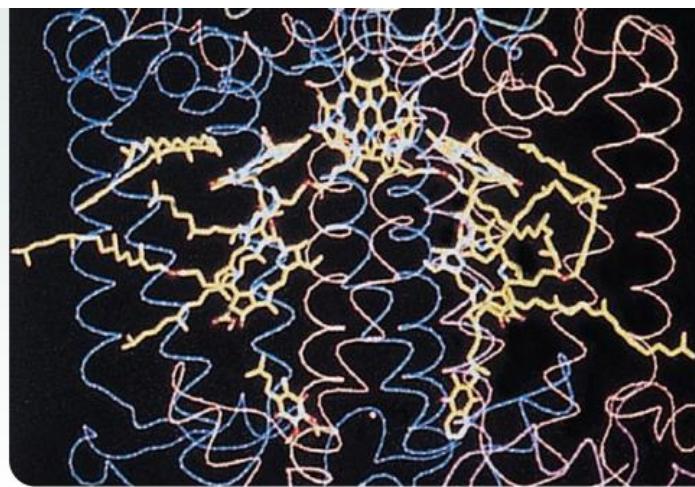


9

Metabolism: Energy Release and Conservation



The reaction center of the purple nonsulfur bacterium, *Rhodopseudomonas viridis*, with the bacteriochlorophylls and other prosthetic groups in yellow. These pigments trap light during photosynthesis.

PREVIEW

- Chemoorganotrophs have three fueling-process options. They are differentiated by the electron acceptor used. Respiration uses exogenous electron acceptors— O_2 for aerobic respiration, and other molecules for anaerobic respiration. Fermentation uses endogenous electron acceptors.
- During catabolism, nutrients are funneled into a few common pathways for more efficient use of enzymes (a few pathways process a wide variety of nutrients).
- The most widely used pathways are the Embden-Meyerhof pathway, the pentose phosphate pathway, and the tricarboxylic acid cycle. These pathways are amphibolic, functioning both catabolically and anabolically. The tricarboxylic acid cycle is the final pathway for the aerobic oxidation of nutrients to CO_2 .
- The majority of energy released during aerobic and anaerobic respiration is generated by the movement of electrons from electron transport carriers with more negative reduction potentials to ones with more positive reduction potentials. Because the O_2/H_2O redox couple has a very positive standard reduction potential, aerobic respiration is much more efficient than anaerobic catabolism.
- Chemolithotrophs use reduced inorganic molecules as electron donors for electron transport and ATP synthesis.
- In chlorophyll-based photosynthesis, trapped light energy boosts electrons to more negative reduction potentials (i.e., higher energy levels). These energized electrons are then used to make ATP. Some prokaryotes carry out rhodopsin-based phototrophy. Electron flow is not involved in this process.
- Proton motive force is a type of potential energy generated by: (1) oxidation of chemical energy sources coupled to electron transport; (2) light-driven electron transport; and (3) light-driven pumping of protons during rhodopsin-based phototrophy; it is used to power the production of ATP and other processes such as transport and bacterial motility.

From the open seas to a eutrophic lake, from a log rotting in a forest to a microbrewery, and from a hydrothermal vent to the tailings near a coal mine, the impact of the fueling reactions of microorganisms can be seen. Phototrophs convert the energy of the sun into chemical energy (figure 9.1), which feeds chemoorganotrophs. Chemoorganotrophs recycle the wastes of other organisms and play important roles in industry; chemolithotrophs oxidize inorganic molecules and in the process contribute to biogeochemical cycles such as the iron and sulfur cycles. All can contribute to pollution and all can help in the maintenance of pristine environments.

This chapter examines the fueling reactions of these diverse nutritional types. We begin with an overview of the metabolism of chemoorganotrophs. This is followed by an introduction to the oxidation of carbohydrates, especially glucose, and a discussion of the generation of ATP by aerobic and anaerobic respiration. Fermentation is then described, followed by a survey of the breakdown of other carbohydrates and organic substances such as lipids, proteins, and amino acids. We end the chapter with sections on chemolithotrophy (the oxidation of inorganic energy sources) and phototrophy (the conversion of light energy into chemical energy).

9.1 CHEMOORGANOTROPHIC FUELING PROCESSES

Recall from chapter 8 that chemoorganotrophs oxidize an organic energy source and conserve the energy released in the form of ATP. The electrons released are accepted by a variety of electron

It is in the fueling reactions that bacteria display their extraordinary metabolic diversity and versatility. Bacteria have evolved to thrive in almost all natural environments, regardless of the nature of available sources of carbon, energy, and reducing power. . . . The collective metabolic capacities of bacteria allow them to metabolize virtually every organic compound on this planet. . . .

—F. C. Neidhardt, J. L. Ingraham, and M. Schaechter

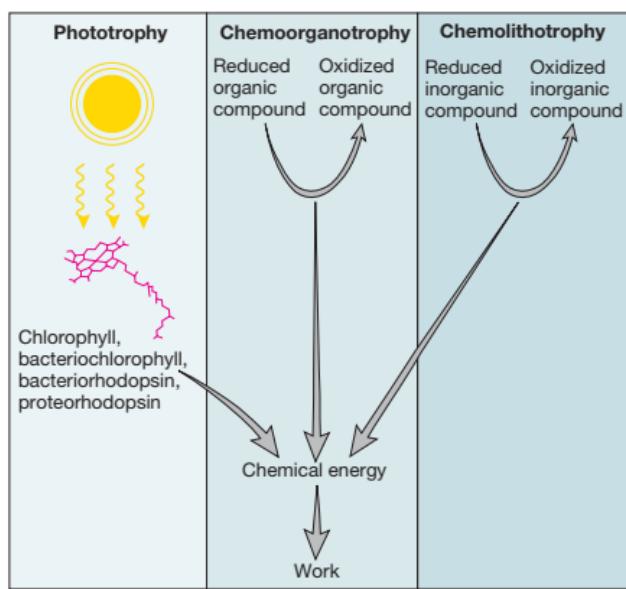


Figure 9.1 Sources of Energy for Microorganisms. Most microorganisms employ one of three energy sources. Phototrophs trap radiant energy from the sun using pigments such as bacteriochlorophyll and chlorophyll. Chemotrophs oxidize reduced organic and inorganic nutrients to liberate and trap energy. The chemical energy derived from these three sources can then be used in work as discussed in chapter 8.

acceptors, and whether the acceptor is exogenous (that is, externally supplied) or endogenous (internally supplied) defines the energy-conserving process used by the organism. When the electron acceptor is exogenous, the metabolic process is called **respiration** and may be divided into two different types (**figure 9.2**). In **aerobic respiration**, the final electron acceptor is oxygen, whereas the terminal acceptor in **anaerobic respiration** is a different exogenous acceptor such as NO_3^- , SO_4^{2-} , CO_2 , Fe^{3+} , and SeO_4^{2-} . Organic acceptors such as fumarate and humic acids also may be used. Respiration involves the activity of an electron transport chain. As electrons pass through the chain to the final electron acceptor, a type of potential energy called the **proton motive force** (PMF) is generated and used to synthesize ATP from ADP and P_i . In contrast, **fermentation** [Latin *fermentare*, to cause to rise or ferment] uses an endogenous electron acceptor and does not involve an electron transport chain or the generation of PMF. The endogenous electron acceptor is usually an intermediate (e.g., pyruvate) of the catabolic pathway used to degrade and oxidize the organic energy source. During fermentation, ATP is synthesized only by **substrate-level phosphorylation**, a process in which a phosphate group is transferred to ADP from a high-energy molecule (e.g., phosphoenolpyruvate) generated by catabolism of the energy source.

In the following sections, we explore the metabolism of chemoorganotrophs in more detail. The discussion begins with aerobic respiration and introduces a number of metabolic pathways and other processes that also occur during anaerobic respi-

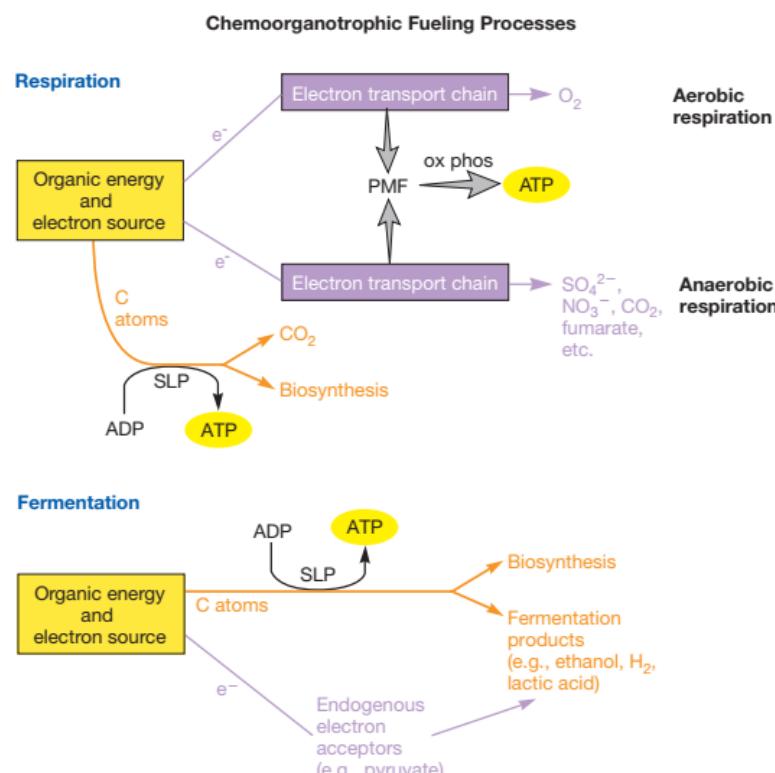


Figure 9.2 Chemoorganotrophic Fueling Processes. Organic molecules serve as energy and electron sources for all three fueling processes used by chemoorganotrophs. In aerobic respiration and anaerobic respiration, the electrons pass through an electron transport system. This generates a proton motive force (PMF), which is used to synthesize most of the cellular ATP by a mechanism called oxidative phosphorylation (ox phos); a small amount of ATP is made by a process called substrate-level phosphorylation (SLP). In aerobic respiration, O_2 is the terminal electron acceptor, whereas in anaerobic respiration exogenous molecules other than O_2 serve as electron acceptors. During fermentation, endogenous organic molecules act as electron acceptors, the electron flow is not coupled with ATP synthesis, and ATP is synthesized only by substrate-level phosphorylation.

ration. Fermentation involves only a subset of the pathways that function during respiration, but is widely used by microbes and has important practical applications.

9.2 AEROBIC RESPIRATION

Aerobic respiration involves several important catabolic pathways. Before learning about some of the more important ones, it is best to look at the “lay of the land” and get our bearings. Albert Lehninger, a biochemist who worked at Johns Hopkins Medical School, helped considerably by pointing out that aerobic respiration may be divided into three stages (figure 9.3). In the first stage, larger nutrient molecules (proteins, polysaccharides, and lipids) are hydrolyzed or otherwise broken down into their constituent parts. The chemical reactions occurring during this stage do not release much energy. Amino acids, monosaccharides, fatty acids, glycerol, and other products of the first stage are degraded to a few simpler molecules in the second stage. Usually

metabolites like acetyl coenzyme A and pyruvate are formed. In addition, the second stage produces some ATP as well as NADH and/or FADH₂. Finally during the third stage of catabolism, partially oxidized carbon is fed into the tricarboxylic acid cycle and oxidized completely to CO₂ with the production of ATP, NADH, and FADH₂. Most of the ATP derived from aerobic respiration comes from the oxidation of NADH and FADH₂ by the electron transport chain, which uses oxygen as the terminal electron acceptor.

Although this picture is somewhat oversimplified, it is useful in discerning the general pattern of respiration. Notice that the microorganism begins with a wide variety of molecules and reduces their number and diversity at each stage. That is, nutrient molecules are funneled into ever fewer metabolic intermediates until they are finally fed into the tricarboxylic acid cycle. A common pathway often degrades many similar molecules (e.g., several different sugars). These metabolic pathways consist of enzyme-catalyzed reactions arranged so that the product of one reaction serves as a substrate for the next. The existence of a few common catabolic pathways, each degrading many nutrients,

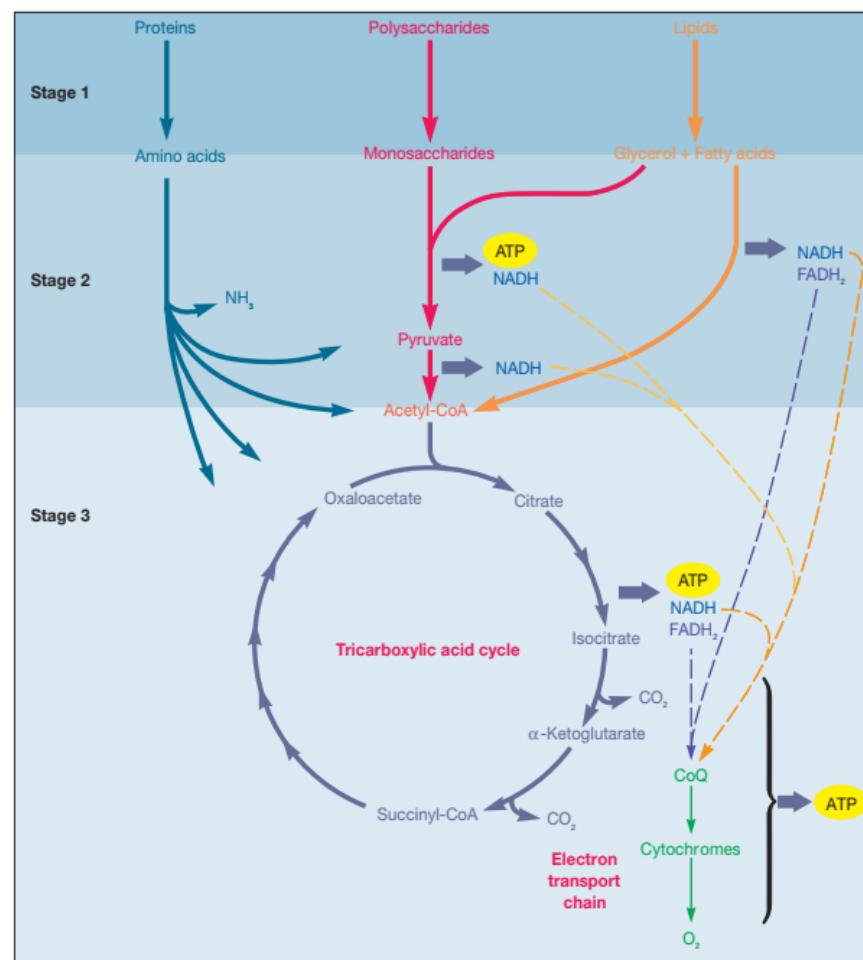


Figure 9.3 The Three Stages of Aerobic Respiration. A general diagram of aerobic respiration in a chemoorganoheterotroph showing the three stages in this process and the central position of the tricarboxylic acid cycle. Although there are many different proteins, polysaccharides, and lipids, they are degraded through the activity of a few common metabolic pathways. The dashed lines show the flow of electrons, carried by NADH and FADH₂, to the electron transport chain.

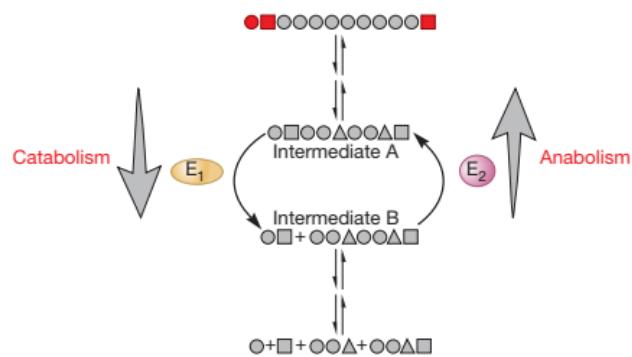


Figure 9.4 Amphibolic Pathway. A simplified diagram of an amphibolic pathway, such as glycolysis. Note that the interconversion of intermediates A and B is catalyzed by two separate enzymes, E_1 operating in the catabolic direction, and E_2 in the anabolic.

greatly increases metabolic efficiency by avoiding the need for a large number of less metabolically flexible pathways.

Although biosynthesis is the topic of chapter 10, it is important to point out that many catabolic pathways also are important in anabolism. They supply materials needed for biosynthesis, including **precursor metabolites** and reducing power. Precursor metabolites serve as the starting molecules for biosynthetic pathways. Reducing power is used to reduce the carbon skeletons provided by the precursor metabolites as they are transformed into amino acids, nucleotides, and the other small molecules needed for synthesis of macromolecules. Pathways that function both catabolically and anabolically are called **amphibolic pathways** [Greek *amphi*, on both sides]. Three of the most important amphibolic pathways are the Embden-Meyerhof pathway, the pentose phosphate pathway, and the tricarboxylic acid (TCA) cycle. Many of the reactions of the Embden-Meyerhof pathway and the TCA cycle are freely reversible and can be used to synthesize or degrade molecules depending on the nutrients available and the needs of the microbe. The few irreversible catabolic steps are bypassed in biosynthesis with alternate enzymes that catalyze the reverse reaction (**figure 9.4**). For example, the enzyme fructose bisphosphatase reverses the phosphofructokinase step when glucose is synthesized from pyruvate. The presence of two separate enzymes, one catalyzing the reversal of the other's reaction, permits independent regulation of the catabolic and anabolic functions of these amphibolic pathways. [The precursor metabolites \(section 10.2\)](#)

1. Compare and contrast fermentation and respiration. Give examples of the types of electron acceptors used by each process. What is the difference between aerobic respiration and anaerobic respiration?
2. Why is it to the cell's advantage to catabolize diverse organic energy sources by funneling them into a few common pathways?
3. What is an amphibolic pathway? Why are amphibolic pathways important?

9.3 THE BREAKDOWN OF GLUCOSE TO PYRUVATE

Microorganisms employ several metabolic pathways to catabolize glucose and other sugars. Because of this metabolic diversity, their metabolism is often confusing. To avoid confusion as much as possible, the ways in which microorganisms degrade sugars to pyruvate and similar intermediates are introduced by focusing on only three routes: (1) the Embden-Meyerhof pathway, (2) the pentose phosphate pathway, and (3) the Entner-Doudoroff pathway. In this text, these three pathways will be referred to collectively as **glycolytic pathways** or as **glycolysis** [Greek *glyko*, sweet, and *lysis*, a loosening]. However, in other texts, the term glycolysis is often reserved for use in reference only to the Embden-Meyerhof pathway. For the sake of simplicity, the detailed structures of metabolic intermediates are not used in pathway diagrams. [Common metabolic pathways \(appendix II\)](#)

The Embden-Meyerhof Pathway

The **Emden-Meyerhof pathway** is undoubtedly the most common pathway for glucose degradation to pyruvate in stage two of aerobic respiration. It is found in all major groups of microorganisms and functions in the presence or absence of O_2 . As noted earlier, it is also an important amphibolic pathway and provides several precursor metabolites. The Embden-Meyerhof pathway occurs in the cytoplasmic matrix of prokaryotes and eukaryotes.

The pathway as a whole may be divided into two parts (**figure 9.5** and [appendix II](#)). In the initial six-carbon phase, energy is consumed as glucose is phosphorylated twice, and is converted to fructose 1,6-bisphosphate. This preliminary phase consumes two ATP molecules for each glucose and "primes the pump" by adding phosphates to each end of the sugar. In essence, the organism invests some of its ATP so that more can be made later in the pathway.

The three-carbon, energy-conserving phase begins when the enzyme fructose 1,6-bisphosphate aldolase catalyzes the cleavage of fructose 1,6-bisphosphate into two halves, each with a phosphate group. One of the products, dihydroxyacetone phosphate, is immediately converted to glyceraldehyde 3-phosphate. This yields two molecules of glyceraldehyde 3-phosphate, which are then converted to pyruvate in a five-step process. Because dihydroxyacetone phosphate can be easily changed to glyceraldehyde 3-phosphate, both halves of fructose 1,6-bisphosphate are used in the three-carbon phase. First, glyceraldehyde 3-phosphate is oxidized with NAD^+ as the electron acceptor (to form NADH), and a phosphate (P_i) is simultaneously incorporated to give a high-energy molecule called 1,3-bisphosphoglycerate. The high-energy phosphate on carbon one is subsequently donated to ADP to produce ATP. This synthesis of ATP is called **substrate-level phosphorylation** because ADP phosphorylation is coupled with the exergonic breakdown of a high-energy bond. [The role of ATP in metabolism \(section 8.5\)](#)

A somewhat similar process generates a second ATP by substrate-level phosphorylation. The phosphate group on 3-phosphoglycerate shifts to carbon two, and 2-phosphoglycerate is

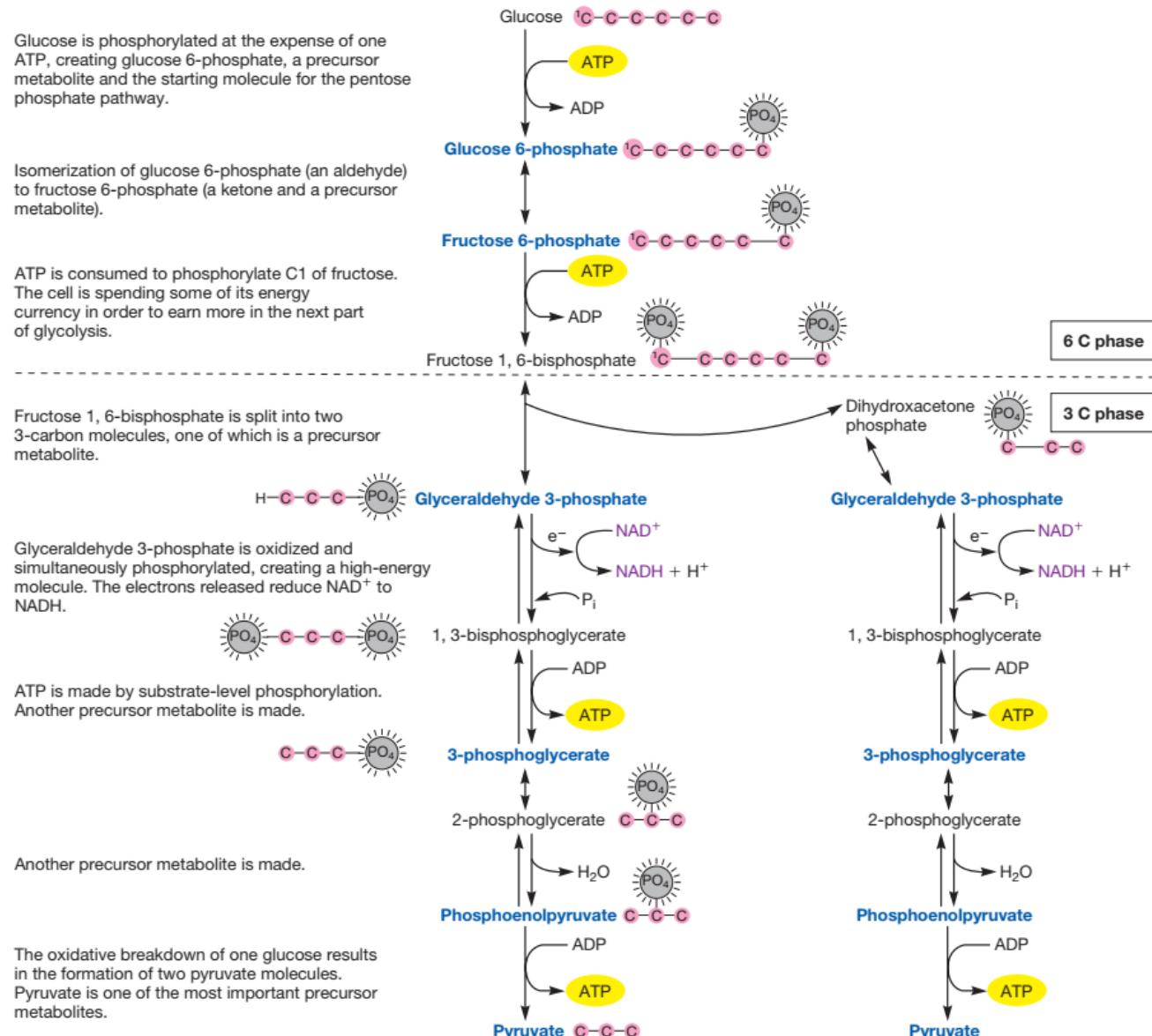


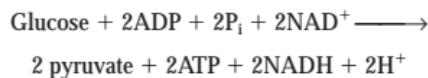
Figure 9.5 Embden-Meyerhof Pathway. This is one of three glycolytic pathways used to catabolize glucose to pyruvate, and it can function during aerobic respiration, anaerobic respiration, and fermentation. When used during a respiratory process, the electrons accepted by NAD⁺ are transferred to an electron transport chain and are ultimately accepted by an exogenous electron acceptor. When used during fermentation, the electrons accepted by NAD⁺ are donated to an endogenous electron acceptor (e.g., pyruvate). The Embden-Meyerhof pathway is also an important amphibolic pathway, as it generates several precursor metabolites (shown in blue).

dehydrated to form a second high-energy molecule, phosphoenolpyruvate. This molecule donates its phosphate to ADP forming a second ATP and pyruvate, the final product of the pathway.

The Embden-Meyerhof pathway degrades one glucose to two pyruvates by the sequence of reactions just outlined and shown in

figure 9.5. ATP and NADH are also produced. The yields of ATP and NADH may be calculated by considering the two phases separately. In the six-carbon phase, two ATPs are used to form fructose 1,6-bisphosphate. For each glyceraldehyde 3-phosphate transformed into pyruvate, one NADH and two ATPs are formed.

Because two glyceraldehyde 3-phosphates arise from a single glucose (one by way of dihydroxyacetone phosphate), the three-carbon phase generates four ATPs and two NADHs per glucose. Subtraction of the ATP used in the six-carbon phase from that produced by substrate-level phosphorylation in the three-carbon phase gives a net yield of two ATPs per glucose. Thus the catabolism of glucose to pyruvate can be represented by this simple equation.



The Pentose Phosphate Pathway

A second pathway, the **pentose phosphate or hexose monophosphate pathway**, may be used at the same time as either the Embden-Meyerhof or the Entner-Doudoroff pathways. It can operate either aerobically or anaerobically and is important in both biosynthesis and catabolism.

The pentose phosphate pathway begins with the oxidation of glucose 6-phosphate to 6-phosphogluconate followed by the oxidation of 6-phosphogluconate to the pentose sugar ribulose 5-phosphate and CO_2 (figure 9.6 and appendix II). NADPH is produced during these oxidations. Ribulose 5-phosphate is then

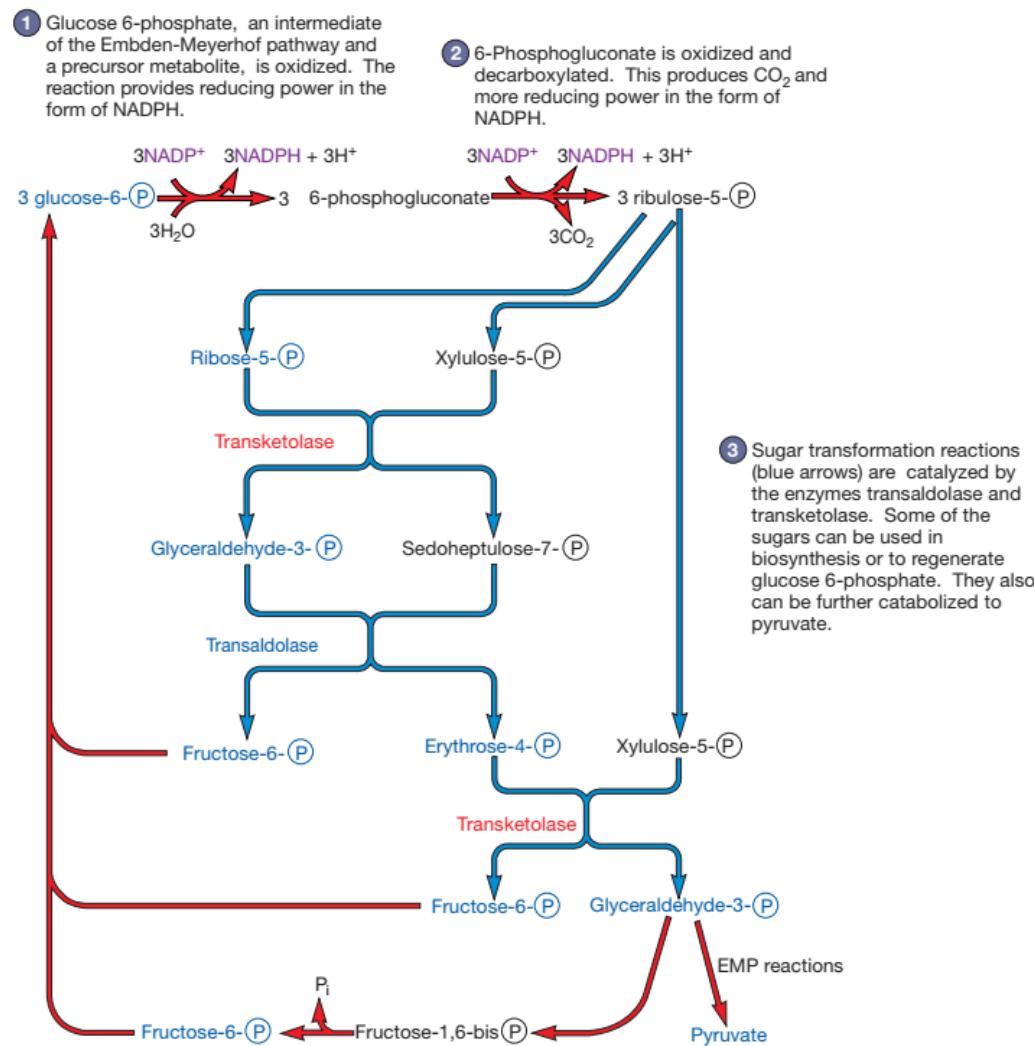
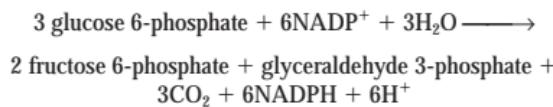


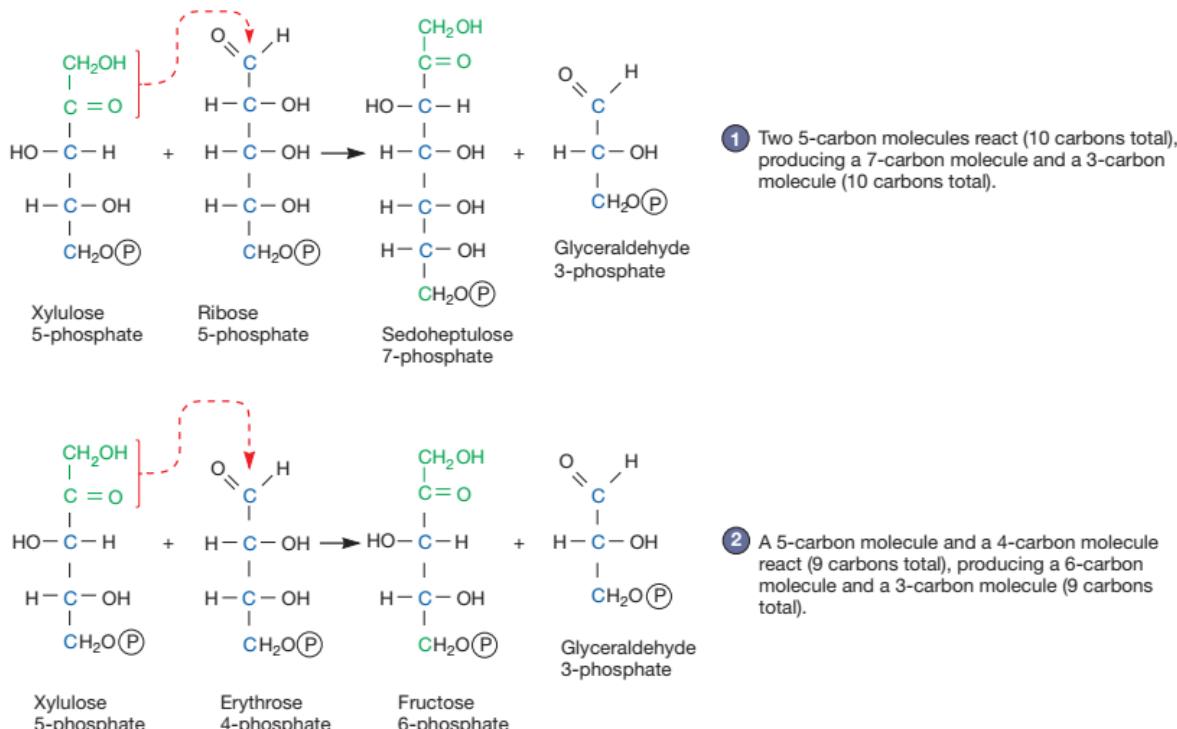
Figure 9.6 The Pentose Phosphate Pathway. The catabolism of three glucose 6-phosphate molecules to two fructose 6-phosphates, a glyceraldehyde 3-phosphate, and three CO_2 molecules is traced. Note that the pentose phosphate pathway generates several intermediates that are also intermediates of the Embden-Meyerhof pathway (EMP). These intermediates can be fed into the EMP with two results: (1) continued degradation to pyruvate or (2) regeneration of glucose 6-phosphate. The pentose phosphate pathway also plays a major role in producing reducing power (NADPH) and several precursor metabolites (shown in blue). The sugar transformations are indicated with blue arrows. These reactions are catalyzed by the enzymes transketolase and transaldolase and are shown in more detail in figure 9.7.

converted to a mixture of three- through seven-carbon sugar phosphates. Two enzymes play a central role in these transformations: (1) transketolase catalyzes the transfer of two-carbon ketol groups, and (2) transaldolase transfers a three-carbon group from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate (**figure 9.7**). The overall result is that three glucose 6-phosphates are converted to two fructose 6-phosphates, glyceraldehyde 3-phosphate, and three CO_2 molecules, as shown in this equation.



These intermediates are used in two ways. The fructose 6-phosphate can be changed back to glucose 6-phosphate while glyceraldehyde 3-phosphate is converted to pyruvate by enzymes of the

The transketolase reactions



The transaldolase reaction

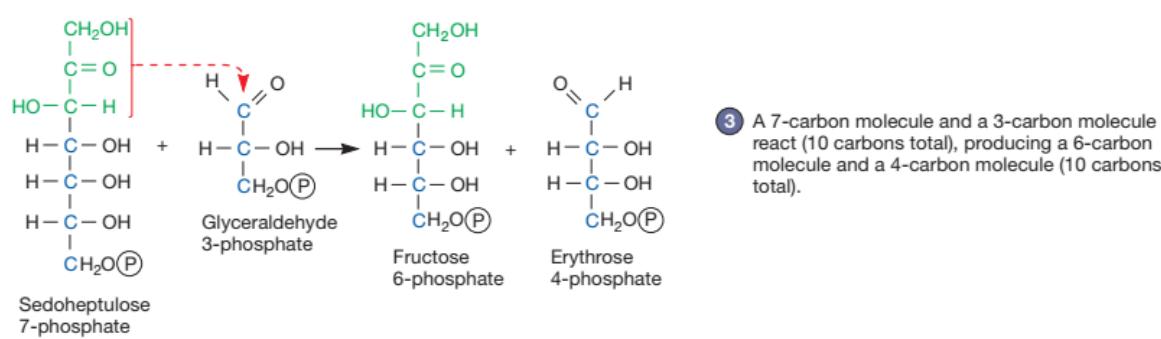
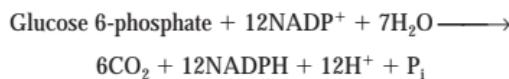


Figure 9.7 Transketolase and Transaldolase Reactions. Examples of the transketolase and transaldolase reactions of the pentose phosphate pathway. The groups transferred in these reactions are in green.

Embden-Meyerhof pathway. Alternatively two glyceraldehyde 3-phosphates may combine to form fructose 1,6-bisphosphate, which is eventually converted back into glucose 6-phosphate. This results in the complete degradation of glucose 6-phosphate to CO_2 and the production of a great deal of NADPH.



The pentose phosphate pathway is a good example of an amphibolic pathway as it has several catabolic and anabolic functions that are summarized as follows:

1. NADPH from the pentose phosphate pathway serves as a source of electrons for the reduction of molecules during biosynthesis.
2. The pathway produces two important precursor metabolites: erythrose 4-phosphate, which is used to synthesize aromatic amino acids and vitamin B₆ (pyridoxal) and ribose 5-phosphate, which is a major component of nucleic acids. Note that when a microorganism is growing on a pentose carbon source, the pathway can function biosynthetically to supply hexose sugars (e.g., glucose needed for peptidoglycan synthesis).
3. Intermediates in the pentose phosphate pathway may be used to produce ATP. Glyceraldehyde 3-phosphate from the pathway can enter the three-carbon phase of the Embden-Meyerhof pathway and be converted to pyruvate, as ATP is produced by substrate-level phosphorylation. Pyruvate may be oxidized in the tricarboxylic acid cycle to provide more energy.

Although the pentose phosphate pathway may be a source of energy in many microorganisms, it is more often of greater importance in biosynthesis. Several functions of the pentose phosphate pathway are mentioned again in chapter 10 when biosynthesis is considered more directly.

The Entner-Doudoroff Pathway

Although the Embden-Meyerhof pathway is the most common route for the conversion of hexoses to pyruvate, the **Entner-Doudoroff pathway** is used by soil microbes, such as *Pseudomonas*, *Rhizobium*, *Azotobacter*, and *Agrobacterium*, and a few other gram-negative bacteria. Very few gram-positive bacteria have this pathway, with the intestinal bacterium *Enterococcus faecalis* being a rare exception.

The Entner-Doudoroff pathway begins with the same reactions as the pentose phosphate pathway: the formation of glucose 6-phosphate, which is then converted to 6-phosphogluconate (figure 9.8 and appendix II). Instead of being further oxidized, 6-phosphogluconate is dehydrated to form 2-keto-3-deoxy-6-phosphogluconate or KDPG, the key intermediate in this pathway. KDPG is then cleaved by KDPG aldolase to pyruvate and glyceraldehyde 3-phosphate. The glyceraldehyde 3-phosphate is converted to pyruvate in the Embden-Meyerhof pathway. If the Entner-Doudoroff pathway degrades glucose to pyruvate in this way, it yields one ATP, one NADPH, and one NADH per glucose metabolized.

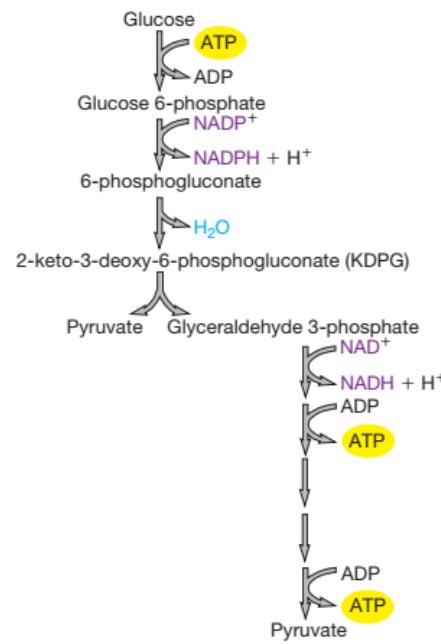


Figure 9.8 The Entner-Doudoroff Pathway. The sequence leading from glyceraldehyde 3-phosphate to pyruvate is catalyzed by enzymes common to the Embden-Meyerhof pathway.

1. Summarize the major features of the Embden-Meyerhof, pentose phosphate, and Entner-Doudoroff pathways. Include the starting points, the products of the pathways, any critical or unique enzymes, the ATP yields, and the metabolic roles each pathway has.
2. What is substrate-level phosphorylation?

9.4 THE TRICARBOXYLIC ACID CYCLE

In the glycolytic pathways, the energy captured by the oxidation of glucose to pyruvate is limited to no more than two ATP generated by substrate-level phosphorylation. During aerobic respiration, the catabolic process continues by oxidizing pyruvate to three CO_2 . The first step of this process employs a multienzyme system called the **pyruvate dehydrogenase complex**. It oxidizes and cleaves pyruvate to form one CO_2 and the two-carbon molecule **acetyl-coenzyme A (acetyl-CoA)** (figure 9.9). Acetyl-CoA is energy-rich because a high-energy thiol links acetic acid to coenzyme A. Note that during stage three of aerobic respiration (figure 9.3), carbohydrates as well as fatty acids and amino acids can be converted to acetyl-CoA.

Acetyl-CoA then enters the **tricarboxylic acid (TCA) cycle**, which is also called the **citric acid cycle** or the **Krebs cycle** (figure 9.9 and appendix II). In the first reaction acetyl-CoA is condensed with (i.e., added to) a four-carbon intermediate, oxaloacetate, to form citrate, a molecule with six carbons. Cit-

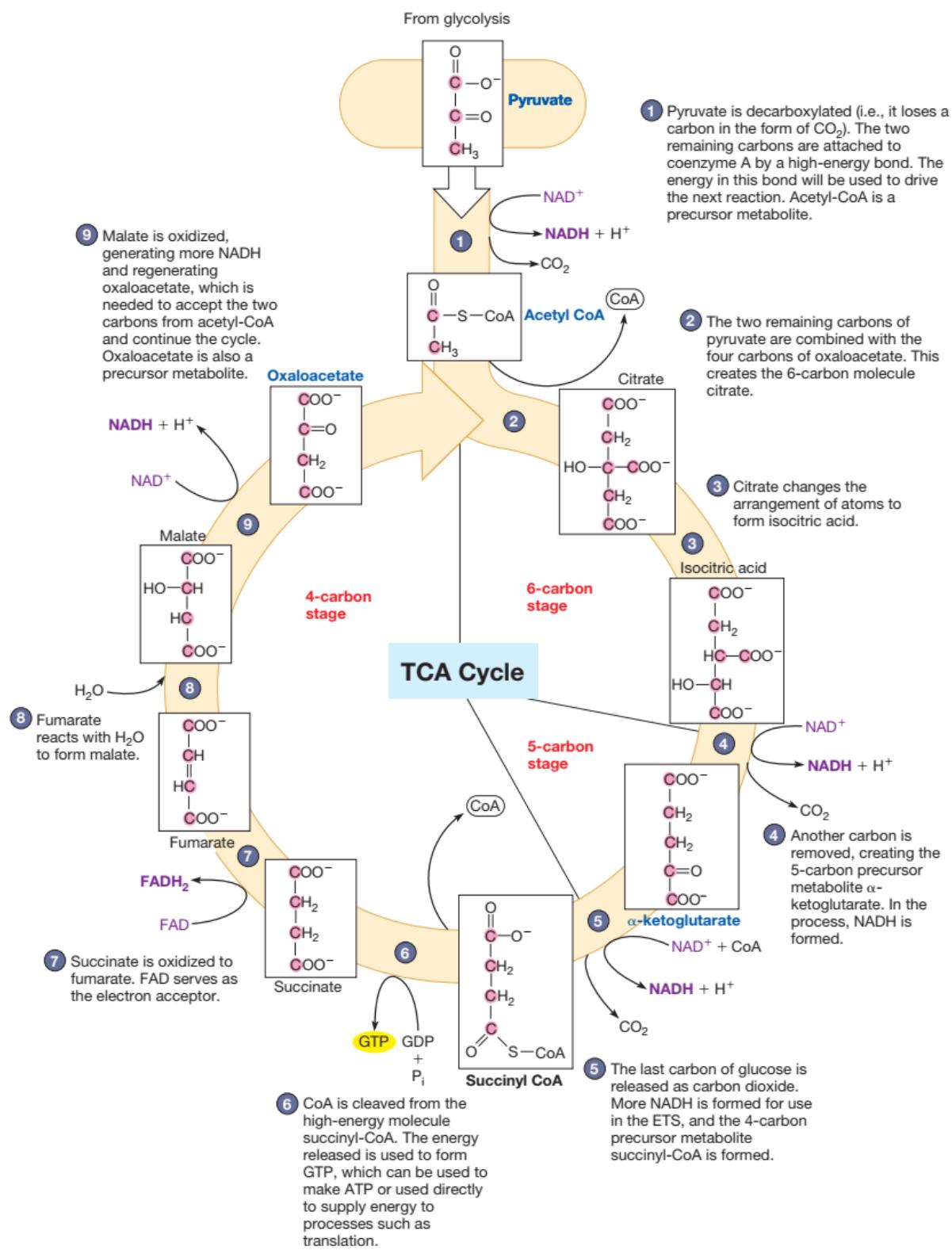


Figure 9.9 The Tricarboxylic Acid Cycle. The TCA cycle is linked to glycolysis by a connecting reaction catalyzed by the pyruvate dehydrogenase complex. The reaction decarboxylates pyruvate (removes a carboxyl group as CO₂) and generates acetyl-CoA. The cycle may be divided into three stages based on the size of its intermediates. The three stages are separated from one another by two decarboxylation reactions. Precursor metabolites, carbon skeletons used in biosynthesis, are shown in blue. NADH and FADH₂ are shown in purple; all can transfer electrons to the electron transport chain (ETC).

rate (a tertiary alcohol) is rearranged to give isocitrate, a more readily oxidized secondary alcohol. Isocitrate is subsequently oxidized and decarboxylated twice to yield α -ketoglutarate (five carbons), and then succinyl-CoA (four carbons), a molecule with a high-energy bond. At this point two NADH molecules have been formed and two carbons lost from the cycle as CO_2 . The cycle continues when succinyl-CoA is converted to succinate. This involves breaking the high-energy bond in succinyl-CoA and using the energy released to form one GTP by substrate-level phosphorylation. GTP is also a high-energy molecule, and it is functionally equivalent to ATP. Two oxidation steps follow, yielding one FADH_2 and one NADH. The last oxidation step regenerates oxaloacetate, and as long as there is a supply of acetyl-CoA the cycle can repeat itself. Inspection of figure 9.9 shows that the TCA cycle generates two CO_2 molecules, three NADH molecules, one FADH_2 , and one GTP for each acetyl-CoA molecule oxidized.

TCA cycle enzymes are widely distributed among microorganisms. In prokaryotes, they are located in the cytoplasmic matrix. In eucaryotes, they are found in the mitochondrial matrix. The complete cycle appears to be functional in many aerobic bacteria, free-living protists, and fungi. This is not surprising because the cycle is such an important source of energy. Even those microorganisms that lack the complete TCA cycle usually have most of the cycle enzymes, because the TCA cycle is also a key source of carbon skeletons for use in biosynthesis. *Synthesis of amino acids: Anaplerotic reactions and amino acid biosynthesis (section 10.5)*

1. Give the substrate and products of the tricarboxylic acid cycle. Describe its organization in general terms. What are its two major functions?
2. What chemical intermediate links pyruvate to the TCA cycle?
3. How many times must the TCA cycle be performed to completely oxidize one molecule of glucose to six molecules of CO_2 ? Why?
4. In what eucaryotic organelle is the TCA cycle found? Where is the cycle located in prokaryotes?
5. Why might it be desirable for a microbe with the Embden-Meyerhof pathway and the TCA cycle also to have the pentose phosphate pathway?
6. Why is GTP functionally equivalent to ATP?

9.5 ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

During the oxidation of glucose to six CO_2 molecules by glycolysis and the TCA cycle, four ATP molecules are generated by substrate-level phosphorylation. Thus at this point, the work done by the cell has yielded relatively little ATP. However, in oxidizing glucose, the cell has also generated numerous molecules of NADH and FADH_2 . Both of these molecules have a relatively negative E'_0 and can be used to conserve energy (see table 8.1). In fact, most of the ATP generated during aerobic respiration comes from the oxidation of these electron carriers in the electron transport chain. The mitochondrial electron transport chain will be examined first because it has been so well studied. Then we will turn to bacterial chains, and finish with a discussion of ATP synthesis.

The Electron Transport Chain

The mitochondrial **electron transport chain** is composed of a series of electron carriers that operate together to transfer electrons from donors, like NADH and FADH_2 , to acceptors, such as O_2 (figure 9.10). The electrons flow from carriers with more negative reduction potentials to those with more positive potentials and eventually combine with O_2 and H^+ to form water. This pattern of electron flow is exactly the same as seen in the electron tower that is described in chapter 8 (see figure 8.8). The electrons move down this potential gradient much like water flowing down a series of rapids. The difference in reduction potentials between O_2 and NADH is large, about 1.14 volts, which makes possible the release of a great deal of energy. The differences in reduction potential at several points in the chain are large enough to provide sufficient energy for ATP production, much like the energy from waterfalls can be harnessed by waterwheels and used to generate electricity. Thus the electron transport chain breaks up the large overall energy release into small steps. As will be seen shortly, electron transport at these points generates proton and electrical

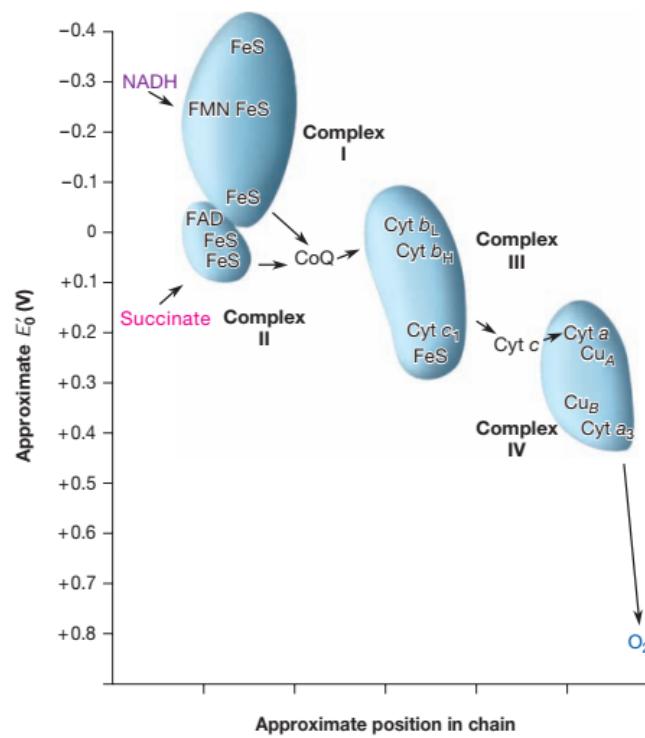


Figure 9.10 The Mitochondrial Electron Transport Chain.

Many of the more important carriers are arranged at approximately the correct reduction potential and sequence. In the eucaryotic mitochondrion, they are organized into four complexes that are linked by coenzyme Q (CoQ) and cytochrome c (Cyt c). Electrons flow from NADH and succinate down the reduction potential gradient to oxygen. See text for details.

gradients. These gradients can drive ATP synthesis and perform other work.

In eucaryotes, the electron transport chain carriers reside within the inner membrane of the mitochondrion. In prokaryotes, they are located within the plasma membrane. The mitochondrial system is arranged into four complexes of carriers, each capable of transporting electrons part of the way to O_2 (figure 9.11). Coenzyme Q and cytochrome *c* connect the complexes with each other. Although some bacterial chains resemble the mitochondrial chain, they are frequently very different. As already noted, bacterial chains are located within the plasma membrane. They also can be composed of different electron carriers (e.g., their cytochromes) and may be extensively branched. Electrons often can enter the chain at several points and leave through several terminal oxidases. Bacterial chains also may be shorter, resulting in the release of less energy. Although prokaryotic and eucaryotic electron transport chains differ in details of construction, they operate using the same fundamental principles.

The electron transport chain of *E. coli* will serve as an example of these differences. A simplified view of the *E. coli*

transport chain is shown in figure 9.12. The NADH generated by the oxidation of organic substrates (during glycolysis and the TCA cycle) is donated to the electron transport chain, where it is oxidized to NAD^+ by the membrane-bound NADH dehydrogenase. The electrons are then transferred to carriers with progressively more positive reduction potentials. As electrons move through the carriers, protons are moved across the plasma membrane to the periplasmic space (i.e., outside the cell) rather than to an intermembrane space as seen in the mitochondria (compare figures 9.11 and 9.12). Another significant difference between the *E. coli* chain and the mitochondrial chain is that the bacterial electron transport chain contains a different array of cytochromes. Furthermore, *E. coli* has evolved two branches of the electron transport chain that operate under different aeration conditions. When oxygen is readily available, the cytochrome *bo* branch is used. When oxygen levels are reduced, the cytochrome *bd* branch is used because it has a higher affinity for oxygen. However, it is less efficient than the *bo* branch because the *bd* branch moves fewer protons into the periplasmic space (figure 9.12).

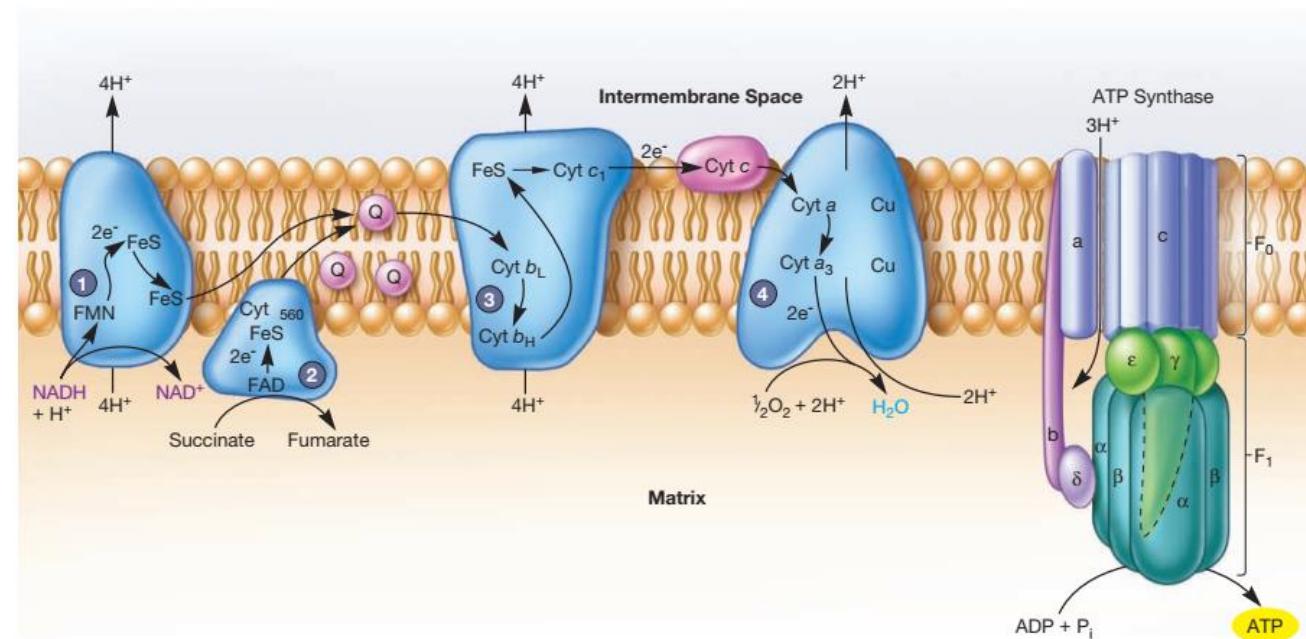


Figure 9.11 The Chemiosmotic Hypothesis Applied to Mitochondria. In this scheme the carriers are organized asymmetrically within the inner membrane so that protons are transported across as electrons move along the chain. Proton release into the intermembrane space occurs when electrons are transferred from carriers, such as FMN and coenzyme Q (Q), that carry both electrons and protons to components like nonheme iron proteins (FeS proteins) and cytochromes (Cyt) that transport only electrons. Complex IV pumps protons across the membrane as electrons pass from cytochrome *a* to oxygen. Coenzyme Q transports electrons from complexes I and II to complex III. Cytochrome *c* moves electrons between complexes III and IV. The number of protons moved across the membrane at each site per pair of electrons transported is still somewhat uncertain; the current consensus is that at least 10 protons must move outward during NADH oxidation. One molecule of ATP is synthesized and released from the enzyme ATP synthase for every three protons that cross the membrane by passing through it.

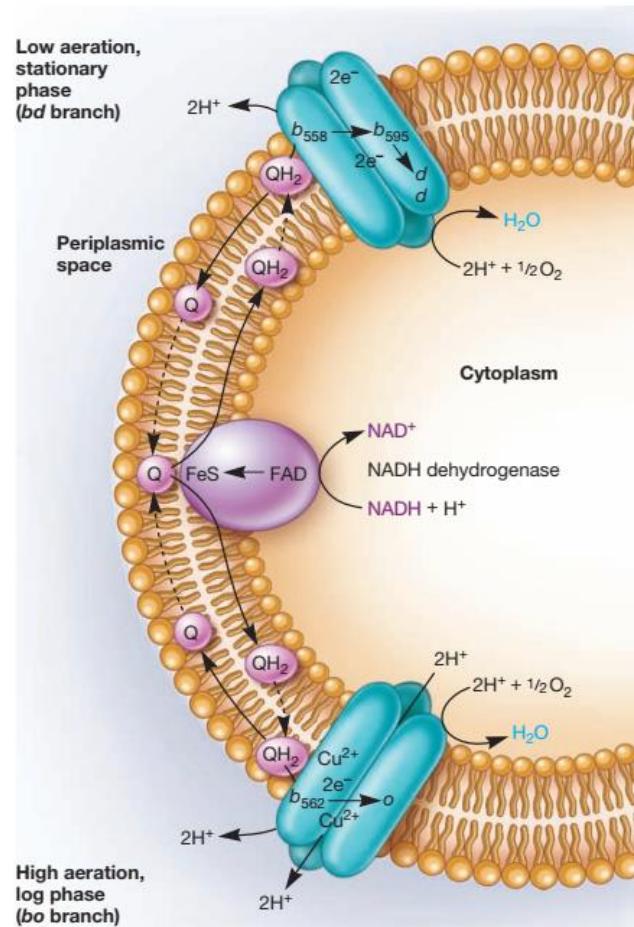


Figure 9.12 The Aerobic Respiratory System of *E. coli*.
NADH is the electron source. Ubiquinone-8 (Q) connects the NADH dehydrogenase with two terminal oxidase systems. The upper branch operates when the bacterium is in stationary phase and there is little oxygen. At least five cytochromes are involved: b_{558} , b_{595} , b_{562} , d , and o . The lower branch functions when *E. coli* is growing rapidly with good aeration.

Oxidative Phosphorylation

Oxidative phosphorylation is the process by which ATP is synthesized as the result of electron transport driven by the oxidation of a chemical energy source. The mechanism by which oxidative phosphorylation takes place has been studied intensively for years. The most widely accepted hypothesis is the chemiosmotic hypothesis, which was formulated by British biochemist **Peter Mitchell**. According to the **chemiosmotic hypothesis**, the electron transport chain is organized so that protons move outward from the mitochondrial matrix as electrons are transported down the chain (figure 9.11).

The movement of protons across the membrane is not completely understood. However, in some cases, the protons are actively pumped across the membrane (e.g., by complex IV of the

mitochondrial chain; figure 9.11). In other cases, translocation of protons results from the juxtaposition of carriers that accept both electrons and protons with carriers that accept only electrons. For instance, coenzyme Q carries two electrons and two protons to cytochrome *b* in complex III of the mitochondrial chain. Cytochrome *b* accepts only one electron at a time, and will not accept protons. Thus for each electron transferred by coenzyme Q, one proton is released to the intermembrane space.

The result of proton expulsion during electron transport is the formation of a concentration gradient of protons (ΔpH ; chemical potential energy) and a charge gradient ($\Delta\psi$; electrical potential energy). Thus the mitochondrial matrix is more alkaline and more negative than the intermembrane space. Likewise with prokaryotes, the cytoplasm is more alkaline and more negative than the periplasmic space. The combined chemical and electrical potential differences make up the **proton motive force (PMF)**. The PMF is used to perform work when protons flow back across the membrane, down the concentration and charge gradients, and into the mitochondrial matrix (or prokaryotic cytoplasm). This flow is exergonic and is often used to phosphorylate ADP to ATP. The PMF is also used to transport molecules into the cell directly (i.e., without the hydrolysis of ATP) and to rotate the flagellar motor. Thus the PMF plays a central role in prokaryotic physiology (figure 9.13). Uptake of nutrients by the cell (section 5.6); Components external to the cell wall: Flagella and motility (section 3.9)

The use of PMF for ATP synthesis is catalyzed by **ATP synthase** (figure 9.14), a multisubunit enzyme also known as F_1F_0 ATPase because it consists of two components and can catalyze ATP hydrolysis. The mitochondrial F_1 component appears as a spherical structure attached to the mitochondrial inner membrane surface by a stalk. The F_0 component is embedded in the membrane. The ATP synthase is on the inner surface of the plasma

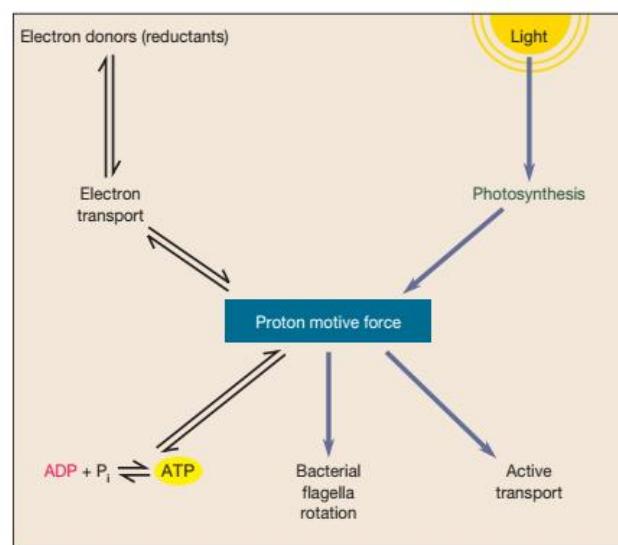


Figure 9.13 The Central Role of Proton Motive Force. It should be noted that active transport is not always driven by PMF.

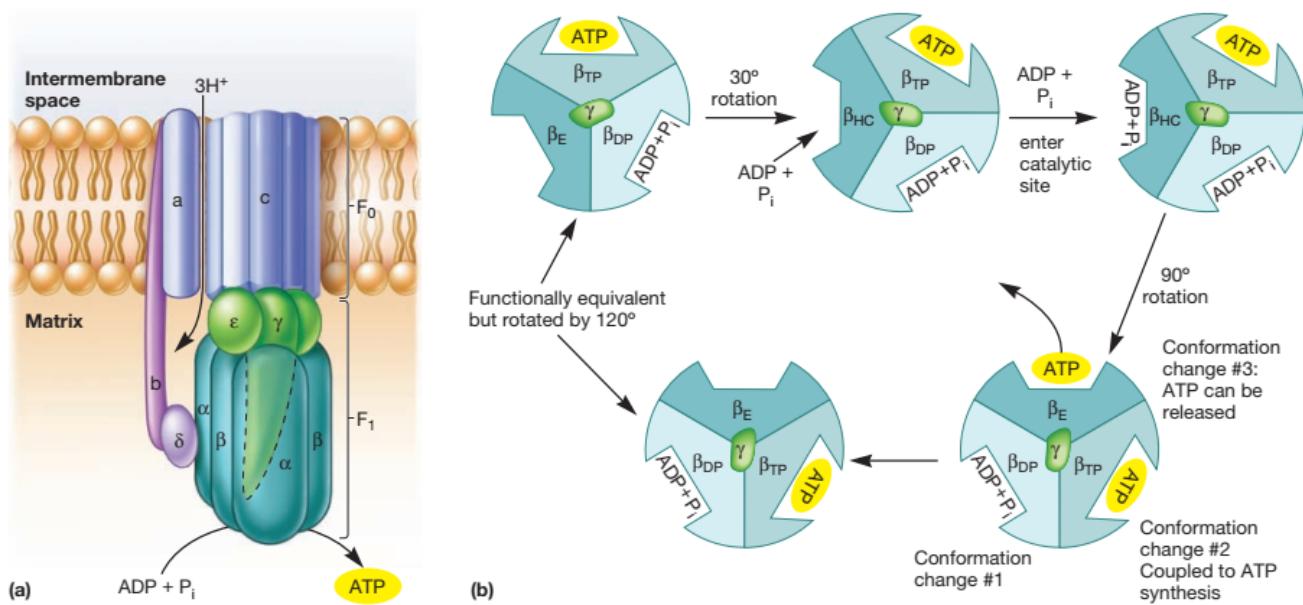


Figure 9.14 ATP Synthase Structure and Function. (a) The major structural features of ATP synthase deduced from X-ray crystallography and other studies. F_1 is a spherical structure composed largely of alternating α and β subunits; the three active sites are on the β subunits. The γ subunit extends upward through the center of the sphere and can rotate. The stalk (γ and ϵ subunits) connects the sphere to F_0 , the membrane embedded complex that serves as a proton channel. F_0 contains one a subunit, two b subunits, and 9–12 c subunits. The stator arm is composed of subunit a , two b subunits, and the δ subunit; it is embedded in the membrane and attached to F_1 . A ring of c subunits in F_0 is connected to the stalk and may act as a rotor and move past the a subunit of the stator. As the c subunit ring turns, it rotates the shaft ($\gamma\epsilon$ subunits). (b) The binding change mechanism is a widely accepted model of ATP synthesis. This simplified drawing of the model shows the three catalytic β subunits and the γ subunit, which is located at the center of the F_1 complex. As the γ subunit rotates, it causes conformational changes in each subunit. The β_E (empty) conformation is an open conformation, which does not bind nucleotides. When the γ subunit rotates 30°, β_E is converted to the β_{HC} (half closed) conformation. P_i and ADP can enter the catalytic site when it is in this conformation. The subsequent 90° rotation by the γ subunit is critical because it brings about three significant conformational changes: (1) β_{HC} to β_{DP} (ADP bound), (2) β_{DP} to β_{TP} (ATP bound), and (3) β_{TP} to β_E . Change from β_{DP} to β_{TP} is accompanied by the formation of ATP; change from β_{TP} to β_E allows for release of ATP from ATP synthase.

membrane in prokaryotes. F_0 participates in proton movement across the membrane. F_1 is a large complex in which three α subunits alternate with three β subunits. The catalytic sites for ATP synthesis are located on the β subunits. At the center of F_1 is the γ subunit. The γ subunit extends through F_1 and interacts with F_0 .

It is now known that ATP synthase functions like a rotary engine. It is thought that the flow of protons down the proton gradient through the F_0 subunit causes F_0 and the γ subunit to rotate. As the γ subunit rotates rapidly within the F_1 (much like a car's crankshaft), the rotation causes conformation changes in the β subunits (figure 9.14b). One conformation change (β_E to β_{HC}) allows entry of ADP and P_i into the catalytic site. Another conformation change (β_{HC} to β_{DP}) loosely binds ADP and P_i in the catalytic site. ATP is synthesized when the β_{DP} conformation is changed to the β_{TP} conformation, and ATP is released when β_{TP} changes to the β_E conformation, to start the synthesis cycle anew.

Much of the evidence supporting the chemiosmotic hypothesis comes from studies using chemicals that inhibit the aerobic synthesis of ATP. These chemicals can even kill cells at sufficiently high concentrations. The inhibitors generally fall into two

categories. Some directly block the transport of electrons. The antibiotic piericidin competes with coenzyme Q; the antibiotic antimycin A blocks electron transport between cytochromes b and c ; and both cyanide and azide stop the transfer of electrons between cytochrome a and O_2 because they are structural analogs of O_2 . Another group of inhibitors known as **uncouplers** stops ATP synthesis without inhibiting electron transport itself. Indeed, they may even enhance the rate of electron flow. Normally electron transport is tightly coupled with oxidative phosphorylation so that the rate of ATP synthesis controls the rate of electron transport. The more rapidly ATP is synthesized during oxidative phosphorylation, the faster the electron transport chain operates to supply the required energy. Uncouplers disconnect oxidative phosphorylation from electron transport; therefore the energy released by the chain is given off as heat rather than as ATP. Many uncouplers like dinitrophenol and valinomycin allow hydrogen ions, potassium ions, and other ions to cross the membrane without activating ATP synthase. In this way they destroy the pH and ion gradients. Valinomycin also may bind directly to ATP synthase and inhibit its activity.

ATP Yield During Aerobic Respiration

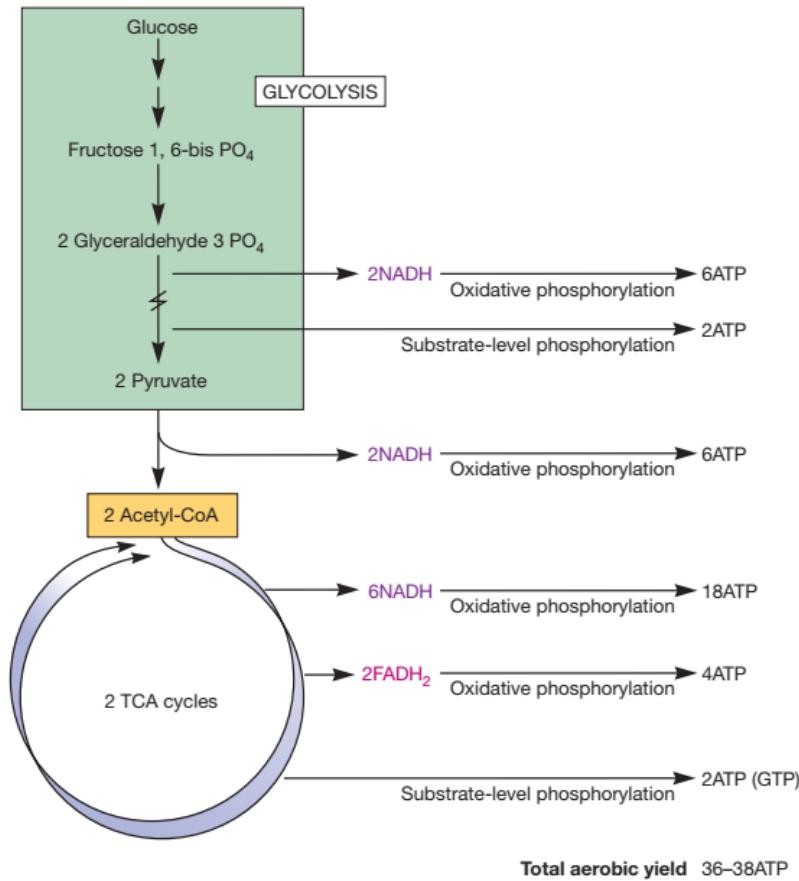
It is possible to estimate the number of ATP molecules synthesized per NADH or FADH₂ oxidized by the electron transport chain. During aerobic respiration, a pair of electrons from NADH is donated to the electron transport chain and ultimately used to reduce an atom of oxygen to H₂O. This releases enough energy to drive the synthesis of three ATP. This is referred to as the **phosphorus to oxygen (P/O) ratio** because it measures the number of ATP (phosphorus) generated per oxygen (O) reduced. Because FADH₂ has a more positive reduction potential than NADH (see figure 8.8), electrons arising from its oxidation flow down a shorter chain, releasing less energy. Thus while the P/O ratio for NADH is 3, only two ATP can be made from the oxidation of a single FADH₂.

We can thus calculate the maximum ATP yield (figure 9.15) of aerobic respiration. Substrate-level phosphorylation during glycolysis yields at most two ATP molecules per glucose converted to pyruvate (figures 9.5, 9.6, and 9.8). Two additional GTP (ATP equivalents) are generated by substrate-level phosphorylation during the two turns of the TCA cycle needed to oxidize two acetyl-CoA molecules (figure 9.9). However, most of the ATP made during aerobic respiration is generated by oxidative phosphorylation. Up to 10 NADH (2 from glycolysis, 2 from pyruvate

conversion to acetyl-CoA, and 6 from the TCA cycle) and 2 FADH₂ (from the TCA cycle) are generated when glucose is oxidized completely to 6 CO₂. Assuming a P/O ratio of 3 for NADH oxidation and 2 for FADH₂ oxidation, the 10 NADH could theoretically drive the synthesis of 30 ATP, while oxidation of the 2 FADH₂ molecules would add another 4 ATP for a maximum of 34 ATP generated via oxidative phosphorylation. Because substrate-level phosphorylation contributes only four ATP per glucose molecule oxidized, oxidative phosphorylation accounts for at least eight times more. The maximum total yield of ATP during aerobic respiration is 38 ATPs. It must be remembered that the calculations just summarized and presented in figure 9.15 are theoretical. In fact, the P/O ratios are more likely about 2.5 for NADH and 1.5 for FADH₂. Thus the total ATP yield from glucose may be closer to 30 ATPs rather than 38.

Because prokaryotic electron transport systems often have lower P/O ratios than eucaryotic systems, prokaryotic ATP yields can be less. For example, *E. coli*, with its truncated electron transport chains, has a P/O ratio around 1.3 when using the cytochrome *bo* path at high oxygen levels and only a ratio of about 0.67 when employing the cytochrome *bd* branch (figure 9.12) at low oxygen concentrations. In this case ATP production varies

Figure 9.15 Maximum Theoretic ATP yield from Aerobic Respiration. To attain the theoretic maximum yield of ATP, one must assume a P/O ratio of 3 for the oxidation of NADH and 2 for FADH₂. The actual yield is probably significantly less and varies between eucaryotes and prokaryotes and among prokaryotic species.



with environmental conditions. Perhaps because *E. coli* normally grows in habitats such as the intestinal tract that are very rich in nutrients, it does not have to be particularly efficient in ATP synthesis. Presumably the electron transport chain functions when *E. coli* is in an oxic freshwater environment between hosts.

1. Briefly describe the structure of the electron transport chain and its role in ATP formation. How do mitochondrial and bacterial chains differ?
2. Describe the current model of oxidative phosphorylation. Briefly describe the structure of ATP synthase and explain how it is thought to function. What is an uncoupler?
3. How do substrate-level phosphorylation and oxidative phosphorylation differ?
4. Calculate the ATP yield when glucose is catabolized completely to six CO₂ by a eucaryotic microbe. How does this value compare to the ATP yield observed for a bacterium? Suppose a bacterium used the Entner-Doudoroff pathway to degrade glucose to pyruvate. How would this impact the total ATP yield? Explain your reasoning.

9.6 ANAEROBIC RESPIRATION

As we have seen, during aerobic respiration sugars and other organic molecules are oxidized and their electrons transferred to NAD⁺ and FAD to generate NADH and FADH₂, respectively. These electron carriers then donate the electrons to an electron transport chain that uses O₂ as the terminal electron acceptor. However, it is also possible for other terminal electron acceptors to be used for electron transport. **Anaerobic respiration**, a process whereby an exogenous terminal electron acceptor other than O₂ is used for electron transport, is carried out by many bacteria and archaea. The most common terminal electron acceptors used during anaerobic respiration are nitrate, sulfate, and CO₂, but metals and a few organic molecules can also be reduced (table 9.1).

Although some bacteria and archaea grow using only anaerobic respiration, many can perform both aerobic and anaerobic respiration, depending on the availability of oxygen. One example is *Paracoccus denitrificans*, a gram-negative, facultative anaerobic soil bacterium that is extremely versatile metabolically. It can degrade a wide variety of organic compounds and can even grow chemolithotrophically. Under anoxic conditions, *P. denitrificans* uses NO₃⁻ as its electron acceptor. As shown in figure 9.16, two different electron transport chains are used by this bacterium, one for aerobic respiration and the second for anaerobic respiration. Notice that during chemoorganotrophic growth, the source of electrons in both chains is NADH. The aerobic chain has four complexes that correspond to the mitochondrial chain (figure 9.16a). When *P. denitrificans* grows without oxygen, using NO₃⁻ as the terminal electron acceptor, the electron transport chain is more complex (figure 9.16b). The chain is highly branched and the cytochrome aa complex is replaced. Electrons are passed from coenzyme Q to cytochrome b for the reduction of nitrate to nitrite (catalyzed by nitrate reductase). Electrons then flow through cytochrome c for the sequential ox-

Table 9.1 Some Electron Acceptors Used in Respiration

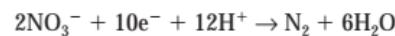
| | Electron Acceptor | Reduced Products | Examples of Microorganisms |
|-----------|---------------------------------|---|---|
| Aerobic | O ₂ | H ₂ O | All aerobic bacteria, fungi, and protists |
| Anaerobic | NO ₃ ⁻ | NO ₂ ⁻ | Enteric bacteria |
| | NO ₃ ⁻ | NO ₂ ⁻ , N ₂ O, N ₂ | <i>Pseudomonas</i> , <i>Bacillus</i> , and <i>Paracoccus</i> |
| | SO ₄ ²⁻ | H ₂ S | <i>Desulfovibrio</i> and <i>Desulfotomaculum</i> |
| | CO ₂ | CH ₄ | All methanogens and acetogens |
| | S ⁰ | H ₂ S | <i>Desulfuromonas</i> and <i>Thermoproteus</i> |
| | Fe ³⁺ | Fe ²⁺ | <i>Pseudomonas</i> , <i>Bacillus</i> , and <i>Geobacter</i> |
| | HAsO ₄ ²⁻ | HAsO ₂ | <i>Bacillus</i> , <i>Desulfotomaculum</i> , <i>Sulfurospirillum</i> |
| | SeO ₄ ²⁻ | Se, HSeO ₃ ⁻ | <i>Aeromonas</i> , <i>Bacillus</i> , <i>Thauera</i> |
| | Fumarate | Succinate | <i>Wolinella</i> |

idation of nitrite to gaseous dinitrogen (N₂). Not as many protons are pumped across the membrane during anaerobic growth, but nonetheless a PMF is established.

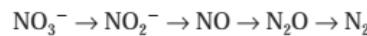
The anaerobic reduction of nitrate makes it unavailable to the cell for assimilation or uptake. Therefore this process is called **dissimilatory nitrate reduction**. Nitrate reductase replaces cytochrome oxidase to catalyze the reaction:



However, reduction of nitrate to nitrite is not a particularly efficient way of making ATP because a large amount of nitrate is required for growth (a nitrate molecule will accept only two electrons). Furthermore, nitrite is quite toxic. Bacteria such as *P. denitrificans* avoid the toxic effects of nitrite by reducing it to nitrogen gas, a process known as **denitrification**. By donating five electrons to a nitrate molecule, NO₃⁻ is converted into a nontoxic product.



As illustrated in figure 9.16, denitrification is a multistep process with four enzymes participating: nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase.



Two types of bacterial nitrite reductases catalyze the formation of NO in bacteria. One contains cytochromes c and d_i (e.g.,

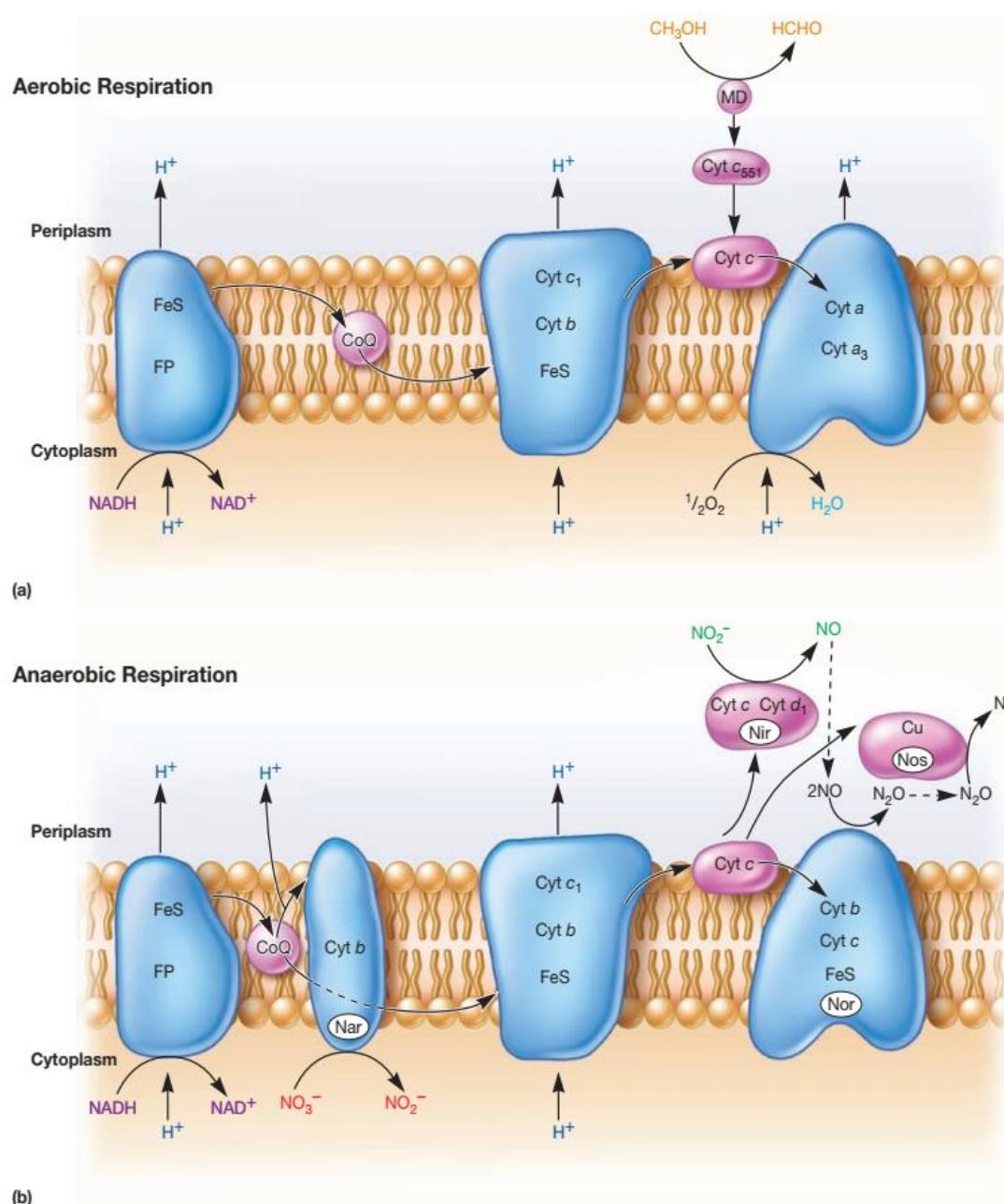


Figure 9.16 *Paracoccus denitrificans* Electron Transport Chains. (a) The aerobic transport chain resembles a mitochondrial electron transport chain and uses oxygen as its acceptor. Methanol and methylamine can contribute electrons at the cytochrome *c* level. (b) The highly branched anaerobic chain is made of both membrane and periplasmic proteins. Nitrate is reduced to diatomic nitrogen by the collective action of four different reductases that receive electrons from CoQ and cytochrome *c*. Locations of proton movement are shown, but the number of protons involved has not been indicated. Abbreviations used: flavoprotein (FP), methanol dehydrogenase (MD), nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos).

Paracoccus and *Pseudomonas aeruginosa*), and the other is a copper-containing protein (e.g., *Alcaligenes*). Nitrite reductase seems to be periplasmic in gram-negative bacteria. Nitric oxide reductase catalyzes the formation of nitrous oxide from NO and is a membrane-bound cytochrome *bc* complex. In *P. denitrificans*, the nitrate reductase and nitric oxide reductase are membrane-bound, whereas nitrite reductase and nitrous oxide reductase are periplasmic (figure 9.16b).

In addition to *P. denitrificans*, some members of the genera *Pseudomonas* and *Bacillus* carry out denitrification. All three genera use denitrification as an alternative to aerobic respiration and may be considered facultative anaerobes. Indeed, if O₂ is present, these bacteria use aerobic respiration, which is far more efficient in capturing energy. In fact, the synthesis of nitrate reductase is repressed by O₂. Denitrification in anoxic soil results in the loss of soil nitrogen and adversely affects soil fertility.

[Biogeochemical cycling: Nitrogen cycle \(section 27.2\)](#)

Not all microbes employ anaerobic respiration facultatively. Some are obligate anaerobes that can carry out only anaerobic respiration. The methanogens are an example. These archaea use CO₂ or carbonate as a terminal electron acceptor. They are called methanogens because the electron acceptor is reduced to methane. Bacteria such as *Desulfovibrio* are another example. They donate eight electrons to sulfate, reducing it to sulfide (S₂⁻ or H₂S).



It should be noted that both methanogens and *Desulfovibrio* are able to function as chemolithotrophs, using H₂ as an energy source (section 9.11).

As we saw for denitrification, anaerobic respiration using sulfate or CO₂ as the terminal electron acceptors is not as efficient in ATP synthesis as is aerobic respiration. Reduction in ATP yield arises from the fact that these alternate electron acceptors have less positive reduction potentials than O₂ (see table 8.1). The difference in standard reduction potential between a donor like NADH and nitrate is smaller than the difference between NADH and O₂. Because energy yield is directly related to the magnitude of the reduction potential difference, less energy is available to make ATP in anaerobic respiration. Nevertheless, anaerobic respiration is useful because it allows ATP synthesis by electron transport and oxidative phosphorylation in the absence of O₂. Anaerobic respiration is prevalent in oxygen-depleted soils and sediments.

The ability of microbes to use a variety of electron acceptors has ecological consequences. Often one sees a succession of microorganisms in an environment when several electron acceptors are present. For example, if O₂, nitrate, manganese ion, ferric ion, sulfate, and CO₂ are available in a particular environment, a predictable sequence of electron acceptor use takes place when an oxidizable substrate is available to the microbial population. Oxygen is employed as an electron acceptor first because it inhibits nitrate

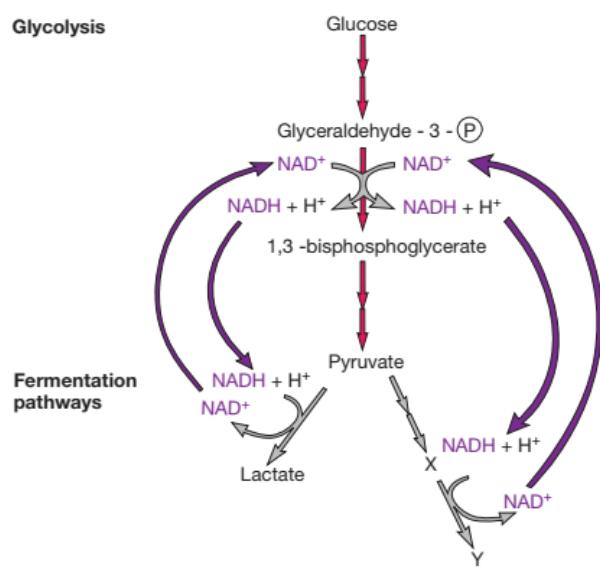
use by microorganisms capable of respiration with either O₂ or nitrate. While O₂ is available, sulfate reducers and methanogens are inhibited because these groups are obligate anaerobes.

Once the O₂ and nitrate are exhausted and fermentation products (section 9.7), including hydrogen, have accumulated, competition for use of other electron acceptors begins. Manganese and iron are used first, followed by competition between sulfate reducers and methanogens. This competition is influenced by the greater energy yield obtained with sulfate as an electron acceptor. Differences in enzymatic affinity for hydrogen, an important energy and electron source used by both groups, also are important. The sulfate reducer *Desulfovibrio* grows rapidly and uses the available hydrogen at a faster rate than *Methanobacterium*. When the sulfate is exhausted, *Desulfovibrio* no longer oxidizes hydrogen, and the hydrogen concentration rises. The methanogens finally dominate the habitat and reduce CO₂ to methane. [The subsurface biosphere \(section 29.7\)](#)

1. Describe the process of anaerobic respiration. Is as much ATP produced in anaerobic respiration as in aerobic respiration? Why or why not?
2. What is denitrification? Why do farmers dislike this process?
3. *E. coli* can use O₂, fumarate²⁻, or nitrate as a terminal electron acceptor under different conditions. What is the order of energy yield from highest to lowest for these electron acceptors? Explain your answer in thermodynamic terms.

9.7 FERMENTATIONS

Despite the tremendous ATP yield obtained by oxidative phosphorylation, some chemoorganotrophic microbes do not respire because either they lack electron transport chains or they repress the synthesis of electron transport chain components under anoxic conditions, making anaerobic respiration impossible. Yet NADH produced by the Embden-Meyerhof pathway reactions during glycolysis (figure 9.5) must still be oxidized back to NAD⁺. If NAD⁺ is not regenerated, the oxidation of glyceraldehyde 3-phosphate will cease and glycolysis will stop. Many microorganisms solve this problem by slowing or stopping pyruvate dehydrogenase activity and using pyruvate or one of its derivatives as an electron acceptor for the reoxidation of NADH in a **fermentation** process (figure 9.17). There are many kinds of fermentations, and they often are characteristic of particular microbial groups (figure 9.18). A few of the more common fermentations are introduced here, and several others are discussed at later points. Three unifying themes should be kept in mind when microbial fermentations are examined: (1) NADH is oxidized to NAD⁺, (2) the electron acceptor is often either pyruvate or a pyruvate derivative, and (3) oxidative phosphorylation cannot operate, reducing the ATP yield per glucose significantly. In fermentation, the substrate is only partially oxidized, ATP is formed exclusively by substrate-level phosphorylation, and oxygen is not needed.



Many fungi, protists, and some bacteria ferment sugars to ethanol and CO_2 in a process called **alcoholic fermentation**. Pyruvate is decarboxylated to acetaldehyde, which is then reduced to ethanol by alcohol dehydrogenase with NADH as the electron donor (figure 9.18, number 2). **Lactic acid fermentation**, the reduction of pyruvate to lactate (figure 9.18, number 1), is even more common. It is present in bacteria (lactic acid bacteria, *Bacillus*), protists (*Chlorella* and some water molds), and even in animal skeletal muscle. Lactic acid fermenters can be separated into two groups. **Homolactic fermenters** use the Embden-Meyerhof pathway and directly reduce almost all their pyruvate to lactate with the enzyme lactate dehydrogenase. **Heterolactic fermenters** form substantial amounts of products other than lactate; many produce lactate, ethanol, and CO_2 . *Class Gammaproteobacteria: Order Enterobacteriales* (section 22.3)

Alcoholic and lactic acid fermentations are quite useful. Alcoholic fermentation by yeasts produces alcoholic beverages; CO_2 from this fermentation causes bread to rise. Lactic acid fermentation can spoil foods, but also is used to make yogurt, sauer-

Figure 9.17 Reoxidation of NADH During Fermentation.
NADH from glycolysis is reoxidized by being used to reduce pyruvate or a pyruvate derivative (X). Either lactate or reduced product Y result.

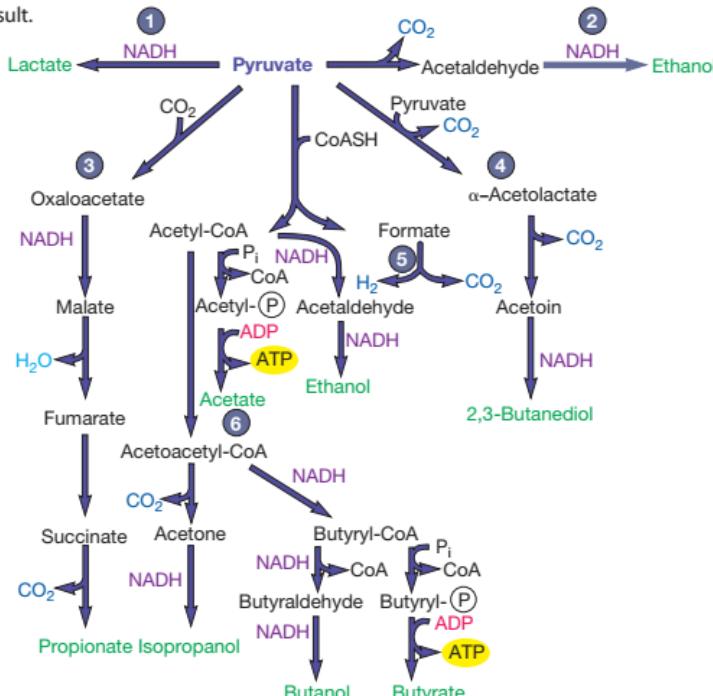


Figure 9.18 Some Common Microbial Fermentations.
Only pyruvate fermentations are shown for the sake of simplicity; many other organic molecules can be fermented. Most of these pathways have been simplified by deletion of one or more steps and intermediates. Pyruvate and major end products are shown in color.

1. Lactic acid bacteria (*Streptococcus*, *Lactobacillus*), *Bacillus*
2. Yeast, *Zymomonas*
3. Propionic acid bacteria (*Propionibacterium*)
4. *Enterobacter*, *Serratia*, *Bacillus*
5. Enteric bacteria (*Escherichia*, *Enterobacter*, *Salmonella*, *Proteus*)
6. *Clostridium*

kraut, cheese, and pickles. The role of fermentations in food production is discussed in chapter 40.

Many bacteria, especially members of the family *Enterobacteriaceae*, can metabolize pyruvate to formic acid and other products in a process sometimes called the formic acid fermentation (figure 9.18, number 5). Formic acid may be converted to H₂ and CO₂ by formic hydrogenlyase (a combination of at least two enzymes).



There are two types of formic acid fermentation. **Mixed acid fermentation** results in the excretion of ethanol and a complex mixture of acids, particularly acetic, lactic, succinic, and formic acids (table 9.2). If formic hydrogenlyase is present, the formic acid will be degraded to H₂ and CO₂. This pattern is seen in *Escherichia*, *Salmonella*, *Proteus*, and other genera. The second type, **butanediol fermentation**, is characteristic of *Enterobacter*, *Serratia*, *Erwinia*, and some species of *Bacillus* (figure 9.18, number 4). Pyruvate is converted to acetoin, which is then reduced to 2,3-butanediol with NADH. A large amount of ethanol is also produced, together with smaller amounts of the acids found in a mixed acid fermentation. [Class Bacilli \(section 23.5\)](#)

Microorganisms carry out a vast array of fermentations using numerous sugars and other organic substrates as their energy source (**Historical Highlights 9.1**). Protozoa and fungi often ferment sugars to lactate, ethanol, glycerol, succinate, formate, acetate, butanediol, and additional products. Some members of the genus *Clostridium* ferment mixtures of amino acids. Proteolytic clostridia such as the pathogens *C. sporogenes* and *C. botulinum* carry out the **Stickland reaction** in which one amino acid is oxidized and a second amino acid acts as the electron acceptor. Figure 9.19 shows the way in which alanine is oxidized and glycine reduced to produce acetate, CO₂, and NH₃. Some ATP is formed from acetyl phosphate by substrate-level phosphorylation, and the fermentation is quite useful for growing in anoxic, protein-rich environments. The Stickland reaction is

Table 9.2

Mixed Acid Fermentation Products of *Escherichia coli*

| | Fermentation Balance (μM Product/100 μM Glucose) | |
|----------------|--|-----------------------------|
| | Acid Growth (pH 6.0) | Alkaline Growth (pH 8.0) |
| Ethanol | 50 | 50 |
| Formic acid | 2 | 86 |
| Acetic acid | 36 | 39 |
| Lactic acid | 80 | 70 |
| Succinic acid | 11 | 15 |
| Carbon dioxide | 88 | 2 |
| Hydrogen gas | 75 | 0.5 |
| Butanediol | 0 | 0 |

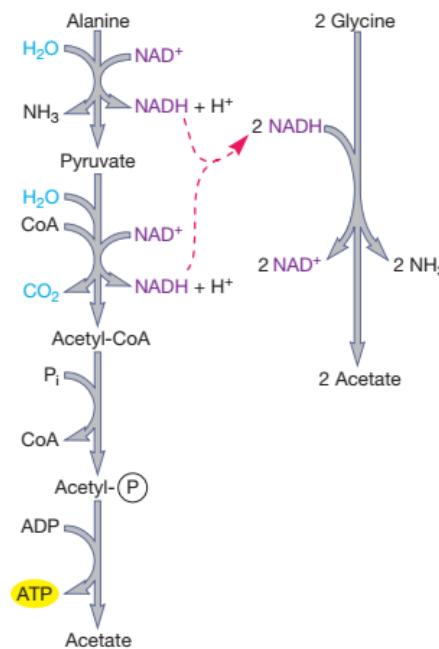


Figure 9.19 The Stickland Reaction. Alanine is oxidized to acetate and glycine is used to reoxidize the NADH generated during alanine degradation. The fermentation also produces some ATP.

used to oxidize several amino acids: alanine, leucine, isoleucine, valine, phenylalanine, tryptophan, and histidine. Bacteria also ferment amino acids (e.g., alanine, glycine, glutamate, threonine, and arginine) by other mechanisms. In addition to sugars and amino acids, organic acids such as acetate, lactate, propionate, and citrate are fermented. Some of these fermentations are of great practical importance. For example, citrate can be converted to diacetyl and give flavor to fermented milk. [Class Clostridia \(section 23.4\); Microbiology of fermented foods \(section 40.6\)](#)

1. What are fermentations and why are they so useful to many microorganisms?
2. How do the electron acceptors used in fermentation differ from the terminal electron acceptors used during either aerobic respiration or anaerobic respiration?
3. Briefly describe alcoholic, lactic acid, and formic acid fermentations. How do homolactic fermenters and heterolactic fermenters differ? How do mixed acid fermenters and butanediol fermenters differ?
4. What is the net yield of ATP during homolactic, acetate, and butyrate fermentations? How do these yields compare to aerobic respiration in terms of both quantity and mechanism of phosphorylation?
5. Some bacteria carry out fermentation only because they lack electron transport chains. Yet these bacteria still have a membrane-bound ATPase. Why do you think this is the case? How do you think these bacteria use the ATPase?
6. When bacteria carry out fermentation, only a few reactions of the TCA cycle operate. What purpose do you think these reactions might serve? Why do you think some parts of the cycle are shut down?



Historical Highlights

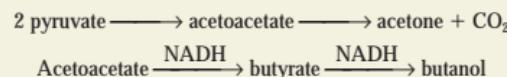
9.1 Microbiology and World War I

The unique economic pressures of wartime sometimes provide incentive for scientific discovery. Two examples from the First World War involve the production of organic solvents by the microbial fermentation of readily available carbohydrates, such as starch or molasses.

The German side needed glycerol to make nitroglycerin. At one time the Germans had imported their glycerol, but such imports were prevented by the British naval blockade. The German scientist Carl Neuberg knew that trace levels of glycerol were usually produced during the alcoholic fermentation of sugar by *Saccharomyces cerevisiae*. He sought to develop a modified fermentation in which the yeasts would produce glycerol instead of ethanol. Normally acetaldehyde is reduced to ethanol by NADH and alcohol dehydrogenase (figure 9.18, pathway 2). Neuberg found that this reaction could be prevented by the addition of 3.5% sodium sulfite at pH 7.0. The bisulfite ions reacted with acetaldehyde and made it unavailable for reduction to ethanol. Because the yeast cells still had to regenerate their NAD⁺ even though acetaldehyde was no longer available, Neuberg suspected that they would simply increase the rate of glycerol synthesis. Glycerol is normally produced by the reduction of dihydroxyacetone phosphate (a glycolytic intermediate) to glycerol phosphate with NADH, followed by the hydrolysis of glycerol phosphate to glycerol. Neuberg's hunch was correct, and German breweries were converted to glycerol manufacture by his procedure, eventually producing 1,000 tons of glycerol per month. Glycerol production by *S. cerevisiae* was not economically competitive under peacetime conditions and was ended. Today glycerol is produced microbially by the halophilic protist *Dunaliella salina*, in which high concentrations of intracellular glycerol accumulate to counterbalance the osmotic pressure from the high level of extra-

cellular salt. *Dunaliella* grows in habitats such as the Great Salt Lake of Utah and seaside rock pools.

The British side needed the organic solvents acetone and butanol. Butanol was required for the production of artificial rubber, whereas acetone was used as a solvent from nitrocellulose in the manufacture of the smokeless explosive powder cordite. Prior to 1914 acetone was made by the dry heating (pyrolysis) of wood. Between 80 and 100 tons of birch, beech, or maple wood were required to make 1 ton of acetone. When war broke out, the demand for acetone quickly exceeded the existing world supply. However, by 1915 Chaim Weizmann, a young Jewish scientist working in Manchester, England, had developed a fermentation process by which the anaerobic bacterium *Clostridium acetobutylicum* converted 100 tons of molasses or grain into 12 tons of acetone and 24 tons of butanol (most clostridial fermentations stop at butyric acid).



This time the British and Canadian breweries were converted until new fermentation facilities could be constructed. Weizmann improved the process by finding a convenient way to select high-solvent producing strains of *C. acetobutylicum*. Because the strains most efficient in these fermentations also made the most heat-resistant spores, Weizmann merely isolated the survivors from repeated 100°C heat shocks. Acetone and butanol were made commercially by this fermentation process until it was replaced by much cheaper petrochemicals in the late 1940s and 1950s. In 1948 Chaim Weizmann became the first president of the State of Israel.

9.8 CATABOLISM OF CARBOHYDRATES AND INTRACELLULAR RESERVE POLYMERS

Thus far our main focus has been on the catabolism of glucose. However, microorganisms can catabolize many other carbohydrates. These carbohydrates may come either from outside the cell or from internal sources generated during normal metabolism. Often the initial steps in the degradation of external carbohydrate polymers differ from those employed with internal reserves.

Carbohydrates

Figure 9.20 outlines some catabolic pathways for the monosaccharides (single sugars) glucose, fructose, mannose, and galactose. The first three are phosphorylated using ATP and easily enter the Embden-Meyerhof pathway. In contrast, galactose must be converted to uridine diphosphate galactose (see figure 10.11) after initial phosphorylation, then changed into glucose 6-phosphate in a three-step process (figure 9.20).

The common disaccharides are cleaved to monosaccharides by at least two mechanisms (figure 9.20). Maltose, sucrose, and lactose can be directly hydrolyzed to their constituent sugars. Many disaccharides (e.g., maltose, cellobiose, and sucrose) are also split by a phosphate attack on the bond joining the two sugars, a process called **phosphorolysis**.

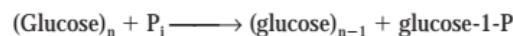
Polysaccharides, like disaccharides, are cleaved by both hydrolysis and phosphorolysis. Prokaryotes and fungi degrade external polysaccharides by secreting hydrolytic enzymes. These exoenzymes cleave polysaccharides that are too large to cross the plasma membrane into smaller molecules that can then be assimilated. Starch and glycogen are hydrolyzed by amylases to glucose, maltose, and other products. Cellulose is more difficult to digest; many fungi and a few bacteria (some gliding bacteria, clostridia, and actinomycetes) produce extracellular cellulases that hydrolyze cellulose to cellobiose and glucose. Some actinomycetes and members of the bacterial genus *Cytophaga*, isolated from marine habitats, excrete an agarase that degrades agar. Many soil bacteria and bacterial

plant pathogens degrade pectin, a polymer of galacturonic acid (a galactose derivative) that is an important constituent of plant cell walls and tissues. Lignin, another important component of plant cell walls, is usually degraded only by certain fungi that release peroxide-generating enzymes. [Microorganisms in the soil environment \(section 29.3\)](#)

In the context of compounds that are recalcitrant or difficult to digest, it should be noted that microorganisms also can degrade xenobiotic compounds (foreign substances not formed by natural biosynthetic processes) such as pesticides and various aromatic compounds. They transform these molecules to normal metabolic intermediates by use of special enzymes and pathways, then continue catabolism in the usual way. Biodegradation and bioremediation are discussed in chapter 41. The fungus *Phanerochaete chrysosporium* is an extraordinary example of the ability to degrade xenobiotics. [Microbial Diversity & Ecology 41.4: A fungus with a voracious appetite](#)

Reserve Polymers

Microorganisms often survive for long periods in the absence of exogenous nutrients. Under such circumstances they catabolize intracellular stores of glycogen, starch, poly- β -hydroxybutyrate, and other carbon and energy reserves. Glycogen and starch are degraded by phosphorylases. Phosphorylases catalyze a phosphorolysis reaction that shortens the polysaccharide chain by one glucose and yields glucose 1-phosphate.



Glucose 1-phosphate can enter glycolytic pathways by way of glucose 6-phosphate (figure 9.20).

Poly- β -hydroxybutyrate (PHB) is an important, wide-spread reserve material. Its catabolism has been studied most thoroughly in the soil bacterium *Azotobacter*. This bacterium hydrolyzes PHB to 3-hydroxybutyrate, then oxidizes the hydroxybutyrate to acetoacetate. Acetoacetate is converted to acetyl-CoA, which can be oxidized in the TCA cycle.

9.9 LIPID CATABOLISM

Chemoorganotrophic microorganisms frequently use lipids as energy sources. Triglycerides or triacylglycerols, esters of glycerol and fatty acids (figure 9.21), are common energy sources and serve as our examples. They can be hydrolyzed to glycerol and fatty acids by microbial lipases. The glycerol is then phosphorylated, oxidized to dihydroxyacetone phosphate, and catabolized in the Embden-Meyerhof pathway (figure 9.5).

Fatty acids from triacylglycerols and other lipids are often oxidized in the β -oxidation pathway after conversion to coenzyme A esters (figure 9.22). In this pathway fatty acids are shortened by two carbons with each turn of the cycle. The two carbon units are released as acetyl-CoA, which can be fed into the TCA cycle or used in biosynthesis. One turn of the cycle produces

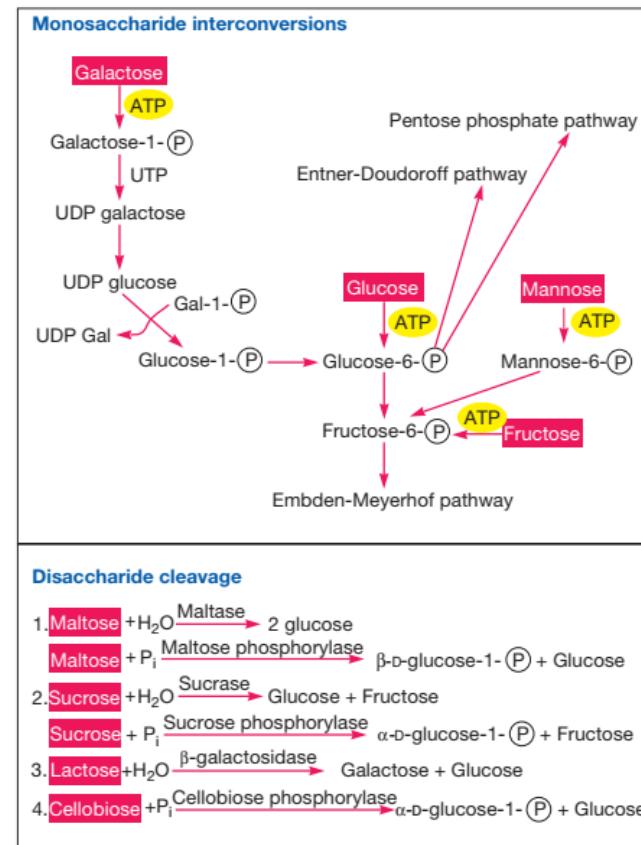


Figure 9.20 Carbohydrate Catabolism. Examples of enzymes and pathways used in disaccharide and monosaccharide catabolism. UDP is an abbreviation for uridine diphosphate.

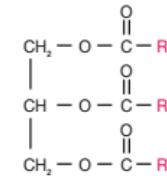


Figure 9.21 A Triacylglycerol or Triglyceride. The R groups represent the fatty acid side chains.

acetyl-CoA, NADH, and FADH₂; NADH and FADH₂ can be oxidized by the electron transport chain to provide more ATP. The fatty acyl-CoA, shortened by two carbons, is ready for another turn of the cycle. Fatty acids are a rich source of energy for microbial growth. In a similar fashion some microorganisms grow well on petroleum hydrocarbons under oxic conditions.

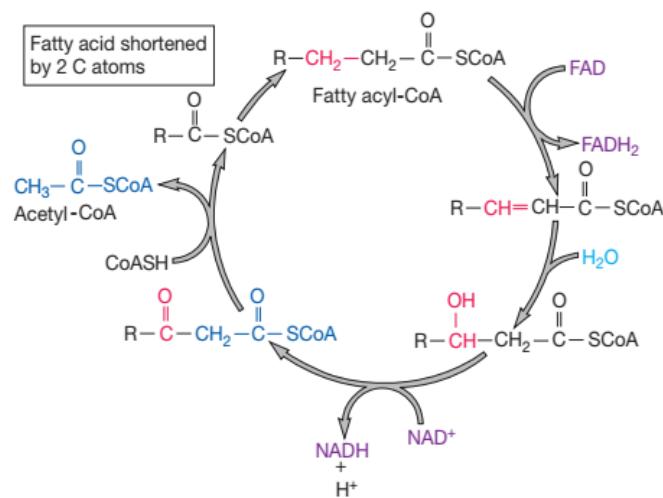


Figure 9.22 Fatty Acid β -Oxidation. The portions of the fatty acid being modified are shown in red.

9.10 PROTEIN AND AMINO ACID CATABOLISM

Some bacteria and fungi—particularly pathogenic, food spoilage, and soil microorganisms—can use proteins as their source of carbon and energy. They secrete **protease** enzymes that hydrolyze proteins and polypeptides to amino acids, which are transported into the cell and catabolized.

The first step in amino acid use is **deamination**, the removal of the amino group from an amino acid. This is often accomplished by **transamination**. The amino group is transferred from an amino acid to an α -keto acid acceptor (figure 9.23). The organic acid resulting from deamination can be converted to pyruvate, acetyl-CoA, or a TCA cycle intermediate and eventually oxidized in the TCA cycle to release energy. It also can be used as a source of carbon for the synthesis of cell constituents. Excess nitrogen from deamination may be excreted as ammonium ion, thus making the medium alkaline.

1. Briefly discuss the ways in which microorganisms degrade and use common monosaccharides, disaccharides, and polysaccharides from both external and internal sources.
2. Describe how a microorganism might derive carbon and energy from the lipids and proteins in its diet. What is β -oxidation? Deamination? Transamination?

9.11 CHEMOLITHOTROPHY

So far, we have considered microbes that synthesize ATP with the energy liberated when they oxidize organic substrates such as carbohydrates, lipids, and proteins. The electron acceptor is: (1) O_2 in aerobic respiration, (2) an oxidized exogenous molecule other than O_2 in anaerobic respiration, or (3) another more oxidized en-

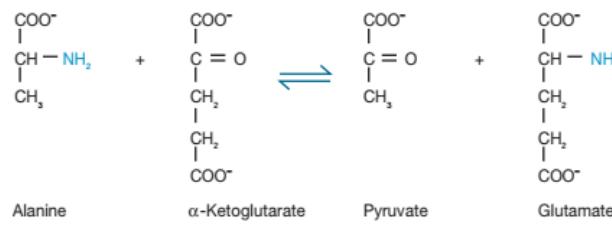
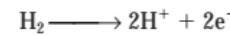


Figure 9.23 Transamination. A common example of this process. The α -amino group (blue) of alanine is transferred to the acceptor α -ketoglutarate forming pyruvate and glutamate. The pyruvate can be catabolized in the tricarboxylic acid cycle or used in biosynthesis.

dogenous organic molecule (usually pyruvate) in fermentation (figure 9.2). In fermentation, ATP is synthesized only by substrate-level phosphorylation; in both aerobic and anaerobic respiration, most of the ATP is formed using the PMF derived from electron transport chain activity. Additional metabolic diversity among bacteria and archaea is reflected in the form of energy metabolism performed by **chemolithotrophs**. These microbes obtain electrons for the electron transport chain from the oxidation of inorganic molecules rather than NADH generated by the oxidation of organic nutrients (figure 9.24). Each species is rather specific in its preferences for electron donors and acceptors (table 9.3). The acceptor is usually O_2 , but sulfate and nitrate are also used. The most common electron donors are hydrogen, reduced nitrogen compounds, reduced sulfur compounds, and ferrous iron (Fe^{2+}).

Much less energy is available from the oxidation of inorganic molecules than from the complete oxidation of glucose to CO_2 , which is accompanied with a standard free energy change of -686 kcal/mole (table 9.4). This is because the NADH that donates electrons to the chain following the oxidation of an organic substrate like glucose has a more negative reduction potential than most of the inorganic substrates that chemolithotrophs use as direct electron donors to their electron transport chains. Thus the P/O ratios for oxidative phosphorylation in chemolithotrophs are probably around 1.0 (although in the oxidation of hydrogen it is considerably higher). Because the yield of ATP is so low, chemolithotrophs must oxidize a large quantity of inorganic material to grow and reproduce. This is particularly true of autotrophic chemolithotrophs, which fix CO_2 into carbohydrates. For each molecule of CO_2 fixed, these microbes expend three ATP and two NADPH molecules. Because they must consume a large amount of inorganic material, chemolithotrophs have significant ecological impact.

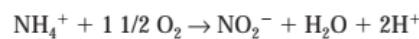
Several bacterial genera can oxidize hydrogen gas to produce energy because they possess a hydrogenase enzyme that catalyzes the oxidation of hydrogen (table 9.3).



Because the $H_2/2H^+, 2e^-$ redox couple has a very negative standard reduction potential, the electrons are donated either to an electron transport chain or to NAD^+ , depending on the hydrogenase. If

NADH is produced, it can be used in ATP synthesis by electron transport and oxidative phosphorylation, with O₂, Fe³⁺, S⁰, and even carbon monoxide (CO) as the terminal electron acceptors. Often these hydrogen-oxidizing microorganisms will use organic compounds as energy sources when such nutrients are available.

Some bacteria use the oxidation of nitrogenous compounds as a source of electrons. Among these chemolithotrophs, the **nitrifying bacteria**, which carry out nitrification are best understood. These are soil and aquatic bacteria of considerable ecological significance. **Nitrification** is the oxidation of ammonia to nitrate. It is a two-step process that depends on the activity of at least two different genera. In the first step, ammonia is oxidized to nitrite by a number of genera including *Nitrosomonas*:



In the second step, the nitrite is oxidized to nitrate by genera such as *Nitrobacter*:

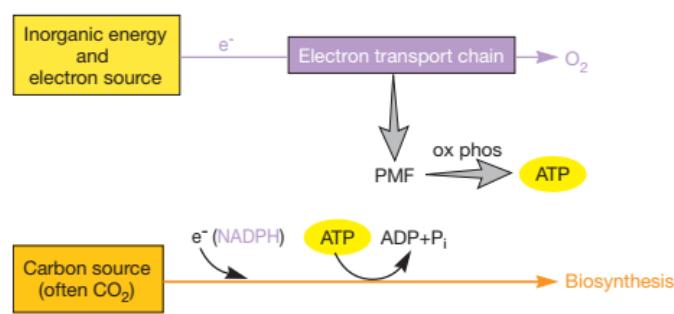


Figure 9.24 Chemolithotrophic Fueling Processes. Chemolithotrophic bacteria and archaea oxidize inorganic molecules (e.g., H₂S and NH₃), which serve as energy and electron sources. The electrons released pass through an electron transport system, generating a proton motive force (PMF). ATP is synthesized by oxidative phosphorylation (ox phos). Most chemolithotrophs use O₂ as the terminal electron acceptor. However, some can use other exogenous molecules as terminal electron acceptors. Note that a molecule other than the energy source provides carbon for biosynthesis. Many chemolithotrophs are autotrophs.

Nitrification differs from denitrification in that nitrification involves the oxidation of inorganic nitrogen compounds to yield nitrate. On the other hand, denitrification is the reduction of oxidized nitrogenous compounds to nitrogen gas (see p. 205). In nitrification, electrons are donated to the electron transport chain, while in denitrification, nitrogen species are used as electron acceptors and nitrogen is lost to the atmosphere. [Biogeochemical cycling: Nitrogen cycle \(section 27.2\)](#)

Energy released upon the oxidation of both ammonia and nitrite is used to make ATP by oxidative phosphorylation. However, autotrophic microorganisms also need NAD(P)H (reducing power) as well as ATP in order to reduce CO₂ and other molecules (figure 9.24). Since molecules like ammonia and nitrite have more positive reduction potentials than NAD⁺, they cannot directly donate their electrons to form the required NADH and NADPH. Recall that electrons spontaneously move only from donors with more negative reduction potentials to acceptors with more positive potentials (see figure 8.8). Sulfur-oxidizing bacteria face the same difficulty. Both types of chemolithotrophs solve this problem by moving the electrons derived from the oxidation of their inorganic substrate (reduced nitrogen or sulfur compounds) up the electron transport chain to reduce NAD(P)⁺ to NAD(P)H (figure 9.25). This is called **reverse electron flow**. Of course, this is not thermodynamically favorable, so energy in the form of the proton motive force must be diverted from performing other cellular work (e.g., ATP synthesis, transport, motility)

| Table 9.4 Energy Yields from Oxidations Used by Chemolithotrophs | |
|---|-------------------------------|
| Reaction | ΔG°' (kcal/mole) ^a |
| H ₂ + 1/2 O ₂ → H ₂ O | -56.6 |
| NO ₂ ⁻ + 1/2 O ₂ → NO ₃ ⁻ | -17.4 |
| NH ₄ ⁺ + 1 1/2 O ₂ → NO ₂ ⁻ + H ₂ O + 2H ⁺ | -65.0 |
| S ⁰ + 1 1/2 O ₂ + H ₂ O → H ₂ SO ₄ | -118.5 |
| S ₂ O ₃ ²⁻ + 2O ₂ + H ₂ O → 2SO ₄ ²⁻ + 2H ⁺ | -223.7 |
| 2Fe ²⁺ + 2H ⁺ + 1/2 O ₂ → 2Fe ³⁺ + H ₂ O | -11.2 |

^aThe ΔG°' for complete oxidation of glucose to CO₂ is -686 kcal/mole. A kcal is equivalent to 4.184 kJ.

| Table 9.3 Representative Chemolithotrophs and Their Energy Sources | | | |
|--|--|------------------------------|---|
| Bacteria | Electron Donor | Electron Acceptor | Products |
| <i>Alcaligenes</i> , <i>Hydrogenophaga</i> , and <i>Pseudomonas</i> spp. | H ₂ | O ₂ | H ₂ O |
| <i>Nitrobacter</i> | NO ₂ ⁻ | O ₂ | NO ₃ ⁻ , H ₂ O |
| <i>Nitrosomonas</i> | NH ₄ ⁺ | O ₂ | NO ₂ ⁻ , H ₂ O |
| <i>Thiobacillus denitrificans</i> | S ⁰ , H ₂ S | NO ₃ ⁻ | SO ₄ ²⁻ , N ₂ |
| <i>Thiobacillus ferrooxidans</i> | Fe ²⁺ , S ⁰ , H ₂ S | O ₂ | Fe ³⁺ , H ₂ O, H ₂ SO ₄ |

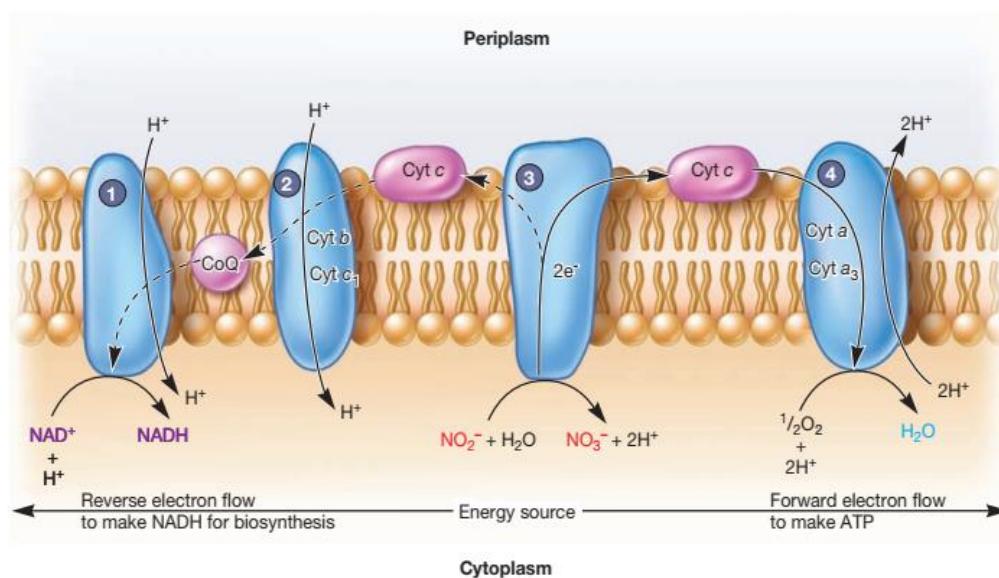


Figure 9.25 Electron Flow in *Nitrobacter* Electron Transport Chain. *Nitrobacter* oxidizes nitrite and carries out normal electron transport to generate proton motive force for ATP synthesis. This is the right-hand branch of the diagram. Some of the proton motive force also is used to force electrons to flow up the reduction potential gradient from nitrite to NAD^+ (left-hand branch). Cytochrome c and four complexes are involved: NADH-ubiquinone oxidoreductase (1), ubiquinol-cytochrome c oxidoreductase (2), nitrite oxidase (3), and cytochrome aa_3 oxidase (4).

to “push” the electrons from molecules of relatively positive reduction potentials to those that are more negative. Because this energy is used to generate NADH as well as ATP, the net yield of ATP is fairly low. Chemolithotrophs can afford this inefficiency as they have no serious competitors for their unique energy sources. [Oxidation-reduction reactions, electron carriers, and electron transport systems \(section 8.6\)](#)

Sulfur-oxidizing microbes are the third major group of chemolithotrophs. The metabolism of *Thiobacillus* has been best studied. These bacteria oxidize sulfur (S^0), hydrogen sulfide (H_2S), thiosulfate ($\text{S}_2\text{O}_3^{2-}$), and other reduced sulfur compounds to sulfuric acid; therefore they have a significant ecological impact ([Microbial Diversity & Ecology 9.2](#)). Interestingly they generate ATP by both oxidative phosphorylation and substrate-level phosphorylation involving **adenosine 5'-phosphosulfate (APS)**. APS is a high-energy molecule formed from sulfite and adenosine monophosphate ([figure 9.26](#)).

Some sulfur-oxidizing prokaryotes are extraordinarily flexible metabolically. For example, *Sulfolobus brierleyi*, an archaeon, and some bacteria can grow aerobically by oxidizing sulfur with oxygen as the electron acceptor; in the absence of O_2 , they carry out anaerobic respiration and oxidize organic material with sulfur as the electron acceptor.

Sulfur-oxidizing bacteria and archaea, like other chemolithotrophs, can use CO_2 as their carbon source. Many will grow heterotrophically if they are supplied with reduced organic carbon sources like glucose or amino acids.

1. How do chemolithotrophs obtain their ATP and NADH? What is their most common source of carbon?
2. Describe energy production by hydrogen-oxidizing bacteria, nitrifying bacteria, and sulfur-oxidizing bacteria.
3. Why can hydrogen-oxidizing bacteria and archaea donate electrons to NAD^+ while sulfur- and ammonia-oxidizing bacteria and archaea cannot?
4. What is reverse electron flow and why do most chemolithotrophs perform it?
5. Arsenate is a compound that inhibits substrate-level phosphorylation. Compare the effect of this compound on a H_2 -oxidizing chemolithotroph, on a sulfite-oxidizing chemolithotroph, and on a chemoorganotroph carrying out fermentation.

9.12 PHOTOTROPHY

Microorganisms derive energy not only from the oxidation of inorganic and organic compounds, but also from light energy, which they capture and use to synthesize ATP and reduce power (e.g., NADPH) ([figure 9.1](#); *see also figure 8.10*). The process by which light energy is trapped and converted to chemical energy is called **photosynthesis**. Usually a phototrophic organism reduces and incorporates CO_2 . Photosynthesis is one of the most significant metabolic processes on Earth because almost all our energy is ultimately derived from solar energy. It provides photosynthetic organisms with the ATP and reducing power necessary to synthesize the organic material required for growth. In turn



Microbial Diversity & Ecology

9.2

Acid Mine Drainage

Each year millions of tons of sulfuric acid flow to the Ohio River from the Appalachian Mountains. This sulfuric acid is of microbial origin and leaches enough metals from the mines to make the river reddish and acidic. The primary culprit is *Thiobacillus ferrooxidans*, a chemolithotrophic bacterium that derives its energy from oxidizing ferrous ion to ferric ion and sulfide ion to sulfate ion. The combination of these two energy sources is important because of the solubility properties of iron. Ferrous ion is somewhat soluble and can be formed at pH values of 3.0 or less in moderately reducing environments. However, when the pH is greater than 4.0 to 5.0, ferrous ion is spontaneously oxidized to ferric ion by O₂ in the water and precipitates as a hydroxide. If the pH drops below about 2.0 to 3.0 because of sulfuric acid production by spontaneous oxidation of sulfur or sulfur oxidation by thiobacilli and other bacteria, the ferrous ion remains reduced, soluble, and available as an energy source. Remarkably, *T. ferrooxidans* grows well at such acidic pHs and actively oxidizes ferrous ion to an insoluble ferric precipitate. The water is rendered toxic for most aquatic life and unfit for human consumption.

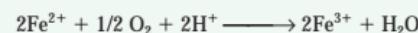
The ecological consequences of this metabolic life-style arise from the common presence of pyrite (FeS₂) in coal mines. The bac-

teria oxidize both elemental components of pyrite for their growth and in the process form sulfuric acid, which leaches the remaining minerals.

Autoxidation or bacterial action



T. ferrooxidans



Pyrite oxidation is further accelerated because the ferric ion generated by bacterial activity readily oxidizes more pyrite to sulfuric acid and ferrous ion. In turn the ferrous ion supports further bacterial growth. It is difficult to prevent *T. ferrooxidans* growth as it requires only pyrite and common inorganic salts. Because *T. ferrooxidans* gets its O₂ and CO₂ from the air, the only feasible method of preventing its damaging growth is to seal the mines to render the habitat anoxic.

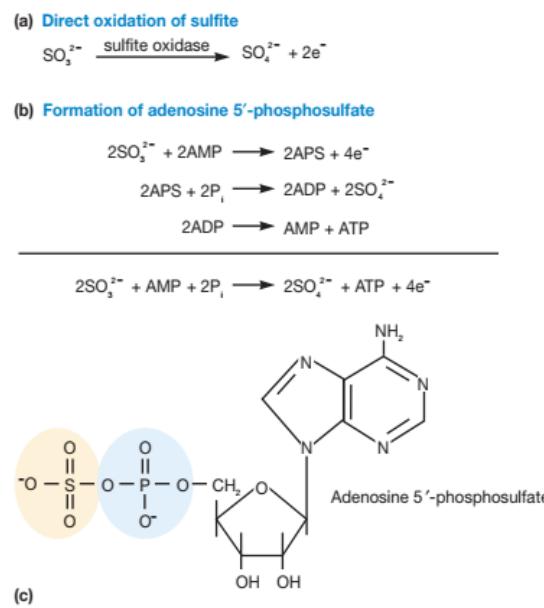


Figure 9.26 Energy Generation by Sulfur Oxidation.

(a) Sulfite can be directly oxidized to provide electrons for electron transport and oxidative phosphorylation. (b) Sulfite can also be oxidized and converted to adenosine 5'-phosphosulfate (APS). This route produces electrons for use in electron transport and ATP by substrate-level phosphorylation with APS. (c) The structure of APS.

Table 9.5 Diversity of Phototrophic Organisms

| Eucaryotic Organisms | Prokaryotic Organisms |
|---|---------------------------------|
| Plants | Cyanobacteria |
| Multicellular green, brown, and red algae | Green sulfur bacteria |
| Unicellular protists (e.g., euglenoids, dinoflagellates, diatoms) | Green nonsulfur bacteria |
| | <i>Halobacterium</i> (archaeon) |
| | Purple sulfur bacteria |
| | Purple nonsulfur bacteria |
| | <i>Prochloron</i> |

these organisms serve as the base of most food chains in the biosphere. One type of photosynthesis is also responsible for replenishing our supply of O₂, a remarkable process carried out by a variety of organisms, both eucaryotic and bacterial (table 9.5). Although most people associate photosynthesis with the larger, more obvious plants, over half the photosynthesis on Earth is carried out by microorganisms.

Photosynthesis as a whole is divided into two parts. In the **light reactions** light energy is trapped and converted to chemical energy. This energy is then used to reduce or fix CO₂ and synthesize cell constituents in the **dark reactions**. In this section three types of phototrophy are discussed: oxygenic photosynthesis,

anoxygenic photosynthesis, and rhodopsin-based phototrophy (**figure 9.27**). The dark reactions of photosynthesis are reviewed in chapter 10. [The fixation of CO₂ by autotrophs \(section 10.3\)](#)

The Light Reaction in Oxygenic Photosynthesis

Phototrophic eucaryotes and the cyanobacteria carry out **oxygenic photosynthesis**, so named because oxygen is generated when light energy is converted to chemical energy. Central to this process, and to all other phototrophic processes, are light-absorbing

pigments (**table 9.6**). In oxygenic phototrophs, the most important pigments are the **chlorophylls**. Chlorophylls are large planar rings composed of four substituted pyrrole rings with a magnesium atom coordinated to the four central nitrogen atoms (**figure 9.28**). Several chlorophylls are found in eucaryotes, the two most important are chlorophyll *a* and chlorophyll *b*. These two molecules differ slightly in their structure and spectral properties. When dissolved in acetone, chlorophyll *a* has a light absorption peak at 665 nm; the corresponding peak for chlorophyll *b* is at 645 nm. In addition to absorbing red light, chlorophylls also absorb

Figure 9.27 Phototrophic Fueling Reactions.

Phototrophs use light to generate a proton motive force (PMF), which is then used to synthesize ATP by a process called photophosphorylation (photo phos). The process requires light-absorbing pigments. When the pigments are chlorophyll or bacteriochlorophyll, the absorption of light triggers electron flow through an electron transport chain, accompanied by the pumping of protons across a membrane. The electron flow can be either cyclic (dashed line) or noncyclic (solid line), depending on the organism and its needs. Rhodopsin-based phototrophy differs in that the PMF is formed directly by the light-absorbing pigment, which is a light-driven proton pump. Many phototrophs are autotrophs and must use much of the ATP and reducing power they make to fix CO₂.

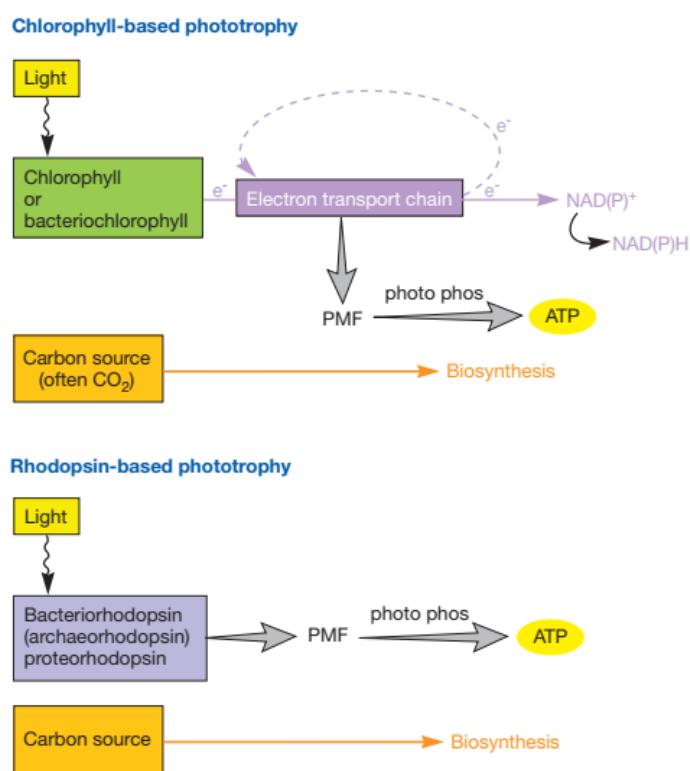


Table 9.6 Properties of Chlorophyll-Based Photosynthetic Systems

| Property | Eucaryotes | Cyanobacteria | Green Bacteria, Purple Bacteria, and Heliobacteria |
|---------------------------------------|----------------------|-----------------------|--|
| Photosynthetic pigment | Chlorophyll <i>a</i> | Chlorophyll <i>a</i> | Bacteriochlorophyll |
| Photosystem II | Present | Present | Absent |
| Photosynthetic electron donors | H ₂ O | H ₂ O | H ₂ , H ₂ S, S, organic matter |
| O ₂ production pattern | Oxygenic | Oxygenic ^a | Anoxygenic |
| Primary products of energy conversion | ATP + NADPH | ATP + NADPH | ATP |
| Carbon source | CO ₂ | CO ₂ | Organic and/or CO ₂ |

^aSome cyanobacteria can function anoxygenically under certain conditions. For example, *Oscillatoria* can use H₂S as an electron donor instead of H₂O.

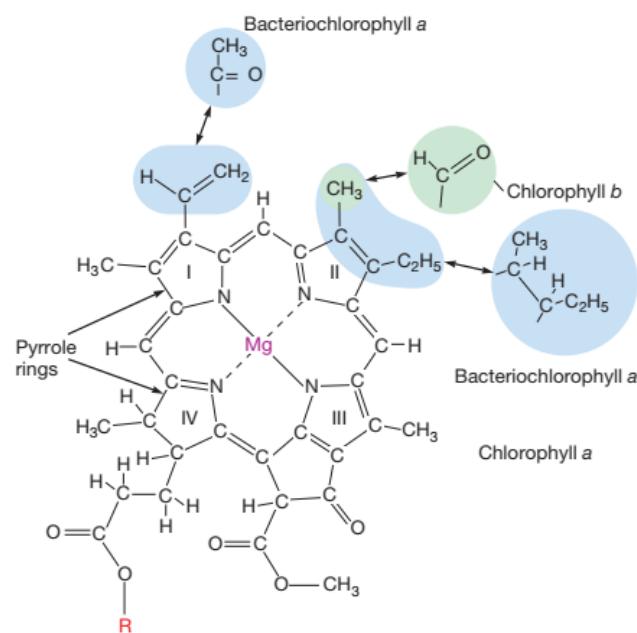


Figure 9.28 Chlorophyll Structure. The structures of chlorophyll *a*, chlorophyll *b*, and bacteriochlorophyll *a*. The complete structure of chlorophyll *a* is given. Only one group is altered to produce chlorophyll *b*, and two modifications in the ring system are required to change chlorophyll *a* to bacteriochlorophyll *a*. The side chain (*R*) of bacteriochlorophyll *a* may be either phytol (a 20-carbon chain also found in chlorophylls *a* and *b*) or geranylgeranyl (a 20-carbon side chain similar to phytol, but with three more double bonds).

blue light strongly (the second absorption peak for chlorophyll *a* is at 430 nm). Because chlorophylls absorb primarily in the red and blue ranges, green light is transmitted. Consequently many oxygenic phototrophs are green in color. The long hydrophobic tail attached to the chlorophyll ring aids in its attachment to membranes, the site of the light reactions.

Other photosynthetic pigments also trap light energy. The most widespread of these are the **carotenoids**, long molecules, usually yellowish in color, that possess an extensive conjugated double bond system (figure 9.29). β -Carotene is present in cyanobacteria belonging to the genus *Prochloron* and most photosynthetic protists; fucoxanthin is found in protists such as diatoms and dinoflagellates. Red algae and cyanobacteria have photosynthetic pigments called **phycobiliproteins**, consisting of a protein with a linear tetrapyrrole attached (figure 9.29). **Phycoerythrin** is a red pigment with a maximum absorption around 550 nm, and **phycocyanin** is blue (maximum absorption at 620 to 640 nm).

Carotenoids and phycobiliproteins are often called **accessory pigments** because of their role in photosynthesis. Accessory pigments are important because they absorb light in the range not absorbed by chlorophylls (the blue-green through yellow range; about

470–630 nm) (see figure 21.4). This light is very efficiently transferred to chlorophyll. In this way accessory pigments make photosynthesis more efficient over a broader range of wavelengths. In addition, this allows organisms to use light not used by other phototrophs in their habitat. For instance, the microbes below a canopy of plants can use light that passes through the canopy. Accessory pigments also protect microorganisms from intense sunlight, which could oxidize and damage the photosynthetic apparatus.

Chlorophylls and accessory pigments are assembled in highly organized arrays called **antennas**, whose purpose is to create a large surface area to trap as many photons as possible. An antenna has about 300 chlorophyll molecules. Light energy is captured in an antenna and transferred from chlorophyll to chlorophyll until it reaches a special **reaction-center chlorophyll pair** directly involved in photosynthetic electron transport. In oxygenic phototrophs, there are two kinds of antennas associated with two different photosystems (figure 9.30). **Photosystem I** absorbs longer wavelength light (≥ 680 nm) and funnels the energy to a special chlorophyll *a* pair called P700. The term P700 signifies that this molecule most effectively absorbs light at a wavelength of 700 nm. **Photosystem II** traps light at shorter wavelengths (≤ 680 nm) and transfers its energy to the special chlorophyll pair P680.

When the photosystem I antenna transfers light energy to the reaction-center P700 chlorophyll pair, P700 absorbs the energy and is excited; its reduction potential becomes very negative. This allows it to donate its excited, high-energy electron to a specific acceptor, probably a special chlorophyll *a* molecule or an iron-sulfur protein. The electron is eventually transferred to ferredoxin and can then travel in either of two directions. In the cyclic pathway (the dashed lines in figure 9.30), the electron moves in a cyclic route through a series of electron carriers and back to the oxidized P700. The pathway is termed cyclic because the electron from P700 returns to P700 after traveling through the photosynthetic electron transport chain. PMF is formed during cyclic electron transport in the region of cytochrome b_6 and used to synthesize ATP. This process is called **cyclic photophosphorylation** because electrons travel in a cyclic pathway and ATP is formed. Only photosystem I participates.

Electrons also can travel in a noncyclic pathway involving both photosystems. P700 is excited and donates electrons to ferredoxin as before. In the noncyclic route, however, reduced ferredoxin reduces NADP⁺ to NADPH (figure 9.30). Because the electrons contributed to NADP⁺ cannot be used to reduce oxidized P700, photosystem II participation is required. It donates electrons to oxidized P700 and generates ATP in the process. The photosystem II antenna absorbs light energy and excites P680, which then reduces pheophytin *a*. Pheophytin *a* is chlorophyll *a* in which two hydrogen atoms have replaced the central magnesium. Electrons subsequently travel to the plastoquinone pool and down the electron transport chain to P700. Although P700 has been reduced, P680 must also be reduced if it is to accept more light energy. Figure 9.30 indicates that the standard reduction potential of P680 is more positive than that of the O₂/H₂O redox couple. Thus H₂O can be used to donate electrons to P680 resulting in the release of oxygen. Because electrons flow from

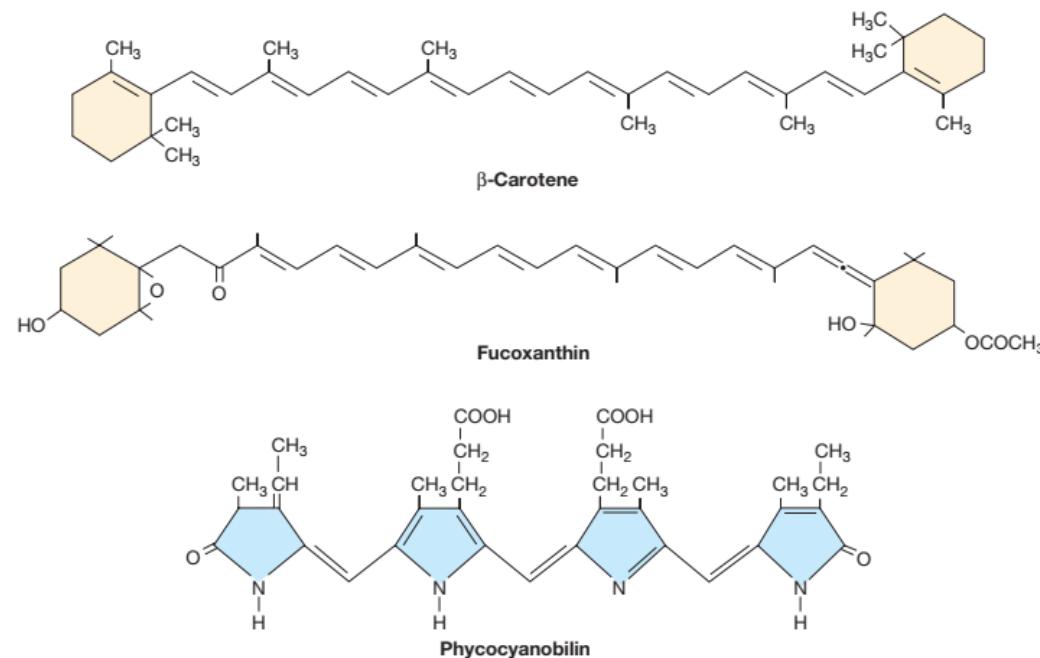
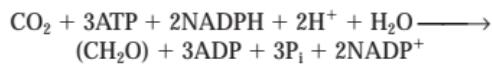


Figure 9.29 Representative Accessory Pigments. Beta-carotene is a carotenoid found in photosynthetic protists and plants. Note that it has a long chain of alternating double and single bonds called conjugated double bonds. Fucoxanthin is a carotenoid accessory pigment in several divisions of algae (the dot in the structure represents a carbon atom). Phycocyanobilin is an example of a linear tetrapyrrole that is attached to a protein to form a phycobiliprotein.

water to NADP^+ with the aid of energy from two photosystems, ATP is synthesized by **noncyclic photophosphorylation**. It appears that one ATP and one NADPH are formed when two electrons travel through the noncyclic pathway.

Just as is true of mitochondrial electron transport, photosynthetic electron transport takes place within a membrane. Chloroplast granal membranes contain both photosystems and their antennas. **Figure 9.31** shows a thylakoid membrane carrying out noncyclic photophosphorylation by the chemiosmotic mechanism. Protons move to the thylakoid interior during photosynthetic electron transport and return to the stroma when ATP is formed. It is believed that stromal lamellae possess only photosystem I and are involved in cyclic photophosphorylation alone. In cyanobacteria, photosynthetic light reactions are located in thylakoid membranes within the cell.

The **dark reactions** require three ATPs and two NADPHs to reduce one CO_2 and use it to synthesize carbohydrate (CH_2O).



The noncyclic system generates one NADPH and one ATP per pair of electrons; therefore four electrons passing through the system will produce two NADPHs and two ATPs. A total of 8 quanta of light energy (4 quanta for each photosystem) is needed to pro-

pel the four electrons from water to NADP^+ . Because the ratio of ATP to NADPH required for CO_2 fixation is 3:2, at least one more ATP must be supplied. Cyclic photophosphorylation probably operates independently to generate the extra ATP. This requires absorption of another 2 to 4 quanta. It follows that around 10 to 12 quanta of light energy are needed to reduce and incorporate one molecule of CO_2 during photosynthesis.

The Light Reaction in Anoxygenic Photosynthesis

Certain bacteria carry out a second type of photosynthesis called **anoxygenic photosynthesis**. This phototrophic process derives its name from the fact that water is not used as an electron source and therefore O_2 is not produced. The process also differs in terms of the photosynthetic pigments used, the participation of just one photosystem, and the mechanisms used to generate reducing power. Three groups of bacteria carry out anoxygenic photosynthesis: phototrophic green bacteria, phototrophic purple bacteria, and heliobacteria. The biology and ecology of these organisms is described in much more detail in chapters 21, 22, and 23.

Anoxygenic phototrophs have photosynthetic pigments called **bacteriochlorophylls** (figure 9.28). The absorption maxima of bacteriochlorophylls (Bchl) are at longer wavelengths than those of chlorophylls. Bacteriochlorophylls *a* and *b* have maxima

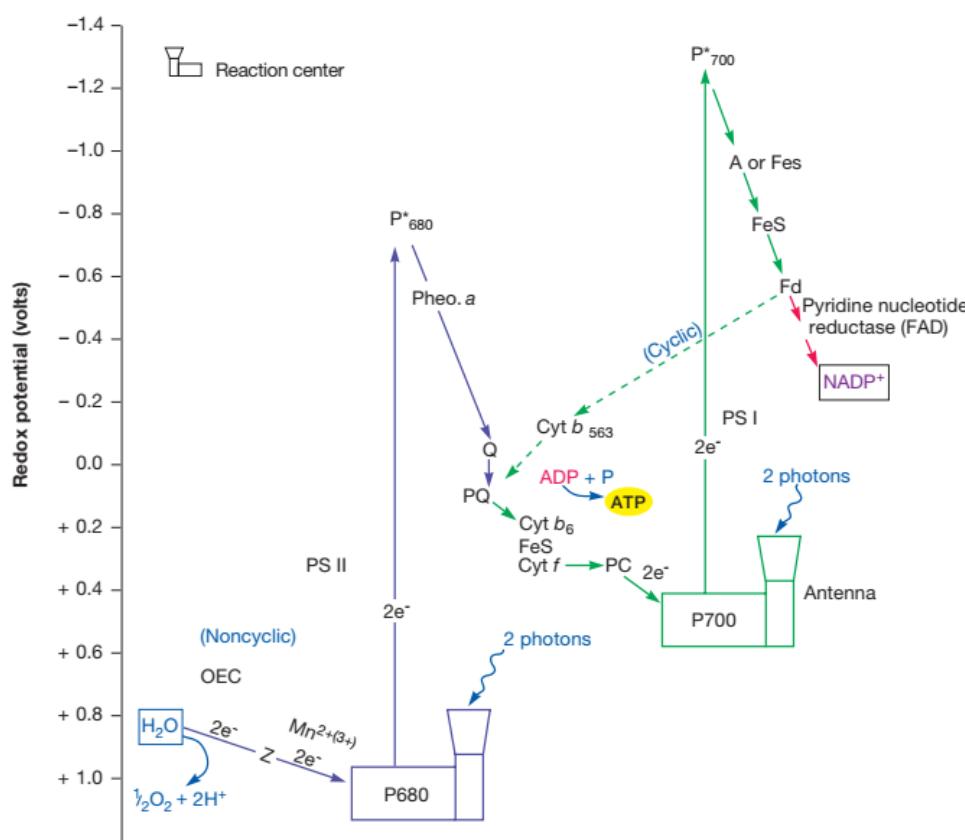


Figure 9.30 Green Plant Photosynthesis. Electron flow during photosynthesis in higher plants. Cyanobacteria and eucaryotic algae are similar in having two photosystems, although they may differ in some details. The carriers involved in electron transport are ferredoxin (Fd) and other FeS proteins; cytochromes *b*₆, *b*₅₆₃, and *f*; plastoquinone (PQ); copper containing plastocyanin (PC); pheophytin *a* (Pheo. *a*); possibly chlorophyll *a* (*A*); and the unknown quinone Q, which is probably a plastoquinone. Both photosystem I (PS I) and photosystem II (PS II) are involved in noncyclic photophosphorylation; only PS I participates in cyclic photophosphorylation. The oxygen evolving complex (OEC) that extracts electrons from water contains manganese ions and the substance Z, which transfers electrons to the PS II reaction center. See the text for further details.

in ether at 775 and 790 nm, respectively. In vivo maxima are about 830 to 890 nm (Bchl *a*) and 1,020 to 1,040 nm (Bchl *b*). This shift of absorption maxima into the infrared region better adapts these bacteria to their ecological niches.

Many differences found in anoxygenic phototrophs are due to their having a single photosystem (figure 9.32). Because of this, they are restricted to cyclic electron flow and are unable to produce O₂ from H₂O. Indeed, almost all anoxygenic phototrophs are strict anaerobes. A tentative scheme for the photosynthetic electron transport chain of a purple nonsulfur bacterium is given in figure 9.33. When the special reaction-center bacteriochlorophyll P870 is excited, it donates an electron to bacteriopheophytin. Electrons then flow to quinones and through an electron transport chain back to P870 while generating sufficient PMF to drive ATP synthesis. Note that although both green and purple bacteria lack two photosystems, the purple bacteria have a photosynthetic ap-

paratus similar to photosystem II, whereas the green sulfur bacteria have a system similar to photosystem I.

Anoxygenic phototrophs face a further problem because they also require reducing power (NAD(P)H or reduced ferredoxin) for CO₂ fixation and other biosynthetic processes. They are able to generate reducing power in at least three ways, depending on the bacterium. Some have hydrogenases that are used to produce NAD(P)H directly from the oxidation of hydrogen gas. This is possible because hydrogen gas has a more negative reduction potential than NAD⁺ (see table 8.1). Others, such as the photosynthetic purple bacteria, must use reverse electron flow to generate NAD(P)H (figure 9.33). In this mechanism, electrons are drawn off the photosynthetic electron transport chain and “pushed” to NAD(P)⁺ using PMF or the hydrolysis of ATP. Electrons from electron donors such as hydrogen sulfide, elemental sulfur, and organic compounds replace the electrons removed from the electron

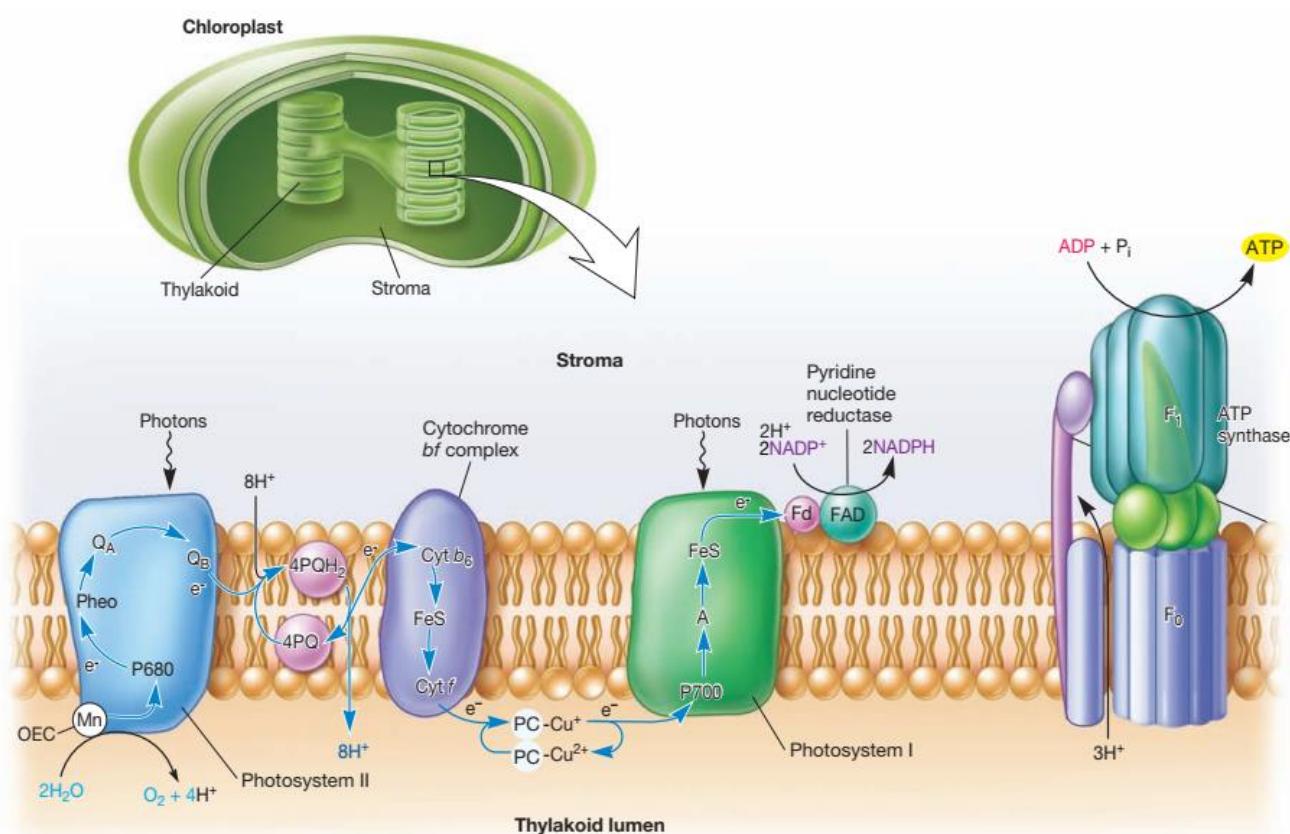


Figure 9.31 The Mechanism of Photosynthesis. An illustration of the chloroplast thylakoid membrane showing photosynthetic electron transport chain function and noncyclic photophosphorylation. The chain is composed of three complexes: PS I, the cytochrome *bf* complex, and PS II. Two diffusible electron carriers connect the three complexes. Plastoquinone (PQ) connects PS I with the cytochrome *bf* complex, and plastocyanin (PC) connects the cytochrome *bf* complex with PS II. The light-driven electron flow pumps protons across the thylakoid membrane and generates an electrochemical gradient, which can then be used to make ATP. Water is the source of electrons and the oxygen-evolving complex (OEC) produces oxygen.

transport chain in this way. Phototrophic green bacteria and heliobacteria also must draw off electrons from their electron transport chains. However, because the reduction potential of the component of the chain where this occurs is more negative than NAD^+ and oxidized ferredoxin, the electrons flow spontaneously to these electron acceptors. Thus these bacteria exhibit a simple form of noncyclic photosynthetic electron flow (figure 9.34).

Rhodopsin-Based Phototrophy

Oxygenic and anoxygenic photosynthesis are chlorophyll-based types of phototrophy—that is, chlorophyll or bacteriochlorophyll is the major pigment used to absorb light and initiate the conversion of light energy to chemical energy. This type of phototrophy is observed only in eucaryotes and bacteria; it has not been observed in any archaea, to date. However, some archaea are able to use light as a source of energy. Instead of using chlorophyll, these

microbes use a membrane protein called **bacteriorhodopsin** (more correctly called archaeorhodopsin). One such archaeon is the halophile *Halobacterium salinarum*.

H. salinarum normally depends on aerobic respiration for the release of energy from an organic energy source. However, under conditions of low oxygen and high light intensity, it synthesizes bacteriorhodopsin, a deep-purple pigment that closely resembles the rhodopsin from the rods and cones of vertebrate eyes. Bacteriorhodopsin's chromophore is retinal, a type of carotenoid. The chromophore is covalently attached to the pigment protein, which is embedded in the plasma membrane in such a way that the retinal is in the center of the membrane.

Bacteriorhodopsin functions as a light-driven proton pump. When retinal absorbs light, a proton is released and the bacteriorhodopsin undergoes a sequence of conformation changes that translocate the proton into the periplasmic space (see figure 20.13). The light-driven proton pumping generates a pH gradient

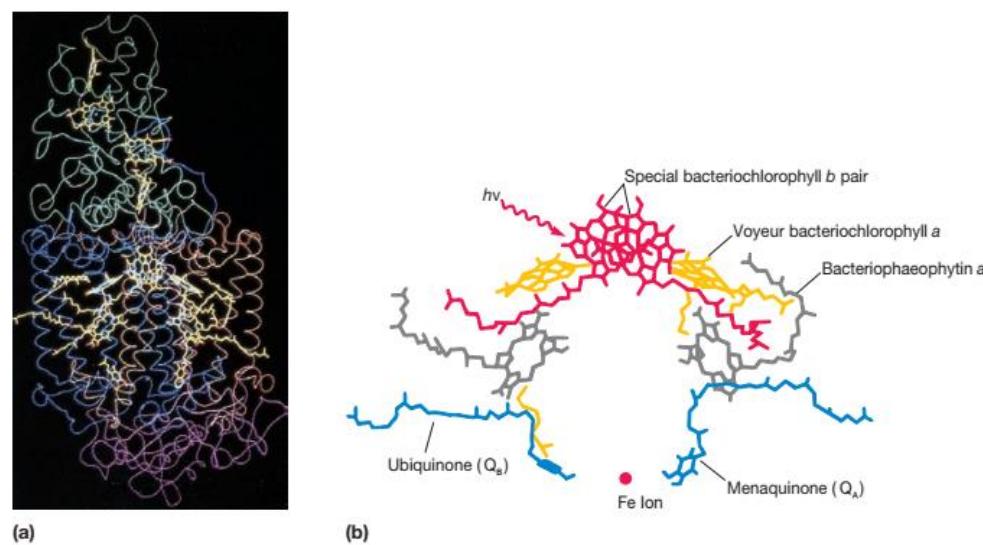


Figure 9.32 A Photosynthetic Reaction Chain. The reaction center of the purple nonsulfur bacterium, *Rhodopseudomonas viridis*.
(a) The structure of the C_a backbone of the center's polypeptide chains with the bacteriochlorophylls and other prosthetic groups in yellow.
(b) A close-up view of the reaction center prosthetic groups. A photon is first absorbed by the "special pair" of bacteriochlorophyll *a* molecules, thus exciting them. An excited electron then moves to the bacteriopheophytin molecule in the right arm of the system.

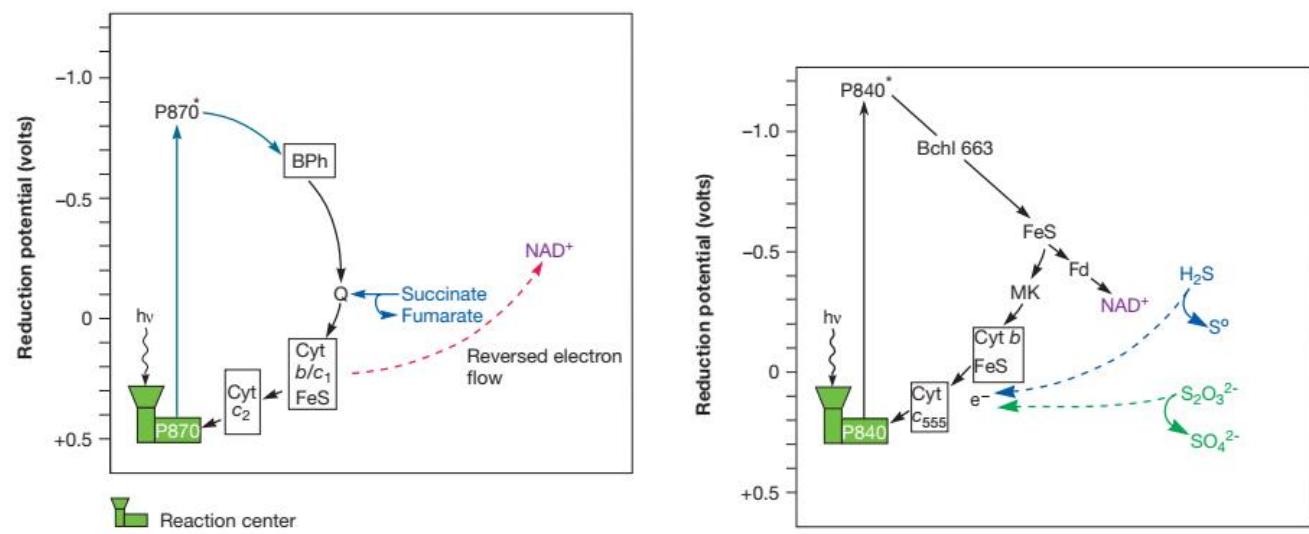


Figure 9.33 Purple Nonsulfur Bacterial Photosynthesis. The photosynthetic electron transport system in the purple nonsulfur bacterium, *Rhodobacter sphaeroides*. This scheme is incomplete and tentative. Ubiquinone (Q) is very similar to coenzyme Q. BPh stands for bacteriopheophytin. The electron source succinate is in blue.

that can be used to power the synthesis of ATP by chemiosmosis. This phototrophic capacity is particularly useful to *Halobacterium* because oxygen is not very soluble in concentrated salt solutions and may decrease to an extremely low level in *Halobacterium*'s habitat. When the surroundings become temporarily anoxic, the archaeon uses light energy to synthesize sufficient ATP to survive until oxygen levels rise again. *Halobacterium* cannot grow anaerobically by anaerobic respiration or fermentation because it needs oxygen for synthesis of retinal. However, it can survive the stress of temporary oxygen limitation by means of phototrophy. Note, however, that this type of phototrophy does not involve electron transport. It had been thought that rhodopsin-based phototrophy is unique to *Archaea*. However, proton-pumping rhodopsins have recently been discovered in some proteobacteria (proteorhodopsin) and a fungus. [Environmental genomics \(section 15.9\)](#)

1. Define the following terms: light reaction, chlorophyll, carotenoid, phycobiliprotein, antenna, and photosystems I and II.
2. What happens to a reaction center chlorophyll pair, like P700, when it absorbs light?
3. What is the function of accessory pigments?
4. What is photophosphorylation? What is the difference between cyclic and noncyclic photophosphorylation?
5. Why is the light reaction in green bacteria, purple bacteria, and heliobacteria termed anoxygenic?
6. Compare and contrast anoxygenic photosynthesis and oxygenic photosynthesis. How do these two types of phototrophy differ from rhodopsin-based phototrophy?
7. Suppose you isolated a bacterial strain that carried out oxygenic photosynthesis. What photosystems would it possess and what group of bacteria would it most likely belong to?

Summary

9.1 Chemoorganotrophic Fueling Processes

- Chemotrophic microorganisms can use three kinds of electron acceptors during energy metabolism ([figure 9.2](#)). The nutrient may be oxidized with an endogenous electron acceptor (fermentation), with oxygen as an exogenous electron acceptor (aerobic respiration), or with another external electron acceptor (anaerobic respiration).

9.2 Aerobic Respiration

- Aerobic respiration can be divided into three stages: (1) breakdown of macromolecules into their constituent parts, (2) catabolism to pyruvate, acetyl-CoA, and other molecules by pathways that converge on glycolytic pathways and the TCA cycle, and (3) completion of catabolism by the TCA cycle. Most energy is produced at this stage and results from oxidation of NADH and FADH₂ by the electron transport chain and oxidative phosphorylation ([figure 9.3](#)).
- The pathways used during aerobic respiration are amphibolic, having both catabolic and anabolic functions ([figure 9.4](#)).

9.3 The Breakdown of Glucose to Pyruvate

- Glycolysis, used in its broadest sense, refers to all pathways used to break down glucose to pyruvate.
- The Embden-Meyerhof pathway has a net production of two NADHs and two ATPs, the latter being produced by substrate-level phosphorylation. It also produces several precursor metabolites ([figure 9.5](#)).
- In the pentose phosphate pathway, glucose 6-phosphate is oxidized twice and converted to pentoses and other sugars. It is a source of NADPH, ATP, and several precursor metabolites ([figure 9.6](#)).
- In the Entner-Doudoroff pathway, glucose is oxidized to 6-phosphogluconate, which is then dehydrated and cleaved to pyruvate and glyceraldehyde 3-phosphate ([figure 9.8](#)). The latter product can be oxidized by glycolytic enzymes to provide ATP, NADH, and another molecule of pyruvate.

9.4 The Tricarboxylic Acid Cycle

- The tricarboxylic acid cycle is the final stage of catabolism in most aerobic cells ([figure 9.9](#)). It oxidizes acetyl-CoA to CO₂ and forms one GTP, three NADHs, and one FADH₂ per acetyl-CoA. It also generates several precursor metabolites.

9.5 Electron Transport and Oxidative Phosphorylation

- The NADH and FADH₂ produced from the oxidation of carbohydrates, fatty acids, and other nutrients can be oxidized in the electron transport chain. Electrons flow from carriers with more negative reduction potentials to those with more positive potentials ([figure 9.10](#) see also [figure 8.8](#)), and free energy is released for ATP synthesis by oxidative phosphorylation.
- Bacterial electron transport chains are often different from eukaryotic chains with respect to such aspects as carriers and branching. In eukaryotes the P/O ratio for NADH is about 3 and that for FADH₂ is around 2; P/O ratios are usually much lower in bacteria.
- ATP synthase catalyzes the synthesis of ATP. In eukaryotes, it is located on the inner surface of the inner mitochondrial membrane. Bacterial ATP synthase is on the inner surface of the plasma membrane.
- The most widely accepted mechanism of oxidative phosphorylation is the chemiosmotic hypothesis in which proton motive force (PMF) drives ATP synthesis ([figure 9.11](#)).
- Aerobic respiration in eukaryotes can yield a maximum of 38 ATPs ([figure 9.15](#)).

9.6 Anaerobic Respiration

- Anaerobic respiration is the process of ATP production by electron transport in which the terminal electron acceptor is an exogenous molecule other than O₂. The most common acceptors are nitrate, sulfate, and CO₂ ([figure 9.16](#)).

9.7 Fermentations

- During fermentation, an endogenous electron acceptor is used to reoxidize any NADH generated by the catabolism of glucose to pyruvate ([figure 9.17](#)).
- Flow of electrons from the electron donor to the electron acceptor does not involve an electron transport chain, and ATP is synthesized only by substrate-level phosphorylation.

9.8 Catabolism of Carbohydrates and Intracellular Reserve Polymers

- Microorganisms catabolize many extracellular carbohydrates. Monosaccharides are taken in and phosphorylated; disaccharides may be cleaved to monosaccharides by either hydrolysis or phosphorolysis.

- b. External polysaccharides are degraded by hydrolysis and the products are absorbed. Intracellular glycogen and starch are converted to glucose 1-phosphate by phosphorolysis (**figure 9.20**).

9.9 Lipid Catabolism

- a. Fatty acids from lipid catabolism are usually oxidized to acetyl-CoA in the β -oxidation pathway (**figure 9.22**).

9.10 Protein and Amino Acid Catabolism

- a. Proteins are hydrolyzed to amino acids that are then deaminated; their carbon skeletons feed into the TCA cycle (**figure 9.23**).

9.11 Chemolithotrophy

- a. Chemolithotrophs synthesize ATP by oxidizing inorganic compounds—usually hydrogen, reduced nitrogen and sulfur compounds, or ferrous iron—with an electron transport chain and O₂ as the electron acceptor (**figure 9.24** and **table 9.3**).
- b. Many of the energy sources used by chemolithotrophs have a more positive standard reduction potential than the NAD⁺/NADH redox pair. These chemolithotrophs must expend energy (PMF or ATP) to drive reverse electron

flow and produce the NADH they need for CO₂ fixation and other processes (**figure 9.25**).

9.12 Phototrophy

- a. In oxygenic photosynthesis, eucaryotes and cyanobacteria trap light energy with chlorophyll and accessory pigments and move electrons through photosystems I and II to make ATP and NADPH (the light reactions).
- b. Cyclic photophosphorylation involves the activity of photosystem I alone and generates ATP only. In noncyclic photophosphorylation photosystems I and II operate together to move electrons from water to NADP⁺ producing ATP, NADPH, and O₂ (**figure 9.30**).
- c. Anoxygenic phototrophs differ from oxygenic phototrophs in possessing bacteriochlorophyll and having only one photosystem (**figures 9.33** and **9.34**). They use cyclic photophosphorylation to make ATP. They are anoxygenic because they do not use water as an electron donor for electron flow through the photosynthetic electron transport chain.
- d. Some archaea and bacteria use a type of phototrophy that involves the proton-pumping pigment bacteriorhodopsin and proteorhodopsin, respectively. This type of phototrophy generates PMF but does not involve an electron transport chain.

Key Terms

| | | | | | | | |
|-----------------------------------|-----|---------------------------------|-----|--------------------------------|-----|----------------------------------|-----|
| accessory pigments | 217 | chemiosmotic hypothesis | 202 | heterolactic fermenters | 208 | photosystem II | 217 |
| acetyl-coenzyme A (acetyl-CoA) | 198 | chemolithotroph | 212 | hexose monophosphate pathway | 196 | phycobiliproteins | 217 |
| adenosine 5'-phosphosulfate (APS) | 214 | chlorophylls | 216 | homolactic fermenters | 208 | phycocyanin | 217 |
| aerobic respiration | 193 | citric acid cycle | 198 | Krebs cycle | 198 | phycocerythrin | 217 |
| alcoholic fermentation | 208 | cyclic photophosphorylation | 217 | lactic acid fermentation | 208 | protease | 212 |
| amphibolic pathways | 194 | dark reactions | 215 | light reactions | 215 | proton motive force (PMF) | 202 |
| anaerobic respiration | 205 | deamination | 212 | mixed acid fermentation | 209 | reaction-center chlorophyll pair | 217 |
| anoxygenic photosynthesis | 218 | denitrification | 205 | nitrification | 213 | respiration | 192 |
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| butanediol fermentation | 209 | glycolysis | 194 | photosynthesis | 214 | uncouplers | 203 |
| carotenoids | 217 | glycolytic pathway | 194 | photosystem I | 217 | | |

Critical Thinking Questions

- Without looking in chapter 21, predict some characteristics that would describe niches occupied by green and purple photosynthetic bacteria.
- From an evolutionary perspective, discuss why most microorganisms use aerobic respiration to generate ATP.
- How would you isolate a thermophilic chemolithotroph that uses sulfur compounds as a source of electrons? What changes in the incubation system would be needed to isolate bacteria using sulfur compounds in anaerobic respiration? How can one tell which process is taking place through an analysis of the sulfur molecules present in the medium?
- Certain uncouplers block ATP synthesis by allowing protons and other ions to “leak across membranes,” disrupting the charge and proton gradients established by electron flow through an electron transport chain. Does this observation support the chemiosmosis hypothesis? Explain your reasoning.
- Two flasks of *E. coli* are grown in batch culture in the same medium (2% glucose and amino acids; no nitrate) and at the same temperature (37°C). Culture #1 is well aerated. Culture #2 is anoxic. After 16 hours the following observations are made:
 - Culture #1 has a high cell density; the cells appear to be in stationary phase, and the glucose level in the medium is reduced to 1.2%.
 - Culture #2 has a low cell density; the cells appear to be in logarithmic phase, although their doubling time is prolonged (over one hour). The glucose level is reduced to 0.2%.
 What type of glucose catabolism was used in each culture? Why does culture #2 have so little glucose remaining relative to culture #1, even though culture #2 displayed slower growth and has less biomass?

Learn More

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