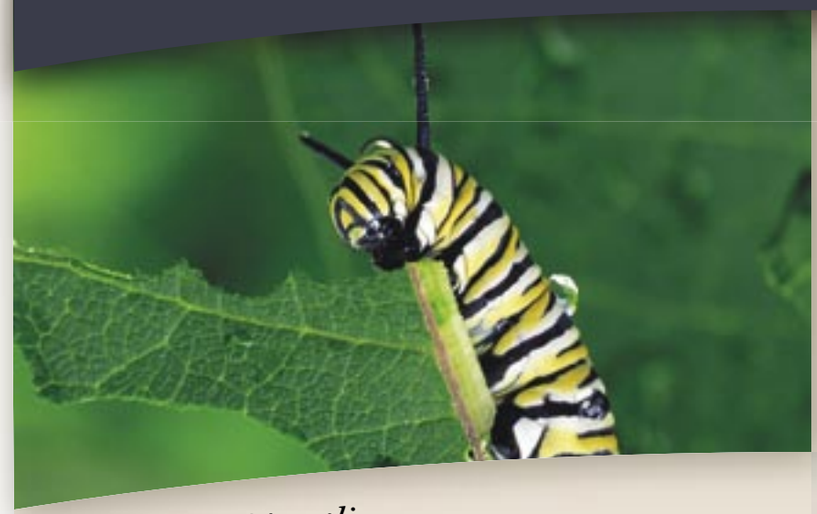


chapter 7

How Cells Harvest Energy

concept outline

introduction

LIFE IS DRIVEN BY ENERGY. All the activities organisms carry out—the swimming of bacteria, the purring of a cat, your thinking about these words—use energy. In this chapter, we discuss the processes all cells use to derive chemical energy from organic molecules and to convert that energy to ATP. Then, in chapter 8, we will examine photosynthesis, which uses light energy to make chemical energy. We consider the conversion of chemical energy to ATP first because all organisms, both the plant, a photosynthesizer, and the caterpillar feeding on the plant, pictured in the photo are capable of harvesting energy from chemical bonds. Energy harvest via respiration is a universal process.

7.1 Overview of Respiration

Plants, algae, and some bacteria harvest the energy of sunlight through photosynthesis, converting radiant energy into chemical energy. These organisms, along with a few others that use chemical energy in a similar way, are called **autotrophs** ("self-feeders"). All other organisms live on the organic compounds autotrophs produce, using them as food, and are called **heterotrophs** ("fed by others"). At least 95% of the kinds of organisms on Earth—all animals and fungi, and most protists and prokaryotes—are heterotrophs. Autotrophs also extract energy from organic compounds—they just have the additional capacity to use the energy from sunlight to synthesize these compounds. The process by which energy is harvested is **cellular respiration**—the oxidation of organic compounds to extract energy from chemical bonds.

Cells oxidize organic compounds to drive metabolism

Most foods contain a variety of carbohydrates, proteins, and fats, all rich in energy-laden chemical bonds. Carbohydrates and fats, as you recall from chapter 3, possess many carbon–hydrogen (C—H) bonds, as well as carbon–oxygen (C—O) bonds.

The job of extracting energy from the complex organic mixture in most foods is tackled in stages. First, enzymes break down the large molecules into smaller ones, a process called **digestion** (chapter 48). Then, other enzymes dismantle these fragments a little at a time, harvesting energy from C—H and other chemical bonds at each stage.

The reactions that break down these molecules share a common feature: They are oxidations. Energy metabolism is therefore concerned with redox reactions, and to understand the process we must follow the fate of the electrons lost from the food molecules.

These reactions are not the simple transfer of electrons, however; they are also **dehydrogenations.** That is, the electrons lost are accompanied by protons, so that what is really lost is a hydrogen atom, and not just an electron.

Cellular respiration is the complete oxidation of glucose

In chapter 6, you learned that an atom that loses electrons is said to be *oxidized*, and an atom accepting electrons is said to be *reduced*. Oxidation reactions are often coupled with reduction reactions in living systems, and these paired reactions are called *redox reactions*. Cells utilize enzyme-facilitated redox reactions to take energy from food sources and convert it to ATP.

Redox reactions

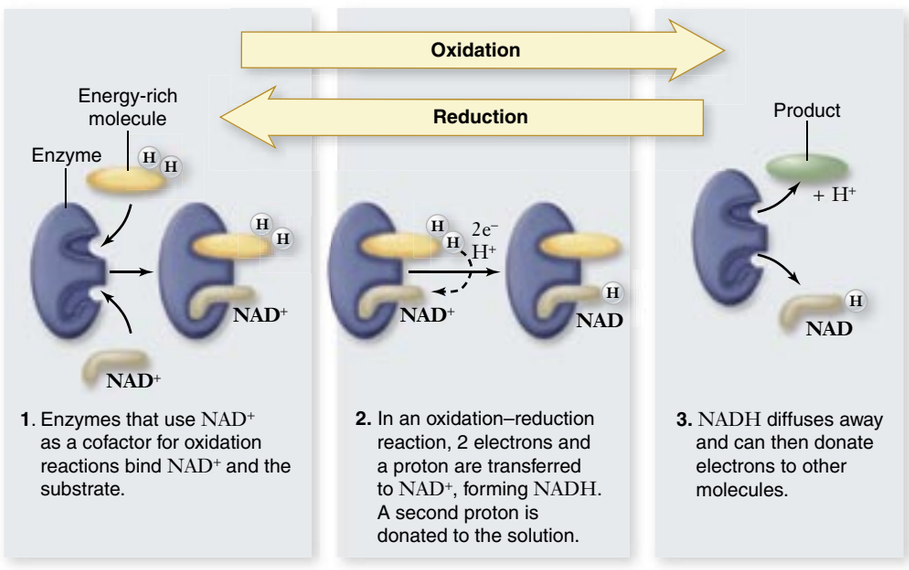
Oxidation–reduction reactions play a key role in the flow of energy through biological systems because the electrons that pass from one atom to another carry energy with them. The amount of energy an electron possesses depends on its orbital position, or energy level, around the atom's nucleus. When this electron departs from one atom and moves to another in a redox reaction, the electron's energy is transferred with it.

Figure 7.1 shows how an enzyme catalyzes a redox reaction involving an energy-rich substrate molecule, with the help of a cofactor, **nicotinamide adenine dinucleotide (NAD^+).** In this reaction, NAD^+ accepts a pair of electrons from the substrate, along with a proton, to form **NADH** (this process is described in more detail shortly). The oxidized product is now released from the enzyme's active site, as is NADH.

In the overall process of cellular energy harvest, dozens of redox reactions take place, and a number of molecules, including NAD^+, act as electron acceptors. During each transfer of electrons energy is released. This energy may be captured and used to make ATP or to form other chemical bonds; the rest is lost as heat.

figure 7.1

OXIDATION–REDUCTION REACTIONS OFTEN EMPLOY COFACTORS. Cells use a chemical cofactor called NAD^+ to carry out many oxidation–reduction reactions. Two electrons and a proton are transferred to NAD^+ with another proton donated to the solution. Molecules that gain energetic electrons are said to be reduced, while ones that lose energetic electrons are said to be oxidized. NAD^+ oxidizes energy-rich molecules by acquiring their electrons (in the figure, this proceeds 1 ⟶ 2 ⟶ 3) and then reduces other molecules by giving the electrons to them (in the figure, this proceeds 3 ⟶ 2 ⟶ 1).



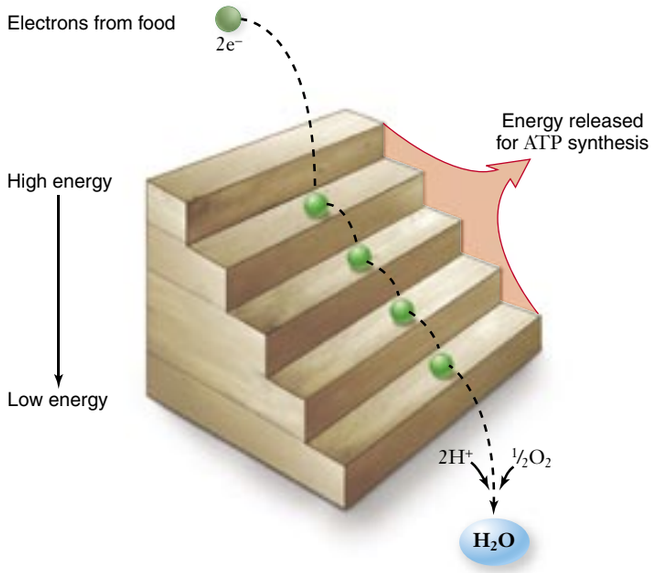


figure 7.2

HOW ELECTRON TRANSPORT WORKS. This diagram shows how ATP is generated when electrons transfer from one energy level to another. Rather than releasing a single explosive burst of energy, electrons "fall" to lower and lower energy levels in steps, releasing stored energy with each fall as they tumble to the lowest (most electronegative) electron acceptor, O_2.

At the end of this process, high-energy electrons from the initial chemical bonds have lost much of their energy, and these depleted electrons are transferred to a final electron acceptor (figure 7.2). When this acceptor is oxygen, the process is called **aerobic respiration.** When the final electron acceptor is an inorganic molecule other than oxygen, the process is called **anaerobic respiration,** and when it is an organic molecule, the process is called **fermentation.**

"Burning" carbohydrates

Chemically, there is little difference between the catabolism of carbohydrates in a cell and the burning of wood in a fireplace. In both instances, the reactants are carbohydrates and oxygen, and the products are carbon dioxide, water, and energy:

$$\underset{\textit{glucose}}{C_6H_{12}O_6} + 6\,\underset{\textit{oxygen}}{O_2} \longrightarrow 6\,\underset{\textit{carbon dioxide}}{CO_2} + 6\,\underset{\textit{water}}{H_2O} + \text{energy (heat and ATP)}$$

The change in free energy in this reaction is –686 kcal/mol (or –2870 kJ/mol) under standard conditions (that is, at room temperature, 1 atm pressure, and so forth). In the conditions that exist inside a cell, the energy released can be as high as –720 kcal/mol (–3012 kJ/mol) of glucose. This means that under actual cellular conditions, more energy is released than under standard conditions.

The same amount of energy is released whether glucose is catabolized or burned, but when it is burned, most of the energy is released as heat. Cells harvest useful energy from the catabolism of glucose by using a portion of the energy to drive the production of ATP.

Electron carriers play a critical role in energy metabolism

During respiration, glucose is oxidized to CO_2, but if the electrons were given directly to O_2, the reaction would be combustion, and cells would burst into flames. Instead, as you have just seen, the cell transfers the electrons to intermediate electron carriers, then eventually to O_2.

Many forms of electron carriers are used in this process: soluble carriers that move electrons from one molecule to another, membrane-bound carriers that form a redox chain, and carriers that move within the membrane. The common feature of all of these carriers is that they can be reversibly oxidized and reduced. Some of these carriers, such as the iron-containing cytochromes, can carry just electrons, and some carry both electrons and protons.

NAD^+ is one of the most important electron (and proton) carriers. As shown on the left in figure 7.3, the NAD^+

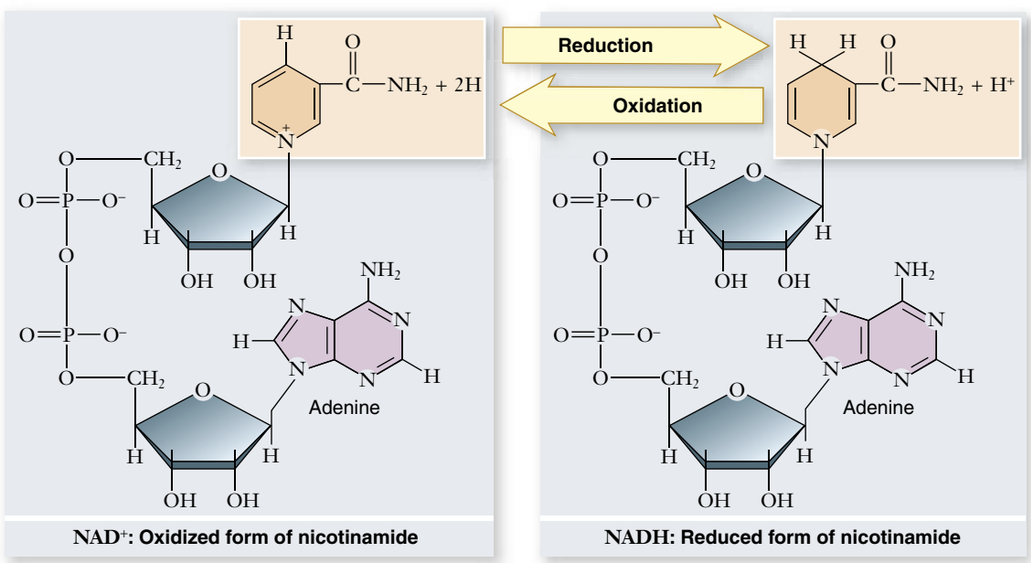


figure 7.3

NAD⁺ AND NADH. This dinucleotide serves as an "electron shuttle" during cellular respiration. NAD^+ accepts a pair of electrons and a proton from catabolized macromolecules and is reduced to NADH.

molecule is composed of two nucleotides bound together. The two nucleotides that make up NAD^+, nicotinamide monophosphate (NMP) and adenine monophosphate (AMP), are joined head-to-head by their phosphate groups. The two nucleotides serve different functions in the NAD^+ molecule: AMP acts as the core, providing a shape recognized by many enzymes; NMP is the active part of the molecule, as it is readily reduced, that is, easily accepts electrons.

When NAD^+ acquires two electrons and a proton from the active site of an enzyme, it is reduced to NADH, shown on the right in figure 7.3. The NADH molecule now carries the two energetic electrons and can supply them to other molecules and reduce them.

This ability to supply high-energy electrons is critical to both energy metabolism and to the biosynthesis of many organic molecules, including fats and sugars. In animals, when ATP is plentiful, the reducing power of the accumulated NADH is diverted to supplying fatty acid precursors with high-energy electrons, reducing them to form fats and storing the energy of the electrons.

Metabolism harvests energy in stages

It is generally true that the larger the release of energy in any single step, the more of that energy is released as heat, and the less is available to be channeled into more useful paths. In the combustion of gasoline, the same amount of energy is released whether all of the gasoline in a car's gas tank explodes at once, or burns in a series of very small explosions inside the cylinders. By releasing the energy in gasoline a little at a time, the harvesting efficiency is greater, and more of the energy can be used to push the pistons and move the car.

The same principle applies to the oxidation of glucose inside a cell. If all of the electrons were transferred to oxygen in one explosive step, releasing all of the free energy at once, the cell would recover very little of that energy in a useful form. Instead, cells burn their fuel much as a car does, a little at a time.

The electrons in the C—H bonds of glucose are stripped off in stages in the series of enzyme-catalyzed reactions collectively referred to as glycolysis and the Krebs cycle. The electrons are removed by transferring them to NAD^+, as described earlier, or to other electron carriers.

The energy released by all of these oxidation reactions is also not all released at once (see figure 7.2). The electrons are passed to another set of electron carriers called the **electron transport chain,** which is located in the mitochondrial inner membrane. Movement of electrons through this chain produces potential energy in the form of an electrochemical gradient. We examine this process in more detail later in this chapter.

ATP plays a central role in metabolism

The previous chapter introduced ATP as the energy currency of the cell. Cells use ATP to power most of those activities that require work. One of the most obvious activities is movement. Tiny fibers within muscle cells pull against one another when muscles contract. Mitochondria can move a meter or more along the narrow nerve cells that extend from your spine to your feet. Chromosomes are pulled apart by microtubules during cell division. All of these movements require the expenditure of energy by ATP hydrolysis. Cells also use ATP to drive endergonic reactions that would otherwise not occur spontaneously (chapter 6).

How does ATP drive an endergonic reaction? The enzyme that catalyzes a particular reaction has two binding sites on its surface: one for the reactant and another for ATP. The ATP site splits the ATP molecule, liberating over 7 kcal ($\Delta G = -7.3$ kcal/mol) of chemical energy. This energy pushes the reactant at the second site "uphill," reaching the activation energy and driving the endergonic reaction. Thus endergonic reactions coupled to ATP hydrolysis become favorable.

The many steps of cellular respiration have as their ultimate goal the production of ATP. ATP synthesis is itself an endergonic reaction, which requires cells to perform exergonic reactions to drive this synthesis. The details of these reactions are presented in the following sections of this chapter.

Cells require energy to maintain their structure, for growth and metabolism. The process of cellular respiration achieves the complete oxidation of glucose. This process utilizes electron carriers that aid in the gradual release of the energy from the oxidation of glucose. The result of energy metabolism is the synthesis of ATP, which cells use as a portable source of energy.

7.2 The Oxidation of Glucose: A Summary

Cells are able to make ATP from the oxidation of glucose by two fundamentally different mechanisms.

1. In **substrate-level phosphorylation,** ATP is formed by transferring a phosphate group directly to ADP from a phosphate-bearing intermediate, or substrate (figure 7.4). During **glycolysis,** the initial breakdown of glucose (discussed later), the chemical bonds of glucose are shifted around in reactions that provide the energy required to form ATP by substrate-level phosphorylation.
2. In **oxidative phosphorylation,** ATP is synthesized by the enzyme **ATP synthase,** using energy from a proton (H^+) gradient. This gradient is formed by high-energy electrons from the oxidation of glucose passing down an electron transport chain (described later). These electrons, with their energy depleted, are then donated to oxygen, hence the term *oxidative phosphorylation*. ATP synthase uses the energy from the proton gradient to catalyze the reaction:

$$ADP + P_i \longrightarrow ATP$$

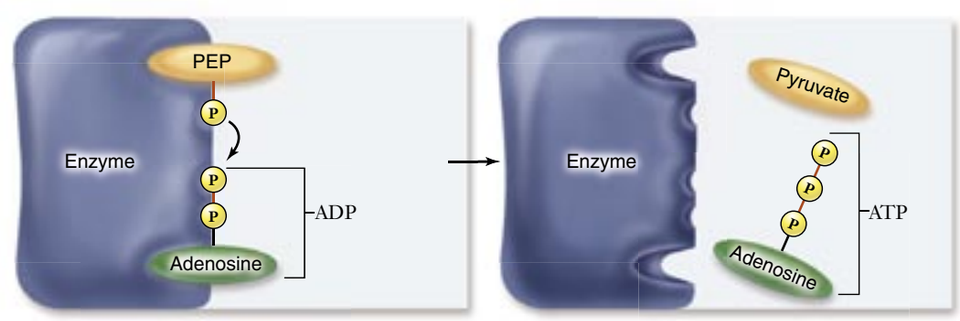


figure 7.4

SUBSTRATE-LEVEL PHOSPHORYLATION. Some molecules, such as phosphoenolpyruvate (PEP), possess a high-energy phosphate bond similar to the bonds in ATP. When PEP's phosphate group is transferred enzymatically to ADP, the energy in the bond is conserved, and ATP is created.

Eukaryotes and aerobic prokaryotes produce the vast majority of their ATP this way.

In most organisms, these two processes are combined. To harvest energy to make ATP from glucose in the presence of oxygen, the cell carries out a complex series of enzyme-catalyzed reactions that occur in four stages: The first stage captures energy by substrate-level phosphorylation through glycolysis; the next three stages carry out aerobic respiration by oxidizing the end-product of glycolysis and producing ATP. In this section, we provide an overview of these stages (figure 7.5); each topic is then discussed in depth in the sections that follow.

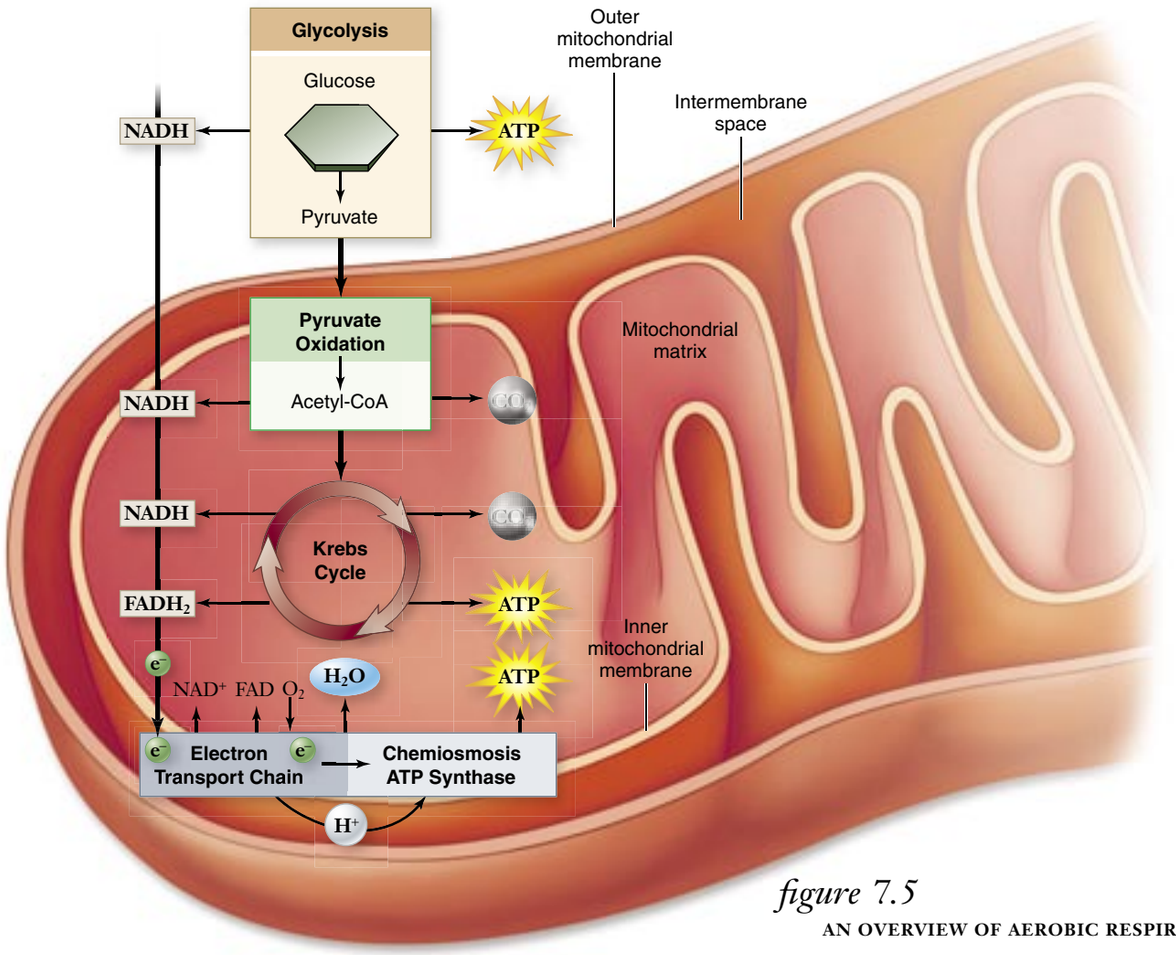


figure 7.5

AN OVERVIEW OF AEROBIC RESPIRATION.

Stage One: Glycolysis The first stage is a 10-reaction biochemical pathway called *glycolysis* that produces ATP by substrate-level phosphorylation. The enzymes that catalyze the glycolytic reactions are in the cytoplasm of the cell, not associated with any membrane or organelle.

For each glucose molecule, two ATP molecules are used up early in the pathway, and four ATP molecules are produced by substrate-level phosphorylation. The net yield is two ATP molecules for each molecule of glucose catabolized. In addition, four electrons are harvested from the chemical bonds of glucose and carried by NADH for oxidative phosphorylation. Glycolysis yields two energy-rich **pyruvate** molecules for each glucose entering the pathway. This remaining energy can be harvested in later stages.

Stage Two: Pyruvate Oxidation In the second stage, pyruvate is converted into carbon dioxide and a two-carbon molecule called **acetyl-CoA.** For each molecule of pyruvate converted, one molecule of NAD^+ is reduced to NADH, again to carry electrons that can be used to make ATP. Remember that two pyruvate molecules result from each glucose.

Stage Three: The Krebs Cycle The third stage introduces acetyl-CoA into a cycle of nine reactions called the **Krebs cycle,** named after the German biochemist Hans Krebs, who discovered it. The Krebs cycle is also called the *citric acid cycle*, for the citric acid, or citrate, formed in its first step, and less commonly, the *tricarboxylic acid cycle*, because citrate has three carboxyl groups.

For each turn of the Krebs cycle, one ATP molecule is produced by substrate-level phosphorylation, and a large number of electrons are removed by the reduction of NAD^+ to NADH and FAD to $FADH_2$. Each glucose provides two acetyl-CoA to the Krebs cycle allowing two turns.

Stage Four: Electron Transport Chain and Chemiosmosis In the fourth stage, energetic electrons carried by NADH are transferred to a series of electron carriers that progressively extract the electrons' energy and use it to pump protons across a membrane.

The proton gradient created by electron transport is used by ATP synthase to produce ATP. This utilization of a proton gradient to drive the synthesis of ATP is called **chemiosmosis** and is the basis for oxidative phosphorylation.

Pyruvate oxidation, the reactions of the Krebs cycle, and ATP production by electron transport chains occur within many forms of prokaryotes and inside the mitochondria of all eukaryotes. Figure 7.5 provides an overview of the complete process of aerobic respiration beginning with glycolysis.

The oxidation of glucose can be broken down into stages. These include glycolysis, which produces pyruvate, the oxidation of pyruvate, and the Krebs cycle. The electrons derived from oxidation reactions are used in the electron transport chain to produce a proton gradient that can be used by the enzyme ATP synthase to make ATP in the process called chemiosmosis.

7.3 Glycolysis: Splitting Glucose

Glucose molecules can be dismantled in many ways, but primitive organisms evolved a glucose-catabolizing process that releases enough free energy to drive the synthesis of ATP in enzyme-coupled reactions. Glycolysis occurs in the cytoplasm and converts glucose into two 3-carbon molecules of pyruvate (figure 7.6). For each molecule of glucose that passes through this transformation, the cell nets two ATP molecules.

Priming changes glucose into an easily cleaved form

The first half of glycolysis consists of five sequential reactions that convert one molecule of glucose into two molecules of the 3-carbon compound **glyceraldehyde 3-phosphate (G3P).** These reactions require the expenditure of ATP, so they are an endergonic process.

Step A: Glucose priming Three reactions "prime" glucose by changing it into a compound that can be cleaved readily into two 3-carbon phosphorylated molecules. Two of these reactions transfer a phosphate from ATP, so this step requires the cell to use two ATP molecules.

Step B: Cleavage and rearrangement In the first of the remaining pair of reactions, the 6-carbon product of step A is split into two 3-carbon molecules. One is G3P, and the other is then converted to G3P by the second reaction (figure 7.7).

ATP is synthesized by substrate-level phosphorylation

In the second half of glycolysis, five more reactions convert G3P into pyruvate in an energy-yielding process that generates ATP.

Step C: Oxidation Two electrons (and one proton) are transferred from G3P to NAD^+, forming NADH. A molecule of P_i is also added to G3P to produce 1,3-bisphosphoglycerate. The phosphate incorporated will later be transferred to ADP by substrate-level phosphorylation to allow a net yield of ATP.

Step D: ATP generation Four reactions convert 1,3-bisphosphoglycerate into pyruvate. This process generates two ATP molecules per G3P (see figures 7.4 and 7.7) produced in Step B.

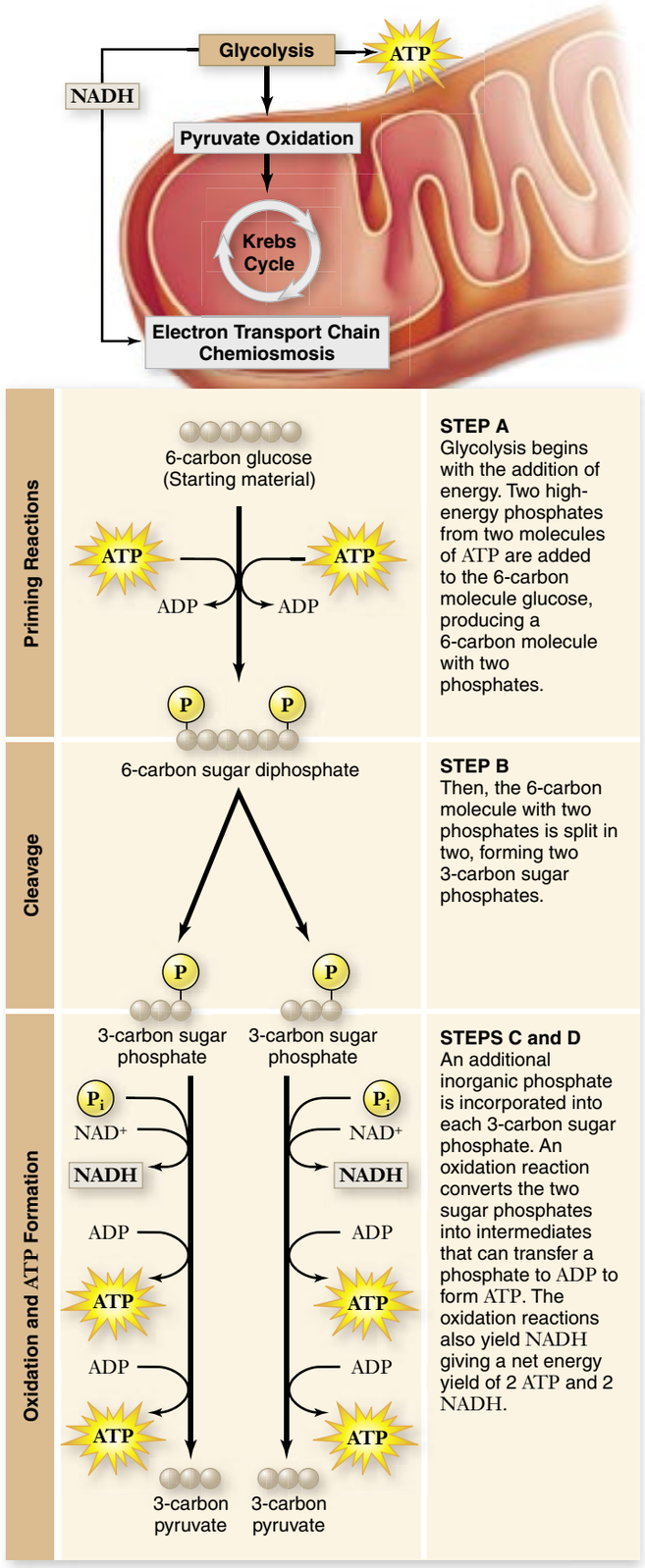


figure 7.6
HOW GLYCOLYSIS WORKS.

Because each glucose molecule is split into two G3P molecules, the overall reaction sequence has a net yield of two molecules of ATP, as well as two molecules of NADH and two of pyruvate:

4 ATP (2 ATP for each of the 2 G3P molecules in step D)
– 2 ATP (used in the two reactions in step A)

2 ATP (net yield for entire process)

The hydrolysis of one molecule of ATP yields a ΔG of –7.3 kcal/mol under standard conditions. Thus cells harvest a maximum of 14.6 kcal of energy per mole of glucose from glycolysis.

A brief history of glycolysis

Although far from ideal in terms of the amount of energy it releases, glycolysis does generate ATP. For more than a billion years during the anaerobic first stages of life on Earth, glycolysis was the primary way heterotrophic organisms generated ATP from organic molecules.

Like many biochemical pathways, glycolysis is believed to have evolved backward, with the last steps in the process being the most ancient. Thus, the second half of glycolysis, the ATP-yielding breakdown of G3P, may have been the original process. The synthesis of G3P from glucose would have appeared later, perhaps when alternative sources of G3P were depleted.

Why does glycolysis take place in modern organisms, since its energy yield in the absence of oxygen is comparatively little? The answer is that evolution is an incremental process: Change occurs by improving on past successes. In catabolic metabolism, glycolysis satisfied the one essential evolutionary criterion—it was an improvement. Cells that could not carry out glycolysis were at a competitive disadvantage, and only cells capable of glycolysis survived. Later improvements in catabolic metabolism built on this success. Metabolism evolved as one layer of reactions added to another. Nearly every present-day organism carries out glycolysis, as a metabolic memory of its evolutionary past.

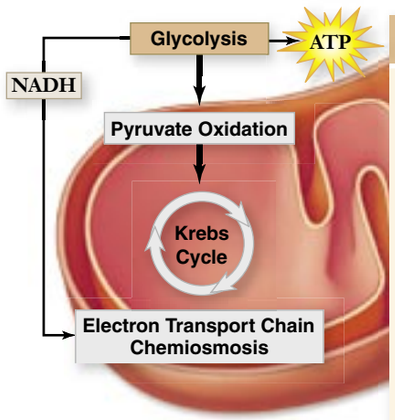
The last section of this chapter discusses the evolution of metabolism in more detail.

NADH must be recycled to continue respiration

Inspect for a moment the net reaction of the glycolytic sequence:

$$\text{glucose} + 2\ \text{ADP} + 2\ P_i + 2\ \text{NAD}^+ \longrightarrow 2\ \text{pyruvate} + 2\ \text{ATP} + 2\ \text{NADH} + 2\ \text{H}^+ + 2\ \text{H}_2\text{O}$$

You can see that three changes occur in glycolysis: (1) Glucose is converted into two molecules of pyruvate; (2) two molecules of ADP are converted into ATP via substrate-level phosphorylation; and (3) two molecules of NAD^+ are reduced to NADH. This leaves the cell with two problems: extracting the energy that remains in the two pyruvate molecules, and regenerating NAD^+ to be able to continue glycolysis.



1. Phosphorylation of glucose by ATP.

2–3. Rearrangement, followed by a second ATP phosphorylation.

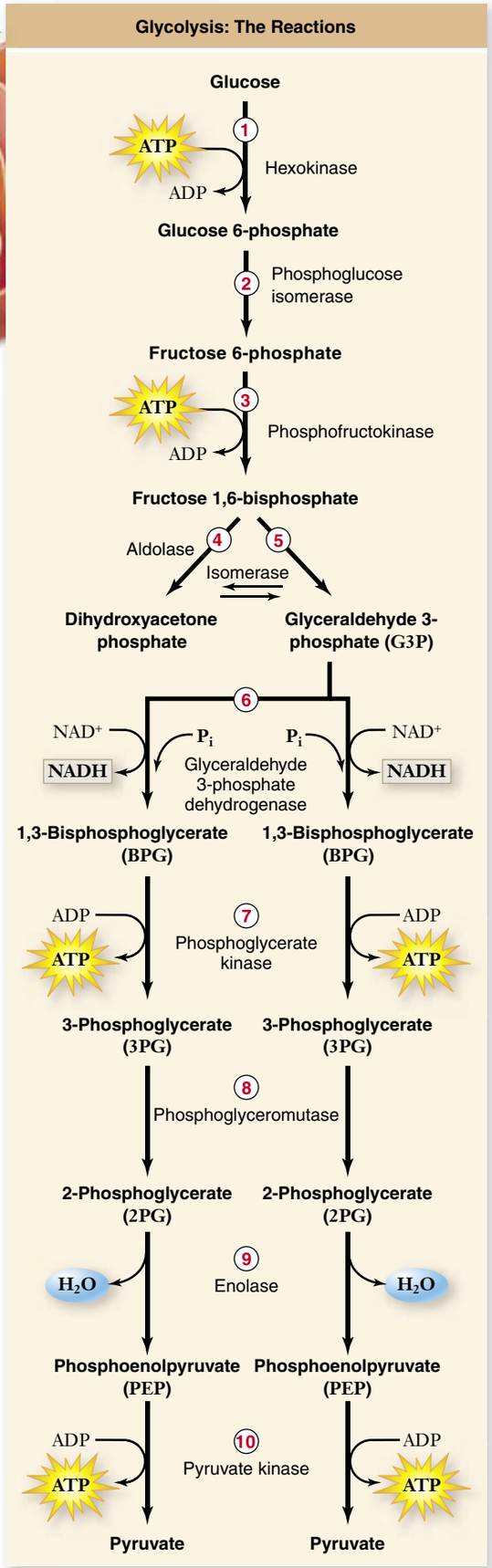
4–5. The 6-carbon molecule is split into two 3-carbon molecules—one G3P, another that is converted into G3P in another reaction.

6. Oxidation followed by phosphorylation produces two NADH molecules and two molecules of BPG, each with one high-energy phosphate bond.

7. Removal of high-energy phosphate by two ADP molecules produces two ATP molecules and leaves two 3PG molecules.

8–9. Removal of water yields two PEP molecules, each with a high-energy phosphate bond.

10. Removal of high-energy phosphate by two ADP molecules produces two ATP molecules and two pyruvate molecules.



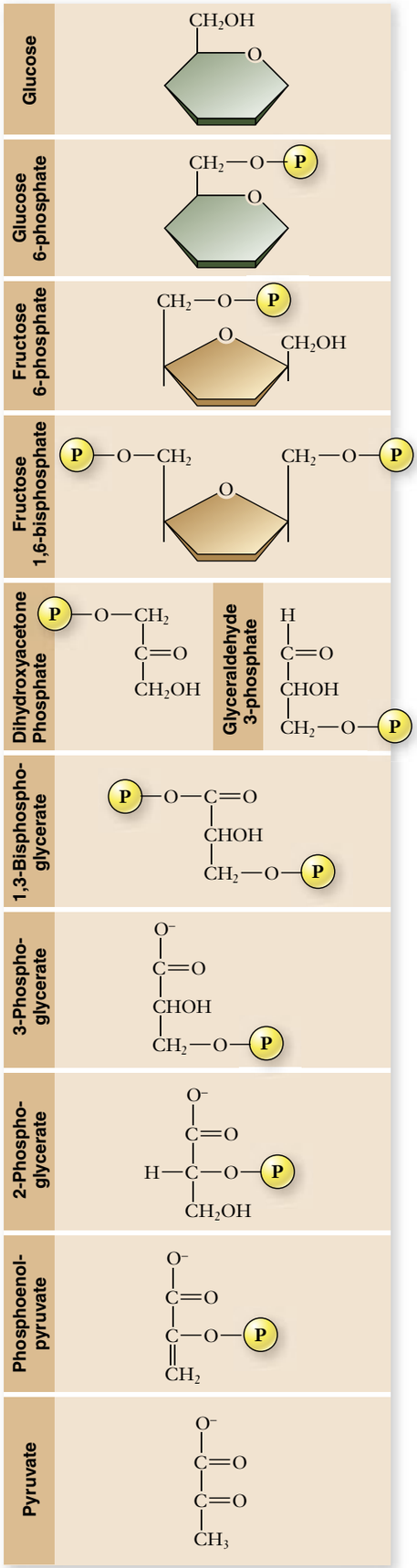


figure 7.7

THE GLYCOLYTIC PATHWAY. The first five reactions convert a molecule of glucose into two molecules of G3P. The second five reactions convert G3P into pyruvate.

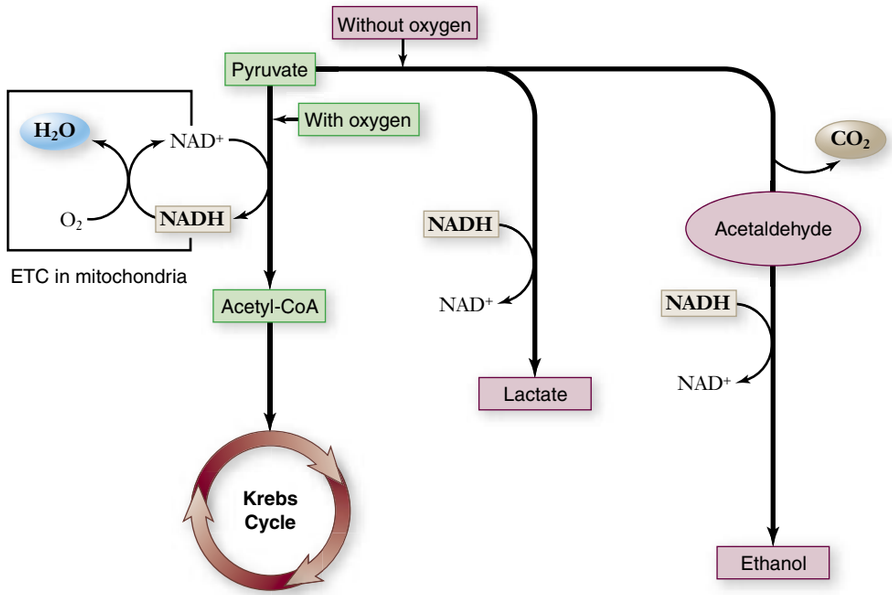


figure 7.8

THE FATE OF PYRUVATE AND NADH PRODUCED BY GLYCOLYSIS. In the presence of oxygen, NADH is oxidized by the electron transport chain (ETC) in mitochondria using oxygen as the final electron acceptor. This regenerates NAD^+ allowing glycolysis to continue. The pyruvate produced by glycolysis is oxidized to acetyl-CoA, which enters the Krebs cycle. In the absence of oxygen, pyruvate is instead reduced, oxidizing NADH and regenerating NAD^+ to allow glycolysis to continue. Direct reduction of pyruvate, as in muscle cells, produces lactate. In yeast, carbon dioxide is first removed from pyruvate producing acetaldehyde, which is then reduced to ethanol.

Recycling NADH

As long as food molecules that can be converted into glucose are available, a cell can continually churn out ATP to drive its activities. In doing so, however, it accumulates NADH and depletes the pool of NAD^+ molecules. A cell does not contain a large amount of NAD^+, and for glycolysis to continue, NADH must be recycled into NAD^+. Some molecule other than NAD^+ must ultimately accept the electrons taken from G3P and be reduced. Two processes can carry out this key task (figure 7.8):

1. **Aerobic respiration.** Oxygen is an excellent electron acceptor. Through a series of electron transfers, electrons taken from G3P can be donated to oxygen, forming water. This process occurs in the mitochondria of eukaryotic cells in the presence of oxygen. Because air is rich in oxygen, this process is also referred to as *aerobic metabolism.* A significant amount of ATP is also produced.
2. **Fermentation.** When oxygen is unavailable, an organic molecule, such as acetaldehyde in wine fermentation, can accept electrons instead (figure 7.9). This reaction plays an important role in the metabolism of most organisms, even those capable of aerobic respiration.

figure 7.9

HOW WINE IS MADE. The conversion of pyruvate to ethanol takes place naturally in grapes left to ferment on vines, as well as in fermentation vats of crushed grapes. Yeasts carry out the process to continue glycolysis under anaerobic conditions. When their conversion increases the ethanol concentration to about 12%, the toxic effects of the alcohol kill the yeast cells. What is left is wine.

The fate of pyruvate

The fate of the pyruvate that is produced by glycolysis depends on which of these two processes takes place. The aerobic respiration path starts with the oxidation of pyruvate to produce acetyl-CoA, which is then further oxidized in a series of reactions called the Krebs cycle. The fermentation path, by contrast, uses the reduction of all or part of pyruvate to oxidize NADH back to NAD^+. We examine aerobic respiration next; fermentation is described in detail in a later section.

Glycolysis splits the 6-carbon molecule glucose into two 3-carbon molecules of pyruvate. This process requires first using two ATP molecules in "priming" reactions eventually producing four molecules of ATP per glucose for a net yield of two ATP. The oxidation reactions of glycolysis require NAD^+ and produce NADH. This NAD^+ must be regenerated either by oxidation in the electron transport chain using O_2, or by using an organic molecule in a fermentation reaction.

7.4 The Oxidation of Pyruvate to Produce Acetyl-CoA

In the presence of oxygen, the oxidation of glucose that begins in glycolysis continues where glycolysis leaves off—with pyruvate. In eukaryotic organisms, the extraction of additional energy from pyruvate takes place exclusively inside mitochondria. In prokaryotes similar reactions take place in the cytoplasm and at the plasma membrane.

The cell harvests pyruvate's considerable energy in two steps. First, pyruvate is oxidized to produce a two-carbon compound and CO_2, with the electrons transferred to NAD^+ to produce NADH. Next, the two-carbon compound is oxidized to CO_2 by the reactions of the Krebs cycle.

Pyruvate is oxidized in a "decarboxylation" reaction that cleaves off one of pyruvate's three carbons. This carbon departs as CO_2 (figure 7.10). The remaining two-carbon compound, called an acetyl group, is then attached to coenzyme A; this entire molecule is called *acetyl-CoA*. A pair of electrons and one associated proton is transferred to the electron carrier NAD^+, reducing it to NADH, with a second proton donated to the solution.

The reaction involves three intermediate stages, and it is catalyzed within mitochondria by a *multienzyme complex*. As chapter 6 noted, a multienzyme complex organizes a series of enzymatic steps so that the chemical intermediates do not diffuse away or undergo other reactions. Within the complex, component polypeptides pass the substrates from one enzyme to the next, without releasing them. *Pyruvate dehydrogenase*, the complex of enzymes that removes CO_2 from pyruvate, is one of the largest enzymes known; it contains 60 subunits! The reaction can be summarized as:

$$\text{pyruvate} + NAD^+ + CoA \longrightarrow \text{acetyl-CoA} + NADH + CO_2 + H^+$$

The molecule of NADH produced is used later to produce ATP. The acetyl group is fed into the Krebs cycle, with the CoA being recycled for another oxidation of pyruvate. The Krebs cycle then completes the oxidation of the original carbons from glucose.

Pyruvate is oxidized in the mitochondria to produce acetyl-CoA and CO_2. This reaction is a link between glycolysis and the reactions of the Krebs cycle as acetyl-CoA is used by the Krebs cycle.

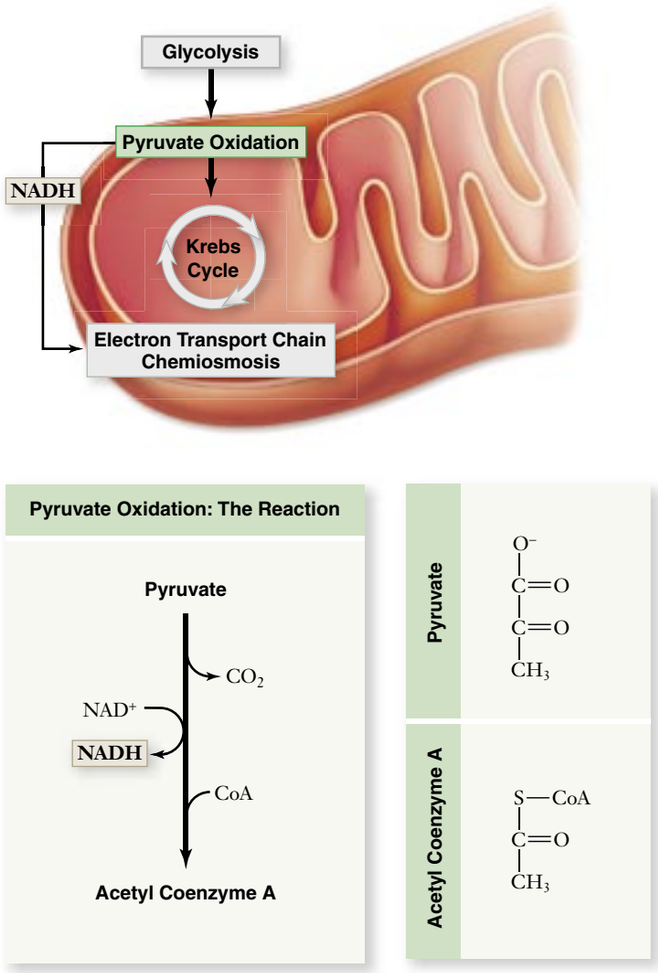


figure 7.10

THE OXIDATION OF PYRUVATE. This complex reaction uses NAD^+ to accept electrons reducing it to NADH. The product, acetyl-CoA, feeds the acetyl unit into the Krebs cycle, and the CoA is recycled for another oxidation of pyruvate. NADH provides energetic electrons for the electron transport chain.

7.5 The Krebs Cycle

In this third stage, the acetyl group from pyruvate is oxidized in a series of nine reactions called the *Krebs cycle*. These reactions occur in the matrix of mitochondria.

In this cycle, the two-carbon acetyl group of acetyl-CoA combines with a four-carbon molecule called oxaloacetate. The resulting six-carbon molecule, citrate, then goes through a several-step sequence of electron-yielding oxidation reactions, during which two CO_2 molecules split off, restoring oxaloacetate. The regenerated oxaloacetate is used to bind to another acetyl group for the next round of the cycle.

In each turn of the cycle, a new acetyl group is added and two carbons are lost as two CO_2 molecules, and more electrons are transferred to electron carriers. These electrons are then used by the electron transport chain to drive *proton pumps* that generate ATP.

The Krebs cycle has three segments: An overview

The nine reactions of the Krebs cycle can be grouped into three overall segments. These are described in the following sections and summarized in figure 7.11.

Segment A: Acetyl-CoA plus oxaloacetate This reaction produces the 6-carbon citrate molecule.

Segment B: Citrate rearrangement and decarboxylation Five more steps, which have been simplified in figure 7.11, reduce citrate to a 5-carbon intermediate and then to 4-carbon succinate. During these reactions, two NADH and one ATP are produced.

Segment C: Regeneration of oxaloacetate Succinate undergoes three additional reactions, also simplified in the figure, to become oxaloacetate. During these reactions, one NADH is produced; in addition, a molecule of **flavin adenine dinucleotide (FAD),** another cofactor, becomes reduced to $FADH_2$.

The specifics of each reaction are described next.

The Krebs cycle is geared to extract electrons and synthesize one ATP

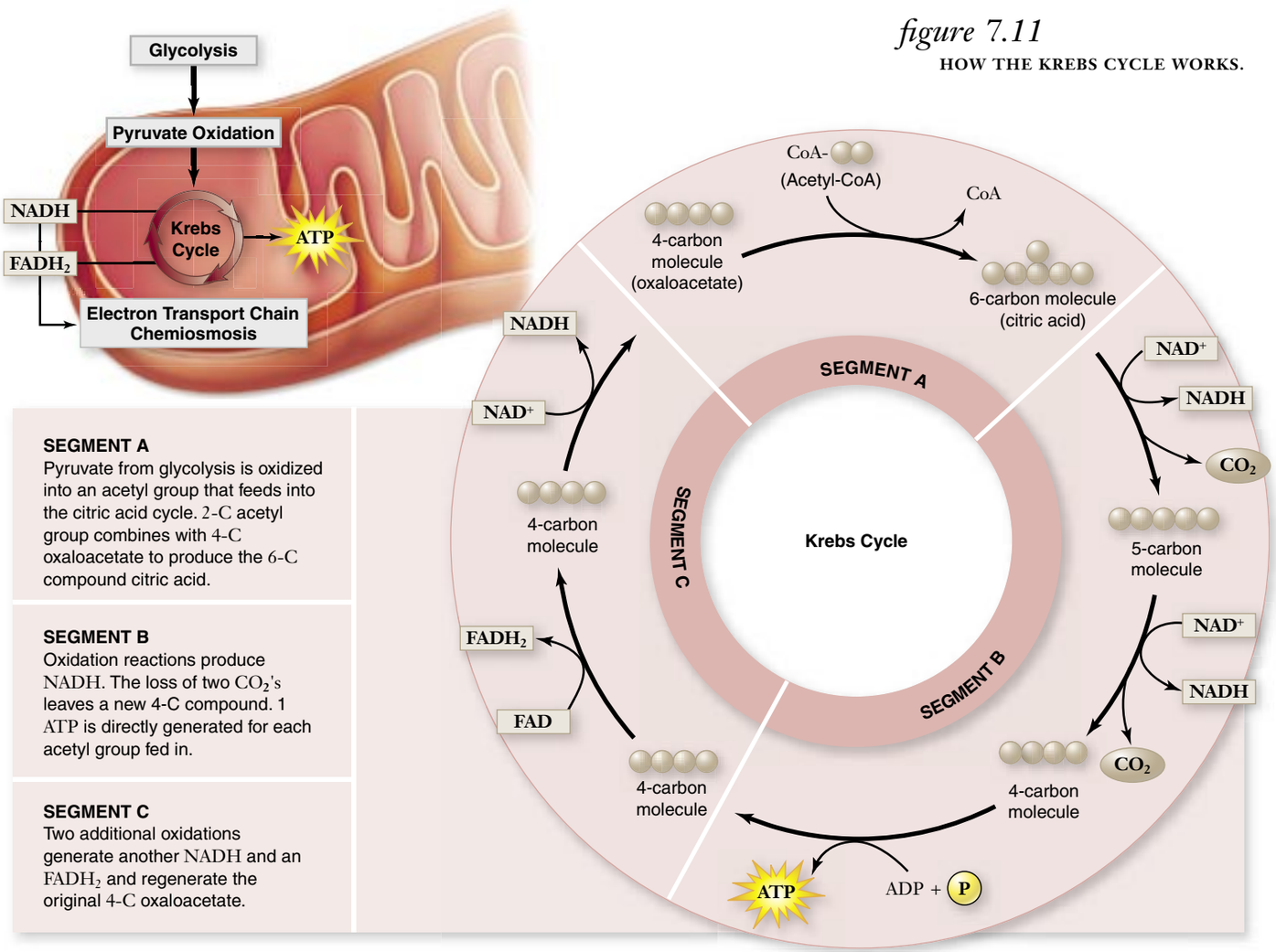
Figure 7.12 summarizes the sequence of the Krebs cycle reactions. A 2-carbon group from acetyl-CoA enters the cycle at the beginning, and two CO_2 molecules, one ATP, and four pairs of electrons are produced.

Reaction 1: Condensation Citrate is formed from acetyl-CoA and oxaloacetate. This condensation reaction is irreversible, committing the 2-carbon acetyl group to the Krebs cycle. The reaction is inhibited when the cell's ATP concentration is high and stimulated when it is low. The result is that when the cell possesses ample amounts of ATP, the Krebs cycle shuts down, and acetyl-CoA is channeled into fat synthesis.

Reactions 2 and 3: Isomerization Before the oxidation reactions can begin, the hydroxyl (—OH) group of citrate must be repositioned. This rearrangement is done in two steps: First, a water molecule is removed from one carbon; then water is added to a different carbon. As a result, an —H group and an —OH group change positions. The product is an isomer

figure 7.11

HOW THE KREBS CYCLE WORKS.



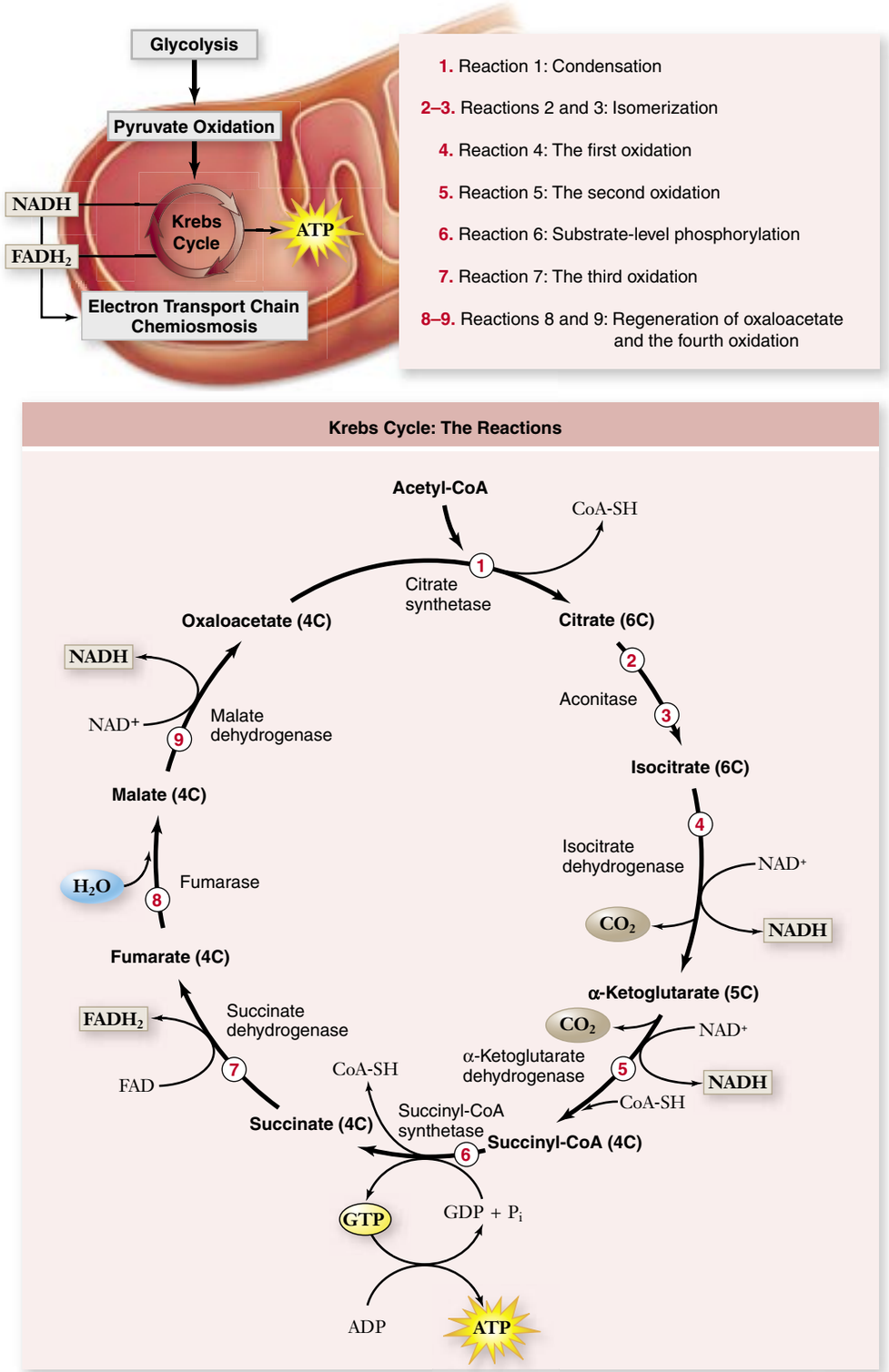


figure 7.12

THE KREBS CYCLE. This series of reactions takes place within the matrix of the mitochondrion. For the complete breakdown of a molecule of glucose, the two molecules of acetyl-CoA produced by glycolysis and pyruvate oxidation each have to make a trip around the Krebs cycle. Follow the different carbons through the cycle, and notice the changes that occur in the carbon skeletons of the molecules and where oxidation reactions take place as they proceed through the cycle.

of citrate called *isocitrate*. This rearrangement facilitates the subsequent reactions.

Reaction 4: The First Oxidation In the first energy-yielding step of the cycle, isocitrate undergoes an oxidative decarboxylation reaction. First, isocitrate is oxidized, yielding a pair of electrons that reduce a molecule of NAD^+ to NADH. Then the oxidized intermediate is decarboxylated; the central carboxyl group splits off to form CO_2, yielding a 5-carbon molecule called *α-ketoglutarate*.

Reaction 5: The Second Oxidation Next, α-ketoglutarate is decarboxylated by a multienzyme complex similar to pyruvate dehydrogenase. The succinyl group left after the removal of CO_2 joins to coenzyme A, forming *succinyl-CoA*. In the process, two electrons are extracted, and they reduce another molecule of NAD^+ to NADH.

Reaction 6: Substrate-Level Phosphorylation The linkage between the 4-carbon succinyl group and CoA is a high-energy bond. In a coupled reaction similar to those that take place in glycolysis, this bond is cleaved, and the energy released drives the phosphorylation of guanosine diphosphate (GDP), forming guanosine triphosphate (GTP). GTP can transfer a phosphate to ADP converting it into ATP. The 4-carbon molecule that remains is called *succinate*.

Reaction 7: The Third Oxidation Next, succinate is oxidized to *fumarate* by an enzyme located in the inner mitochondrial membrane. The free-energy change in this reaction is not large enough to reduce NAD^+. Instead, FAD is the electron acceptor. Unlike NAD^+, FAD is not free to diffuse within the mitochondrion; it is tightly associated with its enzyme in the inner mitochondrial membrane. Its reduced form, $FADH_2$, can only contribute electrons to the electron transport chain in the membrane.

Reactions 8 and 9: Regeneration of Oxaloacetate In the final two reactions of the cycle, a water molecule is added to fumarate, forming *malate*. Malate is then oxidized, yielding a 4-carbon molecule of *oxaloacetate* and two electrons that reduce a molecule of NAD^+ to NADH. Oxaloacetate, the molecule that began the cycle, is now free to combine with another 2-carbon acetyl group from acetyl-CoA and reinitiate the cycle.

Glucose becomes CO_2 and potential energy

In the process of aerobic respiration, glucose is entirely consumed. The six-carbon glucose molecule is cleaved into a pair of 3-carbon pyruvate molecules during glycolysis. One of the carbons of each pyruvate is then lost as CO_2 in the conversion of pyruvate to acetyl-CoA. The two other carbons from acetyl-CoA are lost as CO_2 during the oxidations of the Krebs cycle.

All that is left to mark the passing of a glucose molecule into six CO_2 molecules is its energy, some of which is preserved in four ATP molecules and in the reduced state of 12 electron carriers. Ten of these carriers are NADH molecules; the other two are $FADH_2$.

Following the electrons in the reactions reveals the direction of transfer

As you examine the changes in electrical charge in the reactions that oxidize glucose, a good strategy for keeping the transfers clear is always to *follow the electrons*. For example, in glycolysis, an enzyme extracts two hydrogens—that is, two electrons and two protons—from glucose and transfers both electrons and one of the protons to NAD^+. The other proton is released as a hydrogen ion, H^+, into the surrounding solution. This transfer converts NAD^+ into NADH; that is, two negative electrons ($2e^-$) and one positive proton (H^+) are added to one positively charged NAD^+ to form NADH, which is electrically neutral.

As mentioned earlier, energy captured by NADH is not harvested all at once. The two electrons carried by NADH are passed along the electron transport chain, which consists of a series of electron carriers, mostly proteins, embedded within the inner membranes of mitochondria.

NADH delivers electrons to the beginning of the electron transport chain, and oxygen captures them at the end. The oxygen then joins with hydrogen ions to form water. At each step in the chain, the electrons move to a slightly more electronegative carrier, and their positions shift slightly. Thus, the electrons move *down* an energy gradient.

The entire process of electron transfer releases a total of 53 kcal/mol (222 kJ/mol) under standard conditions. The transfer of electrons along this chain allows the energy to be extracted gradually. Next, we will discuss how this energy is put to work to drive the production of ATP.

The Krebs cycle completes the oxidation of glucose begun with glycolysis. Units of the 2-carbon molecule acetyl-CoA are added to the 2-carbon molecule oxaloacetate to produce citric acid (another name for the cycle is the citric acid cycle). The cycle then uses a series of oxidation, decarboxylation, and rearrangement reactions to return to oxaloacetate. This process produces NADH and $FADH_2$, which provide electrons and protons for the electron transport chain. It also produces one ATP per turn of the cycle.

7.6 The Electron Transport Chain and Chemiosmosis

The NADH and $FADH_2$ molecules formed during aerobic respiration each contain a pair of electrons that were gained when NAD^+ and FAD were reduced. The NADH molecules carry their electrons to the inner mitochondrial membrane, where they transfer the electrons to a series of membrane-associated proteins collectively called the *electron transport chain (ETC)*.

The electron transport chain produces a proton gradient

The first of the proteins to receive the electrons is a complex, membrane-embedded enzyme called **NADH dehydrogenase.** A carrier called *ubiquinone* then passes the electrons to a

protein–cytochrome complex called the *bc_1 complex*. Each complex in the chain operates as a proton pump, driving a proton out across the membrane into the intermembrane space (figure 7.13*a*).

The electrons are then carried by another carrier, *cytochrome c*, to the cytochrome oxidase complex. This complex uses four electrons to reduce a molecule of oxygen. Each oxygen then combines with two protons to form water:

$$O_2 + 4\,H^+ + 4\,e^- \longrightarrow 2\,H_2O$$

In contrast to NADH, which contributes its electrons to NADH dehydrogenase, $FADH_2$, which is located in the inner mitochondrial membrane, feeds its electrons to ubiquinone, which is also in the membrane. Electrons from $FADH_2$ thus "skip" the first step in the electron transport chain.

The plentiful availability of a strong electron acceptor, oxygen, is what makes oxidative respiration possible. As you'll see in chapter 8, the electron transport chain used in aerobic respiration is similar to, and may well have evolved from, the chain employed in photosynthesis.

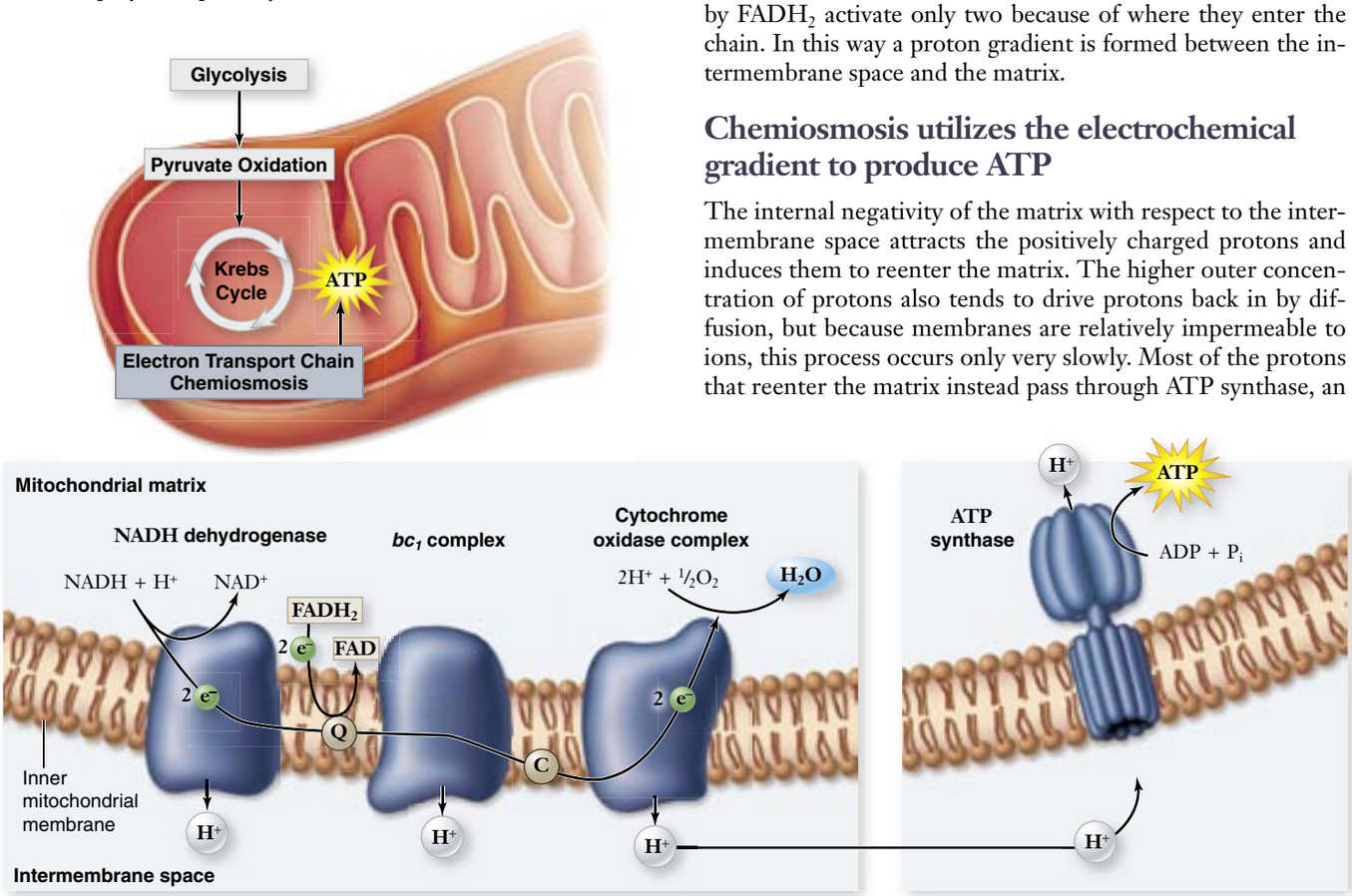
The gradient forms as electrons move through electron carriers

Respiration takes place within the mitochondria present in virtually all eukaryotic cells. The internal compartment, or matrix, of a mitochondrion contains the enzymes that carry out the reactions of the Krebs cycle. As mentioned earlier, protons (H^+) are produced when electrons are transferred to NAD^+. As the electrons harvested by oxidative respiration are passed along the electron transport chain, the energy they release transports protons out of the matrix and into the outer compartment called the intermembrane space.

Three transmembrane complexes of the electron transport chain in the inner mitochondrial membrane actually accomplish the proton transport (see figure 7.13*a*). The flow of highly energetic electrons induces a change in the shape of pump proteins, which causes them to transport protons across the membrane. The electrons contributed by NADH activate all three of these proton pumps, whereas those contributed by $FADH_2$ activate only two because of where they enter the chain. In this way a proton gradient is formed between the intermembrane space and the matrix.

Chemiosmosis utilizes the electrochemical gradient to produce ATP

The internal negativity of the matrix with respect to the intermembrane space attracts the positively charged protons and induces them to reenter the matrix. The higher outer concentration of protons also tends to drive protons back in by diffusion, but because membranes are relatively impermeable to ions, this process occurs only very slowly. Most of the protons that reenter the matrix instead pass through ATP synthase, an



a. The electron transport chain

b. Chemiosmosis

figure 7.13

THE ELECTRON TRANSPORT CHAIN AND CHEMIOSMOSIS. ***a.*** High-energy electrons harvested from catabolized molecules are transported by mobile electron carriers (ubiquinone, marked Q, and cytochrome *c*, marked C) between three complexes of membrane proteins. These three complexes use portions of the electrons' energy to pump protons out of the matrix and into the intermembrane space. The electrons are finally used to reduce oxygen forming water. ***b.*** This creates a concentration gradient of protons across the inner membrane. This electrochemical gradient is a form of potential energy that can be used by ATP synthase. This enzyme couples the reentry of protons to the phosphorylation of ADP to form ATP.

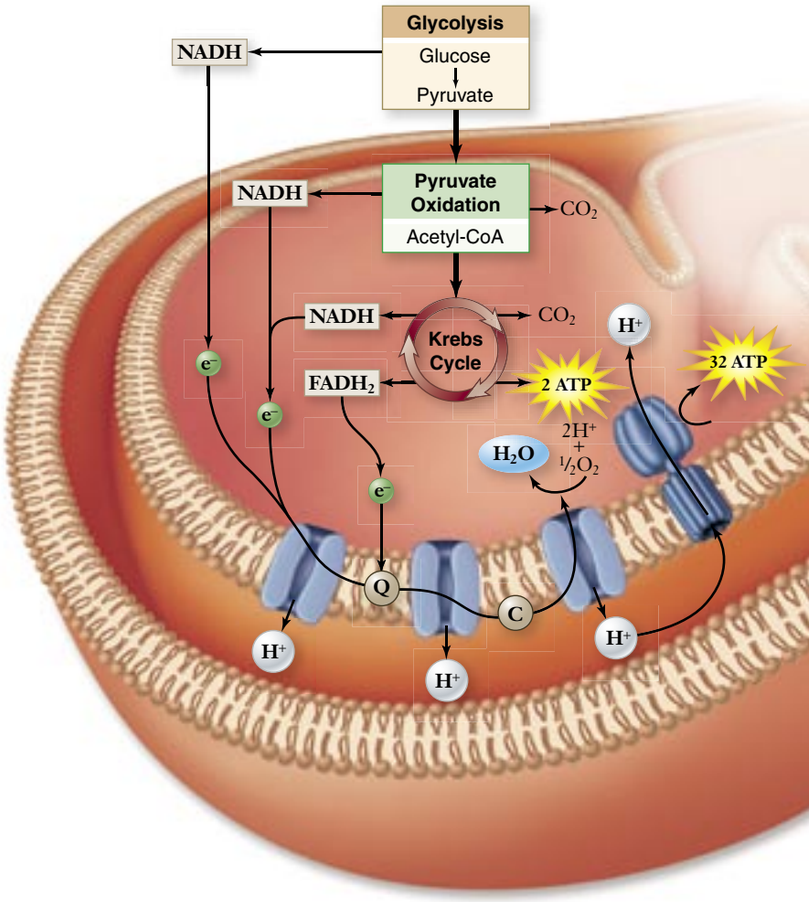


figure 7.14

AEROBIC RESPIRATION IN THE MITOCHONDRIA. The entire process of aerobic respiration is shown in cellular context. Glycolysis occurs in the cytoplasm with the pyruvate and NADH produced entering the mitochondria. Here, pyruvate is oxidized and fed into the Krebs cycle to complete the oxidation process. All the energetic electrons harvested by oxidations in the overall process are transferred by NADH and $FADH_2$ to the electron transport chain. The electron transport chain uses the energy released during electron transport to pump protons across the inner membrane. This creates an electrochemical gradient that contains potential energy. The enzyme ATP synthase uses this gradient to phosphorylate ADP to form ATP.

enzyme that uses the energy of the gradient to catalyze the synthesis of ATP from ADP and P_i. Because the chemical formation of ATP is driven by a diffusion force similar to osmosis, this process is referred to as *chemiosmosis* (figure 7.13*b*). The newly formed ATP is transported by facilitated diffusion to the many places in the cell where enzymes require energy to drive endergonic reactions.

The energy released by the reactions of cellular respiration ultimately drives the proton pumps that produce the proton gradient. The proton gradient provides the energy required for the synthesis of ATP. Figure 7.14 summarizes the overall process.

ATP synthase is a molecular rotary motor

ATP synthase uses a fascinating molecular mechanism to perform ATP synthesis (figure 7.15). Structurally, the enzyme has a membrane-bound portion and a narrow stalk that connects the membrane portion to a knoblike catalytic portion. This complex can be dissociated into two subportions: the F_0 membrane-bound complex, and the F_1 complex composed of the stalk and a knob, or head domain.

The F_1 complex has enzymatic activity. The F_0 complex contains a channel through which protons move across the membrane down their concentration gradient. As they do so, their movement causes part of the F_0 complex and the stalk to rotate relative to the knob. The mechanical energy of this

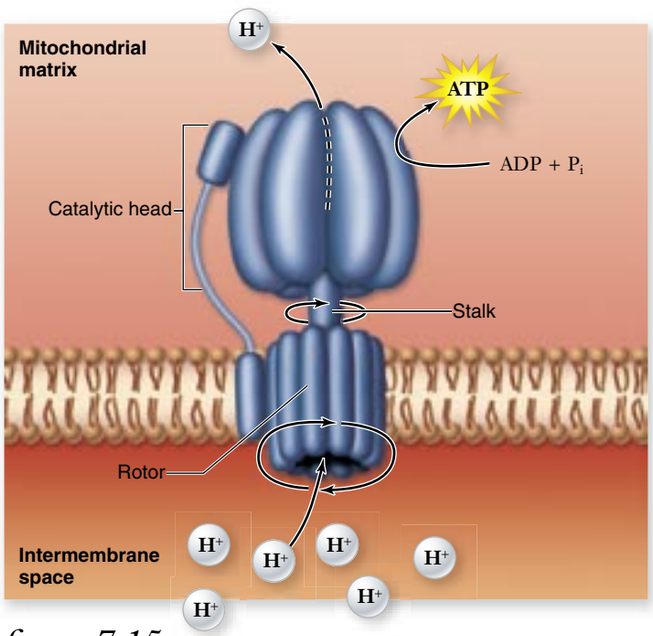


figure 7.15

THE ATP ROTARY ENGINE. Protons move across the membrane down their concentration gradient. The energy released causes the rotor and stalk structures to rotate. This mechanical energy alters the conformation of the ATP synthase enzyme to catalyze the formation of ATP.

rotation is used to change the conformation of the catalytic domain in the F_1 complex.

Thus, the synthesis of ATP is achieved by a tiny rotary motor, the rotation of which is driven directly by a gradient of protons. The process is like that of a water mill, in which the flow of water due to gravity causes a millwheel to turn and accomplish work or produce energy. The flow of protons is similar to the flow of water that causes the wheel to turn. Of course, ATP synthase is a much more complex motor, and it produces a chemical end-product.

The electron transport chain uses electrons from oxidation reactions carried by NADH and $FADH_2$ to create a proton gradient across the inner membrane of the mitochondria. The protein complexes of the electron transport chain are located in the inner membrane and use the energy from electron transfer to pump protons across the membrane, creating an electrochemical gradient. The enzyme ATP synthase can then use this gradient of protons to drive the endergonic reaction of phosphorylating ADP to ATP.

7.7 Energy Yield of Aerobic Respiration

How much metabolic energy in the form of ATP does a cell gain from aerobic breakdown of glucose? Knowing the steps involved in the process, we can calculate the theoretical yield of ATP and compare it with the actual yield.

The theoretical yield for eukaryotes is 36 ATP per glucose molecule

The chemiosmotic model suggests that one ATP molecule is generated for each proton pump activated by the electron transport chain. Because the electrons from NADH activate three pumps and those from $FADH_2$ activate two, we would expect each molecule of NADH and $FADH_2$ to generate three and two ATP molecules, respectively.

In doing this accounting, remember that everything downstream of glycolysis must be multiplied by 2 because two pyruvates are produced per molecule of glucose. A total of 10 NADH molecules is generated by respiration: 2 from glycolysis, 2 from the oxidation of pyruvate (1×2), and another 6 from the Krebs cycle (3×2). Also, two $FADH_2$ are produced (1×2). Finally, two ATP are generated directly by glycolysis and another two ATP from the Krebs cycle (1×2). This gives a total of $10 \times 3 = 30$ ATP from NADH, plus $2 \times 2 = 4$ ATP from $FADH_2$, plus four ATP, for a total of 38 ATP (figure 7.16).

This number is accurate for bacteria, but it does not hold for eukaryotes because the NADH produced in the cytoplasm by glycolysis needs to be transported into the mitochondria by active transport, which costs 1 ATP per NADH transported. This reduces the predicted yield for eukaryotes to 36 ATP.

The actual yield for eukaryotes is 30 ATP per glucose molecule

The amount of ATP actually produced in a eukaryotic cell during aerobic respiration is somewhat lower than 36, for two reasons. First, the inner mitochondrial membrane is somewhat "leaky" to protons, allowing some of them to reenter the matrix without passing through ATP synthase. Second, mitochondria often use the proton gradient generated by chemiosmosis for purposes other than ATP synthesis (such as transporting pyruvate into the matrix).

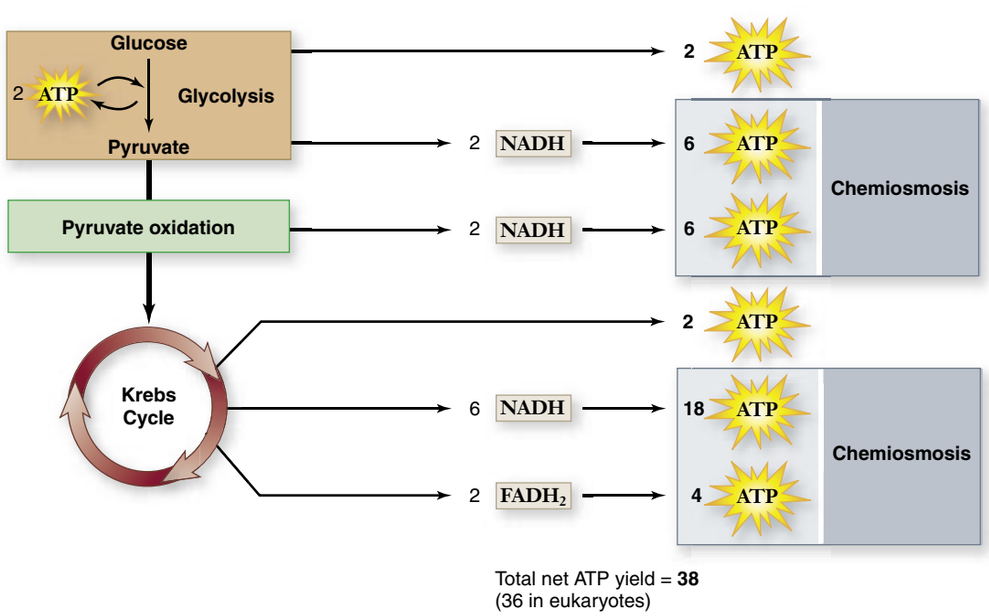


figure 7.16

THEORETICAL ATP YIELD. The theoretical yield of ATP harvested from glucose by aerobic respiration totals 38 molecules. In eukaryotes this is reduced to 36 as the NADH generated by glycolysis in the cytoplasm has to be actively transported into the mitochondria costing the cell 1 ATP per NADH transported.

Consequently, the actual measured values of ATP generated by NADH and $FADH_2$ are closer to 2.5 for each NADH, and 1.5 for each $FADH_2$. With these corrections, the overall harvest of ATP from a molecule of glucose in a eukaryotic cell is calculated as: 4 ATP from substrate-level phosphorylation + 25 ATP from NADH (2.5 × 10) + 3 ATP from $FADH_2$ (1.5 × 2) − 2 ATP for transport of glycolytic NADH = 30 molecules of ATP.

We mentioned earlier that the catabolism of glucose by aerobic respiration, in contrast to that by glycolysis alone, has a large energy yield. Aerobic respiration in a eukaryotic cell harvests about (7.3 × 30)/686 = 32% of the energy available in glucose. (By comparison, a typical car converts only about 25% of the energy in gasoline into useful energy.)

The higher yield of aerobic respiration was one of the key factors that fostered the evolution of heterotrophs. As this mechanism for producing ATP evolved, nonphotosynthetic organisms could more successfully base their metabolism on the exclusive use of molecules derived from other organisms. As long as some organisms captured energy by photosynthesis, others could exist solely by feeding on them.

Passage of electrons down the electron transport chain produces roughly 3 ATP per NADH. This can result in a maximum of 38 ATP for all of the NADH generated by the complete oxidation of glucose, plus the ATP generated by substrate-level phosphorylation. NADH generated in the cytoplasm only lead to 2 ATP/NADH due to the cost of transporting the NADH into the mitochondria, leading to a total of 36 ATP for the mitochondria per glucose

7.8 Regulation of Aerobic Respiration

When cells possess plentiful amounts of ATP, the key reactions of glycolysis, the Krebs cycle, and fatty acid breakdown are inhibited, slowing ATP production. The regulation of these biochemical pathways by the level of ATP is an example of feedback inhibition. Conversely, when ATP levels in the cell are low, ADP levels are high, and ADP activates enzymes in the pathways of carbohydrate catabolism to stimulate the production of more ATP.

Control of glucose catabolism occurs at two key points in the catabolic pathway, namely at a point in glycolysis and at the beginning of the Krebs cycle (figure 7.17). The control point in glycolysis is the enzyme phosphofructokinase, which catalyzes the conversion of fructose phosphate to fructose bisphosphate. This is the first reaction of glycolysis that is not readily reversible, committing the substrate to the glycolytic sequence. ATP itself is an allosteric inhibitor (chapter 6) of phosphofructokinase, as is the Krebs cycle intermediate citrate. High levels of both ATP and citrate inhibit phosphofructokinase. Thus, under conditions when ATP is in excess, or when the Krebs cycle is producing citrate faster than it is being consumed, glycolysis is slowed.

The main control point in the oxidation of pyruvate occurs at the committing step in the Krebs cycle with the enzyme pyruvate dehydrogenase, which converts pyruvate to acetyl-CoA. This enzyme is inhibited by high levels of NADH, a key product of the Krebs cycle.

Another control point in the Krebs cycle is the enzyme citrate synthetase, which catalyzes the first reaction, the conversion of oxaloacetate and acetyl-CoA into citrate. High levels of ATP inhibit citrate synthetase (as well as phosphofructokinase, pyruvate dehydrogenase, and two other Krebs cycle enzymes), slowing down the entire catabolic pathway.

Respiration is controlled by levels of ATP in the cell and levels of key intermediates in the process. The control point for glycolysis is the enzyme phosphofructokinase.

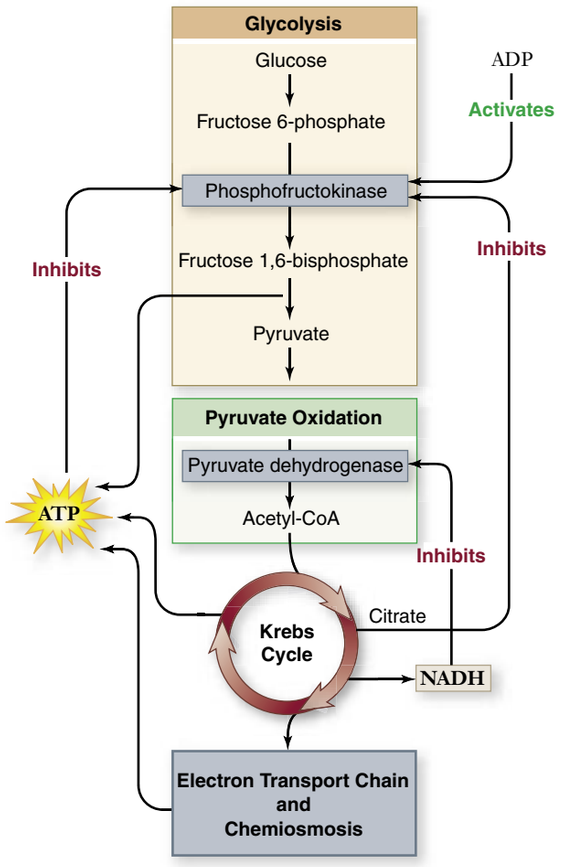


figure 7.17

CONTROL OF GLUCOSE CATABOLISM. The relative levels of ADP and ATP and key intermediates NADH and citrate control the catabolic pathway at two key points: the committing reactions of glycolysis and the Krebs cycle.

7.9 Oxidation Without O_2

In the presence of oxygen, cells can use oxygen to produce a large amount of ATP. But even when no oxygen is present to accept electrons, some organisms can still respire *anaerobically*, using inorganic molecules as final electron acceptors for an electron transport chain.

For example, many prokaryotes use sulfur, nitrate, carbon dioxide, or even inorganic metals as the final electron acceptor in place of oxygen (figure 7.18). The free energy released by using these other molecules as final electron acceptors is not as great as that using oxygen because they have a lower affinity for electrons. Less total ATP is produced, but the process is still respiration and not fermentation.

Methanogens use carbon dioxide

Among the heterotrophs that practice anaerobic respiration are primitive Archaea such as thermophiles and methanogens. Methanogens use carbon dioxide (CO_2) as the electron acceptor, reducing CO_2 to CH_4 (methane). The hydrogens are derived from organic molecules produced by other organisms. Methanogens are found in diverse environments, including soil and the digestive systems of ruminants like cows.

Sulfur bacteria use sulfate

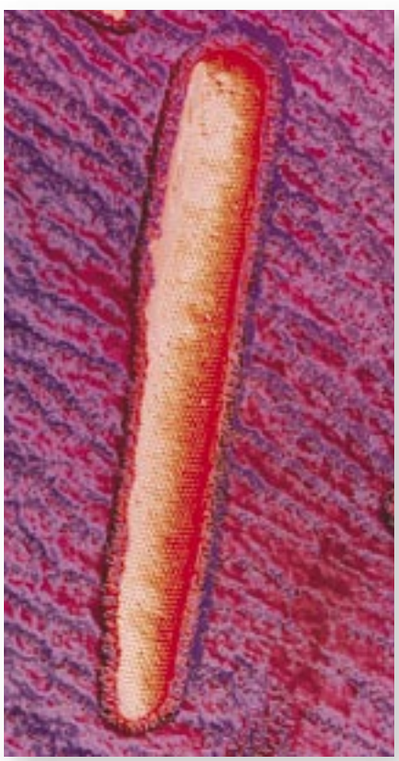
Evidence of a second anaerobic respiratory process among primitive bacteria is seen in a group of rocks about 2.7 BYA, known as the Woman River iron formation. Organic material in these rocks is enriched for the light isotope of sulfur, ^{32}S, relative to the heavier isotope, ^{34}S. No known geochemical process produces such enrichment, but biological sulfur reduction does, in a process still carried out today by certain primitive prokaryotes.

In this sulfate respiration, the prokaryotes derive energy from the reduction of inorganic sulfates (SO_4) to hydrogen sulfide (H_2S). The hydrogen atoms are obtained from organic molecules other organisms produce. These prokaryotes thus are similar to methanogens, but they use SO_4 as the oxidizing (that is, electron-accepting) agent in place of CO_2.

The early sulfate reducers set the stage for the evolution of photosynthesis, creating an environment rich in H_2S. As dis-

figure 7.18

SULFUR-RESPIRING PROKARYOTE. ***a.*** The micrograph shows the archaeal species *Thermoproteus tenax*. This organism can use elemental sulfur as a final electron acceptor for anaerobic respiration. ***b.*** *Thermoproteus* is often found in sulfur containing hot springs such as the Norris Geyser Basin in Yellowstone National Park shown here.



a.

b .

cussed in chapter 8, the first form of photosynthesis obtained hydrogens from H_2S using the energy of sunlight.

Fermentation uses organic compounds as electron acceptors

In the absence of oxygen, cells that cannot utilize an alternative electron acceptor for respiration must rely exclusively on glycolysis to produce ATP. Under these conditions, the electrons generated by glycolysis are donated to organic molecules in a process called *fermentation*. This process recycles NAD^+, the electron acceptor that allows glycolysis to proceed.

Bacteria carry out more than a dozen kinds of fermentation reactions, often using pyruvate or a derivative of pyruvate to accept the electrons from NADH. Organic molecules other than pyruvate and its derivatives can be used as well; the important point is that the process regenerates NAD^+:

organic molecule + NADH ⟶ reduced organic molecule + NAD^+

Often the reduced organic compound is an organic acid—such as acetic acid, butyric acid, propionic acid, or lactic acid—or an alcohol.

Ethanol fermentation

Eukaryotic cells are capable of only a few types of fermentation. In one type, which occurs in yeast, the molecule that accepts electrons from NADH is derived from pyruvate, the end-product of glycolysis.

Yeast enzymes remove a terminal CO_2 group from pyruvate through decarboxylation, producing a 2-carbon molecule called acetaldehyde. The CO_2 released causes bread made with yeast to rise; bread made without yeast (unleavened bread) does not rise. The acetaldehyde accepts a pair of electrons from NADH, producing NAD^+ and ethanol (ethyl alcohol) (figure 7.19).

This particular type of fermentation is of great interest to humans, because it is the source of the ethanol in wine and beer. Ethanol is a by-product of fermentation that is actually toxic to yeast; as it approaches a concentration of about 12%, it begins to kill the yeast. That explains why naturally fermented wine contains only about 12% ethanol.

Lactic acid fermentation

Most animal cells regenerate NAD^+ without decarboxylation. Muscle cells, for example, use the enzyme lactate dehydrogenase to transfer electrons from NADH back to the pyruvate that is produced by glycolysis. This reaction converts pyruvate into lactic acid and regenerates NAD^+ from NADH (see figure 7.19). It therefore closes the metabolic circle, allowing glycolysis to continue as long as glucose is available.

Circulating blood removes excess lactate, the ionized form of lactic acid, from muscles, but when removal cannot keep pace with production, the accumulating lactic acid interferes with muscle function and contributes to muscle fatigue.

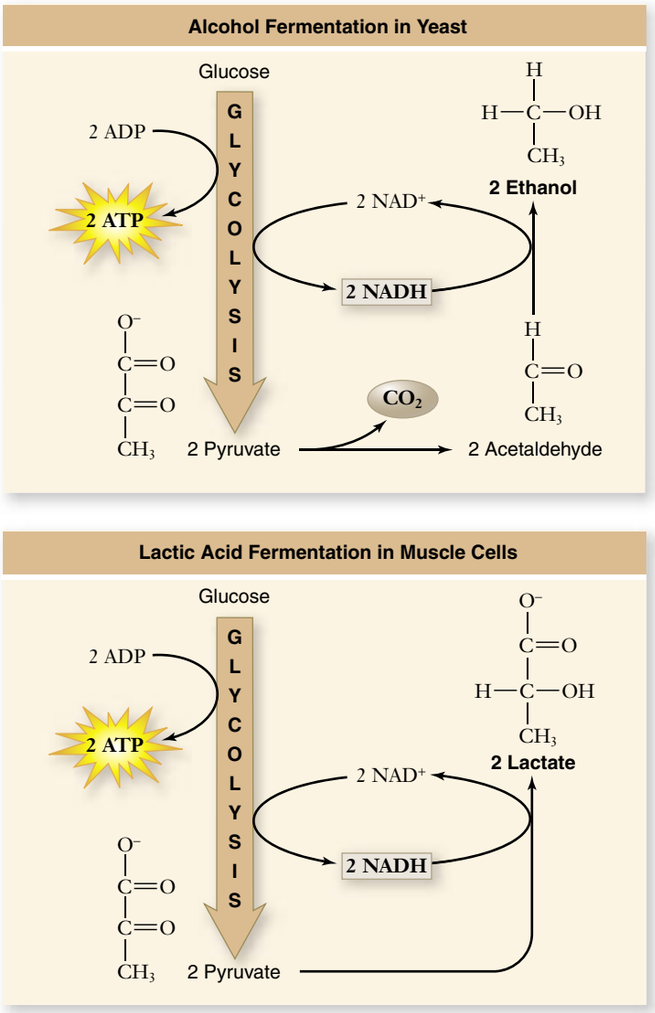


figure 7.19

FERMENTATION. Yeasts carry out the conversion of pyruvate to ethanol. Muscle cells convert pyruvate into lactate, which is less toxic than ethanol. In each case, the reduction of a metabolite of glucose has oxidized NADH back to NAD^+ to allow glycolysis to continue under anaerobic conditions.

O_2 is the electron acceptor for aerobic respiration. Due to oxygen's high affinity for electrons this results in the highest yield of ATP, but this is not the only form of respiration found in living systems. Nitrate, sulfur, and CO_2 are among other terminal electron acceptors used in anaerobic respiration. Organic molecules can also be used in fermentation reactions but allow only a partial oxidation of glucose via glycolysis. Fermentation reactions produce a variety of compounds, including ethanol in yeast and lactic acid in humans.

7.10 Catabolism of Proteins and Fats

Thus far we have focused on the aerobic respiration of glucose, which organisms obtain from the digestion of carbohydrates or from photosynthesis. Organic molecules other than glucose, particularly proteins and fats, are also important sources of energy (figure 7.20).

Catabolism of proteins removes amino groups

Proteins are first broken down into their individual amino acids. The nitrogen-containing side group (the amino group) is then removed from each amino acid in a process called **deamination.** A series of reactions convert the carbon chain that remains into a molecule that enters glycolysis or the Krebs cycle. For example, alanine is converted into pyruvate, glutamate into α-ketoglutarate (figure 7.21), and aspartate into oxaloacetate. The reactions of glycolysis and the Krebs cycle then extract the high-energy electrons from these molecules and put them to work making ATP.

Catabolism of fatty acids produces acetyl groups

Fats are broken down into fatty acids plus glycerol. Long-chain fatty acids typically have an even number of carbons, and the many C—H bonds provide a rich harvest of energy. Fatty acids are oxidized in the matrix of the mitochondrion. Enzymes remove the 2-carbon acetyl groups from the end of each fatty acid until the entire fatty acid is converted into acetyl groups (figure 7.22). Each acetyl group is combined with coenzyme A to form acetyl-CoA. This process is known as **β-oxidation.** This process is oxygen-dependent, which explains why aerobic exercise burns fat, but anaerobic exercise does not.

How much ATP does the catabolism of fatty acids produce? Let's compare a hypothetical 6-carbon fatty acid with the six-carbon glucose molecule, which we've said yields about 30 molecules of ATP in a eukaryotic cell. Two rounds of β-oxidation would convert the fatty acid into three molecules of acetyl-CoA. Each round requires one molecule of ATP to prime the process, but it also produces one molecule of NADH and one of $FADH_2$. These molecules together yield four molecules of ATP (assuming 2.5 ATPs per NADH, and 1.5 ATPs per $FADH_2$).

The oxidation of each acetyl-CoA in the Krebs cycle ultimately produces an additional 10 molecules of ATP. Overall, then, the ATP yield of a 6-carbon fatty acid would be approximately: 8 (from two rounds of β-oxidation) – 2 (for priming those two rounds) + 30 (from oxidizing the three acetyl-CoAs) = 36 molecules of ATP. Therefore, the respiration of a 6-carbon fatty acid yields 20% more ATP than the respiration of glucose.

Moreover, a fatty acid of that size would weigh less than two-thirds as much as glucose, so a gram of fatty acid contains more than twice as many kilocalories as a gram of glucose. You can see from this fact why fat is a storage molecule for excess energy in many types of animals. If excess energy were stored instead as carbohydrate, as it is in plants, animal bodies would have to be much bulkier.

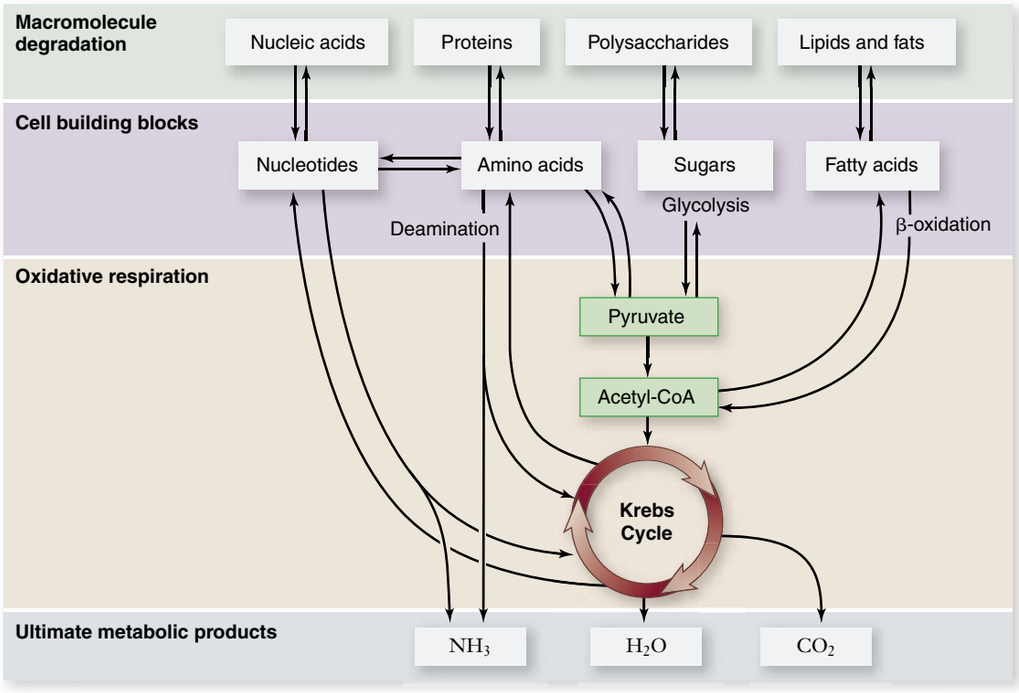


figure 7.20

HOW CELLS EXTRACT CHEMICAL ENERGY. All eukaryotes and many prokaryotes extract energy from organic molecules by oxidizing them. The first stage of this process, breaking down macromolecules into their constituent parts, yields little energy. The second stage, oxidative or aerobic respiration, extracts energy, primarily in the form of high-energy electrons, and produces water and carbon dioxide. Key intermediates in these energy pathways are also used for biosynthetic pathways, shown by reverse arrows.

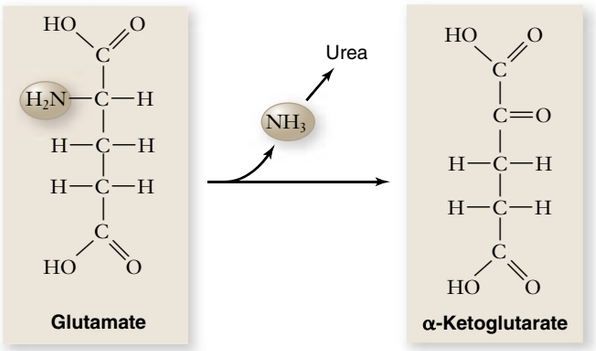


figure 7.21

DEAMINATION. After proteins are broken down into their amino acid constituents, the amino groups are removed from the amino acids to form molecules that participate in glycolysis and the Krebs cycle. For example, the amino acid glutamate becomes α-ketoglutarate, a Krebs cycle intermediate, when it loses its amino group.

A small number of key intermediates connect metabolic pathways

Oxidation pathways of food molecules are interrelated in that a small number of key intermediates, such as pyruvate and acetyl-CoA, link the breakdown from different starting points. These key intermediates allow the interconversion of different types of molecules, such as sugars and amino acids (see figure 7.20).

Cells can make glucose, amino acids, and fats, as well as getting them from external sources, and they use reactions similar to those that break down these substances. In many cases, the reverse pathways even share enzymes if the free-energy changes are small. For example, gluconeogenesis, the process of making new glucose, uses all but three enzymes of the glycolytic pathway. Thus, much of glycolysis runs forward or backward, depending on the concentrations of the intermediates—with only three key steps having different enzymes for forward and reverse directions.

Acetyl-CoA has many roles

Many different metabolic processes generate acetyl-CoA. Not only does the oxidation of pyruvate produce it, but the metabolic breakdown of proteins, fats, and other lipids also generates acetyl-CoA. Indeed, almost all molecules catabolized for energy are converted into acetyl-CoA.

Acetyl-CoA has a role in anabolic metabolism as well. Units of two carbons derived from acetyl-CoA are used to build up the hydrocarbon chains in fatty acids. Acetyl-CoA produced from a variety of sources can therefore be channeled into fatty acid synthesis or into ATP production, depending on the organism's energy requirements. Which of these two options is taken depends on the level of ATP in the cell.

When ATP levels are high, the oxidative pathway is inhibited, and acetyl-CoA is channeled into fatty acid synthesis. This explains why many animals (humans included) develop fat reserves when they consume more food than their activities require. Alternatively, when ATP levels are low, the oxidative pathway is stimulated, and acetyl-CoA flows into energy-producing oxidative metabolism.

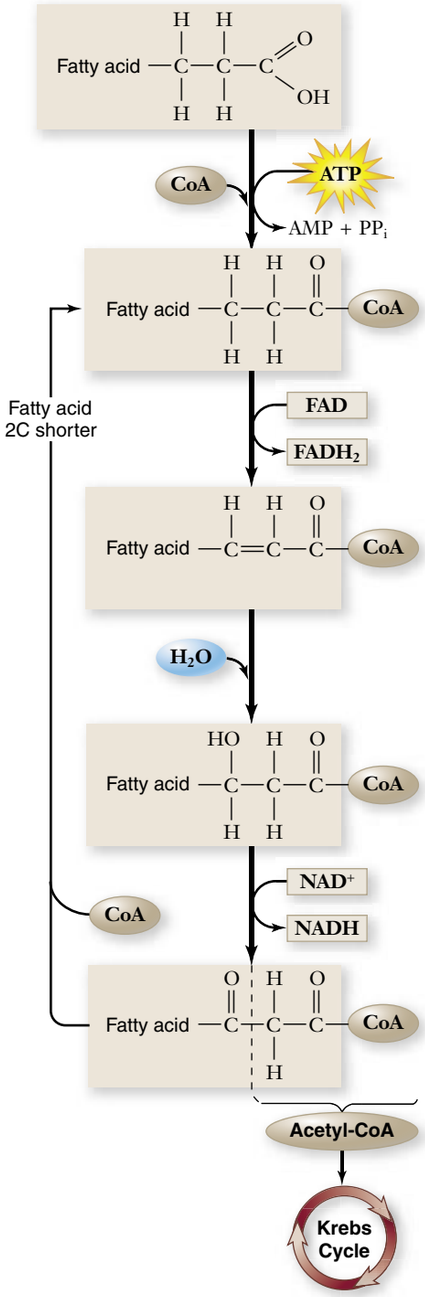


figure 7.22

BETA-OXIDATION. Through a series of reactions known as β-oxidation, the last two carbons in a fatty acid combine with coenzyme A to form acetyl-CoA, which enters the Krebs cycle. The fatty acid, now two carbons shorter, enters the pathway again and keeps reentering until all its carbons have been used to form acetyl-CoA molecules. Each round of β-oxidation uses one molecule of ATP and generates one molecule each of $FADH_2$ and NADH.

inquiry

Given what you have learned in this chapter, how many ATP would be produced by the oxidation of a fatty acid that has 16 carbons?

Fats are a major energy storage molecule that can be broken down into units of acetyl-CoA by β-oxidation and fed into the Krebs cycle. The major metabolic pathways are connected by a number of key intermediates. This allows many processes to be used to either build up (anabolism) or break down (catabolism) the major biological macromolecules and allows interconversion of different types of molecules.

7.11 Evolution of Metabolism

We talk about cellular respiration as a continuous series of stages, but it is important to note that these stages evolved over time, and metabolism has changed a great deal in that time. Both anabolic processes and catabolic processes evolved in concert with each other. We do not know the details of this biochemical evolution, or the order of appearance of these processes. Therefore the following timeline is based on the available geochemical evidence and represents a hypothesis rather than a strict timeline.

The earliest life forms degraded carbon-based molecules present in the environment

The most primitive forms of life are thought to have obtained chemical energy by degrading, or breaking down, organic molecules that were abiotically produced, that is, carbon-containing molecules formed by inorganic processes on the early Earth.

The first major event in the evolution of metabolism was the origin of the ability to harness chemical bond energy. At an early stage, organisms began to store this energy in the bonds of ATP.

The evolution of glycolysis also occurred early

The second major event in the evolution of metabolism was glycolysis, the initial breakdown of glucose. As proteins evolved diverse catalytic functions, it became possible to capture a larger fraction of the chemical bond energy in organic molecules by breaking chemical bonds in a series of steps.

Glycolysis undoubtedly evolved early in the history of life on Earth, because this biochemical pathway has been retained by all living organisms. It is a chemical process that does not appear to have changed for well over 2 billion years.

Anaerobic photosynthesis allowed the capture of light energy

The third major event in the evolution of metabolism was anaerobic photosynthesis. Early in the history of life, a different way of generating ATP evolved in some organisms. Instead of obtaining energy for ATP synthesis by reshuffling chemical bonds, as in glycolysis, these organisms developed the ability to use light to pump protons out of their cells, and to use the resulting proton gradient to power the production of ATP through chemiosmosis.

Photosynthesis evolved in the absence of oxygen and works well without it. Dissolved H_2S, present in the oceans of the early Earth beneath an atmosphere free of oxygen gas, served as a ready source of hydrogen atoms for building organic molecules. Free sulfur was produced as a by-product of this reaction.

Oxygen-forming photosynthesis used a different source of hydrogen

The substitution of H_2O for H_2S in photosynthesis was the fourth major event in the history of metabolism. Oxygen-forming photosynthesis employs H_2O rather than H_2S as a source of hydrogen atoms and their associated electrons. Because it garners its electrons from reduced oxygen rather than from reduced sulfur, it generates oxygen gas rather than free sulfur.

More than 2 BYA, small cells capable of carrying out this oxygen-forming photosynthesis, such as cyanobacteria, became the dominant forms of life on Earth. Oxygen gas began to accumulate in the atmosphere. This was the beginning of a great transition that changed conditions on Earth permanently. Our atmosphere is now 20.9% oxygen, every molecule of which is derived from an oxygen-forming photosynthetic reaction.

Nitrogen fixation provided new organic nitrogen

Nitrogen is available from dead organic matter, and from chemical reactions that generated the original organic molecules. For life to expand, a new source of nitrogen was needed. Nitrogen fixation was the fifth major step in the evolution of metabolism. Proteins and nucleic acids cannot be synthesized from the products of photosynthesis because both of these biologically critical molecules contain nitrogen. Obtaining nitrogen atoms from N_2 gas, a process called *nitrogen fixation*, requires breaking an $N{\equiv}N$ triple bond.

This important reaction evolved in the hydrogen-rich atmosphere of the early Earth, where no oxygen was present. Oxygen acts as a poison to nitrogen fixation, which today occurs only in oxygen-free environments or in oxygen-free compartments within certain prokaryotes.

Aerobic respiration utilized oxygen

Aerobic respiration is the sixth and final event in the history of metabolism. Aerobic respiration employs the same kind of proton pumps as photosynthesis and is thought to have evolved as a modification of the basic photosynthetic machinery.

Biologists think that the ability to carry out photosynthesis without H_2S first evolved among purple nonsulfur bacteria, which obtain their hydrogens from organic compounds instead. It was perhaps inevitable that among the descendants of these respiring photosynthetic bacteria, some would eventually do without photosynthesis entirely, subsisting only on the energy and electrons derived from the breakdown of organic molecules. The mitochondria within all eukaryotic cells are thought to be descendants of these bacteria.

The complex process of aerobic metabolism developed over geological time, as natural selection favored organisms with more efficient methods of obtaining energy from organic molecules. The process of photosynthesis, as you have seen in this concluding section, has also developed over time, and the rise of photosynthesis changed life on Earth forever. The next chapter explores photosynthesis in detail.

Although the evolution of metabolism is not known in detail, major milestones can be recognized. These include the evolution of metabolic pathways that allow extraction of energy from organic compounds, the evolution of photosynthesis, and the evolution of nitrogen fixation. Photosynthesis began as an anoxygenic process that later evolved to produce oxygen, thus allowing the evolution of aerobic metabolism.

7.1 Overview of Respiration

Respiration occurs when carbohydrates and oxygen are converted to carbon dioxide, water, and energy.

- Autotrophs convert energy from sunlight to organic molecules.
- Heterotrophs use organic compounds made by autotrophs.
- Energy-rich molecules are degraded by oxidation reactions.
- Electron carriers can be reversibly oxidized and reduced.
- Energy released from redox reactions is used to make ATP.
- NAD^+ is an important electron carrier that can act as a coenzyme.
- Aerobic respiration uses oxygen as the final electron acceptor.
- Oxidizing food molecules in stages is more efficient than one step.

7.2 The Oxidation of Glucose: A Summary

Cells make ATP from the oxidation of glucose by two fundamentally different mechanisms.

- Substrate-level phosphorylation transfers a phosphate from a phosphate-bearing intermediate directly to ADP (figure 7.4).
- In oxidative phosphorylation ATP is generated by the enzyme ATP synthase, which is powered by a proton gradient.

7.3 Glycolysis: Splitting Glucose (figure 7.6)

Glycolysis is a series of chemical reactions that occur in the cell cytoplasm. Glucose yields 2 pyruvate, 2 NADH and 2 ATP.

- Priming reactions add two phosphates to glucose.
- This 6-carbon diphosphate is cleaved into two 3-carbon molecules of glyceraldehyde-3-phosphate (G3P).
- Oxidation of G3P transfers electrons to NAD^+ yielding NADH.
- The final product is two molecules of pyruvate.
- Glycolysis produces a net of 2 ATP, 2 NADH, and 2 pyruvate.
- NADH must be recycled into NAD^+ to continue glycolysis.
- In the presence of oxygen NADH is oxidized during respiration.

7.4 The Oxidation of Pyruvate to Produce Acetyl-CoA

Pyruvate from glycolysis is transported into the mitochondria where it is oxidized, and the product is fed into the Krebs cycle.

- Pyruvate oxidation results in 1 CO_2, 1 NADH, and 1-acetyl-CoA per pyruvate.
- Acetyl-CoA enters the Krebs cycle as two-carbon acetyl units.

7.5 The Krebs Cycle

Each acetyl compound that enters the Krebs cycle yields 2 CO_2, 1 ATP, 3 NADH and 1 $FADH_2$.

- An acetyl group combines with oxaloacetate producing citrate.
- Citrate is oxidized, removing CO_2 and generating NADH.

7.6 The Electron Transport Chain and Chemiosmosis (figure 7.13)

The electron transport chain is located on the inner membrane of mitochondria. It produces a proton gradient used in ATP synthesis.

- NADH is oxidized to NADH by NADH dehydrogenase.
- The electrons are transferred sequentially through 3 complexes to cytochrome oxidase, where electrons join with H^+ and oxygen.
- As the electrons move down the electron transport chain, three protons are pumped into the intermembrane space.
- This provides sufficient energy to produce 3 ATP.
- Protons diffuse back into the mitochondrial matrix through the ATP synthase channel, which phosphorylates ADP to ATP.
- As each proton passes through ATP synthase the energy causes the rotor and rod to rotate, altering the conformation of ATP synthase and catalyzing the formation of one ATP (figure 7.15).
- $FADH_2$ transfers electrons to ubiquinone. Only two protons are transported into the intermembrane space and 2 ATP are produced.

7.7 Energy Yield of Aerobic Respiration (figure 7.16)

Aerobic respiration theoretically yields 38 ATP per molecule of glucose.

- Eukaryotes yield 36 ATP per glucose molecule because it costs ATP to transport NADH formed during glycolysis into mitochondria.

7.8 Regulation of Aerobic Respiration (figure 7.17)

Glucose catabolism is controlled by the concentration of ATP molecules and products of the Krebs cycle.

- High ATP concentrations inhibit phosphofructokinase, the third enzyme in glycolysis; low ATP levels activate this enzyme.
- High concentrations of NADH inhibit pyruvate dehydrogenase.

7.9 Oxidation Without O_2 (figure 7.8)

In the absence of oxygen another final electron acceptor is necessary for respiration. For normally aerobic organisms, in the absence of oxygen, ATP can only be produced by glycolysis.

- In many prokaryotes inorganic molecules are used as final electron acceptors for an electron transport chain.
- The regeneration of NAD^+ by the oxidation of NADH and reduction of an organic molecule is called fermentation.
- In yeast, pyruvate is decarboxylated, then reduced to ethanol as NADH is oxidized to NAD^+.
- In animals, pyruvate is reduced to lactate as NADH is oxidized.

7.10 Catabolism of Proteins and Fats (figure 7.20)

Proteins, fats, and nucleic acids are built up and broken down through key intermediates.

- Nucleic acids are metabolized through the Krebs cycle.
- Amino acids are deaminated before they are metabolized.
- The fatty acids are converted to acetyl-CoA by β-oxidation.
- With high ATP, acetyl-CoA is converted into fatty acids.

7.11 Evolution of Metabolism

Major milestones are recognized in the evolution of metabolism, the order of events is hypothetical.

- Five major metabolic processes evolved before atmospheric oxygen was present.
  - *Early life-forms metabolized organic molecules that were abiotically produced and began to store energy as ATP.*
  - *Glycolysis evolved incrementally.*
  - *Early photosynthesis used H_2S to make organic molecules from CO_2.*
  - *The substitution of H_2O for H_2S resulted in the formation of oxygen.*
  - *Nitrogen fixation made N available.*

review questions

SELF TEST

1. An *autotroph* is an organism that—
   a. Extracts energy from organic sources
   b. Converts energy from sunlight into chemical energy
   c. Relies on the energy produced by other organisms as an energy source
   d. Both a and b
2. Which of the following processes is (are) required for the complete oxidation of glucose?
   a. The Krebs cycle
   b. Glycolysis
   c. Pyruvate oxidation
   d. All of the above
3. The energy associated with a molecule of glucose is stored in its—
   a. carbon atoms
   b. chemical bonds
   c. electrons
   d. protons
4. How is ATP produced by glycolysis?
   a. Through the priming reactions
   b. Through the production of glyceraldehyde-3-phosphate
   c. By substrate level phosphorylation
   d. As a result of the reduction of NAD^+ to NADH
5. Which of the following is NOT a true statement regarding cellular respiration?
   a. Enzymes catalyze reactions that transfer electrons.
   b. Electrons have a higher potential energy at the end of the process.
   c. Carbon dioxide gas is a by-product.
   d. The process involves multiple redox reactions.
6. The majority of the ATP produced during aerobic respiration is made by—
   a. the electrons carried by NADH
   b. the movement of hydrogen ions through an ATP synthase enzyme
   c. substrate-level phosphorylation
   d. autophosphorylation
7. What is the role of NAD^+ in the process of cellular respiration?
   a. It functions as an electron carrier.
   b. It functions as an enzyme.
   c. It is the final electron acceptor for anaerobic respiration.
   d. It is a nucleotide source for the synthesis of ATP.
8. Which of the following is NOT a product of glycolysis?
   a. ATP
   b. Pyruvate
   c. CO_2
   d. NADH
9. Why is fermentation an important metabolic function in cells?
   a. It generates glucose for the cell in the absence of O_2.
   b. It oxidizes NADH to NAD^+.
   c. It oxidizes pyruvate.
   d. It produces ATP.
10. Which of the following statements is NOT true about the oxidation of pyruvate?
    a. Pyruvate oxidation occurs in the cytoplasm.
    b. Pyruvate oxidation only occurs if oxygen is present.
    c. Pyruvate is converted into acetyl-CoA.
    d. Pyruvate oxidation results in the production of NADH.
11. The Krebs cycle occurs in which region of a mitochondrion?
    a. The inner membrane
    b. The intermembrane space
    c. The outer membrane
    d. The matrix
12. What happens to the electrons carried by NADH and $FADH_2$?
    a. They are pumped into the intermembrane space.
    b. They are transferred to the ATP synthase.
    c. They are moved between proteins in the inner membrane of the mitochondrion.
    d. They are transported into the matrix of the mitochondrion.
13. Can cellular respiration occur in the absence of O_2?
    a. No, O_2 is required as the final electron acceptor.
    b. No, anaerobic organisms only need glycolysis and fermentation.
    c. Yes, because oxygen can be generated by splitting H_2O.
    d. Yes, but only when another final electron acceptor is available.

CHALLENGE QUESTIONS

1. Use the following table to outline the relationship between the molecules and the metabolic reactions.

Molecules	Glycolysis	Cellular Respiration
Glucose		
Pyruvate		
Oxygen		
ATP		
CO_2		

2. Human babies and hibernating or cold-adapted animals are able to maintain body temperature (a process called *thermogenesis*) due to the presence of brown fat. Brown fat is characterized by a high concentration of mitochondria. These brown fat mitochondria have a special protein located within their inner membranes. *Thermogenin* is a protein that functions as a passive proton transporter. Propose a likely explanation for the role of brown fat in thermogenesis based on your knowledge of metabolism, transport, and the structure and function of mitochondria.