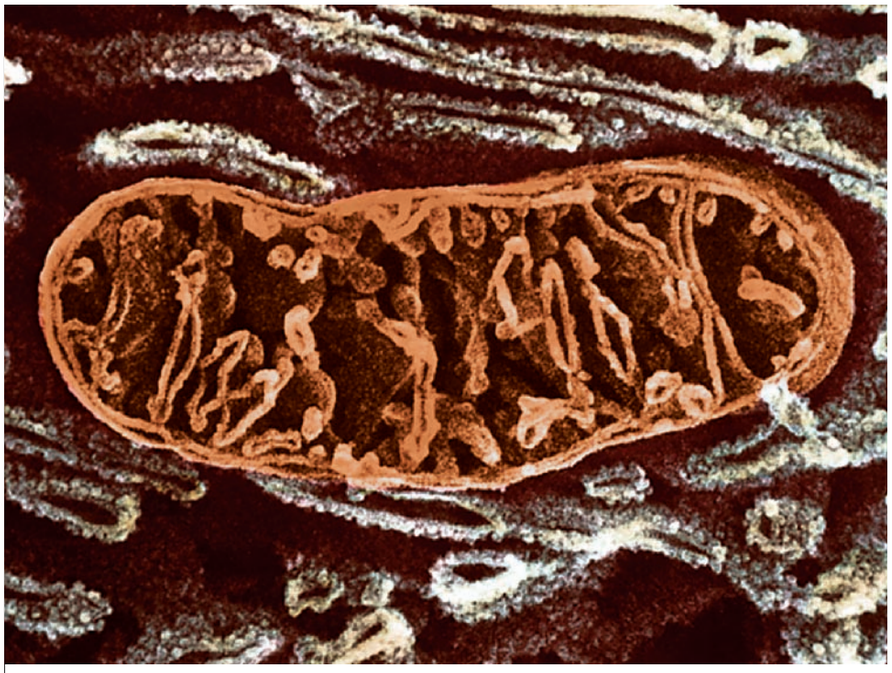


Professors P. Motta and T. Naguro/SPL/Photo Researchers, Inc.

Mitochondrion (colorized SEM). Mitochondria are the sites of cellular respiration.

Study Plan

8.1 Overview of Cellular Energy Metabolism

Coupled oxidation and reduction reactions produce the flow of electrons for energy metabolism

Electrons flow from fuel substances to final electron acceptors

In cellular respiration, cells make ATP by oxidative phosphorylation

8.2 Glycolysis

The reactions of glycolysis include energy-requiring and energy-releasing steps

Glycolysis is regulated at key points

8.3 Pyruvate Oxidation and the Citric Acid Cycle

Pyruvate oxidation produces the two-carbon fuel of the citric acid cycle

The citric acid cycle oxidizes acetyl groups completely to CO_2

Carbohydrates, fats, and proteins can function as electron sources for oxidative pathways

8.4 The Electron Transfer System and Oxidative Phosphorylation

In the electron transfer system, electrons flow through protein complexes in the inner mitochondrial membrane

Ubiquinone and the three major electron transfer complexes pump H^+ across the inner mitochondrial membrane

Chemiosmosis powers ATP synthesis by a proton gradient

Thirty-two ATP molecules are produced for each molecule of glucose completely oxidized to CO_2 and H_2O

Cellular respiration conserves more than 30% of the chemical energy of glucose in ATP

8.5 Fermentation

Fermentation keeps ATP production going when oxygen is unavailable

8 Harvesting Chemical Energy: Cellular Respiration

Why It Matters

In the early 1960s, Swedish physician Rolf Luft mulled over some odd symptoms of a patient. The young woman felt weak and too hot all the time. Even on the coldest winter days, she never stopped perspiring and her skin was always flushed. She was also thin, despite a huge appetite.

Luft inferred that his patient's symptoms pointed to a metabolic disorder. Her cells seemed to be active, but much of their activity was being dissipated as metabolic heat. He decided to order tests to measure her metabolic rates. The patient's oxygen consumption was the highest ever recorded!

Luft also examined a tissue sample from the patient's skeletal muscles. Using a microscope, he found that her muscle cells contained many more mitochondria—the ATP-producing organelles of the cell—than are normal; also, her mitochondria were abnormally shaped. Other studies showed that the mitochondria were engaged in cellular respiration—their prime function—but little ATP was being generated.

The disorder, now called *Luft syndrome,* was the first disorder to be linked directly to a defective cellular organelle. By analogy, someone

with this mitochondrial disorder functions like a city with half of its power plants shut down. Skeletal and heart muscles, the brain, and other hardworking body parts with the highest energy demands are hurt the most. More than 100 mitochondrial disorders are now known.

Defective mitochondria also contribute to many age-related problems, including type 1 diabetes, atherosclerosis, amyotrophic lateral sclerosis (ALS, also called Lou Gehrig disease), as well as Parkinson, Alzheimer, and Huntington diseases.

Clearly, human health depends on mitochondria that are sound structurally and functioning properly. More broadly, every animal, plant, and fungus and most protists depend on mitochondria that are functioning correctly to grow and survive.

In mitochondria, ATP forms as part of the reactions of cellular respiration. The **cellular respiration** pathway breaks down food molecules to produce energy in the form of ATP, releasing water and carbon dioxide in the process. ATP fuels nearly all of the reactions that keep cells, and organisms, metabolically active. Respiration powers metabolism in most eukaryotes and many prokaryotes. This chapter discusses the reactions of cellular respiration.

Photosynthesis, the ultimate source of the chemical energy used by most organisms, is described in Chapter 9. Photosynthesis captures energy from light by splitting water molecules, and hydrogen from the water is combined with carbon dioxide to synthesize carbohydrates. A major by-product of photosynthesis is oxygen, a molecule needed for cellular respiration. Photosynthesis occurs in most plants, many protists, and some prokaryotes.

Respiration and photosynthesis are the major biological steps of the carbon cycle, the global movement of carbon atoms. The physiological connection between respiration and photosynthesis is a consequence of evolution.

8.1 Overview of Cellular Energy Metabolism

Electron-rich food molecules synthesized by plants are used by the plants themselves, and by animals and other eukaryotes. The electrons are removed from fuel substances, such as sugars, and donated to other molecules, such as oxygen, that act as electron acceptors. In the process, some of the energy of the electrons is released and used to drive the synthesis of ATP. ATP provides energy for most of the energy-consuming activities in the cell. Thus, life and its systems are driven by a cycle of electron flow powered by light in photosynthesis and oxidation in cellular respiration.

Coupled Oxidation and Reduction Reactions Produce the Flow of Electrons for Energy Metabolism

The removal of electrons (e^-) from a substance is termed an **oxidation,** and the substance from which the electrons are removed is said to be **oxidized.** The addition of electrons to a substance is termed a **reduction,** and the substance that receives the electrons is said to be **reduced.** A simple mnemonic to remember the direction of electron transfer is OIL RIG—Oxidation Is Loss (of electrons), Reduction Is Gain (of electrons). The term *oxidation* was originally used to describe the reaction that occurs when fuel substances are burned in air, in which oxygen directly accepts electrons removed from the fuels. However, although oxidation suggests that oxygen is involved in electron removal, most cellular oxidations occur without the direct participation of oxygen. The term *reduction* refers to the decrease in positive electrical charge that occurs when electrons, which are negatively charged, are added to a substance. Although reduction suggests that the energy level of molecules is decreased when they accept electrons, molecules typically gain energy from added electrons.

Oxidation and reduction *invariably* are coupled reactions that remove electrons from a donor molecule and simultaneously add them to an acceptor molecule. In such coupled oxidation–reduction reactions, also called **redox reactions,** electrons release some of their energy as they pass from a donor to an acceptor molecule. This free energy is available for cellular work, such as ATP synthesis.

Frequently, protons (hydrogen atoms stripped of electrons, symbolized as H^+) are also removed from a molecule during oxidation. (Recall from Chapter 2 that a hydrogen atom, H, consists of a proton and an electron: $H = H^+ + e^-$.) The molecules that accept electrons may also combine with protons, as oxygen does when it is reduced to form water.

The gain or loss of an electron in a redox reaction is not always complete. That is, depending on the redox reaction, electrons are transferred completely from one atom to another, or alternatively, the degree of electron sharing in covalent bonds changes. The latter condition is said to involve a relative loss or gain of electrons; most redox reactions in the electron transfer system discussed later in the chapter are of this type. The redox reaction between methane and oxygen (the burning of natural gas in air) that produces carbon dioxide and water illustrates a change in the degree of electron sharing. The dots in **Figure 8.1** indicate the positions of the electrons involved in the covalent bonds of the reactants and products.

Compare the reactant methane with the product carbon dioxide. In methane, the covalent electrons are shared essentially equally between bonded C and H atoms because C and H are almost equally electronegative. In carbon dioxide, electrons are closer to the O at-

oms than to the C atom in the C=O bonds because O atoms are highly electronegative. Overall, this means that the C atom has partially "lost" its shared electrons in the reaction. In short, methane has been oxidized. Now compare the oxygen reactant with the product water. In the oxygen molecule, the two O atoms share their electrons equally. The oxygen reacts with the hydrogen from methane, producing water, in which the electrons are closer to the O atom than to the H atoms. This means that each O atom has partially "gained" electrons; in short, oxygen has been reduced.

The movement of electrons away from an atom requires energy. The more electronegative an atom is, the greater the force that holds the electrons to that atom and therefore the greater the energy required to remove an electron. The changes in electron positions in a redox reaction consequently change the amount of chemical energy in the reactants and products. In our example of methane burning in oxygen, electrons are held more tightly in the product molecules (by being closer to the highly electronegative O atoms) than in the reactant molecules. Therefore, in this redox reaction, the potential energy of the reactants has dropped and chemical energy that can be used for cellular work is released.

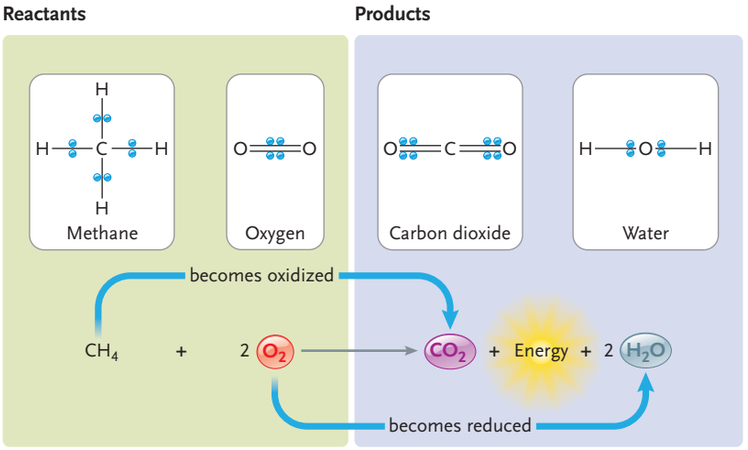


Figure 8.1
Relative loss and gain of electrons in a redox reaction, the burning of methane (natural gas) in oxygen. Compare the positions of the electrons in the covalent bonds of reactants and products. In this redox reaction, methane is oxidized and oxygen is reduced.

Electrons Flow from Fuel Substances to Final Electron Acceptors

The energy of the electrons removed during cellular oxidations originates in the reactions of photosynthesis **(Figure 8.2a).** During photosynthesis, electrons derived from water are pushed to very high energy levels using energy from the absorption of light. The high-energy electrons, together with H^+ from water, are combined with carbon dioxide to form sugar molecules and then are removed by the oxidative reactions that release energy for cellular activities **(Figure 8.2b).** As electrons pass to acceptor molecules, they lose much of their energy; some of this energy drives the synthesis of ATP from ADP and P_i (a phosphate group from an inorganic source) (see Section 4.2).

The total amount of energy obtained from electrons flowing through cellular oxidative pathways depends on the difference between their high energy level in fuel substances and the lower energy level in the molecule that acts as the *final acceptor* for electrons, that is, the last molecule reduced in cellular pathways. The lower the energy level in the final acceptor, the greater the yield of energy for cellular activities. Oxygen is the final acceptor in the most efficient and highly developed form of cellular oxidation: cellular respiration (see Figure 8.2b). The very low energy level of the electrons added to oxygen allows a maximum output of energy for ATP synthesis. As part of the final reduction, oxygen combines with protons and electrons to form water.

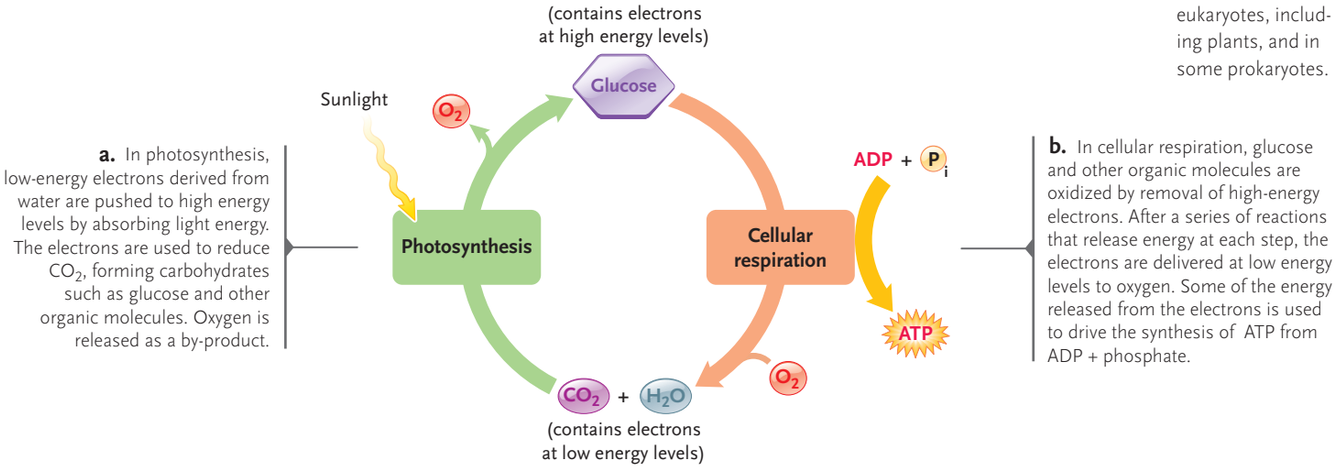


Figure 8.2
Flow of energy from sunlight to ATP. **(a)** Photosynthesis occurs in plants, many protists, and some prokaryotes; **(b)** cellular respiration occurs in all eukaryotes, including plants, and in some prokaryotes.

In Cellular Respiration, Cells Make ATP by Oxidative Phosphorylation

Cellular respiration includes both the reactions that transfer electrons from organic molecules to oxygen and the reactions that make ATP. These reactions are often written in a summary form that uses glucose ($C_6H_{12}O_6$) as the initial reactant:

$$C_6H_{12}O_6 + 6\ O_2 + 32\ ADP + 32\ P_i \rightarrow 6\ H_2O + 6\ CO_2 + 32\ ATP$$

In this overall reaction, electrons and protons are transferred from glucose to oxygen, forming water, and the carbons left after this transfer are released as carbon dioxide. How we derive the 32 ATP molecules is explained later in this chapter.

ATP synthesis is the key part of this reaction. As discussed in Section 4.2, phosphorylation is a reaction that adds a phosphate group to a substance such as ADP. The process by which ATP is synthesized using the energy released by electrons as they are transferred to oxygen is called oxidative phosphorylation.

The entire process of cellular respiration can be divided into three stages **(Figure 8.3):**

1. In **glycolysis**, enzymes break down a molecule of glucose (containing six carbon atoms) into two molecules of pyruvate (an organic compound with a backbone of three C atoms). Some ATP is synthesized during glycolysis.
2. In **pyruvate oxidation**, enzymes convert the three-carbon molecule pyruvate into a two-carbon acetyl group, which enters the **citric acid cycle**, where it is completely oxidized to carbon dioxide. Some ATP is synthesized during the citric acid cycle.
3. In the **electron transfer system**, high-energy electrons produced from glycolysis, pyruvate oxidation, and the citric acid cycle are delivered to oxygen by a sequence of electron carriers. Free energy released by the electron flow generates an H^+ gradient. In **oxidative phosphorylation**, the enzyme **ATP synthase** uses the H^+ gradient built by the electron transfer system as the energy source to make ATP.

In eukaryotes, most of the reactions of cellular respiration occur in various regions of the mitochondrion **(Figure 8.4);** only glycolysis is located in the cytosol. Pyruvate oxidation and the citric acid cycle take place in the mitochondrial matrix. The inner mitochondrial membrane houses the electron transfer system and the ATP synthase enzymes. Transport proteins, concentrated primarily in the inner membrane, control the substances that enter and leave mitochondria.

The locations of the reactions in mitochondria were determined by studies of mitochondria that had been isolated from cells by **cell fractionation**—a technique that divides cells into fractions containing a single type of organelle, such as mitochondria or chloroplasts, or other structures, such as ribosomes **(Figure 8.5).** The

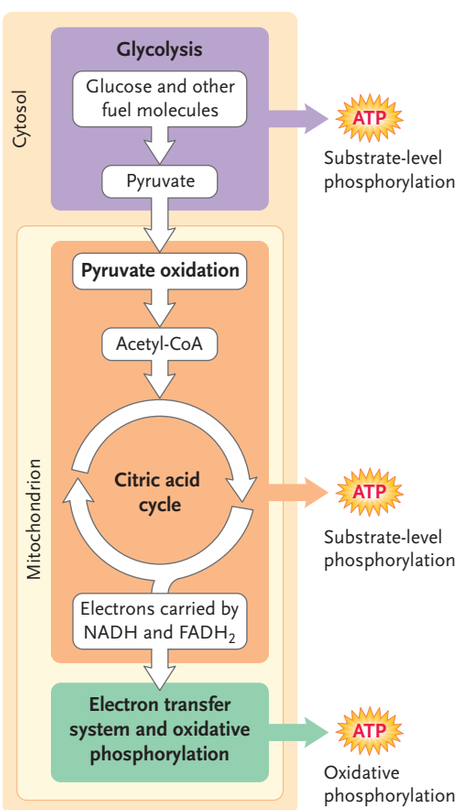


Figure 8.3
The three stages of cellular respiration: (1) glycolysis, (2) pyruvate oxidation and the citric acid cycle, and (3) the electron transfer system and oxidative phosphorylation.

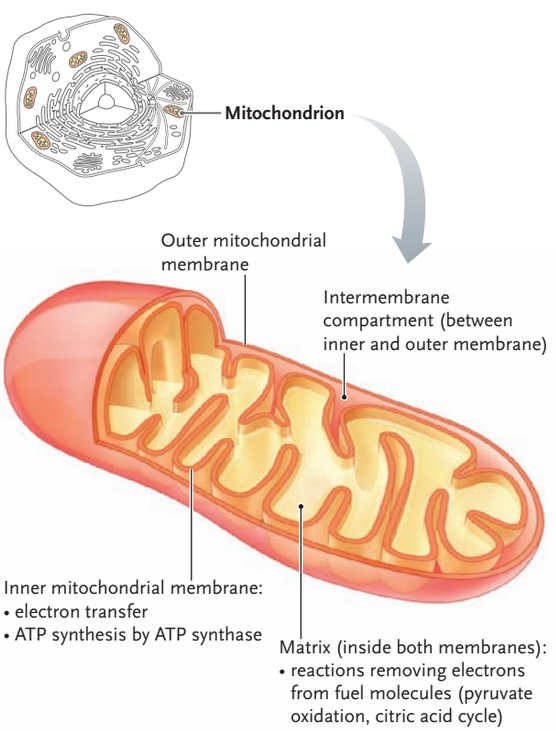


Figure 8.4
Membranes and compartments of mitochondria. Label lines that end in a dot indicate a compartment enclosed by the membranes.

Figure 8.5 Research Method

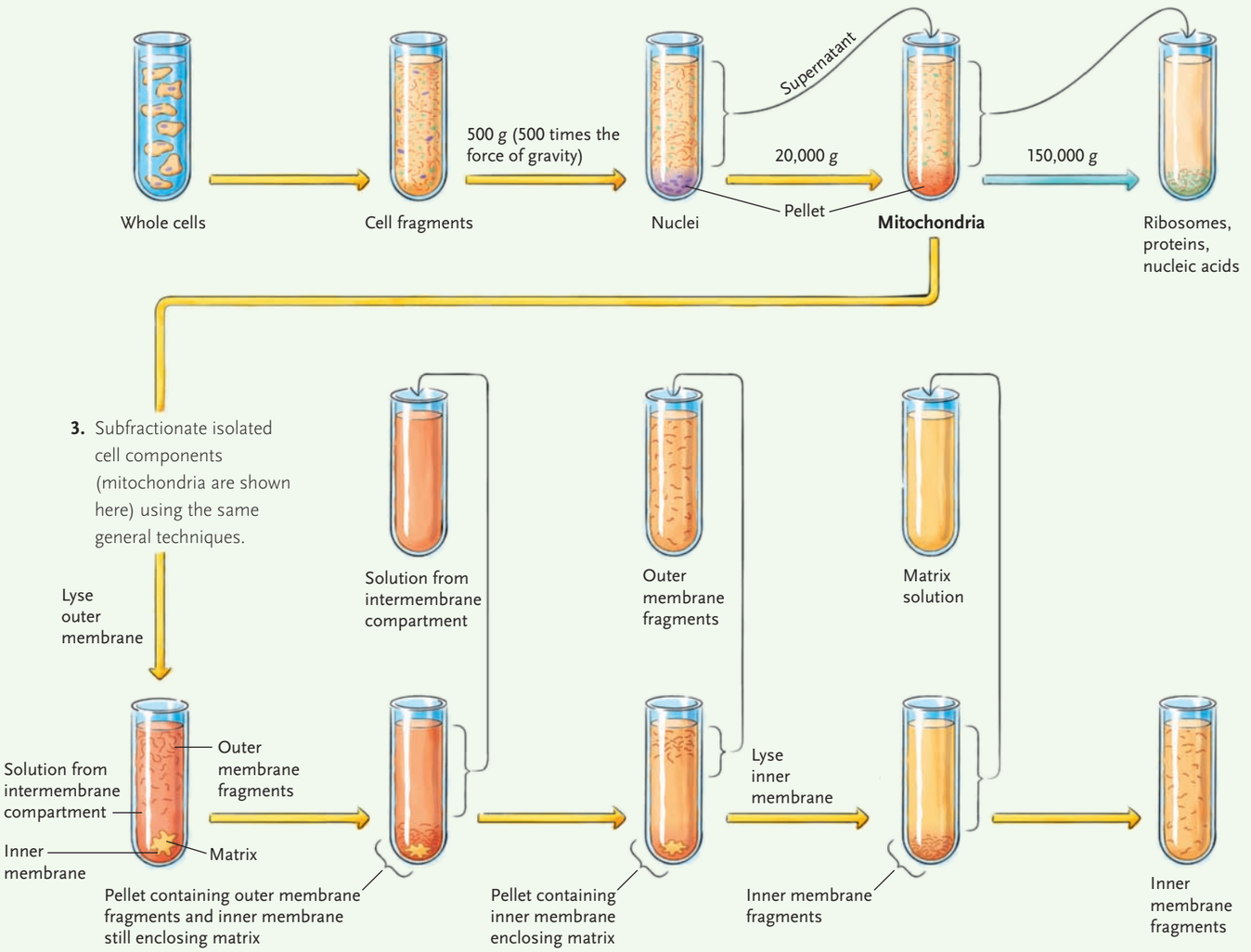
Cell Fractionation

PURPOSE: Cell fractionation breaks cells into fractions containing a single cell component, such as mitochondria or ribosomes. Once isolated, the cell component can be disassembled by the same general techniques to analyze its structure and function. This example shows the isolation and subfractionation of mitochondria.

PROTOCOL:

1. Break open intact cells by sonication (high-frequency sound waves), grinding in fine glass beads, or exposure to detergents that disrupt plasma membranes.

2. Use sequential centrifugations at increasing speeds to separate and purify cell structures. The spinning centrifuge drives cellular structures to bottom of tube at a rate that depends on their shape and density. With each centrifugation, the largest and densest components are isolated and concentrated into a pellet; the remaining solution, the supernatant, is drawn off and can be centrifuged again at higher speed.



4. Centrifuge to concentrate outer membrane fragments and inner membrane enclosing matrix into a pellet.

5. Resuspend pellet and centrifuge it to separate outer membrane fragments and inner membrane enclosing matrix.

6. Sonicate pellet to break inner membrane and release matrix contents. Centrifuge to separate inner membrane fragments and matrix solution.

INTERPRETING THE RESULTS: Many of the cell or organelle subfractions generated by cell fractionation retain their biological activity, making them useful in studies of various cellular processes. For example, mitochondrial subfractions were used to work out the structure and function of the electron transfer system. Cell fractionation is still used to determine the cellular location of a protein or biological reaction, such as whether it is free in the cytosol or associated with a membrane.

collected mitochondria were, in turn, fractionated into different subfractions using experimental treatments. For example, the outer and inner mitochondrial membranes react differently to particular detergents, permitting each membrane, as well as the solutions in the matrix and intermembrane compartment, to be purified individually and then studied in detail. Each subfraction was then analyzed to identify the locations of the individual reactions of cellular respiration.

In prokaryotes, glycolysis, pyruvate oxidation, and the citric acid cycle are all located in the cytosol. The other reactions of cellular respiration occur in the plasma membrane.

The following three sections examine the three stages of cellular respiration in turn.

Study Break

1. Distinguish between oxidation and reduction.
2. Distinguish between cellular respiration and oxidative phosphorylation.

8.2 Glycolysis

Glycolysis, the first series of oxidative reactions that remove electrons from cellular fuel molecules, takes place in the cytosol of all organisms. In glycolysis (*glykys* = sweet; *lysis* = breakdown), sugars such as glucose are partially oxidized and broken down into smaller molecules, and a relatively small amount of ATP is produced. Glycolysis is also known as the Embden–Meyerhof pathway in honor of Gustav Embden and Otto Meyerhof, two German physiological chemists who (separately) made the most important contributions to determining the sequence of reactions in the pathway. Meyerhof received a Nobel Prize in 1922 for his work.

Glycolysis starts with the six-carbon sugar glucose and produces two molecules of the three-carbon organic substance *pyruvate* or *pyruvic acid* in 10 sequential enzyme-catalyzed reactions. (The *-ate* suffix indicates the ionized form of organic acids such as pyruvate, in which the carboxyl group —COOH dissociates to $—COO^- + H^+$, as is usual under cellular conditions.) Pyruvate still contains many electrons that can be removed by oxidation, and it is the primary fuel substance for the second stage of cellular respiration.

The Reactions of Glycolysis Include Energy-Requiring and Energy-Releasing Steps

The initial steps of glycolysis (red in **Figure 8.6**) are energy-requiring reactions—2 ATP are hydrolyzed; they convert glucose into an unstable phosphorylated derivative. In the subsequent energy-releasing part of

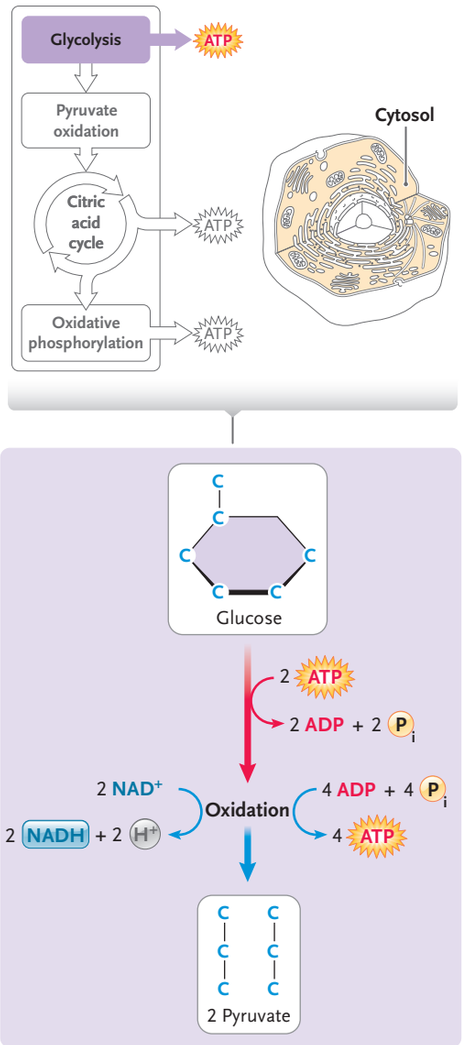


Figure 8.6
Overall reactions of glycolysis. Glycosis splits glucose (six carbons) into pyruvate (three carbons) and yields ATP and NADH.

glycolysis (blue in Figure 8.6), electrons are removed from the phosphorylated derivatives of glucose and 4 ATP are produced, giving a net gain of 2 ATP. Two molecules of pyruvate are generated in the final reaction of the pathway.

The electrons removed from fuel molecules in glycolysis are accepted by the electron carrier molecule *nicotinamide adenine dinucleotide* **(Figure 8.7).** The oxidized form of this electron carrier is NAD^+; the reduced form, NADH, carries a pair of electrons and a proton removed from fuel molecules. Nicotinamide adenine dinucleotide is one of many nucleotide-based carriers that shuttle electrons, protons, or metabolic products between major reaction systems (nucleotides are discussed in Section 3.6).

The reactions of glycolysis are shown in **Figure 8.8.** The major oxidation of glycolysis, which occurs in reac-

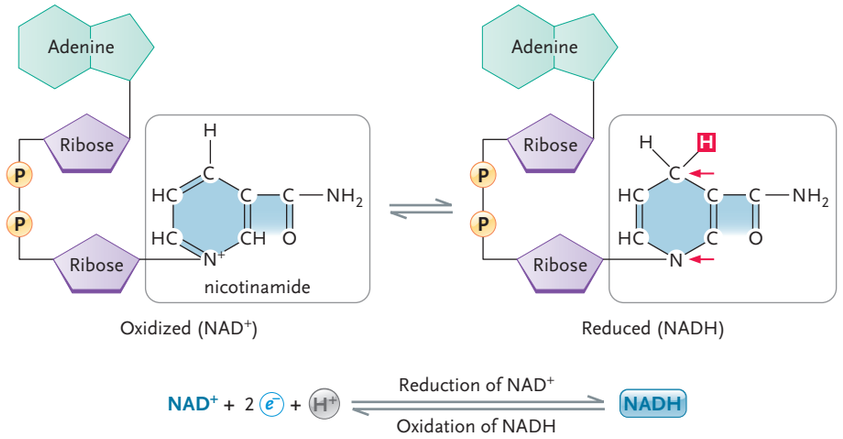


Figure 8.7
Electron carrier NAD^+. As the carrier is reduced to NADH, an electron is added at each of the two positions marked by a red arrow; a proton is also added at the position boxed in red. The nitrogenous base (blue) that adds and releases electrons and protons is nicotinamide, which is derived from the vitamin niacin (nicotinic acid).

tion 6, removes two electrons and two protons from the three-carbon substance *glyceraldehyde-3-phosphate* (G3P). Both electrons and one proton are picked up by NAD^+ to form NADH (see Figure 8.7). The other proton is released into the cytosol.

For each molecule of glucose that enters the pathway (see Figure 8.8), reactions 1 to 5 generate 2 molecules of G3P using 2 ATP, and reactions 6 to 10 convert the 2 molecules of G3P to 2 molecules of pyruvate, producing 4 ATP and 2 NADH. The net reactants and products of glycolysis are:

$$\text{glucose} + 2\ \text{ADP} + 2\ P_i + 2\ NAD^+ \rightarrow$$
$$2\ \text{pyruvate} + 2\ \text{NADH} + 2\ H^+ + 2\ \text{ATP}$$

The total of six carbon atoms in the two molecules of pyruvate is the same as in glucose; no carbons are released as CO_2 by glycolysis.

Each ATP molecule produced in the energy-releasing steps of glycolysis—steps 8 and 10 (see Figure 8.8)—results from **substrate-level phosphorylation**, an enzyme-catalyzed reaction that transfers a phosphate group from a substrate to ADP **(Figure 8.9).**

Glycolysis Is Regulated at Key Points

The rate of sugar oxidation by glycolysis is closely regulated by several mechanisms to match the cell's need for ATP. For example, if excess ATP is present in the cytosol, it binds to *phosphofructokinase*, the enzyme that catalyzes reaction 3 in Figure 8.8, inhibiting its action. This is an example of feedback inhibition (introduced in Section 4.5). The resulting decrease in the concentration of the product of reaction 3, fructose-1,6-bisphosphate, slows or stops the subsequent reactions of glycolysis. Thus, glycolysis does not oxidize fuel substances needlessly when ATP is in adequate supply.

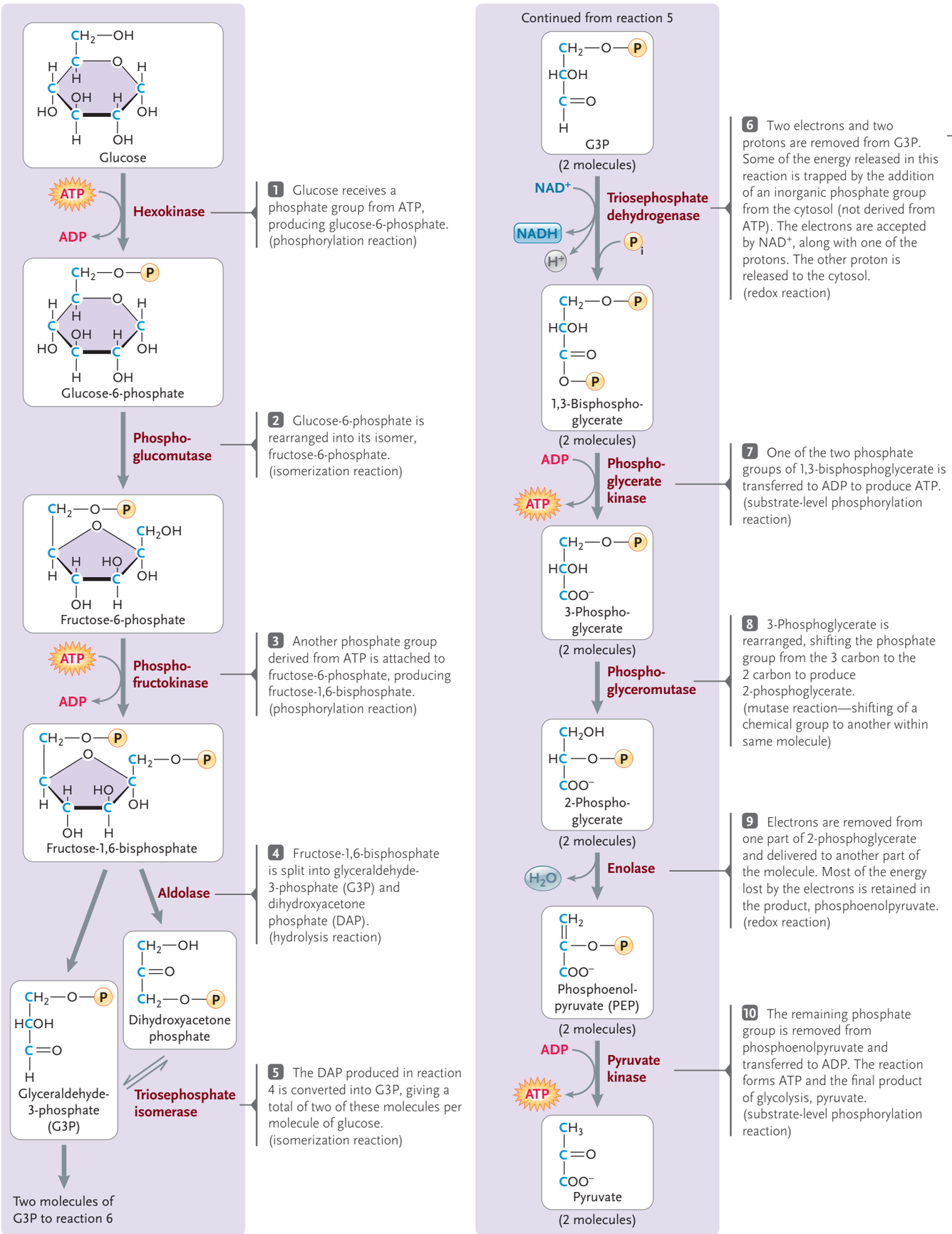
If energy-requiring activities then take place in the cell, ATP concentration decreases and ADP concentration increases in the cytosol. As a result, ATP is released from phosphofructokinase, relieving inhibition of the enzyme. In addition, ADP activates the enzyme. Therefore, the rates of glycolysis and ATP production increase proportionately as cellular activities convert ATP to ADP.

NADH also inhibits phosphofructokinase. This inhibition slows glycolysis if excess NADH is present, such as when oxidative phosphorylation has been slowed by limited oxygen supplies. The systems that regulate phosphofructokinase and other enzymes of glycolysis closely balance the rate of the pathway to produce adequate supplies of ATP and NADH without oxidizing excess quantities of glucose and other sugars.

Our discussion of the oxidative reactions that supply electrons now moves from the cytosol to mitochondria, the locale of pyruvate oxidation and the citric acid cycle. These reactions complete the breakdown of fuel substances into carbon dioxide and provide most of the electrons that drive electron transfer and ATP synthesis.

Study Break

1. What are the energy-requiring and energy-releasing steps of glycolysis?
2. Why is phosphofructokinase a target for inhibition by ATP?


Glucose
ATP
Hexokinase
ADP
1 Glucose receives a phosphate group from ATP, producing glucose-6-phosphate. (phosphorylation reaction)
Glucose-6-phosphate
Phospho-glucomutase
2 Glucose-6-phosphate is rearranged into its isomer, fructose-6-phosphate. (isomerization reaction)
Fructose-6-phosphate
ATP
Phospho-fructokinase
ADP
3 Another phosphate group derived from ATP is attached to fructose-6-phosphate, producing fructose-1,6-bisphosphate. (phosphorylation reaction)
Fructose-1,6-bisphosphate
Aldolase
4 Fructose-1,6-bisphosphate is split into glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DAP). (hydrolysis reaction)
Dihydroxyacetone phosphate
Glyceraldehyde-3-phosphate (G3P)
Triosephosphate isomerase
5 The DAP produced in reaction 4 is converted into G3P, giving a total of two of these molecules per molecule of glucose. (isomerization reaction)
Two molecules of G3P to reaction 6
Continued from reaction 5
G3P
(2 molecules)
NAD+
NADH
H+
Pi
Triosephosphate dehydrogenase
6 Two electrons and two protons are removed from G3P. Some of the energy released in this reaction is trapped by the addition of an inorganic phosphate group from the cytosol (not derived from ATP). The electrons are accepted by NAD+, along with one of the protons. The other proton is released to the cytosol. (redox reaction)
1,3-Bisphospho-glycerate
(2 molecules)
ADP
ATP
Phospho-glycerate kinase
7 One of the two phosphate groups of 1,3-bisphosphoglycerate is transferred to ADP to produce ATP. (substrate-level phosphorylation reaction)
3-Phospho-glycerate
(2 molecules)
Phospho-glyceromutase
8 3-Phosphoglycerate is rearranged, shifting the phosphate group from the 3 carbon to the 2 carbon to produce 2-phosphoglycerate. (mutase reaction—shifting of a chemical group to another within same molecule)
2-Phospho-glycerate
(2 molecules)
H2O
Enolase
9 Electrons are removed from one part of 2-phosphoglycerate and delivered to another part of the molecule. Most of the energy lost by the electrons is retained in the product, phosphoenolpyruvate. (redox reaction)
Phosphoenol-pyruvate (PEP)
(2 molecules)
ADP
ATP
Pyruvate kinase
10 The remaining phosphate group is removed from phosphoenolpyruvate and transferred to ADP. The reaction forms ATP and the final product of glycolysis, pyruvate. (substrate-level phosphorylation reaction)
Pyruvate
(2 molecules)

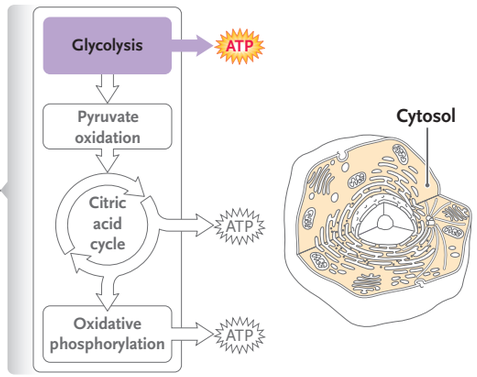


Figure 8.8
Reactions of glycolysis, which occur in the cytosol. Because two molecules of G3P are produced in reaction 5, all the reactions from 6 to 10 are doubled (not shown). The names of the enzymes that catalyze each reaction are in rust.

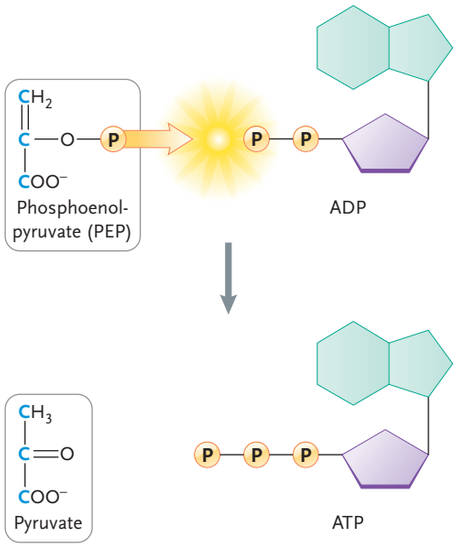


Figure 8.9
Mechanism that synthesizes ATP by substrate-level phosphorylation. A phosphate group is transferred from a high-energy donor directly to ADP, forming ATP.

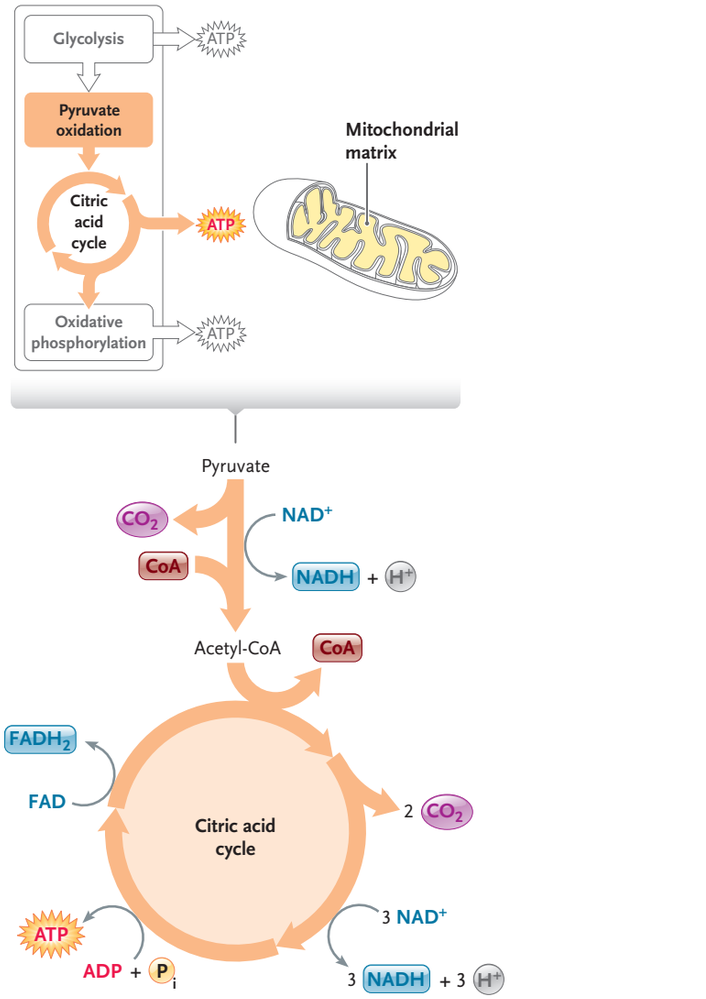


Figure 8.10
Overall reactions of pyruvate oxidation and the citric acid cycle. Each turn of the cycle oxidizes an acetyl group of acetyl-CoA to 2 CO_2. Acetyl-CoA, NAD^+, FAD, and ADP enter the cycle; CoA, NADH, $FADH_2$, ATP, and CO_2 are released as products.

8.3 Pyruvate Oxidation and the Citric Acid Cycle

Glycolysis produces pyruvate molecules in the cytosol, and an active transport mechanism moves them into the mitochondrial matrix, where pyruvate oxidation and the citric acid cycle proceed. An overview of these two processes is presented in **Figure 8.10.** Oxidation of pyruvate generates CO_2, *acetyl-coenzyme A* (acetyl-CoA), and NADH. The acetyl group of acetyl-CoA enters the citric acid cycle. As the citric acid cycle turns, every available electron carried into the cycle from pyruvate oxidation is transferred to NAD^+ or to another nucleotide-based molecule, *flavin adenine dinucleotide* (FAD; the reduced form is $FADH_2$). With each turn of the cycle, substrate-level phosphorylation produces 1 ATP. The combined action of pyruvate oxidation and the citric acid cycle oxidizes the three-carbon products of glycolysis completely to carbon dioxide. The NADH and $FADH_2$ produced during this stage carry high-energy electrons to the electron transfer system in the mitochondrion.

Pyruvate Oxidation Produces the Two-Carbon Fuel of the Citric Acid Cycle

In pyruvate oxidation (also called **pyruvic acid oxidation**), a multienzyme complex removes the —COO^- from pyruvate as CO_2 and then oxidizes the remaining two-carbon fragment of pyruvate to an acetyl group (CH_3CO—) **(Figure 8.11).** Two electrons and two protons are released by these reactions; the electrons and

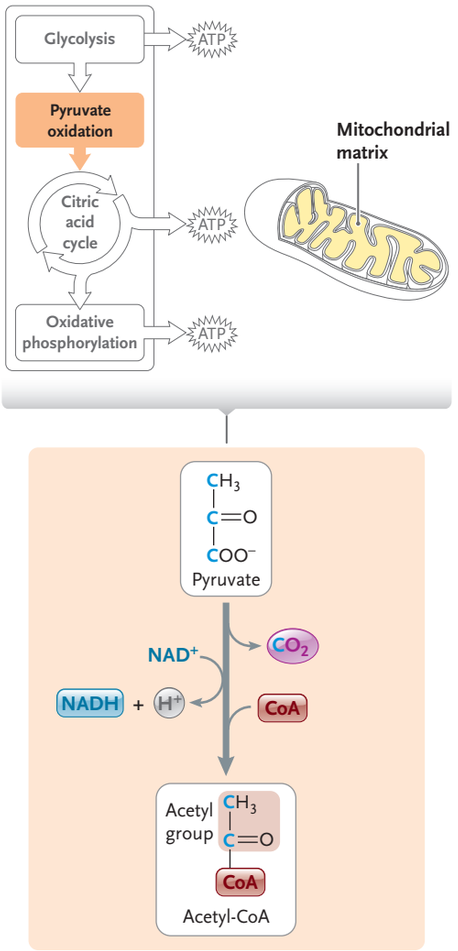


Figure 8.11
Reactions of pyruvate oxidation. Pyruvate (three carbons) is oxidized to an acetyl group (two carbons), which is carried from the cycle by CoA. The third carbon is released as CO_2. NAD^+ accepts two electrons and one proton removed in the oxidation. The acetyl group carried from the reaction by CoA is the fuel for the citric acid cycle.

one proton are accepted by NAD^+, reducing it to NADH, and the other proton is released as free H^+. The acetyl group is transferred to the nucleotide-based carrier *coenzyme A* (CoA). As acetyl-CoA, it carries acetyl groups to the citric acid cycle.

In summary, the pyruvate oxidation reaction is:

$$\text{pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{NADH} + \text{H}^+ + \text{CO}_2$$

Because each glucose molecule that enters glycolysis produces two molecules of pyruvate, all the reactants and products in this equation are doubled when pyruvate oxidation is considered as a continuation of glycolysis.

The Citric Acid Cycle Oxidizes Acetyl Groups Completely to CO_2

The reactions of the citric acid cycle **(Figure 8.12)** oxidize acetyl groups completely to CO_2 and synthesize some ATP molecules. The citric acid cycle gets its name from citrate, the product of the first reaction of the cycle. It is also called the **tricarboxylic acid cycle** or **Krebs cycle**, the latter after Hans Krebs, a German-born scientist who worked out the majority of the reactions in the cycle in research he conducted in England beginning in 1932. Using slices of fresh liver and kidney tissue, he tested various compounds thought to be important in cellular energy metabolism and discovered that a number of organic acids, including citrate, succinate, fumarate, and acetate, are oxidized rapidly. Several other scientists pieced together segments of the reaction series, but Krebs found the key reaction that linked the series into a cycle (see reaction 1 in Figure 8.12). Krebs was awarded a Nobel Prize in 1953 for his elucidation of the citric acid cycle.

The citric acid cycle has eight reactions, each catalyzed by a specific enzyme. All of the enzymes are located in the mitochondrial matrix except the enzyme for reaction 6, which is bound to the inner mitochondrial membrane on the matrix side. In a complete turn of the cycle, one two-carbon acetyl unit is consumed and two molecules of CO_2 are released (at reactions 3 and 4), thereby completing the conversion of all the C atoms originally in glucose to CO_2. The CoA molecule that carried the acetyl group to the cycle is released and participates again in pyruvate oxidation to pick up another acetyl group. Electron pairs are removed at each of four oxidations in the cycle (reactions 3, 4, 6, and 8). Three of the oxidations use NAD^+ as the electron acceptor, producing 3 NADH, and one uses FAD, producing 1 $FADH_2$. Substrate-level phosphorylation generates 1 ATP as part of reaction 5. Therefore, the net reactants and products of one turn of the citric acid cycle are:

$$1\ \text{acetyl-CoA} + 3\ \text{NAD}^+ + 1\ \text{FAD} + 1\ \text{ADP} + 1\ \text{P}_i + 2\ \text{H}_2\text{O} \rightarrow 2\ \text{CO}_2 + 3\ \text{NADH} + 1\ \text{FADH}_2 + 1\ \text{ATP} + 3\ \text{H}^+ + 1\ \text{CoA}$$

Because one molecule of glucose is converted to two molecules of pyruvate by glycolysis and each molecule of pyruvate is converted to one acetyl group, all the reactants and products in this equation are doubled when the citric acid cycle is considered as a continuation of glycolysis and pyruvate oxidation.

Most of the energy released by the four oxidations of the cycle is associated with the high-energy electrons carried by the 3 NADH and 1 $FADH_2$. These high-energy electrons enter the electron transfer system, where their energy is used to make most of the ATP produced in cellular respiration.

Like glycolysis, the citric acid cycle is regulated at several steps to match its rate to the cell's requirements for ATP. For example, the enzyme that catalyzes the first reaction of the citric acid cycle, *citrate synthase,* is inhibited by elevated ATP concentrations. The inhibitions automatically slow or stop the cycle when ATP production exceeds the demands of the cell and, by doing so, conserve cellular fuels.

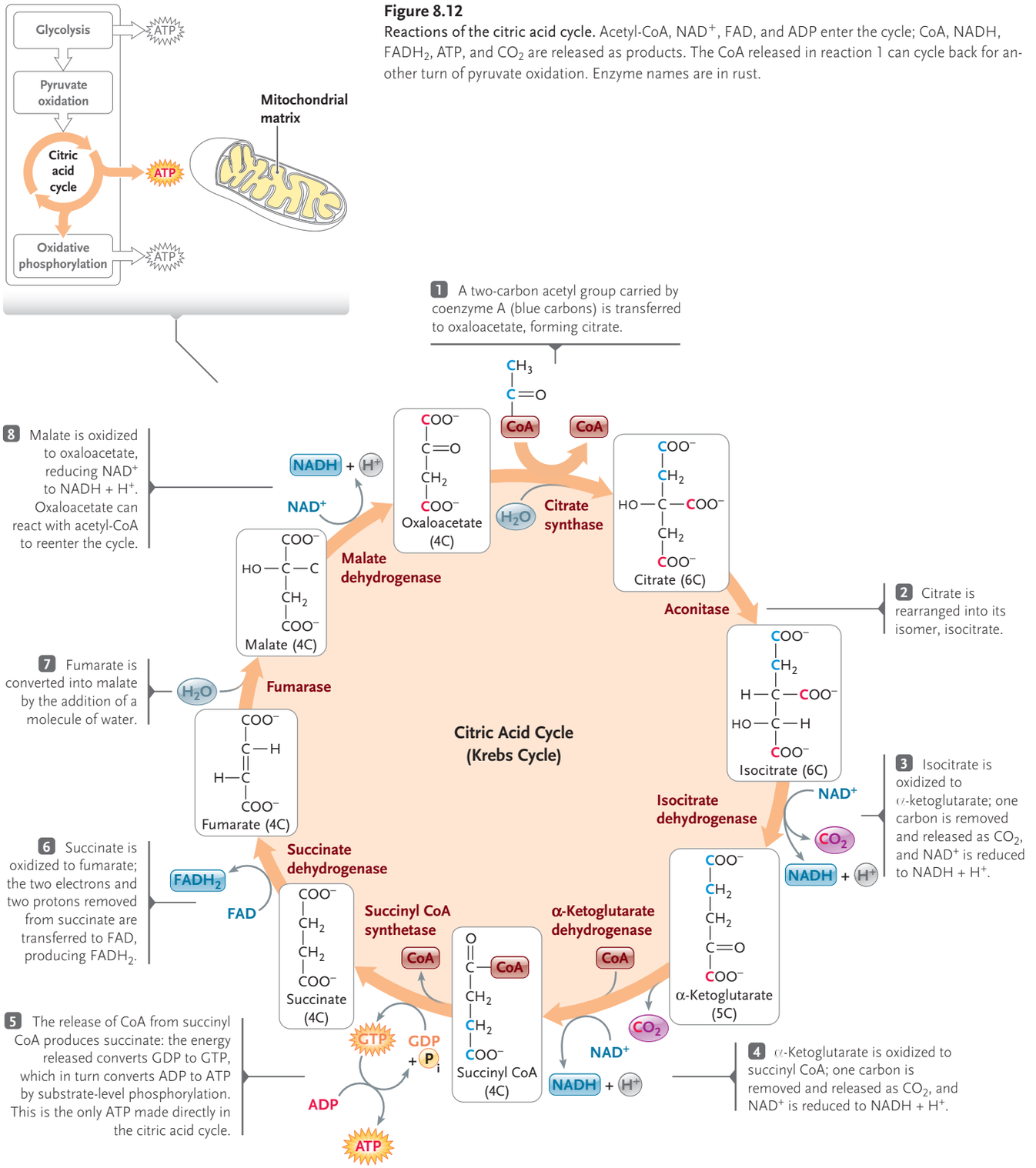


Figure 8.12

Reactions of the citric acid cycle. Acetyl-CoA, NAD^+, FAD, and ADP enter the cycle; CoA, NADH, $FADH_2$, ATP, and CO_2 are released as products. The CoA released in reaction 1 can cycle back for another turn of pyruvate oxidation. Enzyme names are in rust.

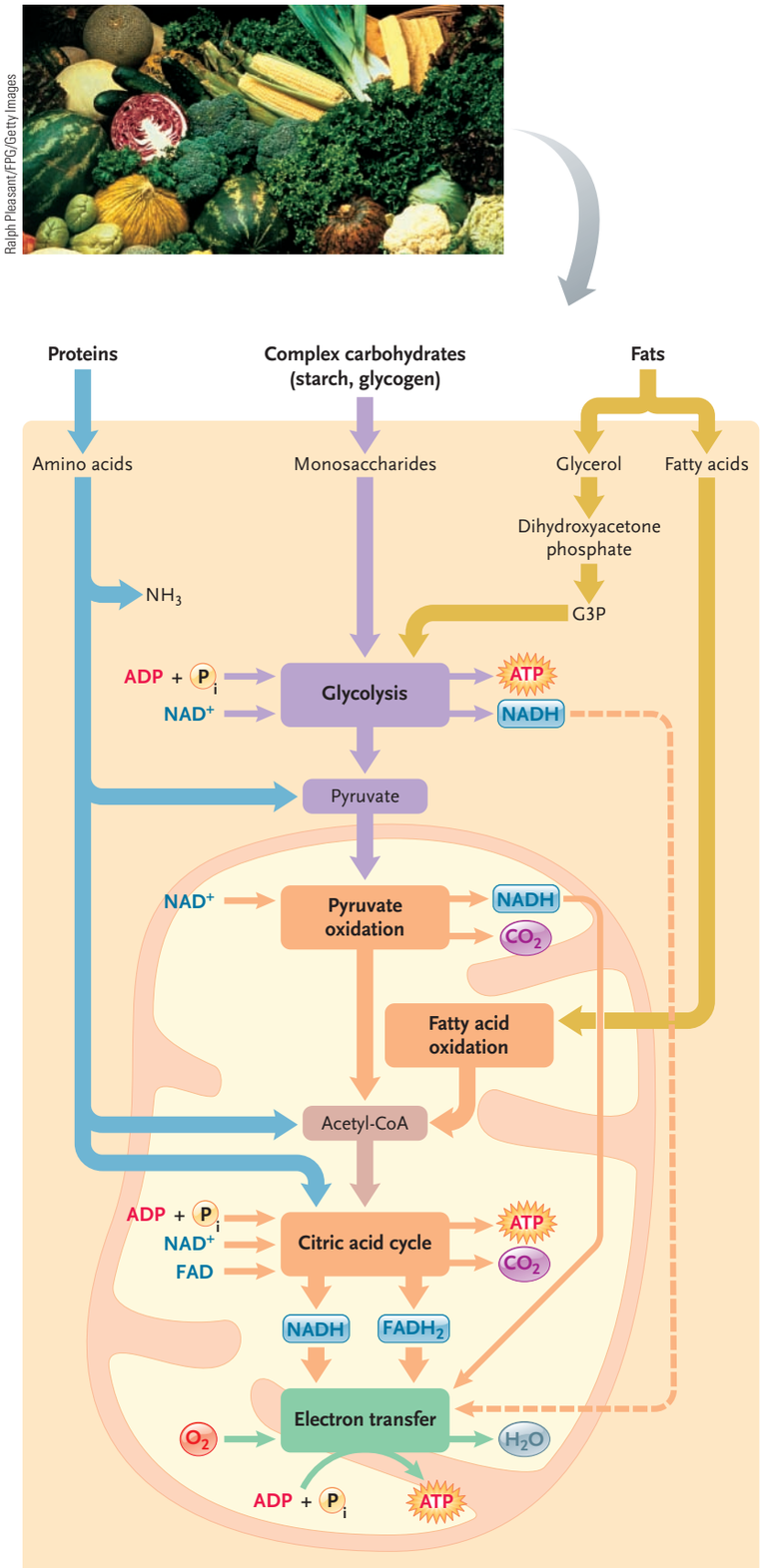


Figure 8.13
Major pathways that oxidize carbohydrates, fats, and proteins. Reactions that occur in the cytosol are shown against a tan background; reactions that occur in mitochondria are shown inside the organelle. CoA funnels the products of many oxidative pathways into the citric acid cycle.

Carbohydrates, Fats, and Proteins Can Function as Electron Sources for Oxidative Pathways

In addition to glucose and other six-carbon sugars, reactions leading from glycolysis through pyruvate oxidation also oxidize a wide range of carbohydrates, lipids, and proteins, which enter the reaction pathways at various points. **Figure 8.13** summarizes the cellular pathways involved; it shows the central role of CoA in funneling acetyl groups from different pathways into the citric acid cycle and of the mitochondrion as the site where most of these groups are oxidized.

Carbohydrates such as sucrose and other disaccharides are easily broken into monosaccharides such as glucose and fructose, which enter glycolysis at early steps. Starch (see Figure 3.7a) is hydrolyzed by digestive enzymes into individual glucose molecules, which enter the first reaction of glycolysis. Glycogen, a more complex carbohydrate that consists of glucose subunits (see Figure 3.7b), is broken down and converted by enzymes into glucose-6-phosphate, which enters glycolysis at reaction 2 of Figure 8.8.

Among the fats, triglycerides (see Figure 3.9) are major sources of electrons for ATP synthesis. Before entering the oxidative reactions, they are hydrolyzed into glycerol and individual fatty acids. The glycerol is converted to G3P and enters glycolysis at reaction 6 of Figure 8.8, in the ATP-producing portion of the pathway. The fatty acids—and many other types of lipids—are split into two-carbon fragments, which enter the citric acid cycle as acetyl-CoA. The energy released by the oxidation of fats, by weight, is comparatively high—about twice the energy yield of carbohydrates. This fact explains why fats are an excellent source of energy in the diet.

Proteins are hydrolyzed to amino acids before oxidation. The amino group (—NH_2) is removed, and the remainder of the molecule enters the pathway of carbohydrate oxidation as either pyruvate, acetyl units carried by CoA, or intermediates of the citric acid cycle. For example, the amino acid alanine is converted into pyruvate; leucine, into acetyl units; and phenylalanine, into fumarate, which enters the citric acid cycle at reaction 7 of Figure 8.12.

Study Break

Summarize the fate of pyruvate molecules produced by glycolysis.

8.4 The Electron Transfer System and Oxidative Phosphorylation

From the standpoint of ATP synthesis, the most significant products of glycolysis, pyruvate oxidation, and the citric acid cycle are the many high-energy electrons

removed from fuel molecules and picked up by the carrier molecules NAD^+ or FAD. These electrons are released by the carriers into the electron transfer system of mitochondria.

The **mitochondrial electron transfer system** consists of a series of electron carriers that alternately pick up and release electrons, ultimately transferring them to their final acceptor, oxygen. As the electrons flow through the system, they release free energy, which is used to build a gradient of H^+ across the inner mitochondrial membrane. The gradient goes from a high H^+ concentration in the intermembrane compartment to a low concentration in the matrix. The H^+ gradient supplies the energy that drives ATP synthesis by mitochondrial ATP synthase.

In the Electron Transfer System, Electrons Flow through Protein Complexes in the Inner Mitochondrial Membrane

The mitochondrial electron transfer system includes three major protein complexes, numbered I, III, and IV, which serve as electron carriers **(Figure 8.14).** These protein complexes are integral membrane proteins located in the inner mitochondrial membrane. In addition, a smaller complex, complex II, is bound to the inner mitochondrial membrane on the matrix side. Associated with the system are two small, highly mobile electron carriers, *cytochrome c* and *ubiquinone* (also known as coenzyme Q, or CoQ), which shuttle electrons between the major complexes. (**Cytochromes** are proteins with a heme prosthetic group that contains an iron atom. The iron atom accepts and donates electrons.)

Electrons flow through the major complexes as shown in Figure 8.14. Complex I picks up high-energy electrons from NADH on its side facing the mitochondrial matrix and conducts them via two electron carriers within the mitochondrial membrane, FMN (flavin mononucleotide) and an Fe/S (iron–sulfur) protein, to ubiquinone molecules. Complex II also contributes high-energy electrons to ubiquinone. Complex II is a succinate dehydrogenase complex that catalyzes two reactions. One is reaction 6 of the citric acid cycle, the conversion of succinate to fumarate (see Figure 8.12). In that reaction, FAD accepts two protons and two electrons and is reduced to $FADH_2$. The other reaction is the transfer to ubiquinone of two electrons obtained from the oxidation of $FADH_2$ to FAD. Two protons are also released in this reaction, and they are released back into the mitochondrial matrix. Electrons that pass to ubiquinone by the complex II reaction bypass complex I of the electron transfer system. Complex III accepts electrons from ubiquinone and transfers them through the electron carriers in the complex—cytochrome *b,* an Fe/S protein, and cytochrome c_1—to cytochrome *c*, which diffuses freely in the intermembrane space. Complex IV accepts electrons from cytochrome *c* and delivers them via electron carriers cytochromes *a* and a_3 to oxygen. Four protons are added to a molecule of O_2 as it accepts four electrons, forming 2 H_2O.

The gas carbon monoxide inhibits complex IV activity, leading to abnormalities in mitochondrial function. In this way, the carbon monoxide in tobacco smoke contributes to the development of diseases associated with smoking.

Ubiquinone and the Three Major Electron Transfer Complexes Pump H^+ across the Inner Mitochondrial Membrane

Ubiquinone and the proteins of complexes I, III, and IV pump (actively transport) H^+ (protons) using energy from electron flow. Complex II, which does not pump H^+, works primarily as an entry point for the electrons removed from succinate.

The electron transfer system pumps protons from the matrix to the intermembrane compartment, resulting in an H^+ gradient with a high concentration in the intermembrane compartment and a low concentration in the matrix. Because protons carry a positive charge, the asymmetric distribution of protons generates an electrical and chemical gradient across the inner mitochondrial membrane, with the intermembrane compartment more positively charged than the matrix. The combination of a proton gradient and voltage gradient across the membrane produces stored energy known as the **proton-motive force.** This force contributes energy for ATP synthesis, as well as for cotransport of substances to and from mitochondria (see Section 6.4).

In our explanation of cellular respiration, electrons have been transferred to oxygen and the H^+ gradient has been generated across the inner mitochondrial membrane. We now focus on the use of this gradient to power the synthesis of ATP.

Chemiosmosis Powers ATP Synthesis by a Proton Gradient

Within the mitochondrion, ATP is synthesized by ATP synthase, an enzyme embedded in the inner mitochondrial membrane. In 1961, British scientist Peter Mitchell of Glynn Research Laboratories proposed that mitochondrial electron transfer produces an H^+ gradient and that the gradient powers ATP synthesis by ATP synthase. He called this pioneering model the **chemiosmotic hypothesis;** the process is commonly called *chemiosmosis* (see Figure 8.14). At the time, this hypothesis was a radical proposal because most researchers thought that the energy of electron transfer was stored as a high-energy chemical intermediate. No such intermediate was ever found, and eventually, Mitchell's hypothesis was supported by the results of many experiments. Mitchell received a Nobel Prize in 1978 for his model and supporting research.

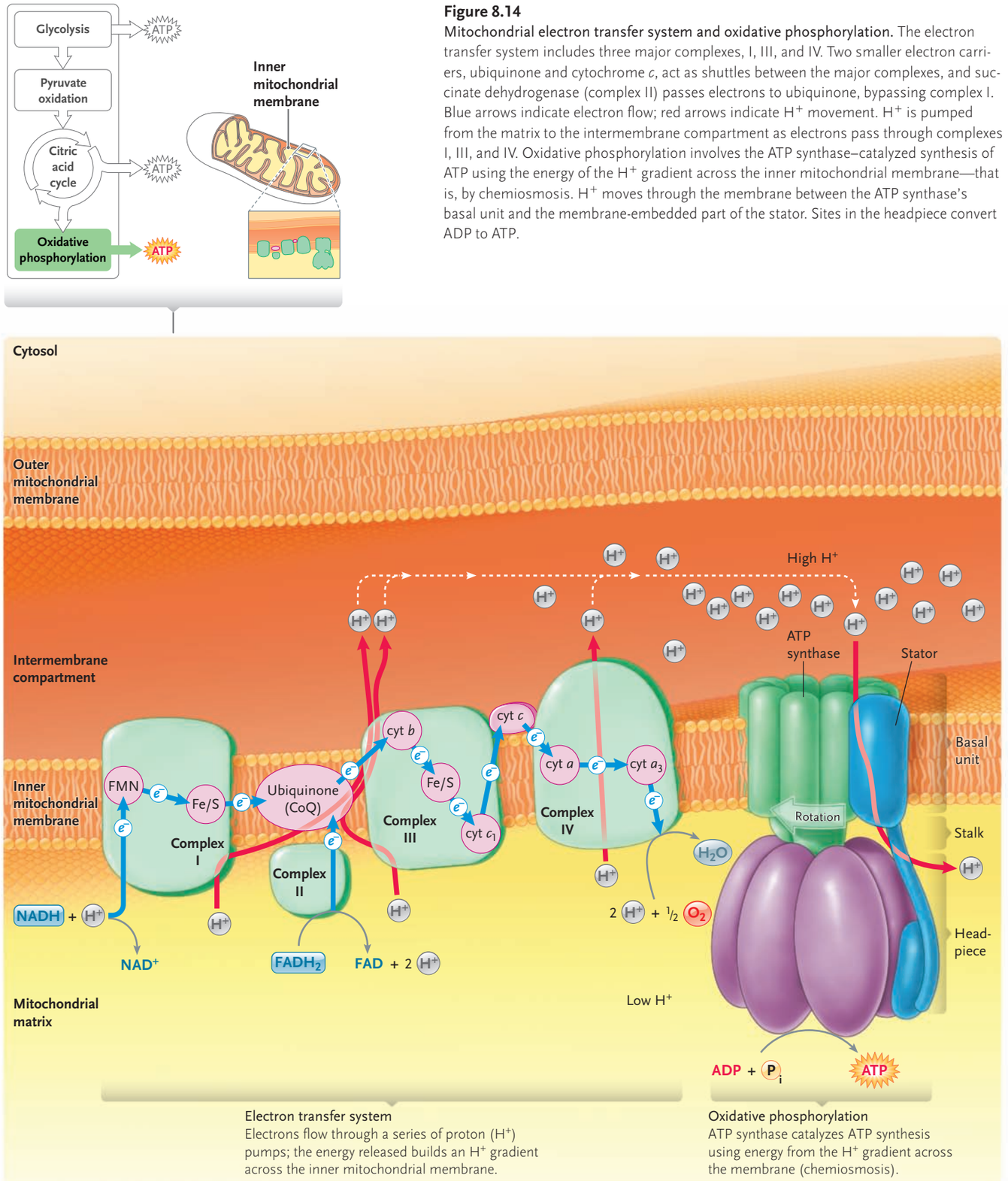


Figure 8.14
Mitochondrial electron transfer system and oxidative phosphorylation. The electron transfer system includes three major complexes, I, III, and IV. Two smaller electron carriers, ubiquinone and cytochrome *c*, act as shuttles between the major complexes, and succinate dehydrogenase (complex II) passes electrons to ubiquinone, bypassing complex I. Blue arrows indicate electron flow; red arrows indicate H^+ movement. H^+ is pumped from the matrix to the intermembrane compartment as electrons pass through complexes I, III, and IV. Oxidative phosphorylation involves the ATP synthase–catalyzed synthesis of ATP using the energy of the H^+ gradient across the inner mitochondrial membrane—that is, by chemiosmosis. H^+ moves through the membrane between the ATP synthase's basal unit and the membrane-embedded part of the stator. Sites in the headpiece convert ADP to ATP.

How does ATP synthase use the H^+ gradient to power ATP synthesis in chemiosmosis? ATP synthase consists of a *basal unit,* which is embedded in the inner mitochondrial membrane, connected to a *headpiece* by a *stalk,* and with a peripheral stalk called a *stator* bridging the basal unit and headpiece (see Figure 8.14). The headpiece extends into the mitochondrial matrix. Protons move between the basal unit and the membrane-embedded part of the stator. ATP synthase functions like an active transport ion pump. In Chapter 6, we described active transport pumps that use the energy created by hydrolysis of ATP to ADP and P_i to transport ions across membranes against their concentration gradients (see Figure 6.11). However, if the concentration of an ion is very high on the side toward which it is normally transported, the pump runs in reverse—that is, the ion is transported backward through the pump, and the pump adds phosphate to ADP to generate ATP. That is how ATP synthase operates in mitochondrial membranes. Proton-motive force moves protons in the intermembrane space through the channel in the enzyme's basal unit down their concentration gradient into the matrix. The flow of protons powers ATP synthesis by the headpiece; this phosphorylation reaction is oxidative phosphorylation. ATP synthase occurs in similar form and works in the same way in mitochondria, chloroplasts, and prokaryotes capable of oxidative phosphorylation.

Many details of the chemiosmotic mechanism are still being investigated. Paul D. Boyer of UCLA, one of the major contributors to this research, proposed the novel idea that passage of protons through the channel of the basal unit makes the stalk and headpiece spin like a top, just as the flow of water makes a waterwheel turn. The turning motion cycles each of three catalytic sites on the headpiece through sequential conformational changes that pick up ADP and phosphate, combine them, and release the ATP product. Another researcher, John Walker of the Laboratory of Molecular Biology (Cambridge, United Kingdom) used X-ray diffraction to create a three-dimensional picture of ATP synthase that clearly verified Boyer's model by showing the head in different rotational positions as ATP synthesis proceeds. Boyer and Walker jointly received a Nobel Prize in 1997 for their research into the mechanisms by which ATP synthase makes ATP.

Thirty-Two ATP Molecules Are Produced for Each Molecule of Glucose Completely Oxidized to CO_2 and H_2O

How many ATP molecules are produced as electrons flow through the mitochondrial electron transfer system? The most recent research indicates that approximately 2.5 ATP are synthesized as a pair of electrons released by NADH travels through the entire electron transfer pathway to oxygen. The shorter pathway, followed by an electron pair released from $FADH_2$ by complex II to oxygen, synthesizes about 1.5 ATP. (Some accounts of ATP production round these numbers to 3 and 2 molecules of ATP, respectively.)

These numbers allow us to estimate the total amount of ATP that would be produced by the complete oxidation of glucose to CO_2 and H_2O if the entire H^+ gradient produced by electron transfer is used for ATP synthesis **(Figure 8.15).** During glycolysis, substrate-level phosphorylation produces 2 ATP. Glycolysis also produces 2 NADH, which leads to 5 ATP (see earlier discussion). In pyruvate oxidation, 2 NADH are produced from the two molecules of pyruvate, again leading to 5 ATP. In summary, glycolysis and pyruvate oxidation together yield 2 ATP, 4 NADH, and 2 CO_2 and, in the end, are responsible for 12 of the ATP produced by oxidation of glucose.

The subsequent citric acid cycle turns twice for each molecule of glucose that enters glycolysis, yielding a total of 2 ATP produced by substrate-level phosphorylation, as well as 6 NADH, 2 $FADH_2$, and 4 CO_2. The 6 NADH lead to 15 ATP, and the 2 $FADH_2$ lead to 3 ATP, for a total of 20 ATP from the citric acid cycle. With the ATP from glycolysis and pyruvate oxidation,

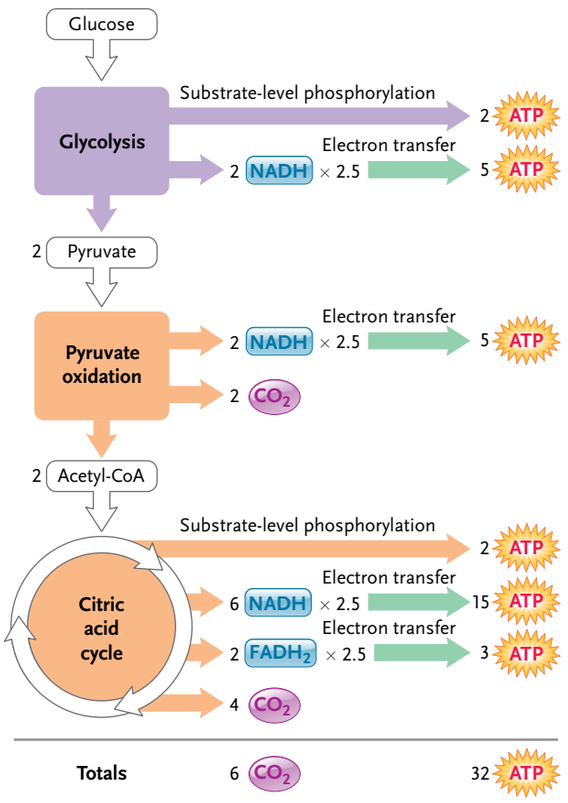


Figure 8.15
Summary of ATP production from the complete oxidation of a molecule of glucose. The total of 32 ATP assumes that electrons carried from glycolysis by NADH are transferred to NAD^+ inside mitochondria. If the electrons from glycolysis are instead transferred to FAD inside mitochondria, total production will be 30 ATP.

the total yield is 32 ATP from each molecule of glucose oxidized to carbon dioxide and water.

The combination of glycolysis, pyruvate oxidation, and the citric acid cycle has the following summary reaction:

$$\text{glucose} + 4\ \text{ADP} + 4\ P_i + 10\ NAD^+ + 2\ FAD \rightarrow 4\ \text{ATP} + 10\ \text{NADH} + 10\ H^+ + 2\ FADH_2 + 6\ CO_2$$

The total of 32 ATP assumes that the two pairs of electrons carried by the 2 NADH reduced in glycolysis each drive the synthesis of 2.5 ATP when traversing the mitochondrial electron transfer system. However, because NADH cannot penetrate the mitochondrial membranes, its electrons are transferred inside by one of two shuttle systems. The more efficient shuttle mechanism transfers the electrons to NAD^+ as the acceptor inside mitochondria. These electron pairs, when passed through the electron transfer system, result in the synthesis of 2.5 ATP each, producing the grand total of 32 ATP. The less efficient shuttle transfers the electrons to FAD as the acceptor inside mitochondria. These electron pairs, when passed through the electron transfer system, result in the synthesis of only 1.5 ATP each and produce a grand total of 30 ATP instead of 32.

Which shuttle predominates depends on the particular species and cell types involved. For example, heart, liver, and kidney cells in mammals use the more efficient shuttle; skeletal muscle and brain cells use the less efficient shuttle. Regardless, the numbers are ideal, because mitochondria also use the H^+ gradient to drive cotransport; any of the energy in the gradient used for this activity would reduce ATP production proportionately.

Cellular Respiration Conserves More Than 30% of the Chemical Energy of Glucose in ATP

Cellular respiration is not 100% efficient in converting the chemical energy of glucose to ATP. Using the estimate of 32 ATP produced for each molecule of glucose oxidized under ideal conditions, we can estimate the overall efficiency of cellular glucose oxidation—that is, the percentage of the chemical energy of glucose conserved as ATP energy.

Under standard conditions, including neutral pH (pH = 7) and a temperature of 25°C, the hydrolysis of ATP to ADP yields about 7.0 kilocalories per mole (kcal/mol). Assuming that complete glucose oxidation produces 32 ATP, the total energy conserved in ATP production would be about 224 kcal/mol. By contrast, if glucose is simply burned in air, it releases 686 kcal/mol. On this basis, the efficiency of cellular glucose oxidation would be about 32% (224/686 × 100 = about 32%). This value is considerably better than that of most devices designed by human engineers—for example, an automobile extracts only about 25% of the energy in the fuel it burns.

The chemical energy released by cellular oxidations that is not captured in ATP synthesis is released as heat. In mammals and birds, this source of heat maintains body temperature at a constant level. In certain mammalian tissues, including *brown fat* (see Chapter 46), the inner mitochondrial membranes contain *uncoupling proteins* (UCPs) that make the inner mitochondrial membrane "leaky" to H^+. As a result, electron transfer runs without building an H^+ gradient or synthesizing ATP and releases all the energy extracted from the electrons as heat. Brown fat with UCPs occurs in significant quantities in hibernating mammals and in very young offspring, including human infants. (*Insights from the Molecular Revolution* describes research showing that some plants also use UCPs in mitochondrial membranes to heat tissues.)

Study Break

1. What distinguishes the four complexes of the mitochondrial electron transfer system?
2. Explain how the proton pumps of complexes I, III, and IV relate to ATP synthesis.

8.5 Fermentation

Fermentation Keeps ATP Production Going When Oxygen Is Unavailable

When oxygen is plentiful, electrons carried by the 2 NADH produced by glycolysis are passed to the electron transfer system inside mitochondria, and the released energy drives the synthesis of ATP. If, instead, oxygen is absent or in short supply, the electrons may be used in fermentation. In **fermentation**, electrons carried by NADH are transferred to an organic acceptor molecule rather than to the electron transfer system. This transfer converts the NADH to NAD^+, which is required to accept electrons in reaction 6 of glycolysis (see Figure 8.8). As a result, glycolysis continues to supply ATP by substrate-level phosphorylation.

Two types of fermentation reactions exist: lactate fermentation and alcoholic fermentation **(Figure 8.16). Lactate fermentation** converts pyruvate into lactate (Figure 8.16a). This reaction occurs in the cytosol of muscle cells in animals whenever vigorous or strenuous activity calls for more oxygen than breathing and circulation can supply. For example, significant quantities of lactate accumulate in the leg muscles of a sprinter during a 100-meter race. The lactate temporarily stores electrons, and when the oxygen content of the muscle cells returns to normal levels, the reverse of the reaction in Figure 8.16a regenerates pyruvate and NADH. The pyruvate can be used in the second stage

Insights from the Molecular Revolution

Keeping the Potatoes Hot

Mammals use several biochemical and molecular processes to maintain body heat. One process is shivering; the muscular activity of shivering releases heat that helps keep body temperature at normal levels. Another mechanism operates through uncoupling proteins (UCPs), which eliminate the mitochondrial H^+ gradient by making the inner mitochondrial membrane leaky to protons. Electron transfer and the oxidative reactions then run at high rates in mitochondria without trapping energy in ATP. The energy is released as heat that helps maintain body temperature.

Until recently, production of body heat by UCPs was thought to be confined to animals. But research by Maryse Laloi and her colleagues at the Max Planck Institute for Molecular Plant Physiology in Germany shows that some tissues in plants may use the same process to generate heat. The research team used molecular techniques to show that potato plants *(Solanum tuberosum)* have a gene with a DNA sequence similar to that of a mammalian UCP gene. The potato gene encodes a protein of the same size as the two known UCPs of mammalian mitochondria. Enough sequence similarities exist to indicate that the potato and mammalian proteins are related and have the same overall three-dimensional structure.

The investigators then used the DNA of the potato UCP gene to probe for the presence of messenger RNA (mRNA), the molecules that serve as instructions for making proteins in the cytoplasm. This test determined whether the UCP genes were actually active in the potatoes. Potato plants grown at 20°C showed a low level of UCP mRNA in leaves and tubers, a moderate level in stems and fruits, and a very high level in roots and flowers. These results indicate that the gene encoding the plant UCP is active at different levels in various plant tissues, suggesting that certain tissues naturally need warming for optimal function.

Laloi and her coworkers then used the same method to test whether exposing potato plants to cold temperatures could induce greater synthesis of the UCP mRNA. After potato plants were kept for 1 to 3 days at 4°C, the UCP mRNA in leaves rose to a level comparable with the high level found in the flowers of plants kept at 20°C.

The research indicates that although potato plants cannot shiver to keep warm, they probably use the mitochondrial uncoupling process to warm tissues when they are stressed by low temperatures. Thus, mechanisms for warming body tissues, once thought to be the province only of animals, appear to be much more widespread. In particular, UCPs, which were believed to have evolved in relatively recent evolutionary times with the appearance of birds and mammals, may be a much more ancient development.

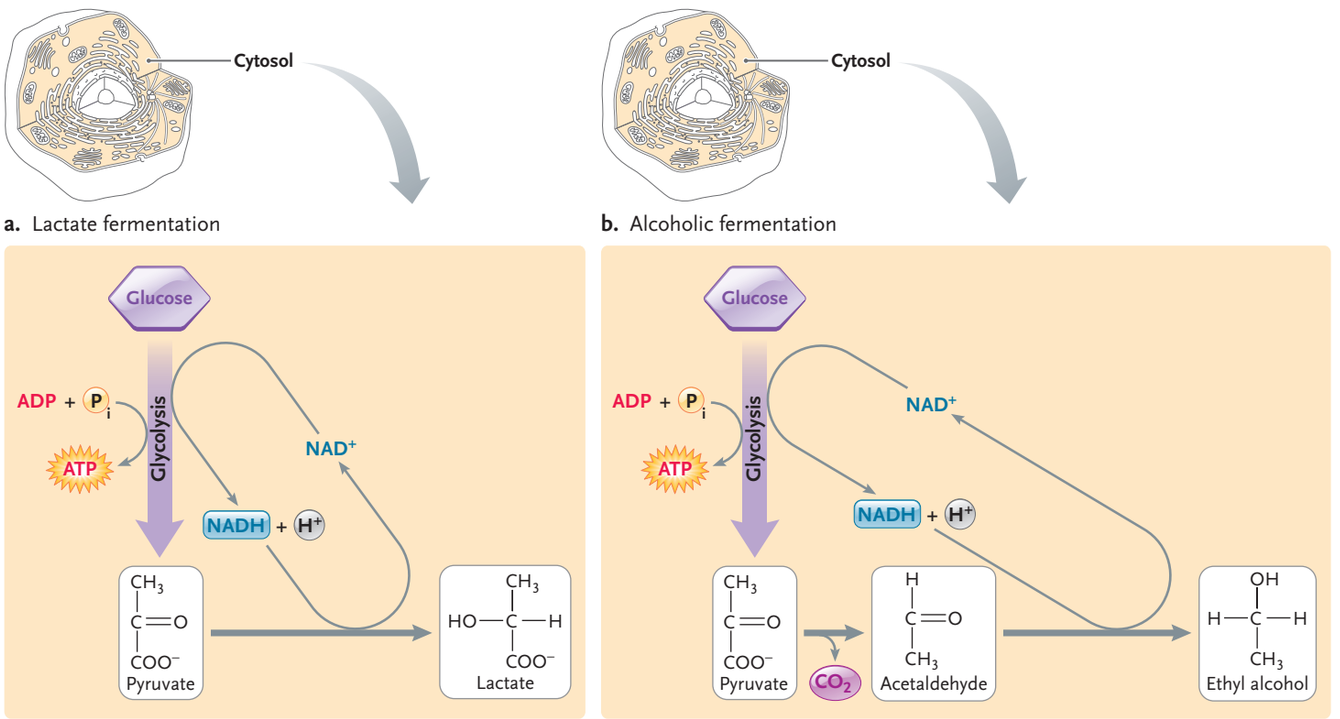


Figure 8.16
Fermentation reactions that produce (a) lactate and (b) ethyl alcohol. The fermentations, which occur in the cytosol, convert NADH to NAD^+, allowing the electron carrier to cycle back to glycolysis. This process keeps glycolysis running, with continued production of ATP.

David M. Phillips/Visuals Unlimited

Figure 8.17
Alcoholic fermentation in nature: wild yeast cells, visible as a dust-like coating on grapes.

of cellular respiration, and the NADH contributes its electron pair to the electron transfer system. Some bacteria also produce lactate as their fermentation product; the sour taste of buttermilk, yogurt, and dill pickles is a sign of their activity.

Alcoholic fermentation (Figure 8.16b) occurs in microorganisms such as yeasts, which are single-celled fungi. In this reaction, pyruvate is converted into ethyl alcohol (which has two carbons) and CO_2 in a two-step series that also converts NADH into NAD^+. Alcoholic fermentation by yeasts has widespread commercial applications. Bakers use the yeast *Saccharomyces cerevisiae* to make bread dough rise. They mix the yeast with a small amount of sugar and blend the mixture into the dough where oxygen levels are low. As the yeast cells convert the sugar into ethyl alcohol and carbon dioxide, the gaseous CO_2 expands and creates bubbles that cause the dough to rise. Oven heat evaporates the alcohol and causes further expansion of the bubbles, producing a light-textured product. Alcoholic fermentation is also the mainstay of beer and wine brewing. Fruits are a natural home to wild yeasts **(Figure 8.17);** for example, winemakers rely on a mixture of wild and cultivated yeasts to produce wine. Alcoholic fermentation also occurs naturally in the environment; for example, overripe or rotting fruit frequently will start to ferment, and birds that eat the fruit may become too drunk to fly.

Fermentation is a lifestyle for some organisms. In bacteria and fungi that lack the enzymes and factors to carry out oxidative phosphorylation, fermentation is the only source of ATP. These organisms are called **strict anaerobes** (*an* = without; *aero* = air; *bios* = life). In general, these organisms require an oxygen-free environment; they cannot utilize oxygen as a final electron acceptor. Among these organisms are the bacteria that cause botulism, tetanus, and some other serious diseases. For example, the bacterium that causes botulism thrives in the oxygen-free environment of canned foods that prevents the growth of most other microorganisms.

Other organisms, called **facultative anaerobes**, can switch between fermentation and full oxidative pathways, depending on the oxygen supply. Facultative anaerobes include *Escherichia coli,* the bacterium that inhabits the digestive tract of humans; the *Lactobacillus* bacteria used to produce buttermilk and yogurt; and *S. cerevisiae,* the yeast used in brewing

Unanswered Questions

Glycolysis and energy metabolism are crucial for the normal functioning of an animal. Research of many kinds is being conducted in this area, such as characterizing the molecular components in detail and determining how the reactions are regulated. The goal is to generate comprehensive models of cellular respiration and its regulation. Following are two specific examples of ongoing research related to human disease caused by defects in cellular respiration.

How do mitochondrial proteins change in patients with Alzheimer disease?

Alzheimer disease (AD) is an age-dependent, irreversible, neurodegenerative disorder in humans. Symptoms include a progressive deterioration of cognitive functions and, in particular, a significant loss of memory. Reduced brain metabolism occurs early in the onset of AD. One of the mechanisms for this physiological change appears to be damage to or reduction of key mitochondrial components, including enzymes of the citric acid cycle and the oxidative phosphorylation system. However, the complete scope of mitochondrial protein changes has not been established, nor have detailed comparisons been made in mitochondrial protein changes among AD patients. Currently, Gail Breen at the University of Texas, Dallas, is performing research to detail qualitatively and quantitatively all mitochondrial proteins and their levels in healthy and AD brains. A mouse model of AD is being used for this research. Breen's group hopes that the information they obtain will provide a better understanding of how mitochondrial dysfunction contributes to AD. With such information in hand, it may be possible to develop interventions that slow or halt the progression of AD in humans.

How are the oxidative phosphorylation complexes in the mitochondrion assembled?

Defects in oxidative phosphorylation may cause disorders in which several systems of the human body are adversely affected. Often, these disorders involve the nervous system and the skeletal and cardiac muscles. The enzyme complexes of the oxidative phosphorylation system consist of about 80 different protein subunits, some of which are encoded by nuclear genes and some by mitochondrial genes. The protein subunits are assembled into complexes in the mitochondria. This assembly process requires a large number of accessory proteins, and many important mitochondrial diseases are caused by defects in the assembly protein genes.

Eric Shoubridge of McGill University in Canada is studying the molecular genetics of assembly of oxidative phosphorylation complexes. His focus is identifying and characterizing the assembly genes with long-term goals of understanding how the complexes are assembled and how defects in complex assembly lead to disease. Shoubridge's group has identified mutations in four different assembly genes in infants with a fatal disease caused by cytochrome *c* deficiency (a defect in the assembly of complex IV). They have also identified complex I assembly proteins, and they were the first to show an association between a defect in one of the proteins and a human disease. Unexpectedly, the biochemical deficiencies caused by the mutant assembly proteins tend to be tissue-specific, even though the assembly protein genes are expressed in all tissues. As a result, clinical symptoms caused by defective assembly proteins vary based on the extent of the enzyme deficiencies in different tissues. Understanding how the tissue-specific differences occur and how they are regulated will be important in developing therapies for patients with the diseases.

Peter J. Russell

and baking. Many cell types in higher organisms, including vertebrate muscle cells, are also facultative anaerobes.

Some prokaryotic and eukaryotic cells are **strict aerobes**—that is, they have an absolute requirement for oxygen to survive and are unable to live solely by fermentations. Vertebrate brain cells are key examples of strict aerobes.

This chapter traced the flow of high-energy electrons from fuel molecules to ATP. As part of the process, the fuels are broken into molecules of carbon dioxide. The next chapter shows how photosynthetic organisms use these inorganic raw materials to produce organic molecules through a process that pushes the electrons back to high energy levels by absorbing the energy of sunlight.

Study Break

What is fermentation, and when does it occur? What are the two types of fermentation?

Review

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8.1 Overview of Cellular Energy Metabolism

- Oxidation–reduction reactions, called redox reactions, partially or completely transfer electrons from donor to acceptor atoms; the donor is oxidized as it releases electrons, and the acceptor is reduced (Figure 8.1).
- Plants and almost all other organisms obtain energy for cellular activities through cellular respiration, the process of transferring electrons from donor organic molecules to a final acceptor molecule such as oxygen; the energy that is released drives ATP synthesis (Figure 8.2).
- Cellular respiration occurs in three stages: (1) In glycolysis, glucose is converted to two molecules of pyruvate through a series of enzyme-catalyzed reactions; (2) in pyruvate oxidation and the citric acid cycle, pyruvate is converted to an acetyl compound that is oxidized completely to carbon dioxide; and (3) in the electron transfer system and oxidative phosphorylation, high-energy electrons produced from the first two stages pass through the transfer system, with much of their energy being used to establish an H^+ gradient across the membrane that drives the synthesis of ATP from ADP and P_i (Figure 8.3).
- In eukaryotes, most of the reactions of cellular respiration occur in mitochondria (Figure 8.4).

Animation: The functional zones in mitochondria

8.2 Glycolysis

- In glycolysis, which occurs in the cytosol, glucose (six carbons) is oxidized into two molecules of pyruvate (three carbons each). Electrons removed in the oxidations are delivered to NAD^+, producing NADH. The reaction sequence produces a net gain of 2 ATP, 2 NADH, and 2 pyruvate molecules for each molecule of glucose oxidized (Figures 8.6 and 8.8).
- ATP molecules produced in the energy-releasing steps of glycolysis result from substrate-level phosphorylation, an enzyme-catalyzed reaction that transfers a phosphate group from a substrate to ADP (Figure 8.9).

Animation: The overall reactions of glycolysis

Practice: Recreating the reactions of glycolysis

8.3 Pyruvate Oxidation and the Citric Acid Cycle

- In pyruvate oxidation, which occurs inside mitochondria, 1 pyruvate (three carbons) is oxidized to 1 acetyl group (two carbons) and 1 CO_2. Electrons removed in the oxidation are accepted by 1 NAD^+ to produce 1 NADH. The acetyl group is transferred to coenzyme A, which carries it to the citric acid cycle (Figure 8.11).
- In the citric acid cycle, acetyl groups are oxidized completely to CO_2. Electrons removed in the oxidations are accepted by NAD^+ or FAD, and substrate-level phosphorylation produces ATP. For each acetyl group oxidized by the cycle, 2 CO_2, 1 ATP, 3 NADH, and 1 $FADH_2$ are produced (Figure 8.12).

Animation: Pyruvate oxidation and the citric acid cycle

Animation: Major pathways oxidizing carbohydrates, fats, and proteins

8.4 The Electron Transfer System and Oxidative Phosphorylation

- Electrons are passed from NADH and $FADH_2$ to the electron transfer system, which consists of four protein complexes and two smaller shuttle carriers. As the electrons flow from one carrier to the next through the system, some of their energy is used by the complexes to pump protons across the inner mitochondrial membrane (Figure 8.14).
- Ubiquinone and the three major protein complexes (I, III, and IV) pump H^+ from the matrix to the intermembrane compartment, generating an H^+ gradient with a high concentration in the intermembrane compartment and a low concentration in the matrix (Figure 8.14).
- The H^+ gradient produced by the electron transfer system is used by ATP synthase as an energy source for synthesis of ATP from ADP and P_i. The ATP synthase is embedded in the inner mitochondrial membrane together with the electron transfer system (Figure 8.14).
- An estimated 2.5 ATP are synthesized as each electron pair travels from NADH to oxygen through the mitochondrial electron transfer system; about 1.5 ATP are synthesized as each electron pair travels through the system from $FADH_2$ to oxygen. Using these totals gives an efficiency of more than 30% for the utilization of energy released by glucose oxidation if the H^+ gradient is used only for ATP production (Figure 8.15).

Animation: The mitochondrial electron transfer system and oxidative phosphorylation

8.5 Fermentation

- Fermentations are reaction pathways that deliver electrons carried from glycolysis by NADH to organic acceptor molecules, thereby converting NADH back to NAD^+. The NAD^+ can accept electrons generated by glycolysis, allowing glycolysis to supply ATP by substrate-level phosphorylation (Figure 8.16).

Animation: The fermentation reactions

Questions

Self-Test Questions

1. In glycolysis:
   a. free oxygen is required for the reactions to occur.
   b. ATP is used when glucose and fructose-6-phosphate are catabolized, and ATP is synthesized when 3-phosphoglycerate and pyruvate are formed.
   c. the enzymes that move phosphate groups on and off the molecules are uncoupling proteins.
   d. the product with the highest potential energy in the pathway is pyruvate.
   e. the end product of glycolysis moves to the electron transfer system.
2. Which of the following statements about phosphofructokinase is *false*?
   a. It is located and has its main activity on the inner mitochondrial membrane.
   b. It catalyzes a reaction to form a product with the highest potential energy in the pathway.
   c. It can be inactivated by ATP at an inhibitory site on its surface.
   d. It can be activated by ADP at an excitatory site on its surface.
   e. It can cause ADP to form.
3. Which of the following statements is *false*? Imagine that you ingested three chocolate bars just before sitting down to study this chapter. Most likely:
   a. your brain cells are using ATP.
   b. there is no deficit of the initial substrate to begin glycolysis.
   c. the respiratory processes in your brain cells are moving atoms from glycolysis through the citric acid cycle to the electron transfer system.
   d. after a couple of hours, you change position and stretch to rest certain muscle cells, which removes lactate from these muscles.
   e. after 2 hours, your brain cells are oxygen-deficient.
4. If ADP produced throughout the respiratory reactions is in excess, this excess ADP will:
   a. bind glucose to turn off glycolysis.
   b. bind glucose-6-phosphate to turn off glycolysis.
   c. bind phosphofructokinase to turn on or keep glycolysis turned on.
   d. cause lactate to form.
   e. increase oxaloacetate binding to increase NAD^+ production.
5. Which of the following statements is *false*? In cellular respiration:
   a. one molecule of glucose can produce about 32 ATP.
   b. oxygen unites directly with glucose to form carbon dioxide.
   c. a series of energy-requiring reactions is coupled to a series of energy-releasing reactions.
   d. NADH and $FADH_2$ allow H^+ to be pumped across the inner mitochondrial membrane.
   e. the electron transfer system occurs on the inner mitochondrial membrane.
6. You are reading this text while breathing in oxygen and breathing out carbon dioxide. The carbon dioxide arises from:
   a. glucose in glycolysis.
   b. NAD^+ redox reactions in the mitochondrial matrix.
   c. NADH redox reactions on the inner mitochondrial membrane.
   d. $FADH_2$ in the electron transfer system.
   e. the oxidation of pyruvate, isocitrate, and α-ketoglutarate in the citric acid cycle.
7. In the citric acid cycle:
   a. NADH and H^+ are produced when α-ketoglutarate is both produced and metabolized.
   b. ATP is produced by oxidative phosphorylation.
   c. to progress from a four-carbon molecule to a six-carbon molecule, CO_2 enters the cycle.
   d. $FADH_2$ is formed when succinate is converted to oxaloacetate.
   e. for each molecule of glucose metabolized, the cycle "turns" once.
8. For each NADH produced from the citric acid cycle, about how many ATP are formed?
   a. 38 b. 36 c. 32 d. 2.5 e. 2.0
9. In the 1950s, a diet pill that had the effect of "poisoning" ATP synthase was tried. The person taking it could not use glucose and "lost weight"—and ultimately his or her life. Today, we know that the immediate effect of poisoning ATP synthase is:
   a. ATP would not be made at the electron transfer system.
   b. there would be an increase in H^+ movement across the inner mitochondrial membrane.
   c. more than 32 ATP could be produced from a molecule of glucose.
   d. ADP would be united with phosphate more readily in the mitochondria.
   e. ATP would react with oxygen.
10. Amino acids and fats enter the respiration pathway:
    a. by joining to NADH.
    b. by joining to glucose.
    c. at the citric acid cycle.
    d. on the inner mitochondrial membrane.
    e. on the electron transfer system.

Questions for Discussion

1. Why do you think nucleic acids are not oxidized extensively as a cellular energy source?
2. A hospital patient was regularly found to be intoxicated. He denied that he was drinking alcoholic beverages. The doctors and nurses made a special point to eliminate the possibility that the patient or his friends were smuggling alcohol into his room, but he was still regularly intoxicated. Then, one of the doctors had an idea that turned out to be correct and cured the patient of his intoxication. The idea involved the patient's digestive system and one of the oxidative reactions covered in this chapter. What was the doctor's idea?

Experimental Analysis

There are several ways to measure cellular respiration experimentally. For example, CO_2 and O_2 gas sensors measure changes over time in the concentration of carbon dioxide or oxygen, respectively. Design two experiments to test the effects of changing two different variables or conditions (one per experiment) on the respiration of a research organism of your choice.

Evolution Link

Which of the two phosphorylation mechanisms, oxidative phosphorylation or substrate-level phosphorylation, is likely to have appeared first in evolution? Why?

How Would You Vote?

Developing new drugs is costly. There is little incentive for pharmaceutical companies to target ailments that affect relatively few individuals, such as Luft syndrome. Should the federal government allocate some funds to private companies that search for cures for diseases affecting a relatively small number of people? Go to www.thomsonedu.com/login to investigate both sides of the issue and then vote.