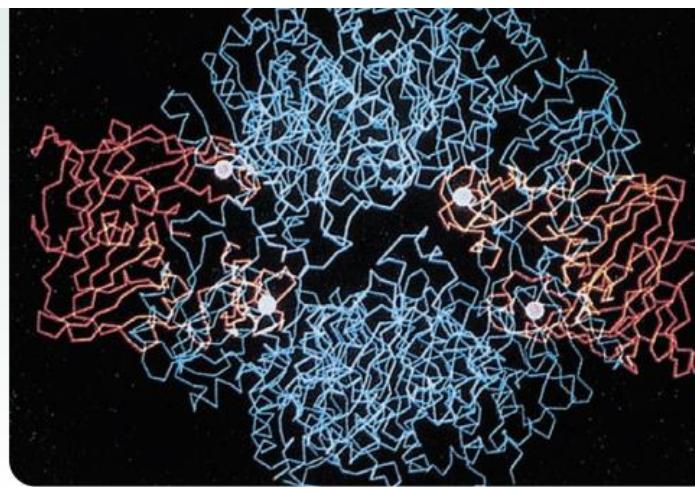


8

Metabolism: Energy, Enzymes, and Regulation



This model shows *Escherichia coli* aspartate carbamoyltransferase in the less active T state. The catalytic polypeptide chains are in blue and the regulatory chains are colored red.

PREVIEW

- Metabolism is the total of all chemical reactions that occur in cells. It is divided into two major parts: energy-conserving reactions that release and conserve the energy provided by an organism's energy source; and anabolism, the reactions that consume energy in order to build large, complex molecules from smaller, simpler molecules.
- Cells use energy to do cellular work. Living organisms do three major types of work: chemical work, transport work, and mechanical work.
- All living organisms obey the laws of thermodynamics. These laws can be used to predict the spontaneity of chemical reactions that occur in cells, and the amount of energy released or energy consumed during a reaction.
- ATP is a high-energy molecule that serves as the cell's energy currency. It links energy-yielding exergonic reactions to energy-consuming endergonic reactions.
- Oxidation-reduction reactions are important in the energy-conserving processes that cells carry out. When electrons are transferred from an electron donor with a more negative reduction potential to an electron acceptor with a more positive potential, energy is made available for work.
- Enzymes are protein catalysts that make life possible by increasing the rate of reactions. They do this by lowering the activation energy of the reactions they catalyze.
- Metabolic pathways are regulated to maintain cell components in proper balance, even in the face of a changing environment, and to conserve energy and raw materials. Metabolic pathways are regulated by one of three methods: metabolic channeling, regulating the activity of certain enzymes, and regulating the amount of an enzyme that is synthesized.
- The activity of enzymes and proteins involved in complex behaviors such as chemotaxis can be regulated by the same mechanisms used to control metabolic pathways.

In the early chapters of this text, we focus on a series of "what" questions about microorganisms: what are they; what do they look like; what are they made of? In chapters 8 through 13 we begin to consider a number of "how" questions: how do microbes extract energy from their energy source; how do they use the nutrients obtained from their environment; how do they build themselves? To begin to answer these "how" questions, we must turn our attention more fully to the chemistry of cells; that is, their metabolism. Chapters 8 through 10 introduce metabolism, focusing on those processes that conserve the energy supplied by an organism's energy source and on how that energy is used to synthesize the building blocks from which an organism is constructed. Chapters 11 through 13 consider the synthesis of three important macromolecules: DNA, RNA, and proteins.

This chapter begins with a brief overview of metabolism. In order to understand metabolism, the nature of energy and the laws of thermodynamics must be considered, so a discussion of these topics follows. As will be seen, microorganisms display an amazing array of metabolic diversity, especially in terms of the energy sources and energy-conserving processes they employ. Yet, despite this diversity, there are several basic principles and processes common to the metabolism of all microbes. These will be the focus of most of the remaining sections of the chapter. The chapter ends with a discussion of metabolic regulation.

8.1 AN OVERVIEW OF METABOLISM

Metabolism is the total of all chemical reactions occurring in the cell. These chemical reactions are summarized in figure 8.1. Metabolism may be divided into two major parts: energy-conserving

*Fresh oxygen flows
From the open stomata
The whole world inhales*

—Crystal Cunningham

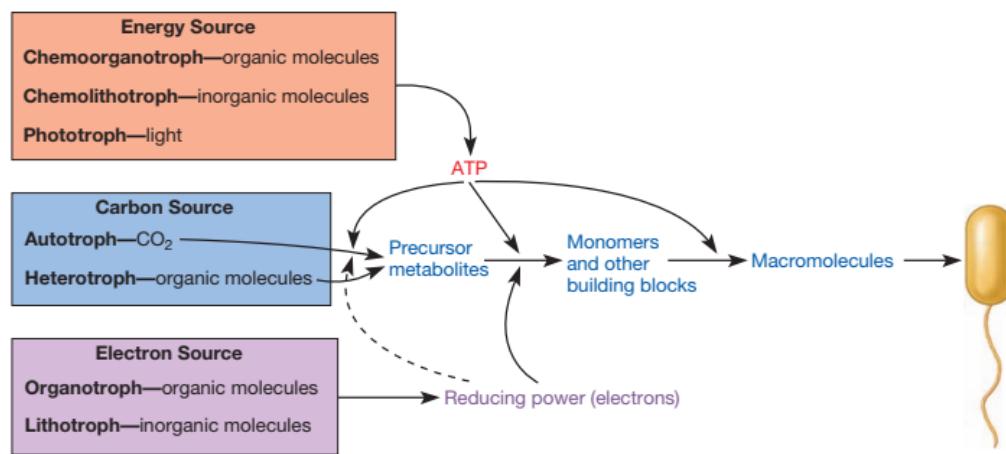


Figure 8.1 Overview of Metabolism. The cell structures of organisms are assembled from various macromolecules (e.g., nucleic acids and proteins). Macromolecules are synthesized from monomers and other building blocks (e.g., nucleotides and amino acids), which are the products of biochemical pathways that begin with precursor metabolites (e.g., pyruvate and α -ketoglutarate). In autotrophs, the precursor metabolites arise from CO_2 -fixation pathways and related pathways; in heterotrophs, they arise from reactions of the central metabolic pathways. Reducing power and ATP are consumed in many metabolic pathways. All organisms can be defined metabolically in terms of their energy source, carbon source, and electron source. In the case of chemoorganotrophs, the energy source is an organic molecule that is also the source of carbon and electrons. For chemolithotrophs, the energy source is an inorganic molecule that is also the electron source; the carbon source can be either CO_2 (autotrophs) or an organic molecule (heterotrophs). For phototrophs, the energy source is light, the carbon source can be CO_2 or organic molecules, and the electron source can be water (oxygenic phototrophs) or another reduced molecule such as hydrogen sulfide (anoxygenic phototrophs).

reactions and anabolism. In the **energy-conserving reactions** or **fueling reactions**, the energy provided to the cell by its energy source is released and conserved as ATP. These reactions are sometimes referred to as **catabolism** [Greek *cata*, down, and *ballein*, to throw], since they can involve the breakdown of relatively large, complex organic molecules into smaller, simpler molecules. **Anabolism** [Greek, *ana*, up] is the synthesis of complex organic molecules from simpler ones. It involves a series of steps: (1) conversion of the organism's carbon source into a set of small molecules called **precursor metabolites**; (2) synthesis of monomers and other building blocks (i.e., amino acids, nucleotides, simple carbohydrates, and simple lipids) from the precursor metabolites; (3) synthesis of macromolecules (i.e., proteins, nucleic acids, complex carbohydrates, and complex lipids); and (4) assembly of macromolecules into cellular structures. Anabolism requires energy, which is transferred from the energy source to the synthetic systems of the cell by ATP. Anabolism also requires a source of electrons stored in the form of **reducing power**. Reducing power is needed because anabolism is a reductive process; that is, electrons are added to small molecules as they are used to build macromolecules (figure 8.1). Energy conservation and the provision of reducing power are the focus of chapter 9; the initial steps in anabolism are the focus of chapter 10.

As discussed in chapter 5, there are five major nutritional types of microorganisms based on their sources of energy, carbon, and electrons (figure 8.1). Animals and many microbes are **chemoorganotrophs**.

heterotrophs. These organisms use organic molecules as their source of energy, carbon, and electrons. In other words, the same molecule that supplies them with energy also supplies them with carbon and electrons. Chemoorganoheterotrophs (often simply referred to as chemoorganotrophs or chemoheterotrophs) can use one or more of the following catabolic processes: fermentation, aerobic respiration, or anaerobic respiration. **Chemolithoautotrophs** use CO_2 as a carbon source and reduced inorganic molecules as sources of both energy and electrons. Their energy-conserving processes are sometimes referred to as respiration because they are similar to the respiratory processes carried out by chemoorganoheterotrophs. **Photolithotrophic** microbes use light as their source of energy and inorganic molecules as a source of electrons. When they use water as their electron source, as do plants, they release oxygen into the atmosphere by a process called **oxygenic photosynthesis**. Certain photosynthetic bacteria do not use water as an electron source; they do not release oxygen into the atmosphere and are called **anoxygenic phototrophs**. Photolithotrophs are usually autotrophic, using CO_2 as a carbon source. However, some phototrophic microbes are heterotrophic. **Aerobic respiration** (section 9.2); **Anaerobic respiration** (section 9.6); **Fermentation** (section 9.7); **Chemolithotrophy** (section 9.11); **Phototrophy** (section 9.12)

The interactions of the nutritional types of microorganisms are critical to the functioning of the biosphere. The ultimate source of most biological energy is visible sunlight. Light energy is trapped and reducing power is generated by photoautotrophs and used to transform CO_2 into organic molecules such as glucose. The or-

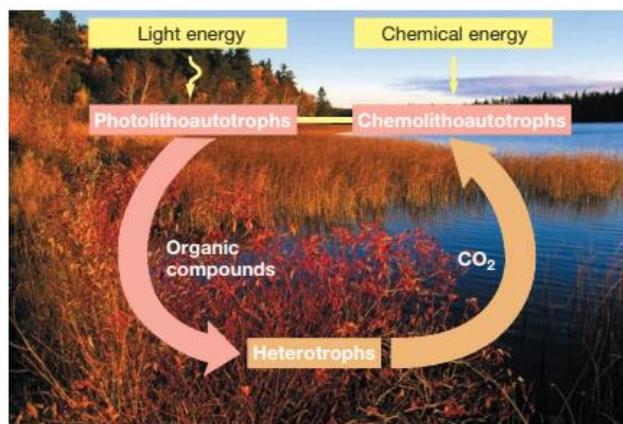


Figure 8.2 The Flow of Carbon and Energy in an Ecosystem. This diagram depicts the flow of energy and carbon in general terms. See text for discussion.

ganic molecules then serve as energy, carbon, and electron sources for chemoorganoheterotrophs. The breakdown of the organic molecules by chemoorganotrophs releases CO₂ back into the atmosphere (figure 8.2). In a similar cycle, chemolithoautotrophs use the energy and reducing power derived from inorganic energy sources to synthesize organic molecules, which “feed” chemoorganoheterotrophs (figure 8.2). Thus the flows of carbon and energy in ecosystems are intimately related.

8.2 ENERGY AND WORK

Energy may be most simply defined as the capacity to do work. This is because all physical and chemical processes are the result of the application or movement of energy. Living cells carry out three major types of work, and all are essential to life processes. **Chemical work** involves the synthesis of complex biological molecules from much simpler precursors (i.e., anabolism); energy is needed to increase the molecular complexity of a cell. **Transport work** requires energy in order to take up nutrients, eliminate wastes, and maintain ion balances. Energy input is needed because molecules and ions often must be transported across cell membranes against an electrochemical gradient. For example, molecules move into a cell even though their concentration is higher internally. Similarly a solute may be expelled from the cell against a concentration gradient. The third type of work is **mechanical work**, perhaps the most familiar of the three. Energy is required for cell motility and to move structures within cells.

- Define metabolism, energy, energy-conserving reactions, catabolism, anabolism, and reducing power.
- Describe in general terms how energy from sunlight is spread throughout the biosphere. What sources of energy, other than sunlight, do microorganisms use?

- What kinds of work are carried out in a cell? Suppose a bacterium was doing the following: synthesizing peptidoglycan, rotating its flagellum and swimming, and secreting siderophores. What type of work is the bacterium doing in each case?

8.3 THE LAWS OF THERMODYNAMICS

To understand how energy is trapped as ATP and how ATP is used to do cellular work, some knowledge of the basic principles of thermodynamics is required. The science of **thermodynamics** analyzes energy changes in a collection of matter (e.g., a cell or a plant) called a **system**. All other matter in the universe is called the **surroundings**. Thermodynamics focuses on the energy differences between the initial state and the final state of a system. It is not concerned with the rate of the process. For instance, if a pan of water is heated to boiling, only the condition of the water at the start and at boiling is important in thermodynamics, not how fast it is heated or on what kind of stove.

Two important laws of thermodynamics must be understood. The **first law of thermodynamics** says that energy can be neither created nor destroyed. The total energy in the universe remains constant although it can be redistributed, as it is during the many energy exchanges that occur during chemical reactions. For example, heat is given off by exothermic reactions and absorbed during endothermic reactions. However, the first law alone cannot explain why heat is released by one chemical reaction and absorbed by another. Nor does it explain why gas will flow from a full cylinder to an empty cylinder until the gas pressure is equal in both (figure 8.3). Explanations for these phenomena require the **second law of thermodynamics** and a condition of matter called **entropy**. **Entropy** may be considered a measure of the randomness or disorder of a system. The greater the disorder of a system, the greater is its entropy. The second law states that physical and chemical processes proceed in such a way that the randomness or disorder of the universe (the system and its surroundings)

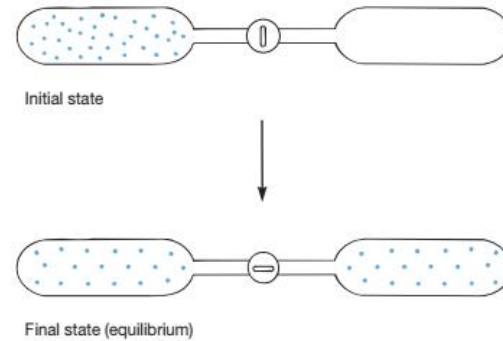


Figure 8.3 A Second Law Process. The expansion of gas into an empty cylinder simply redistributes the gas molecules until equilibrium is reached. The total number of molecules remains unchanged.

increases to the maximum possible. Gas will always expand into an empty cylinder.

It is necessary to specify quantitatively the amount of energy used in or evolving from a particular process, and two types of energy units are employed. A **calorie** (cal) is the amount of heat energy needed to raise one gram of water from 14.5 to 15.5°C. The amount of energy also may be expressed in terms of **joules** (J), the units of work capable of being done. One cal of heat is equivalent to 4.1840 J of work. One thousand calories or a kilocalorie (kcal) is enough energy to boil 1.9 ml of water. A kilojoule is enough energy to boil about 0.44 ml of water, or enable a person weighing 70 kg to climb 35 steps. The joule is normally used by chemists and physicists. Because biologists most often speak of energy in terms of calories, this text will employ calories when discussing energy changes.

8.4 FREE ENERGY AND REACTIONS

The first and second laws can be combined in a useful equation, relating the changes in energy that can occur in chemical reactions and other processes.

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

ΔG is the change in free energy, ΔH is the change in enthalpy, T is the temperature in Kelvin ($^{\circ}\text{C} + 273$), and ΔS is the change in entropy occurring during the reaction. The change in **enthalpy** is the change in heat content. Cellular reactions occur under conditions of constant pressure and volume. Thus the change in enthalpy is about the same as the change in total energy during the reaction. The **free energy change** is the amount of energy in a system (or cell) available to do useful work at constant temperature and pressure. Therefore the change in entropy (ΔS) is a measure of the proportion of the total energy change that the system cannot use in performing work. Free energy and entropy changes do not depend on how the system gets from start to finish. A reaction will occur spontaneously if the free energy of the system decreases during the reaction or, in other words, if ΔG is negative. It follows from the equation that a reaction with a large positive change in entropy will normally tend to have a negative ΔG value and therefore occur spontaneously. A decrease in entropy will tend to make ΔG more positive and the reaction less favorable.

It can be helpful to think of the relationship between entropy (ΔS) and change in free energy (ΔG) in terms that are more concrete. Consider the Greek myth of Sisyphus, king of Corinth. For his assorted crimes against the gods, he was condemned to roll a large boulder to the top of a steep hill for all eternity. This represents a very negative change in entropy—a boulder poised at the top of a hill is neither random nor disordered—and this activity (reaction) has a very positive ΔG . That is to say, Sisyphus had to put a lot of energy into the system. Unfortunately for Sisyphus, as soon as the boulder was at the top of the hill, it *spontaneously* rolled back down the hill. This represents a positive change in entropy and a negative ΔG . Sisyphus did not need to put energy into

the system. He probably just stood at the top of the hill and watched the reaction proceed.

The change in free energy also has a definite, concrete relationship to the direction of chemical reactions. Consider this simple reaction.



If the molecules A and B are mixed, they will combine to form the products C and D. Eventually C and D will become concentrated enough to combine and produce A and B at the same rate as C and D are formed from A and B. The reaction is now at **equilibrium**: the rates in both directions are equal and no further net change occurs in the concentrations of reactants and products. This situation is described by the **equilibrium constant** (K_{eq}), relating the equilibrium concentrations of products and substrates to one another.

$$K_{\text{eq}} = \frac{[\text{C}][\text{D}]}{[\text{A}][\text{B}]}$$

If the equilibrium constant is greater than one, the products are in greater concentration than the reactants at equilibrium—that is, the reaction tends to go to completion as written.

The equilibrium constant of a reaction is directly related to its change in free energy. When the free energy change for a process is determined at carefully defined standard conditions of concentration, pressure, pH, and temperature, it is called the **standard free energy change** (ΔG°). If the pH is set at 7.0 (which is close to the pH of living cells), the standard free energy change is indicated by the symbol $\Delta G'$. The change in standard free energy may be thought of as the maximum amount of energy available from the system for useful work under standard conditions. Using $\Delta G'$ values allows one to compare reactions without worrying about variations in the ΔG due to differences in environmental conditions. The relationship between $\Delta G'$ and K_{eq} is given by this equation.

$$\Delta G' = -2.303RT \cdot \log K_{\text{eq}}$$

R is the gas constant (1.9872 cal/mole-degree or 8.3145 J/mole-degree), and T is the absolute temperature. Inspection of this equation shows that when $\Delta G'$ is negative, the equilibrium constant is greater than one and the reaction goes to completion as written. It is said to be an **exergonic reaction** (figure 8.4). In an **endergonic**

Exergonic reactions	Endergonic reactions
$\text{A} + \text{B} \rightleftharpoons \text{C} + \text{D}$	$\text{A} + \text{B} \rightleftharpoons \text{C} + \text{D}$
$K_{\text{eq}} = \frac{[\text{C}][\text{D}]}{[\text{A}][\text{B}]} > 1.0$	$K_{\text{eq}} = \frac{[\text{C}][\text{D}]}{[\text{A}][\text{B}]} < 1.0$
$\Delta G'$ is negative.	$\Delta G'$ is positive.

Figure 8.4 $\Delta G'$ and Equilibrium. The relationship of $\Delta G'$ to the equilibrium of reactions. Note the differences between exergonic and endergonic reactions.

reaction ΔG° is positive and the equilibrium constant is less than one. That is, the reaction is not favorable, and little product will be formed at equilibrium under standard conditions. Keep in mind that the ΔG° value shows only where the reaction lies at equilibrium, not how fast the reaction reaches equilibrium.

8.5 THE ROLE OF ATP IN METABOLISM

As already noted, considerable metabolic diversity exists in the microbial world. However, there are several biochemical principles common to all types of metabolism. These are (1) the use of ATP to store energy captured during exergonic reactions so it can be used to drive endergonic reactions; (2) the organization of metabolic reactions into pathways and cycles; (3) the catalysis of metabolic reactions by enzymes; and (4) the importance of oxidation-reduction reactions in energy conservation. This section considers the role of ATP in metabolism.

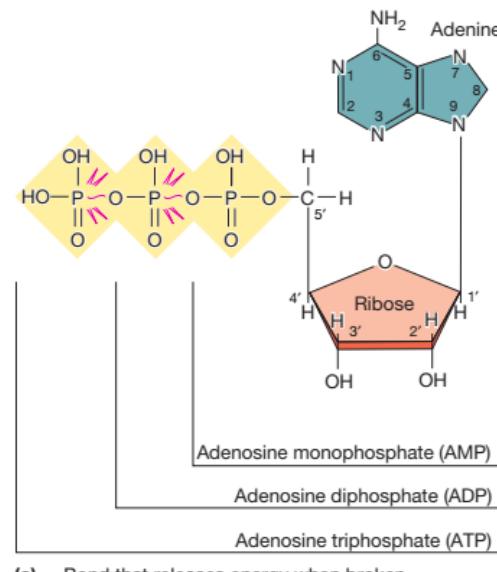
Energy is released from a cell's energy source in exergonic reactions (i.e., those reactions with a negative ΔG). Rather than wasting this energy, much of it is trapped in a practical form that allows its transfer to the cellular systems doing work. These systems carry out endergonic reactions (i.e., anabolism), and the energy captured by the cell is used to drive these reactions to completion. In living organisms, this practical form of energy is **adenosine 5'-triphosphate (ATP)**; **figure 8.5**. In a sense, cells carry out certain processes so that they can "earn" ATP and carry out other processes in which they "spend" their ATP. Thus ATP is often referred to as the cell's energy currency. In the cell's economy, ATP serves as the link between exergonic reactions and endergonic reactions (**figure 8.6**).

What makes ATP suited for this role as energy currency? ATP is a **high-energy molecule**. That is, it breaks down or hydrolyzes almost completely to the products **adenosine diphosphate (ADP)** and orthophosphate (P_i) with a ΔG° of -7.3 kcal/mole .

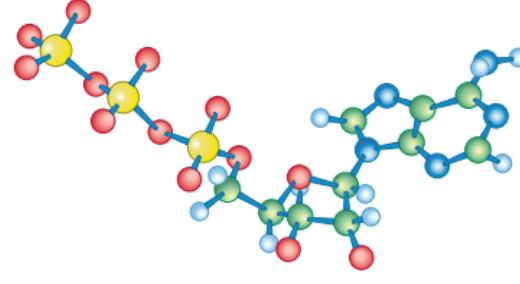


The reference to ATP as a high-energy molecule does not mean that there is a great deal of energy stored in a particular bond of ATP. It simply indicates that the removal of the terminal phosphate goes to completion with a large negative standard free energy change; that is, the reaction is strongly exergonic. Because ATP readily transfers its phosphate to water, it is said to have a **high phosphate group transfer potential**, defined as the negative of ΔG° for the hydrolytic removal of phosphate. A molecule with a higher group transfer potential will donate phosphate to one with a lower potential.

Although the free energy change for the hydrolysis of ATP is quite large, there are numerous reactions that release even greater amounts of free energy. This energy is used to resynthesize ATP from ADP and P_i during catabolism and other energy-conserving processes. Likewise, catabolism can generate molecules with a phosphate group transfer potential that is even higher than that of ATP. Cells use these molecules to regenerate ATP from ADP by a mechanism called **substrate-level phos-**



(a) — Bond that releases energy when broken



(b)

Figure 8.5 Adenosine Triphosphate and Adenosine Diphosphate. (a) Structure of ATP, ADP, and AMP. The two red bonds (—) are more easily broken or have a high phosphate group transfer potential (see text). The pyrimidine ring atoms have been numbered as have the carbon atoms in ribose. (b) A model of ATP. Carbon is in green; hydrogen in light blue; nitrogen in dark blue; oxygen in red; and phosphorus in yellow.

phorylation. Thus ATP, ADP, and P_i form an energy cycle (**figure 8.7**). The fueling reactions conserve energy released from an energy source by using it to synthesize ATP from ADP and P_i . When ATP is hydrolyzed, the energy released drives endergonic processes such as anabolism, transport, and mechanical work. The mechanisms for synthesizing ATP will be described in more detail in chapter 9.

1. What is thermodynamics? Summarize the first and second laws of thermodynamics.
2. Define entropy and enthalpy. Do living cells increase entropy within themselves? Do they increase entropy in the environment?

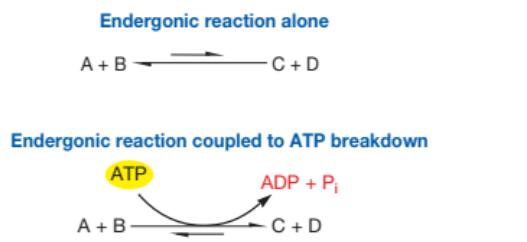


Figure 8.6 ATP as a Coupling Agent. The use of ATP to make endergonic reactions more favorable. It is formed by exergonic reactions and then used to drive endergonic reactions.

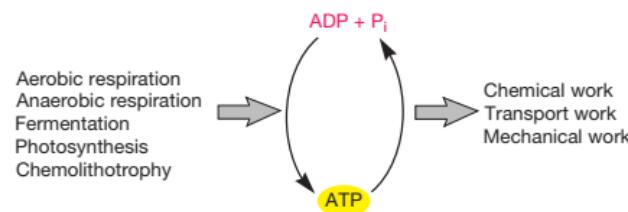


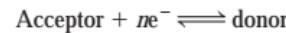
Figure 8.7 The Cell's Energy Cycle. ATP is formed from energy made available during aerobic respiration, anaerobic respiration, fermentation, chemolithotrophy, and photosynthesis. Its breakdown to ADP and phosphate (P_i) makes chemical, transport, and mechanical work possible.

3. Define free energy. What are exergonic and endergonic reactions?
4. Suppose that a chemical reaction had a large negative ΔG° value. Is the reaction endergonic or exergonic? What would this indicate about its equilibrium constant?
5. Describe the energy cycle and ATP's role in it. What characteristics of ATP make it suitable for this role? Why is ATP called a high-energy molecule?

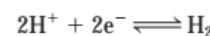
8.6 OXIDATION-REDUCTION REACTIONS, ELECTRON CARRIERS, AND ELECTRON TRANSPORT SYSTEMS

Free energy changes are related to the equilibria of all chemical reactions including the equilibria of oxidation-reduction reactions. The release of energy from an energy source normally involves oxidation-reduction reactions. **Oxidation-reduction (redox) reactions** are those in which electrons move from an **electron donor** to an **electron acceptor**.¹ By convention such a reaction is written with the donor to the right of the acceptor and the number (n) of electrons (e^-) transferred.

¹In an oxidation-reduction reaction, the electron donor is often called the reducing agent or reductant because it is donating electrons to the acceptor and thus reducing it. The electron acceptor is called the oxidizing agent or oxidant because it is removing electrons from the donor and oxidizing it.



The acceptor and donor pair is referred to as a redox couple (table 8.1). When an acceptor accepts electrons, it then becomes the donor of the couple. The equilibrium constant for the reaction is called the **standard reduction potential** (E_0) and is a measure of the tendency of the donor to lose electrons. The reference standard for reduction potentials is the hydrogen system with an E_0 (the reduction potential at pH 7.0) of -0.42 volts or -420 millivolts.



In this reaction each hydrogen atom provides one proton (H^+) and one electron (e^-). As just noted, the standard reduction potential is measured in volts or millivolts. The volt is a unit of electrical potential or electromotive force. Therefore redox couples like the hydrogen system are a potential source of energy.

The reduction potential has a concrete meaning. Redox couples with more negative reduction potentials will donate electrons to couples with more positive potentials and greater affinity for electrons. Thus electrons tend to move from donors at the top of the list in table 8.1 to acceptors at the bottom because the latter have more positive potentials. This may be expressed visually in the form of an electron tower in which the most negative reduc-

Table 8.1 Selected Biologically Important Redox Couples

Redox Couple	E'_0 (Volts) ^a
$2\text{H}^+ + 2e^- \rightarrow \text{H}_2$	-0.42
Ferredoxin (Fe^{3+}) + e^- → ferredoxin (Fe^{2+})	-0.42
$\text{NAD(P)}^+ + \text{H}^+ + 2e^- \rightarrow \text{NAD(P)}\text{H}$	-0.32
$\text{S} + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{S}$	-0.274
Acetaldehyde + $2\text{H}^+ + 2e^- \rightarrow$ ethanol	-0.197
Pyruvate ⁻ + $2\text{H}^+ + 2e^- \rightarrow$ lactate ²⁻	-0.185
$\text{FAD} + 2\text{H}^+ + 2e^- \rightarrow \text{FADH}_2$	-0.18 ^b
Oxaloacetate ²⁻ + $2\text{H}^+ + 2e^- \rightarrow$ malate ²⁻	-0.166
Fumarate ²⁻ + $2\text{H}^+ + 2e^- \rightarrow$ succinate ²⁻	0.031
Cytochrome <i>b</i> (Fe^{3+}) + $e^- \rightarrow$ cytochrome <i>b</i> (Fe^{2+})	0.075
Ubiquinone + $2\text{H}^+ + 2e^- \rightarrow$ ubiquinone H_2	0.10
Cytochrome <i>c</i> (Fe^{3+}) + $e^- \rightarrow$ cytochrome <i>c</i> (Fe^{2+})	0.254
Cytochrome <i>a</i> (Fe^{3+}) + $e^- \rightarrow$ cytochrome <i>a</i> (Fe^{2+})	0.29
Cytochrome <i>a₃</i> (Fe^{3+}) + $e^- \rightarrow$ cytochrome <i>a₃</i> (Fe^{2+})	0.35
$\text{NO}_3^- + 2\text{H}^+ + 2e^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$	0.421
$\text{NO}_2^- + 8\text{H}^+ + 6e^- \rightarrow \text{NH}_4^+ + 2\text{H}_2\text{O}$	0.44
$\text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+}$	0.771 ^c
$\text{O}_2 + 4\text{H}^+ + 4e^- \rightarrow 2\text{H}_2\text{O}$	0.815

^a E'_0 is the standard reduction potential at pH 7.0.

^b The value for FAD/FADH₂ applies to the free cofactor because it can vary considerably when bound to an apoenzyme.

^c The value for free Fe, not Fe complexed with proteins (e.g., cytochromes).

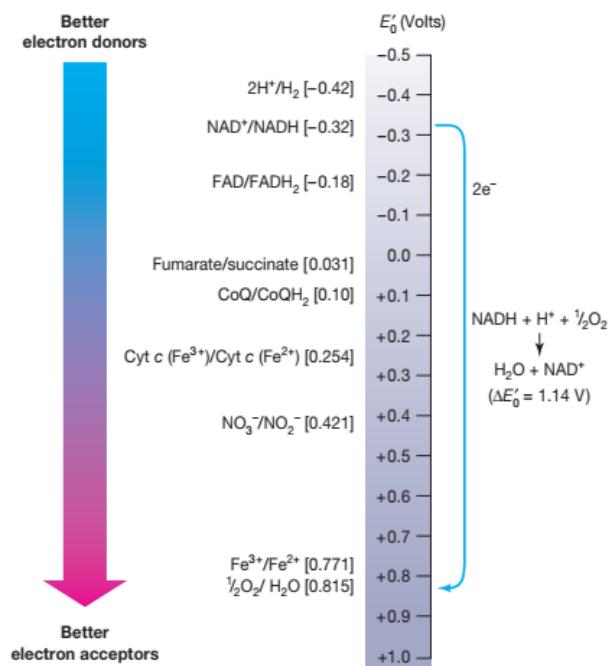
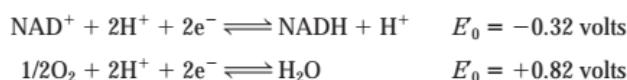


Figure 8.8 Electron Movement and Reduction Potentials. The vertical electron tower in this illustration has the most negative reduction potentials at the top. Electrons will spontaneously move from donors higher on the tower (more negative potentials) to acceptors lower on the tower (more positive potentials). That is, the donor is always higher on the tower than the acceptor. For example, NADH will donate electrons to oxygen and form water in the process. Some typical donors and acceptors are shown on the left, and their redox potentials are given in brackets.

tion potentials are at the top (figure 8.8). Electrons move from donors to acceptors down the potential gradient or fall down the tower to more positive potentials. Consider the case of the electron carrier **nicotinamide adenine dinucleotide** (NAD^+). The NAD^+/NADH couple has a very negative E'_0 and can therefore give electrons to many acceptors, including O_2 .



Because the reduction potential of NAD^+/NADH is more negative than that of $\frac{1}{2}\text{O}_2/\text{H}_2\text{O}$, electrons will flow from NADH (the donor) to O_2 (the acceptor) as shown in figure 8.8.



Because the NAD^+/NADH couple has a relatively negative E'_0 , it stores more potential energy than redox couples with less negative (or more positive) E'_0 values. It follows that when electrons move from a donor to an acceptor with a more positive redox po-

tential, free energy is released. The $\Delta G^\circ'$ of the reaction is directly related to the magnitude of the difference between the reduction potentials of the two couples ($\Delta E'_0$). The larger the $\Delta E'_0$, the greater the amount of free energy made available, as is evident from the equation

$$\Delta G^\circ' = -nF \cdot \Delta E'_0$$

in which n is the number of electrons transferred and F is the Faraday constant (23,062 cal/mole-volt or 96,494 J/mole-volt). For every 0.1 volt change in $\Delta E'_0$, there is a corresponding 4.6 kcal change in $\Delta G^\circ'$ when a two-electron transfer takes place. This is similar to the relationship of $\Delta G^\circ'$ and K_{eq} in other chemical reactions—the larger the equilibrium constant, the greater the $\Delta G^\circ'$. The difference in reduction potentials between NAD^+/NADH and $\frac{1}{2}\text{O}_2/\text{H}_2\text{O}$ is 1.14 volts, a large $\Delta E'_0$ value. When electrons move from NADH to O_2 , a large amount of free energy is made available to synthesize ATP.

We have focused our attention on the reduction of O_2 by NADH because NADH plays a central role in the metabolism of many organisms, especially chemoorganotrophs. Many chemoorganotrophs use glucose as a source of energy. As glucose is catabolized, it is oxidized. Many of the electrons released from glucose are accepted by NAD^+ , which is then reduced to NADH. NADH next transfers the electrons to O_2 . However, it does not do so directly. Instead, the electrons are transferred to O_2 via a series of electron carriers. The electron carriers are organized into a system called an **electron transport system** (ETS) or **electron transport chain** (ETC). The carriers are organized such that the first electron carrier has the most negative E'_0 , and each successive carrier is slightly less negative (figure 8.9). In this way, the potential energy stored in the redox couple whose electrons initiate electron flow is released and used to form ATP.

The ETSs of chemoorganotrophs are located in the plasma membrane in prokaryotes and the internal mitochondrial membranes in eucaryotes. Electron transport systems also play a pivotal role in the metabolism of chemolithotrophs and phototrophs, where they are used to conserve energy from inorganic energy sources and light, respectively. The ETSs are located in the plasma membrane or internal membrane systems of chemolithotrophs, which are all prokaryotes. They are located in the plasma membrane and internal membrane systems of procaryotic phototrophs and in the thylakoid membranes of chloroplasts in eucaryotic phototrophs (figure 8.9).

The carriers that make up ETSs differ in terms of their chemical nature and the way they carry electrons. NAD^+ , and its chemical relative **nicotinamide adenine dinucleotide phosphate** (NADP^+), contain a nicotinamide ring (figure 8.10). This ring accepts two electrons and one proton from a donor (e.g., an intermediate formed during the catabolism of glucose), and a second proton is released. **Flavin adenine dinucleotide** (FAD) and **flavin mononucleotide** (FMN) bear two electrons and two protons on the complex ring system shown in figure 8.11. Proteins bearing FAD and FMN are often called flavoproteins. **Coenzyme Q (CoQ)** or **ubiquinone** is a quinone that transports two electrons and two protons in many electron transport chains

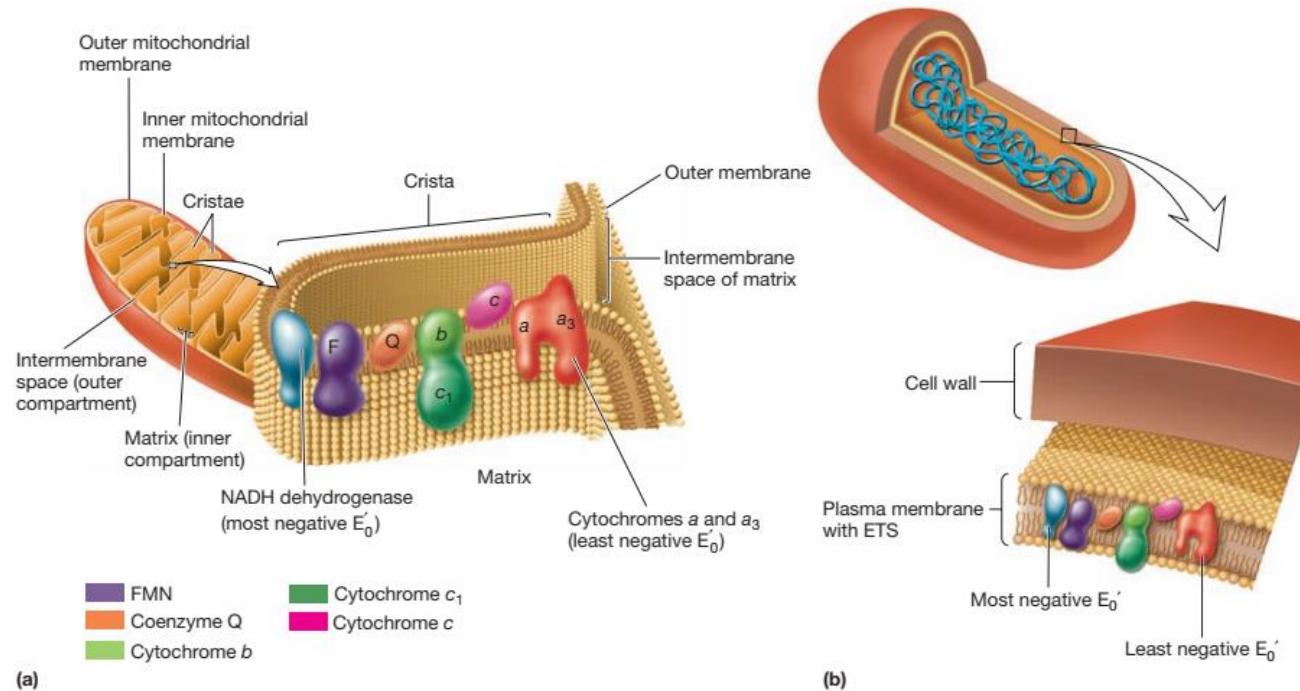


Figure 8.9 Electron Transport Systems. Electron transport systems (ETSs) are located in membranes. Electrons flow from the electron carrier having the most negative reduction potential to the carrier having the most positive reduction potential. During respiratory processes (aerobic respiration, anaerobic respiration, and chemolithotrophy), an exogenous molecule such as oxygen serves as the terminal electron acceptor. **(a)** The mitochondrial ETS. **(b)** A typical bacterial ETS.

(figure 8.12). **Cytochromes** and several other carriers use iron atoms to transport electrons one electron at a time by reversible oxidation and reduction reactions.



In the cytochromes these iron atoms are part of a heme group (figure 8.13) or other similar iron-porphyrin rings. Several different cytochromes, each of which consists of a protein and an ironporphyrin ring, are a prominent part of electron transport chains. Some iron containing electron-carrying proteins lack a heme group and are called **nonheme iron proteins**. **Ferrodoxin** is a nonheme iron protein active in photosynthetic electron transport and several other electron transport processes. Even though its iron atoms are not bound to a heme group, they still undergo reversible oxidation and reduction reactions. Like cytochromes, they carry only one electron at a time. This difference in the number of electrons and protons carried is of great importance in the operation of electron transport chains and is discussed further in chapter 9.

1. Write a generalized equation for a redox reaction. Define standard reduction potential.
2. How is the direction of electron flow between redox couples related to the standard reduction potential and the release of free energy?

3. When electrons flow from the NAD^+/NADH redox couple to the $\text{O}_2/\text{H}_2\text{O}$ redox couple, does the reaction begin with NAD^+ or with NADH ? What is produced— O_2 or H_2O ?
4. Which among the following would be the best electron donor? Which would be the worst? ubiquinone/ubiquinone H_2 , NAD^+/NADH , FAD/FADH_2 , $\text{NO}_3^-/\text{NO}_2^-$. Explain your answers.
5. In general terms, how is ΔG° related to ΔE° ? What is the ΔE° when electrons flow from the NAD^+/NADH redox couple to the $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple? How does this compare to the ΔE° when electrons flow from the $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple to the $\text{O}_2/\text{H}_2\text{O}$ couple? Which will yield the largest amount of free energy to the cell?
6. Name and briefly describe the major electron carriers found in cells. Why is NAD^+ a good electron carrier? Why is ferredoxin an even better electron carrier?

8.7 ENZYMES

Recall that an exergonic reaction is one with a negative ΔG° and an equilibrium constant greater than one. An exergonic reaction will proceed to completion in the direction written (that is, toward the right of the equation). Nevertheless, one often can combine the reactants for an exergonic reaction with no obvious result. For instance, the hydrolysis of polysaccharides into

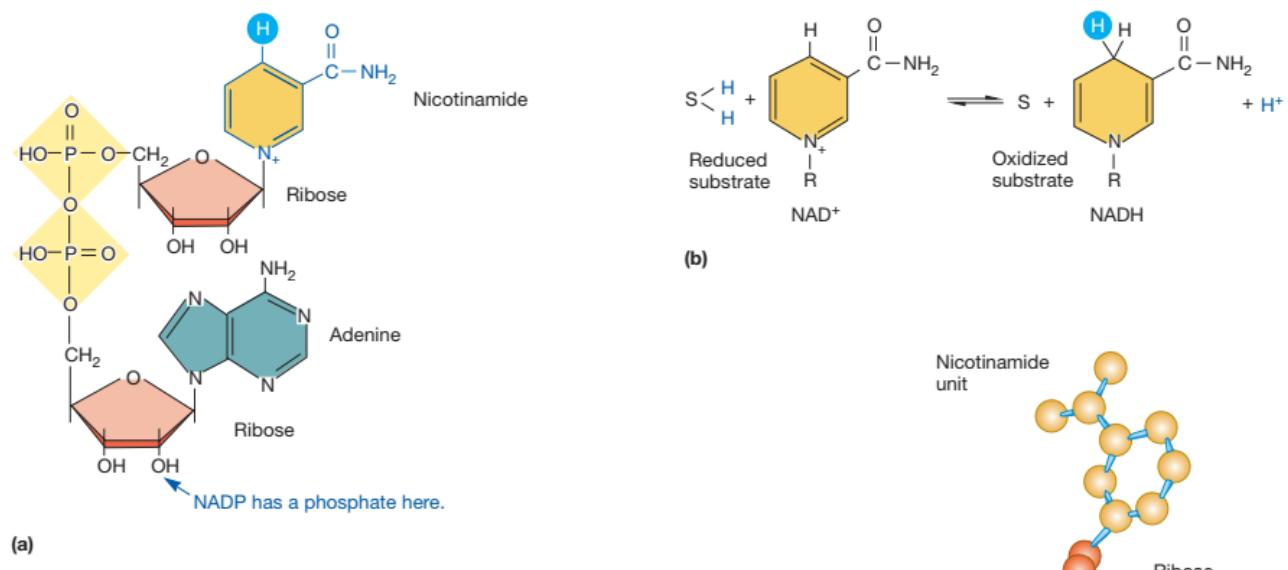


Figure 8.10 The Structure and Function of NAD. (a) The structure of NAD and NADP. NADP differs from NAD in having an extra phosphate on one of its ribose sugar units. (b) NAD can accept electrons and a hydrogen from a reduced substrate (SH_2). These are carried on the nicotinamide ring. (c) Model of NAD^+ when bound to the enzyme lactate dehydrogenase.

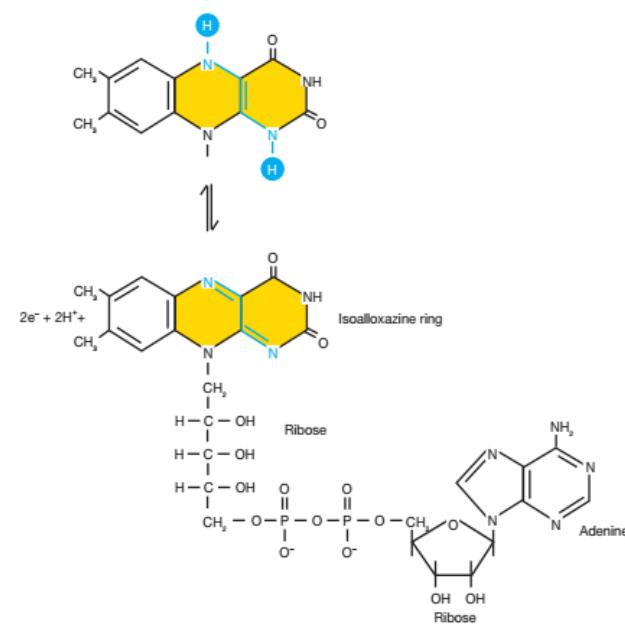


Figure 8.11 The Structure and Function of FAD. The vitamin riboflavin is composed of the isoalloxazine ring and its attached ribose sugar. FMN is riboflavin phosphate. The portion of the ring directly involved in oxidation-reduction reactions is in color.

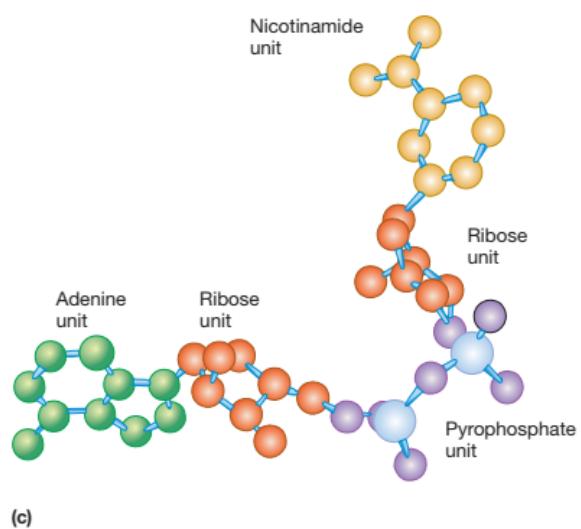


Figure 8.12 The Structure and Function of Coenzyme Q or Ubiquinone. The length of the side chain varies among organisms from $n = 6$ to $n = 10$.

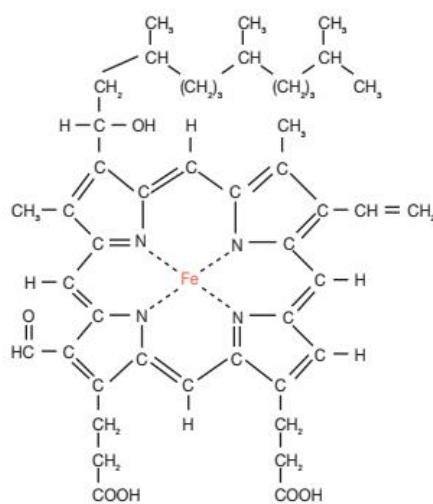


Figure 8.13 The Structure of Heme. Heme is composed of a porphyrin ring and an attached iron atom. It is the nonprotein component of many cytochromes. The iron atom alternatively accepts and releases an electron.

their component monosaccharides is exergonic and will occur spontaneously. However, an organic chemist would have to carry out this reaction in 6 M HCl and at 100°C for several hours to get it to go to completion. A cell, on the other hand, can accomplish the same reaction at neutral pH, at a much lower temperature, and in just fractions of a second. How are cells able to do this? They can do so because they manufacture proteins called enzymes that speed up chemical reactions. Enzymes are critically important to cells, since most biological reactions occur very slowly without them. Indeed, enzymes make life possible.

Structure and Classification of Enzymes

Enzymes may be defined as protein catalysts that have great specificity for the reaction catalyzed and the molecules acted on. A **catalyst** is a substance that increases the rate of a chemical reaction without being permanently altered itself. Thus enzymes speed up cellular reactions. The reacting molecules are called **substrates**, and the substances formed are the **products**. [Proteins \(appendix I\)](#)

Many enzymes are composed only of proteins. However, some enzymes consist of a protein, the **apoenzyme**, and a non-protein component, a **cofactor**, required for catalytic activity. The complete enzyme consisting of the apoenzyme and its cofactor is called the **holoenzyme**. If the cofactor is firmly attached to the apoenzyme it is a **prosthetic group**. If the cofactor is loosely attached to the apoenzyme and can dissociate from the protein after products have been formed, it is called a **coenzyme**. Many coenzymes can carry one of the products to another enzyme (**figure 8.14**). For example, NAD⁺ is a coenzyme that carries electrons within the cell. Many vitamins that humans require serve as

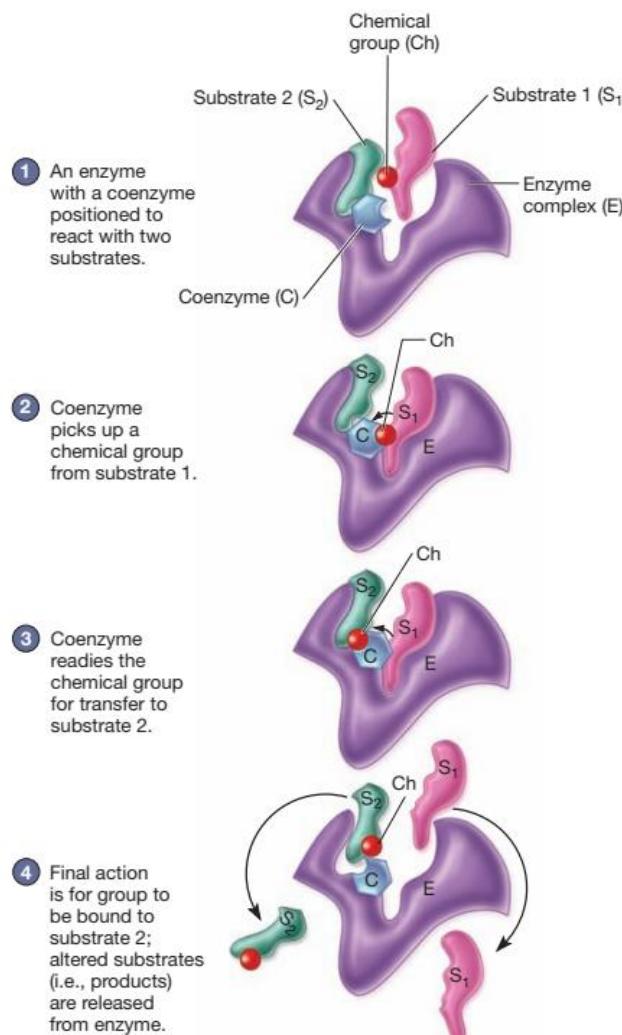
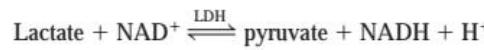


Figure 8.14 Coenzymes as Carriers.

coenzymes or as their precursors. Niacin is incorporated into NAD⁺ and riboflavin into FAD. Metal ions may also be bound to apoenzymes and act as cofactors.

Despite the large number and bewildering diversity of enzymes present in cells, they may be placed in one of six general classes (**table 8.2**). Enzymes usually are named in terms of the substrates they act on and the type of reaction catalyzed. For example, lactate dehydrogenase (LDH) removes hydrogens from lactate.



Lactate dehydrogenase can also be given a more complete and detailed name, L-lactate:NAD oxidoreductase. This name describes the substrates and reaction type with even more precision.

Table 8.2 Enzyme Classification

Type of Enzyme	Reaction Catalyzed by Enzyme	Example of Reaction
Oxidoreductase	Oxidation-reduction reactions	Lactate dehydrogenase: Pyruvate + NADH + H \rightleftharpoons lactate + NAD ⁺
Transferase	Reactions involving the transfer of groups between molecules	Aspartate carbamoyltransferase: Aspartate + carbamoylphosphate \rightleftharpoons carbamoylaspartate + phosphate
Hydrolase	Hydrolysis of molecules	Glucose-6-phosphatase: Glucose-6-phosphate + H ₂ O \rightarrow glucose + P _i
Lyase	Removal of groups to form double bonds or addition of groups to double bonds	Fumarate hydratase: L-malate \rightleftharpoons fumarate + H ₂ O
	$\begin{array}{c} \diagup \quad \diagdown \\ c = c + x - y \rightleftharpoons -c - c - \\ \quad \quad \quad \end{array}$	
Isomerase	Reactions involving isomerizations	Alanine racemase: L-alanine \rightleftharpoons D-alanine
Ligase	Joining of two molecules using ATP energy (or that of other nucleoside triphosphates)	Glutamine synthetase: Glutamate + NH ₃ + ATP \rightarrow glutamine + ADP + P _i

The Mechanism of Enzyme Reactions

It is important to keep in mind that enzymes increase the rates of reactions but do not alter their equilibrium constants. If a reaction is endergonic, the presence of an enzyme will not shift its equilibrium so that more products can be formed. Enzymes simply speed up the rate at which a reaction proceeds toward its final equilibrium.

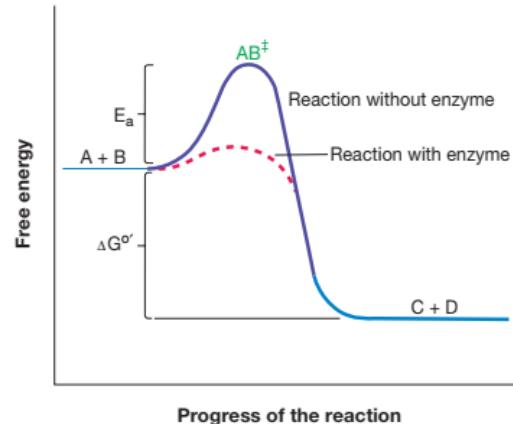
How do enzymes catalyze reactions? Although a complete answer would be long and complex, some understanding of the mechanism can be gained by considering the course of a simple exergonic chemical reaction.



When molecules A and B approach each other to react, they form a **transition-state complex**, which resembles both the substrates and the products (figure 8.15). **Activation energy** is required to bring the reacting molecules together in the correct way to reach the transition state. The transition-state complex can then resolve to yield the products C and D. The difference in free energy level between reactants and products is ΔG° . Thus the equilibrium in our example will lie toward the products because ΔG° is negative (i.e., the products are at a lower energy level than the substrates).

As seen in figure 8.15, A and B will not be converted to C and D if they are not supplied with an amount of energy equivalent to the activation energy. Enzymes accelerate reactions by lowering the activation energy; therefore more substrate molecules will have sufficient energy to come together and form products. Even though the equilibrium constant (or ΔG°) is unchanged, equilibrium will be reached more rapidly in the presence of an enzyme because of this decrease in the activation energy.

Researchers have worked hard to discover how enzymes lower the activation energy of reactions, and the process is becoming clearer. Enzymes bring substrates together at a specific

**Figure 8.15 Enzymes Lower the Energy of Activation.**

This figure traces the course of a chemical reaction in which A and B are converted to C and D. The transition-state complex is represented by AB[‡], and the activation energy required to reach it, by E_a. The red line represents the course of the reaction in the presence of an enzyme. Note that the activation energy is much lower in the enzyme-catalyzed reaction.

place on their surface called the **active site** or **catalytic site** to form an **enzyme-substrate complex** (figures 8.16, 8.17; see also appendix figure A1.19). An enzyme can interact with its substrate in two general ways. It may be rigid and shaped to precisely fit the substrate so that the correct substrate binds specifically and is positioned properly for reaction. This mechanism is referred to as the **lock-and-key model** (figure 8.16). An enzyme also may change shape when it binds the substrate so that the active site surrounds and precisely fits the substrate. This has been called the

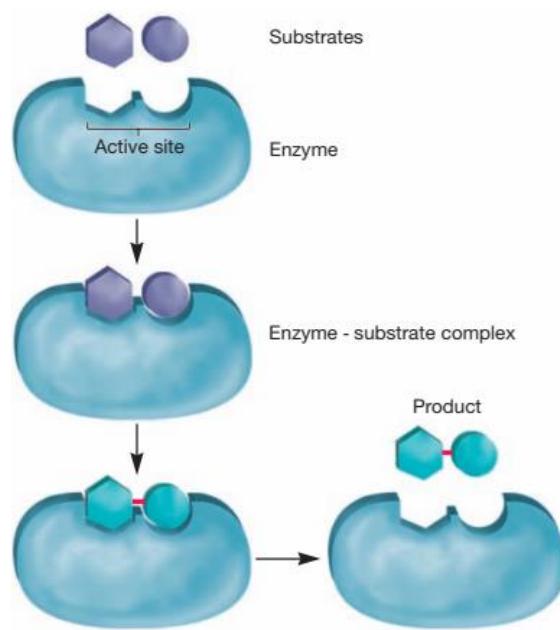
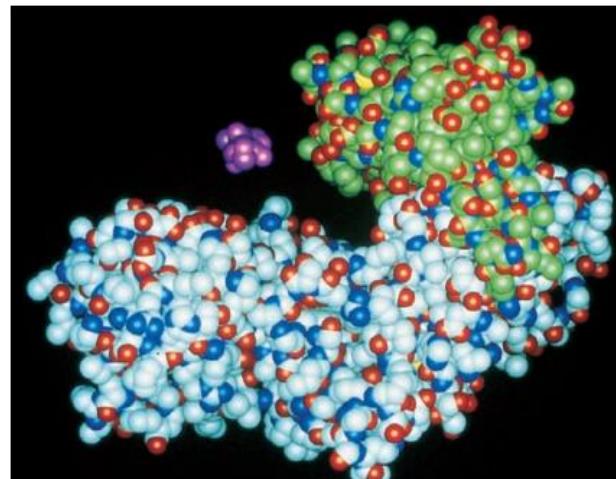


Figure 8.16 Lock-and-Key Model of Enzyme Function. In this model, the active site is a relatively rigid structure that accommodates only those molecules with the correct corresponding shape. The formation of the enzyme-substrate complex and its conversion to product is shown.

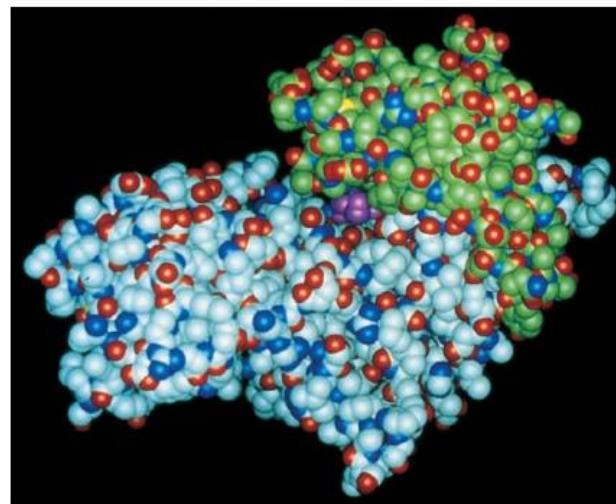
induced fit model and is used by hexokinase and many other enzymes (figure 8.17). The formation of an enzyme-substrate complex can lower the activation energy in many ways. For example, by bringing the substrates together at the active site, the enzyme is, in effect, concentrating them and speeding up the reaction. An enzyme does not simply concentrate its substrates, however. It also binds them so that they are correctly oriented with respect to each other in order to form a transition-state complex. Such an orientation lowers the amount of energy that the substrates require to reach the transition state. These and other catalytic site activities speed up a reaction hundreds of thousands of times.

The Effect of Environment on Enzyme Activity

Enzyme activity varies greatly with changes in environmental factors, one of the most important being the substrate concentration. As will be emphasized later, substrate concentrations are usually low within cells. At very low substrate concentrations, an enzyme makes product slowly because it seldom contacts a substrate molecule. If more substrate molecules are present, an enzyme binds substrate more often, and the reaction velocity (usually expressed in terms of the rate of product formation) is greater than at a lower substrate concentration. Thus the rate of an enzyme-catalyzed reaction increases with substrate concentration (figure 8.18). Eventually further increases



(a)



(b)

Figure 8.17 The Induced Fit Model of Enzyme Function. (a) A space-filling model of yeast hexokinase and its substrate glucose (purple). The active site is in the cleft formed by the enzyme's small lobe (green) and large lobe (blue). (b) When glucose binds to form the enzyme-substrate complex, hexokinase changes shape and surrounds the substrate.

in substrate concentration do not result in a greater reaction velocity because the available enzyme molecules are binding substrate and converting it to product as rapidly as possible. That is, the enzyme is saturated with substrate and operating at maximal velocity (V_{max}). The resulting substrate concentration curve is a hyperbola (figure 8.18). It is useful to know the substrate concentration an enzyme needs to function adequately. Usually the **Michaelis constant (K_m)**, the substrate concentra-

tion required for the enzyme to achieve half maximal velocity, is used as a measure of the apparent affinity of an enzyme for its substrate. The lower the K_m value, the lower the substrate concentration at which an enzyme catalyzes its reaction. Enzymes with a low K_m value are said to have a high affinity for their substrates.

Enzymes also change activity with alterations in pH and temperature (figure 8.19). Each enzyme functions most rapidly at a specific pH optimum. When the pH deviates too greatly from an enzyme's optimum, activity slows and the enzyme may be damaged. Enzymes likewise have temperature optima for maximum activity. If the temperature rises too much above the optimum, an enzyme's structure will be disrupted and its activity lost. This phenomenon, known as **denaturation**, may be caused by extremes of pH and temperature or by other factors. The pH and temperature optima of a microorganism's enzymes often reflect the pH and temperature of its habitat. Not surprisingly bacteria growing best at high tempera-

tures often have enzymes with high temperature optima and great heat stability. [The influence of environmental factors on growth \(section 6.5\)](#)

Enzyme Inhibition

Microorganisms can be poisoned by a variety of chemicals, and many of the most potent poisons are enzyme inhibitors. A **competitive inhibitor** directly competes with the substrate at an enzyme's catalytic site and prevents the enzyme from forming product (figure 8.20). Competitive inhibitors usually resemble normal substrates, but they cannot be converted to products.

Competitive inhibitors are important in the treatment of many microbial diseases. Sulfa drugs like sulfanilamide (figure 8.20b) resemble *p*-aminobenzoate, a molecule used in the formation of the coenzyme folic acid. The drugs compete with *p*-aminobenzoate for the catalytic site of an enzyme involved in folic acid synthesis. This blocks the production of folic acid and inhibits bacterial growth. Humans are not harmed because they do not synthesize folic acid but rather obtain it in their diet. [Antimicrobial drugs: Metabolic antagonists \(section 34.4\)](#)

Noncompetitive inhibitors also can affect enzyme activity by binding to the enzyme at some location other than the active site. This alters the enzyme's shape, rendering it inactive or less active. These inhibitors are called noncompetitive because they do not directly compete with the substrate. Heavy metal poisons like mercury frequently are noncompetitive inhibitors of enzymes.

1. What is an enzyme? How does it speed up reactions? How are enzymes named? Define apoenzyme, holoenzyme, cofactor, coenzyme, prosthetic group, active or catalytic site, and activation energy.
2. Draw a diagram showing how enzymes catalyze reactions by altering the activation energy. What is a transition state complex? Use the diagram to explain why enzymes do not change the equilibria of the reactions they catalyze.
3. What is the difference between the lock-and-key and the induced-fit models of enzyme-substrate complex formation?
4. Define the terms Michaelis constant and maximum velocity. How does enzyme activity change with substrate concentration, pH, and temperature?
5. What special properties might an enzyme isolated from a psychrophilic bacterium have? Will enzymes need to lower the activation energy more or less in thermophiles than in psychrophiles?
6. What are competitive and noncompetitive inhibitors and how do they inhibit enzymes?

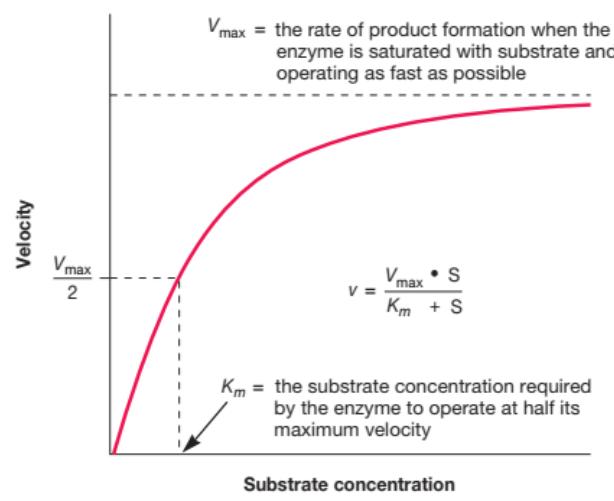


Figure 8.18 Michaelis-Menten Kinetics. The dependence of enzyme activity upon substrate concentration. This substrate curve fits the Michaelis-Menten equation given in the figure, which relates reaction velocity (v) to the substrate concentration (S) using the maximum velocity and the Michaelis constant (K_m).

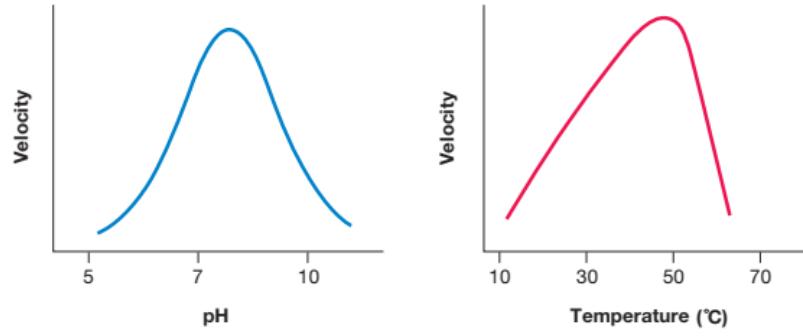


Figure 8.19 pH, Temperature, and Enzyme Activity. The variation of enzyme activity with changes in pH and temperature. The ranges in pH and temperature are only representative. Enzymes differ from one another with respect to the location of their optima and the shape of their pH and temperature curves.

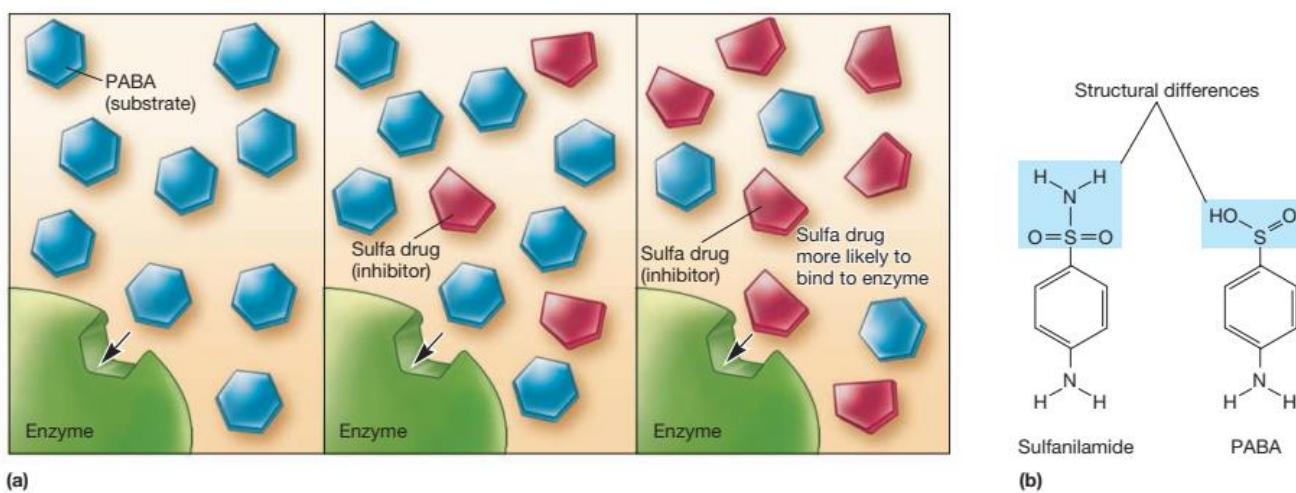


Figure 8.20 Competitive Inhibition of Enzyme Activity. (a) A competitive inhibitor is usually similar in shape to the normal substrate of the enzyme, and therefore can bind the active site of the enzyme. This prevents the substrate from binding, and the reaction is blocked. (b) Structure of sulfanilamide, a structural analog of PABA. PABA is the substrate of an enzyme involved in folic acid biosynthesis. When sulfanilamide binds the enzyme, activity of the enzyme is inhibited and synthesis of folic acid is stopped.

8.8 THE NATURE AND SIGNIFICANCE OF METABOLIC REGULATION

Microorganisms must regulate their metabolism to conserve raw materials and energy and to maintain a balance among various cell components. Because they live in environments where the nutrients, energy sources, and physical conditions often change rapidly, they must continuously monitor internal and external conditions and respond accordingly. This involves activating or inactivating pathways as needed. For instance, if a particular energy source is unavailable, the enzymes required for its use are not needed and their further synthesis is a waste of carbon, nitrogen, and energy. Similarly it would be extremely wasteful for a microorganism to synthesize the enzymes required to manufacture a certain end product if that end product were already present in adequate amounts.

The drive to maintain balance and conserve energy and material is evident in the regulatory responses of a bacterium like *E. coli*. If the bacterium is grown in a very simple medium containing only glucose as a carbon and energy source, it will synthesize all needed cell components in balanced amounts. However, if the amino acid tryptophan is added to the medium, the pathway synthesizing tryptophan will be immediately inhibited and synthesis of the pathway's enzymes also will slow or cease. Likewise, if *E. coli* is transferred to a medium containing only the sugar lactose, it will synthesize the enzymes required for catabolism of this nutrient. In contrast, when *E. coli* grows in a medium possessing both glucose and lactose, glucose (the sugar supporting most rapid growth) is catabolized first. The culture will use lactose only after the glucose supply has been exhausted.

Metabolic pathways can be regulated in three major ways:

1. **Metabolic channeling**—this phenomenon influences pathway activity by localizing metabolites and enzymes into different parts of a cell.
2. **Regulation of the amount of synthesis of a particular enzyme**—in other words, transcription and translation can be regulated. These two processes function in synthesizing enzymes. Regulation at this level is relatively slow, but it saves the cell considerable energy and raw material.
3. **Direct stimulation or inhibition of the activity of critical enzymes**—this type of regulation rapidly alters pathway activity. It is often called **posttranslational regulation** because it occurs after the enzyme has been synthesized.

In this chapter we introduce metabolic channeling and direct control of enzyme activity. Discussion of the regulation enzyme synthesis follows the descriptions of DNA, RNA, and protein synthesis and can be found in chapter 12.

8.9 METABOLIC CHANNELING

One of the most common metabolic channeling mechanisms is that of **compartmentation**, the differential distribution of enzymes and metabolites among separate cell structures or organelles. Compartmentation is particularly important in eucaryotic microorganisms with their many membrane-bound organelles. For example, fatty acid catabolism is located within the mitochondrion, whereas fatty acid synthesis occurs in the cytoplasmic matrix. The periplasm in prokaryotes can also be considered an example of compartmentation. Compartmentation makes possible the simultaneous, but sep-

arate, operation and regulation of similar pathways. Furthermore, pathway activities can be coordinated through regulation of the transport of metabolites and coenzymes between cell compartments. Suppose two pathways in different cell compartments require NAD for activity. The distribution of NAD between the two compartments will then determine the relative activity of these competing pathways, and the pathway with access to the most NAD will be favored. [The bacterial cell wall \(section 3.6\); Archaeal cell wall \(section 3.7\)](#)

Channeling also occurs within compartments such as the cytoplasmic matrix. The matrix is a structured dense material with many subcompartments. In eucaryotes it also is subdivided by the endoplasmic reticulum and cytoskeleton. Metabolites and coenzymes do not diffuse rapidly in such an environment, and metabolite gradients will build up near localized enzymes or enzyme systems. This occurs because enzymes at a specific site convert their substrates to products, resulting in decreases in the concentration of one or more metabolites and increases in others. For example, product concentrations will be high near an enzyme and decrease with increasing distance from it. [The cytoplasmic matrix, microfilaments, intermediate filaments, and microtubules \(section 4.3\)](#)

Channeling can generate marked variations in metabolite concentrations and therefore directly affect enzyme activity. Substrate levels are generally around 10^{-3} moles/liter (M) to 10^{-6} M or even lower. Thus they may be in the same range as enzyme concentrations and equal to or less than the Michaelis constants (K_m) of many enzymes (figure 8.18). Under these conditions the concentration of an enzyme's substrate may control its activity because the substrate concentration is in the rising portion of the hyperbolic substrate saturation curve (figure 8.21). As the substrate level increases, it is converted to product more rapidly; a decline in substrate concentration automatically leads to lower enzyme activity. If two enzymes in different pathways use the same metabolite, they may directly compete for it. The pathway winning this competition—the one with the enzyme having the lowest K_m value for the metabolite—will operate closer to full capacity. Thus channeling within a cell compartment can regulate and coordinate metabolism through variations in metabolite and coenzyme levels.

1. Give three ways in which a metabolic pathway may be regulated.
2. Define the terms metabolic channeling and compartmentation. How are they involved in the regulation of metabolism?

8.10 CONTROL OF ENZYME ACTIVITY

Adjustment of the activity of regulatory enzymes and other proteins controls the functioning of many metabolic pathways and cellular processes. This type of regulation is an example of posttranslational regulation because it occurs after the protein is synthesized. There are a number of posttranslational regulatory mechanisms. Some are irreversible—for instance, cleavage of a protein can either activate or inhibit its activity. Other types of posttranslational control are reversible. In this section, we consider examples of two important, reversible control measures: allosteric regulation and covalent modification. Our focus will be on the regulation of metabolic path-

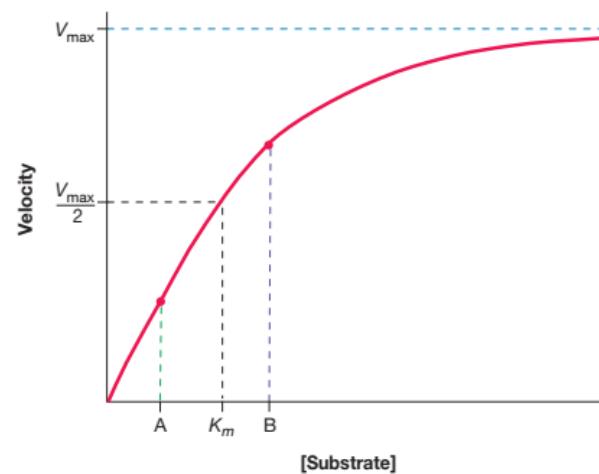


Figure 8.21 Control of Enzyme Activity by Substrate Concentration. An enzyme-substrate saturation curve with the Michaelis constant (K_m) and the velocity equivalent to half the maximum velocity (V_{max}) indicated. The initial velocity of the reaction (v) is plotted against the substrate concentration [Substrate]. The maximum velocity is the greatest velocity attainable with a fixed amount of enzyme under defined conditions. When the substrate concentration is equal to or less than the K_m , the enzyme's activity will vary almost linearly with the substrate concentration. Suppose the substrate increases in concentration from level A to B. Because these concentrations are in the range of the K_m , a significant increase in enzyme activity results. A drop in concentration from B to A will lower the rate of product formation.

ways, but it is important to remember that not all proteins or enzymes function in metabolic pathways. Instead, they are involved in cellular behaviors. At the end of this section, we will consider the regulation of one of these behaviors—chemotaxis.

Allosteric Regulation

Most regulatory enzymes are **allosteric enzymes**. The activity of an allosteric enzyme is altered by a small molecule known as an **effector** or **modulator**. The effector binds reversibly by noncovalent forces to a **regulatory site** separate from the catalytic site and causes a change in the shape or conformation of the enzyme (figure 8.22). The activity of the catalytic site is altered as a result. A positive effector increases enzyme activity, whereas a negative effector decreases activity or inhibits the enzyme. These changes in activity often result from alterations in the apparent affinity of the enzyme for its substrate, but changes in maximum velocity also can occur.

The substrate saturation curve for an allosteric enzyme is often sigmoidal rather than hyperbolic like that of a nonregulatory enzyme. Therefore the substrate concentration required for a regulatory enzyme to function at half its maximal velocity is given its own name: $[S]_{0.5}$ or $K_{0.5}$. The impact of positive effectors and negative effectors on the $K_{0.5}$ of an allosteric enzyme can be readily

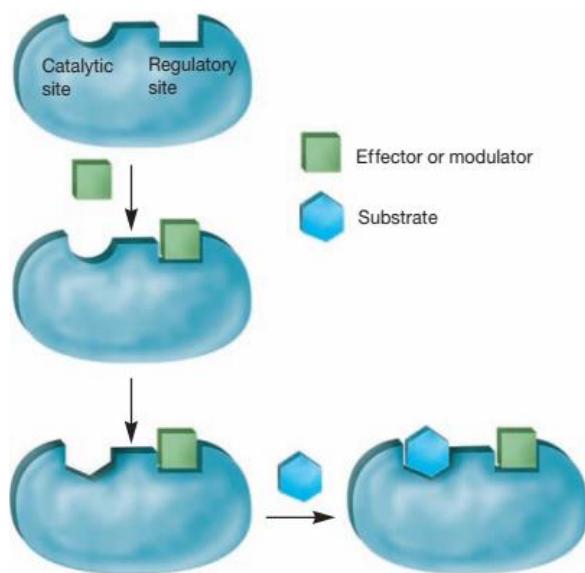


Figure 8.22 Allosteric Regulation. The structure and function of an allosteric enzyme. In this example the effector or modulator first binds to a separate regulatory site and causes a change in enzyme conformation that results in an alteration in the shape of the active site. The active site can now more effectively bind the substrate. This effector is a positive effector because it stimulates substrate binding and catalytic activity.

seen with one of the best-studied allosteric regulatory enzymes—*aspartate carbamoyltransferase* (ACTase) from *E. coli*. The enzyme catalyzes the condensation of carbamoyl phosphate with aspartate to form carbamoylaspartate (**figure 8.23**). This is the rate-determining reaction of the pyrimidine nucleotide biosynthetic pathway in *E. coli*. The substrate saturation curve is sigmoidal when the concentration of either substrate is varied (**figure 8.24**). This is because the enzyme has more than one active site, and the binding of a substrate molecule to an active site increases the binding of substrate at the other sites. In addition, cytidine triphosphate (CTP), an end product of pyrimidine biosynthesis, inhibits the enzyme, while the purine ATP activates it. Both effectors alter the $K_{0.5}$ value of the enzyme but not its maximum velocity. CTP inhibits by increasing $K_{0.5}$ (i.e., by shifting the substrate saturation curve to higher values). This causes the enzyme to operate more slowly at a particular substrate concentration when CTP is present. ATP activates the enzyme by moving the curve to lower substrate concentration values so that the enzyme is maximally active over a wider substrate concentration range. Thus when the pathway is so active that the CTP concentration rises too high, CTP acts as a brake to decrease ACTase activity. In contrast, when the purine end product ATP increases relative to CTP, it stimulates CTP synthesis through its effects on ACTase. *Synthesis of purine, pyrimidines and nucleotides (section 10.6)*

E. coli aspartate carbamoyltransferase provides a clear example of separate regulatory and catalytic sites in allosteric enzymes. The enzyme is a large protein composed of two catalytic subunits and three regulatory subunits (**figure 8.25**). The catalytic

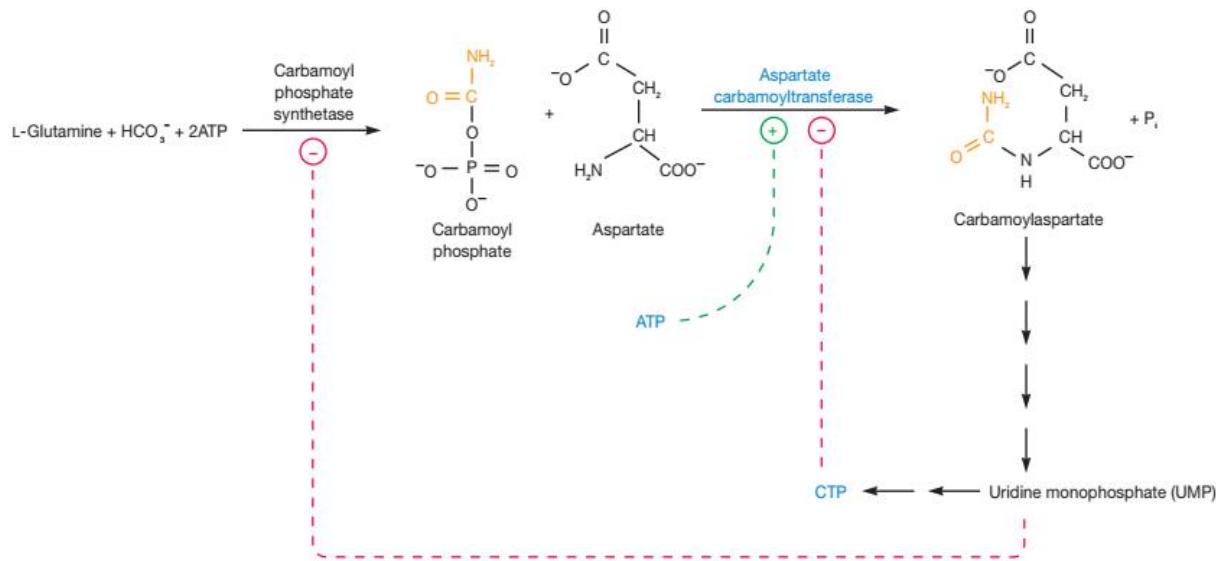


Figure 8.23 ACTase Regulation. The aspartate carbamoyltransferase reaction and its role in the regulation of pyrimidine biosynthesis. The end product CTP inhibits its activity (−) while ATP activates the enzyme (+). Carbamoyl phosphate synthetase is also inhibited by pathway end products such as UMP.

subunits contain only catalytic sites and are unaffected by CTP and ATP. Regulatory subunits do not catalyze the reaction but possess regulatory sites to which CTP and ATP bind. When these effectors bind to the regulatory subunits, they cause conformational changes in both the regulatory and catalytic subunits. The enzyme can change reversibly between a less active T form and a more active R form (figure 8.25b, c). Thus the regulatory site influences a catalytic site that is about 6.0 nm away.

Covalent Modification of Enzymes

Regulatory enzymes also can be switched on and off by **reversible covalent modification**. Usually this occurs through the addition and removal of a particular group, typically a phospho-

ryl, methyl or adenyl group. The enzyme with an attached group can be either activated or inhibited.

One of the most intensively studied regulatory enzymes is *E. coli* glutamine synthetase, an enzyme involved in nitrogen assimilation. It is a large, complex enzyme consisting of 12 subunits, each of which can be covalently modified by an adenylic acid residue (figure 8.26). When an adenylic acid residue is attached to all of its 12 subunits, glutamine synthetase is not very active. Removal of AMP groups produces more active deadenylylated glutamine synthetase, and glutamine is formed. [Synthesis of amino acids: Nitrogen assimilation \(section 10.5\)](#)

There are some advantages to using covalent modification for the regulation of enzyme activity. These interconvertible enzymes often are also allosteric. For instance, glutamine synthetase also is regulated allosterically. Because each form can respond differently to allosteric effectors, systems of covalently modified enzymes are able to respond to more stimuli in varied and sophisticated ways. Regulation can also be exerted on the enzymes that catalyze the covalent modifications, which adds a second level of regulation to the system.

Feedback Inhibition

The rate of many metabolic pathways is adjusted through control of the activity of the regulatory enzymes described in the preceding section. Every pathway has at least one **pacemaker enzyme** that catalyzes the slowest or rate-limiting reaction in the pathway. Because other reactions proceed more rapidly than the pacemaker reaction, changes in the activity of this enzyme directly alter the speed with which a pathway operates. Usually the first step in a pathway is a pacemaker reaction catalyzed by a regulatory enzyme. The end product of the pathway often inhibits this regulatory enzyme, a process known as **feedback inhibition** or **end product inhibition**. Feedback inhibition ensures balanced production of a pathway end product. If the end product becomes too concentrated, it inhibits the regulatory enzyme and slows its own synthesis. As the end product concentration decreases, pathway

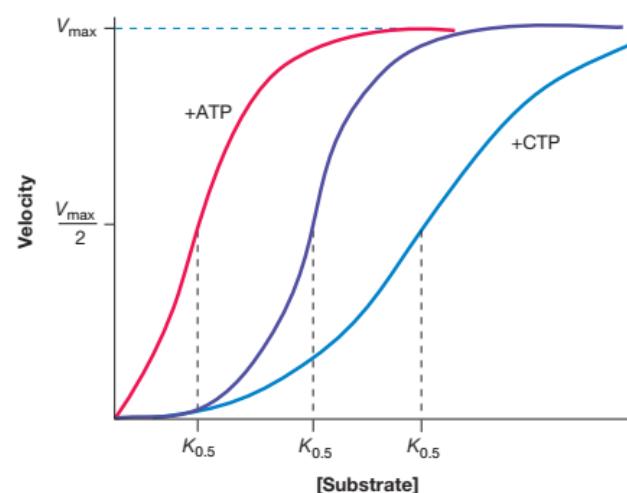


Figure 8.24 The Kinetics of *E. coli* Aspartate Carbamoyltransferase. CTP, a negative effector, increases the $K_{0.5}$ value while ATP, a positive effector, lowers the $K_{0.5}$. The V_{max} remains constant.

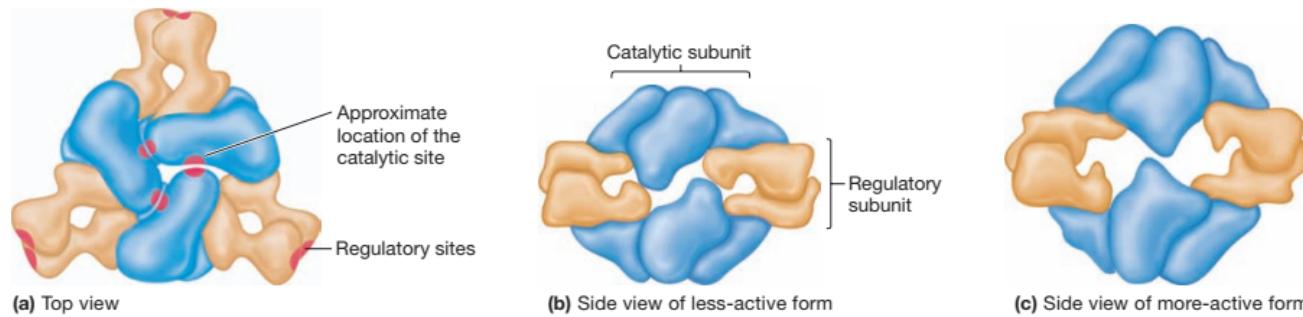


Figure 8.25 The Structure and Regulation of *E. coli* Aspartate Carbamoyltransferase. (a) A schematic diagram of the enzyme showing the six catalytic polypeptide chains (blue), the six regulatory chains (tan), and the catalytic and regulatory sites. The enzyme is viewed from the top. Each catalytic subunit contains three catalytic chains, and each regulatory subunit has two chains. (b) The less active T state of ACTase viewed from the side. (c) The more active R state of ACTase. The regulatory subunits have rotated and pushed the catalytic subunits apart.

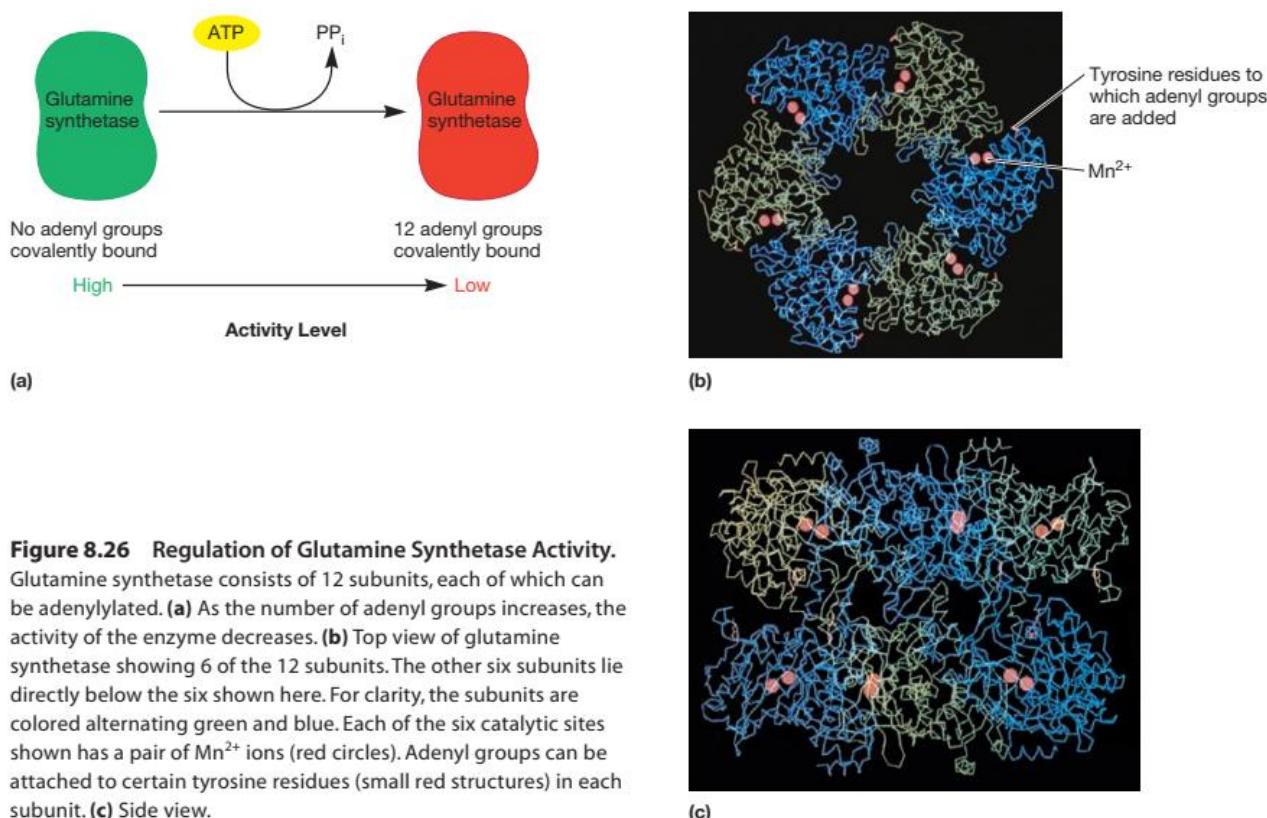


Figure 8.26 Regulation of Glutamine Synthetase Activity. Glutamine synthetase consists of 12 subunits, each of which can be adenylated. (a) As the number of adenyl groups increases, the activity of the enzyme decreases. (b) Top view of glutamine synthetase showing 6 of the 12 subunits. The other six subunits lie directly below the six shown here. For clarity, the subunits are colored alternating green and blue. Each of the six catalytic sites shown has a pair of Mn²⁺ ions (red circles). Adenyl groups can be attached to certain tyrosine residues (small red structures) in each subunit. (c) Side view.

activity again increases and more product is formed. In this way feedback inhibition automatically matches end product supply with the demand. The previously discussed *E. coli* aspartate carbamoyltransferase is an excellent example of end product or feedback inhibition.

Frequently a biosynthetic pathway branches to form more than one end product. In such a situation the synthesis of pathway end products must be coordinated precisely. It would not do to have one end product present in excess while another is lacking. Branching biosynthetic pathways usually achieve a balance between end products through the use of regulatory enzymes at branch points (figure 8.27). If an end product is present in excess, it often inhibits the branch-point enzyme on the sequence leading to its formation, in this way regulating its own formation without affecting the synthesis of other products. In figure 8.27 notice that both products also inhibit the initial enzyme in the pathway. An excess of one product slows the flow of carbon into the whole pathway while inhibiting the appropriate branch-point enzyme. Because less carbon is required when a branch is not functioning, feedback inhibition of the initial pacemaker enzyme helps match the supply with the demand in branching pathways. The regulation of multiple branched pathways is often made even more sophisticated by the presence of **isoenzymes**, different forms of an enzyme that catalyze the same reaction. The initial pacemaker step may be catalyzed by several isoenzymes, each under separate and

independent control. In such a situation an excess of a single end product reduces pathway activity but does not completely block pathway function because some isoenzymes are still active.

1. Define the following: allosteric enzyme, effector or modulator, and $[S]_{0.5}$ or $K_{0.5}$.
2. How can regulatory enzymes be influenced by reversible covalent modification? What group is used for this purpose with glutamine synthetase, and which form of this enzyme is active?
3. What is a pacemaker enzyme? Feedback inhibition? How does feedback inhibition automatically adjust the concentration of a pathway end product?
4. What is the significance of the fact that regulatory enzymes often are located at pathway branch points? What are isoenzymes and why are they important in pathway regulation?

Chemotaxis

Thus far in our discussion of the regulation of enzyme activity, we have focused on the control of metabolic pathways brought about by modulating the activity of certain regulatory enzymes that function in the pathway. However, not all enzymes function in metabolic pathways. Rather, some enzymes are involved in processes that are more complex. These include behavioral changes made by microbes in response to their environment. Chemotaxis is an example of the roles enzymes play in micro-

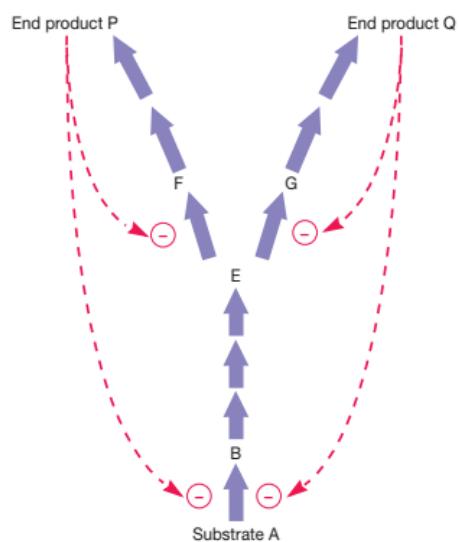


Figure 8.27 Feedback Inhibition. Feedback inhibition in a branching pathway with two end products. The branch-point enzymes, those catalyzing the conversion of intermediate E to F and G, are regulated by feedback inhibition. Products P and Q also inhibit the initial reaction in the pathway. A colored line with a minus sign at one end indicates that an end product, P or Q, is inhibiting the enzyme catalyzing the step next to the minus. See text for further explanation.

bial behavior, and how controlling enzyme activity changes that behavior.

In chapter 3, chemotaxis was briefly introduced. Recall that microorganisms are able to sense chemicals in their environment and either move toward them or away from them, depending on whether the chemical is an attractant or a repellent. For simplicity of discussion, we will only concern ourselves with movement toward an attractant. The best-studied chemotactic system is that of *E. coli*, which, like many other bacteria, exhibits two movement modalities: a forward-swimming motion called a run and a tumbling motion called a tumble. A run occurs when the flagellum rotates in a counterclockwise direction (CCW), and a tumble occurs when the flagellum rotates clockwise (CW) (see figures 3.41 and 3.45). The cell alternates between these two types of movements, with the tumble establishing the direction of movement in the run that follows. When *E. coli* is in an environment that is homogenous—that is, the concentration of all chemicals in the environment is the same throughout its habitat—the cell moves about randomly, with no apparent direction or purpose; this is called a **random walk**. However, if a chemical gradient exists in its environment, the frequency of tumbles decreases as long as the cell is moving toward the attractant. In other words, the length of time spent moving toward the attractant is increased and eventually the cell gets closer to the attractant. The process is not perfect, however. Because bacteria are

small, they often can be knocked off course by the movement of molecules in their environment. Therefore they must continually readjust their direction through a trial and error process that is mediated by tumbling. When one examines the path taken by the cell, it is similar to a random walk, but is biased toward the attractant. Thus the movement of the bacterium toward the attractant often is referred to as a **biased random walk**.

For over three decades, scientists have been dissecting this complex behavior in order to understand how *E. coli* senses the presence of an attractant, how it switches from a run to a tumble and back again, and how it knows it is heading in the correct direction. Many aspects of chemotaxis are now understood, at least superficially, but many questions remain. However, one thing is clear: the chemotactic response of *E. coli* involves a number of enzymes and other proteins that are regulated by covalent modification. One important component is a phosphorelay system. **Phosphorelay systems** consist of at least two proteins: a **sensor kinase** and a **response regulator**. As described here, a phosphorelay system is used to regulate enzyme activity. Other phosphorelay systems are used to regulate protein synthesis and generally use only these two components. These systems are described in chapter 12.

In order for chemotaxis to occur, *E. coli* must determine if an attractant is present and then modulate the activity of the phosphorelay system that dictates the rotational direction of the flagellum (i.e., either run or tumble). *E. coli* senses chemicals in its environment when they bind to chemoreceptors (figure 8.28). Numerous chemoreceptors have been identified. We will focus on one class of receptors called **methyl-accepting chemotaxis proteins (MCPs)**. The phosphorelay system that controls direction of flagellar rotation consists of the sensor kinase CheA and the response regulator CheY. When activated, CheA phosphorylates itself using ATP (figure 8.28c). The phosphoryl group is then quickly transferred to CheY. Phosphorylated CheY diffuses through the cytoplasm to the flagellar motor. Upon interacting with the motor, the direction of rotation is switched from CCW to CW, and a tumble ensues. When CheA is inactive, the flagellum rotates in its default mode (CCW), and the cell moves forward in a smooth run.

As implied by the preceding discussion, the state of the MCPs must be communicated to the CheA/CheY phosphorelay system. How is this accomplished? The MCPs are buried in the plasma membrane with different parts exposed on each side of the membrane (figure 8.28c). The periplasmic side of each MCP has a binding site for one or more attractant molecules. The cytoplasmic side of an MCP interacts with two proteins, CheW and CheA. The CheW protein binds to the MCP and helps attach the CheA protein. Together with CheW and CheA, the MCP receptors form large clusters at one or both poles of the cell (figure 8.28b). It is thought that smaller aggregations of the MCPs, CheA and CheW, which function as signaling teams, are the building blocks of the receptor clusters. The number of each of these molecules in the signaling team is not clear, but it has been suggested that each team includes three receptors, often of different types (figure 8.29), two CheW molecules, and one CheA dimer. It has also been suggested that the signaling teams aggregate with each other to form “signaling leagues.” Finally, the signaling teams (and perhaps signaling

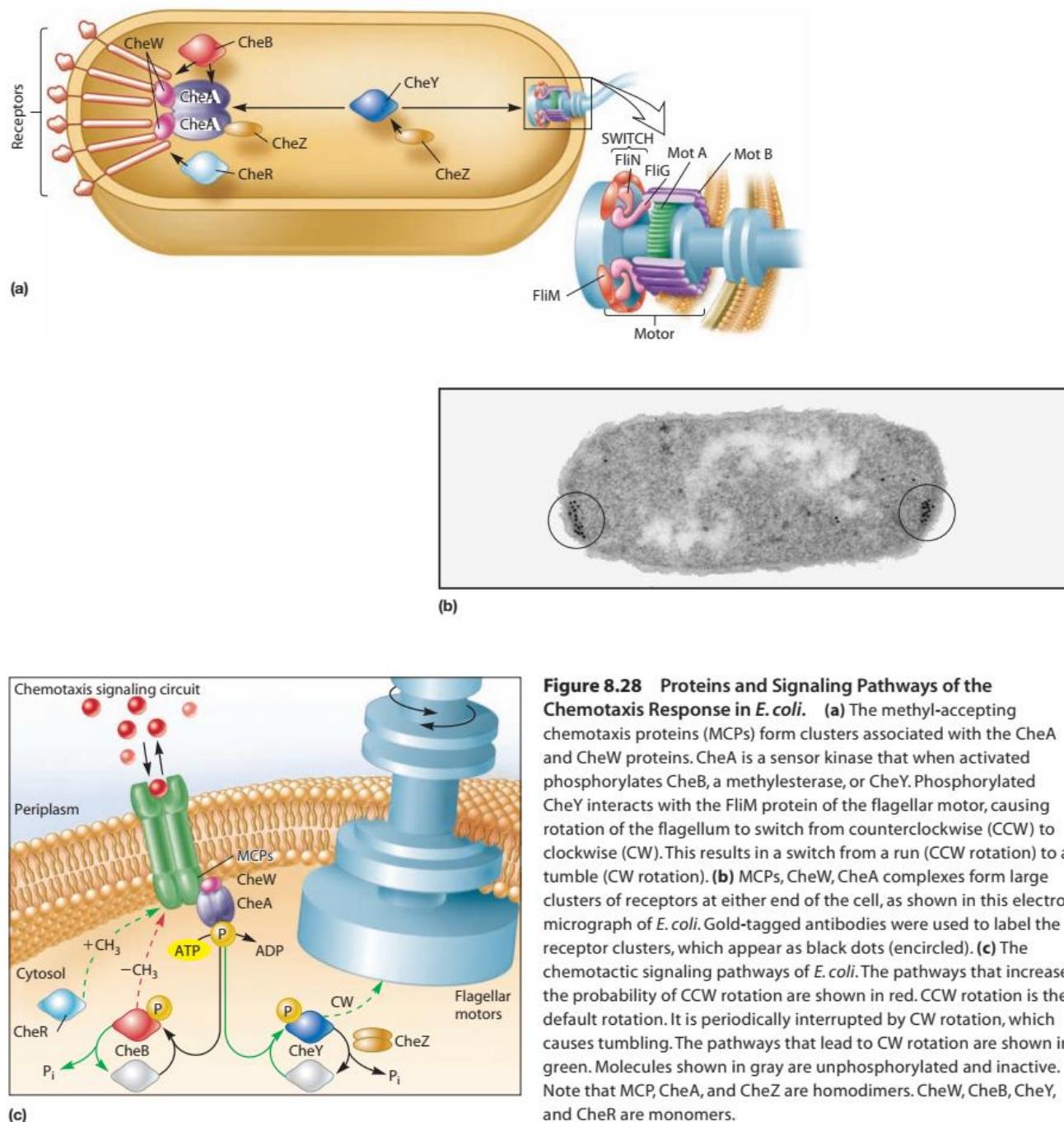


Figure 8.28 Proteins and Signaling Pathways of the Chemotaxis Response in *E. coli*. **(a)** The methyl-accepting chemotaxis proteins (MCPs) form clusters associated with the CheA and CheW proteins. CheA is a sensor kinase that when activated phosphorylates CheB, a methyl esterase, or CheY. Phosphorylated CheY interacts with the FliM protein of the flagellar motor, causing rotation of the flagellum to switch from counterclockwise (CCW) to clockwise (CW). This results in a switch from a run (CCW rotation) to a tumble (CW rotation). **(b)** MCPs, CheW, CheA complexes form large clusters of receptors at either end of the cell, as shown in this electron micrograph of *E. coli*. Gold-tagged antibodies were used to label the receptor clusters, which appear as black dots (encircled). **(c)** The chemotactic signaling pathways of *E. coli*. The pathways that increase the probability of CCW rotation are shown in red. CCW rotation is the default rotation. It is periodically interrupted by CW rotation, which causes tumbling. The pathways that lead to CW rotation are shown in green. Molecules shown in gray are unphosphorylated and inactive. Note that MCP, CheA, and CheZ are homodimers. CheW, CheB, CheY, and CheR are monomers.

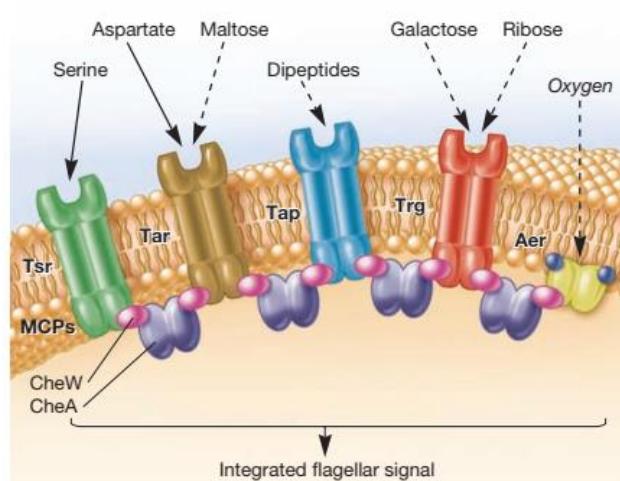


Figure 8.29 The Methyl-Accepting Chemotaxis Proteins of *E. coli*. The attractants sensed by each methyl-accepting chemotaxis protein (MCP) are shown. Some are sensed directly, when the attractant binds the MCP (solid lines). Others are sensed indirectly (dashed lines). The attractants maltose, dipeptides, galactose, and ribose are detected by their interaction with periplasmic binding proteins. Oxygen is detected indirectly by the Aer chemoreceptor, which differs from other MCPs in that it lacks a periplasmic sensing domain. Instead, the cytoplasmic domain has a binding site for FAD. FAD is an important electron carrier found in many electron transport systems. The redox state of the MCP-bound FAD molecule is used to monitor the functioning of the electron transport system. This in turn mediates a tactic response to oxygen.

leagues) become interconnected by an unknown mechanism to form the receptor clusters visible at the poles of the cell.

No matter what the precise stoichiometry or architecture of the receptor clusters, there is evidence that the MCPs in each signaling team work cooperatively to modulate CheA activity. When any one of the MCPs in the signaling team is bound to an attractant, CheA autophosphorylation is inhibited, the flagellum continues rotating CCW, and the cell continues in its run. Because of this cooperation, the cell can respond to very low concentrations of attractant. Furthermore, it can integrate signals from all receptors in the team (figure 8.29). On the other hand, if attractant levels decrease, so that the level of attractant bound to the MCPs in a signaling team decreases, CheA is stimulated to autophosphorylate, the phosphorelay is set into motion, and the cell begins to tumble. However, tumbling does not continue indefinitely. About 10 sec-

onds after the switch to CW rotation occurs, the phosphoryl group is removed from CheY by the CheZ protein, and CCW rotation is resumed.

But how does *E. coli* measure the concentration of attractant in its environment, and how does it know when it is moving toward the attractant? *E. coli* measures the concentration of an attractant every few seconds and determines if the concentration is increasing or decreasing over time. As long as the concentration increases, the cell continues a run. If the concentration decreases, a tumble is triggered. In order to compare concentrations of the attractant over time, *E. coli* must have a mechanism for remembering the previous concentration. *E. coli* accomplishes this by comparing the overall methylation level of the MCPs (on the cytoplasmic side) with the overall amount of attractant bound (on the periplasmic face). The cytoplasmic portion of each MCP has four to six glutamic acid residues that can be methylated. Addition and removal of methyl groups is catalyzed by two different enzymes. Methylation is catalyzed by the MCP-specific methyltransferase CheR. Demethylation is catalyzed by the MCP-specific methylesterase CheB. Methylation occurs at a fairly steady rate regardless of the attractant level. However, an MCP-attractant complex is a better substrate for CheR than is an MCP that is not bound to attractant. Thus when attractant is bound, methylation of the MCP is favored. The methylesterase activity of CheB is also modified by the CheA protein. As long as the concentration of the attractant keeps increasing, the number of MCPs bound to attractant remains high, and the MCP methylation level remains high. However, if the attractant concentration decreases, the level of methylation will exceed the level of attractant bound. This disparity in methylation level and MCP-bound attractant stimulates CheA to autophosphorylate. As a result, the phosphorelay signal for CW flagellar rotation is initiated and the cell tumbles in an attempt to reorient itself in the gradient so that it is moving up the gradient (toward the attractant) rather than down the gradient (away from the attractant). At the same time, some of the phosphoryl groups on CheA are transferred to CheB. This activates CheB, and it removes methyl groups from the MCPs. This lowers the methylation level so that it is commensurate with the number of MCPs bound to attractant. A few seconds later, the number of MCPs bound to attractant will be compared to this new methylation level. Based on the correspondence of the two, the cell will determine if it is again moving up the gradient. If it is, tumbling will be suppressed (as will methylesterase activity) and the run will continue.

1. What is a phosphorelay system?
2. Describe the MCP-CheW-CheA receptor complex. What two proteins are phosphorylated by CheA? What is the role of each?
3. How does the MCP regulate the rate of CheA autophosphorylation? How does this mediate chemotaxis?

Summary

8.1 An Overview of Metabolism

- a. Metabolism is the total of all chemical reactions that occur in cells. It can be divided into two parts: energy-conserving reactions (sometimes called catabolism) and anabolism.
- b. Organisms are defined nutritionally based on how they fulfill their carbon, energy, and electron needs. The interactions of organisms belonging to different nutritional types is the basis for the flow of energy, carbon, and electrons in the biosphere. The ultimate source of energy for most microbes is sunlight trapped by photoautotrophs and used to form organic material from CO_2 . Photoautotrophs and the organic molecules they have synthesized are consumed by chemoorganoheterotrophs (figures 8.1 and 8.2).

8.2 Energy and Work

- a. Energy is the capacity to do work. Living cells carry out three major kinds of work: chemical work of biosynthesis, transport work, and mechanical work.

8.3 The Laws of Thermodynamics

- a. The first law of thermodynamics states that energy is neither created nor destroyed.
- b. The second law of thermodynamics states that changes occur in such a way that the randomness or disorder of the universe increases to the maximum possible. That is, entropy always increases during spontaneous processes.

8.4 Free Energy and Reactions

- a. The first and second laws can be combined to determine the amount of energy made available for useful work.

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

In this equation the change in free energy (ΔG) is the energy made available for useful work, the change in enthalpy (ΔH) is the change in heat content, and the change in entropy is ΔS .

- b. The standard free energy change (ΔG°) for a chemical reaction is directly related to the equilibrium constant.
- c. In exergonic reactions ΔG° is negative and the equilibrium constant is greater than one; the reaction goes to completion as written. Endergonic reactions have a positive ΔG° and an equilibrium constant less than one (figure 8.4).

8.5 The Role of ATP in Metabolism

- a. ATP is a high-energy molecule that serves as an energy currency; it transports energy in a useful form from one reaction or location in a cell to another. (figure 8.5)
- b. ATP is readily synthesized from ADP and P_i using energy released from exergonic reactions; when hydrolyzed back to ADP and P_i , it releases the energy, which is used to drive endergonic reactions. This cycling of ATP with ADP and P_i is called the cell's energy cycle (figure 8.7).

8.6 Oxidation-Reduction Reactions, Electron Carriers, and Electron Transport Systems

- a. In oxidation-reduction (redox) reactions, electrons move from an electron donor to an electron acceptor. The standard reduction potential measures the tendency of the donor to give up electrons.

- b. Redox couples with more negative reduction potentials donate electrons to those with more positive potentials, and energy is made available during the transfer (figure 8.8, table 8.1).
- c. Some of the most important electron carriers in cells are NAD^+ , NADP^+ , FAD, FMN, coenzyme Q, cytochromes, and the nonheme iron proteins.
- d. Electron carriers are often organized into electron transport systems that are located in membranes. These systems are critical to the energy-conserving processes observed during aerobic respiration, anaerobic respiration, chemolithotrophy, and photosynthesis (figure 8.9).

8.7 Enzymes

- a. Enzymes are protein catalysts that catalyze specific reactions.
- b. Enzymes consist of a protein component, the apoenzyme, and often a nonprotein cofactor that may be a prosthetic group, a coenzyme, or a metal activator.
- c. Enzymes speed reactions by binding substrates at their active sites and lowering the activation energy (figure 8.15).
- d. The rate of an enzyme-catalyzed reaction increases with substrate concentration at low substrate levels and reaches a plateau (the maximum velocity) at saturating substrate concentrations. The Michaelis constant is the substrate concentration that the enzyme requires to achieve half maximal velocity (figure 8.18).
- e. Enzymes have pH and temperature optima for activity (figure 8.19).
- f. Enzyme activity can be slowed by competitive and noncompetitive inhibitors (figure 8.20).

8.8 The Nature and Significance of Metabolic Regulation

- a. The regulation of metabolism keeps cell components in proper balance and conserves metabolic energy and material.

8.9 Metabolic Channeling

- a. The localization of metabolites and enzymes in different parts of the cell, called metabolic channeling, influences pathway activity. A common channeling mechanism is compartmentation.

8.10 Control of Enzyme Activity

- a. Many regulatory enzymes are allosteric enzymes, enzymes in which an effector or modulator binds noncovalently and reversibly to a regulatory site separate from the catalytic site and causes a conformational change in the enzyme to alter its activity (figure 8.22).
- b. Enzyme activity also can be regulated by reversible covalent modification. Usually a phosphoryl, methyl, or adenyl group is attached to the enzyme.
- c. The first enzyme in a pathway and enzymes at branch points often are subject to feedback inhibition by one or more end products. Excess end product slows its own synthesis (figure 8.27).
- d. Complex behaviors such as chemotaxis can also be regulated by altering enzyme activity (figure 8.28 and 8.29).

Key Terms

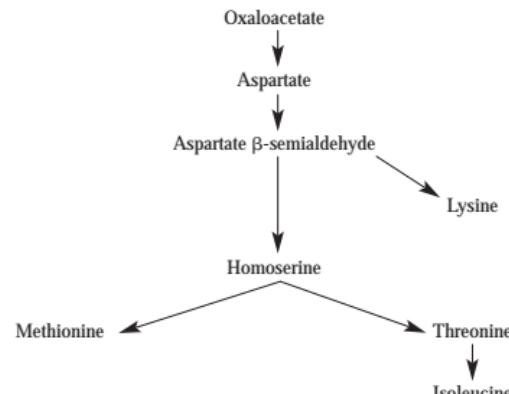
activation energy 177	effector or modulator 181	flavin adenine dinucleotide (FAD) 173	oxidation-reduction (redox) reaction 172
active site 177	electron acceptor 172	flavin mononucleotide (FMN) 173	pacemaker enzyme 183
adenosine diphosphate (ADP) 171	electron donor 172	free energy change 170	phosphate group transfer potential 171
adenosine 5'-triphosphate (ATP) 171	electron transport chain (ETC) 173	high-energy molecule 171	phosphorelay system 185
allosteric enzymes 181	electron transport system (ETS) 173	holoenzyme 176	posttranslational regulation 181
anabolism 168	endergonic reaction 170	isoenzymes 184	product 176
apoenzyme 176	end product inhibition 183	joule 170	prosthetic group 176
calorie 170	energy 169	mechanical work 169	reducing power 168
catabolism 168	energy-conserving reactions 168	metabolic channeling 180	regulatory site 181
catalyst 176	enthalpy 170	metabolism 167	response regulator 185
catalytic site 177	entropy 169	methyl-accepting chemotaxis proteins (MCPs) 185	reversible covalent modification 183
chemical work 169	enzyme 176	Michaelis constant (K_m) 178	second law of thermodynamics 169
coenzyme 176	enzyme-substrate complex 177	nicotinamide adenine dinucleotide (NAD ⁺) 173	sensor kinase 185
coenzyme Q or CoQ (ubiquinone) 173	equilibrium 170	nicotinamide adenine dinucleotide phosphate (NADP ⁺) 173	standard free energy change 170
cofactor 176	equilibrium constant (K_{eq}) 170	noncompetitive inhibitor 179	standard reduction potential 172
compartmentation 180	exergonic reaction 170	nonheme iron protein 174	substrate 176
competitive inhibitor 179	feedback inhibition 183		thermodynamics 169
cytochrome 174	ferredoxin 174		transition-state complex 177
denaturation 179	first law of thermodynamics 169		transport work 169

Critical Thinking Questions

- How could electron transport be driven in the opposite direction? Why would it be desirable to do this?
- Suppose that a chemical reaction had a large negative $\Delta G^\circ'$ value. What would this indicate about its equilibrium constant? If displaced from equilibrium, would it proceed rapidly to completion? Would much or little free energy be made available?
- Take a look at the structures of macromolecules (appendix I). Which type has the most electrons to donate? Why are carbohydrates usually the primary source of electrons for chemoorganotrophic bacteria?
- Most enzymes do not operate at their biochemical optima inside cells. Why not?
- Examine the branched pathway shown here for the synthesis of the amino acids aspartate, methionine, lysine, threonine, and isoleucine. For each of these two scenarios answer the following questions:
 - Which portion(s) of the pathway would need to be shut down in this situation?
 - How might allosteric control be used to accomplish this?

Scenario 1: The microbe is cultured in a medium containing aspartate and lysine, but lacking methionine, threonine, and isoleucine.

Scenario 2: The microbe is cultured in a medium containing a rich supply of all five amino acids.



Learn More

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