Лекция 4.
Обмен энергией

Bioenergetics and Metabolism

Metabolism is a highly coordinated and directed cell activity, in which many multienzyme systems cooperate to accomplish four functions: (1) to obtain chemical energy by capturing solar energy or by degrading energy-rich nutrients from the environment, (2) to convert nutrient molecules into the cell's own characteristic molecules, including macromolecular precursors, (3) to polymerize monomeric precursors into proteins, nucleic acids, lipids, polysaccharides, and other cell components, and (4) to synthesize and degrade biomolecules required in specialized cellular functions.

Although metabolism embraces hundreds of different enzyme-catalyzed reactions, the central metabolic pathways—our major concern—are few in number and are remarkably similar in all forms of life. Living organisms can be divided into two large groups according to the chemical form in which they obtain carbon from the environment. **Autotrophs** (such as photosynthetic bacteria and higher plants) can use carbon dioxide from the atmosphere as their sole source of carbon, from which they construct all their carbon-containing biomolecules (see Fig. 2–4). Some autotrophic organisms, such as cyanobacteria, can also use atmospheric nitrogen to generate all their nitrogenous components. **Heterotrophs** cannot use atmospheric carbon dioxide and must obtain carbon from their environment in the form of relatively complex organic molecules, such as glucose. The cells of higher animals and most microorganisms are heterotrophic. Autotrophic cells are relatively self-sufficient, whereas heterotrophic cells, with their requirements for carbon in more complex forms, must subsist on the products of other cells.

Many autotrophic organisms are photosynthetic and obtain their energy from sunlight, whereas heterotrophic cells obtain their energy from the degradation of organic nutrients made by autotrophs. In our biosphere, autotrophs and heterotrophs live together in a vast, interdependent cycle in which autotrophic organisms use atmospheric CO_2 to build their organic biomolecules, some of them generating oxygen from H_2O in the process. Heterotrophs in turn use the organic products of autotrophs as nutrients and return CO_2 to the atmosphere. The oxidation reactions that produce CO_2 also consume O_2, converting it to H_2O. Thus carbon, oxygen, and water are constantly cycled between the heterotrophic and autotrophic worlds, solar energy ultimately providing the driving force for this massive process (Fig. 1).

Facing page: The active site of glyceraldehyde-3-phosphate dehydrogenase, with the bound cofactor nicotinamide adenine dinucleotide (NAD) shown in red. This enzyme catalyzes the oxidation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate, a step in glycolysis, a central pathway in glucose metabolism. This is the earliest known example of an enzymatic reaction in which the energy released by electron transfer (oxidation) drives the formation of a high-energy phosphate compound.

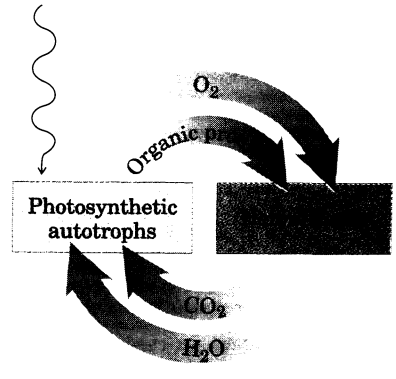


Figure 1 The cycling of carbon dioxide and oxygen between the autotrophic (photosynthetic) and the heterotrophic domains in the biosphere. The flow of mass through this cycle is enormous; about 4×10^{11} metric tons of carbon are turned over in the biosphere annually.

All living organisms also require a source of nitrogen, which is necessary for the synthesis of amino acids, nucleotides, and other compounds. Plants are generally able to use either ammonia or soluble nitrates as their sole source of nitrogen, but vertebrate animals must obtain some nitrogen in the form of amino acids or other organic compounds. Only a few organisms—the cyanobacteria and a few species of soil bacteria that live symbiotically on the roots of certain plants (legumes)—are capable of converting ("fixing") atmospheric nitrogen (N_2) into ammonia. Other microbial organisms (nitrifying bacteria) carry out the oxidation of ammonia to nitrites and nitrates. Thus, in addition to the global carbon and oxygen cycle (Fig. 1), a nitrogen cycle operates in the biosphere in which huge amounts of nitrogen undergo cycling and turnover (Fig. 2). The cycling of carbon, oxygen, and nitrogen, which involves many species of living organisms, depends on a proper balance between the activities of the producers (autotrophs) and consumers (heterotrophs) in our biosphere.

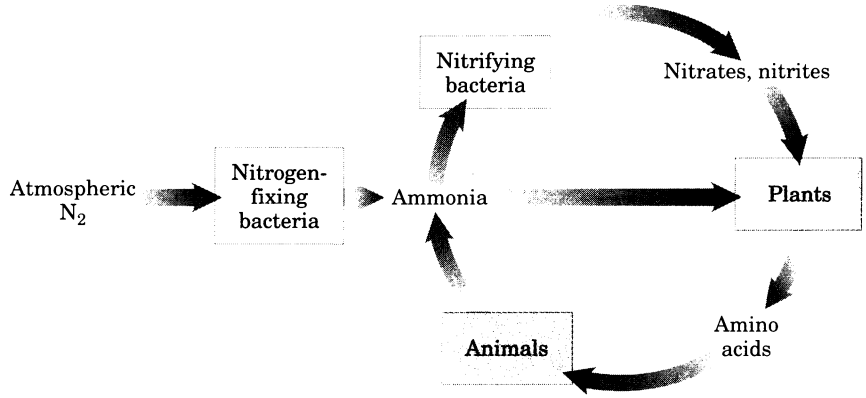


Figure 2 The cycling of nitrogen in the biosphere. Gaseous nitrogen (N_2) makes up 80% of our atmosphere.

These great cycles of matter are driven by an enormous flow of energy through the biosphere, which begins with the capture of solar energy by photosynthetic organisms and its use to generate energy-rich carbohydrates and other organic nutrients; these nutrients are then used as energy sources by heterotrophic organisms. In the metabolic processes of each organism participating in these cycles, and in all energy-requiring activities, there is a loss of useful energy (free energy) and an inevitable increase in the amount of unavailable energy as heat and entropy. In contrast to the cycling of matter, therefore, energy flows one-way through the biosphere; useful energy can never be regenerated in living organisms from energy dissipated as heat and entropy. Carbon, oxygen, and nitrogen recycle continuously, but energy is constantly transformed into unusable forms.

Metabolism, the sum of all of the chemical transformations that occur in a cell or organism, occurs in a series of enzyme-catalyzed reactions that constitute metabolic pathways. Each of the consecutive steps in such a pathway brings about a small, specific chemical change, usually the removal, transfer, or addition of a specific atom, functional group, or molecule. In this sequence of steps (the **pathway**), a precursor is converted into a product through a series of metabolic intermediates (**metabolites**). The term intermediary metabolism is often applied to the combined activities of all of the metabolic pathways that interconvert precursors, metabolites, and products of low molecular weight (not including macromolecules).

Catabolism is the degradative phase of metabolism, in which organic nutrient molecules (carbohydrates, fats, and proteins) are converted into smaller, simpler end products (e.g., lactic acid, CO_2, NH_3). Catabolic pathways release free energy, some of which is conserved in the formation of ATP and reduced electron carriers (NADH and NADPH). In **anabolism,** also called biosynthesis, small, simple precursors are built up into larger and more complex molecules, including lipids, polysaccharides, proteins, and nucleic acids. Anabolic reactions require the input of energy, generally in the forms of the free energy of hydrolysis of ATP and the reducing power of NADH and NADPH (Fig. 3).

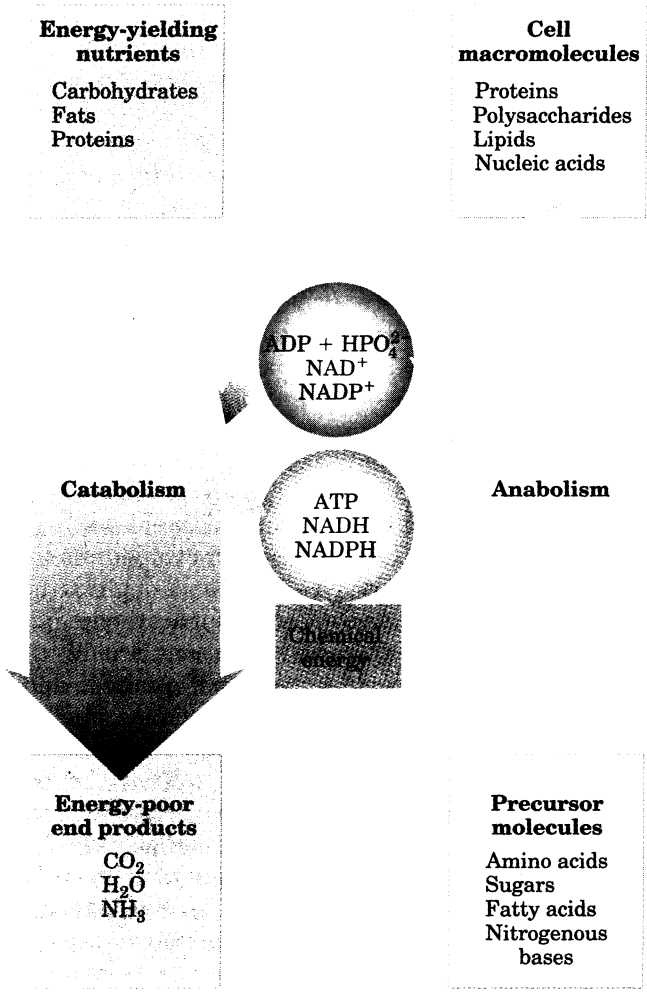


Figure 3 Energy relationships between catabolic and anabolic pathways. Catabolic pathways deliver chemical energy in the form of ATP, NADH, and NADPH. These are used in anabolic pathways to convert small precursor molecules into cell macromolecules.

Metabolic pathways are sometimes linear and sometimes branched, yielding several useful end products from a single precursor or converting several starting materials into a single product. In general, catabolic pathways are convergent and anabolic pathways divergent (Fig. 4). Some pathways are even cyclic: one of the starting components of the pathway is regenerated in the series of reactions that converts another starting component into a product. We shall see examples of each type of pathway in the following chapters.

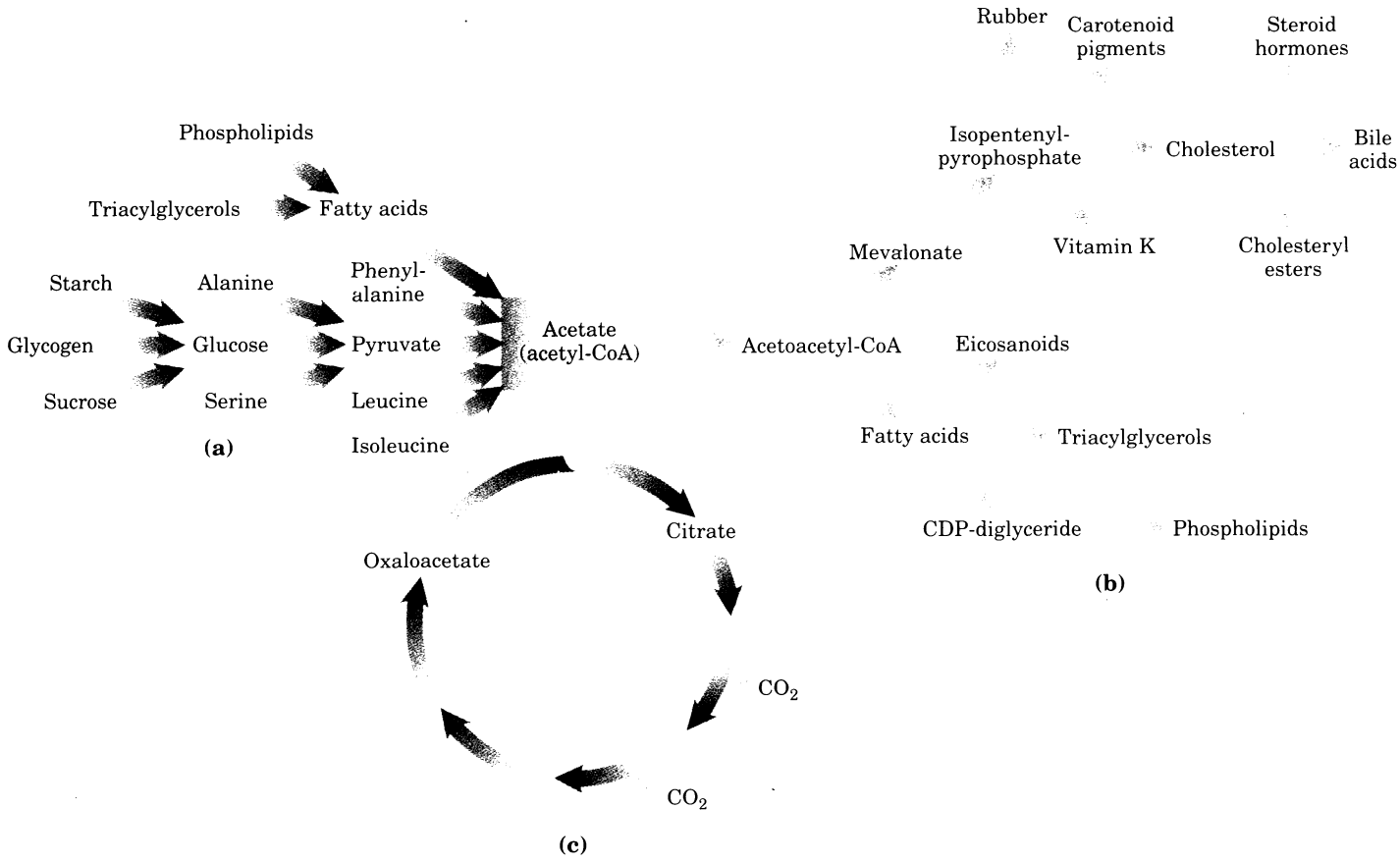


Figure 4 Three types of nonlinear metabolic pathways: **(a)** converging, catabolic; **(b)** diverging, anabolic; and **(c)** a cyclic pathway, in which one of the starting materials (oxaloacetate) is regenerated and reenters the pathway. Acetate, a key metabolic intermediate, can be produced by the breakdown of a variety of fuels **(a),** can serve as the precursor for the biosynthesis of an array of products **(b),** or can be consumed in the catabolic pathway known as the citric acid cycle **(c).**

Most organisms have the enzymatic equipment to carry out both the degradation and the synthesis of certain compounds (fatty acids, for example). The simultaneous synthesis and degradation of fatty acids would be wasteful and is prevented by separately regulating anabolic and catabolic reaction sequences: when one occurs, the other is suppressed. Such regulation could not occur if anabolic and catabolic pathways were catalyzed by the same set of enzymes, operating in one direction for anabolism, the opposite for catabolism. Inhibition of an enzyme involved in catabolism would also inhibit the reaction sequence in the anabolic direction. Catabolic and anabolic pathways that connect the same two end points (a fatty acid and acetate, for example) may employ many of the same enzymes, but invariably at least one of the steps is catalyzed by different enzymes in the catabolic and the anabolic directions, and these enzymes are the sites of separate regulation. It is also common for such paired catabolic and anabolic pathways to occur in different cellular compartments. Fatty acid catabolism, for example, occurs in mitochondria, whereas the synthesis of fatty acids takes place in the cytosol. The concentrations of intermediates, enzymes, and regulators can be maintained at different levels in different compartments, further contributing to the separate regulation of catabolic and anabolic reaction sequences. These devices for separation of anabolic and catabolic processes will be of particular interest in our discussions of metabolism.

Metabolic pathways are regulated at three levels. The first and most immediately responsive form of regulation is through the action

of allosteric enzymes, which are capable of changing their catalytic activity in response to stimulatory or inhibitory modulators (p. 230). We shall meet examples of allosteric regulation throughout the following chapters. Metabolic control is exerted at a second level in higher organisms by hormonal regulation. Hormones are chemical messengers released by one tissue that stimulate or inhibit some process in another tissue. Hormones serve to coordinate the metabolic activities of different tissues, and their actions and effects are generally on a somewhat longer time scale than those of allosteric effectors. The third level of metabolic regulation is control of the rate of a metabolic step by regulating the concentration of its enzyme in the cell. The concentration of an enzyme at any given time is the result of a balance between its rate of synthesis and its rate of degradation, both of which are subject to regulation on a time scale of minutes to hours.

The number of metabolic transformations that occur in a typical cell can seem overwhelming to a beginning student. Fortunately, there are recurring patterns in the metabolic pathways that make learning easier. Certain types of reactions occur in many different metabolic pathways but always employ the same coenzyme(s) and the same general mechanism. Many of the coenzymes are derived from vitamins (see Table 8–2), compounds essential in the diets of animals. The coenzymes are critical to the reaction mechanisms in which they participate. Once you have learned the general mechanism of a reaction, including the role of the cofactor, the recurring pattern in a variety of metabolic pathways will be easily recognizable. In the chapters that follow, we will usually discuss the general mechanism for each of these reactions when we first encounter the cofactor in its typical role.

In the first half of Part III we consider the major catabolic pathways by which cells obtain energy from the oxidation of various fuels: first, the central pathways of hexose conversion to triose (Chapter 14) and triose oxidation to carbon dioxide (Chapter 15); then the pathways of fatty acid oxidation (Chapter 16) and amino acid oxidation (Chapter 17). Chapter 18 is the pivotal point of our discussion of metabolism; it concerns chemiosmotic energy coupling, the universal mechanism in which a transmembrane electrochemical potential, produced either by substrate oxidation or by light absorption, drives the synthesis of ATP.

The second half of this part describes the major anabolic pathways by which cells use ATP to produce carbohydrates (Chapter 19), lipids (Chapter 20), and amino acids and nucleotides (Chapter 21) from simpler precursors. Finally, in Chapter 22 we step back from the details of the metabolic pathways and consider how those pathways are regulated and integrated in mammals by hormonal mechanisms.

We begin our study of intermediary metabolism with an introduction to bioenergetics (Chapter 13). But before we begin, a final word. Try not to forget that the myriad reactions described on these pages take place in, and play crucial roles in, living organisms. Ask of each reaction and of each pathway, "What is accomplished for the cell or the organism by this reaction or pathway? How does this pathway interconnect with the other pathways occurring simultaneously in the same cell to produce the energy and products required for cell maintenance and growth? How do the multilayered regulatory mechanisms cooperate to balance metabolic and energetic inputs and outputs, achieving the dynamic steady state of life?" Learned with this perspective, metabolism provides fascinating and revealing insights into life.

CHAPTER

Principles of Bioenergetics

Living cells and organisms must perform work to stay alive, to grow, and to reproduce themselves. The ability to harness energy from various sources and to channel it into biological work is a fundamental property of all living organisms; it must have been acquired very early in the process of cellular evolution. Modern organisms carry out a remarkable variety of energy transductions, conversions of one form of energy to another. They use chemical energy in fuels to bring about the synthesis of complex molecules from simple precursors, producing macromolecules with highly ordered structure. They also convert the chemical energy of various fuels into concentration gradients and electrical gradients, motion, heat, and even, in a few organisms such as fireflies, light. Photosynthetic organisms transduce light energy into all of these other forms of energy.

The chemical mechanisms that underlie biological energy transductions have fascinated and challenged biologists for centuries. Antoine Lavoisier, before he lost his head in the French Revolution, recognized that animals somehow transform chemical fuels (foods) into heat and that this process of respiration is essential to life. He observed that

> . . . in general, respiration is nothing but a slow combustion of carbon and hydrogen, which is entirely similar to that which occurs in a lighted lamp or candle, and that, from this point of view, animals that respire are true combustible bodies that burn and consume themselves. . . . One may say that this analogy between combustion and respiration has not escaped the notice of the poets, or rather the philosophers of antiquity, and which they had expounded and interpreted. This fire stolen from heaven, this torch of Prometheus, does not only represent an ingenious and poetic idea, it is a faithful picture of the operations of nature, at least for animals that breathe; one may therefore say, with the ancients, that the torch of life lights itself at the moment the infant breathes for the first time, and it does not extinguish itself except at death.*

Antoine Lavoisier
1743–1794

In this century, biochemical studies have revealed much of the chemistry of energy transductions in living organisms. Biological energy transductions obey the same physical laws that govern all other natural processes. It is therefore essential for a student of biochemistry to understand these laws and the ways in which they apply to the flow of energy in the biosphere. In this chapter we first review the laws of

* From a memoir by Armand Seguin and Antoine Lavoisier, dated 1789, quoted in Lavoisier, A. (1862) *Oeuvres de Lavoisier*, Imprimerie Impériale, Paris.

thermodynamics and the quantitative relationships among free energy, enthalpy, and entropy. We then describe the special role of ATP in biological energy exchanges. Finally, we consider the importance of oxidation–reduction reactions in living cells, the energetics of such electron transfer reactions, and the electron carriers commonly employed as cofactors of the enzymes that catalyze these reactions.

Bioenergetics and Thermodynamics

Bioenergetics is the quantitative study of the energy transductions that occur in living cells and of the nature and function of the chemical processes underlying these transductions. Although many of the principles of thermodynamics have been introduced in earlier chapters and may be familiar to you, it is worth reviewing the quantitative aspects of these principles.

Biological Energy Transformations Follow the Laws of Thermodynamics

Many quantitative observations made by physicists and chemists on the interconversion of different forms of energy led to the formulation, in the nineteenth century, of two fundamental laws of thermodynamics. The first law is the principle of the conservation of energy: *in any physical or chemical change, the total amount of energy in the universe remains constant, although the form of the energy may change.* The second law of thermodynamics, which can be stated in several forms, says that the universe always tends toward more and more disorder: *in all natural processes, the entropy of the universe increases.*

"Now, in the *second* law of thermodynamics . . . "

Living organisms consist of collections of molecules much more highly organized than the surrounding materials from which they are constructed, and they maintain and produce order, seemingly oblivious to the second law of thermodynamics. Living organisms do not violate the second law; they operate strictly within it. To discuss the application of the second law to biological systems, we must first define those systems and the universe in which they occur. The reacting system is the collection of matter that is undergoing a particular chemical or physical process; it may be an organism, a cell, or two reacting compounds. The reacting system and its surroundings together constitute the universe. Some chemical or physical processes can be made to take place in isolated or closed systems, in which no material or energy is exchanged with the surroundings. Living cells and organisms are open systems, which exchange both material and energy with their surroundings; living systems are never at equilibrium with their surroundings.

We have defined earlier in this text three thermodynamic quantities that describe the energy changes occurring in a chemical reaction. Gibbs free energy (G) expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure (p. 8). When a reaction proceeds with the release of free energy (i.e., when the system changes so as to possess less free energy), the free-energy change, ΔG, has a negative sign and the reaction is said to be exergonic. In endergonic reactions, the system gains free energy and ΔG is positive. Enthalpy, H, is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and

BOX 13–1 Entropy: The Advantages of Being Disorganized

The term entropy, which literally means "a change within," was first used in 1851 by Rudolf Clausius, one of the promulgators of the second law. A rigorous quantitative definition of entropy involves statistical and probability considerations. However, its nature can be illustrated qualitatively by three simple examples, each of which shows one aspect of entropy. The key descriptors of entropy are *randomness* or *disorder,* manifested in different ways.

Case 1: The Teakettle and the Randomization of Heat

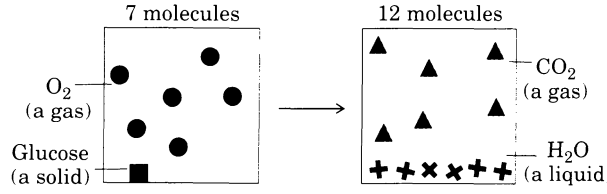
We know that steam generated from boiling water can do useful work. But suppose we turn off the burner under a teakettle full of water at 100 °C (the "system") in the kitchen (the "surroundings") and allow it to cool. As it cools, no work will be done, but heat will pass from the teakettle to the surroundings, raising the temperature of the surroundings (the kitchen) by an infinitesimally small amount until complete equilibrium is attained. At this point all parts of the teakettle and the kitchen will be at precisely the same temperature. The free energy that was once concentrated in the teakettle of hot water at 100 °C, *potentially* capable of doing work, has disappeared. Its equivalent in heat energy is still present in the teakettle + kitchen (i.e., the "universe") but has become completely randomized throughout. This energy is no longer available to do work because there is no temperature differential within the kitchen. Moreover, the increase in entropy of the kitchen (the surroundings) is irreversible. We know from everyday experience that heat will never spontaneously pass back from the kitchen into the teakettle to raise the temperature of the water to 100 °C again.

Case 2: The Oxidation of Glucose

Entropy is a state or condition not only of energy but also of matter. Aerobic organisms extract free energy from glucose obtained from their surroundings. To extract this energy they oxidize the glucose with molecular oxygen, also obtained from the surroundings. The end products of the oxidative metabolism of glucose are CO_2 and H_2O, which are returned to the surroundings. In this process the surroundings undergo an increase in entropy, whereas the organism itself remains in a steady state and undergoes no change in its internal order. Although some of the entropy arises from the dissipation of heat, entropy also arises from another kind of disorder, illustrated by the equation for the oxidation of glucose by living organisms, which we can write as

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

or represent schematically as



products. When a chemical reaction releases heat, it is said to be exothermic; the heat content of the products is less than that of the reactants and ΔH has a negative value. Reacting systems that take up heat from their surroundings are endothermic and have positive values of ΔH (p. 66). Entropy, S, is a quantitative expression for the randomness or disorder in a system (Box 13–1). When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy (p. 72). The units of ΔG and ΔH are joules/mole or calories/mole (recall that 1 cal equals 4.18 J); units of entropy are joules/mole • degree Kelvin (J/mol • K) (Table 13–1).

Under the conditions existing in biological systems (at constant temperature and pressure), changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation

$$\Delta G = \Delta H - T\,\Delta S \qquad (13\text{–}1)$$

in which ΔG is the change in Gibbs free energy of the reacting system,

Table 13–1 Some physical constants and units frequently used in thermodynamics

Boltzmann constant, $\mathbf{k} = 1.381 \times 10^{-23}$ J/K
Avogadro's number, $N = 6.022 \times 10^{23}$ mol^{-1}
Faraday constant, $\mathcal{F} = 96{,}480$ J/V • mol
Gas constant, $R = 8.315$ J/mol • K
(= 1.987 cal/mol • K)

Units of ΔG and ΔH are J/mol (or cal/mol)
Units of ΔS are J/mol • K (or cal/mol • K)
1 cal = 4.184 J

Units of absolute temperature, T, are degrees Kelvin, K
25 °C = 298 K
At 25 °C, RT = 2.479 kJ/mol
(= 0.592 kcal/mol)
$\ln x = 2.303 \log x$

The atoms contained in 1 molecule of glucose plus 6 molecules of oxygen, a total of 7 molecules, are more randomly dispersed by the oxidation reaction and are now present in a total of 12 molecules ($6CO_2 + 6H_2O$).

Whenever a chemical reaction proceeds so that there is an increase in the number of molecules—or when a solid substance, such as glucose, is converted into liquid or gaseous products, which have more freedom to move or fill space than a solid—there is an increase in molecular disorder and thus an increase in entropy.

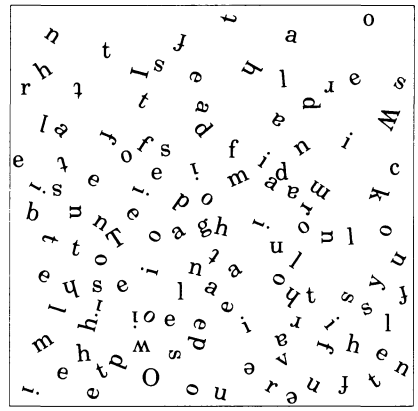
Case 3: Information and Entropy

The following short passage from *Julius Caesar,* Act IV, Scene 3, is spoken by Brutus, when he realizes that he must face Mark Antony's army. It is an information-rich nonrandom arrangement of 125 letters of the English alphabet:

> There is a tide in the affairs of men,
> Which, taken at the flood, leads on to fortune;
> Omitted, all the voyage of their life
> Is bound in shallows and in miseries.

In addition to what this quotation says overtly, it has many hidden meanings. It not only reflects a complex sequence of events in the play, it also echoes the play's ideas on conflict, ambition, and the demands of leadership. Permeated with Shakespeare's understanding of human nature, it is very rich in information.

However, if the 125 letters making up this quotation were allowed to fall into a completely random, chaotic pattern, as shown in the following box, they would have no meaning whatsoever.



In this form the 125 letters would contain little or no information, but would be very rich in entropy. Such considerations have led to the conclusion that information is a form of energy; information has been called "negative entropy." In fact, the branch of mathematics called information theory, which is basic to the programming logic of computers, is closely related to thermodynamic theory. Living organisms are highly ordered, nonrandom structures, immensely rich in information and thus entropy-poor.

ΔH is the change in enthalpy of the system, T is the absolute temperature, and ΔS is the change in entropy of the reacting system. By convention ΔS has a positive sign when entropy increases and ΔH has a negative sign when heat is released by the system to its surroundings. Either of these conditions, which are typical of favorable processes, will tend to make ΔG negative. In fact, ΔG of a spontaneously reacting system is always negative.

The second law of thermodynamics states that the entropy *of the universe* increases during all chemical and physical processes, but it does not require that the entropy increase take place *in the reacting system* itself. The order produced within cells as they grow and divide is more than compensated for by the disorder they create in their surroundings in the course of growth and division (Box 13–1, case 2). In short, living organisms preserve their internal order by taking from the surroundings free energy in the form of nutrients or sunlight, and returning to their surroundings an equal amount of energy as heat and entropy.

Cells Require Sources of Free Energy

Cells are isothermal systems—they function at essentially constant temperature (and at constant pressure). Heat flow is not a source of energy for cells because heat can do work only as it passes from a zone or object at one temperature to a zone or object at a lower temperature. The energy that cells can and must use is free energy, described by the Gibbs free-energy function G, which allows prediction of the direction of chemical reactions, their exact equilibrium position, and the amount of work they can in theory perform at constant temperature and pressure. Heterotrophic cells acquire free energy from nutrient molecules, and photosynthetic cells acquire it from absorbed solar radiation. Both kinds of cells transform this free energy into ATP and other energy-rich compounds, capable of providing energy for biological work at constant temperature.

Standard Free-Energy Change Is Directly Related to the Equilibrium Constant

The composition of a reacting system (a mixture of chemical reactants and products) will tend to continue changing until equilibrium is reached. At the equilibrium concentration of reactants and products, the rates of the forward and reverse reactions are exactly equal and no further net change occurs in the system. The concentrations of reactants and products *at equilibrium* define the equilibrium constant (p. 90). In the general reaction $aA + bB \rightleftharpoons cC + dD$, where a, b, c, and d are the number of molecules of A, B, C, and D participating, the equilibrium constant is given by

$$K_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b} \qquad (13\text{–}2)$$

where [A], [B], [C], and [D] are the molar concentrations of the reaction components at the point of equilibrium.

When a reacting system is not at equilibrium, the tendency to move toward equilibrium represents a driving force, the magnitude of which can be expressed as the free-energy change for the reaction, ΔG. Under standard conditions (298 K (25 °C)), when reactants and products are initially present at 1 M concentrations or, for gases, at partial pressures of 101.3 kPa (1 atm), the force driving the system toward equilibrium is defined as the standard free-energy change, ΔG°. By this definition, the standard state for reactions that involve hydrogen ions is $[H^+] = 1$ M, or pH is 0. Most biochemical reactions occur in well-buffered aqueous solutions near pH 7; both the pH and the concentration of water (55.5 M) are essentially constant. For convenience of calculations, biochemists therefore define a slightly different standard state, in which the concentration of H^+ is 10^{-7} M (pH is 7) and that of water is 55.5 M. Physical constants based on this biochemical standard state are written with a prime (e.g., $\Delta G^{\circ\prime}$ and K'_{eq}) to distinguish them from the constants used by chemists and physicists. Under this convention, when H_2O or H^+ are reactants or products, their concentrations are not included in equations such as Equation 13–2, but are instead incorporated into the constants $\Delta G^{\circ\prime}$ and K'_{eq}.

Just as K'_{eq} is a physical constant characteristic for each reaction, so too is $\Delta G^{\circ\prime}$ a constant. As we noted in Chapter 8 (p. 204), there is a simple relationship between K'_{eq} and $\Delta G^{\circ\prime}$:

$$\Delta G^{\circ\prime} = -RT \ln K'_{eq}$$

The standard free-energy change of a chemical reaction is simply an alternative mathematical way of expressing its equilibrium constant. Table 13–2 shows the relationship between $\Delta G^{\circ\prime}$ and K'_{eq}. If the equilibrium constant for a given chemical reaction is 1.0, the standard free-energy change of that reaction is 0.0 (the natural logarithm of 1.0 is zero). If K'_{eq} of a reaction is greater than 1.0, its $\Delta G^{\circ\prime}$ is negative. If K'_{eq} is less than 1.0, $\Delta G^{\circ\prime}$ is positive. Because the relationship between $\Delta G^{\circ\prime}$ and K'_{eq} is exponential, relatively small changes in $\Delta G^{\circ\prime}$ correspond to large changes in K'_{eq}.

It may be helpful to think of the standard free-energy change in another way. $\Delta G^{\circ\prime}$ is the difference between the free-energy content of the products and the free-energy content of the reactants under standard conditions. When $\Delta G^{\circ\prime}$ is negative, the products contain less free energy than the reactants. The reaction will therefore proceed spontaneously to form the products under standard conditions, because all chemical reactions tend to go in the direction that results in a decrease in the free energy of the system. A positive value of $\Delta G^{\circ\prime}$ means that the products of the reaction contain more free energy than the reactants. The reaction will therefore tend to go in the reverse direction if we start with 1.0 M concentrations of all components. Table 13–3 summarizes these points.

Table 13–2 Relationship between the equilibrium constants of chemical reactions and their standard free-energy changes

K'_{eq}	$\Delta G^{\circ\prime}$ (kJ/mol)
0.001	17.1
0.01	11.4
0.1	5.7
1.0	0.0
10.0	−5.7
100.0	−11.4
1,000.0	−17.1

Table 13–3 Relationships among K'_{eq}, $\Delta G^{\circ\prime}$, and the direction of chemical reactions under standard conditions

When K'_{eq} is	$\Delta G^{\circ\prime}$ is	Starting with 1 M components the reaction
>1.0	Negative	Proceeds forward
1.0	Zero	Is at equilibrium
<1.0	Positive	Proceeds in reverse

As an example, let us make a simple calculation of the standard free-energy change of the reaction catalyzed by the enzyme phosphoglucomutase:

$$\text{Glucose-1-phosphate} \rightleftharpoons \text{glucose-6-phosphate}$$

Chemical analysis shows that whether we start with, say, 20 mM glucose-1-phosphate (but no glucose-6-phosphate) in the presence of phosphoglucomutase, or with 20 mM glucose-6-phosphate, the final equilibrium mixture in either case will contain 1 mM glucose-1-phosphate and 19 mM glucose-6-phosphate at 25 °C and pH 7.0. (Remember that enzymes do not affect the point of equilibrium of a reaction; they merely hasten its attainment.) From these data we can calculate the equilibrium constant:

$$K'_{eq} = \frac{[\text{glucose-6-phosphate}]}{[\text{glucose-1-phosphate}]} = \frac{19\ \text{mM}}{1\ \text{mM}} = 19$$

From this value of K'_{eq} we can calculate the standard free-energy change:

$$\begin{aligned}\Delta G^{\circ\prime} &= -RT \ln K'_{eq} \\ &= -(8.315\ \text{J/mol} \cdot \text{K})(298\ \text{K})(\ln 19) \\ &= -7{,}296\ \text{J/mol} = -7.3\ \text{kJ/mol}\end{aligned}$$

Because the standard free-energy change is negative, when the reaction starts with 1.0 M glucose-1-phosphate and 1.0 M glucose-6-phosphate, the conversion of glucose-1-phosphate into glucose-6-phosphate

proceeds with a loss (release) of free energy. For the reverse reaction (the conversion of glucose-6-phosphate to glucose-1-phosphate), $\Delta G^{\circ\prime}$ has the same magnitude but the opposite sign.

Table 13–4 gives the standard free-energy changes for several representative chemical reactions. Note that hydrolysis of simple esters, amides, peptides, and glycosides, as well as rearrangements and eliminations, proceed with relatively small standard free-energy changes, whereas hydrolysis of acid anhydrides occurs with relatively large decreases in standard free energy. The oxidation of organic compounds to CO_2 and H_2O proceeds with especially large decreases in standard free energy. However, standard free-energy changes such as those in Table 13–4 tell how much free energy is available from a reaction under *standard conditions*. To describe the energy released under the conditions that exist within cells, an expression for the *actual* free-energy change is essential.

Table 13–4 Standard free-energy changes of some chemical reactions at pH 7.0 and 25 °C (298 K)

	$\Delta G^{\circ\prime}$	
Reaction type	(kJ/mol)	(kcal/mol)*
Hydrolysis reactions		
Acid anhydrides		
Acetic anhydride + H_2O ⟶ 2 acetate	−91.1	−21.8
ATP + H_2O ⟶ ADP + P_i	−30.5	−7.3
Esters		
Ethyl acetate + H_2O ⟶ ethanol + acetate	−19.6	−4.7
Glucose-6-phosphate + H_2O ⟶ glucose + P_i	−13.8	−3.3
Amides and peptides		
Glutamine + H_2O ⟶ glutamate + NH_4^+	−14.2	−3.4
Glycylglycine + H_2O ⟶ 2 glycine	−9.2	−2.2
Glycosides		
Maltose + H_2O ⟶ 2 glucose	−15.5	−3.7
Lactose + H_2O ⟶ glucose + galactose	−15.9	−3.8
Rearrangements		
Glucose-1-phosphate ⟶ glucose-6-phosphate	−7.3	−1.74
Fructose-6-phosphate ⟶ glucose-6-phosphate	−1.7	−0.40
Elimination of water		
Malate ⟶ fumarate + H_2O	3.1	0.75
Oxidations with molecular oxygen		
Glucose + $6O_2$ ⟶ $6CO_2$ + $6H_2O$	−2,840	−686
Palmitic acid + $23O_2$ ⟶ $16CO_2$ + $16H_2O$	−9,770	−2,338

* Although joules and kilojoules are the standard units of energy and are used throughout this text, biochemists sometimes express $\Delta G^{\circ\prime}$ values in kilocalories per mole. We have therefore included values in both kilojoules and kilocalories in this table and in Table 13–5. To convert kilojoules to kilocalories, divide the number of kilojoules by 4.184.

The Actual Free-Energy Change Depends on the Reactant and Product Concentrations

We must be careful to distinguish between two different quantities, the free-energy change, ΔG, and the standard free-energy change, $\Delta G^{\circ\prime}$. Each chemical reaction has a characteristic standard free-energy

change, which may be positive, negative, or zero, depending on the equilibrium constant of the reaction. The standard free-energy change tells us in which direction and how far a given reaction will go to reach equilibrium *when the initial concentration of each component is 1.0 M, the pH is 7.0, and the temperature is 25 °C.* Thus $\Delta G^{\circ\prime}$ is a constant: it has a characteristic, unchanging value for a given reaction. But the *actual* free-energy change, ΔG, of a given chemical reaction is a function of the concentrations and of the temperature actually prevailing during the reaction, which are not necessarily the standard conditions as defined above. Moreover, the ΔG of any reaction proceeding spontaneously toward its equilibrium is always negative, becomes less negative as the reaction proceeds, and is zero at the point of equilibrium, indicating that no more work can be done by the reaction.

ΔG and $\Delta G^{\circ\prime}$ for any reaction A + B $\rightleftharpoons$ C + D are related by the equation

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[C][D]}{[A][B]} \qquad (13\text{–}3)$$

in which the terms in red are those *actually prevailing* in the system under observation. The concentration terms in this equation express the effects commonly called mass action. As an example, let us suppose that the reaction A + B $\rightleftharpoons$ C + D is taking place at the standard conditions of temperature (25 °C) and pressure (101.3 kPa) but that the concentrations of A, B, C, and D are *not* equal and that none of them is present at the standard concentration of 1.0 M. To determine the actual free-energy change, ΔG, that will occur under these nonstandard conditions of concentration as the reaction proceeds from left to right, we simply put in the *actual* concentrations of A, B, C, and D; the values of R, T, and $\Delta G^{\circ\prime}$ are the standard values. ΔG will be negative and will approach zero as the reaction proceeds because the actual concentrations of A and B will be getting smaller and the concentrations of C and D will be getting larger. Notice that when a reaction is at equilibrium, where there is no force driving the reaction in either direction and ΔG is equal to zero, Equation 13–3 reduces to

$$0 = \Delta G^{\circ\prime} + RT \ln \frac{[C]_{eq}[D]_{eq}}{[A]_{eq}[B]_{eq}}$$

or

$$\Delta G^{\circ\prime} = -RT \ln K'_{eq}$$

the equation that, as we noted above (p. 368), relates the standard free-energy change and the equilibrium constant.

Even a reaction for which $\Delta G^{\circ\prime}$ is positive can go in the forward direction, *if* ΔG *is negative.* This is possible if the term RT ln ([products]/[reactants]) in Equation 13–3 is negative and has a larger absolute value than $\Delta G^{\circ\prime}$. For example, the immediate removal of the products of a reaction can keep the ratio [products]/[reactants] well below 1, giving the term RT ln ([products]/[reactants]) a large, negative value.

$\Delta G^{\circ\prime}$ and ΔG are expressions of the *maximum* amount of free energy that a given reaction can *theoretically* deliver. This amount of energy could be realized only if there were a perfectly efficient device available to trap or harness it. Given that no such device is available, the amount of work done by the reaction at constant temperature and pressure is always less than the theoretical amount.

It is also essential to understand that some reactions that are ther-

modynamically favorable (i.e., for which ΔG is large and negative) nevertheless do not occur at measurable rates. For example, firewood can be converted into CO_2 and H_2O by combustion in a reaction that is very favorable thermodynamically. Nevertheless, firewood is stable for years, because the activation energy (see Fig. 8–4) for its combustion is higher than that provided by room temperature. If the necessary activation energy is provided (with a lighted match, for example), combustion will begin, converting the wood to the more stable products CO_2 and H_2O and releasing energy as heat and light.

In living cells, reactions that would be extremely slow if uncatalyzed are caused to occur, not by supplying additional heat but by lowering the activation energy with an enzyme (see Fig. 8–4). *The free-energy change* ΔG *for a reaction is independent of the pathway by which the reaction occurs;* it depends only on the nature and concentration of the initial reactants and the final products. An enzyme provides an alternative reaction pathway with a lower activation energy, so that at room temperature a large fraction of the substrate molecules have enough thermal energy to overcome the activation barrier, and the reaction rate increases dramatically. Enzymes cannot change equilibrium constants; but they can and do increase the rate at which a reaction proceeds in the direction dictated by thermodynamics.

Standard Free-Energy Changes Are Additive

In the case of two sequential chemical reactions, A $\rightleftharpoons$ B and B $\rightleftharpoons$ C, each reaction has its own equilibrium constant and each has its characteristic standard free-energy change, $\Delta G_1^{\circ\prime}$ and $\Delta G_2^{\circ\prime}$. As the two reactions are sequential, B cancels out and the overall reaction is A $\rightleftharpoons$ C. Reaction A $\rightleftharpoons$ C will also have its own equilibrium constant and thus will also have its own standard free-energy change, $\Delta G_{\text{total}}^{\circ\prime}$. *The* $\Delta G^{\circ\prime}$ *values of sequential chemical reactions are additive.* For the overall reaction A $\rightleftharpoons$ C, $\Delta G_{\text{total}}^{\circ\prime}$ is the algebraic sum of the individual standard free-energy changes, $\Delta G_1^{\circ\prime}$ and $\Delta G_2^{\circ\prime}$, of the two separate reactions: $\Delta G_{\text{total}}^{\circ\prime} = \Delta G_1^{\circ\prime} + \Delta G_2^{\circ\prime}$. This principle of bioenergetics explains how a thermodynamically unfavorable (endergonic) reaction can be driven in the forward direction by coupling it to a highly exergonic reaction through a common intermediate. For example, the synthesis of glucose-6-phosphate is the first step in the utilization of glucose by many organisms:

$$\text{Glucose} + P_i \longrightarrow \text{glucose-6-phosphate} + H_2O \qquad \Delta G^{\circ\prime} = 13.8 \text{ kJ/mol}$$

The positive value of $\Delta G^{\circ\prime}$ predicts that under standard conditions the reaction will tend not to proceed spontaneously in the direction written. Another cellular reaction, the hydrolysis of ATP to ADP and P_i, is very exergonic:

$$\text{ATP} + H_2O \longrightarrow \text{ADP} + P_i \qquad \Delta G^{\circ\prime} = -30.5 \text{ kJ/mol}$$

These two reactions share the common intermediates P_i and H_2O and may be expressed as sequential reactions:

$$\begin{array}{ll} (1) & \text{Glucose} + P_i \longrightarrow \text{glucose-6-phosphate} + H_2O \\ (2) & \text{ATP} + H_2O \longrightarrow \text{ADP} + P_i \\ \hline \textit{Sum:} & \text{ATP} + \text{glucose} \longrightarrow \text{ADP} + \text{glucose-6-phosphate} \end{array}$$

The overall standard free-energy change is obtained by adding the $\Delta G^{\circ\prime}$ values for individual reactions:

$$\Delta G^{\circ\prime} = +13.8 \text{ kJ/mol} + (-30.5 \text{ kJ/mol}) = -16.7 \text{ kJ/mol}$$

The overall reaction is exergonic. In this case, energy stored in the bonds of ATP is used to drive the synthesis of glucose-6-phosphate, a product whose formation from glucose and phosphate is endergonic. The *pathway* of glucose-6-phosphate formation by phosphate transfer from ATP is different from reactions (1) and (2) above, but the net result is the same as the sum of the two reactions. In thermodynamic calculations, all that matters is the initial and final states; the route between them is immaterial.

We have said that $\Delta G^{\circ\prime}$ is a way of expressing the equilibrium constant for a reaction. For reaction (1) above,

$$K'_{eq_1} = \frac{[\text{glucose-6-phosphate}]}{[\text{glucose}][P_i]} = 3.9 \times 10^{-3}\ \text{M}^{-1}$$

Notice that H_2O is not included in this expression. The equilibrium constant for the hydrolysis of ATP is

$$K'_{eq_2} = \frac{[\text{ADP}][P_i]}{[\text{ATP}]} = 2 \times 10^{5}\ \text{M}$$

The equilibrium constant for the two coupled reactions is

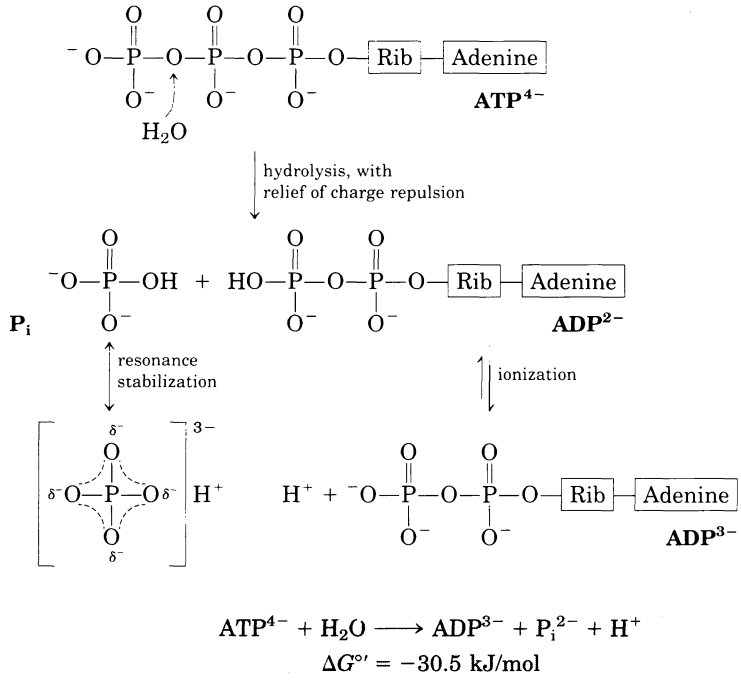
$$\begin{aligned} K'_{eq_3} &= \frac{[\text{glucose-6-phosphate}][\text{ADP}][P_i]}{[\text{glucose}][P_i][\text{ATP}]} \\ &= (K'_{eq_1})(K'_{eq_2}) = (3.9 \times 10^{-3}\ \text{M}^{-1})(2.0 \times 10^{5}\ \text{M}) \\ &= 7.8 \times 10^{2} \end{aligned}$$

By coupling ATP hydrolysis to glucose-6-phosphate synthesis, the K_{eq} for formation of glucose-6-phosphate has been raised by a factor of about 2×10^5.

This strategy is employed by all living cells in the synthesis of metabolic intermediates and cellular components. Obviously, the strategy only works if compounds such as ATP are continuously available. In the following chapters we consider several of the most important cellular pathways for producing ATP.

Phosphate Group Transfers and ATP

Having developed some fundamental principles of energy changes in chemical systems, we can now examine the energy cycle in cells and the special role of ATP in linking catabolism and anabolism (see Fig. 1–13). Heterotrophic cells obtain free energy in a chemical form by the catabolism of nutrient molecules and use that energy to make ATP from ADP and P_i. ATP then donates some of its chemical energy to endergonic processes such as the synthesis of metabolic intermediates and macromolecules from smaller precursors, transport of substances across membranes against concentration gradients, and mechanical motion. This donation of energy from ATP generally involves the covalent participation of ATP in the reaction that is to be driven, with the result that ATP is converted to ADP and P_i or to AMP and $2P_i$. We discuss here the chemical basis for the large free-energy changes that accompany hydrolysis of ATP and other high-energy phosphate compounds, and show that most cases of energy donation by ATP involve group transfer, not simple hydrolysis of ATP. To illustrate the range of energy transductions in which ATP provides energy, we consider the synthesis of information-rich macromolecules, the transport of solutes across membranes, and motion produced by muscle contraction.



$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$
$$\Delta G^{\circ\prime} = -30.5 \text{ kJ/mol}$$

Figure 13–1 The chemical basis for the large free-energy change associated with ATP hydrolysis. (1) Electrostatic repulsion among the four negative charges on ATP is relieved by charge separation after hydrolysis. (2) Inorganic phosphate (P_i) released by hydrolysis is stabilized by formation of a resonance hybrid (left), in which each of the four P—O bonds has the same degree of double-bond character and the hydrogen ion is not permanently associated with any one of the oxygens. (3) The other direct product of hydrolysis, ADP^{2-}, also immediately ionizes (right), releasing a proton into a medium of very low $[H^+]$ (pH 7). A fourth factor (not shown) that favors ATP hydrolysis is the greater degree of solvation (hydration) of the products P_i and ADP relative to ATP, which further stabilizes the products relative to the reactants.

The Free-Energy Change for ATP Hydrolysis Is Large and Negative

Figure 13–1 summarizes the chemical basis for the relatively large, negative, standard free energy of hydrolysis of ATP. The hydrolytic cleavage of the terminal phosphoric acid anhydride (phosphoanhydride) bond in ATP separates off one of the three negatively charged phosphates and thus relieves some of the electrostatic repulsion in ATP; the P_i (HPO_4^{2-}) released by hydrolysis is stabilized by the formation of several resonance forms not possible in ATP; and ADP^{2-}, the other direct product of hydrolysis, immediately ionizes, releasing H^+ into a medium of very low $[H^+]$ ($\sim 10^{-7}$ M). The low concentration of the direct products favors, by mass action, the hydrolysis reaction.

Although its hydrolysis is highly exergonic ($\Delta G^{\circ\prime} = -30.5$ kJ/mol), ATP is kinetically stable toward nonenzymatic breakdown at pH 7 because the activation energy for ATP hydrolysis is relatively high. Rapid cleavage of the phosphoric acid anhydride bonds occurs only when catalyzed by an enzyme.

Although the $\Delta G^{\circ\prime}$ for ATP hydrolysis is -30.5 kJ/mol under standard conditions, the *actual* free energy of hydrolysis (ΔG) of ATP in living cells is very different. This is because the concentrations of ATP, ADP, and P_i in living cells are not identical and are much lower than the standard 1.0 M concentrations (Table 13–5). Furthermore, the cytosol contains Mg^{2+}, which binds to ATP and ADP (Fig. 13–2). In most enzymatic reactions that involve ATP as phosphoryl donor, the true substrate is $MgATP^{2-}$ and the relevant $\Delta G^{\circ\prime}$ is that for $MgATP^{2-}$ hydrolysis. Box 13–2 shows how ΔG for ATP hydrolysis in the intact erythrocyte can be calculated from the data in Table 13–4. ΔG for ATP hydrolysis in intact cells, usually designated ΔG_p, is much more negative than $\Delta G^{\circ\prime}$; in most cells ΔG_p ranges from -50 to -65 kJ/mol. ΔG_p is often called the **phosphorylation potential.** In the following discussion we use the standard free-energy change for ATP hydrolysis, because this allows convenient comparison with the energetics of other cellular reactions for which the actual free-energy changes within cells are not known with certainty.

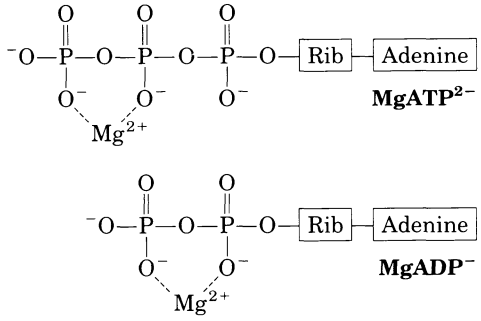


Figure 13–2 Formation of Mg^{2+} complexes partially shields the negative charges and influences the conformation of the phosphate groups in nucleotides such as ATP and ADP.

BOX 13–2 The Free Energy of Hydrolysis of ATP within Cells: The Real Cost of Doing Metabolic Business

The standard free energy of hydrolysis of ATP has the value −30.5 kJ/mol. In the cell, however, the concentrations of ATP, ADP, and P_i are not only unequal but are also much lower than the standard 1 M concentrations (see Table 13–5). Moreover, the pH inside cells may differ somewhat from the standard pH of 7.0. Thus the *actual* free energy of hydrolysis of ATP under intracellular conditions (ΔG_p) differs from the standard free-energy change, $\Delta G^{\circ\prime}$. We can easily calculate ΔG_p. For example, in human erythrocytes the concentrations of ATP, ADP, and P_i are 2.25, 0.25, and 1.65 mM, respectively (Table 13–5). Let us assume for simplicity that the pH is 7.0 and the temperature is 25 °C, the standard pH and temperature. The actual free energy of hydrolysis of ATP in the erythrocyte under these conditions is given by the relationship

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[\text{ADP}][P_i]}{[\text{ATP}]}$$

Substituting the appropriate values we obtain

$$\Delta G = -30{,}500 \text{ J/mol} + (8.315 \text{ J/mol} \cdot \text{K})(298 \text{ K}) \ln \frac{(2.50 \times 10^{-4})(1.65 \times 10^{-3})}{2.25 \times 10^{-3}}$$

$$= -30{,}500 \text{ J/mol} + (2{,}480 \text{ J/mol}) \ln (1.83 \times 10^{-4})$$

$$= -30{,}500 \text{ J/mol} - 21{,}300 \text{ J/mol} = -51{,}800 \text{ J/mol}$$

$$= -51.8 \text{ kJ/mol}$$

Thus ΔG_p, the actual free-energy change for ATP hydrolysis in the intact erythrocyte (−51.8 kJ/mol), is much larger than the standard free-energy change (−30.5 kJ/mol). By the same token, the free energy required to *synthesize* ATP from ADP and P_i under the conditions prevailing in the erythrocyte would be 51.8 kJ/mol.

Because the concentrations of ATP, ADP, and P_i may differ from one cell type to another (Table 13–5), ΔG_p for ATP hydrolysis likewise differs. Moreover, in any given cell ΔG_p can vary from time to time, depending on the metabolic conditions in the cell and how they influence the concentrations of ATP, ADP, P_i, and H^+ (pH). We can calculate the actual free-energy change for any given metabolic reaction as it occurs in the cell, providing we know the concentrations of all the reactants and products of the reaction and other factors (such as pH, temperature, and the concentration of Mg^{2+}) that may affect the equilibrium constant and thus the free-energy change.

Table 13–5 Adenine nucleotide, inorganic phosphate, and phosphocreatine concentrations in some cells*

	Concentration (mM)				
	ATP	ADP	AMP	P_i	PCr
Rat hepatocyte	3.38	1.32	0.29	4.8	0
Rat myocyte	8.05	0.93	0.04	8.05	28
Human erythrocyte	2.25	0.25	0.02	1.65	0
Rat neuron	2.59	0.73	0.06	2.72	4.7
E. coli cell	7.90	1.04	0.82	7.9	0

* For erythrocytes the concentrations are those of the cytosol (human erythrocytes lack a nucleus and mitochondria). In the other types of cells the data are for the entire cell contents, although the cytosol and the mitochondria have very different concentrations of ADP. Phosphocreatine (PCr) is discussed later in this chapter.

To summarize, compounds with large, negative, standard free energies of hydrolysis give products that are more stable than the reactants because of one or more of the following: (1) the bond strain in reactants due to electrostatic repulsion is relieved by charge separation, as in the case of ATP (described earlier), (2) the products are stabilized by ionization, as in the case of ATP, acyl phosphates, and thioesters, (3) the products are stabilized by isomerization (tautomerization), as for phosphoenolpyruvate, and/or (4) the products are stabilized by resonance, as for creatine from phosphocreatine, the carboxylate ion from acyl phosphates and thioesters, and phosphate (P_i) from all of the phosphorylated compounds.

ATP Provides Energy by Group Transfers, Not by Simple Hydrolysis

Throughout this book we will refer to reactions or processes for which ATP supplies energy, and the contribution of ATP to these reactions will commonly be indicated as in Figure 13–8a, with a single arrow showing the conversion of ATP into ADP and P_i, or of ATP into AMP and PP_i (pyrophosphate). When written this way, these reactions of ATP appear to be simple hydrolysis reactions in which water displaces either P_i or PP_i, and one is tempted to say that an ATP-dependent reaction is "driven by the hydrolysis of ATP." This is *not* the case. ATP hydrolysis per se usually accomplishes nothing but the liberation of heat, which cannot drive a chemical process in an isothermal system.

Single reaction arrows such as those in Figure 13–8a almost invariably represent two-step processes (Fig. 13–8b) in which part of the ATP molecule, either a phosphoryl group or the adenylate moiety (AMP), is first transferred to a substrate molecule or to an amino acid residue in an enzyme, becoming covalently attached to and raising the free-energy content of the substrate or enzyme. In the second step, the phosphate-containing moiety transferred in the first step is displaced,

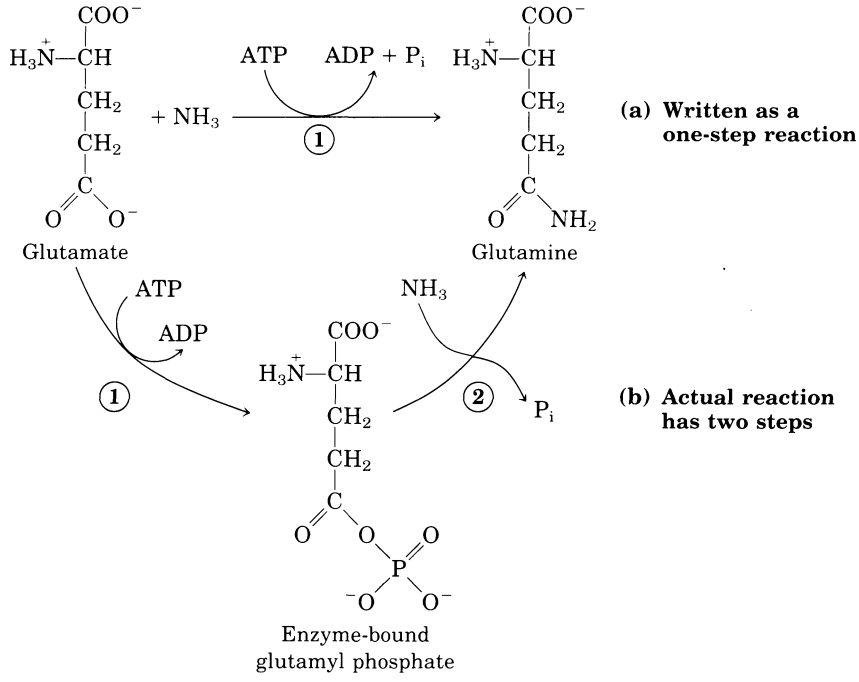


Figure 13–8 The contribution of ATP to a reaction is often shown with a single arrow **(a),** but is almost always a two-step process, such as that shown here for the reaction catalyzed by ATP-dependent glutamine synthetase **(b).**

generating either P_i or AMP. Thus ATP participates in the enzyme-catalyzed reaction to which it contributes free energy. There is one important class of exceptions to this generalization: those processes in which noncovalent binding of ATP (or of GTP), followed by its hydrolysis to ADP and P_i, provides the energy to cycle a protein between two conformations, producing mechanical motion, as in muscle contraction or in the movement of enzymes along DNA (discussed below).

The phosphate compounds found in living organisms can be divided arbitrarily into two groups, based on their standard free energies of hydrolysis (Fig. 13–9). "High-energy" compounds have a $\Delta G^{\circ\prime}$ of hydrolysis more negative than −25 kJ/mol; "low-energy" compounds have a less negative $\Delta G^{\circ\prime}$. ATP, for which $\Delta G^{\circ\prime}$ of hydrolysis is −30.5 kJ/mol (−7.3 kcal/mol), is a high-energy compound; glucose-6-phosphate, with a standard free energy of hydrolysis of −13.8 kJ/mol (−3.3 kcal/mol), is a low-energy compound.

The term "high-energy phosphate bond," although long used by biochemists, is incorrect and misleading, as it wrongly suggests that the bond itself contains the energy. In fact, the breaking of chemical bonds requires an *input* of energy. The free energy released by hydrolysis of phosphate compounds thus does not come from the specific bond that is broken but results from the products of the reaction having a smaller free-energy content than the reactants. For simplicity, we will sometimes use the term "high-energy phosphate compound" when referring to ATP or other phosphate compounds with a large, negative, standard free energy of hydrolysis.

From the additivity of free-energy changes of sequential reactions, one can see that the synthesis of any phosphorylated compound can be accomplished by coupling it to the breakdown of another phosphorylated compound with a more negative free energy of hydrolysis (Fig. 13–9). One can therefore describe phosphorylated compounds as having a high or low **phosphate group transfer potential.** The phosphate group transfer potential of phosphoenolpyruvate is very high, that of ATP is high, and that of glucose-6-phosphate is low.

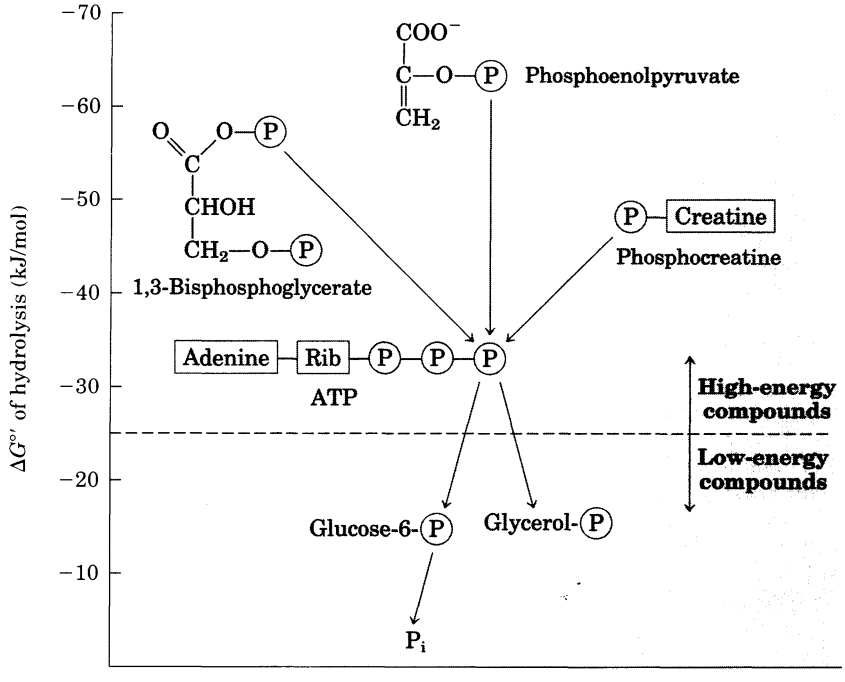


Figure 13–9 Flow of phosphate groups, represented by Ⓟ, from high-energy phosphate donors via ATP to acceptor molecules (such as glucose and glycerol) to form their low-energy phosphate derivatives. This flow of phosphate groups, which is catalyzed by enzymes called kinases, proceeds with an overall loss of free energy under intracellular conditions. Hydrolysis of low-energy phosphate compounds releases P_i, which has an even lower group transfer potential.

Much of catabolism is directed toward the synthesis of high-energy phosphate compounds, but their formation is not an end in itself; it is the means of activating a very wide variety of compounds for further chemical transformation. The transfer of a phosphoryl group to a compound effectively puts free energy into that compound, so that it has more free energy to give up during subsequent metabolic transformations. We described above how the synthesis of glucose-6-phosphate is accomplished by phosphoryl group transfer from ATP. We shall see in the next chapter that this phosphorylation of glucose activates or "primes" the glucose for catabolic reactions that occur in nearly every living cell.

In some reactions that involve ATP, both of its terminal phosphate groups are released in one piece as PP_i. Simultaneously, the remainder of the ATP molecule (adenylate) is joined to another compound, which is thereby activated. For example, the first step in the activation of a fatty acid either for energy-yielding oxidation (Chapter 16) or for use in the synthesis of more complex lipids (Chapter 20) is its attachment to the carrier coenzyme A (Fig. 13–10). The direct condensation of a fatty acid with coenzyme A is endergonic, but the formation of fatty acyl–CoA is made exergonic by coupling it to the net breakdown, in two steps, of ATP.

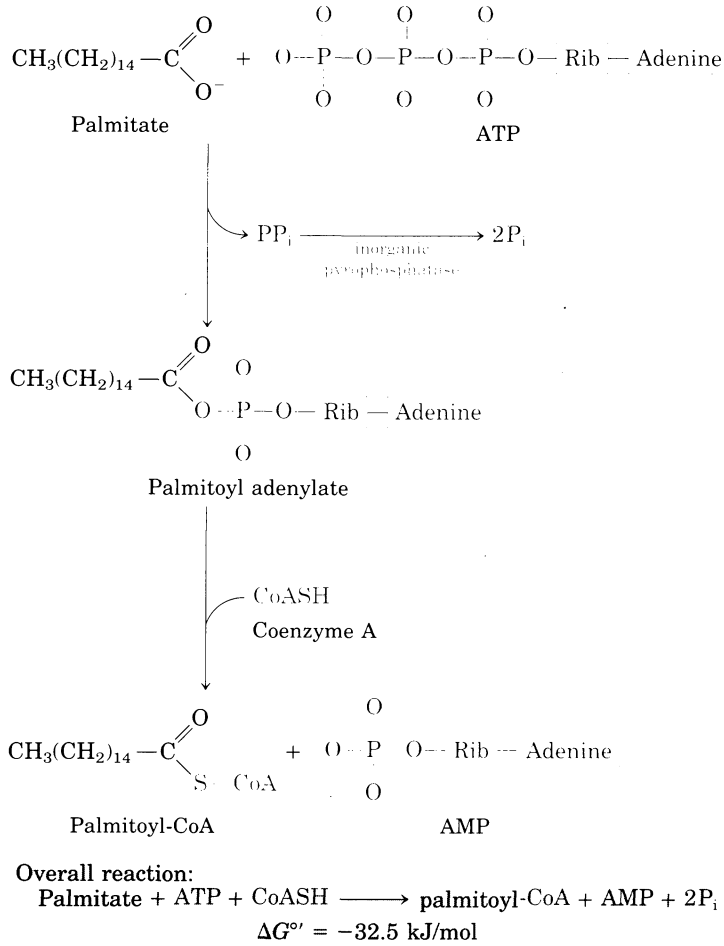


Figure 13–10 Both phosphoric acid anhydride bonds in ATP are eventually broken in the formation of palmitoyl-coenzyme A. In the first step of the reaction, ATP donates adenylate (AMP), forming the fatty acyl adenylate and releasing PP_i. The pyrophosphate is subsequently hydrolyzed by inorganic pyrophosphatase. The "energized" fatty acyl group is then transferred to coenzyme A.

In the first step, adenylate (AMP) is transferred from ATP to the carboxyl group of the fatty acid, forming a mixed anhydride (fatty acyl adenylate) and liberating PP_i. In the second step, the thiol group of coenzyme A displaces the adenylate group and forms a thioester with the fatty acid. The sum of these two reactions is the exergonic hydrolysis of ATP to AMP and PP_i ($\Delta G^{\circ\prime} = -32.2$ kJ/mol) and the endergonic formation of fatty acyl–CoA ($\Delta G^{\circ\prime} = 31.4$ kJ/mol).

The formation of fatty acyl–CoA is made energetically favorable by a third step, in which the PP_i formed in the first step is hydrolyzed by the ubiquitous enzyme **inorganic pyrophosphatase** to yield $2P_i$:

$$PP_i + H_2O \longrightarrow 2P_i \qquad \Delta G^{\circ\prime} = -33.4 \text{ kJ/mol}$$

Thus, in the activation of a fatty acid, both of the phosphoric acid anhydride bonds of ATP are broken. The resulting $\Delta G^{\circ\prime}$ is the sum of the $\Delta G^{\circ\prime}$ values for the breakage of these bonds:

$$ATP + 2H_2O \longrightarrow AMP + 2P_i \qquad \Delta G^{\circ\prime} = -65.6 \text{ kJ/mol}$$

The activation of amino acids before their polymerization into proteins (Chapter 26) is accomplished by an analogous set of reactions. An aminoacyl adenylate is first formed from the amino acid and ATP, with the elimination of PP_i. The adenylate group is then displaced by a transfer RNA, which is thereby joined to the amino acid. In this case, too, the PP_i formed in the first step is hydrolyzed by inorganic pyrophosphatase. An unusual use of the cleavage of ATP to AMP and PP_i occurs in the firefly, which uses ATP as an energy source to produce light flashes (Box 13–3, p. 382).

The AMP produced in adenylate transfers is returned to the ATP cycle by the action of **adenylate kinase,** which catalyzes the reversible reaction

$$ATP + AMP \underset{}{\overset{Mg^{2+}}{\rightleftharpoons}} ADP + ADP \qquad \Delta G^{\circ\prime} \approx 0$$

The ADP so formed can be phosphorylated to ATP, using reactions described in detail in later chapters.

Assembly of Informational Macromolecules Requires Energy

When simple precursors are assembled into high molecular weight polymers with defined sequences (DNA, RNA, proteins), as described in detail in Part IV, energy is required both for the condensation of monomeric units and for the creation of *ordered* sequences. The precursors for DNA and RNA synthesis are nucleoside triphosphates, and polymerization is accompanied by cleavage of the phosphoric acid anhydride linkage between the α- and β-phosphates, with the release of PP_i (Fig. 13–11). The moieties transferred to the growing polymer in these polymerization reactions are adenylate (AMP), guanylate (GMP), cytidylate (CMP), or uridylate (UMP) for RNA synthesis, and their deoxy analogs for DNA synthesis. We have seen that the activation of amino acids for protein synthesis involves the donation of adenylate groups from ATP, and we shall see later that the formation of peptide bonds on the ribosome is also accompanied by GTP hydrolysis (Chapter 26). In all of these cases, the exergonic breakdown of a nucleoside triphosphate is coupled to the endergonic process of synthesizing a polymer of a specific sequence.

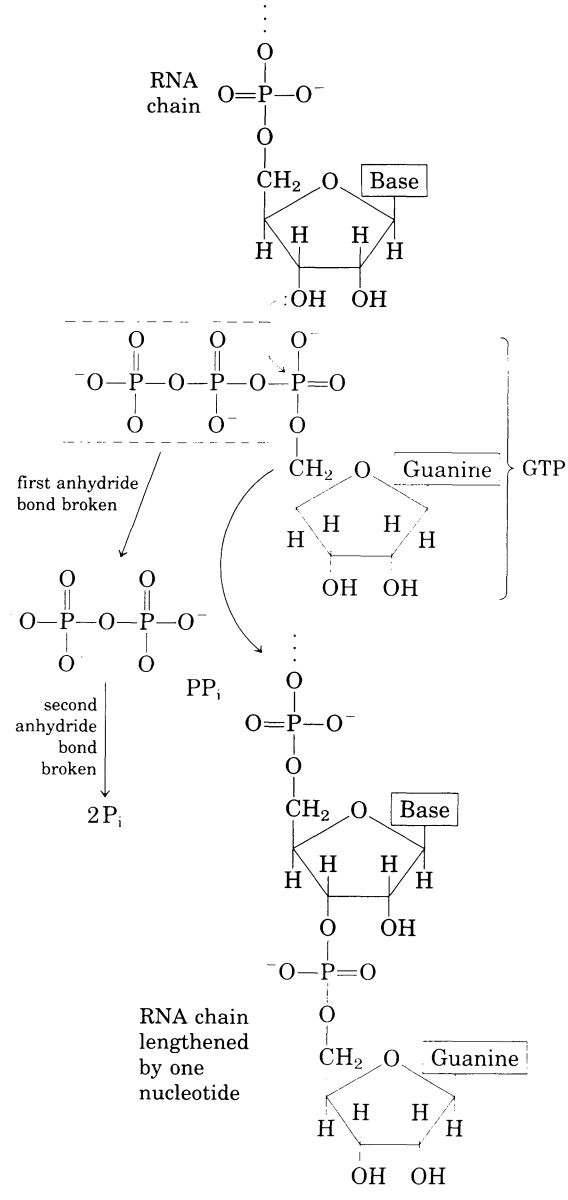


Figure 13–11 Nucleoside triphosphates are the substrates for RNA synthesis. With each nucleoside monophosphate added to the growing chain, one PP_i is released and then hydrolyzed to two P_i. The hydrolysis of two phosphoric acid anhydride bonds for each nucleotide added provides energy for forming the bonds in the RNA polymer and for assembling a specific sequence of nucleotides.

BOX 13–3 Firefly Flashes: Glowing Reports of ATP

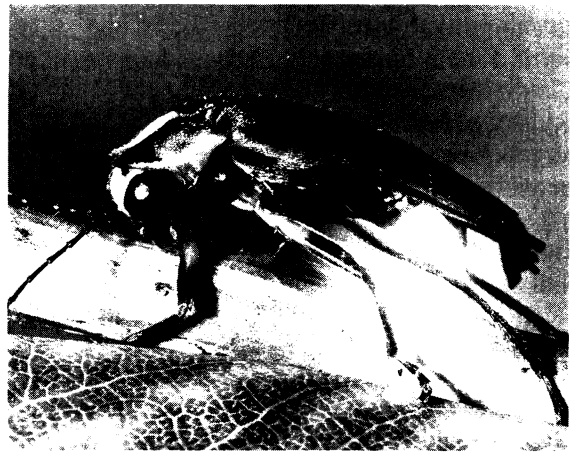
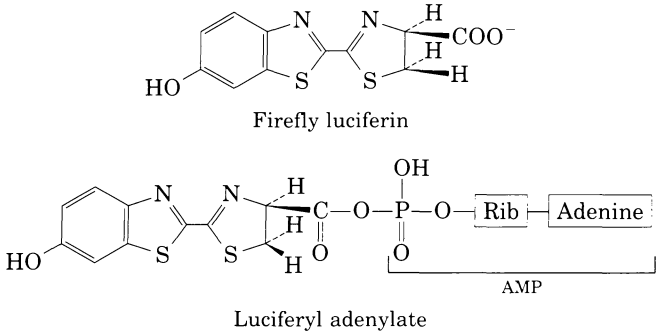


Figure 1 The firefly, a beetle of the Lampyridae family.

Many fungi, marine microorganisms, jellyfish, and crustaceans as well as the firefly (Fig. 1) are capable of generating bioluminescence, which requires considerable amounts of energy. In the firefly, ATP is used in a set of reactions that converts chemical energy into light energy. From many thousands of firefly lanterns collected by children in and around Baltimore, William McElroy and his colleagues at The Johns Hopkins University isolated the principal biochemical components involved, luciferin (Fig. 2), a complex carboxylic acid, and luciferase, an enzyme. The generation of a light flash requires activation of luciferin by an enzymatic reaction with ATP in which a pyrophosphate cleavage of ATP occurs, to form luciferyl adenylate (Fig. 2). This compound is then acted upon by molecular oxygen and luciferase to bring about the oxidative decarboxylation of the luciferin to yield oxyluciferin. This reaction, which has intermediate steps, is accompanied by emission of light (Fig. 2). The color of the light flash differs with firefly species and appears to be determined by differences in the structure of the luciferase. Luciferin is then regenerated from oxyluciferin in a subsequent series of reactions. Other bioluminescent organisms use other types of enzymatic reactions to generate light.

In the laboratory, pure firefly luciferin and luciferase are used to measure minute quantities of ATP by the intensity of the light flash produced. As little as a few picomoles (10^{-12} mol) of ATP can be measured in this way.



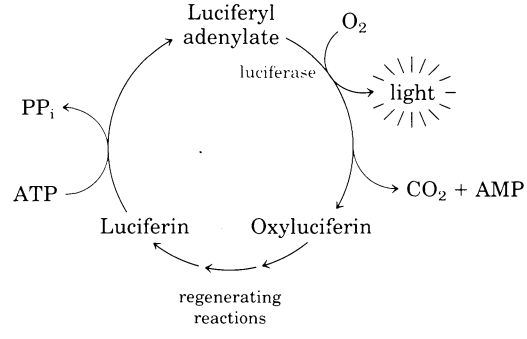


Figure 2 Important components in firefly bioluminescence, and the firefly bioluminescence cycle.

ATP Energizes Active Transport across Membranes

ATP can supply the energy for transporting an ion or a molecule across a membrane into another aqueous compartment where its concentration is higher. Recall from Chapter 10 that the free-energy change (ΔG_t) for the transport of a nonionic solute from one compartment to another is given by

$$\Delta G_t = RT \ln (C_2/C_1) \qquad (13\text{–}4)$$

where C_1 is the molar concentration of the solute in the compartment from which the ion or molecule moves and C_2 is its molar concentration in the compartment into which it moves. When a proton or other charged species moves across a membrane without a counterion, the separation of electrical charge requires extra electrical work beyond the osmotic work against a concentration gradient. The extra electrical work is $Z\mathcal{F}\Delta\psi$, where Z is the (unitless) electrical charge of the transported species, $\Delta\psi$ is the transmembrane electrical potential (in volts), and $\mathcal{F}$ is the Faraday constant (96.48 kJ/V • mol). The total energy cost of moving a charged species against an electrochemical gradient is

$$\Delta G_t = RT \ln (C_2/C_1) + Z\mathcal{F}\Delta\psi \qquad (13\text{–}5)$$

Transport processes are major consumers of energy; in tissues such as human kidney and brain, as much as two-thirds of the energy consumed at rest is used to pump Na^+ and K^+ across plasma membranes via the Na^+K^+ ATPase. Na^+ and K^+ transport is driven by cyclic phosphorylation and dephosphorylation of the transporter protein, with ATP as the phosphate donor (see Fig. 10–23). Na^+-dependent phosphorylation of the Na^+K^+ ATPase forces a change in the protein's conformation, and K^+-dependent dephosphorylation favors return to the original conformation. Each cycle in the transport process results in the conversion of ATP to ADP and P_i, and it is the free-energy change of ATP hydrolysis that drives the pumping of Na^+ and K^+. In animal cells, the net hydrolysis of one ATP is accompanied by the outward transport of three Na^+ ions and the uptake of two K^+ ions.

ATP Is the Energy Source for Muscle Contraction

In the contractile system of skeletal muscle cells, myosin and actin are specialized to transduce the chemical energy of ATP into motion. ATP binds tightly but noncovalently to the head portion of one conformation of myosin, holding the protein in that conformation. When myosin (which is also an ATPase) catalyzes the hydrolysis of its bound ATP, the ADP and P_i produced dissociate from the protein, allowing it to relax into a second conformation until another molecule of ATP binds (Fig. 13–12). The binding and subsequent hydrolysis of ATP thus provide the energy that forces cyclic changes in the conformation of the myosin head. The change in conformation of many individual myosin molecules results in the sliding of myosin fibrils along actin filaments (see Fig. 7–32), which translates into macroscopic contraction of the muscle fiber.

This production of mechanical motion at the expense of ATP is one of the few cases in which ATP hydrolysis per se, and not group transfer from ATP, is the source of the chemical energy in a coupled process. The energy-dependent reactions catalyzed by helicases, RecA protein, and some topoisomerases (Chapter 24) and by certain GTP-binding proteins (Chapter 22) also involve direct hydrolysis of phosphoric acid anhydride bonds.

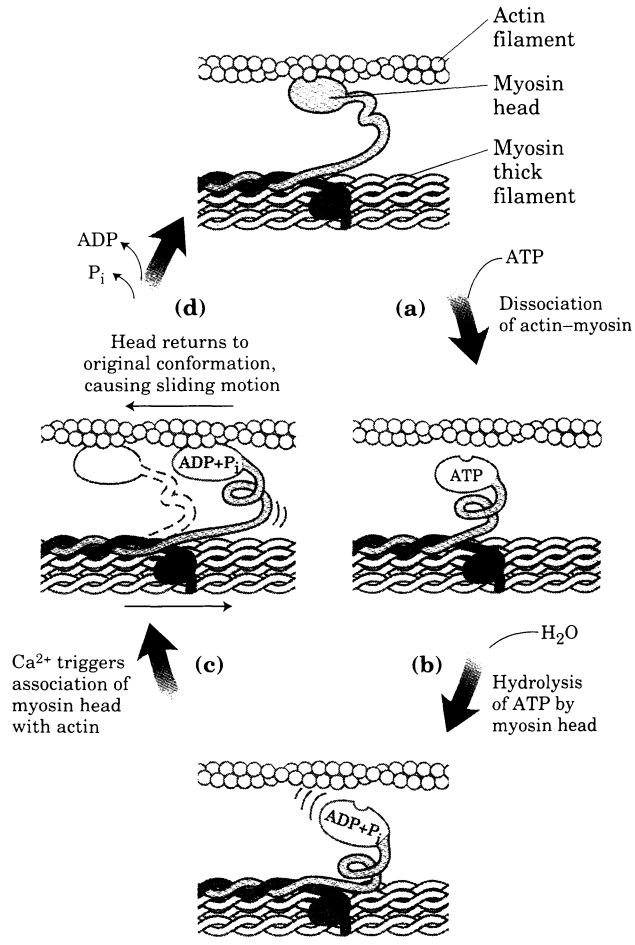


Figure 13–12 ATP hydrolysis drives the cross-bridge cycle during the sliding motion of actin–myosin complexes in muscle. This proposed mechanism begins with each myosin head bound to an actin filament. Binding of ATP to myosin **(a)** causes dissociation of the actin–myosin cross-bridge. ATP hydrolysis **(b)** leaves myosin with bound ADP and P_i, which favors a different conformation of the myosin head. In this conformation, the myosin head binds to an adjacent actin filament **(c)** when elevated cytosolic Ca^{2+} signals contraction. This cross-bridge formation induces the release of bound ADP and P_i **(d),** which provides the free energy for a conformational change in the myosin head; the head tilts, forcing the thin (actin) filament to slide relative to the thick (myosin) filament, producing contraction. ATP then binds to the myosin head to dissociate the cross-bridge and start another cycle. Each cycle occurs in about 1 msec.

free flavin nucleotide. Flavoproteins are often very complex; some have, in addition to a flavin nucleotide, tightly bound inorganic ions (iron or molybdenum, for example) capable of participating in electron transfers.

Summary

Living cells constantly perform work and thus require energy for the maintenance of highly organized structures, for the synthesis of cellular components, for movement, for the generation of electrical currents, for the production of light, and for many other processes. Bioenergetics is the quantitative study of energy relationships and energy conversions in biological systems. Biological energy transformations obey the laws of thermodynamics. All chemical reactions are influenced by two forces: the tendency to achieve the most stable bonding state (for which enthalpy, H, is a useful expression) and the tendency to achieve the highest degree of randomness, expressed as entropy, S. The net driving force in a reaction is ΔG, the free-energy change, which represents the net effect of these two factors: $\Delta G = \Delta H - T\,\Delta S$. Cells require sources of free energy to perform work.

The standard free-energy change, $\Delta G^{\circ\prime}$, is a physical constant characteristic for a given reaction, and can be calculated from the equilibrium constant for the reaction: $\Delta G^{\circ\prime} = -RT \ln K'_{eq}$. The actual free-energy change, ΔG, is a variable, which depends on $\Delta G^{\circ\prime}$ and on the concentrations of reactants and products: $\Delta G = \Delta G^{\circ\prime} + RT \ln$ ([products]/[reactants]). When ΔG is large and negative, the reaction tends to go in the forward direction; when it is large and positive, the reaction tends to go in the reverse direction; and when $\Delta G = 0$, the system is at equilibrium. The free-energy change for a reaction is independent of the pathway by which the reaction occurs. Free-energy changes are also additive; the net chemical reaction that results from the successive occurrence of reactions sharing a common intermediate has an overall free-energy change that is the sum of the ΔG values for the individual reactions.

ATP is the chemical link between catabolism and anabolism. Its exergonic conversion to ADP and P_i, or to AMP and PP_i, is coupled to a large number of endergonic reactions and processes. In general, it is not ATP hydrolysis, but the transfer of phosphate or adenylate from ATP to a substrate or enzyme molecule that couples the energy of ATP breakdown to endergonic transformations of substrates. By these group transfer reactions ATP provides the energy for anabolic reactions, including the synthesis of informational molecules, and for the transport of molecules and ions across membranes against concentration and electrical potential gradients. Muscle contraction is one of several exceptions to this generalization; ATP hydrolysis drives the conformational changes in myosin that produce contraction in muscle.

Cells contain other metabolites with large, negative, free energies of hydrolysis, including phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine. These high-energy compounds, like ATP, have a high phosphate group transfer potential; they are good donors of the phosphate group. Thioesters also have high free energies of hydrolysis.

Biological oxidation–reduction reactions can be described in terms of two half-reactions, each with a characteristic standard reduction potential, E_0'. When two electrochemical half-cells, each containing the components of a half-reaction, are connected, electrons tend to flow to the half-cell with the higher reduction potential. The strength of this tendency is proportional to the difference between the two reduction potentials (ΔE), and is a function of the concentrations of oxidized and reduced species. The standard free-energy change for an oxidation–reduction reaction is directly proportional to the difference in standard reduction potentials of the two half-cells: $\Delta G^{\circ\prime} = -n\mathcal{F}\Delta E'_0$.

Many biological oxidation reactions are dehydrogenations in which one or two hydrogen atoms (electron and proton) are transferred from a substrate to a hydrogen acceptor. Oxidation–reduction reactions in cells involve specialized electron carrier cofactors. NAD and NADP are the freely diffusible cofactors of many dehydrogenases of cells. Both cofactors accept two electrons and one proton. FAD and FMN, the flavin nucleotides, serve as tightly bound prosthetic groups of flavoproteins. They can accept either one or two electrons. In many organisms, a central energy-conserving process is the stepwise oxidation of glucose to CO_2, in which the energy of oxidation is conserved in ATP as electrons are passed to O_2.

Further Reading

Bioenergetics and Thermodynamics

Atkins, P.W. (1984) *The Second Law,* Scientific American Books, Inc., New York.
A well-illustrated and elementary discussion of the second law and its implications.

Blum, H.F. (1968) *Time's Arrow and Evolution,* 3rd edn, Princeton University Press, Princeton, NJ.

Cantor, C.R. & Schimmel, P.R. (1980) *Biophysical Chemistry,* W.H. Freeman and Company, San Francisco.
This and the next two books are outstanding advanced treatments of thermodynamics.

Dickerson, R.E. (1969) *Molecular Thermodynamics,* W.A. Benjamin, Inc., Menlo Park, CA.

Edsall, J.T. & Gutfreund, H. (1983) *Biothermodynamics: The Study of Biochemical Processes at Equilibrium,* John Wiley & Sons, Inc., New York.

Ingraham, L.L. & Pardee, A.B. (1967) Free energy and entropy in metabolism. In *Metabolic Pathways,* 3rd edn, Vol. I (Greenberg, D.M., ed), pp. 1–46, Academic Press, Inc., New York.

Klotz, I.M. (1967) *Energy Changes in Biochemical Reactions,* Academic Press, Inc., New York.
Brief and nonmathematical introduction to thermodynamics for biochemists, with many illustrative examples.

Morowitz, H.J. (1970) *Entropy for Biologists: An Introduction to Thermodynamics,* Academic Press, Inc., New York.
A good introduction to thermodynamics in biology, not limited to a discussion of entropy.

Rothman, T. (1989) *Science à la Mode,* Princeton University Press, Princeton, NJ.
Chapter 4, "The Evolution of Entropy," is an excellent discussion of entropy in biology.

van Holde, K.E. (1985) *Physical Biochemistry,* 2nd edn, Prentice-Hall, Inc., Englewood Cliffs, NJ.
Chapters 1 through 3 cover the thermodynamic concepts discussed in this chapter.

Phosphate Group Transfers and ATP

Alberty, R.A. (1969) Standard Gibbs free energy, enthalpy and entropy changes as a function of pH and pMg for several reactions involving adenosine phosphates. *J. Biol. Chem.* **244,** 3290–3302.
This research paper documents the strong dependence of the free energy of ATP hydrolysis on the

Bock, R.M. (1960) Adenine nucleotides and properties of pyrophosphate compounds. In *The Enzymes,* 2nd edn, Vol. 2 (Boyer, P.D., Lardy, H., & Myrbäck, K., eds), pp. 3–38, Academic Press, Inc., New York.

Bridger, W.A. & Henderson, J.F. (1983) *Cell ATP,* John Wiley & Sons, Inc., New York.
The chemistry of ATP, the role of ATP in metabolic regulation, and the catabolic and anabolic roles of ATP.

Hanson, R.W. (1989) The role of ATP in metabolism. *Biochem. Educ.* **17,** 86–92.
Excellent summary of the chemistry and biology of ATP.

Harold, F.M. (1986) *The Vital Force: A Study of Bioenergetics,* W.H. Freeman and Company, New York.
A beautifully clear discussion of thermodynamics in biological processes.

Jencks, W.P. (1990) How does ATP make work? *Chemtracts—Biochem. Mol. Biol.* **1,** 1–13.
A clear and sophisticated description of ATP energy transductions in ion transport, muscle contraction, oxidative phosphorylation, and photophosphorylation.

Kalckar, H.M. (1969) *Biological Phosphorylations: Development of Concepts,* Prentice-Hall, Inc., Englewood Cliffs, NJ.
An historical account by one of the central participants in the study of biological phosphorylations.

Lipmann, F. (1941) Metabolic generation and utilization of phosphate bond energy. *Adv. Enzymol.* **11,** 99–162.
The classic description of the role of high-energy phosphate compounds in biology.

Pullman, B. & Pullman, A. (1960) Electronic structure of energy-rich phosphates. *Radiat. Res.* Suppl. 2, pp. 160–181.
An advanced discussion of the chemistry of ATP and other "energy-rich" compounds.

Westheimer, F.H. (1987) Why nature chose phosphates. *Science* **235,** 1173–1178.
A chemist's description of the unique suitability of phosphate esters and anhydrides for metabolic transformations.

Biological Oxidation–Reduction Reactions

Dolphin, D., Avramovic, O., & Poulson, R. (eds) (1987) *Pyridine Nucleotide Coenzymes: Chemical, Biochemical, and Medical Aspects,* John Wiley & Sons, Inc., New York.

An excellent two-volume collection of authoritative reviews. Among the most useful of these are the chapters by Kaplan, Westheimer, Veech, and Ohno and Ushio.

Latimer, W.M. (1952) *Oxidation Potentials,* 2nd edn, Prentice-Hall, Inc., New York.

Montgomery, R. & Swenson, C.A. (1976) *Quantitative Problems in the Biochemical Sciences,* 2nd edn, W.H. Freeman and Company, San Francisco.

Segel, I.H. (1976) *Biochemical Calculations,* 2nd edn, John Wiley & Sons, Inc., New York.

Problems

1. *Entropy Changes during Egg Development* Consider an ecosystem consisting of an egg in an incubator. The white and yolk of the egg contain proteins, carbohydrates, and lipids. If fertilized, the egg is transformed from a single cell to a complex organism. Discuss this irreversible process in terms of the entropy changes in the system, surroundings, and universe. Be sure that you first clearly define the system and surroundings.

2. *Calculation of* $\Delta G^{\circ\prime}$ *from Equilibrium Constants* Calculate the standard free-energy changes of the following metabolically important enzyme-catalyzed reactions at 25 °C and pH 7.0 from the equilibrium constants given.

(a) Glutamate + oxaloacetate $\overset{\text{aspartate aminotransferase}}{\rightleftharpoons}$ aspartate + α-ketoglutarate $\quad K'_{eq} = 6.8$

(b) Dihydroxyacetone phosphate $\overset{\text{triose phosphate isomerase}}{\rightleftharpoons}$ glyceraldehyde-3-phosphate $\quad K'_{eq} = 0.0475$

(c) Fructose-6-phosphate + ATP $\overset{\text{phosphofructokinase}}{\rightleftharpoons}$ fructose-1,6-bisphosphate + ADP $\quad K'_{eq} = 254$

3. *Calculation of Equilibrium Constants from* $\Delta G^{\circ\prime}$ Calculate the equilibrium constants K'_{eq} for each of the following reactions at pH 7.0 and 25 °C, using the $\Delta G^{\circ\prime}$ values of Table 13–4:

(a) Glucose-6-phosphate + H_2O $\overset{\text{glucose-6-phosphatase}}{\rightleftharpoons}$ glucose + P_i

(b) Lactose + H_2O $\overset{\beta\text{-galactosidase}}{\rightleftharpoons}$ glucose + galactose

(c) Malate $\overset{\text{fumarase}}{\rightleftharpoons}$ fumarate + H_2O

4. *Experimental Determination of* K'_{eq} *and* $\Delta G^{\circ\prime}$ If a 0.1 M solution of glucose-1-phosphate is incubated with a catalytic amount of phosphoglucomutase, the glucose-1-phosphate is transformed to glucose-6-phosphate until equilibrium is established. The equilibrium concentrations are

Glucose-1-phosphate $\rightleftharpoons$ glucose-6-phosphate

4.5×10^{-3} M $\qquad$ 9.6×10^{-2} M

Calculate K'_{eq} and $\Delta G^{\circ\prime}$ for this reaction at 25 °C.

5. *Experimental Determination of* $\Delta G^{\circ\prime}$ *for ATP Hydrolysis* A direct measurement of the standard free-energy change associated with the hydrolysis of ATP is technically demanding because the minute amount of ATP remaining at equilibrium is difficult to measure accurately. The value of $\Delta G^{\circ\prime}$ can be calculated indirectly, however, from the equilibrium constants of two other enzymatic reactions having less favorable equilibrium constants:

Glucose-6-phosphate + H_2O $\longrightarrow$ glucose + P_i $\quad K'_{eq} = 270$

ATP + glucose $\longrightarrow$ ADP + glucose-6-phosphate $\quad K'_{eq} = 890$

Using this information, calculate the standard free energy of hydrolysis of ATP. Assume a temperature of 25 °C.

6. *Difference between* $\Delta G^{\circ\prime}$ *and* ΔG Consider the following interconversion, which occurs in glycolysis (Chapter 14):

Fructose-6-phosphate $\rightleftharpoons$ glucose-6-phosphate $\quad K'_{eq} = 1.97$

(a) What is $\Delta G^{\circ\prime}$ for the reaction (assuming that the temperature is 25 °C)?

(b) If the concentration of fructose-6-phosphate is adjusted to 1.5 M and that of glucose-6-phosphate is adjusted to 0.5 M, what is ΔG?

(c) Why are $\Delta G^{\circ\prime}$ and ΔG different?

7. *Dependence of* ΔG *on pH* The free energy released by the hydrolysis of ATP under standard

conditions at pH 7.0 is −30.5 kJ/mol. If ATP is hydrolyzed under standard conditions but at pH 5.0, is more or less free energy released? Why?

8. *The $\Delta G^{\circ\prime}$ for Coupled Reactions* Glucose-1-phosphate is converted into fructose-6-phosphate in two successive reactions:

Glucose-1-phosphate ⟶ glucose-6-phosphate
Glucose-6-phosphate ⟶ fructose-6-phosphate

Using the $\Delta G^{\circ\prime}$ values in Table 13–4, calculate the equilibrium constant, K'_{eq}, for the sum of the two reactions at 25 °C:

Glucose-1-phosphate ⟶ fructose-6-phosphate

9. *Strategy for Overcoming an Unfavorable Reaction: ATP-Dependent Chemical Coupling* The phosphorylation of glucose to glucose-6-phosphate is the initial step in the catabolism of glucose. The direct phosphorylation of glucose by P_i is described by the equation

$$\text{Glucose} + P_i \longrightarrow \text{glucose-6-phosphate} + H_2O \qquad \Delta G^{\circ\prime} = 13.8 \text{ kJ/mol}$$

(a) Calculate the equilibrium constant for the above reaction. In the rat hepatocyte the physiological concentrations of glucose and P_i are maintained at approximately 4.8 mM. What is the equilibrium concentration of glucose-6-phosphate obtained by the direct phosphorylation of glucose by P_i? Does this route represent a reasonable metabolic route for the catabolism of glucose? Explain.

(b) In principle, at least, one way to increase the concentration of glucose-6-phosphate is to drive the equilibrium reaction to the right by increasing the intracellular concentrations of glucose and P_i. Assuming a fixed concentration of P_i at 4.8 mM, how high would the intracellular concentration of glucose have to be to have an equilibrium concentration of glucose-6-phosphate of 250 μM (normal physiological concentration)? Would this route be a physiologically reasonable approach, given that the maximum solubility of glucose is less than 1 M?

(c) The phosphorylation of glucose in the cell is coupled to the hydrolysis of ATP; that is, part of the free energy of ATP hydrolysis is utilized to effect the endergonic phosphorylation of glucose:

(1) $\text{Glucose} + P_i \longrightarrow \text{glucose-6-phosphate} + H_2O \qquad \Delta G^{\circ\prime} = 13.8 \text{ kJ/mol}$

(2) $\text{ATP} + H_2O \longrightarrow \text{ADP} + P_i \qquad \Delta G^{\circ\prime} = -30.5 \text{ kJ/mol}$

Sum: $\text{Glucose} + \text{ATP} \longrightarrow \text{glucose-6-phosphate} + \text{ADP}$

Calculate K'_{eq} for the overall reaction. When the ATP-dependent phosphorylation of glucose is carried out, what concentration of glucose is needed to achieve a 250 μM intracellular concentration of glucose-6-phosphate when the concentrations of ATP and ADP are 3.38 and 1.32 mM, respectively? Does this coupling process provide a feasible route, at least in principle, for the phosphorylation of glucose as it occurs in the cell? Explain.

(d) Although coupling ATP hydrolysis to glucose phosphorylation makes thermodynamic sense, how this coupling is to take place has not been specified. Given that coupling requires a common intermediate, one conceivable route is to use ATP hydrolysis to raise the intracellular concentration of P_i and thus drive the unfavorable phosphorylation of glucose by P_i. Is this a reasonable route? Explain.

(e) The ATP-coupled phosphorylation of glucose is catalyzed in the hepatocyte by the enzyme glucokinase. This enzyme binds ATP and glucose to form a glucose–ATP–enzyme complex, and the phosphate is transferred directly from ATP to glucose. Explain the advantages of this route.

10. *Calculations of $\Delta G^{\circ\prime}$ for ATP-Coupled Reactions* From data in Table 13–6 calculate the $\Delta G^{\circ\prime}$ value for the reactions

(a) $\text{Phosphocreatine} + \text{ADP} \longrightarrow \text{creatine} + \text{ATP}$

(b) $\text{ATP} + \text{fructose} \longrightarrow \text{ADP} + \text{fructose-6-phosphate}$

11. *Coupling ATP Cleavage to an Unfavorable Reaction* This problem explores the consequences of coupling ATP hydrolysis under physiological conditions to a thermodynamically unfavorable biochemical reaction. Because we want to explore these consequences in stages, we shall consider the hypothetical transformation, X ⟶ Y, a reaction for which $\Delta G^{\circ\prime} = 20$ kJ/mol.

(a) What is the ratio [Y]/[X] at equilibrium?

(b) Suppose X and Y participate in a sequence of reactions during which ATP is hydrolyzed to ADP and P_i. The overall reaction is

$$X + ATP + H_2O \longrightarrow Y + ADP + P_i$$

Calculate [Y]/[X] for this reaction at equilibrium. Assume for the purposes of this calculation that the concentrations of ATP, ADP, and P_i are all 1 M when the reaction is at equilibrium.

(c) We know that [ATP], [ADP], and [P_i] are *not* 1 M under physiological conditions. Calculate the ratio [Y]/[X] for the ATP-coupled reaction when the values of [ATP], [ADP], and [P_i] are those found in rat myocytes (Table 13–5).

12. *Calculations of ΔG at Physiological Concentrations* Calculate the physiological ΔG (not $\Delta G^{\circ\prime}$) for the reaction

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

at 25 °C as it occurs in the cytosol of neurons, in which phosphocreatine is present at 4.7 mM, creatine at 1.0 mM, ADP at 0.20 mM, and ATP at 2.6 mM.

13. *Free Energy Required for ATP Synthesis under Physiological Conditions* In the cytosol of rat hepatocytes, the mass-action ratio is

$$\frac{[ATP]}{[ADP][P_i]} = 5.33 \times 10^2\ M^{-1}$$

Calculate the free energy required to synthesize ATP in the rat hepatocyte.

14. *Daily ATP Utilization by Human Adults*

(a) A total of 30.5 kJ/mol of free energy is needed to synthesize ATP from ADP and P_i when the reactants and products are at 1 M concentration (standard state). Because the actual physiological concentrations of ATP, ADP, and P_i are not 1 M, the free energy required to synthesize ATP under physiological conditions is different from $\Delta G^{\circ\prime}$. Calculate the free energy required to synthesize ATP in the human hepatocyte when the physiological concentrations of ATP, ADP, and P_i are 3.5, 1.50, and 5.0 mM, respectively.

(b) A normal 68 kg (150 lb) adult requires a caloric intake of 2,000 kcal (8,360 kJ) of food per day (24 h). This food is metabolized and the free energy used to synthesize ATP, which is then utilized to do the body's daily chemical and mechanical work. Assuming that the efficiency of converting food energy into ATP is 50%, calculate the weight of ATP utilized by a human adult in a 24 h period. What percentage of the body weight does this represent?

(c) Although adults synthesize large amounts of ATP daily, their body weight, structure, and composition do not change significantly during this period. Explain this apparent contradiction.

15. *ATP Reserve in Muscle Tissue* The ATP concentration in muscle tissue (approximately 70% water) is about 8.0 mM. During strenuous activity each gram of muscle tissue uses ATP at the rate of 300 μmol/min for contraction.

(a) How long would the reserve of ATP last during a 100 meter dash?

(b) The phosphocreatine level in muscle is about 40.0 mM. How does this help extend the reserve of muscle ATP?

(c) Given the size of the reserve ATP pool, how can a person run a marathon?

16. *Rates of Turnover of γ- and β-Phosphates of ATP* If a small amount of ATP labeled with radioactive phosphorus in the terminal position, [γ-^{32}P]ATP, is added to a yeast extract, about half of the ^{32}P activity is found in P_i within a few minutes, but the concentration of ATP remains unchanged. Explain. If the same experiment is carried out using ATP labeled with ^{32}P in the central position, [β-^{32}P]ATP, the ^{32}P does not appear in P_i within the same number of minutes. Why?

17. *Cleavage of ATP to AMP and PP_i during Metabolism* The synthesis of the activated form of acetate (acetyl-CoA) is carried out in an ATP-dependent process:

$$\text{Acetate} + \text{CoA} + \text{ATP} \longrightarrow \text{acetyl-CoA} + \text{AMP} + PP_i$$

(a) The $\Delta G^{\circ\prime}$ for the hydrolysis of acetyl-CoA to acetate and CoA is −32.2 kJ/mol and that for hydrolysis of ATP to AMP and PP_i is −30.5 kJ/mol. Calculate $\Delta G^{\circ\prime}$ for the ATP-dependent synthesis of acetyl-CoA.

(b) Almost all cells contain the enzyme inorganic pyrophosphatase, which catalyzes the hydrolysis of PP_i to P_i. What effect does the presence of this enzyme have on the synthesis of acetyl-CoA? Explain.

18. *Are All Metabolic Reactions at Equilibrium?*

(a) Phosphoenolpyruvate is one of the two phosphate donors in the synthesis of ATP during glycolysis. In human erythrocytes, the steady-state concentration of ATP is 2.24 mM, that of ADP is 0.25 mM, and that of pyruvate is 0.051 mM. Calculate the concentration of phosphoenolpyruvate at 25 °C, assuming that the pyruvate kinase reaction (Fig. 13–3) is at equilibrium in the cell.

(b) The physiological concentration of phosphoenolpyruvate in human erythrocytes is 0.023 mM. Compare this with the value obtained in (a). What is the significance of this difference? Explain.

19. *Standard Reduction Potentials* The standard reduction potential, E'_0, of any redox pair is defined for the half-cell reaction:

$$\text{Oxidizing agent} + n\ \text{electrons} \rightleftharpoons \text{reducing agent}$$

The E'_0 values for the NAD^+/NADH and pyruvate/lactate conjugate redox pairs are −0.32 and −0.19 V, respectively.

(a) Which conjugate pair has the greater tendency to lose electrons? Explain.

(b) Which is the stronger oxidizing agent? Explain.

(c) If we begin with 1 M concentrations of each reactant and product at pH 7, in which direction will the following reaction proceed?

$$\text{Pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{lactate} + NAD^+$$

(d) What is the standard free-energy change ($\Delta G^{\circ\prime}$) at 25 °C for this reaction?

(e) What is the equilibrium constant (K'_{eq}) for this reaction?

20. *Energy Span of the Respiratory Chain* Electron transfer in the mitochondrial respiratory chain may be represented by the net reaction equation

$$\text{NADH} + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons H_2O + NAD^+$$

(a) Calculate the value of $\Delta E_0'$ for the net reaction of mitochondrial electron transfer.

(b) Calculate $\Delta G^{\circ\prime}$ for this reaction.

(c) How many ATP molecules can *theoretically* be generated by this reaction if the standard free energy of ATP synthesis is 30.5 kJ/mol?

21. *Dependence of Electromotive Force on Concentrations* Calculate the electromotive force (in volts) registered by an electrode immersed in a solution containing the following mixtures of NAD^+ and NADH at pH 7.0 and 25 °C, with reference to a half-cell of $E_0' = 0.00$ V.

(a) 1.0 mM NAD^+ and 10 mM NADH
(b) 1.0 mM NAD^+ and 1.0 mM NADH
(c) 10 mM NAD^+ and 1.0 mM NADH

22. *Electron Affinity of Compounds* List the following substances in order of increasing tendency to accept electrons: (a) α-ketoglutarate + CO_2 (yielding isocitrate), (b) oxaloacetate, (c) O_2, (d) $NADP^+$.

23. *Direction of Oxidation–Reduction Reactions* Which of the following reactions would be expected to proceed in the direction shown under standard conditions, assuming that the appropriate enzymes are present to catalyze them?

(a) Malate + NAD^+ $\longrightarrow$ oxaloacetate + NADH + H^+
(b) Acetoacetate + NADH + H^+ $\longrightarrow$ β-hydroxybutyrate + NAD^+
(c) Pyruvate + NADH + H^+ $\longrightarrow$ lactate + NAD^+
(d) Pyruvate + β-hydroxybutyrate $\longrightarrow$ lactate + acetoacetate
(e) Malate + pyruvate $\longrightarrow$ oxaloacetate + lactate
(f) Acetaldehyde + succinate $\longrightarrow$ ethanol + fumarate

lyze ATP synthesis from ADP and P_i as protons flow back into the cell through proton channels formed by F_o.

Certain bacterial transport systems bring about uptake of extracellular nutrients (lactose, for example) against a concentration gradient, in symport with protons (see Fig. 10–25). The respiration-linked transmembrane proton extrusion provides the driving force for this uptake. The rotary motion of bacterial flagella, which move cells through their surroundings, is provided by "proton turbines," molecular rotary motors driven not by ATP but directly by the transmembrane electrochemical potential generated by respiration-linked proton pumping (Fig. 18–31). It appears likely that the chemiosmotic mechanism evolved early (before the emergence of eukaryotes). The proton-motive force can clearly be used to power processes other than ATP synthesis.

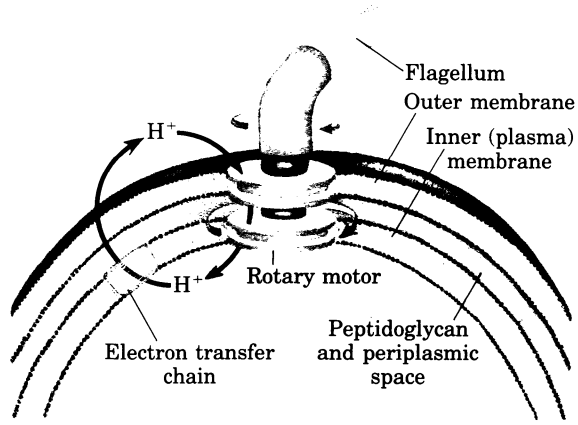


Figure 18–31 Rotation of bacterial flagella by proton-motive force. The shaft and rings at the base of the flagellum make up a rotary motor that has been called a "proton turbine." Protons ejected by electron transfer flow back into the cell through the "turbine," causing rotation of the shaft of the flagellum. This motion differs fundamentally from the motion of muscle or of eukaryotic flagella and cilia, for which ATP hydrolysis is the energy source.

Photosynthesis: Harvesting Light Energy

We now turn to another reaction sequence in which the flow of electrons is coupled to the synthesis of ATP: light-driven phosphorylation. The capture of solar energy by photosynthetic organisms and its conversion into the chemical energy of reduced organic compounds is the ultimate source of nearly all biological energy. Photosynthetic and heterotrophic organisms live in a balanced steady state in the biosphere (Fig. 18–32). Photosynthetic organisms trap solar energy and form ATP and NADPH, which they use as energy sources to make carbohydrates and other organic components from CO_2 and H_2O; simultaneously, they release O_2 into the atmosphere. Aerobic heterotrophs (we humans, for example) use the O_2 so formed to degrade the energy-rich organic products of photosynthesis to CO_2 and H_2O, generating ATP for their own activities. The CO_2 formed by respiration in heterotrophs returns to the atmosphere, to be used again by photosynthetic organisms. Solar energy thus provides the driving force for the continuous cycling of atmospheric CO_2 and O_2 through the biosphere and provides the reduced substrates (fuels), such as glucose, on which nonphotosynthetic organisms depend.

Enormous amounts of energy are stored as products of photosynthesis. Each year at least 10^{17} kJ of free energy from sunlight is captured and used for biosynthesis by photosynthetic organisms. This is more than ten times the fossil-fuel energy used each year by people the world over. Even fossil fuels (coal, oil, and natural gas) are the products of photosynthesis that took place millions of years ago. Because of our global dependence upon solar energy, past and present, for both energy and food, discovering the mechanism of photosynthesis is a central goal of biochemical research.

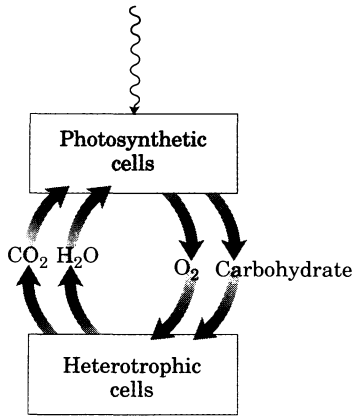


Figure 18–32 Solar energy is the ultimate source of all biological energy. Photosynthetic organisms use the energy of sunlight to manufacture glucose and other organic cell products, which heterotrophic cells use as energy and carbon sources.

The overall equation of photosynthesis describes an oxidation–reduction reaction in which H_2O donates electrons (as hydrogen) for the reduction of CO_2 to carbohydrate (CH_2O):

$$CO_2 + H_2O \xrightarrow{\text{light}} O_2 + (CH_2O)$$

Unlike NADH (the hydrogen donor in oxidative phosphorylation), H_2O is a poor electron donor; its standard reduction potential is 0.82 V, compared with −0.32 V for NADH. The central difference between photophosphorylation and oxidative phosphorylation is that the latter process begins with a good electron donor, whereas the former requires the input of energy in the form of light to *create* a good electron donor. Except for this crucial difference, the two processes are remarkably similar. In photophosphorylation, electrons flow through a series of membrane-bound carriers including cytochromes, quinones, and iron–sulfur proteins, while protons are pumped across a membrane to create an electrochemical potential. This potential is the driving force for ATP synthesis from ADP and P_i by a membrane-bound ATP synthase complex closely similar to that which functions in oxidative phosphorylation.

Photosynthesis encompasses two processes: the **light reactions,** which occur only when plants are illuminated, and the **carbon fixation reactions,** or so-called dark reactions, which occur in both light and darkness (Fig. 18–33). In the light reactions, chlorophyll and other pigments of the photosynthetic cells absorb light energy and conserve it in chemical form as the two energy-rich products ATP and NADPH; simultaneously, O_2 is evolved. In the carbon fixation reactions, ATP and NADPH are used to reduce CO_2 to form glucose and other organic products. The formation of O_2, which occurs only in the light, and the reduction of CO_2, which does not require light, thus are distinct and separate processes. In this chapter we are concerned only with the light reactions; the reduction of CO_2 is described in Chapter 19.

In photosynthetic eukaryotic cells, both the light and carbon fixation reactions take place in the chloroplasts (Fig. 18–34). When solar energy is not available, mitochondria in the plant cell generate ATP by oxidizing carbohydrates originally produced in chloroplasts in the light.

Chloroplasts may assume many different shapes in different species, and they usually have a much larger volume than mitochondria.

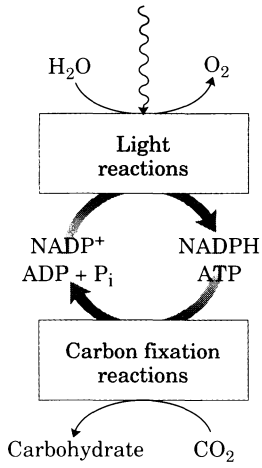
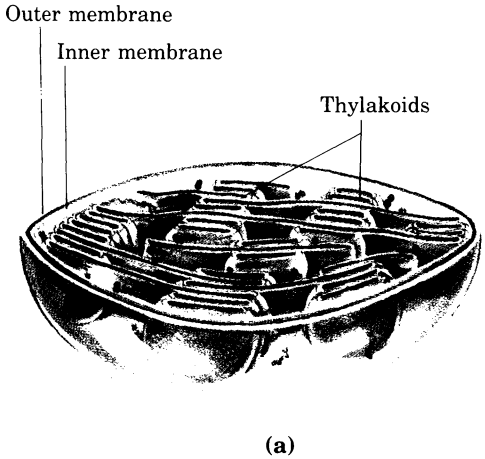
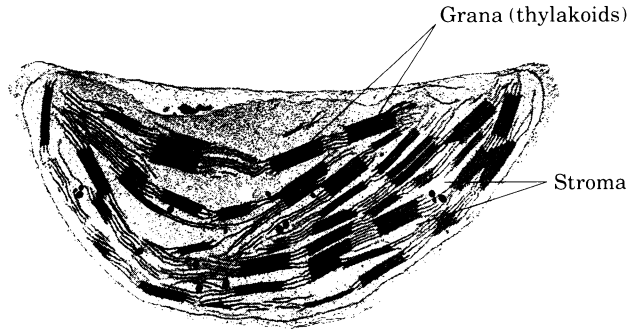


Figure 18–33 The light reactions generate energy-rich NADPH and ATP at the expense of solar energy. These products are used in the carbon fixation reactions, which occur in light or darkness, to reduce CO_2 to form trioses and more complex compounds (such as glucose) derived from trioses.

Figure 18–34 Schematic diagram **(a)** and electron micrograph **(b)** of a single chloroplast at high magnification.



(a)



(b)

They are surrounded by a continuous outer membrane, which, like the outer mitochondrial membrane, is permeable to small molecules and ions. An inner membrane system encloses the internal compartment, in which there are many flattened, membrane-surrounded vesicles or sacs, called **thylakoids,** which are usually arranged in stacks called **grana.** The thylakoid membranes are separate from the inner chloroplast membrane. Embedded in the thylakoid membranes are the photosynthetic pigments and all the enzymes required for the primary light reactions. The fluid in the compartment surrounding the thylakoids, the **stroma,** contains most of the enzymes required for the carbon fixation reactions, in which CO_2 is reduced to form triose phosphates and, from them, glucose.

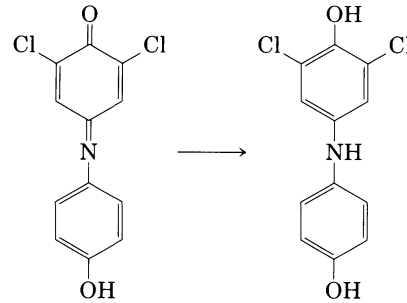
Our discussion here focuses on the nature of the light-absorbing systems and their roles in photosynthesis.

Light Produces Electron Flow in Chloroplasts

How do the pigment molecules of the thylakoid membranes transduce absorbed light energy into chemical energy? The key to answering this question came from a discovery made in 1937 by Robert Hill, a pioneer in photosynthesis research. He found that when leaf extracts containing chloroplasts were supplemented with a nonbiological hydrogen acceptor and then illuminated, evolution of O_2 and simultaneous reduction of the hydrogen acceptor took place, according to an equation now known as the **Hill reaction:**

$$2H_2O + 2A \xrightarrow{\text{light}} 2AH_2 + O_2$$

where A is the artificial hydrogen acceptor. One of the nonbiological hydrogen acceptors used by Hill was the dye 2,6-dichlorophenolindophenol, now called a **Hill reagent,** which in its oxidized form (A) is blue and in its reduced form (AH_2) is colorless. When the leaf extract supplemented with the dye was illuminated, the blue dye became colorless and O_2 was evolved. In the dark neither O_2 evolution nor dye reduction took place. This was the first specific clue to how absorbed light energy is converted into chemical energy: it causes electrons to flow from H_2O to an electron acceptor. Moreover, Hill found that CO_2 was not required for this reaction, nor was it reduced to a stable form under these conditions. He therefore concluded that O_2 evolution can be dissociated from CO_2 reduction. Several years later it was found that $NADP^+$ is the biological electron acceptor in chloroplasts, according to the equation



Oxidized form (blue) → Reduced form (colorless)

Dichlorophenolindophenol

$$2H_2O + 2NADP^+ \xrightarrow{\text{light}} 2NADPH + 2H^+ + O_2$$

This equation shows an important distinction between mitochondrial oxidative phosphorylation and the analogous process in chloroplasts: in chloroplasts electrons flow from H_2O to $NADP^+$, whereas in mitochondrial respiration electrons flow in the opposite direction, from NADH or NADPH to O_2, with the release of free energy. Because light-induced electron flow in chloroplasts is in the reverse or "uphill" direction, from H_2O to $NADP^+$, it cannot occur without the input of free energy; this energy comes from light. To understand how this occurs, we must first consider the effects of light absorption on molecular structure.

Absorption of Light Excites Molecules

Visible light is electromagnetic radiation of wavelengths 400 to 700 nm, a small part of the electromagnetic spectrum (Fig. 18–35), ranging from violet to red. The energy of a single **photon** (a quantum of light) is greater at the violet end of the spectrum than at the red end. The energy in a "mole" of photons (one einstein; 6×10^{23} photons) is 170 to 300 kJ, about an order of magnitude more than the energy required to synthesize a mole of ATP from ADP and P_i (about 30 kJ under standard conditions). The energy of an einstein in the infrared or microwave regions of the spectrum is too small to be useful in the kinds of photochemical events that occur in photosynthesis. UV light and x rays, on the other hand, have so much energy that they damage proteins and nucleic acids and induce mutations that are often lethal.

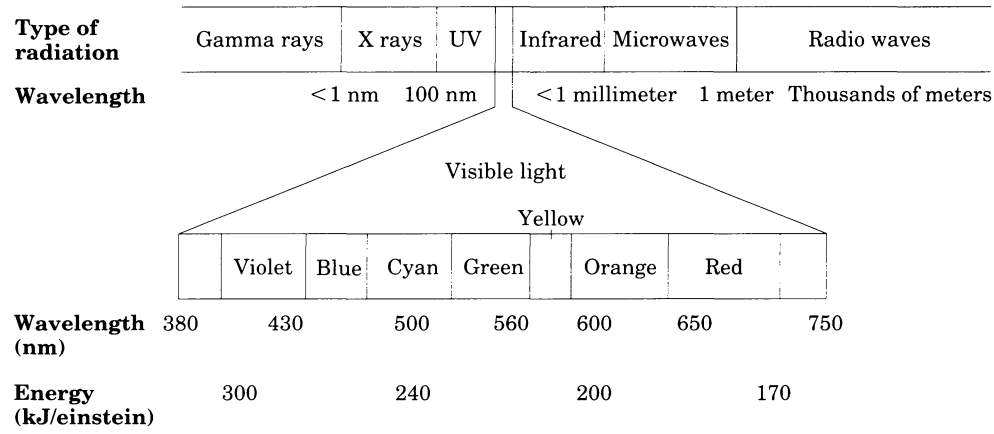
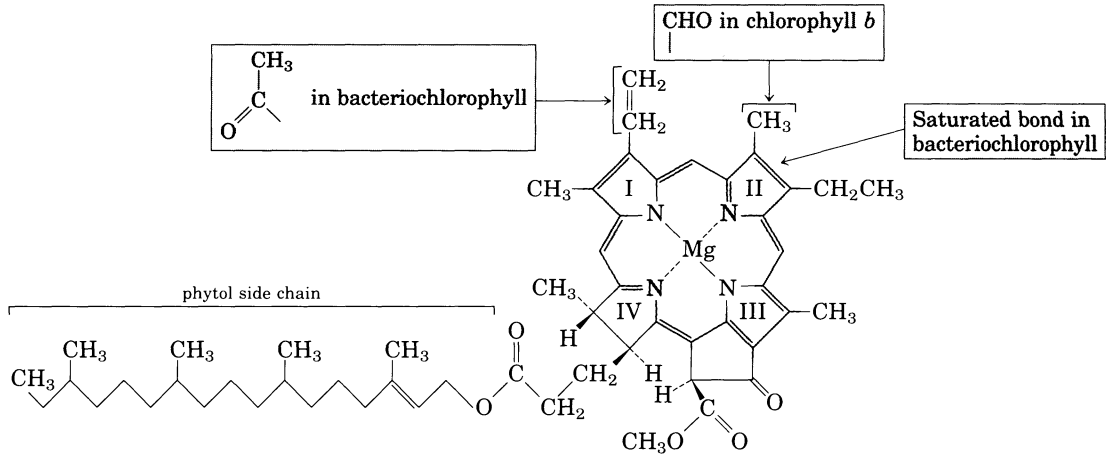


Figure 18–35 The spectrum of electromagnetic radiation, and the energy of photons in the visible range of the spectrum. One einstein is 6×10^{23} photons.

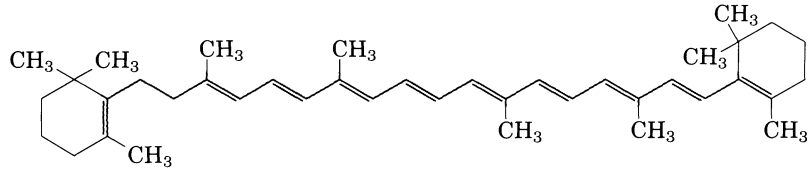
The ability of a molecule to absorb light depends upon the arrangement of electrons around the atomic nuclei in its structure. When a photon is absorbed, an electron is lifted to a higher energy level. This happens on an all-or-none basis; to be absorbed, the photon must contain a quantity of energy (a **quantum**) that exactly matches the energy of the electronic transition. A molecule that has absorbed a photon is in an **excited state,** which is generally unstable. The electrons lifted into higher-energy orbitals usually return rapidly to their normal lower-energy orbitals; the excited molecule reverts (decays) to the stable **ground state,** giving up the absorbed quantum as light or heat or using it to do chemical work. The light emitted upon decay of excited molecules, called **fluorescence,** is always of a longer wavelength (lower energy) than the absorbed light. Excitation of molecules by light and their fluorescent decay are extremely fast processes, occurring in about 10^{-15} and 10^{-12} s, respectively. The initial events in photosynthesis are therefore very rapid.

Chlorophylls Absorb Light Energy for Photosynthesis

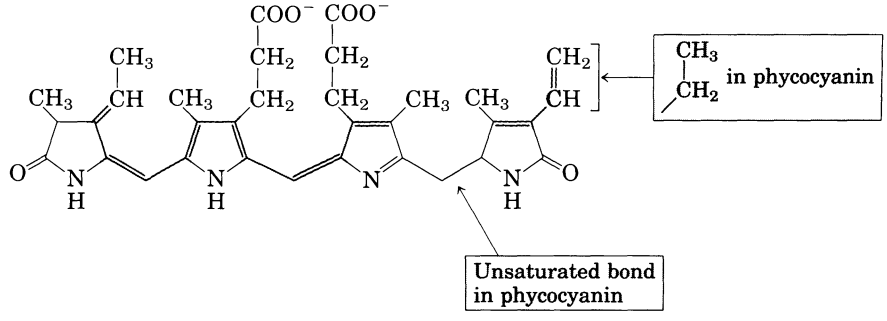
The most important light-absorbing pigments in the thylakoid membranes are the **chlorophylls,** green pigments with polycyclic, planar structures resembling the protoporphyrin of hemoglobin (see Fig. 7–18), except that Mg^{2+}, not Fe^{2+}, occupies the central position (Fig. 18–36). Chlorophyll *a*, present in the chloroplasts of all green plant cells, contains four substituted pyrrole rings, one of which (ring IV) is reduced, and a fifth ring that is not a pyrrole. All chlorophylls have a long **phytol** side chain, esterified to a carboxyl-group substituent in ring IV. The four inward-oriented nitrogen atoms of chlorophyll *a* are coordinated with the Mg^{2+}.



Chlorophyll *a*



β-Carotene



Phycoerythrin

Figure 18–36 Structures of the primary photopigments chlorophylls *a* and *b* and bacteriochlorophyll, and of the accessory pigments β-carotene (a carotenoid) and phycoerythrin and phycocyanin (phycobilins). The areas shaded pink represent conjugated systems (alternating single and double bonds), which largely account for the absorption of visible light.

The heterocyclic five-ring system that surrounds the Mg^{2+} has an extended polyene structure, with alternating single and double bonds. Such polyenes characteristically show strong absorption in the visible region of the spectrum (Fig. 18–37); the chlorophylls have unusually high molar absorption coefficients (see Box 5–1) and are therefore particularly well-suited for absorbing visible light during photosynthesis.

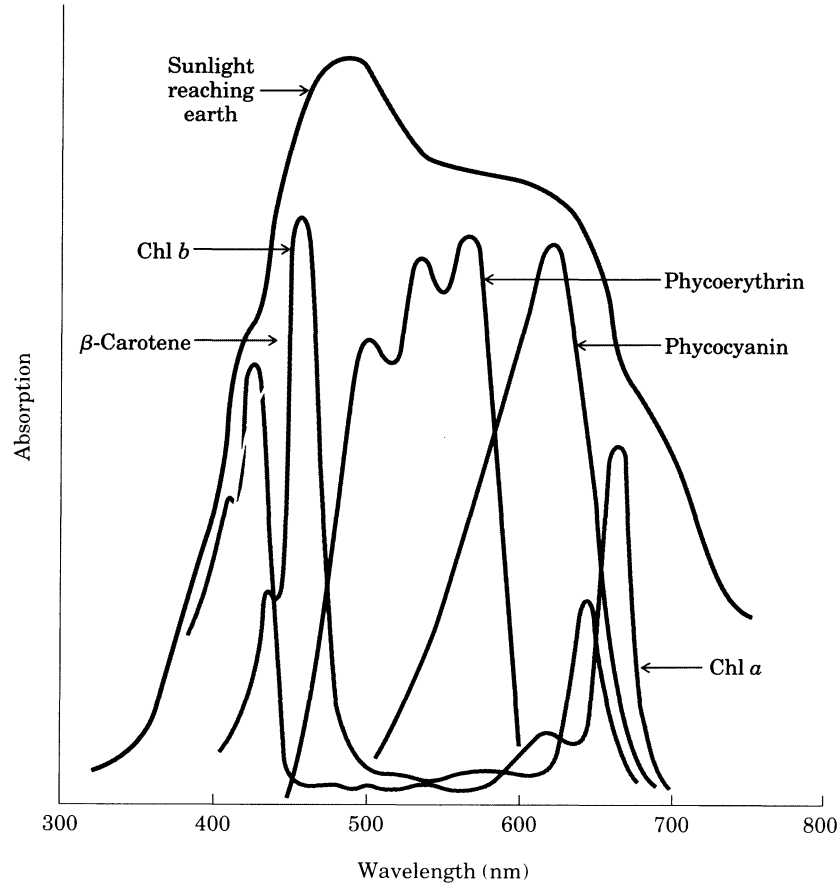


Figure 18–37 Absorption of visible light by photopigments shown in Fig. 18–36. Plants are green because their pigments absorb light from the red and violet regions of the spectrum, leaving primarily green light to be reflected or transmitted. Compare the absorption spectra of the pigments with the spectrum of sunlight reaching the earth's surface; the combination of chlorophylls (chl *a* and chl *b*) and accessory pigments enables plants to harvest most of the energy available from sunlight.

Chloroplasts of higher plants always contain two types of chlorophyll. One is invariably chlorophyll *a*, and the second in many species is chlorophyll *b*, which has an aldehyde group instead of a methyl group attached to ring II (Fig. 18–36). Although both are green, their absorption spectra are slightly different (Fig. 18–37), allowing the two pigments to complement each other's range of light absorption in the visible region. Most higher plants contain about twice as much chlorophyll *a* as chlorophyll *b*. The bacterial chlorophylls differ only slightly from the plant pigments (Fig. 18–36).

Accessory Pigments Also Absorb Light

In addition to chlorophylls, the thylakoid membranes contain secondary light-absorbing pigments, together called the **accessory pigments,** the carotenoids and phycobilins. **Carotenoids** may be yellow, red, or purple. The most important are **β-carotene** (Fig. 18–36), a red-orange isoprenoid compound that is the precursor of vitamin A in animals, and the yellow carotenoid **xanthophyll.** The carotenoid pig-

ments absorb light at wavelengths other than those absorbed by the chlorophylls (Fig. 18–37) and thus are supplementary light receptors. **Phycobilins** are linear tetrapyrroles that have the extended polyene system found in chlorophylls, but not their cyclic structure or central Mg^{2+}. Examples are phycoerythrin and phycocyanin (Fig. 18–36).

The relative amounts of the chlorophylls and the accessory pigments are characteristic for different plant species. It is variation in the proportions of these pigments that is responsible for the range of colors of photosynthetic organisms, which vary from the deep blue-green of spruce needles, to the greener green of maple leaves, to the red, brown, or even purple color of different species of multicellular algae and the leaves of some decorative plants.

Experimental determination of the effectiveness of light of different colors in promoting photosynthesis yields an **action spectrum** (Fig. 18–38), often useful in identifying the pigment primarily responsible for a biological effect of light. By capturing light in a region of the spectrum not used by other plants, a photosynthetic organism can claim its unique ecological niche. For example, the phycobilins, present only in red algae and cyanobacteria, absorb in the region 520 to 630 nm, allowing these organisms to live in niches where light of lower or higher wavelength has been filtered out by the pigments of other organisms living in the water above them, or by the water itself.

Chlorophyll Funnels Absorbed Energy to Reaction Centers

The light-absorbing pigments of thylakoid membranes are arranged in functional sets or arrays called **photosystems.** In spinach chloroplasts each photosystem contains about 200 molecules of chlorophylls and about 50 molecules of carotenoids. The clusters can absorb light over the entire visible spectrum but especially well between 400 to 500 nm and 600 to 700 nm (Fig. 18–37). All the pigment molecules in a photosystem can absorb photons, but only a few can transduce the light energy into chemical energy. A transducing pigment consists of several chlorophyll molecules combined with a protein complex also containing tightly bound quinones; this complex is called a **photochemical reaction center.** The other pigment molecules in a photosystem are called **light-harvesting** or **antenna molecules.** They function to absorb light energy and transmit it at a very high rate to the reaction center where the photochemical reactions occur (Fig. 18–39, p. 578), which will be described in detail later.

The chlorophyll molecules in thylakoid membranes are bound to integral membrane proteins (chlorophyll *a*/*b*-binding, or CAB, proteins) that orient the chlorophyll relative to the plane of the membrane and confer light absorption properties that are subtly different from those of free chlorophyll. When isolated chlorophyll molecules in vitro are excited by light, the absorbed energy is quickly released as fluorescence and heat, but when chlorophyll in intact spinach leaves is excited by visible light (Fig. 18–40 (step ①), p. 579), very little fluorescence is observed. Instead, a direct transfer of energy from the excited chlorophyll (an antenna chlorophyll) to a neighboring chlorophyll molecule occurs, exciting the second molecule and allowing the first to return to its ground state (step ②). This **resonance energy transfer** is repeated to a third, fourth, or subsequent neighbor, until the chlorophyll at the photochemical reaction center becomes excited (step ③). In this special chlorophyll molecule, an electron is promoted by excitation to a higher-energy orbital. This electron then passes to a nearby electron

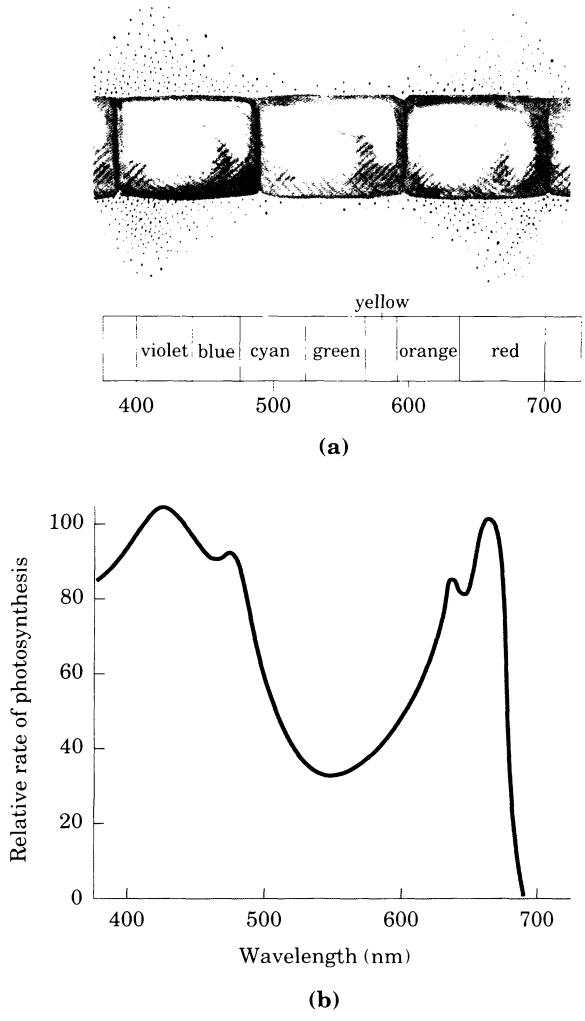
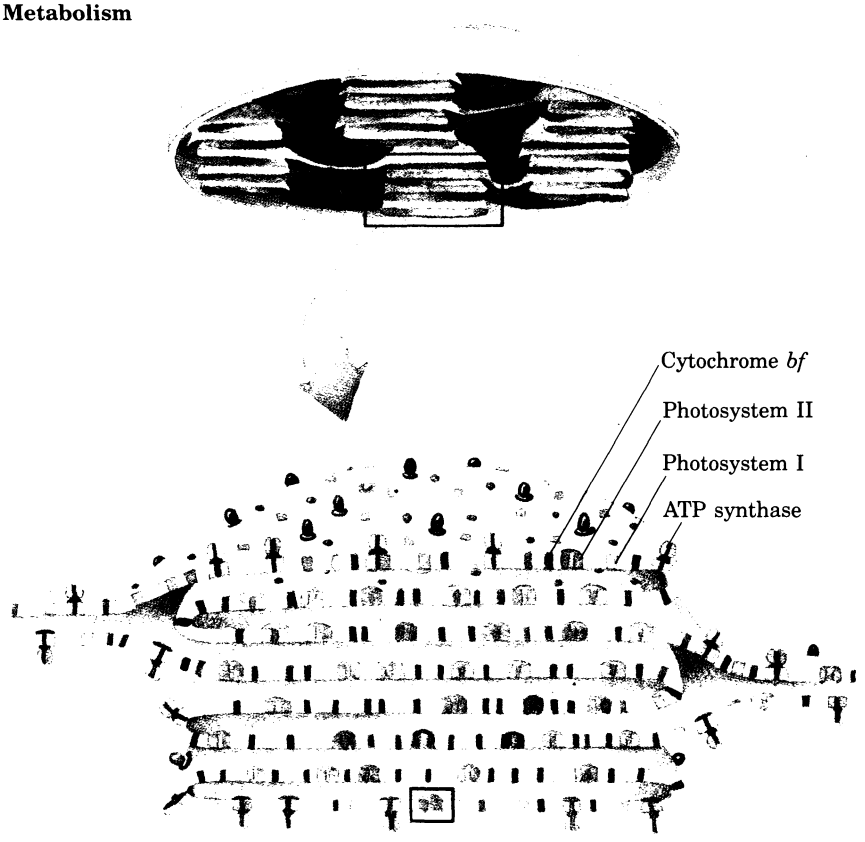
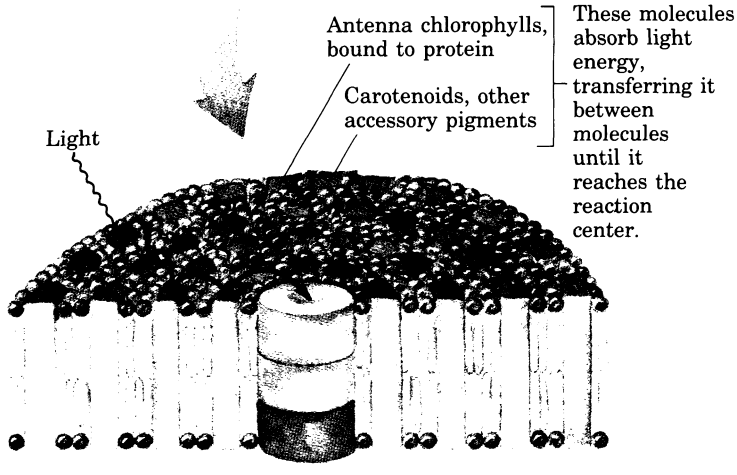


Figure 18–38 Two ways to determine the action spectrum for photosynthesis. **(a)** The results of a classic experiment done by T.W. Englemann in 1882 to determine what wavelength of light was most effective in supporting photosynthesis. Englemann placed a filamentous, photosynthetic alga on a microscope stage and illuminated it with light from a prism, so that cells in one part of the filament received mainly blue light, another yellow, another red. To determine which cells carried out photosynthesis most actively, bacteria known to migrate toward regions of high O_2 concentration were also placed on the microscope slide. The distribution of bacteria showed highest O_2 levels (produced by photosynthesis) in the regions illuminated with violet and red light. **(b)** A similar experiment using modern techniques for the measurement of O_2 production yields the same result. An action spectrum describes the relative rate of photosynthesis for illumination with a constant number of photons of different wavelengths. Such an action spectrum is useful because it suggests (by comparison with absorption spectra such as those in Fig. 18–37) which pigments are able to channel energy into photosynthesis.



(a)



(b)

Figure 18–39 Organization of the photosystem components in the thylakoid membrane. **(a)** The distribution of photosystems I and II, ATP synthase, and the cytochrome *bf* complex in the thylakoid membranes is not random. Photosystem I and ATP synthase are almost completely excluded from the regions with tightly stacked membranes, whereas photosystem II and the cytochrome *bf* complex are enriched in these regions of tight packing. This separation of photosystems I and II prevents energy absorbed by photosystem II from being transferred directly to photosystem I, and also places photosystem I in the regions most accessible to $NADP^+$ from the stroma. **(b)** An enlargement of a photosystem showing the reaction center, antenna chlorophylls, and accessory pigments. Cytochrome *bf* and ATP synthase of chloroplasts are described later in this chapter.

acceptor that is part of the electron transfer chain of the chloroplast, leaving the excited chlorophyll molecule with an empty orbital (an "electron hole") (step ④). The electron acceptor thus acquires a negative charge. The electron lost by the reaction-center chlorophyll is replaced by an electron from a neighboring electron donor molecule (step ⑤), which becomes positively charged. In this way, *excitation by light causes electric charge separation and initiates an oxidation–reduction chain*. Coupled to the light-dependent electron flow along this chain are processes that generate ATP and NADPH.

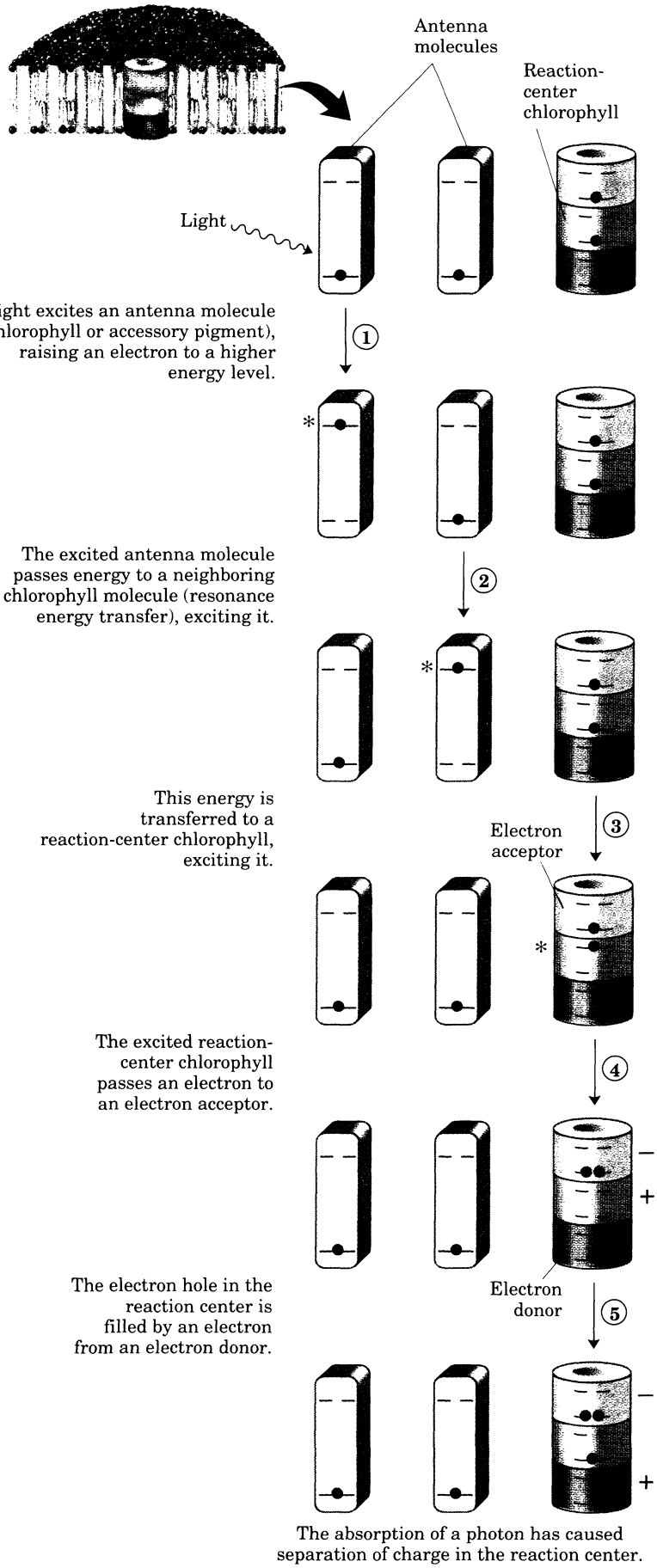


Figure 18–40 A generalized scheme showing the conversion of energy from an absorbed photon into separation of charges at the photosystem reaction center. The steps are further described in the text. Note that step (2) may be repeated a number of times between successive antenna molecules until a reaction-center chlorophyll is reached. The asterisk (*) represents the excited state of an antenna molecule.

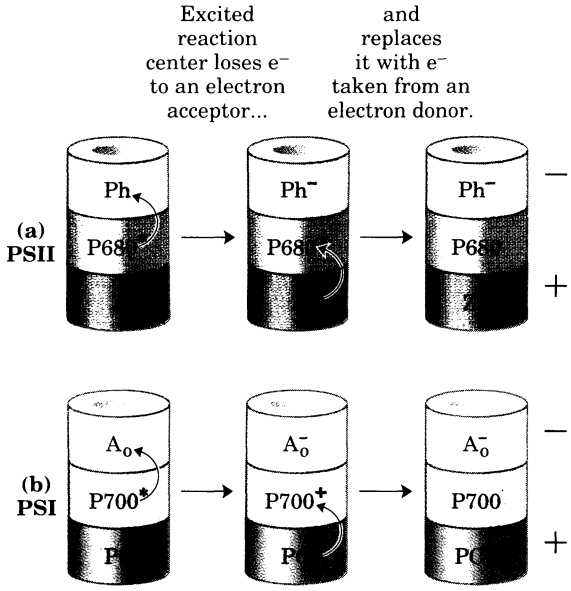


Figure 18–41 Photochemical events following excitation of photosystems by light absorption. (The steps shown here are equivalent to steps ④ and ⑤ in Fig. 18–40.) **(a)** Photosystem II (PSII). Z represents a Tyr residue in the D1 protein of PSII; Ph, pheophytin. **(b)** Photosystem I (PSI). A_0 is a chlorophyll molecule near the reaction center of PSI; it accepts an electron from P700 to become the powerful reducing agent A_0^-. PC, plastocyanin.

Light-Driven Electron Flow

Thylakoid membranes have two different kinds of photosystems, each with its own type of photochemical reaction center and a set of antenna molecules. The two systems have distinct and complementary functions. **Photosystem I** has a reaction center designated **P700** and a high ratio of chlorophyll *a* to chlorophyll *b*. **Photosystem II,** with its reaction center **P680,** contains roughly equal amounts of chlorophyll *a* and *b* and may also contain a third type, chlorophyll *c*. The thylakoid membranes of a single spinach chloroplast have many hundreds of each kind of photosystem. All O_2-evolving photosynthetic cells—those of higher plants, algae, and cyanobacteria—contain both photosystems I and II; all other species of photosynthetic bacteria, which do not evolve O_2, contain only photosystem I.

It is between photosystems I and II that light-driven electron flow occurs, producing NADPH and a transmembrane proton gradient.

Light Absorption by Photosystem II Initiates Charge Separation

How can light energy captured by chloroplasts induce electrons to flow energetically "uphill"? Excitation briefly creates a chemical species of very low standard reduction potential—an excellent electron donor. Excited P680, designated P680*, within picoseconds transfers an electron to **pheophytin** (a chlorophyll-like accessory pigment lacking Mg^{2+}), giving it a negative charge (designated Ph^-) (Fig. 18–41a). With the loss of its electron, P680* is transformed into a radical cation, designated $P680^+$. Thus excitation, in creating Ph^- and $P680^+$, causes charge separation. Ph^- very rapidly passes its extra electron to a protein-bound **plastoquinone,** Q_A, which in turn passes its electron to another, more loosely bound quinone, Q_B (Fig. 18–42; see also Fig. 18–44). When Q_B has acquired two electrons in two such transfers from Q_A and two protons from the solvent water, it is in its fully reduced quinol form, Q_BH_2. This molecule dissociates from its protein and diffuses away from the photochemical reaction center, carrying in its chemical bonds some of the energy of the photons that originally excited P680. The overall reaction initiated by light in photosystem II is therefore

$$4\ P680 + 4H^+ + 2Q_B + \text{light (4 photons)} \longrightarrow 4\ P680^+ + 2Q_BH_2 \quad (18\text{–}4)$$

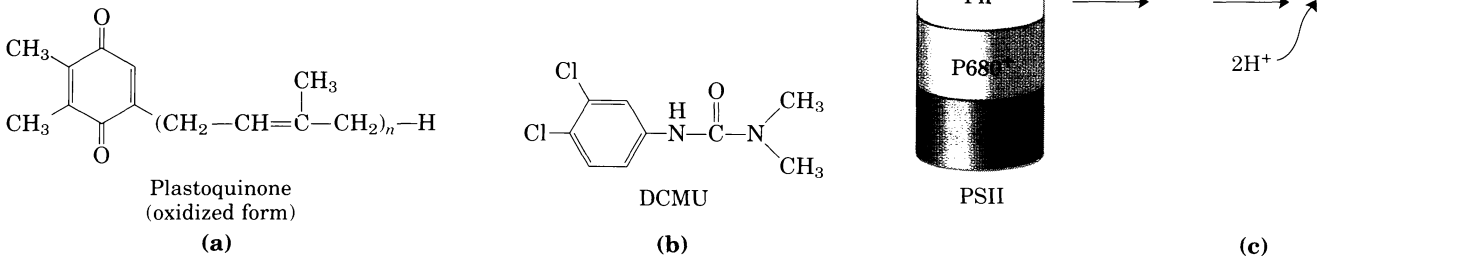


Figure 18–42 **(a)** Plastoquinone (Q_A). **(b)** The herbicide DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), which displaces Q_B from its binding site in photosystem II and blocks electron transfer from photosystem II to photosystem I. **(c)** The role of Q_A and Q_B in transferring electrons away from photosystem II. Q_BH_2 carries some of the energy of light absorbed by PSII.

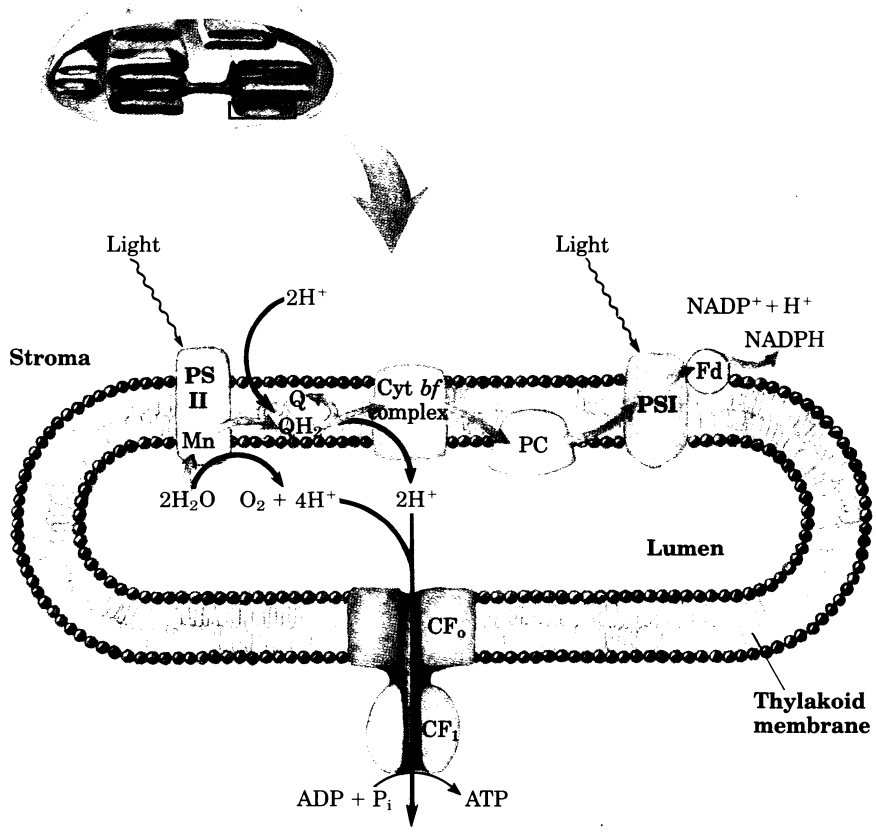


Figure 18–45 Proton and electron circuits in chloroplast thylakoids. Electrons (blue arrows) move from H_2O through photosystem II, the intermediate chain of carriers, photosystem I, and finally to $NADP^+$. Protons (red arrows) are pumped into the thylakoid lumen by the flow of electrons through the chain of carriers between photosystem II and photosystem I, and reenter the stroma through proton channels formed by the F_o portion of the ATP synthase, designated CF_o in the chloroplast enzyme. The F_1 subunit (CF_1) catalyzes synthesis of ATP.

this complex involves a Q cycle (see Fig. 18–10) in which electrons pass from Q_BH_2 to cytochrome *b* one at a time. As in the mitochondrial Complex III, this cycle results in the pumping of protons across the membrane; in chloroplasts, the direction of proton movement is from the stromal compartment to the thylakoid lumen. The result is the production of a proton gradient across the thylakoid membrane as electrons pass from photosystem II to photosystem I (Fig. 18–45). Because the volume of the flattened thylakoid lumen is small, the influx of a small number of protons has a relatively large effect on lumenal pH. The measured difference in pH between the stroma (pH 8) and the thylakoid lumen (pH 4.5) represents a 3,000-fold difference in proton concentration—a powerful driving force for ATP synthesis.

Daniel Arnon

Coupling ATP Synthesis to Light-Driven Electron Flow

We have now seen how one of the two energy-rich products formed in the light reactions, NADPH, is generated by photosynthetic electron transfer from H_2O to $NADP^+$. What about the other energy-rich product, ATP?

In 1954 Daniel Arnon and his colleagues discovered that ATP is generated from ADP and P_i during photosynthetic electron transfer in illuminated spinach chloroplasts. Support for these findings came from the work of Albert Frenkel who detected light-dependent ATP production in membranous pigment-containing structures called **chromatophores,** derived from photosynthetic bacteria. They concluded that some of the light energy captured by the photosynthetic systems of these organisms is transformed into the phosphate bond energy of ATP. This process is called **photophosphorylation** or **photosyn-**

thetic phosphorylation, to distinguish it from oxidative phosphorylation in respiring mitochondria.

Recall that oxidative phosphorylation of ADP to ATP in mitochondria occurs at the expense of the free energy released as high-energy electrons flow downhill along the electron transfer chain from substrates to O_2. In a similar way, photophosphorylation of ADP to ATP is coupled to the energy released as high-energy electrons flow down the photosynthetic electron transfer chain from excited photosystem II to the electron-deficient photosystem I. The direct effect of electron flow is the formation of a proton gradient, which then provides the energy for ATP synthesis by an ATP synthase.

ATP synthesis in chloroplasts can be coupled to two types of electron flow—cyclic and noncyclic—as we shall see. We will also turn our attention to photophosphorylation in organisms other than green plants, and to the possible bacterial origins of chloroplasts. The chapter concludes with the development of an overall equation for photosynthesis in plants.

A Proton Gradient Couples Electron Flow and Phosphorylation

Several properties of photosynthetic electron transfer and photophosphorylation in chloroplasts show a role for a proton gradient as in mitochondrial oxidative phosphorylation: (1) the reaction centers, electron carriers, and ATP-forming enzymes are located in a membrane—the thylakoid membrane; (2) photophosphorylation requires intact thylakoid membranes; (3) the thylakoid membrane is impermeable to protons; (4) photophosphorylation can be uncoupled from electron flow by reagents that promote the passage of protons through the thylakoid membrane; (5) photophosphorylation can be blocked by venturicidin and similar agents that inhibit the formation of ATP from ADP and P_i by ATP synthase of mitochondria (see Fig. 18–13); and (6) ATP synthesis is catalyzed by F_oF_1 complexes, located on the outer surface of the thylakoid membranes, that are very similar in structure and function to the F_oF_1 complexes of mitochondria.

Electron-transferring molecules in the connecting chain between photosystem II and photosystem I are oriented asymmetrically in the thylakoid membrane, so that photoinduced electron flow results in the net movement of protons across the membrane, from the *outside* of the thylakoid membrane to the inner compartment (Fig. 18–45).

In 1966 André Jagendorf showed that a pH gradient across the thylakoid membrane (alkaline outside) could furnish the driving force to generate ATP. Jagendorf's early observations provided some of the most important experimental evidence in support of Mitchell's chemiosmotic hypothesis. In the dark, he soaked chloroplasts in a pH 4 buffer, which slowly penetrated into the inner compartment of the thylakoids, lowering their internal pH. He added ADP and P_i to the dark suspension of chloroplasts and then suddenly raised the pH of the outer medium to 8, momentarily creating a large pH gradient across the membrane. As protons moved out of the thylakoids into the medium, ATP was generated from ADP and P_i. Because the formation of ATP occurred in the dark (with no input of energy from light), this experiment showed that a pH gradient across the membrane is a high-energy state that can, as in mitochondrial oxidative phosphorylation, mediate the transduction of energy from electron transfer into the chemical energy of ATP.

André Jagendorf

The stoichiometry for this process (protons transported per electron) is not well established. Electron transfer between photosystems II and I through the cytochrome *bf* complex contributes to the proton gradient an amount of proton-motive force roughly equivalent to one to two ATP formed per pair of electrons.

The ATP Synthase of Chloroplasts Is Like That of Mitochondria

The enzyme responsible for ATP synthesis in chloroplasts is a large complex with two functional components, CF_o and CF_1 (the C denoting chloroplast origin). CF_o is a transmembrane proton pore composed of several integral membrane proteins and is homologous with mitochondrial F_o. CF_1 is a peripheral membrane protein complex very similar in subunit composition, structure, and function to mitochondrial F_1 (Table 18–8). Together these proteins constitute the ATP synthase of chloroplasts. (Bacteria also contain ATP synthases remarkably similar in structure and function to those of chloroplasts and mitochondria (Table 18–8).)

Table 18–8 Equivalent subunits in ATP synthase of mitochondria, chloroplasts, and bacteria (*E. coli*)*

Portion of ATP synthase	Mitochondria		Chloroplasts		*E. coli*	
	Subunit	Number	Subunit	Number	Subunit	Number
F_1	α	3	α	3	α	3
	β	3	β	3	β	3
	γ	1	γ	1	γ	1
	OSCP	1	δ	1	δ	1
	δ	1	ϵ	1	ϵ	1
	ϵ	1	—	—	—	—
F_o	a	1	a (IV)	2	a	1
	b	1	b and b′ (I and II)	1 + 1	b	2
	c	6–12	c (III)	6–12	c	10–12

Source: Data primarily from Walker, J.E., Lutter, R., Dupuis, A., & Runswick, M.J. (1991) Identification of the subunits of F_1F_o-ATPase from bovine heart mitochondria. *Biochemistry* **30,** 5369–5378.

* Subunits on the same horizontal line are structurally related and are believed to be functionally homologous. Chloroplasts contain two nonidentical subunits, b and b′, that are together the homologs of mitochondrial b; *E. coli* has two identical b subunits. OSCP is oligomycin sensitivity-conferring protein. Alternative nomenclatures for the four chloroplast F_o subunits are shown in parentheses. In addition to the subunits shown here, the mitochondrial enzyme complex contains at least five more proteins with no apparent homologs in the chloroplast or bacterium: F6, inhibitor protein, A6L, d, and e. These subunits are released as soluble proteins when F_1 and F_o are dissociated, but they are believed to be part of the intact, functioning complex in mitochondria.

Electron microscopy of sectioned chloroplasts shows ATP synthase complexes as knoblike projections on the *outside* (stromal) surface of thylakoid membranes; these correspond to the ATP synthase complexes seen to project on the *inside* (matrix) surface of the inner mitochondrial membrane (see Fig. 18–15). Thus both the orientation of the ATP synthase and the direction of proton pumping in chloroplasts are opposite to those in mitochondria. In both cases, the F_1 portion of ATP synthase is located on the more alkaline side of the membrane through which protons flow down their concentration gradient; the direction of proton flow relative to F_1 is the same in both cases (Fig. 18–46).

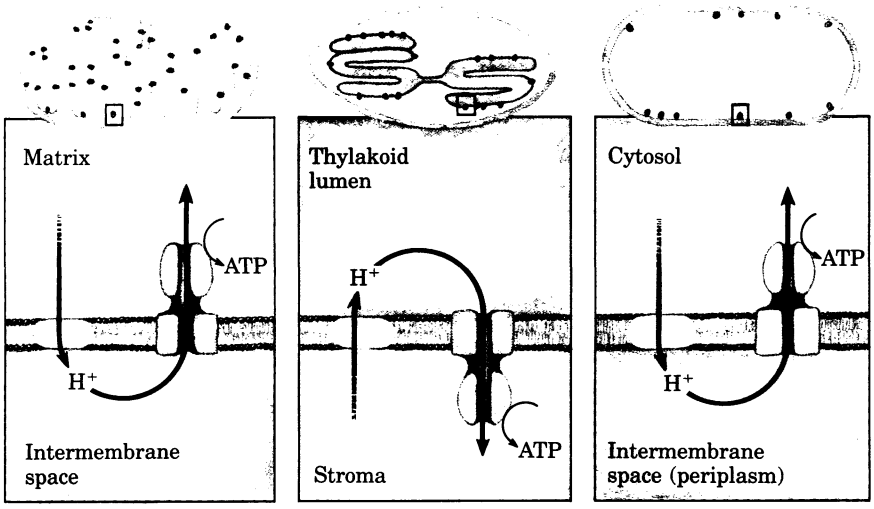


Figure 18–46 Comparison of the topology of proton movement and ATP synthase orientation in the membranes of mitochondria, chloroplasts, and the bacterium *E. coli*. In each case, the orientation of the proton gradient relative to the ATP synthase is the same.

The mechanism of chloroplast ATP synthase is also believed to be essentially identical to that of its mitochondrial analog; ADP and P_i readily condense to form ATP on the enzyme surface, but the release of this enzyme-bound ATP requires a proton-motive force (see Fig. 18–23). As for the mitochondrial ATP synthase, the details of this mechanism remain to be determined.

Cyclic Electron Flow Produces ATP but Not NADPH or O_2

There is an alternative path of light-induced electron flow that allows chloroplasts to vary the ratio of NADPH and ATP formed during illuminations; this is called **cyclic electron flow** to differentiate it from the normally unidirectional or **noncyclic electron flow** that proceeds from H_2O to $NADP^+$, as we have discussed thus far. Cyclic electron flow involves only photosystem I (Fig. 18–44). Electrons passed from P700 to ferredoxin do not continue to $NADP^+$, but move back through the cytochrome *bf* complex to plastocyanin. Plastocyanin donates electrons to P700, the illumination of which promotes electron transfer to ferredoxin. Thus illumination of photosystem I can cause electrons to cycle continuously out of the reaction center of photosystem I and back into it, each electron being propelled around the cycle by the energy yielded by absorption of one photon. Cyclic electron flow is not accompanied by net formation of NADPH or the evolution of O_2. However, it is accompanied by proton pumping and by the phosphorylation of ADP to ATP, referred to as **cyclic photophosphorylation.** The overall reaction equation for cyclic electron flow and photophosphorylation is simply

$$ADP + P_i \xrightarrow{\text{light}} ATP + H_2O$$

Cyclic electron flow and photophosphorylation are believed to occur when the plant cell is already amply supplied with reducing power in the form of NADPH but requires additional ATP for other metabolic needs. By regulating the partitioning of electrons between $NADP^+$ reduction and cyclic photophosphorylation, a plant adjusts the ratio of NADPH and ATP produced in the light reactions to match the needs for these products in the carbon fixation reactions and in other energy-requiring processes.

in which H_2D symbolizes a hydrogen donor and D is its oxidized form. H_2D thus may be water, hydrogen sulfide, lactate, or some other organic compound, depending upon the species.

The Structure of a Bacterial Photosystem Reaction Center Has Been Determined

The three-dimensional structure of the photoreaction center of a photosynthetic bacterium, *Rhodopseudomonas viridis*, which is analogous in some ways to photosystem II in higher plants, is known from x-ray crystallographic studies (Fig. 18–47). This structure sheds light on how phototransduction takes place in the bacterium, and presumably in higher plants as well.

The bacterial reaction center has four types of proteins: a *c*-type cytochrome, two subunits (L and M) associated with bacteriochlorophyll, and a fourth protein, the H subunit. A single reaction center contains four hemes (cytochromes), four bacteriochlorophylls that are similar to the chlorophylls of chloroplasts, two bacteriopheophytins, one inorganic Fe, and a quinone. From a variety of physical studies, the extremely rapid sequence of events shown in Figure 18–47b has been deduced.

A pair of closely spaced bacteriochlorophylls (the "special pair") constitutes the site of the initial photochemistry in the bacterial reaction center. The "electron hole" that develops in the chlorophyll is filled with electrons from the *c*-type cytochrome, a role played by H_2O in photosystem II of higher plants.

The bacteriochlorophylls, bacteriopheophytins, and quinone are held rigidly in a fixed orientation relative to each other by the proteins of the reaction center. The photochemical reactions among these components therefore take place in a virtually solid state, accounting for the high efficiency and rapidity of the reactions; nothing is left to chance collision or dependent upon random diffusion.

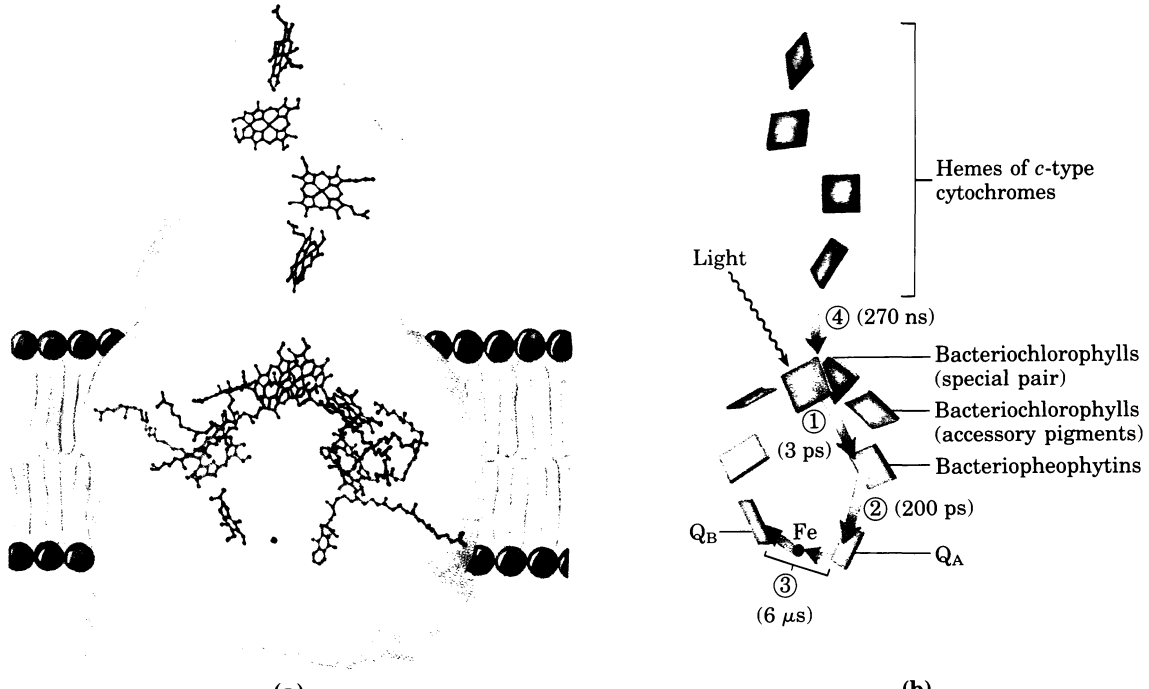


Figure 18–47 The purple sulfur bacterium *Rhodopseudomonas viridis* performs light-driven ATP synthesis using machinery closely similar to photosystem II of higher plants, although the bacterium has no analog of photosystem I. **(a)** Superimposed on the structure of the reaction center proteins (see Fig. 10–11) are the prosthetic groups that participate in the photochemical events. There are four molecules of bacteriochlorophyll, two of which (the "special pair," dark blue) constitute the site of the first photochemical changes after light absorption. The other two bacteriochlorophylls (orange) are called accessory pigments; their role in the photochemical events is not well understood. Two bacterial pheophytins (light blue) lie beneath the bacteriochlorophylls, and beneath them, two bacterial quinones, Q_A and Q_B (green), separated by a non-heme iron atom (red). Shown at the top of the figure are four heme groups (red) associated with *c*-type cytochromes of the reaction center. **(b)** The sequence of events that occurs upon excitation of the special pair of bacteriochlorophylls and the time scale of the electron transfers (in parentheses). The excited special pair passes an electron to bacteriopheophytin ①, from which the electron moves rapidly to the tightly bound quinone Q_A ②. This quinone much more slowly passes electrons to the diffusible quinone Q_B through the non-heme iron atom ③. Meanwhile, the "electron hole" in the special pair is filled by an electron from one of the hemes of the four *c*-type cytochromes ④.

Although the reaction centers of plants have not yet been seen in such detail, the similarities between the bacterial and eukaryotic reaction centers suggest that the principles deduced from studies of the geometry and mechanism of the bacterial reaction center will also apply to the reaction centers of plants.

Salt-Loving Bacteria Use Light Energy to Make ATP

The halophilic ("salt-loving") bacterium *Halobacterium halobium* conserves energy derived from absorbed sunlight by an interesting variation on the principle employed by true photosynthetic organisms. These unusual bacteria live only in brine ponds and salt lakes (Great Salt Lake and the Dead Sea, for example), where the high salt concentration results from water loss by evaporation; indeed, they cannot live in NaCl concentrations lower than 3 M. Halobacteria are aerobes and normally use O_2 to oxidize organic fuel molecules. However, the solubility of O_2 is so low in brine ponds, in which the NaCl concentration may exceed 4 M, that these bacteria must sometimes call on another source of energy, namely sunlight. The plasma membrane of *H. halobium* contains patches of light-absorbing pigments, called **purple patches.** These patches are made up of closely packed molecules of the protein **bacteriorhodopsin** (see Fig. 10–10), which contains retinal (vitamin A aldehyde; see Fig. 9–18) as a prosthetic group. When the cells are illuminated, the bacteriorhodopsin molecules are excited by an absorbed photon. As the excited molecules revert to their initial ground state, an induced conformational change results in the release of protons outside the cell, forming an acid-outside pH gradient across the plasma membrane. Protons tend to diffuse back into the cell through an ATP synthase complex in the membrane, very similar to that of mitochondria and of chloroplasts, supplying the energy for ATP synthesis (Fig. 18–48). Thus halobacteria can use light to supplement the ATP synthesized by oxidative phosphorylation with the O_2 that is available. However, halobacteria do not evolve O_2, nor do they carry out photoreduction of $NADP^+$; their phototransducing machinery is therefore much simpler than that of cyanobacteria or higher plants.

Bacteriorhodopsin, with only 247 amino acid residues, is the simplest light-driven proton pump known. The determination of its molecular structure should yield important insights into light-dependent energy transduction and the action of the proton pumps that function in respiration and photosynthesis.

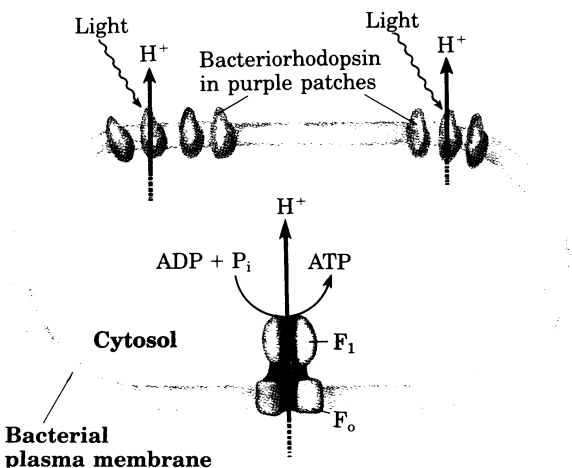


Figure 18–48 Light-driven proton currents in *Halobacterium halobium* provide the proton-motive force for ATP synthesis. Illumination of the phototransducing protein bacteriorhodopsin results in outward proton movement, generating a proton-motive force. Reentry of protons through the F_oF_1 complex provides the energy for ATP synthesis.

Photosynthesis Uses the Energy in Light Very Efficiently

The standard free-energy change for the synthesis of glucose from CO_2 and H_2O during photosynthesis by the reaction

$$6CO_2 + 6H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6O_2 \qquad (18\text{–}9)$$

is 2,840 kJ/mol. (Recall that oxidation of glucose by the reverse of this equation proceeds with a *decrease* of 2,840 kJ/mol.) Now let us compare this energy requirement with the energy yielded by the light reactions of plant photosynthesis.

Recall that two photons must be absorbed, one by each photosystem, to cause flow of one electron from H_2O to $NADP^+$. To generate one molecule of O_2, four electrons must be transferred. Therefore, production of six molecules of O_2 (as in Eqn 18–9) requires the absorption and use of 48 photons: (2 photons/e^-)(4e^-/O_2)(6O_2) = 48 photons. Because the energy of one einstein may range from 300 kJ at 400 nm to about 170 kJ at 700 nm (Fig. 18–35), anywhere from 8,160 to 14,400 kJ (depending upon the wavelength of the absorbed light) is required under standard conditions to make 1 mol of glucose "costing" 2,840 kJ.

In the next chapter we turn to a consideration of the carbon fixation reactions by which photosynthetic organisms use the ATP and NADPH produced in the light reactions to carry out the reduction of CO_2 to carbohydrates.

Summary

Chemiosmotic theory provides the intellectual framework for understanding many biological energy transductions, including the processes of oxidative phosphorylation in mitochondria and photophosphorylation in chloroplasts. The mechanism of energy coupling is similar in both cases. The conservation of free energy involves the passage of electrons through a chain of membrane-bound oxidation–reduction (redox) carriers and the concomitant pumping of protons across the membrane, producing an electrochemical gradient, the protonmotive force. This force drives the synthesis of ATP by membrane-bound enzyme complexes through which protons flow back across the membrane, down their electrochemical gradient. Protonmotive force also drives other energy-requiring processes of cells.

In mitochondria, H atoms removed from substrates by the action of NAD-linked dehydrogenases donate their electrons to the respiratory (electron transfer) chain, which transfers them to molecular O_2, reducing it to H_2O. Shuttle systems convey reducing equivalents from cytosolic NADH to mitochondrial NADH. Reducing equivalents from all NAD-linked dehydrogenations are transferred to mitochondrial NADH dehydrogenase (Complex I), which contains FMN as its prosthetic group. They are then passed via a series of Fe–S centers to ubiquinone, which transfers the electrons to cytochrome *b*, the first carrier in Complex III. In this complex, electrons pass through two *b*-type cytochromes and cytochrome c_1 before reaching an Fe–S center. The Fe–S center passes electrons, one at a time, through cytochrome *c* and into Complex IV, cytochrome oxidase. This copper-containing enzyme, which also contains cytochromes *a* and a_3, accumulates electrons, then passes them to O_2, reducing it to H_2O.

There are alternative paths of entry of electrons into this chain of carriers. Succinate, for example, is oxidized by succinate dehydrogenase (Complex II), which contains a flavoprotein (with FAD) that passes electrons through several Fe–S centers and into the chain at the level of ubiquinone. Electrons derived from the oxidation of fatty acids pass into ubiquinone via the electron-transferring flavoprotein (ETFP).

The flow of electrons through Complexes I, III, and IV results in the pumping of protons across the mitochondrial inner membrane, making the matrix alkaline relative to the extramitochondrial space. This proton gradient provides the energy (proton-motive force) for ATP synthesis from ADP and P_i by an inner-membrane protein complex, ATP synthase, also called F_oF_1 ATPase. The details of this ATP-synthesizing mechanism are still under investigation. Bacteria carry out oxidative phosphorylation by essentially the same mechanism, using electron carriers and an ATP synthase in the plasma membrane. Oxidative phosphorylation produces most of the ATP required by aerobic cells; it is regulated by cellular energy demands. In brown fat tissue, which is specialized for the production of metabolic heat, electron transfer is uncoupled from ATP synthesis; the energy of fatty acid oxidation is therefore dissipated as heat.

Photophosphorylation in the chloroplasts of green plants and in cyanobacteria also involves electron flow through a series of membrane-bound carriers. In the light reactions of plants, the absorption of a photon excites chlorophyll molecules and other (accessory) pigments that funnel the energy into reaction centers in the thylakoid membranes of chloroplasts. At the reaction centers, photoexcitation results in a charge separation that produces one chemical species that is a good electron donor (reducing agent) and another that is a good electron acceptor. In chloroplasts there are two different photoreaction centers, which function together. Photosystem I passes electrons from its excited reaction center, P700, through a series of carriers to ferredoxin, which then reduces $NADP^+$ to NADPH. The reaction center, P680, of photosystem II passes electrons to plastoquinone, reducing it to the quinol form. The electrons lost from P680 are replaced by electrons abstracted from H_2O (hydrogen donors other than H_2O are used in other organisms). This light-driven splitting of H_2O is catalyzed by a Mn-containing protein complex; O_2 is produced. Reduced plastoquinone carries electrons from photosystem II to the cytochrome *bf* complex; these electrons pass to the soluble protein plastocyanin, and then to P700 to replace those lost during its photoexcitation. Electron flow through the cytochrome *bf* complex is accompanied by proton pumping across the thylakoid membrane, and the proton-motive force thus created drives ATP synthesis by a CF_oCF_1 complex closely similar to the F_oF_1 complex of mitochondria. This flow of electrons through photosystems II and I thus produces both NADPH and ATP. A second type of electron flow (cyclic flow) produces ATP only.

Both mitochondria and chloroplasts contain their own genomes and are believed to have originated from prokaryotic endosymbionts of early eukaryotic cells. Oxidative phosphorylation in aerobic bacteria and photophosphorylation in photosynthetic bacteria are closely similar, in machinery and mechanism, to the homologous processes in mitochondria and chloroplasts.

Further Reading

History and Background

Arnon, D.I. (1984) The discovery of photosynthetic phosphorylation. *Trends Biochem. Sci.* **9,** 258–262.

Harold, F.M. (1986) *The Vital Force: A Study in Bioenergetics,* W.H. Freeman and Company, New York.

A very readable synthesis of the principles of bioenergetics and their application to energy transductions.

Kalckar, H.M. (1991) 50 years of biological research—from oxidative phosphorylation to energy requiring transport regulation. *Annu. Rev. Biochem.* **60,** 1–37.

A delightful autobiographical account by one of the pioneers in the field.

Keilin, D. (1966) *The History of Cell Respiration and Cytochrome,* Cambridge University Press, London.

An authoritative and absorbing account of the discovery of cytochromes and of their roles in respiration, written by the discoverer of cytochromes.

Lehninger, A.L. (1964) *The Mitochondrion: Molecular Basis of Structure and Function,* The Benjamin Co., Inc., New York.

A classic description of early work on mitochondria.

Mitchell, P. (1979) Keilin's respiratory chain concept and its chemiosmotic consequences. *Science* **206,** 1148–1159.

The author's Nobel lecture, outlining the evolution of the chemiosmotic hypothesis.

Skulachev, V.P. (1992) The laws of cell energetics. *Eur. J. Biochem.* **208,** 203–209.

On the interconvertibility of ATP and ion gradients.

Slater, E.C. (1987) The mechanism of the conservation of energy of biological oxidations. *Eur. J. Biochem.* **166,** 489–504.

A clear and critical account of the evolution of the chemiosmotic model.

Staehelin, L.A. & Arntzen, C.J. (eds) (1986) *Photosynthesis III: Photosynthetic Membranes and Light Harvesting Systems,* Encyclopedia of Plant Physiology, Vol. 19, Springer-Verlag, Berlin.

Authoritative reviews of many aspects of photosynthesis.

Respiratory Electron Flow

Babcock, G.T. & Wickström, M. (1992) Oxygen activation and the conservation of energy in cell respiration. *Nature* **356,** 301–309.

An advanced discussion of the reduction of water and pumping of protons by cytochrome oxidase.

Douce, R. & Neuburger, M. (1989) The uniqueness of plant mitochondria. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40,** 371–414.

A focus on the features of plant mitochondria that distinguish them from mitochondria of animal cells.

Hinkle, P.C. & McCarty, R.E. (1978) How cells make ATP. *Sci. Am.* **238** (March), 104–123.

Although not recent, this is an excellent, readable, and well-illustrated description of oxidative phosphorylation.

Lehninger, A.L., Reynafarje, B., Alexandre, A., & Villalobo, A. (1980) Respiration-coupled H^+ ejection by mitochondria. *Ann. N. Y. Acad. Sci.* **341,** 585–592.

The methods and problems in measuring proton efflux stoichiometry.

Malmström, B.G. (1989) The mechanism of proton translocation in respiration and photosynthesis. *FEBS Lett.* **250,** 9–21.

Comparative review of the electron-transferring complexes of mitochondria and chloroplasts.

Trumpower, B.L. (1990) The protonmotive Q cycle: energy transduction by coupling of proton translocation to electron transfer by the cytochrome bc_1 complex. *J. Biol. Chem.* **265,** 11409–11412.

Short, clear description of the Q cycle and electron flow through Complex III.

Coupling ATP Synthesis to Respiratory Electron Flow

Boyer, P.D. (1989) A perspective of the binding change mechanism for ATP synthesis. *FASEB J.* **3,** 2164–2178.

An article on the historical development and current state of the binding-change model, by its principal architect.

Futai, M., Noumi, T., & Maeda, M. (1987) Molecular biological studies on structure and mechanism of proton translocating ATPase (H^+-ATPase, F_oF_1). *Adv. Biophys.* **23,** 1–37.

Insight into the mechanism of ATP synthase from studies of the genes that encode its subunits.

Pedersen, P.L. & Carafoli, E. (1987) Ion motive ATPases. I. Ubiquity, properties, and significance to cell function. *Trends Biochem. Sci.* **12,** 145–150. II. Energy coupling and work output. *Trends Biochem. Sci.* **12,** 186–189.

Two short reviews that place ATP synthase within the family of ATP-dependent proton pumps; include their general mechanisms.

Penefsky, H.S. & Cross, R.L. (1991) Structure and mechanism of F_oF_1-type ATP synthases and ATPases. *Adv. Enzymol. Relat. Areas Mol. Bio.* **64,** 173–214.

An advanced discussion.

Ricquier, D., Casteilla, L., & Bouillaud, F. (1991) Molecular studies of the uncoupling protein. *FASEB J.* **5,** 2237–2242.

A discussion of the protein and its role in thermogenesis.

Senior, A.E. (1988) ATP synthesis by oxidative phosphorylation. *Physiol. Rev.* **68,** 177–231.

An advanced but very clear review, with an emphasis on the mechanism of ATP synthase.

Regulation of Mitochondrial Oxidative Phosphorylation

Brand, M.D. & Murphy, M.P. (1987) Control of electron flux through the respiratory chain in mitochondria and cells. *Biol. Rev. Cambridge Phil. Soc.* **62,** 141–193.

An advanced description of respiratory control.

Harris, D.A. & Das, A.M. (1991) Control of mitochondrial ATP synthesis in the heart. *Biochem. J.* **280,** 561–573.

An advanced discussion of regulation of the ATP synthase by Ca^{2+} and other factors.

Photosynthesis: Harvesting Light Energy

Green, B.R., Pichersky, E., & Kloppstech, K. (1991) Chlorophyll *a*/*b*-binding proteins: an extended family. *Trends Biochem. Sci.* **16,** 181–186.
An intermediate-level description of the proteins that orient chlorophyll molecules in chloroplasts.

Huber, R. (1990) A structural basis of light energy and electron transfer in biology. *Eur. J. Biochem.* **187,** 283–305.
The author's Nobel lecture, describing the physics and chemistry of phototransductions. An exceptionally clear and well-illustrated discussion, based on crystallographic studies of reaction centers.

Zuber, H. (1986) Structure of light-harvesting antenna complexes of photosynthetic bacteria, cyanobacteria and red algae. *Trends Biochem. Sci.* **11,** 414–419.

Light-Driven Electron Flow

Andréasson, L.-E. & Vänngård, T. (1988) Electron transport in photosystems I and II. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39,** 379–411.
An advanced description of the path of electron flow in chloroplasts, studied with spectroscopic techniques.

Blankenship, R.E. & Prince, R.C. (1985) Excited-state redox potentials and the Z scheme of photosynthesis. *Trends Biochem. Sci.* **10,** 382–383.
A concise and lucid statement of the redox properties of excited states.

Deisenhofer, J. & Michel, H. (1991) Structures of bacterial photosynthetic reaction centers. *Annu. Rev. Cell Biol.* **7,** 1–23.
The structure of the reaction center of purple bacteria, and implications for the function of bacterial and plant reaction centers.

Glazer, A.N. & Melis, A. (1987) Photochemical reaction centers: structure, organization, and function. *Annu. Rev. Plant Physiol.* **38,** 11–45.
An advanced description of the structure and function of reaction centers of green plants, cyanobacteria, and purple and green bacteria.

Golbeck, J.H. (1992) Structure and function of photosystem I. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **43,** 293–324.

Govindjee & Coleman, W.J. (1990) How plants make oxygen. *Sci. Am.* **262** (February), 50–58.
An exceptionally clear account of the water-splitting activity of photosystem II.

Nitschke, W. & Rutherford, A.W. (1991) Photosynthetic reaction centres: variations on a common structural theme? *Trends Biochem. Sci.* **16,** 241–245.
A comparison of the structure and function of photosystems I and II and the reaction centers of several photosynthetic bacteria.

Coupling ATP Synthesis to Light-Driven Electron Flow

Cramer, W.A., Widger, W.R., Herrmann, R.G., & Trebst, A. (1985) Topography and function of thylakoid membrane proteins. *Trends Biochem. Sci.* **10,** 125–129.

Jagendorf, A.T. (1967) Acid-base transitions and phosphorylation by chloroplasts. *Fed. Proc.* **26,** 1361–1369.
The classic experiment establishing the ability of a proton gradient to drive ATP synthesis in the dark.

Youvan, D.C. & Marrs, B.L. (1987) Molecular mechanisms of photosynthesis. *Sci. Am.* **256** (June), 42–48.
An excellent description of the chemical basis for light reactions.

Problems

1. *Oxidation–Reduction Reactions* The NADH dehydrogenase complex of the mitochondrial respiratory chain promotes the following series of oxidation–reduction reactions, in which Fe^{3+} and Fe^{2+} represent the iron in iron–sulfur centers, UQ is ubiquinone, UQH_2 is ubiquinol, and E is the enzyme:

(1) $NADH + H^+ + E\text{–}FMN \rightarrow NAD^+ + E\text{–}FMNH_2$
(2) $E\text{–}FMNH_2 + 2Fe^{3+} \rightarrow E\text{–}FMN + 2Fe^{2+} + 2H^+$
(3) $2Fe^{2+} + 2H^+ + UQ \rightarrow 2Fe^{3+} + UQH_2$
Sum: $NADH + H^+ + UQ \rightarrow NAD^+ + UQH_2$

For each of the three reactions catalyzed by the NADH dehydrogenase complex, identify (a) the

electron donor, (b) the electron acceptor, (c) the conjugate redox pair, (d) the reducing agent, and (e) the oxidizing agent.

2. *Standard Reduction Potentials* The standard reduction potential of any redox couple is defined for the half-cell reaction (or half-reaction):

$$\text{Oxidizing agent} + n \text{ electrons} \longrightarrow \text{reducing agent}$$

The standard reduction potentials of the NAD^+/NADH and pyruvate/lactate redox pairs are -0.320 and -0.185 V, respectively.

(a) Which redox pair has the greater tendency to lose electrons? Explain.

(b) Which is the stronger oxidizing agent? Explain.

(c) Beginning with 1 M concentrations of each reactant and product at pH 7, in which direction will the following reaction proceed?

$$\text{Pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{lactate} + NAD^+$$

(d) What is the standard free-energy change, $\Delta G^{\circ\prime}$, at 25 °C for this reaction?

(e) What is the equilibrium constant for this reaction at 25 °C?

3. *Energy Span of the Respiratory Chain* Electron transfer in the mitochondrial respiratory chain may be represented by the net reaction equation

$$\text{NADH} + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons H_2O + NAD^+$$

(a) Calculate the value of the change in standard reduction potential, $\Delta E'_0$, for the net reaction of mitochondrial electron transfer.

(b) Calculate the standard free-energy change, $\Delta G^{\circ\prime}$, for this reaction.

(c) How many ATP molecules could *theoretically* be generated per molecule of NADH oxidized by this reaction, given a standard free energy of ATP synthesis of 30.5 kJ/mol?

(d) How many ATP molecules could be synthesized under typical cellular conditions (see Box 13–2)?

4. *Use of FAD Rather Than NAD^+ in the Oxidation of Succinate* All the dehydrogenation steps in glycolysis and the citric acid cycle use NAD^+ (E'_0 for NAD^+/NADH = -0.32 V) as the electron acceptor except succinate dehydrogenase, which uses covalently bound FAD (E'_0 for FAD/$FADH_2$ in this enzyme = 0.05 V). Why is FAD a more appropriate electron acceptor than NAD^+ in the dehydrogenation of succinate? Give a possible explanation based on a comparison of the E'_0 values of the fumarate/succinate pair ($E'_0 = 0.03$), the NAD^+/NADH pair, and the succinate dehydrogenase FAD/$FADH_2$ pair.

5. *Degree of Reduction of Electron Carriers in the Respiratory Chain* The degree of reduction of each electron carrier in the respiratory chain is determined by the conditions existing in the mitochondrion. For example, when the supply of NADH and O_2 is abundant, the steady-state degree of reduction of the carriers decreases as electrons pass from the substrate to O_2. When electron transfer is blocked, the carriers before the block become more reduced while those beyond the block become more oxidized (Fig. 18–7). For each of the conditions below, predict the state of oxidation of each carrier in the respiratory chain (ubiquinone and cytochromes b, c_1, c, and $a + a_3$).

(a) Abundant supply of NADH and O_2 but cyanide added

(b) Abundant supply of NADH but O_2 exhausted

(c) Abundant supply of O_2 but NADH exhausted

(d) Abundant supply of NADH and O_2

6. *The Effect of Rotenone and Antimycin A on Electron Transfer* Rotenone, a toxic natural product from plants, strongly inhibits NADH dehydrogenase of insect and fish mitochondria. Antimycin A, a toxic antibiotic, strongly inhibits the oxidation of ubiquinol.

(a) Explain why rotenone ingestion is lethal to some insect and fish species.

(b) Explain why antimycin A is a poison.

(c) Assuming that rotenone and antimycin A are equally effective in blocking their respective sites in the electron transfer chain, which would be a more potent poison? Explain.

7. *Uncouplers of Oxidative Phosphorylation* In normal mitochondria the rate of electron transfer is tightly coupled to the demand for ATP. Thus when the rate of utilization of ATP is relatively low, the rate of electron transfer is also low. Conversely, when ATP is demanded at a high rate, electron transfer is rapid. Under such conditions of tight coupling, the number of ATP molecules produced per atom of oxygen consumed when NADH is the electron donor—known as the P/O ratio—is close to 3.

(a) Predict the effect of a relatively low and a relatively high concentration of an uncoupling agent on the rate of electron transfer and the P/O ratio.

(b) The ingestion of uncouplers causes profuse sweating and an increase in body temperature. Explain this phenomenon in molecular terms. What happens to the P/O ratio in the presence of uncouplers?

(c) The uncoupler 2,4-dinitrophenol was once prescribed as a weight-reducing drug. How can this agent, in principle, serve as a reducing aid? Such uncoupling agents are no longer prescribed because some deaths occurred following their use. Why can the ingestion of uncouplers lead to death?

8. *Mode of Action of Dicyclohexylcarbodiimide (DCCD)* When DCCD is added to a suspension of tightly coupled, actively respiring mitochondria, the rate of electron transfer (measured by O_2 consumption) and the rate of ATP production dramatically decrease. If a solution of 2,4-dinitrophenol is

now added to the inhibited mitochondrial preparation, O_2 consumption returns to normal but ATP production remains inhibited.

(a) What process in electron transfer or oxidative phosphorylation is affected by DCCD?

(b) Why does DCCD affect the O_2 consumption of mitochondria? Explain the effect of 2,4-dinitrophenol on the inhibited mitochondrial preparation.

(c) Which of the following inhibitors does DCCD most resemble in its action: antimycin A, rotenone, or oligomycin?

9. *The Malate–α-Ketoglutarate Transport System of Mitochondria* The inner mitochondrial membrane transport system that promotes the transport of malate and α-ketoglutarate across the membrane (Fig. 18–25) is inhibited by *n*-butylmalonate. Suppose *n*-butylmalonate is added to an aerobic suspension of kidney cells using glucose exclusively as fuel. Predict the effect of this inhibitor on

(a) Glycolysis
(b) Oxygen consumption
(c) Lactate formation
(d) ATP synthesis

10. *The Pasteur Effect* When O_2 is added to an anaerobic suspension of cells using glucose at a high rate, the rate of glucose consumption declines dramatically as the added O_2 is consumed. In addition, the accumulation of lactate ceases. This effect, first observed by Louis Pasteur in the 1860s, is characteristic of most cells capable of both aerobic and anaerobic utilization of glucose.

(a) Why does the accumulation of lactate cease after O_2 is added?

(b) Why does the presence of O_2 decrease the rate of glucose consumption?

(c) How does the onset of O_2 consumption slow down the rate of glucose consumption? Explain in terms of specific enzymes.

11. *How Many Protons in a Mitochondrion?* Electron transfer functions to translocate protons from the mitochondrial matrix to the external medium to establish a pH gradient across the inner membrane, the outside more acidic than the inside. The tendency of protons to diffuse from the outside into the matrix, where $[H^+]$ is lower, is the driving force for ATP synthesis via the ATP synthase. During oxidative phosphorylation by a suspension of mitochondria in a medium of pH 7.4, the internal pH of the matrix has been measured as 7.7.

(a) Calculate $[H^+]$ in the external medium and in the matrix under these conditions.

(b) What is the outside:inside ratio of $[H^+]$? Comment on the energy inherent in this concentration. (Hint: See p. 383, Eqn 13–5.)

(c) Calculate the number of protons in a respiring liver mitochondrion, assuming its inner matrix compartment is a sphere of diameter 1.5 μm.

(d) From these data would you think the pH gradient alone is sufficiently great to generate ATP?

(e) If not, can you suggest how the necessary energy for synthesis of ATP arises?

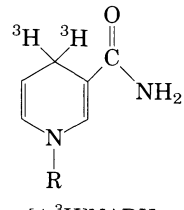
12. *Rate of ATP Turnover in Rat Heart Muscle* Rat heart muscle operating aerobically fills more than 90% of its ATP needs by oxidative phosphorylation. This tissue consumes O_2 at the rate of 10 μmol/min • g of tissue, with glucose as the fuel source.

(a) Calculate the rate at which this tissue consumes glucose and produces ATP.

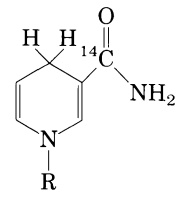
(b) If the steady-state concentration of ATP in rat heart muscle is 5 μmol/g of tissue, calculate the time required (in seconds) to completely turn over the cellular pool of ATP. What does this result indicate about the need for tight regulation of ATP production? (Note: Concentrations are expressed as micromoles per gram of muscle tissue because the tissue is mostly water.)

13. *Rate of ATP Breakdown in Flight Muscle* ATP production in the flight muscles of the fly *Lucilia sericata* results almost exclusively from oxidative phosphorylation. During flight, 187 ml of O_2/h • g of fly body weight is needed to maintain an ATP concentration of 7 μmol/g of flight muscle. Assuming that the flight muscles represent 20% of the weight of the fly, calculate the rate at which the flight-muscle ATP pool turns over. How long would the reservoir of ATP last in the absence of oxidative phosphorylation? Assume that reducing equivalents are transferred by the glycerol-3-phosphate shuttle and that O_2 is at 25 °C and 101.3 kPa (1 atm). (Note: Concentrations are expressed in micromoles per gram of flight muscle.)

14. *Transmembrane Movement of Reducing Equivalents* Under aerobic conditions, extramitochondrial NADH must be oxidized by the mitochondrial electron transfer chain. Consider a preparation of rat hepatocytes containing mitochondria and all the enzymes of the cytosol. If [4–^{3}H]NADH is introduced, radioactivity appears quickly in the mitochondrial matrix. However, if [7–^{14}C]NADH is introduced, no radioactivity appears in the matrix. What do these observations tell us about the oxidation of extramitochondrial NADH by the electron transfer chain?



[4-^{3}H]NADH



[7-^{14}C]NADH

15. *Photochemical Efficiency of Light at Different Wavelengths* The rate of photosynthesis, measured by O_2 production, is higher when a green plant is illuminated with light of wavelength 680 nm than with light of 700 nm. However, illumination by a combination of light of 680 nm and 700 nm gives a higher rate of photosynthesis than light of either wavelength alone. Explain.

16. *Role of H_2S in Some Photosynthetic Bacteria* Illuminated purple sulfur bacteria carry out photosynthesis in the presence of H_2O and $^{14}CO_2$, but only if H_2S is added and O_2 is absent. During the course of photosynthesis, measured by formation of [^{14}C]carbohydrate, H_2S is converted into elemental sulfur, but no O_2 is evolved. What is the role of the conversion of H_2S into sulfur? Why is no O_2 evolved?

17. *Boosting the Reducing Power of Photosystem I by Light Absorption* When photosystem I absorbs red light at 700 nm, the standard reduction potential of P700 changes from 0.4 to about −1.2 V. What fraction of the absorbed light is trapped in the form of reducing power?

18. *Mode of Action of the Herbicide DCMU* When chloroplasts are treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, or Diuron), a potent herbicide, O_2 evolution and photophosphorylation cease. Oxygen evolution but not photophosphorylation can be restored by the addition of an external electron acceptor, or Hill reagent. How does this herbicide act as a weed killer? Suggest a location for the inhibitory site of this herbicide in the scheme shown in Figure 18–44. Explain.

19. *Bioenergetics of Photophosphorylation* The steady-state concentrations of ATP, ADP, and P_i in isolated spinach chloroplasts under full illumination at pH 7.0 are 120, 6, and 700 μM, respectively.

(a) What is the free-energy requirement for the synthesis of 1 mol of ATP under these conditions?

(b) The energy for ATP synthesis is furnished by light-induced electron transfer in the chloroplasts. What is the minimum voltage drop necessary during the transfer of a pair of electrons to synthesize ATP under these conditions? (You may need to refer to p. 389, Eqn 13–8.)

20. *Equilibrium Constant for Water-Splitting Reactions* The coenzyme $NADP^+$ is the terminal electron acceptor in chloroplasts, according to the reaction

$$2H_2O + 2NADP^+ \longrightarrow 2NADPH + 2H^+ + O_2$$

Use the information in Table 18–2 to calculate the equilibrium constant at 25 °C for this reaction. (The relationship between K'_{eq} and $\Delta G^{\circ\prime}$ is discussed on p. 368.) How can the chloroplast overcome this unfavorable equilibrium?

21. *Energetics of Phototransduction* During photosynthesis, eight photons of light must be absorbed (four by each photosystem) for every O_2 molecule produced:

$$2H_2O + 2NADP^+ + 8 \text{ photons} \longrightarrow 2NADPH + 2H^+ + O_2$$

Assuming that these photons have a wavelength of 700 nm (red) and that the absorption and utilization of light energy are 100% efficient, calculate the free-energy change for the process.

22. *Electron Transfer to a Hill Reagent* Isolated spinach chloroplasts evolve O_2 when illuminated in the presence of potassium ferricyanide (the Hill reagent), according to the equation

$$2H_2O + 4Fe^{3+} \longrightarrow O_2 + 4H^+ + 4Fe^{2+}$$

where Fe^{3+} represents ferricyanide and Fe^{2+}, ferrocyanide. Is NADPH produced in this process? Explain.

23. *How Often Does a Chlorophyll Molecule Absorb a Photon?* The amount of chlorophyll *a* (M_r 892) in a spinach leaf is about 20 μg/cm^2 of leaf. In noonday sunlight (average energy 5.4 J/cm^2 • min), the leaf absorbs about 50% of the radiation. How often does a single chlorophyll molecule absorb a photon? If the average lifetime of an excited chlorophyll molecule in vivo is 1 ns, what fraction of chlorophyll molecules are excited at any one time?

24. *Effect of Monochromatic Light on Electron Flow* The extent to which an electron carrier is oxidized or reduced during photosynthetic electron transfer can sometimes be observed directly with a spectrophotometer. When chloroplasts are illuminated with 700 nm light, cytochrome *f*, plastocyanin, and plastoquinone are oxidized. When chloroplasts are illuminated with 680 nm light, however, these electron carriers are reduced. Explain.

25. *Function of Cyclic Photophosphorylation* When the [NADPH]/[$NADP^+$] ratio in chloroplasts is high, photophosphorylation is predominantly cyclic (Fig. 18–44). Is O_2 evolved during cyclic photophosphorylation? Explain. Can the chloroplast produce NADPH this way? What is the main function of cyclic photophosphorylation?