required for success in the microbiology laboratory, and it is one of the first methods learned by the novice microbiologist. Airborne contaminants are the most common problem because the dust in laboratory air contains microorganisms. When containers are opened, they must be handled in such a way that contaminant-laden air does not enter (Figures 4.4 and 4.5).

Aseptic transfer of a culture from one tube of medium to another is typically accomplished with an inoculating loop or needle that has previously been sterilized in a flame (Figure 4.4). Cells from liquid cultures can also be transferred to the surface of agar plates where colonies develop from the growth and division of single cells (Figure 4.5). Picking an isolated colony and restreaking it is the main method for obtaining pure cultures from samples containing several different organisms.

MiniQuiz

- What is meant by the word sterile? What would happen if freshly prepared culture media were not sterilized and then left at room temperature?
- Why is aseptic technique necessary for successful cultivation of pure cultures in the laboratory?

II Energetics and Enzymes

Regardless of how a microorganism makes a living—whether by chemoorganotrophy, chemolithotrophy, or phototrophy—it must be able to conserve some of the energy released in its energy-yielding reactions. Here we discuss the principles of energy conservation, using some simple laws of chemistry and physics to guide our understanding. We then consider enzymes, the cell's catalysts.

4.4 Bioenergetics

Energy is the ability to do work. In microbiology, energy is measured in kilojoules (kJ), a unit of heat energy. All chemical reactions in a cell are accompanied by *changes* in energy, energy either being required for or released during the reaction.

Basic Energetics

Although in any chemical reaction some energy is lost as heat, in microbiology we are interested in **free energy** (abbreviated ***G***), which is the energy available to do work. The *change* in free energy during a reaction is expressed as $\Delta G^{0\prime}$, where the symbol Δ is read as "change in." The "0" and "prime" superscripts indicate that the free-energy value is for standard conditions: pH 7, 25°C, 1 atmosphere of pressure, and all reactants and products at molar concentrations.

Consider the reaction

$$A + B \rightarrow C + D$$

If $\Delta G^{0\prime}$ for this reaction is *negative* in arithmetic sign, then the reaction will proceed with the *release* of free energy, energy that the cell may conserve as ATP. Such energy-yielding reactions are called **exergonic**. However, if $\Delta G^{0\prime}$ is *positive*, the reaction *requires* energy in order to proceed. Such reactions are called **endergonic**. Thus, exergonic reactions *release* energy whereas endergonic reactions *require* energy.

Table 4.3 *Free energy of formation for a few compounds of biological interest*

Compound	*Free energy of formation (G_f^0)*[a]
Water (H_2O)	−237.2
Carbon dioxide (CO_2)	−394.4
Hydrogen gas (H_2)	0
Oxygen gas (O_2)	0
Ammonium (NH_4^+)	−79.4
Nitrous oxide (N_2O)	+104.2
Acetate ($C_2H_3O_2^-$)	−369.4
Glucose ($C_6H_{12}O_6$)	−917.3
Methane (CH_4)	−50.8
Methanol (CH_3OH)	−175.4

[a]The free energy of formation values are in kJ/mol. See Table A1.1 in Appendix 1 for a more complete list of free energies of formation.

Free Energy of Formation and Calculating $\Delta G^{0\prime}$

To calculate the free-energy yield of a reaction, one first needs to know the free energy of its reactants and products. This is the free energy of formation (G_f^0), the energy released or required during the formation of a given molecule from the elements. Table 4.3 gives a few examples of G_f^0. By convention, the free energy of formation of the elements in their elemental and electrically neutral form (for instance, C, H_2, N_2) is zero. The free energies of formation of compounds, however, are not zero. If the formation of a compound from its elements proceeds exergonically, then the G_f^0 of the compound is negative (energy is released). If the reaction is endergonic, then the G_f^0 of the compound is positive (energy is required).

For most compounds G_f^0 is negative. This reflects the fact that compounds tend to form spontaneously (that is, with energy being released) from their elements. However, the positive G_f^0 for nitrous oxide (N_2O) (+104.2 kJ/mol, Table 4.3) indicates that this compound does not form spontaneously. Instead, over time it decomposes spontaneously to yield N_2 and O_2. The free energies of formation of more compounds of microbiological interest are given in Appendix 1.

Using free energies of formation, it is possible to calculate $\Delta G^{0\prime}$ of a given reaction. For the reaction $A + B \rightarrow C + D$, $\Delta G^{0\prime}$ is calculated by subtracting the sum of the free energies of formation of the reactants (A + B) from that of the products (C + D). Thus

$$\Delta G^{0\prime} = G_f^0[C + D] - G_f^0[A + B]$$

The value obtained for $\Delta G^{0\prime}$ tells us whether the reaction is exergonic or endergonic. The phrase "products minus reactants" is a simple way to recall how to calculate changes in free energy during chemical reactions. However, before free-energy calculations can be made, it is first necessary to balance the reaction.

Appendix 1 details the steps in balancing reactions both electrically and atomically and calculating free energies for any hypothetical reaction.

$\Delta G^{0\prime}$ versus ΔG

Although calculations of $\Delta G^{0\prime}$ are usually reasonable estimates of actual free-energy changes, under some circumstances they are not. We will see later in this book that the actual concentrations of products and reactants in nature, which are rarely at molar levels, can alter the bioenergetics of reactions, sometimes in significant ways. Thus, what may be most relevant to a bioenergetic calculation is not $\Delta G^{0\prime}$, but ΔG, the free-energy change that occurs under the actual conditions in which the organism is growing. The equation for ΔG takes into account the actual concentrations of reactants and products in the reaction and is

$$\Delta G = \Delta G^{0\prime} + RT \ln K$$

where R and T are physical constants and K is the equilibrium constant for the reaction (Appendix 1). We distinguish between $\Delta G^{0\prime}$ and ΔG in important ways in Chapter 14, where we consider metabolic diversity in more detail, but for now, we only need to focus on the expression $\Delta G^{0\prime}$ and what it tells us about a chemical reaction catalyzed by a microorganism. Only reactions that are exergonic yield energy that can be conserved by the cell as ATP.

MiniQuiz

- What is free energy?
- Using the data in Table 4.3, calculate $\Delta G^{0\prime}$ for the reaction $CH_4 + \frac{1}{2}O_2 \rightarrow CH_3OH$. How does $\Delta G^{0\prime}$ differ from ΔG?
- Does glucose formation from the elements release or require energy?

4.5 Catalysis and Enzymes

Free-energy calculations reveal only whether energy is released or required in a given reaction. The value obtained says nothing about the *rate* of the reaction. Consider the formation of water from gaseous oxygen (O_2) and hydrogen (H_2). The energetics of this reaction are quite favorable: $H_2 + \frac{1}{2}O_2 \rightarrow H_2O$, $\Delta G^{0\prime} = -237$ kJ. However, if we were to mix O_2 and H_2 together in a sealed bottle and leave it for years, no measurable amount of water would form. This is because the bonding of oxygen and hydrogen atoms to form water requires that their chemical bonds first be broken. The breaking of these bonds requires some energy, and this energy is called **activation energy**.

Activation energy is the energy required to bring all molecules in a chemical reaction into the reactive state. For a reaction that proceeds with a net release of free energy (that is, an exergonic reaction), the situation is as diagrammed in Figure 4.6. Although the activation energy barrier is virtually insurmountable in the absence of a catalyst, in the presence of the proper catalyst, this barrier is greatly reduced.

Enzymes

The concept of activation energy leads us to consider catalysis and enzymes. A **catalyst** is a substance that lowers the activation energy of a reaction, thereby increasing the reaction rate. Catalysts facilitate reactions but are not consumed or transformed by them. Moreover, catalysts do not affect the energetics or the equilibrium of a reaction; catalysts affect only the *rate* at which reactions proceed.

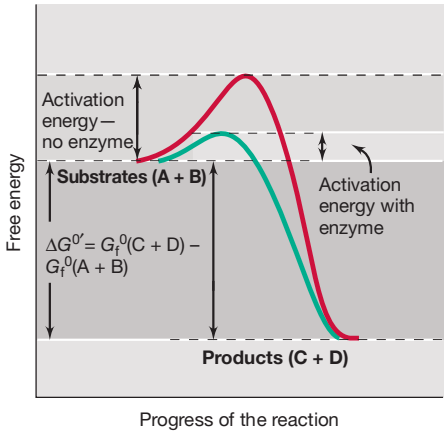


Figure 4.6 Activation energy and catalysis. Even chemical reactions that release energy may not proceed spontaneously, because the reactants must first be activated. Once they are activated, the reaction proceeds spontaneously. Catalysts such as enzymes lower the required activation energy.

Most cellular reactions do not proceed at useful rates without catalysis. Biological catalysts are called **enzymes**. Enzymes are proteins (or in a few cases, RNAs) that are highly specific for the reactions they catalyze. That is, each enzyme catalyzes only a single type of chemical reaction, or in the case of some enzymes, a single class of closely related reactions. This specificity is a function of the precise three-dimensional structure of the enzyme molecule.

In an enzyme-catalyzed reaction, the enzyme (E) combines with the reactant, called a *substrate* (S), forming an enzyme–substrate complex (E—S). Then, as the reaction proceeds, the *product* (P) is released and the enzyme is returned to its original state:

$$E + S \leftrightarrows E\text{—}S \leftrightarrows + P$$

The enzyme is generally much larger than the substrate(s), and the portion of the enzyme to which substrate binds is called the *active site*; the entire enzymatic reaction, from substrate binding to product release, may take only a few milliseconds.

Many enzymes contain small nonprotein molecules that participate in catalysis but are not themselves substrates. These small molecules can be divided into two classes based on the way they associate with the enzyme: *prosthetic groups* and *coenzymes*. Prosthetic groups bind very tightly to their enzymes, usually covalently and permanently. The heme group present in cytochromes (Section 4.9) is an example of a prosthetic group. **Coenzymes**, by contrast, are loosely bound to enzymes, and a single coenzyme molecule may associate with a number of different enzymes. Most coenzymes are derivatives of vitamins, and NAD^+/NADH, a derivative of the vitamin niacin, is a good example.

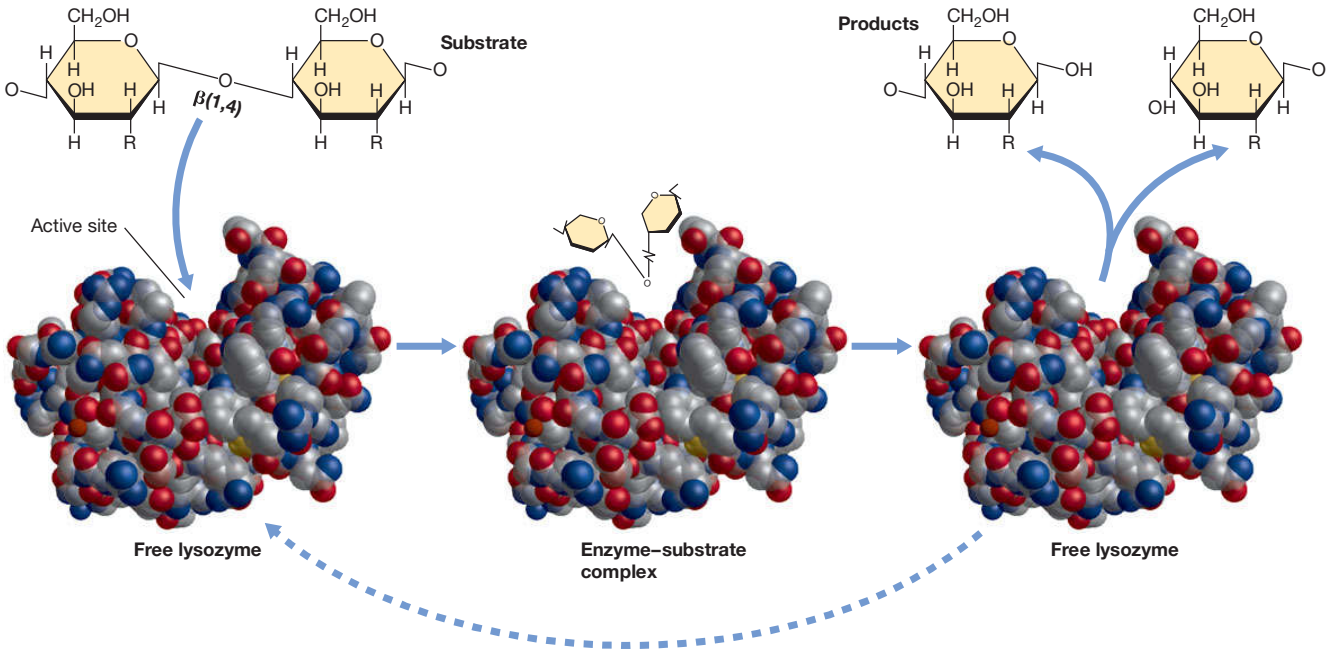


Figure 4.7 The catalytic cycle of an enzyme. The enzyme depicted here, lysozyme, catalyzes the cleavage of the β-1,4-glycosidic bond in the polysaccharide backbone of peptidoglycan. Following binding in the enzyme's active site, strain is placed on the bond, and this favors breakage. Space-filling model of lysozyme courtesy of Richard Feldmann.

Enzyme Catalysis

The catalytic power of enzymes is impressive. Enzymes increase the rate of chemical reactions anywhere from 10^8 to 10^{20} times over that which would occur spontaneously. To catalyze a specific reaction, an enzyme must do two things: (1) bind its substrate and (2) position the substrate relative to the catalytically active amino acids in the enzyme's active site. The enzyme–substrate complex (**Figure 4.7**) aligns reactive groups and places strain on specific bonds in the substrate(s). The net result is a reduction in the activation energy required to make the reaction proceed from substrate(s) to product(s) (Figure 4.6). These steps are shown in Figure 4.7 for the enzyme lysozyme, an enzyme whose substrate is the polysaccharide backbone of the bacterial cell wall polymer, peptidoglycan (Figure 3.16).

The reaction depicted in Figure 4.6 is exergonic because the free energy of formation of the substrates is greater than that of the products. Enzymes can also catalyze reactions that require energy, converting energy-poor substrates into energy-rich products. In these cases, however, not only must an activation energy barrier be overcome, but sufficient free energy must also be put into the reaction to raise the energy level of the substrates to that of the products. This is done by coupling the energy-*requiring* reaction to an energy-*yielding* one, such as the hydrolysis of ATP.

Theoretically, all enzymes are reversible in their activity. However, enzymes that catalyze highly exergonic or highly endergonic reactions typically act only unidirectionally. If a particularly exergonic or endergonic reaction needs to be reversed, a different enzyme usually catalyzes the reverse reaction.

MiniQuiz

- What is the function of a catalyst? What are enzymes made of?
- Where on an enzyme does the substrate bind?
- What is activation energy?

III Oxidation–Reduction and Energy-Rich Compounds

The energy released in oxidation–reduction (redox) reactions is conserved in cells by the simultaneous synthesis of energy-rich compounds, such as ATP. Here we first consider oxidation–reduction reactions and the major electron carriers present in the cell. We then examine the compounds that actually conserve the energy released in oxidation–reduction reactions.

4.6 Electron Donors and Electron Acceptors

An *oxidation* is the removal of an electron or electrons from a substance, and a *reduction* is the addition of an electron or electrons to a substance. Oxidations and reductions are common in cellular biochemistry and can involve just electrons or an electron plus a proton (a hydrogen atom; H).

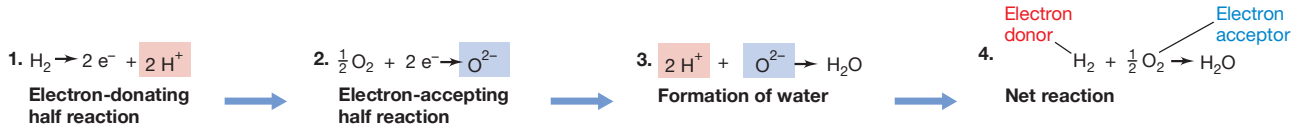


Figure 4.8 **Example of an oxidation–reduction reaction.** The formation of H_2O by reaction of the electron donor H_2 and the electron acceptor O_2.

Redox Reactions

Redox reactions occur in pairs. For example, hydrogen gas (H_2) can release electrons and protons and become oxidized (**Figure 4.8**). However, electrons cannot exist alone in solution; they must be part of atoms or molecules. Thus, the equation as drawn does not itself represent an independent reaction. The reaction is only a *half reaction*, a term that implies the need for a second half reaction. This is because for any substance to be oxidized, another substance must be reduced.

The oxidation of H_2 can be coupled to the reduction of many different substances, including oxygen (O_2), in a second half reaction. This reduction half reaction, when coupled to the oxidation of H_2, yields the overall balanced reaction in step 4 of Figure 4.8. In reactions of this type, we refer to the substance *oxidized* (in this case, H_2) as the **electron donor**, and the substance *reduced* (in this case, O_2) as the **electron acceptor**. The concept of electron donors and electron acceptors is very important in microbiology and underlies virtually all aspects of energy metabolism.

Reduction Potentials and Redox Couples

Substances differ in their tendency to be electron donors or electron acceptors. This tendency is expressed as their **reduction potential** (E_0', standard conditions), measured in volts (V) in reference to that of a standard substance, H_2 (**Figure 4.9**). By convention, reduction potentials are given for half reactions written as *reductions*, with reactions at pH 7 because the cytoplasm of most cells is neutral, or nearly so.

A substance can be either an electron donor or an electron acceptor under different circumstances, depending on the substances with which it reacts. The constituents on each side of the arrow in half reactions are called a *redox couple*, such as $2\ H^+/H_2$, or $\frac{1}{2}\ O_2/H_2O$ (Figure 4.8). By convention, when writing a redox couple, the *oxidized* form of the couple is always placed on the left, before the forward slash, followed by the *reduced* form after the forward slash. In the example of Figure 4.8, the E_0' of the $2\ H^+/H_2$ couple is -0.42 V and that of the $\frac{1}{2}\ O_2/H_2O$ couple is $+0.82$ V. We will learn shortly that these values mean that O_2 is an excellent electron *acceptor* and H_2 is an excellent electron *donor*.

In redox reactions, the *reduced* substance of a redox couple whose E_0' is more negative donates electrons to the *oxidized* substance of a redox couple whose E_0' is more positive. Thus, in the couple $2\ H^+/H_2$, H_2 has a greater tendency to donate electrons than the tendency of $2\ H^+$ to accept them, and in the couple $\frac{1}{2}O_2/H_2O$, H_2O has a very weak tendency to donate electrons, whereas O_2 has a great tendency to accept them. It then follows that in a reaction of H_2 and O_2, H_2 will be the electron donor and become oxidized, and O_2 will be the electron acceptor and become reduced (Figure 4.8).

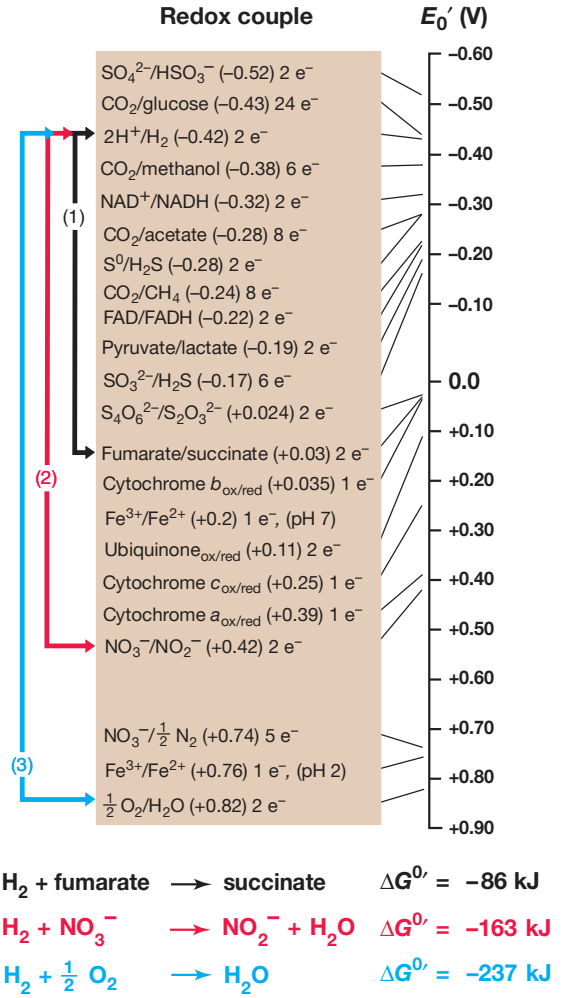


Figure 4.9 **The redox tower.** Redox couples are arranged from the strongest donors at the top to the strongest acceptors at the bottom. Electrons can be "caught" by acceptors at any intermediate level as long as the donor couple is more negative than the acceptor couple. The greater the difference in reduction potential between electron donor and electron acceptor, the more free energy is released. Note the differences in energy yield when H_2 reacts with three different electron acceptors, fumarate, nitrate, and oxygen.

As previously mentioned, all half reactions are written as reductions. However, in an actual reaction between two redox couples, the half reaction with the more negative E_0' proceeds as an oxidation and is therefore written in the opposite direction. In the reaction between H_2 and O_2 shown in Figure 4.8, H_2 is thus oxidized and is written in the reverse direction from its formal half reaction.

The Redox Tower and Its Relationship to $\Delta G^{o\prime}$

A convenient way of viewing electron transfer reactions in biological systems is to imagine a vertical tower (Figure 4.9). The tower represents the range of reduction potentials possible for redox couples in nature, from those with the most negative E_0' on the top to those with the most positive E_0' at the bottom; thus, we can call the tower a *redox tower*. The *reduced* substance in the redox couple at the top of the tower has the greatest tendency to donate electrons, whereas the *oxidized* substance in the redox couple at the bottom of the tower has the greatest tendency to accept electrons.

Using the tower analogy, imagine electrons from an electron donor near the top of the tower falling and being "caught" by electron acceptors at various levels. The difference in reduction potential between the donor and acceptor redox couples is expressed as $\Delta E_0'$. The further the electrons drop from a donor before they are caught by an acceptor, the greater the amount of energy released. That is, $\Delta E_0'$ is *proportional* to $\Delta G_0'$ (Figure 4.9). Oxygen, at the bottom of the redox tower, is the strongest electron acceptor of any significance in nature. In the middle of the redox tower, redox couples can be either electron donors or acceptors depending on which redox couples they react with. For instance, the $2\ H^+/H_2$ couple (−0.42 V) can react with the fumarate/succinate (+0.03 V), NO_3^-/NO_2^- (+0.42 V), or $\frac{1}{2}\ O_2/H_2O$(+8.82 V) couples, with increasing amounts of energy being released, respectively (Figure 4.9).

Electron donors used in energy metabolism are also called *energy sources* because energy is released when they are oxidized (Figure 4.9). The point is not that the electron donor per se contains energy but that the chemical reaction in which the electron donor participates releases energy. The presence of a suitable electron acceptor is just as important as the presence of a suitable electron donor. Lacking one or the other, the energy-releasing reaction cannot proceed. Many potential electron donors exist in nature, including a wide variety of organic and inorganic compounds.

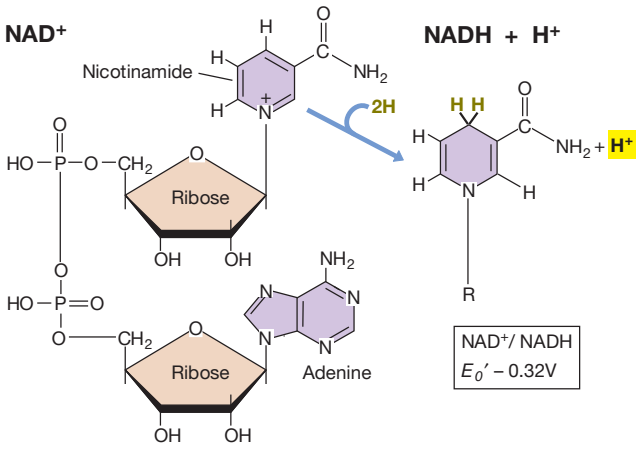


Figure 4.10 The oxidation–reduction coenzyme nicotinamide adenine dinucleotide (NAD^+). NAD^+ undergoes oxidation–reduction as shown and is freely diffusible. "R" is the adenine dinucleotide portion of NAD^+.

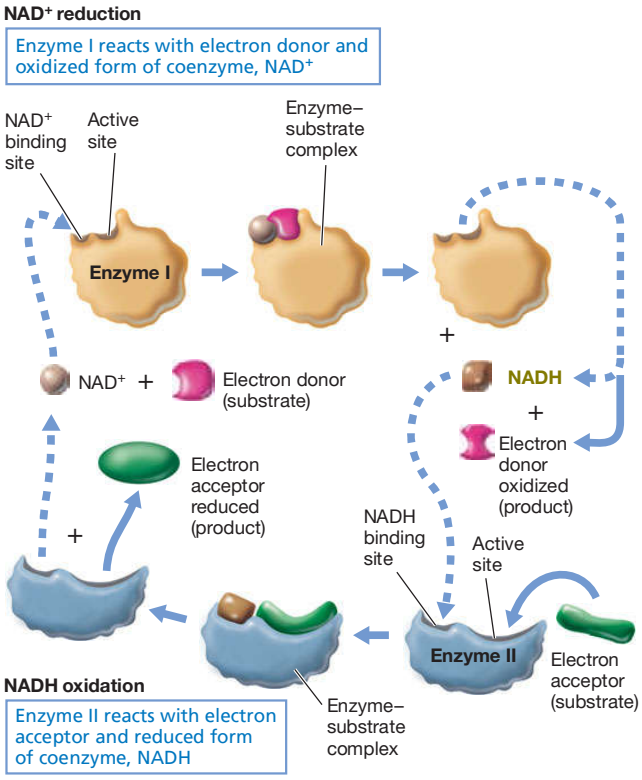


Figure 4.11 NAD^+/NADH cycling. A schematic example of redox reactions in two different enzymes linked by their use of either NAD^+ or NADH.

Electron Carriers and NAD/NADH Cycling

Redox reactions in microbial cells are typically mediated by one or more small molecules. A very common carrier is the coenzyme nicotinamide adenine dinucleotide (NAD^+) (**Figure 4.10**). NAD^+ is an electron plus proton carrier, transporting $2\ e^-$ and $2\ H^+$ at the same time.

The reduction potential of the NAD^+/NADH couple is −0.32 V, which places it fairly high on the electron tower; that is, NADH is a good electron donor (Figure 4.10). Coenzymes such as NADH increase the diversity of redox reactions possible in a cell by allowing chemically dissimilar electron donors and acceptors to interact, with the coenzyme acting as the intermediary. For example, electrons removed from an electron donor can reduce NAD^+ to NADH, and the latter can be converted back to NAD^+ by donating electrons to the electron acceptor. **Figure 4.11** shows an example of such electron shuttling by NAD^+/NADH. In the reaction, NAD^+ and NADH facilitate the redox reaction without being

consumed in the process. Recall that the cell requires large amounts of a primary electron donor (the substance that was oxidized to yield NADH) and a final electron acceptor (such as O_2). But the cell needs only a tiny amount of NAD^+/NADH because they are constantly being recycled. All that is needed is an amount sufficient to service the redox enzymes in the cell that use these coenzymes in their reaction mechanisms (Figure 4.11).

$NADP^+$/NADPH is a related redox coenzyme in which a phosphate group is added to NAD^+/NADH. $NADP^+$/NADPH typically participate in redox reactions distinct from those that use NAD^+/NADH, most commonly in anabolic (biosynthetic) reactions in which oxidations and reductions occur.

MiniQuiz

- In the reaction $H_2 + \frac{1}{2}O_2 \rightarrow H_2O$, what is the electron donor and what is the electron acceptor?
- Why is nitrate (NO_3^-) a better electron acceptor than fumarate?
- Is NADH a better electron donor than H_2? Is NAD^+ a better acceptor than H^+? How do you determine this?

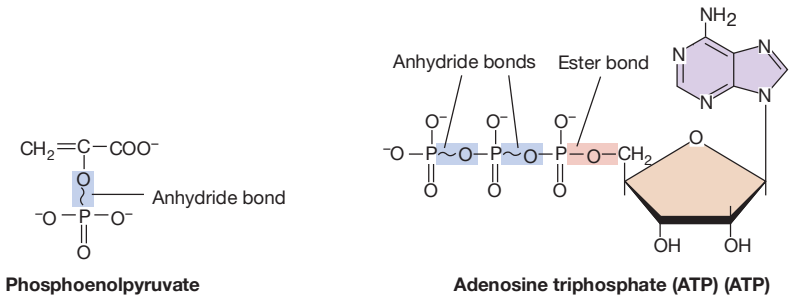
4.7 Energy-Rich Compounds and Energy Storage

Energy released from redox reactions must be conserved by the cell if it is to be used later to drive energy-requiring cell functions. In living organisms, chemical energy released in redox reactions is conserved primarily in phosphorylated compounds. The free energy released upon hydrolysis of the phosphate in these *energy-rich compounds* is significantly greater than that of the average covalent bond in the cell, and it is this released energy that is conserved by the cell.

Phosphate can be bonded to organic compounds by either ester or anhydride bonds, as illustrated in **Figure 4.12**. However, not all phosphate bonds are energy-rich. As seen in the figure, the $\Delta G^{0\prime}$ of hydrolysis of the phosphate *ester* bond in glucose 6-phosphate is only −13.8 kJ/mol. By contrast, the $\Delta G^{0\prime}$ of hydrolysis of the phosphate *anhydride* bond in phosphoenolpyruvate is −51.6 kJ/mol, almost four times that of glucose 6-phosphate. Although either compound could be hydrolyzed to yield energy, cells typically use a small group of compounds whose $\Delta G^{0\prime}$ of hydrolysis is greater than −30 kJ/mol as energy "currencies" in the cell. Thus, phosphoenolpyruvate is energy-rich whereas glucose 6-phosphate is not. Notice in Figure 4.12 that ATP contains three phosphates, but only two of them have free energies of hydrolysis of >30 kJ. Also notice that the thioester bond between the C and S atoms of coenzyme A has a free energy of hydrolysis of >30 kJ.

Adenosine Triphosphate

The most important energy-rich phosphate compound in cells is **adenosine triphosphate (ATP)**. ATP consists of the ribonucleoside adenosine to which three phosphate molecules are bonded in series. ATP is the prime energy currency in all cells, being generated during exergonic reactions and consumed in endergonic reactions. From the structure of ATP (Figure 4.12), it can be seen that two of the phosphate bonds are phosphoanhydrides that have free energies of hydrolysis greater than 30 kJ. Thus, the reactions ATP → ADP + P_i and ADP → AMP + P_i each release roughly 32 kJ/mol of energy. By contrast, AMP is not energy-rich because its free energy of hydrolysis is only about half that of ADP or ATP (Figure 4.12).



Compound	$G^{0\prime}$ kJ/mol
$\Delta G^{0\prime}$ > 30kJ	
Phosphoenolpyruvate	−51.6
1,3-Bisphosphoglycerate	−52.0
Acetyl phosphate	−44.8
ATP	−31.8
ADP	−31.8
Acetyl-CoA	−35.7
$\Delta G^{0\prime}$ < 30kJ	
AMP	−14.2
Glucose 6-phosphate	−13.8

Figure 4.12 Phosphate bonds in compounds that conserve energy in bacterial metabolism. Notice, by referring to the table, the range in free energy of hydrolysis of the phosphate bonds highlighted in the compounds. The "R" group of acetyl-CoA is a 3′ phospho ADP group.

Although the energy released in ATP hydrolysis is −32 kJ, a caveat must be introduced here to define more precisely the energy requirements for the synthesis of ATP. In an actively growing *Escherichia coli* cell, the ratio of ATP to ADP is about 7.5:1. This deviation from equilibrium affects the energy requirements for ATP synthesis. In such a cell, the actual energy expenditure (that is, the ΔG, Section 4.4) for the synthesis of 1 mole of ATP is on the order of −55 to −60 kJ. Nevertheless, for the purposes of learning and applying the basic principles of bioenergetics, we assume that reactions conform to "standard conditions" ($\Delta G^{0\prime}$), and thus we assume that the energy required for synthesis or hydrolysis of ATP is 32 kJ/mol.

Coenzyme A

Cells can use the free energy available in the hydrolysis of other energy-rich compounds as well as phosphorylated compounds. These include, in particular, derivatives of *coenzyme A* (for example, acetyl-CoA; see structure in Figure 4.12). Coenzyme A derivatives contain thioester bonds. Upon hydrolysis, these yield sufficient free energy to drive the synthesis of an energy-rich phosphate bond. For example, in the reaction

$$\text{acetyl-S-CoA} + H_2O + \text{ADP} + P_i \rightarrow \text{acetate}^- + \text{HS-CoA} + \text{ATP} + H^+$$

the energy released in the hydrolysis of coenzyme A is conserved in the synthesis of ATP. Coenzyme A derivatives (acetyl-CoA is just one of many) are especially important to the energetics of anaerobic microorganisms, in particular those whose energy metabolism depends on fermentation. We return to the importance of coenzyme A derivatives many times in Chapter 14.

Energy Storage

ATP is a dynamic molecule in the cell; it is continuously being broken down to drive anabolic reactions and resynthesized at the expense of catabolic reactions. For longer-term energy storage, microorganisms produce insoluble polymers that can be catabolized later for the production of ATP.

Examples of energy storage polymers in prokaryotes include glycogen, poly-β-hydroxybutyrate and other polyhydroxyalkanoates, and elemental sulfur, stored from the oxidation of H_2S by sulfur chemolithotrophs. These polymers are deposited within the cell as large granules that can be seen with the light or electron microscope (↻ Section 3.10). In eukaryotic microorganisms, polyglucose in the form of starch and lipids in the form of simple fats are the major reserve materials. In the absence of an external energy source, a cell can break down these polymers to make new cell material or to supply the very low amount of energy, called *maintenance energy*, needed to maintain cell integrity when it is in a nongrowing state.

MiniQuiz

- How much energy is released per mole of ATP converted to ADP + P_i under standard conditions? Per mole of AMP converted to adenosine and P_i?
- During periods of nutrient abundance, how can cells prepare for periods of nutrient starvation?

IV Essentials of Catabolism

Two series of reactions—fermentation and respiration—are linked to energy conservation in chemoorganotrophs: **Fermentation** is the form of anaerobic catabolism in which an organic compound is both an electron donor and an electron acceptor, and ATP is produced by substrate-level phosphorylation; and **respiration** is the catabolism in which a compound is oxidized with O_2 (or an O_2 substitute) as the terminal electron acceptor, usually accompanied by ATP production by oxidative phosphorylation. In both series of reactions, ATP synthesis is coupled to energy released in oxidation–reduction reactions.

One can look at fermentation and respiration as alternative metabolic choices available to some microorganisms. In organisms that can both ferment and respire, such as yeast, fermentation is necessary when conditions are anoxic and terminal electron acceptors are absent. When O_2 is available, respiration can take place. We will see that much more ATP is produced in respiration than in fermentation and thus respiration is the preferred choice (see the Microbial Sidebar, "Yeast Fermentation, the Pasteur Effect, and the Home Brewer"). But many microbial habitats lack O_2 or other electron acceptors that can substitute for O_2 in respiration (see Figure 4.22), and in such habitats, fermentation is the only option for energy conservation by chemoorganotrophs.

4.8 Glycolysis

In fermentation, ATP is produced by a mechanism called **substrate-level phosphorylation**. In this process, ATP is synthesized directly from energy-rich intermediates during steps in the catabolism of the fermentable substrate (**Figure 4.13*a***). This

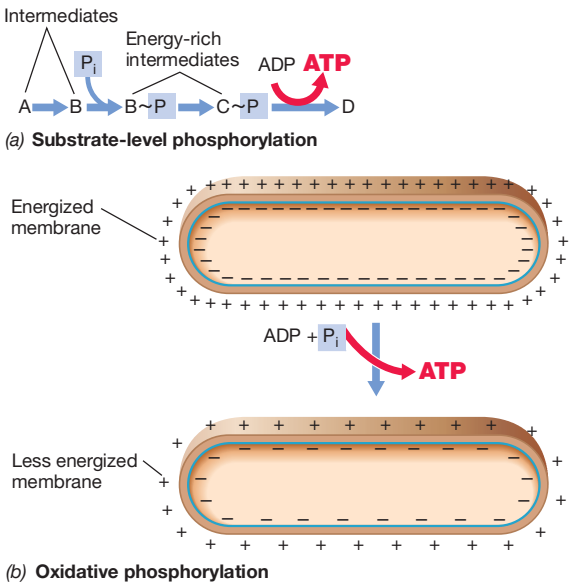


Figure 4.13 Energy conservation in fermentation and respiration. *(a)* In fermentation, substrate-level phosphorylation produces ATP. *(b)* In respiration, the cytoplasmic membrane, energized by the proton motive force, dissipates energy to synthesize ATP from ADP + P_i by oxidative phosphorylation.

Yeast Fermentation, the Pasteur Effect, and the Home Brewer

Every home wine maker, brewer, and baker is an amateur microbiologist, perhaps without even realizing it. Indeed, anaerobic mechanisms of microbial energy generation are at the heart of some of the most commonly consumed fermented foods and beverages (Figure 1).

In the production of breads and most alcoholic beverages, the yeast *Saccharomyces cerevisiae* or a related species is exploited to produce ethanol (ethyl alcohol) and carbon dioxide (CO_2). Found in various sugar-rich environments such as fruit juices and nectar, yeasts can carry out the two opposing modes of chemoorganotrophic metabolism discussed in this chapter, *fermentation* and *respiration*. When oxygen (O_2) is present in high amounts, yeast grows efficiently on various sugars, making yeast cells and CO_2 (the latter from the citric acid cycle, Section 4.11) in the process. However, when conditions are anoxic, yeasts switch to fermentative metabolism using the glycolytic pathway. This reduces the production of new cells but yields significant amounts of the fermentation products ethanol and CO_2.

During his studies on fermentation, the early microbiologist Louis Pasteur (Section 1.7) recognized that yeast switch between aerobic and anaerobic metabolism. He showed that the ratio of glucose consumed by a yeast suspension to the weight of cells produced varied with the concentration of O_2 supplied; the ratio was maximal in the absence of O_2. In Pasteur's own words, "the ferment lost its fermentative abilities in proportion to the concentration of this gas." He referred to the yeast cells as "the ferment" because it had not yet been established that the yeast in the fermenting mixture were actually living cells! He described what has come to be known as the "Pasteur effect," a phenomenon that occurs in any organism (even humans) that can both ferment and respire glucose. The fermentation of glucose is maximal under anoxic conditions and is incrementally inhibited by O_2 because respiration yields much more energy per glucose than does fermentation. As a rule, cells carry out the metabolism that is most energetically beneficial to them.

The Pasteur effect occurs in alcoholic beverage fermentation. When grapes are squeezed to make juice, called *must*, small numbers of yeast cells present on the grapes are transferred to the must. During the first several days of the wine-making process, yeast grow primarily by respiration and consume O_2, making the juice anoxic. The yeast respire the glucose in the juice rather than fermenting it because more energy is available from the respiration of glucose than from its fermentation. However, as soon as the O_2 in the grape juice is depleted, fermentation begins along with alcohol formation. This switch from aerobic to anaerobic metabolism is crucial in wine making, and care must be taken to ensure that O_2 is kept out of the fermentation vessel. The vessel is thus sealed against the introduction of air. Laboratory studies of yeast have shown that the introduction of O_2 to a fermenting yeast culture triggers the expression of hundreds of genes necessary for respiration, and such events would interrupt ethanol formation and other desirable reactions in wine production.

Barton Spear

Figure 1 Major food and beverage products of fermentation by the yeast *Saccharomyces cerevisiae*.

Wine is only one of many alcoholic products made with yeast. Others include beer and distilled spirits such as brandy, whisky, vodka, and gin (Chapter 15). In distilled spirits, the ethanol, produced in relatively low amounts (10–15% by volume) by the yeast, is concentrated by distilling to make a beverage containing 40–70% alcohol. Even alcohol for motor fuel is made with yeast in parts of the world where sugar is plentiful but petroleum is in short supply (such as Brazil). In the United States, ethyl alcohol for use as an industrial solvent and motor fuel is produced using corn starch as a source of the fermentable substrate (glucose). Yeast also serves as the leavening agent in bread, although here it is not the alcohol that is important, but CO_2, the *other* product of the alcohol fermentation (see Figure 4.14). The CO_2 raises the dough, and the alcohol produced along with it is volatilized during the baking process. We discuss yeast and yeast products in Chapters 15 and 20.

The yeast cell, forced to carry out a fermentative lifestyle because the O_2 it needs for respiration is absent, has had a considerable impact on the lives of humans. Substances that from the physiological standpoint of the yeast cell are "waste products" of the glycolytic pathway—ethanol and CO_2—are, respectively, the foundation of the alcoholic beverage and baking industries.

is in contrast to **oxidative phosphorylation**, typical of respiration, in which ATP is produced at the expense of the proton motive force (Figure 4.13*b*).

The fermentable substrate in a fermentation is both the electron donor and electron acceptor; not all compounds can be fermented, but sugars, especially hexoses such as glucose, are excellent fermentable substrates. A common pathway for the catabolism of glucose is **glycolysis**, which breaks down glucose into pyruvate. Glycolysis is also called the *Embden–Meyerhof–Parnas pathway* for its major discoverers. Whether glucose is fermented or respired, it travels through this pathway. Here we focus on the reactions of glycolysis and the reactions that follow under anoxic conditions.

Glycolysis can be divided into three stages, each involving a series of enzymatic reactions. Stage I comprises "preparatory" reactions; these are not redox reactions and do not release energy but instead lead to the production of a key intermediate of the pathway. In Stage II, redox reactions occur, energy is conserved in the form of ATP, and two molecules of pyruvate are formed. The reactions of glycolysis are finished at this point. However, redox balance has not yet been achieved. So, in Stage III, redox reactions occur once again and fermentation products are formed (Figure 4.14).

Stage I: Preparatory Reactions

In Stage I glucose is phosphorylated by ATP, yielding glucose 6-phosphate; the latter is then isomerized to fructose 6-phosphate. A second phosphorylation leads to the production of fructose 1,6-bisphosphate. The enzyme aldolase then splits fructose 1,6-bisphosphate into two 3-carbon molecules, *glyceraldehyde 3-phosphate* and its isomer, *dihydroxyacetone phosphate*, which can be converted into glyceraldehyde 3-phosphate. To this point, all of the reactions, including the consumption of ATP, have proceeded without redox reactions.

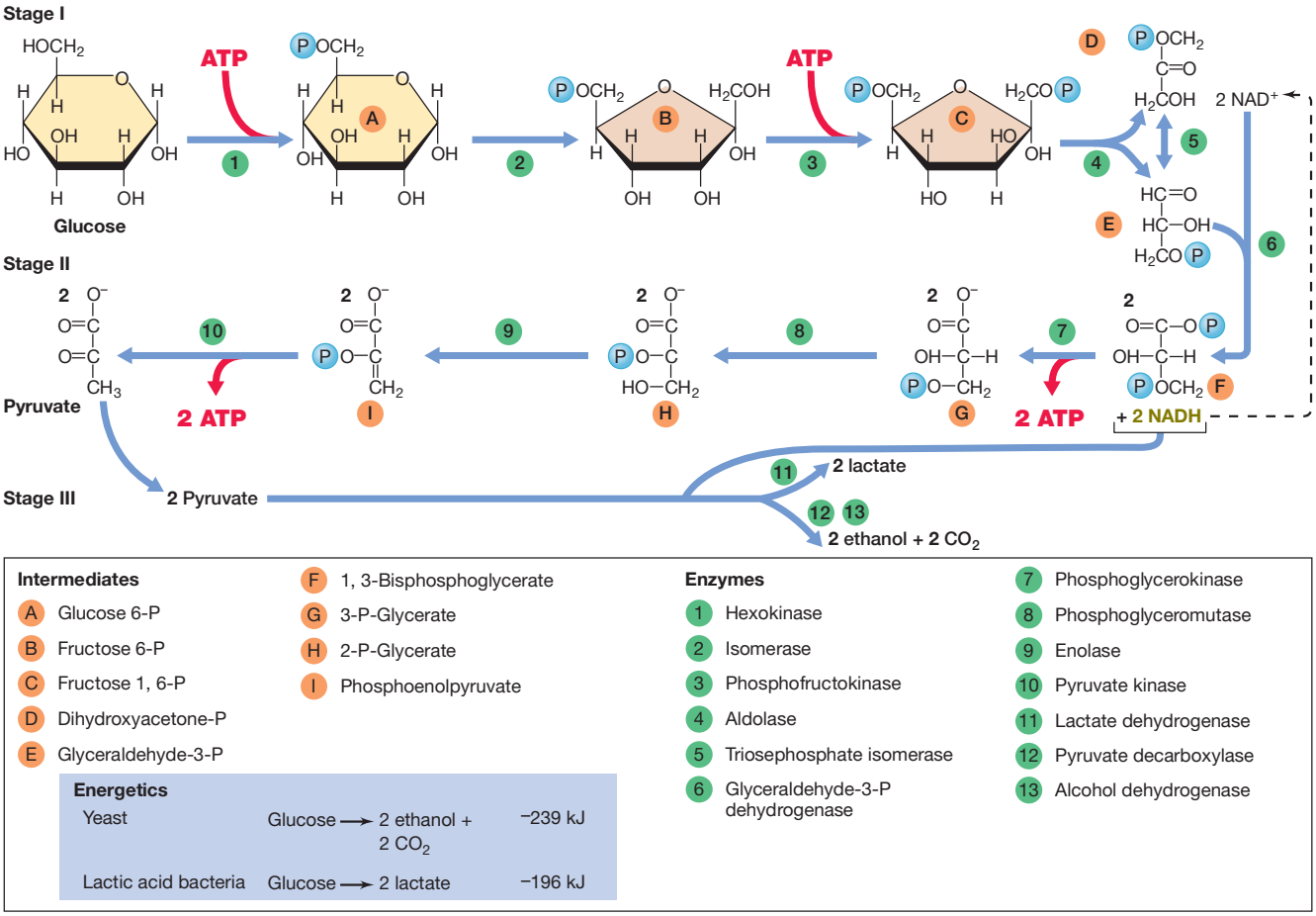


Figure 4.14 Embden–Meyerhof–Parnas pathway (glycolysis). The sequence of reactions in the catabolism of glucose to pyruvate and then on to fermentation products. Pyruvate is the end product of glycolysis, and fermentation products are made from it. The blue table at the bottom left lists the energy yields from the fermentation of glucose by yeast or lactic acid bacteria.

Stage II: Production of NADH, ATP, and Pyruvate

The first redox reaction of glycolysis occurs in Stage II during the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglyceric acid. In this reaction (which occurs twice, once for each of the two molecules of glyceraldehyde 3-phosphate produced from glucose), the enzyme glyceraldehyde-3-phosphate dehydrogenase reduces its coenzyme NAD^+ to NADH. Simultaneously, each glyceraldehyde 3-phosphate molecule is phosphorylated by the addition of a molecule of inorganic phosphate. This reaction, in which inorganic phosphate is converted to organic form, sets the stage for energy conservation. ATP formation is possible because 1,3-bisphosphoglyceric acid is an energy-rich compound (Figure 4.12). ATP is then synthesized when (1) each molecule of 1,3-bisphosphoglyceric acid is converted to 3-phosphoglyceric acid, and (2) each molecule of phosphoenolpyruvate is converted to pyruvate (Figure 4.14).

During Stages I and II of glycolysis, *two* ATP molecules have been consumed and *four* ATP molecules have been synthesized (Figure 4.14). Thus, the net energy yield in glycolysis is *two molecules of ATP per molecule of glucose fermented*.

Stage III: Consumption of NADH and Production of Fermentation Products

During the formation of two molecules of 1,3-bisphosphoglyceric acid, two NAD^+ are reduced to NADH (Figure 4.14). However, as previously discussed (Section 4.6 and Figure 4.11), NAD^+ is only an electron shuttle, not a net (terminal) acceptor of electrons. Thus, the NADH produced in glycolysis must be oxidized back to NAD^+ in order for glycolysis to continue, and this is accomplished when pyruvate is reduced (by NADH) to fermentation products (Figure 4.14). For example, in fermentation by yeast, pyruvate is reduced to ethanol with the subsequent production of carbon dioxide (CO_2). By contrast, lactic acid bacteria reduce pyruvate to lactate. Many other possibilities for pyruvate reduction are possible depending on the organism (see sections on fermentative diversity in Chapter 14), but the net result is the same: NADH is reoxidized to NAD^+ during the production of fermentation products, allowing reactions of the pathway that depend on NAD^+ to continue.

Glucose Fermentation: Net and Practical Results

During glycolysis, glucose is consumed, two ATPs are made, and fermentation products are generated. For the organism the crucial product is ATP, which is used in energy-requiring reactions; fermentation products are merely waste products. However, fermentation products are not considered wastes by the distiller, the brewer, the cheese maker, or the baker (see the Microbial Sidebar). Thus, fermentation is more than just an energy-yielding process for a cell; it is also a means of making natural products useful to humans.

MiniQuiz

- Which reactions in glycolysis involve oxidations and reductions?
- What is the role of NAD^+/NADH in glycolysis?
- Why are fermentation products made during glycolysis?

4.9 Respiration and Electron Carriers

We have just seen that fermentation is an anaerobic process and releases only a small amount of energy. As a result, only a few ATP molecules are synthesized. Why is more energy not conserved in fermentation? The simple answer is that, although the fermentation products excreted still contain a large amount of potential energy, the organism cannot oxidize these further because O_2 is absent. By contrast, if O_2 (or other usable terminal acceptors, see Figure 4.22) are present, pyruvate can be oxidized to CO_2 instead of being reduced to fermentation products and excreted. When pyruvate is oxidized to CO_2, a far higher yield of ATP is possible. Oxidation using O_2 as the terminal electron acceptor is called *aerobic respiration*; oxidation using other acceptors under anoxic conditions is called *anaerobic respiration* (Section 4.12).

Our discussion of respiration covers both carbon transformations and redox reactions and focuses on two issues: (1) how electrons are transferred from the organic compound to the terminal electron acceptor and how this is coupled to energy conservation, and (2) the pathway by which organic carbon is oxidized into CO_2. During the former, ATP is synthesized at the expense of the proton motive force (Figure 4.13*b*); thus we begin with a consideration of electron transport, the series of reactions that lead to the proton motive force.

Electron transport is a membrane-mediated process and has two basic functions: (1) facilitating the transfer of electrons from primary donor to terminal acceptor and (2) participating in membrane events whose end result is energy conservation. Several types of oxidation–reduction enzymes participate in electron transport. These include *NADH dehydrogenases*, *flavoproteins* (**Figure 4.15**), *iron–sulfur proteins*, and *cytochromes*. Also participating are nonprotein electron carriers called *quinones*. The carriers are arranged in the membrane in order of increasingly more positive reduction potential, with NADH dehydrogenase first and the cytochromes last (see Figure 4.19).

NADH dehydrogenases are proteins bound to the inside surface of the cytoplasmic membrane. They have an active site that binds NADH and accepts two electrons plus two protons ($2\ e^- + 2\ H^+$) when NADH is oxidized to NAD^+ (Figures 4.10

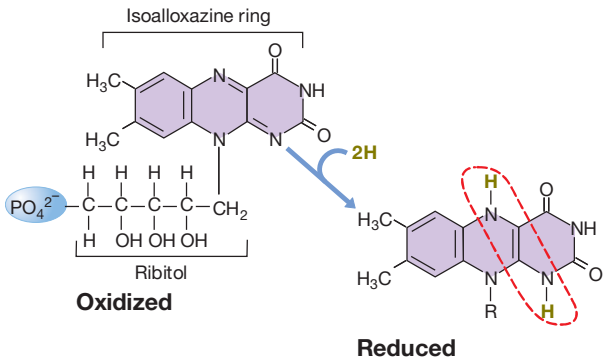


Figure 4.15 Flavin mononucleotide (FMN), a hydrogen atom carrier. The site of oxidation–reduction (dashed red circle) is the same in FMN and the related coenzyme flavin adenine dinucleotide (FAD, not shown). FAD contains an adenosine group bonded through the phosphate group on FMN.

and 4.11). The 2 e^- + 2 H^+ are then transferred to a flavoprotein, the next carrier in the chain.

Flavoproteins contain a derivative of the vitamin riboflavin. The flavin portion, which is bound to a protein, is a prosthetic group that is reduced as it accepts 2 e^- + 2 H^+ and oxidized when 2 e^- are passed on to the next carrier in the chain. Note that flavoproteins *accept* 2 e^- + 2 H^+ but *donate* only electrons. We will consider what happens to the 2 H^+ later. Two flavins are commonly found in cells, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). In the latter, FMN is bonded to ribose and adenine through a second phosphate. Riboflavin, also called vitamin B_2, is a source of the parent flavin molecule in flavoproteins and is a required growth factor for some organisms.

The cytochromes are proteins that contain heme prosthetic groups (**Figure 4.16**). Cytochromes undergo oxidation and reduction through loss or gain of a single electron by the iron atom in the heme of the cytochrome:

$$\text{Cytochrome—Fe}^{2+} \leftrightarrows \text{Cytochrome—Fe}^{3+} + e^-$$

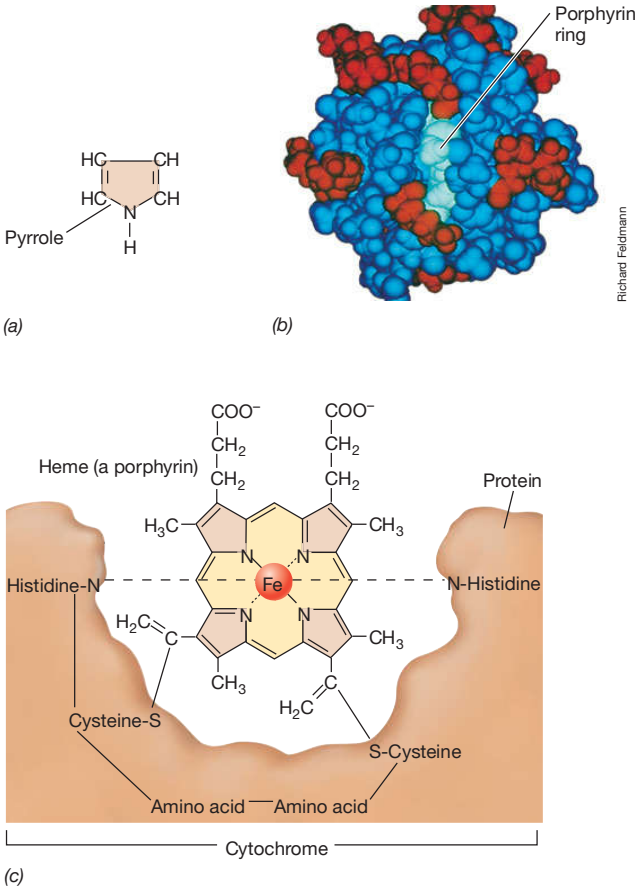


Figure 4.16 **Cytochrome and its structure.** *(a)* Structure of pyrrole, which is the building block of porphyrins such as heme in part c. *(b)* Space-filling model of cytochrome c; the porphyrin (light blue) is covalently linked via disulfide bridges to cysteine residues in the protein. *(c)* Schematic of cytochrome *c* model. Cytochromes carry electrons only; the redox site is the iron atom, which can alternate between the Fe^{2+} and Fe^{3+} oxidation states.

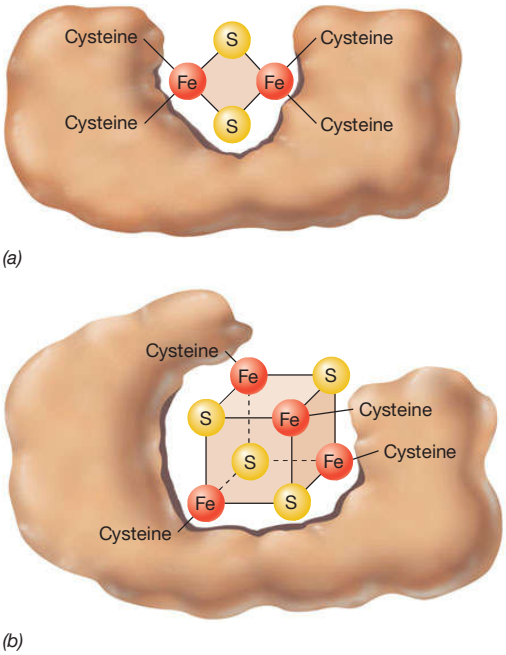


Figure 4.17 **Arrangement of the iron–sulfur centers of nonheme iron–sulfur proteins.** *(a)* Fe_2S_2 center. *(b)* Fe_4S_4 center. The cysteine linkages are from the protein portion of the molecule.

Several classes of cytochromes are known, differing widely in their reduction potentials (Figure 4.9). Different classes of cytochromes are designated by letters, such as cytochrome *a*, cytochrome *b*, cytochrome *c*, and so on, depending upon the type of heme they contain. The cytochromes of a given class in one organism may differ slightly from those of another, and so there are designations such as cytochromes a_1, a_2, a_3, and so on among cytochromes of the same class. Occasionally, cytochromes form complexes with other cytochromes or with iron–sulfur proteins. An important example is the cytochrome bc_1 complex, which contains two different *b*-type cytochromes and one *c*-type cytochrome. The cytochrome bc_1 complex plays an important role in energy metabolism, as we will see later.

In addition to the cytochromes, in which iron is bound to heme, one or more proteins with nonheme iron are typically present in electron transport chains. Centered in these proteins are clusters of iron and sulfur atoms, with Fe_2S_2 and Fe_4S_4 clusters being the most common (**Figure 4.17**). *Ferredoxin*, a common nonheme iron–sulfur protein, has an Fe_2S_2 configuration.

The reduction potentials of iron–sulfur proteins vary over a wide range depending on the number of iron and sulfur atoms present and how the iron centers are embedded in the protein. Thus, different iron–sulfur proteins can function at different locations in the electron transport chain. Like cytochromes, nonheme iron–sulfur proteins carry electrons only.

Quinones (**Figure 4.18**) are hydrophobic molecules that lack a protein component. Because they are small and hydrophobic, quinones are free to move about within the membrane. Like the

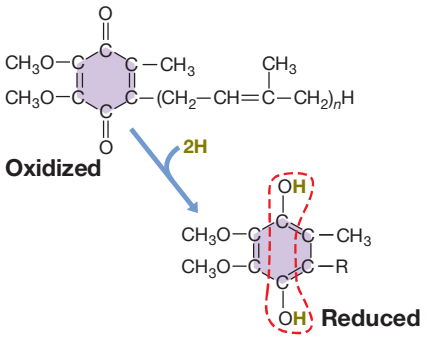


Figure 4.18 **Structure of oxidized and reduced forms of coenzyme Q, a quinone.** The five-carbon unit in the side chain (an isoprenoid) occurs in a number of multiples, typically 6–10. Oxidized quinone requires 2 e^- and 2 H^+ (2 H) to become fully reduced (dashed red circles).

flavoproteins, quinones accept 2 e^- + 2 H^+ but transfer only 2 e^- to the next carrier in the chain; quinones typically participate as links between iron–sulfur proteins and the first cytochromes in the electron transport chain.

MiniQuiz

- In what major way do quinones differ from other electron carriers in the membrane?
- Which electron carriers described in this section accept 2 e^- + 2 H^+? Which accept electrons only?

4.10 The Proton Motive Force

The conservation of energy by oxidative phosphorylation is linked to an energized state of the membrane (Figure 4.13*b*). This energized state is established by electron transport reactions between the electron carriers just discussed. To understand how electron transport is linked to ATP synthesis, we must first understand how the electron transport system is oriented in the cytoplasmic membrane. Electron transport carriers are oriented in the membrane in such a way that, as electrons are transported, protons are separated from electrons. Two electrons plus two protons enter the electron transport chain from NADH through NADH dehydrogenase to initiate the process. Carriers in the electron transport chain are arranged in the membrane in order of their increasingly positive reduction potential, with the final carrier in the chain donating the electrons plus protons to a terminal electron acceptor such as O_2 (**Figure 4.19**).

During electron transport, H^+ are extruded to the outer surface of the membrane. These H^+ originate from two sources: (1) NADH and (2) the dissociation of water (H_2O) into H^+ and OH^- in the cytoplasm. The extrusion of H^+ to the environment results in the accumulation of OH^- on the inside of the membrane. However, despite their small size, neither H^+ nor OH^- can diffuse through the membrane because they are charged (↻ Section 3.4). As a result of the separation of H^+ and OH^-, the two sides of the membrane differ in both charge and pH.

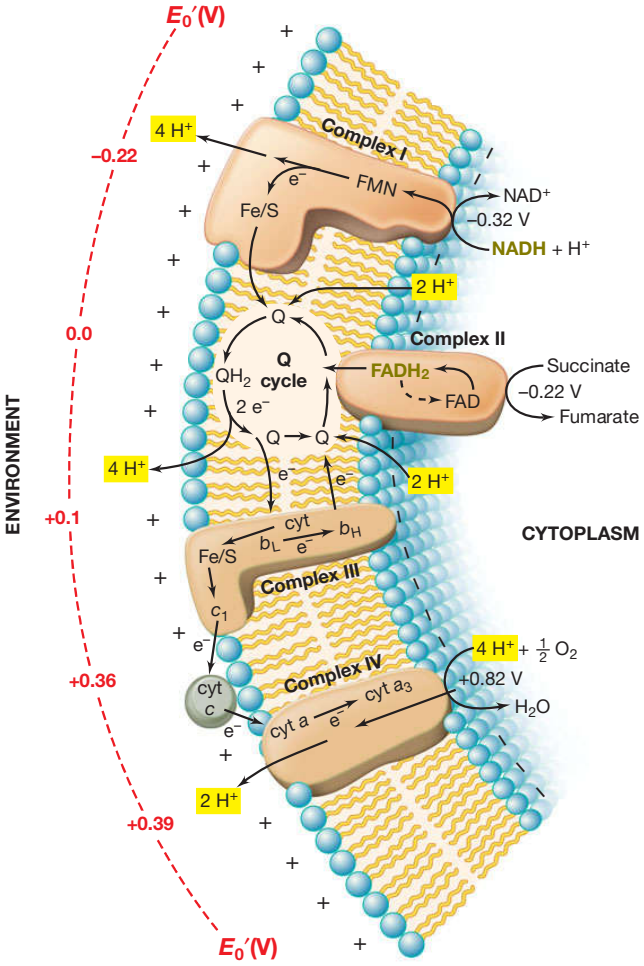


Figure 4.19 **Generation of the proton motive force during aerobic respiration.** The orientation of electron carriers in the membrane of *Paracoccus denitrificans*, a model organism for studies of respiration. The + and – charges at the edges of the membrane represent H^+ and OH^-, respectively. E_0' values for the major carriers are shown. Note how when a hydrogen atom carrier (for example, FMN in Complex I) reduces an electron-accepting carrier (for example, the Fe/S protein in Complex I), protons are extruded to the outer surface of the membrane. Abbreviations: FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; Q, quinone; Fe/S, iron–sulfur–protein; cyt *a*, *b*, *c*, cytochromes (b_L and b_H, low- and high-potential *b*-type cytochromes, respectively). At the quinone site, electrons are recycled during the "Q cycle." This is because electrons from QH_2 can be split in the bc_1 complex (Complex III) between the Fe/S protein and the *b*-type cytochromes. Electrons that travel through the cytochromes reduce Q (in two, one-electron steps) back to QH_2, thus increasing the number of protons pumped at the Q-bc_1 site. Electrons that travel to Fe/S proceed to reduce cytochrome c_1, then cytochrome *c*, and then *a*-type cytochromes in Complex IV, eventually reducing O_2 to H_2O (2 electrons and 4 protons are required to reduce $\frac{1}{2}$ O_2 to H_2O along with 2 H^+ extruded, and these come from electrons through cyt *c* and cytoplasmic protons, respectively). Complex II, the succinate dehydrogenase complex, bypasses Complex I and feeds electrons directly into the quinone pool at a more positive E_0' than NADH (see the electron tower in Figure 4.9).

The result of electron transport is thus the formation of an electrochemical potential across the membrane (Figure 4.19). This potential, along with the difference in pH across the membrane, is called the **proton motive force (pmf)** and causes the membrane to be energized much like a battery. Some of the potential energy in the pmf is then conserved in the formation of ATP. However, besides driving ATP synthesis, the pmf can also be tapped to do other forms of work, such as ion transport, flagellar rotation, and a few other energy-requiring reactions in the cell.

We now consider the individual electron transport reactions that lead to formation of the proton motive force.

Generation of the Proton Motive Force: Complexes I and II

The proton motive force develops from the activities of flavin enzymes, quinones, the cytochrome bc_1 complex, and the terminal cytochrome oxidase. Following the donation of NADH + H^+ to form $FMNH_2$, 4 H^+ are extruded to the outer surface of the membrane when $FMNH_2$ donates 2 e^- to a series of nonheme iron proteins (Fe/S), forming the membrane protein section of *Complex I* (shown in Figure 4.19). These electron carriers are called *complexes* because each consists of several proteins that function together. For example, Complex I in *Escherichia coli* contains 14 different proteins and the equivalent complex in the mitochondrion contains at least 44 proteins. Complex I is also called *NADH:quinone oxidoreductase* because the reaction is one in which NADH is initially oxidized and quinone is ultimately reduced. Notably, 2 H^+ are taken up from the dissociation of H_2O in the cytoplasm when coenzyme Q is reduced at a catalytic site of Complex 1 formed by Fe/S centers (Figure 4.19).

Complex II simply bypasses Complex I and feeds e^- and H^+ from FADH directly into the quinone pool. Complex II is also called the *succinate dehydrogenase complex* because of the specific substrate, succinate (a product of the citric acid cycle, Section 4.11), that it oxidizes. However, because Complex II bypasses Complex I, fewer H^+ are pumped per 2 e^- that enter the electron transport chain here than for 2 e^- that enter from NADH (Figure 4.19).

Complexes III and IV: bc_1 and *a*-Type Cytochromes

Reduced coenzyme Q passes electrons one at a time to the cytochrome bc_1 complex (*Complex III*, Figure 4.19). The cytochrome bc_1 complex consists of several proteins that contain hemes (Figure 4.16) or other metal cofactors. These include two *b*-type hemes (b_L and b_H), one *c*-type heme (c_1), and one iron–sulfur protein. The bc_1 complex is present in the electron transport chain of almost all organisms that can respire. It also plays a fundamental role in photosynthetic electron flow of phototrophic organisms (Sections 13.4 and 13.5).

The major function of the cytochrome bc_1 complex is to transfer e^- from quinones to cytochrome *c*. Electrons travel from the bc_1 complex to a molecule of cytochrome *c*, located in the periplasm. Cytochrome *c* functions as a shuttle to transfer e^- to the high-potential cytochromes *a* and a_3 (*Complex IV*, Figure 4.19). Complex IV is the terminal oxidase and reduces O_2 to H_2O in the final step of the electron transport chain. Complex IV also pumps protons to the outer surface of the membrane, thereby increasing the strength of the proton motive force (Figure 4.19).

Besides transferring e^- to cytochrome *c*, the cytochrome bc_1 complex can also interact with quinones in such a way that on average, two additional H^+ are pumped at the Q-bc_1 site. This happens in a series of electron exchanges between cytochrome bc_1 and Q, called the *Q cycle*. Because quinone and bc_1 have roughly the same E_0' (near 0 V, Figure 4.19), quinone molecules can alternately become oxidized and reduced using e^- fed back to quinones from the bc_1 complex. This mechanism allows on average a total of 4 H^+ (instead of 2 H^+) to be pumped to the outer surface of the membrane at the Q-bc_1 site for every 2 e^- that enter the chain in Complex I.

The electron transport chain shown in Figure 4.19 is one of many different sequences of electron carriers known from different organisms. However, three features are characteristic of all electron transport chains: (1) arrangement of carriers in order of increasingly more positive E_0', (2) alternation of electron-only and electron-plus-proton carriers in the chain, and (3) generation of a proton motive force.

As we will see now, it is this last characteristic, the proton motive force, that drives ATP synthesis.

ATP Synthase

How does the proton motive force generated by electron transport actually drive ATP synthesis? Interestingly, a strong parallel exists between the mechanism of ATP synthesis and the mechanism of the motor that drives rotation of the bacterial flagellum (Section 3.13). In analogy to how dissipation of the pmf applies torque that rotates the bacterial flagellum, the pmf also creates torque in a large protein complex that makes ATP. This complex is called **ATP synthase**, or **ATPase** for short.

ATPases consist of two components, a multiprotein cytoplasmic complex called F_1 that carries out the chemical function (ATP synthesis), connected to a membrane-integrated component called F_o that carries out the ion-translocating function (**Figure 4.20**). ATPase catalyzes a reversible reaction between ATP and ADP + P_i as shown in the figure. The structure of ATPase proteins is highly conserved throughout all the domains of life, suggesting that this mechanism of energy conservation was a very early evolutionary invention (Section 16.2).

F_1 and F_o are actually two rotary motors. Pmf-driven H^+ movement through F_o causes rotation of its *c* proteins. This generates a torque that is transmitted to F_1 via the coupled rotation of the γε subunits (Figure 4.20). The latter activity causes conformational changes in the β subunits that allows them to bind ADP + P_i. ATP is synthesized when the β subunits return to their original conformation, releasing the free energy needed to drive the synthesis.

ATPase-catalyzed ATP synthesis is called *oxidative phosphorylation* if the proton motive force originates from respiration reactions and *photophosphorylation* if it originates from photosynthetic reactions. Quantitative measures (stoichiometry) of H^+ consumed by ATPase per ATP produced yield a number between 3 and 4.

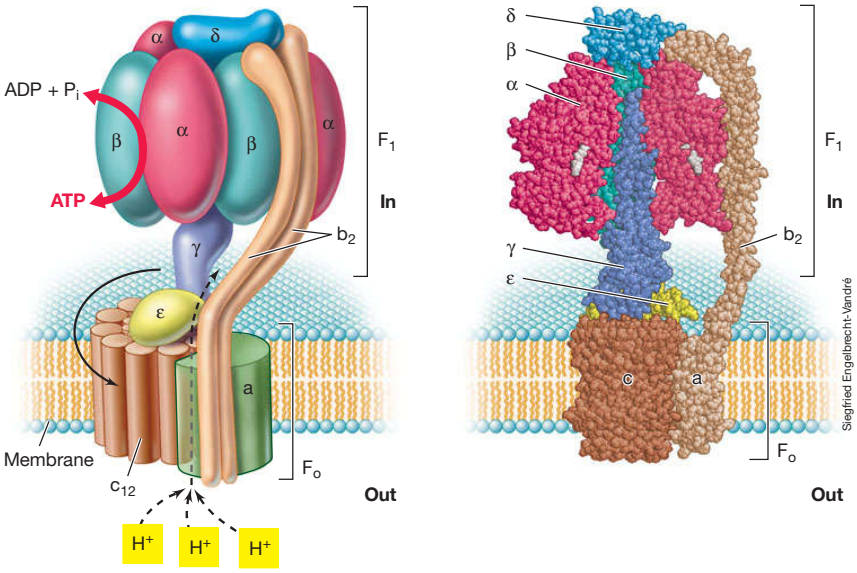


Figure 4.20 **Structure and function of ATP synthase (ATPase) in *Escherichia coli.*** *(a)* Schematic. F_1 consists of five different polypeptides forming an $\alpha_3\beta_3\gamma\varepsilon\delta$ complex, the stator. F_1 is the catalytic complex responsible for the interconversion of ADP + P_i and ATP. F_o, the rotor, is integrated in the membrane and consists of three polypeptides in an ab_2c_{12} complex. As protons enter, the dissipation of the proton motive force drives ATP synthesis (3 H^+/ATP). ATPase is reversible in that ATP hydrolysis can drive formation of a proton motive force. *(b)* Space-filling model. The color-coding corresponds to the art in part *a*. Since proton translocation from outside the cell to inside the cell leads to ATP synthesis by ATPase, it follows that proton translocation from inside to outside in the electron transport chain (Figure 4.19) represents work done on the system and a source of potential energy.

Reversibility of ATPase

ATPase is reversible. The hydrolysis of ATP supplies torque for γε to rotate in the opposite direction from that in ATP synthesis, and this catalyzes the pumping of H^+ from the inside to the outside of the cell through F_o. The net result is *generation* instead of *dissipation* of the proton motive force. Reversibility of the ATPase explains why strictly fermentative organisms that lack electron transport chains and are unable to carry out oxidative phosphorylation still contain ATPases. As we have said, many important reactions in the cell, such as motility and transport, require energy from the pmf rather than from ATP. Thus, ATPase in organisms incapable of respiration, such as the strictly fermentative lactic acid bacteria, for example, functions unidirectionally to generate the pmf necessary to drive these important cell functions.

MiniQuiz

- How do electron transport reactions generate the proton motive force?
- What is the ratio of H^+ extruded per NADH oxidized through the electron transport chain of *Paracoccus* shown in Figure 4.19? At which sites in the chain is the proton motive force being established?
- What structure in the cell converts the proton motive force to ATP? How does it function?

4.11 The Citric Acid Cycle

Now that we have a grasp of how ATP is made in respiration, we need to consider the important reactions in carbon metabolism associated with formation of ATP. Our focus here is on the citric acid cycle, also called the Krebs cycle, a key pathway in virtually all cells.

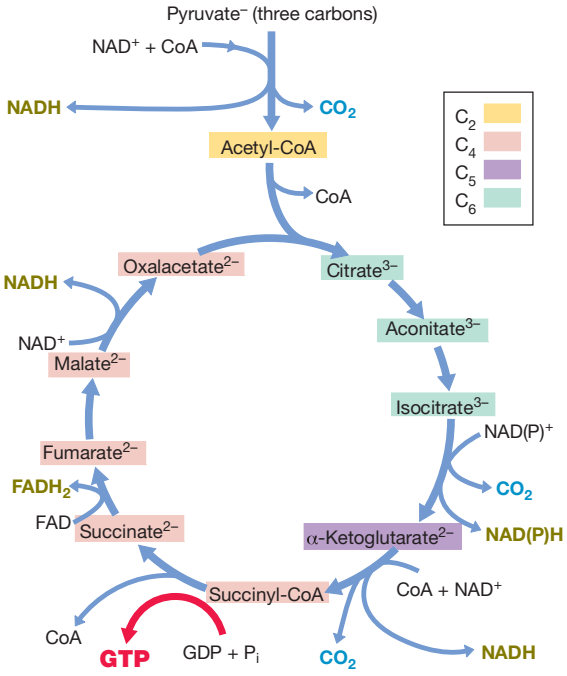
Respiration of Glucose

The early biochemical steps in the respiration of glucose are the same as those of glycolysis; all steps from glucose to pyruvate (Figure 4.14) are the same. However, whereas in fermentation pyruvate is reduced and converted into products that are excreted, in respiration pyruvate is oxidized to CO_2. The pathway by which pyruvate is completely oxidized to CO_2 is called the **citric acid cycle** (CAC), summarized in **Figure 4.21**.

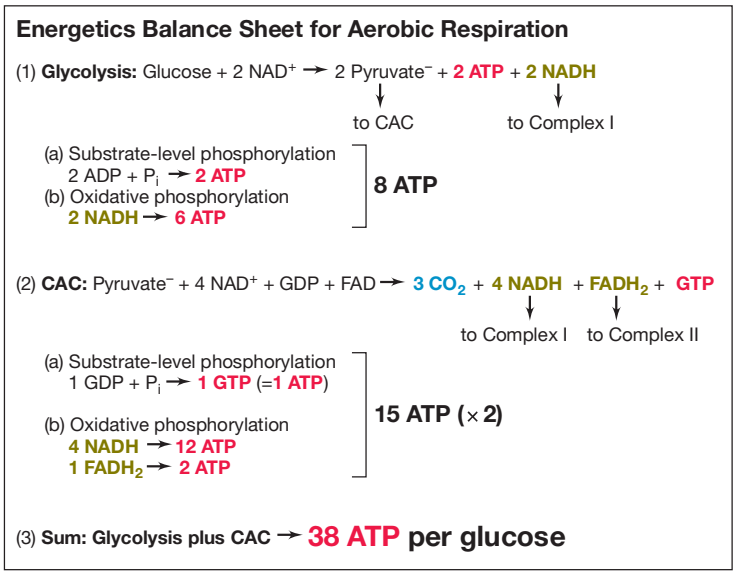
Pyruvate is first decarboxylated, leading to the production of CO_2, NADH, and the energy-rich substance *acetyl-CoA* (Figure 4.12). The acetyl group of acetyl-CoA then combines with the four-carbon compound oxalacetate, forming the six-carbon compound citric acid. A series of reactions follow, and two additional CO_2 molecules, three more NADH, and one FADH are formed. Ultimately, oxalacetate is regenerated to return as an acetyl acceptor, thus completing the cycle (Figure 4.21).

CO_2 Release and Fuel for Electron Transport

The oxidation of pyruvate to CO_2 requires the concerted activity of the citric acid cycle and the electron transport chain. For each pyruvate molecule oxidized through the citric acid cycle, three CO_2 molecules are released (Figure 4.21). Electrons released during the oxidation of intermediates in the citric acid cycle are transferred to NAD^+ to form NADH, or to FAD to form $FADH_2$. This is where respiration and fermentation differ in a major way. Instead of being used in the reduction of pyruvate as in fermentation (Figure 4.14), in respiration, electrons from NADH and $FADH_2$ are fuel for the electron transport chain, ultimately resulting in the reduction of an electron acceptor (O_2) to H_2O. This allows for the complete oxidation of glucose to CO_2 along with a much greater yield of energy. Whereas only *2 ATP* are produced per glucose fermented in alcoholic or lactic acid fermentations (Figure 4.14), a total of *38 ATP* can be made by aerobically respiring the same glucose molecule to CO_2 + H_2O (Figure 4.21*b*).



(a)



(b)

Figure 4.21 The citric acid cycle. *(a)* The citric acid cycle (CAC) begins when the two-carbon compound acetyl-CoA condenses with the four-carbon compound oxalacetate to form the six-carbon compound citrate. Through a series of oxidations and transformations, this six-carbon compound is ultimately converted back to the four-carbon compound oxalacetate, which then begins another cycle with addition of the next molecule of acetyl-CoA. *(b)* The overall balance sheet of fuel ($NADH/FADH_2$) for the electron transport chain and CO_2 generated in the citric acid cycle. NADH and $FADH_2$ feed into electron transport chain Complexes I and II, respectively (Figure 4.19).

Biosynthesis and the Citric Acid Cycle

Besides playing a key role in catabolism, the citric acid cycle plays another important role in the cell. The cycle generates several key compounds, small amounts of which can be drawn off for biosynthetic purposes when needed. Particularly important in this regard are α-ketoglutarate and oxalacetate, which are precursors of several amino acids (Section 4.14), and succinyl-CoA, needed to form cytochromes, chlorophyll, and several other tetrapyrrole compounds (Figure 4.16). Oxalacetate is also important because it can be converted to phosphoenolpyruvate, a precursor of glucose. In addition, acetate provides the starting material for fatty acid biosynthesis (Section 4.15, and see Figure 4.27). The citric acid cycle thus plays two major roles in the cell: *bioenergetic* and *biosynthetic*. Much the same can be said about the glycolytic pathway, as certain intermediates from this pathway are drawn off for various biosynthetic needs as well (Section 4.13).

MiniQuiz

- How many molecules of CO_2 and pairs of electrons are released per pyruvate oxidized in the citric acid cycle?
- What two major roles do the citric acid cycle and glycolysis have in common?

4.12 Catabolic Diversity

Thus far in this chapter we have dealt only with catabolism by chemoorganotrophs. We now briefly consider catabolic diversity, some of the alternatives to the use of organic compounds as electron donors, with emphases on both electron and carbon flow. **Figure 4.22** summarizes the mechanisms by which cells generate energy other than by fermentation and aerobic respiration. These include *anaerobic respiration*, *chemolithotrophy*, and *phototrophy*.

Anaerobic Respiration

Under anoxic conditions, electron acceptors other than oxygen can be used to support respiration in certain prokaryotes. These processes are called **anaerobic respiration**. Some of the electron acceptors used in anaerobic respiration include nitrate (NO_3^-), reduced to nitrite, NO_2^-, by *Escherichia coli* or to N_2 by *Pseudomonas* species), ferric iron (Fe^{3+}, reduced to Fe^{2+} by *Geobacter* species), sulfate (SO_4^{2-}, reduced to hydrogen sulfide, H_2S, by *Desulfovibrio* species), carbonate (CO_3^{2-}, reduced to methane, CH_4, by methanogens or to acetate by acetogens), and even certain organic compounds. Some of these acceptors, for example Fe^{3+}, are often only available in the form of insoluble

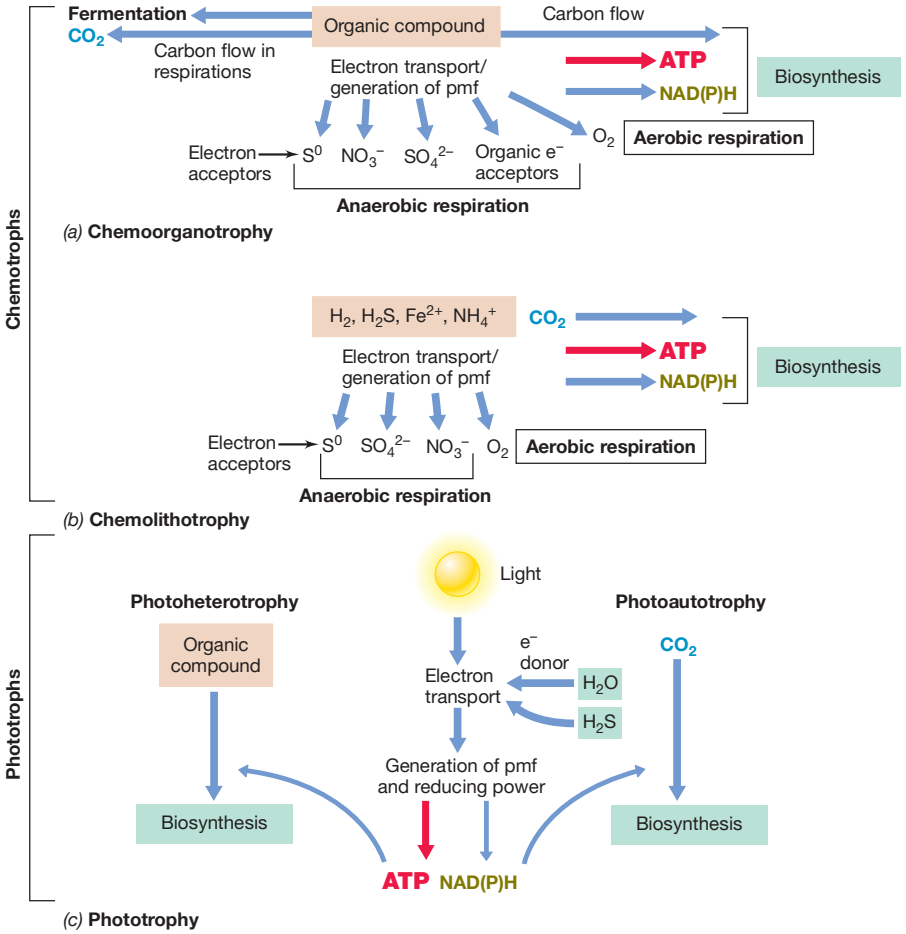


Figure 4.22 Catabolic diversity. *(a)* Chemoorganotrophs. *(b)* Chemolithotrophs. *(c)* Phototrophs. Chemoorganotrophs differ from chemolithotrophs in two important ways: (1) The nature of the electron donor (organic versus inorganic compounds, respectively), and (2) The nature of the source of cellular carbon (organic compounds versus CO_2 respectively). However, note the importance of electron transport driving proton motive force formation in all forms of respiration and in photosynthesis.

minerals, such as metal oxides. These common minerals, widely distributed in nature, allow for anaerobic respiration in a wide variety of microbial habitats.

Because of the positions of these alternative electron acceptors on the redox tower (none has an E_0' as positive as the O_2/H_2O couple; Figure 4.9), less energy is released when they are reduced instead of oxygen (recall that $\Delta G^{0\prime}$ is proportional to $\Delta E_0'$; Section 4.6). Nevertheless, because O_2 is often limiting or absent in many microbial habitats, anaerobic respirations can be very important means of energy generation. As in aerobic respiration, anaerobic respirations involve electron transport, generation of a proton motive force, and the activity of ATPase.

Chemolithotrophy

Organisms able to use *inorganic* chemicals as electron donors are called **chemolithotrophs**. Examples of relevant inorganic electron donors include H_2S, hydrogen gas (H_2), Fe^{2+}, and NH_3.

Chemolithotrophic metabolism is typically aerobic and begins with the oxidation of the inorganic electron donor (Figure 4.22). Electrons from the inorganic donor enter an electron transport chain and a proton motive force is formed in exactly the same way as for chemoorganotrophs (Figure 4.19). However, one important distinction between chemolithotrophs and chemoorganotrophs, besides their electron donors, is their source of carbon for biosynthesis. Chemoorganotrophs use organic compounds (glucose, acetate, and the like) as carbon sources. By contrast, chemolithotrophs use carbon dioxide (CO_2) as a carbon source and are therefore **autotrophs** (organisms capable of biosynthesizing all cell material from CO_2 as the sole carbon source). We consider many examples of chemolithotrophy in Chapter 13.

Phototrophy

Many microorganisms are **phototrophs**, using light as an energy source in the process of photosynthesis. The mechanisms by which light is used as an energy source are complex, but the end result is the same as in respiration: generation of a proton motive force that is used to drive ATP synthesis. Light-mediated ATP synthesis is called **photophosphorylation**. Most phototrophs use energy conserved in ATP for the assimilation of CO_2 as the carbon source for biosynthesis; they are called *photoautotrophs*. However, some phototrophs use organic compounds as carbon

sources with light as the energy source; these are the *photoheterotrophs* (Figure 4.22).

As we discussed in Chapter 2, there are two types of photosynthesis: *oxygenic* and *anoxygenic*. Oxygenic photosynthesis, carried out by cyanobacteria and their relatives and also by green plants, results in O_2 evolution. Anoxygenic photosynthesis is a simpler process used by purple and green bacteria that does not evolve O_2. The reactions leading to proton motive force formation in both forms of photosynthesis have strong parallels, as we see in Chapter 13.

The Proton Motive Force and Catabolic Diversity

Microorganisms show an amazing diversity of bioenergetic strategies. Thousands of organic compounds, many inorganic compounds, and light can be used by one or another microorganism as an energy source. With the exception of fermentations, in which substrate-level phosphorylation occurs (Section 4.8), energy conservation in respiration and photosynthesis is driven by the proton motive force.

Whether electrons come from the oxidation of organic or inorganic chemicals or from phototrophic processes, in all forms of respiration and photosynthesis, energy conservation is linked to the pmf through ATPase (Figure 4.20). Considered in this way, respiration and anaerobic respiration are simply metabolic variations employing different electron acceptors. Likewise, chemoorganotrophy, chemolithotrophy, and photosynthesis are simply metabolic variations upon a theme of different electron donors. Electron transport and the pmf link all of these processes, bringing these seemingly quite different forms of metabolism into a common focus. We pick up on this theme in Chapters 13 and 14.

MiniQuiz

- In terms of their electron donors, how do chemoorganotrophs differ from chemolithotrophs?
- What is the carbon source for autotrophic organisms?
- Why can it be said that the proton motive force is a unifying theme in most of bacterial metabolism?

V Essentials of Anabolism

We close this chapter with a brief consideration of biosynthesis. Our focus here will be on biosynthesis of the building blocks of the four classes of macromolecules—sugars, amino acids, nucleotides, and fatty acids. Collectively, these biosyntheses are called *anabolism*. In Chapters 6 and 7 we consider synthesis of the macromolecules themselves, in particular, nucleic acids and proteins.

Many detailed biochemical pathways support the metabolic patterns we present here, but we will keep our focus on the essential principles. We finish with a glimpse at how the enzymes that drive these biosynthetic processes are controlled by the cell. For a cell to be competitive, it must regulate its metabolism. This happens in several ways and at several levels, one of which, the control of enzyme activity, is relevant to our discussion here.

4.13 Biosynthesis of Sugars and Polysaccharides

Polysaccharides are key constituents of the cell walls of many organisms, and in *Bacteria*, the peptidoglycan cell wall (↻ Section 3.6) has a polysaccharide backbone. In addition, cells often store carbon and energy reserves in the form of the polysaccharides glycogen and starch (↻ Section 3.10). The monomeric units of these polysaccharides are six-carbon sugars called *hexoses*, in particular, glucose or glucose derivatives. In addition to hexoses, five-carbon sugars called *pentoses* are common in the cell. Most notably, these include ribose and deoxyribose, present in the backbone of RNA and DNA, respectively.

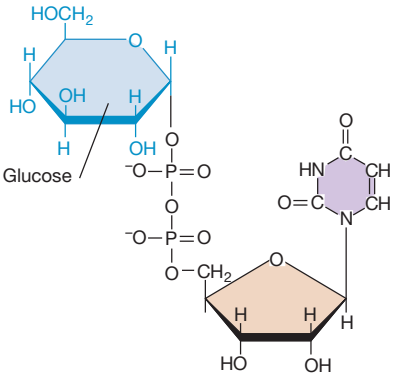
In prokaryotes, polysaccharides are synthesized from either uridine diphosphoglucose (UDPG; **Figure 4.23**) or adenosine diphosphoglucose (ADPG), both of which are *activated* forms of glucose. ADPG is the precursor for the biosynthesis of glycogen. UDPG is the precursor of various glucose derivatives needed for the biosynthesis of other polysaccharides in the cell, such as *N*-acetylglucosamine and *N*-acetylmuramic acid in peptidoglycan or the lipopolysaccharide component of the gram-negative outer membrane (↻ Sections 3.6 and 3.7). Polysaccharides are produced by adding glucose (from the activated form) to the preexisting polymer; for example, ADPG + glycogen → ADP + glycogen-glucose.

When a cell is growing on a hexose such as glucose, obtaining glucose for polysaccharide synthesis is obviously not a problem. But when the cell is growing on other carbon compounds, glucose must be synthesized. This process, called *gluconeogenesis*, uses phosphoenolpyruvate, one of the intermediates of glycolysis (Figure 4.14), as starting material. Phosphoenolpyruvate can be synthesized from oxalacetate, a citric acid cycle intermediate (Figure 4.21). An overview of gluconeogenesis is shown in Figure 4.23*b*.

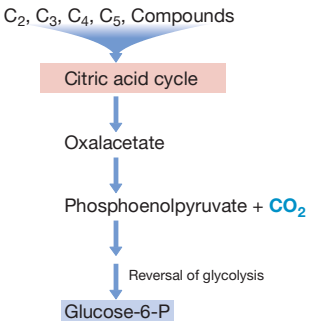
Pentoses are formed by the removal of one carbon atom from a hexose, typically as CO_2. The pentoses needed for nucleic acid synthesis, ribose and deoxyribose, are formed as shown in Figure 4.23*c*. The enzyme ribonucleotide reductase converts ribose into deoxyribose by reduction of the hydroxyl (−OH) group on the 2′ carbon of the 5-carbon sugar ring. Interestingly, this reaction occurs after, not before, synthesis of nucleotides. Thus, *ribo*nucleotides are biosynthesized, and some of them are later reduced to *deoxy*ribonucleotides for use as precursors of DNA.

MiniQuiz

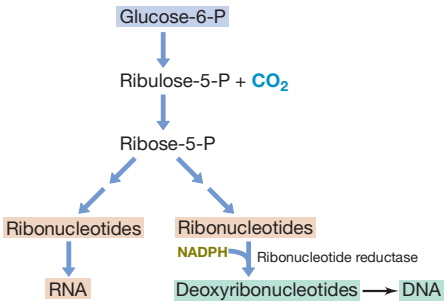
- How does anabolism differ from catabolism? Give an example of each.
- What form of activated glucose is used in the biosynthesis of glycogen by bacteria?
- What is gluconeogenesis?



(a)



(b)



(c)

Figure 4.23 **Sugar metabolism.** *(a)* Polysaccharides are synthesized from activated forms of hexoses such as UDPG. Glucose is shown here in blue. *(b)* Gluconeogenesis. When glucose is needed, it can be biosynthesized from other carbon compounds, generally by the reversal of steps in glycolysis. *(c)* Pentoses for nucleic acid synthesis are formed by decarboxylation of hexoses such as glucose-6-phosphate. Note how the precursors of DNA are produced from the precursors of RNA by the enzyme ribonucleotide reductase. This enzyme reduces the 2′ hydroxyl group of the sugar, converting ribose to deoxyribose and reducing the hydroxyl group to water, and is active on all four ribonucleotides.

4.14 Biosynthesis of Amino Acids and Nucleotides

The monomers in proteins and nucleic acids are amino acids and nucleotides, respectively. Their biosyntheses are often long, multistep pathways and so we approach their biosyntheses here by identifying the key carbon skeletons needed to begin the biosynthetic pathways.

Monomers of Proteins: Amino Acids

Organisms that cannot obtain some or all of their amino acids preformed from the environment must synthesize them from other sources. Amino acids are grouped into structurally related *families* that share several biosynthetic steps. The carbon skeletons for amino acids come almost exclusively from intermediates of glycolysis (Figure 4.14) or the citric acid cycle (Figure 4.21; **Figure 4.24**).

The amino group of amino acids is typically derived from some inorganic nitrogen source in the environment, such as ammonia (NH_3). Ammonia is most often incorporated in formation of the amino acids glutamate or glutamine by the enzymes *glutamate dehydrogenase* and *glutamine synthetase*, respectively (**Figure 4.25**). When NH_3 is present at high levels, glutamate dehydrogenase or other amino acid dehydrogenases are used. However, when NH_3 is present at low levels, glutamine synthetase, with its energy-consuming reaction mechanism (Figure 4.25*b*) and high affinity for substrate, is employed. We discuss control of the activity of the important enzyme glutamine synthetase in Section 4.16.

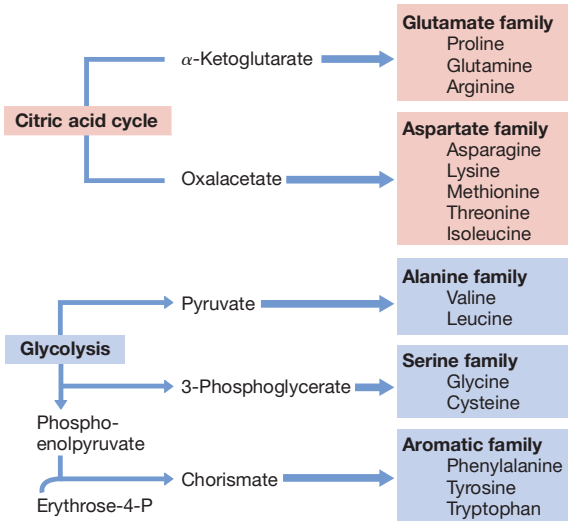


Figure 4.24 **Amino acid families.** The citric acid cycle and glycolysis provide the carbon skeletons for most amino acids. Synthesis of the various amino acids in a family may require many steps starting with the parent amino acid (shown in bold as the name of the family). Glycolysis is discussed in Section 4.8 (see Figure 4.14) and the citric acid cycle is discussed in Section 4.11 (see Figure 4.21).

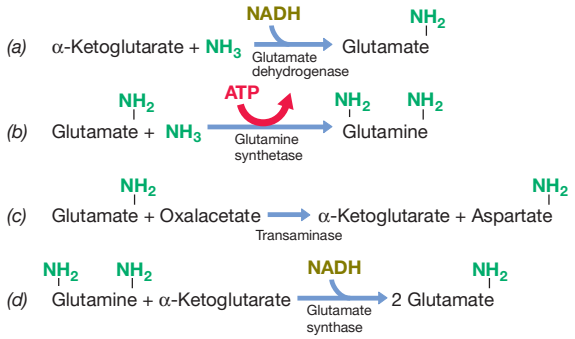


Figure 4.25 **Ammonia incorporation in bacteria.** To emphasize the flow of nitrogen, both free ammonia (NH_3) and the amino groups of all amino acids are shown in green. Two major pathways for NH_3 assimilation in bacteria are those catalyzed by the enzymes *(a)* glutamate dehydrogenase and *(b)* glutamine synthetase. *(c)* Transaminase reactions transfer an amino group from an amino acid to an organic acid. *(d)* The enzyme glutamate synthase forms two glutamates from one glutamine and one α-ketoglutarate.

Once ammonia is incorporated into glutamate or glutamine, the amino group can be transferred to form other nitrogenous compounds. For example, glutamate can donate its amino group to oxalacetate in a transaminase reaction, producing α-ketoglutarate and aspartate (Figure 4.25*c*). Alternatively, glutamine can react with α-ketoglutarate to form two molecules of glutamate in an aminotransferase reaction (Figure 4.25*d*). The end result of these types of reactions is the shuttling of ammonia into various carbon skeletons from which further biosynthetic reactions can occur to form all 22 amino acids (↻ Figure 6.29) needed to make proteins.

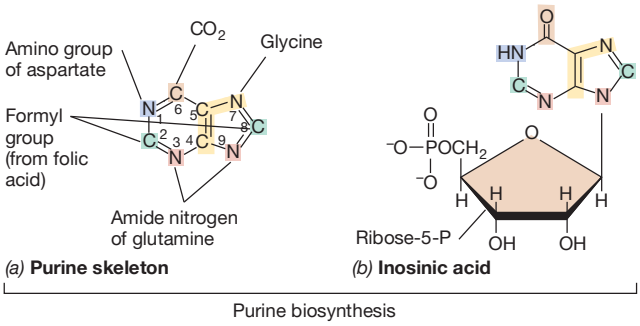
Monomers of Nucleic Acids: Nucleotides

The biochemistry behind purine and pyrimidine biosynthesis is quite complex. Purines are constructed literally atom by atom from several carbon and nitrogen sources, including even CO_2 (**Figure 4.26**). The first key purine, inosinic acid (Figure 4.26*b*), is the precursor of the purine nucleotides adenine and guanine. Once these are synthesized (in their triphosphate forms) and attached to ribose, they are ready to be incorporated into DNA (following ribonucleotide reductase activity) or RNA.

Like the purine ring, the pyrimidine ring is also constructed from several sources (Figure 4.26*c*). The first key pyrimidine is the compound uridylate (Figure 4.26*d*), and from this the pyrimidines thymine, cytosine, and uracil are derived. Structures of all of the purines and pyrimidines are shown in Figure 6.1.

MiniQuiz

- What is an amino acid family?
- List the steps required for the cell to incorporate NH_3 into amino acids.
- Which nitrogen bases are purines and which are pyrimidines?



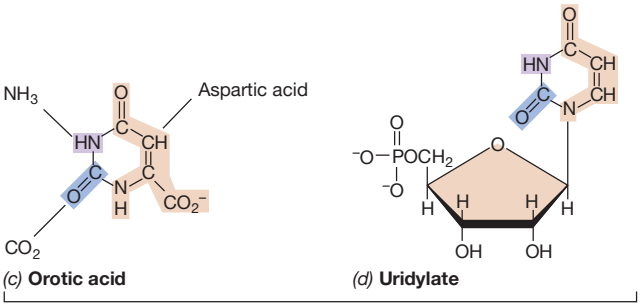


Figure 4.26 **Composition of purines and pyrimidines.** *(a)* Components of the purine skeleton. *(b)* Inosinic acid, the precursor of all purine nucleotides. *(c)* Components of the pyrimidine skeleton, orotic acid. *(d)* Uridylate, the precursor of all pyrimidine nucleotides. Uridylate is formed from orotate following a decarboxylation and the addition of ribose 5-phosphate.

4.15 Biosynthesis of Fatty Acids and Lipids

Lipids are important constituents of cells, as they are major structural components of membranes. Lipids can also be carbon and energy reserves. Other lipids function in and around the cell surface, including, in particular, the lipopolysaccharide layer of the outer membrane of gram-negative bacteria (↻ Section 3.7). A cell can make many different types of lipids, some of which are produced only under certain conditions or have special functions in the cells. The biosynthesis of fatty acids is thus a major series of reactions in cells. Recall that *Archaea* do not contain fatty acids in their membrane lipids, but have instead branched side chains constructed of multiples of isoprene, a C_5 branched chained hydrocarbon (↻ Figure 3.7).

Fatty Acid Biosynthesis

Fatty acids are biosynthesized two carbon atoms at a time with the help of a protein called *acyl carrier protein* (ACP). ACP holds the growing fatty acid as it is being synthesized and releases it once it has reached its final length (**Figure 4.27**). Although fatty acids are constructed *two* carbons at a time, each C_2 unit originates from the C_3 compound malonate, which is attached to the ACP to form malonyl-ACP. As each malonyl residue is donated, one molecule of CO_2 is released (Figure 4.27).

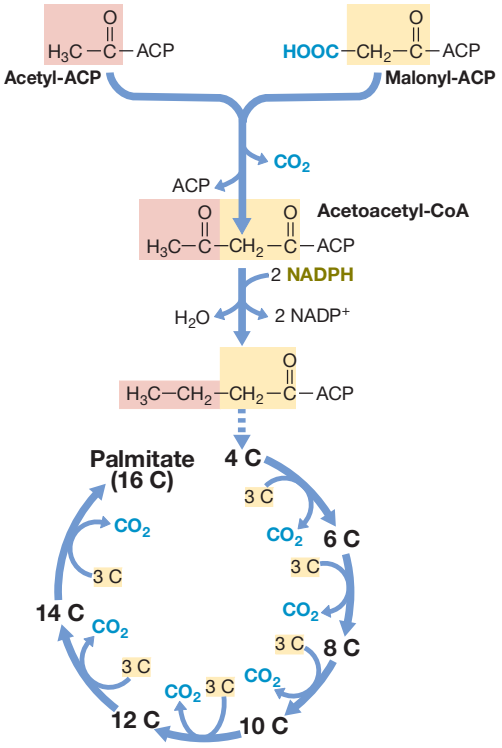


Figure 4.27 **The biosynthesis of the C_{16} fatty acid palmitate.** The condensation of acetyl-ACP and malonyl-ACP forms acetoacetyl-CoA. Each successive addition of an acetyl unit comes from malonyl-ACP.

The fatty acid composition of cells varies from species to species and can also vary within a species due to differences in temperature. Growth at low temperatures promotes the biosynthesis and insertion in membrane lipids of shorter-chain fatty acids whereas growth at higher temperatures promotes longer-chain fatty acids. The most common fatty acids in lipids of *Bacteria* are those with chain lengths of C_{12}–C_{20}.

In addition to saturated, even-carbon-number fatty acids, fatty acids can also be unsaturated, branched, or have an odd number of carbon atoms. Unsaturated fatty acids contain one or more double bonds in the long hydrophobic portion of the molecule. The number and position of these double bonds is often species-specific or group-specific, and double bonds typically form by desaturation reactions after the saturated fatty acid has formed. Branched-chain fatty acids are biosynthesized using an initiating molecule that contains a branched-chain fatty acid, and odd-carbon-number fatty acids are biosynthesized using an initiating molecule that contains a propionyl (C_3) group.

Lipid Biosynthesis

In the assembly of lipids in cells of *Bacteria* and *Eukarya*, fatty acids are added to glycerol. For simple triglycerides (fats), all three glycerol carbons are esterified with fatty acids. In complex lipids, one of the carbon atoms in glycerol contains a molecule of phosphate, ethanolamine, carbohydrate, or some other polar substance (↻ Figure 3.4*a*). In *Archaea*, membrane lipids contain phytanyl (C_{15}) or biphytanyl (C_{30}) side chains (↻ Figure 3.7) instead of fatty acids, and the biosynthesis of phytanyl is distinct from that described here for fatty acids. However, as for the lipids of *Bacteria* or *Eukarya*, the glycerol backbone of archaeal membrane lipids also contains a polar group (a sugar, phosphate, sulfate, or polar organic compound) that facilitates formation of the typical membrane architecture: a hydrophobic interior with hydrophilic surfaces (↻ Figure 3.7).

MiniQuiz

- Explain why in fatty acid synthesis fatty acids are constructed two carbon atoms at a time even though the immediate donor for these carbons contains three carbon atoms.

4.16 Regulating the Activity of Biosynthetic Enzymes

We have just reviewed some of the key cellular biosyntheses. Anabolism requires hundreds of different enzymatic reactions, and many of the enzymes that catalyze these reactions are highly regulated. The advantage of regulation is clear: If the compound to be biosynthesized is available from the environment, neither carbon nor energy need be wasted in its biosynthesis.

There are two major modes of enzyme regulation in cells, one that controls the *amount* (or even the complete presence or absence) of an enzyme and another that controls the *activity* of an enzyme. In prokaryotic cells, the amount of a given enzyme is regulated at the gene level, and we reserve discussion of this until after we have considered some principles of molecular biology. Here we focus on what the cell can do to control the activity of enzymes already present in the cell.

Inhibition of an enzyme's activity is typically the result of either covalent or noncovalent changes in its structure. We begin with feedback inhibition and isoenzymes, both examples of noncovalent interactions, and end with the example of covalent modification of the enzyme glutamine synthetase.

Feedback Inhibition

A major means of controlling enzymatic activity is by **feedback inhibition**. This mechanism temporarily shuts off the reactions in an entire biosynthetic pathway. The reactions are shut off because an excess of the end product of the pathway inhibits activity of an early (typically the *first*) enzyme of the pathway. Inhibiting an early step effectively shuts down the entire pathway because no intermediates are generated for enzymes farther down the pathway (**Figure 4.28**). Feedback inhibition is reversible, however, because once levels of the end product become limiting, the pathway again becomes functional.

How can the end product of a pathway inhibit the activity of an enzyme whose substrate is quite unrelated to it? This occurs because the inhibited enzyme is an **allosteric enzyme**, an enzyme that has two binding sites, the *active site* (where substrate binds, Section 4.5), and the *allosteric site*, where the end product of the pathway binds. When the end product is in excess, it binds at the