



## Energy Exists in Many Forms

To understand why a chemical reaction occurs, we first need to consider **energy**, which we will define as the ability to promote change or do work. Physicists often consider energy in two forms: kinetic energy and potential energy (Figure 6.1). **Kinetic energy** is energy associated with movement, such as the movement of a baseball bat from one location to another. By comparison, **potential energy** is the energy that a substance possesses due to its structure or location. The energy contained within covalent bonds in molecules is also a type of potential energy called **chemical energy**. The breakage of those bonds is one way that living cells can harness this energy to perform cellular functions. Table 6.1 summarizes chemical and other forms of energy important in biological systems.

An important issue in biology is the ability of energy to be converted from one form to another. The study of energy interconversions is called **thermodynamics**. Physicists have determined that two laws govern energy interconversions:

1. **The first law of thermodynamics**—The first law states that energy cannot be created or destroyed; it is also called the law of conservation of energy. However, energy can be transferred from one place to another and can be transformed from one type to another (as when, for example, chemical energy is transformed into heat).
2. **The second law of thermodynamics**—The second law states that the transfer of energy or the transformation of energy from one form to another increases the **entropy**, or degree of disorder of a system (Figure 6.2). Entropy is a measure of the randomness of molecules in a system. When a physical system becomes more disordered, the entropy increases. As the energy becomes more evenly distributed, that energy is less able to promote change or do work. When energy is converted from one form to another, some energy may become unusable by living organisms. For example, unusable heat may be released during a chemical reaction.



(a) Kinetic energy



(b) Potential energy

**Figure 6.1** Examples of energy. (a) Kinetic energy, such as swinging a bat, is energy associated with motion. (b) Potential energy is stored energy, as in a bow that is ready to fire an arrow.

Next, we will see how the two laws of thermodynamics place limits on the ways that living cells can use energy for their own needs.

## The Change in Free Energy Determines the Direction of a Chemical Reaction or Any Other Cellular Process

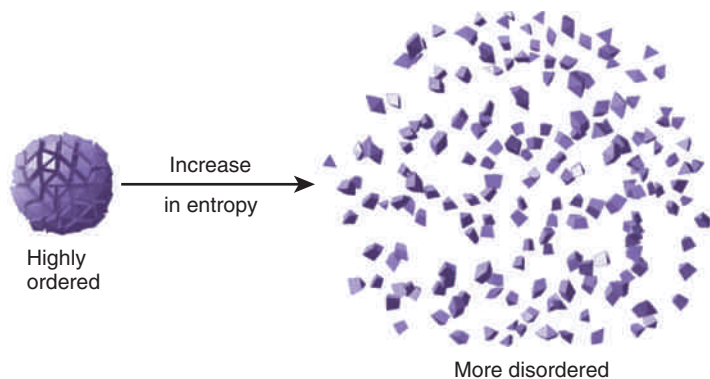
Energy is necessary for living organisms to exist. Energy is required for many cellular processes, including chemical reactions, cellular movements such as those occurring in muscle contraction, and the maintenance of cell organization. To understand how living organisms use energy, we need to distinguish between the energy that can be used to promote change or do work (usable energy) and the energy that cannot (unusable energy).

$$\text{Total energy} = \text{Usable energy} + \text{Unusable energy}$$

Why is some energy unusable? The main culprit is entropy. As stated by the second law of thermodynamics, energy transformations involve an increase in entropy, a measure of the disorder that cannot be harnessed in a useful way. The total energy is termed **enthalpy** ( $H$ ), and the usable energy—the

**Table 6.1** Types of Energy That Are Important in Biology

Energy type	Description	Biological example
Light	Light is a form of electromagnetic radiation. The energy of light is packaged in photons.	During photosynthesis, light energy is captured by pigments (see Chapter 8). Ultimately, this energy is used to reduce carbon and produce organic molecules.
Heat	Heat is the transfer of kinetic energy from one object to another or from an energy source to an object. In biology, heat is often viewed as energy that can be transferred due to a difference in temperature between two objects or locations.	Many organisms, such as humans, maintain their bodies at a constant temperature. This is achieved, in part, by chemical reactions that generate heat.
Mechanical	Mechanical energy is the energy possessed by an object due to its motion or its position relative to other objects.	In animals, mechanical energy is associated with movements due to muscle contraction, such as walking.
Chemical	Chemical energy is stored in the chemical bonds of molecules. When the bonds are broken and rearranged, large amounts of energy can be released.	The covalent bonds in organic molecules, such as glucose and ATP, store large amounts of energy. When bonds are broken in larger molecules to form smaller molecules, the chemical energy that is released can be used to drive cellular processes.
Electrical/ion gradient	The movement of charge or the separation of charge can provide energy. Also, a difference in ion concentration across a membrane constitutes an electrochemical gradient, which is a source of potential energy.	During oxidative phosphorylation (described in Chapter 7), a $H^+$ gradient provides the energy to drive ATP synthesis.



**Figure 6.2** Entropy. Entropy is a measure of the disorder of a system. An increase in entropy means an increase in disorder.

**Concept check:** Which do you think has more entropy, a NaCl crystal at the bottom of a beaker of water or the same beaker of water after the  $\text{Na}^+$  and  $\text{Cl}^-$  in the crystal have dissolved in the water?

amount of available energy that can be used to promote change or do work—is called the **free energy (G)**. The letter G is in recognition of J. Willard Gibbs, who proposed the concept of free energy in 1878. The unusable energy is the system's entropy (S). Gibbs proposed that these three factors are related to each other in the following way:

$$H = G + TS$$

where  $T$  is the absolute temperature in Kelvin (K). Because our focus is on free energy, we can rearrange this equation as

$$G = H - TS$$

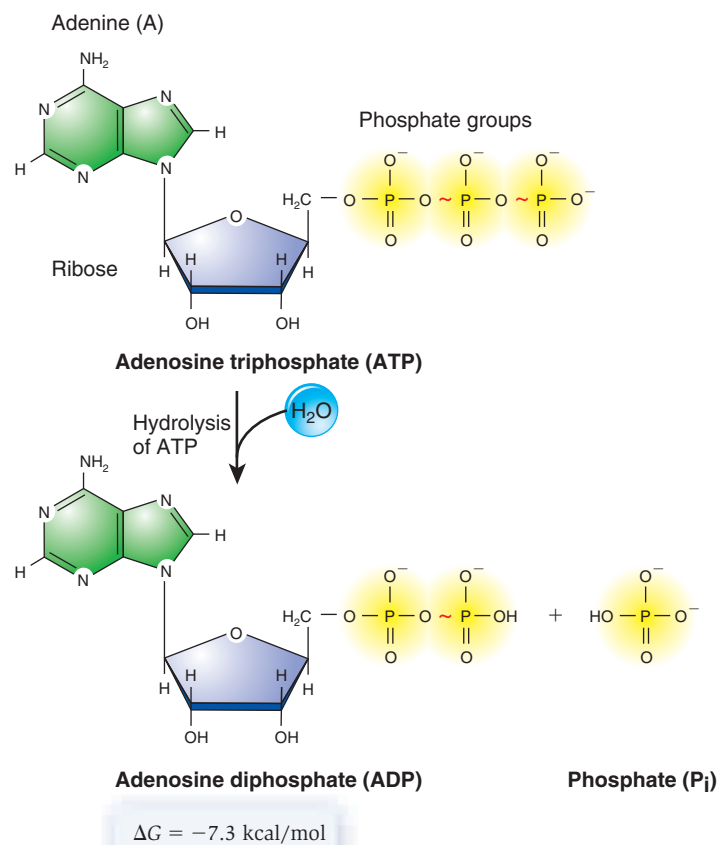
A critical issue in biology is whether a process will or will not occur spontaneously. For example, will glucose be broken down into carbon dioxide and water? Another way of framing this question is to ask: Is the breakdown of glucose a spontaneous reaction? A spontaneous reaction or process is one that will occur without being driven by an input of energy. However, a spontaneous reaction does not necessarily proceed quickly. In some cases, the rate of a spontaneous reaction can be quite slow. For example, the breakdown of sugar is a spontaneous reaction, but the rate at which sugar in a sugar bowl would break down into  $\text{CO}_2$  and  $\text{H}_2\text{O}$  would be very slow.

The key way to evaluate if a chemical reaction is spontaneous is to determine the free-energy change that occurs as a result of the reaction:

$$\Delta G = \Delta H - T\Delta S$$

where the  $\Delta$  sign (the Greek letter delta) indicates a change, such as before and after a chemical reaction. If a chemical reaction has a negative free-energy change ( $\Delta G < 0$ ), this means that the products have less free energy than the reactants, and, therefore, free energy is released during product formation. Such a reaction is said to be **exergonic**. Exergonic reactions are spontaneous. Alternatively, if a reaction has a positive free-energy change ( $\Delta G > 0$ ), requiring the addition of free energy from the environment, it is termed **endergonic**. An endergonic reaction is not a spontaneous reaction.

If  $\Delta G$  for a chemical reaction is negative, the reaction favors the formation of products, whereas a reaction with a positive  $\Delta G$  favors the formation of reactants. Chemists have determined



**Figure 6.3** The hydrolysis of ATP to ADP and  $\text{P}_i$ . As shown in this figure, ATP has a net charge of  $-4$ , and ADP and  $\text{P}_i$  are shown with net charges of  $-2$  each. When these compounds are shown in chemical reactions with other molecules, the net charges will also be indicated. Otherwise, these compounds will simply be designated ATP, ADP, and  $\text{P}_i$ . At neutral pH,  $\text{ADP}^{2-}$  will dissociate to  $\text{ADP}^{3-}$  and  $\text{H}^+$ .

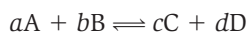
**Concept check:** Because  $\Delta G$  is negative, what does that tell us about the direction of this chemical reaction? What does it tell us about the rate?

free-energy changes for a variety of chemical reactions, which allows them to predict their direction. As an example, let's consider **adenosine triphosphate (ATP)**, which is a molecule that is a common energy source for all cells. ATP is broken down to adenosine diphosphate (ADP) and inorganic phosphate ( $\text{P}_i$ ). Because water is used to remove a phosphate group, chemists refer to this as the hydrolysis of ATP (Figure 6.3). In the reaction of converting 1 mole of ATP to 1 mole of ADP and  $\text{P}_i$ ,  $\Delta G$  equals  $-7.3$  kcal/mole. Because this is a negative value, the reaction strongly favors the formation of products. As discussed later in this chapter, the energy liberated by the hydrolysis of ATP is used to drive a variety of cellular processes.

## Chemical Reactions Will Eventually Reach a State of Equilibrium

Even when a chemical reaction is associated with a negative free-energy change, not all of the reactants are converted to

products. The reaction reaches a state of **chemical equilibrium** in which the rate of formation of products equals the rate of formation of reactants. Let's consider the generalized reaction

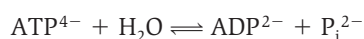


where again A and B are the reactants, C and D are the products, and  $a$ ,  $b$ ,  $c$ , and  $d$  are the number of moles of reactants and products. An equilibrium occurs, such that:

$$K_{\text{eq}} = \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

where  $K_{\text{eq}}$  is the equilibrium constant. Each type of chemical reaction will have a specific value for  $K_{\text{eq}}$ .

Biologists make two simplifying assumptions when determining values for equilibrium constants. First, the concentration of water does not change during the reaction, and the pH remains constant at pH 7. The equilibrium constant under these conditions is designated  $K_{\text{eq}}'$  (' is the prime symbol). If water is one of the reactants, as in a hydrolysis reaction, it is not included in the chemical equilibrium equation. As an example, let's consider the chemical equilibrium for the hydrolysis of ATP.

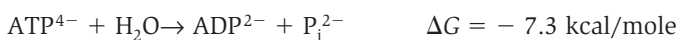
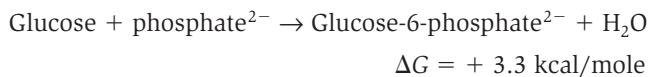


$$K_{\text{eq}}' = \frac{[\text{ADP}][\text{P}_i]}{[\text{ATP}]}$$

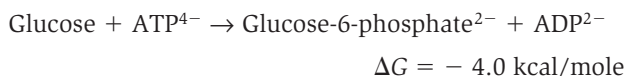
Experimentally, the value for  $K_{\text{eq}}'$  for this reaction has been determined and found to be approximately 1,650,000 M. Such a large value indicates that the equilibrium greatly favors the formation of products—ADP and  $\text{P}_i$ .

## Cells Use ATP to Drive Endergonic Reactions

In living organisms, many vital processes require the addition of free energy; that is, they are endergonic and will not occur spontaneously. Fortunately, organisms have a way to overcome this problem. Rather than catalyzing exothermic reactions that release energy in the form of unusable heat, cells often couple exergonic reactions with endergonic reactions. If an exergonic reaction is coupled to an endergonic reaction, the endergonic reaction will proceed spontaneously if the net free-energy change for both processes combined is negative. For example, consider the following reactions:



Coupled reaction:



The first reaction, in which phosphate is covalently attached to glucose, is endergonic, whereas the second, the hydrolysis

of ATP, is exergonic. By itself, the first reaction would not be spontaneous. If the two reactions are coupled, however, the net free-energy change for both reactions combined is exergonic. In the coupled reaction, a phosphate is directly transferred from ATP to glucose in a process called **phosphorylation**. This coupled reaction proceeds spontaneously because the net free-energy change is negative. Exergonic reactions, such as the breakdown of ATP, are commonly coupled to cellular processes that would otherwise be endergonic or require energy.

## 6.2 Enzymes and Ribozymes

For most chemical reactions in cells to proceed at a rapid pace, such as the breakdown of sugar, a catalyst is needed. A **catalyst** is an agent that speeds up the rate of a chemical reaction without being permanently changed or consumed. In living cells, the most common catalysts are enzymes. The term was coined in 1876 by a German physiologist, Wilhelm Kühne, who discovered trypsin, an enzyme in pancreatic juice that is needed for digestion of food proteins. In this section, we will explore how enzymes are able to increase the rate of chemical reactions. Interestingly, some biological catalysts are RNA molecules called ribozymes. We will also examine a few examples in which RNA molecules carry out catalytic functions.

### Enzymes Increase the Rates of Chemical Reactions

Thus far, we have examined aspects of energy and considered how the laws of thermodynamics are related to the direction of chemical reactions. If a chemical reaction has a negative free-energy change, the reaction will be spontaneous; it will tend to proceed in the direction of reactants to products. Although thermodynamics governs the direction of an energy transformation, it does not control the rate of a chemical reaction. For example, the breakdown of the molecules in gasoline to smaller molecules is a highly exergonic reaction. Even so, we could place gasoline and oxygen in a container and nothing much would happen (provided it wasn't near a flame). If we came back several days later, we would expect to see the gasoline still sitting there. Perhaps if we came back in a few million years, the gasoline would have been broken down. On a time-scale of months or a few years, however, the chemical reaction would proceed very slowly.

In living cells, the rates of enzyme-catalyzed reactions typically occur millions of times faster than the corresponding uncatalyzed reactions. An extreme example is the enzyme catalase, which is found in peroxisomes (see Chapter 4). This enzyme catalyzes the breakdown of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) into water and oxygen. Catalase speeds up this reaction  $10^{15}$ -fold faster than the uncatalyzed reaction!

Why are catalysts necessary to speed up a chemical reaction? When a covalent bond is broken or formed, this process initially involves the straining or stretching of one or more bonds in the starting molecule(s), and/or it may involve the positioning of two molecules so they interact with each other

properly. Let's consider the reaction in which ATP is used to phosphorylate glucose.

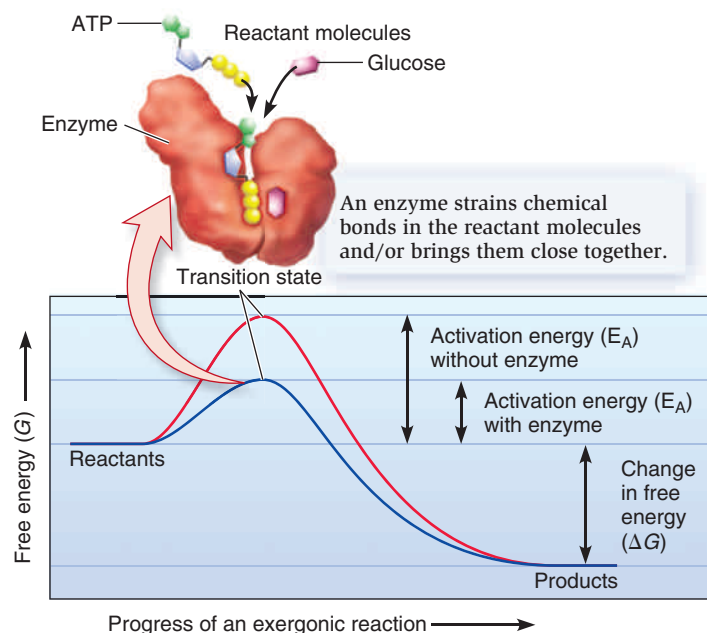


For a reaction to occur between glucose and ATP, the molecules must collide in the correct orientation and possess enough energy so that chemical bonds can be changed. As glucose and ATP approach each other, their electron clouds cause repulsion. To overcome this repulsion, an initial input of energy, called the **activation energy**, is required (Figure 6.4). Activation energy allows the molecules to get close enough to cause a rearrangement of bonds. With the input of activation energy, glucose and ATP can achieve a **transition state** in which the original bonds have stretched to their limit. Once the reactants have reached the transition state, the chemical reaction can readily proceed to the formation of products, which in this case is glucose-phosphate and ADP.

The activation energy required to achieve the transition state is a barrier to the formation of products. This barrier is the reason why the rate of many chemical reactions is very slow. There are two common ways to overcome this barrier and thereby accelerate a chemical reaction. First, the reactants could be exposed to a large amount of heat. For example, as we noted previously, if gasoline is sitting at room temperature, nothing much happens. However, if the gasoline is exposed to a flame or spark, it breaks down rapidly, perhaps at an explosive rate! Alternatively, a second strategy is to lower the activation energy barrier. Enzymes lower the activation energy to a point where a small amount of available heat can push the reactants to a transition state (Figure 6.4).

How do enzymes lower the activation energy barrier of chemical reactions? Enzymes are generally large proteins that bind relatively small reactants (Figure 6.4). When bound to an enzyme, the bonds in the reactants can be strained, thereby making it easier for them to achieve the transition state. This is one way that enzymes lower the activation energy. In addition, when a chemical reaction involves two or more reactants, the enzyme provides a site in which the reactants are positioned very close to each other in an orientation that facilitates the formation of new covalent bonds. This also lowers the necessary activation energy for a chemical reaction.

Straining the reactants and bringing them close together are two common ways that enzymes lower the activation energy barrier. In addition, enzymes may facilitate a chemical reaction by changing the local environment of the reactants. For example, amino acids in an enzyme may have charges that affect the chemistry of the reactants. In some cases, enzymes lower the activation energy by directly participating in the chemical reaction. For example, certain enzymes that hydrolyze ATP form a covalent bond between phosphate and an amino acid in the enzyme. However, this is a temporary condition. The covalent bond between phosphate and the amino acid is quickly broken, releasing the phosphate and returning the amino acid back to its original condition. An example of such an enzyme is  $\text{Na}^+/\text{K}^+$ -ATPase, described in Chapter 5 (refer back to Figure 5.25).



**Figure 6.4** Activation energy of a chemical reaction.

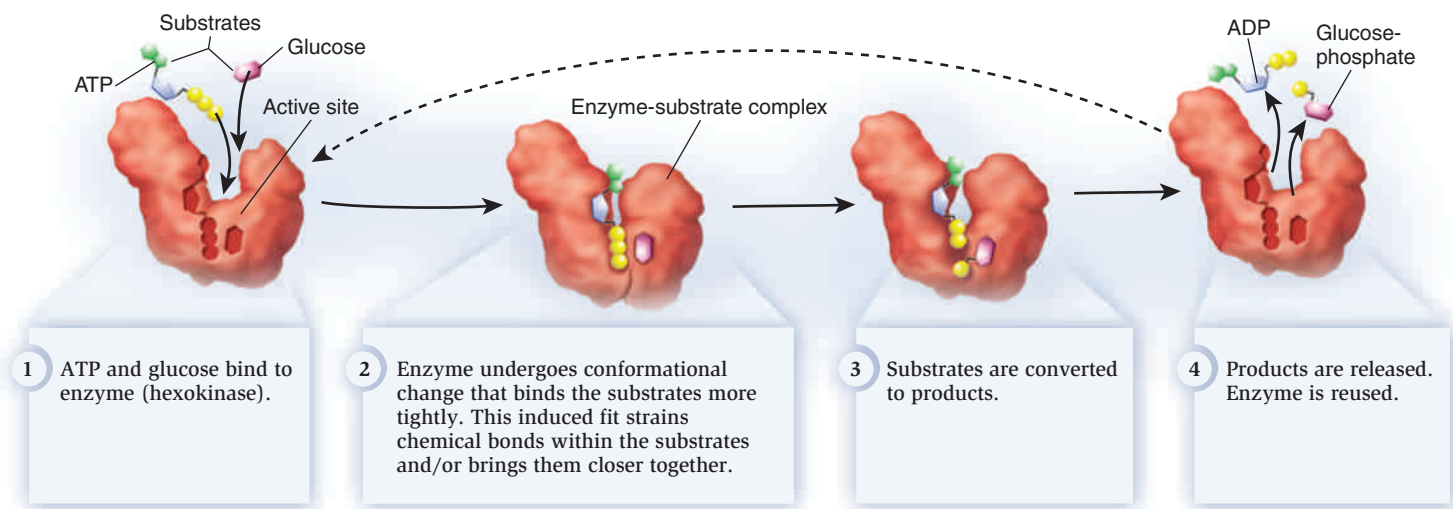
This figure depicts an exergonic reaction. The activation energy is needed for molecules to achieve a transition state. One way that enzymes lower the activation energy is by straining the reactants so that less energy is required to attain the transition state. A second way is by binding two reactants so they are close to each other and in a favorable orientation.

**Concept check:** How does lowering the activation energy affect the rate of a chemical reaction? How does it affect the direction?

## Enzymes Recognize Their Substrates with High Specificity and Undergo Conformational Changes

Thus far, we have considered how enzymes lower the activation energy of a chemical reaction and thereby increase its rate. Let's consider some other features of enzymes that enable them to serve as effective catalysts in chemical reactions. The **active site** is the location in an enzyme where the chemical reaction takes place. The **substrates** for an enzyme are the reactant molecules that bind to an enzyme at the active site and participate in the chemical reaction. For example, hexokinase is an enzyme whose substrates are glucose and ATP (Figure 6.5). The binding between an enzyme and substrate produces an **enzyme-substrate complex**.

A key feature of nearly all enzymes is they bind their substrates with a high degree of **specificity**. For example, hexokinase recognizes glucose but does not recognize other similar sugars very well, such as fructose and galactose. In 1894, the German scientist Emil Fischer proposed that the recognition of a substrate by an enzyme resembles the interaction between a lock and key: only the right-sized key (the substrate) will fit into the keyhole (active site) of the lock (the enzyme). Further research revealed that the interaction between an enzyme and its substrates also involves movements or conformational changes in the enzyme itself. As shown in Figure 6.5, these



**Figure 6.5** The steps of an enzyme-catalyzed reaction. The example shown here involves the enzyme hexokinase, which binds glucose and ATP. The products are glucose-phosphate and ADP, which are released from the enzyme.

**Concept check:** During which step is the activation energy lowered?

conformational changes cause the substrates to bind more tightly to the enzyme, a phenomenon called **induced fit**, which was proposed by American biochemist Daniel Koshland in 1958. Only after this conformational change takes place does the enzyme catalyze the conversion of reactants to products.

### Competitive and Noncompetitive Inhibitors Affect Enzyme Function

Molecules or ions may bind to enzymes and inhibit their function. To understand how such inhibitors work, researchers compare the function of enzymes in the absence or presence of inhibitors. Let's first consider enzyme function in the absence of an inhibitor. In the experiment of **Figure 6.6a**, tubes labeled A, B, C, and D each contained one microgram of enzyme. This enzyme recognizes a single type of substrate and converts it to a product. For each data point, the substrate concentration added to each tube was varied from a low to a high level. The samples were incubated for 60 seconds, and then the amount of product in each tube was measured. In this example, the velocity of the chemical reaction is expressed as the amount of product produced per second. As we see in **Figure 6.6a**, the velocity increases as the substrate concentration increases, but eventually reaches a plateau. Why does the plateau occur? At high substrate concentrations, nearly all of the active sites of the enzyme are occupied with substrate, so increasing the substrate concentration further has a negligible effect. At this point, the enzyme is saturated with substrate, and the velocity of the chemical reaction is near its maximal rate, called its  $V_{\max}$ .

**Figure 6.6a** also helps us understand the relationship between substrate concentration and velocity. The  $K_M$  is the substrate concentration at which the velocity is half its maximal value. The  $K_M$  is also called the Michaelis constant in honor

of the German biochemist Leonor Michaelis, who carried out pioneering work with the Canadian biochemist Maud Menten on the study of enzymes. The  $K_M$  is a measure of the substrate concentration required for catalysis to occur. An enzyme with a high  $K_M$  requires a higher substrate concentration to achieve a particular reaction velocity compared to an enzyme with a lower  $K_M$ .

For an enzyme-catalyzed reaction, we can view the formation of product as occurring in two steps: (1) binding or release of substrate and (2) formation of product.



where

$E$  is the enzyme

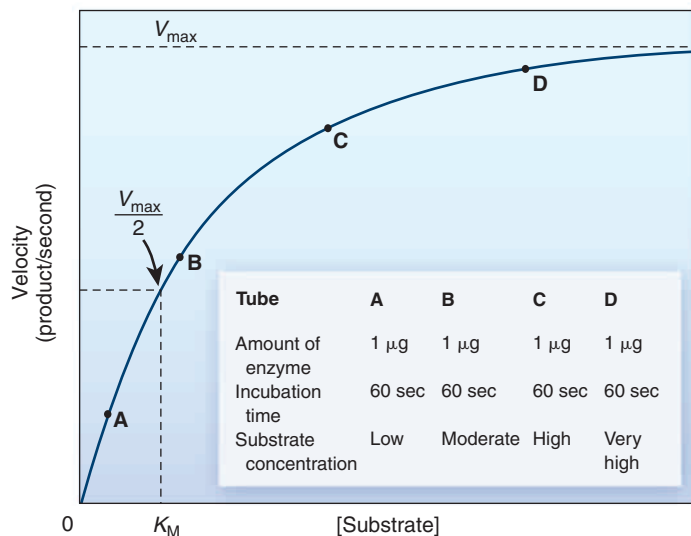
$S$  is the substrate

$ES$  is the enzyme-substrate complex

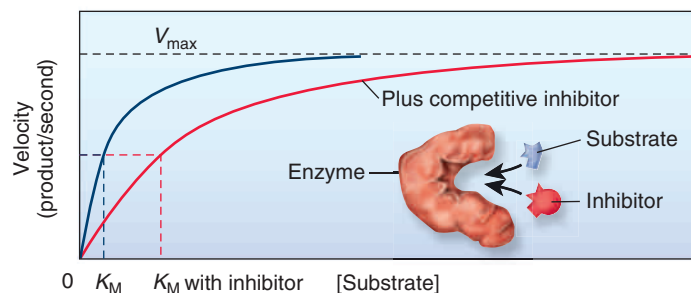
$P$  is the product

If the second step—the rate of product formation—is much slower than the rate of substrate release, the  $K_M$  is inversely related to the **affinity**—degree of attraction—between the enzyme and substrate. For example, let's consider an enzyme that breaks down ATP into ADP and  $P_i$ . If the rate of formation of ADP and  $P_i$  is much slower than the rate of ATP release, the  $K_M$  for such an enzyme is a measure of its affinity for ATP. In such cases, the  $K_M$  and affinity show an inverse relationship. Enzymes with a high  $K_M$  have a low affinity for their substrates—they bind them more weakly. By comparison, enzymes with a low  $K_M$  have a high affinity for their substrates—they bind them more tightly.

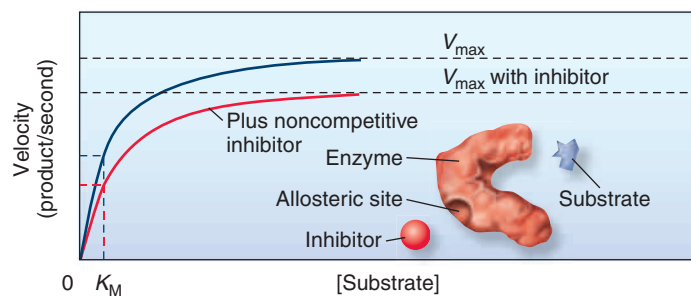
Now that we understand the relationship between substrate concentration and the velocity of an enzyme-catalyzed



(a) Reaction velocity in the absence of inhibitors



(b) Competitive inhibition



(c) Noncompetitive inhibition

**Figure 6.6** The relationship between velocity and substrate concentration in an enzyme-catalyzed reaction, and the effects of inhibitors. (a) In the absence of an inhibitor, the maximal velocity ( $V_{max}$ ) is achieved when the substrate concentration is high enough to be saturating. The  $K_M$  value is the substrate concentration where the velocity is half the maximal velocity. (b) A competitive inhibitor binds to the active site of an enzyme and raises the  $K_M$  for the substrate. (c) A noncompetitive inhibitor binds outside the active site to an allosteric site and lowers the  $V_{max}$  for the reaction.

**Concept check:** Enzyme A has a  $K_M$  of 0.1 mM, whereas enzyme B has a  $K_M$  of 1.0 mM. They both have the same  $V_{max}$ . If the substrate concentration was 0.5 mM, which reaction—the one catalyzed by enzyme A or B—would have the higher velocity?

reaction, we can explore how inhibitors may affect enzyme function. **Competitive inhibitors** are molecules that bind to the active site of an enzyme and inhibit the ability of the substrate to bind. Such inhibitors compete with the substrate for the ability to bind to the enzyme. Competitive inhibitors usually have a structure or a portion of their structure that mimics the structure of the enzyme's substrate. As seen in **Figure 6.6b**, when competitive inhibitors are present, the apparent  $K_M$  for the substrate increases—a higher concentration of substrate is needed to achieve the same velocity of the chemical reaction. In this case, the effects of the competitive inhibitor can be overcome by increasing the concentration of the substrate.

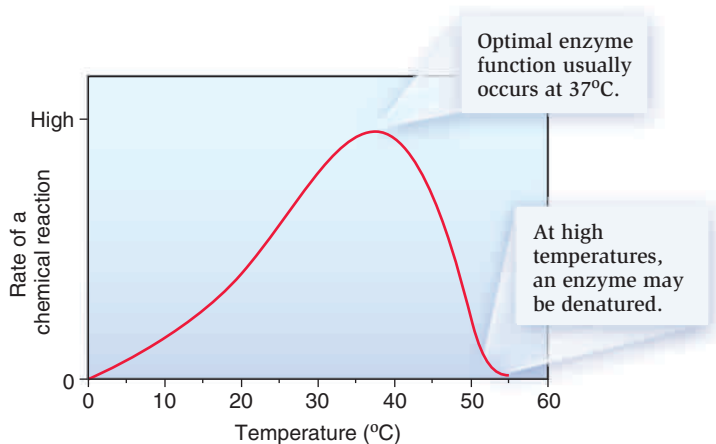
By comparison, **Figure 6.6c** illustrates the effects of a **noncompetitive inhibitor**. As seen here, this type of inhibitor lowers the  $V_{max}$  for the reaction without affecting the  $K_M$ . A noncompetitive inhibitor binds noncovalently to an enzyme at a location outside the active site, called an **allosteric site**, and inhibits the enzyme's function. In this example, a molecule binding to the allosteric site inhibits the enzyme's function, but for other enzymes, such binding can enhance their function.

## Additional Factors Influence Enzyme Function

Enzymes, which are composed of protein, sometimes require additional nonprotein molecules or ions to carry out their functions. **Prosthetic groups** are small molecules that are permanently attached to the surface of an enzyme and aid in catalysis. **Cofactors** are usually inorganic ions, such as  $Fe^{3+}$  or  $Zn^{2+}$ , that temporarily bind to the surface of an enzyme and promote a chemical reaction. Finally, some enzymes use **coenzymes**, organic molecules that temporarily bind to an enzyme and participate in the chemical reaction but are left unchanged after the reaction is completed. Some of these coenzymes can be synthesized by cells, but many of them are taken in as dietary vitamins by animal cells.

The ability of enzymes to increase the rate of a chemical reaction is also affected by the surrounding conditions. In particular, the temperature, pH, and ionic conditions play an important role in the proper functioning of enzymes. Most enzymes function maximally in a narrow range of temperature and pH. For example, many human enzymes work best at 37°C (98.6°F), which is the body's normal temperature. If the temperature was several degrees above or below this value due to infection or environmental causes, the function of many enzymes would be greatly inhibited (**Figure 6.7**). Increasing the temperature may have more severe effects on enzyme function if the protein structure of an enzyme is greatly altered. Very high temperatures may denature a protein—cause it to become unfolded. Denaturing an enzyme is expected to inhibit its function.

Enzyme function is also sensitive to pH. Certain enzymes in the stomach function best at the acidic pH found in this organ. For example, pepsin is a protease—an enzyme that digests proteins—that is released into the stomach. Its function is to degrade food proteins into shorter peptides. The optimal pH for pepsin function is around pH 2.0, which is extremely



acidic. By comparison, many cytosolic enzymes function optimally at a more neutral pH, such as pH 7.2, which is the pH normally found in the cytosol of human cells. If the pH was significantly above or below this value, enzyme function would be decreased for cytosolic enzymes.

**Figure 6.7** Effects of temperature on a typical human enzyme. Most enzymes function optimally within a narrow range of temperature. Many human enzymes function best at 37°C, which is body temperature.

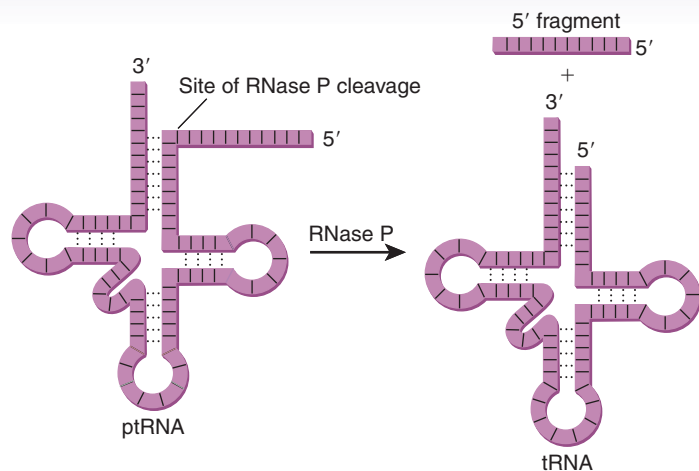
## FEATURE INVESTIGATION

### The Discovery of Ribozymes by Sidney Altman Revealed That RNA Molecules May Also Function as Catalysts

Until the 1980s, scientists thought that all biological catalysts are proteins. One avenue of study that dramatically changed this view came from the analysis of ribonuclease P (RNase P), a catalyst initially found in the bacterium *Escherichia coli* and later identified in all species examined. RNase P is involved in the processing of tRNA molecules—a type of molecule required for protein synthesis. Such tRNA molecules are synthesized as longer precursor molecules called ptRNAs, which have 5' and 3' ends. (The 5' and 3' directionality of RNA molecules is described in Chapter 11.) RNase P breaks a covalent bond at a specific site in precursor tRNAs, which releases a fragment at the 5' end and makes them shorter (Figure 6.8).

Sidney Altman and his colleagues became interested in the processing of tRNA molecules and turned their attention to RNase P in *E. coli*. During the course of their studies, they purified this enzyme and, to their surprise, discovered it has two subunits—one is an RNA molecule that contains 377 nucleotides, and the other is a small protein with a mass of 14 kDa. A complex between RNA and a protein is called a **ribonucleoprotein**. In 1990, the finding that a catalyst has an RNA subunit was very unexpected. Even so, a second property of RNase P would prove even more exciting.

Altman and colleagues were able to purify RNase P and study its properties *in vitro*. As mentioned earlier in this chapter, the functioning of enzymes is affected by the surrounding conditions. Cecilia Guerrier-Takada in Altman's laboratory determined that  $Mg^{2+}$  had a stimulatory effect on RNase P function. In the experiment described in Figure 6.9, the effects of  $Mg^{2+}$  were studied in greater detail. The researchers analyzed the effects of low (10 mM  $MgCl_2$ ) and high (100 mM  $MgCl_2$ ) magnesium concentrations on the processing of a ptRNA. At low or high magnesium concentrations, the ptRNA was incubated without RNase P (as a control); with the RNA subunit alone; or with intact RNase P (RNA subunit and protein sub-



**Figure 6.8** The function of RNase P. A specific bond in a precursor tRNA (ptRNA) is cleaved by RNase P, which releases a small fragment at the 5' end. This results in the formation of a mature tRNA.

unit). Following incubation, they performed gel electrophoresis on the samples to determine if the ptRNAs had been cleaved into two pieces—the tRNA and a 5' fragment.

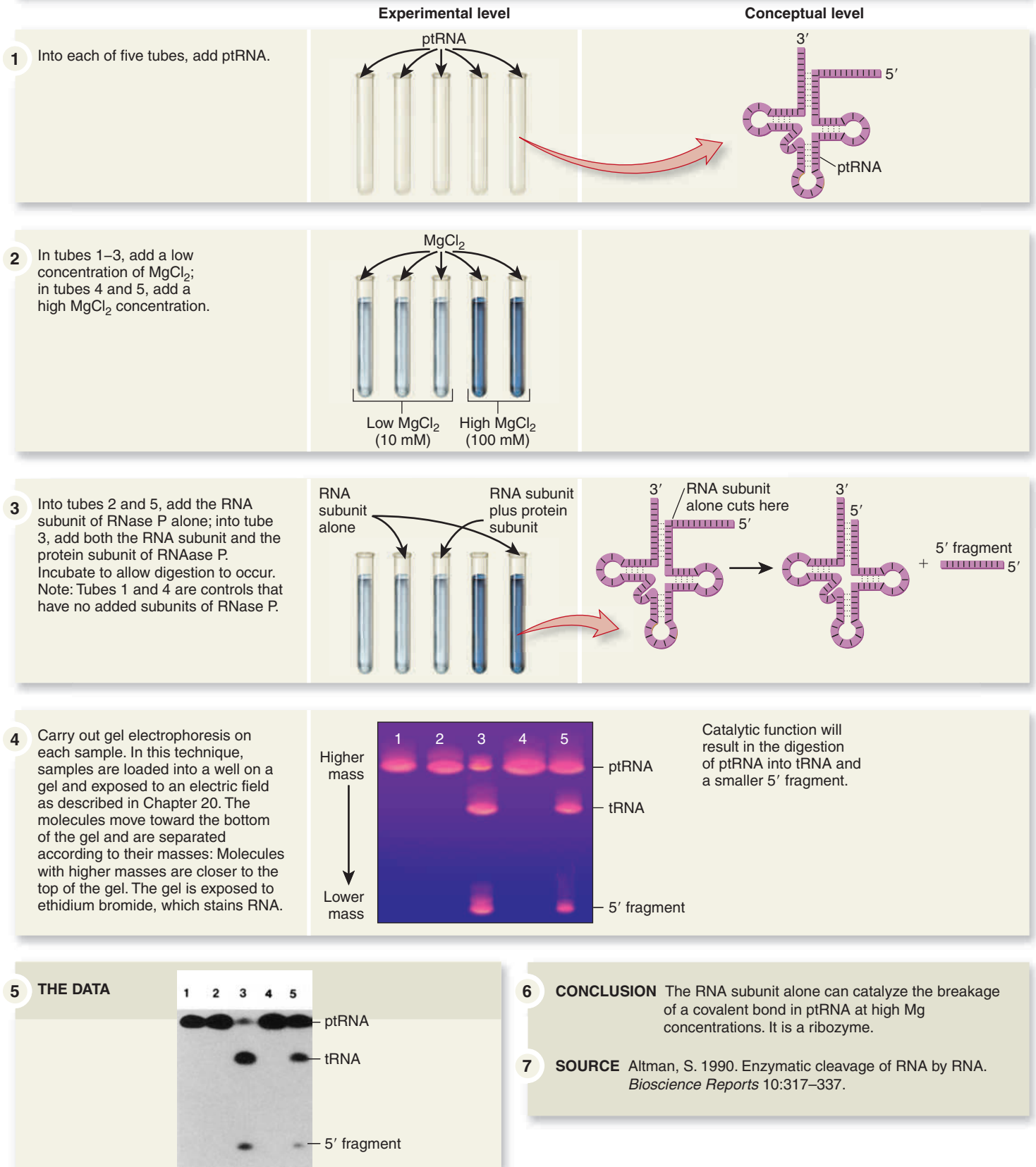
Let's now look at the data. As a control, ptRNAs were incubated with low (lane 1) or high (lane 4)  $MgCl_2$  in the absence of RNase P. As expected, no processing to a lower molecular mass tRNA was observed. When the RNA subunit alone was incubated with ptRNA molecules in the presence of low  $MgCl_2$  (lane 2), no processing occurred, but it did occur if the protein subunit was also included (lane 3).

The surprising result is shown in lane 5. In this case, the RNA subunit alone was incubated with ptRNAs in the presence of high  $MgCl_2$ . The RNA subunit by itself was able to cleave the ptRNA to a smaller tRNA and a 5' fragment! These results indicate that RNA molecules alone can act as catalysts that facilitate the breakage of a covalent bond. In this case, the RNA subunit

**Figure 6.9** The discovery that the RNA subunit of RNase P is a catalyst.

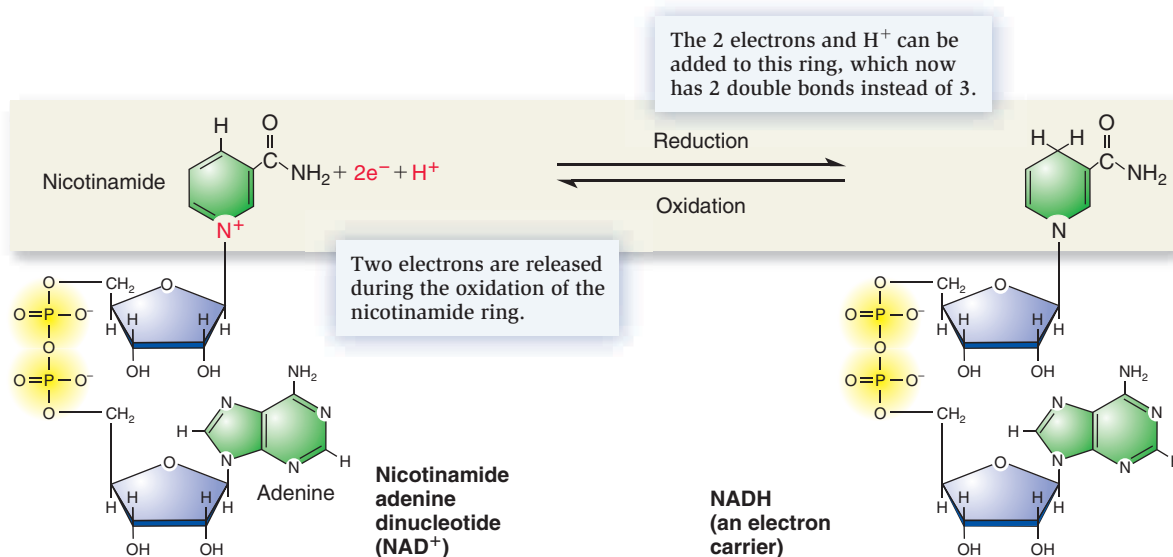
**HYPOTHESIS** The catalytic function of RNase P could be carried out by its RNA subunit or by its protein subunit.

**KEY MATERIALS** Purified precursor tRNA (ptRNA) and purified RNA and protein subunits of RNase P from *E. coli*.





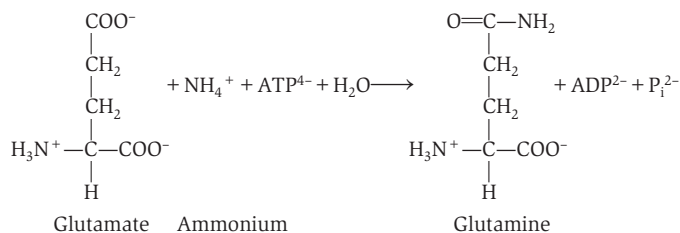




**Figure 6.11** The reduction of  $NAD^+$  to create NADH.  $NAD^+$  is composed of two nucleotides, one with an adenine base and one with a nicotinamide base. The oxidation of organic molecules releases electrons that can bind to  $NAD^+$ , and along with a hydrogen ion, result in the formation of NADH. The two electrons and  $H^+$  are incorporated into the nicotinamide ring. Note: The actual net charges of  $NAD^+$  and NADH are minus one and minus two, respectively. They are designated  $NAD^+$  and NADH to emphasize the net charge of the nicotinamide ring, which is involved in oxidation-reduction reactions.

**Concept check:** Which is the oxidized form,  $NAD^+$  or NADH?

Subsequently, another amino acid, glutamine, is made from glutamate and ammonium.



In both reactions, an energy intermediate molecule such as NADH or ATP is needed to drive the reaction forward.

## Genomes & Proteomes Connection

### Many Proteins Use ATP as a Source of Energy

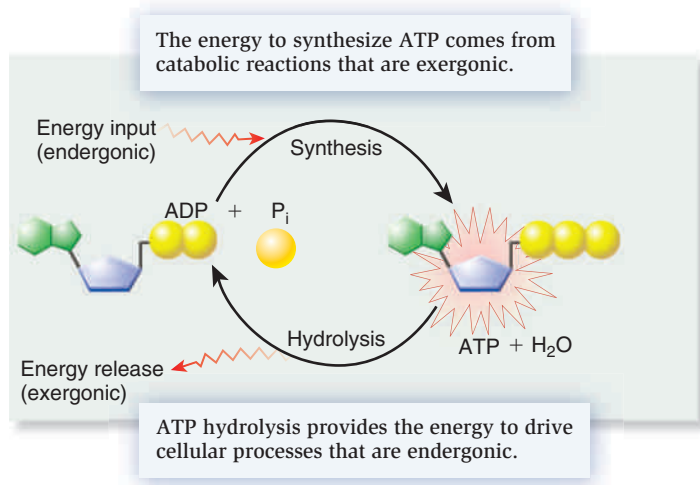
Over the past several decades, researchers have studied the functions of many types of proteins and discovered numerous examples in which a protein uses the hydrolysis of ATP to drive a cellular process (Table 6.3). In humans, a typical cell uses millions of ATP molecules per second. At the same time, the breakdown of food molecules to form smaller molecules releases energy that allows us to make more ATP from ADP and  $P_i$ . The turnover of ATP occurs at a remarkable pace. An average person hydrolyzes about 100 pounds of ATP per day, yet at any given time we do not have 100 pounds of ATP in our bodies. For this to happen, each ATP undergoes about

**Table 6.3** Examples of Proteins That Use ATP for Energy

Type	Description
Metabolic enzymes	Many enzymes use ATP to catalyze endergonic reactions. For example, hexokinase uses ATP to attach phosphate to glucose.
Transporters	Ion pumps, such as the $\text{Na}^+/\text{K}^+$ -ATPase, use ATP to pump ions against a gradient (see Chapter 5).
Motor proteins	Motor proteins such as myosin use ATP to facilitate cellular movement, as in muscle contraction (see Chapter 46).
Chaperones	Chaperones are proteins that use ATP to aid in the folding and unfolding of cellular proteins (see Chapter 4).
Protein kinases	Protein kinases are regulatory proteins that use ATP to attach a phosphate to proteins, thereby phosphorylating the protein and affecting its function (see Chapter 9).

10,000 cycles of hydrolysis and resynthesis during an ordinary day (Figure 6.12).

By studying the structures of many proteins that use ATP, biochemists have discovered that particular amino acid sequences within proteins function as ATP-binding sites. This information has allowed researchers to predict whether a newly discovered protein uses ATP or not. When an entire genome sequence of a species has been determined, the genes that encode proteins can be analyzed to find out if the encoded proteins have ATP-binding sites in their amino acid sequences. Using this approach, researchers have been able to analyze



**Figure 6.12** The ATP cycle. Living cells continuously recycle ATP. The breakdown of food molecules into smaller molecules is used to synthesize ATP from ADP and  $P_i$ . The hydrolysis of ATP to ADP and  $P_i$  is used to drive many different endergonic reactions and processes that occur in cells.

**Concept check:** If a large amount of ADP was broken down in the cell, how would this affect the ATP cycle?

**proteomes**—all of the proteins that a given cell can make—and estimate the percentage of proteins that are able to bind ATP. This approach has been applied to the proteomes of bacteria, archaea, and eukaryotes.

On average, over 20% of all proteins bind ATP. However, this number is likely to be an underestimate of the total percentage of ATP-utilizing proteins because we may not have identified all of the types of ATP-binding sites in proteins. In humans, who have an estimated genome size of 20,000 to 25,000 different genes, a minimum of 4,000 to 5,000 of those genes encode proteins that use ATP. From these numbers, we can see the enormous importance of ATP as a source of energy for living cells.

## Metabolic Pathways Are Regulated in Three General Ways

The regulation of metabolic pathways is important for a variety of reasons. Catabolic pathways are regulated so that organic molecules are broken down only when they are no longer needed or when the cell requires energy. During anabolic reactions, regulation assures that a cell synthesizes molecules only when they are needed. The regulation of catabolic and anabolic pathways occurs at the genetic, cellular, and biochemical levels.

**Gene Regulation** Because enzymes in every metabolic pathway are encoded by genes, one way that cells control chemical reactions is via gene regulation. For example, if a bacterial cell is not exposed to a particular sugar in its environment, it will turn off the genes that encode the enzymes that are needed to break down that sugar. Alternatively, if the sugar becomes available, the genes are switched on. Chapter 13 examines the steps of gene regulation in detail.

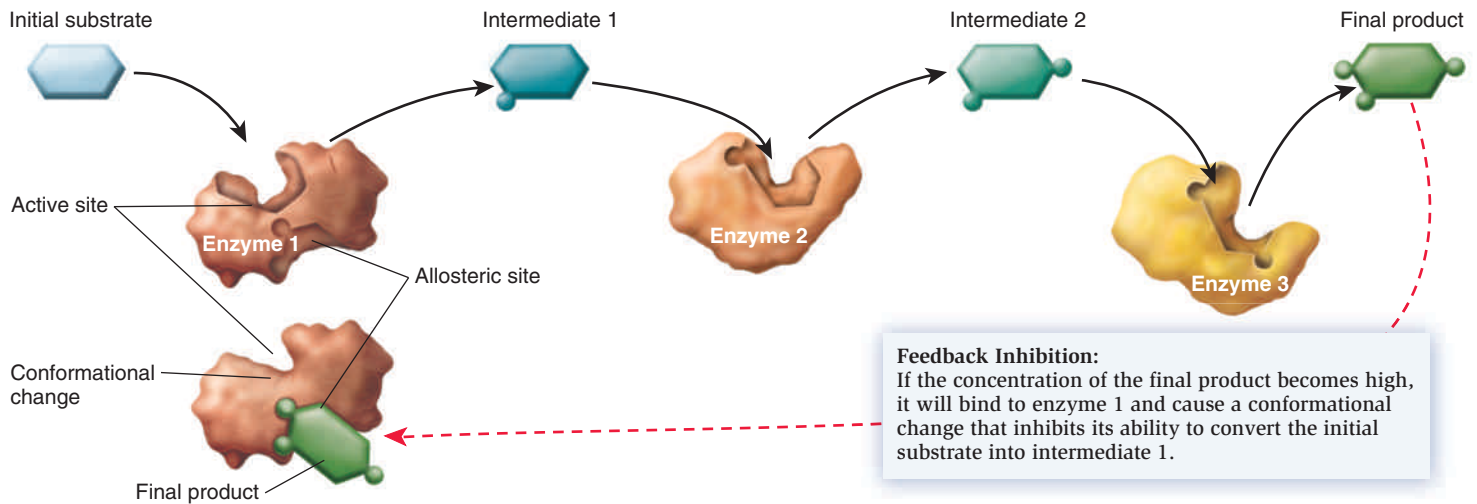
**Cellular Regulation** Metabolism is also coordinated at the cellular level. Cells integrate signals from their environment and adjust their chemical reactions to adapt to those signals. As discussed in Chapter 9, cell-signaling pathways often lead to the activation of protein kinases—enzymes that covalently attach a phosphate group to target proteins. For example, when people are frightened, they secrete a hormone called epinephrine into their bloodstream. This hormone binds to the surface of muscle cells and stimulates an intracellular pathway that leads to the phosphorylation of several intracellular proteins, including enzymes involved in carbohydrate metabolism. These activated enzymes promote the breakdown of carbohydrates, an event that supplies the frightened individual with more energy. Epinephrine is sometimes called the “fight-or-flight” hormone because the added energy prepares an individual to either stay and fight or run away. After a person is no longer frightened, hormone levels drop, and other enzymes called phosphatases remove the phosphate groups from enzymes, thereby restoring the original level of carbohydrate metabolism.

Another way that cells control metabolic pathways is via compartmentalization. The membrane-bound organelles of eukaryotic cells, such as the endoplasmic reticulum and mitochondria, serve to compartmentalize the cell. As discussed in Chapter 7, this allows specific metabolic pathways to occur in one compartment in the cell but not in others.

**Biochemical Regulation** A third and very prominent way that metabolic pathways are controlled is at the biochemical level. In this case, the binding of a molecule to an enzyme directly regulates its function. As discussed earlier, one form of biochemical regulation involves the binding of molecules such as competitive or noncompetitive inhibitors (see Figure 6.6). An example of noncompetitive inhibition is a type of regulation called **feedback inhibition**, in which the product of a metabolic pathway inhibits an enzyme that acts early in the pathway, thus preventing the overaccumulation of the product (Figure 6.13).

Many metabolic pathways use feedback inhibition as a form of biochemical regulation. In such cases, the inhibited enzyme has two binding sites. One site is the active site, where the reactants are converted to products. In addition, enzymes controlled by feedback inhibition also have an allosteric site, where a molecule can bind noncovalently and affect the function of the active site. The binding of a molecule to an allosteric site causes a conformational change in the enzyme that inhibits its catalytic function. Allosteric sites are often found in the enzymes that catalyze the early steps in a metabolic pathway. Such allosteric sites typically bind molecules that are the products of the metabolic pathway. When the products bind to these sites, they inhibit the function of these enzymes and thereby prevent the formation of too much product.

Cellular and biochemical regulation are important and rapid ways to control chemical reactions in a cell. For a metabolic pathway composed of several enzymes, which enzyme in a pathway should be controlled? In many cases, a metabolic pathway has a **rate-limiting step**, which is the slowest step in a pathway. If the rate-limiting step is inhibited or enhanced, such



**Figure 6.13** Feedback inhibition. In this process, the product of a metabolic pathway inhibits an enzyme that functions in the pathway, thereby preventing the overaccumulation of the product.

**Concept check:** What would be the consequences if a mutation had no effect on the active site on enzyme 1 but altered its allosteric site so that it no longer recognized the final product?

changes will have the greatest impact on the formation of the product of the metabolic pathway. Rather than affecting all of the enzymes in a metabolic pathway, cellular and biochemical regulation are often directed at the enzyme that catalyzes the rate-limiting step. This is an efficient and rapid way to control the amount of product of a pathway.

## 6.4 Recycling of Macromolecules

Except for DNA, which is stably maintained and inherited from cell to cell, other large molecules such as RNA, proteins, lipids, and polysaccharides typically exist for a relatively short period of time. Biologists often speak of the **half-life** of molecules, which is the time it takes for 50% of the molecules to be broken down and recycled. For example, a population of messenger RNA molecules in prokaryotes has an average half-life of about 5 minutes, whereas mRNAs in eukaryotes tend to exist for longer periods of time, on the order of 30 minutes to 24 hours or even several days.

Why is recycling important? To compete effectively in their native environments, all living organisms must efficiently use and recycle the organic molecules that are needed as building blocks to construct larger molecules and macromolecules. Otherwise, they would waste a great deal of energy making such building blocks. For example, organisms conserve an enormous amount of energy by re-using the amino acids that are needed to construct cellular proteins.

As discussed in Chapters 1 and 4, the characteristics of cells are controlled by the genome and the resulting proteome. The genome of every cell contains many genes that are transcribed into RNA. Most of these RNA molecules, called messenger RNA, or mRNA, encode proteins that ultimately determine the structure and function of cells. The expression of the genome is a very dynamic process, allowing cells to

respond to changes in their environment. RNA and proteins are made when they are needed and then broken down when they are not. After they are broken down, the building blocks of RNA and proteins—nucleotides and amino acids—are recycled to make new RNAs and proteins. In this section, we will explore how RNAs and proteins are recycled and consider a mechanism for the recycling of materials found in an entire organelle.

### Messenger RNA Molecules in Eukaryotes Are Broken Down by 5' → 3' Cleavage or by the Exosome

The degradation of mRNA serves two important functions. First, the proteins that are encoded by particular mRNAs may be needed only under certain conditions. A cell conserves energy by degrading mRNAs when such proteins are no longer necessary. Second, mRNAs may be faulty. For example, mistakes during mRNA synthesis can result in mRNAs that produce aberrant proteins. The degradation of faulty mRNAs is beneficial to the cell to prevent the potentially harmful effects of such aberrant proteins.

As described in Chapter 12, eukaryotic mRNAs contain a cap at their 5' end. A tail is found at their 3' end consisting of many adenine bases (look ahead to Figure 12.11). In most cases, degradation of mRNA begins with the removal of nucleotides in the poly A tail at the 3' end (Figure 6.14). After the tail gets shorter, two mechanisms of degradation may occur.

In one mechanism, the 5' cap is removed, and the mRNA is degraded by an **exonuclease**—an enzyme that cleaves off nucleotides, one at a time, from the end of the RNA. In this case, the exonuclease removes nucleotides starting at the 5' end and moving toward the 3'. The nucleotides can then be used to make new RNA molecules.

The other mechanism involves mRNA being degraded by an **exosome**, a multiprotein complex discovered in 1997. Exosomes are found in eukaryotic cells and some archaea, whereas in bacteria a simpler complex called the degradosome carries out similar functions. The core of the exosome has a six-membered protein ring to which other proteins are attached (see inset to Figure 6.14). Certain proteins within the exosome are exonucleases that degrade the mRNA starting at the 3' end and moving toward the 5' end, thereby releasing nucleotides that can be recycled.

## Proteins in Eukaryotes and Archaea Are Broken Down in the Proteasome

Cells continually degrade proteins that are faulty or no longer needed. To be degraded, proteins are recognized by **proteases**—enzymes that cleave the bonds between adjacent amino acids. The primary pathway for protein degradation in archaea and eukaryotic cells is via a protein complex called a **proteasome**. Similar to the exosome that has a central cavity surrounded by a ring of proteins, the core of the proteasome is formed from four stacked rings, each composed of seven protein subunits (Figure 6.15a). The proteasomes of eukaryotic cells also contain cap structures at each end that control the entry of proteins into the proteasome.

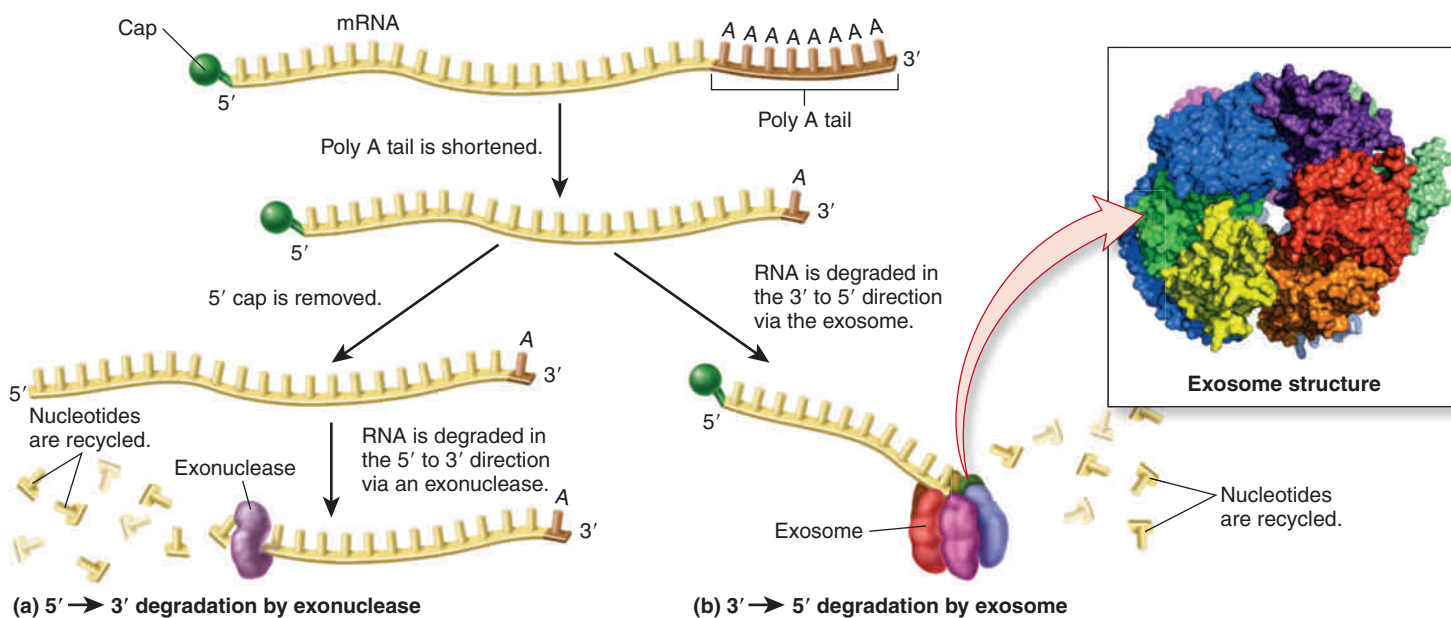
In eukaryotic cells, unwanted proteins are directed to a proteasome by the covalent attachment of a small protein called **ubiquitin**. Figure 6.15b describes the steps of protein degradation via eukaryotic proteasomes. First, a string of ubiquitin proteins are attached to the target protein. This event directs the protein to a proteasome cap, which has binding sites for

ubiquitin. The cap also has enzymes that unfold the protein and inject it into the internal cavity of the proteasome core. The ubiquitin proteins are removed during entry and are returned to the cytosol for reuse. Inside the proteasome, proteases degrade the protein into small peptides and amino acids. The process is completed when the peptides and amino acids are recycled back into the cytosol. The amino acids can be used to make new proteins.

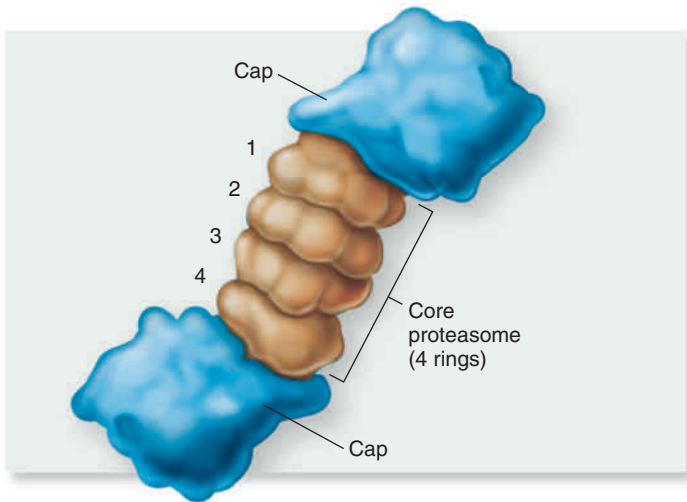
Ubiquitin targeting has two advantages. First, the enzymes that attach ubiquitin to its target recognize improperly folded proteins, allowing cells to identify and degrade nonfunctional proteins. Second, changes in cellular conditions may warrant the rapid breakdown of particular proteins. For example, cell division requires a series of stages called the cell cycle, which depends on the degradation of specific proteins. After these proteins perform their functions in the cycle, ubiquitin targeting directs them to the proteasome for degradation.

## Autophagy Recycles the Contents of Entire Organelles

As described in Chapter 4, lysosomes contain many different types of acid hydrolases that break down proteins, carbohydrates, nucleic acids, and lipids. This enzymatic function enables lysosomes to break down complex materials. One function of lysosomes involves the digestion of substances that are taken up from outside the cell. This process, called endocytosis, is described in Chapter 5. In addition, lysosomes help digest intracellular materials. In a process known as **autophagy** (from the Greek, meaning eating one's self), cellular material, such as a worn-out organelle, becomes enclosed in a double membrane



**Figure 6.14** Two pathways for mRNA degradation in eukaryotic cells. Degradation usually begins with a shortening of the poly A tail. After tail shortening, either (a) the 5' cap is removed and the RNA degraded in a 5' to 3' direction by an exonuclease, or (b) the mRNA is degraded in the 3' to 5' direction via an exosome. The reason why cells have two different mechanisms for RNA degradation is not well understood.

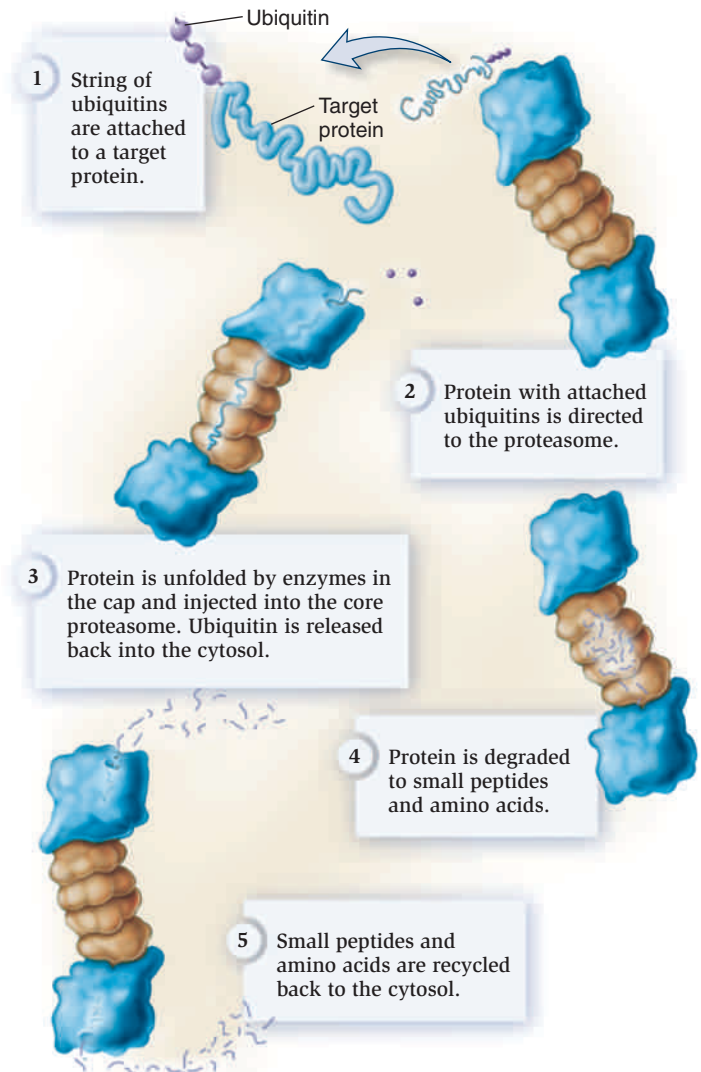


(a) Structure of the eukaryotic proteasome

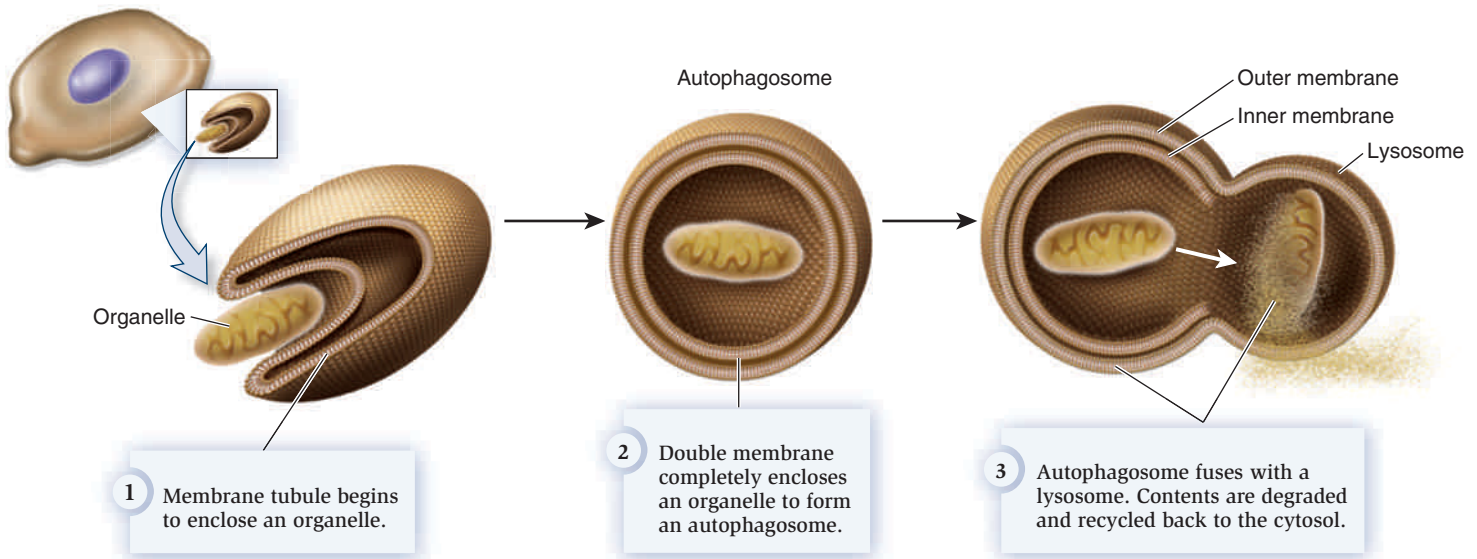
**Figure 6.15** Protein degradation via the proteasome.

**Concept check:** What are advantages of protein degradation?

(Figure 6.16). This double membrane is formed from a tubule that elongates and eventually wraps around the organelle to form an **autophagosome**. The autophagosome then fuses with a lysosome, and the material inside the autophagosome is digested. The small molecules released from this digestion are recycled back into the cytosol.



(b) Steps of protein degradation in eukaryotic cells



**Figure 6.16** Autophagy.

## Summary of Key Concepts

### 6.1 Energy and Chemical Reactions

- The fate of a chemical reaction is determined by its direction and rate.
- Energy, the ability to promote change or do work, exists in many forms. According to the first law of thermodynamics, energy cannot be created or destroyed, but it can be converted from one form to another. The second law of thermodynamics states that energy interconversions involve an increase in entropy. (Figures 6.1, 6.2, Table 6.1)
- Free energy is the amount of available energy that can be used to promote change or do work. Spontaneous reactions, which release free energy, have a negative free-energy change. (Figure 6.3)
- An exergonic reaction has a negative free-energy change, whereas an endergonic reaction has a positive change. Chemical reactions proceed until they reach a state of chemical equilibrium, where the rate of formation of products equals the rate of formation of reactants.
- Exergonic reactions, such as the breakdown of ATP, are commonly coupled to cellular processes that would otherwise be endergonic.

### 6.2 Enzymes and Ribozymes

- Proteins that speed up the rate of a chemical reaction are called enzymes. They lower the activation energy that is needed to achieve a transition state. (Figure 6.4)
- Enzymes recognize reactants, also called substrates, with a high specificity. Conformational changes lower the activation energy for a chemical reaction. (Figure 6.5)
- Each enzyme-catalyzed reaction exhibits a maximal velocity ( $V_{\max}$ ). The  $K_M$  is the substrate concentration at which the velocity of the chemical reaction is half of the  $V_{\max}$ . Competitive inhibitors raise the apparent  $K_M$  for the substrate, whereas noncompetitive inhibitors lower the  $V_{\max}$ . (Figure 6.6)
- Enzyme function may be affected by a variety of other factors, including prosthetic groups, cofactors, coenzymes, temperature, and pH. (Figure 6.7)
- Altman and colleagues discovered that RNase P is a ribozyme—the RNA molecule within RNase P is a catalyst. Other ribozymes also play key roles in the cell. (Figures 6.8, 6.9, Table 6.2)

### 6.3 Overview of Metabolism

- Metabolism is the sum of the chemical reactions in a living organism. Enzymes often function in pathways that lead to the formation of a particular product. (Figure 6.10)
- Catabolic reactions involve the breakdown of larger molecules into smaller ones. These reactions regenerate small molecules that are used as building blocks to make new molecules. The small molecules are also broken down to make energy intermediates such as ATP and NADH. Such reactions are often redox reactions in which electrons are transferred from one molecule to another. (Figure 6.11)

- Anabolic reactions involve the synthesis of larger molecules and macromolecules.
- Cells continuously synthesize ATP from ADP and  $P_i$  and then hydrolyze it to drive endergonic reactions. Estimates from genome analysis indicate that over 20% of a cell's proteins use ATP. (Table 6.3, Figure 6.12)
- Metabolic pathways are controlled by gene regulation, cell signaling, compartmentalization, and feedback inhibition. (Figure 6.13)

### 6.4 Recycling of Macromolecules

- Large molecules in cells have a finite half-life.
- Recycling of macromolecules is important because it saves a great deal of energy for living organisms.
- Messenger RNAs in eukaryotes are degraded by 5' to 3' exonucleases or by the exosome. (Figure 6.14)
- Proteins in eukaryotes and archaea are degraded by the proteasome. (Figure 6.15)
- During autophagy in eukaryotes, an entire organelle is surrounded by a double membrane and then fuses with a lysosome. The internal contents are degraded, and the smaller building blocks are recycled to the cytosol. (Figure 6.16)

## Assess and Discuss

### Test Yourself

1. According to the second law of thermodynamics,
  - a. energy cannot be created or destroyed.
  - b. each energy transfer decreases the disorder of a system.
  - c. energy is constant in the universe.
  - d. each energy transfer increases the level of disorder in a system.
  - e. chemical energy is a form of potential energy.
2. Reactions that release free energy are
  - a. exergonic.
  - b. spontaneous.
  - c. endergonic.
  - d. endothermic.
  - e. both a and b.
3. Enzymes speed up reactions by
  - a. providing chemical energy to fuel a reaction.
  - b. lowering the activation energy necessary to initiate the reaction.
  - c. causing an endergonic reaction to become an exergonic reaction.
  - d. substituting for one of the reactants necessary for the reaction.
  - e. none of the above.
4. Which of the following factors may alter the function of an enzyme?
 

a. pH	d. all of the above
b. temperature	e. b and c only
c. cofactors	

5. In biological systems, ATP functions by
  - a. providing the energy to drive endergonic reactions.
  - b. acting as an enzyme and lowering the activation energy of certain reactions.
  - c. adjusting the pH of solutions to maintain optimal conditions for enzyme activity.
  - d. regulating the speed at which endergonic reactions proceed.
  - e. interacting with enzymes as a cofactor to stimulate chemical reactions.
6. In a chemical reaction, NADH is converted to  $\text{NAD}^+ + \text{H}^+$ . We would say that NADH has been
  - a. reduced.
  - b. phosphorylated.
  - c. oxidized.
  - d. decarboxylated.
  - e. methylated.
7. Currently, scientists are identifying proteins that use ATP as an energy source by
  - a. determining whether those proteins function in anabolic or catabolic reactions.
  - b. determining if the protein has a known ATP-binding site.
  - c. predicting the free energy necessary for the protein to function.
  - d. determining if the protein has an ATP synthase subunit.
  - e. all of the above.
8. With regard to its effects on an enzyme-catalyzed reaction, a competitive inhibitor
  - a. lowers the  $K_M$  only.
  - b. lowers the  $K_M$  and lowers the  $V_{max}$ .
  - c. raises the  $K_M$  only.
  - d. raises the  $K_M$  and lowers the  $V_{max}$ .
  - e. raises the  $K_M$  and raises the  $V_{max}$ .
9. In eukaryotes, mRNAs may be degraded by
  - a. a 5' to 3' exonuclease.
  - b. the exosome.
  - c. the proteasome.
  - d. all of the above.
  - e. a and b only.
10. Autophagy provides a way for cells to
  - a. degrade entire organelles and recycle their components.
  - b. automatically control the level of ATP.
  - c. engulf bacterial cells.
  - d. export unwanted organelles out of the cell.
  - e. inhibit the first enzyme in a metabolic pathway.

## Conceptual Questions

1. With regard to rate and direction, discuss the differences between endergonic and exergonic reactions.
2. Describe the mechanism and purpose of feedback inhibition in a metabolic pathway.
3. Why is recycling of amino acids and nucleotides an important metabolic function of cells? Explain how eukaryotic cells recycle amino acids found in worn-out proteins.

## Collaborative Questions

1. Living cells are highly ordered units, yet the universe is heading toward higher entropy. Discuss how life can maintain its order in spite of the second law of thermodynamics. Are we defying this law?
2. What is the advantage of using ATP as a common energy source? Another way of asking this question is, Why is ATP an advantage over using a bunch of different food molecules? For example, instead of just having a  $\text{Na}^+/\text{K}^+$ -ATPase in a cell, why not have a bunch of different ion pumps each driven by a different food molecule, like a  $\text{Na}^+/\text{K}^+$ -glucosase (a pump that uses glucose), a  $\text{Na}^+/\text{K}^+$ -sucrase (a pump that uses sucrose), a  $\text{Na}^+/\text{K}^+$ -fatty acidase (a pump that uses fatty acids), and so on?

## Online Resource

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Stay a step ahead in your studies with animations that bring concepts to life and practice tests to assess your understanding. Your instructor may also recommend the interactive ebook, individualized learning tools, and more.