AIDS. AZT is converted in cells to the triphosphate form and is incorporated into the growing viral DNA. Because the drug lacks a 3′-OH group, it blocks DNA synthesis and replication of the virus. AZT inhibits viral replication preferentially because reverse transcriptase has a higher affinity for the drug than for thymidine triphosphate; human cellular DNA polymerases do not show this preference.

C. Dideoxycytosine monophosphate (ddCMP) lacks the 5′-triphosphate group as well as the 3′-hydroxyl group of the sugar ring. It therefore cannot provide the energy that drives the polymerization reaction of nucleotides into DNA and therefore will not be incorporated into the replicating DNA. Addition of this compound should not affect DNA replication.

ANSWER 6–10 See **Figure A6–10**.

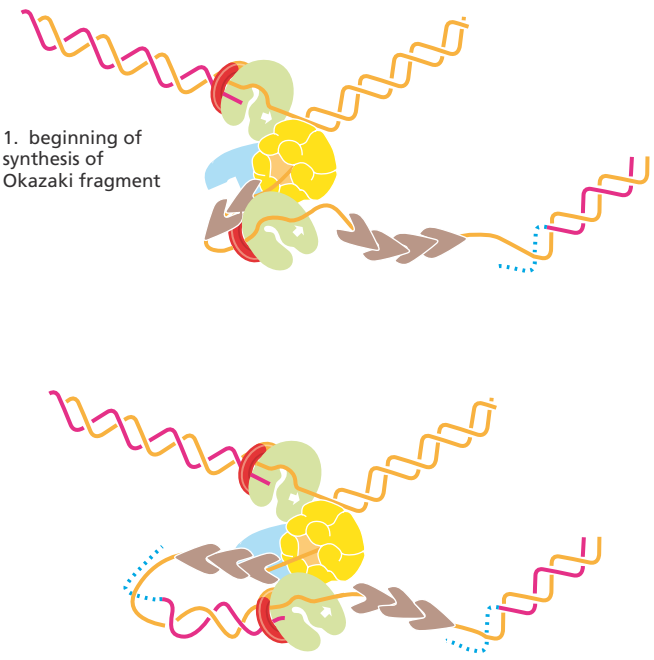


Figure A6–10

ANSWER 6–11 Both strands of the bacterial chromosome contain 6×10^6 nucleotides. During the polymerization of nucleoside triphosphates into DNA, two phosphoanhydride bonds are broken for each nucleotide added: the nucleoside triphosphate is hydrolyzed to produce the nucleoside monophosphate added to the growing DNA strand, and the released pyrophosphate is hydrolyzed to phosphate. Therefore, 1.2×10^7 high-energy bonds are hydrolyzed during each round of bacterial DNA replication. This requires 4×10^5 (= $1.2 \times 10^7/30$) glucose molecules, which weigh 1.2×10^{-16} g (= 4×10^5 molecules × 180 g/mole/6×10^{23} molecules/mole), which is 0.01% of the total weight of the cell.

ANSWER 6–12 The statement is correct. If the DNA in somatic cells is not sufficiently stable (that is, if it accumulates mutations too rapidly), the organism dies (of cancer, for example), and because this may often happen before the organism can reproduce, the species will die out. If the DNA in reproductive cells is not sufficiently stable, many mutations will accumulate and be passed on to future generations, so that the species will not be maintained.

ANSWER 6–13 As shown in **Figure A6–13**, thymine and uracil lack amino groups and therefore cannot be deaminated. Deamination of adenine and guanine produces

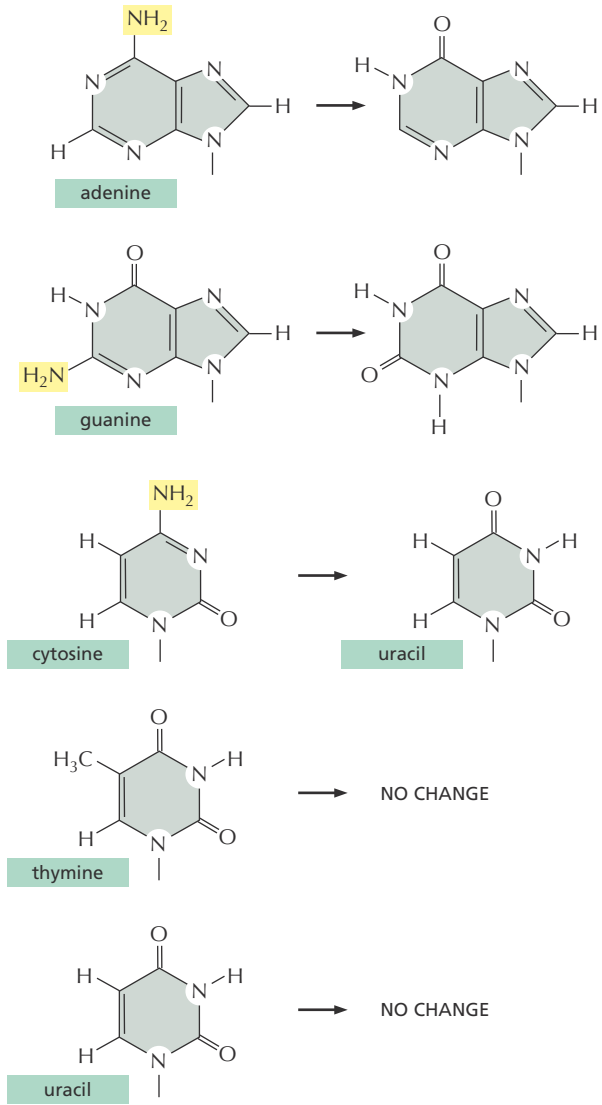


Figure A6–13

purine rings that are not found in conventional nucleic acids. In contrast, deamination of cytosine produces uracil. Therefore, if uracil were a naturally occurring base in DNA (as it is in RNA), repair enzymes could not distinguish whether a uracil is the appropriate base or whether it arose through spontaneous deamination of cytosine. This dilemma is not encountered, however, because thymine, rather than uracil, is used in DNA. Therefore, if a uracil base is found in DNA, it can be automatically recognized as a damaged base and then excised and replaced by cytosine.

ANSWER 6–14

A. Because DNA polymerase requires a 3′-OH to synthesize DNA, without telomeres and telomerase, the ends of linear chromosomes would shrink during each round of DNA replication (**Figure A6–14**). For bacterial chromosomes, which have no ends, the problem does not arise; there will always be a 3′-OH group available to prime the DNA polymerase that replaces the RNA primer with DNA. Telomeres and telomerase prevent the shrinking of chromosomes because they extend the 3′ end of a DNA strand (see Figure 6–22). This extension of the lagging-strand template provides the "space" to begin the final Okazaki fragments.

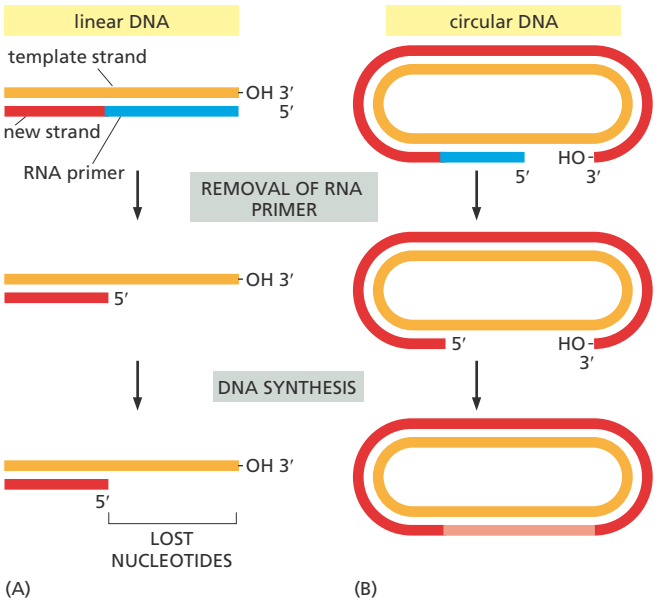


Figure A6–14

B. As shown in Figure A6–14, telomeres and telomerase are still needed even if the last fragment of the lagging strand were initiated by primase at the very 3′ end of chromosomal DNA, inasmuch as the RNA primer must be removed.

ANSWER 6–15

A. If the single origin of replication were located exactly in the center of the chromosome, it would take more than 8 days to replicate the DNA [= 75×10^6 nucleotides/(100 nucleotides/sec)]. The rate of replication would therefore severely limit the rate of cell division. If the origin were located at one end, the time required to replicate the chromosome would be approximately double this.

B. A chromosome end that is not "capped" with a telomere would lose nucleotides during each round of DNA replication and would gradually shrink. Eventually, essential genes would be lost, and the chromosome's ends might be recognized by the DNA damage-response mechanisms, which would stop cell division or induce cell death.

C. Without centromeres, which attach mitotic chromosomes to the mitotic spindle, the two new chromosomes that result from chromosome duplication would not be partitioned accurately between the two daughter cells. Therefore, many daughter cells would die, because they would not receive a full set of chromosomes.

ANSWER 6–16 The addition of each nucleotide by a hypothetical polymerase that synthesized DNA in the reverse 3′-to-5′ direction would require the energy provided by hydrolysis of the high-energy phosphate bond at the 5′ end of the growing chain—rather than at the 5′ end of the incoming nucleotide, as do the actual DNA polymerases. If an incorrectly incorporated nucleotide were removed from such a growing chain, DNA synthesis would grind to a halt, as there would be no high-energy bonds remaining at the 5′ end of the chain to fuel further polymerization (see Figure A6–16).

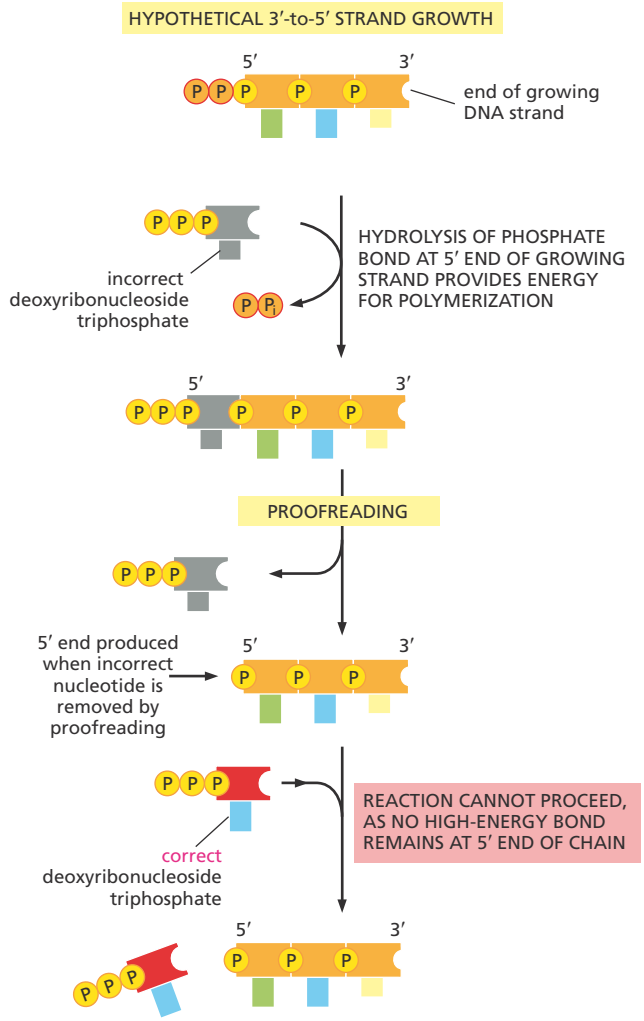


Figure A6–16

Chapter 7

ANSWER 7–1 Perhaps the best answer was given by Francis Crick himself, who coined the term in the mid-1950s: "I called this idea the central dogma for two reasons, I suspect. I had already used the obvious word hypothesis in the sequence hypothesis, which proposes that genetic information is encoded in the sequence of the DNA bases, and in addition I wanted to suggest that this new assumption was more central and more powerful…. As it turned out, the use of the word dogma caused more trouble than it was worth. Many years later Jacques Monod pointed out to me that I did not appear to understand the correct use of the word dogma, which is a belief that cannot be doubted. I did appreciate this in a vague sort of way but since I thought that all religious beliefs were without serious foundation, I used the word in the way I myself thought about it, not as the world does, and simply applied it to a grand hypothesis that, however plausible, had little direct experimental support at the time." (Francis Crick, *What Mad Pursuit: A Personal View of Scientific Discovery.* Basic Books, 1988.)

ANSWER 7–2 Actually, the RNA polymerases are not moving at all in the micrograph, because they have been fixed and coated with metal to prepare the sample for

viewing in the electron microscope. However, before they were fixed, they were moving from left to right, as indicated by the gradual lengthening of the RNA transcripts.

The RNA transcripts are shorter because they begin to fold up (i.e., to acquire a three-dimensional structure) as they are synthesized (see, for example, Figure 7–5), whereas the DNA is an extended double helix.

ANSWER 7–3 At first glance, the catalytic activities of an RNA polymerase used for transcription could replace the DNA primase. Upon further reflection, however, there are some serious problems. (1) The RNA polymerase used to make primers would need to initiate every few hundred bases, which is much more often than promoters are spaced on the DNA. Initiation would therefore need to occur in a promoter-independent fashion or many more promoters would have to be present in the DNA, both of which would be problematic for the control of transcription. (2) Similarly, the RNA primers used in DNA replication are much shorter than mRNAs. The RNA polymerase would therefore need to terminate much more frequently than during transcription. Termination would need to occur spontaneously, i.e., without requiring a terminator sequence in the DNA, or many more terminators would need to be present. Again, both of these scenarios would be problematic for the control of transcription.

Although it might be possible to overcome this problem if special control proteins became attached to RNA polymerase during replication, the problem has been solved during evolution by using separate enzymes with specialized properties. Some small DNA viruses, however, do utilize the host RNA polymerase to make DNA primers for their replication.

ANSWER 7–4 This experiment demonstrates that the ribosome does not check the amino acid that is attached to a tRNA. Once an amino acid has been coupled to a tRNA, the ribosome will "blindly" incorporate that amino acid into the position according to the match between the codon and anticodon. We can therefore conclude that a significant part of the correct reading of the genetic code, i.e., the matching of a codon in an mRNA with the correct amino acid, is performed by the synthetase enzymes that correctly match tRNAs and amino acids.

ANSWER 7–5 The mRNA will have a 5′-to-3′ polarity, opposite to that of the DNA strand that serves as the template. Thus the mRNA sequence will read 5′-GAAAAAAGCCGUUAA-3′. The N-terminal amino acid coded for by GAA is glutamic acid. UAA specifies a stop codon, so the C-terminal amino acid is coded for by CGU and is an arginine. Note that the convention in describing the sequence of a gene is to give the sequence of the DNA strand that is *not* used as a template for RNA synthesis; this sequence is the same as that of the RNA transcript, with T written in place of U.

ANSWER 7–6 The first statement is probably correct: RNA is thought to have been the first self-replicating catalyst and, in modern cells, is no longer self-replicating. We can debate, however, whether this represents a "loss." RNA now serves many roles in the cell: as messengers, as adaptors for protein synthesis, as primers for DNA replication, and as catalysts for some of the most fundamental reactions, especially RNA splicing and protein synthesis.

ANSWER 7–7

A. False. Ribosomes can make any protein that is specified by the particular mRNA that they are translating. After translation, ribosomes are released from the mRNA and can then start translating a different mRNA. It is true, however, that a ribosome can only make one type of protein at a time.
B. False. mRNAs are translated as linear polymers; there is no requirement that they have any particular folded structure. In fact, such structures that are formed by mRNA can inhibit its translation, because the ribosome has to unfold the mRNA in order to read the message it contains.
C. False. Ribosomal subunits exchange partners after each round of translation. After a ribosome is released from an mRNA, its two subunits dissociate and enter a pool of free small and large subunits from which new ribosomes assemble around a new mRNA.
D. False. Ribosomes are cytoplasmic organelles, but they are not individually enclosed in a membrane.
E. False. The position of the promoter determines the direction in which transcription proceeds and therefore which DNA strand is used as the template. Transcription in the opposite direction would produce an mRNA with a completely different (and probably meaningless) sequence.
F. False. RNA contains uracil but not thymine.
G. False. The level of a protein depends on its rate of synthesis and degradation but not on its catalytic activity.

ANSWER 7–8 Because the deletion in the Lacheinmal mRNA is internal, it is likely that the deletion arises from an mRNA splicing defect. The simplest interpretation is that the *Lacheinmal* gene contains a 173-nucleotide-long exon (labeled "E2" in **Figure A7–8**), and that this exon is lost during the processing of the mutant precursor mRNA (pre-mRNA). This could occur, for example, if the mutation changed the 3′ splice site in the preceding intron ("I1") so that it was no longer recognized by the splicing machinery (a change in the CAG sequence shown in Figure 7–19 could do this). The snRNP would search for the next available 3′ splice site, which is found at the 3′ end of the next intron ("I2"), and the splicing reaction would therefore remove E2 together with I1 and I2, resulting in a shortened mRNA. The mRNA is then translated into a defective protein, resulting in the Lacheinmal deficiency.

Because 173 nucleotides do not amount to an integral number of codons, the lack of this exon in the mRNA will shift the reading frame at the splice junction. Therefore, the Lacheinmal protein would be made correctly only through exon E1. As the ribosome begins translating sequences in exon E3, it will be in a different reading frame and therefore will produce a protein sequence that is unrelated to the Lacheinmal sequence normally encoded by exon E3. Most likely, the ribosome will soon encounter a stop codon, which in RNA sequences that do not code for protein would be expected to occur on average about once in every 21 codons (there are 3 stop codons in the 64 codons of the genetic code).

ANSWER 7–9 Sequence 1 and sequence 4 both code for the peptide Arg-Gly-Asp. Because the genetic code is redundant, different nucleotide sequences can encode the same amino acid sequence.

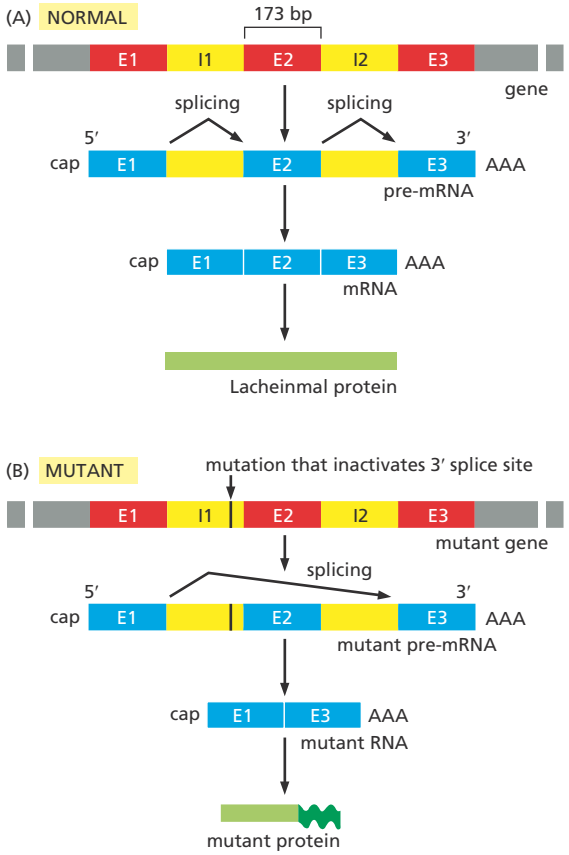


Figure A7–8

ANSWER 7–10

A. Incorrect. The bonds are not covalent, and their formation does not require input of energy.
B. Correct. The aminoacyl-tRNA enters the ribosome at the A site and forms hydrogen bonds with the codon in the mRNA.
C. Correct. As the ribosome moves along the mRNA, the tRNAs that have donated their amino acid to the growing polypeptide chain are ejected from the ribosome and the mRNA. The ejection takes place two cycles after the tRNA first enters the ribosome (see Figure 7–34).

ANSWER 7–11 *Replication*. Dictionary definition: the creation of an exact copy; molecular biology definition: the act of duplicating DNA. *Transcription*. Dictionary definition: the act of writing out a copy, especially from one physical form to another; molecular biology definition: the act of copying the information stored in DNA into RNA. *Translation*. Dictionary definition: the act of putting words into a different language; molecular biology definition: the act of polymerizing amino acids into a defined linear sequence using the information provided by the linear sequence of nucleotides in mRNA. (Note that "translation" is also used in a quite different sense, both in ordinary language and in scientific contexts, to mean a movement from one place to another.)

ANSWER 7–12 With four different nucleotides to choose from, a code of two nucleotides could specify 16 different amino acids (= 4^2), and a triplet code in which the position of the nucleotides is not important could specify 20 different amino acids (= 4 possibilities of 3 of the same bases + 12 possibilities of 2 bases the same and one different + 4 possibilities of 3 different bases). In both cases, these maximal amino acid numbers would need to be reduced by at least 1, because of the need to specify translation stop codons. It is relatively easy to envision how a doublet code could be translated by a mechanism similar to that used in our world by providing tRNAs with only two relevant bases in the anticodon loop. It is more difficult to envision how the nucleotide composition of a stretch of three nucleotides could be translated without regard to their order, because base-pairing can then no longer be used: AUG, for example, will not base-pair with the same anticodon as UGA.

ANSWER 7–13 It is likely that in early cells the matching between codons and amino acids was less accurate than it is in present-day cells. The feature of the genetic code described in the question may have allowed early cells to tolerate this inaccuracy by allowing a blurred relationship between sets of roughly similar codons and roughly similar amino acids. One can easily imagine how the matching between codons and amino acids could have become more accurate, step by step, as the translation machinery evolved into that found in modern cells.

ANSWER 7–14 The codon for Trp is 5′-UGG-3′. Thus a normal Trp-tRNA contains the sequence 5′-CCA-3′ as its anticodon (see Figure 7–30). If this tRNA contains a mutation so that its anticodon is changed to UCA, it will recognize a UGA codon and lead to the incorporation of a tryptophan residue instead of causing translation to stop. Many other protein-encoding sequences, however, contain UGA codons as their natural stop sites, and these stops would also be affected by the mutant tRNA. Depending on the competition between the altered tRNA and the normal translation release factors (Figure 7–38), some of these proteins would be made with additional amino acids at their C-terminal end. The additional lengths would depend on the number of codons before the ribosomes encounter a non-UGA stop codon in the mRNA in the reading frame in which the protein is translated.

ANSWER 7–15 One effective way of driving a reaction to completion is to remove one of the products, so that the reverse reaction cannot occur. ATP contains *two* high-energy bonds that link the three phosphate groups. In the reaction shown, PP_i is released, consisting of two phosphate groups linked by one of these high-energy bonds. Thus PP_i can be hydrolyzed with a considerable gain of free energy, and thereby can be efficiently removed. This happens rapidly in cells, and reactions that produce and further hydrolyze PP_i are therefore virtually irreversible (see Figure 3–40).

ANSWER 7–16

A. A titin molecule is made of 25,000 (3,000,000/120) amino acids. It therefore takes about 3.5 hours [(25,000/2)× (1/60) × (1/60)] to synthesize a single molecule of titin in muscle cells.
B. Because of its large size, the probability of making a titin molecule without any mistakes is only 0.08 [= $(1 - 10^{-4})^{25,000}$]; i.e., only 8 in 100 titin molecules synthesized are free of mistakes. In contrast, over 97% of newly synthesized proteins of average size are made correctly.
C. The error rate limits the sizes of proteins that can be synthesized accurately. Similarly, if a eukaryotic

ribosomal protein were synthesized as a single molecule, a large portion (87%) of this hypothetical giant ribosomal protein would be expected to contain at least one mistake. It is therefore more advantageous to make ribosomal proteins individually, because in this way only a small proportion of each type of protein will be defective, and these few bad molecules can be individually eliminated by proteolysis to ensure that there are no defects in the ribosome as a whole.

D. To calculate the time it takes to transcribe a titin mRNA, you would need to know the size of its gene, which is likely to contain many introns. Transcription of the exons alone (25,000 × 3 = 75,000 nucleotides) requires about 42 minutes [(75,000/30) × (1/60)]. Because introns can be quite large, the time required to transcribe the entire gene is likely to be considerably longer.

ANSWER 7–17 Mutations of the type described in (B) and (D) are often the most harmful. In both cases, the reading frame would be changed, and because this frameshift occurs near the beginning or in the middle of the coding sequence, much of the protein will contain a nonsensical and/or truncated sequence of amino acids. In contrast, a reading-frame shift that occurs toward the end of the coding sequence, as described in (A), will result in a largely correct protein that may be functional. Deletion of three consecutive nucleotides, as described in (C), leads to the deletion of an amino acid but does not alter the reading frame. The deleted amino acid may or may not be important for the folding or activity of the protein; in many cases such mutations are silent, i.e., have no or only minor consequences for the organism. Substitution of one nucleotide for another, as in (E), is often completely harmless. In some cases, it will not change the amino acid sequence of the protein; in other cases it will change a single amino acid; at worst, it may create a new stop codon, giving rise to a truncated protein.

Chapter 8

ANSWER 8–1

A. Transcription of the tryptophan operon would no longer be regulated by the absence or presence of tryptophan; the enzymes would be permanently turned on in scenarios (1) and (2) and permanently shut off in scenario (3).

B. In scenarios (1) and (2), the normal tryptophan repressor molecules would completely restore the regulation of the tryptophan biosynthesis enzymes. In contrast, expression of the normal protein would have no effect in scenario (3), because the tryptophan operator would remain occupied by the mutant protein, even in the presence of tryptophan.

ANSWER 8–2 Contacts can form between the protein and the edges of the base pairs that are exposed in the major groove of the DNA (**Figure A8–2**). These sequence-specific contacts can include hydrogen bonds with the highlighted oxygen, nitrogen, and hydrogen atoms, as well as hydrophobic interactions with the methyl group on thymine (*yellow*). Note that the arrangement of hydrogen-bond donors (*blue*) and hydrogen-bond acceptors (*red*) of a T-A pair is different from that of a C-G pair. Similarly, the

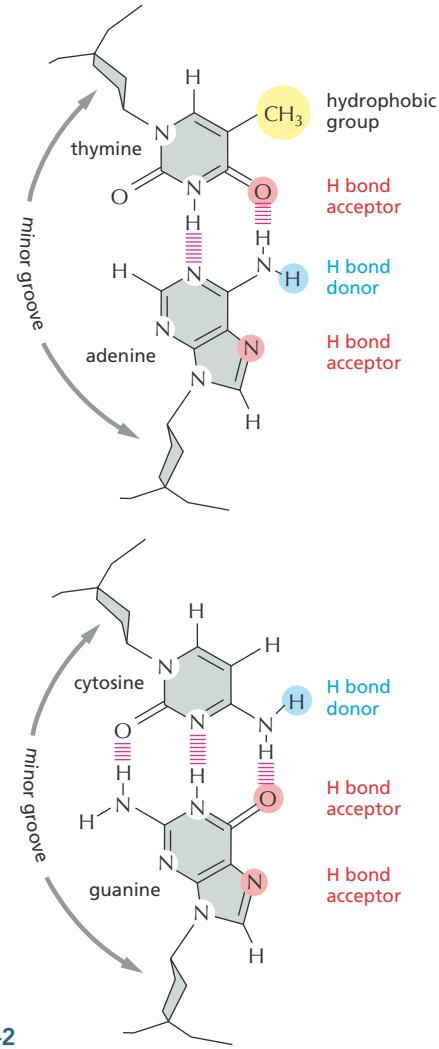


Figure A8–2

arrangement of hydrogen-bond donors and hydrogen-bond acceptors of A-T and G-C pairs would be different from one another and from the two pairs shown in the figure. These differences allow recognition of specific DNA sequences via the major groove. In addition to the contacts shown in the figure, electrostatic attractions between the positively charged amino acid side chains of the protein and the negatively charged phosphate groups in the DNA backbone usually stabilize DNA–protein interactions.

ANSWER 8–3 Bending proteins can help to bring distant DNA regions together that normally would contact each other only inefficiently (**Figure A8–3**). Such proteins are found in both prokaryotes and eukaryotes and are involved in many examples of transcriptional regulation.

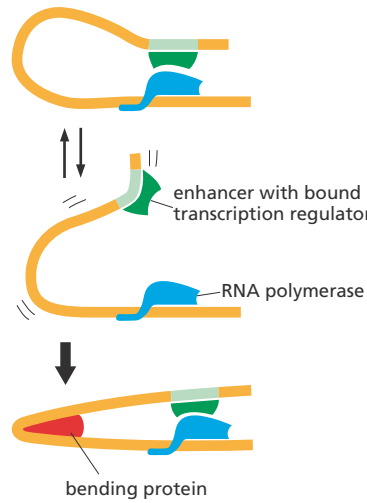


Figure A8–3

ANSWER 8–4

A. UV light throws the switch from the prophage to the lytic state: when cI protein is destroyed, Cro is made and turns off the further production of cI. The virus produces coat proteins, and new virus particles are made.
B. When the UV light is switched off, the virus remains in the lytic state. Thus, cI and Cro form a gene regulatory switch that "memorizes" its previous setting.
C. This switch makes sense in the viral life cycle: UV light tends to damage the bacterial DNA (see Figure 6–24), thereby rendering the bacterium an unreliable host for the virus. A prophage will therefore switch to the lytic state and leave the "sinking ship" in search for new host cells to infect.

ANSWER 8–5

A. True. Prokaryotic mRNAs are often transcripts of entire operons. Ribosomes can initiate translation at internal AUG start sites of these "polycistronic" mRNAs (see Figures 7–36 and 8–6).
B. True. The major groove of double-stranded DNA is sufficiently wide to allow a protein surface, such as one face of an α helix, access to the base pairs. The sequence of H-bond donors and acceptors in the major groove can then be "read" by the protein to determine the sequence and orientation of the DNA.
C. True. It is advantageous to exert control at the earliest possible point in a pathway. This conserves metabolic energy because unnecessary products are not made in the first place.

ANSWER 8–6 From our knowledge of enhancers, one would expect their function to be relatively independent of their distance from the promoter—possibly weakening as this distance increases. The surprising feature of the data (which have been adapted from an actual experiment) is the periodicity: the enhancer is maximally active at certain distances from the promoter (50, 60, or 70 nucleotides), but almost inactive at intermediate distances (55 or 65 nucleotides). The periodicity of 10 suggests that the mystery can be explained by considering the structure of double-helical DNA, which has 10 base pairs per turn. Thus, placing an enhancer on the side of the DNA opposite to that of the promoter (**Figure A8–6**) would make it more difficult for the activator that binds to it to interact with the proteins bound at the promoter. At longer distances, there is more DNA to absorb the twist, and the effect is diminished.

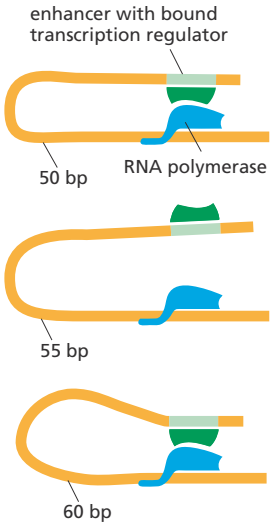


Figure A8–6

ANSWER 8–7 The affinity of the dimeric λ repressor for its binding site is the sum of the interactions made by each of the two DNA-binding domains. A single DNA-binding domain can make only half the contacts and provide just half the binding energy compared with the dimer. Thus, although the concentration of binding domains is unchanged, they are no longer coupled, and their individual affinities for DNA are sufficiently weak that they cannot remain bound. As a result, the genes for lytic growth are turned on.

ANSWER 8–8 The function of these *Arg* genes is to synthesize arginine. When arginine is abundant, expression of the biosynthetic genes should be turned off. If ArgR acts as a gene repressor (which it does in reality), then binding of arginine should increase its affinity for its regulatory sites, allowing it to bind and shut off gene expression. If ArgR acted as a gene activator instead, then the binding of arginine would be predicted to reduce its affinity for its regulatory DNA, preventing its binding and thereby shutting off expression of the *Arg* genes.

ANSWER 8–9 The results of this experiment favor DNA looping, which should not be affected by the protein bridge (so long as it allowed the DNA to bend, which it does). The scanning or entry-site model, however, is predicted to be affected by the nature of the linkage between the enhancer and the promoter. If the proteins enter at the enhancer and scan to the promoter, they would have to traverse the protein linkage. If such proteins are geared to scan on DNA, they would likely have difficulty scanning across such a barrier.

ANSWER 8–10 The most definitive result is one showing that a single differentiated cell taken from a specialized tissue can re-create a whole organism. This proves that the cell must contain all the information required to produce a whole organism, including all of its specialized cell types. Experiments of this type are summarized in Figure 8–2.

ANSWER 8–11 In principle, you could create 16 different cell types with 4 different transcription regulators (all the 8 cell types shown in Figure 8–17, plus another 8 created by adding an additional transcription regulator). MyoD by itself is sufficient to induce muscle-specific gene expression only in certain cell types, such as some kinds of fibroblasts. The action of MyoD is therefore consistent with the model shown in Figure 8–17: if muscle cells were specified, for example, by the combination of transcription regulators 1, 3, and MyoD, then the addition of MyoD would convert only two of the cell types of Figure 8–17 (cells F and H) to muscle.

ANSWER 8–12 The induction of a transcriptional activator protein that stimulates its own synthesis can create a positive feedback loop that can produce cell memory. The continued self-stimulated synthesis of activator A can in principle last for many cell generations, serving as a constant reminder of an event that took place in the past. By contrast, the induction of a transcriptional repressor that inhibits its own synthesis creates a negative feedback loop which ensures that the response to the transient stimulus will be similarly transient. Because repressor R shuts off its

own synthesis, the cell will quickly return to the state that existed before the signal.

ANSWER 8–13 Many transcription regulators are continually made in the cell; that is, their expression is constitutive and the activity of the protein is controlled by signals from inside or outside the cell (e.g., the availability of nutrients, as for the tryptophan repressor, or by hormones, as for the glucocorticoid receptor), thereby adjusting the transcriptional program to the physiological needs of the cell. Moreover, a given transcription regulator usually controls the expression of many different genes. Transcription regulators are often used in various combinations and can affect each other's activity, thereby further increasing the possibilities for regulation with a limited set of proteins. Nevertheless, most cells devote a large fraction of their genomes to the control of transcription: about 10% of genes in eukaryotic cells code for transcription regulators.

Chapter 9

ANSWER 9–1 When it comes to genetic information, a balance must be struck between stability and change. If the mutation rate were too high, a species would eventually die out because all its individuals would accumulate mutations in genes essential for survival. And for a species to be successful—in evolutionary terms—individual members must have a good genetic memory; that is, there must be high fidelity in DNA replication. At the same time, occasional changes are needed if the species is to adapt to changing conditions. If the change leads to an improvement, it will persist by selection; if it is neutral, it may or may not accumulate; but if the change proves disastrous, the individual organism that was the unfortunate subject of nature's experiment will die, but the species will survive.

ANSWER 9–2 In single-celled organisms, the genome is the germ line and any modification is passed on to the next generation. By contrast, in multicellular organisms, most of the cells are somatic cells and make no contribution to the next generation; thus, modification of those cells by horizontal gene transfer would have no consequence for the next generation. The germ-line cells are usually sequestered in the interior of multicellular organisms, minimizing their contact with foreign cells, viruses, and DNA, thus insulating the species from the effects of horizontal gene transfer. Nevertheless, horizontal gene transfer is possible for multicellular organisms. For example, the genomes of some insect species contain DNA that was horizontally transferred from bacteria that infect them.

ANSWER 9–3 It is unlikely that any gene came into existence perfectly optimized for its function. Ribosomal RNA genes presumably varied a great deal when they first appeared on Earth. But this would have been at the very early stage of a common ancestral cell (see Figure 9–23). Since then there has been much less leeway for change since ribosomal RNA (and other highly conserved genes) play such a fundamental role in living processes. Nonetheless, the environment an organism finds itself in is changeable, so no gene can be optimal indefinitely. Thus we find there are indeed significant differences in ribosomal RNAs among species.

ANSWER 9–4 Each time another copy of a transposon is inserted into a chromosome, the change can be either neutral, beneficial, or detrimental for the organism. Because individuals that accumulate detrimental insertions would be selected against, the proliferation of transposons is controlled by natural selection. If a transposon arose that proliferated uncontrollably, it is unlikely that a viable host organism could be maintained. For this reason, most transposons have evolved to transpose only rarely. Many transposons, for example, synthesize only infrequent bursts of very small amounts of the transposase that is required for their movement.

ANSWER 9–5 Viruses cannot exist as free-living organisms: they have no metabolism, do not communicate with other viruses, and cannot reproduce themselves. They thus have none of the attributes that one normally associates with life. Indeed, they can even be crystallized. Only inside cells can they redirect normal cellular biosynthetic activities to the task of making more copies of themselves. Thus, the only aspect of "living" that viruses display is their capacity to direct their own reproduction once inside a cell.

ANSWER 9–6 Mobile genetic elements could provide opportunities for homologous recombination events, thereby causing genomic rearrangements. They could insert into genes, possibly obliterating splicing signals and thereby changing the protein produced by the gene. They could also insert into the regulatory region of a gene, where insertion between an enhancer and a transcription start site could block the function of the enhancer and therefore reduce the level of expression of a gene. In addition, the mobile genetic element could itself contain an enhancer and thereby change the time and place in the organism where the gene is expressed.

ANSWER 9–7 With their ability to facilitate genetic recombination, mobile genetic elements have almost certainly played an important part in the evolution of modern-day organisms. They can facilitate gene duplication and the creation of new genes via exon shuffling, and they can change the way in which existing genes are expressed. Although the transposition of a mobile genetic element can be harmful for an individual organism—if, for example, it disrupts the activity of a critical gene—these agents of genetic change may well be beneficial to the species as a whole.

ANSWER 9–8 About 7.6% of each gene is converted to mRNA [(5.4 exons/gene × 266 nucleotide pairs/exon)/(19,000 nucleotide pairs/gene) = 7.6%]. Protein-coding genes occupy about 28% of Chromosome 22 [(700 genes × 19,000 nucleotide pairs/gene)/(48 × 10^6 nucleotide pairs) = 27.7%]. However, over 90% of this DNA is made of introns.

ANSWER 9–9 This statement is probably true. For example, nearly half our DNA is composed of defunct mobile genetic elements. And only about 9% of the human genome appears to be under positive selection. However, it is possible that future research will uncover a function for some portion of our seemingly unimportant DNA.

ANSWER 9–10 The HoxD cluster is packed with complex and extensive regulatory sequences that direct each of its genes to be expressed at the correct time and place during development. Insertion of mobile genetic elements into the

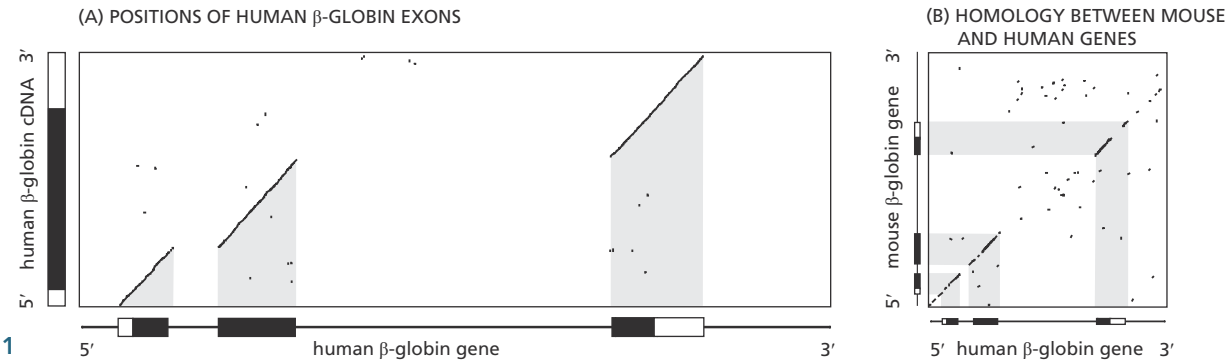


Figure A9–11

HoxD cluster is thought to be selected against because it would disrupt proper regulation of its resident genes.

ANSWER 9–11

A. The exons in the human β-globin gene correspond to the positions of sequence similarity (in this case identity) with the cDNA, which is a direct copy of the mRNA and thus contains no introns. The introns correspond to the regions between the exons. The positions of the introns and exons in the human β-globin gene are indicated in **Figure A9–11A**. Also shown (in open bars) are sequences present in the mature β-globin mRNA (and in the gene) that are not translated into protein.

B. From the positions of the exons, as defined in Figure A9–11A, it is clear that the first two exons of the human β-globin gene have counterparts, with similar sequence, in the mouse β-globin gene (Figure A9–11B). However, only the first half of the third exon of the human β-globin gene is similar to the mouse β-globin gene. The similar portion of the third exon contains sequences that encode protein, whereas the portion that is different represents the 3′ untranslated region of the gene. Because this portion of the gene does not encode protein (nor does it contain extensive regulatory sequences), its sequence is not constrained and the mouse and human sequences have drifted apart.

C. The human and mouse β-globin genes are also similar at their 5′ ends, as indicated by the cluster of points along the same diagonal as the first exon (Figure A9–11B). These sequences correspond to the regulatory regions upstream of the start sites for transcription. Functional sequences, which are under selective pressure, diverge much more slowly than sequences without function.

D. The diagon plot shows that the first intron is nearly the same length in the human and mouse genes, but the length of the second intron is noticeably different (Figure A9–11B). If the introns were the same length, the line segments that represent sequence similarity would fall on the same diagonal. The easiest way to test for the colinearity of the line segments is to tilt the page and sight along the diagonal. It is impossible to tell from this comparison if the change in length is due to a shortening of the mouse intron or to a lengthening of the human intron, or some combination of those possibilities.

ANSWER 9–12 Computer algorithms that search for exons are complex affairs, as you might imagine. To identify unknown genes, these programs combine statistical information derived from known genes, such as:

1. An exon that encodes protein will have an open reading frame. If the amino acid sequence specified by this open reading frame matches a protein sequence in any database, there is a high likelihood that it is an authentic exon.
2. The reading frames of adjacent exons in the same gene will match up when the intron sequences are omitted.
3. Internal exons (excluding the first and the last) will have splicing signals at each end; most of the time (98.1%) these will be AG at the 5′ ends of the exons and GT at the 3′ ends.
4. The multiple codons for most individual amino acids are not used with equal frequency. This so-called coding bias can be factored in to aid in the recognition of true exons.
5. Exons and introns have characteristic length distributions. The median length of exons in human genes is about 120 nucleotide pairs. Introns tend to be much larger: a median length of about 2 kb in genomic regions of 30–40% GC content, and a median length of about 500 nucleotide pairs in regions above 50% GC.
6. The initiation codon for protein synthesis (nearly always an ATG) has a statistical association with adjacent nucleotides that seem to enhance its recognition by translation factors.
7. The terminal exon will have a signal (most commonly AATAAA) for cleavage and polyadenylation close to its 3′ end.

The statistical nature of these features, coupled with the low frequency of coding information in the genome (2–3%) and the frequency of alternative splicing (an estimated 95% of human genes), makes it especially impressive that current algorithms can identify more than 70% of individual exons in the human genome. As shown in Figure 9–37, bioinformatic approaches are usually coupled with direct experimental data, such as those obtained from RNA Seq.

ANSWER 9–13 It is not a simple matter to determine the function of a gene from scratch, nor is there a universal recipe for doing so. Nevertheless, there are a variety of standard questions whose answers help to narrow down the possibilities. Below we list some of these questions.

In what tissues is the gene expressed? If the gene is expressed in all tissues, it is likely to have a general function. If it is expressed in one or a few tissues, its function is likely to be more specialized, perhaps related to the specialized functions of the tissues. If the gene is expressed in the embryo but not the adult, it probably functions in development.

In what compartment of the cell is the protein found? Knowing the subcellular localization of the protein—nucleus, plasma membrane, mitochondria, etc.—can also help to

suggest categories of potential function. For example, a protein that is localized to the plasma membrane is likely to be a transporter, a receptor or other component of a signaling pathway, a cell-adhesion molecule, etc.

What are the effects of mutations in the gene? Mutations that eliminate or modify the function of the gene product can also provide clues to function. For example, if the gene product is critical at a certain time during development, mutant embryos will often die at that stage or develop obvious abnormalities. Unless the abnormality is highly specific, it is usually difficult to deduce its function. And often the links are indirect, becoming apparent only after the gene's function is known.

With what other proteins does the encoded protein interact? In carrying out their function, proteins often interact with other proteins involved in the same or closely related processes. If an interacting protein can be identified, and if its function is already known (through previous research or through the searching of databases), the range of possible functions can be narrowed dramatically.

Can mutations in other genes alter effects of mutation in the unknown gene? Searching for such mutations can be a very powerful approach to investigating gene function, especially in organisms such as bacteria and yeast, which have simple genetic systems. Although much more difficult to perform in the mouse, this type of approach can nonetheless be used. The rationale for this strategy is analogous to that of looking for interacting proteins: genes that interact genetically—so that the double mutant phenotype is more selective than either of the individual mutants—are often involved in the same process or in closely related processes. Identification of such an interacting gene (and knowledge of its function) would provide an important clue to the function of the unknown gene.

Addressing each of these questions requires specialized experimental expertise and a substantial time commitment from the investigator. It is no wonder that progress is made much more rapidly when a clue to a gene's function can be found simply by identifying a similar gene of known function in the database. As more and more genes are studied, this strategy will become increasingly successful.

ANSWER 9–14 In a very long, random sequence of DNA, each of the 64 different codons will occur with equal frequency. Because 3 of the 64 are stop codons, they will be expected to occur on average every 21 codons (64/3 = 21.3).

ANSWER 9–15 On the surface, its resistance to mutation suggests that the genetic code was shaped by forces of natural selection. An underlying assumption, which seems reasonable, is that resistance to mutation is a valuable feature of a genetic code. This reasoning suggests that it would have been a lucky accident indeed—roughly a one-in-a-million chance—to stumble on a code as error-proof as our own.

But all is not so simple. If resistance to mutation is an essential feature of any code that can support complex organisms such as ourselves, then the only codes we *could* observe are ones that are error-resistant. A less favorable frozen accident, giving rise to a more error-prone code, might have limited the complexity of life to organisms too simple to contemplate their own genetic code. This is akin to the anthropic principle of cosmology: many universes may be possible, but few are compatible with life that can ponder the nature of the universe.

Beyond these considerations, there is ample evidence that the code is not static, and thus could respond to the forces of natural selection. Altered versions of the standard genetic code have been identified in the mitochondrial and nuclear genomes of several organisms. In each case, one or a few codons have taken on a new meaning.

ANSWER 9–16 All of these mechanisms contribute to the evolution of new protein-coding genes. A, B, C, and E were discussed in the text. Recent studies indicate that certain short protein-coding genes arose from previously untranslated regions of genomes, so choice D is also correct.

ANSWER 9–17

A. Because synonymous changes do not alter the amino acid sequence of the protein, they usually do not affect the overall fitness of the organism and are therefore not selected against. By contrast, nonsynonymous changes, which substitute a new amino acid in place of the original one, can alter the function of the encoded protein and change the fitness of the organism. Since most amino acid substitutions probably harm the protein, they tend to be selected against.
B. Virtually all amino acid substitutions in the histone H3 protein are deleterious and are therefore selected against. The extreme conservation of histone H3 argues that its function is very tightly constrained, probably because of extensive interactions with other proteins and with DNA.
C. Histone H3 is clearly not in a "privileged" site in the genome because it undergoes synonymous nucleotide changes at about the same rate as other genes.

ANSWER 9–18

A. The data embodied in the phylogenetic tree (Figure Q9–18) refutes the hypothesis that plant hemoglobin genes were acquired by horizontal transfer. Looking at the more familiar parts of the tree, we see that the hemoglobins of vertebrates (fish to human) have approximately the same phylogenetic relationships as do the species themselves. Plant hemoglobins also form a distinct group that displays accepted evolutionary relationships, with barley, a monocot, diverging before bean, alfalfa, and lotus, which are all dicots (and legumes). The basic hemoglobin gene, therefore, was in place long ago in evolution. The phylogenetic tree of Figure Q9–18 indicates that the hemoglobin genes in modern plant and animal species were inherited from a common ancestor.
B. Had the plant hemoglobin genes arisen by horizontal transfer from a nematode, then the plant sequences would have clustered with the nematode sequences in the phylogenetic tree in Figure Q9–18.

ANSWER 9–19 In each human lineage, new mutations will be introduced at a rate of 10^{-10} alterations per nucleotide per cell generation, and the differences between two human lineages will accumulate at twice this rate. To accumulate 10^{-3} differences per nucleotide will thus take $10^{-3}/(2 \times 10^{-10})$ cell generations, corresponding to $(1/200) \times 10^{-3}/(2 \times 10^{-10}) = 25{,}000$ human generations, or 750,000 years. In reality, we are not descended from one pair of genetically identical ancestral humans; rather, it is likely that

we are descended from a relatively small founder population of humans who were already genetically diverse. More sophisticated analysis suggests that this founder population existed about 150,000 years ago.

ANSWER 9–20 The AIDS virus (the human immunodeficiency virus, HIV) is a retrovirus, and thus synthesizes DNA from an RNA template using reverse transcriptase. This leads to frequent mutation of the viral genome. In fact, AIDS patients often carry many different genetic variants of HIV that are distinct from the original virus that infected them. This poses great problems in treating the infection: drugs that block essential viral enzymes work only temporarily, because new strains of the virus resistant to these drugs arise rapidly by mutation.

RNA replicases (enzymes that synthesize RNA using RNA as a template) do not proofread either. Thus, RNA viruses that replicate their RNA genomes directly (that is, without using DNA as an intermediate) also mutate frequently. In such a virus, this tends to produce changes in the coat proteins that cause the mutated virus to appear "new" to our immune systems; the virus is therefore not suppressed by immunity that has arisen to the previous version. This is part of the explanation for the new strains of the influenza (flu) virus and the common cold virus that regularly appear.

Chapter 10

ANSWER 10–1 The presence of a mutation in a gene does not necessarily mean that the protein expressed from it is defective. For example, the mutation could change one codon into another that still specifies the same amino acid, and so does not change the amino acid sequence of the protein. Or, the mutation may cause a change from one amino acid to another in the protein, but in a position that is not important for the folding or function of the protein. In assessing the likelihood that such a mutation might cause a defective protein, information on the known β-globin mutations that are found in humans is essential. You would therefore want to know the precise nucleotide change in your mutant gene, and whether this change has any known or predictable consequences for the function of the encoded protein. If your mate has two normal copies of the globin gene, 50% of your children would be carriers of your mutant gene.

ANSWER 10–2

A. Digestion with EcoRI produces two products:

5′-AAGAATTGCGG AATTCGGGCCTTAAGCGCCGCGTCGAGGCCTTAAA-3′
3′-TTCTTAACGCCTTAA GCCCGGAATTCGCGGCGCAGCTCCGGAATTT-5′

B. Digestion with HaeIII produces three products:

5′-AAGAATTGCGGAATTCGGG CCTTAAGCGCCGCGTCGAGG CCTTAAA-3′
3′-TTCTTAACGCCTTAAGCCC GGAATTCGCGGCGCAGCTCC GGAATTT-5′

C. The sequence lacks a HindIII cleavage site.

D. Digestion with all three enzymes therefore produces:

5′-AAGAATTGCGG AATTCGGG CCTTAAGCGCCGCGTCGAGG CCTTAAA-3′
3′-TTCTTAACGCCTTAA GCCC GGAATTCGCGGCGCAGCTCC GGAATTT-5′

ANSWER 10–3 Protein biochemistry is still very important because it provides the link between the amino acid sequence (which can be deduced from DNA sequences) and the functional properties of the protein. We are still not able to infallibly predict the folding of a polypeptide chain from its amino acid sequence, and in most cases information regarding the function of the protein, such as its catalytic activity, cannot be deduced from the gene sequence alone. Instead, such information must be obtained experimentally by analyzing the properties of proteins biochemically. Furthermore, the structural information that can be deduced from DNA sequences is necessarily incomplete. We cannot, for example, accurately predict covalent modifications of the protein, proteolytic processing, the presence of tightly bound small molecules, or the association of the protein with other subunits. Moreover, we cannot accurately predict the effects these modifications might have on the activity of the protein.

ANSWER 10–4

A. After an additional round of amplification there will be 2 gray, 4 green, 4 red, and 22 yellow-outlined fragments; after a second additional round there will be 2 gray, 5 green, 5 red, and 52 yellow-outlined fragments. Thus the DNA fragments outlined in yellow increase exponentially and will eventually overrun the other reaction products. Their length is determined by the DNA sequence that spans the distance between the two primers plus the length of the primers.

B. The mass of one DNA molecule 500 nucleotide pairs long is 5.5×10^{-19} g [$= 2 \times 500 \times 330$ (g/mole)$/6 \times 10^{23}$ (molecules/mole)]. Ignoring the complexities of the first few steps of the amplification reaction (which produce longer products that eventually make an insignificant contribution to the total DNA amplified), this amount of product approximately doubles for every amplification step. Therefore, 100×10^{-9} g $= 2^N \times 5.5 \times 10^{-19}$ g, where N is the number of amplification steps of the reaction. Solving this equation for $N = \log(1.81 \times 10^{11})/\log(2)$ gives $N = 37.4$. Thus, only about 40 cycles of PCR amplification are sufficient to amplify DNA from a single molecule to a quantity that can be readily handled and analyzed biochemically. This whole procedure is automated and takes only a few hours in the laboratory.

ANSWER 10–5 If the ratio of dideoxyribonucleoside triphosphates to deoxyribonucleoside triphosphates is increased, DNA polymerization will be terminated more frequently and thus shorter DNA strands will be produced. Such conditions are favorable for determining nucleotide sequences that are close to the DNA primer used in the reaction. In contrast, decreasing the ratio of dideoxyribonucleoside triphosphates to deoxyribonucleoside triphosphates will produce longer DNA fragments, thus allowing one to determine nucleotide sequences more distant from the primer.

ANSWER 10–6 Although several explanations are possible, the simplest is that the DNA probe has hybridized predominantly with its corresponding mRNA, which is typically present in many more copies per cell than is the gene. The different extents of hybridization probably reflect different levels of gene expression. Perhaps each of the different cell types that make up the tissue expresses the gene at a different level.

ANSWER 10–7 Like the vast majority of mammalian genes, the attractase gene likely contains introns. Bacteria do not have the splicing machinery required to remove introns, and therefore the correct protein would not be expressed from the gene. For expression of most mammalian genes in bacterial cells, a cDNA version of the gene must be used.

ANSWER 10–8

A. False. Restriction sites are found at random throughout the genome, within as well as between genes.
B. True. DNA bears a negative charge at each phosphate, giving DNA an overall negative charge.
C. False. Clones isolated from cDNA libraries do not contain promoter sequences. These sequences are not transcribed and are therefore not part of the mRNAs that are used as the templates to make cDNAs.
D. True. Each polymerization reaction produces double-stranded DNA that must, at each cycle, be denatured to allow new primers to hybridize so that the DNA strand can be copied again.
E. False. Digestion of genomic DNA with restriction nucleases that recognize four-nucleotide sequences produces fragments that are *on average* 256 nucleotides long. However, the actual lengths of the fragments produced will vary considerably on both sides of the average.
F. True. Reverse transcriptase is first needed to copy the mRNA into single-stranded DNA, and DNA polymerase is then required to make the second DNA strand.
G. True. Using a sufficient number of STRs, individuals can be uniquely "fingerprinted" (see Figure 10–18).
H. True. If cells of the tissue do not transcribe the gene of interest, it will not be represented in a cDNA library prepared from this tissue. However, it will be represented in a genomic library prepared from the same tissue.

ANSWER 10–9

A. The DNA sequence, from its 5′ end to its 3′ end, is read starting from the bottom of the gel, where the smallest DNA fragments migrate. Each band results from the incorporation of the appropriate dideoxyribonucleoside triphosphate, and as expected there are no two bands that have the same mobility. This allows one to determine the DNA sequence by reading off the bands in strict order, proceeding upward from the bottom of the gel, and assigning the correct nucleotide according to which lane the band is in.

   The nucleotide sequence of the top strand (**Figure A10–9A**) was obtained directly from the data of Figure Q10–9, and the bottom strand was deduced from the complementary base-pairing rules.

B. The DNA sequence can then be translated into an amino acid sequence using the genetic code. However, there are two strands of DNA that could be transcribed into RNA and three possible reading frames for each strand. Thus there are six amino acid sequences that can in principle be encoded by this stretch of DNA. Of the three reading frames possible from the top strand, only one is not interrupted by a stop codon (*yellow* blocks in **Figure A10–9B**).

   From the bottom strand, two of the three reading frames also have stop codons (not shown). The third frame gives the following sequence:

   `SerAlaLeuGlySerSerGluAsnArgProArgThrProAlaArg`
   `ThrGlyCysProValTyr`

   It is not possible from the information given to tell which of the two open reading frames corresponds to the actual protein encoded by this stretch of DNA. What additional experiment could distinguish between these two possibilities?

ANSWER 10–10

A. Cleavage of human genomic DNA with HaeIII would generate about 11×10^6 different fragments [$= 3 \times 10^9/4^4$] and with EcoRI about 730,000 different fragments [$= 3 \times 10^9/4^6$]. There will also be some additional fragments generated because the maternal and paternal chromosomes are very similar but not identical in DNA sequence.
B. A set of overlapping DNA fragments will be generated. Libraries constructed from sets of overlapping fragments are valuable because they can be used to order cloned sequences in relation to their original order in the genome and thus obtain the DNA sequence of a long stretch of DNA (see Figure 10–26).

ANSWER 10–11 By comparison with the positions of the size markers, we find that EcoRI treatment gives two fragments of 4 kb and 6 kb; HindIII treatment gives one fragment of 10 kb; and treatment with EcoRI + HindIII gives three fragments of 6 kb, 3 kb, and 1 kb. This gives a total length of 10 kb calculated as the sum of the fragments in each lane. Thus the original DNA molecule must be 10 kb (10,000 nucleotide pairs) long. Because treatment with HindIII gives a fragment 10 kb long it could be that the original DNA is a linear molecule with no cutting site for HindIII. But we can rule that out by the results of the EcoRI + HindIII digestion. We know that EcoRI cleavage alone produces two fragments of 6 kb and 4 kb, and in the double digest this 4-kb fragment is further cleaved by HindIII into a 3-kb and a 1-kb fragment. The DNA therefore contains a single HindIII cleavage site, and thus it must be circular, as a single fragment of 10 kb is produced when it is cut with HindIII alone. Arranging the cutting sites on a circular DNA to give the appropriate sizes of fragments produces the map illustrated in **Figure A10–11**.

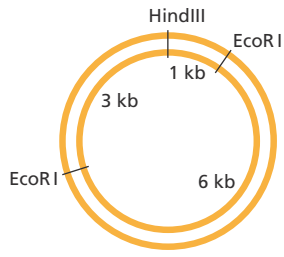


Figure A10–11

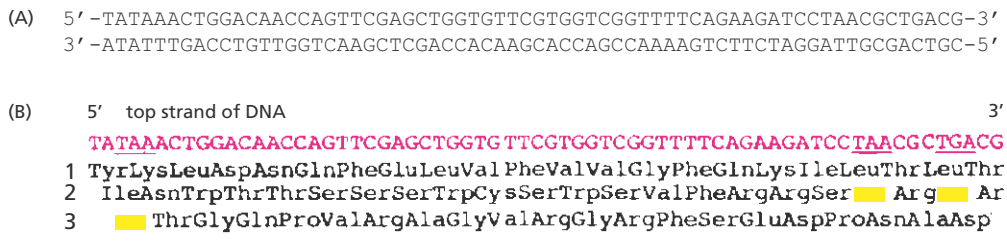


Figure A10–9

ANSWER 10–12

A. The genetic code is degenerate, and there is more than one possible codon for each amino acid, with the exception of tryptophan and methionine. Therefore, to detect the nucleotide sequence that codes for the amino acid sequence of the protein, many DNA molecules must be made and pooled to ensure that the mixture will contain the one that exactly matches the DNA sequence of the gene. For the three peptide sequences given in this question, the following probes need to be made (alternative bases at the same position are given in parentheses):

Peptide 1:
5′-TGGATGCA(C,T)CA(C,T)AA(A,G)-3′

Because of the three twofold degeneracies, you would need eight (= 2^3) different DNA sequences in the mixture.

Peptide 2:
5′(T,C)T(G,A,T,C)(A,T)(G,C)(G,A,T,C)(A,C)G(G,A,T,C)(T,C)T(G,A,T,C)(A,C)G(G,A,T,C)-3′

The mixture representing peptide sequence #2 is much more complicated. Leu, Ser, and Arg are each encoded by six different codons; you would therefore need to synthesize a mixture of 7776 (= 6^5) different DNA molecules.

Peptide 3:
5′-TA(C,T)TT(C,T)GG(G,A,T,C)ATGCA(A,G)3′

Because of three twofold and one fourfold degeneracies, you would need 32 (= $2^3 \times 4$) different sequences in the mixture.

You would probably first use probe #1 to screen your library by hybridization. Because there are only eight possible DNA sequences, the ratio of the one correct sequence to the incorrect ones is highest, giving you the best chance of finding a matching clone. Probe #2 is practically useless, because only 1/7776 of the DNA in the mixture would perfectly hybridize to your gene of interest. You could use probe #3 to verify that the clone you obtained is correct. Any library clones that hybridize to probes #1 and #3 are very likely to contain the gene of interest.

B. Knowing that peptide sequence #3 contains the last amino acid of the protein is valuable information because it tells you that the other two peptide sequences must precede it, that is, they must be located farther toward the N-terminal end of the protein. Knowing this order is important, because DNA primers can be extended by DNA polymerases only from their 3′ ends; thus, the 3′ ends of two primers need to "face" each other during a PCR amplification reaction (see Figure 10–14). A PCR primer based on peptide sequence #3 must therefore be the complementary sequence of probe #3 (so that its 3′ end corresponds to the first nucleotide of the sequence complementary to the Trp codon):

5′-(TC)TGCAT(G,A,T,C)CC(G,A)AA(G,A)TA-3′

As before, this "primer" would contain 32 different DNA sequences, only one of which will perfectly match the gene. Probe #1 could be your choice for the second primer. Probe #2, again because of its high degeneracy, would be a much less suitable choice.

C. The ends of the final amplification product are derived from the primers, which are each 15 nucleotides long. Therefore, a 270-nucleotide segment of the cDNA of the gene has been amplified. This will encode 90 amino acids; adding the amino acids encoded by the primers gives you a protein-coding sequence of 100 amino acids. This is unlikely to represent the whole gene. To your satisfaction, however, you note that CTATCACGCTTAGG encodes peptide sequence #2. This information therefore confirms that your PCR product indeed encodes a fragment of the protein you originally isolated.

ANSWER 10–13

The products will comprise a large number of different single-stranded DNA molecules, one for each nucleotide in the sequence. However, each DNA molecule will be one of four colors, depending on which of the four dideoxyribonucleotides terminated the polymerization reaction of that chain. Separation by gel electrophoresis will generate a ladder of bands, each one nucleotide apart, and the sequence can be read from the order of colors (**Figure A10–13**). The method described here forms the basis for the DNA sequencing strategy used in most automated DNA sequencing machines (see Figure 10–21).

Figure A10–13

ANSWER 10–14

A. cDNA clones could not be used because there is no overlap between cDNA clones from adjacent genes.

B. Such repetitive DNA sequences can confuse chromosome walks, because the walk would appear to branch off in many different directions at once. The general strategy for avoiding these problems is to use genomic clones that are sufficiently long to span beyond the repetitive DNA sequences.

ANSWER 10–15

A. Infants 2 and 8 have identical STR patterns and therefore must be identical twins. Infants 3 and 6 also have identical STR patterns and must also be identical twins. The other two sets of twins must be fraternal twins because their STR patterns are not identical. Fraternal twins, like any pair of siblings born to the same parents, will have roughly half their genome in common. Thus, roughly half the STR polymorphisms in fraternal twins will be identical. Using this criterion, you can identify infants 1 and 7 as fraternal twins and infants 4 and 5 as fraternal twins.

B. You can match infants to their parents by using the same sort of analysis of STR polymorphisms. Every band present in the analysis of an infant should have a matching band in one or the other of the parents, and, on average, each infant will share half of its

polymorphisms with each parent. Thus, the degree of match between each child and each parent will be approximately the same as that between fraternal twins.

ANSWER 10–16 Mutant bacteria that do not produce ice-protein have probably arisen many times in nature. However, bacteria that produce ice-protein have a slight growth advantage over bacteria that do not, so it would be difficult to find such mutants in the wild. Recombinant DNA technology makes these mutants much easier to obtain. In this case, the consequences, both advantageous and disadvantageous, of using a genetically modified organism are therefore nearly indistinguishable from those of a natural mutant. Indeed, bacterial and yeast strains have been selected for centuries for desirable genetic traits that make them suitable for industrial-scale applications such as cheese and wine production. The possibilities of recombinant DNA technology are endless, however, and as with any technology, there is a finite risk of unforeseen consequences. Recombinant DNA experimentation, therefore, is regulated, and the risks of individual projects are carefully assessed by review panels before permissions are granted. The state of our knowledge is sufficiently advanced that the consequences of some changes, such as the disruption of a bacterial gene in the example above, can be predicted with reasonable certainty. Other applications, such as germ-line gene therapy to correct human disease, may have far more complex outcomes, and it will take many more years of research and ethical debate to determine whether such treatments will eventually be used.

Chapter 11

ANSWER 11–1 Water is a liquid, and thus hydrogen bonds between water molecules are not static; they are continually formed and broken again by thermal motion. When a water molecule happens to be next to a hydrophobic molecule, it is more restricted in motion and has fewer neighbors with which it can interact because it cannot form any hydrogen bonds in the direction of the hydrophobic molecule. It will therefore form hydrogen bonds to the more limited number of water molecules in its proximity. Bonding to fewer partners results in a more ordered water structure, which represents the cagelike structure in Figure 11–9. This structure has been likened to ice, although it is a more transient, less organized, and less extensive network than even a tiny ice crystal. The formation of any ordered structure decreases the entropy of the system and is thus energetically unfavorable (discussed in Chapter 3).

ANSWER 11–2 (B) is the correct analogy for lipid bilayer assembly because exclusion from water rather than attractive forces between the lipid molecules is involved. If the lipid molecules formed bonds with one another, the bilayer would be less fluid, and might even become rigid, depending on the strength of the interaction.

ANSWER 11–3 The fluidity of the bilayer is strictly confined to one plane: lipid molecules can diffuse laterally in their own monolayer but do not readily flip from one monolayer to the other. Specific types of lipid molecules inserted into one monolayer therefore remain in it unless they are actively transferred by an enzyme—called a flippase.

ANSWER 11–4 In both an α helix and a β barrel the polar peptide bonds of the polypeptide backbone can be completely shielded from the hydrophobic environment of the lipid bilayer by the hydrophobic amino acid side chains. Internal hydrogen bonds between the peptide bonds stabilize the α helix and β barrel.

ANSWER 11–5 The sulfate group in SDS is charged and therefore hydrophilic. The OH group and the C–O–C groups in Triton X-100 are polar; they can form hydrogen bonds with water molecules and are therefore hydrophilic. In contrast, the blue portions of the detergents are either hydrocarbon chains or aromatic rings, neither of which has polar groups that could form hydrogen bonds with water molecules; they are therefore hydrophobic. (See **Figure A11–5**.)

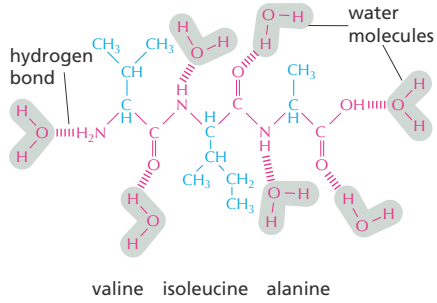


Figure A11–5

ANSWER 11–6 Some of the molecules of the two different transmembrane proteins are anchored to the spectrin filaments of the cell cortex. These molecules are not free to rotate or diffuse within the plane of the membrane. There is an excess of transmembrane proteins over the available attachment sites in the cortex, however, so that some of the transmembrane protein molecules are not anchored and are free to rotate and diffuse within the plane of the membrane. Indeed, measurements of protein mobility show that there are two populations of each transmembrane protein, corresponding to those proteins that are anchored and those that are not.

ANSWER 11–7 The different ways in which membrane proteins can be restricted to different regions of the membrane are summarized in Figure 11–31. The mobility of the membrane proteins is drastically reduced if they are bound to other proteins such as those of the cell cortex or the extracellular matrix. Some membrane proteins are confined to membrane domains by barriers, such as tight junctions. The fluidity of the lipid bilayer is not significantly affected by the anchoring of membrane proteins; the sea of lipid molecules flows around anchored membrane proteins like water around the posts of a pier.

ANSWER 11–8 All of the statements are correct.

A, B, C, D. The lipid bilayer is fluid because its lipid molecules can undergo these motions.

E. Glycolipids are mostly restricted to the monolayer of membranes that faces away from the cytosol. Some special glycolipids, such as phosphatidylinositol (discussed in Chapter 16), are found specifically in the cytosolic monolayer.

F. The reduction of double bonds (by hydrogenation) allows the resulting saturated lipid molecules to pack

more tightly against one another and therefore increases viscosity—that is, it turns oil into margarine.

G. Examples include the many membrane enzymes involved in cell signaling (discussed in Chapter 16).

H. Polysaccharides are the main constituents of mucus and slime; the carbohydrate coat of a cell, which is made up of polysaccharides and oligosaccharides, is a very important lubricant—for cells that line blood vessels or circulate in the bloodstream, for example.

ANSWER 11–9 In a two-dimensional fluid, the molecules are free to move only in one plane; the molecules in a normal fluid, in contrast, can move in three dimensions.

ANSWER 11–10

A. You would have a detergent. The diameter of the lipid head would be much larger than that of the hydrocarbon tail, so that the shape of the molecule would be a cone rather than a cylinder, and the molecules would aggregate to form micelles rather than bilayers.

B. Lipid bilayers formed would be much more fluid, as the tails would have less tendency to interact with one another. The bilayers would also be less stable, as the shorter hydrocarbon tails would be less hydrophobic, so the forces that drive the formation of the bilayer would be reduced.

C. The lipid bilayers formed would be much less fluid. Whereas a normal lipid bilayer has the viscosity of olive oil, a bilayer made of the same lipids but with saturated hydrocarbon tails would have the consistency of bacon fat.

D. The lipid bilayers formed would be much more fluid. Also, because the lipids would pack together less well, there would be more gaps and the bilayer would be more permeable to small, water-soluble molecules.

E. If we assume that the lipid molecules are completely intermixed, the fluidity of the membrane would be unchanged. In such bilayers, however, the saturated lipid molecules would tend to aggregate with one another because they can pack so much more tightly and would therefore form patches of much-reduced fluidity. The bilayer would not, therefore, have uniform properties over its surface. Because, normally, one saturated and one unsaturated hydrocarbon tail are linked to the same hydrophilic head in membrane phospholipid molecules, such segregation does not occur in cell membranes.

F. The lipid bilayers formed would have virtually unchanged properties. Each lipid molecule would now span the entire membrane, with one of its two head groups exposed at each surface. Such lipid molecules are found in the membranes of thermophilic bacteria, which can live at temperatures approaching boiling water. Their bilayers do not come apart at elevated temperatures, as usual bilayers do, because the original two monolayers are now covalently linked into a single structure.

ANSWER 11–11 Phospholipid molecules are approximately cylindrical in shape. Detergent molecules, by contrast, are conical or wedge-shaped. A phospholipid molecule with only one hydrocarbon tail, for example, would be a detergent. To make a phospholipid molecule into a detergent, you would have to make its hydrophilic head larger or remove one of its tails so that it could form a micelle. Detergent molecules also usually have shorter hydrocarbon tails than phospholipid molecules. This makes them slightly water-soluble, so that detergent molecules leave and re-enter micelles frequently in aqueous solution. Because of this, some monomeric detergent molecules are always present in aqueous solution and therefore can enter the lipid bilayer of a cell membrane to solubilize the proteins (see Figure 11–26).

ANSWER 11–12

A. There are about 4000 lipid molecules, each 0.5 nm wide, between one end of the bacterial cell and the other. So if a lipid molecule at one end moved directly in a straight line it would require only 4×10^{-4} sec ($= 4000 \times 10^{-7}$) to reach the other end. In reality, however, the lipid molecule would move in a random path so that it would take considerably longer. We can calculate the approximate time required from the equation: $t = x^2/2D$ where x is the average distance moved, t is the time taken, and D is a constant called the diffusion coefficient. Inserting step values x = 0.5 nm and $t = 10^{-7}$ sec we obtain $D = 1.25 \times 10^{-7}$ cm^2/sec. Using this value in the same equation but with distance $x = 2 \times 10^{-4}$ cm ($= 2$ μm) gives the time taken $t = 1.6$ seconds.

B. Similarly, if a 4-cm ping-pong ball exchanged partners every 10^{-7} seconds and moved in a linear fashion it would reach the opposite wall in 1.5×10^{-5} sec (traveling at 1,440,000 km/hr. But a random walk would take longer. Using the equation above, we calculate the constant D in this case to be 8×10^7 cm^2/sec and the time required to travel 6 m about 2 msec ($= 600^2/(1.6 \times 10^8)$).

ANSWER 11–13 Transmembrane proteins anchor the plasma membrane to the underlying cell cortex, strengthening the membrane so that it can withstand the forces on it when the red blood cell is pumped through small blood vessels. Transmembrane proteins also transport nutrients and ions across the plasma membrane.

ANSWER 11–14 The hydrophilic faces of the five membrane-spanning α helices, each contributed by a different subunit, are thought to come together to form a pore across the lipid bilayer that is lined with the hydrophilic amino acid side chains (**Figure A11–14**). Ions can pass through this hydrophilic pore without coming into contact with the hydrophobic lipid tails of the bilayer. The hydrophobic side chains on the opposite face of the α helices interact with the hydrophobic lipid tails.

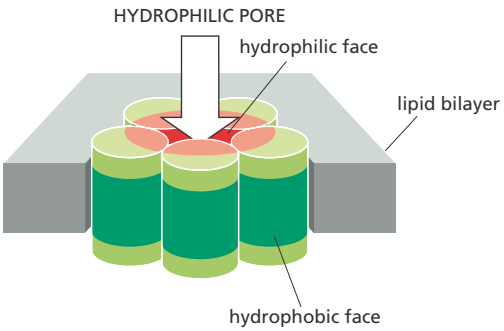


Figure A11–14

ANSWER 11–15 There are about 100 lipid molecules (i.e., phospholipid + cholesterol) for every protein molecule in

the membrane [= (2/50,000)/(1/800 + 1/386)]. A similar protein/lipid ratio is seen in many cell membranes.

ANSWER 11–16 Membrane fusion does not alter the orientation of the membrane proteins with their attached color tags: the portion of each transmembrane protein that is exposed to the cytosol always remains exposed to the cytosol, and the portion exposed to the outside always remains exposed to the outside despite diffusional mixing (**Figure A11–16**). At 0°C, the fluidity of the membrane is reduced, and the mixing of the membrane proteins is significantly slowed.

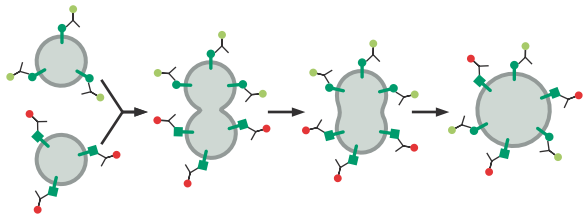


Figure A11–16

ANSWER 11–17 The exposure of hydrophobic amino acid side chains to water is energetically unfavorable. There are two ways that such side chains can be sequestered away from water to achieve an energetically more favorable state. First, they can form transmembrane segments that span a lipid bilayer. This requires about 20 of them to be located sequentially in the polypeptide chain. Second, the hydrophobic amino acid side chains can be sequestered in the interior of the folded polypeptide chain. This is one of the major forces that lock the polypeptide chain into a unique three-dimensional structure. In either case, the hydrophobic forces in the lipid bilayer or in the interior of a protein are based on the same principles.

ANSWER 11–18 (A) Antarctic fish live at subzero temperatures and are cold-blooded. To keep their membranes fluid at these temperatures, they have an unusually high percentage of unsaturated phospholipids.

ANSWER 11–19 Sequence B is most likely to form a transmembrane helix. It is composed primarily of hydrophobic amino acids, and therefore can be stably integrated into a lipid bilayer. In contrast, sequence A contains many polar amino acids (S, T, N, Q), and sequence C contains many charged amino acids (K, R, H, E, D), which would be energetically disfavored in the hydrophobic interior of the lipid bilayer.

Chapter 12

ANSWER 12–1

A. The movement of a solute mediated by a transporter can be described by a strictly analogous equation:

equation 1: $T + S \leftrightarrow TS \rightarrow T + S^*$

where S is the solute, S* is the solute on the other side of the membrane (i.e., although it is still the same molecule, it is now located in a different environment), and T is the transporter.

B. This equation is useful because it describes a binding step, followed by a delivery step. The mathematical treatment of this equation would be very similar to that described for enzymes (see Figure 3–24); thus transporters are characterized by a K_M value that describes their affinity for a solute and a V_{max} value that describes their maximal rate of transfer.

To be more accurate, one could include the conformational change of the transporter in the reaction scheme:

equation 2: $T + S \leftrightarrow TS \leftrightarrow T^*S^* \rightarrow T^* + S^*$

equation 3: $T \leftrightarrow T^*$

where T* is the transporter after the conformational change that exposes its solute-binding site on the other side of the membrane. This account requires a second equation (3) that allows the transporter to return to its starting conformation.

C. The equations do not describe the behavior of channels because solutes passing through channels do not bind to them in the way that a substrate binds to an enzyme.

ANSWER 12–2 If the Na^+ pump is not working at full capacity because it is partially inhibited by ouabain or digitalis, the electrochemical gradient of Na^+ that the pump generates is less steep than that in untreated cells. Consequently, the Ca^{2+}-Na^+ antiport works less efficiently, and Ca^{2+} is removed from the cell more slowly. When the next cycle of muscle contraction begins, there is still an elevated level of Ca^{2+} left in the cytosol. The entry of the same number of Ca^{2+} ions into the cell therefore leads to a higher Ca^{2+} concentration than in untreated cells, which in turn leads to a stronger and longer-lasting muscle contraction. Because the Na^+ pump fulfills essential functions in all animal cells, both to maintain osmotic balance and to generate the Na^+ gradient used to power many transporters, the drugs are deadly poisons if too much is taken.

ANSWER 12–3

A. The properties define a transporter acting as a symport.

B. No additional properties need to be specified. The important feature that provides the coupling of the two solutes is that the protein cannot switch its conformation if only one of the two solutes is bound. Solute B, which is driving the transport of solute A, is in excess on the side of the membrane from which transport initiates and therefore occupies its binding site most of the time. In this state, the transporter is prevented from switching its conformation until a solute A molecule binds, which it will occasionally. With both binding sites occupied, the transporter switches conformation. Now exposed to the other side of the membrane, the binding site for solute B is mostly empty because there is little of it in the solution on this side of the membrane. Although the binding site for A is now more frequently occupied, the transporter can switch back only after solute A is unloaded as well.

C. An antiport could be similarly constructed with a transmembrane protein with the following properties. It has two binding sites, one for solute A and one for solute B. The protein can undergo a conformational change to switch between two states: either both binding sites are exposed exclusively on one side of the membrane or both are exposed exclusively on the other side. The protein can switch between the two conformational states only if one binding site is occupied, but not if both binding sites are either occupied or empty.

Note that these rules described in B and C provide an alternative model to that shown in Figure 12–14.

Thus, in principle, there are two possible ways to couple the transport of two solutes: (1) provide cooperative solute-binding sites and allow the transporter to switch between the two states randomly as shown in Figure 12–14 or (2) allow independent binding of both solutes and make the switch between the two states conditional on the occupancy of the binding sites. As the structure of a coupled transporter has not yet been determined, we do not know which of the two mechanisms such transporters use.

ANSWER 12–4

A. Each of the rectangular peaks corresponds to the opening of a single channel that allows a small current to pass. You note from the recording that the channels present in the patch of membrane open and close frequently. Each channel remains open for a very short, somewhat variable time, averaging about 10 milliseconds. When open, the channels allow a small current with a unique amplitude (4 pA; one picoampere = 10^{-12} A) to pass. In one instance, the current doubles, indicating that two channels in the same membrane patch opened simultaneously.
B. If acetylcholine is omitted or is added to the solution outside the pipette, you would measure only the baseline current. Acetylcholine must bind to the extracellular portion of the acetylcholine receptor in the membrane patch to allow the channel to open frequently enough to detect; in the membrane patch shown in Figure 12–24, only, the cytoplasmic side of the receptor is exposed to the solution outside the microelectrode.

ANSWER 12–5 The equilibrium potential of K^+ is –90 mV [= 62 mV $\log_{10}$ (5 mM/140 mM)], and that of Na^+ is +72 mV [= 62 mV $\log_{10}$ (145 mM/10 mM)]. The K^+ leak channels are the main ion channels open in the plasma membrane of a resting cell, and they allow K^+ to come to equilibrium; the membrane potential of the cell is therefore close to –90 mV. When Na^+ channels open, Na^+ rushes in, and, as a result, the membrane potential reverses its polarity to a value nearer to +72 mV, the equilibrium value for Na^+. Upon closure of the Na^+ channels, the K^+ leak channels allow K^+, now no longer at equilibrium, to exit from the cell until the membrane potential is restored to the equilibrium value for K^+, about –90 mV.

ANSWER 12–6 When the resting membrane potential of an axon (inside negative) rises to a threshold value, voltage-gated Na^+ channels in the immediate neighborhood open and allow an influx of Na^+. This depolarizes the membrane further, causing more voltage-gated Na^+ channels to open, including those in the adjacent plasma membrane. This creates a wave of depolarization that spreads rapidly along the axon, called the action potential. Because Na^+ channels become inactivated soon after they open, the outward flow of K^+ through voltage-gated K^+ channels and K^+ leak channels is rapidly able to restore the original resting membrane potential. (96 words)

ANSWER 12–7 If the number of functional acetylcholine receptors is reduced by the antibodies, the neurotransmitter (acetylcholine) that is released from the nerve terminals cannot (or can only weakly) stimulate the muscle to contract.

ANSWER 12–8 Although the concentration of Cl^- outside cells is much higher than inside, when transmitter-gated Cl^- channels open in the plasma membrane of a postsynaptic neuron in response to an inhibitory neurotransmitter, very little Cl^- enters the cell. This is because the driving force for the influx of Cl^- across the membrane is close to zero at the resting membrane potential, which opposes the influx. If, however, the excitatory neurotransmitter opens Na^+ channels in the postsynaptic membrane at the same time that an inhibitory neurotransmitter opens Cl^- channels, the resulting depolarization caused by the Na^+ influx will cause Cl^- to move into the cell through the open Cl^- channels, neutralizing the effect of the Na^+ influx. In this way, inhibitory neurotransmitters suppress the production of an action potential by making the target cell membrane much harder to depolarize.

ANSWER 12–9 By analogy to the Na^+ pump shown in Figure 12–9, ATP might be hydrolyzed and donate a phosphate group to the transporter when—and only when—it has the solute bound on the cytosolic face of the membrane (step 1 → 2). The attachment of the phosphate would trigger an immediate conformational change (step 2 → 3), thereby capturing the solute and exposing it to the other side of the membrane. The phosphate would be removed from the protein when—and only when—the solute had dissociated, and the now empty, nonphosphorylated transporter would switch back to the starting conformation (step 3 → 4) (**Figure A12–9**).

ANSWER 12–10

A. False. The plasma membrane contains transport proteins that confer selective permeability to many but not all charged molecules. In contrast, a pure lipid bilayer lacking proteins is highly impermeable to all charged molecules.
B. False. Channels do not have binding pockets for the solute that passes through them. Selectivity of a channel is achieved by the size of the internal pore and by charged regions at the entrance of the pore that attract or repel ions of the appropriate charge.
C. False. Transporters are slower. They have enzymelike properties, i.e., they bind solutes and need to undergo

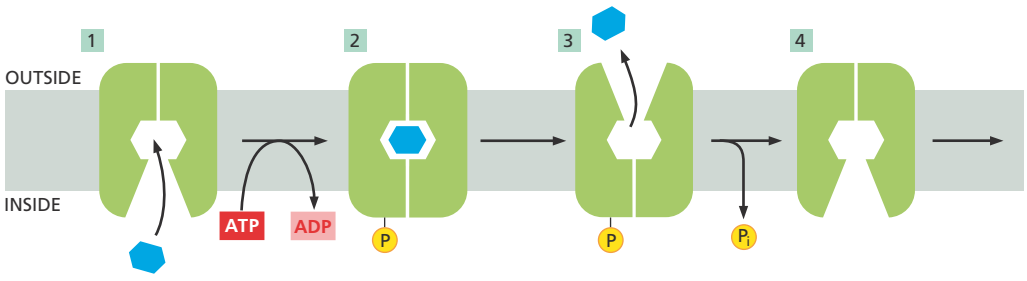


Figure A12–9

conformational changes during their functional cycle. This limits the maximal rate of transport to about 1000 solute molecules per second, whereas channels can pass up to 1,000,000 solute molecules per second.

D. True. The bacteriorhodopsin of some photosynthetic bacteria pumps H^+ out of the cell, using energy captured from visible light.

E. True. Most animal cells contain K^+ leak channels in their plasma membrane that are predominantly open. The K^+ concentration inside the cell still remains higher than outside, because the membrane potential is negative and therefore inhibits the positively charged K^+ from leaking out. K^+ is also continually pumped into the cell by the Na^+ pump.

F. False. A symport binds two different solutes on the same side of the membrane. Turning it around would not change it into an antiport, which must also bind to different solutes, but on opposing sides of the membrane.

G. False. The peak of an action potential corresponds to a transient shift of the membrane potential from a negative to a positive value. The influx of Na^+ causes the membrane potential first to move toward zero and then to reverse, rendering the cell positively charged on its inside. Eventually, the resting potential is restored by an efflux of K^+ through voltage-gated K^+ channels and K^+ leak channels.

ANSWER 12–11 The permeabilities are N_2 (small and nonpolar) > ethanol (small and slightly polar) > water (small and polar) > glucose (large and polar) > Ca^{2+} (small and charged) > RNA (very large and charged).

ANSWER 12–12

A. Both couple the movement of two different solutes across a cell membrane. Symports transport both solutes in the same direction, whereas antiports transport the solutes in opposite directions.

B. Both are mediated by membrane transport proteins. Passive transport of a solute occurs downhill, in the direction of its concentration or electrochemical gradient, whereas active transport occurs uphill and therefore needs an energy source. Active transport can be mediated by transporters but not by channels, whereas passive transport can be mediated by either.

C. Both terms describe gradients across a membrane. The membrane potential refers to the voltage gradient; the electrochemical gradient is a composite of the voltage gradient and the concentration gradient of a specific charged solute (ion). The membrane potential is defined independently of the solute of interest, whereas an electrochemical gradient refers to the particular solute.

D. A pump is a specialized transporter that uses energy to transport a solute uphill—against an electrochemical gradient for a charged solute or a concentration for an uncharged solute.

E. Both transmit electrical signals by means of electrons in wires and ion movements across the plasma membrane in axons. Wires are made of copper, axons are not. The signal passing down an axon does not diminish in strength, because it is self-amplifying, whereas the signal in a wire decreases over distance (by leakage of current across the insulating sheath).

F. Both affect the osmotic pressure in a cell. An ion is a solute that bears a charge.

ANSWER 12–13 A bridge allows vehicles to pass over water in a steady stream; the entrance can be designed to exclude, for example, oversized trucks, and it can be intermittently closed to traffic by a gate. By analogy, gated channels allow ions to pass across a cell membrane, imposing size and charge restrictions.

A ferry, in contrast, loads vehicles on one side of the body of water, crosses, and unloads on the other side—a slower process. During loading, particular vehicles could be selected from the waiting line because they fit particularly well on the car deck. By analogy, transporters bind solutes on one side of the membrane and then, after a conformational movement, release them on the other side. Specific binding selects the molecules to be transported. As in the case of coupled transport, sometimes you have to wait until the ferry is full before you can go.

ANSWER 12–14 Acetylcholine is being transported into the vesicles by an H^+–acetylcholine antiport in the vesicle membrane. The H^+ gradient that drives the uptake is generated by an ATP-driven H^+ pump in the vesicle membrane, which pumps H^+ into the vesicle (hence the dependence of the reaction on ATP). Raising the pH of the solution surrounding the vesicles decreases the H^+ concentration of the solution, thereby increasing the outward gradient across the vesicle membrane, explaining the enhanced rate of acetylcholine uptake.

ANSWER 12–15 The voltage gradient across the membrane is about 150,000 V/cm (70×10^{-3} V/4.5×10^{-7} cm). This extremely powerful electric field is close to the limit at which insulating materials—such as the lipid bilayer—break down and cease to act as insulators. The large field indicates what a large amount of energy can be stored in electrical gradients across the membrane, as well as the extreme electrical forces that proteins can experience in a membrane. A voltage of 150,000 V would instantly discharge in an arc across a 1-cm-wide gap (that is, air would be an insufficient insulator for this strength of field).

ANSWER 12–16

A. Nothing. You require ATP to drive the Na^+ pump.

B. The ATP becomes hydrolyzed, and Na^+ is pumped into the vesicles, generating a concentration gradient of Na^+ across the membrane. At the same time, K^+ is pumped out of the vesicles, generating a concentration gradient of K^+ of opposite polarity. When all the K^+ is pumped out of the vesicle or the ATP runs out, the pump would stop.

C. The pump would initiate a transport cycle and then cease. Because all reaction steps must occur strictly sequentially, dephosphorylation and the accompanying conformational switch cannot occur in the absence of K^+. The Na^+ pump will therefore become stuck in the phosphorylated state, waiting indefinitely for a potassium ion. The number of sodium ions transported would be minuscule, because each pump molecule would have functioned only a single time.

Similar experiments, leaving out individual ions and analyzing the consequences, were used to determine the sequence of steps by which the Na^+ pump works.

D. ATP would become hydrolyzed, and Na^+ and K^+ would be pumped across the membrane as described in (B). However, the pump molecules that sit in the membrane in the reverse orientation would be completely inactive

(i.e., they would not—as one might have erroneously assumed—pump ions in the opposite direction), because ATP would not have access to the site on these molecules where phosphorylation occurs, which is normally exposed to the cytosol. ATP is highly charged and cannot cross membranes without the help of specific transporters.

E. ATP becomes hydrolyzed, and Na^+ and K^+ are pumped across the membrane, as described in (B). K^+, however, immediately flows back into the vesicles through the K^+ leak channels. K^+ moves down the K^+ concentration gradient formed by the action of the Na^+ pump. With each K^+ that moves into the vesicle through a leak channel, a positive charge is moved across the membrane, generating a membrane potential that is positive on the inside of the vesicles. Eventually, K^+ will stop flowing through the leak channels when the membrane potential balances the K^+ concentration gradient. The scenario described here is a slight oversimplification: the Na^+ pump in mammalian cells actually moves three sodium ions out of cells for each two potassium ions that it pumps, thereby driving an electric current across the membrane and making a small additional contribution to the resting membrane potential (which therefore corresponds only approximately to a state of equilibrium for K^+ moving via K^+ leak channels).

ANSWER 12–17 Ion channels can be ligand-gated, voltage-gated, or mechanically (stress) gated.

ANSWER 12–18 The cell has a volume of 10^{-12} liters (= 10^{-15} m^3) and thus contains 6×10^4 calcium ions (= 6×10^{23} molecules/mole × 100×10^{-9} moles/liter × 10^{-12} liters). Therefore, to raise the intracellular Ca^{2+} concentration fiftyfold, another 2,940,000 calcium ions have to enter the cell (note that at 5 μM concentration there are 3×10^6 ions in the cell, of which 60,000 are already present before the channels are opened). Because each of the 1000 channels allows 10^6 ions to pass per second, each channel has to stay open for only 3 milliseconds.

ANSWER 12–19 Animal cells drive most transport processes across the plasma membrane with the electrochemical gradient of Na^+. ATP is needed to fuel the Na^+ pump to maintain the Na^+ gradient.

ANSWER 12–20

A. If H^+ is pumped across the membrane into the endosomes, an electrochemical gradient of H^+ results—composed of both an H^+ concentration gradient and a membrane potential, with the interior of the vesicle positive. Both of these components add to the energy that is stored in the gradient and that must be supplied to generate it. The electrochemical gradient will limit the transfer of more H^+. If, however, the membrane also contains Cl^- channels, the negatively charged Cl^- in the cytosol will flow into the endosomes and diminish their membrane potential. It therefore becomes energetically less expensive to pump more H^+ across the membrane, and the interior of the endosomes can become more acidic.

B. No. As explained in (A), some acidification would still occur in their absence.

ANSWER 12–21

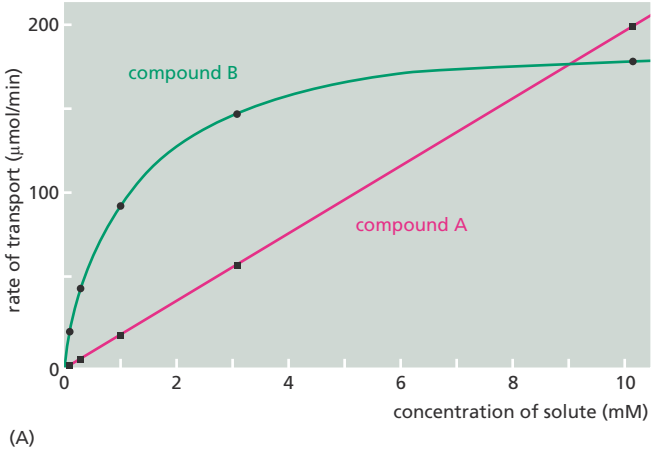
A. See **Figure A12–21A**.

B. The transport rates of compound A are proportional to its concentration, indicating that compound A can diffuse through membranes on its own. Compound A is likely to be ethanol, because it is a small and relatively nonpolar molecule that can diffuse readily through the lipid bilayer (see Figure 12–2).

In contrast, the transport rates of compound B saturate at high concentrations, indicating that compound B is transported across the membrane by some sort of membrane transport protein. Transport rates cannot increase beyond a maximal rate at which this protein can function. Compound B is likely to be acetate, because it is a charged molecule that could not cross the membrane without the help of a membrane transport protein.

C. For ethanol, the graph shows a linear relationship between concentration and transport rate. Thus, at 0.5 mM the transport rate would be 10 μmol/min, and at 100 mM the transport rate would be 2000 μmol/min (2 mmol/min).

For the transport-protein-mediated movement of acetate, the relationship between concentration, S, and transport rate can be described by the Michaelis–Menten equation, which describes simple enzyme reactions:



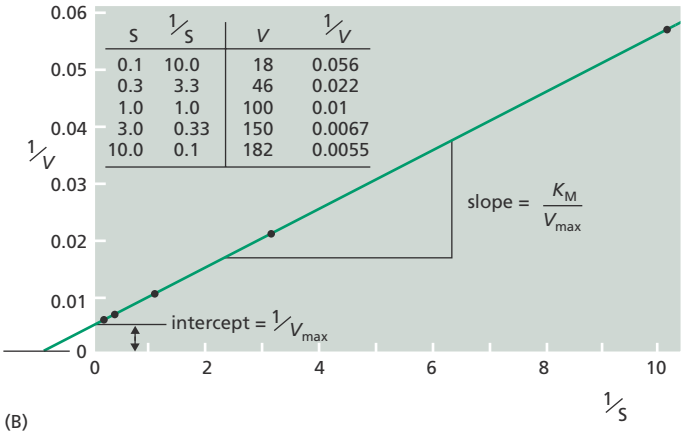


Figure A12–21

equation 1: transport rate = $V_{max} \times S/[K_M + S]$

Recall from Chapter 3 (see Question 3–20, p. 118) that to determine the V_{max} and K_M, a trick is used in which the Michaelis–Menten equation is transformed so that it is possible to plot the data as a straight line. A simple transformation yields

equation 2: **1/rate** = (K_M/V_{max}) **(1/S)** + $1/V_{max}$
(i.e., an equation of the form $y = ax + b$)

Calculation of 1/rate and 1/S for the given data and plotting them in a new graph as in **Figure A12–21B** gives a straight line. The K_M (= 1.0 mM) and V_{max} (= 200 μmol/min) are determined from the intercept of the line with the *y* axis ($1/V_{max}$) and from its slope (K_M/V_{max}). Knowing the values for K_M and V_{max} allows you to calculate the transport rates for 0.5 mM and 100 mM acetate using equation (1). The results are 67 μmol/min and 198 μmol/min, respectively.

ANSWER 12–22 The membrane potential and the steep extracellular Na^+ concentration provide a large inward electrochemical driving force and a large reservoir of Na^+ ions, so that mostly Na^+ ions enter the cell as acetylcholine receptors open. Ca^{2+} ions will also enter the cell, but their influx is much more limited because of their lower extracellular concentration. (Most of the Ca^{2+} that enters the cytosol to stimulate muscle contraction is released from intracellular stores, as we discuss in Chapter 17). Because of the high intracellular K^+ concentration and the opposing direction of the membrane potential, there will be little if any movement of K^+ ions upon opening of a cation channel.

ANSWER 12–23 The diversity of neurotransmitter-gated ion channels is a good thing for the industry, as it raises the possibility of developing new drugs specific for each channel type. Each of the diverse subtypes of these channels is expressed in a narrow subset of neurons. This narrow range of expression should make it possible, in principle, to discover or design drugs that affect particular receptor subtypes present in a selected set of neurons, thus to target particular brain functions with greater specificity.

Chapter 13

ANSWER 13–1 To keep glycolysis going, cells need to regenerate NAD^+ from NADH. There is no efficient way to do this without fermentation. In the absence of regenerated NAD^+, step 6 of glycolysis (the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (Panel 13–1, pp. 428–429) could not occur and the product glyceraldehyde 3-phosphate would accumulate. The same thing would happen in cells unable to make either lactate or ethanol: neither would be able to regenerate NAD^+, and so glycolysis would be blocked at the same step.

ANSWER 13–2 Arsenate instead of phosphate becomes attached in step 6 of glycolysis to form 1-arseno-3-phosphoglycerate (**Figure A13–2**). Because of its sensitivity to hydrolysis in water, the high-energy bond is destroyed before the molecule that contains it can diffuse to reach the next enzyme. The product of the hydrolysis, 3-phosphoglycerate, is the same product normally formed in step 7 by the action of phosphoglycerate kinase. But because hydrolysis occurs nonenzymatically, the energy liberated by breaking the high-energy bond cannot be

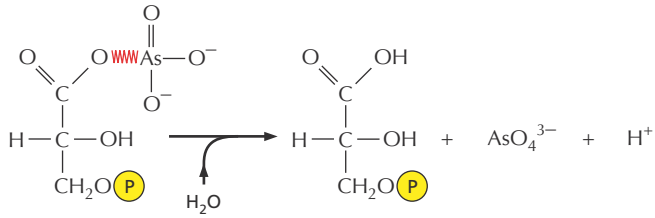


Figure A13–2

captured to generate ATP. In Figure 13–7, therefore, the reaction corresponding to the downward-pointing arrow would still occur, but the wheel that provides the coupling to ATP synthesis is missing. Arsenate wastes metabolic energy by uncoupling many phosphotransfer reactions by the same mechanism, which is why it is so poisonous.

ANSWER 13–3 The oxidation of fatty acids breaks the carbon chain into two-carbon units (acetyl groups) that become attached to CoA. Conversely, during biogenesis, fatty acids are constructed by linking together acetyl groups. Most fatty acids therefore have an even number of carbon atoms.

ANSWER 13–4 Because the function of the citric acid cycle is to harvest the energy released during the oxidation, it is advantageous to break the overall reaction into as many steps as possible (see Figure 13–1). Using a two-carbon compound, the available chemistry would be much more limited, and it would be impossible to generate as many intermediates.

ANSWER 13–5 It is true that oxygen atoms are returned as part of CO_2 to the atmosphere. The CO_2 released from the cells, however, does not contain those specific oxygen atoms that were consumed as part of the oxidative phosphorylation process and converted into water. One can show this directly by incubating living cells in an atmosphere that includes molecular oxygen containing the ^{18}O isotope of oxygen instead of the naturally abundant isotope, ^{16}O. In such an experiment, one finds that all the CO_2 released from cells contains only ^{16}O. Therefore, the oxygen atoms in the released CO_2 molecules do not come directly from the atmosphere but from organic molecules that the cell has first made and then oxidized as fuel (see top of first page of Panel 13–2, pp. 434–435).

ANSWER 13–6 The cycle continues because intermediates are replenished as necessary by reactions leading into the citric acid cycle (instead of away from it). One of the most important reactions of this kind is the conversion of pyruvate to oxaloacetate by the enzyme pyruvate carboxylase:

$$\text{pyruvate} + CO_2 + ATP + H_2O \rightarrow \text{oxaloacetate} + ADP + P_i + 2H^+$$

This is one of the many examples of how metabolic pathways are carefully coordinated to work together to maintain appropriate concentrations of all metabolites required by the cell (see **Figure A13–6**).

ANSWER 13–7 The carbon atoms in sugar molecules are already partially oxidized, in contrast to all but the very first carbon atoms in the acyl chains of fatty acids. Thus,

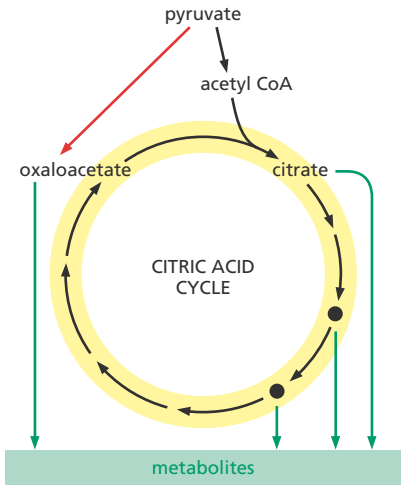


Figure A13–6

two carbon atoms from glucose are lost as CO_2 during the conversion of pyruvate to acetyl CoA, and only four of the six carbon atoms of the sugar molecule are recovered and can enter the citric acid cycle, where most of the energy is captured. In contrast, all carbon atoms of a fatty acid are converted into acetyl CoA.

ANSWER 13–8

A. False. If this were the case, then the reaction would be useless for the cell. No chemical energy would be harvested in a useful form (e.g., ATP) to be used for metabolic processes. (Cells would be nice and warm, though!)
B. False. No energy-conversion process can be 100% efficient. Recall that entropy in the universe always has to increase, and for most reactions this is accomplished by releasing heat.
C. True. The carbon atoms in glucose are in a reduced state compared with those in CO_2, in which they are fully oxidized.
D. False. The reaction does indeed produce some water, but water is so abundant in the biosphere that this is no more than "a drop in the ocean."
E. True. If it had occurred in only one step, then all the energy would be released at once and it would be impossible to harness it efficiently to drive other reactions, such as the synthesis of ATP.
F. False. Molecular oxygen (O_2) is used only in the very last step of the reaction.
G. True. Plants convert CO_2 into sugars by harvesting the energy of light in photosynthesis. O_2 is produced in the process and released into the atmosphere by plant cells.
H. True. Anaerobically growing cells use glycolysis to oxidize sugars to pyruvate. Animal cells convert pyruvate to lactate, and no CO_2 is produced; yeast cells, however, convert pyruvate to ethanol and CO_2. It is this CO_2 gas, released from yeast cells during fermentation, that makes bread dough rise and that carbonates beer and champagne.

ANSWER 13–9 Darwin exhaled the carbon atom, which therefore must be the carbon atom of a CO_2 molecule. After spending some time in the atmosphere, the CO_2 molecule must have entered a plant cell, where it became "fixed" by photosynthesis and converted into part of a sugar molecule. While it is certain that these early steps must have happened this way, there are many different paths from there that the carbon atom could have taken. The sugar could have been broken down by the plant cell into pyruvate or acetyl CoA, for example, which then could have entered biosynthetic reactions to build an amino acid. The amino acid might have been incorporated into a plant protein, maybe an enzyme or a protein that builds the cell wall. You might have eaten the delicious leaves of the plant in your salad, and digested the protein in your gut to produce amino acids again. After circulating in your bloodstream, the amino acid might have been taken up by a developing red blood cell to make its own protein, such as the hemoglobin in question. If we wish, of course, we can make our food chain scenario more complicated. The plant, for example, might have been eaten by an animal that in turn was consumed by you during lunch break. Moreover, because Darwin died more than 100 years ago, the carbon atom could have traveled such a route many times. In each round, however, it would have started again as fully oxidized CO_2 gas and entered the living world following its reduction during photosynthesis.

ANSWER 13–10 Yeast cells grow much better aerobically. Under anaerobic conditions they cannot perform oxidative phosphorylation and therefore have to produce all their ATP by glycolysis, which is less efficient. Whereas one glucose molecule yields a net gain of two ATP molecules by glycolysis, the additional use of the citric acid cycle and oxidative phosphorylation boosts the energy yield up to about 30 ATP molecules.

ANSWER 13–11 The amount of free energy stored in the phosphate bond in creatine phosphate is larger than that of the anhydride bonds in ATP. Hydrolysis of creatine phosphate can therefore be directly coupled to the production of ATP.

$$\text{creatine phosphate} + \text{ADP} \rightarrow \text{creatine} + \text{ATP}$$

The $\Delta G°$ for this reaction is –3 kcal/mole, indicating that it proceeds rapidly to the right, as written.

ANSWER 13–12 The extreme conservation of glycolysis is some of the evidence that all present cells are derived from a single founder cell as discussed in Chapter 1. The elegant reactions of glycolysis would therefore have evolved only once, and then they would have been inherited as cells evolved. The later invention of oxidative phosphorylation allowed follow-up reactions to capture 15 times more energy than is possible by glycolysis alone. This remarkable efficiency is close to the theoretical limit and hence virtually eliminates the opportunity for further improvements. Thus, the generation of alternative pathways would result in no obvious reproductive advantage that would have been selected in evolution.

ANSWER 13–13 If one glucose produces 30 ATPs, then to generate 10^9 ATP molecules will require $1 \times 10^9/30 = 3.3 \times 10^7$ glucose molecules and $6 \times 3.3 \times 10^7 = 2 \times 10^8$ molecules of oxygen. Thus in one minute the cell will consume $2 \times 10^8/(6 \times 10^{23})$ or 3.3×10^{-16} moles of oxygen, which would occupy $3.3 \times 10^{-16} \times 22.4 = 7.4 \times 10^{-15}$ liters in gaseous form. The volume of the cell is 10^{-15} cubic meters $(= (10^{-5})^3)$, which is 10^{-12} liter. The cell therefore consumes about 0.7% of its volume of O_2 gas every minute, or its own volume of O_2 gas in 2 hours and 15 minutes.

ANSWER 13–14 The reactions each have negative ΔG values and are therefore energetically favorable (see **Figure A13–14** for energy diagrams).

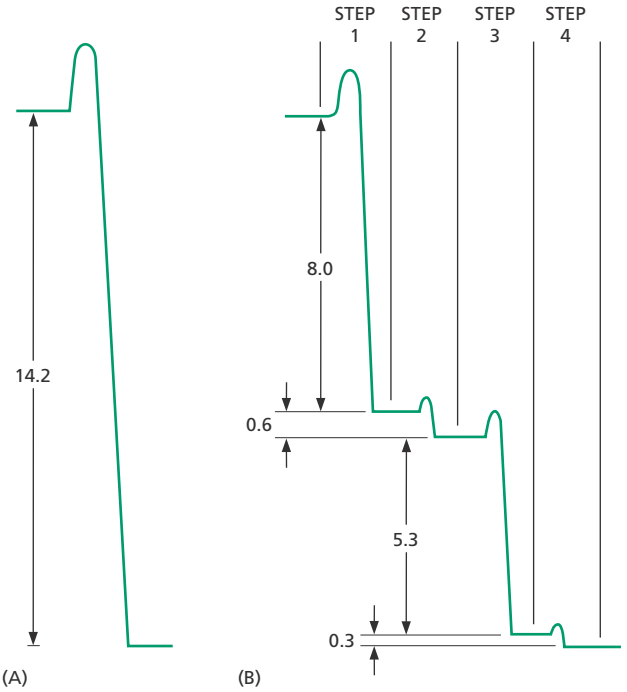


Figure A13–14

ANSWER 13–15

A. Pyruvate is converted to acetyl CoA, and the labeled ^{14}C atom is released as $^{14}CO_2$ gas (see Figure 13–10A).

B. By following the ^{14}C-labeled atom through every reaction in the cycle, shown in Panel 13–2 (pp. 434–435), you find that the added ^{14}C label would be quantitatively recovered in oxaloacetate. The analysis also reveals, however, that it is no longer in the keto group but in the methylene group of oxaloacetate (**Figure A13–15**).

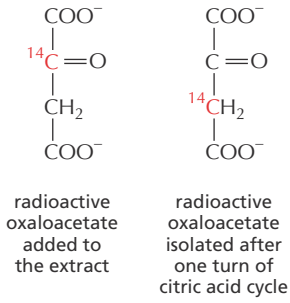


Figure A13–15

ANSWER 13–16 In the presence of molecular oxygen, oxidative phosphorylation converts most of the cellular NADH to NAD^+. Since fermentation requires NADH, it is severely inhibited by the availability of oxygen gas.

Chapter 14

ANSWER 14–1 By making membranes permeable to protons, DNP collapses—or at very small concentrations diminishes—the proton gradient across the inner mitochondrial membrane. Cells continue to oxidize food molecules to feed high-energy electrons into the electron-transport chain, but H^+ ions pumped across the membrane flow back into the mitochondria in a futile cycle. As a result, the energy of the electrons cannot be tapped to drive ATP synthesis, and instead is released as heat. Patients who have been given small doses of DNP lose weight because their fat reserves are used more rapidly to feed the electron-transport chain, and the whole process simply "wastes" energy as heat.

A similar mechanism of heat production is used naturally in a specialized tissue composed of brown fat cells, which is abundant in newborn humans and in hibernating animals. These cells are packed with mitochondria that leak part of their H^+ gradient futilely back across the membrane for the sole purpose of warming up the organism. These cells are brown because they are packed with mitochondria, which contain high concentrations of pigmented proteins, such as cytochromes.

ANSWER 14–2 The inner mitochondrial membrane is the site of oxidative phosphorylation, and it produces most of the cell's ATP. Cristae are portions of the mitochondrial inner membrane that are folded inward. Mitochondria that have a higher density of cristae have a larger area of inner membrane and therefore a greater capacity to carry out oxidative phosphorylation. Heart muscle expends a lot of energy during its continuous contractions, whereas skin cells have a smaller energy demand. An increased density of cristae therefore increases the ATP-production capacity of the heart muscle cell. This is a remarkable example of how cells adjust the abundance of their individual components according to need.

ANSWER 14–3

A. The DNP collapses the electrochemical proton gradient completely. H^+ ions that are pumped to one side of the membrane flow back freely, and therefore no energy to drive ATP synthesis can be stored across the membrane.

B. An electrochemical gradient is made up of two components: a concentration gradient and an electrical potential. If the membrane is made permeable to K^+ with nigericin, K^+ will be driven into the matrix by the electrical potential of the inner membrane (negative inside, positive outside). The influx of positively charged K^+ will abolish the membrane's electrical potential. In contrast, the concentration component of the H^+ gradient (the pH difference) is unaffected by nigericin. Therefore, only part of the driving force that makes it energetically favorable for H^+ ions to flow back into the matrix is lost.

ANSWER 14–4

A. Such a turbine running in reverse is an electrically driven water pump, which is analogous to what the ATP synthase becomes when it uses the energy of ATP hydrolysis to pump protons against their electrochemical gradient across the inner mitochondrial membrane.

B. The ATP synthase should stall when the energy that it can draw from the proton gradient is just equal to the ΔG required to make ATP; at this equilibrium point there will be neither net ATP synthesis nor net ATP consumption.

C. As the cell uses up ATP, the ATP/ADP ratio in the matrix

falls below the equilibrium point just described, and ATP synthase uses the energy stored in the proton gradient to synthesize ATP in order to restore the original ATP/ADP ratio. Conversely, when the electrochemical proton gradient drops below that at the equilibrium point, ATP synthase uses ATP in the matrix to restore this gradient.

ANSWER 14–5 An electron pair causes 10 H^+ to be pumped across the membrane when passing from NADH to O_2 through the three respiratory complexes. Four H^+ are needed to make each ATP: three for synthesis from ADP and one for ATP export to the cytosol. Therefore, 2.5 ATP molecules are synthesized from each NADH molecule.

ANSWER 14–6 One can describe four essential roles for the proteins in the process. First, the chemical environment provided by a protein's amino acid side chains sets the redox potential of each Fe ion such that electrons can be passed in a defined order from one component to the next, giving up their energy in small steps and becoming more firmly bound as they proceed. Second, the proteins position the Fe ions so that the electrons can move efficiently between them. Third, the proteins prevent electrons from skipping an intermediate step; thus, as we have learned for other enzymes (discussed in Chapter 4), they channel the electron flow along a defined path. Fourth, the proteins couple the movement of the electrons down their energy ladder to the pumping of protons across the membrane, thereby harnessing the energy that is released and storing it in a proton gradient that is then used for ATP production.

ANSWER 14–7 It would not be productive to use the same carrier in two steps. If ubiquinone, for example, could transfer electrons directly to the cytochrome *c* oxidase, the cytochrome *c* reductase complex would often be skipped when electrons are collected from NADH dehydrogenase. Given the large difference in redox potential between ubiquinone and cytochrome *c* oxidase, a large amount of energy would be released as heat and thus be wasted. Electron transfer directly between NADH dehydrogenase and cytochrome *c* would similarly allow the cytochrome *c* reductase complex to be bypassed.

ANSWER 14–8 Protons pumped across the inner mitochondrial membrane into the intermembrane space equilibrate with the cytosol, which functions as a huge H^+ sink. Both the mitochondrial matrix and the cytosol support many metabolic reactions that require a pH around neutrality. The H^+ concentration difference, ΔpH, that can be achieved between the mitochondrial matrix and the cytosol is therefore relatively small (less than one pH unit). Much of the energy stored in the mitochondrial electrochemical proton gradient is instead due to the membrane potential (see Figure 14–15).

In contrast, chloroplasts have a smaller, dedicated compartment into which H^+ ions are pumped. Much higher concentration differences can be achieved (up to a thousandfold, or 3 pH units), and much of the energy stored in the thylakoid H^+ gradient is due to the H^+ concentration difference between the thylakoid space and the stroma.

ANSWER 14–9 NADH and NADPH differ by the presence of a single phosphate group. That phosphate gives NADPH a slightly different shape from NADH, which allows these molecules to be recognized by different enzymes, and thus to deliver their electrons to different destinations. Such a division of labor is useful because NADPH tends to be involved in biosynthetic reactions, where high-energy electrons are used to produce energy-rich biological molecules. NADH, on the other hand, is involved in reactions that oxidize energy-rich food molecules to produce ATP. Inside the cell, the ratio of NAD^+ to NADH is kept high, whereas the ratio of $NADP^+$ to NADPH is kept low. This provides plenty of NAD^+ to act as an oxidizing agent and plenty of NADPH to act as a reducing agent—as required for their special roles in catabolism and anabolism, respectively.

ANSWER 14–10

A. Photosynthesis produces sugars, most importantly sucrose, that are transported from the photosynthetic cells through the sap to root cells. There, the sugars are oxidized by glycolysis in the root cell cytoplasm and by oxidative phosphorylation in the root cell mitochondria to produce ATP, as well as being used as the building blocks for many other metabolites.
B. Mitochondria are required even during daylight hours in chloroplast-containing cells to supply the cell with ATP derived by oxidative phosphorylation. Glyceraldehyde 3-phosphate made by photosynthesis in chloroplasts moves to the cytosol and is eventually used as a source of energy to drive ATP production in mitochondria.

ANSWER 14–11 All statements are correct.

A. This is a necessary condition. If it were not true, electrons could not be removed from water and the reaction that splits water molecules ($H_2O \rightarrow 2H^+ + \frac{1}{2}O_2 + 2e^-$) would not occur.
B. Only when excited by light energy does chlorophyll have a low enough affinity for an electron to pass it to an electron carrier with a low electron affinity. This transfer allows the energy of the photon to be harnessed as energy that can be utilized in chemical conversions.
C. It can be argued that this is one of the most important obstacles that had to be overcome during the evolution of photosynthesis: partially reduced oxygen molecules, such as the superoxide radical O_2^-, are dangerously reactive and will attack and destroy almost any biologically active molecule. These intermediates therefore have to remain tightly bound to the metals in the active site of the enzyme until all four electrons have been removed from two water molecules. This requires the sequential capture of four photons by the same reaction center.

ANSWER 14–12

A. True. NAD^+ and quinones are examples of compounds that do not have metal ions but can participate in electron transfer.
B. False. The potential is due to protons (H^+) that are pumped across the membrane from the matrix to the intermembrane space. Electrons remain bound to electron carriers in the inner mitochondrial membrane.
C. True. Both components add to the driving force that makes it energetically favorable for H^+ to flow back into the matrix.
D. True. Both move rapidly in the plane of the membrane.
E. False. Not only do plants need mitochondria to make ATP in cells that do not have chloroplasts, such as root cells, but mitochondria make most of the cytosolic ATP in all plant cells.

F. True. Chlorophyll's physiological function requires it to absorb light; heme just happens to be a colored compound from which blood derives its red color.
G. False. Chlorophyll absorbs light and transfers energy in the form of an energized electron, whereas the iron in heme is a simple electron carrier.
H. False. Most of the dry weight of a tree comes from carbon derived from the CO_2 that has been fixed during photosynthesis.

ANSWER 14–13 It takes three protons. The precise value of the ΔG for ATP synthesis depends on the concentrations of ATP, ADP, and P_i (as described in Chapter 3). The higher the ratio of the concentration of ATP to ADP, the more energy it takes to make additional ATP. The lower value of 11 kcal/mole therefore applies to conditions where cells have expended a lot of energy and have therefore decreased the normal ATP/ADP ratio.

ANSWER 14–14 If no O_2 is available, all components of the mitochondrial electron-transport chain will accumulate in their *reduced* form. This is the case because electrons derived from NADH enter the chain but cannot be transferred to O_2. The electron-transport chain therefore stalls with all of its components in the reduced form. If O_2 is suddenly added again, the electron carriers in cytochrome *c* oxidase will become *oxidized before* those in NADH dehydrogenase. This is true because, after O_2 addition, cytochrome *c* oxidase will donate its electrons directly to O_2, thereby becoming oxidized. A wave of increasing oxidation then passes backward with time from cytochrome *c* oxidase through the components of the electron-transport chain, as each component regains the opportunity to pass on its electrons to downstream components.

ANSWER 14–15 As oxidized ubiquinone becomes reduced, it picks up two electrons but also two protons from water (Figure 14–23). Upon oxidation, these protons are released. If reduction occurs on one side of the membrane and oxidation at the other side, a proton is pumped across the membrane for each electron transported. Electron transport by ubiquinone thereby contributes directly to the generation of the H^+ gradient.

ANSWER 14–16 Photosynthetic bacteria and plant cells use the electrons derived in the reaction $2H_2O \rightarrow 4e^- + 4H^+ + O_2$ to reduce $NADP^+$ to NADPH, which is then used to produce useful metabolites. If the electrons were used instead to produce H_2 in addition to O_2, the cells would lose any benefit they derive from carrying out the reaction, because the electrons could not take part in metabolically useful reactions.

ANSWER 14–17
A. The switch in solutions creates a pH gradient across the thylakoid membrane. The flow of H^+ ions down its electrochemical potential drives ATP synthase, which converts ADP to ATP.
B. No light is needed, because the H^+ gradient is established artificially without a need for the light-driven electron-transport chain.
C. Nothing. The H^+ gradient would be in the wrong direction; ATP synthase would not work.
D. The experiment provided early supporting evidence for the chemiosmotic model by showing that an H^+ gradient alone is sufficient to drive ATP synthesis.

ANSWER 14–18
A. When the vesicles are exposed to light, H^+ ions (derived from H_2O) pumped into the vesicles by the bacteriorhodopsin flow back out through the ATP synthase, causing ATP to be made in the solution surrounding the vesicles in response to light.
B. If the vesicles are leaky, no H^+ gradient can form and thus ATP synthase cannot work.
C. Using components from widely divergent organisms can be a very powerful experimental tool. Because the two proteins come from such different sources, it is very unlikely that they form a direct functional interaction. The experiment therefore strongly suggests that electron transport and ATP synthesis are separate events. This approach is therefore a valid one.

ANSWER 14–19 The redox potential of $FADH_2$ is too low to transfer electrons to the NADH dehydrogenase complex, but high enough to transfer electrons to ubiquinone (Figure 14–24). Therefore, electrons from $FADH_2$ can enter the electron-transport chain only at this step (**Figure A14–19**). Because the NADH dehydrogenase complex is bypassed, fewer H^+ ions are pumped across the membrane and less ATP is made. This example shows the versatility of the electron-transport chain. The ability to use vastly different sources of electrons from the environment to feed electron transport is thought to have been an essential feature in the early evolution of life.

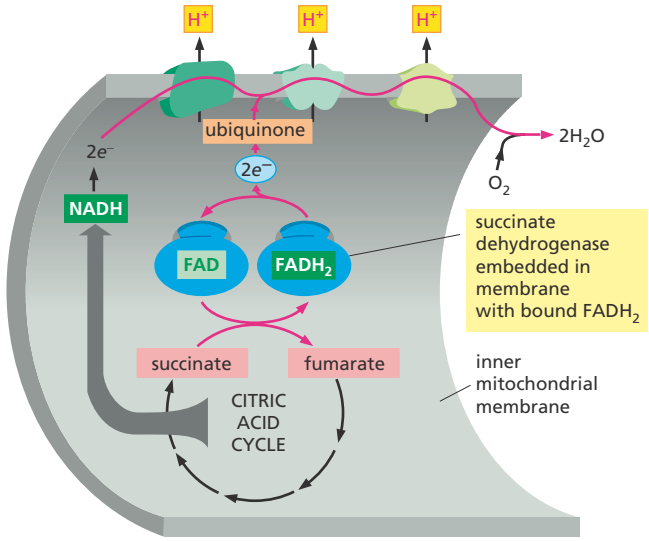


Figure A14–19

ANSWER 14–20 If these bacteria used a proton gradient to make their ATP in a fashion analogous to that in other bacteria (that is, fewer protons inside than outside), they would need to raise their cytoplasmic pH even higher than that of their environment (pH 10). Cells with a cytoplasmic pH greater than 10 would not be viable. These bacteria must therefore use gradients of ions other than H^+, such as Na^+ gradients, in the chemiosmotic coupling between electron transport and an ATP synthase.

ANSWER 14–21 Statements A and B are accurate. Statement C is incorrect, because the chemical reactions that are carried out in each cycle are completely different, even though the net effect is the same as that expected for simple reversal.

ANSWER 14–22 This experiment would suggest a two-step model for ATP synthase function. According to this model, the flow of protons through the base of the synthase drives rotation of the head, which in turn causes ATP synthesis. In their experiment, the authors have succeeded in uncoupling these two steps. If rotating the head mechanically is sufficient to produce ATP in the absence of any applied proton gradient, the ATP synthase is a protein machine that indeed functions like a "molecular turbine." This would be a very exciting experiment indeed, because it would directly demonstrate the relationship between mechanical movement and enzymatic activity. There is no doubt that it should be published and that it would become a "classic."

ANSWER 14–23 Only under condition (E) is electron transfer observed, with cytochrome *c* becoming reduced. A portion of the electron-transport chain has been reconstituted in this mixture, so that electrons can flow in the energetically favored direction from reduced ubiquinone to the cytochrome *c* reductase complex to cytochrome *c*. Although energetically favorable, the transfer in (A) cannot occur spontaneously in the absence of the cytochrome *c* reductase complex to catalyze this reaction. No electron flow occurs in the other experiments, whether the cytochrome *c* reductase complex is present or not: in experiments (B) and (F) both ubiquinone and cytochrome *c* are oxidized; in experiments (C) and (G) both are reduced; and in experiments (D) and (H) electron flow is energetically disfavored because an electron in reduced cytochrome *c* has a lower free energy than an electron added to oxidized ubiquinone.

Chapter 15

ANSWER 15–1 Although the nuclear envelope forms one continuous membrane, it has specialized regions that contain special proteins and have a characteristic appearance. One such specialized region is the inner nuclear membrane. Membrane proteins can indeed diffuse between the inner and outer nuclear membranes, at the connections formed around the nuclear pores. Those proteins with particular functions in the inner membrane, however, are usually anchored there by their interaction with other components such as chromosomes and the nuclear lamina (a protein meshwork underlying the inner nuclear membrane that helps give structural integrity to the nuclear envelope).

ANSWER 15–2 Eukaryotic gene expression is more complicated than prokaryotic gene expression. In particular, prokaryotic cells do not have introns that interrupt the coding sequences of their genes, so that an mRNA can be translated immediately after it is transcribed, without a need for further processing (discussed in Chapter 7). In fact, in prokaryotic cells, ribosomes start translating most mRNAs before transcription is finished. This would have disastrous consequences in eukaryotic cells, because most RNA transcripts have to be spliced before they can be translated. The nuclear envelope separates the transcription and translation processes in space and time: a primary RNA transcript is held in the nucleus until it is properly processed to form an mRNA, and only then is it allowed to leave the nucleus so that ribosomes can translate it.

ANSWER 15–3 An mRNA molecule is attached to the ER membrane by the ribosomes translating it. This ribosome population, however, is not static; the mRNA is continuously moved through the ribosome. Those ribosomes that have finished translation dissociate from the 3′ end of the mRNA and from the ER membrane, but the mRNA itself remains bound by other ribosomes, newly recruited from the cytosolic pool, that have attached to the 5′ end of the mRNA and are still translating the mRNA. Depending on its length, there are about 10–20 ribosomes attached to each membrane-bound mRNA molecule.

ANSWER 15–4

A. The internal signal sequence functions as a membrane anchor, as shown in Figure 15–17. Because there is no stop-transfer sequence, however, the C-terminal end of the protein continues to be translocated into the ER lumen. The resulting protein therefore has its N-terminal domain in the cytosol, followed by a single transmembrane segment, and a C-terminal domain in the ER lumen (**Figure A15–4A**).
B. The N-terminal signal sequence initiates translocation of the N-terminal domain of the protein until translocation is stopped by the stop-transfer sequence. A cytosolic domain is synthesized until the start-transfer sequence initiates translocation again. The situation now resembles that described in (A), and the C-terminal domain of the protein is translocated into the lumen of the ER. The resulting protein therefore spans the membrane twice. Both its N-terminal and C-terminal domains are in the ER lumen, and a loop domain between the two transmembrane regions is exposed in the cytosol (**Figure A15–4B**).
C. It would need a cleaved signal sequence, followed by an internal stop-transfer sequence, followed by pairs of start- and stop-transfer sequences (**Figure A15–4C**).

These examples demonstrate that complex protein topologies can be achieved by simple variations and combinations of the two basic mechanisms shown in Figures 15–16 and 15–17.

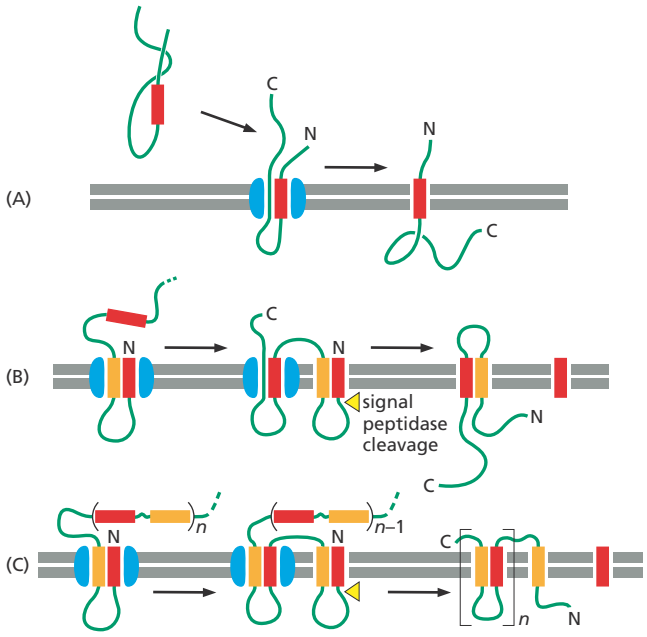


Figure A15–4

ANSWER 15–5

A. Clathrin coats cannot assemble in the absence of

adaptins that link the clathrin to the membrane. At high clathrin concentrations and under the appropriate ionic conditions, clathrin cages assemble in solution, but they are empty shells, lacking other proteins, and they contain no membrane. This shows that the information to form clathrin baskets is contained in the clathrin molecules themselves, which are therefore able to self-assemble.

B. Without clathrin, adaptins still bind to receptors in the membrane, but no clathrin coat can form and thus no clathrin-coated pits or vesicles are produced.
C. Deeply invaginated clathrin-coated pits form on the membrane, but they do not pinch off to form closed vesicles (see Figure A15–13).
D. Prokaryotic cells do not perform endocytosis. A prokaryotic cell therefore does not contain any receptors with appropriate cytosolic tails that could mediate adaptin binding. Therefore, no clathrin can bind and no clathrin coats can assemble.

ANSWER 15–6 The preassembled sugar chain allows better quality control. The assembled oligosaccharide chains can be checked for accuracy before they are added to the protein; if a mistake were made in adding sugars individually to the protein, the whole protein would have to be discarded. Because far more energy is used in building a protein than in building a short oligosaccharide chain, this is a much more economical strategy. This difficulty becomes apparent as the protein moves to the cell surface: although sugar chains are continually modified by enzymes in various compartments of the secretory pathway, these modifications are often incomplete and result in considerable heterogeneity of the glycoproteins that leave the cell. This heterogeneity is largely due to the restricted access that the enzymes have to the sugar trees attached to the surface of proteins. The heterogeneity also explains why glycoproteins are more difficult to study and purify than nonglycosylated proteins.

ANSWER 15–7 Aggregates of the secretory proteins would form in the ER, just as they do in the *trans* Golgi network. As the aggregation is specific for secretory proteins, ER proteins would be excluded from the aggregates. The aggregates would eventually be degraded.

ANSWER 15–8 Transferrin without Fe bound does not interact with its receptor and circulates in the bloodstream until it catches an Fe ion. Once iron is bound, the iron–transferrin complex can bind to the transferrin receptor on the surface of a cell and be endocytosed. Under the acidic conditions of the endosome, the transferrin releases its iron, but the transferrin remains bound to the transferrin receptor, which is recycled back to the cell surface, where it encounters the neutral pH environment of the blood. The neutral pH causes the receptor to release the transferrin into the circulation, where it can pick up another Fe ion to repeat the cycle. The iron released in the endosome, like the LDL in Figure 15–33, moves on to lysosomes, from where it is transported into the cytosol.

The system allows cells to take up iron efficiently even though the concentration of iron in the blood is extremely low. The iron bound to transferrin is concentrated at the cell surface by binding to transferrin receptors; it becomes further concentrated in clathrin-coated pits, which collect the transferrin receptors. In this way, transferrin cycles between the blood and endosomes, delivering the iron that cells need to grow.

ANSWER 15–9

A. True.
B. False. The signal sequences that direct proteins to the ER contain a core of eight or more hydrophobic amino acids. The sequence shown here contains many hydrophilic amino acid side chains, including the charged amino acids His, Arg, Asp, and Lys, and the uncharged hydrophilic amino acids Gln and Ser.
C. True. Otherwise they could not dock at the correct target membrane or recruit a fusion complex to a docking site.
D. True.
E. True. Lysosomal proteins are selected in the *trans* Golgi network and packaged into transport vesicles that deliver them to the late endosome. If not selected, they would enter by default into transport vesicles that move constitutively to the cell surface.
F. False. Lysosomes also digest internal organelles by autophagy.
G. False. Mitochondria do not participate in vesicular transport, and therefore *N*-linked glycoproteins, which are exclusively assembled in the ER, cannot be transported to mitochondria.

ANSWER 15–10 They must contain a nuclear localization signal as well. Proteins with nuclear export signals shuttle between the nucleus and the cytosol. An example is the A1 protein, which binds to mRNAs in the nucleus and guides them through the nuclear pores. Once in the cytosol, a nuclear localization signal ensures that the A1 protein is re-imported so that it can participate in the export of further mRNAs.

ANSWER 15–11 Influenza virus enters cells by endocytosis and is delivered to endosomes, where it encounters an acidic pH that activates its fusion protein. The viral membrane then fuses with the membrane of the endosome, releasing the viral genome into the cytosol (**Figure A15–11**). NH_3 is a small molecule that readily penetrates membranes. Thus, it can enter all intracellular compartments, including endosomes, by diffusion. Once in a compartment that has an acidic pH, NH_3 binds H^+ to form NH_4^+, which is a charged ion and therefore cannot cross the membrane by diffusion. NH_4^+ ions therefore accumulate in acidic compartments, raising their pH. When the pH of the endosome is raised, viruses are still endocytosed, but

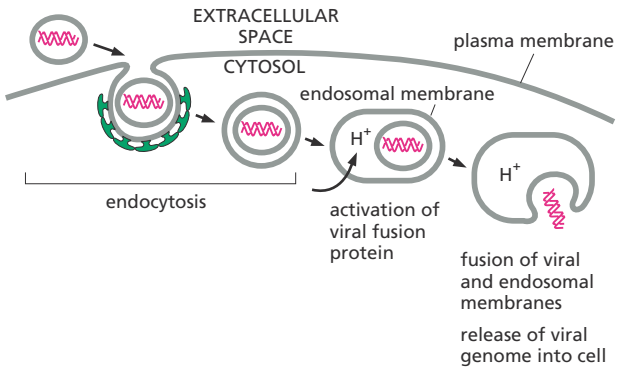


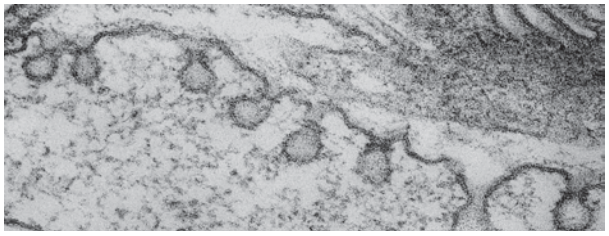
Figure A15–11

because the viral fusion protein cannot be activated, the virus cannot enter the cytosol. Remember this the next time you have the flu and have access to a stable.

ANSWER 15–12

A. The problem is that vesicles having two different kinds of v-SNAREs in their membrane could dock on either of two different membranes.
B. The answer to this puzzle is currently not known, but we can predict that cells must have ways of turning the docking ability of SNAREs on and off. This may be achieved through other proteins that are, for example, co-packaged in the ER with SNAREs into transport vesicles and facilitate the interactions of the correct v-SNARE with the t-SNARE in the *cis* Golgi network.

ANSWER 15–13 Synaptic transmission involves the release of neurotransmitters by exocytosis. During this event, the membrane of the synaptic vesicle fuses with the plasma membrane of the nerve terminals. To make new synaptic vesicles, membrane must be retrieved from the plasma membrane by endocytosis. This endocytosis step is blocked if dynamin is defective, as the protein is required to pinch off the clathrin-coated endocytic vesicles. The first clue to deciphering the role of dynamin came from electron micrographs of synapses of the mutant flies (**Figure A15–13**). Note that there are many flasklike invaginations of the plasma membrane, representing deeply invaginated clathrin-coated pits that cannot pinch off. The collars visible around the necks of these invaginations are made of mutant dynamin.



From J.H. Koenig and K. Ikeda, *J. Neurosci.* 9:3844–3860, 1989. With permission from The Society for Neuroscience.

Figure A15–13

ANSWER 15–14 The first two sentences are correct. The third is not. It should read: "Because the contents of the lumen of the ER or any other compartment in the secretory or endocytic pathways never mix with the cytosol, proteins that enter these pathways will never need to be imported again."

ANSWER 15–15 The protein is translocated into the ER. Its ER signal sequence is recognized as soon as it emerges from the ribosome. The ribosome then becomes bound to the ER membrane, and the growing polypeptide chain is transferred through the ER translocation channel. The nuclear localization sequence is therefore never exposed to the cytosol. It will never encounter nuclear import receptors, and the protein will not enter the nucleus.

ANSWER 15–16 (1) Proteins are imported into the nucleus after they have been synthesized, folded, and, if appropriate, assembled into complexes. In contrast, unfolded polypeptide chains are translocated into the ER as they are being made by the ribosomes. Ribosomes are assembled in the nucleus yet function in the cytosol, and the enzyme complexes that catalyze RNA transcription and splicing are assembled in the cytosol yet function in the nucleus. Thus, both ribosomes and these enzyme complexes need to be transported through the nuclear pores intact. (2) Nuclear pores are gates, which are always open to small molecules; in contrast, translocation channels in the ER membrane are normally closed, and open only after the ribosome has attached to the membrane and the translocating polypeptide chain has sealed the channel from the cytosol. It is important that the ER membrane remain impermeable to small molecules during the translocation process, as the ER is a major store for Ca^{2+} in the cell, and Ca^{2+} release into the cytosol must be tightly controlled (discussed in Chapter 16). (3) Nuclear localization signals are not cleaved off after protein import into the nucleus; in contrast, ER signal peptides are usually cleaved off. Nuclear localization signals are needed to repeatedly re-import nuclear proteins after they have been released into the cytosol during mitosis, when the nuclear envelope breaks down.

ANSWER 15–17 The transient intermixing of nuclear and cytosolic contents during mitosis supports the idea that the nuclear interior and the cytosol are indeed evolutionarily related. In fact, one can consider the nucleus as a subcompartment of the cytosol that has become surrounded by the nuclear envelope, with access only through the nuclear pores.

ANSWER 15–18 The actual explanation is that the single amino acid change causes the protein to misfold slightly so that, although it is still active as a protease inhibitor, it is prevented by chaperone proteins in the ER from exiting this organelle. It therefore accumulates in the ER lumen and is eventually degraded. Alternative interpretations might have been that (1) the mutation affects the stability of the protein in the bloodstream so that it is degraded much faster in the blood than the normal protein, or (2) the mutation inactivates the ER signal sequence and prevents the protein from entering the ER. (3) Another explanation could have been that the mutation altered the sequence to create an ER retention signal, which would have retained the mutant protein in the ER. One could distinguish between these possibilities by using fluorescently tagged antibodies against the protein or by expressing the protein as a fusion with GFP to follow its transport in the cells (see How We Know, pp. 512–513).

ANSWER 15–19 Critique: "Dr. Outonalimb proposes to study the biosynthesis of forgettin, a protein of significant interest. The main hypothesis on which this proposal is based, however, requires further support. In particular, it is questionable whether forgettin is indeed a secreted protein, as proposed. ER signal sequences are normally found at the N-terminus. C-terminal hydrophobic sequences will be exposed outside the ribosome only after protein synthesis has already terminated and can therefore not be recognized by an SRP during translation. It is therefore unlikely that forgettin will be translocated by an SRP-dependent mechanism; it is more likely that it will remain in the cytosol. Dr. Outonalimb should take these considerations into account when submitting a revised application."

ANSWER 15–20 The Golgi apparatus may have evolved from specialized patches of ER membrane. These regions of the ER might have pinched off, forming a new compartment

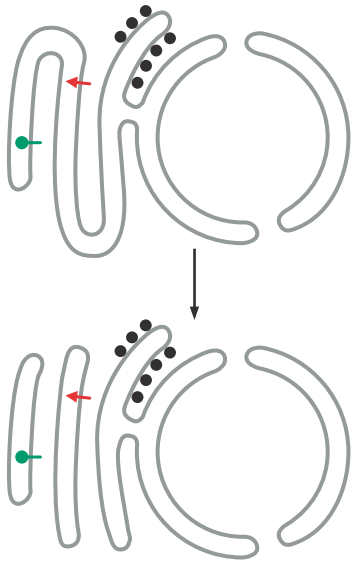


Figure A15–20

(Figure A15–20), which still communicates with the ER by vesicular transport. For the newly evolved Golgi compartment to be useful, transport vesicles would also have to have evolved.

ANSWER 15–21 This is a chicken-and-egg question. In fact, the situation never arises in present-day cells, although it must have posed a considerable problem for the first cells that evolved. New cell membranes are made by expansion of existing membranes, and the ER is never made *de novo*. There will always be an existing piece of ER with translocation channels to integrate new translocation channels. Inheritance is therefore not limited to the propagation of the genome; a cell's organelles must also be passed from generation to generation. In fact, the ER translocation channels can be traced back to structurally related translocation channels in the prokaryotic plasma membrane.

ANSWER 15–22

A. Extracellular space
B. Cytosol
C. Plasma membrane
D. Clathrin coat
E. Membrane of deeply invaginated, clathrin-coated pit
F. Captured cargo particles
G. Lumen of deeply invaginated, clathrin-coated pit

ANSWER 15–23 A single, incomplete round of nuclear import would occur. Because nuclear transport is fueled by GTP hydrolysis, under conditions of insufficient energy, GTP would be used up and no Ran-GTP would be available to unload the cargo protein from its nuclear import receptor upon arrival in the nucleus (see Figure 15–10). Unable to release its cargo, the nuclear import receptor would be stuck at the nuclear pore and not return to the cytosol. Because the nuclear cargo protein is not released, it would not be functional, and no further import could occur.

Chapter 16

ANSWER 16–1 Most paracrine signaling molecules are very short-lived after they are released from a signaling cell: they are either degraded by extracellular enzymes or are rapidly taken up by neighboring target cells. In addition, some become attached to the extracellular matrix and are thus prevented from diffusing too far.

ANSWER 16–2 Polar groups are hydrophilic, so cholesterol, with only one polar –OH group, would be too hydrophobic to be an effective hormone by itself. Because it is virtually insoluble in water, it could not move readily as a messenger from one cell to another via the extracellular fluid, unless carried by specific proteins.

ANSWER 16–3 The protein could be an enzyme that produces a large number of small intracellular signaling molecules such as cyclic AMP or cyclic GMP. Or, it could be an enzyme that modifies a large number of intracellular target proteins—for example, by phosphorylation.

ANSWER 16–4 In the case of the steroid-hormone receptor, a one-to-one complex of steroid and receptor binds to DNA to activate or inactivate gene transcription; there is thus no amplification between ligand binding and transcriptional regulation. Amplification occurs later, because transcription of a gene gives rise to many mRNAs, each of which is translated to give many copies of the protein it encodes (discussed in Chapter 7). For the ion-channel-coupled receptors, a single ion channel will let through thousands of ions in the time it remains open; this serves as the amplification step in this type of signaling system.

ANSWER 16–5 The mutant G protein would be almost continuously activated, because GDP would dissociate spontaneously, allowing GTP to bind even in the absence of an activated GPCR. The consequences for the cell would therefore be similar to those caused by cholera toxin, which modifies the α subunit of G_s so that it cannot hydrolyze GTP to shut itself off. In contrast to the cholera toxin case, however, the mutant G protein would not stay permanently activated: it would switch itself off normally, but then it would instantly become activated again as the GDP dissociated and GTP re-bound.

ANSWER 16–6 Rapid breakdown keeps the intracellular cyclic AMP concentrations low. The lower the cAMP levels are, the larger and faster the increase achieved upon activation of adenylyl cyclase, which makes new cyclic AMP. If you have \$100 in the bank and you deposit another \$100, you have doubled your wealth; if you have only \$10 to start with and you deposit \$100, you have increased your wealth tenfold, a much larger proportional increase resulting from the same deposit.

ANSWER 16–7 Recall that the plasma membrane constitutes a rather small area compared with the total membrane surfaces in a cell (discussed in Chapter 15). The endoplasmic reticulum is especially abundant and spans the entire volume of the cell as a vast network of membrane tubes and sheets. The Ca^{2+} stored in the endoplasmic reticulum can therefore be released throughout the cytosol. This is important because the rapid clearing of Ca^{2+} ions from the cytosol by Ca^{2+} pumps prevents Ca^{2+} from diffusing any significant distance in the cytosol.

ANSWER 16–8 Each reaction involved in the amplification scheme must be turned off to reset the signaling pathway to a resting level. Each of these off switches is equally important.

ANSWER 16–9 Because each antibody has two antigen-binding sites, it can cross-link the receptors and cause them to cluster on the cell surface. This clustering is likely to activate RTKs, which are usually activated by dimerization. For RTKs, clustering allows the individual kinase domains of the receptors to phosphorylate adjacent receptors in the cluster. The activation of GPCRs is more complicated, because the ligand has to induce a particular conformational change; only very special antibodies mimic receptor ligands sufficiently well to induce the conformational change that activates a GPCR.

ANSWER 16–10 The more steps there are in an intracellular signaling pathway, the more places the cell has to regulate the pathway, amplify the signal, integrate signals from different pathways, and spread the signal along divergent paths (see Figure 16–13).

ANSWER 16–11

A. True. Acetylcholine, for example, slows the beating of heart muscle cells by binding to a GPCR and stimulates the contraction of skeletal muscle cells by binding to a different acetylcholine receptor, which is an ion-channel-coupled receptor.
B. False. Acetylcholine is short-lived and exerts its effects locally. Indeed, the consequences of prolonging its lifetime can be disastrous. Compounds that inhibit the enzyme acetylcholinesterase, which normally breaks down acetylcholine at a nerve–muscle synapse, are extremely toxic: for example, the nerve gas sarin, used in chemical warfare, is an acetylcholinesterase inhibitor.
C. True. Nucleotide-free $\beta\gamma$ complexes can activate ion channels, and GTP-bound α subunits can activate enzymes. The GDP-bound form of trimeric G proteins is the inactive state.
D. True. The inositol phospholipid that is cleaved to produce IP_3 contains three phosphate groups, one of which links the sugar to the diacylglycerol lipid. IP_3 is generated by a simple hydrolysis reaction (see Figure 16–27).
E. False. Calmodulin senses but does not regulate intracellular Ca^{2+} levels.
F. True. See Figure 16–40.
G. True. See Figure 16–32.

ANSWER 16–12

1. You would expect a high background level of Ras activity, because Ras cannot be turned off efficiently.
2. Because some Ras molecules are already GTP-bound, Ras activity in response to an extracellular signal would be greater than normal, but this activity would be liable to saturate when all Ras molecules are converted to the GTP-bound form.
3. The response to a signal would be much less rapid, because the signal-dependent increase in GTP-bound Ras would occur over an elevated background of preexisting GTP-bound Ras (see Question 16–6).
4. The increase in Ras activity in response to a signal would also be prolonged compared to the response in normal cells.

ANSWER 16–13

A. Both types of signaling can occur over a long range: neurons can send action potentials along very long axons (think of the axons in the neck of a giraffe, for example), and hormones are carried via the bloodstream throughout the organism. Because neurons secrete large amounts of neurotransmitters at a synapse, a small, well-defined space between two cells, the concentrations of these signal molecules are high; neurotransmitter receptors, therefore, need to bind to neurotransmitters with only low affinity. Hormones, in contrast, are vastly diluted in the bloodstream, where they circulate at often minuscule concentrations; hormone receptors therefore generally bind their hormone with extremely high affinity.
B. Whereas neuronal signaling is a private affair, with one neuron talking to a select group of target cells through specific synaptic connections, endocrine signaling is a public announcement, with any target cell with appropriate receptors able to respond to the hormone in the blood. Neuronal signaling is very fast, limited only by the speed of propagation of the action potential and the workings of the synapse, whereas endocrine signaling is slower, limited by blood flow and diffusion over larger distances.

ANSWER 16–14

A. There are 100,000 molecules of X and 10,000 molecules of Y in the cell (= rate of synthesis × average lifetime).
B. After one second, the concentration of X will have increased by 10,000 molecules. The concentration of X, therefore, one second after its synthesis is increased, is about 110,000 molecules per cell—which is a 10% increase over the concentration of X before the boost of its synthesis. The concentration of Y will also increase by 10,000 molecules, which, in contrast to X, represents a full twofold increase in its concentration (for simplicity, we can neglect the breakdown in this estimation because X and Y are relatively stable during the one-second stimulation).
C. Because of its larger proportional increase, Y is the preferred signaling molecule. This calculation illustrates the surprising but important principle that the time it takes to switch a signal on is determined by the lifetime of the signaling molecule.

ANSWER 16–15 The information transmitted by a cell signaling pathway is contained in the *concentration* of the messenger, be it a small molecule or a phosphorylated protein. Therefore, to allow the detection of a change in concentration, the original messenger has to be both rapidly destroyed and rapidly resynthesized. The shorter the average lifetime of the messenger population, the faster the system can respond to changes. Human communication relies on messages that are delivered only once and that are generally not interpreted by their abundance but by their *content*. So it is a mistake to kill the human messengers; they can be used more than once.

ANSWER 16–16

A. The mutant RTK lacking its extracellular ligand-binding domain is inactive. It cannot bind extracellular signals, and its presence has no consequences for the function of the normal RTK (**Figure A16–16A**).
B. The mutant RTK lacking its intracellular domain is also inactive, but its presence will block signaling by the normal receptors. When a signal molecule binds to either receptor, it will induce their dimerization. Two

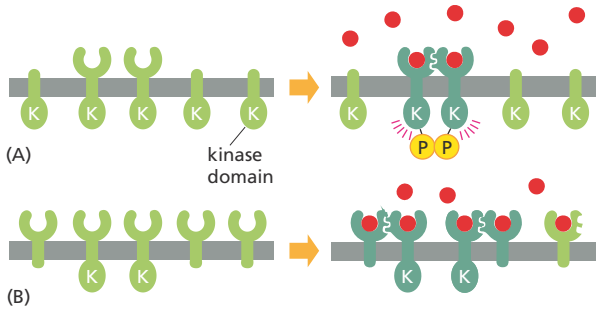


Figure A16–16

normal receptors have to come together to activate each other by phosphorylation. In the presence of an excess of mutant receptors, however, normal receptors will usually form mixed dimers, in which their intracellular domain cannot be activated because their partner is a mutant and lacks a kinase domain (Figure A16–16B).

ANSWER 16–17 The statement is correct. Upon ligand binding, transmembrane helices of multispanning receptors, like the GPCRs, shift and rearrange with respect to one another (Figure A16–17A). This conformational change is sensed on the cytosolic side of the membrane because of a change in the arrangement of the cytoplasmic loops. A single transmembrane segment is not sufficient to transmit a signal across the membrane directly; no rearrangements in the membrane are possible upon ligand binding. Upon ligand binding, single-span receptors such as most RTKs tend to dimerize, thereby bringing their intracellular kinase domains into proximity so that they can cross-phosphorylate and activate each other (Figure A16–17B).

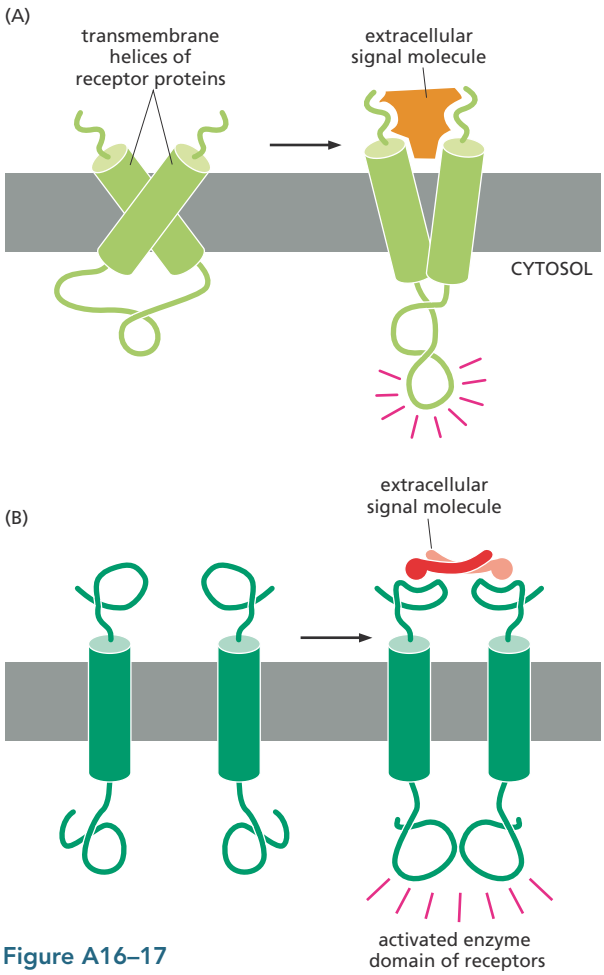


Figure A16–17

ANSWER 16–18 Activation in both cases depends on proteins that catalyze GDP–GTP exchange on the G protein or Ras protein. Whereas activated GPCRs perform this function directly for G proteins, enzyme-linked receptors assemble multiple signaling proteins into a signaling complex when the receptors are activated by phosphorylation; one of these is an adaptor protein that recruits a guanine nucleotide exchange factor that fulfills this function for Ras.

ANSWER 16–19 Because the cytosolic concentration of Ca^{2+} is so low, an influx of relatively few Ca^{2+} ions leads to large changes in its cytosolic concentration. Thus, a tenfold increase in cytosolic Ca^{2+} can be achieved by raising its concentration into the micromolar range, which would require far fewer ions than would be required to change significantly the cytosolic concentration of a more abundant ion such as Na^+. In muscle, a greater than tenfold change in cytosolic Ca^{2+} concentration can be achieved in microseconds by releasing Ca^{2+} from the sarcoplasmic reticulum, a task that would be difficult to accomplish if changes in the millimolar range were required.

ANSWER 16–20 In a multicellular organism such as an animal, it is important that cells survive only when and where they are needed. Having cells depend on signals from other cells may be a simple way of ensuring this. A misplaced cell, for example, would probably fail to get the survival signals it needs (as its neighbors would be inappropriate) and would therefore kill itself. This strategy can also help regulate cell numbers: if cell type A depends on a survival signal from cell type B, the number of B cells could control the number of A cells by making a limited amount of the survival signal, so that only a certain number of A cells could survive. There is indeed evidence that such a mechanism does operate to help regulate cell numbers—in both developing and adult tissues (see Figure 18–41).

ANSWER 16–21 Ca^{2+}-activated Ca^{2+} channels create a positive feedback loop: the more Ca^{2+} that is released, the more Ca^{2+} channels open. The Ca^{2+} signal in the cytosol is therefore propagated explosively throughout the entire muscle cell, thereby ensuring that all myosin–actin filaments contract almost synchronously.

ANSWER 16–22 K2 activates K1. If K1 is permanently activated, a response is observed regardless of the status of K2. If the order were reversed, K1 would need to activate K2, which cannot occur because in our example K2 contains an inactivating mutation.

ANSWER 16–23

A. Three examples of extended signaling pathways to the nucleus are (1) extracellular signal → RTK → adaptor protein → Ras-activating protein → MAP kinase kinase kinase → MAP kinase kinase → MAP kinase → transcription regulator; (2) extracellular signal → GPCR → G protein → phospholipase C → IP_3 → Ca^{2+} → calmodulin → CaM-kinase → transcription regulator; (3) extracellular signal → GPCR → G protein → adenylyl cyclase → cyclic AMP → PKA → transcription regulator.

B. An example of a direct signaling pathway to the nucleus is Delta → Notch → cleaved Notch tail → transcription.

ANSWER 16–24 When PI 3-kinase is activated by an activated RTK, it phosphorylates a specific inositol phospholipid in the plasma membrane. The resulting phosphorylated inositol phospholipid then recruits to the plasma membrane both Akt and another protein kinase that helps phosphorylate and activate Akt. A third kinase that is permanently associated with the membrane also helps activate Akt (see Figure 16–35).

ANSWER 16–25 Animals and plants are thought to have evolved multicellularity independently and therefore will be expected to have evolved some distinct signaling mechanisms for their cells to communicate with one another. On the other hand, animal and plant cells are thought to have evolved from a common eukaryotic ancestor cell, and so plants and animals would be expected to share some intracellular signaling mechanisms that the common ancestor cell used to respond to its environment.

Chapter 17

ANSWER 17–1 Cells that migrate rapidly from one place to another, such as amoebae (A) and sperm cells (F), do not in general need intermediate filaments in their cytoplasm, since they do not develop or sustain large tensile forces. Plant cells (G) are pushed and pulled by the forces of wind and water, but they resist these forces by means of their rigid cell walls rather than by their cytoskeleton. Epithelial cells (B), smooth muscle cells (C), and the long axons of nerve cells (E) are all rich in cytoplasmic intermediate filaments, which prevent them from rupturing as they are stretched and compressed by the movements of their surrounding tissues.

All of the above eukaryotic cells possess at least intermediate filaments in their nuclear lamina. Bacteria, such as *Escherichia coli* (D), have none whatsoever.

ANSWER 17–2 Two tubulin dimers have a lower affinity for each other (because of a more limited number of interaction sites) than a tubulin dimer has for the end of a microtubule (where there are multiple possible interaction sites, both end-to-end of tubulin dimers adding to a protofilament and side-to-side of the tubulin dimers interacting with tubulin subunits in adjacent protofilaments forming the ringlike cross section). Thus, to initiate a microtubule from scratch, enough tubulin dimers have to come together and remain bound to one another for long enough for other tubulin molecules to add to them. Only when a number of tubulin dimers have already assembled will the binding of the next subunit be favored. The formation of these initial "nucleating sites" is therefore rare and will not occur spontaneously at cellular concentrations of tubulin.

Centrosomes contain preassembled rings of γ-tubulin (in which the γ-tubulin subunits are held together in much tighter side-to-side interactions than αβ-tubulin can form) to which αβ-tubulin dimers can bind. The binding conditions of αβ-tubulin dimers resemble those of adding to the end of an assembled microtubule. The γ-tubulin rings in the centrosome can therefore be thought of as permanently preassembled nucleation sites.

ANSWER 17–3

A. The microtubule is shrinking because it has lost its GTP cap, i.e., the tubulin subunits at its end are all in their GDP-bound form. GTP-loaded tubulin subunits from solution will still add to this end, but they will be short-lived—either because they hydrolyze their GTP or because they fall off as the microtubule rim around them disassembles. If, however, enough GTP-loaded subunits are added quickly enough to cover up the GDP-containing tubulin subunits at the microtubule end, a new GTP cap can form and regrowth is favored.
B. The rate of addition of GTP-tubulin will be greater at higher tubulin concentrations. The frequency with which shrinking microtubules switch to the growing mode will therefore increase with increasing tubulin concentration. The consequence of this regulation is that the system is self-balancing: the more microtubules shrink (resulting in a higher concentration of free tubulin), the more frequently microtubules will start to grow again. Conversely, the more microtubules grow, the lower the concentration of free tubulin will become and the rate of GTP-tubulin addition will slow down; at some point GTP hydrolysis will catch up with new GTP-tubulin addition, the GTP cap will be destroyed, and the microtubule will switch to the shrinking mode.
C. If only GDP were present, microtubules would continue to shrink and eventually disappear, because tubulin dimers with GDP have very low affinity for each other and will not add stably to microtubules.
D. If GTP is present but cannot be hydrolyzed, microtubules will continue to grow until all free tubulin subunits have been used up.

ANSWER 17–4 If all the dynein arms were equally active, there could be no significant relative motion of one microtubule to the other as required for bending (think of a circle of nine weightlifters, each trying to lift his neighbor off the ground: if they all succeeded, the group would levitate!). Thus, a few ciliary dynein molecules must be activated selectively on one side of the cilium. As they move their neighboring microtubules toward the tip of the cilium, the cilium bends away from the side containing the activated dyneins.

ANSWER 17–5 Any actin-binding protein that stabilizes complexes of two or more actin monomers without blocking the ends required for filament growth will facilitate the initiation of a new filament (nucleation).

ANSWER 17–6 Only fluorescent actin molecules assembled into filaments are visible, because unpolymerized actin molecules diffuse so rapidly they produce a dim uniform background. Since, in your experiment, so few actin molecules are labeled (1:10,000), there should be at most one labeled actin monomer per filament (see Figure 17–29). The lamellipodium as a whole has many actin filaments, some of which overlap and therefore show a random speckled pattern of actin molecules, each marking a different filament.

This technique (called "speckle fluorescence") can be used to follow the movement of polymerized actin in a migrating cell. If you watch this pattern with time, you will see that individual fluorescent spots move steadily back from the leading edge toward the interior of the cell, a movement that occurs whether or not the cell is actually migrating. Rearward movement takes place because actin monomers are added to filaments at the plus end and are lost from the minus end (where they are depolymerized) (see Figure

17–35B). In effect, actin monomers "move through" the actin filaments, a phenomenon termed "treadmilling." Treadmilling has been demonstrated to occur in isolated actin filaments in solution and also in dynamic microtubules, such as those within a mitotic spindle.

ANSWER 17–7 Cells contain actin-binding proteins that bundle and cross-link actin filaments (see Figure 17–32). The filaments extending from lamellipodia and filopodia become firmly connected to the filamentous meshwork of the cell cortex, thus providing the mechanical anchorage required for the growing rodlike filaments to deform the cell membrane.

ANSWER 17–8 Although the subunits are indeed held together by noncovalent bonds that are individually weak, there are a very large number of them, distributed among a very large number of filaments. As a result, the stress a human being exerts by lifting a heavy object is dispersed over so many subunits that their interaction strength is not exceeded. By analogy, a single thread of silk is not nearly strong enough to hold a human, but a rope woven of such fibers is.

ANSWER 17–9 Both filaments are composed of subunits in the form of protein dimers that are held together by coiled-coil interactions. Moreover, in both cases, the dimers polymerize through their coiled-coil domains into filaments. Whereas intermediate filament dimers assemble head-to-head, however, and thereby create a filament that has no polarity, all myosin molecules in the same half of the myosin filament are oriented with their heads pointing in the same direction. This polarity is necessary for them to be able to develop a contractile force in muscle.

ANSWER 17–10

A. Successive actin molecules in an actin filament are identical in position and conformation. After a first protein (such as troponin) had bound to the actin filament, there would be no way in which a second protein could recognize every seventh monomer in a naked actin filament. Tropomyosin, however, binds along the length of an actin filament, spanning precisely seven monomers, and thus provides a molecular "ruler" that measures the length of seven actin monomers. Troponin becomes localized by binding to the evenly spaced ends of tropomyosin molecules.
B. Calcium ions influence force generation in the actin–myosin system only if both troponin (to bind the calcium ions) and tropomyosin (to transmit the information that troponin has bound calcium to the actin filament) are present. (i) Troponin cannot bind to actin without tropomyosin. The actin filament would be permanently exposed to the myosin, and the system would be continuously active, independently of whether calcium ions were present or not (a muscle cell would therefore be continuously contracted with no possibility of regulation). (ii) Tropomyosin would bind to actin and block binding of myosin completely; the system would be permanently inactive, no matter whether calcium ions were present, because tropomyosin is not affected by calcium. (iii) The system will contract in response to calcium ions.

ANSWER 17–11

A. True. A continual outward movement of ER is required; in the absence of microtubules, the ER collapses toward the center of the cell.
B. True. Actin is needed to make the contractile ring that causes the physical cleavage between the two daughter cells, whereas the mitotic spindle that partitions the chromosomes is composed of microtubules.
C. True. Both extensions are associated with transmembrane proteins that protrude from the plasma membrane and enable the cell to form new anchor points on the substratum.
D. False. To cause bending, ATP is hydrolyzed by the dynein motor proteins that are attached to the outer microtubules in the flagellum.
E. False. Cells could not divide without rearranging their intermediate filaments, but many terminally differentiated and long-lived cells, such as nerve cells, have stable intermediate filaments that are not known to depolymerize.
F. False. The rate of growth is independent of the size of the GTP cap. The plus and minus ends have different growth rates because they have physically distinct binding sites for the incoming tubulin subunits; the rate of addition of tubulin subunits differs at the two ends.
G. True. Both are nice examples of how the same membrane can have regions that are highly specialized for a particular function.
H. False. Myosin movement is activated by the phosphorylation of myosin, or by calcium binding to troponin.

ANSWER 17–12 The average time taken for a small molecule (such as ATP) to diffuse a distance of 10 μm is given by the calculation

$$(10^{-3})^2 / (2 \times 5 \times 10^{-6}) = 0.1 \text{ seconds}$$

Similarly, a protein takes 1 second and a vesicle 10 seconds on average to travel 10 μm. A vesicle would require on average 10^9 seconds, or more than 30 years, to diffuse to the end of a 10-cm axon. This calculation makes it clear why kinesin and other motor proteins evolved to carry molecules and organelles along microtubules.

ANSWER 17–13 (1) Animal cells are much larger and more diversely shaped, and do not have a cell wall. Cytoskeletal elements are required to provide mechanical strength and shape in the absence of a cell wall. (2) Animal cells, and all other eukaryotic cells, have a nucleus that is shaped and held in place in the cell by intermediate filaments; the nuclear lamins attached to the inner nuclear membrane support and shape the nuclear membrane, and a meshwork of intermediate filaments surrounds the nucleus and spans the cytosol. (3) Animal cells can move by a process that requires a change in cell shape. Actin filaments and myosin motor proteins are required for these activities.
(4) Animal cells have a much larger genome than bacteria; this genome is fragmented into many chromosomes. For cell division, chromosomes need to be accurately distributed to the daughter cells, requiring the function of the microtubules that form the mitotic spindle. (5) Animal cells have internal organelles. Their localization in the cell is dependent on motor proteins that move them along microtubules. A remarkable example is the long-distance travel of membrane-enclosed vesicles (organelles) along microtubules in an axon that can be up to 1 m (≈3 ft) long in the case of the nerve cells that extend from your spinal cord to your feet.

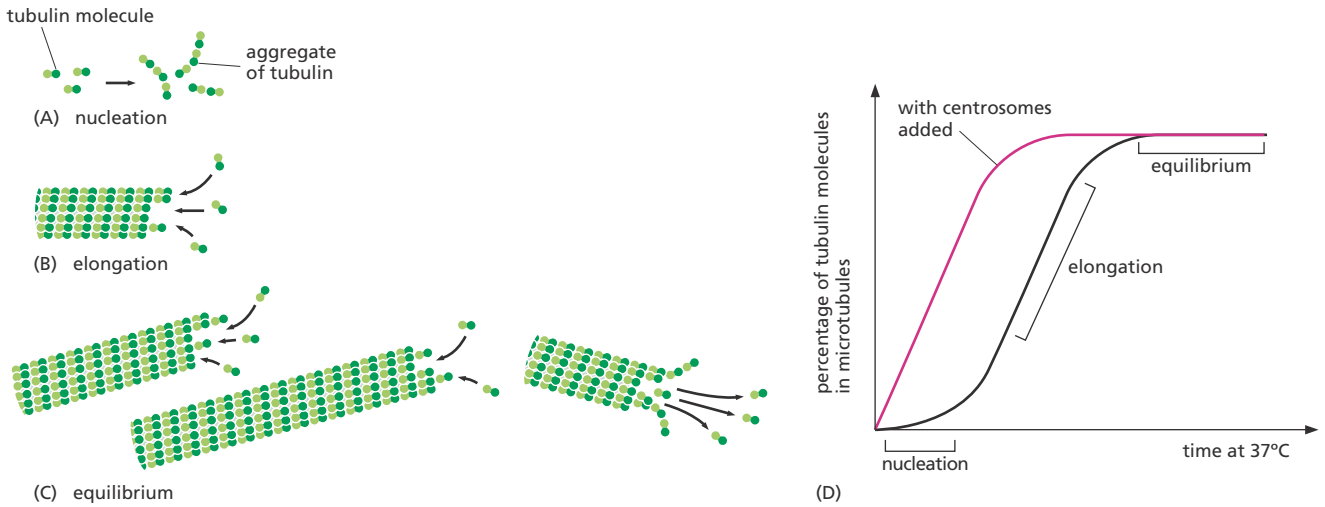


Figure A17–19

ANSWER 17–14 The ends of an intermediate filament are indistinguishable from each other, because the filaments are built by the assembly of symmetrical tetramers made from two coiled-coil dimers. In contrast to microtubules and actin filaments, intermediate filaments therefore have no polarity.

ANSWER 17–15 Intermediate filaments have no polarity; their ends are chemically indistinguishable. It would therefore be difficult to envision how a hypothetical motor protein that bound to the middle of the filament could sense a defined direction. Such a motor protein would be equally likely to attach to the filament facing one end or the other.

ANSWER 17–16 Katanin breaks microtubules along their length, and at positions remote from their GTP caps. The fragments that form therefore contain GDP-tubulin at their exposed ends and rapidly depolymerize. Katanin thus provides a very quick means of destroying existing microtubules.

ANSWER 17–17 Cell division depends on the ability of microtubules both to polymerize and to depolymerize. This is most obvious when one considers that the formation of the mitotic spindle requires the prior depolymerization of other cellular microtubules to free up the tubulin required to build the spindle. This rearrangement is not possible in Taxol-treated cells, whereas in colchicine-treated cells, division is blocked because a spindle cannot be assembled. On a more subtle but no less important level, both drugs block the dynamic instability of microtubules and would therefore interfere with the workings of the mitotic spindle, even if one could be properly assembled.

ANSWER 17–18 Motor proteins are unidirectional in their action; kinesin always moves toward the plus end of a microtubule and dynein toward the minus end. Thus if kinesin molecules are attached to glass, only those individual motors that have the correct orientation in relation to the microtubule that settles on them can attach to the microtubule and exert force on it to propel it forward. Since kinesin moves toward the plus end of the microtubule, the microtubule will always crawl minus-end first over the coverslip.

ANSWER 17–19

A. Phase A corresponds to a lag phase, during which tubulin molecules assemble to form nucleation centers (**Figure A17–19A**). Nucleation is followed by a rapid rise (phase B) to a plateau value as tubulin dimers add to the ends of the elongating microtubules (**Figure A17–19B**). At phase C, equilibrium is reached with some microtubules in the population growing while others are rapidly shrinking (**Figure A17–19C**). The concentration of free tubulin is constant at this point, because polymerization and depolymerization are balanced (see also Question 17–3, p. 577).
B. The addition of centrosomes introduces nucleation sites that eliminate the lag phase A as shown by the red curve in Figure A17–19D. The rate of microtubule growth (i.e., the slope of the curve in the elongation phase B) and the equilibrium level of free tubulin remain unchanged, because the presence of centrosomes does not affect the rates of polymerization and depolymerization.

ANSWER 17–20 The ends of the shrinking microtubule are visibly frayed, and the individual protofilaments appear to come apart and curl as the end depolymerizes. This micrograph therefore suggests that the GTP cap (which is lost from shrinking microtubules) holds the protofilaments properly aligned with each other, perhaps by strengthening the side-to-side interactions between αβ-tubulin subunits when they are in their GTP-bound form.

ANSWER 17–21 Cytochalasin interferes with actin filament formation, and its effect on the cell demonstrates the importance of actin to cell locomotion. The experiment with colchicine shows that microtubules are required to give a cell a polarity that then determines which end becomes the leading edge (see Figure 17–14). In the absence of microtubules, cells still go through the motions normally associated with cell movement, such as the extension of lamellipodia, but in the absence of cell polarity these are futile exercises because they happen indiscriminately in all directions.

Antibodies bind tightly to the antigen (in this case vimentin) to which they were raised (see Panel 4–2, pp. 146–147). When bound, an antibody can interfere with

the function of the antigen by preventing it from interacting properly with other cell components. The antibody injection experiment therefore suggests that intermediate filaments are not required for the maintenance of cell polarity or for the motile machinery.

ANSWER 17–22 Either (B) or (C) would complete the sentence correctly. The direct result of the action potential in the plasma membrane is the release of Ca^{2+} into the cytosol from the sarcoplasmic reticulum; muscle cells are triggered to contract by this rapid rise in cytosolic Ca^{2+}. Calcium ions at high concentrations bind to troponin, which in turn causes tropomyosin to move to expose myosin-binding sites on the actin filaments. (A) and (D) would be wrong because Ca^{2+} has no effect on the detachment of the myosin head from actin, which is the result of ATP hydrolysis. Nor does it have any role in maintaining the structure of the myosin filament.

ANSWER 17–23 Only (D) is correct. Upon contraction, the Z discs move closer together, and neither actin nor myosin filaments contract (see Figures 17–41 and 17–42).

Chapter 18

ANSWER 18–1 Because all cells arise by division of another cell, this statement is correct, assuming that "first cell division" refers to the division of the successful founder cell from which all life as we know it has derived. There were probably many other unsuccessful attempts to start the chain of life.

ANSWER 18–2 Cells in peak B contain twice as much DNA as those in peak A, indicating that they contain replicated DNA, whereas the cells in peak A contain unreplicated DNA. Peak A therefore contains cells that are in G_1, and peak B contains cells that are in G_2 and mitosis. Cells in S phase have begun but not finished DNA synthesis; they therefore have various intermediate amounts of DNA and are found in the region between the two peaks. Most cells are in G_1, indicating that it is the longest phase of the cell cycle (see Figure 18–2).

ANSWER 18–3 For multicellular organisms, the control of cell division is extremely important. Individual cells must not proliferate unless it is to the benefit of the whole organism. The G_0 state offers protection from aberrant activation of cell division, because the cell-cycle control system is largely dismantled. If, on the other hand, a cell just paused in G_1, it would still contain all of the cell-cycle control system and could readily be induced to divide. The cell would also have to remake the "decision" not to divide almost continuously. To re-enter the cell cycle from G_0, a cell has to resynthesize all of the components that have disappeared.

ANSWER 18–4 The cell would replicate its damaged DNA and therefore would introduce mutations to the two daughter cells when the cell divides. Such mutations could increase the chances that the progeny of the affected daughter cells would eventually become cancer cells.

ANSWER 18–5 Before injection, the frog oocytes must contain inactive M-Cdk. Upon injection of the M-phase cytoplasm, the small amount of the active M-Cdk in the injected cytoplasm activates the inactive M-Cdk by switching on the activating phosphatase (Cdc25), which removes the inhibitory phosphate groups from the inactive M-Cdk (see Figure 18–17). An extract of the second oocyte, now in M phase itself, will therefore contain as much active M-Cdk as the original cytoplasmic extract, and so on.

ANSWER 18–6 The experiment shows that kinetochores are not preassigned to one or other spindle pole; microtubules attach to the kinetochores that they are able to reach. For the chromosomes to remain attached to a microtubule, however, tension has to be exerted. Tension is normally achieved by the opposing pulling forces from opposite spindle poles. The requirement for such tension ensures that if two sister kinetochores ever become attached to the same spindle pole, so that tension is not generated, one or both of the connections would be lost, and microtubules from the opposing spindle pole would have another chance to attach properly.

ANSWER 18–7 Recall from Figure 18–30 that the new nuclear envelope reassembles on the surface of the chromosomes. The close apposition of the envelope to the chromosomes prevents cytosolic proteins from being trapped between the chromosomes and the envelope. Nuclear proteins are then selectively imported through the nuclear pores, causing the nucleus to expand while maintaining its characteristic protein composition.

ANSWER 18–8 The membranes of the Golgi vesicles fuse to form part of the plasma membranes of the two daughter cells. The interiors of the vesicles, which are filled with cell-wall material, become the new cell-wall matrix separating the two daughter cells. Proteins in the membranes of the Golgi vesicles thus become plasma membrane proteins. Those parts of the proteins that were exposed to the lumen of the Golgi vesicle will end up exposed to the new cell wall (**Figure A18–8**).

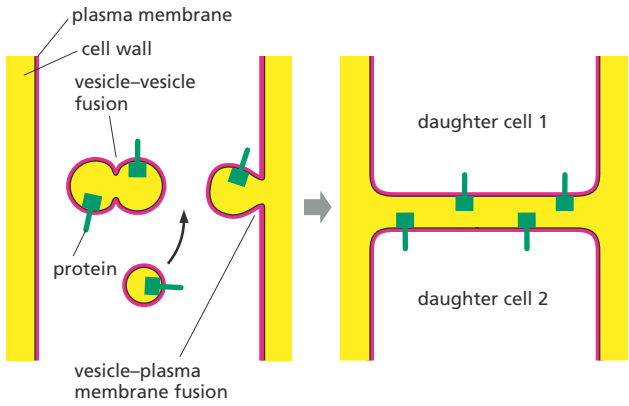


Figure A18–8

ANSWER 18–9 In a eukaryotic organism, the genetic information that the organism needs to survive and reproduce is distributed between multiple chromosomes. It is therefore crucial that each daughter cell receives a copy of each chromosome when a cell divides; if a daughter cell receives too few or too many chromosomes, the effects are usually deleterious or even lethal. Only two copies of each chromosome are produced by chromosome replication in mitosis. If the cell were to randomly distribute the chromosomes when it divided, it would be very unlikely that each daughter cell would receive precisely one copy

of each chromosome. In contrast, the Golgi apparatus fragments into tiny vesicles that are all alike, and by random distribution it is very likely that each daughter cell will receive an approximately equal number of them.

ANSWER 18–10 As apoptosis occurs on a large scale in both developing and adult tissues, it must not trigger alarm reactions that are normally associated with cell injury. Tissue injury, for example, leads to the release of signal molecules that stimulate the proliferation of surrounding cells so that the wound heals. It also causes the release of signals that can cause a destructive inflammatory reaction. Moreover, the release of intracellular contents could elicit an immune response against molecules that are normally not encountered by the immune system. Such reactions would be self-defeating if they occurred in response to the massive cell death that occurs in normal development.

ANSWER 18–11 Because the cell population is increasing exponentially, doubling its weight at every cell division, the weight of the cell cluster after N cell divisions is $2^N \times 10^{-9}$ g. Therefore, 70 kg (70×10^3 g) = $2^N \times 10^{-9}$ g, or $2^N = 7 \times 10^{13}$. Taking the logarithm of both sides allows you to solve the equation for N. Therefore, $N = \ln (7 \times 10^{13}) / \ln 2 = 46$; i.e., it would take only 46 days if cells proliferated exponentially. Cell division in animals is tightly controlled, however, and most cells in the human body stop dividing when they become highly specialized. The example demonstrates that exponential cell proliferation occurs only for very brief periods, even during embryonic development.

ANSWER 18–12 The egg cells of many animals are big and contain stores of enough cell components to last for many cell divisions. The daughter cells that form during the first cell divisions after fertilization are progressively smaller in size and thus can be formed without a need for new protein or RNA synthesis. Whereas normally dividing cells would grow continuously in G_1, G_2, and S phases, until their size doubled, there is no cell growth in these early cleavage divisions, and both G_1 and G_2 are virtually absent. As G_1 is usually longer than G_2 and S phase, G_1 is the most drastically reduced in these divisions.

ANSWER 18–13

A. Radiation leads to DNA damage, which activates a checkpoint mechanism (mediated by p53 and p21; see Figure 18–15) that arrests the cell cycle until the DNA has been repaired.
B. The cell will replicate damaged DNA and thereby introduce mutations in the daughter cells when the cell divides.
C. The cell will be able to divide normally, but it will be prone to mutations, because some DNA damage always occurs as the result of natural irradiation caused, for example, by cosmic rays. The checkpoint mechanism mediated by p53 is mainly required as a safeguard against the devastating effects of accumulating DNA damage, but not for the natural progression of the cell cycle in undamaged cells.
D. Cell division in humans is an ongoing process that does not cease upon reaching maturity, and it is required for survival. Blood cells and epithelial cells in the skin or lining the gut, for example, are being constantly produced by cell division to meet the body's needs; each day, your body produces about 10^{11} new red blood cells alone.

ANSWER 18–14

A. Only the cells that were in the S phase of their cell cycle (i.e., those cells making DNA) during the 30-minute labeling period contain any radioactive DNA.
B. Initially, mitotic cells contain no radioactive DNA because these cells were not engaged in DNA synthesis during the labeling period. Indeed, it takes about two hours before the first labeled mitotic cells appear.
C. The initial rise of the curve corresponds to cells that were just finishing DNA replication when the radioactive thymidine was added. The curve rises as more labeled cells enter mitosis; the peak corresponds to those cells that had just started S phase when the radioactive thymidine was added. The labeled cells then exit from mitosis, and are replaced by unlabeled mitotic cells, which were not yet in S phase during the labeling period. After 20 hours the curve starts rising again, because the labeled cells enter their second round of mitosis.
D. The intial two-hour lag before any labeled mitotic cells appear corresponds to the G_2 phase, which is the time between the end of S phase and the beginning of mitosis. The first labeled cells seen in mitosis were those that were just finishing S phase (DNA synthesis) when the radioactive thymidine was added.

ANSWER 18–15 Loss of M cyclin leads to inactivation of M-Cdk. As a result, the M-Cdk target proteins become dephosphorylated by phosphatases, and the cells exit from mitosis: they disassemble the mitotic spindle, reassemble the nuclear envelope, decondense their chromosomes, and so on. The M cyclin is degraded by ubiquitin-dependent destruction in proteasomes, and the activation of M-Cdk leads to the activation of the APC, which ubiquitylates the cyclin, but with a substantial delay. As discussed in Chapter 7, ubiquitylation tags proteins for degradation in proteasomes.

ANSWER 18–16 M cyclin accumulates gradually as it is steadily synthesized. As it accumulates, it will tend to form complexes with the mitotic Cdk molecules that are present. After a certain threshold level has been reached, a sufficient amount of M-Cdk has been formed so that it is activated by the appropriate kinases and phosphatases that phosphorylate and dephosphorylate it. Once activated, M-Cdk acts to enhance the activity of the activating phosphatase; this positive feedback leads to the explosive activation of M-Cdk (see Figure 18–17). Thus, M-cyclin accumulation acts like a slow-burning fuse, which eventually helps trigger the explosive self-activation of M-Cdk. The precipitous destruction of M cyclin terminates M-Cdk activity, and a new round of M-cyclin accumulation begins.

ANSWER 18–17 The order is G, C, B, A, D. Together, these five steps are referred to as mitosis (F). No step in mitosis is influenced by the phases of the moon (E). Cytokinesis is the last step in M phase, which overlaps with anaphase and telophase. Mitosis and cytokinesis are both part of M phase.

ANSWER 18–18 If the growth rate of microtubules is the same in mitotic and in interphase cells, their length is proportional to their lifetime. Thus, the average length of microtubules in mitosis is 1 μm (= 20 μm × 15 s/300 s).

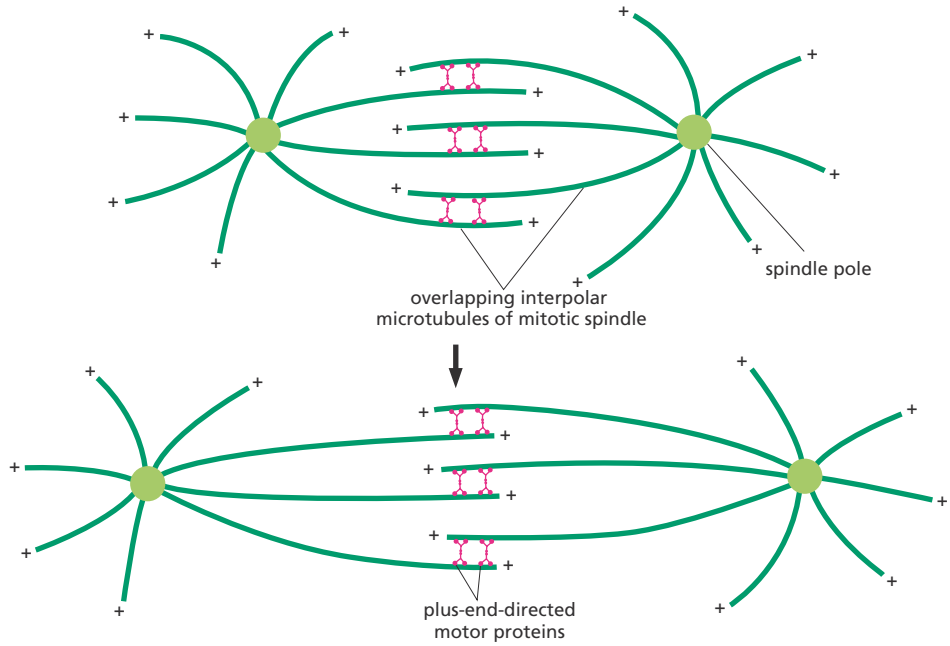


Figure A18–19

ANSWER 18–19 As shown in **Figure A18–19**, the overlapping interpolar microtubules from opposite poles of the spindle have their plus ends pointing in opposite directions. Plus-end-directed motor proteins cross-link adjacent antiparallel microtubules together and tend to move the microtubules in the direction that will push the two poles of the spindle apart, as shown in the figure. Minus-end-directed motor proteins also cross-link adjacent antiparallel microtubules together but move in the opposite direction, tending to pull the spindle poles together (not shown).

ANSWER 18–20 The sister chromatid becomes committed when a microtubule from one of the spindle poles attaches to the kinetochore of the chromatid. Microtubule attachment is still reversible until a second microtubule from the other spindle pole attaches to the kinetochore of its partner sister chromatid so that the duplicated chromosome is now put under mechanical tension by pulling forces from both poles. The tension ensures that both microtubules remain attached to the chromosome. The position of a chromatid in the cell at the time that the nuclear envelope breaks down will influence which spindle pole it will be pulled to, as its kinetochore is most likely to become attached to the spindle pole toward which it is facing.

ANSWER 18–21 It is still not certain what drives the poleward movement of chromosomes during anaphase. In principle, two possible models could explain it (**Figure A18–21**). In the model shown in (A), microtubule motor proteins associated with the kinetochore dash toward the minus end of the depolymerizing microtubule, dragging the chromosome toward the pole. Although this model is appealingly simple, there is little evidence that motor proteins are required for chromosome movement during anaphase. Instead, current experimental evidence greatly supports the model outlined in (B). In this model, chromosome movement is driven by kinetochore proteins

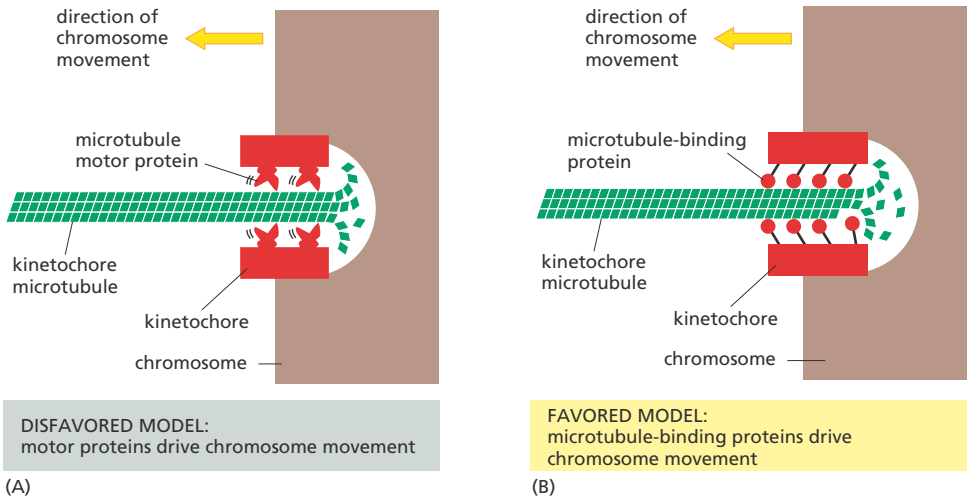


Figure A18–21

that cling to the sides of the depolymerizing microtubule. These proteins frequently detach from—and reattach to—the kinetochore microtubule. As tubulin subunits continue to dissociate, the kinetochore must slide poleward to maintain its grip on the retreating end of the shrinking microtubule.

ANSWER 18–22 Both sister chromatids could end up in the same daughter cell for any of a number of reasons. (1) If the microtubules or their connections with a kinetochore were to break during anaphase, both sister chromatids could be drawn to the same pole, and hence into the same daughter cell. (2) If microtubules from the same spindle pole attached to both kinetochores, the chromosome would be pulled to the same pole. (3) If the cohesins that link sister chromatids were not degraded, the pair of chromatids might be pulled to the same pole. (4) If a duplicated chromosome never engaged microtubules and was left out of the spindle, it would also end up in one daughter cell.

Some of these errors in the mitotic process would be expected to activate a checkpoint mechanism that delays the onset of anaphase until all chromosomes are attached properly to both poles of the spindle. This "spindle assembly checkpoint" mechanism should allow most chromosome attachment errors to be corrected, which is one reason why such errors are rare.

The consequences of both sister chromatids ending up in one daughter cell are usually dire. One daughter cell would contain only one copy of all the genes carried on that chromosome and the other daughter cell would contain three copies. The altered gene dosage, leading to correspondingly changed amounts of the mRNAs and proteins produced, is often detrimental to the cell. In addition, there is the possibility that the single copy of the chromosome may contain a defective gene with a critical function, which would normally be taken care of by the good copy of the gene on the other chromosome that is now missing.

ANSWER 18–23

A. True. Centrosomes replicate during interphase, before M phase begins.
B. True. Sister chromatids separate completely only at the start of anaphase.
C. False. The ends of interpolar microtubules overlap and attach to one another via proteins (including motor proteins) that bridge between the microtubules.
D. False. Microtubules and their motor proteins play no role in DNA replication.
E. False. To be a correct statement, the terms "centromere" and "centrosome" must be switched.

ANSWER 18–24 Antibodies bind tightly to the antigen (in this case myosin) to which they were raised. When bound, an antibody can interfere with the function of the antigen by preventing it from interacting properly with other cell components. (A) The movement of chromosomes at anaphase depends on microtubules and their motor proteins and does not depend on actin or myosin. Injection of an anti-myosin antibody into a cell will therefore have no effect on chromosome movement during anaphase. (B) Cytokinesis, on the other hand, depends on the assembly and contraction of a ring of actin and myosin filaments, which forms the cleavage furrow that splits the cell in two. Injection of anti-myosin antibody will therefore block cytokinesis.

ANSWER 18–25 The plasma membrane of the cell that died by necrosis in Figure 18–37A is ruptured; a clear break is visible, for example, at a position corresponding to the 12 o'clock mark on a watch. The cell's contents, mostly membranous and cytoskeletal debris, are seen spilling into the surroundings through these breaks. The cytosol stains lightly, because most soluble cell components were lost before the cell was fixed. In contrast, the cell that underwent apoptosis in Figure 18–37B is surrounded by an intact membrane, and its cytosol is densely stained, indicating a normal concentration of cell components. The cell's interior is remarkably different from a normal cell, however. Particularly characteristic are the large "blobs" that extrude from the nucleus, probably as the result of the breakdown of the nuclear lamina. The cytosol also contains many large, round, membrane-enclosed vesicles of unknown origin, which are not normally seen in healthy cells. The pictures visually confirm the notion that necrosis involves cell lysis, whereas cells undergoing apoptosis remain relatively intact until they are phagocytosed and digested by another cell.

ANSWER 18–26

A. False. There is no G_1 to M phase transition. The statement is correct, however, for the G_1 to S phase transition, in which cells commit themselves to a division cycle.
B. True. Apoptosis is an active process carried out by special proteases (caspases).
C. True. This mechanism is thought to adjust the number of neurons to the number of specific target cells to which the neurons connect.
D. True. An amazing evolutionary conservation!
E. True. Association of a Cdk protein with a cyclin is required for its activity (hence its name cyclin-dependent kinase). Furthermore, phosphorylation at a specific site and dephosphorylation at other sites on the Cdk protein are required for the cyclin–Cdk complex to be active.

ANSWER 18–27 Cells in an animal must behave for the good of the organism as a whole—to a much greater extent than people generally act for the good of society as a whole. In the context of an organism, unsocial behavior would lead to a loss of organization and to cancer. Many of the rules that cells have to obey would be unacceptable in a human society. Most people, for example, would be reluctant to kill themselves for the good of society, yet our cells do it all the time.

ANSWER 18–28 The most likely approach to success (if that is what the goal should be called) is plan C, which should result in an increase in cell numbers. The problem is, of course, that cell numbers of each tissue must be increased similarly to maintain balanced proportions in the organism, yet different cells respond to different growth factors. As shown in **Figure A18–28**, however, the approach has indeed met with limited success. A mouse producing very large quantities of growth hormone (*left*)—which acts to stimulate the production of a secreted protein that acts as a survival factor, growth factor, or mitogen, depending on the cell type—grows to almost twice the size of a normal mouse (*right*). To achieve this twofold change in size, however, growth hormone was massively overproduced (about fiftyfold). And note that the mouse did not even attain the size of a rat, let alone a dog.

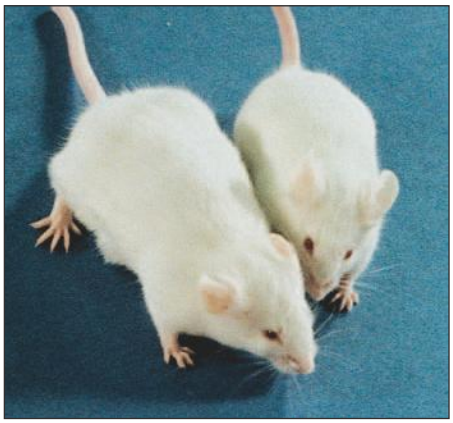


Figure A18–28 Courtesy of Ralph Brinster

The other approaches have conceptual problems:

A. Blocking all apoptosis would lead to defects in development, as rat development requires the selective death of many cells. It is unlikely that a viable animal would be obtained.
B. Blocking p53 function would eliminate an important checkpoint of the cell cycle that detects DNA damage and stops the cycle so that the cell can repair the damage; removing p53 would increase mutation rates and lead to cancer. Indeed, mice without p53 usually develop normally but die of cancer at a young age.
D. Given the circumstances, switching careers might not be a bad option.

ANSWER 18–29 The on-demand, limited release of PDGF at a wound site triggers cell division of neighboring cells for a limited amount of time, until the PDGF is degraded. This is different from the continuous release of PDGF from mutant cells, where PDGF is made in an uncontrolled way at high levels. Moreover, the mutant cells that make PDGF often express their own PDGF receptor inappropriately, so that they can stimulate their own proliferation, thereby promoting the development of cancer.

ANSWER 18–30 All three types of mutant cells would be unable to divide. The cells

A. would enter mitosis but would not be able to exit mitosis.
B. would arrest permanently in G_1 because the cyclin–Cdk complexes that act in G_1 would be inactivated.
C. would not be able to activate the transcription of genes required for cell division because the required transcription regulators would be constantly inhibited by unphosphorylated Rb.

ANSWER 18–31 In alcoholism, liver cells proliferate because the organ is overburdened and becomes damaged by the large amounts of alcohol that have to be metabolized. This need for more liver cells activates the control mechanisms that normally regulate cell proliferation. Unless badly damaged and full of scar tissue, the liver will usually shrink back to a normal size after the patient stops drinking excessively. In liver cancer, in contrast, mutations abolish normal cell proliferation control and, as a result, cells divide and keep on dividing in an uncontrolled manner, which is usually fatal.

Chapter 19

ANSWER 19–1 Although each daughter cell ends up with a diploid amount of DNA after the first meiotic division, each cell has effectively only a haploid set of chromosomes (albeit in two copies), representing only one or other homolog of each type of chromosome (although some mixing will have occurred during crossing-over). Because the maternal and paternal chromosomes of a pair will carry different versions of many of the genes, these daughter cells will not be genetically identical; each one will, however, have lost either the paternal or the maternal version of each chromosome. In contrast, somatic cells dividing by mitosis inherit a diploid set of chromosomes, and all daughter cells are genetically identical and inherit both maternal and paternal gene copies. The role of gametes produced by meiosis is to mix and reassort gene pools during sexual reproduction, and thus it is a definite advantage for each of them to have a slightly different genetic constitution. The role of somatic cells on the other hand is to build an organism that contains the same genes in all its cells and retains in each cell both maternal and paternal genetic information.

ANSWER 19–2 A typical human female produces fewer than 1000 mature eggs in her lifetime (12 per year over about 40 years); this is less than one-tenth of a percent of the possible gametes, excluding the effects of meiotic crossing-over. A typical human male produces billions of sperm during a lifetime, so in principle, every possible chromosome combination is sampled many times.

ANSWER 19–3 For simplicity, consider the situation where a father carries genes for two dominant traits, M and N, on one of his two copies of human Chromosome 1. If these two genes were located at opposite ends of this chromosome, and there were one and only one crossover event per chromosome as postulated in the question, half of his children would express trait M and the other half would express trait N—with no child resembling the father in carrying both traits. This is very different from the actual situation, where there are multiple crossover events per chromosome, causing the traits M and N to be inherited as if they were on separate chromosomes. By constructing a Punnett square like that in Figure 19–27, one can see that in this latter, more realistic case, we would actually expect one-fourth of the children of this father to inherit both traits, one-fourth to inherit trait M only, one-fourth to inherit trait N only, and one-fourth to inherit neither trait.

ANSWER 19–4 Inbreeding tends to give rise to individuals who are homozygous for many genes. To see why, consider the extreme case where inbreeding takes the form of brother–sister matings (as among the Pharaohs of ancient Egypt): because the parents are closely related, there is a high probability that the maternal and paternal alleles inherited by the offspring will be the same. Inbreeding continued over many generations gives rise to individuals who are all alike and homozygous for almost every gene. Because of the randomness of the mechanism of inheritance, there is a large chance that some deleterious alleles will become prevalent in the population in this way, giving all individuals a reduced fitness. In another, separate inbred population, the same thing will happen, but chances are a different set of deleterious alleles will become

prevalent. When individuals from the two separate inbred populations mate, their offspring will inherit deleterious alleles of genes *A*, *B*, and *C* for example, from the mother, but good alleles of those genes from the father; conversely, they will inherit deleterious alleles of genes *D*, *E*, and *F* from the father, but good alleles of those genes from the mother. Most deleterious mutations are recessive. The hybrid offspring, because they are heterozygous for these genes, will thus escape the deleterious effects seen in the parents.

ANSWER 19–5 Although any one of the three explanations could in principle account for the observed result, A and B can be ruled out as being implausible.

A. There is no precedent for any instability in DNA so great as to be detectable in such a SNP analysis; in any case, the hypothesis would predict a steady decrease in the frequency of the SNP with age, not a drop in frequency that begins only at age 50.
B. Human genes change only very slowly over time (unless a massive population migration brings an influx of individuals who are genetically different). People born 50 years ago will be, on average, virtually the same genetically as the population being born today.
C. This hypothesis is correct. A SNP with these properties has been used to discover a gene that appears to cause a substantial increase in the probability of death from cardiac abnormalities.

ANSWER 19–6 Natural selection alone is not sufficient to eliminate recessive lethal genes from the population. Consider the following line of reasoning. Homozygous defective individuals can arise only as the offspring of a mating between two heterozygous individuals. By the rules of Mendelian genetics, offspring of such a mating will be in the ratio of 1 homozygous normal: 2 heterozygous: 1 homozygous defective. Thus, statistically, heterozygous individuals should always be more numerous than the homozygous, defective individuals. And although natural selection effectively eliminates the defective genes in homozygous individuals through death, it cannot act to eliminate the defective genes in heterozygous individuals because they do not affect the phenotype. Natural selection will keep the frequency of the defective gene low in the population, but, in the absence of any other effect, there will always be a reservoir of defective genes in the heterozygous individuals.

At low frequencies of the defective gene, another important factor—chance—comes into play. Chance variation can increase or decrease the frequency of heterozygous individuals (and thereby the frequency of the defective gene). By chance, the offspring of a mating between heterozygotes could all be normal, which would eliminate the defective gene from that lineage. Increases in the frequency of a deleterious gene are opposed by natural selection; however, decreases are unopposed and can, by chance, lead to elimination of the defective gene from the population. On the other hand, new mutations are continually occurring, albeit at a low rate, creating fresh copies of the deleterious recessive allele. In a large population, a balance will be struck between the creation of new copies of the allele in this way, and their elimination through the death of homozygotes.

ANSWER 19–7

A. True.
B. True.
C. False. Mutations that occur during meiosis can be propagated, unless they give rise to nonviable gametes.

ANSWER 19–8 Two copies of the same chromosome can end up in the same daughter cell if one of the microtubule connections breaks before sister chromatids are separated. Alternatively, microtubules from the same spindle pole could attach to both kinetochores of the chromosome. As a consequence, one daughter cell would receive only one copy of all the genes carried on that chromosome, and the other daughter cell would receive three copies. The imbalance of the genes on this chromosome compared with the genes on all the other chromosomes would produce imbalanced levels of protein which, in most cases, is detrimental to the cell. If the mistake happens during meiosis, in the process of gamete formation, it will be propagated in all cells of the organism. A form of mental retardation called Down syndrome, for example, is due to the presence of three copies of Chromosome 21 in all of the nucleated cells in the body.

ANSWER 19–9 Meiosis begins with DNA replication, producing a tetraploid cell containing four copies of each chromosome. These four copies have to be distributed equally during the two sequential meiotic divisions into four haploid cells. Sister chromatids remain paired so that (1) the cells resulting from the first division receive two complete sets of chromosomes and (2) the chromosomes can be evenly distributed again in the second meiotic division. If the sister chromatids did not remain paired, it would not be possible in the second division to distinguish which chromatids belong together, and it would therefore be difficult to ensure that precisely one copy of each chromatid is pulled into each daughter cell. Keeping two sister chromatids paired in the first meiotic division is therefore an easy way to keep track of which chromatids belong together.

This biological principle suggests that you might consider clamping your socks together in matching pairs before putting them into the laundry. In this way, the cumbersome process of sorting them out afterward—and the seemingly inevitable mistakes that occur during that process—could be avoided.

ANSWER 19–10

A. A gene is a stretch of DNA that codes for a protein or functional RNA. An allele is an alternative form of a gene. Within the population, there are often several "normal" alleles, whose functions are indistinguishable. In addition, there may be many rare alleles that are defective to varying degrees. An individual, however, normally carries a maximum of two alleles of each gene.
B. An individual is said to be homozygous if the two alleles of a gene are the same. An individual is said to be heterozygous if the two alleles of a gene are different. An individual can be heterozygous for gene A and homozygous for gene B.
C. The genotype is the specific set of alleles present in the genome of an individual. In practice, for organisms studied in a laboratory, the genotype is usually specified as a list of the known differences between the individual and the wild type, which is the standard, naturally occurring type. The phenotype is a description of the visible characteristics of the individual. In practice, the

phenotype is usually a list of the differences in visible characteristics between the individual and the wild type.

D. An allele *A* is dominant (relative to a second allele *a*) if the presence of even a single copy of *A* is enough to affect the phenotype; that is, if heterozygotes (with genotype *Aa*) appear different from *aa* homozygotes. An allele *a* is recessive (relative to a second allele *A*) if the presence of a single copy makes no difference to the phenotype, so that *Aa* individuals look just like *AA* individuals. If the phenotype of the heterozygous individual differs from the phenotypes of individuals that are homozygous for either allele, the alleles are said to be co-dominant.

ANSWER 19–11

A. Since the pea plant is diploid, any true-breeding plant must carry two mutant copies of the same gene—both of which have lost their function.

B. No, the same phenotype can be produced by mutations in different genes.

C. If each plant carries a mutation in a different gene, this will be revealed by complementation tests (see Panel 19–1, p. 669). When plant A is crossed with plant B, all of the F_1 plants will produce only round peas. And the same result will be obtained when plant B is crossed with plant C, or when plant A is crossed with plant C. In contrast, a cross between any two true-breeding plants that carry loss-of-function mutations in the same gene should produce only plants with wrinkled peas. This is true if the mutations themselves lie in different parts of the gene.

ANSWER 19–12

A. The mutation is likely to be dominant, because roughly half of the progeny born to an affected parent—in each of three marriages to hearing partners—are deaf, and it is unlikely that all these hearing partners were heterozygous carriers of the mutation.

B. The mutation is present on an autosome. If it were instead carried on a sex chromosome, either only the female progeny should be affected (expected if the mutation arose in a gene on the grandfather's X chromosome), or only the male progeny should be affected (expected if the mutation arose in a gene on the grandfather's Y chromosome). In fact, the pedigree reveals that both males and females have inherited the mutant form of the gene.

C. Suppose that the mutation was present on one of the two copies of the grandfather's Chromosome 12. Each of these copies of Chromosome 12 would be expected to carry a different pattern of SNPs, since one of them was inherited from his father and the other was inherited from his mother. Each of the copies of Chromosome 12 that was passed to his grandchildren will have gone through two meioses—one meiosis per generation.

Because two or three crossover events occur per chromosome during a meiosis, each chromosome inherited by a grandchild will have been subjected to about five crossovers since it left the grandfather, dividing it into six segments. An identical pattern of SNPs should surround whatever gene causes the deafness in each of the four affected grandchildren; moreover, this SNP pattern should be clearly different from that surrounding the same gene in each of the seven grandchildren who are normal. These SNPs would form an unusually long haplotype block—one that extends for about one-sixth of the length of Chromosome 12. (One-quarter of the DNA of each grandchild will have been inherited from the grandfather, in roughly 70 segments of this length scattered among the grandchild's 46 chromosomes.)

ANSWER 19–13 Individual 1 might be either heterozygous (+/–) or homozygous for the normal allele (+/+). Individual 2 must be homozygous for the recessive deafness allele (–/–). (Both his parents must have been heterozygous because they produced a deaf son.) Individual 3 is almost certainly heterozygous (+/–) and responsible for transmitting the mutant allele to his children and grandchildren. Given that the mutant allele is rare, individual 4 is most probably homozygous for the normal allele (+/+).

ANSWER 19–14 Your friend is wrong.

A. Mendel's laws, and the clear understanding that we now have concerning the mechanisms that produce them, rule out many false ideas concerning human heredity. One of them is that a first-born child has a different chance of inheriting particular traits from its parents than its siblings.

B. The probability of this type of pedigree arising by chance is one-fourth for each generation, or one in 64 for the three generations shown.

C. Data from an enlarged sampling of family members, or from more generations, would quickly reveal that the regular pattern observed in this particular pedigree arose by chance.

D. An opposite result, if it had strong statistical significance, would suggest that some process of selection was involved: for example, parents who had had a first child that was affected might regularly opt for screening of subsequent pregnancies and selectively terminate those pregnancies in which the fetus was found to be affected. Fewer second children would then be born with the abnormality.

ANSWER 19–15 Each carrier is a heterozygote, and 50% of his sperm or her eggs will carry the lethal allele. When two carriers marry, there is therefore a 25% chance that any baby will inherit the lethal allele from both parents and so will show the fatal phenotype. Because one person in 100 is a carrier, one partnership in 10,000 (100 × 100) will be a partnership of carriers (assuming that people choose their partners at random). Other things being equal, one baby in 40,000 will then be born with the defect, or 25 babies per year out of a total of a million babies born.

ANSWER 19–16 A dominant-negative mutation gives rise to a mutant gene product that interferes with the function of the normal gene product, causing a loss-of-function phenotype even in the presence of a normal copy of the gene. For example, if a protein forms a hexamer, and the mutant protein can interact with the normal subunits and inhibit the function of the hexamer, the mutation will be dominant. This ability of a single defective allele to determine the phenotype is the reason why such an allele is dominant. A gain-of-function mutation increases the activity of the gene or makes it active in inappropriate circumstances. The change in activity often has a phenotypic consequence, which is why such mutations are usually dominant.

ANSWER 19–17 This statement is largely true. Diabetes is one of the oldest diseases described by humans, dating at least back to the time of the ancient Greeks. Diabetes itself comes from the Greek word for siphon, which was used to describe the main symptoms: "The disease was called diabetes, as though it were a siphon, because it converts the human body into a pipe for the transflux of liquid humors" (in other words, untreated patients have constant thirst, balanced by high output of urine). If there were no human disease, the role of insulin would not have come to our attention in so demanding a way. We would have ultimately understood its role—and by now may have. Yet it is difficult to overstate the case for the role of disease in focusing our efforts toward a molecular understanding. Even today, the quest to understand and alleviate human disease is a principal driving force in biomedical research.

ANSWER 19–18

A. As outlined in Figure A19–18, if flies that are defective in different genes mate, their progeny will have one normal gene at each locus. In the case of a mating between a ruby-eyed fly and a white-eyed fly, every progeny fly will inherit one functional copy of the white gene from one parent and one functional copy of the ruby gene from the other parent. Note that the normal white allele produces brick-red eyes and the mutated form of the gene produces white eyes. Because each of the mutant alleles is recessive to the corresponding wild-type allele, the progeny will have the wild-type phenotype—brick-red eyes.

B. Garnet, ruby, vermilion, and carnation complement one another and the various alleles of the white gene (that is, when these mutant flies are mated with each other, they produce flies with a normal eye color); thus each of these mutants defines a separate gene. In contrast, white, cherry, coral, apricot, and buff do not complement each other; thus, they must be alleles of the same gene, which has been named the white gene. Thus, these nine different eye-color mutants define five different genes.

C. Different alleles of the same gene, like the five alleles of the white gene, often have different phenotypes. Different mutations compromise the function of the gene product to different extents, depending on the location of the mutation. Alleles that do not produce any functional product (null alleles), even if they result from different DNA sequence changes, do have the same phenotype.

ANSWER 19–19 SNPs are single-nucleotide differences between individuals for which two or more variants are each found at high frequency in the population. In the human population, SNPs occur roughly once per 1000 nucleotides of sequence. Many have been identified and mapped in various organisms, including several million in the human genome. SNPs, which can be detected by sequencing or oligonucleotide hybridization, serve as physical markers whose genomic locations are known. By tracking a mutant gene through different matings, and correlating the presence of the gene with the co-inheritance of particular SNP variants, one can narrow down the potential location of a gene to a chromosomal region that may contain only a few genes. These candidate genes can then be tested for the presence of a mutation that could account for the original mutant phenotype (see Figure 19–38).

Chapter 20

ANSWER 20–1 The horizontal orientation of the microtubules will be associated with a horizontal orientation of cellulose microfibrils deposited in the cell walls. The growth of the cells will therefore be in a vertical direction, expanding the distance between the cellulose microfibrils without stretching them. In this way, the stem will rapidly

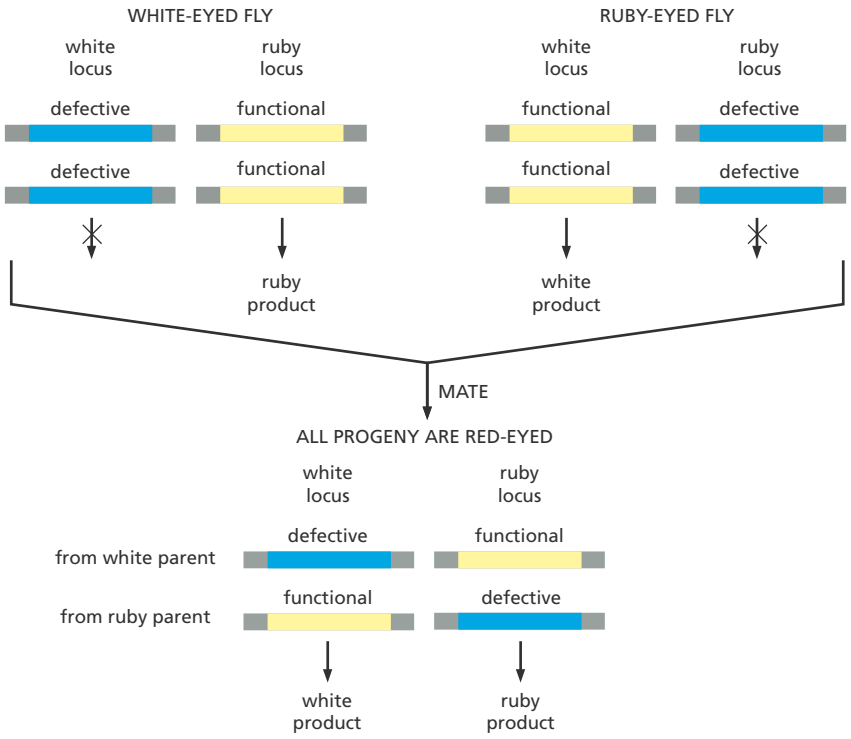


Figure A19–18

elongate; in a typical natural environment, this will hasten emergence from darkness into light.

ANSWER 20–2

A. As three collagen chains have to come together to form the triple helix, a defective molecule will impair assembly, even if normal collagen chains are present at the same time. Collagen mutations are therefore dominant; that is, they have a deleterious effect even in the presence of a normal copy of the gene.
B. The different severity of the mutations results from a polarity in the assembly process. Collagen monomers assemble into the triple-helical rod starting from their amino-terminal ends. A mutation in an "early" glycine therefore allows only short rods to form, whereas a mutation farther downstream allows for longer, more normal rods.

ANSWER 20–3 The remarkable ability to swell and thus occupy a large volume of space depends on the negative charges. These attract a cloud of positive ions, chiefly Na^+, which by osmosis draw in large amounts of water, thus giving proteoglycans their unique properties. Uncharged polysaccharides such as cellulose, starch, and glycogen, by contrast, are easily compacted into fibers or granules.

ANSWER 20–4 Focal contact sites are common in connective tissue, where fibroblasts exert traction forces on the extracellular matrix, and in cell culture, where cell crawling is observed. The forces for pulling on matrix or for driving crawling movement are generated by the actin cytoskeleton. In mature epithelium, focal contact sites are presumably less prominent because the cells are largely fixed in place and have no need to crawl over the basal lamina or actively pull on it.

ANSWER 20–5 Suppose a cell is damaged so that its plasma membrane becomes leaky. Ions present in high concentration in the extracellular fluid, such as Na^+ and Ca^{2+}, then rush into the cell, and valuable metabolites leak out. If the cell were to remain connected to its healthy neighbors, these too would suffer from the damage. But the influx of Ca^{2+} into the sick cell causes its gap junctions to close immediately, effectively isolating the cell and preventing damage from spreading in this way.

ANSWER 20–6 Ionizing (high-energy) radiation tears through matter, knocking electrons out of their orbits and breaking chemical bonds. In particular, it creates breaks and other damage in DNA, and thus causes cells to arrest in the cell cycle (discussed in Chapter 18). If the damage is so severe that it cannot be repaired, cells become permanently arrested and undergo apoptosis; that is, they activate a suicide program.

ANSWER 20–7 Cells in the gut epithelium are exposed to a quite hostile environment, containing digestive enzymes and many other substances that vary drastically from day to day depending on the food intake of the organism. The epithelial cells also form a first line of defense against potentially hazardous compounds and mutagens that are ubiquitous in our environment. The rapid turnover protects the organism from harmful consequences, as wounded and sick cells are discarded. If an epithelial cell started to divide inappropriately as the result of a mutation, for example, it and its unwanted progeny would most often simply be discarded by natural disposal from the tip of a villus: even though such mutations must occur often, they rarely give rise to a cancer.

A neuron, on the other hand, lives in a very protected environment, insulated from the outside world. Its function depends on a complex system of connections with other neurons—a system that is created during development and is not easy to reconstruct if the neuron subsequently dies.

ANSWER 20–8 Every cell division generates one additional cell; so if the cells were never lost or discarded from the body, the number of cells in the body should equal the number of divisions plus one. The number of divisions is 1000-fold greater than the number of cells because, in the course of a lifetime, 1000 cells are discarded by mechanisms such as apoptosis for every cell that is retained in the body.

ANSWER 20–9

A. False. Gap junctions are not connected to the cytoskeleton; their role is to provide cell–cell communication by allowing small molecules to pass from one cell to another.
B. True. Upon wilting, the turgor pressure in the plant cell is reduced, and consequently the cell walls, having tensile but little compressive strength, like a rubber tire, no longer provide rigidity.
C. False. Proteoglycans can withstand a large amount of compressive force but do not have a rigid structure. Their space-filling properties result from their tendency to absorb large amounts of water.
D. True.
E. True.
F. True. Stem cells stably express control genes that ensure that their daughter cells will be of the appropriate differentiated cell types.

ANSWER 20–10 Small cytosolic molecules, such as glutamic acid, cyclic AMP, and Ca^{2+} ions, pass readily through both gap junctions and plasmodesmata, whereas large cytosolic macromolecules, such as mRNA and G proteins, are excluded. Plasma membrane phospholipids diffuse in the plane of the membrane through plasmodesmata because the plasma membranes of adjacent cells are continuous through these junctions. This traffic is not possible through gap junctions, because the membranes of the connected cells remain separate.

ANSWER 20–11 Plants are exposed to extreme changes in the environment, which often are accompanied by huge fluctuations in the osmotic properties of their surroundings. An intermediate filament network as we know it from animal cells would not be able to provide full osmotic support for cells: the sparse, rivetlike attachment points would not be able to prevent the membrane from bursting in response to a huge osmotic pressure applied from the inside of the cell.

ANSWER 20–12 Action potentials can, in fact, be passed from cell to cell through gap junctions. Indeed, heart muscle cells are connected in this way, which ensures that they contract synchronously when stimulated. This mechanism of passing the signal from cell to cell is rather limited, however. As we discuss in Chapter 12, synapses are far more sophisticated and allow signals to be modulated and to be integrated with other signals received by the cell. Thus, gap junctions are like simple soldered joints between electrical

components, while synapses are like complex relay devices, enabling systems of neurons to perform computations.

ANSWER 20–13 To make jello, gelatin is boiled in water, which denatures the collagen fibers. Upon cooling, the disordered fibers form a tangled mess that solidifies into a gel. This gel actually resembles the collagen as it is initially secreted by fibroblasts. It is not until the fibers have been aligned, bundled, and cross-linked that they acquire their ability to resist tensile forces.

ANSWER 20–14 The evidence that DNA is the blueprint that specifies all the structural characteristics of an organism is based on observations that small changes in the DNA by mutation result in changes in the organism. Although DNA provides the plans that specify structure, these plans need to be executed during development. This requires a suitable environment (a human baby would not fit into a stork's egg shell), suitable nourishment, suitable tools (such as the appropriate transcription regulators required for early development), suitable spatial organization (such as the asymmetries in the egg cell required to allow for appropriate cell differentiation during the early cell divisions), and so on. Thus inheritance is not restricted to the passing on of the organism's DNA, because development requires appropriate conditions to be set up by the parent. Nevertheless, when all these conditions are met, the plans that are archived in the genome will determine the structure of the organism to be built.

ANSWER 20–15 White blood cells circulate in the bloodstream and migrate into and out of tissues in performance of their normal function of defending the body against infection: they are naturally invasive. Once mutations have occurred to upset the normal controls on production of these cells, there is no need for additional mutations to enable the cells to spread through the body. Thus, the number of mutations that have to be accumulated in order to give rise to leukemia is smaller than for other types of cancer.

ANSWER 20–16 The shape of the curve reflects the need for multiple mutations to accumulate in a cell before a cancer results. If a single mutation were sufficient, the graph would be a straight horizontal line: the likelihood of occurrence of a particular mutation, and therefore of cancer, would be the same at any age. If two specific mutations were required, the graph would be a straight line sloping upward from the origin: the second mutation has an equal chance of occurring at any time, but will tip the cell into cancerous behavior only if the first mutation has already occurred in the same cell lineage; and the likelihood that the first mutation has already occurred will be proportional to the age of the individual. The steeply curved graph shown in the figure goes up approximately as the fifth power of the age, and this indicates that far more than two mutations have to be accumulated before cancer sets in. It is not easy to say precisely how many, because of the complex ways in which cancers develop. Successive mutations can alter cell numbers and cell behavior, and thereby change both the probability of subsequent mutations and the selection pressures that drive the evolution of cancer.

ANSWER 20–17 During exposure to the carcinogen, mutations are induced, but the number of relevant mutations in any one cell is usually not enough to convert it directly into a cancer cell. Over the years, the cells that have become predisposed to cancer through the induced mutations accumulate progressively more mutations. Eventually, one of them will turn into a cancer cell. The long delay between exposure and cancer has made it extremely difficult to hold cigarette manufacturers or producers of industrial carcinogens legally responsible for the damage that is caused by their products.

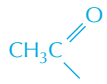
ANSWER 20–18 By definition, a carcinogen is any substance that promotes the occurrence of one or more types of cancer. The sex hormones can therefore be classified as naturally occurring carcinogens. Although most carcinogens act by directly causing mutations, carcinogenic effects are also often exerted in other ways. The sex hormones increase both the rate of cell division and the numbers of cells in hormone-sensitive organs such as breast, uterus, and prostate. The first effect increases the mutation rate per cell, because mutations, regardless of environmental factors, are spontaneously generated in the course of DNA replication and chromosome segregation; the second effect increases the number of cells at risk. In these and possibly other ways, the hormones can favor the development of cancer, even though they do not directly cause mutations.

ANSWER 20–19 The short answer is no—cancer in general is not a hereditary disease. It arises from new mutations occurring in our own somatic cells, rather than mutations we inherit from our parents. In some rare types of cancer, however, there is a strong heritable risk factor, so that parents and their children both show the same predisposition to a specific form of the disease. This occurs, for example, in families carrying a mutation that knocks out one of the two copies of the tumor suppressor gene *APC*; the children then inherit a propensity to colorectal cancer. Much weaker heritable tendencies are also seen in several other cancers, including breast cancer, but the genes responsible for these effects are still mostly unknown.

Glossary

acetyl CoA (**acetyl coenzyme A**)
Activated carrier that donates the carbon atoms in its readily transferable acetyl group to many metabolic reactions, including the citric acid cycle and fatty acid biosynthesis; the acetyl group is linked to coenzyme A (CoA) by a thioester bond that releases a large amount of energy when hydrolyzed.

acetyl group
Chemical group derived from acetic acid.



acid
A molecule that releases a proton when dissolved in water; this dissociation generates hydronium (H_3O^+) ions, thereby lowering the pH.

actin filament
Thin, flexible protein filament

made from a chain of globular actin molecules; a major constituent of all eukaryotic cells, this cytoskeletal element is essential for cell movement and for the contraction of muscle cells.

actin-binding protein
Protein that interacts with actin monomers or filaments to control the assembly, structure, and behavior of actin filaments and networks.

action potential
Traveling wave of electrical excitation caused by rapid, transient, self-propagating depolarization of the plasma membrane in a neuron or other excitable cell; also called a nerve impulse.

activated carrier
A small molecule that stores energy or chemical groups in a form that can be donated to many different metabolic reactions. Examples include ATP, acetyl CoA, and NADH.

activation energy
The energy that must be acquired by a molecule to undergo a chemical reaction.

activator
A protein that binds to a specific regulatory region of DNA to permit transcription of an adjacent gene.

active site
Region on the surface of an enzyme that binds to a substrate molecule and catalyzes its chemical transformation.

active transport
The movement of a solute across a membrane against its electrochemical gradient; requires an input of energy, such as that provided by ATP hydrolysis.

acyl group
Functional group derived from a carboxylic acid.

adaptation
Adjustment of sensitivity following repeated stimulation; allows a cell or organism to register small changes in a signal despite a high background level of stimulation.

adenylyl cyclase
Enzyme that catalyzes the formation of cyclic AMP from ATP; an important component in some intracellular signaling pathways.

adherens junction
Cell junction that helps hold together epithelial cells in a sheet of epithelium; actin filaments inside the cell attach to its cytoplasmic face.

ADP (**adenosine 5′-diphosphate**)
Nucleoside diphosphate produced by hydrolysis of the terminal phosphate of ATP. (*See* Figure 3–31.)

alcohol
Organic compound containing a hydroxyl group (–OH) bound to a saturated carbon atom, for example, ethanol. (*See* Panel 2–1, pp. 66–67.)

aldehyde
Reactive organic compound that contains the HC=O group, for example, glyceraldehyde. (*See* Panel 2–1, pp. 66–67.)

alkyl group
Functional group consisting solely of single-bonded carbon and hydrogen atoms, such as methyl ($–CH_3$) or ethyl ($–CH_2CH_3$) groups.

allele
An alternative form of a gene; for a given gene, many alleles may exist in the gene pool of the species.

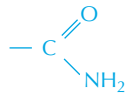
allosteric
Describes a protein that can exist in multiple conformations depending on the binding of a molecule (ligand) at a site other than the catalytic site; changes from one conformation to another often alter the protein's activity or ligand affinity.

alpha helix (**α helix**)
Folding pattern, common in many proteins, in which a single polypeptide chain twists around itself to form a rigid cylinder stabilized by hydrogen bonds between every fourth amino acid.

alternative splicing
The production of different mRNAs (and proteins) from the same gene by splicing its RNA transcripts in different ways.

***Alu* sequence**
Family of mobile genetic elements that comprises about 10% of the human genome; this short, repetitive sequence is no longer mobile on its own, but requires enzymes encoded by other elements to transpose.

amide
Molecule containing the functional group –$CONH_2$. (*See* Panel 2–1, pp. 66–67.)



amine
Molecule containing an amino group (–NH_2). (*See* Panel 2–1, pp. 66–67.)

amino acid
Small organic molecule containing both an amino group and a carboxyl group; it serves as the building block of proteins. (*See* Panel 2–5, pp. 74–75.)

amino acid sequence
The order of the amino acid subunits in a protein chain. Sometimes called the primary structure of a protein.

amino group
Functional group (–NH_2) derived from ammonia. Can accept a proton and carry a positive charge in aqueous solution. (*See* Panel 2–1, pp. 66–67.)

amino terminus—*see* **N-terminus**

aminoacyl-tRNA synthetase
During protein synthesis, an enzyme that attaches the correct amino acid to a tRNA molecule to form a "charged" aminoacyl-tRNA.

AMP (**adenosine 5′ monophosphate**)
Nucleotide produced by the energetically favorable hydrolysis of the final two phosphate groups from ATP, a reaction that drives the synthesis of DNA and RNA. (*See* Figure 3–40.)

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

anabolic pathway
Series of enzyme-catalyzed reactions by which large biological molecules are synthesized from smaller subunits; usually requires an input of energy.

anabolism
Set of metabolic pathways by which large molecules are made from smaller ones.

anaerobic
Describes a cell, organism, or metabolic process that operates in the absence of air or, more precisely, in the absence of molecular oxygen.

anaphase
Stage of mitosis during which the two sets of chromosomes separate and are pulled toward opposite ends of the dividing cell.

anaphase-promoting complex (**APC**)
A protein complex that triggers the separation of sister chromatids and orchestrates the carefully timed destruction of proteins that control progress through the cell cycle; the complex catalyzes the ubiquitylation of its targets.

anion
Negatively charged ion, such as Cl^- or CH_3COO^-.

antenna complex
In chloroplasts and photosynthetic bacteria, the part of the membrane-bound photosystem that captures energy from sunlight; contains an array of proteins that bind hundreds of chlorophyll molecules and other photosensitive pigments.

antibody
Protein produced by B lymphocytes in response to a foreign molecule or invading organism. Binds to the foreign molecule or cell extremely tightly, thereby inactivating it or marking it for destruction.

anticodon
Set of three consecutive nucleotides in a transfer RNA molecule that recognizes, through base-pairing, the three-nucleotide codon on a messenger RNA molecule; this interaction helps to deliver the correct amino acid to a growing polypeptide chain.

antigen
Molecule or fragment of a molecule that is recognized by an antibody.

antiparallel
Describes two similar structures arranged in opposite orientations, such as the two strands of a DNA double helix.

antiport
Type of coupled transporter that transfers two different ions or small molecules across a membrane in opposite directions, either simultaneously or in sequence.

APC—*see* **anaphase-promoting complex**

apical
Describes the top or the tip of a cell, structure, or organ; in an epithelial cell, for example, this surface is opposite the base, or basal surface.

apoptosis
A tightly controlled form of programmed cell death that allows cells that are unneeded or unwanted to be eliminated from an adult or developing organism.

archaea
One of the two divisions of prokaryotes, often found in hostile environments such as hot springs or concentrated brine. (*See also* **bacteria**.)

asexual reproduction
Mode of reproduction in which offspring arise from a single parent, producing an individual genetically identical to that parent; includes budding, binary fission, and parthenogenesis.

aster
Star-shaped array of microtubules emanating from a centrosome or from a pole of a mitotic spindle.

atom
The smallest particle of an element that still retains its distinctive chemical properties; consists of a positively charged nucleus surrounded by a cloud of negatively charged electrons.

atomic mass
The mass of an atom expressed in daltons, the atomic mass unit that closely approximates the mass of a hydrogen atom.

ATP (**adenosine 5′-triphosphate**)
Molecule that serves as the principal carrier of energy in cells; this nucleoside triphosphate is composed of adenine, ribose, and three phosphate groups. (*See* Figure 2–24.)

ATP synthase
Membrane-associated enzyme complex that catalyzes the formation of ATP from ADP and inorganic phosphate during oxidative phosphorylation and photosynthesis.

autophagy
Mechanism by which a cell "eats itself," digesting molecules and organelles that are damaged or obsolete.

Avogadro's number
The number of molecules in a mole, the quantity of a substance equal to its molecular weight in grams; approximately 6×10^{23}.

axon
Long, thin extension that conducts electrical signals away from a nerve cell body toward remote target cells.

bacteria (singular bacterium)
One of the two divisions of prokaryotes; some species cause disease. The term is sometimes used to refer to any prokaryotic microorganism, although the world of prokaryotes also includes archaea, which are only distantly related. (*See also* **archaea**.)

bacteriorhodopsin
Pigmented protein found in abundance in the plasma membrane of the salt-loving archaeon *Halobacterium halobium*; pumps protons out of the cell in response to light.

basal
Situated near the base; opposite of apical.

basal body—*see* **centriole**

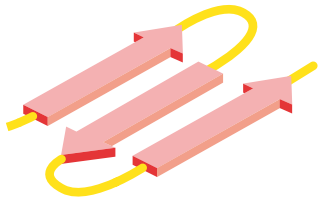
basal lamina
Thin mat of extracellular matrix, secreted by epithelial cells, upon which the cells sit.

base
Molecule that accepts a proton when dissolved in water; also used to refer to the nitrogen-containing purines or pyrimidines in DNA and RNA.

base pair
Two complementary nucleotides in an RNA or a DNA molecule that are held together by hydrogen bonds—for example, G with C, and A with T or U.

Bcl2 family
Related group of intracellular proteins that regulates apoptosis; some family members promote cell death, others inhibit it.

beta sheet
(**β sheet**)



Folding pattern found in many proteins in which neighboring regions of the polypeptide chain associate side by side with each other through hydrogen bonds to give a rigid, flattened structure.

bi-orientation
The symmetrical attachment of a sister chromatid pair on the mitotic spindle, such that one chromatid in the duplicated chromosome is attached to one spindle pole and the other is attached to the opposite pole.

binding site
Region on the surface of a protein, typically a cavity or groove, that interacts with another molecule (a ligand) through the formation of multiple noncovalent bonds.

biosynthesis
An enzyme-catalyzed process by which complex molecules are formed from simple substances by living cells; also called anabolism.

bivalent
Structure formed when a duplicated chromosome pairs with its homolog at the beginning of meiosis; contains four sister chromatids.

bond—*see* **chemical bond**

bond energy
The strength of the chemical linkage between two atoms, measured by the energy in kilocalories needed to break it.

bond length
Average distance between two interacting atoms in a molecule, usually those linked covalently.

buffer
Mixture of weak acids and bases that maintains the pH of a solution by releasing and taking up protons.

C-terminus (**carboxyl terminus**)
The end of a polypeptide chain that carries a free carboxyl group (–COOH).

Ca^{2+} pump
An active transporter that uses energy supplied by ATP hydrolysis to actively expel Ca^{2+} from the cell cytosol.

Ca^{2+}/calmodulin-dependent protein kinase
(**CaM kinase**)
Enzyme that phosphorylates target proteins in response to an increase in Ca^{2+} ion concentration through its interaction with the Ca^{2+}-binding protein calmodulin.

cadherin
A member of a family of Ca^{2+}-dependent proteins that mediates the attachment of one cell to another in animal tissues.

calmodulin (**CaM**)
Small Ca^{2+}-binding protein that modifies the activity of many target proteins in response to changes in Ca^{2+} concentration.

calorie
Unit of heat. Equal to the amount of heat needed to raise the temperature of 1 gram of water by 1°C.

CaM—*see* calmodulin

cancer
Disease caused by abnormal and uncontrolled cell proliferation, followed by invasion and colonization of body sites normally reserved for other cells.

carbohydrate
General term for sugars and related compounds with the general formula $(CH_2O)_n$. (*See* Panel 2–3, pp. 70–71.)

carbohydrate layer
Protective layer of sugar residues, including the polysaccharide portions of proteoglycans and oligosaccharides attached to protein or lipid molecules, on the outer surface of a cell. Also called the glycocalyx.

carbon fixation
Process by which green plants and other photosynthetic organisms incorporate carbon atoms from atmospheric carbon dioxide into sugars. The second stage of photosynthesis.

carbonyl group
Carbon atom linked to an oxygen atom by a double bond. (*See* Panel 2–1, pp. 66–67.)

carboxyl group
Carbon atom linked to an oxygen atom by a double bond and to a hydroxyl group (–COOH). In aqueous solution, acts as a weak acid. (*See* Panel 2–1, pp. 66–67.)

carboxyl terminus—*see* C-terminus

cascade—*see* signaling cascade

caspase
A family of proteases that, when activated, mediates the destruction of the cell by apoptosis.

catabolism
Set of enzyme-catalyzed reactions by which complex molecules are degraded to simpler ones with release of energy; intermediates in these reactions are sometimes called catabolites.

catalysis
The acceleration of a chemical reaction brought about by the action of a catalyst; virtually all reactions in a cell require such assistance to occur under conditions present in living organisms.

catalyst
Substance that accelerates a chemical reaction by lowering its activation energy; enzymes perform this role in cells.

cation
Positively charged ion, such as Na^+ or $CH_3NH_3^+$.

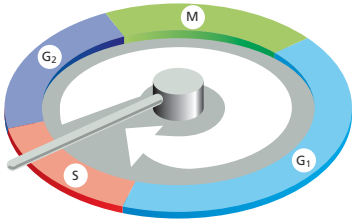
Cdk inhibitor protein
Regulatory protein that blocks the assembly or activity of cyclin–Cdk complexes, delaying progression primarily through the G_1 and S phases of the cell cycle.

cDNA library
Collection of DNA fragments synthesized using all of the mRNAs present in a particular type of cell as a template.

cell
The basic unit from which a living organism is made; consists of an aqueous solution of organic molecules enclosed by a membrane.

cell cortex
Specialized layer of cytoplasm on the inner face of the plasma membrane. In animal cells, it is rich in the actin filaments that govern cell shape and drive cell movement.

cell cycle
The orderly sequence of events by which a cell duplicates its contents and divides into two.



cell division
Separation of a cell into two daughter cells. In eukaryotic cells, entails the splitting of the nucleus (mitosis) closely followed by cleavage of the cytoplasm (cytokinesis).

cell junction
Specialized region of connection between two cells or between a cell and the extracellular matrix.

cell line
Population of cells derived from a plant or animal capable of dividing indefinitely in culture.

cell locomotion
Active movement of a cell from one location to another.

cell memory
The ability of differentiated cells and their descendants to maintain their identity.

cell respiration
Process by which cells harvest the energy stored in food molecules; usually accompanied by the uptake of O_2 and the release of CO_2.

cell signaling
The molecular mechanisms by which cells detect and respond to external stimuli and send messages to other cells.

cell wall
Mechanically strong fibrous layer deposited by a cell outside its plasma membrane. Prominent in most plants, bacteria, algae, and fungi, but not present in most animal cells.

cell-cycle control system
Network of regulatory proteins that govern the orderly progression of a eukaryotic cell through the stages of cell division.

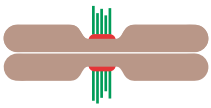
cellulose
Structural polysaccharide consisting of long chains of covalently linked glucose units. Provides tensile strength in plant cell walls.

cellulose microfibril
Long, thin strand of cellulose that helps strengthen plant cell walls.

central dogma
The principle that genetic information flows from DNA to RNA to protein.

centriole
Cylindrical array of microtubules usually found in pairs at the center of a centrosome in animal cells. Also found at the base of cilia and flagella, where they are called basal bodies.

centromere
Specialized DNA sequence that allows duplicated chromosomes to be separated during M phase; can be seen as the constricted region of a mitotic chromosome.



centrosome
Microtubule-organizing center that sits near the nucleus in an animal cell; during the cell cycle, this structure duplicates to form the two poles of the mitotic spindle.

centrosome cycle
Process by which the centrosome duplicates (during interphase) and the two new centrosomes separate (at the beginning of mitosis) to form the poles of the mitotic spindle.

channel
A protein that forms a hydrophilic pore across a membrane, through which selected small molecules or ions can passively diffuse.

chaperone protein
Molecule that steers proteins along productive folding pathways, helping them to fold correctly and preventing them from forming aggregates inside the cell.

checkpoint
Mechanism by which the cell-cycle control system can regulate progression through the cycle, ensuring that conditions are favorable and each process has been completed before proceeding to the next stage.

chemical bond
An exchange of electrons that holds two atoms together. Types found in living cells include ionic bonds, covalent bonds, and hydrogen bonds.

chemical group
Combination of atoms, such as a hydroxyl group (–OH) or an amino group ($–NH_2$), with distinct chemical and physical properties that influences the behavior of the molecule in which it resides.

chemiosmotic coupling
Mechanism that uses the energy stored in a transmembrane proton gradient to drive an energy-requiring process, such as the synthesis of ATP or the transport of a molecule across a membrane.

chiasma (plural chiasmata)
X-shaped connection between paired homologous chromosomes during meiosis; represents a site of crossing-over between two non-sister chromatids.

chlorophyll
Light-absorbing green pigment that plays a central part in photosynthesis.

chloroplast
Specialized organelle in algae and plants that contains chlorophyll and serves as the site in which photosynthesis takes place.

cholesterol
Short, rigid lipid molecule present in large amounts in the plasma membranes of animal cells, where it makes the lipid bilayer less flexible.

chromatid—*see* sister chromatid

chromatin
Complex of DNA and proteins that makes up the chromosomes in a eukaryotic cell.

chromatin-remodeling complex
Enzyme (typically multisubunit) that uses the energy of ATP hydrolysis to alter the arrangement of nucleosomes in eukaryotic chromosomes, changing the accessibility of the underlying DNA to other proteins, including those involved in transcription.

chromatography
Technique used to separate the individual molecules in a complex mixture on the basis of their size, charge, or their ability to bind to a particular chemical group. In a common form of the technique, the mixture is run through a column filled with a material that either binds or lets through the desired molecule.

chromosome
Long, threadlike structure composed of DNA and proteins that carries the genetic information of an organism; becomes visible as a distinct entity when a plant or animal cell prepares to divide.

chromosome condensation
Process by which a duplicated chromosome becomes packed into a more compact structure prior to cell division.

cilium (plural cilia)
Hairlike structure made of microtubules found on the surface of many eukaryotic cells; when present in large numbers, its rhythmic beating can drive the movement of fluid over the cell surface, as in the epithelium of the lungs.

cis
On the same side as.

***cis* Golgi network**
Section of the Golgi apparatus that receives materials from the endoplasmic reticulum.

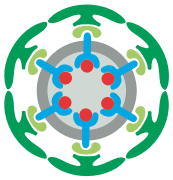
citric acid cycle
Series of reactions that generates large amounts of NADH by oxidizing acetyl groups derived from food molecules to CO_2. In eukaryotic cells, this central metabolic pathway takes place in the mitochondrial matrix.

classical genetic approach
Experimental techniques used to isolate the genes responsible for an interesting phenotype.

clathrin
Protein that makes up the coat of a type of transport

vesicle that buds from either the Golgi apparatus (on the outward secretory pathway) or from the plasma membrane (on the inward endocytic pathway).

coated vesicle
Small membrane-enclosed sac that wears a distinctive layer of proteins on its cytosolic surface. It is formed by pinching-off of a protein-coated region of cell membrane.



codon
Group of three consecutive nucleotides that specifies a particular amino acid or that starts or stops protein synthesis; applies to the nucleotides in an mRNA or in a coding sequence of DNA.

coenzyme A
Small molecule used to carry and transfer acetyl groups needed for a variety of metabolic reactions, such as the synthesis of fatty acids. (*See also* **acetyl CoA** and Figure 3–36.)

cohesin
Protein complex that holds sister chromatids together after DNA has been replicated in the cell cycle.

coiled-coil
Stable, rodlike protein structure formed when two or more α helices twist around each other.

collagen
Triple-stranded, fibrous protein that is a major component of the extracellular matrix and connective tissues; it is the main protein in animal tissues, and different forms can be found in skin, tendon, bone, cartilage, and blood vessels.

combinatorial control
Describes the way in which groups of transcription regulators work together to regulate the expression of a single gene.

complementary
Describes two molecular surfaces that fit together closely and form noncovalent bonds with each other. Examples include complementary base pairs, such as A and T, and the two complementary strands of a DNA molecule.

complementary DNA (**cDNA**)
DNA molecule synthesized from an mRNA molecule and therefore lacking the introns that are present in genomic DNA.

complementation test
Genetic experiment that determines whether two mutations that are associated with the same phenotype lie in the same gene or in different genes.

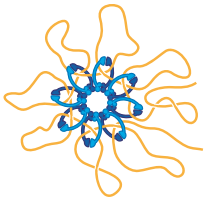
complex
A collection of macromolecules that are bound to each other by noncovalent bonds to form a large structure with a specific function.

complex trait
A heritable characteristic whose transmission to progeny does not appear to obey Mendel's laws. Such characteristics, for example height, usually result from the interaction of multiple genes.

condensation—*see* **chromosome condensation**

condensation reaction
Chemical reaction in which a covalent bond is formed between two molecules as water is expelled; used to build polymers, such as proteins, polysaccharides, and nucleic acids.

condensin
Protein complex that helps configure duplicated chromosomes for segregation by making them more compact.



conformation
Precise, three-dimensional shape of a protein or other macromolecule, based on the spatial location of its atoms in relation to one another.

connective tissue
Tissues such as bone, tendons, and the dermis of the skin, in which extracellular matrix makes up the bulk of the tissue and carries the mechanical load.

conserved synteny
The preservation of gene order and location in the genomes of different species.

contractile ring
Structure made of actin and myosin filaments that forms a belt around a dividing cell, pinching it in two.

copy-number variation (**CNV**)
Large segment of DNA, 1000 nucleotide pairs or greater, that has been duplicated or lost in an individual genome (compared to the "reference" genome sequence).

coupled pump
Active transporter that uses the movement of one solute down its electrochemical gradient to drive the uphill transport of another solute across the same membrane.

coupled reaction
Linked pair of chemical reactions in which free energy released by one reaction serves to drive the other reaction.

covalent bond
Stable chemical link between two atoms produced by sharing one or more pairs of electrons.

crossing-over
Process whereby two homologous chromosomes break at corresponding sites and rejoin to produce two recombined chromosomes that have physically exchanged segments of DNA.

cyclic AMP (**cAMP**)
Small intracellular signaling molecule generated from ATP in response to hormonal stimulation of cell-surface receptors.

cyclic-AMP-dependent protein kinase (**protein kinase A**, **PKA**)
Enzyme that phosphorylates target proteins in response to a rise in intracellular cyclic AMP concentration.

cyclin
Regulatory protein whose concentration rises and falls at specific times during the eukaryotic cell cycle;

cyclins help control progression from one stage of the cell cycle to the next by binding to cyclin-dependent protein kinases (Cdks).

cyclin-dependent protein kinase (**Cdk**)
Enzyme that, when complexed with a regulatory cyclin protein, can trigger various events in the cell-division cycle by phosphorylating specific target proteins.

cytochrome
Membrane-bound, colored, heme-containing protein that transfers electrons during cellular respiration and photosynthesis.

cytochrome *c* oxidase
Protein complex that serves as the final electron carrier in the respiratory chain; removes electrons from cytochrome *c* and passes them to O_2 to produce H_2O.

cytokine
Small signaling molecule, made and secreted by cells, that acts on neighboring cells to alter their behavior. Usually a protein, polypeptide, or glycoprotein.

cytokinesis
Process by which the cytoplasm of a plant or animal cell divides in two to form individual daughter cells.

cytoplasm
Contents of a cell that are contained within its plasma membrane but, in the case of eukaryotic cells, contained outside the nucleus.

cytoskeleton
System of protein filaments in the cytoplasm of a eukaryotic cell that gives the cell shape and the capacity for directed movement. Its most abundant components are actin filaments, microtubules, and intermediate filaments.

cytosol
Contents of the main compartment of the cytoplasm, excluding membrane-enclosed organelles such as endoplasmic reticulum and mitochondria. The cell fraction remaining after membranes, cytoskeletal components, and other organelles have been removed.

dalton
Unit of molecular mass. Defined as one-twelfth the mass of an atom of carbon 12 (1.66×10^{-24} g); approximately equal to the mass of a hydrogen atom.

dark reactions
In photosynthesis, the set of reactions that produce sugars from CO_2; these reactions, also called carbon fixation, can occur in the absence of sunlight.

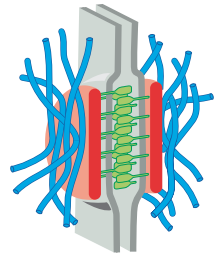
denature
To cause a dramatic change in the structure of a macromolecule by exposing it to extreme conditions, such as high heat or harsh chemicals. Usually results in the loss of biological function.

dendrite
Short, branching structure that extends from the surface of a nerve cell and receives signals from other neurons.

deoxyribonucleic acid—*see* **DNA**

depolarization
A shift in the membrane potential, making it less negative.

desmosome
Specialized cell–cell junction, usually formed between two epithelial cells, that serves to connect the ropelike keratin filaments of the adjoining cells, providing tensile strength.



detergent
Soapy substance used to solubilize membrane proteins.

diacylglycerol (**DAG**)
Small messenger molecule produced by the cleavage of membrane inositol phospholipids in response to extracellular signals. Helps activate protein kinase C.

dideoxy sequencing or Sanger sequencing
The standard method of determining the nucleotide sequence of DNA; utilizes DNA polymerase and a set of chain-terminating nucleotides.

differentiation
Process by which a cell undergoes a progressive, coordinated change to a more specialized cell type, brought about by large-scale changes in gene expression.

diffusion
Process by which molecules and small particles move from one location to another by random, thermally driven motion.

dimer
A molecule composed of two structurally similar subunits.

diploid
Describes a cell or organism containing two sets of homologous chromosomes, one inherited from each parent. (*See also* **haploid**.)

disulfide bond
Covalent cross-link formed between the sulfhydryl groups on two cysteine side chains; often used to reinforce a secreted protein's structure or to join two different proteins together.

divergence
Differences in sequence that accumulate over time in DNA segments derived from a common ancestral sequence.

DNA (**deoxyribonucleic acid**)
Double-stranded polynucleotide formed from two separate chains of covalently linked deoxyribonucleotide units. It serves as the cell's store of genetic information that is transmitted from generation to generation.

DNA cloning
Production of many identical copies of a DNA sequence.

DNA library
Collection of cloned DNA molecules, representing either an entire genome (genomic library) or copies of the mRNA produced by a cell (cDNA library).

DNA ligase
Enzyme that reseals nicks that arise in the backbone of a DNA molecule; in the laboratory, can be used to join together two DNA fragments.

DNA methylation
The enzymatic addition of methyl groups to cytosine bases in DNA; this covalent modification generally turns off genes by attracting proteins that block gene expression.

DNA microarray
A surface on which a large number of short DNA molecules (typically in the tens of thousands) have been immobilized in an orderly pattern. Each of these DNA fragments acts as a probe for a specific gene, allowing the activities of thousands of genes to be monitored at the same time.

DNA repair
Collective term for the enzymatic processes that correct deleterious changes affecting the continuity or sequence of a DNA molecule.

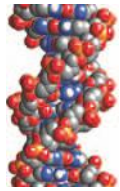
DNA replication
The process by which a copy of a DNA molecule is made.

DNA transcription—*see* **transcription**

domain
Small discrete region of a structure; in a protein, a segment that folds into a compact and stable structure. In a membrane, a region of the bilayer with a characteristic lipid and protein composition.

double bond
Chemical linkage formed when two atoms share four electrons.

double helix
The typical structure of a DNA molecule in which the two complementary polynucleotide strands are wound around each other with base-pairing between the strands.



dynamic instability
The rapid switching between growth and shrinkage shown by microtubules.

dynein
Motor protein that uses the energy of ATP hydrolysis to move toward the minus end of a microtubule. One form of the protein is responsible for the bending of cilia.

electrochemical gradient
Driving force that determines which way an ion will move across a membrane; consists of the combined influence of the ion's concentration gradient and the membrane potential.

electron
Negatively charged subatomic particle that occupies space around an atomic nucleus (e^-).

electron acceptor
Atom or molecule that readily takes up electrons, thereby becoming reduced.

electron carrier
Molecule capable of picking up an electron from a molecule with weak electron affinity and transferring it to a molecule with a higher electron affinity.

electron donor
Molecule that easily gives up an electron, thereby becoming oxidized.

electron microscope
Instrument that illuminates a specimen using beams of electrons to reveal and magnify the structures of very small objects, such as organelles and large molecules.

electron-transport chain
A series of membrane-embedded electron carrier molecules that facilitate the movement of electrons from a higher to a lower energy level, as in oxidative phosphorylation and photosynthesis.

electrophoresis
Technique for separating a mixture of proteins or DNA fragments by placing them on a polymer gel and subjecting them to an electric field. The molecules migrate through the gel at different speeds depending on their size and net charge.

electrostatic attraction
Force that draws together oppositely charged atoms. Examples include ionic bonds and the attractions between molecules containing polar covalent bonds.

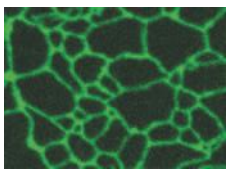
element
Substance that cannot be broken down to any other chemical form; composed of a single type of atom.

embryonic stem cell (**ES cell**)
An undifferentiated cell type derived from the inner cell mass of an early mammalian embryo and capable of differentiating to give rise to any of the specialized cell types in the adult body.

endocytosis
Process by which cells take in materials through an invagination of the plasma membrane, which surrounds the ingested material in a membrane-enclosed vesicle. (*See also* **pinocytosis** and **phagocytosis**.)

endomembrane system
Interconnected network of membrane-enclosed organelles in a eukaryotic cell; includes the endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, and endosomes.

endoplasmic reticulum (**ER**)
Labyrinthine membrane-enclosed compartment in the cytoplasm of eukaryotic cells where lipids and proteins are made.



endosome
Membrane-enclosed compartment of a eukaryotic cell through which material ingested by endocytosis passes on its way to lysosomes.

enhancer
Regulatory DNA sequence to which transcription regulators bind, influencing the rate of transcription of a gene that may be many thousands of base pairs away.

entropy
Thermodynamic quantity that measures the degree of disorder in a system.

enzyme
A protein that catalyzes a specific chemical reaction.

enzyme-coupled receptor
Transmembrane protein that, when stimulated by the binding of a ligand, activates an intracellular enzyme (either a separate enzyme or part of the receptor itself).

epigenetic inheritance
The transmission of a heritable pattern of gene expression from one cell to its progeny that does not involve altering the nucleotide sequence of the DNA.

epithelium (plural epithelia)
Sheet of cells covering an external surface or lining an internal body cavity.

equilibrium
State in which the forward and reverse rates of a chemical reaction are equal so that no net chemical change occurs.

equilibrium constant (***K***)
For a reversible chemical reaction, the ratio of substrate to product when the rates of the forward and reverse reactions are equal. (*See* Table 3–1, p. 98.)

Escherichia coli (***E. coli***)
Rodlike bacterium normally found in the colon of humans and other mammals and widely used in biomedical research.

eubacteria
The proper term for the bacteria of common occurrence, used to distinguish them from archaea.

euchromatin
One of the two main states in which chromatin exists within an interphase cell. Prevalent in gene-rich areas, its less compact structure allows access for proteins involved in transcription. (*See also* **heterochromatin**.)

eukaryote
An organism whose cells have a distinct nucleus and cytoplasm.

evolution
Process of gradual modification and adaptation that occurs in living organisms over generations.

exocytosis
Process by which most molecules are secreted from a eukaryotic cell. These molecules are packaged in membrane-enclosed vesicles that fuse with the plasma membrane, releasing their contents to the outside.

exon
Segment of a eukaryotic gene that is transcribed into RNA and dictates the amino acid sequence of part of a protein.

exon shuffling
Mechanism for the evolution of new genes; in the process, coding sequences from different genes are brought together to generate a protein with a novel combination of domains.

extracellular matrix
Complex network of polysaccharides (such as glycosaminoglycans or cellulose) and proteins (such as collagen) secreted by cells. A structural component of tissues that also influences their development and physiology.

extracellular signal molecule
Any molecule present outside the cell that can elicit a response inside the cell when the molecule binds to a receptor.

FAD—*see* **$FADH_2$**

$FADH_2$ (**reduced flavin adenine dinucleotide**)
A high-energy electron carrier produced by reduction of FAD during the breakdown of molecules derived from food, including fatty acids and acetyl CoA.

fat
Type of lipid used by living cells to store metabolic energy. Mainly composed of triacylglycerols. (*See* Panel 2–4, pp. 72–73.)

fatty acid

Molecule that consists of a carboxylic acid attached to a long hydrocarbon chain. Used as a major source of energy during metabolism and as a starting point for the synthesis of phospholipids. (*See* Panel 2–4, pp. 72–73.)

feedback inhibition
A form of metabolic control in which the end product of a chain of enzymatic reactions reduces the activity of an enzyme early in the pathway.

fermentation
The breakdown of organic molecules without the involvement of molecular oxygen. This form of oxidation yields less energy than aerobic cell respiration.

fertilization
The fusion of two gametes—sperm and egg—to produce a new individual organism.

fibroblast
Cell type that produces the collagen-rich extracellular matrix in connective tissues such as skin and tendon. Proliferates readily in wounded tissue and in tissue culture.

fibronectin
Extracellular matrix protein that helps cells attach to the matrix by acting as a "linker" that binds to a cell-surface integrin molecule on one end and to a matrix component, such as collagen, on the other.

fibrous protein
A protein with an elongated, rodlike shape, such as collagen or a keratin filament.

filopodium (plural filopodia)
Long, thin, actin-containing extension on the surface of an animal cell. Sometimes has an exploratory function, as in a growth cone.

flagellum (plural flagella)
Long, whiplike structure capable of propelling a cell through a fluid medium with its rhythmic beating.

Eukaryotic flagella are longer versions of cilia; bacterial flagella are completely different, being smaller and simpler in construction.

fluorescence microscope
Instrument used to visualize a specimen that has been labeled with a fluorescent dye; samples are illuminated with a wavelength of light that excites the dye, causing it to fluoresce.

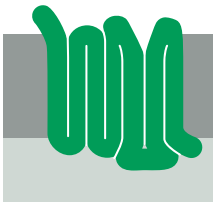
free energy (***G***)
Energy that can be harnessed to do work, such as driving a chemical reaction.

free-energy change (**Δ*G***)
"Delta *G*": in a chemical reaction, the difference in free energy between reactant and product molecules. A large negative value of Δ*G* indicates that the reaction has a strong tendency to occur. The standard free-energy change (Δ*G*°) is the free-energy change measured at defined concentration, temperature, and pressure.

***G*, Δ*G*, Δ*G*°**—*see* **free energy**, **free-energy change**

G protein
A membrane-bound GTP-binding protein involved in intracellular signaling; composed of three subunits, this intermediary is usually activated by the binding of a hormone or other ligand to a transmembrane receptor.

G-protein-coupled receptor (**GPCR**)
Cell-surface receptor that associates with an intracellular trimeric GTP-binding protein (G protein) after activation by an extracellular ligand. These receptors are embedded in the membrane by seven transmembrane α helices.



G_1 cyclin
Regulatory protein that helps drive a cell through the first gap phase of the cell cycle and toward S phase.

G_1 phase
Gap 1 phase of the eukaryotic cell cycle; falls between the end of cytokinesis and the start of DNA synthesis.

G_1-Cdk
Protein complex whose activity drives the cell through the first gap phase of the cell cycle; consists of a G_1 cyclin plus a cyclin-dependent protein kinase (Cdk).

G_1/S cyclin
Regulatory protein that helps to launch the S phase of the cell cycle.

G_1/S-Cdk
Protein complex whose activity triggers entry into S phase of the cell cycle; consists of a G_1/S cyclin plus a cyclin-dependent protein kinase (Cdk).

G_2 phase
Gap 2 phase of the eukaryotic cell cycle; falls between the end of DNA synthesis and the beginning of mitosis.

gain-of-function mutation
Genetic change that increases the activity of a gene or makes it active in inappropriate circumstances; such mutations are usually dominant.

gamete
Cell type in a diploid organism that carries only one set of chromosomes and is specialized for sexual reproduction. A sperm or an egg; also called germ cell.

gap junction
In animal tissues, specialized connection between juxtaposed cells through which ions and small molecules can pass from one cell to the other.

GDP (**guanosine 5′-diphosphate**)
Nucleotide that is produced by the hydrolysis of the terminal phosphate of GTP, a reaction that also produces inorganic phosphate.

gene
Unit of heredity containing the instructions that dictate the characteristics or phenotype of an organism; in molecular terms, a segment of DNA that directs the production of a protein or functional RNA molecule.

gene duplication and divergence
A process by which new genes can form; involves the accidental generation of an additional copy of a stretch of DNA containing one or more genes, followed by an accumulation of mutations that over time can alter the function of either the original or its copy.

gene expression
The process by which a gene makes a product that is useful to the cell or organism by directing the synthesis of a protein or an RNA molecule with a characteristic activity.

gene family
A set of related genes that has arisen through a process of gene duplication and divergence.

gene knockout
A genetically engineered animal in which a specific gene has been inactivated.

gene replacement
Technique that substitutes a mutant form of a gene for its normal counterpart to investigate the gene's function.

general transcription factors
Proteins that assemble on the promoters of many eukaryotic genes near the start site of transcription and load the RNA polymerase in the correct position.

genetic code
Set of rules by which the information contained in the nucleotide sequence of a gene and its corresponding RNA molecule is translated into the amino acid sequence in a protein.

genetic engineering—*see* **recombinant DNA technology**

genetic instability
An increased rate of mutation often caused by defects

in the systems that govern the accurate replication and maintenance of the genome; the resulting mutations sometimes drive the evolution of cancer.

genetic map
A graphic representation of the order of genes in chromosomes spaced according to the amount of recombination that occurs between them.

genetic screen
Experimental technique used to search through a collection of mutants for a particular phenotype.

genetics
The study of genes, heredity, and the variation that gives rise to differences between one living organism and another.

genome
The total genetic information carried by all the chromosomes of a cell or organism.

genomic DNA library
Collection of cloned DNA molecules that represents the entire genome of a cell.

genotype
The genetic makeup of a cell or organism, including which alleles (gene variants) it carries.

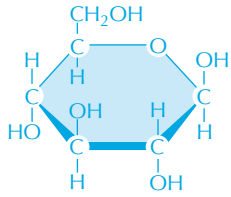
germ cell
Cell type in a diploid organism that carries only one set of chromosomes and is specialized for sexual reproduction. A sperm or an egg; also called gamete.

germ line
The lineage of reproductive cells that contributes to the formation of a new generation of organisms, as distinct from somatic cells, which form the body and leave no descendants in the next generation.

globular protein
Any protein in which the polypeptide chain folds into a compact, rounded shape. Includes most enzymes.

gluconeogenesis
Set of enzyme-catalyzed reactions by which glucose is synthesized from small organic molecules such as pyruvate, lactate, or amino acids; in effect, the reverse of glycolysis.

glucose
Six-carbon sugar that plays a major role in the metabolism of living cells. Stored in polymeric form as glycogen in animal cells and as starch in plant cells. (*See* Panel 2–3, pp. 70–71.)



glycocalyx
Protective layer of carbohydrates on the outside surface of the plasma membrane formed by the sugar residues of membrane glycoproteins, proteoglycans, and glycolipids.

glycogen
Branched polymer composed exclusively of glucose units used to store energy in animal cells. Granules of this material are especially abundant in liver and muscle cells.

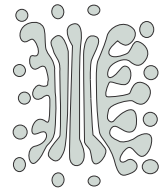
glycolipid
Membrane lipid molecule that has a short carbohydrate chain attached to its hydrophilic head.

glycolysis
Series of enzyme-catalyzed reactions in which sugars are partially degraded and their energy captured by the activated carriers ATP and NADH. (Literally, "sugar splitting.")

glycoprotein
Any protein with one or more covalently linked oligosaccharide chains. Includes most secreted proteins and most proteins exposed on the outer surface of the plasma membrane.

glycosaminoglycan (GAG)
Polysaccharide chain that can form a gel that acts as a "space filler" in the extracellular matrix of connective tissues; helps animal tissues resist compression.

Golgi apparatus
Membrane-enclosed organelle in eukaryotic cells that modifies the proteins and lipids made in the endoplasmic reticulum and sorts them for transport to other sites.



green fluorescent protein (GFP)
Fluorescent protein, isolated from a jellyfish, that is used experimentally as a marker for monitoring the location and movement of proteins in living cells.

group—*see* chemical group

growth factor
Extracellular signaling molecule that stimulates a cell to increase in size and mass. Examples include epidermal growth factor (EGF) and platelet-derived growth factor (PDGF).

GTP (guanosine 5′-triphosphate)
Nucleoside triphosphate used in the synthesis of RNA and DNA. Like the closely related ATP, serves as an activated carrier in some energy-transfer reactions. Also has a special role in microtubule assembly, protein synthesis, and cell signaling.

GTP-binding protein
Intracellular signaling protein whose activity is determined by its association with either GTP or GDP. Includes both trimeric G proteins and monomeric GTPases, such as Ras.

haploid
Describes a cell or organism with only one set of chromosomes, such as a sperm cell or a bacterium. (*See also* **diploid**.)

haplotype block
A combination of alleles or other DNA markers that has been inherited as a unit, undisturbed by genetic recombination, across many generations.

helix
An elongated structure whose subunits twist in a regular fashion around a central axis, like a spiral staircase.

hemidesmosome
Structure that anchors epithelial cells to the basal lamina beneath them.

heredity
The genetic transmission of traits from parents to offspring.

heterochromatin
Highly condensed region of an interphase chromosome; generally gene-poor and transcriptionally inactive. (*See also* **euchromatin**.)

heterozygous
Possessing dissimilar alleles for a given gene.

high-energy bond
Covalent bond whose hydrolysis releases an unusually large amount of free energy under the conditions existing in a cell. Examples include the phosphodiester bonds in ATP and the thioester linkage in acetyl CoA.

histone
One of a group of abundant highly conserved proteins around which DNA wraps to form nucleosomes, structures that represent the most fundamental level of chromatin packing.

histone deacetylase
Enzyme that removes acetyl groups from lysines present in histones; its action often allows chromatin to pack more tightly.

homolog
A gene, chromosome, or any structure that has a close similarity to another as a result of common ancestry. (*See also* **homologous chromosome**.)

homologous
Describes genes, chromosomes, or any structures that are similar because of their common evolutionary origin. Can also refer to similarities between protein sequences or nucleic acid sequences.

homologous chromosome
In a diploid cell, one of the two copies of a particular chromosome, one of which comes from the father and the other from the mother.

homologous gene—*see* homologous

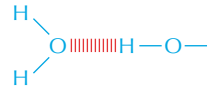
homologous recombination
Mechanism by which double-strand breaks in a DNA molecule can be repaired flawlessly; uses an undamaged, duplicated, or homologous chromosome to guide the repair. During meiosis, the mechanism results in an exchange of genetic information between the maternal and paternal homologs.

homozygous
Possessing identical alleles for a given gene.

horizontal gene transfer
Process by which DNA is passed from the genome of one organism to that of another, even to an individual from another species. This contrasts with "vertical" gene transfer, which refers to the transfer of genetic information from parent to progeny.

hormone
Extracellular signal molecule that is secreted and transported via the bloodstream (in animals) or the sap (in plants) to target tissues on which it exerts a specific effect.

hybridization
Experimental technique in which two complementary nucleic acid strands come together and form hydrogen bonds to produce a double helix; used to detect specific nucleotide sequences in either DNA or RNA.

hydrogen bond


A weak noncovalent interaction between a positively charged hydrogen atom in one molecule and a negatively charged atom, such as nitrogen or oxygen, in another; these interactions are key to the structure and properties of water.

hydrogen ion
Positively charged ion generated by the removal of an electron from a hydrogen atom; often used to refer to a proton (H^+) in aqueous solution. Its presence is the basis of acidity. (*See* Panel 2–2, pp. 68–69.)

hydrolysis
Chemical reaction that involves cleavage of a covalent bond with the accompanying consumption of water (its –H being added to one product of the cleavage and its –OH to the other); the reverse of condensation.

hydronium ion (**H_3O^+**)
The form taken by a proton (H^+) in aqueous solution.

hydrophilic
Molecule or part of a molecule that readily forms hydrogen bonds with water, allowing it to dissolve; literally, "water loving."

hydrophobic
Nonpolar, uncharged molecule or part of a molecule that forms few or no hydrogen bonds with water molecules and therefore does not dissolve; literally, "water fearing."

hydrophobic interaction
Type of noncovalent bond that forces together the hydrophobic portions of dissolved molecules to minimize their disruption of the hydrogen-bonded network of water; helps push together membrane phospholipids and fold proteins into a compact, globular shape.

hydroxyl (**–OH**)
Chemical group consisting of a hydrogen atom linked to an oxygen, as in an alcohol. (*See* Panel 2–1, pp. 66–67.)

***in situ* hybridization**
Technique in which a single-stranded RNA or DNA probe is used to locate a complementary nucleotide sequence in a chromosome, cell, or tissue; used to diagnose genetic disorders or to track gene expression.

in vitro
Term used by biochemists to describe a process that takes place in an isolated cell-free extract. Also used by cell biologists to refer to cells growing in culture, as opposed to in an organism.

in vivo
In an intact cell or organism. (Latin for "in life.")

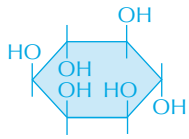
induced pluripotent stem cell (**iPS cell**)
Somatic cell that has been reprogrammed to resemble and behave like a pluripotent embryonic stem (ES) cell through the artificial introduction of a set of genes encoding particular transcription regulators.

initiation factor
Protein that promotes the proper association of ribosomes with mRNA and is required for the initiation of protein synthesis.

initiator tRNA
Special tRNA that initiates the translation of an mRNA in a ribosome. It always carries the amino acid methionine.

inorganic
Not composed of carbon and hydrogen.

inositol
Sugar molecule with six hydroxyl groups that forms the structural basis for inositol phospholipids, which can act as membrane-bound signaling molecules.



inositol 1,4,5-trisphosphate (IP_3)
Small intracellular signaling molecule that triggers the release of Ca^{2+} from the endoplasmic reticulum into the cytosol; produced when a signal molecule activates a membrane-bound protein called phospholipase C.

inositol phospholipid
Minor lipid component of plasma membranes that plays a part in signal transduction in eukaryotic cells; cleavage yields two small messenger molecules, IP_3 and diacylglycerol.

integrin
Family of transmembrane proteins present on cell surfaces that enable cells to make and break attachments to the extracellular matrix, allowing them to crawl through a tissue.

intermediate filament
Fibrous cytoskeletal element, about 10 nm in diameter, that forms ropelike networks in animal cells; helps cells resist tension applied from outside.

interphase
Long period of the cell cycle between one mitosis and the next. Includes G_1 phase, S phase, and G_2 phase.

interphase chromosome
State in which a eukaryotic chromosome exists when the cell is between divisions; more extended and transcriptionally active than mitotic chromosomes.

intracellular signaling molecule
Molecule that is part of the mechanism for transducing and transmitting signals inside a cell.

intracellular signaling pathway
A set of proteins and small-molecule second messengers that interact with each other to relay a signal from the cell membrane to its final destination in the cytoplasm or nucleus.

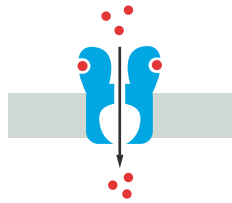
intrinsically disordered sequence
Region in a polypeptide chain that lacks a definite structure.

intron
Noncoding sequence within a eukaryotic gene that is transcribed into an RNA molecule but is then excised by RNA splicing to produce an mRNA.

ion
An atom carrying an electrical charge, either positive or negative.

ion channel
Transmembrane protein that forms a pore across the lipid bilayer through which specific inorganic ions can diffuse down their electrochemical gradients.

ion-channel-coupled receptor
Transmembrane receptor protein or protein complex that opens in response to the binding of a ligand to its external face, allowing the passage of a specific inorganic ion.



ionic bond
Interaction formed when one atom donates electrons to another; this transfer of electrons causes both atoms to become electrically charged.

iron–sulfur center
Metal complex found in electron carriers that operate early in the electron-transport chain; has a relatively weak affinity for electrons.

isomer (stereoisomer)
One of two or more substances that contains the same atoms and has the same molecular formula (such as $C_6H_{12}O_6$) as the other, but differs from the other in the spatial arrangement of these atoms. Optical isomers are mirror images of each other.

isotope
A variant of an element that has the same number of protons but a different atomic weight. Some are radioactive.

K^+
Potassium ion—the most abundant positively charged ion in living cells.

K^+ leak channel
Ion channel permeable to K^+ that randomly flickers between an open and closed state; largely responsible for the resting membrane potential in animal cells.

karyotype
An ordered display of the full set of chromosomes of a cell arranged with respect to size, shape, and number.

keratin filament
Class of intermediate filament abundant in epithelial cells, where it provides tensile strength; main structural component of hair, feathers, and claws.

kilocalorie (kcal)
Unit of heat equal to 1000 calories. Often used to express the energy content of food or molecules: bond strengths, for example, are measured in kcal/mole. An alternative unit in wide use is the kilojoule.

kilojoule (kJ)
Standard unit of energy equal to 0.239 kilocalories.

kinase—*see* protein kinase

kinesin
A large family of motor proteins that uses the energy

of ATP hydrolysis to move toward the plus end of a microtubule.

kinetochore
Protein complex that assembles on the centromere of a condensed mitotic chromosome; the site to which spindle microtubules attach.

K_M
The concentration of substrate at which an enzyme works at half its maximum rate. Large values of K_M usually indicate that the enzyme binds to its substrate with relatively low affinity.

knockout mouse
Genetically engineered mouse in which a specific gene has been inactivated, for example, by introducing a deletion in its DNA.

***L1* element**
Type of retrotransposon that constitutes 15% of the human genome; also called *LINE-1*.

lagging strand
At a replication fork, the DNA strand that is made discontinuously in short separate fragments that are later joined together to form one continuous new strand.

lamellipodium (plural lamellipodia)
Dynamic sheetlike extension on the surface of an animal cell, especially one migrating over a surface.

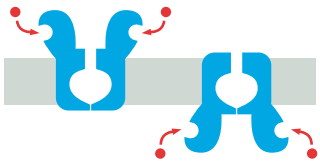
law of independent assortment
Principle that, during gamete formation, the alleles for different traits segregate independently of one another; Mendel's second law of inheritance.

law of segregation
Principle that the maternal and paternal alleles for a trait separate from one another during gamete formation and then reunite during fertilization; Mendel's first law of inheritance.

leading strand
At a replication fork, the DNA strand that is made by continuous synthesis in the 5′-to-3′ direction.

ligand
General term for a molecule that binds to a specific site on a protein.

ligand-gated channel
An ion channel that is stimulated to open by the binding of a small molecule such as a neurotransmitter.

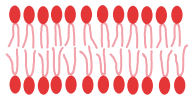


ligase
Enzyme that reseals nicks that arise in the backbone of a DNA molecule; in the laboratory, can be used to join together two DNA fragments.

light reactions
In photosynthesis, the set of reactions that converts the energy of sunlight into chemical energy in the form of ATP and NADPH.

lipid
Organic molecule that is insoluble in water but dissolves readily in nonpolar organic solvents; typically contains long hydrocarbon chains or multiple rings. One class, the phospholipids, forms the structural basis of biological membranes.

lipid bilayer
Thin pair of closely juxtaposed sheets, composed mainly of phospholipid molecules, that forms the structural basis for all cell membranes.



local mediator
Secreted signal molecule that acts at a short range on adjacent cells.

long noncoding RNA
Class of RNA molecules more than 200 nucleotides in length that does not encode proteins.

loss-of-function mutation
A genetic alteration that reduces or eliminates the activity of a gene. Such mutations are usually recessive: the organism can function normally as long as it retains at least one normal copy of the affected gene.

lumen
The space inside a hollow or tubular structure; can refer to the cavity in a tissue or within an organelle.

lymphocyte
White blood cell that mediates the immune response to foreign molecules (antigens). Can be an antibody-secreting B cell type or the T cell type that recognizes and ultimately eliminates infected cells.

lysosome
Membrane-enclosed organelle that breaks down worn-out proteins and organelles and other waste materials, as well as molecules taken up by endocytosis; contains digestive enzymes that are typically most active at the acid pH found inside these organelles.

lysozyme
Enzyme that severs the polysaccharide chains that form the cell walls of bacteria; found in many secretions including saliva and tears.

M cyclin
Regulatory protein that binds to mitotic Cdk to form M-Cdk, the protein complex that triggers the M phase of the cell cycle.

M phase
Period of the eukaryotic cell cycle during which the nucleus and cytoplasm divide.

M-Cdk
Protein complex that triggers the M phase of the cell cycle; consists of an M cyclin plus a mitotic cyclin-dependent protein kinase (Cdk).

macromolecule
Polymer built from covalently linked subunits; includes proteins, nucleic acids, and polysaccharides with a molecular mass greater than a few thousand daltons.

macrophage
Cell found in animal tissues that defends against infections by ingesting invading microbes by a process of phagocytosis; derived from a type of white blood cell.

MAP kinase
Mitogen-activated protein kinase. Signaling molecule that is the final kinase in a three-kinase sequence called the MAP-kinase signaling module.

MAP-kinase signaling module
Set of three functionally interlinked protein kinases that allows cells to respond to extracellular signal molecules that stimulate proliferation; includes a mitogen-activated protein kinase (MAP kinase), a MAP kinase kinase, and a MAP kinase kinase kinase.

mass spectrometry
Technique for determining the exact mass of every peptide present in a sample of purified protein or protein mixture.

matrix
Large internal compartment within a mitochondrion.

mechanically gated channel
An ion channel that allows the passage of select ions across a membrane in response to a physical perturbation.

meiosis
Specialized type of cell division by which eggs and sperm cells are made. Two successive nuclear divisions with only one round of DNA replication generate four haploid cells from an initial diploid cell.

membrane
Thin sheet of lipid molecules and associated proteins that encloses all cells and forms the boundaries of many eukaryotic organelles.

membrane domain
Functionally and structurally specialized region in the membrane of a cell or organelle; typically characterized by the presence of specific proteins.

membrane potential
Voltage difference across a membrane due to a slight excess of positive ions on one side and of negative ions on the other.

membrane protein
A protein associated with the lipid bilayer of a cell membrane.

membrane transport protein
Any transmembrane protein that provides a passageway for the movement of select substances across a cell membrane.

membrane-enclosed organelle
Any organelle in a eukaryotic cell that is surrounded by a lipid bilayer, for example, the endoplasmic reticulum, Golgi apparatus, and lysosome.

membrane-enclosed organelle
Any organelle in the eukaryotic cell that is surrounded by a lipid bilayer; for example, the endoplasmic reticulum, Golgi apparatus, and lysosome.

messenger RNA (mRNA)
RNA molecule that specifies the amino acid sequence of a protein.

metabolic pathway
Interconnected sequence of enzymatic reactions in which the product of one reaction is the substrate of the next.

metabolism
The sum total of the chemical reactions that take place in the cells of a living organism.

metaphase
Stage of mitosis in which chromosomes are firmly attached to the mitotic spindle at its equator but have not yet segregated toward opposite poles.

metastasis
The spread of cancer cells from the initial site of the tumor to form secondary tumors at other sites in the body.

methyl ($-CH_3$) group
Hydrophobic chemical group derived from methane (CH_4). (*See* Panel 2–1, pp. 66–67.)

Michaelis constant (K_M)
Concentration of substrate at which an enzyme works at half its maximum velocity; serves as a measure of how tightly the substrate is bound.

micro-
In the metric system, prefix denoting 10^{-6}.

micrograph
Any photograph or digital image taken through a microscope. Can be a light micrograph or an electron micrograph, depending on the type of microscope used.

micrometer
Unit of length equal to one millionth (10^{-6}) of a meter or 10^{-4} centimeter.

microRNA (miRNA)
Small noncoding RNA that controls gene expression by base-pairing with a specific mRNA to regulate its stability and its translation.

microscope
Instrument for viewing extremely small objects. A light microscope utilizes a focused beam of visible light and is used to examine cells and organelles. An electron microscope utilizes a beam of electrons and can be used to examine objects as small as individual molecules.

microtubule

Long, stiff, cylindrical structure composed of the protein tubulin. Used by eukaryotic cells to organize their cytoplasm and guide the intracellular transport of macromolecules and organelles.

microtubule-associated protein
Accessory protein that binds to microtubules; can stabilize microtubule filaments, link them to other cell structures, or transport various components along their length.

milli-
In the metric system, prefix denoting 10^{-3}.

mismatch repair
Mechanism for recognizing and correcting incorrectly paired nucleotides—those that are noncomplementary.

mitochondrion (plural mitochondria)
Membrane-enclosed organelle, about the size of a

bacterium, that carries out oxidative phosphorylation and produces most of the ATP in eukaryotic cells.

mitogen
An extracellular signal molecule that stimulates cell proliferation.

mitosis
Division of the nucleus of a eukaryotic cell.

mitotic chromosome
Highly condensed duplicated chromosome in which the two new chromosomes (also called sister chromatids) are still held together at the centromere. The structure chromosomes adopt during mitosis.

mitotic spindle
Array of microtubules and associated molecules that forms between the opposite poles of a eukaryotic cell during mitosis and pulls duplicated chromosome sets apart.

mobile genetic element
Short segment of DNA that can move, sometimes through an RNA intermediate, from one location in a genome to another; an important source of genetic variation in most genomes. Also called a transposon.

model organism
A living thing selected for intensive study as a representative of a large group of species. Examples include the mouse (representing mammals), the yeast *Saccharomyces cerevisiae* (representing a unicellular eukaryote), and *Escherichia coli* (representing bacteria).

mole
The amount of a substance, in grams, that is equal to its molecular weight; this quantity will contain 6×10^{23} molecules of the substance.

molecular mass
The weight of a molecule expressed in daltons, the atomic mass unit that closely approximates the mass of a hydrogen atom.

molecular switch
Intracellular signaling protein that toggles between an active and inactive state in response to receiving a signal.

molecular weight
Sum of the atomic weights of the atoms in a molecule; as a ratio of molecular masses, it is a number without units.

molecule
Group of atoms joined together by covalent bonds.

monomer
Small molecule that can be linked to others of a similar type to form a larger molecule (polymer).

monomeric GTPase
Small, single-subunit GTP-binding protein. Proteins of this family, such as Ras and Rho, are part of many different signaling pathways.

motor protein
Protein such as myosin or kinesin that uses energy derived from ATP hydrolysis to propel itself along a protein filament or polymeric molecule.

mutation
A randomly produced, permanent change in the nucleotide sequence of DNA.

myofibril
Long, cylindrical structure that constitutes the contractile element of a muscle cell; constructed of arrays of highly organized bundles of actin, myosin, and other accessory proteins.

myosin
Type of motor protein that uses ATP to drive movements along actin filaments. One subtype interacts with actin to form the thick contractile bundles of skeletal muscle.

myosin filament
Polymer composed of interacting molecules of myosin-II; interaction with actin promotes contraction in muscle and nonmuscle cells.

myosin-I
Simplest type of myosin, present in all cells; consists of a single actin-binding head and a tail that can attach to other molecules or organelles.

myosin-II
Type of myosin that exists as a dimer with two actin-binding heads and a coiled-coil tail; can associate to form long myosin filaments.

N-terminus (**amino terminus**)
The end of a polypeptide chain that carries a free α-amino group.

Na^+
Sodium ion—a positively charged ion that is a major constituent of living cells.

Na^+ pump (**sodium pump**)
Transporter found in the plasma membrane of most animal cells that actively pumps Na^+ out of the cell and K^+ in using the energy derived from ATP hydrolysis.

NAD^+ (**nicotine adenine dinucleotide**)
Activated carrier that accepts a hydride ion (H^-) from a donor molecule, thereby producing NADH. Widely used in the energy-producing breakdown of sugar molecules. (*See* Figure 3–34.)

NADH
Activated carrier widely used in the energy-producing breakdown of sugar molecules. (*See* Figure 3–34.)

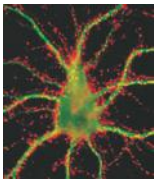
NADPH (**nicotine adenine dinucleotide phosphate**)
Activated carrier closely related to NADH and used as an electron donor in biosynthetic pathways. In the process it is oxidized to $NADP^+$. (*See* Figure 3–35.)

nanometer
Unit of length that represents 10^{-9} (one billionth of a) meter; commonly used to measure molecules and organelles.

Nernst equation
An equation that relates the concentrations of an inorganic ion on the two sides of a permeable membrane to the membrane potential at which there would be no net movement of the ion across the membrane.

nerve terminal
Structure at the end of an axon that signals to another neuron or target cell.

neuron
An electrically excitable cell that integrates and transmits information as part of the nervous system; a nerve cell.



neurotransmitter
Small signaling molecule secreted by a nerve cell at a synapse to transmit information to a postsynaptic cell. Examples include acetylcholine, glutamate, GABA, and glycine.

nitric oxide (NO)
Locally acting gaseous signal molecule that diffuses across cell membranes to affect the activity of intracellular proteins.

nitrogen fixation
Conversion of nitrogen gas from the atmosphere into nitrogen-containing molecules by soil bacteria and cyanobacteria.

noncovalent bond
Chemical association that does not involve the sharing of electrons; singly are relatively weak, but can sum together to produce strong, highly specific interactions between molecules. Examples are hydrogen bonds and van der Waals attractions.

nonhomologous end joining
A quick-and-dirty mechanism for repairing double-strand breaks in DNA that involves quickly bringing together, trimming, and rejoining the two broken ends; results in a loss of information at the site of repair.

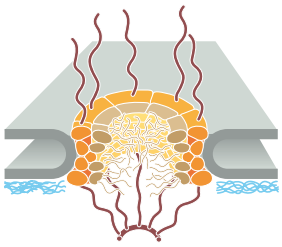
nonpolar
Describes a molecule that lacks a local accumulation of positive or negative charge; generally insoluble in water.

nuclear envelope
Double membrane surrounding the nucleus. Consists of outer and inner membranes, perforated by nuclear pores.

nuclear lamina
Fibrous layer on the inner surface of the inner nuclear membrane formed as a network of intermediate filaments made from nuclear lamins.

nuclear magnetic resonance (NMR) spectroscopy
Technique used for determining the three-dimensional structure of a protein in solution.

nuclear pore
Channel through which selected large molecules move between the nucleus and the cytoplasm.



nuclear receptor
Protein inside a eukaryotic cell that, on binding to a signal molecule, enters the nucleus and regulates transcription.

nucleic acid
Macromolecule that consists of a chain of nucleotides joined together by phosphodiester bonds; RNA or DNA.

nucleolus
Large structure within the nucleus where ribosomal RNA is transcribed and ribosomal subunits are assembled.

nucleoside
Molecule made of a nitrogen-containing ring compound attached to a sugar, either ribose (in RNA) or deoxyribose (in DNA).

nucleosome
Beadlike structural unit of a eukaryotic chromosome composed of a short length of DNA wrapped around a core of histone proteins; includes a nucleosomal core particle (DNA plus histone protein) along with a segment of linker DNA that ties the core particles together.

nucleotide
Basic building block of the nucleic acids, DNA and RNA; includes a nucleoside with a series of one or more phosphate groups linked to its sugar.

nucleus
In biology, refers to the prominent, rounded structure that contains the DNA of a eukaryotic cell. In chemistry, refers to the dense, positively charged center of an atom.

Okazaki fragment
Short length of DNA produced on the lagging strand during DNA replication. Adjacent fragments are rapidly joined together by DNA ligase to form a continuous DNA strand.

oligo-
Prefix that denotes a short polymer (oligomer). May be made of amino acids (oligopeptide), sugars (oligosaccharide), or nucleotides (oligonucleotide).

oncogene
A gene that, when activated, can potentially make a cell cancerous. Typically a mutant form of a normal gene (proto-oncogene) involved in the control of cell growth or division.

open reading frame (ORF)
Long sequence of nucleotides that contains no stop codon; used to identify potential protein-coding sequences in DNA.

optogenetics
Technique that uses light to control the activity of neurons into which light-gated ion channels have been artificially introduced.

organelle
A discrete structure or subcompartment of a eukaryotic cell that is specialized to carry out a particular function. Examples include mitochondria and the Golgi apparatus.

organic chemistry
The branch of chemistry concerned with compounds made of carbon. Includes essentially all of the molecules from which living cells are made, apart from water and metal ions such as Na^+.

organic molecule
Chemical compound that contains carbon and hydrogen.

origin recognition complex (ORC)
Assembly of proteins that is bound to the DNA at origins of replication in eukaryotic chromosomes throughout the cell cycle.

osmosis
Passive movement of water across a cell membrane from a region where the concentration of water is high (because the concentration of solutes is low) to a region where the concentration of water is low (and the concentration of solutes is high).

oxidation
Removal of electrons from an atom, as occurs during the addition of oxygen to a carbon atom or when a hydrogen is removed from a carbon atom. The opposite of reduction. (*See* Figure 3–11.)

oxidative phosphorylation
Process in bacteria and mitochondria in which ATP formation is driven by the transfer of electrons from food molecules to molecular oxygen.

p53
Transcription regulator that controls the cell's response to DNA damage, preventing the cell from entering S phase until the damage has been repaired or inducing the cell to commit suicide if the damage is too extensive; mutations in the gene encoding this protein are found in many human cancers.

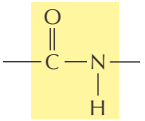
pairing
In meiosis, the process by which a pair of duplicated homologous chromosomes attach to one another to form a structure containing four sister chromatids.

passive transport
The spontaneous movement of a solute down its concentration gradient across a cell membrane via a membrane transport protein, such as a channel or a transporter.

patch-clamp recording
Technique used to monitor the activity of ion channels in a membrane; involves the formation of a tight seal between the tip of a glass electrode and a small region of cell membrane, and manipulation of the membrane potential by varying the concentrations of ions in the electrode.

pedigree
Chart showing the line of descent, or ancestry, of an individual organism.

peptide bond
Chemical bond between the carbonyl group of one amino acid and the amino group of a second amino acid. (*See* Panel 2–5, pp. 74–75.)



peroxisome
Small membrane-enclosed organelle that contains enzymes that degrade lipids and destroy toxins.

pH scale
Concentration of hydrogen ions in a solution, expressed as a logarithm. Thus, an acidic solution with pH 3 will contain 10^{-3} M hydrogen ions.

phagocytic cell
A cell such as a macrophage or neutrophil that is specialized to take up particles and microorganisms by phagocytosis.

phagocytosis
The process by which particulate material is engulfed ("eaten") by a cell. Prominent in predatory cells, such as *Amoeba proteus*, and in cells of the vertebrate immune system such as macrophages.

phenotype
The observable characteristics of a cell or organism.

phosphatidylcholine
Common phospholipid present in abundance in most cell membranes; uses choline attached to a phosphate as its head group.

phosphodiester bond
Strong covalent bond that forms the backbone of DNA and RNA molecules; links the 3′ carbon of one sugar to the 5′ carbon of another. (*See* Figure 2–26.)

phosphoinositide 3-kinase (PI 3-kinase)
Enzyme that phosphorylates inositol phospholipids in the plasma membrane, which generates docking sites for intracellular signaling proteins that promote cell growth and survival.

phospholipase C
Enzyme associated with the plasma membrane that generates two small messenger molecules in response to activation.

phospholipid
A major type of lipid molecule in many cell membranes. Generally composed of two fatty acid tails linked to one of a variety of phosphate-containing polar groups.

phosphorylation—*see* protein phosphorylation

photosynthesis
The process by which plants, algae, and some bacteria use the energy of sunlight to drive the synthesis of organic molecules from carbon dioxide and water.

photosystem
Large multiprotein complex containing chlorophyll that captures light energy and converts it into chemical energy; consists of a set of antenna complexes and a reaction center.

phragmoplast
In a dividing plant cell, structure made of microtubules and membrane vesicles that guides the formation of a new cell wall.

phylogenetic tree
Diagram or "family tree" showing the evolutionary history of a group of organisms or proteins.

pinocytosis
Type of endocytosis in which soluble materials are taken up from the environment and incorporated into vesicles for digestion. (Literally, "cell drinking.")

plasma membrane
The protein-containing lipid bilayer that surrounds a living cell.

plasmid
Small circular DNA molecule that replicates

independently of the genome. Used extensively as a vector for DNA cloning.

plasmodesma (plural plasmodesmata)
Cell–cell junction that connects one plant cell to the next; consists of a channel of cytoplasm lined by membrane.

pluripotent
Capable of giving rise to any type of cell or tissue.

pluripotent stem cell
Cell capable of giving rise to any of the specialized cell types in the body.

point mutation
Change in a single nucleotide pair in a DNA sequence.

polar
In chemistry, describes a molecule or bond in which electrons are distributed unevenly.

polarity
An inherent asymmetry that allows one end of an object to be distinguished from another; can refer to a molecule, a polymer (such as an actin filament), or even a cell (for example, an epithelial cell that lines the mammalian small intestine).

polyadenylation
The addition of multiple adenine nucleotides to the 3′ end of a newly synthesized mRNA molecule.

polymer
Long molecule made by covalently linking multiple identical or similar subunits (monomers).

polymerase
General term for an enzyme that catalyzes addition of subunits to a nucleic acid polymer. DNA polymerase, for example, makes DNA, and RNA polymerase makes RNA.

polymerase chain reaction (**PCR**)
Technique for amplifying selected regions of DNA by multiple cycles of DNA synthesis; can produce billions of copies of a given sequence in a matter of hours.

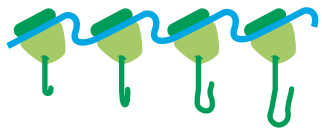
polymorphism
DNA sequence for which two or more variants are present at high frequency in the general population.

polynucleotide
A molecular chain of nucleotides chemically linked by a series of phosphodiester bonds. A strand of RNA or DNA.

polypeptide backbone
Repeating sequence of atoms (–N–C–C–) that forms the core of a protein molecule and to which the amino acid side chains are attached.

polypeptide; polypeptide chain
Linear polymer composed of multiple amino acids. Proteins are composed of one or more long polypeptide chains.

polyribosome
Messenger RNA molecule to which multiple ribosomes are attached and engaged in protein synthesis.



polysaccharide
Linear or branched polymer composed of sugars. Examples are glycogen, hyaluronic acid, and cellulose.

positive feedback loop
An important form of regulation in which the end product of a reaction or pathway stimulates continued activity; controls a variety of biological processes, including enzyme activity, cell signaling, and gene expression.

post-transcriptional control
Regulation of gene expression that occurs after transcription of the gene has begun; examples include RNA splicing and RNA interference.

primary structure
The amino acid sequence of a protein.

primary transcript—*see* **transcription**

primase
An RNA polymerase that uses DNA as a template to produce an RNA fragment that serves as a primer for DNA synthesis.

primer
In DNA replication, a short length of RNA made at the beginning of the synthesis of each DNA fragment; these RNA segments are subsequently removed and filled in with DNA.

processive
Describes an ability to catalyze consecutive reactions or undergo multiple conformational changes without releasing a substrate. Examples include replication by DNA polymerase or the movement of motor proteins involved in transport, such as kinesin.

programmed cell death
A tightly controlled form of cell suicide that allows cells that are unneeded or unwanted to be eliminated from an adult or developing organism; also called apoptosis.

prokaryote
Major category of living cells distinguished by the absence of a nucleus. Prokaryotes include the archaea and the eubacteria (commonly called bacteria).

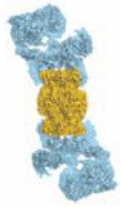
prometaphase
Stage of mitosis in which the nuclear envelope breaks down and duplicated chromosomes are captured by the spindle microtubules; precedes metaphase.

promoter
DNA sequence that initiates gene transcription; includes sequences recognized by RNA polymerase.

proofreading
The process by which DNA polymerase corrects its own errors as it moves along DNA.

prophase
First stage of mitosis, during which the duplicated chromosomes condense and the mitotic spindle forms.

protease
Enzyme that degrades proteins by hydrolyzing their peptide bonds.

proteasome

Large protein machine that degrades proteins that are damaged, misfolded, or no longer needed by the cell; its target proteins are marked for destruction primarily by the attachment of a short chain of ubiquitin.

protein
Polymer built from amino acids that provides cells with their shape and structure and performs most of their activities.

protein complex—*see* **complex**

protein domain
Segment of a polypeptide chain that can fold into a compact stable structure and that usually carries out a specific function.

protein family
A group of polypeptides that shares a similar amino acid sequence or three-dimensional structure, reflecting a common evolutionary origin. Individual members often have related but distinct functions, such as kinases that phosphorylate different target proteins.

protein kinase
Enzyme that catalyzes the transfer of a phosphate group from ATP to a specific amino acid side chain on a target protein.

protein kinase C (**PKC**)
Enzyme that phosphorylates target proteins in response to a rise in diacylglycerol and Ca^{2+} ions.

protein machine
Large assembly of protein molecules that operates as a unit to perform a complex series of biological activities, such as replicating DNA.

protein phosphatase
Enzyme that catalyzes the removal of a phosphate group from a protein, often with high specificity for the phosphorylated site.

protein phosphorylation
The covalent addition of a phosphate group to a side chain of a protein, catalyzed by a protein kinase; serves as a form of regulation that usually alters the activity or properties of the target protein.

proteoglycan
Molecule consisting of one or more glycosaminoglycan chains attached to a core protein; these aggregates can form gels that regulate the passage of molecules through the extracellular medium and guide cell migration.

proteolysis
Degradation of a protein by means of a protease.

proteomics
The large-scale study of the structure and function of proteins.

proto-oncogene
Gene that when mutated or overexpressed can transform a normal cell into a cancerous one.

proton
Positively charged particle found in the nucleus of every atom; also, another name for a hydrogen ion (H^+).

proton (H^+) **pump**
A transporter that actively moves H^+ across a cell membrane, thereby generating a gradient that can be used by the cell, for example, to import other solutes.

protozoan (plural protozoa)
A free-living, nonphotosynthetic, single-celled, motile eukaryote.

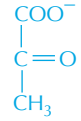
pump
Transporter that uses a source of energy, such as ATP hydrolysis or sunlight, to actively move a solute across a membrane against its electrochemical gradient.

purifying selection
Preservation of a specific nucleotide sequence driven by the elimination of individuals carrying mutations that interfere with its functions.

purine
A double-ringed, nitrogen-containing compound found in DNA and RNA. Examples are adenine and guanine. (*See* Panel 2–6, pp. 76–77.)

pyrimidine
A nitrogen-containing, six-membered ring compound found in DNA and RNA. Examples are thymine, cytosine, and uracil. (*See* Panel 2–6, pp. 76–77.)

pyruvate
Three-carbon metabolite that is the end product of the glycolytic breakdown of glucose; provides a crucial link to the citric acid cycle and many biosynthetic pathways.



quaternary structure
Complete structure formed by multiple, interacting polypeptide chains within a protein molecule.

quinone
Small, lipid-soluble, mobile electron carrier molecule found in the respiratory and photosynthetic electron-transport chains. (*See* Figure 14–23.)

Rab protein
A family of small GTP-binding proteins present on the surfaces of transport vesicles and organelles that serves as a molecular marker to help ensure that transport vesicles fuse only with the correct membrane.

Ras
One of a large family of small GTP-binding proteins (the monomeric GTPases) that helps relay signals from cell-surface receptors to the nucleus. Many human cancers contain an overactive mutant form of the protein.

reaction center
In photosynthetic membranes, a protein complex that contains a specialized pair of chlorophyll molecules that performs photochemical reactions to convert the energy of photons (light) into high-energy electrons for transport down the photosynthetic electron-transport chain.

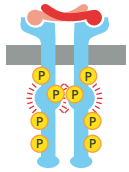
reading frame
One of the three possible ways in which a set of successive nucleotide triplets can be translated into

protein, depending on which nucleotide serves as the starting point.

receptor
Protein that recognizes and responds to a specific signal molecule.

receptor serine/threonine kinase
Enzyme-coupled receptor that phosphorylates target proteins on serine or threonine.

receptor tyrosine kinase (RTK)
Enzyme-coupled receptor in which the intracellular domain has a tyrosine kinase activity, which is activated by ligand binding to the receptor's extracellular domain.



receptor-mediated endocytosis
Mechanism of selective uptake of material by animal cells in which a macromolecule binds to a receptor in the plasma membrane and enters the cell in a clathrin-coated vesicle.

recombinant DNA molecule
A DNA molecule that is composed of DNA sequences from different sources.

recombinant DNA technology
The collection of techniques by which DNA segments from different sources are combined to make new DNA. Recombinant DNAs are widely used in the cloning of genes, in the genetic modification of organisms, and in molecular biology generally.

recombination
Process in which an exchange of genetic information occurs between two chromosomes or DNA molecules. Enzyme-mediated recombination can occur naturally in living cells or in a test tube using purified DNA and enzymes that break and re-ligate DNA strands.

redox pair
Two molecules that can be interconverted by the gain or loss of an electron; for example, NADH and NAD^+.

redox potential
A measure of the tendency of a given redox pair to donate or accept electrons.

redox reaction
A reaction in which electrons are transferred from one chemical species to another. An oxidation–reduction reaction.

reduction
Addition of electrons to an atom, as occurs during the addition of hydrogen to a carbon atom or the removal of oxygen from it. The opposite of oxidation. (*See* Figure 3–11.)

regulatory DNA sequence
DNA sequence to which a transcription regulator binds to determine when, where, and in what quantities a gene is to be transcribed into RNA.

regulatory RNA
RNA molecule that plays a role in controlling gene expression.

replication fork
Y-shaped junction that forms at the site where DNA is being replicated.

replication origin
Nucleotide sequence at which DNA replication is initiated.

reporter gene
Gene encoding a protein whose activity is easy to monitor experimentally; used to study the expression pattern of a target gene or the localization of its protein product.

repressor
A protein that binds to a specific regulatory region of DNA to prevent transcription of an adjacent gene.

reproductive cloning
The artificial production of genetically identical copies of an animal by, for example, the transplantation of a somatic cell nucleus into an enucleated fertilized egg.

respiration
General term for any process in a cell in which the uptake of molecular oxygen (O_2) is coupled to the production of CO_2.

respiratory enzyme complex
Set of proteins in the inner mitochondrial membrane that facilitates the transfer of high-energy electrons from NADH to water while pumping protons into the intermembrane space.

resting membrane potential
Voltage difference across the plasma membrane when a cell is not stimulated.

restriction nuclease
Enzyme that can cleave a DNA molecule at a specific, short sequence of nucleotides. Extensively used in recombinant DNA technology.

retrotransposon
Type of mobile genetic element that moves by being first transcribed into an RNA copy that is reconverted to DNA by reverse transcriptase and inserted elsewhere in the chromosomes.

retrovirus
RNA-containing virus that replicates in a cell by first making a double-stranded DNA intermediate that becomes integrated into the cell's chromosome.

reverse transcriptase
Enzyme that makes a double-stranded DNA copy from a single-stranded RNA template molecule. Present in retroviruses and as part of the transposition machinery of retrotransposons.

Rho protein family
Family of small, monomeric GTPases that controls the organization of the actin cytoskeleton.

ribosomal RNA (rRNA)
RNA molecule that forms the structural and catalytic core of the ribosome.

ribosome
Large macromolecular complex, composed of ribosomal RNAs and ribosomal proteins, that translates messenger RNA into protein.

ribozyme
An RNA molecule with catalytic activity.

RNA (ribonucleic acid)
Molecule produced by the transcription of DNA; usually single-stranded, it is a polynucleotide composed of covalently linked ribonucleotide subunits. Serves a variety of structural, catalytic, and regulatory functions in cells.

RNA capping
The modification of the 5′ end of a maturing RNA transcript by the addition of an atypical nucleotide.

RNA interference (RNAi)
Cellular mechanism activated by double-stranded RNA molecules that results in the destruction of RNAs containing a similar nucleotide sequence. It is widely exploited as an experimental tool for preventing the expression of selected genes (gene silencing).

RNA polymerase
Enzyme that catalyzes the synthesis of an RNA molecule from a DNA template using nucleoside triphosphate precursors.

RNA primer—*see* primer

RNA processing
Broad term for the modifications that a precursor mRNA undergoes as it matures into an mRNA. It typically includes 5′ capping, RNA splicing, and 3′ polyadenylation.

RNA splicing
Process in which intron sequences are excised from RNA molecules in the nucleus during the formation of a mature messenger RNA.

RNA transcript
RNA molecule produced by transcription that is complementary to one strand of DNA.

RNA world
Hypothetical period in Earth's early history in which life-forms were thought to use RNA both to store genetic information and to catalyze chemical reactions.

RNA-Seq
Sequencing technique used to determine directly the nucleotide sequence of a collection of RNAs.

rough endoplasmic reticulum
Region of the endoplasmic reticulum associated with ribosomes and involved in the synthesis of secreted and membrane-bound proteins.

S cyclin
Regulatory protein that helps to launch the S phase of the cell cycle.

S phase
Period during a eukaryotic cell cycle in which DNA is synthesized.

S-Cdk
Protein complex whose activity initiates DNA replication; consists of an S cyclin plus a cyclin-dependent protein kinase (Cdk).

sarcomere
Highly organized assembly of actin and myosin filaments that serves as the contractile unit of a myofibril in a muscle cell.

saturated
Describes an organic molecule that contains a full complement of hydrogen; in other words, no double or triple carbon–carbon bonds.

second messenger
Small intracellular signaling molecule generated or released in response to an extracellular signal. Examples include cAMP, IP_3, and Ca^{2+}.

secondary structure
Regular local folding pattern of a polymeric molecule. In proteins, it refers to α helices and β sheets.

secretion
Production and release of a substance from a cell.

secretory vesicle
Membrane-enclosed organelle in which molecules destined for secretion are stored prior to release. Sometimes called a secretory granule because darkly staining contents make the organelle visible as a small solid object.

segregation
During cell division, the process by which duplicated chromosomes are organized and then separated into the chromosome sets that will be inherited by each of the daughter cells.

sequence
The linear order of monomers in a large molecule, for example amino acids in a protein or nucleotides in DNA; encodes information that specifies a macromolecule's precise biological function.

serine/threonine kinase
Enzyme that phosphorylates target proteins on serines or threonines.

sex chromosome
Type of chromosome that determines the sex of an individual and directs the development of sexual characteristics. In mammals, the X and Y chromosomes.

sexual reproduction
Mode of reproduction in which the genomes of two individuals are mixed to produce an individual that is genetically distinct from its parents.

side chain
Portion of an amino acid not involved in forming peptide bonds; its chemical identity gives each amino acid its unique properties.

signal sequence
Amino acid sequence that directs a protein to a specific location in the cell, such as the nucleus or mitochondria.

signal transduction
Conversion of an impulse or stimulus from one physical or chemical form to another. In cell biology, the process by which a cell responds to an extracellular signal.

signaling cascade
Sequence of linked reactions, often including phosphorylation and dephosphorylation, that carries information within a cell, often amplifying an initial signal.

single-nucleotide polymorphism (SNP)
Form of genetic variation in which one portion of the population differs from another in terms of which nucleotide is found at a particular position in the genome.

sister chromatid
Copy of a chromosome, produced by DNA replication, that remains bound to the other copy.

site-directed mutagenesis
Technique by which a mutation can be made at a particular site in DNA.

site-specific recombination
Type of genetic exchange in which one segment of DNA is inserted into another at a particular nucleotide sequence; does not require extensive similarity between the two participating DNA sequences, which can be on different DNA molecules or within a single DNA molecule.

small interfering RNA (siRNA)
Short length of RNA produced from double-stranded RNA during the process of RNA interference. It base-pairs with identical sequences in other RNAs, leading to the inactivation or destruction of the target RNA.

small intracellular signaling molecule
Nucleotide, lipid, ion, or other small molecule generated or released in response to an extracellular signal. Examples include cAMP, IP_3, and Ca^{2+}. Also called second messengers.

small messenger—*see* second messenger

small nuclear ribonucleoprotein (snRNP)
Complex made of RNA and protein that recognizes RNA splice sites and participates in the chemistry of splicing; together these complexes form the core of the spliceosome.

small nuclear RNA (snRNA)
RNA molecule of around 200 nucleotides that participates in RNA splicing.

smooth endoplasmic reticulum (SER)
Region of the endoplasmic reticulum not associated with ribosomes; involved in the synthesis of lipids.

SNARE
One of a family of membrane proteins responsible for the selective fusion of vesicles with a target membrane inside the cell.

sodium pump—*see* Na^+ pump

solute
Any substance that is dissolved in a liquid. The liquid is called the solvent.

somatic cell
Any cell that forms part of the body of a plant or animal that is not a germ cell or germ-line precursor.

specificity
Selective affinity of one molecule for another that permits the two to bind or react, even in the presence of a vast excess of unrelated molecular species.

spindle pole
Centrosome from which microtubules radiate to form the mitotic spindle.

spliceosome
Large assembly of RNA and protein molecules that splices introns out of pre-mRNA in the nucleus of eukaryotic cells.

starch
Polysaccharide composed exclusively of glucose units, used as an energy store in plant cells.

stem cell
Relatively undifferentiated, self-renewing cell that produces daughter cells that can either differentiate into more specialized cell types or can retain the developmental potential of the parent cell.

steroid hormone
Hydrophobic signal molecule related to cholesterol; can pass through the plasma membrane to interact with intracellular receptors that affect gene expression in the target cell. Examples include estrogen and testosterone.

stroma
In a chloroplast, the large interior space that contains the enzymes needed to incorporate CO_2 into sugars during the carbon-fixation stage of photosynthesis; equivalent to the matrix of a mitochondrion.

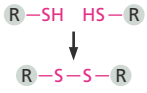
substrate
A molecule on which an enzyme acts.

substratum
Solid surface to which a cell adheres.

subunit
A monomer that forms part of a larger molecule, such as an amino acid residue in a protein or a nucleotide residue in a nucleic acid. Can also refer to a complete molecule that forms part of a larger molecule. Many proteins, for example, are composed of multiple polypeptide chains, each of which is called a protein subunit.

sugar
A substance made of carbon, hydrogen, and oxygen with the general formula $(CH_2O)_n$. A carbohydrate or saccharide. The "sugar" of everyday use is sucrose, a sweet-tasting disaccharide made of glucose and fructose.

sulfhydryl group (–SH, thiol)
Chemical group containing sulfur and hydrogen found in the amino acid cysteine and other molecules. Two can join together to produce a disulfide bond.

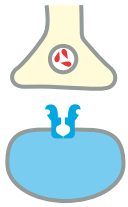


survival factor
Extracellular signal molecule that must be present to suppress apoptosis.

symbiosis
Intimate association between two organisms of different species from which both derive a long-term selective advantage.

symport
A transporter that transfers two different solutes across a cell membrane in the same direction.

synapse
Specialized junction where a nerve cell communicates with another cell (such as a nerve cell, muscle cell, or gland cell), usually via a neurotransmitter secreted by the nerve cell.



synaptic plasticity
The ability of a synapse to adjust its strength for a prolonged period, either up or down, depending on its use; thought to play an important role in learning and memory.

synaptic vesicle
Small membrane-enclosed sac filled with neurotransmitter that releases its contents by exocytosis at a synapse.

telomerase
Enzyme that elongates telomeres, synthesizing the repetitive nucleotide sequences found at the ends of eukaryotic chromosomes.

telomere
Repetitive nucleotide sequence that caps the ends of linear chromosomes. Counteracts the tendency of the chromosome otherwise to shorten with each round of replication.

telophase
Final stage of mitosis in which the two sets of separated chromosomes decondense and become enclosed by a nuclear envelope.

template
A molecular structure that serves as a pattern for the production of other molecules. For example, one strand of DNA directs the synthesis of the complementary DNA strand.

tertiary structure
Complete three-dimensional structure of a fully folded protein.

therapeutic cloning
Procedure that uses nuclear transplantation to generate cells for tissue repair and other such purposes, as opposed to producing whole multicellular individuals.

thioester bond
High-energy bond formed by a condensation reaction between an acid (acyl) group and a thiol group (–SH); seen, for example, in acetyl CoA and in many enzyme–substrate complexes.

thylakoid
In a chloroplast, the flattened disclike sac whose membranes contain the proteins and pigments that convert light energy into chemical energy during photosynthesis.

tight junction
Cell–cell junction that seals adjacent epithelial cells together, preventing the passage of most dissolved molecules from one side of the epithelial sheet to the other.

tissue
Cooperative assembly of cells and matrix woven together to form a distinctive multicellular fabric with a specific function.

trans
Beyond, or on the other side.

***trans* Golgi network (TGN)**
Portion of the Golgi apparatus furthest from the endoplasmic reticulum and from which proteins and lipids leave for lysosomes, secretory vesicles, or the cell surface.

transcription
Process in which RNA polymerase uses one strand of DNA as a template to synthesize a complementary RNA sequence.

transcription factor
Term loosely applied to any protein required to initiate or regulate transcription in eukaryotes. Includes transcription regulators as well as the general transcription factors.

transcription regulator
Protein that binds specifically to a regulatory DNA sequence and is involved in controlling whether a gene is switched on or off.

transcriptional activator
A protein that binds to a specific regulatory region of DNA to permit transcription of an adjacent gene.

transcriptional repressor
A protein that binds to a specific regulatory region of DNA to prevent transcription of an adjacent gene.

transfer RNA (tRNA)
Small RNA molecule that serves as an adaptor that "reads" a codon in mRNA and adds the correct amino acid to the growing polypeptide chain.

transformation
Process by which cells take up DNA molecules from their surroundings and then express genes on that DNA.

transgenic organism
A plant or animal that has stably incorporated into its genome one or more genes derived from another cell or organism.

transition state
Structure that forms transiently during the course of a chemical reaction; in this configuration, a molecule has the highest free energy, and is no longer a substrate, but is not yet a product.

translation
Process by which the sequence of nucleotides in a messenger RNA molecule directs the incorporation of amino acids into protein.

translation initiation factor
Protein that promotes the proper association of ribosomes with mRNA and is required for the initiation of protein synthesis.

transmitter-gated ion channel
Transmembrane receptor protein or protein complex that opens in response to the binding of a neurotransmitter, allowing the passage of a specific inorganic ion; its activation can trigger an action potential in a postsynaptic cell.

transport vesicle
Membrane vesicle that carries proteins from one

intracellular compartment to another, for example, from the endoplasmic reticulum to the Golgi apparatus.

transporter
Membrane transport protein that moves a solute across a cell membrane by undergoing a series of conformational changes.

transposon
General name for short segments of DNA that can move from one location to another in the genome. Also known as mobile genetic elements.

triacylglycerol
Compound made of three fatty acid tails covalently attached to glycerol. A storage form of fat, the main constituent of fat droplets in animal tissues (in which the fatty acids are saturated) and of vegetable oil from plants (in which the fatty acids are mainly unsaturated).

tryptophan repressor
In bacteria, a transcription regulator that, in the presence of tryptophan, shuts off production of the tryptophan biosynthetic enzymes by binding to the promoter region that controls expression of those genes.

tubulin
Protein from which microtubules are made.

γ-tubulin ring
Protein complex in centrosomes from which microtubules grow.

tumor suppressor gene
A gene that in a normal tissue cell inhibits cancerous behavior. Loss or inactivation of both copies of such a gene from a diploid cell can cause it to behave as a cancer cell.

turgor pressure
Force exerted on a plant cell wall when water enters the cell by osmosis; keeps plant from wilting.

turnover number
The number of substrate molecules an enzyme can convert into product per second.

tyrosine kinase
Enzyme that phosphorylates target proteins on tyrosines.

unfolded protein response (UPR)
Molecular program triggered by the accumulation of misfolded proteins in the endoplasmic reticulum. Allows cells to expand the endoplasmic reticulum and produce more of the molecular machinery needed to restore proper protein folding and processing.

unsaturated
Describes an organic molecule that contains one or more double or triple bonds between its carbon atoms.

valence
The number of electrons an atom must gain or lose (either by electron sharing or electron transfer) to achieve a filled outer shell. For example, Na must lose one electron, and Cl must gain one electron. This number is also equal to the number of single bonds that the atom can form.

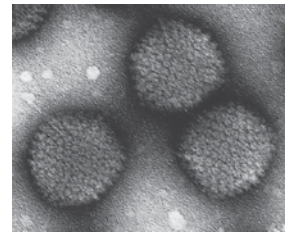
van der Waals attraction
Weak noncovalent interaction, due to fluctuating electrical charges, that comes into play between two atoms within a short distance of each other.

vector
DNA molecule that is used as a vehicle to carry a fragment of DNA into a recipient cell for the purpose of gene cloning; examples include plasmids, engineered viruses, and artificial chromosomes.

vesicle
Small, membrane-enclosed, spherical sac in the cytoplasm of a eukaryotic cell.

vesicular transport
Movement of material between organelles in the eukaryotic cell via membrane-enclosed vesicles.

virus
Particle consisting of nucleic acid (RNA or DNA) enclosed in a protein coat and capable of replicating within a host cell and spreading from cell to cell. Often the cause of disease.



V_{max}
The maximum rate of an enzymatic reaction, reached when the active sites of the enzyme molecules in a sample are fully occupied by substrate.

voltage-gated channel
Channel protein that permits the passage of selected ions, such as Na^+, across a membrane in response to changes in the membrane potential. Found primarily in electrically excitable cells such as nerve and muscle.

voltage-gated ion channel
Protein that selectively allows particular ions to cross a membrane in response to a change in membrane potential. Found mainly in electrically excitable cells such as nerve and muscle cells.

voltage-gated Na^+ channel
Protein in the plasma membrane of electrically excitable cells that opens in response to membrane depolarization, allowing Na^+ to enter the cell. It is responsible for action potentials in these cells.

wild type
Typical non-mutant form of a species, gene, or characteristic as it occurs in nature.

Wnt protein
Member of a family of extracellular signal molecules that regulates cell proliferation and migration during embryonic development and that maintains stem cells in a proliferative state.

X chromosome
Larger of the two sex chromosomes in mammals. The cells of males contain one, and females possess two.

X-ray crystallography
Technique used to determine the three-dimensional structure of a protein molecule by analyzing the pattern produced when a beam of X-rays is passed through an ordered array of the protein.

Y chromosome
Smaller of the two sex chromosomes of mammals. Present in a single copy only in the cells of males, contains genes that direct the development of male sex organs and characteristics.

yeast
Common term for several families of eukaryotic unicellular fungi used as model organisms. Includes species used for brewing beer and making bread, as well as species that cause disease.

zygote
Diploid cell produced by fusion of a male and a female gamete. A fertilized egg.

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