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 is, 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 (and less dangerous). 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 containing a nutrient broth that could support microbial growth 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 heat-sterilized nutrient broth in these flasks never showed any signs of life; however, it was capable of supporting life, as could be demonstrated by washing some of the "dust" from the neck of the flask into the broth.

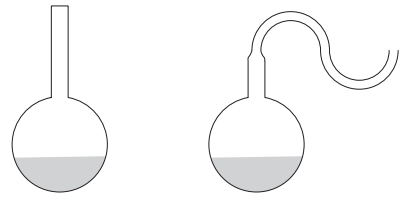


Figure A1–3

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—that is, 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 organisms 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 nature of the instrument and in the preparation of the sample (which needs to be extremely thinly sliced, stained with an electron-dense heavy metal, and completely dehydrated). 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 either by electron microscopy or by using specific dyes to make them visible by confocal or super-resolution fluorescence microscopy (although no form of fluorescence microscopy can match the resolution of an electron microscope).

ANSWER 1–7 Because the basic workings of all cells are so similar, a great deal has been learned from studying model systems. Brewer's yeast is a good model for eukaryotic cells because yeast cells are much simpler than human cancer cells. We can grow them 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 grow and divide. Cancer cells grow and divide when they should not (and therefore give rise to tumors), and a basic understanding of how cell growth and division are 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 growth and 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, like animals, are composed of eukaryotic cells, but unlike animal cells, they contain chloroplasts as cytoplasmic organelles. The chloroplasts are thought to be evolutionarily derived from engulfed photosynthetic bacteria.
D. True. The number of chromosomes varies from one organism to another, but is constant in all nucleated cells (except germ cells) within the same multicellular 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 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 1–11 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 many 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 1–12 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 1–13 Bacteria continually acquire mutations in their DNA. In the population of cells exposed to the poison, one or a few cells may already harbor a mutation that makes them resistant to the action of the poison. 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 1–14 $10^{13} = 2^{(t/1)}$. 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, and many die because of their internal abnormalities or because they do not have a sufficient blood supply, and so the actual progression of cancer is usually slower.

ANSWER 1–15 Living cells evolved from nonliving matter, but they 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 1–16 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 division 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 1–17 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 area of membrane, which—as we discuss in more detail in later chapters—is required for many essential cell functions.

ANSWER 1–18 There are many lines of evidence for a common ancestor cell. 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 type to another, and the organic compounds that make up polynucleotides (DNA and RNA) 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 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 trial 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 1–19 A quick inspection might reveal the characteristic beating of cilia on the cell surface; their presence would tell you that the cell was eukaryotic (prokaryote flagella have entirely different structures and motions compared to eukaryote cilia and flagella). 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 the DNA 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 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 we describe more fully in Chapter 10. If the nucleic acid sequences encode molecules that are highly conserved in eukaryotes, such as those that form the core components of the nuclear pore, you can be sure your cell is a eukaryote. If there are no eukaryote-specific sequences, you should still be able to distinguish whether you are looking at a bacterium or an archaeon. If you can't detect any DNA or 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 a "Greek" 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 6 (= 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, carbon-14 is chemically identical to carbon-12.

ANSWER 2–3 The statement is correct. Both ionic and covalent bonds are based on the same principles: an exchange of electrons. In polar covalent bonds, the electrons are shared unequally between the interacting atoms. In ionic bonds, the electrons are completely lost by one atom and gained by the other. And at the other end of the spectrum, electrons can be shared equally between two interacting atoms to form a nonpolar covalent 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 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—that is, 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); that is, only 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 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" has two different meanings. In one meaning, polarity refers to a 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 bonds 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, nucleic acids and polypeptides always have two different ends, which give the chains 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;" that is, used to build new macromolecules.

ANSWER 2–9 Many of the functions that macromolecules perform rely on their ability to associate and dissociate readily. This chemical flexibility 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 strong and too permanent 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), 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^3). 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. The 1.7-fold difference in the two calculations reflects (1) that carbon is not the only atom in cellulose and (2) that paper is not an atomic lattice of precisely arranged molecules (as a diamond would be for precisely arranged carbon atoms), but a random meshwork of fibers containing many voids.

ANSWER 2–12

A. The occupancies of the three innermost electron levels are 2, 8, 8.
B.

helium	already has full outer electron shell
oxygen	gain 2
carbon	gain 4 or lose 4
sodium	lose 1
chlorine	gain 1

C. Helium, with its fully occupied outer electron shell, is chemically unreactive. Sodium and chlorine, on the other hand, are extremely reactive and readily form stable Na^+ and Cl^- ions that form ionic bonds, as in table salt.

ANSWER 2–13 Whether a substance is a liquid or a 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 an H_2S molecule is not strongly attracted to the hydrogen atoms in an adjacent H_2S molecule, and the hydrogen bonds that 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.

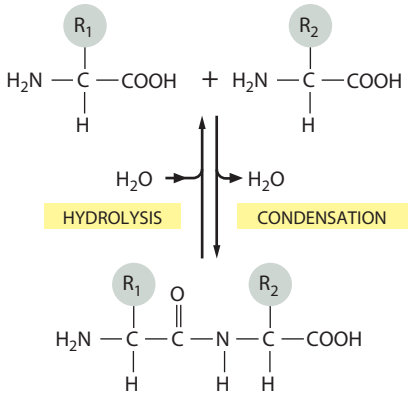


Figure A2–14

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. Lipids assemble into bilayers by noncovalent bonds. A membrane is therefore not a macromolecule.
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 the folded-up proteins, because they are repelled from water.
E. True. Hydrophobic hydrocarbon tails contain only nonpolar covalent 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 mass 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 each weighs 9×10^{31} g (= 8×10^{-21} g × 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 very large container indeed.
C. Given that most cellular 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 is 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 biology. In the course of this book, 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 require specific groups to interact; one is always a hydrogen atom linked by a polar covalent bond to an oxygen or a nitrogen, 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 close. Both types of bonds can therefore be thought of as a means of "fine-tuning" an interaction; that is, 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 form in all three examples. Hydrogen bonds would form in (c) only.

ANSWER 2–19 Noncovalent bonds form between the subunits of macromolecules—e.g., the side chains of amino acids in a polypeptide chain—and cause the polymer chain to assume a unique shape. These interactions include hydrogen bonds, ionic bonds, van der Waals attractions, and hydrophobic forces. 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 ends can hydrogen-bond to water, but their hydrophobic ends are repelled from water because they interfere with the water structure. Consequently, the hydrophobic ends of amphipathic molecules tend to be exposed to air at air–water interfaces, or will always cluster together to minimize their contact with water molecules—both at this interface and in the interior of an aqueous solution. (See **Figure A2–20**.)

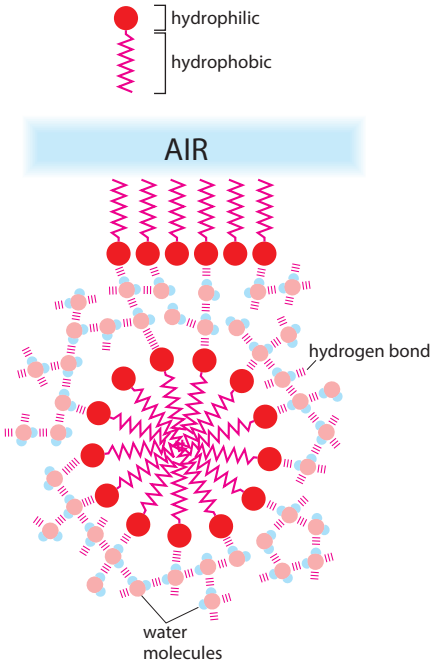


Figure A2–20

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 solution in water, 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; that is, 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—that is, 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—that is, 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, that is, 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 $\Delta G°_{X \rightarrow Z} = \Delta G°_{X \rightarrow Y} + \Delta G°_{Y \rightarrow Z}$.

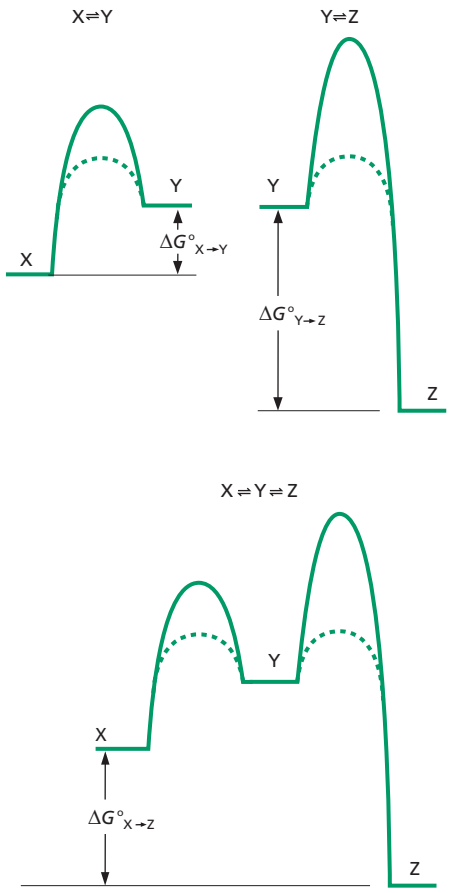


Figure A3–4

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—that is, 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 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.

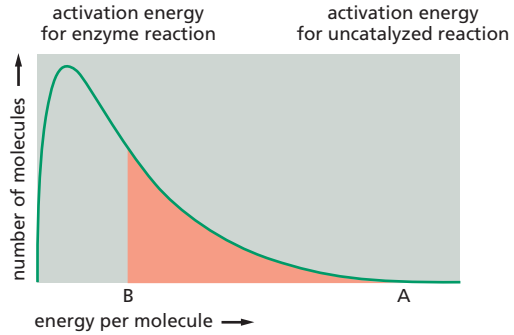


Figure A3–5

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 concentrations of A, B, and AB (as discussed in Figure 3–19). Presumably, when this enzyme was isolated, its activity was detected by 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 3–29B provide the energy to lift the bucket of water. (i) In the reaction X + ATP ⟶ Y + ADP + P_i, ATP hydrolysis is driving the reaction; thus ATP corresponds to the rocks on top of the cliff. (ii) The broken debris in Figure 3–29B corresponds to ADP and P_i, the products of ATP hydrolysis. (iii) and (iv) 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 rocks 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

$$\Delta G = -50 \text{ kJ/mole} = -30.5 \text{ kJ/mole} + 2.58 \ln \frac{[\text{ADP}] \times [P_i]}{[\text{ATP}]}$$

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 3–42).

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; that is, they are 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 –12.6 kJ/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 $X \rightarrow Y$ is therefore negative.) The equilibrium constant for the reaction is therefore about 100 (from Table 3–1, p. 96); that is, there are about 100 times more molecules of Y than of X at equilibrium. An additional three hydrogen bonds would increase $\Delta G°$ to –25.2 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 –17.8 kJ/mole 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; that is, 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 (= 2867/50) 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 number 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, 1347 kJ (the remaining 47% of the available 2867 kJ 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 (1347 kJ × 0.24 = 323 kcal; 323 kcal/75 kg = 4.3). 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 part (C) 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 4200 kJ 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 about 2850 kJ (= 57 × 50 kJ) 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. 96) we know that a free-energy difference of 17.8 kJ/mole corresponds to an equilibrium constant of 10^{-3}; that is, $[A^*]/[A] = 10^{-3}$. 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; that is, 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 to A* at equilibrium.

ANSWER 3–17

A. The mutant mushroom would probably be safe to eat. ATP hydrolysis can provide approximately –50 kJ/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. 96, we see that –23.8 kJ/mole corresponds to an equilibrium constant of 10^4; thus, –50 kJ/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 a high concentration in the cells so as to allow the synthesis of nucleotides, fatty acids, and other essential molecules. Because 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.

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 a more efficient utilization of a food source, enhanced resistance to environmental insults, or an improved 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**.)

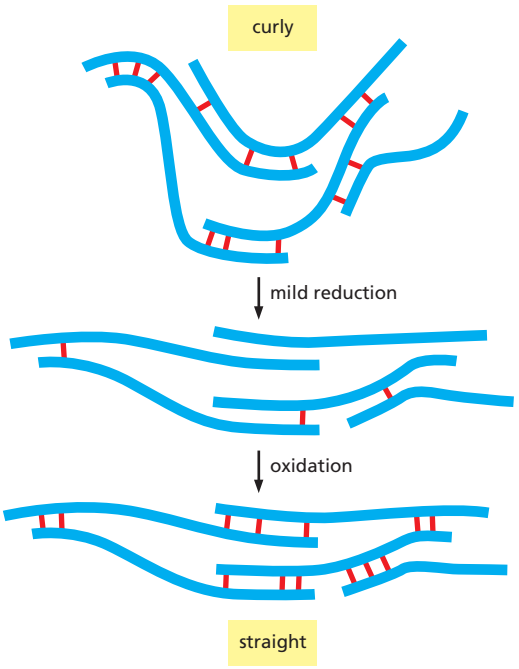


Figure A4–4

ANSWER 4–5 See Figure A4–5.

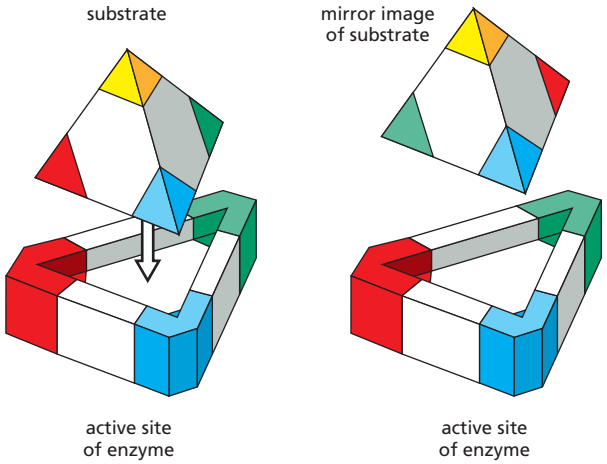


Figure A4–5

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 4–42.
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 4–39); 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 major contributors 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 acids 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 ones 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 an EP complex) are unavailable for catalysis; excess P therefore can inhibit 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 side chains 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 molecules 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 4–20 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).

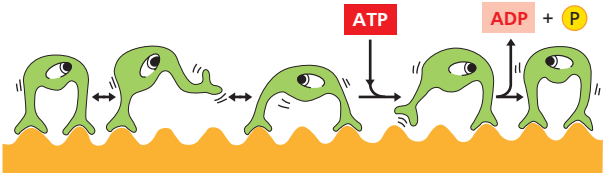


Figure A4–19

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.

ANSWER 4–23

A. When [S] << K_M, the term (K_M + [S]) 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]/(K_M + [S]) equals ½. Therefore, the reaction rate is half of the maximal rate V_{max}.
C. If [S] >> K_M, the term (K_M + [S]) approaches [S]. Therefore, [S]/(K_M + [S]) equals 1 and the reaction occurs at its maximal rate V_{max}.

ANSWER 4–24 The substrate concentration is 1 mM. This value can be obtained by substituting values into 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 A4–24.

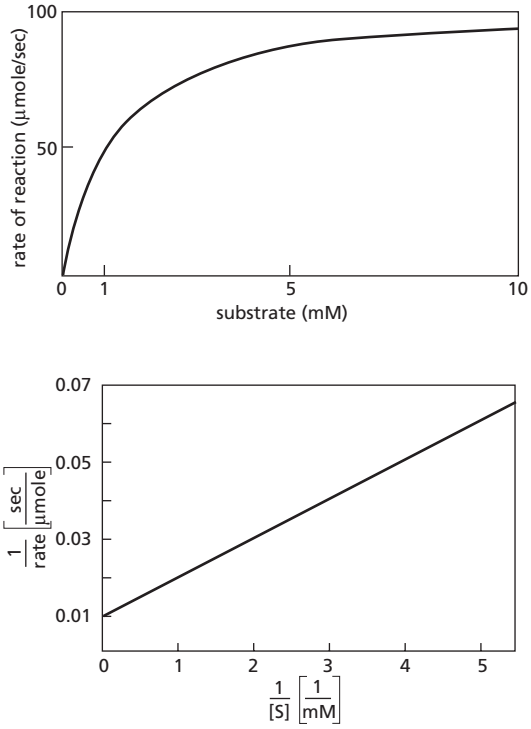


Figure A4–24

A plot of 1/rate versus 1/[S] is a straight line because rearranging the standard equation yields the equation given in Question 4–26B.

ANSWER 4–25 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).

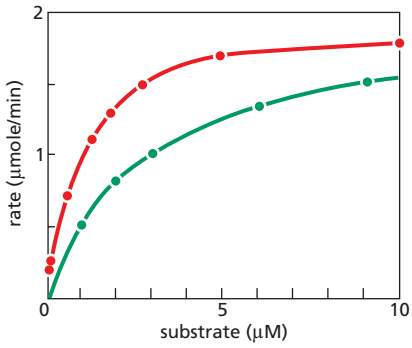
ANSWER 4–26

A,B. The data in the boxes have been used to plot the *red* curve and *red* line in Figure A4–26. 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 A4–26.

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.



DATA FOR A AND B

[S] (μM)	$\frac{1}{[S]}\left[\frac{1}{\mu M}\right]$	rate (μmole/min)	$\frac{1}{\text{rate}}\left[\frac{\text{min}}{\mu\text{mole}}\right]$
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	0.55	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

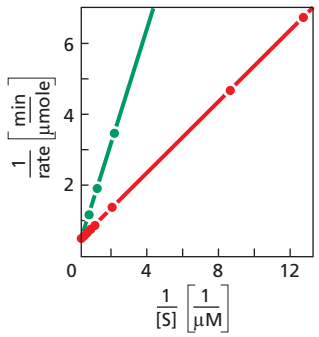


Figure A4–26

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 5–28). This inactivation 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 process 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% thymine. This leaves 74% [= 100% – (13% + 13%)] for G and C, which also form base pairs and hence are equimolar. Thus $y = z = 74/2 = 37\%$ of each.

C. A single-stranded DNA molecule that is *N* nucleotides long can have any one of 4^N possible sequences.

D. To specify a unique sequence that 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 at which the A base is attached to

the sugar–phosphate backbone is vastly different in the A-C pair compared with A-T, 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 macromolecule 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 5–4). 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.

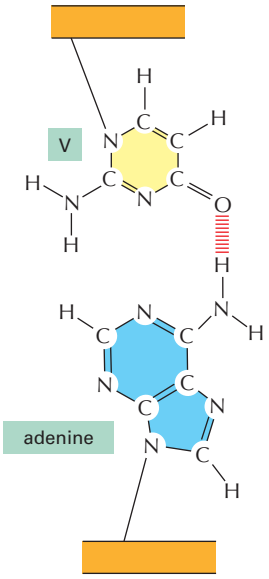


Figure A5–7

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 5–4, 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*. A nucleosome includes the DNA wrapped around this histone core plus a segment of linker DNA. 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 for a hypothetical primase with proofreading activity to distinguish the correct from incorrect bases; 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, adjacent DNA—which has already been proofread—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 so time-consuming that the cell will be unable to divide.
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 be made 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 You cannot transform an individual from one species into another species 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 made in pieces while the leading strand is synthesized continuously.
B. False. Okazaki fragments initially contain both RNA primers and DNA, but only the RNA primers are removed by RNA nucleases.

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 these 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 sufficient to satisfy this minimum requirement.

ANSWER 6–9

A. Dideoxycytidine 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 no further extension of this strand will occur. This strategy 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′-hydroxyl group, it blocks further 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 and therefore still function in the presence of the drug.
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 nucleotides 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).
C. Dideoxycytidine 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 thus not affect DNA replication.

ANSWER 6–10 See Figure A6–10.

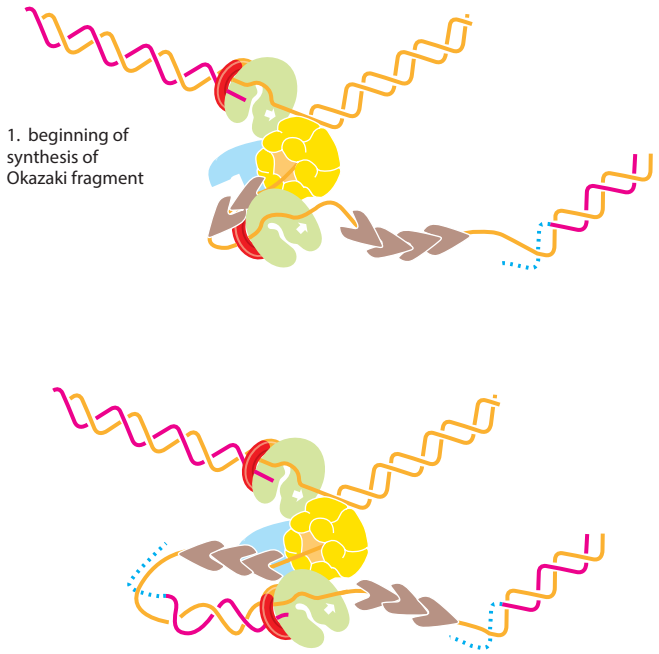


Figure A6–10

ANSWER 6–11 The two strands of the bacterial chromosome contain 6×10^6 nucleotides in total. 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 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

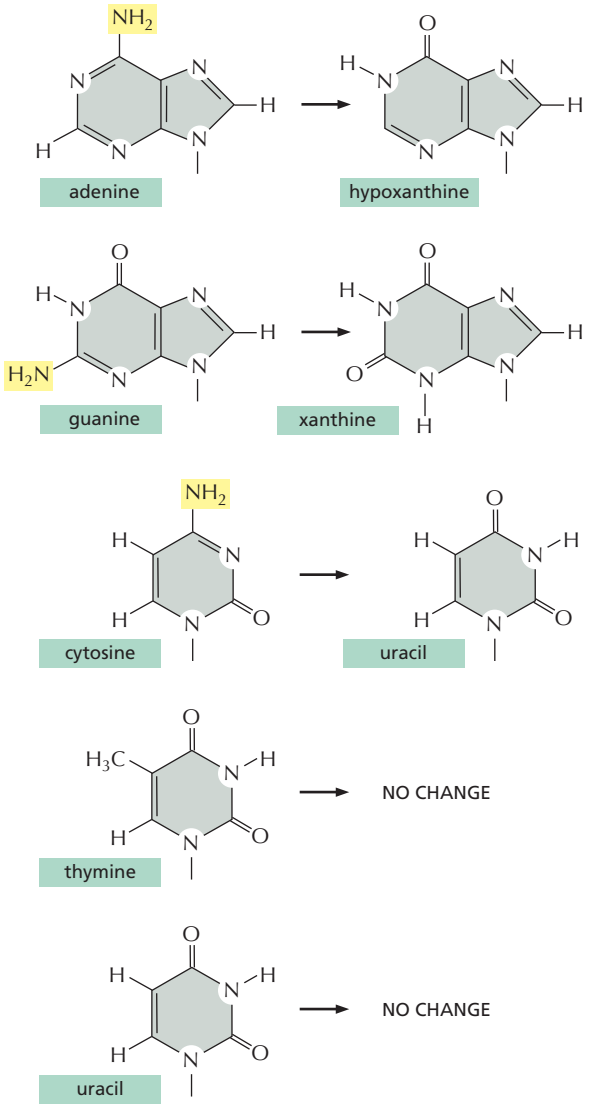


Figure A6–13

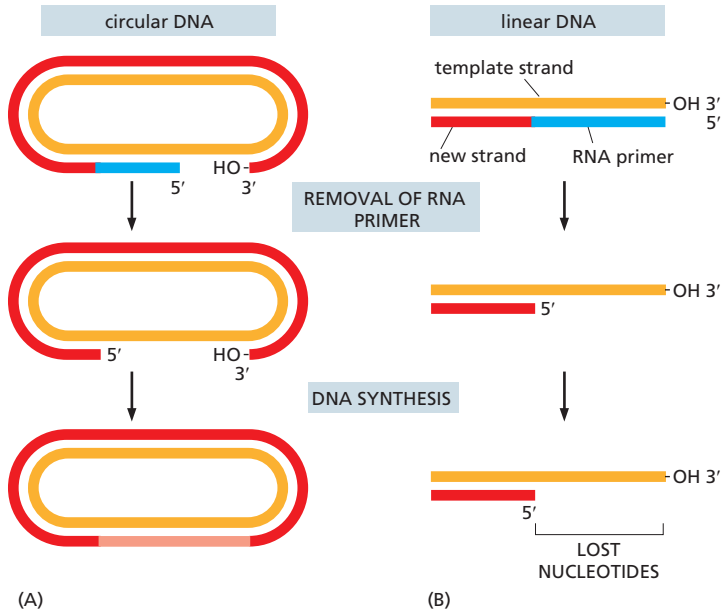


Figure A6–14

(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. 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. 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 (Figure A6–14). Telomeres and telomerase prevent the shrinking of chromosomes because they extend the 3′ end of the template DNA strand (see Figure 6–23). This extension of the lagging-strand template provides the "space" to begin the final Okazaki fragments.

B. As shown in Figure A6–14A, 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.

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 not fully extended because they begin to fold up and interact with proteins as they are synthesized; this is why they are shorter than the corresponding DNA segments.

ANSWER 7–3 At first glance, the catalytic activities of an RNA polymerase used for transcription could replace the primase that operates during DNA replication. Upon further reflection, however, there would be 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 synthesis of mRNA. In addition, RNA polymerase normally begins transcription on double-stranded DNA, whereas the DNA replication primers are synthesized using single-stranded DNA. (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 else many more terminators would need to be present. Again, both of these scenarios would be problematic for mRNA production. 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 by the evolution of separate enzymes with specialized properties. Some small DNA viruses, however, do utilize the host RNA polymerase to make RNA primers for their replication.

ANSWER 7–4 This experiment demonstrates that, once an amino acid has been coupled to a tRNA, the ribosome will trust the tRNA and "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—that is, 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 usual 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, as regulators of gene expression, and as catalysts for some of the most important reactions, including 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 can 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 not individually enclosed in a membrane.
E. False. The position of the promoter determines the direction in which transcription proceeds and therefore which of the two DNA strands is used as the template. Transcription of the other strand would produce an mRNA with a completely different (and in most cases 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 likely arose from incorrect splicing of the pre-mRNA. 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 ("skipped") 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 7–20 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

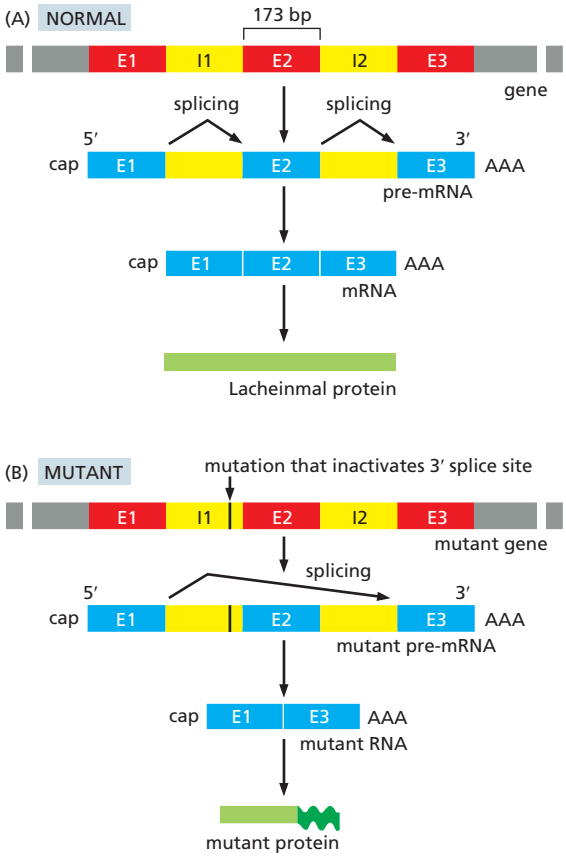


Figure A7–8

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 the wrong reading frame and will 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 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.

ANSWER 7–10

A. Incorrect. The bonds are not covalent, and their formation does not require an 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 7–37).

ANSWER 7–11 *Replication*. Dictionary definition: the creation of an exact copy; molecular biology definition: the act of copying a DNA sequence. *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 $tRNA^{Trp}$ contains the sequence 5′-CCA-3′ as its anticodon (see Figure 7–33). 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 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 7–41), 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 3–41).

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 [= $(1 - 10^{-4})^{25,000}$]; that is, 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. 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—that is, they 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.

ANSWER 7–18 The RNA transcripts that are growing from the DNA template like bristles on a bottlebrush tend to be shorter at the left-hand side of each gene and longer on the right-hand side. Because RNA polymerase synthesizes in the 5′-to-3′ direction it must move along the DNA template strand in the 3′-to-5′ direction (see Figure 7–7). The longest RNAs, therefore, should appear at the 5′ end of the template strand—when transcription is nearly complete. Hence the 3′ end of the template strand is toward the left of the image (**Figure A7–18**). The RNA transcripts, meanwhile, are synthesized in the 5′-to-3′ direction. Thus, the 5′ end of each transcript can be found at the end of each bristle (see Figure A7–18); the 3′ end of each transcript can be found within the RNA polymerase molecules that dot the spine of the DNA template molecule.

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 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 arrangements of hydrogen-bond donors and hydrogen-bond acceptors of A-T and G-C pairs are 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. Finally, some DNA-binding proteins also contact bases from the minor

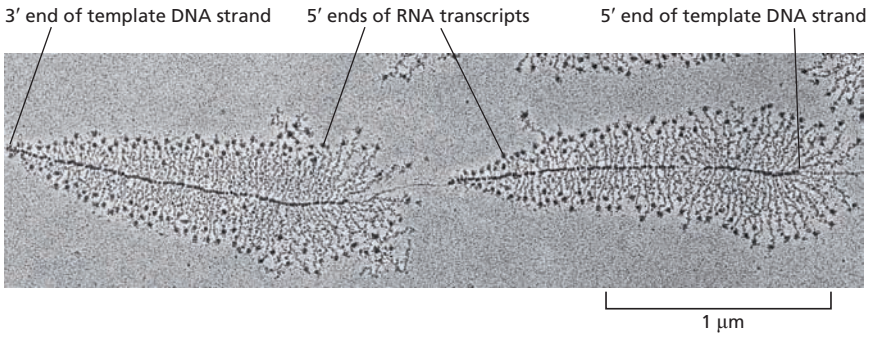


Figure A7–18

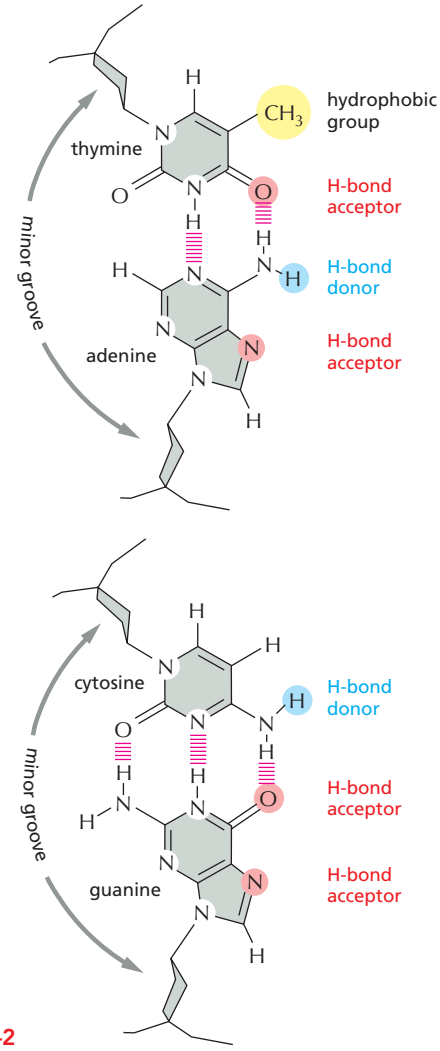


Figure A8–2

groove (see Figure 8–4). The minor groove, however, contains fewer features that distinguish one base from another than does the major groove.

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.

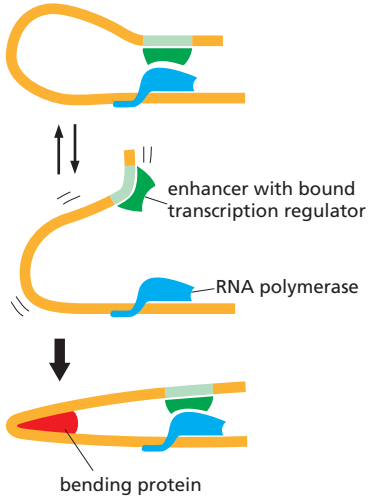


Figure A8–3

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 transcription 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 6–25), 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 of new host cells to infect.

ANSWER 8–5

A. True. Prokaryotic mRNAs are often transcripts of entire operons. Ribosomes can initiate translation at the internal AUG start sites of these "polycistronic" mRNAs (see Figures 7–40 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 of the DNA (see Figure A8–2).
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.

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.

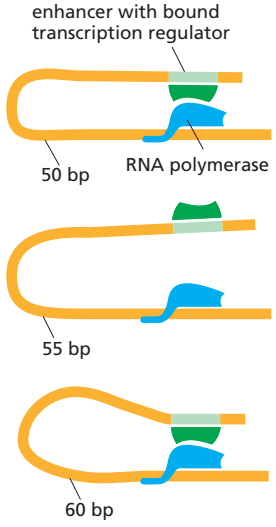


Figure A8–6

ANSWER 8–7 The affinity of the dimeric λ repressor for its binding site depends on the interactions made by each of the two DNA-binding domains. A single DNA-binding domain can make only half the contacts and therefore provide just half the binding energy compared with the dimer. Although cleavage of the repressor does not change the concentration of binding domains, the affinity that each repressor monomer has for DNA is sufficiently weak that the repressors do not remain bound. As a result, the genes for lytic growth are turned on.

ANSWER 8–8 The function of these *Arg* genes is to encode the enzymes that synthesize arginine. When arginine is abundant, expression of these 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 creates 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 that 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). In this way, the transcriptional program is adjusted 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 protein-coding 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 extremely unlikely that any gene came into existence perfectly optimized for its function. Ribosomal RNA sequences have been highly conserved because this molecule plays such an important role in protein synthesis in the cell. 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 move 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 internal metabolism, 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 Although they can harm individuals, mobile genetic elements do 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 DNA sequences 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 10% of the human genome appears to be under positive selection. However, it is possible that future research will uncover functions for some portion of our DNA that now seems unimportant.

ANSWER 9–10 The HoxD cluster is packed with complex and extensive regulatory DNA sequences that direct each of its genes to be expressed at the correct time and place during development. Insertions of mobile genetic elements into the HoxD cluster were probably selected against because they would disrupt proper regulation of these 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 DNA sequences), its sequence is probably 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 DNA sequences 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, although it is not conserved in sequence, it is nearly the same length in the human and mouse genes; however, the length of

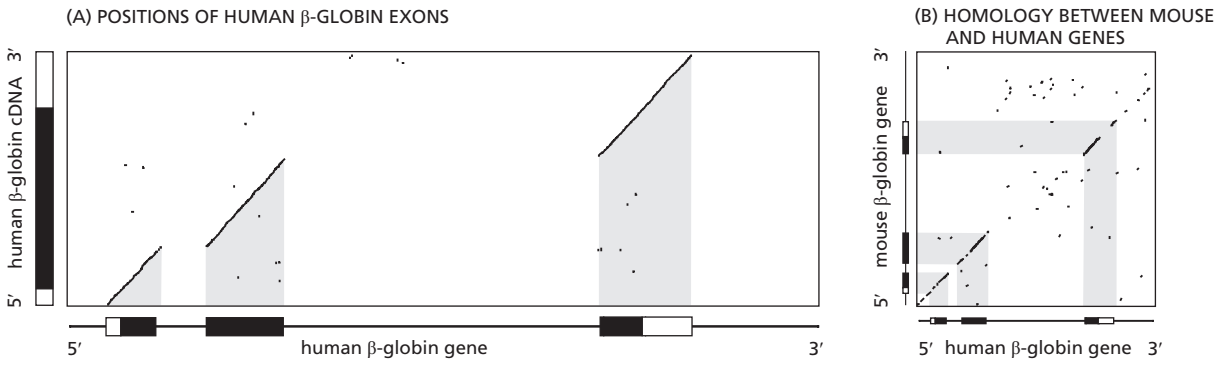


Figure A9–11

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, 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%) 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, which varies from one species to the next, 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 (1.5% for humans) and the high frequency of alternative splicing (estimated to occur in 95% of human genes), makes it difficult for an algorithm to correctly identify all exons. As shown in Figure 9–36, these bioinformatic approaches are usually coupled with direct experimental data, such as those obtained from full-genome RNA sequencing (RNA-Seq).

ANSWER 9–13 It is often not a simple matter to determine the function of a gene, 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 specific 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 rule out or support potential functions. 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 provide important 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.

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 often be narrowed.

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 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 All of these mechanisms contribute to the evolution of new protein-coding genes. A, C, D, 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 B is also correct.

ANSWER 9–16

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 9–17

A. The data embodied in the phylogenetic tree (Figure Q9–17) refutes the hypothesis that plant hemoglobin genes were acquired by horizontal transfer from animals. 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 Q9–17 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 Q9–17.

ANSWER 9–18 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 200,000 years ago.

ANSWER 9–19 The virus that causes AIDS in humans, 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, people who are HIV-positive often carry many different genetic variants of HIV that are distinct from the original virus that infected them. This posed a problem in treating the infection: drugs that block essential viral enzymes would work only temporarily, because new strains of the virus resistant to these drugs arose rapidly by mutation. Today's strategy employs multiple drugs simultaneously, which greatly decreases the likelihood that a fully resistant mutant virus could arise.

Like reverse transcriptases, RNA replicases (enzymes that synthesize RNA using RNA as a template) do not proofread. 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 enzymes 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 10–15).
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 (underlined in the DNA sequence and represented by *yellow* blocks in the three amino acid sequences 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

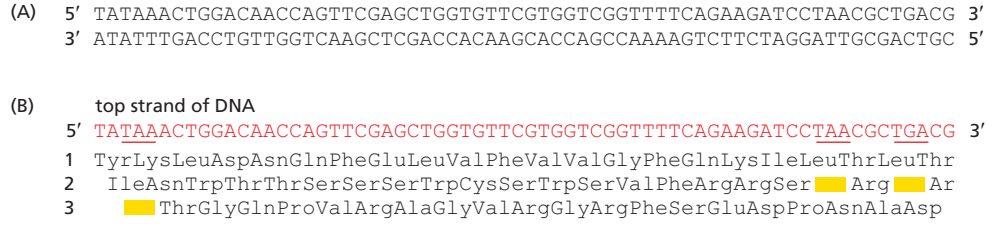


Figure A10–9

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 10–20).

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**.

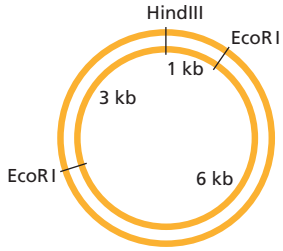


Figure A10–11

ANSWER 10–12

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 10–13 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 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 the hydrogen bonds that form between water molecules are not static; they are continually broken and remade 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. Lipid molecules inserted into one monolayer therefore remain in it unless they are actively transferred to the other monolayer by a transporter such as a scramblase or 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 also form hydrogen bonds with water molecules and are therefore hydrophilic. In contrast, the red 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. (One example of a tripeptide with hydrophobic side chains is shown in **Figure A11–5**.)

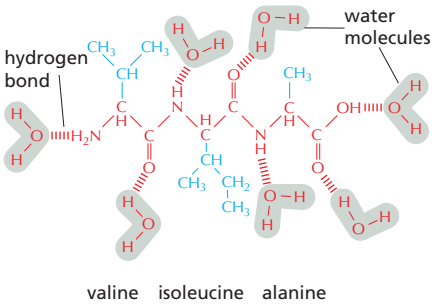


Figure A11–5

ANSWER 11–6 Some of the 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 some of the transmembrane protein molecules are not anchored. These proteins 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. The lipid bilayer is fluid because its lipid can undergo these motions.
B. The lipid bilayer is fluid because its lipid can undergo these motions.
C. Such exchanges require the action of a transporter.
D. Hydrogen bonds are formed and broken by thermal motion.
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 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, particularly for cells that line blood vessels or circulate in the bloodstream.

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. The lipid bilayers formed would be much more fluid. 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 in membrane lipid molecules, one saturated and one unsaturated hydrocarbon tail are typically linked to the same hydrophilic head, 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 membrane.

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 lipid molecules. This makes them

slightly water-soluble, so that detergent molecules leave and reenter 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 11–27).

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 (= 4000×10^{-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 $D = 1.25 \times 10^{-8}$ cm^2/sec. Using this value in the same equation but with distance $x = 2 \times 10^{-4}$ cm (= μm) gives the time taken $t = 0.16$ seconds.

B. Similarly, if a 4-cm-diameter 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 lipid tails of the bilayer. The hydrophobic side chains interact with the hydrophobic lipid tails.

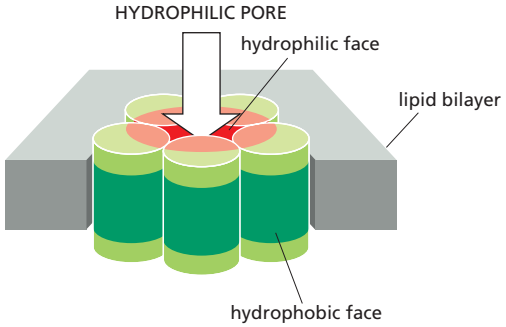


Figure A11–14

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 (Figure A11–16). At 0°C, the fluidity of the membrane is reduced, and the mixing of the membrane proteins is significantly slowed.

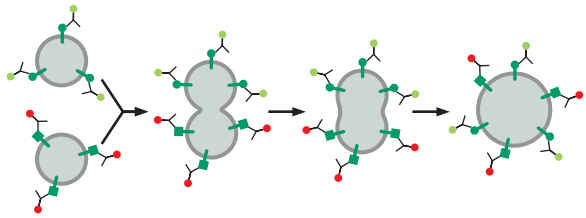


Figure A11–16

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 a 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 a 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.

ANSWER 11–20 Triacylglycerol is an entirely hydrophobic molecule. Without a hydrophilic portion, it is unable to form favorable interactions with water. Thus, triacylglycerol would be unlikely to become part of a lipid bilayer. Instead, such purely hydrophobic molecules cluster together to limit their contact with surrounding water molecules (see Figure 11–9). In this way, triacylglycerols—which are major components of animal fats and plant oils—coalesce into fat droplets in an aqueous environment, including those in fat cells and plant seeds.

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 4–35); 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. 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 5 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 changes in the currents; in the membrane patch shown in Figure 12–25B, only the cytoplasmic side of the receptor is exposed to the solution outside the microelectrode.

ANSWER 12–4 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 12–5 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 12–6 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 12–7 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 12–8 By analogy to the Na^+ pump shown in Figure 12–12, 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 A12–8**).

ANSWER 12–9

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.

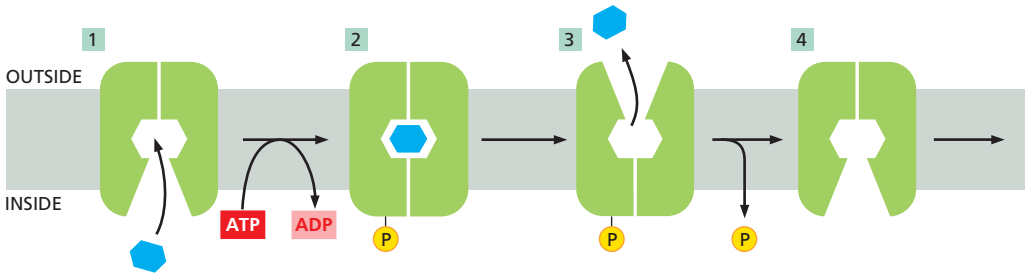


Figure A12–8

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 enzyme-like properties; that is, they bind solutes and need to undergo 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 two 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 12–10 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 12–11

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 gradient for an uncharged solute.
E. Both transmit electrical signals, by means of electrons in wires and by 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 12–12 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 12–13 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 12–14 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 12–15

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 12–16 Ion channels can be ligand-gated, voltage-gated, or mechanically- (stress-) gated.

ANSWER 12–17 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 $\times$ 100 $\times$ 10^{-9} moles/liter $\times$ 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 12–18 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 12–19

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 12–20

A. See Figure A12–20.
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.

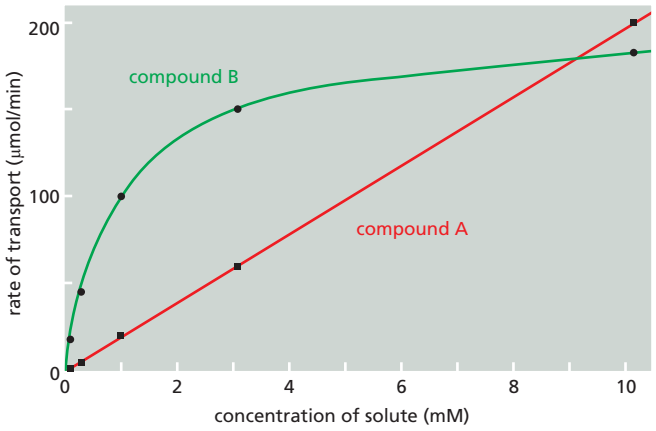


Figure A12–20

ANSWER 12–21 The membrane potential and the high 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 12–22 The diversity of neurotransmitter-gated ion channels raises the hope 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 targeting particular brain functions with greater specificity.

Chapter 13

ANSWER 13–1 To keep glycolysis going, cells need to regenerate NAD^+ from NADH. In the absence of oxygen, there is no efficient way to do this without fermentation. Without regenerated NAD^+, step 6 of glycolysis [the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (Panel 13–1, pp. 436–437)] 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 captured to generate ATP. In Figure 13–7, therefore, the reaction corresponding to the downward-pointing arrow in step 7 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.

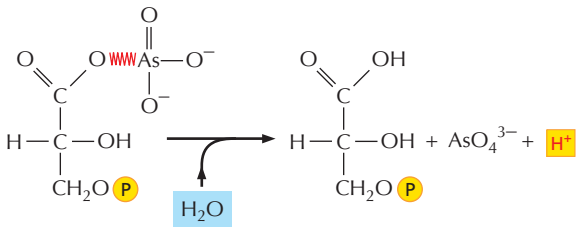


Figure A13–2

ANSWER 13–3 The oxidation of fatty acids breaks the carbon chain down into two-carbon units at a time (acetyl groups that had 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 (acetyl CoA), 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 to the atmosphere as part of CO_2 during the oxidative degradation of glucose (see Figure 13–3). The CO_2 released from the cells, however, does not contain the specific oxygen atoms consumed as part of the oxidative phosphorylation process; these are 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. 442–443).

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:

$$\text{pyruvate} + CO_2 + \text{ATP} + H_2O \rightarrow \text{oxaloacetate} + \text{ADP} + P_i + 2H^+$$

This reaction feeds oxaloacetate into the citric acid cycle. It 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**).

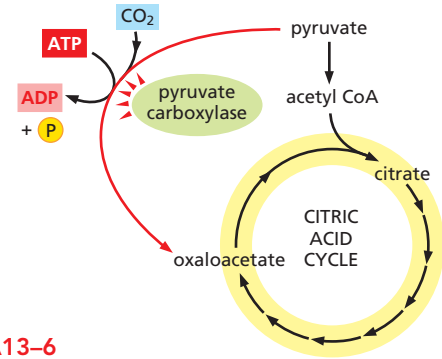


Figure A13–6

ANSWER 13–7 The carbon atoms in sugar molecules are already partially oxidized. In contrast, only the very first carbon atom in the acyl chains of fatty acids is oxidized. Thus, two carbon atoms from glucose are lost as CO_2 during the conversion of pyruvate to acetyl CoA (see Figure 13–3), leaving only four carbons to 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 (see Figure 13–11).

ANSWER 13–8

A. False. If this were the case, 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. (The cells would certainly be 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 occurs 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 final steps of oxidative phosphorylation do indeed produce some water (see Figure 13–3). 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 (see Figure 13–3).
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 the pyruvate into lactate, and no CO_2 is produced; yeast cells, however, convert the pyruvate into 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. 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 a 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 through photosynthesis in a plant.

ANSWER 13–10 Yeast cells proliferate 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. The citric acid cycle depends on O_2 because it needs NAD^+ to continue running.

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.

$$\text{creatine phosphate} + \text{ADP} \longrightarrow \text{creatine} + \text{ATP}$$

The $\Delta G°$ for this reaction is –12.6 kJ/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-day 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 organisms evolved. The later invention of oxidative phosphorylation allowed organisms to capture 15 times more energy from fuel molecules 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 molecule 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 an amount of O_2 gas equivalent to about 0.7% of the cell volume every minute, or an amount of O_2 gas equivalent to the cell volume 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).

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 13–10).
B. By following the ^{14}C-labeled atom through every reaction in the citric acid cycle, shown in Panel 13–2 (pp. 442–443), 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**).

ANSWER 13–16 In the presence of molecular oxygen, oxidative phosphorylation converts most of the cellular NADH to NAD^+ (see Figure 13–19). Since fermentation requires NADH (see Figure 13–6), it is severely inhibited by the availability of oxygen gas.

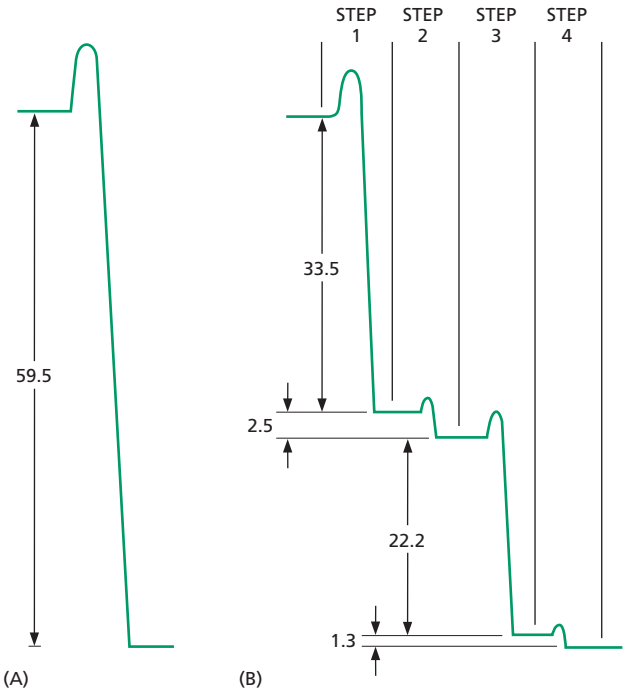


Figure A13–14

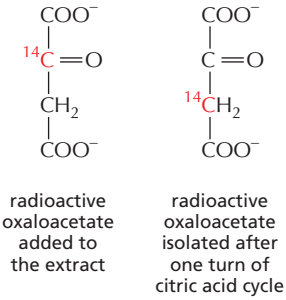


Figure A13–15

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 across that membrane 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, when passing from NADH to O_2 through the three respiratory complexes, causes 10 H^+ to be pumped across the membrane. 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 46 kJ/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 14–21). 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 the electrochemical proton gradient 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 (see How We Know, pp. 476–477).

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.
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 14–22). 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.

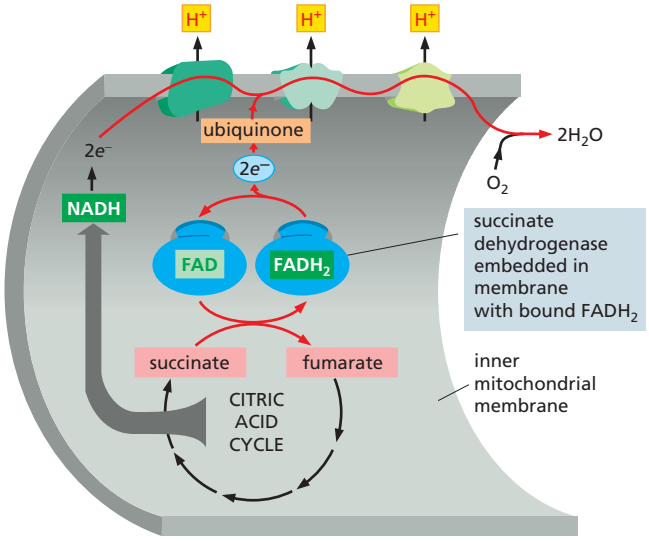


Figure A14–19

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).

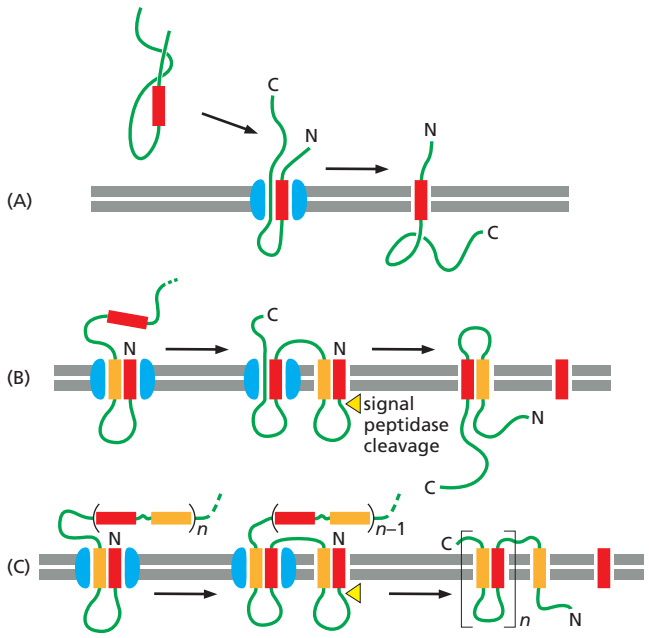


Figure A15–4

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.

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 A15–21B).

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. The difficulty of modifying oligosaccharides precisely 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.
H. False. The outer nuclear membrane is continuous with the ER and all proteins made by ribosomes bound there end up in the ER lumen.

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 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.

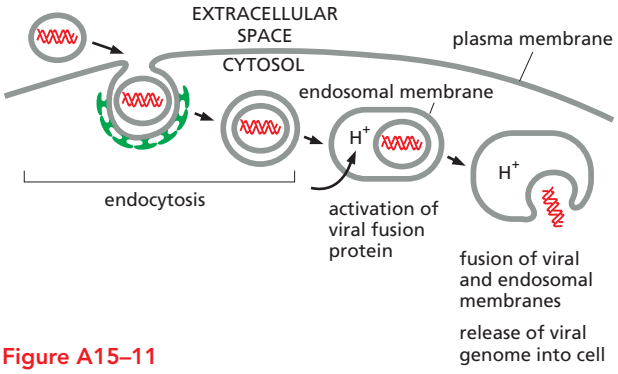


Figure A15–11

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.

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. 520–521).

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), 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.

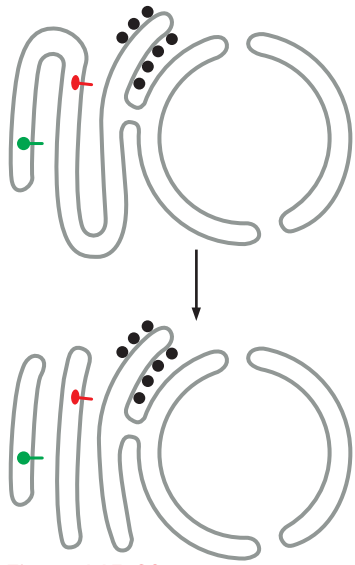


Figure A15–20

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 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 16–3 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 16–4 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 16–5 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 16–6 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 16–7 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 16–8

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 16–23).
E. False. Calmodulin senses but does not regulate intracellular Ca^{2+} levels.
F. True. See Figure 16–35.
G. True. See Figure 16–29.

ANSWER 16–9

1. You would expect a high background level of Ras activity, because Ras cannot be turned off efficiently.
2. Because many 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.
4. The increase in Ras activity in response to a signal would also be prolonged compared to the response in normal cells.

ANSWER 16–10

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 16–11

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 per cell. 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 per cell, 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 16–12

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 A16–12A). If the mutant receptors are present at extremely high levels, however, they might dimerize in the absence of the extracellular signal molecule, causing activation of signaling.

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 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 A16–12B).

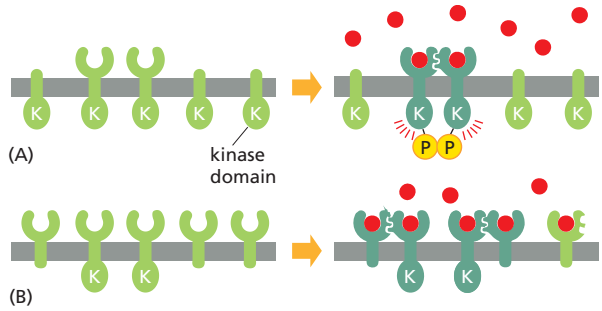


Figure A16–12

ANSWER 16–13 The statement is largely correct. Upon ligand binding, transmembrane helices of multispanning receptors, like the GPCRs, shift and rearrange with respect to one another (Figure A16–13A). 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. Thus, 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 A16–13B).

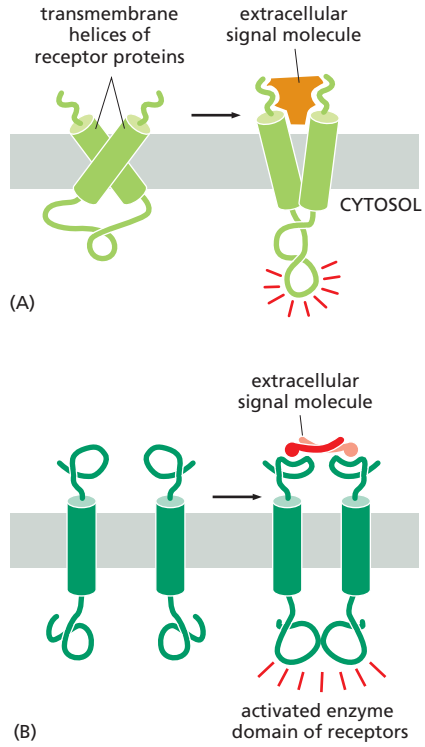


Figure A16–13

ANSWER 16–14 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 proteins is an adaptor protein that recruits a guanine nucleotide exchange factor that fulfills this function for Ras.

ANSWER 16–15 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 16–16 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 16–17 Ca^{2+}-activated Ca^{2+} channels create a positive feedback loop: the more Ca^{2+} that is released, the more Ca^{2+} channels that open. The Ca^{2+} signal in the cytosol is therefore propagated explosively throughout the cardiac muscle cell, thereby ensuring that all myosin–actin filaments contract almost synchronously.

ANSWER 16–18 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 16–19

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 16–20 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 16–32).

ANSWER 16–21 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 16–22 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 receptor, 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 16–23 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 16–9).

ANSWER 16–24 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 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 for tubulin dimers adding to a protofilament, and side-to-side for 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 does 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; that is 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, sufficient 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 that 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 17–30). The lamellipodium as a whole has many actin filaments, some of which overlap, and it therefore shows 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 the lamellipodia and filopodia are firmly anchored in 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) has 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 to the actin filament that troponin has bound calcium) 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. Motorized transport at 1 μm/sec would require 10^5 seconds, or 28 hours. These calculations make 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 than bacteria, and they 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 long in the case of the nerve cells that extend from your spinal cord to your feet.

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 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 less obvious 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 cover slip.

ANSWER 17–19

A. Phase A corresponds to a lag phase, during which tubulin dimers 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. 586).
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 17–15). 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. 140–141). 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).

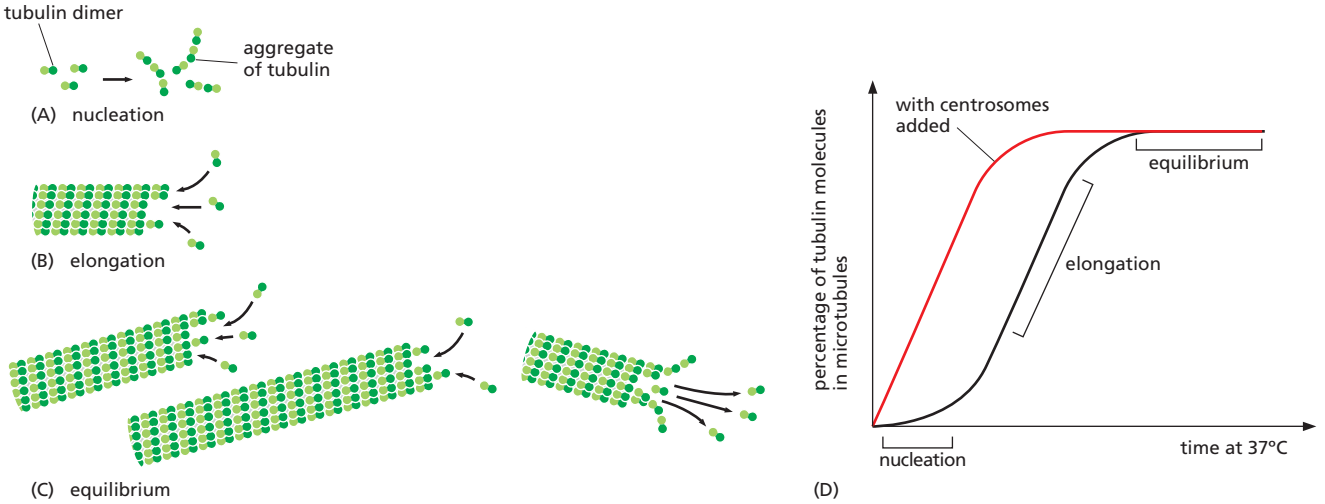


Figure A17–19

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 chromosome 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).

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, $N = \ln (7 \times 10^{13}) / \ln 2 = 46$; that is 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 phase in these divisions.

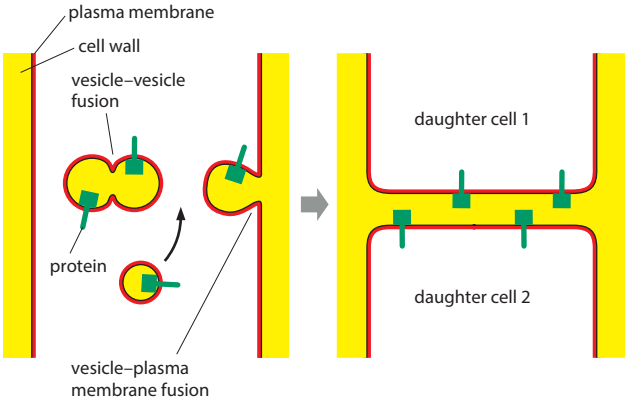


Figure A18–8

ANSWER 18–13

A. Radiation leads to DNA damage, which activates a regulatory 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 mechanism mediated by p53 is mainly required as a safeguard against the devastating effects of accumulating DNA damage; this mechanism is not required 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 initial 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 APC/C, 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. The Cdk in these complexes is inhibited by phosphorylation (see Figure 18–10). After a certain threshold level has been reached, M-Cdk is activated by the phosphatase Cdc25. Once activated, M-Cdk acts to enhance the activity of the activating phosphatase; this positive feedback leads to the complete 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 F, C, B, A, D. Together, these five steps are referred to as mitosis (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).

ANSWER 18–19 As shown in Figure A18–19, the

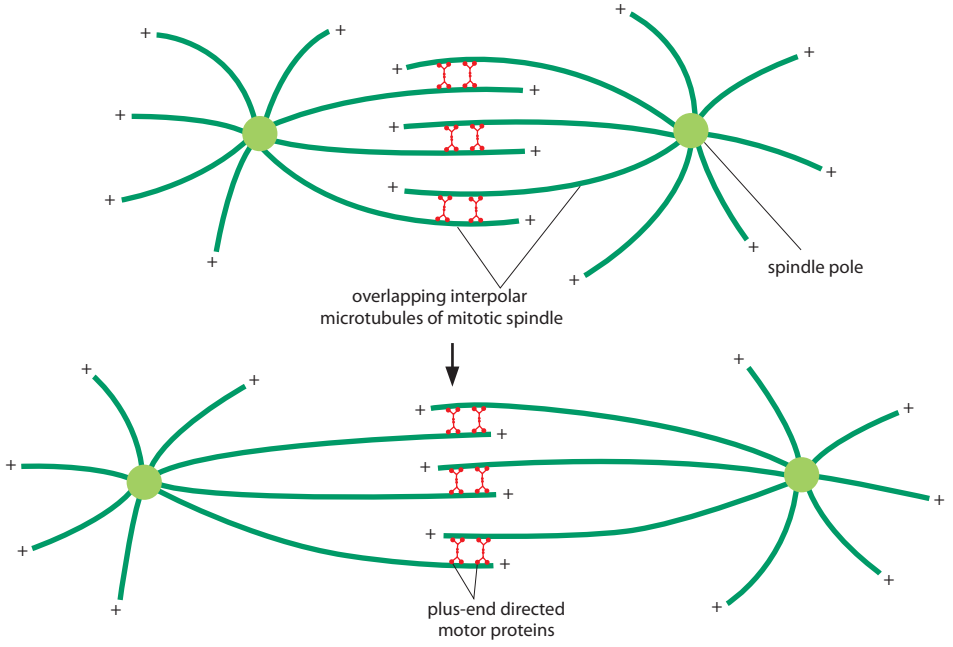


Figure A18–19

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 that cling to the sides of the depolymerizing microtubule (see Figure 18–23). 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.

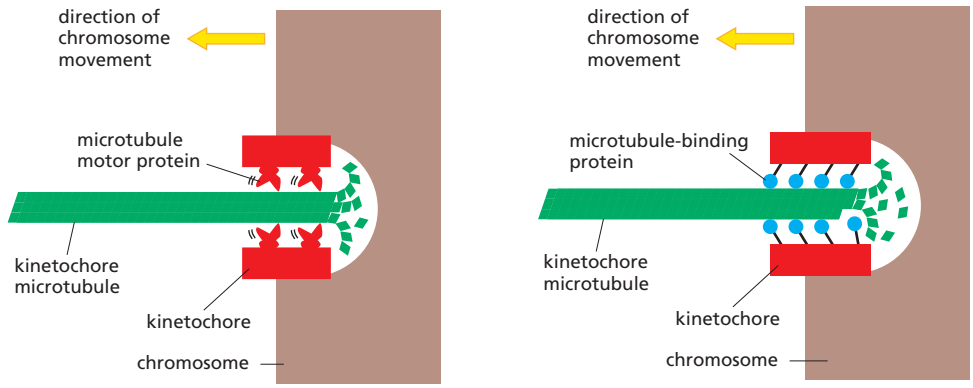


Figure A18–21

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 an anti-myosin antibody will therefore block cytokinesis.

ANSWER 18–25 The plasma membrane of the cell that died by necrosis in Figure 18–38A 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 18–38B 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, dephosphorylation at specific sites on the Cdk protein is 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 possibly 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.

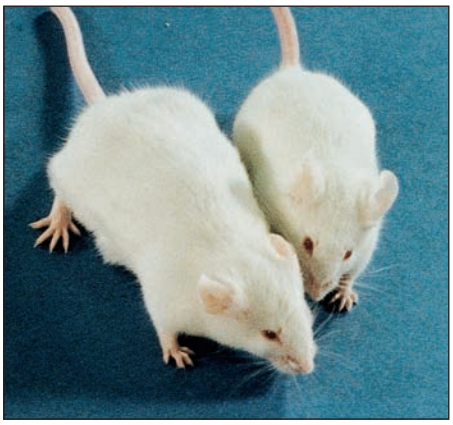


Figure A18–28 Courtesy of Ralph Brinster

The other two 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 mechanism in 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.

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 After the first meiotic division, each nucleus has a diploid amount of DNA; however, that DNA effectively contains 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 was 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 the consanguineous relationship takes the form of brother–sister inbreeding (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 homozygous for almost every gene. Because of the randomness of the mechanism of inheritance, some deleterious alleles will become prevalent in the descendants. If the gene is important, individuals that inherit two defective copies will be unhealthy—often severely so. 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 functional alleles of those genes from the father; conversely, they will inherit deleterious alleles of genes *D*, *E*, and *F* from the father, but functional alleles of those genes from the mother. Because most deleterious mutations are recessive, the hybrid offspring—who are heterozygous for these genes—will thus escape the deleterious effects.

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 In mitosis, 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. If each plant carries a mutation in a different gene, this will be revealed by complementation tests (see Panel 19–1, p. 675). 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 not present on a sex chromosome. If it were, 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. If statistical tests showed that the pattern was not due to chance, it 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

A. As outlined in Figure A19–17, if flies that are defective in different genes mate, their progeny will have one normal gene. In the case of a mating between a ruby-

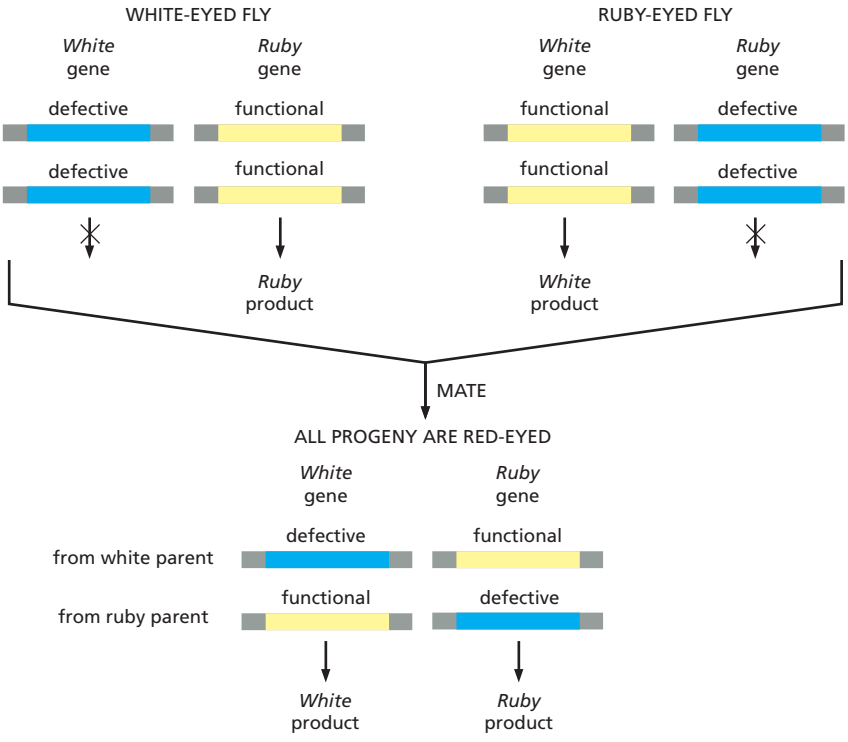


Figure A19–17

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 19–18 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 millions in the human genome. SNPs, which are detected by sequencing, 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 (see Figure 20–6). In this way, the stem will rapidly elongate; in a typical natural environment, this will hasten emergence from darkness into light.

ANSWER 20–2 As three collagen polypeptide chains have to come together to form the triple helix, a single defective polypeptide chain will impair assembly, even if normal 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.

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. With fewer negative charges, proteoglycans will attract less water and occupy less space. By contrast, uncharged polysaccharides such as cellulose, starch, and glycogen (all composed entirely of glucose subunits) are easily compacted into fibers or granules.

ANSWER 20–4 Focal contacts 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 the matrix or for crawling are generated by the actin cytoskeleton. In mature epithelia, focal contacts are presumably rare 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 by open gap junctions, these cells 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 to allow time to repair the damaged DNA before proceeding to cell division (discussed in Chapter 18). If the damage is so severe that it cannot be repaired, cells usually kill themselves by undergoing apoptosis.

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. These epithelial cells form a first line of defense against potentially hazardous compounds and mutagens that we consume or are ubiquitous in our environment. Rapid turnover of epithelial cells protects the organism from harmful consequences, as wounded and sick epithelial cells are discarded (along with undamaged ones during the normal course of gut epithelium renewal). 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 the villus: even though such mutations must occur often, they rarely give rise to a cancer.

A neuron, on the other hand, lives in a highly protected environment, largely 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; they form cell–cell channels that allow 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 deflated 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 and ability to resist compression 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 can only develop into certain 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. Some proteins and mRNAs can pass through plasmodesmata, but all such macromolecules are excluded from gap junctions. Plasma membrane phospholipids diffuse in the plane of the membrane through plasmodesmata because the plasma membranes and smooth ER 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 contract synchronously by this mechanism. This way 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 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 can result in large 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 molecular tools present in the egg (such as the appropriate transcription regulators required for early embryo 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 therefore 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 accumulate 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 driver mutations to accumulate in a cell before a cancer results. If a single driver 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 driver 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 driver mutations have to accumulate 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 a cancer.

ANSWER 20–17 During exposure to the carcinogen, mutations are induced, but the number of relevant (driver) 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 the mutant cells 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 survival of

cells, thereby increasing cell numbers in hormone-sensitive organs such as breast, uterus, and prostate. The increase in cell division boosts the mutation rate per cell, because mutations, regardless of environmental factors, are spontaneously generated in the course of DNA replication and chromosome segregation. The increase in cell numbers increases the total pool 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 from 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.