# 分子细胞生物学

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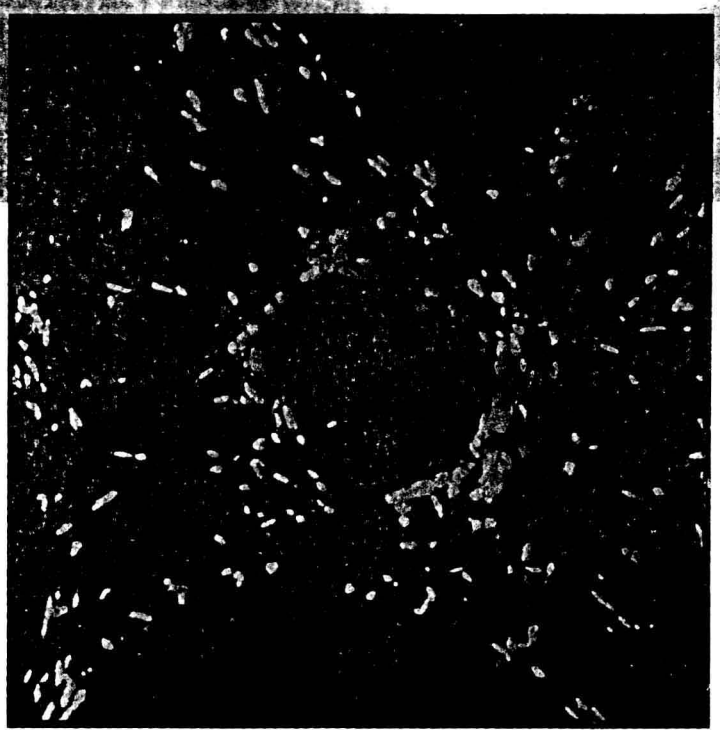
# 17 Cellular Energetics: Formation of ATP by Glycolysis and Oxidative Phosphorylation



▲ Two types of mitochondria (yellow or green fluorescing) in a living cultured mink fibroblast.

All processes involved in the growth of cells require energy. In most cases, free energy is supplied by the hydrolysis of one of the high-energy phosphoanhydride bonds in adenosine triphosphate (ATP) by the reaction

$$ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + P_i^{2-} + H^+$$

where the change in free energy under standard conditions $\Delta G°'$ is $-7.3$ kcal/mol, and where ADP = adenosine diphosphate and $P_i$ = inorganic phosphate, $HPO_4^{2-}$.

The energy released by the hydrolysis of phosphoanhydride bonds powers many otherwise energetically unfavorable events, such as the transport of molecules against a concentration gradient, exemplified by the $Na^+$-$K^+$ ATPase, the movement (beating) of cilia, and the contraction of muscle. ATP is needed in a multitude of pathways, including the synthesis of nucleic acids and proteins from amino acids and nucleotides. ATP is a universal "currency" of chemical energy, found in all types of organisms. ATP must have occurred in the earliest life forms.

This chapter and the next focus on how cells generate the high-energy phosphoanhydride bond of ATP by the endergonic reaction (one requiring the input of free energy, $G$, in order to proceed): $P_i^{2-} + H^+ + ADP^{3-} \rightleftharpoons ATP^{4-} + H_2O$ where the change in free energy, $\Delta G°'$, is $+7.3$ kcal/mol.

We shall concentrate on two of the most important processes. In Chapter 18, we discuss photosynthesis, detailing how light energy is converted to the chemical energy of a phosphoanhydride bond and how $CO_2$ and $H_2O$ are converted to six-carbon sugars. Here, we examine the metabolic pathways by which glucose and fatty acids (the principal sources of energy in animal and most other nonphotosynthetic cells, including plant cells such as roots) are metabolized to $CO_2$. The complete aerobic degradation of glucose to $CO_2$ and $H_2O$ is coupled to the synthesis of as many as 32 molecules of ATP:

$$C_6H_{12}O_6 + 6O_2 + 32P_i^{2-} + 32ADP^{3-} + 32H^+ \longrightarrow 6CO_2 + 32ATP^{4-} + 38H_2O$$

In eukaryotic cells and in bacteria, the initial enzymatically catalyzed chemical reactions in the pathway of glucose degradation occur in the cytosol. In eukaryotes, the final steps occur in the mitochondria, together with generation of most of the ATP, while in bacteria, which lack mitochondria, many of the final steps occur on the plasma membrane. Similarly, the final stages of metabolism of fatty acids can also occur in mitochondria, though in most cells metabolism of fatty acids occurs in peroxisomes without production of ATP. Localizing different metabolic pathways to different compartments of the cell prevents the pathways from interfering with each other. An important focus of this chapter is the way in which mitochondria convert the energy released by the oxidation of metabolic products of glucose and lipids to ATP phosphoanhydride bonds.

At first glance, photosynthesis and aerobic oxidation appear to have little similarity. However, a revolutionary finding in cell biology is that bacteria, mitochondria, and chloroplasts all use the same (or very nearly the same) process, called *chemiosmosis* (Figure 17-1), to generate ATP from ADP and $P_i$. The immediate energy sources that power this reaction are the proton concentration gradient and the membrane electric potential, collectively termed the *proton-motive force.* In photosynthesis, formation of this gradient is generated by the energy absorbed from light. In mitochondria, energy from the metabolism of sugars, fatty acids, and other substances, culminating in their oxidation by $O_2$, is used to pump protons across a mitochondrial membrane, generating a proton-motive force.

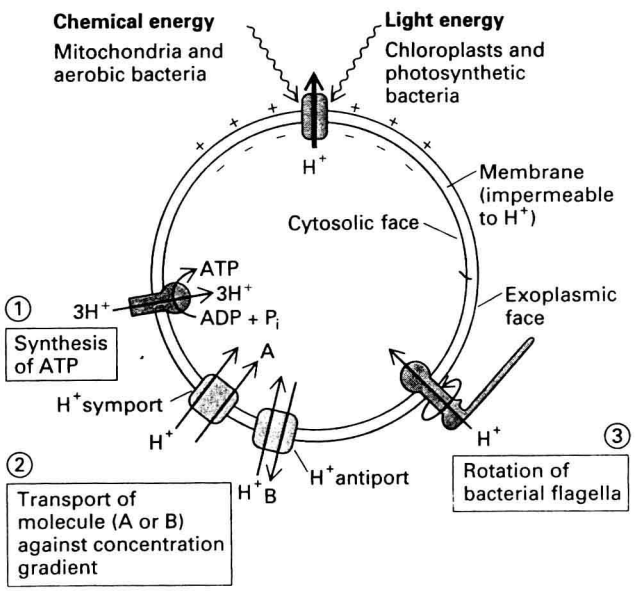
We have already seen that the proton-motive force is used in many ways. Proton concentration gradients supply energy for the transport of small molecules across a membrane against a concentration gradient; for example, the uptake of lactose by certain bacteria is catalyzed by a proton-sugar symport (see Figure 15-23), as is the concentration of ions and sucrose by plant vacuoles (see Figure 15-22). The rotation of bacterial flagella is also powered by the transmembrane proton concentration gradient (in contrast to the beating of eukaryotic cilia, which is powered by ATP hydrolysis). Conversely, ATP-powered proton pumps (see Figure 15-10) utilize the energy released by the hydrolysis of a phosphoanhydride bond to transport protons against the proton concentration gradient. The *chemiosmotic theory* is based on the principle introduced previously in the discussion of active transport: *the membrane potential, the concentration gradients of protons (and other ions) across a membrane, and the phosphoanhydride bonds in ATP are equivalent and interconvertible forms of chemical potential energy.*

Because of the central importance of the cytosol in energy metabolism, we begin our discussion of cellular energetics with the initial steps in glucose metabolism, which take place in the cytosol. We then discuss mitochondria and some details of the chemiosmotic process.

## ➤ *Energy Metabolism in the Cytosol*

### Glycolysis Results in the Net Production of Two ATP Molecules from the Conversion of One Glucose Molecule to Two Pyruvate Molecules

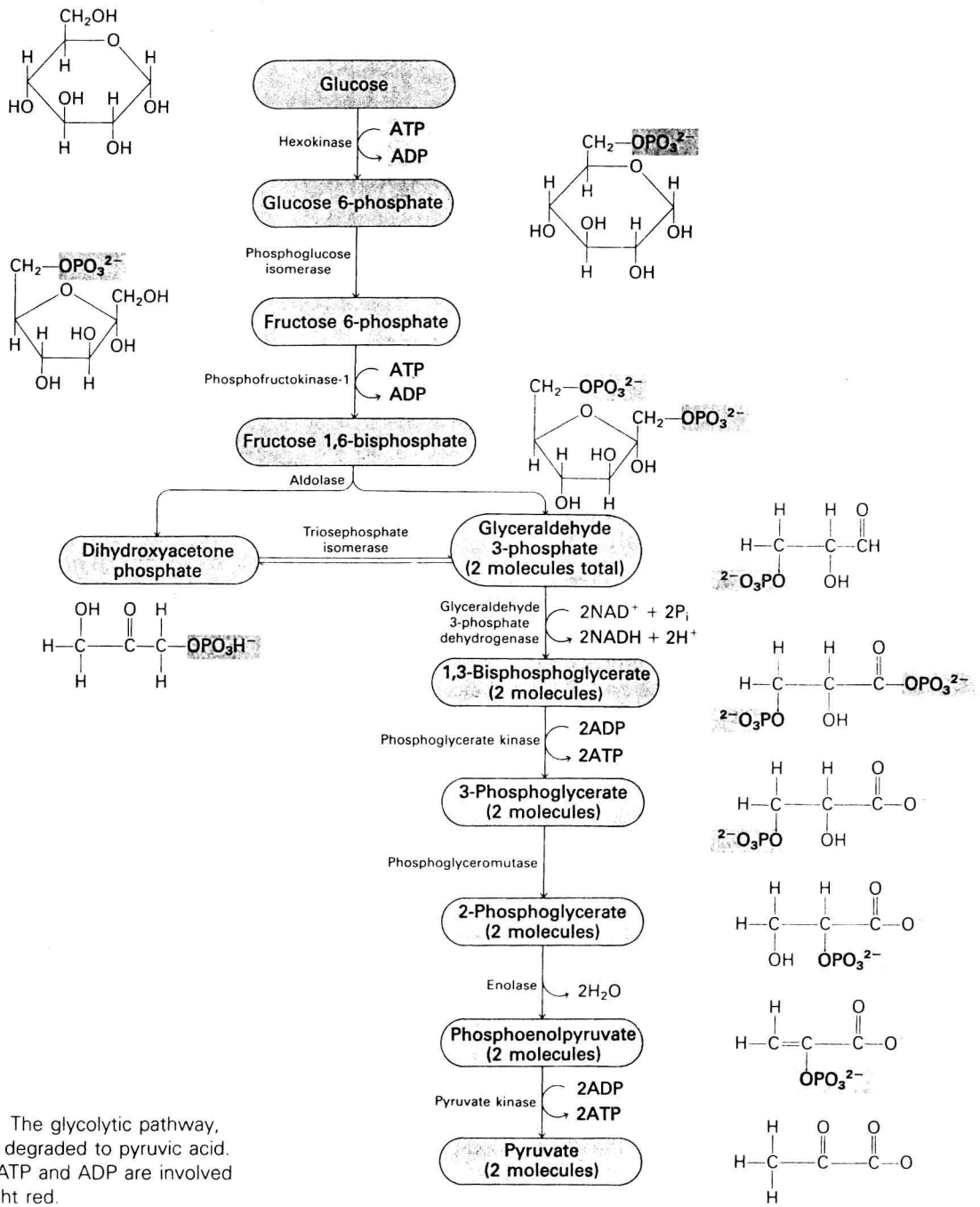
In the initial stage of glucose metabolism, *glycolysis,* each glucose molecule is converted to two molecules of the three-carbon compound pyruvate $C_3H_3O_3^-$ (Figure




▲ FIGURE 17-1 The chemiosmotic process. Chemiosmosis requires sealed, closed membrane vesicles that are relatively impermeable to $H^+$. In photosynthesis, energy absorbed from light is used to move protons across the membrane, generating a transmembrane proton (a gradient of proton concentration and/or -motive force electric potential across the membrane). In mitochondria and aerobic bacteria, energy liberated by the oxidation of carbon compounds is used to move protons across the membrane. In all cases, the protons are moved across the membrane to the exoplasmic face. (In mitochondria and chloroplasts, the cytosolic face contacts the matrix or stromal space respectively. The exoplasmic face contacts the intermembrane space of mitochondria and the thylakoid lumen of chloroplasts; see Figure 14-13). The energy stored in the $H^+$ gradient can be used (1) to move protons from the exoplasmic face down their concentration gradient across the membrane, coupled to the synthesis of ATP from ADP and $P_i$, which occurs on the cytosolic membrane face in mitochondria, chloroplasts, and bacteria; (2) to pump metabolites across the membrane against their concentration gradient; and (3) to power the rotation of flagella in bacteria.

17-2). These chemical reactions, called the *glycolytic pathway,* take place in the cytosol and do not involve molecular oxygen. Glycolysis is highly regulated: just enough glucose to meet the cell's need for ATP is transported into the cell (Figure 15-5) or is generated by the hydrolysis of polymers such as glycogen (Figure 20-24).

All of the metabolic intermediates between the initial carbohydrate and the final product, pyruvate, are phosphorylated compounds. Four molecules of ATP are formed from ADP in glycolysis: two in the step catalyzed by phosphoglycerate kinase, when two molecules of 1,3-bisphosphoglycerate are converted to 3-phosphoglycerate, and two in the step catalyzed by pyruvate kinase (see Figure 17-2). However, two ATP molecules are consumed during earlier steps of this pathway: the first by the addition of a phosphate residue to glucose in the reaction catalyzed by hexokinase, and the second by the addition of a second phosphate to fructose 6-phosphate in the reaction catalyzed by phosphofructokinase-1. Thus there is a net gain of two ATP molecules. The balanced chemical equa-




▲ FIGURE 17-2 The glycolytic pathway, by which glucose is degraded to pyruvic acid. Reactions in which ATP and ADP are involved are highlighted in light red.

tion for this series of reactions shows that four hydrogen atoms (four protons and four electrons) are also formed:

$$C_6H_{12}O_6 \longrightarrow 2C_3H_4O_3 + 4H$$

or

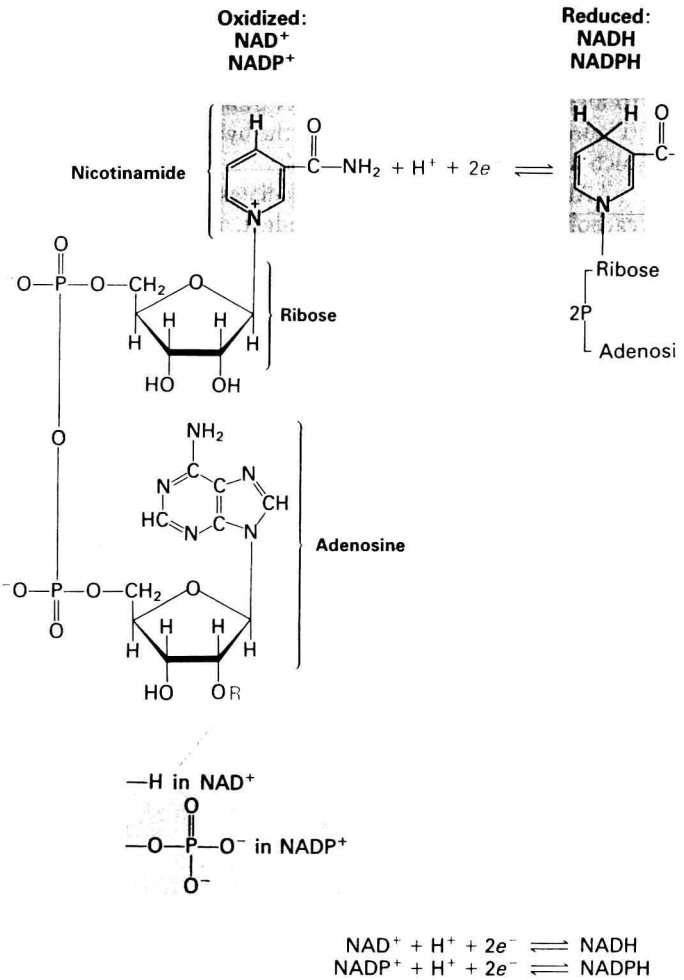
$$C_6H_{12}O_6 \longrightarrow 2C_3H_4O_3 + 4H^+ + 4e^-$$

The reaction that generates these H atoms is catalyzed by the enzyme glyceraldehyde 3-phosphate dehydrogenase. All four electrons and two of the four protons are transferred to two molecules of the oxidized form of the electron carrier nicotinamide adenine dinucleotide, $NAD^+$, to produce the reduced form, NADH (Figure 17-3):

$$2H^+ + 4e^- + 2NAD^+ \longrightarrow 2NADH$$

Thus the overall reaction for this first stage of glucose metabolism is

$$C_6H_{12}O_6 + 2NAD^+ + 2ADP^{3-} + 2P_i^{2-} \longrightarrow 2C_3H_4O_3 + 2NADH + 2ATP^{4-}$$

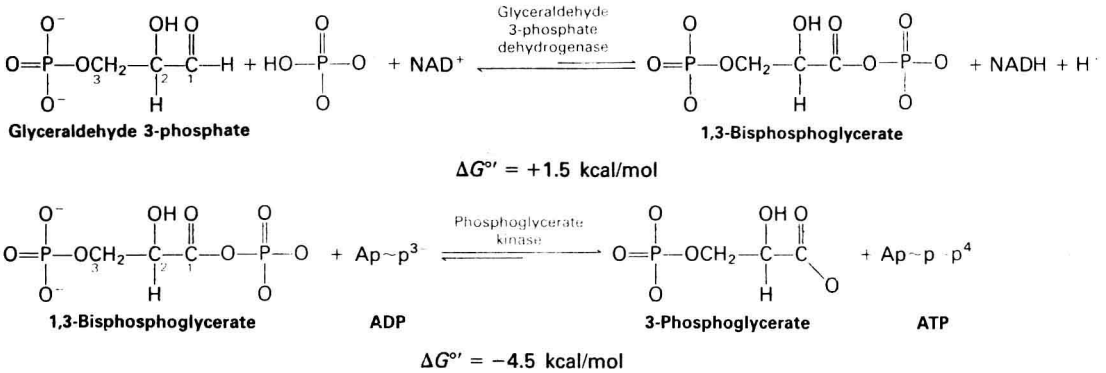



▲ FIGURE 17-3 Structure of $NAD^+$ and NADH. Nicotinamide adenine dinucleotide ($NAD^+$) and the related nicotinamide adenine dinucleotide phosphate ($NADP^+$) accept only pairs of electrons; reduction to NADH or NADPH involves the transfer of two electrons simultaneously. In most oxidation-reduction reactions in biological systems, a pair of hydrogen atoms (two protons and two electrons) are removed from a molecule. One of the protons and both electrons are transferred to $NAD^+$; the other proton is released into solution. Thus the overall reaction is sometimes written $NAD^+ + 2H^+ + 2e^- \rightleftharpoons NADH + H^+$. NADP is identical in structure to NAD except for the presence of an additional phosphate group. However, NAD and NADP participate in different sets of enzymatically catalyzed reactions.

## In Glycolysis, ATP Is Generated by Substrate-Level Phosphorylation

Cells synthesize ATP in two basically different ways. The immediate energy source for ATP synthesis in chloroplasts and mitochondria is provided by the proton-motive force across a membrane. In the other process, *substrate-level phosphorylation*, metabolites in the cytosol are chemically transformed by aqueous enzymes, so that membranes and ion gradients are not involved.

Two substrate-level phosphorylations occur in glycolysis. The first results from a pair of reactions catalyzed by glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase (Figure 17-4). In the first of these reactions (Figures 17-4 and 17-5), the aldehyde (CHO) group on glyceraldehyde 3-phosphate is oxidized by $NAD^+$; the oxidation is coupled to the addition of a phosphate group, forming 1,3-bisphosphoglycerate with a single high-energy phosphate bond. The $\Delta G^{\circ\prime}$ for the hydrolysis of the phosphoanhydride bond to carbon 1 in 1,3-bisphosphoglycerate is more negative than the $\Delta G^{\circ\prime}$ for the hydrolysis of the terminal phosphoanhydride bond in ATP (−12 kcal/mol versus −7.3 kcal/mol). The second reaction, which is strongly *exergonic* (accompanied by the release of free energy), catalyzed by phosphoglycerate kinase (see Figure 17-4), transfers this high-energy phosphate group to ADP, forming ATP. The net change in standard free energy of these two reactions is negative ($\Delta G^{\circ\prime} = -4.5 + 1.5 = -3.0$ kcal/mol), so the two reactions overall are strongly exergonic. In the preceding stages of glycolysis, each molecule of fructose 1,6-bisphosphate generated two molecules

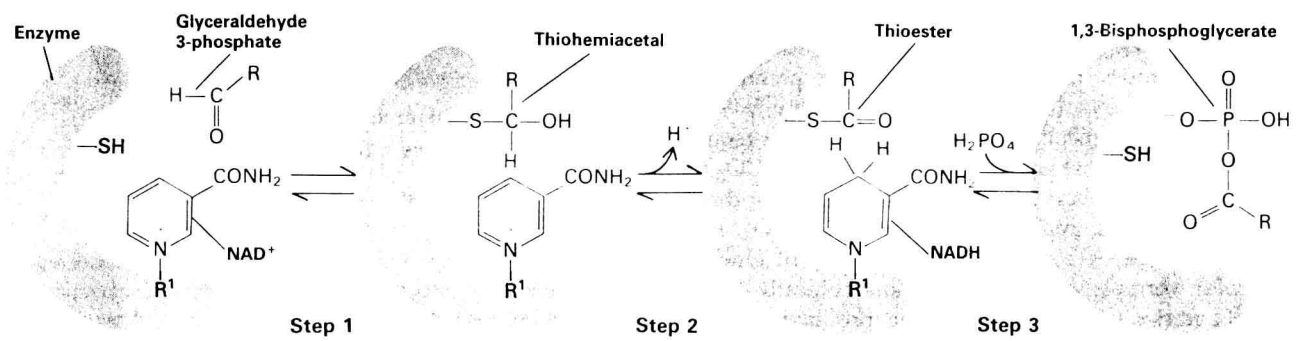



▲ FIGURE 17-4 The first of two substrate-level phosphorylation reactions in glycolysis. Glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase are water-soluble enzymes that catalyze ATP synthesis in the cytosol. The three C atoms of the glycerol are numbered.

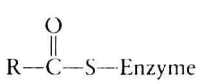
of glyceraldehyde 3-phosphate (see Figure 17-2); therefore, the catabolism of one glucose molecule has now generated two ATPs.

The second high-energy phosphate bond is formed when the product of the phosphoglycerate kinase reaction, 3-phosphoglycerate, is converted in a series of reactions to pyruvate (Figure 17-6). In the first of these reactions, catalyzed by phosphoglyceromutase, 3-phosphoglycerate is converted to 2-phosphoglycerate. In the next reaction, catalyzed by enolase, loss of water converts the phosphate-carbon bond in 2-phosphoglycerate from a low-energy bond ($\Delta G^{\circ\prime}$ of hydrolysis = −2 kcal/mol) to the high-energy bond ($\Delta G^{\circ\prime}$ of hydrolysis = −15 kcal/mol) in phosphoenolpyruvate. In the third reaction, catalyzed by pyruvate kinase, the phosphate in phosphoenolpyruvate is transferred to ADP—a strongly exergonic reaction ($\Delta G^{\circ\prime}$ = −7.5 kcal/mol)—and pyruvate is produced.

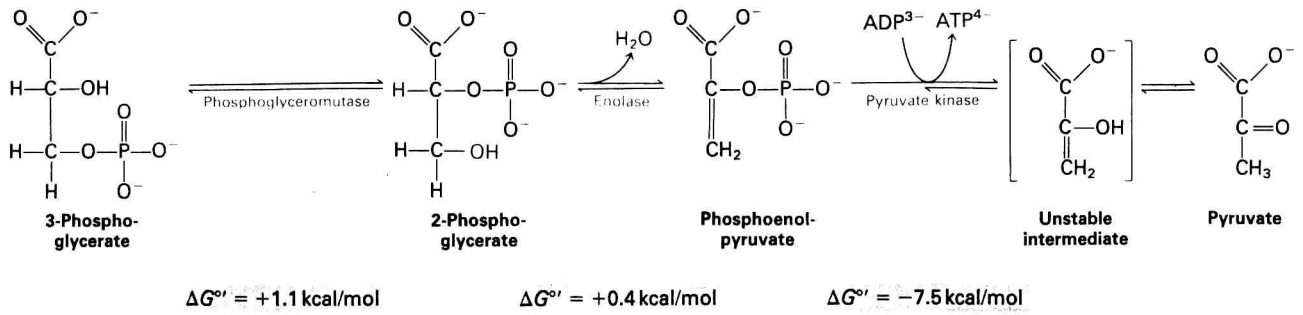
These examples illustrate how interconversions of soluble chemicals by soluble enzymes are coupled to generate ATP. However, only two of the 32 ATP molecules gener-




▲ FIGURE 17-5 The mechanism of action of glyceraldehyde 3-phosphate dehydrogenase. A thioester



is an energy-rich intermediate in the reaction. The sulfhydryl (SH) group is the side chain of cysteine at the active site; R symbolizes the rest of the glyceraldehyde 3-phosphate molecule. R′ is the rest of the NAD molecule. *Step 1:* The enzyme has bound $NAD^+$, and the -SH group on the enzyme reacts with glyceraldehyde 3-phosphate to form a thiohemiacetal. *Step 2:* A hydrogen atom (red) and two electrons are transferred to $NAD^+$, forming the reduced form NADH, and a proton from the O atom of the thiohemiacetal is simultaneously lost to the medium; the products are a thioester and NADH + $H^+$. *Step 3:* The thioester reacts with phosphate to produce 1,3-bisphosphoglycerate; NADH is freed from the enzyme surface, and the free enzyme with its -SH group is regenerated.




▲ FIGURE 17-6 Formation of the second pair of ATP molecules during glycolysis. This reaction, catalyzed by pyruvate kinase, is the second of the two substrate-level phosphorylations in glycolysis.

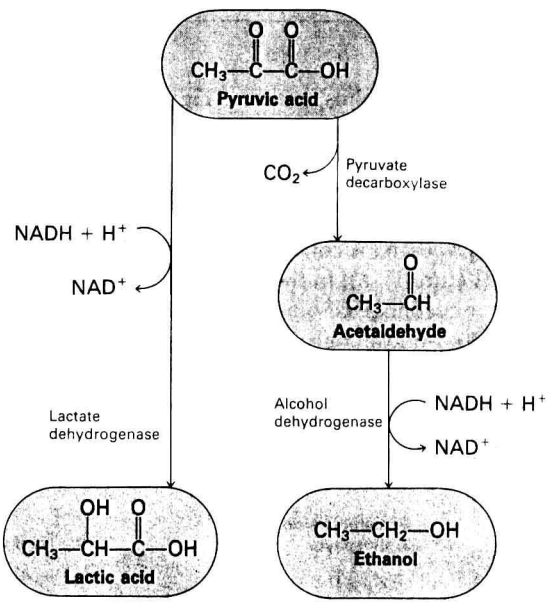
ated during the complete oxidation of glucose to $CO_2$ and $H_2O$ are created during the conversion of glucose to pyruvate. The remaining 30 are synthesized in the mitochondria by a fundamentally different type of process that involves the generation and utilization of proton concentration gradients across the inner mitochondrial membrane.

## Some Eukaryotic and Prokaryotic Cells Metabolize Glucose Anaerobically

Most eukaryotic cells are *obligate aerobes:* they grow only in the presence of oxygen and they metabolize glucose (or related sugars) completely to $CO_2$, with the concomitant production of a large amount of ATP. Most of these cells also generate some ATP by anaerobic metabolism. A few eukaryotes, including certain yeasts, are *facultative anaerobes:* they grow in either the presence or absence of oxygen. Annelids and mollusks, as examples, can live and grow for days without oxygen. Many prokaryotic cells are *obligate anaerobes:* they cannot grow in the presence of oxygen.

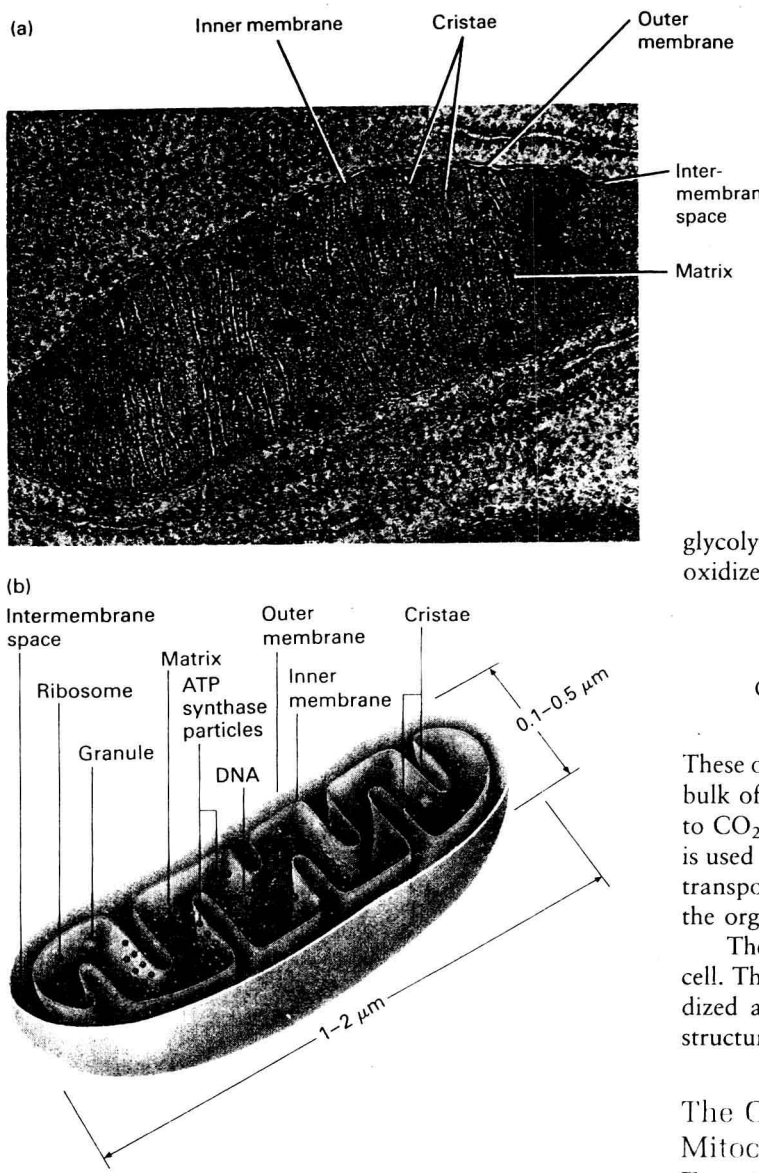
The anaerobic metabolism of glucose does not require mitochondria. Glucose is not converted entirely to $CO_2$ (as it is in obligate aerobes) but to one or more two- or three-carbon compounds, and only in some cases to $CO_2$. As a result, much less ATP is produced per mole of glucose. Yeasts, for example, ferment glucose anaerobically to two ethanol and two $CO_2$ molecules; the net production is only two ATP molecules per glucose molecule (Figure 17-7; see also Figure 17-2). Recall that during the initial stages of glycolysis (that is, during the conversion of glucose to pyruvate), two NAD molecules are reduced to NADH; as the pyruvate is converted into ethanol, the NADH from the initial stages is reoxidized to NAD. This *anaerobic fermentation* is the basis of the beer and wine industry.

During the prolonged contraction of mammalian skeletal muscle cells, oxygen becomes limited and glucose cannot be oxidized completely to $CO_2$ and $H_2O$. The cells ferment glucose to two molecules of lactic acid—again, with the net production of only two molecules of ATP per




▲ FIGURE 17-7 The anaerobic metabolism of glucose. In the formation of pyruvate from glucose, one molecule of $NAD^+$ is reduced to NADH for each molecule of pyruvate formed (see Figure 17-2). To regenerate $NAD^+$, two electrons are transferred from each NADH molecule to an acceptor molecule. When oxygen supplies are low in muscle cells, the acceptor is pyruvic acid, and lactic acid is formed. In yeasts, acetaldehyde is the acceptor, and ethanol is formed.

glucose molecule (see Figures 17-2 and 17-7). The lactic acid causes muscle and joint aches. It is largely secreted into the blood; some passes into the liver, where it is reoxidized to pyruvate and either further metabolized to $CO_2$ or converted to glucose. Much lactate is metabolized to $CO_2$ by the heart, which is highly perfused by blood and can




◄ FIGURE 17-8 The structure of the mitochondrion. (a) Electron micrograph of a mitochondrion from a bat pancreas, showing the inner membrane with extensive cristae, the outer membrane, the intermembrane space, and small granules in the matrix. (b) A three-dimensional diagram of a mitochondrion cut longitudinally. The matrix contains the mitochondrial DNA and ribosomes. [Part (a) from D. W. Fawcett, *The Cell,* 2d ed., Saunders, 1981, p. 421. Courtesy of Don Fawcett.]

continue aerobic metabolism at times when exercising skeletal muscles secrete lactate. Lactic acid bacteria (the organisms that "spoil" milk) and other prokaryotes also generate ATP by the fermentation of glucose to lactate.

## ➤ *Mitochondria and the Metabolism of Carbohydrates and Lipids*

In aerobic cells, glucose is oxidized completely to $CO_2$ by $O_2$. In the latter stages of this process, pyruvate formed in glycolysis is transported to the mitochondria, where it is oxidized by $O_2$ to $CO_2$:

$$CH_3{-}\overset{O}{\overset{\|}{C}}{-}\overset{O}{\overset{\|}{C}}{-}OH + 2\tfrac{1}{2}O_2 \longrightarrow 3CO_2 + 2H_2O$$

These oxidation reactions in the mitochondria generate the bulk of the ATP produced from the conversion of glucose to $CO_2$. Some energy released in mitochondrial oxidation is used for other purposes, such as heat generation and the transport of ADP into the mitochondrion and ATP out of the organelle into the cytosol.

The mitochondrion is really the "power plant" of the cell. The details of the reactions by which pyruvate is oxidized and ATP is formed depend on understanding the structure of the mitochondrion, which we now address.

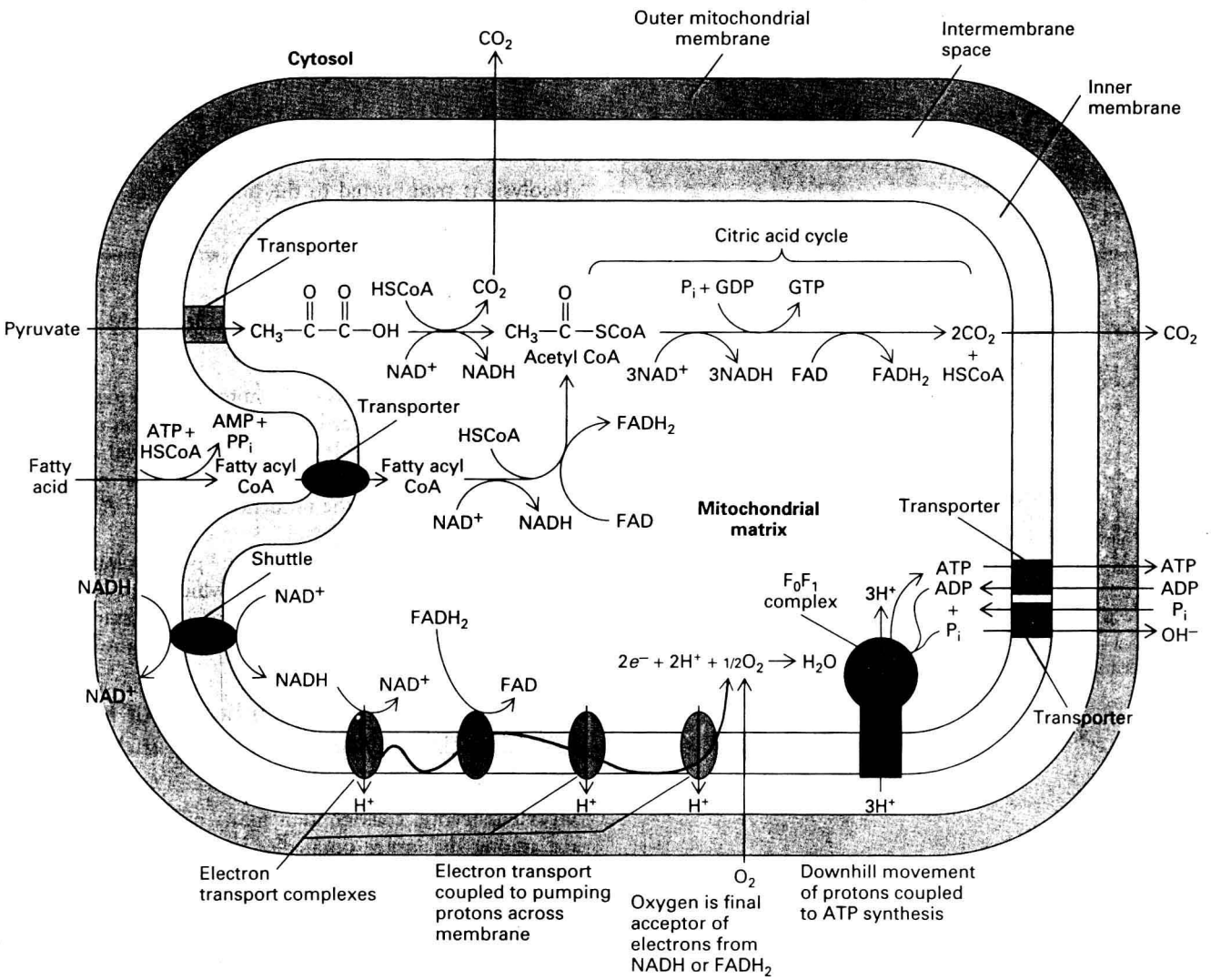
### The Outer and Inner Membranes of the Mitochondrion Are Structurally and Functionally Distinct

Most eukaryotic cells contain many mitochondria. They are among the larger organelles in the cell—each one is about the size of an *E. coli* bacterium—and they occupy as much as 25 percent of the volume of the cytoplasm. They are large enough to be seen under a light microscope, but the details of their structure can be viewed only with the electron microscope (Figure 17-8).

Mitochondria contain two very different membranes—the *outer membrane* and the *inner membrane*—which define two submitochondrial compartments: the *intermembrane space* between the two membranes, and the *matrix* or central compartment. The fractionation and purification of these membranes and compartments has made it possible to determine their protein and phospholipid compositions and to localize each reaction to a specific membrane or space.

The outer membrane defines the smooth outer perimeter of the mitochondrion. Transmembrane channels formed by the protein *mitochondrial porin* make this membrane freely permeable to most small molecules (<4,000 to 5,000 MW), including protons. While flow of metabolites across the outer membrane may limit their rate of mitochondrial oxidation, the inner membrane is the major permeability barrier between the cytosol and the mitochondrial matrix.

Freeze-fracture studies indicate that the inner membrane contains many protein-rich intramembrane particles that are laterally mobile in the membrane plane (see Figure 14-33). Some of these particles function in electron transport from NADH or reduced flavin adenine dinucleotide ($FADH_2$) to $O_2$ (Figure 17-9) and in ATP synthesis. Some particles are transporters that allow otherwise impermeable molecules, such as ADP and phosphate, to pass from the cytosol to the matrix, and other molecules, such as




▲ FIGURE 17-9 Outline of the major metabolic reactions in mitochondria. The substrates of oxidative phosphorylation—pyruvate, fatty acids, ADP, and $P_i$—are transported to the matrix from the cytosol by transporters; $O_2$ diffuses into the matrix. NADH, which is generated in the cytosol during glycolysis, is not transported directly to the matrix because the inner membrane is impermeable to $NAD^+$ or NADH; instead, a shuttle system (see Figure 17-21) transports electrons from cytosolic NADH to the electron transport chain. ATP is transported to the cytosol in exchange for ADP and $P_i$, $CO_2$ diffuses into the cytosol across the mitochondrial membranes. HSCoA denotes free coenzyme A (CoA), and SCoA denotes CoA when it is esterified (see Figure 17-11). Fatty acids are linked to CoA on the outer mitochondrial membrane. Subsequently, the fatty-acyl group is removed from the CoA, linked to a carnitine carrier that transports it across the inner membrane, and then the fatty acid is reattached to a CoA on the matrix side of the inner membrane. The blue ovals depict the electron carrier protein complexes that transfer electrons from NADH and $FADH_2$ to $O_2$.

ATP, to move from the matrix into the cytosol. Protein constitutes 76 percent of the total inner membrane weight—a higher fraction than in any other cellular membrane. Cardiolipin (diphosphatidylglycerol, see Figure 14-2), a lipid concentrated in the inner membrane, reduces the permeability of the phospholipid bilayer to protons and thus enables a proton-motive force to be established across the inner membrane.

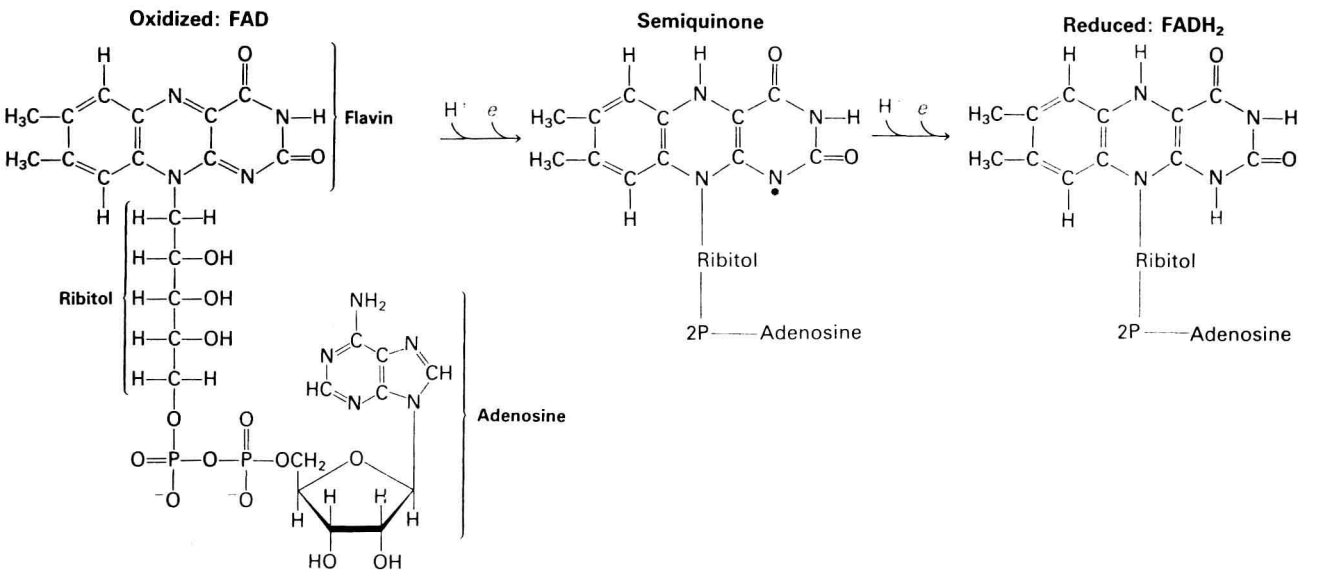
The inner membrane and the matrix are the sites of most reactions involving the oxidation of pyruvate and fatty acids to $CO_2$ and $H_2O$ and the coupled synthesis of ATP from ADP and $P_i$. These complex processes involve many steps but can be subdivided into three groups of reactions, each of which occurs in a discrete membrane or space in the mitochondrion (see Figure 17-9):

1. Oxidation of pyruvate or fatty acids to $CO_2$, coupled to the reduction of the electron carriers $NAD^+$ and FAD (Figure 17-10) to NADH and $FADH_2$, respectively. These reactions occur in the matrix or on inner-membrane proteins facing it.
2. Electron transfer from NADH and $FADH_2$ to $O_2$. These reactions occur in the inner membrane and are coupled to the generation of a proton-motive force across it.
3. Harnessing of the energy stored in the electrochemical proton gradient for ATP synthesis by the $F_0F_1$ ATPase complex in the inner membrane.

The last two groups of reactions involve multisubunit proteins that are asymmetrically oriented in the inner mitochondrial membrane.

The highly convoluted foldings, or *cristae,* of the inner membrane greatly expand its surface area, enhancing its ability to generate ATP. In typical liver mitochondria, for example, the area of the inner membrane is about five times that of the outer membrane. In fact, the total area of all mitochondrial inner membranes in liver cells is about 17 times that of the plasma membrane. In heart and skeletal muscles, mitochondria contain three times as many cristae as are found in typical liver mitochondria—presumably reflecting the greater demand for ATP by muscle cells.

In plants, mitochondria are essential for ATP production in photosynthetic cells during dark periods when photosynthesis is not possible, and all of the time in roots and other nonphotosynthetic tissues. Stored carbohydrates, mostly in the form of starch, are hydrolyzed to glucose and then metabolized to pyruvate; as in animal mitochondria, pyruvate is oxidized to $CO_2$, with concomitant generation of ATP.




▲ FIGURE 17-10 Reduction of FAD to $FADH_2$. The cofactor flavin adenine dinucleotide (FAD) contains a three-ring flavin component that can accept one or two hydrogen atoms. The addition of one electron together with a proton (i.e., a hydrogen atom) generates a semiquinone intermediate. The semiquinone is a free radical because it contains an unpaired electron (denoted here by a blue dot), which is delocalized by resonance to all of the flavin ring atoms. The addition of a second electron and proton (i.e., a second hydrogen atom) generates the reduced form, $FADH_2$. Flavin mononucleotide (FMN) is a cofactor related to FAD that contains only the flavin–ribitol phosphate part of FAD (shown enclosed in the color screen).

## Acetyl CoA Is a Key Intermediate in the Mitochondrial Metabolism of Pyruvate and Fatty Acids

*Pyruvate,* which is generated in the cytosol during glycolysis, is transported across the mitochondrial membranes to the matrix. The complete oxidation of pyruvate to form $CO_2$ and $H_2O$ occurs in the mitochondrion and utilizes $O_2$ as the final electron acceptor (oxidizer).

Figure 17-9 traces the metabolism of pyruvate in the mitochondrion. Immediately on entering the matrix, pyruvate reacts with coenzyme A (HSCoA) to form $CO_2$ and the intermediate acetyl CoA (Figure 17-11)—a reaction catalyzed by the enzyme *pyruvate dehydrogenase,* a component of the matrix:

$$CH_3{-}\overset{\overset{O}{\|}}{C}{-}\overset{\overset{O}{\|}}{C}{-}O^- + HSCoA + NAD^+ \longrightarrow CH_3{-}\overset{\overset{O}{\|}}{C}{-}SCoA + CO_2 + NADH$$

This reaction is highly exergonic ($\Delta G^{\circ\prime} = -8.0$ kcal/mol) and essentially irreversible. Pyruvate dehydrogenase is one of the most complex enzymes known. It is a giant molecule 30 nm in diameter (4.6 million MW), even larger than a ribosome, and contains 60 subunits composed of three different enzymes, several regulatory polypeptides, and five different coenzymes.

Fatty acids are also oxidized in the mitochondrion to produce acetyl CoA; the energy released is used to synthesize ATP from ADP and $P_i$. In most mammalian cells, in yeasts and in plants, and probably in most eukaryotes, fatty acids are degraded chiefly in peroxisomes; fatty acids containing over ≈20 $CH_2$ groups are degraded only in these organelles. In peroxisomes, fatty acids are converted to acetyl CoA, but since $H_2O_2$ is generated, not ATP, we discuss peroxisomal oxidation at the end of this chapter.

Fatty acids are stored as triacylglycerols, primarily as droplets in adipose (fat-storing) cells. In response to hormones such as adrenaline, triacylglycerols are hydrolyzed in the cytosol to free fatty acids and glycerol:

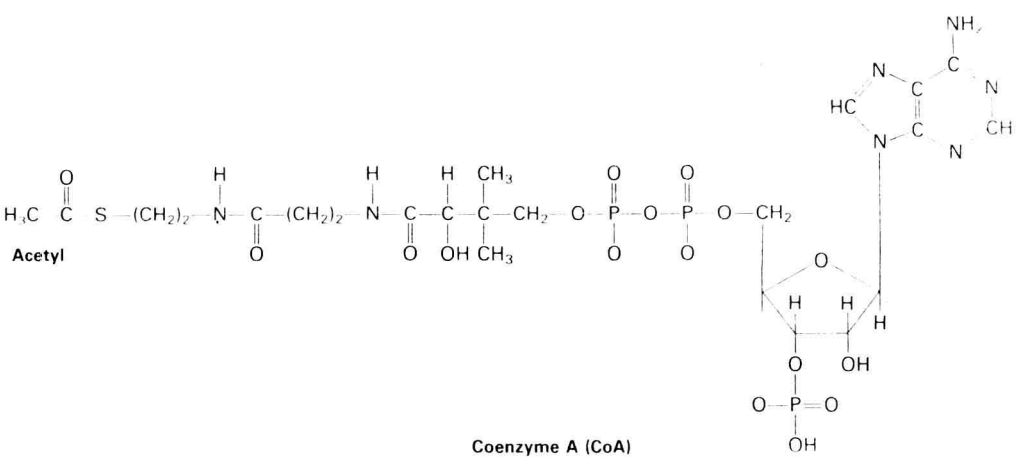
$$CH_3{-}(CH_2)_n{-}\overset{\overset{O}{\|}}{C}{-}O{-}CH_2$$
$$CH_3{-}(CH_2)_n{-}\overset{\overset{O}{\|}}{C}{-}O{-}CH \quad + 3H_2O \longrightarrow$$
$$CH_3{-}(CH_2)_n{-}\overset{\overset{O}{\|}}{C}{-}O{-}CH_2$$

$$3\,CH_3{-}(CH_2)_n{-}\overset{\overset{O}{\|}}{C}{-}OH \quad + \quad \begin{matrix} HO{-}CH_2 \\ | \\ HO{-}CH \\ | \\ HO{-}CH_2 \end{matrix}$$

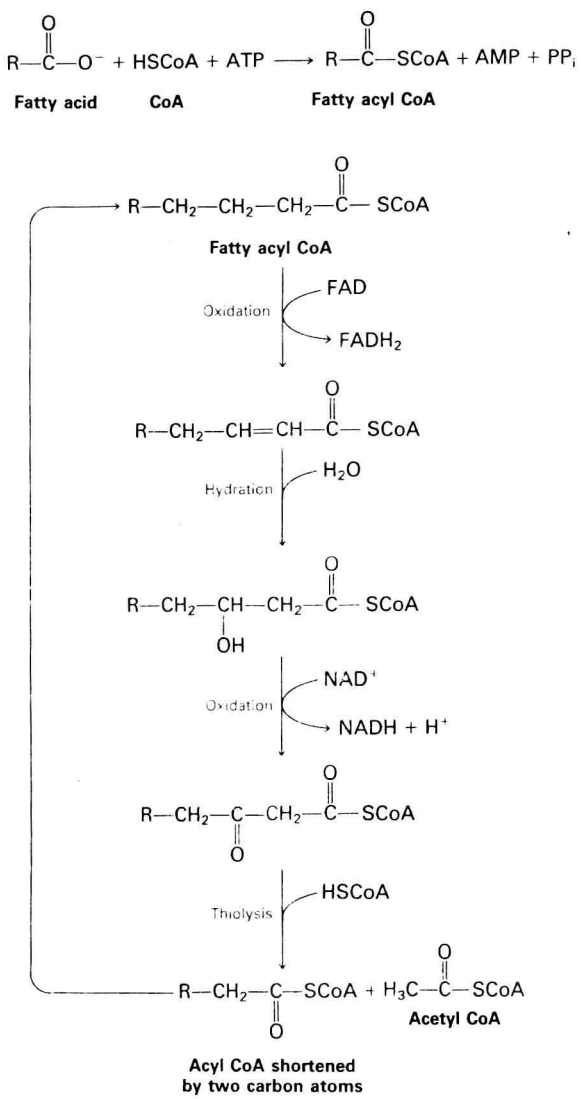
Fatty acid Glycerol

Fatty acids are released into the blood, from which they are taken up and oxidized by most cells. They are the major energy source for many tissues, particularly for heart muscle. In humans, the oxidation of fats is quantitatively more important than the oxidation of glucose as a source of ATP. In part, this is because the oxidation of 1 g of triacylglycerol to $CO_2$ generates about six times as much ATP as does the oxidation of 1 g of hydrated glycogen.

In the cytosol, free fatty acids are linked to coenzyme A to form an acyl CoA (Figure 17-12) in an exergonic reaction coupled to the hydrolysis of ATP to AMP and $PP_i$ (inorganic pyrophosphate). $PP_i$ is hydrolyzed to two molecules of phosphate ($P_i$), drawing this reaction to comple-




◀ FIGURE 17-11
The structure of acetyl CoA—an important intermediate in the metabolism of pyruvate, fatty acids, and many amino acids.




▲ FIGURE 17-12 Oxidation of fatty acids in mitochondria. Four enzymatically catalyzed reactions convert a fatty acyl CoA molecule to acetyl CoA and a fatty acyl CoA shortened by two carbon atoms. Concomitantly, one $NAD^+$ molecule is reduced to NADH and one FAD molecule is reduced to $FADH_2$. The cycle is repeated on the shortened acyl CoA until fatty acids with an even number of carbon atoms are completely converted to acetyl CoA. Fatty acids with an odd number of C atoms are rare; they are metabolized to one molecule of propionyl CoA and multiple acetyl CoAs.

tion. Then the fatty acyl group is transported across the inner mitochondrial membrane by a transporter protein and is reattached to another CoA molecule on the matrix side. Each molecule of acyl CoA in the mitochondrion is then oxidized to form one molecule of acetyl CoA and an acyl CoA shortened by two carbon atoms (see Figure 17-12). Concomitantly, one molecule apiece of $NAD^+$ and FAD are reduced, respectively, to NADH and $FADH_2$. This set of reactions is repeated on the shortened acyl CoA until all C atoms are converted to acetyl CoA. For stearoyl CoA, the overall reaction is

$$\begin{aligned} &CH_3{-}(CH_2)_{16}{-}\overset{\overset{O}{\|}}{C}{-}SCoA \\ &\quad + 8HSCoA + 8FAD + 8NAD^+ + 8H_2O \longrightarrow \\ &\quad 9CH_3{-}\overset{\overset{O}{\|}}{C}{-}SCoA + 8FADH_2 + 8NADH + 8H^+ \end{aligned}$$

In addition to its role in the oxidation of fatty acids and carbohydrates, acetyl CoA occupies a central position in the mitochondrial oxidation of many amino acids. It is also an intermediate in many biosynthetic reactions, such as the transfer of an acetyl group to lysine residues in histone proteins and to the N-termini of many mammalian proteins. Acetyl CoA is also a biosynthetic precursor of cholesterol and other steroids, and also of the isoprenyl groups that anchor proteins such as Ras to membranes (see Figure 14-20). In respiring mitochondria, however, the fate of the acetyl group of acetyl CoA is almost always oxidation to $CO_2$.

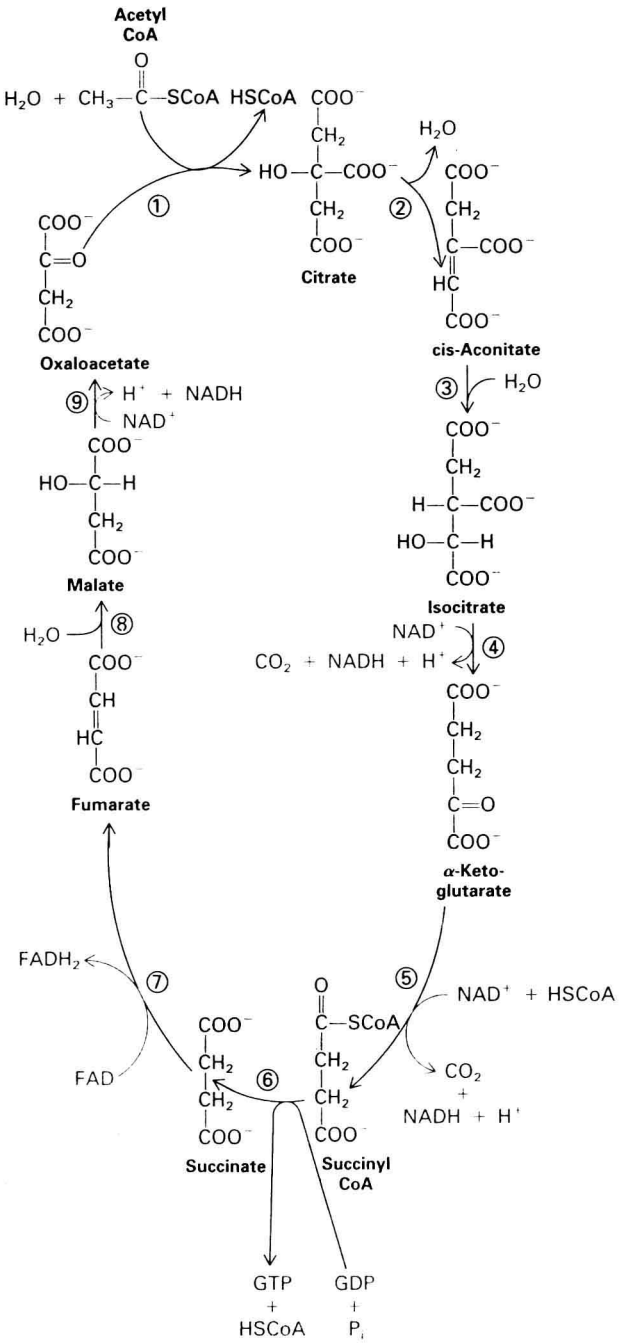
## The Citric Acid Cycle Oxidizes the Acetyl Group of Acetyl CoA to $CO_2$ and Reduces NAD and FAD to NADH and $FADH_2$

The final stage of the oxidation of carbohydrates and lipids—the *citric acid cycle* (also called the *tricarboxylic acid cycle* and the *Krebs cycle*)—is a complex set of nine reactions (Figure 17-13). First consider the net reaction:

$$\begin{aligned} &CH_3{-}\overset{\overset{O}{\|}}{C}{-}SCoA \\ &\quad + 3NAD^+ + FAD + GDP^{3-} + P_i^{2-} + 2H_2O \longrightarrow \\ &\quad 2CO_2 + 3NADH + FADH_2 + GTP^{4-} + 2H^+ + HSCoA \end{aligned}$$

Note that there is no involvement of molecular $O_2$ in this cycle and that only one high-energy phosphate bond is synthesized by substrate-level phosphorylation (in GTP). The two carbon atoms in acetyl CoA are oxidized to two molecules of $CO_2$. Concomitantly, the released electrons are transferred to $NAD^+$ and FAD to form the reduced molecules NADH and $FADH_2$.

Now let us look at the cycle in detail. Note that Figure 17-13 depicts the oxidation of a single molecule of acetyl CoA, but we must keep in mind that the oxidation of one glucose molecule will have generated two molecules of acetyl CoA, and the oxidation of one molecule of stearic acid generates nine of acetyl CoA.




▲ FIGURE 17-13 The citric acid cycle. First, a two-carbon acetyl residue from acetyl CoA condenses with the four-carbon molecule oxaloacetate (reaction 1) to form the six-carbon molecule citrate. Through a sequence of enzymatically catalyzed reactions (2–9), each molecule of citrate is eventually converted to oxaloacetate, losing two $CO_2$ molecules in the process. In four of the reactions, four pairs of electrons are removed from the carbon atoms: three pairs are transferred to three molecules of $NAD^+$ to form $3NADH + 3H^+$; one pair is transferred to the acceptor FAD to form $FADH_2$.

As shown in Figure 17-13, the cycle begins with the condensation of the two-carbon acetyl group from acetyl CoA with the four-carbon molecule oxaloacetate. The product of reaction 1 is the six-carbon citric acid, for which the cycle is named. In reactions 2 and 3, citrate is isomerized to the six-carbon molecule isocitrate by the single enzyme aconitase. In reaction 4, isocitrate is oxidized to the five-carbon $\alpha$-ketoglutarate, generating one $CO_2$ molecule and reducing one molecule of $NAD^+$ to NADH. In reaction 5, the $\alpha$-ketoglutarate is oxidized to the four-carbon molecule succinyl CoA, generating the second $CO_2$ molecule formed during each turn of the cycle and reducing another $NAD^+$ molecule to NADH. In reactions 6–9, succinyl CoA is oxidized to oxaloacetate, regenerating the molecule that was initially condensed with acetyl CoA. Concomitantly, one FAD molecule is reduced to $FADH_2$ and one $NAD^+$ molecule to NADH. The conversion of succinyl CoA to succinate (reaction 6) is coupled to the synthesis of one GTP molecule (from GDP and $P_i$); this reaction is slightly exergonic ($\Delta G^{\circ\prime} = -0.8$ kcal/mol).

Most enzymes and small molecules involved in the citric acid cycle are soluble in aqueous solution and are localized to the matrix of the mitochondrion. This includes the water-soluble molecules CoA, acetyl CoA, and succinyl CoA, as well as $NAD^+$ and NADH. Succinate dehydrogenase (reaction 7) together with the FAD/$FADH_2$ and $\alpha$-ketoglutarate dehydrogenase (reaction 5) are localized to the inner membrane.

The protein concentration of the mitochondrial matrix is 500 mg/ml (a 50 percent protein solution), and the matrix must have a viscous, gel-like consistency. When mitochondria are disrupted by gentle ultrasonic vibration or osmotic lysis, the six non–membrane-bound enzymes in the citric acid cycle are released as a very large multiprotein complex. The reaction product of one enzyme, it is believed, passes directly to the next enzyme without diffusing through the solution. However, much work is needed to determine the structure of the enzyme complex: biochemists generally study the properties of enzymes in dilute aqueous solutions of less than 1 mg/ml, and weak interactions between enzymes are often difficult to detect.

## Electrons Are Transferred from NADH and $FADH_2$ to Molecular $O_2$ by Electron-Carrier Proteins

In summary, the reactions in the glycolytic pathway and citric acid cycle result in the conversion of one glucose molecule to six $CO_2$ molecules and the concomitant reduction of 10 $NAD^+$ to 10 NADH molecules and of two FAD to two $FADH_2$ molecules (Table 17-1). The reduced coenzymes are reoxidized by molecular $O_2$ in a multistep process. NADH and $FADH_2$ first transfer electrons to acceptor molecules in the inner mitochondrial membrane; the loss of electrons regenerates the oxidized forms of $NAD^+$ and FAD as well as the reduced form of the acceptor. The

**TABLE 17-1 Net Result of the Glycolytic Pathway and the Citric Acid Cycle**

| Reaction | $CO_2$ Molecules Produced | NADH Molecules Produced | $FADH_2$ Molecules Produced |
|---|---|---|---|
| 1 Glucose molecule to 2 pyruvates | 0 | 2 | 0 |
| 2 Pyruvates to 2 acetyl CoAs | 2 | 2 | 0 |
| 2 Acetyl CoAs to 4 $CO_2$s | 4 | 6 | 2 |
| Total | 6 | 10 | 2 |

electrons released from NADH and $FADH_2$ are transferred along the electron transport chain, a group of electron carriers all but one of which are integral components of the inner membrane. Eventually, they are transferred to $O_2$, forming $H_2O$.

The following overall reactions summarize these steps:

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

$$\Delta G^{\circ\prime} = -52.6 \text{ kcal/mol}$$

$$FADH_2 + \tfrac{1}{2}O_2 \longrightarrow FAD + H_2O$$

$$\Delta G^{\circ\prime} = -43.4 \text{ kcal/mol}$$

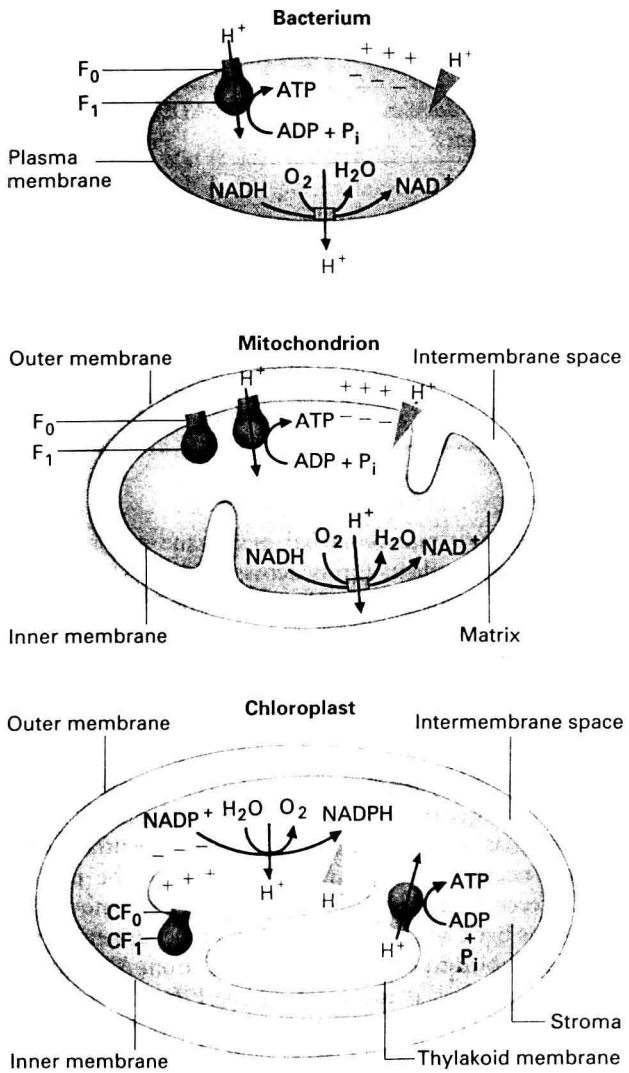
As the negative $\Delta G^{\circ\prime}$ values indicate, the oxidation of these reduced coenzymes by $O_2$ are strongly exergonic reactions. More importantly, most of the free energy released during the oxidation of glucose to $CO_2$ is retained in the reduced coenzymes generated during glycolysis and the citric acid cycle. To see this, recall the large total change in standard free energy for the oxidation of glucose to $CO_2$ ($\Delta G^{\circ\prime} = -680$ kcal/mol). The oxidation of the 10 NADH and 2 $FADH_2$ molecules yields an almost equivalent change of $\Delta G^{\circ\prime} = 10\,(-52.6) + 2\,(-43.4) = -613$ kcal/mol of glucose. Thus over 90 percent of the potential free energy present in the glucose bonds that become oxidized is conserved in the reduced coenzymes. The reoxidation of these coenzymes by $O_2$ generates the vast majority of ATP phosphoanhydride bonds.

The free energy released during the oxidation of a single NADH or $FADH_2$ molecule by $O_2$ is sufficient to drive the synthesis of several molecules of ATP from ADP and $P_i$ ($\Delta G^{\circ\prime} = +7.3$ kcal/mol for the reaction ADP + $P_i \rightarrow$ ATP). Thus it is not surprising that NADH oxidation and ATP synthesis do not occur in a single reaction. Rather, a step-by-step transfer of electrons from NADH to $O_2$, via the electron transport chain, allows the free energy to be released in small increments. At several sites during electron transport from NADH to $O_2$, protons are transported across the inner mitochondrial membrane and a proton concentration gradient forms across it. Because the outer membrane is freely permeable to protons, the pH of the mitochondrial matrix is higher (i.e., the proton concentration is lower) than that of the cytosol and intermembrane space (see Figure 17-9). An electric potential across the inner membrane also results from the pumping of positively charged protons outward from the matrix, which becomes negative with respect to the intermembrane space. Thus free energy released during the oxidation of NADH or $FADH_2$ is stored both as an electric potential and a proton concentration gradient—collectively, the proton-motive force—across the inner membrane. The movement of protons back across the inner membrane, driven by this force, is coupled to the synthesis of ATP from ADP and $P_i$.

ATP synthesis is catalyzed by the $F_0F_1$ ATPase complex, also called the ATP synthase, a member of the F class of ATP-powered proton pumps (see Figure 15-10). Such complexes form the knob-like particles that protrude from the inner mitochondrial membrane (see Figure 17-9).

## A Similar Electrochemical Proton Gradient Is Used to Generate ATP from ADP and $P_i$ in Mitochondria, Bacteria, and Chloroplasts

Bacteria lack mitochondria, yet aerobic bacteria carry out the same processes of oxidative phosphorylation that occur in eukaryotic mitochondria. Bacterial enzymes that catalyze the reactions of both the glycolytic pathway and the citric acid cycle are localized to the bacterial cytosol; enzymes that oxidize NADH to $NAD^+$ and transfer the electrons to the ultimate acceptor $O_2$ are localized to the bacterial plasma membrane. The movement of electrons through these membrane carriers is coupled to the pumping of protons out of the cell (Figure 17-14). The movement of protons back into the cell, down their concentration gradient, is coupled to the synthesis of ATP; the membrane proteins involved in ATP synthesis are essentially identical in structure and function to those of the




▲ FIGURE 17-14 Membrane orientation and the direction of proton movement in bacteria, mitochondria, and chloroplasts. The membrane surface facing a shaded area is a cytosolic face; the surface facing an unshaded area is an exoplasmic face. In bacteria, mitochondria, and chloroplasts, the $F_0F_1$ complexes always face the cytosolic face of the membrane. During electron transport, protons are always moved from the cytosolic face to the exoplasmic face, creating a proton concentration gradient (exoplasmic face > cytosolic face) and an electric potential (negative cytosolic face) across the membrane. During the generation of ATP, protons flow in the reverse direction (down their electrochemical proton gradient) through the $F_0F_1$ complexes.

mitochondrial $F_0F_1$ ATPase complex, which we examine in some detail later in this chapter. The proton-motive force across the bacterial plasma membrane is also used to power the uptake of nutrients such as sugars (see Figure 15-23). Chloroplasts also utilize an $F_0F_1$ complex to synthesize ATP; these particles are localized to the thylakoid membranes (see Figure 17-14).

Recall that every cellular membrane has a cytosolic face and an exoplasmic face. In chloroplasts, mitochondria, and bacteria, the $F_0F_1$ ATPase complex is always positioned so that the globular $F_1$ segment is on the cytosolic face. The $F_1$ segment catalyzes ATP synthesis from ADP and $P_i$. Thus in all cases, ATP is formed on the cytosolic face of the membrane. In the mitochondrion, for instance, ATP is synthesized on the matrix side of the inner membrane. (Note that the cytosolic face is toward the matrix or stroma, respectively, in the inner mitochondrial membrane and the thylakoid membrane.)

Protons always flow through the $F_0F_1$ ATPase complex across the membrane from the exoplasmic to the cytosolic face, driven by a combination of the proton concentration gradient (exoplasmic face > cytosolic face) and the membrane electric potential (the cytosolic face is negative with respect to the exoplasmic face). The polarity of this proton gradient and membrane potential is established by electron transport. During electron transport in mitochondria, protons are translocated from the cytosolic face to the exoplasmic face (see Figure 17-14), since the direction of proton movement is from the matrix to the intermembrane space. Similarly, in aerobic bacteria, the oxidation of carbohydrates is coupled to electron transport and to translocation of protons from the cytosol into the external medium.

During oxidative phosphorylation in both bacteria and mitochondria, the movement of electrons through the membrane is coupled to the generation of a proton-motive force, making the processes of electron transport, proton pumping, and ATP formation interdependent. The generation of this electrochemical proton gradient and its simultaneous dissipation in ATP formation normally occur and the processes are closely coupled.

Through the combined efforts of biochemists, biophysicists, microscopists, and geneticists, much has been learned about the process of oxidative phosphorylation. The following sections describe the way in which a proton concentration gradient is used to generate ATP in oxidative phosphorylation and the ways in which electron transport is coupled to the generation of a proton-motive force.

## ➢ *The Proton-Motive Force, ATP Generation, and Transport of Metabolites*

### Closed Vesicles Are Required for the Generation of ATP

Much evidence shows that in mitochondria the coupling between the oxidation of NADH and $FADH_2$ by $O_2$ and the synthesis of ATP from ADP and $P_i$ occurs only via the electrochemical proton gradient across the inner membrane. In the laboratory, adding oxygen and an oxidizable substrate such as pyruvate or succinate to isolated intact

mitochondria results in a net synthesis of ATP. However, ATP production is absolutely dependent on the integrity of the inner mitochondrial membrane. In the presence of minute amounts of detergents that make the membrane leaky, the oxidation of these metabolites by $O_2$ still occurs, but no ATP is made. Under these conditions, no transmembrane proton concentration gradient or membrane electric potential can be maintained.

## The Proton-Motive Force Is Composed of a Proton Concentration Gradient and a Membrane Electric Potential

The proton-motive force that propels protons down their electrochemical gradient is a combination, or sum, of the proton concentration gradient and the membrane electric potential. A similar combination of forces drives the movement of $Na^+$ ions into a cell (see Figure 15-9).

Since protons are positively charged, moving them across the membrane generates an electric potential only if the membrane is poorly permeable to other cations and to anions. A proton concentration (pH) gradient can develop only if the membrane is permeable to a major anion, such as $Cl^-$ (chloride), or if the protons are exchanged for another cation, such as $K^+$ (potassium). In the latter case, proton movement does not lead to a difference in electric potential across the membrane because there is always an equal concentration of positive and negative ions on each side of the membrane. However, proton movement does produce a pH gradient across the membrane, making the proton concentration gradient the major component of the proton-motive force in such cases. This occurs in the chloroplast thylakoid membrane during photosynthesis (Chapter 18). By contrast, the mitochondrial inner membrane is relatively impermeable to both anions and cations: a greater proportion of energy is stored as a membrane electric potential, and the actual pH gradient is smaller. In respiring mitochondria, the electric potential across the inner membrane is about 200 mV (the matrix being negative with respect to the intermembrane space), so that the membrane electric potential is the more significant component of the proton-motive force.

Since a difference of one pH unit represents a tenfold difference in $H^+$ concentration, a pH gradient of one unit across a membrane is equivalent to an electric potential of 59 mV (at 20° C). Thus we can define the proton-motive force, pmf, as

$$\text{pmf} = \Psi - \left(\frac{RT}{\mathcal{F}}\right) \cdot \Delta\text{pH} = \Psi - 59\Delta\text{pH}$$

where R is the gas constant of 1.987 cal/(degree-mol); T is the temperature (in degrees Kelvin); $\mathcal{F}$ is the Faraday constant (23,062 cal $\cdot$ $V^{-1}$ $\cdot$ $mol^{-1}$); and $\Psi$ is the transmembrane electric potential; $\Psi$ and pmf are measured in millivolts. In respiring mitochondria, pmf $\simeq$ 220 mV; $\Psi \simeq 160$ mV, and a pH of one unit (equivalent to $\sim$60 mV) accounts for the remaining pmf.

Mitochondria and chloroplasts are much too small to be impaled with electrodes, so how can the electric potential and the pH gradient across the inner mitochondrial membrane be determined? The inner membrane is normally impermeable to potassium ions, but the antibiotic valinomycin (see Figure 15-6) is a potassium ionophore: a lipid-soluble peptide that selectively binds one $K^+$ ion in its hydrophilic interior, thus allowing $K^+$ ions to be transported across the otherwise impermeable phospholipid membrane (see Figure 17-28c). At equilibrium, the concentration of $K^+$ ions on both sides of the membrane is determined by the membrane electric potential, $E$ (in mV), according to the Nernst equation (page 642):

$$E = -\frac{RT}{\mathcal{F}} \ln \frac{K_{in}}{K_{out}} = -59 \log \frac{K_{in}}{K_{out}}$$

When trace amounts of valinomycin and radioactive potassium ($^{42}K^+$) ions are added to a suspension of respiring mitochondria, oxidative phosphorylation proceeds and is largely unaffected. The $^{42}K^+$ ions accumulate inside the mitochondria in a $K_{matrix}$:$K_{cytosol}$ ratio of about 2500. By the Nernst equation

$$E = -59 \log \frac{K_{in}}{K_{out}} = -59 \log 2500 = -200 \text{ mV}$$

Thus the electric potential across the inner membrane is $\sim$200 mV (negative inside matrix).

The fluorescent properties of a number of dyes are dependent on pH (Chapter 5). By trapping such dyes inside inner-membrane vesicles of mitochondria, we can measure the inside pH during oxidative phosphorylation; as noted, the matrix pH is typically one unit higher than that of the cytosol.

## The $F_0F_1$ Complex Couples ATP Synthesis to Proton Movement down the Electrochemical Gradient

The $F_0F_1$ ATPase complex (Figure 17-15) couples proton movement down its electrochemical gradient with the synthesis of ATP from ADP and $P_i$. The enzyme complex has two principal components: $F_0$ and $F_1$.

The integral membrane complex $F_0$ contains three types of subunits, *a*, *b*, and *c*. In bacteria, these have the composition $a_1b_2c_{10}$. Each *a* subunit is thought to span the membrane eight times, each *b* once, and each *c* twice. In mitochondria, each $F_0$ complex also contains, depending on the species, two to five additional peptides of unknown function (Table 17-2).