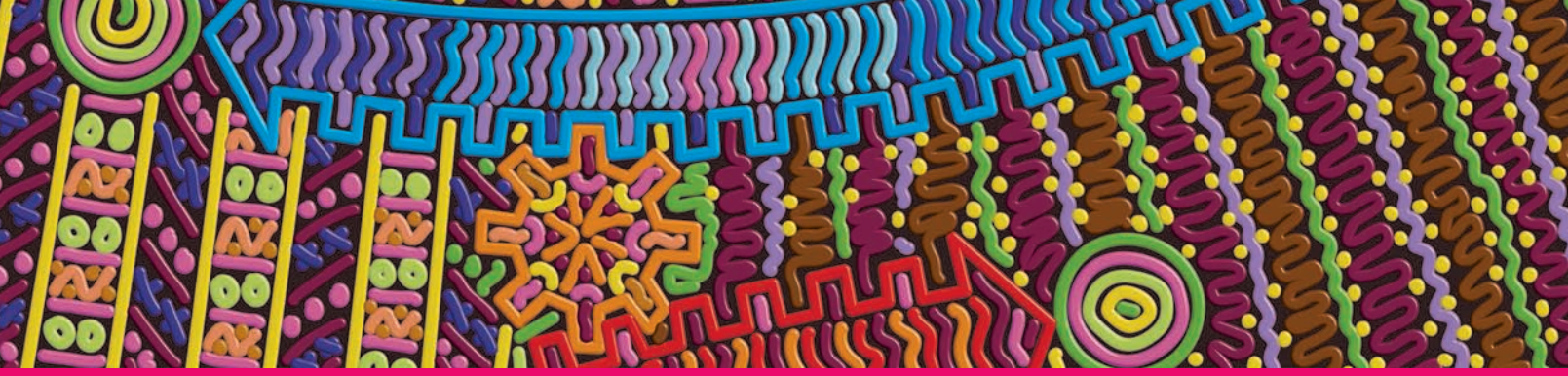


CHAPTER **THREE** **3**

Energy, Catalysis, and Biosynthesis

One property above all makes living things seem almost miraculously different from nonliving matter: they create and maintain order in a universe that is tending always toward greater disorder. To accomplish this remarkable feat, the cells in a living organism must continuously carry out a never-ending stream of chemical reactions to maintain their structure, meet their metabolic needs, and stave off unrelenting chemical decay. In these reactions, small organic molecules—amino acids, sugars, nucleotides, and lipids—can be taken apart or modified to supply the many other small molecules that the cell requires. These molecules are also used to construct an enormously diverse range of large molecules, including the proteins, nucleic acids, and other macromolecules that constitute most of the mass of living systems and endow them with their distinctive properties.

Each cell can be viewed as a tiny chemical factory, performing many millions of reactions every second. This incessant activity requires both a source of atoms in the form of food molecules and a source of energy. Both the atoms and the energy must come, ultimately, from the nonliving environment. In this chapter, we discuss why cells require energy, and how they use energy and atoms from their environment to create and maintain the molecular order that makes life possible.

Most of the chemical reactions that cells perform would normally occur only at temperatures that are much higher than those inside a cell. Each reaction therefore requires a major boost in chemical reactivity to enable it to proceed rapidly within the cell. This boost is provided by a large set of specialized proteins called *enzymes*, each of which accelerates, or *catalyzes*, just one of the many possible reactions that a particular

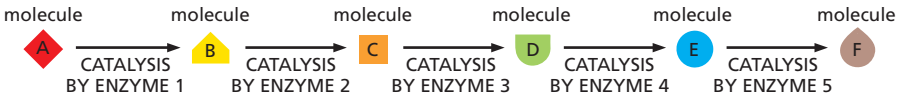


Figure 3–1 A series of enzyme-catalyzed reactions forms a linked pathway. Each chemical reaction is catalyzed by a distinct enzyme. Together, this set of enzymes, acting in series, converts molecule A to molecule F.

molecule could in principle undergo. These enzyme-catalyzed reactions are usually connected in series, so that the product of one reaction becomes the starting material for the next (**Figure 3–1**). The long, linear reaction pathways that result are in turn linked to one another, forming a complex web of interconnected reactions.

Rather than being an inconvenience, the necessity for *catalysis* is a benefit, as it allows the cell to precisely control its **metabolism**—the sum total of all the chemical reactions it needs to carry out to survive, grow, and reproduce. This control is central to the chemistry of life.

Two opposing streams of chemical reactions occur in cells: the *catabolic* pathways and the *anabolic* pathways. The catabolic pathways (**catabolism**) break down foodstuffs into smaller molecules, thereby generating both a useful form of energy for the cell and some of the small molecules that the cell needs as building blocks. The anabolic, or *biosynthetic*, pathways (**anabolism**) use the energy harnessed by catabolism to drive the synthesis of the many molecules that form the cell. Together, these two sets of reactions constitute the metabolism of the cell (**Figure 3–2**).

The details of the reactions that comprise cell metabolism are part of the subject matter of *biochemistry*, and they need not concern us here. But the general principles by which cells obtain energy from their environment and use it to create order are central to cell biology. We therefore begin this chapter by explaining why a constant input of energy is needed to sustain living organisms. We then discuss how enzymes catalyze the reactions that produce biological order. Finally, we describe the molecules inside cells that carry the energy that makes life possible.

THE USE OF ENERGY BY CELLS

Left to themselves, nonliving things eventually become disordered: buildings crumble and dead organisms decay. Living cells, by contrast, not only maintain but actually generate order at every level, from the large-scale structure of a butterfly or a flower down to the organization of the molecules that make up such organisms (**Figure 3–3**). This property of life is made possible by elaborate molecular mechanisms that extract energy from the environment and convert it into the energy stored in chemical bonds. Biological structures are therefore able to maintain their form, even though the materials that form them are continually being broken down, replaced, and recycled. Your body has the same basic structure it had 10 years ago, even though you now contain atoms that, for the most part, were not part of your body then.

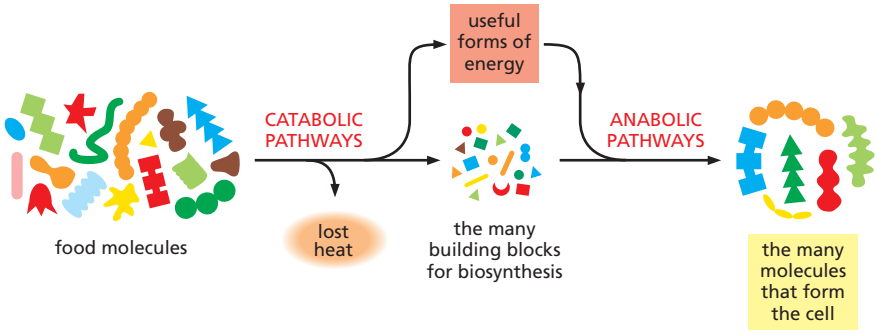


Figure 3–2 Catabolic and anabolic pathways together constitute the cell's metabolism. During catabolism, a major portion of the energy stored in the chemical bonds of food molecules is dissipated as heat. But some of this energy is converted to the useful forms of energy needed to drive the synthesis of new molecules in anabolic pathways, as indicated.

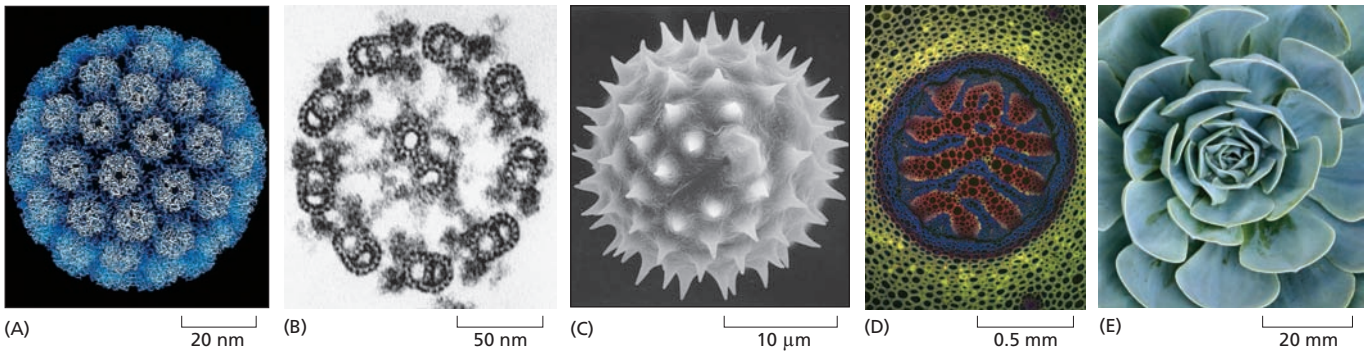


Figure 3–3 Biological structures are highly ordered. Well-defined, ornate, and beautiful spatial patterns can be found at every level of organization in living organisms. Shown are: (A) protein molecules in the coat of a virus (a parasite that, although not technically alive, contains the same types of molecules as those found in living cells); (B) the regular array of microtubules seen in a cross section of a sperm tail; (C) surface contours of a pollen grain; (D) cross section of a fern stem, showing the patterned arrangement of cells; and (E) a spiral array of leaves, each made of millions of cells. (A, courtesy of Robert Grant, Stéphane Crainic, and James M. Hogle; B, courtesy of Lewis Tilney; C, courtesy of Colin MacFarlane and Chris Jeffree; D, courtesy of Jim Haseloff.)

Biological Order Is Made Possible by the Release of Heat Energy from Cells

The universal tendency of things to become disordered is expressed in a fundamental law of physics called the *second law of thermodynamics.* This law states that in the universe as a whole, or in any isolated system (a collection of matter that is completely cut off from the rest of the universe), the degree of disorder can only increase. The second law of thermodynamics has such profound implications for living things that it is worth restating in several ways.

We can express the second law in terms of probability by stating that *systems will change spontaneously toward those arrangements that have the greatest probability.* Consider a box in which 100 coins are all lying heads up. A series of events that disturbs the box—for example, someone jiggling it a bit—will tend to move the arrangement toward a mixture of 50 heads and 50 tails. The reason is simple: there is a huge number of possible arrangements of the individual coins that can achieve the 50–50 result, but only one possible arrangement that keeps them all oriented heads up. Because the 50–50 mixture accommodates a greater number of possibilities and places fewer constraints on the orientation of each individual coin, we say that it is more "disordered." For the same reason, one's living space will become increasingly disordered without an intentional effort to keep it organized. Movement toward disorder is a spontaneous process, and requires a periodic input of energy to reverse it (**Figure 3–4**).

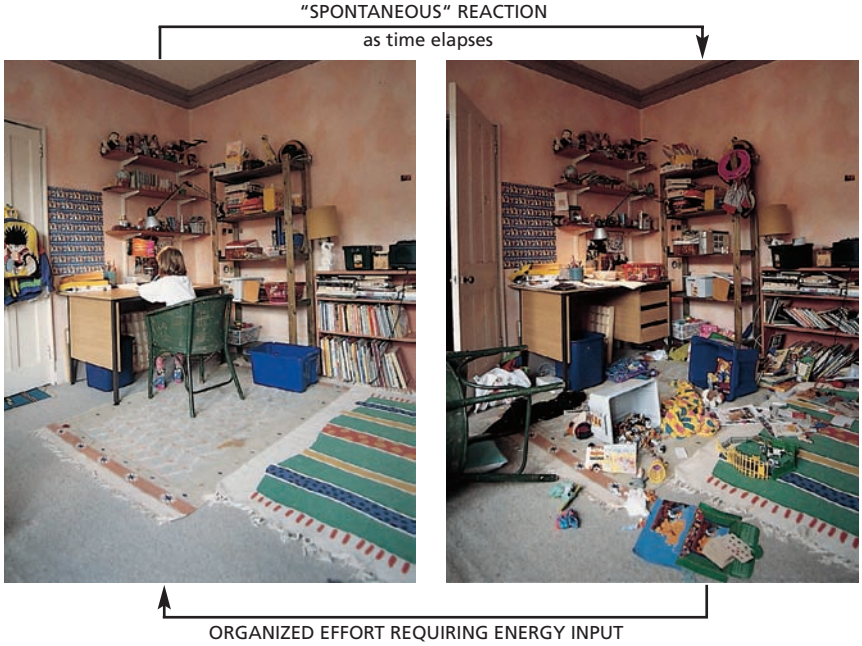


Figure 3–4 The spontaneous tendency toward disorder is an everyday experience. Reversing this natural tendency toward disorder requires an intentional effort and an input of energy. In fact, from the second law of thermodynamics, we can be certain that the human intervention required will release enough heat to the environment to more than compensate for the reestablishment of order in this room.

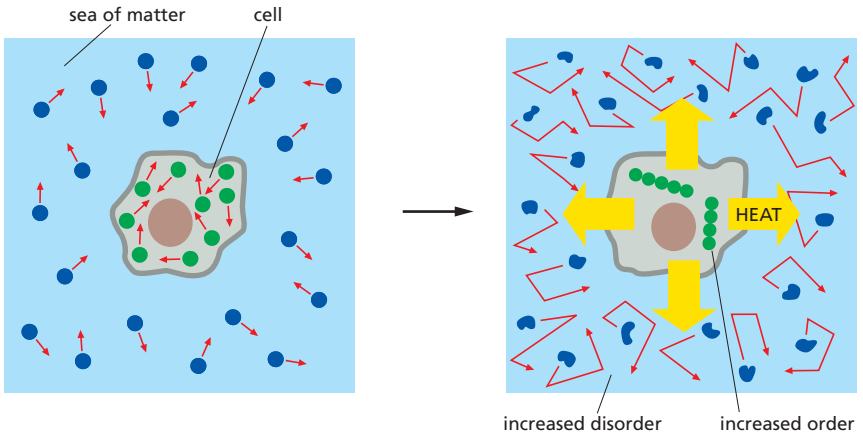


Figure 3–5 Living cells do not defy the second law of thermodynamics. In the diagram on the left, the molecules of both the cell and the rest of the universe (the environment) are depicted in a relatively disordered state. In addition, *red* arrows suggest the relative amount of thermal motion of the molecules both inside and outside the cell. In the diagram on the right, the cell has taken in energy from food molecules, carried out a reaction that gives order to the molecules that the cell contains, and released heat (*yellow* arrows) into the environment. The released heat increases the disorder in the cell's surroundings—as depicted here by the increase in thermal motion of the molecules in the environment and the distortion of those molecules due to enhanced vibration and rotation. The second law of thermodynamics is thereby satisfied, even as the cell grows and constructs larger molecules.

The measure of a system's disorder is called the **entropy** of the system, and the greater the disorder, the greater the entropy. Thus another way to express the second law of thermodynamics is to say that systems will change spontaneously toward arrangements with greater entropy. Living cells—by surviving, growing, and forming complex communities and even whole organisms—generate order and thus might appear to defy the second law of thermodynamics. This is not the case, however, because a cell is not an isolated system. Rather, a cell takes in energy from its environment—in the form of food, inorganic molecules, or photons of light from the sun—and uses this energy to generate order within itself, forging new chemical bonds and building large macromolecules. In the course of performing the chemical reactions that generate order, some energy is inevitably lost in the form of heat (see Figure 3–2). Heat is energy in its most disordered form—the random jostling of molecules (analogous to the random jostling of the coins in the box). Because the cell is not an isolated system, the heat energy produced by metabolic reactions is quickly dispersed into the cell's surroundings. There, the heat increases the intensity of the thermal motions of nearby molecules, thereby increasing the entropy of the cell's environment (**Figure 3–5**).

To satisfy the second law of thermodynamics, the amount of heat released by a cell must be great enough that the increased order generated inside the cell is more than compensated for by the increased disorder generated in the environment. In other words, the chemical reactions inside a cell must increase the total entropy of the entire system: that of the cell plus its environment. Thanks to the cell's activity, the universe thereby becomes more disordered—and the second law of thermodynamics is obeyed.

Cells Can Convert Energy from One Form to Another

Where does the heat released by cells as they generate order come from? To understand that, we need to consider another important physical law. According to the *first law of thermodynamics*, energy cannot be created or destroyed—but it can be converted from one form to another (**Figure 3–6**). Cells take advantage of this law of thermodynamics, for example, when they convert the energy from sunlight into the energy in the chemical bonds of sugars and other small organic molecules during photosynthesis. Although the chemical reactions that power such energy conversions can change how much energy is present in one form or another, the first law tells us that the total amount of energy in the universe must always be the same.

Heat, too, is a product of energy conversion. When an animal cell breaks down foodstuffs, some of the energy in the chemical bonds in the food

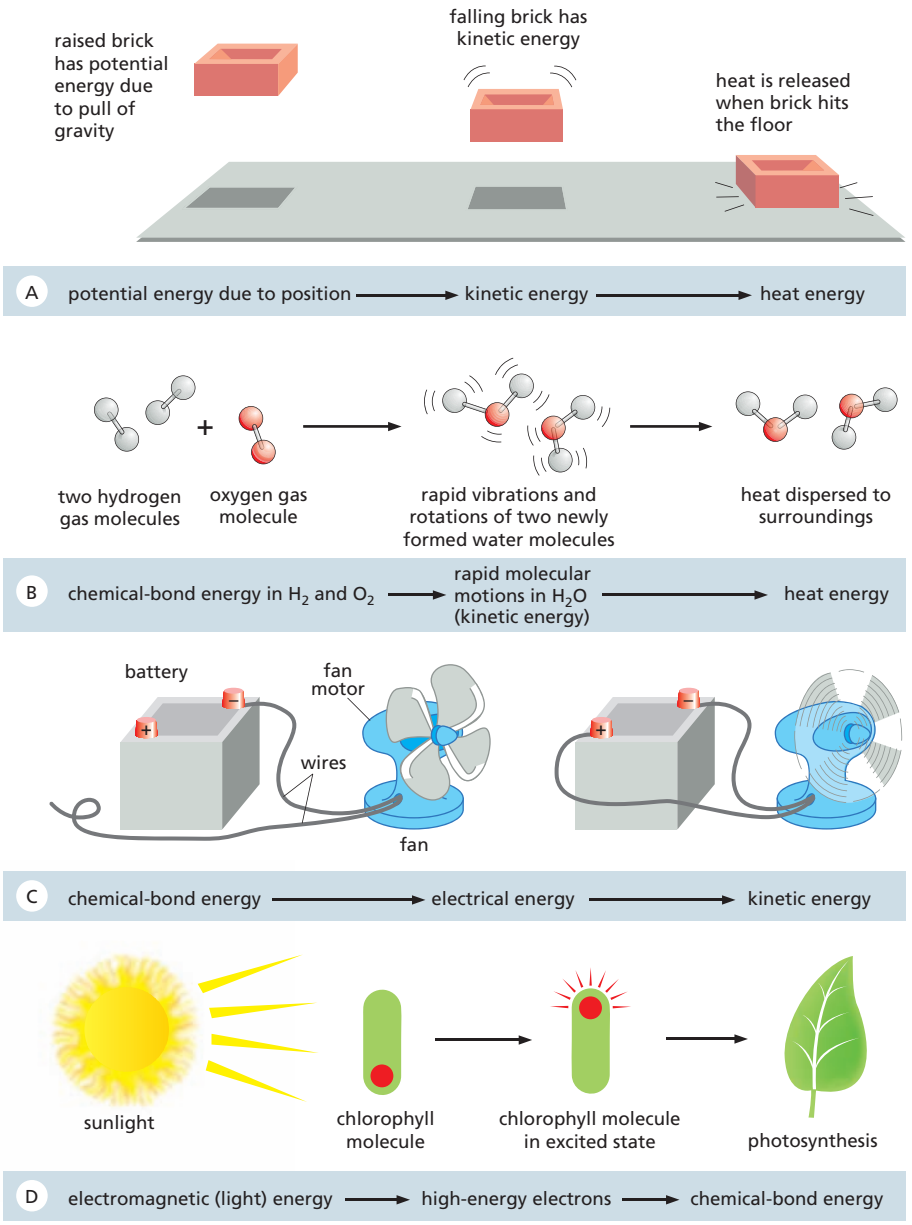


Figure 3–6 Different forms of energy are interconvertible, but the total amount of energy must be conserved. (A) We can use the height and weight of the brick to predict exactly how much heat will be released when it hits the floor. (B) The large amount of chemical-bond energy released when water (H_2O) is formed from H_2 and O_2 is initially converted to very rapid thermal motions in the two new H_2O molecules; however, collisions with other H_2O molecules almost instantaneously spread this kinetic energy evenly throughout the surroundings (heat transfer), making the new H_2O molecules indistinguishable from all the rest. (C) Cells can convert chemical-bond energy into kinetic energy to drive, for example, molecular motor proteins; however, this occurs without the intermediate conversion of chemical energy to electrical energy that a man-made appliance such as this fan requires. (D) Some cells can also harvest the energy from sunlight to form chemical bonds via photosynthesis.

molecules (chemical-bond energy) is converted into the thermal motion of molecules (heat energy). This conversion of chemical energy into heat energy causes the universe as a whole to become more disordered—as required by the second law of thermodynamics. But a cell cannot derive any benefit from the heat energy it produces unless the heat-generating reactions are directly linked to processes that maintain molecular order inside the cell. It is the tight coupling of heat production to an increase in order that distinguishes the metabolism of a cell from the wasteful burning of fuel in a fire. Later in this chapter, we illustrate how this coupling occurs. For the moment, it is sufficient to recognize that—by directly linking the "burning" of food molecules to the generation of biological order—cells are able to create and maintain an island of order in a universe tending toward chaos.

Photosynthetic Organisms Use Sunlight to Synthesize Organic Molecules

All animals live on energy stored in the chemical bonds of organic molecules, which they take in as food. These food molecules also provide the

Figure 3–7 With few exceptions, the radiant energy of sunlight sustains all life. Trapped by plants and some microorganisms through photosynthesis, light from the sun is the ultimate source of all energy for humans and other animals. (*Wheat Field Behind Saint-Paul Hospital with a Reaper* by Vincent van Gogh. Courtesy of Museum Folkwang, Essen.)

QUESTION 3–1

Consider the equation

light energy + CO_2 + H_2O → sugars + O_2 + heat energy

Would you expect this reaction to occur in a single step? Why must heat be generated in the reaction? Explain your answers.

atoms that animals need to construct new living matter. Some animals obtain their food by eating other animals, others by eating plants. Plants, by contrast, obtain their energy directly from sunlight. Thus, the energy animals obtain by eating plants—or by eating animals that have eaten plants—ultimately comes from the sun (**Figure 3–7**).

Solar energy enters the living world through **photosynthesis**, a process that converts the electromagnetic energy in sunlight into chemical-bond energy in cells. Photosynthetic organisms—including plants, algae, and some bacteria—use the energy they derive from sunlight to synthesize small chemical building blocks such as sugars, amino acids, nucleotides, and fatty acids. These small molecules in turn are converted into the macromolecules—the proteins, nucleic acids, and polysaccharides—that form the plant.

We describe the elegant mechanisms that underlie photosynthesis in detail in Chapter 14. Generally speaking, the reactions of photosynthesis take place in two stages. In the first stage, energy from sunlight is captured and transiently stored as chemical-bond energy in specialized molecules called *activated carriers*, which we discuss in more detail later in the chapter. All of the oxygen (O_2) in the air we breathe is generated by the splitting of water molecules during this first stage of photosynthesis.

In the second stage, the activated carriers are used to help drive a *carbon-fixation* process, in which sugars are manufactured from carbon dioxide gas (CO_2). In this way, photosynthesis generates an essential source of stored chemical-bond energy and other organic materials—for the plant itself and for any animals that eat it. The two stages of photosynthesis are summarized in **Figure 3–8**.

Cells Obtain Energy by the Oxidation of Organic Molecules

To live, grow, and reproduce, all organisms rely on the energy stored in the chemical bonds of organic molecules—either the sugars that a plant has produced by photosynthesis as food for itself or the mixture of large and small molecules that an animal has eaten. In both plants and animals, this chemical energy is extracted from food molecules by a process of gradual *oxidation*, or controlled burning.

Earth's atmosphere is about 21% oxygen. In the presence of oxygen, the most energetically stable form of carbon is CO_2 and that of hydrogen is H_2O; the oxidation of carbon-containing molecules is therefore energetically very favorable. A cell is able to obtain energy from sugars or other organic molecules by allowing the carbon and hydrogen atoms in these molecules to combine with oxygen—that is, become *oxidized*—to produce CO_2 and H_2O, respectively. This complex step-wise process by which food molecules are broken down to produce energy is known as **cell respiration**.

Photosynthesis and cell respiration are complementary processes (**Figure 3–9**). Plants, animals, and microorganisms have existed together on this

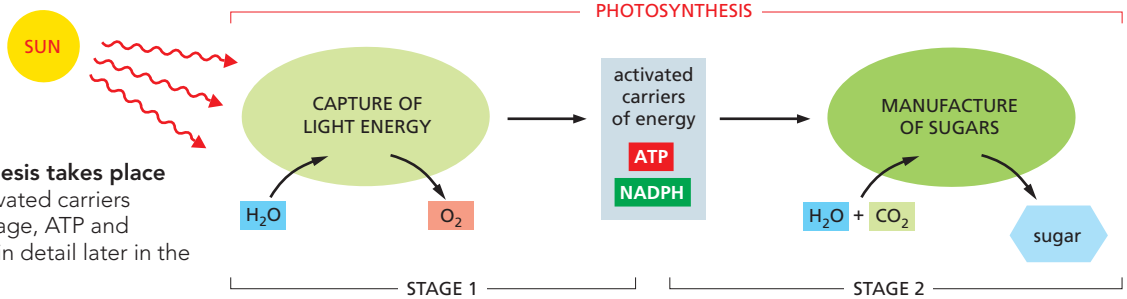


Figure 3–8 Photosynthesis takes place in two stages. The activated carriers generated in the first stage, ATP and NADPH, are described in detail later in the chapter.

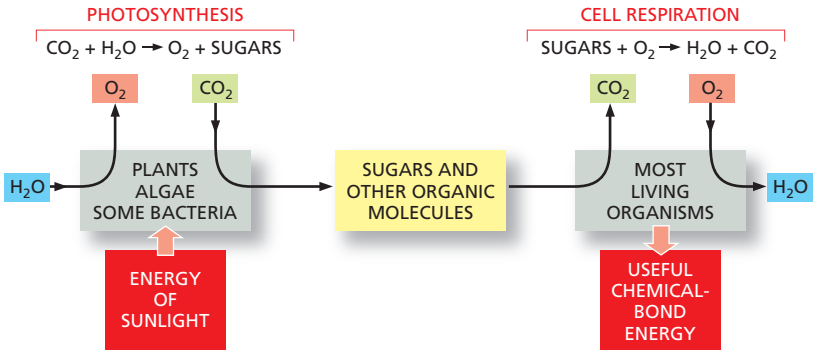


Figure 3–9 Photosynthesis and cell respiration are complementary processes in the living world. The left side of the diagram shows how photosynthesis—carried out by plants and photosynthetic microorganisms—uses the energy of sunlight to produce sugars and other organic molecules from the carbon atoms in CO_2 in the atmosphere. In turn, these molecules serve as food for other organisms. The right side of the diagram shows how cell respiration in most organisms—including plants and other photosynthetic organisms—uses O_2 to oxidize food molecules, releasing the same carbon atoms in the form of CO_2 back to the atmosphere. In the process, the organisms obtain the useful chemical-bond energy that they need to survive.

The first cells on Earth are thought to have been capable of neither photosynthesis nor cell respiration (discussed in Chapter 14). However, photosynthesis must have preceded cell respiration on the Earth, because there is strong evidence that billions of years of photosynthesis were required to release enough O_2 to create an atmosphere that could support respiration.

planet for so long that they have become an essential part of each other's environments. The oxygen released by photosynthesis is consumed by nearly all organisms for the oxidative breakdown of organic molecules. And some of the CO_2 molecules that today are incorporated into organic molecules by photosynthesis in a green leaf were released yesterday into the atmosphere by the respiration of an animal, a fungus, or the plant itself—or by the burning of fossil fuels. Carbon atoms therefore pass through a huge cycle that involves the entire *biosphere*—the collection of living things on Earth—as they move between individual organisms (**Figure 3–10**).

Oxidation and Reduction Involve Electron Transfers

The cell does not oxidize organic molecules in one step, as occurs when organic material is burned in a fire. Through the use of enzyme catalysts, metabolism directs the molecules through a series of chemical reactions, few of which actually involve the direct addition of oxygen. Before we consider these reactions, we need to explain what is meant by oxidation.

Although the term **oxidation** literally means the addition of oxygen atoms to a molecule, oxidation is said to occur in any reaction in which electrons are transferred between atoms. Oxidation, in this sense, involves the removal of electrons from an atom. Thus, Fe^{2+} is oxidized when it loses an electron to become Fe^{3+}. The converse reaction, called **reduction**, involves the addition of electrons to an atom. Fe^{3+} is reduced when it gains an electron to become Fe^{2+}, and a chlorine atom is reduced when it gains an electron to become Cl^-.

Because the number of electrons is conserved in a chemical reaction (there is no net loss or gain), oxidation and reduction always occur simultaneously: that is, if one molecule gains an electron in a reaction (reduction), a second molecule must lose the electron (oxidation).

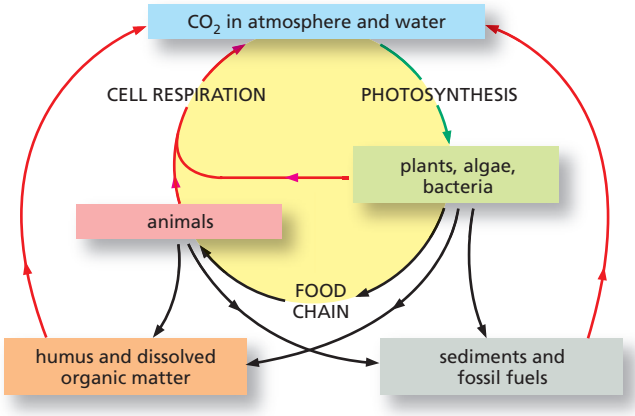


Figure 3–10 Carbon atoms cycle continuously through the biosphere. Individual carbon atoms are incorporated into organic molecules of the living world by the photosynthetic activity of plants, algae, and bacteria. They then pass to animals and microorganisms—as well as into organic material in soil and oceans—and are ultimately restored to the atmosphere in the form of CO_2 when organic molecules are oxidized by cells during respiration or burned by humans as fossil fuels. In this diagram, the *green* arrow denotes an uptake of CO_2, whereas the *red* arrows indicate CO_2 release.

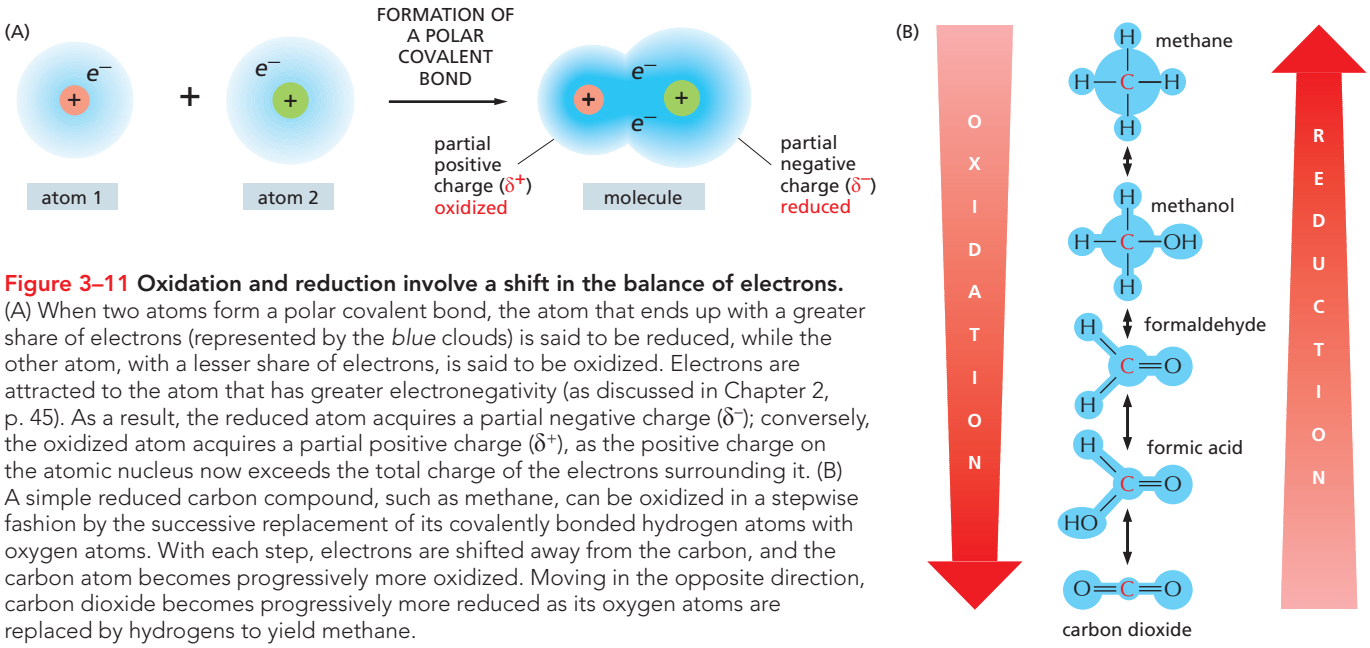


Figure 3–11 Oxidation and reduction involve a shift in the balance of electrons. (A) When two atoms form a polar covalent bond, the atom that ends up with a greater share of electrons (represented by the *blue* clouds) is said to be reduced, while the other atom, with a lesser share of electrons, is said to be oxidized. Electrons are attracted to the atom that has greater electronegativity (as discussed in Chapter 2, p. 45). As a result, the reduced atom acquires a partial negative charge (δ^-); conversely, the oxidized atom acquires a partial positive charge (δ^+), as the positive charge on the atomic nucleus now exceeds the total charge of the electrons surrounding it. (B) A simple reduced carbon compound, such as methane, can be oxidized in a stepwise fashion by the successive replacement of its covalently bonded hydrogen atoms with oxygen atoms. With each step, electrons are shifted away from the carbon, and the carbon atom becomes progressively more oxidized. Moving in the opposite direction, carbon dioxide becomes progressively more reduced as its oxygen atoms are replaced by hydrogens to yield methane.

Why is a "gain" of electrons referred to as a "reduction"? The term arose before anything was known about the movement of electrons. Originally, reduction reactions involved a liberation of oxygen—for example, when metals are extracted from ores by heating—which caused the samples to become lighter; in other words, "reduced" in mass.

It is important to recognize that the terms oxidation and reduction apply even when there is only a partial shift of electrons between atoms. When a carbon atom becomes covalently bonded to an atom with a strong affinity for electrons—oxygen, chlorine, or sulfur, for example—it gives up more than its equal share of electrons to form a *polar covalent bond*. The positive charge of the carbon nucleus now slightly exceeds the negative charge of its electrons, so that the carbon atom acquires a partial positive charge (δ^+) and is said to be oxidized. Conversely, the carbon atom in a C–H bond has somewhat more than its share of electrons; it acquires a partial negative charge (δ^-) and so is said to be reduced (**Figure 3–11A**).

In such oxidation–reduction reactions, electrons generally do not travel alone. When a molecule in a cell picks up an electron (e^-), it often picks up a proton (H^+) at the same time (protons being freely available in water). The net effect in this case is to add a hydrogen atom to the molecule:

$$A + e^- + H^+ \rightarrow AH$$

Even though a proton is involved (in addition to the electron), such *hydrogenation* reactions are reductions, and the reverse *dehydrogenation* reactions are oxidations. An easy way to tell whether an organic molecule is being oxidized or reduced is to count its C–H bonds: an increase in the number of C–H bonds indicates a reduction, whereas a decrease indicates an oxidation (**Figure 3–11B**).

As we will see later in this chapter—and again in Chapter 13—cells use enzymes to catalyze the oxidation of organic molecules in small steps, through a sequence of reactions that allows much of the energy that is released to be harvested in useful forms, instead of being liberated as heat.

FREE ENERGY AND CATALYSIS

Life depends on the highly specific chemical reactions that take place inside cells. The vast majority of these reactions are catalyzed by proteins called **enzymes**. Enzymes, like cells, must obey the second law of

thermodynamics. Although an individual enzyme can greatly accelerate an energetically favorable reaction—one that produces disorder in the universe—it cannot force an energetically unfavorable reaction to occur. Cells, however, must do just that in order to grow and divide—or just to survive. They must build highly ordered and energy-rich molecules from small and simple ones—a process that requires an input of energy.

To understand how enzymes promote the acceleration of the specific chemical reactions needed to sustain life, we first need to examine the energetics involved. In this section, we consider how the free energy of molecules contributes to their chemistry, and we see how free-energy changes—which reflect how much total disorder is generated in the universe by a reaction—influence whether and how a reaction will proceed. Examining these energetic concepts will reveal how enzymes working together can exploit the free-energy changes of different reactions to drive the energetically unfavorable reactions that produce biological order. This type of enzyme-assisted catalysis is crucial for cells: without it, life could not exist.

Chemical Reactions Proceed in the Direction That Causes a Loss of Free Energy

Paper burns readily, releasing into the atmosphere water and carbon dioxide as gases, while simultaneously releasing energy as heat:

$$\text{paper} + O_2 \rightarrow \text{smoke} + \text{ashes} + \text{heat} + CO_2 + H_2O$$

This reaction occurs in only one direction: smoke and ashes never spontaneously gather carbon dioxide and water from the heated atmosphere and reconstitute themselves into paper. When paper burns, most of its chemical energy is dissipated as heat. This heat is not lost from the universe, since energy can never be created or destroyed; instead, it is irretrievably dispersed in the chaotic random thermal motions of molecules. In the language of thermodynamics, there has been a release of *free energy*—that is, energy that can be harnessed to do work or drive chemical reactions. This release reflects a loss of orderliness in the way the energy and molecules had been stored in the paper; the greater the free-energy change, the greater the amount of disorder created in the universe when the reaction occurs.

We will discuss free energy in more detail shortly, but a general principle can be summarized as follows: chemical reactions proceed only in the direction that leads to a loss of free energy. In other words, the spontaneous direction for any reaction is the direction that goes "downhill." A "downhill" reaction in this sense is said to be energetically favorable.

Enzymes Reduce the Energy Needed to Initiate Spontaneous Reactions

Although the most energetically favorable form of carbon under ordinary conditions is CO_2, and that of hydrogen is H_2O, a living organism will not disappear in a puff of smoke, and the book in your hands will not burst spontaneously into flames. This is because the molecules in both the living organism and the book are in a relatively stable state, and they cannot be changed to lower-energy states without an initial input of energy. In other words, a molecule requires a boost over an energy barrier before it can undergo a chemical reaction that moves it to a lower-energy (more stable) state. This boost is known as the **activation energy** (**Figure 3–12A**). In the case of a burning book, the activation energy is provided by the heat of a lighted match. But cells can't raise their temperature to drive biological reactions. Inside cells, the push over the energy barrier is aided by enzymes.

QUESTION 3–2

In which of the following reactions does the *red* atom undergo an oxidation?

A. $Na \rightarrow Na^+$ (Na atom → Na^+ ion)
B. $Cl \rightarrow Cl^-$ (Cl atom → Cl^- ion)
C. $CH_3CH_2OH \rightarrow CH_3CHO$ (ethanol → acetaldehyde)
D. $CH_3CHO \rightarrow CH_3COO^-$ (acetaldehyde → acetic acid)
E. $CH_2{=}CH_2 \rightarrow CH_3CH_3$ (ethene → ethane)

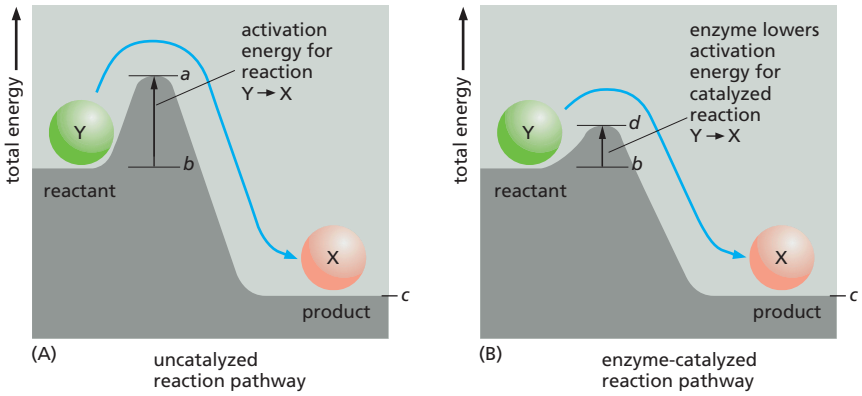


Figure 3–12 Even energetically favorable reactions require activation energy to get them started. (A) Compound Y (a reactant) is in a relatively stable state; thus energy is required to convert it to compound X (a product), even though X is at a lower overall energy level than Y. This conversion will not take place, therefore, unless compound Y can acquire enough *activation energy* (energy *a* minus energy *b*) from its surroundings to undergo the reaction that converts it into compound X. This energy may be provided by means of an unusually energetic collision with other molecules. For the reverse reaction, X ⟶ Y, the activation energy required will be much larger (energy *a* minus energy *c*); this reaction will therefore occur much more rarely. The total energy change for the energetically favorable reaction Y ⟶ X is energy *c* minus energy *b*, a negative number, which corresponds to a loss of free energy. (B) Energy barriers for specific reactions can be lowered by catalysts, as indicated by the line marked *d*. Enzymes are particularly effective catalysts because they greatly reduce the activation energy for the reactions they catalyze. Note that activation energies are always positive.

Each enzyme binds tightly to one or two molecules, called **substrates**, and holds them in a way that greatly reduces the activation energy needed to facilitate a specific chemical interaction between them (**Figure 3–12B**). A substance that can lower the activation energy of a reaction is termed a **catalyst**; catalysts increase the rate of chemical reactions because they allow a much larger proportion of the random collisions with surrounding molecules to kick the substrates over the energy barrier, as illustrated in **Figure 3–13** and **Figure 3–14A**. Enzymes are among the most effective catalysts known. They can speed up reactions by a factor of as much as 10^{14}—that is, trillions of times faster than the same reactions would proceed without an enzyme catalyst. Enzymes therefore allow reactions that would not otherwise occur to proceed rapidly at the normal temperature inside cells.

Unlike the effects of temperature, enzymes are highly selective. Each enzyme usually speeds up—or *catalyzes*—only one particular reaction out of the several possible reactions that its substrate molecules could undergo. In this way, enzymes direct each of the many different molecules in a cell along specific reaction pathways (**Figure 3–14B** and **C**), thereby producing the compounds that the cell actually needs.

Like all catalysts, enzyme molecules themselves remain unchanged after participating in a reaction and can therefore act over and over again (**Figure 3–15**). In Chapter 4, we will discuss further how enzymes work, after we have looked in detail at the molecular structure of proteins.

The Free-Energy Change for a Reaction Determines Whether It Can Occur

According to the second law of thermodynamics, a chemical reaction can proceed only if it results in a net (overall) increase in the disorder of

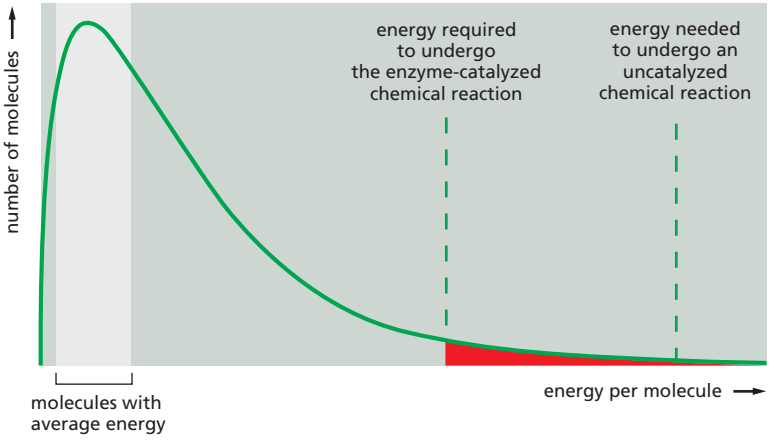


Figure 3–13 Lowering the activation energy greatly increases the probability that a reaction will occur. At any given instant, a population of identical substrate molecules will have a range of energies, distributed as shown on the graph. The varying energies come from collisions with surrounding molecules, which make the substrate molecules jiggle, vibrate, and spin. For a molecule to undergo a chemical reaction, the energy of the molecule must exceed the activation-energy barrier for that reaction (*dashed* lines); for most biological reactions, this almost never happens without enzyme catalysis. Even with enzyme catalysis, only a small fraction of substrate molecules (*red* shaded area) will experience the highly energetic collisions needed to reach an energy state high enough for them to undergo a reaction.

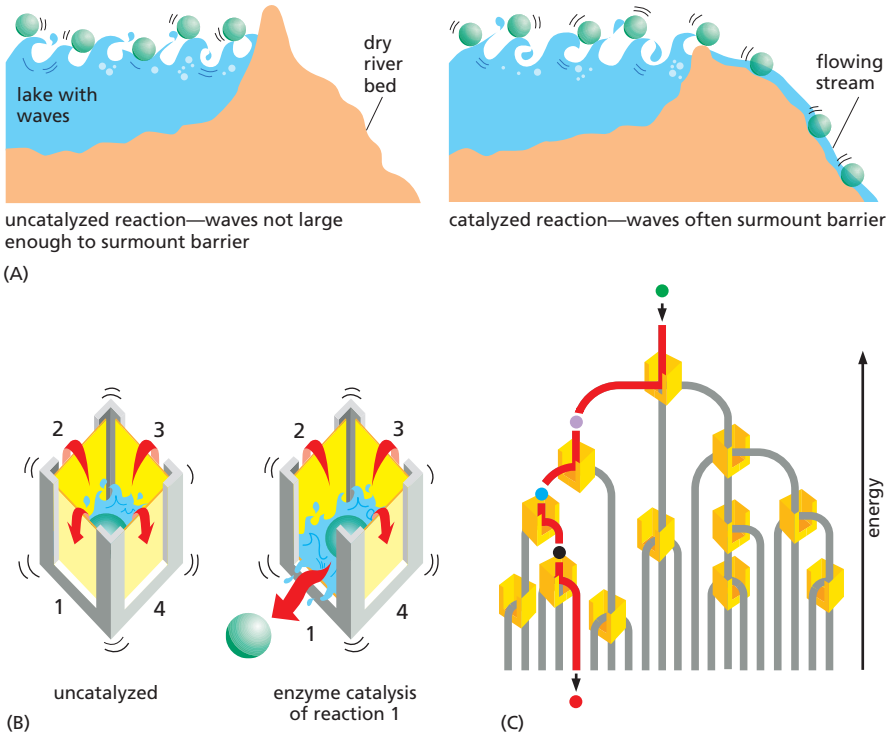


Figure 3–14 Enzymes catalyze reactions by lowering the activation-energy barrier. (A) The dam represents the activation energy, which is lowered by enzyme catalysis. Each *green* ball represents a potential substrate molecule that is bouncing up and down in energy level owing to constant encounters with waves, an analogy for the thermal bombardment of substrate molecules by surrounding water molecules. When the barrier—the activation energy—is lowered significantly, the balls (substrate molecules) with sufficient energy can roll downhill, an energetically favorable movement. (B) The four walls of the box represent the activation-energy barriers for four different chemical reactions that are all energetically favorable because the products are at lower energy levels than the substrates. In the left-hand box, none of these reactions occurs because even the largest waves are not large enough to surmount any of the energy barriers. In the right-hand box, enzyme catalysis lowers the activation energy for reaction number 1 only; now the jostling of the waves allows the substrate molecule to pass over this energy barrier, allowing reaction 1 to proceed (Movie 3.1). (C) A branching set of reactions with a selected set of enzymes (*yellow* boxes) serves to illustrate how a series of enzyme-catalyzed reactions—by controlling which reaction will take place at each junction—determines the exact reaction pathway followed by each molecule inside the cell.

the universe (see Figure 3–5). Disorder increases when useful energy that could be harnessed to do work is dissipated as heat. The useful energy in a system is known as its **free energy**, or ***G***. And because chemical reactions involve a transition from one molecular state to another, the term that is of most interest to chemists and cell biologists is the **free-energy change**, denoted **ΔG** ("Delta *G*").

Let's consider a collection of molecules. ΔG measures the amount of disorder created in the universe when a reaction involving these molecules takes place. *Energetically favorable* reactions, by definition, are those that create disorder in the universe by decreasing the free energy of the system to which they belong; in other words, they have a *negative* ΔG (**Figure 3–16**).

A reaction can occur spontaneously only if ΔG is negative. On a macroscopic scale, an energetically favorable reaction with a negative ΔG is the relaxation of a compressed spring into an expanded state, which releases its stored elastic energy as heat to its surroundings. On a microscopic scale, an energetically favorable reaction—one with a negative ΔG—occurs when salt (NaCl) dissolves in water. Note that just because a reaction can occur spontaneously does not mean it will occur quickly. The decay of diamonds into graphite is a spontaneous process—but it takes millions of years.

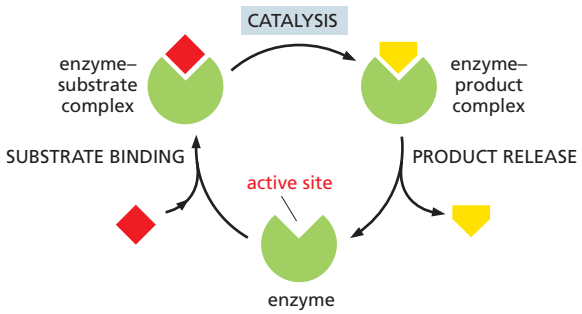


Figure 3–15 Enzymes convert substrates to products while remaining unchanged themselves. Catalysis takes place in a cycle in which a substrate molecule (*red*) binds to an enzyme and undergoes a reaction to form a product molecule (*yellow*), which then gets released. Although the enzyme participates in the reaction, it remains unchanged.

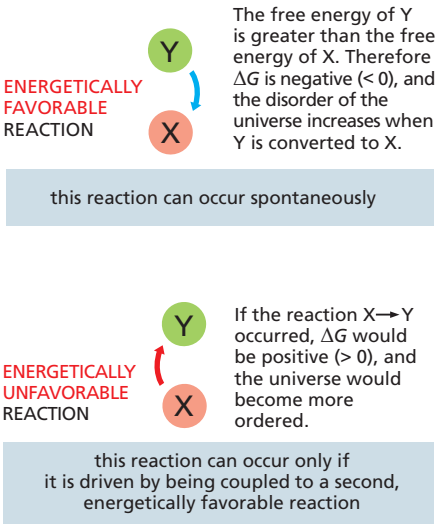


Figure 3–16 Energetically favorable reactions have a negative ΔG, whereas energetically unfavorable reactions have a positive ΔG. Imagine, for example, that molecule Y has a free energy (*G*) of 10 kilojoules (kJ) per mole, whereas X has a free energy of 4 kJ/mole. The reaction Y ⟶ X therefore has a ΔG *of* −6 kJ/mole, making it energetically favorable.

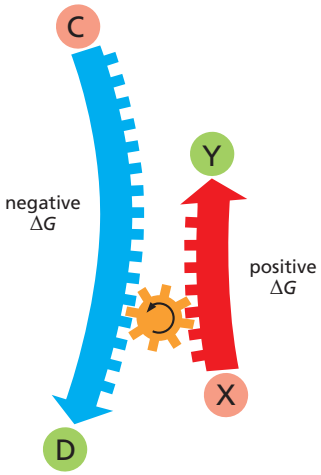


Figure 3–17 Reaction coupling can drive an energetically unfavorable reaction. The energetically unfavorable ($\Delta G > 0$) reaction X ⟶ Y cannot occur unless it is coupled to an energetically favorable ($\Delta G < 0$) reaction C ⟶ D, such that the net free-energy change for the pair of reactions is negative (less than 0).

Energetically unfavorable reactions, by contrast, create order in the universe; they have a *positive* ΔG. Such reactions—for example, the formation of a peptide bond between two amino acids—cannot occur spontaneously; they take place only when they are coupled to a second reaction with a negative ΔG large enough that the net ΔG of the entire process is negative (**Figure 3–17**). Life is possible because enzymes can create biological order by coupling energetically unfavorable reactions with energetically favorable ones. These critical concepts are summarized, with examples, in **Panel 3–1** (pp. 94–95).

ΔG Changes as a Reaction Proceeds Toward Equilibrium

It's easy to see how a tensed spring, when left to itself, will relax and release its stored energy to the environment as heat. But chemical reactions are a bit more complex—and harder to intuit. That's because whether a reaction will proceed in a particular direction depends not only on the energy stored in each individual molecule, but also on the concentrations of the molecules in the reaction mixture. Going back to our jiggling box of coins, more coins will flip from a head to a tail orientation when the box contains 90 heads and 10 tails than when the box contains 10 heads and 90 tails.

The same is true for a chemical reaction. As the energetically favorable reaction Y ⟶ X proceeds, the concentration of the product X will increase and the concentration of the substrate Y will decrease. This change in relative concentrations of substrate and product will cause the ratio of Y to X to shrink, making the initially favorable ΔG less and less negative. Unless more Y is added, the reaction will slow and eventually stop.

Because ΔG changes as products accumulate and substrates are depleted, chemical reactions will generally proceed until they reach a state of **equilibrium**. At that point, the rates of the forward and reverse reactions are equal, and there is no further net change in the concentrations of substrate or product (**Figure 3–18**). For reactions at chemical equilibrium, $\Delta G = 0$, so the reaction will not proceed forward or backward, and no work can be done.

Such a state of chemical inactivity would be incompatible with life, inevitably allowing chemical decay to overcome the cell. Living cells work hard to avoid reaching a state of complete chemical equilibrium. They are constantly exchanging materials with their environment: replenishing nutrients and eliminating waste products. In addition, many of the individual reactions in the cell's complex metabolic network also exist in disequilibrium because the products of one reaction are continually being siphoned off to become the substrates in a subsequent reaction. Rarely do products and substrates reach concentrations at which the forward and reverse reaction rates are equal.

The Standard Free-Energy Change, $\Delta G°$, Makes It Possible to Compare the Energetics of Different Reactions

Because ΔG depends on the concentrations of the molecules in the reaction mixture at any given time, it is not a particularly useful value for comparing the relative energies of different types of chemical reactions. But such energetic assessments are necessary, for example, to predict whether an energetically favorable reaction is likely to have a ΔG negative enough to drive an energetically unfavorable reaction. To compare reactions in this way, we need to turn to the **standard free-energy change** of a reaction, $\Delta G°$. A reaction's $\Delta G°$ is independent of concentration; it depends only on the intrinsic characters of the reacting molecules, based

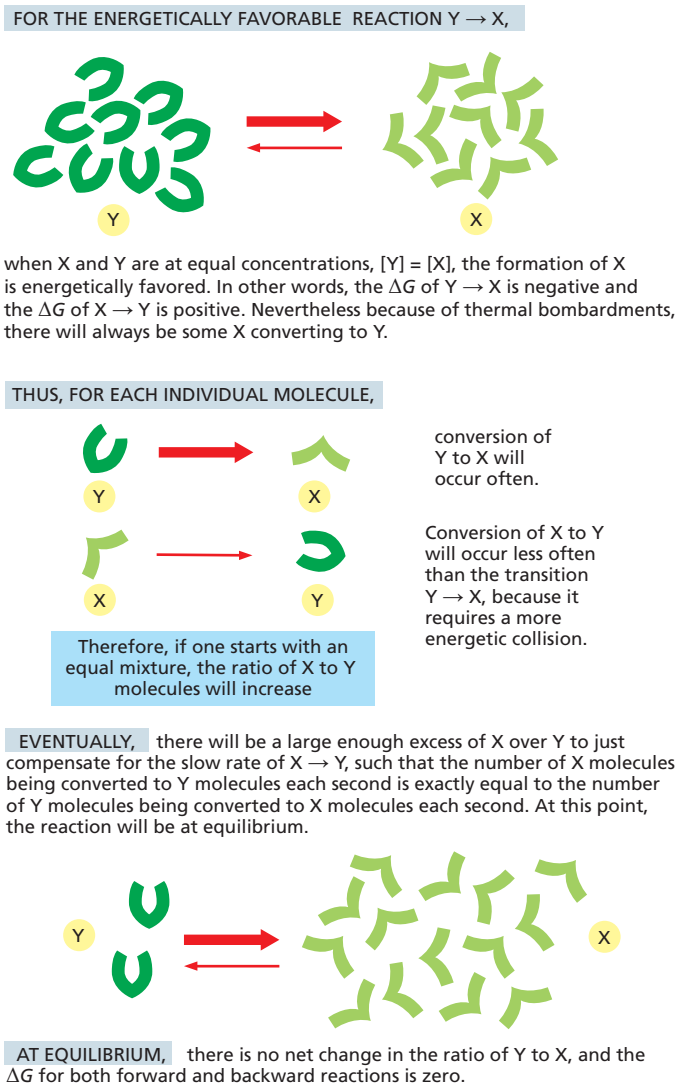


Figure 3–18 Reactions will eventually reach a chemical equilibrium. At that point, the forward and the backward fluxes of reacting molecules are equal and opposite. The widths of the arrows indicate the relative rates at which an *individual molecule* converts.

on their behavior under ideal conditions where the concentrations of all the reactants are set to the same fixed value of 1 mole/liter in aqueous solution.

A large body of thermodynamic data has been collected from which ΔG° can be calculated for most metabolic reactions. Some common reactions are compared in terms of their ΔG° in Panel 3–1 (pp. 94–95).

The ΔG of a reaction can be calculated from ΔG° if the concentrations of the reactants and products are known. For the simple reaction Y → X, their relationship follows this equation:

$$\Delta G = \Delta G^\circ + RT \ln \frac{[X]}{[Y]}$$

where ΔG° is in kilojoules per mole, [Y] and [X] denote the concentrations of Y and X in moles/liter (a mole is 6×10^{23} molecules of a substance), ln is the natural logarithm, and RT is the product of the gas constant, R, and the absolute temperature, T. At 37°C, $RT = 2.58$.

From this equation, we can see that when the concentrations of reactants and products are equal—in other words, [X]/[Y] = 1—the value of ΔG equals the value of ΔG° (because ln 1 = 0). Thus when the reactants and products are present in equal concentrations, the direction of the reaction depends entirely on the intrinsic properties of the molecules.

QUESTION 3–3

Consider the analogy of the jiggling box containing coins that was described on page 83. The reaction, the flipping of coins that either face heads up (H) or tails up (T), is described by the equation H ↔ T, where the rate of the forward reaction equals the rate of the reverse reaction.

A. What are ΔG and ΔG° in this analogy?

B. What corresponds to the temperature at which the reaction proceeds? What corresponds to the activation energy of the reaction? Assume you have an "enzyme," called jigglase, which catalyzes this reaction. What would the effect of jigglase be and what, mechanically, might jigglase do in this analogy?

PANEL 3–1 FREE ENERGY AND BIOLOGICAL REACTIONS

FREE ENERGY

This panel reviews the concept of free energy and offers examples showing how changes in free energy determine whether—and how—biological reactions occur.

The molecules of a living cell possess energy because of their vibrations, rotations, and movement through space, and because of the energy that is stored in the bonds between individual atoms.

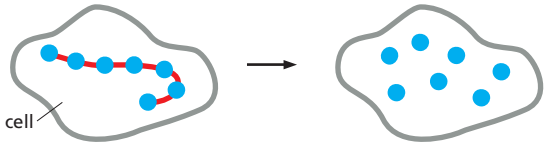
The free energy, *G* (in kJ/mole), measures the energy of a molecule that could in principle be used to do useful work at constant temperature, as in a living cell. Energy can also be expressed in calories (1 joule = 0.24 calories).

REACTIONS CAUSE DISORDER

Think of a chemical reaction occurring in a cell that has a constant temperature and volume. This reaction can produce disorder in two ways.

1 Changes of bond energy of the reacting molecules can cause heat to be released, which disorders the environment around the cell.

2 The reaction can decrease the amount of order in the cell—for example, by breaking apart a long chain of molecules, or by disrupting an interaction that prevents bond rotations.



PREDICTING REACTIONS

To predict the outcome of a reaction (Will it proceed to the right or to the left? At what point will it stop?), we must determine its standard free-energy change ($\Delta G°$). This quantity represents the gain or loss of free energy as one mole of reactant is converted to one mole of product under "standard conditions" (all molecules present in aqueous solution at a concentration of 1 M and pH 7.0).

driving force

$\Delta G°$ for some reactions

glucose 1-P → glucose 6-P	–7.3 kJ/mole
sucrose → glucose + fructose	–23 kJ/mole
ATP → ADP + P	–30.5 kJ/mole
glucose + $6O_2$ → $6CO_2$ + $6H_2O$	–2867 kJ/mole

ΔG ("DELTA G")

Changes in free energy occurring in a reaction are denoted by ΔG, where "Δ" indicates a difference. Thus, for the reaction

$$A + B \longrightarrow C + D$$

ΔG = free energy (C + D) minus free energy (A + B)

ΔG measures the amount of disorder caused by a reaction: the change in order inside the cell, plus the change in order of the surroundings caused by the heat released.

ΔG is useful because it measures how far away from equilibrium a reaction is. The reaction

$$ATP \longrightarrow ADP + P$$

has a large negative ΔG because cells keep the reaction a long way from equilibrium by continually making fresh ATP. However, if the cell dies, then most of its ATP will be hydrolyzed until equilibrium is reached; at equilibrium, the forward and backward reactions occur at equal rates and $\Delta G = 0$.

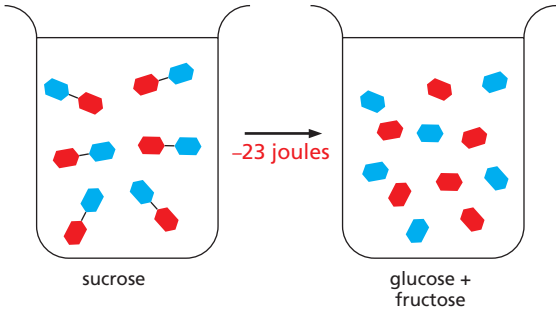
SPONTANEOUS REACTIONS

From the second law of thermodynamics, we know that the disorder of the universe can only increase. ΔG is *negative* if the disorder of the universe (reaction plus surroundings) *increases*.

In other words, a chemical reaction that occurs spontaneously must have a negative ΔG:

$$G_{products} - G_{reactants} = \Delta G < 0$$

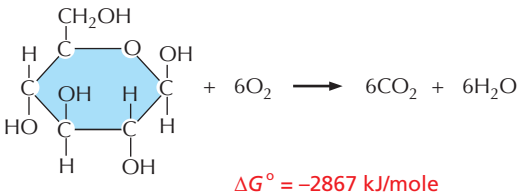
EXAMPLE: The difference in free energy of 100 mL of 10 mM sucrose (common sugar) and 100 mL of 10 mM glucose plus 10 mM fructose is about –23 joules. Therefore, the hydrolysis reaction that produces two monosaccharides from a disaccharide (sucrose → glucose + fructose) can proceed spontaneously.



In contrast, the reverse reaction (glucose + fructose → sucrose), which has a ΔG of +23 joules, could not occur without an input of energy from a coupled reaction.

REACTION RATES

A spontaneous reaction is not necessarily a rapid reaction: a reaction with a negative free-energy change (ΔG) will not necessarily occur rapidly by itself. Consider, for example, the combustion of glucose in oxygen:



$\Delta G^\circ = -2867$ kJ/mole

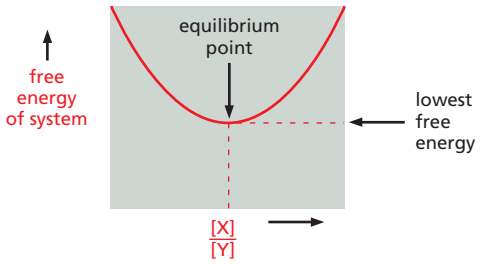
Even this highly favorable reaction may not occur for centuries unless enzymes are present to speed up the process. Enzymes are able to catalyze reactions and speed up their rate, but they cannot change the ΔG° of a reaction.

CHEMICAL EQUILIBRIA

A fixed relationship exists between the standard free-energy change of a reaction, ΔG°, and its equilibrium constant K. For example, the reversible reaction

$$Y \rightleftarrows X$$

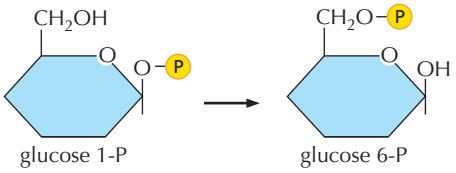
will proceed until the ratio of concentrations [X]/[Y] is equal to K (note: square brackets [ ] indicate concentration). At this point, the free energy of the system will have its lowest value.



At 37°C, $\Delta G^\circ = -5.94 \log_{10} K$ (see text, p. 96)

$$K = 10^{-\Delta G^\circ/5.94}$$

For example, the reaction



has $\Delta G^\circ = -7.3$ kJ/mole. Therefore, its equilibrium constant

$$K = 10^{(7.3/5.94)} = 10^{(1.23)} = 17$$

So the reaction will reach steady state when

[glucose 6-P]/[glucose 1-P] = 17

COUPLED REACTIONS

Reactions can be "coupled" together if they share one or more intermediates. In this case, the overall free-energy change is simply the sum of the individual ΔG° values. A reaction that is unfavorable (has a positive ΔG°) can for this reason be driven by a second, highly favorable reaction.

SINGLE REACTION

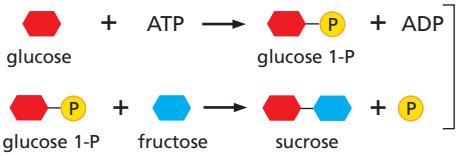
$\Delta G^\circ = +23$ kJ/mole

NET RESULT: reaction will not occur

$\Delta G^\circ = -30.5$ kJ/mole

NET RESULT: reaction is highly favorable

COUPLED REACTIONS



$\Delta G^\circ = 23 - 30.5 = -7.5$ kJ/mole

NET RESULT: sucrose is made in a reaction driven by the hydrolysis of ATP

HIGH-ENERGY BONDS

One of the most common reactions in the cell is hydrolysis, in which a covalent bond is split by adding water.

The ΔG° for this reaction is sometimes loosely termed the "bond energy." Compounds such as acetyl phosphate and ATP, which have a large negative ΔG° of hydrolysis in an aqueous solution, are said to have "high-energy" bonds.

	ΔG° (kJ/mole)
acetyl–P → acetate + P	–43.1
ATP → ADP + P	–30.5
glucose 6-P → glucose + P	–13.8

(Note that, for simplicity, H_2O is omitted from the above equations.)

The Equilibrium Constant Is Directly Proportional to $\Delta G°$

As mentioned earlier, all chemical reactions tend to proceed toward equilibrium. Knowing where that equilibrium lies for any given reaction will reveal which way the reaction will proceed—and how far it will go. For example, if a reaction is at equilibrium when the concentration of the product is ten times the concentration of the substrate, and we begin with a surplus of substrate and little or no product, the reaction will continue to proceed forward. The ratio of substrate to product at this equilibrium point is called the reaction's **equilibrium constant**, ***K***. For the simple reaction Y → X,

$$K = \frac{[X]}{[Y]}$$

where [X] is the concentration of the product and [Y] is the concentration of the substrate at equilibrium. In the example we just described, $K = 10$.

The equilibrium constant depends on the intrinsic properties of the molecules involved, as expressed by $\Delta G°$. In fact, the equilibrium constant is directly proportional to $\Delta G°$. Let's see why.

At equilibrium, the rate of the forward reaction is exactly balanced by the rate of the reverse reaction. At that point, $\Delta G = 0$, and there is no net change of free energy to drive the reaction in either direction (see Panel 3–1, pp. 94–95).

Now, if we return to the equation presented on page 93,

$$\Delta G = \Delta G° + RT \ln \frac{[X]}{[Y]}$$

we can see that, at equilibrium at 37°C, where $\Delta G = 0$ and the constant $RT = 2.58$, this equation becomes:

$$\Delta G° = -2.58 \ln \frac{[X]}{[Y]}$$

In other words, $\Delta G°$ is directly proportional to the equilibrium constant, K:

$$\Delta G° = -2.58 \ln K$$

If we convert this equation from natural log (ln) to the more commonly used base-10 logarithm (log), we get

$$\Delta G° = -5.94 \log K$$

This equation reveals how the equilibrium ratio of Y to X, expressed as the equilibrium constant K, depends on the intrinsic character of the molecules, as expressed in the value of $\Delta G°$. Thus, for the reaction we presented, Y → X, where $K = 10$, $\Delta G° = -5.94$ kJ/mole. In fact, for every 5.94 kJ/mole difference in free energy at 37°C, the equilibrium constant for a reaction changes by a factor of 10, as shown in **Table 3–1**. Thus, the more energetically favorable the reaction, the more product will accumulate when the reaction proceeds to equilibrium. For a reaction with a $\Delta G°$ of –17.8 kJ/mole, K will equal 1000, which means that at equilibrium, there will be 1000 molecules of product for every molecule of substrate present.

TABLE 3–1 RELATIONSHIP BETWEEN THE STANDARD FREE-ENERGY CHANGE, $\Delta G°$, AND THE EQUILIBRIUM CONSTANT

Equilibrium Constant [X]/[Y]	Standard Free-Energy Change ($\Delta G°$) for Reaction Y → X (kJ/mole)
10^5	–29.7
10^4	–23.8
10^3	–17.8
10^2	–11.9
10	–5.9
1	0
10^{-1}	5.9
10^{-2}	11.9
10^{-3}	17.8
10^{-4}	23.8
10^{-5}	29.7

Values of the equilibrium constant were calculated for the simple chemical reaction Y → X, using the equation given in the text.

The $\Delta G°$ values given here are in kilojoules per mole at 37°C. As explained in the text, $\Delta G°$ represents the free-energy difference under standard conditions (where all components are present at a concentration of 1 mole/liter).

From this table, we see that if there is a favorable free-energy change of –17.8 kJ/mole for the transition Y → X, there will be 1000 times more molecules of X than of Y at equilibrium ($K = 1000$).

In Complex Reactions, the Equilibrium Constant Includes the Concentrations of All Reactants and Products

We have so far discussed the simplest of reactions, Y → X, in which a single substrate is converted into a single product. But inside cells, it is more common for two reactants to combine to form a single product: A + B → AB. How can we predict how this reaction will proceed?

The same principles apply, except that in this case the equilibrium constant K includes the concentrations of both of the reactants, in addition

to the concentration of the product:

$$K = [AB]/[A][B]$$

The concentrations of both reactants are multiplied in the denominator because the formation of product AB depends on the collision of A and B, and these encounters occur at a rate that is proportional to [A] × [B] (**Figure 3–19**). As with single-substrate reactions, $\Delta G° = -5.94 \log K$ at 37°C. Thus, the relationship between K and $\Delta G°$ is the same as that shown in Table 3–1.

The Equilibrium Constant Also Indicates the Strength of Noncovalent Binding Interactions

The concept of free-energy change does not apply only to chemical reactions where covalent bonds are being broken and formed. It is also used to quantitate the strength of interactions in which one molecule binds to another by means of noncovalent interactions (discussed in Chapter 2, p. 48). Two molecules will bind to each other if the free-energy change for the interaction is negative; that is, the free energy of the resulting complex is lower than the sum of the free energies of the two partners when unbound. Noncovalent interactions are immensely important to cells. They include the binding of substrates to enzymes, the binding of transcription regulators to DNA, and the binding of one protein to another to make the many different structural and functional protein complexes that operate in a living cell.

The equilibrium constant, K, used to describe reactions in which covalent bonds are formed and broken, also reflects the binding strength of a noncovalent interaction between two molecules. This binding strength is a very useful quantity because it indicates how specific the interaction is between the two molecules. When molecule A binds to molecule B to form the complex AB, the reaction proceeds until it reaches equilibrium. At which point the number of association events precisely equals the number of dissociation events; at this point, the concentrations of reactants A and B, and of the complex AB, can be used to determine the equilibrium constant K (see Figure 3–19).

K becomes larger as the *binding energy*—that is, the energy released in the binding interaction—increases. In other words, the larger K is, the greater is the drop in free energy between the dissociated and associated states, and the more tightly the two molecules will bind. Even a

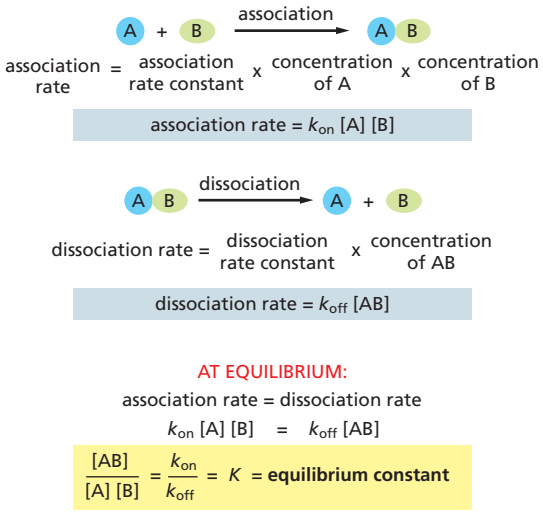


Figure 3–19 The equilibrium constant, *K*, for the reaction A + B → AB depends on the concentrations of A, B, and AB. Molecules A and B must collide in order to interact, and the association rate is therefore proportional to the product of their individual concentrations [A] × [B]. As shown, the ratio of the rate constants k_{on} and k_{off} for the association (bond formation) and the dissociation (bond breakage) reactions, respectively, is equal to the equilibrium constant, K.

Consider 1000 molecules of A and 1000 molecules of B in the cytosol of a eukaryotic cell. The concentration of both will be about 10^{-9} M.

If the equilibrium constant (K) for A + B → AB is 10^{10} liters/mole, then at equilibrium there will be

270	270	730
A molecules	B molecules	AB complexes

If the equilibrium constant is a little weaker, say 10^{8} liters/mole—a value that represents a loss of 11.9 kJ/mole of binding energy from the example above, or 2–3 fewer hydrogen bonds—then there will be

915	915	85
A molecules	B molecules	AB complexes

Figure 3–20 Small changes in the number of weak bonds can have drastic effects on a binding interaction. This example illustrates the dramatic effect of the presence or absence of a few weak noncovalent bonds in the interaction between two cytosolic proteins.

change of a few noncovalent bonds can have a striking effect on a binding interaction, as illustrated in **Figure 3–20**. In this example, a loss of 11.9 kJ/mole of binding energy, equivalent to eliminating a few hydrogen bonds from a binding interaction, can be seen to cause a dramatic decrease in the amount of complex that exists at equilibrium.

For Sequential Reactions, the Changes in Free Energy Are Additive

Now we return to our original concern regarding how cells can generate and maintain order. And more specifically: how can enzymes catalyze reactions that are energetically unfavorable?

One way they do so is by directly coupling energetically unfavorable reactions with energetically favorable ones. Consider, for example, two sequential reactions,

$$X \rightarrow Y \text{ and } Y \rightarrow Z$$

where the $\Delta G°$ values are +21 and –54 kJ/mole, respectively. (Recall that a mole is 6×10^{23} molecules of a substance.) The unfavorable reaction, X → Y, will not occur spontaneously. However, it can be driven by the favorable reaction Y → Z, provided that the second reaction follows the first. That's because the overall free-energy change for the coupled reaction is equal to the sum of the free-energy changes for each individual reaction. In this case, the $\Delta G°$ for the coupled reaction, X → Y → Z, will be –33 kJ/mole, making the overall pathway energetically favorable.

Cells can therefore cause the energetically unfavorable transition, X → Y, to occur if an enzyme catalyzing the X → Y reaction is supplemented by a second enzyme that catalyzes the energetically favorable reaction, Y → Z. In effect, the reaction Y → Z acts as a "siphon," pulling the conversion of all of molecule X to molecule Y, and then to molecule Z (**Figure 3–21**). Several of the reactions in the long pathway that converts sugars into CO_2 and H_2O are energetically unfavorable. This pathway nevertheless

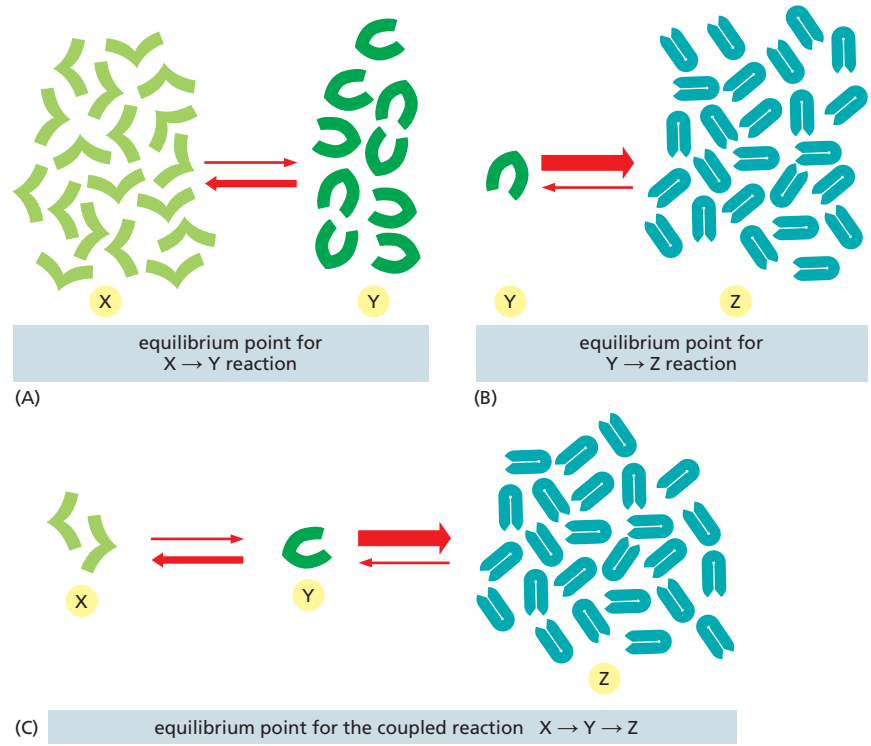


Figure 3–21 An energetically unfavorable reaction can be driven by an energetically favorable follow-on reaction that acts as a chemical siphon. (A) At equilibrium, there are twice as many X molecules as Y molecules. (B) At equilibrium, there are 25 times more Z molecules than Y molecules. (C) If the reactions in (A) and (B) are coupled, nearly all of the X molecules will be converted to Z molecules, as shown. In terms of energetics, the $\Delta G°$ of the Y → Z reaction is so negative that, when coupled to the X → Y reaction, it lowers the ΔG of X → Y. This is because the ΔG of X → Y decreases as the ratio of Y to X declines (see Figure 3–18).

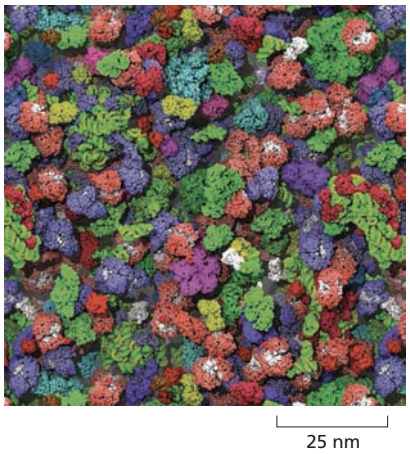


Figure 3–22 The cytosol is crowded with various molecules. Only the macromolecules, which are drawn to scale and displayed in different colors, are shown. Enzymes and other macromolecules diffuse relatively slowly in the cytosol, in part because they interact with so many other macromolecules. Small molecules, by contrast, can diffuse nearly as rapidly as they do in water (see **Movie 1.2**). (From S.R. McGuffee and A.H. Elcock, *PLoS Comput. Biol.* 6(3): e1000694, 2010.)

proceeds rapidly to completion because the total ΔG° for the series of sequential reactions has a large negative value.

Forming a sequential pathway, however, is not the answer for many other metabolic needs. Often the desired reaction is simply X → Y, without further conversion of Y to some other product. Fortunately, there are other, more general ways of using enzymes to couple reactions together, involving the production of activated carriers that can shuttle energy from one reaction site to another, as we discuss shortly.

Enzyme-catalyzed Reactions Depend on Rapid Molecular Collisions

Thus far we have talked about chemical reactions as if they take place in isolation. But the cytosol of a cell is densely packed with molecules of various shapes and sizes (**Figure 3–22**). So how do enzymes and their substrates, which are present in relatively small amounts in the cytosol of a cell, manage to find each other? And how do they do it so quickly? Observations indicate that a typical enzyme can capture and process about a thousand substrate molecules every second.

Rapid binding is possible because molecular motions are enormously fast—very much faster than the human mind can easily imagine. Because of heat energy, molecules are in constant motion and consequently will explore the cytosolic space very efficiently by wandering randomly through it—a process called **diffusion**. In this way, every molecule in the cytosol collides with a huge number of other molecules each second. As these molecules in solution collide and bounce off one another, an individual molecule moves first one way and then another, its path constituting a *random walk* (**Figure 3–23**).

Although the cytosol of a cell is densely packed with molecules of various shapes and sizes, experiments in which fluorescent dyes and other labeled molecules are injected into the cell cytosol show that small organic molecules diffuse through this aqueous gel nearly as rapidly as they do through water. A small organic molecule, such as a substrate, takes only about one-fifth of a second on average to diffuse a distance of 10 μm. Diffusion is therefore an efficient way for small molecules to move limited distances in the cell.

Because proteins diffuse through the cytosol much more slowly than do small molecules, the rate at which an enzyme will encounter its substrate depends on the concentration of the substrate. The most abundant substrates are present in the cell at a concentration of about 0.5 mM. Because pure water is 55 M, there is only about one such substrate molecule in the cell for every 10^5 water molecules. Nevertheless, the site on an enzyme that binds this substrate will be bombarded by about 500,000 random collisions with the substrate every second! For a substrate concentration tenfold lower (0.05 mM), the number of collisions drops to 50,000 per second, and so on. These incredibly numerous collisions play a critical role in life's chemistry.

QUESTION 3–4

For the reactions shown in Figure 3–21, sketch an energy diagram similar to that in Figure 3–12 for the two reactions alone and for the combined reactions. Indicate the standard free-energy changes for the reactions X → Y, Y → Z, and X → Z in the graph. Indicate how enzymes that catalyze these reactions would change the energy diagram.

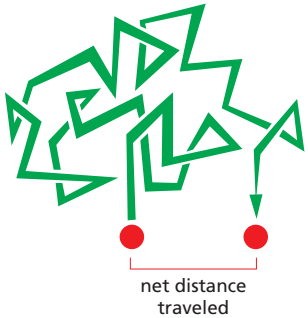


Figure 3–23 A molecule traverses the cytosol by taking a random walk. Molecules in solution move in a random fashion due to the continual buffeting they receive in collisions with other molecules. This movement allows small molecules to diffuse rapidly throughout the cell cytosol (**Movie 3.2**).

QUESTION 3–5

The enzyme carbonic anhydrase is one of the speediest enzymes known. It catalyzes the rapid conversion of CO_2 gas into the much more soluble bicarbonate ion (HCO_3^-). The reaction:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

is very important for the efficient transport of CO_2 from tissues, where CO_2 is produced by respiration, to the lungs, where it is exhaled. Carbonic anhydrase accelerates the reaction 10^7-fold, hydrating 10^5 CO_2 molecules per second at its maximal speed. What do you suppose limits the speed of the enzyme? Sketch a diagram analogous to the one shown in Figure 3–13 and indicate which portion of your diagram has been designed to display the 10^7-fold acceleration.

QUESTION 3–6

In cells, an enzyme catalyzes the reaction AB ⟶ A + B. It was isolated, however, as an enzyme that carries out the opposite reaction A + B ⟶ AB. Explain the paradox.

Noncovalent Interactions Allow Enzymes to Bind Specific Molecules

The first step in any enzyme-catalyzed chemical reaction is the binding of the substrate. Once this step has taken place, the substrate must remain bound to the enzyme long enough for the chemistry to occur. The association of enzyme and substrate is stabilized by the formation of multiple, weak bonds between the participating molecules. These weak interactions—which can include hydrogen bonds, van der Waals attractions, and electrostatic attractions (discussed in Chapter 2)—persist until random thermal motion causes the molecules to dissociate again.

When two colliding molecules have poorly matching surfaces, few noncovalent bonds are formed, and their total energy is negligible compared with that of thermal motion. In this case, the two molecules dissociate as rapidly as they come together (see Figure 2–35). As we saw in Figure 3–20, even small changes in the number of noncovalent bonds made between two interacting molecules can have a dramatic effect on their ability to form a complex. Poor noncovalent bond formation is what prevents unwanted associations from forming between mismatched molecules, such as those between an enzyme and the wrong substrate. Only when the enzyme and substrate are well matched do they form many weak interactions. It is these numerous noncovalent bonds that keep them together long enough for a covalent bond in the substrate molecule to be formed or broken, converting substrate to product.

Enzymes are remarkable catalysts, capturing substrates and releasing products in mere milliseconds. But though an enzyme can lower the activation energy for a reaction, such as Y ⟶ X (see Figure 3–12), it is important to note that the same enzyme will also lower the activation energy for the reverse reaction X ⟶ Y to exactly the same degree. That's because the same noncovalent bonds are formed with the enzyme whether the reaction goes forward or backward. The forward and backward reactions will therefore be accelerated by the same factor by an enzyme, and the equilibrium point for the reaction—and thus its $\Delta G°$—remains unchanged (**Figure 3–24**).

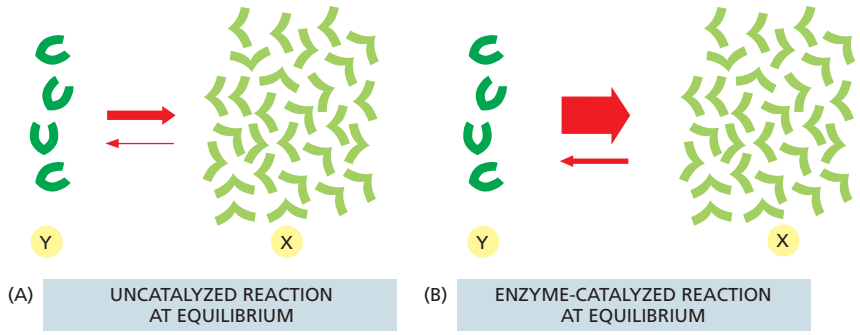


Figure 3–24 Enzymes cannot change the equilibrium point for reactions. Enzymes, like all catalysts, speed up the forward and reverse rates of a reaction by the same amount. Therefore, for both the (A) uncatalyzed and (B) catalyzed reactions shown here, the number of molecules undergoing the transition Y ⟶ X is equal to the number of molecules undergoing the transition X ⟶ Y when the ratio of X molecules to Y molecules is 7 to 1, as illustrated. In other words, both the catalyzed and uncatalyzed reactions will eventually reach the same equilibrium point, although the catalyzed reaction will reach equilibrium much faster.

ACTIVATED CARRIERS AND BIOSYNTHESIS

Much of the energy released by an energetically favorable reaction such as the oxidation of a food molecule must be stored temporarily before it can be used by cells to fuel energetically unfavorable reactions, such as the synthesis of all the other molecules needed by the cell. In most cases, the energy is stored as chemical-bond energy in a set of **activated carriers**, small organic molecules that contain one or more energy-rich covalent bonds. These molecules diffuse rapidly and carry their bond energy from the sites of energy generation to the sites where energy is used either for **biosynthesis** or for the many other energy-requiring activities that a cell must perform (**Figure 3–25**). In a sense, cells use activated carriers like money to pay for the energetically unfavorable reactions that otherwise would not take place.

Activated carriers store energy in an easily exchangeable form, either as a readily transferable chemical group or as readily transferable ("high-energy") electrons. They can serve a dual role as a source of both energy and chemical groups for biosynthetic reactions. As we shall discuss shortly, the most important activated carriers are *ATP* and two molecules that are close chemical cousins, *NADH* and *NADPH*.

An understanding of how cells transform the energy locked in food molecules into a form that can be used to do work required the dedicated effort of the world's finest chemists (**How We Know**, pp. 102–103). Their discoveries, amassed over the first half of the twentieth century, marked the dawn of the study of biochemistry.

The Formation of an Activated Carrier Is Coupled to an Energetically Favorable Reaction

When a fuel molecule such as glucose is oxidized inside a cell, enzyme-catalyzed reactions ensure that a large part of the free energy released is captured in a chemically useful form, rather than being released wastefully as heat. When your cells oxidize the sugar from a chocolate bar, that energy allows you to power metabolic reactions; burning that same chocolate bar in the street will get you nowhere, warming the environment while producing no metabolically useful energy.

In cells, energy capture is achieved by means of a special form of **coupled reaction**, in which an energetically favorable reaction is used to drive an energetically unfavorable one, so that an activated carrier or some other useful molecule is produced. Such coupling requires enzyme catalysis, which is fundamental to all of the energy transactions in the cell.

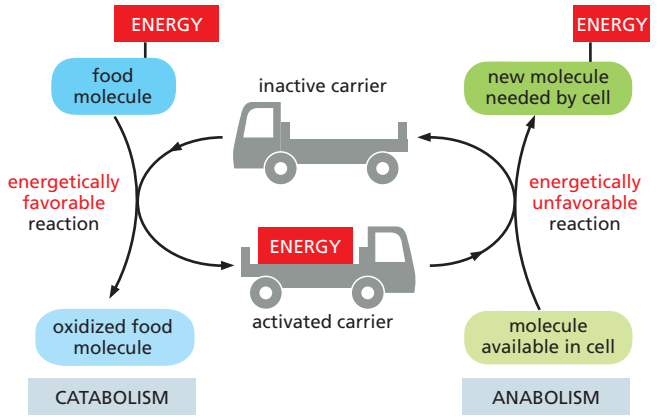


Figure 3–25 Activated carriers can store and transfer energy in a form that cells can use. By serving as intracellular energy shuttles, activated carriers perform their function as go-betweens that link the release of energy from the breakdown of food molecules (catabolism) to the energy-requiring biosynthesis of small and large organic molecules (anabolism).

"HIGH-ENERGY" PHOSPHATE BONDS POWER CELL PROCESSES

Cells require a continuous stream of energy to generate and maintain order, while acquiring the materials they need to survive, grow, and reproduce. But even as late as 1921, very little was known about how energy—which for animal cells is derived from the breakdown of nutrients—is biochemically transformed, stored, and released for work in the cell. It would take the efforts of a handful of biochemists, many of whom worked with Otto Meyerhof—a pioneer in the field of cell metabolism—to get a handle on this fundamental problem.

Muscling in

Meyerhof was trained as a physician in Heidelberg, Germany, and he had a strong interest in physiological chemistry; in particular, he wondered how energy is transformed during chemical reactions in cells. He recognized that between its initial entry in the form of food and its final dissipation as heat, a large amount of energy must be made available by a series of intermediate chemical steps that allow the cell or organism to maintain itself in a state of dynamic equilibrium.

To explore how these mysterious chemical transformations power the work done by cells, Meyerhof focused his attention on muscle. Muscle tissue could be isolated from an animal, such as a frog, and stimulated to contract with a pulse of electricity. And contraction provided a dramatic demonstration of the conversion of energy to a usable, mechanical form.

When Meyerhof got started, all that was known about the chemistry of contraction is that, in active muscle tissue, lactic acid is generated by a process of fermentation. As Meyerhof's first order of business, he demonstrated that this lactic acid comes from the breakdown of glycogen—a branched polymer made of glucose units that serves as an energy store in animal cells, particularly in muscle (see Panel 2–4, p. 73).

While Meyerhof focused on the chemistry, English physiologist Archibald "A.V." Hill determined that working muscles give off heat, both as they contract and as they recover; further, he found that the amount of heat correlates with how hard the muscle is working.

Hill and Meyerhof then showed that the heat produced during muscle relaxation was linked to the resynthesis of glycogen. A portion of the lactic acid made by the muscle would be completely oxidized to CO_2 and water, and the energy from this oxidative breakdown would be used to convert the remaining lactic acid back to glycogen. This conversion of glycogen to lactic acid—and back again—provided the first evidence of cyclical energy transformation in cells (**Figure 3–26**). And in 1922, it earned Meyerhof and Hill a Nobel Prize.

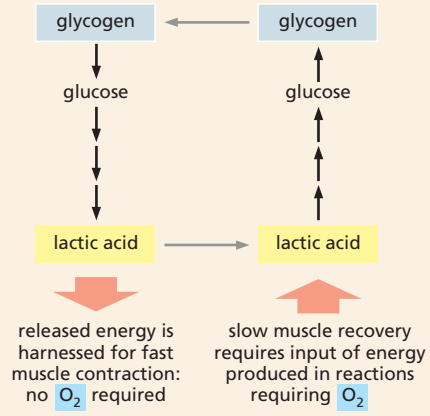


Figure 3–26 A "lactic acid cycle" was thought to supply the energy needed to power muscle contraction. Preparations of frog muscle were stimulated to contract while being held at constant length (isometric contraction). As shown, contraction was accompanied by the breakdown of glycogen and the formation of lactic acid. The energy released by this oxidation was thought to somehow power muscle contraction. Lactic acid is converted back to glycogen as the muscle recovers.

In the mail

But did the conversion of glycogen into lactic acid directly power the mechanical work of muscle contraction? Meyerhof had thought so—until 1927, when a letter arrived from Danish physiologist Einar Lundsgaard. In it, Lundsgaard told Meyerhof of the surprising results of some experiments he had performed both on isolated muscles and in living rabbits and frogs. Lundsgaard had injected muscles with iodoacetate, a compound that inhibits an enzyme involved in the breakdown of sugars (as we discuss in Chapter 13). In these iodoacetate-treated muscles, fermentation was blocked and no lactic acid could be made.

What Lundsgaard discovered was that the poisoned muscles continued to contract. Indeed, animals injected with the compound at first "behaved quite normally," wrote Fritz Lipmann, a biochemist who was working in Meyerhof's laboratory. But after a few minutes, they suddenly keeled over, their muscles frozen in rigor.

But if the formation of lactic acid was not providing fuel for muscle contraction, what was? Lundsgaard went on to show that the source of energy for muscle contraction in poisoned muscles appeared to be a recently discovered molecule called creatine phosphate. When lactic acid formation was blocked by iodoacetate, muscle contraction was accompanied by the hydrolysis of creatine phosphate. When the supply of creatine phosphate was exhausted, muscles seized up permanently.

"The turmoil that this news created in Meyerhof's laboratory is difficult to realize today," wrote Lipmann. The finding contradicted Meyerhof's theory that lactic acid formation powered muscle contraction. And it pointed toward not just an alternative molecule, but a whole new idea: that certain phosphate bonds, when hydrolyzed, could provide energy. "Lundsgaard had discovered that the muscle machine can be driven by phosphate-bond energy, and he shrewdly realized that this type of energy was 'nearer,' as he expressed it, to the conversion of metabolic energy into mechanical energy than lactic acid," wrote Lipmann.

But rather than being upset, Meyerhof welcomed Lundsgaard to his lab in Heidelberg, where he was serving as director of the Kaiser Wilhelm Physiology Institute. There, Lundsgaard made very careful measurements showing that the breakdown of creatine phosphate—and the heat it generated—closely tracked the amount of tension generated by intact muscle.

The most direct conclusion that could be drawn from these observations is that the hydrolysis of creatine phosphate supplied the energy that powers muscle contraction. But in one of his papers published in 1930, Lundsgaard was careful to note that there was another possibility: that in normal muscle, both lactic acid formation and creatine phosphate hydrolysis transferred energy to a third, yet-to-be identified system. This is where ATP comes in.

Squiggle P

Even before Lundsgaard's eye-opening observations, Meyerhof had an interest in the amount of energy contained in various metabolic compounds, particularly those that contained phosphate. He thought that metabolic energy sources might be identified by finding naturally occurring molecules that release unusually large amounts of heat when hydrolyzed. Creatine phosphate was one of those compounds. Another was ATP, which had been discovered in 1929—by Meyerhof's assistant, chemist Karl Lohmann, and, at the same time, by biochemists Cyrus Fiske and Yellapragada Subbarow working in America.

By 1935, Lohmann had demonstrated that the hydrolytic breakdown of creatine phosphate occurs through the transfer of its phosphate group to ADP to form ATP. It is the hydrolysis of ATP that serves as the direct source of energy for muscle contraction; creatine phosphate provides a reservoir of "high-energy" phosphate groups that replenish depleted ATP and maintain the needed ratio of ATP to ADP (**Figure 3–27**).

In 1941, Lipmann published a 63-page review in the inaugural issue of *Advances in Enzymology*. Entitled "The metabolic generation and utilization of phosphate bond

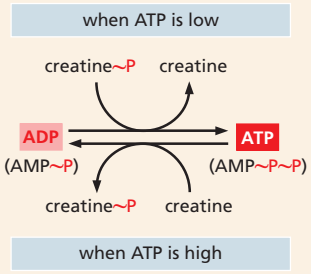


Figure 3–27 Creatine phosphate serves as an intermediate energy store. An enzyme called creatine kinase transfers a phosphate group from creatine phosphate to ADP when ATP concentrations are low; the same enzyme can catalyze the reverse reaction to generate a pool of creatine phosphate when ATP concentrations are high. Here, the "high-energy" phosphate bonds are symbolized by ~P. AMP is adenosine monophosphate (see Figure 3–41).

energy," this article introduced the symbol ~P (or "squiggle P") to denote an energy-rich phosphate bond—one whose hydrolysis yields enough energy to drive energetically unfavorable reactions and processes (**Figure 3–28**).

Although several molecules contain such high-energy phosphate bonds (see Panel 3–1, p. 95), it is the hydrolysis of ATP that provides the driving force for most of the energy-requiring reactions in living systems, including the contraction of muscles, the transport of substances across membranes, and the synthesis of macromolecules including proteins, nucleic acids, and carbohydrates. Indeed, in a memorial written after the death of Meyerhof in 1951, Lipmann—who would shortly win his own Nobel Prize for work on a different activated carrier—wrote: "The discovery of ATP thus was the key that opened the gates to the understanding of the conversion mechanisms of metabolic energy."

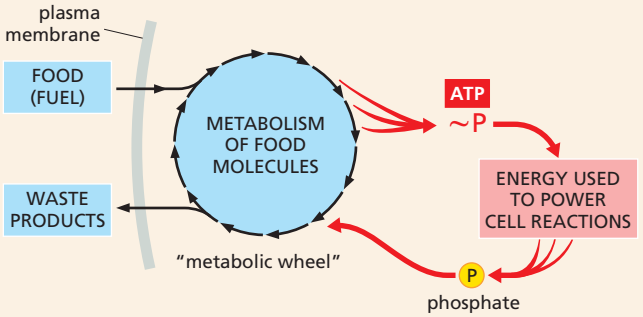


Figure 3–28 High-energy phosphate bonds generate an energy current (*red*) that powers cell reactions. This diagram, modeled on a figure published in Lipmann's 1941 article in *Advances in Enzymology,* shows how energy released by the metabolism of food molecules (represented by the "metabolic wheel") is captured in the form of high-energy phosphate bonds (~P) of ATP, which are used to power all other cell reactions. After the high-energy bonds are hydrolyzed, the inorganic phosphate released is recycled and reused, as indicated.

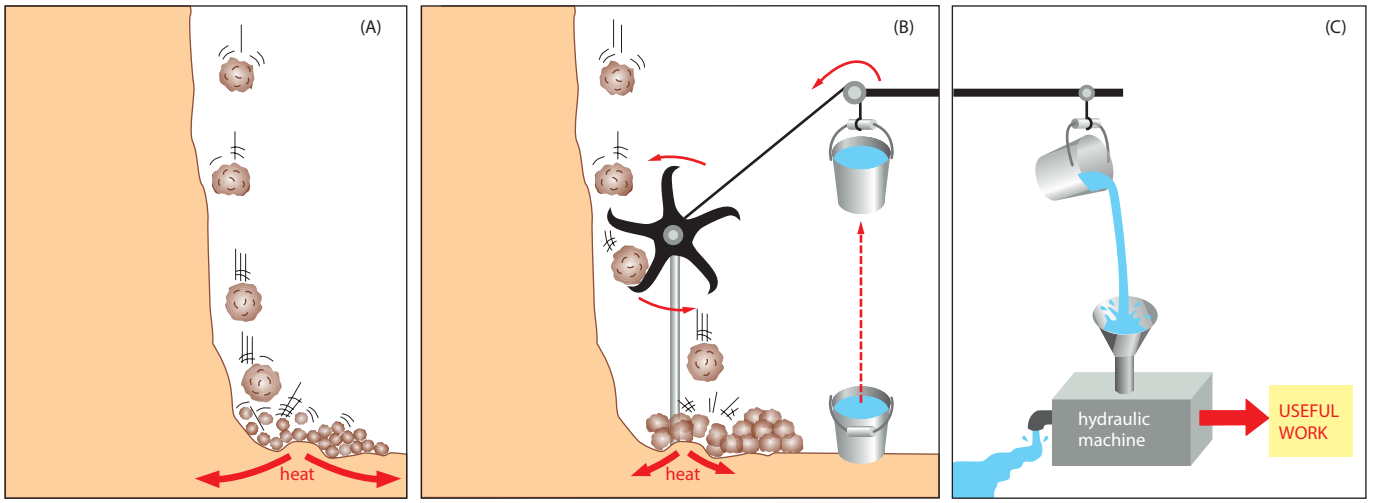


Figure 3–29 A mechanical model illustrates the principle of coupled chemical reactions. (A) The spontaneous reaction shown could serve as an analogy for the direct oxidation of glucose to CO_2 and H_2O, which produces only heat. (B) The same reaction is coupled to a second reaction, which could serve as an analogy for the synthesis of activated carriers. (C) The energy produced in (B) is in a more useful form than in (A) and can be used to drive a variety of otherwise energetically unfavorable reactions.

To provide an everyday representation of how coupled reactions work, let's consider a mechanical analogy in which an energetically favorable chemical reaction is represented by rocks falling from a cliff. The kinetic energy of falling rocks would normally be entirely wasted in the form of heat generated by friction when the rocks hit the ground (**Figure 3–29A**). By careful design, however, part of this energy could be used to drive a paddle wheel that lifts a bucket of water (**Figure 3–29B**). Because the rocks can now reach the ground only after moving the paddle wheel, we say that the energetically favorable reaction of rocks falling has been directly coupled to the energetically unfavorable reaction of lifting the bucket of water. Because part of the energy is used to do work in (B), the rocks hit the ground with less velocity than in (A), and correspondingly less energy is wasted as heat. The energy saved in the elevated bucket of water can then be used to do useful work (**Figure 3–29C**).

Analogous processes occur in cells, where enzymes play the role of the paddle wheel in Figure 3–29B. By mechanisms that we discuss in Chapter 13, enzymes couple an energetically favorable reaction, such as the oxidation of food molecules, to an energetically unfavorable reaction, such as the generation of activated carriers. As a result, the amount of heat released by the oxidation reaction is reduced by exactly the amount of energy that is stored in the energy-rich covalent bonds of the activated carrier. That saved energy can then be used to power a chemical reaction elsewhere in the cell.

QUESTION 3–7

Use Figure 3–29B to illustrate the following reaction driven by the hydrolysis of ATP:

$$X + ATP \rightarrow Y + ADP + P_i$$

A. In this case, which molecule or molecules would be analogous to (i) rocks at the top of the cliff, (ii) broken debris at the bottom of the cliff, (iii) the bucket at its highest point, and (iv) the bucket on the ground?

B. What would be analogous to (i) the rocks hitting the ground in the absence of the paddle wheel in Figure 3–29A and (ii) the hydraulic machine in Figure 3–29C?

ATP Is the Most Widely Used Activated Carrier

The most important and versatile of the activated carriers in cells is **ATP** (adenosine 5′-triphosphate). Just as the energy stored in the raised bucket of water in Figure 3–29B can be used to drive a wide variety of hydraulic machines, ATP serves as a convenient and versatile store, or currency, of energy that can be used to drive a variety of chemical reactions in cells. As shown in **Figure 3–30**, ATP is synthesized in an energetically unfavorable *phosphorylation* reaction, in which a phosphate group is added to **ADP** (adenosine 5′-diphosphate). When required, ATP gives up this energy packet in an energetically favorable hydrolysis to ADP and inorganic phosphate (P_i). The regenerated ADP is then available to be used for another round of the phosphorylation reaction that forms ATP, creating an ATP cycle in the cell.

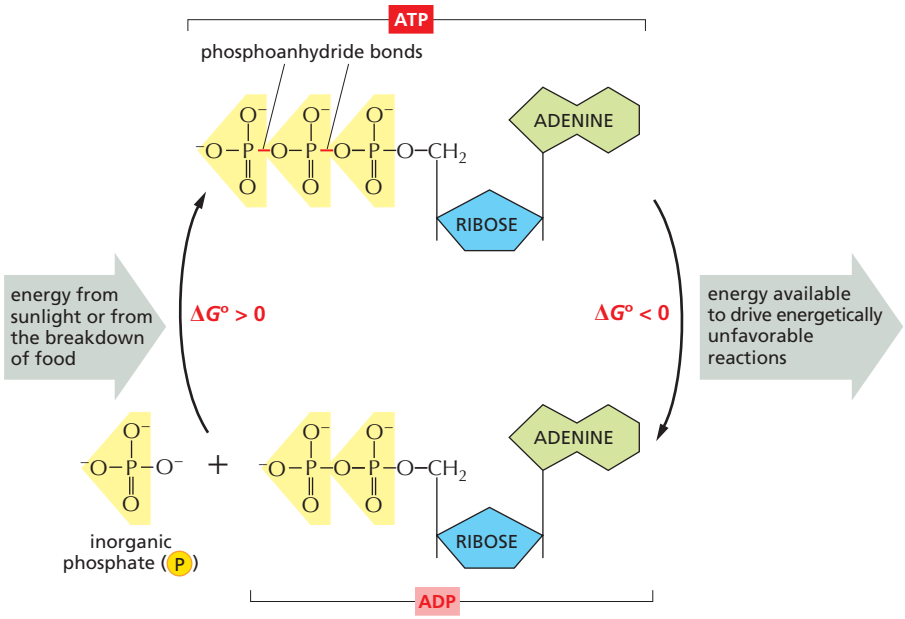


Figure 3–30 The interconversion of ATP and ADP occurs in a cycle. The two outermost phosphate groups in ATP are held to the rest of the molecule by "high-energy" phosphoanhydride bonds and are readily transferred to other organic molecules. Water can be added to ATP to form ADP and inorganic phosphate (P_i). Inside a cell, this hydrolysis of the terminal phosphate of ATP yields between 46 and 54 kJ/mole of usable energy. (Although the $\Delta G°$ of this reaction is –30.5 kJ/mole, its ΔG inside cells is much more negative, because the ratio of ATP to the products ADP and P_i is kept so high.)

The formation of ATP from ADP and P_i reverses the hydrolysis reaction; because this condensation reaction is energetically unfavorable, it must be coupled to a highly energetically favorable reaction to occur.

The large negative $\Delta G°$ of the ATP hydrolysis reaction arises from a number of factors. Release of the terminal phosphate group removes an unfavorable repulsion between adjacent negative charges; in addition, the inorganic phosphate ion (P_i) released is stabilized by favorable hydrogen-bond formation with water.

The energetically favorable reaction of ATP hydrolysis is coupled to many otherwise unfavorable reactions through which other molecules are synthesized. We will encounter several of these reactions in this chapter, where we will see exactly how this coupling is carried out. ATP hydrolysis is often accompanied by a transfer of the terminal phosphate in ATP to another molecule, as illustrated in **Figure 3–31**. Any reaction that involves the transfer of a phosphate group to a molecule is termed a *phosphorylation* reaction. Phosphorylation reactions are examples of condensation reactions (see Figure 2–19), and they occur in many important cell processes: they activate substrates for a subsequent reaction, mediate movement, and serve as key constituents of intracellular signaling pathways (discussed in Chapter 16).

ATP is the most abundant activated carrier in cells. It is used to supply energy for many of the pumps that actively transport substances into

QUESTION 3–8

The phosphoanhydride bond that links two phosphate groups in ATP in a high-energy linkage has a $\Delta G°$ of –30.5 kJ/mole. Hydrolysis of this bond in a cell liberates from 46 to 54 kJ/mole of usable energy. How can this be? Why do you think a range of energies is given, rather than a precise number as for $\Delta G°$?

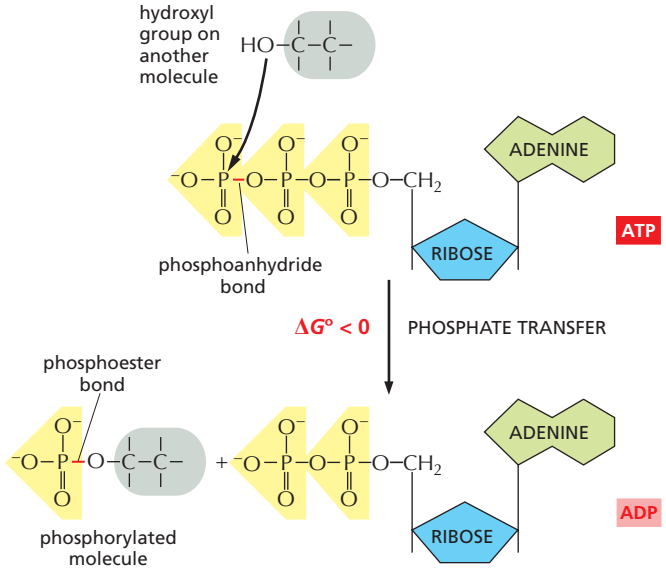


Figure 3–31 The terminal phosphate of ATP can be readily transferred to other molecules. Because an energy-rich phosphoanhydride bond in ATP is converted to a less energy-rich phosphoester bond in the phosphate-accepting molecule, this reaction is energetically favorable, having a large negative $\Delta G°$ (see Panel 3–1, pp. 94–95). Phosphorylation reactions of this type are involved in the synthesis of phospholipids and in the initial steps of the breakdown of sugars, as well as in many other metabolic and intracellular signaling pathways.

or out of the cell (discussed in Chapter 12) and to power the molecular motors that enable muscle cells to contract and nerve cells to transport materials along their lengthy axons (discussed in Chapter 17), to name just two important examples. Why evolution selected this particular nucleoside triphosphate over the others as the major carrier of energy, however, remains a mystery. GTP, although chemically similar to ATP, is involved in a different set of functions in the cell, as we discuss in later chapters.

Energy Stored in ATP Is Often Harnessed to Join Two Molecules Together

A common type of reaction that is needed for biosynthesis is one in which two molecules, A and B, are joined together by a covalent bond to produce A–B in an energetically unfavorable condensation reaction:

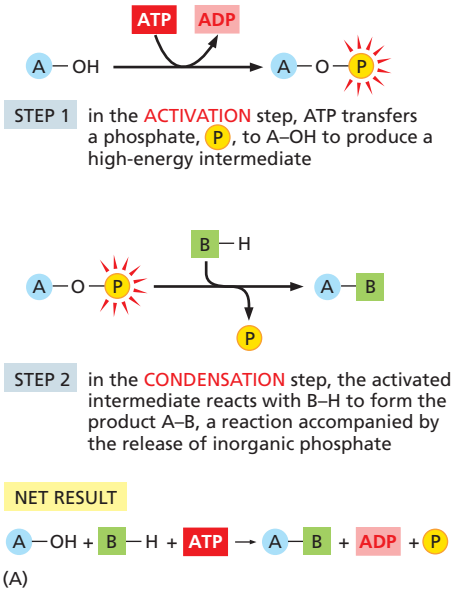
$$A\text{–}OH + B\text{–}H \rightarrow A\text{–}B + H_2O$$

ATP hydrolysis can be coupled indirectly to this reaction to make it go forward. In this case, energy from ATP hydrolysis is first used to convert A–OH to a higher-energy intermediate compound, which then reacts directly with B–H to give A–B. The simplest mechanism involves the transfer of a phosphate from ATP to A–OH to make A–O–PO_3, in which case the reaction pathway contains only two steps (**Figure 3–32A**). The condensation reaction, which by itself is energetically unfavorable, has been forced to occur by being coupled to ATP hydrolysis in an enzyme-catalyzed reaction pathway.

A biosynthetic reaction of exactly this type is employed to synthesize the amino acid glutamine, as illustrated in **Figure 3–32B**. We will see later in the chapter that very similar (but more complex) mechanisms are also used to produce nearly all of the large molecules of the cell.

NADH and NADPH Are Both Activated Carriers of Electrons

Other important activated carriers participate in oxidation–reduction reactions and are also commonly part of coupled reactions in cells. These



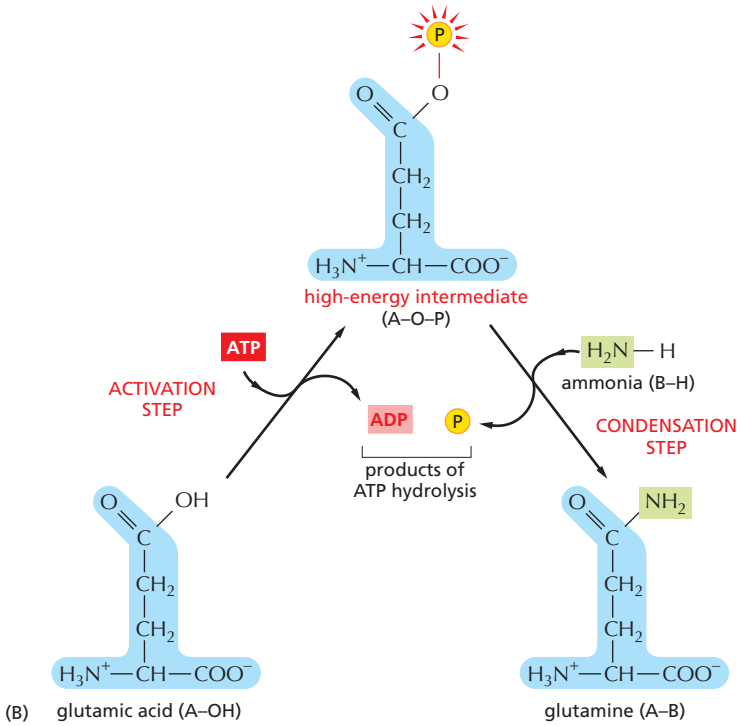


Figure 3–32 An energetically unfavorable biosynthetic reaction can be driven by ATP hydrolysis. (A) Schematic illustration of the condensation reaction described in the text. In this set of reactions, a phosphate group is first donated by ATP to form a high-energy intermediate, A–O–PO_3, which then reacts with the other substrate, B–H, to form the product A–B. (B) Reaction showing the biosynthesis of the amino acid glutamine from glutamic acid. Glutamic acid, which corresponds to the A–OH shown in (A), is first converted to a high-energy phosphorylated intermediate, which corresponds to A–O–PO_3. This intermediate then reacts with ammonia (which corresponds to B–H) to form glutamine. In this example, both steps occur on the surface of the same enzyme, glutamine synthetase (not shown). ATP hydrolysis can drive this energetically unfavorable reaction because it produces a favorable free-energy change ($\Delta G°$ of –30.5 kJ/mole) that is larger in magnitude than the energy required for the synthesis of glutamine from glutamic acid plus NH_3 ($\Delta G°$ of +14.2 kJ/mole). For clarity, the glutamic acid side chain is shown in its uncharged form.

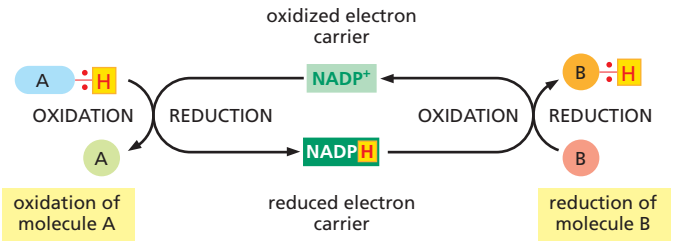


Figure 3–33 NADPH is an activated carrier of electrons that participates in oxidation–reduction reactions. NADPH is produced in reactions of the general type shown on the left, in which two electrons are removed from a substrate (A–H). The oxidized form of the carrier molecule, $NADP^+$, receives these two electrons as one hydrogen atom plus an electron (a hydride ion). Because NADPH holds its hydride ion in a high-energy linkage, this ion can easily be transferred to other molecules, such as B, as shown on the right. In this reaction, NADPH is re-oxidized to yield $NADP^+$, thus completing the cycle.

activated carriers are specialized to carry both high-energy electrons and hydrogen atoms. The most important of these *electron carriers* are **NADH** (nicotinamide adenine dinucleotide) and the closely related molecule **NADPH** (nicotinamide adenine dinucleotide phosphate). Both NADH and NADPH carry energy in the form of two high-energy electrons plus a proton (H^+), which together form a hydride ion (H^-). When these activated carriers pass their hydride ion to a donor molecule, they become oxidized to form **NAD^+** and **$NADP^+$**, respectively.

Like ATP, NADPH is an activated carrier that participates in many important biosynthetic reactions that would otherwise be energetically unfavorable. NADPH is produced according to the general scheme shown in **Figure 3–33**. During a special set of energy-yielding catabolic reactions, a hydride ion is removed from the substrate molecule and added to the nicotinamide ring of $NADP^+$ to form NADPH. This is a typical oxidation–reduction reaction: the substrate is oxidized and $NADP^+$ is reduced.

The hydride ion carried by NADPH is given up readily in a subsequent oxidation–reduction reaction, because the nicotinamide ring can achieve a more stable arrangement of electrons without it (**Figure 3–34**). In this subsequent reaction, which regenerates $NADP^+$, the NADPH becomes oxidized and the substrate becomes reduced—thus completing the NADPH cycle (see Figure 3–33). NADPH is efficient at donating its hydride ion to other molecules for the same reason that ATP readily transfers a phosphate: in both cases, the transfer is accompanied by a large negative free-energy change. One example of the use of NADPH in biosynthesis is shown in **Figure 3–35**.

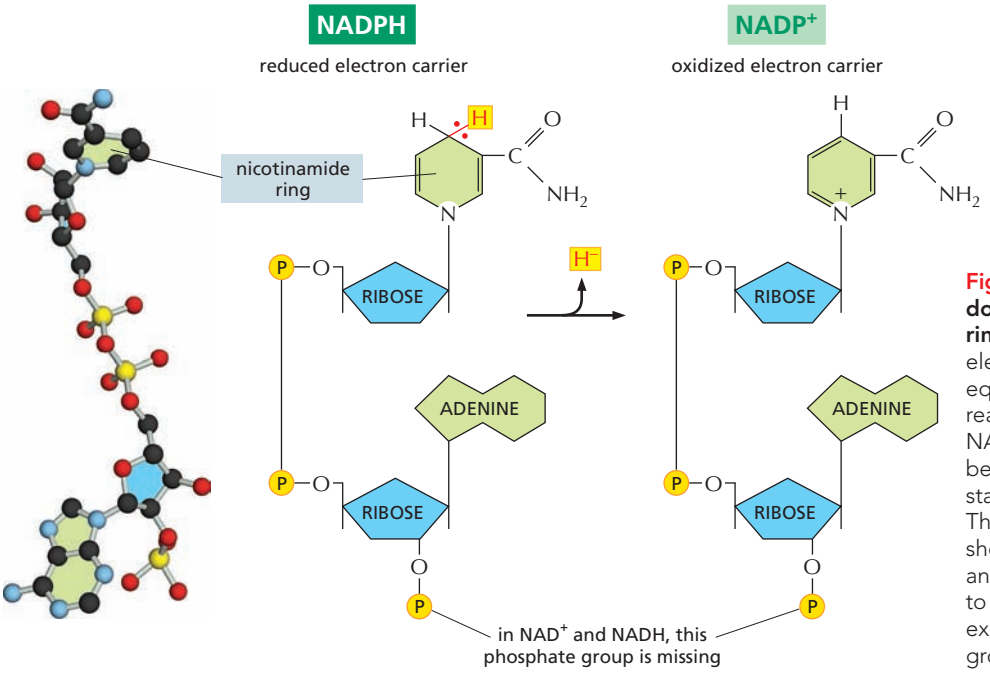


Figure 3–34 NADPH accepts and donates electrons via its nicotinamide ring. NADPH donates its high-energy electrons together with a proton (the equivalent of a hydride ion, H^-). This reaction, which oxidizes NADPH to $NADP^+$, is energetically favorable because the nicotinamide ring is more stable when these electrons are absent. The ball-and-stick model on the left shows the structure of $NADP^+$. NAD^+ and NADH are identical in structure to $NADP^+$ and NADPH, respectively, except that they lack the phosphate group, as indicated.

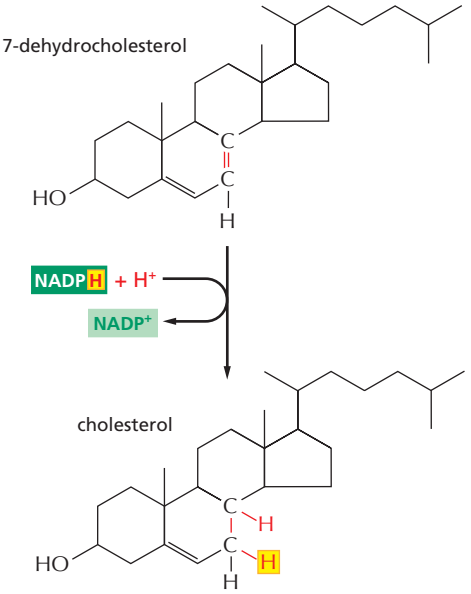


Figure 3–35 NADPH participates in the final stage of one of the biosynthetic routes leading to cholesterol. As in many other biosynthetic reactions, the reduction of the C=C bond is achieved by the transfer of a hydride ion from the activated carrier NADPH, plus a proton (H^+) from solution.

NADPH and NADH Have Different Roles in Cells

NADPH and NADH differ in a single phosphate group, which is located far from the region involved in electron transfer in NADPH (see Figure 3–34). Although this phosphate group has no effect on the electron-transfer properties of NADPH compared with NADH, it is nonetheless crucial for their distinctive roles, as it gives NADPH a slightly different shape from NADH. This subtle difference in conformation makes it possible for the two carriers to bind as substrates to different sets of enzymes and thereby deliver electrons (in the form of hydride ions) to different target molecules.

Why should there be this division of labor? The answer lies in the need to regulate two sets of electron-transfer reactions independently. NADPH operates chiefly with enzymes that catalyze anabolic reactions, supplying the high-energy electrons needed to synthesize energy-rich biological molecules. NADH, by contrast, has a special role as an intermediate in the catabolic system of reactions that generate ATP through the oxidation of food molecules, as we discuss in Chapter 13. The genesis of NADH from NAD^+ and that of NADPH from $NADP^+$ occurs by different pathways that are independently regulated, so that the cell can adjust the supply of electrons for these two contrasting purposes. Inside the cell, the ratio of NAD^+ to NADH is kept high, whereas the ratio of $NADP^+$ to NADPH is kept low. This arrangement 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 (**Figure 3–36**).

Cells Make Use of Many Other Activated Carriers

In addition to ATP (which transfers a phosphate) and NADPH and NADH (which transfer electrons and hydrogen), cells make use of other activated carriers that pick up and carry a chemical group in an easily transferred, high-energy linkage. $FADH_2$, like NADH and NADPH, carries hydrogen and high-energy electrons (see Figure 13–13B). But other important reactions involve the transfers of acetyl, methyl, carboxyl, and glucose groups from activated carriers for the purpose of biosynthesis (**Table 3–2**). Coenzyme A, for example, can carry an acetyl group in a readily transferable linkage. This activated carrier, called **acetyl CoA** (acetyl coenzyme A), is shown in **Figure 3–37**. It is used, for example, to sequentially add two-carbon units in the biosynthesis of the hydrocarbon tails of fatty acids.

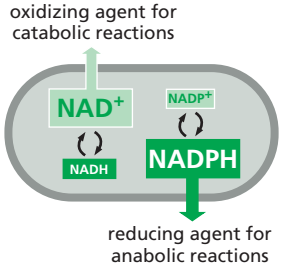


Figure 3–36 NADPH and NADH have different roles in the cell, and the relative concentrations of these carrier molecules influence their affinity for electrons. Keeping reduced NADPH at a higher concentration than its oxidized counterpart, $NADP^+$, makes NADPH a stronger electron donor. This arrangement ensures that NADPH can serve as a reducing agent for anabolic reactions. The reverse is true for NADH. Cells keep the amount of reduced NADH lower than that of NAD^+, which makes NAD^+ a better electron acceptor. Thus NAD^+ acts as an effective oxidizing agent, accepting electrons generated during oxidative breakdown of food molecules.

TABLE 3–2 SOME ACTIVATED CARRIERS WIDELY USED IN METABOLISM

Activated Carrier	Group Carried in High-Energy Linkage
ATP	phosphate
NADH, NADPH, $FADH_2$	electrons and hydrogens
Acetyl CoA	acetyl group
Carboxylated biotin	carboxyl group
S-adenosylmethionine	methyl group
Uridine diphosphate glucose	glucose

In acetyl CoA and the other activated carriers in Table 3–2, the transferable group makes up only a small part of the molecule. The rest consists of a large organic portion that serves as a convenient "handle," facilitating the recognition of the carrier molecule by specific enzymes. As with acetyl CoA, this handle portion very often contains a nucleotide. This curious fact may be a relic from an early stage of cell evolution. It is thought that the main catalysts for early life-forms on Earth were RNA molecules (or their close relatives) and that proteins were a later evolutionary addition. It is therefore tempting to speculate that many of the activated carriers that we find today originated in an earlier RNA world, where their nucleotide portions would have been useful for binding these carriers to RNA-based catalysts, or *ribozymes* (discussed in Chapter 7).

Activated carriers are usually generated in reactions coupled to ATP hydrolysis, as shown for biotin in **Figure 3–38**. Therefore, the energy that enables their groups to be used for biosynthesis ultimately comes from the catabolic reactions that generate ATP. The same principle applies to the synthesis of large macromolecules—nucleic acids, proteins, and polysaccharides—as we discuss next.

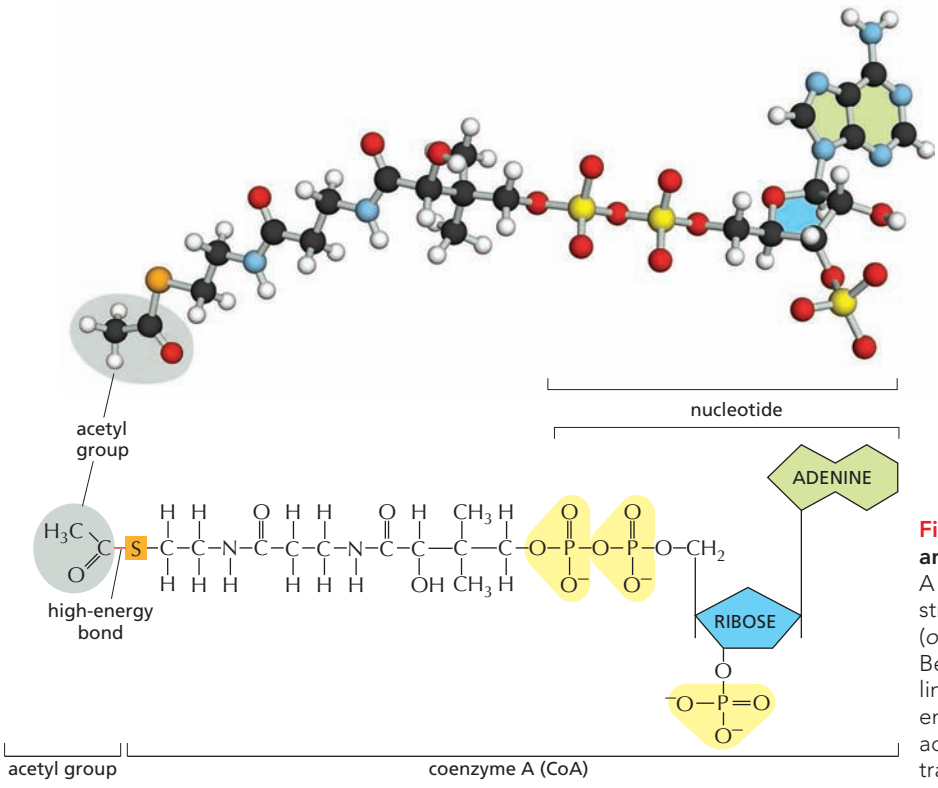


Figure 3–37 Acetyl coenzyme A (CoA) is another important activated carrier. A ball-and-stick model is shown above the structure of acetyl CoA. The sulfur atom (*orange*) forms a thioester bond to acetate. Because the thioester bond is a high-energy linkage, it releases a large amount of free energy when it is hydrolyzed. Thus the acetyl group carried by CoA can be readily transferred to other molecules.

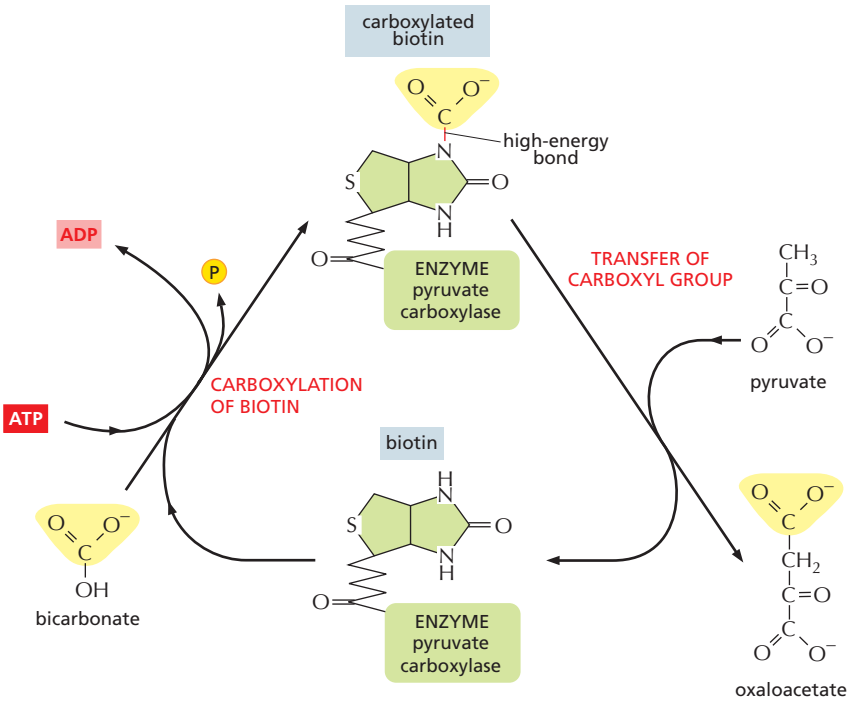


Figure 3–38 Biotin transfers a carboxyl group to a substrate. Biotin is a vitamin that is used by a number of enzymes to transfer a carboxyl group to a substrate. Shown here is the reaction in which biotin, held by the enzyme *pyruvate carboxylase*, *accepts* a carboxyl group from bicarbonate and transfers it to pyruvate, producing oxaloacetate, a molecule required in the citric acid cycle (discussed in Chapter 13). Other enzymes use biotin to transfer carboxyl groups to other molecules. Note that the synthesis of carboxylated biotin requires energy derived from ATP hydrolysis—a general feature that applies to many activated carriers.

The Synthesis of Biological Polymers Requires an Energy Input

The macromolecules of the cell constitute the vast majority of its dry mass—that is, the mass not due to water. These molecules are made from *subunits* (or monomers) that are linked together by bonds formed during an enzyme-catalyzed *condensation* reaction. The reverse reaction—the breakdown of polymers—occurs through enzyme-catalyzed *hydrolysis* reactions. These hydrolysis reactions are energetically favorable, whereas the corresponding biosynthetic reactions require an energy input and are more complex (**Figure 3–39**).

The nucleic acids (DNA and RNA), proteins, and polysaccharides are all polymers that are produced by the repeated addition of a subunit onto one end of a growing chain. The mode of synthesis of each of these macromolecules is outlined in **Figure 3–40**. As indicated, the condensation step in each case depends on energy provided by the hydrolysis of a nucleoside triphosphate. And yet, except for the nucleic acids, there are no phosphate groups left in the final product molecules. How, then, is the energy of ATP hydrolysis coupled to polymer synthesis?

Each type of macromolecule is generated by an enzyme-catalyzed pathway that resembles the one discussed previously for the synthesis of the amino acid glutamine (see Figure 3–32). The principle is exactly the same, in that the –OH group that will be removed in the condensation reaction is first activated by forming a high-energy linkage to a second molecule. The mechanisms used to link ATP hydrolysis to the synthesis of proteins and polysaccharides, however, are more complex than that used for glutamine synthesis. In the biosynthetic pathways leading

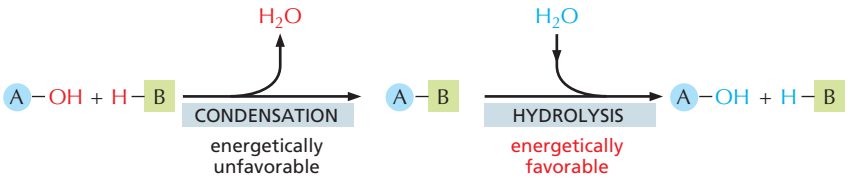


Figure 3–39 In cells, macromolecules are synthesized by condensation reactions and broken down by hydrolysis reactions. Condensation reactions are all energetically unfavorable, whereas hydrolysis reactions are all energetically favorable.

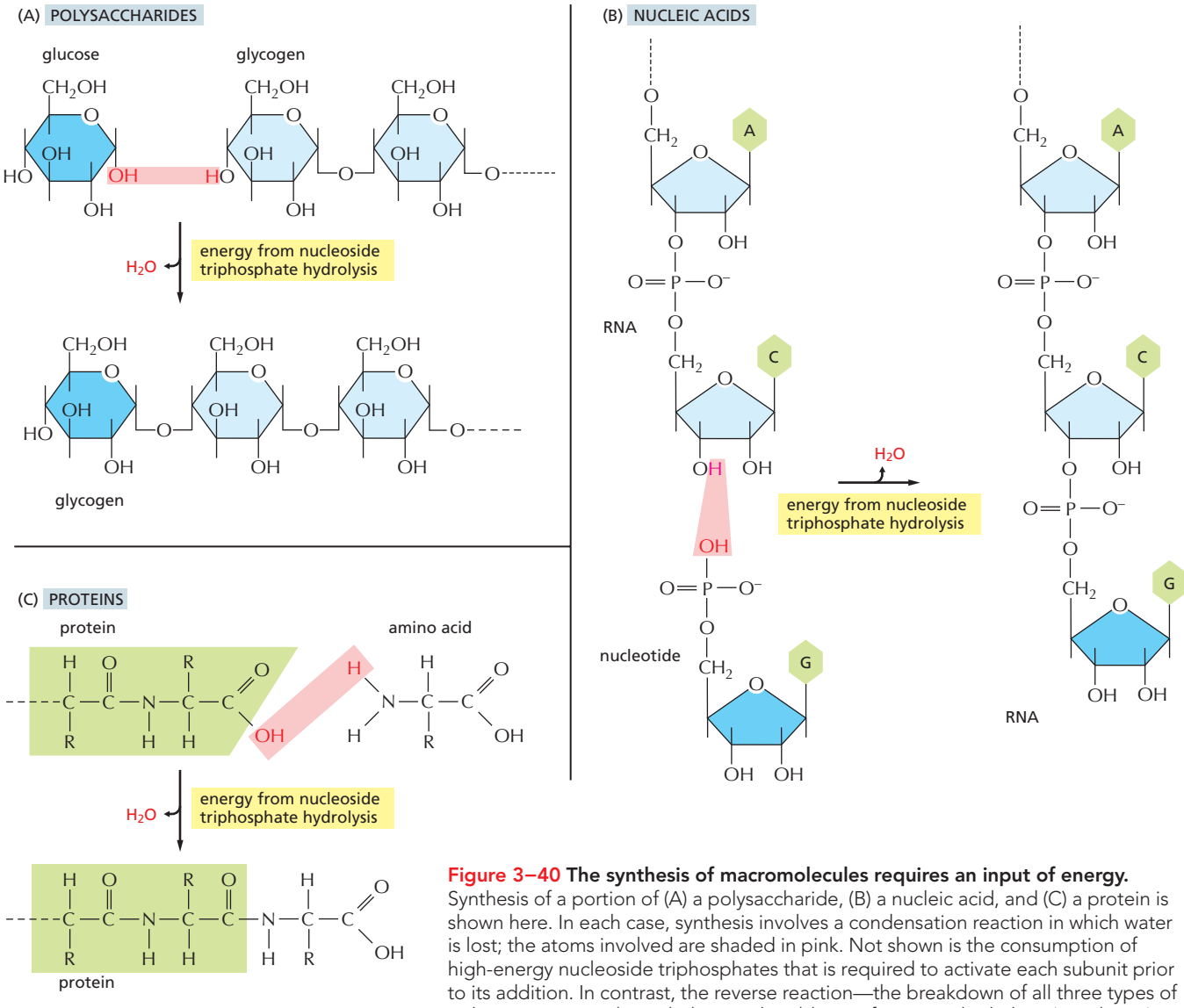


Figure 3–40 The synthesis of macromolecules requires an input of energy. Synthesis of a portion of (A) a polysaccharide, (B) a nucleic acid, and (C) a protein is shown here. In each case, synthesis involves a condensation reaction in which water is lost; the atoms involved are shaded in pink. Not shown is the consumption of high-energy nucleoside triphosphates that is required to activate each subunit prior to its addition. In contrast, the reverse reaction—the breakdown of all three types of polymers—occurs through the simple addition of water, or hydrolysis (not shown).

to these macromolecules, several high-energy intermediates are consumed in series to generate the final high-energy bond that will be broken during the condensation step. One important example of such a biosynthetic reaction, that of protein synthesis, is discussed in detail in Chapter 7.

There are limits to what each activated carrier can do in driving biosynthesis. For example, the ΔG for the hydrolysis of ATP to ADP and inorganic phosphate (P_i) depends on the concentrations of all of the reactants, and under the usual conditions in a cell, it is between –46 and –54 kJ/mole. In principle, this hydrolysis reaction can be used to drive an unfavorable reaction with a ΔG of, perhaps, +40 kJ/mole, provided that a suitable reaction path is available. For some biosynthetic reactions, however, even –54 kJ/mole may be insufficient. In these cases, the path of ATP hydrolysis can be altered so that it initially produces AMP and pyrophosphate (PP_i), which is itself then hydrolyzed in solution in a subsequent step (**Figure 3–41**). The whole process makes available a total ΔG of about –109 kJ/mole. The biosynthetic reaction involved in the synthesis of nucleic acids (polynucleotides) is driven in this way (**Figure 3–42**).

QUESTION 3–9

Which of the following reactions will occur only if coupled to a second, energetically favorable reaction?

A. glucose + $O_2 \rightarrow CO_2 + H_2O$
B. $CO_2 + H_2O \rightarrow$ glucose + O_2
C. nucleoside triphosphates → DNA
D. nucleotide bases → nucleoside triphosphates
E. ADP + $P_i \rightarrow$ ATP

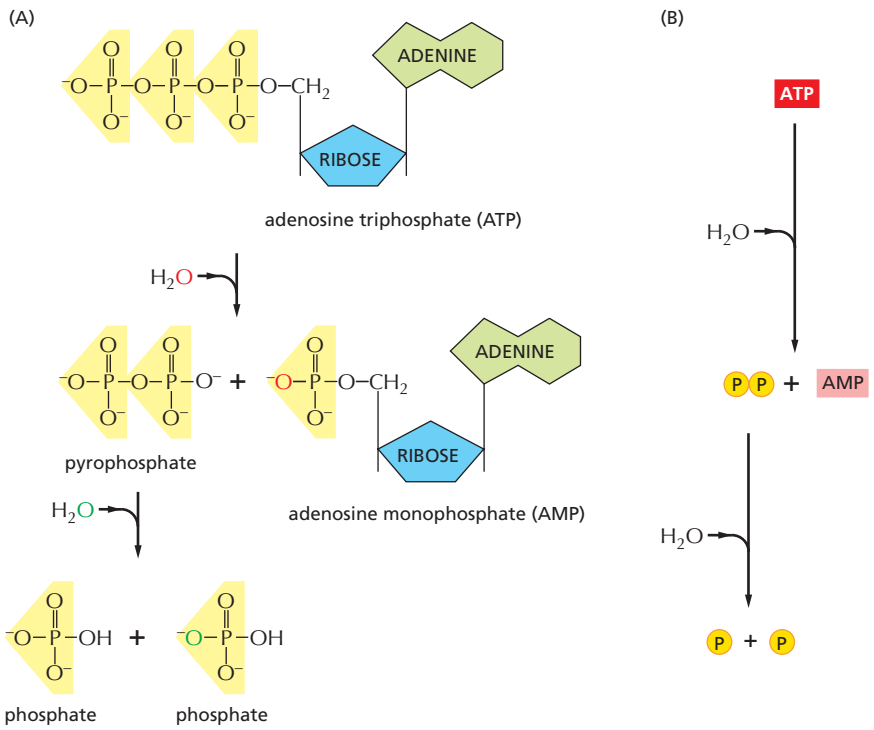


Figure 3–41 In an alternative route for the hydrolysis of ATP, pyrophosphate is first formed and then hydrolyzed in solution. This route releases about twice as much free energy as the reaction shown earlier in Figure 3–30. (A) In each of the two successive hydrolysis reactions, an oxygen atom from the participating water molecule is retained in the products, whereas the hydrogen atoms from water form free hydrogen ions, H^+. (B) The overall reaction shown in summary form.

ATP will make many appearances throughout the book as a molecule that powers reactions in the cell. And in Chapters 13 and 14, we discuss how the cell uses the energy from food to generate ATP. In the next chapter, we learn more about the proteins that make such reactions possible.

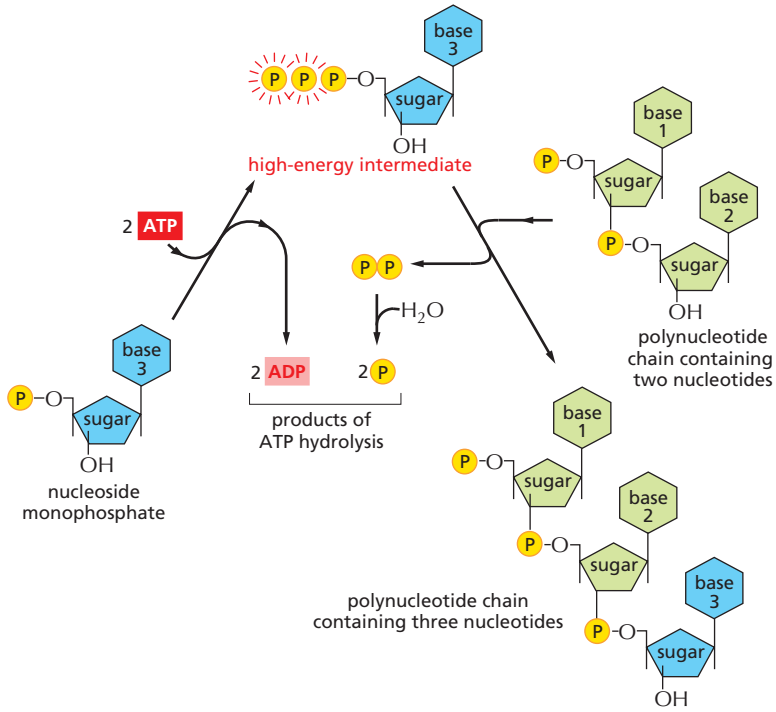


Figure 3–42 Synthesis of a polynucleotide, RNA or DNA, is a multistep process driven by ATP hydrolysis. In the first step, a nucleoside monophosphate is activated by the sequential transfer of the terminal phosphate groups from two ATP molecules. The high-energy intermediate formed—a nucleoside triphosphate—exists free in solution until it reacts with the growing end of an RNA or a DNA chain, with release of pyrophosphate. Hydrolysis of the pyrophosphate to inorganic phosphate is highly favorable and helps to drive the overall reaction in the direction of polynucleotide synthesis.

ESSENTIAL CONCEPTS

- Living organisms are able to exist because of a continual input of energy. Part of this energy is used to carry out essential reactions that support cell metabolism, growth, movement, and reproduction; the remainder is lost in the form of heat.
- The ultimate source of energy for most living organisms is the sun. Plants, algae, and photosynthetic bacteria use solar energy to produce organic molecules from carbon dioxide. Animals obtain food by eating plants or by eating animals that feed on plants.
- Each of the many hundreds of chemical reactions that occur in a cell is specifically catalyzed by an enzyme. Large numbers of different enzymes work in sequence to form chains of reactions, called metabolic pathways, each performing a different function in the cell.
- Catabolic reactions release energy by breaking down organic molecules, including foods, through oxidative pathways. Anabolic reactions generate the many complex organic molecules needed by the cell, and they require an energy input. In animal cells, both the building blocks and the energy required for the anabolic reactions are obtained through catabolic reactions.
- Enzymes catalyze reactions by binding to particular substrate molecules in a way that lowers the activation energy required for making and breaking specific covalent bonds.
- The rate at which an enzyme catalyzes a reaction depends on how rapidly it finds its substrates and how quickly the product forms and then diffuses away. These rates vary widely from one enzyme to another.
- The only chemical reactions possible are those that increase the total amount of disorder in the universe. The free-energy change for a reaction, ΔG, measures this disorder, and it must be less than zero for a reaction to proceed spontaneously.
- The ΔG for a chemical reaction depends on the concentrations of the reacting molecules, and it may be calculated from these concentrations if the equilibrium constant (K) of the reaction (or the standard free-energy change, ΔG°, for the reactants) is known.
- Equilibrium constants govern all of the associations (and dissociations) that occur between macromolecules and small molecules in the cell. The larger the binding energy between two molecules, the larger the equilibrium constant and the more likely that these molecules will be found bound to each other.
- By creating a reaction pathway that couples an energetically favorable reaction to an energetically unfavorable one, enzymes can make otherwise impossible chemical transformations occur. Large numbers of such coupled reactions make life possible.
- A small set of activated carriers, particularly ATP, NADH, and NADPH, plays a central part in these coupled reactions in cells. ATP carries high-energy phosphate groups, whereas NADH and NADPH carry high-energy electrons.
- Food molecules provide the carbon skeletons for the formation of macromolecules. The covalent bonds of these larger molecules are produced by condensation reactions that are coupled to energetically favorable bond changes in activated carriers such as ATP and NADPH.

KEY TERMS

acetyl CoA
activated carrier
activation energy
ADP, ATP
anabolism
biosynthesis
catabolism
catalyst
cell respiration
coupled reaction
diffusion
entropy
enzyme
equilibrium
equilibrium constant, *K*
free energy, *G*
free-energy change, ΔG
metabolism
NAD^+, NADH
$NADP^+$, NADPH
oxidation
photosynthesis
reduction
standard free-energy change, $\Delta G°$
substrate

QUESTIONS

QUESTION 3–10

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

A. Some enzyme-catalyzed reactions cease completely if their enzyme is absent.

B. High-energy electrons (such as those found in the activated carriers NADH and NADPH) move faster around the atomic nucleus.

C. Hydrolysis of ATP to AMP can provide about twice as much energy as hydrolysis of ATP to ADP.

D. A partially oxidized carbon atom has a somewhat smaller diameter than a more reduced one.

E. Some activated carrier molecules can transfer both energy and a chemical group to a second molecule.

F. The rule that oxidations release energy, whereas reductions require energy input, applies to all chemical reactions, not just those that occur in living cells.

G. Cold-blooded animals have an energetic disadvantage because they release less heat to the environment than warm-blooded animals do. This slows their ability to make ordered macromolecules.

H. Linking the reaction X → Y to a second, energetically favorable reaction Y → Z will shift the equilibrium constant of the first reaction.

QUESTION 3–11

Consider a transition of X → Y. Assume that the only difference between X and Y is the presence of three hydrogen bonds in Y that are absent in X. What is the ratio of X to Y when the reaction is in equilibrium? Approximate your answer by using Table 3–1 (p. 96), with 4.2 kJ/mole as the energy of each hydrogen bond. If Y instead has six hydrogen bonds that distinguish it from X, how would that change the ratio?

QUESTION 3–12

Protein A binds to protein B to form a complex, AB. At equilibrium in a cell the concentrations of A, B, and AB are all at 1 μM.

A. Referring to Figure 3–19, calculate the equilibrium constant for the reaction A + B ↔ AB.

B. What would the equilibrium constant be if A, B, and AB were each present in equilibrium at the much lower concentrations of 1 nM each?

C. How many extra hydrogen bonds would be needed to hold A and B together at this lower concentration so that a similar proportion of the molecules are found in the AB complex? (Remember that each hydrogen bond contributes about 4.2 kJ/mole.)

QUESTION 3–13

Discuss the following statement: "Whether the ΔG for a reaction is larger, smaller, or the same as $\Delta G°$ depends on the concentration of the compounds that participate in the reaction."

QUESTION 3–14

A. How many ATP molecules could maximally be generated from one molecule of glucose, if the complete oxidation of 1 mole of glucose to CO_2 and H_2O yields 2867 kJ of free energy and the useful chemical energy available in the high-energy phosphate bond of 1 mole of ATP is 50 kJ?

B. As we will see in Chapter 14 (Table 14–1), respiration produces 30 moles of ATP from 1 mole of glucose. Compare this number with your answer in part (A). What is the overall efficiency of ATP production from glucose?

C. If the cells of your body oxidize 1 mole of glucose, by how much would the temperature of your body (assume that your body consists of 75 kg of water) increase if the heat were not dissipated into the environment? [Recall that

a kilocalorie (kcal) is defined as that amount of energy that heats 1 kg of water by 1°C. And 1 kJ equals 0.24 kcal.]

D. What would the consequences be if the cells of your body could convert the energy in food substances with only 20% efficiency? Would your body—as it is presently constructed—work just fine, overheat, or freeze?

E. A resting human hydrolyzes about 40 kg of ATP every 24 hours. The oxidation of how much glucose would produce this amount of energy? (Hint: Look up the structure of ATP in Figure 2–26 to calculate its molecular weight; the atomic weights of H, C, N, O, and P are 1, 12, 14, 16, and 31, respectively.)

QUESTION 3–15

A prominent scientist claims to have isolated mutant cells that can convert 1 molecule of glucose into 57 molecules of ATP. Should this discovery be celebrated, or do you suppose that something might be wrong with it? Explain your answer.

QUESTION 3–16

In a simple reaction A ↔ A*, a molecule is interconvertible between two forms that differ in standard free energy $G°$ by 18 kJ/mole, with A* having the higher $G°$.

A. Use Table 3–1 (p. 96) to find how many more molecules will be in state A* compared with state A at equilibrium.

B. If an enzyme lowered the activation energy of the reaction by 11.7 kJ/mole, how would the ratio of A to A* change?

QUESTION 3–17

In a mushroom, a reaction in a single-step biosynthetic pathway that converts a metabolite into a particularly vicious poison (metabolite ↔ poison) is energetically highly unfavorable. The reaction is normally driven by ATP hydrolysis. Assume that a mutation in the enzyme that catalyzes the reaction prevents it from utilizing ATP, but still allows it to catalyze the reaction.

A. Do you suppose it might be safe for you to eat a mushroom that bears this mutation? Base your answer on an estimation of how much less poison the mutant mushroom would produce, assuming the reaction is in equilibrium and most of the energy stored in ATP is used to drive the unfavorable reaction in nonmutant mushrooms.

B. Would your answer be different for another mutant mushroom whose enzyme couples the reaction to ATP hydrolysis but works 100 times more slowly?

QUESTION 3–18

Consider the effects of two enzymes, A and B. Enzyme A catalyzes the reaction

$$ATP + GDP \leftrightarrow ADP + GTP$$

and enzyme B catalyzes the reaction

$$NADH + NADP^+ \leftrightarrow NAD^+ + NADPH$$

Discuss whether the enzymes would be beneficial or detrimental to cells.

QUESTION 3–19

Discuss the following statement: "Enzymes and heat are alike in that both can speed up reactions that—although thermodynamically feasible—do not occur at an appreciable rate because they require a high activation energy. Diseases that seem to benefit from the careful application of heat—in the form of hot chicken soup, for example—are therefore likely to be due to the insufficient function of an enzyme."