CHAPTER 7

How Cells Harvest Energy

Chapter Contents

AP Essential Knowledge

1.B.1	Organisms share many conserved core processes and features that evolved and are widely distributed among organisms today. **(7.1)**	Big Idea 1
1.D.2	Scientific evidence from many different disciplines supports models of the origin of life. **(7.10)**	Big Idea 1
2.A.1	All living systems require constant input of free energy. **(7.1, 7.2, 7.4)**	Big Idea 2
2.A.2	Organisms capture and store free energy for use in biological processes. **(7.1, 7.2, 7.3, 7.4, 7.5, 7.8, 7.9)**	Big Idea 2
4.A.2	The structure and function of subcellular components, and their interactions, provide essential cellular processes. **(7.5)**	Big Idea 4
4.B.1	Interactions between molecules affect their structure and function. **(7.7)**	Big Idea 4

7.1 Overview of Respiration

Learning Outcomes

1. *Characterize oxidation–dehydrogenation reactions in biological systems.*
2. *Explain the role of electron carriers in energy metabolism.*
3. *Describe the role of ATP in biological systems.*

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.

Cellular oxidations are usually also dehydrogenations

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 (see chapter 47). Then, other enzymes dismantle these fragments a bit 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, 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 adenosine 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.

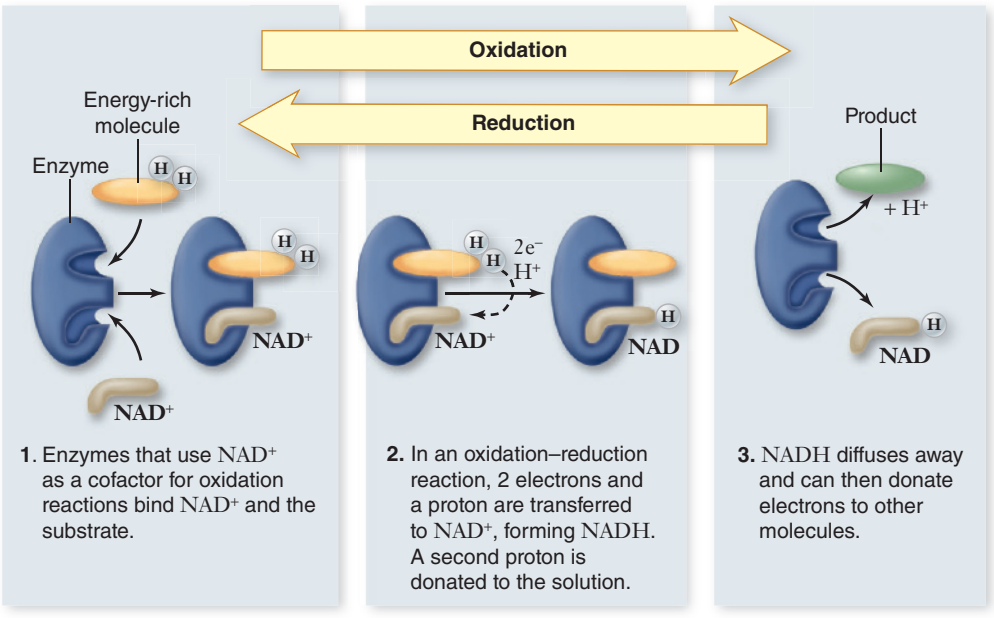


Figure 7.1 Oxidation–reduction reactions often employ cofactors. Cells use a chemical cofactor called nicotinamide adenosine dinucleotide (NAD^+) to carry out many oxidation–reduction reactions. Two electrons and a proton are transferred to NAD^+ with another proton donated to the solution. Molecules that gain electrons are said to be reduced, and 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). NADH is the reduced form of NAD^+.

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.

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:

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

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.

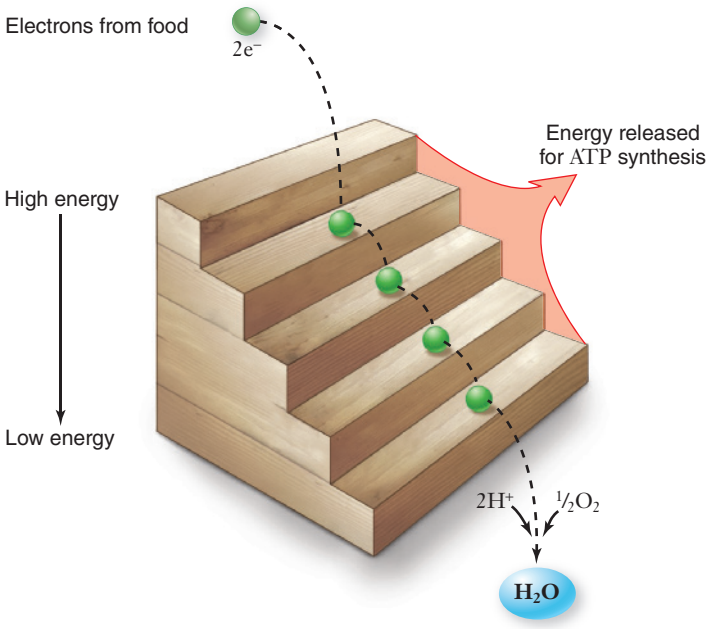


Figure 7.2 How electron transport works. This diagram shows how ATP is generated when electrons transfer from one energy level to another. Rather than releasing a single explosive burst of energy, electrons "fall" to lower and lower energy levels in steps, releasing stored energy with each fall as they tumble to the lowest (most electronegative) electron acceptor, O_2.

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. 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: (1) soluble carriers that move electrons from one molecule to another, (2) membrane-bound carriers that form a redox chain, and (3) 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^+ molecule is composed of two nucleotides bound together. The two nucleotides that make up NAD^+, nicotinamide monophosphate (NMP) and adenosine 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, because it is readily reduced—that is, it 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.

Respiration 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.

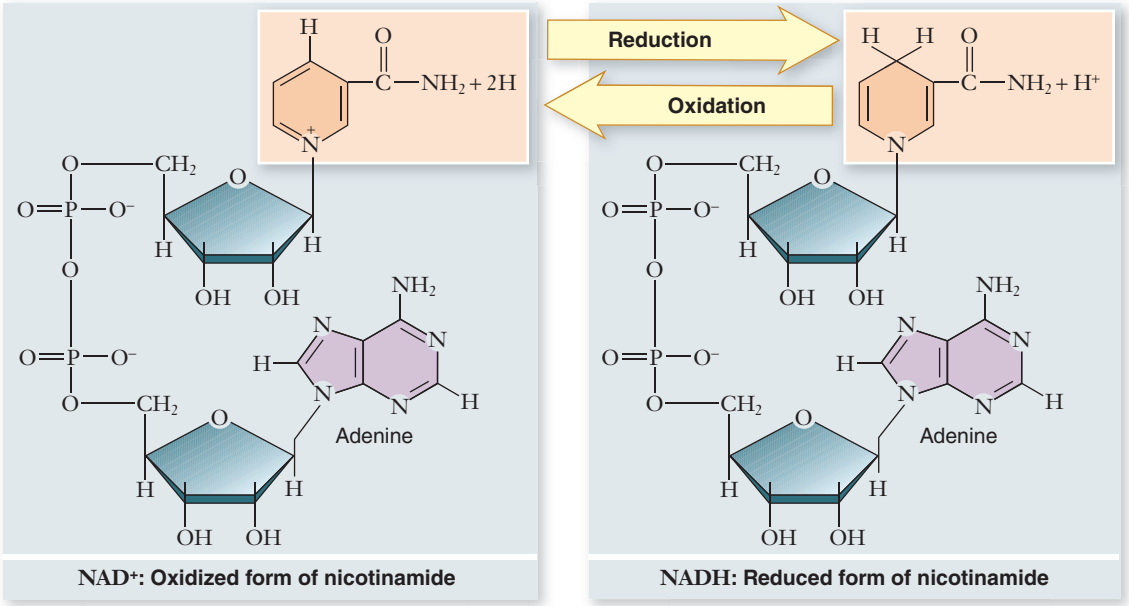


Figure 7.3 NAD⁺ and NADH. This dinucleotide serves as an "electron shuttle" during cellular respiration. NAD^+ accepts a pair of electrons and a proton from catabolized macromolecules and is reduced to NADH.

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 section 7.5.

ATP plays a central role in metabolism

Chapter 6 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 of which 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 (see 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 hydrolyzes the terminal phosphate of ATP, releasing over 7 kcal ($\Delta G = -7.3$ kcal/mol) of energy. This provides the energy absorbed by 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 uses energy from the exergonic reactions of cellular respiration.

Cells make ATP by two fundamentally different mechanisms

The synthesis of ATP can be accomplished by two distinct mechanisms: one that involves chemical coupling with an intermediate bound to phosphate, and another that relies on an electrochemical gradient of protons for the potential energy to phosphorylate ADP.

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 (see section 7.2), the chemical bonds of glucose are shifted

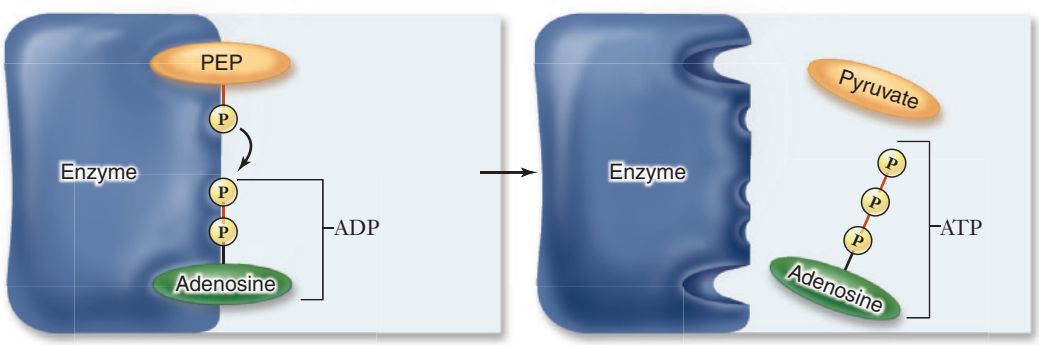


Figure 7.4 Substrate-level phosphorylation. Some molecules, such as phosphoenolpyruvate (PEP), possess a high-energy phosphate (P) bond similar to the bonds in ATP. When PEP's phosphate group is transferred enzymatically to ADP, the energy in the bond is conserved, and ATP is created.

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 (see section 7.5). 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:

$$ADP + P_i \longrightarrow ATP$$

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

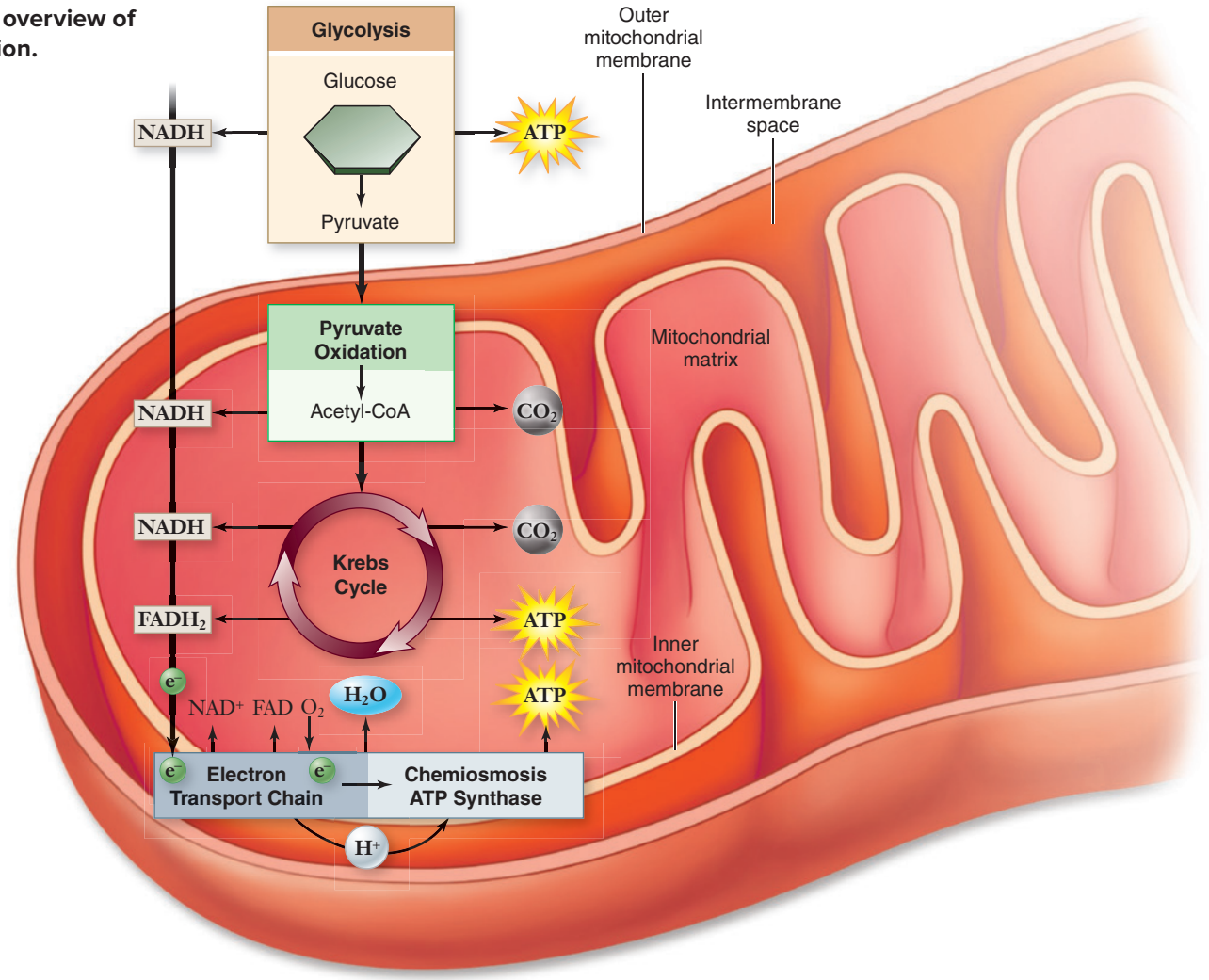
In most organisms, these two processes are combined. To harvest energy to make ATP from glucose in the presence of oxygen, the cell carries out a complex series of enzyme-catalyzed reactions that remove energetic electrons via oxidation reactions. These electrons are then used in an electron transport chain that passes the electrons down a series of carriers while translocating protons into the intermembrane space. The final electron acceptor in aerobic respiration is oxygen, and the resulting proton gradient provides energy for the enzyme ATP synthase to phosphorylate ADP to ATP (figure 7.5). The details of this complex process will be covered in the remainder of this chapter.

Learning Outcomes Review 7.1

Cells acquire energy from the complete oxidation of glucose. In these redox reactions, protons as well as electrons are transferred, and thus they are dehydrogenation reactions. Electron carriers aid in the gradual, stepwise release of the energy from oxidation, rather than rapid combustion. The result is the synthesis of ATP, a portable source of energy. ATP synthesis can occur by two mechanisms: substrate-level phosphorylation and oxidative phosphorylation.

- ***Why don't cells just link the oxidation of glucose directly to cellular functions that require the energy?***

Figure 7.5 **An overview of aerobic respiration.**



7.2 Glycolysis: Splitting Glucose

Learning Outcomes

1. *Describe the process of glycolysis.*
2. *Calculate the energy yield from glycolysis.*
3. *Distinguish between aerobic respiration and fermentation.*

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.

Glycolysis converts glucose into two pyruvate, forming two ATP and two NADH in the process

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

Priming reactions The first three reactions "prime" glucose by changing it into a compound that can be readily cleaved 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.

Cleavage This 6-carbon diphosphate sugar is then split into two 3-carbon monophosphate sugars. One of these is G3P, and the other is converted into G3P. The G3P then undergoes a series of reactions that eventually yields more energy than was spent priming (figure 7.7).

Oxidation and ATP formation Each G3P is oxidized, transferring two electrons (and one proton) to NAD^+, thus forming NADH. A molecule of P_i is also added to G3P to produce 1,3-bisphosphoglycerate (BPG). The phosphate incorporated can be transferred to ADP by substrate-level phosphorylation (see figure 7.4) to allow a positive yield of ATP at the end of the process.

Another four reactions convert BPG into pyruvate. In the process, the phosphates are transferred to ADP to yield two ATP per G3P. The entire process is shown in detail in figure 7.7.

Each glucose molecule is split into two G3P molecules, so 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)
– 2 ATP (used in the two reactions in the first step)

2 ATP (net yield for entire process)

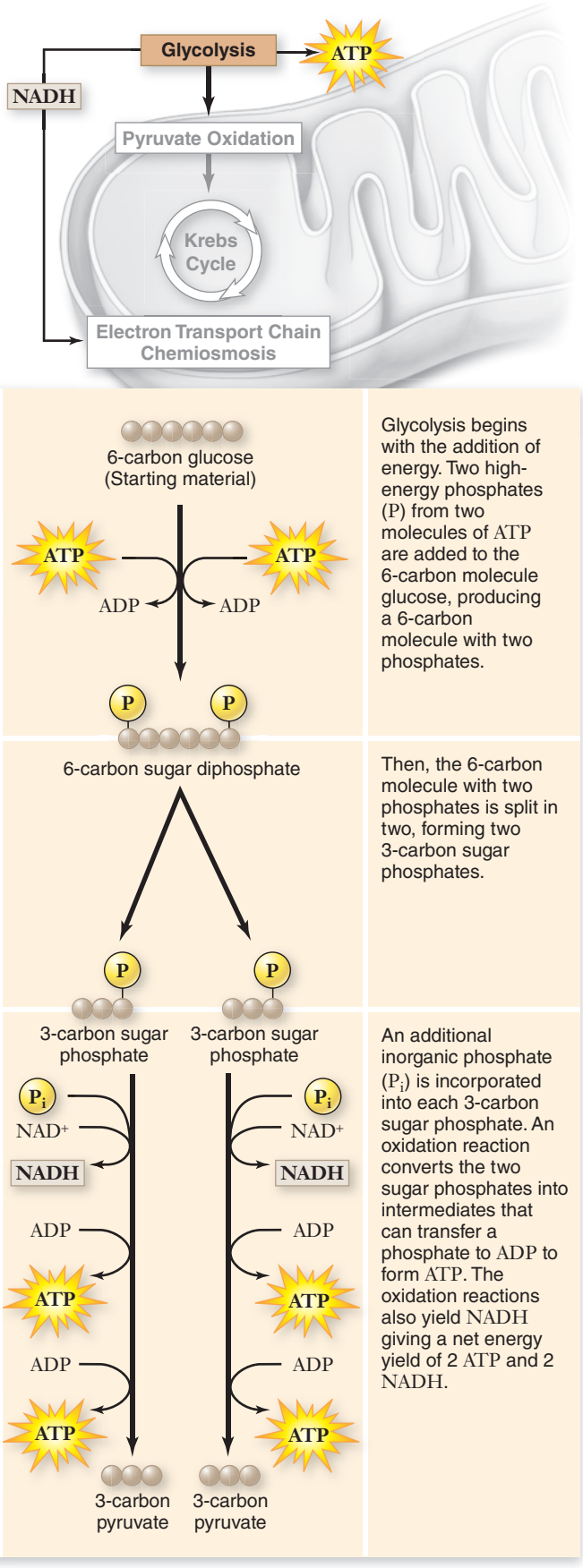
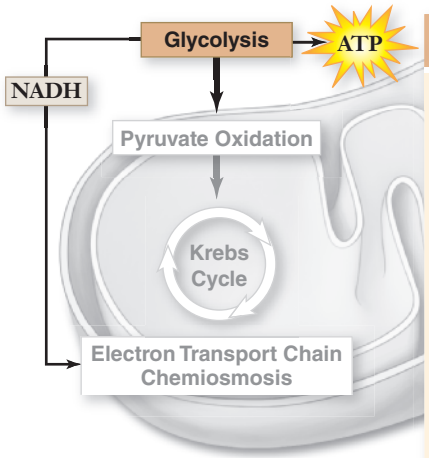


Figure 7.6 An overview of glycolysis.



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.

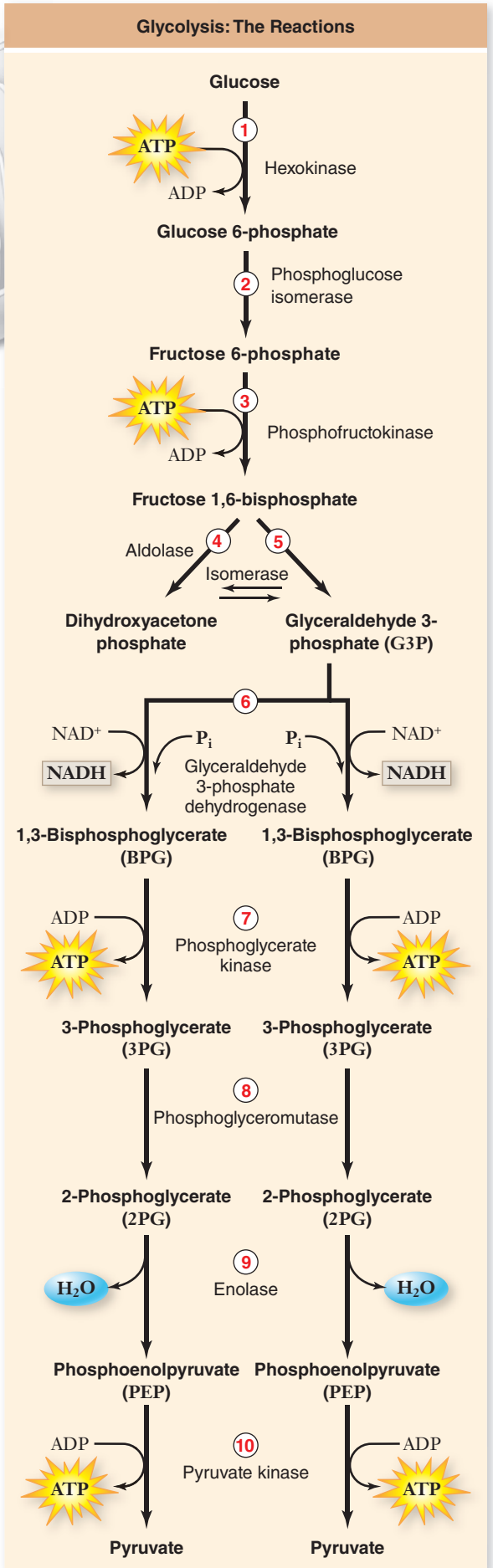
6. Oxidation followed by phosphorylation produces two NADH molecules and two molecules of BPG, each with one high-energy phosphate bond.

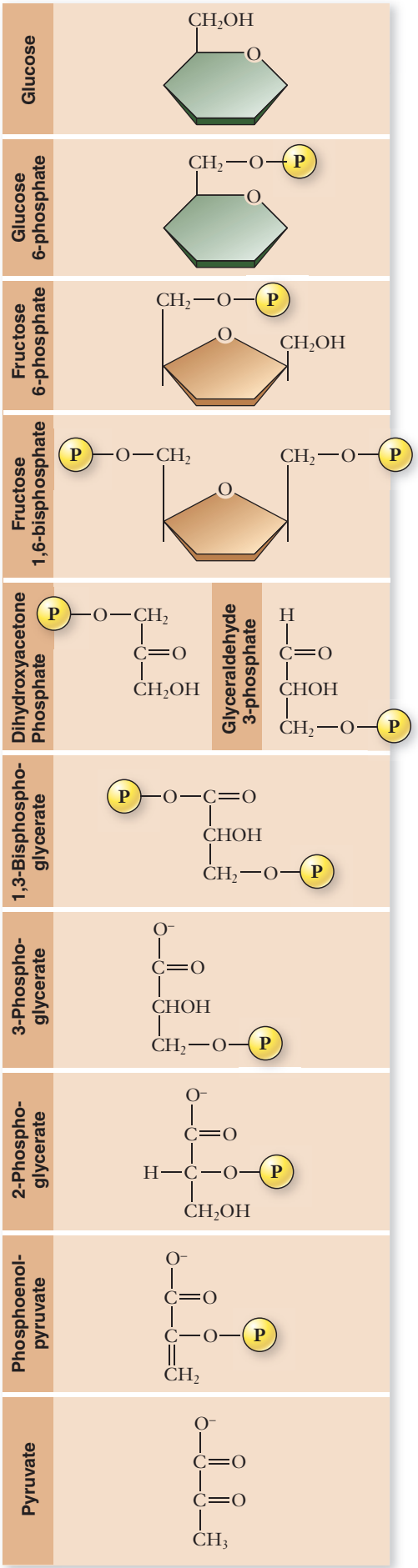
7. Removal of high-energy phosphate by two ADP molecules produces two ATP molecules and leaves two 3PG molecules.

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.





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 the ATP yield from glycolysis is low, it is actually quite efficient, with just under 40% of the energy released being trapped as 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—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? There are several possible answers. First, the process is energetically efficient, and better than the alternative—no ATP. Second, 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 framework to increase the yield of ATP as oxygen became available as an oxidizing agent. 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

Consider the net reaction of the glycolytic sequence:

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

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.

Recycling NADH

As long as glucose is available, a cell can continually churn out ATP by glycolysis to drive its activities. However, this process accumulates NADH and depletes the pool of NAD^+ molecules. Cells do not contain a large amount of NAD^+ so for glycolysis to continue, NADH must be recycled into NAD^+. The NADH is oxidized back to NAD^+ by reducing another molecule. Cells can do this in two ways, and which one is used depends on whether O_2 is available (figure 7.8):

1. **Aerobic respiration.** Oxygen has a high affinity for electrons, making it an excellent electron acceptor. Electrons are transferred through a series of membrane carriers, ultimately reducing oxygen and 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 can accept electrons. The organic molecules used are quite varied and include acetaldehyde in ethanolic fermentation or pyruvate itself in lactic acid fermentation. This reaction plays an important role in the metabolism of most organisms, even those capable of aerobic respiration.

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 coenzyme

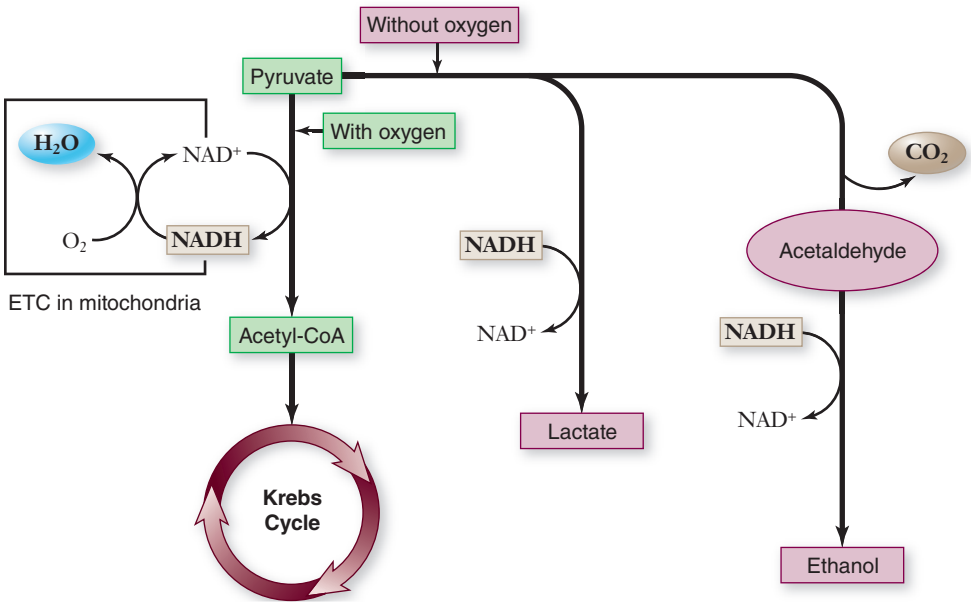


Figure 7.8 The fate of pyruvate and NADH produced by glycolysis. In the presence of oxygen, NADH is oxidized by the electron transport chain (ETC) in mitochondria using oxygen as the final electron acceptor. This regenerates NAD^+, allowing glycolysis to continue. The pyruvate produced by glycolysis is oxidized to acetyl-CoA, which enters the Krebs cycle. In the absence of oxygen, pyruvate is instead reduced, oxidizing NADH and regenerating NAD^+, thus allowing 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.

A (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 section 7.8.

Learning Outcomes Review 7.2

Glycolysis splits the 6-carbon molecule glucose into two 3-carbon molecules of pyruvate. This process uses two ATP molecules in "priming" reactions and eventually produces four molecules of ATP per glucose for a net yield of two ATP. The oxidation reactions of glycolysis require NAD^+ and produce NADH. When oxygen is abundant, NAD^+ is regenerated in the electron transport chain, using O_2 as an acceptor. When oxygen is absent, NAD^+ is regenerated in a fermentation reaction using an organic molecule as an electron receptor.

- *Does glycolysis taking place in the cytoplasm argue for or against the endosymbiotic origin of mitochondria?*

7.3 The Oxidation of Pyruvate Produces Acetyl-CoA

Learning Outcome

1. *Diagram how the oxidation of pyruvate links glycolysis with the Krebs cycle.*

In the presence of oxygen, the pyruvate produced by glycolysis can be further oxidized. 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 2-carbon compound and CO_2, while reducing NAD^+ to NADH. Next, the 2-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 in the form of CO_2 (figure 7.9). The remaining 2-carbon compound, called an acetyl group, becomes bound to coenzyme A, producing *acetyl-CoA*. This oxidation is also a dehydrogenation, so a pair of electrons and one associated proton are transferred to 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, subunits pass the substrates from one enzyme to the next without releasing them. The enzyme that performs these concerted reactions is called *pyruvate*

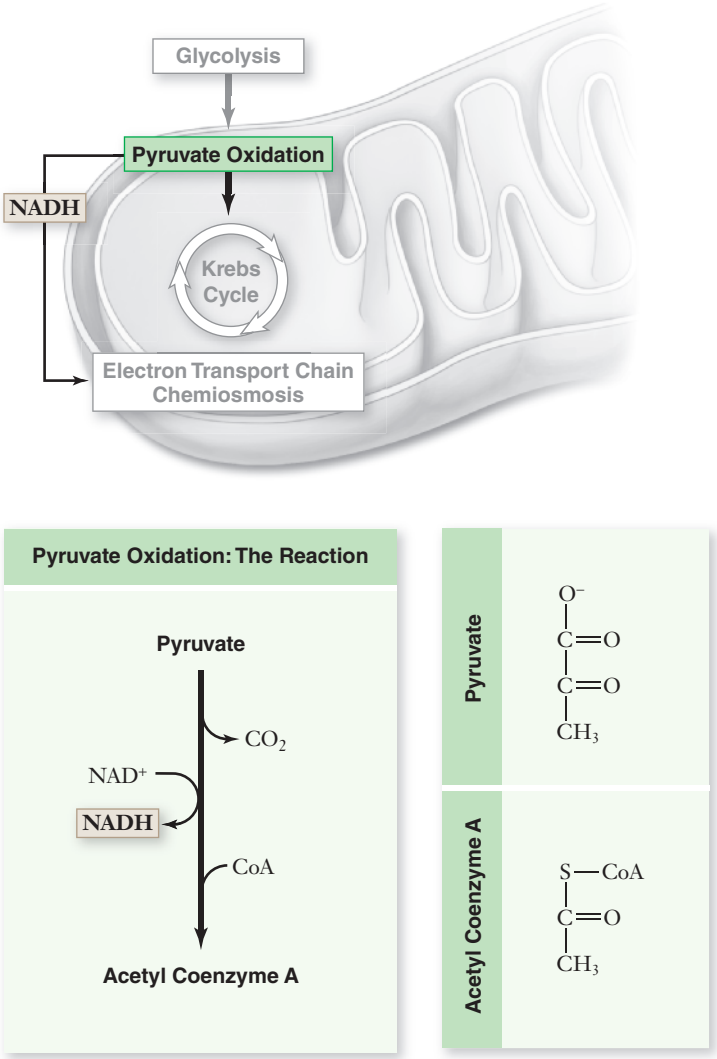


Figure 7.9 The oxidation of pyruvate. This complex reaction uses NAD^+ to accept electrons, reducing it to NADH. The product, acetyl coenzyme A (acetyl-CoA), feeds the acetyl unit into the Krebs cycle, and the CoA is recycled for another oxidation of pyruvate. NADH provides energetic electrons for the electron transport chain.

dehydrogenase, and it is one of the largest enzymes known; it contains 60 subunits! The reaction can be summarized as follows:

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

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.

Learning Outcome Review 7.3

Pyruvate is oxidized in the mitochondria to produce acetyl-CoA and CO_2. Acetyl-CoA is the molecule that links glycolysis and the reactions of the Krebs cycle.

- *What are the advantages and disadvantages of a multienzyme complex?*

7.4 The Krebs Cycle

Learning Outcomes

1. *Relate the nine reactions of the Krebs cycle to the flow of carbon and electrons in the cycle.*
2. *Diagram the oxidation reactions in the Krebs cycle.*

The *Krebs cycle* allows the oxidation of 2-carbon units in the form of acetyl groups bound to CoA (acetyl-CoA). These can come from the oxidation of pyruvate, or from the oxidation of fatty acids (see section 7.9). The acetyl group is added to a 4-carbon acid, oxaloacetate. The resulting 6-carbon molecule is citric acid, thus the cycle is also called the citric acid cycle, and the TCA cycle (for tricarboxylic acid). The reactions of the Krebs cycle convert citric back to oxaloacetate, generating CO_2 and transferring electrons and protons to the electron carriers NADH and $FADH_2$. A single ATP is generated during the cycle as well, but most of the energy released is retained in the form of the electrons in NADH and $FADH_2$ that can be used by the electron transport chain to generate a *proton gradient* to drive ATP synthesis.

An overview of the Krebs cycle

The reactions of the Krebs cycle take place in the mitochondrial matrix. They take in acetyl units from acetyl-CoA, convert them into CO_2, transferring electrons and protons to NADH and $FADH_2$ (figure 7.10).

The first reaction combines the 4-carbon oxaloacetate with the acetyl group to produce the 6-carbon citrate molecule. Five more steps, which have been simplified in figure 7.10, convert citrate to a 5-carbon intermediate and then to the 4-carbon succinate. During these reactions, two NADH and one ATP are produced.

Succinate undergoes three additional reactions, also simplified in the figure, to become oxaloacetate. During these reactions, one more 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.

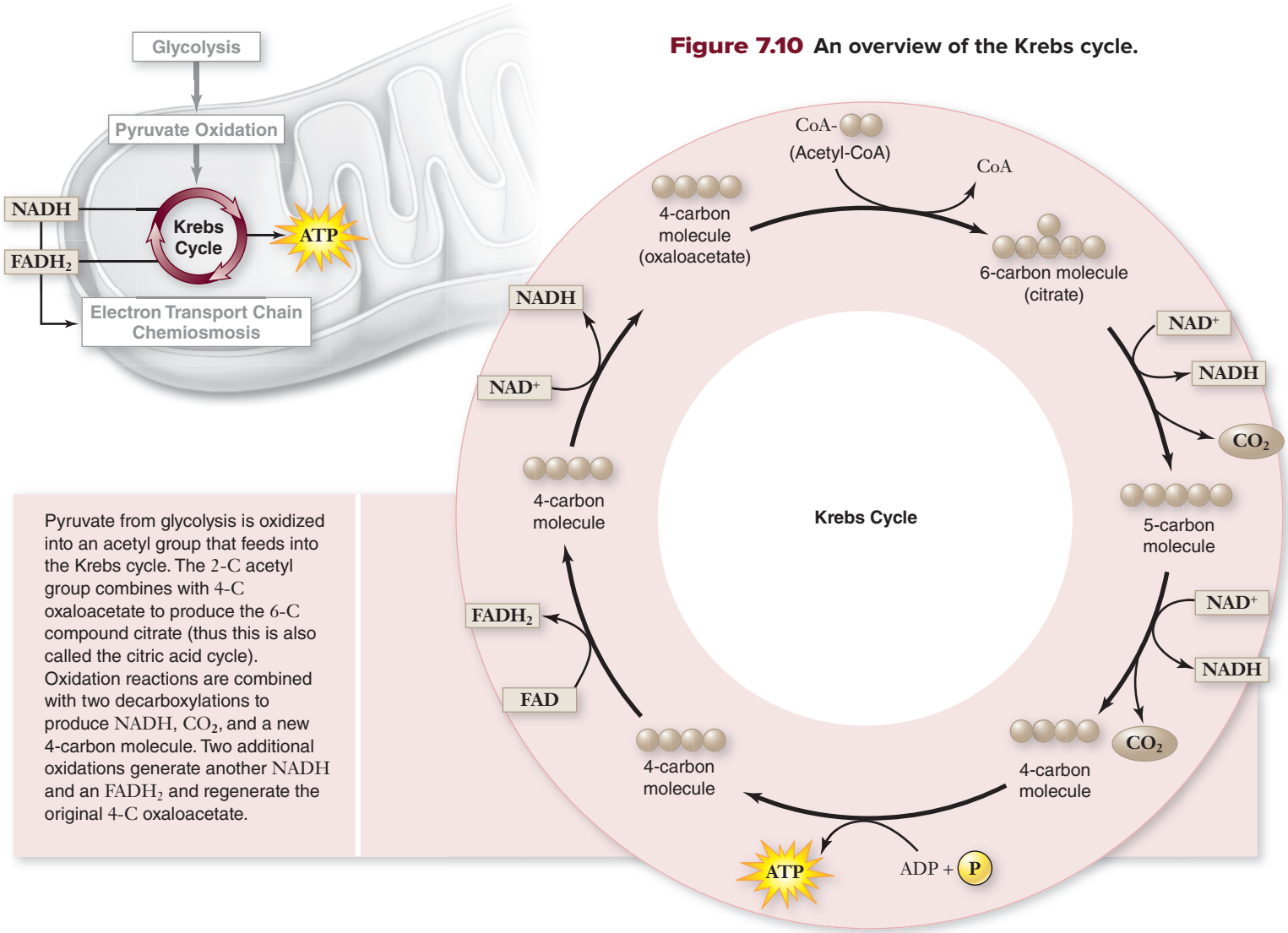


Figure 7.10 An overview of the Krebs cycle.

Pyruvate from glycolysis is oxidized into an acetyl group that feeds into the Krebs cycle. The 2-C acetyl group combines with 4-C oxaloacetate to produce the 6-C compound citrate (thus this is also called the citric acid cycle). Oxidation reactions are combined with two decarboxylations to produce NADH, CO_2, and a new 4-carbon molecule. Two additional oxidations generate another NADH and an $FADH_2$ and regenerate the original 4-C oxaloacetate.

The Krebs cycle extracts electrons and synthesizes one ATP

Figure 7.11 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 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 begin the cycle again.

Glucose becomes CO_2 and potential energy

In the process of aerobic respiration, glucose is entirely consumed. The 6-carbon glucose molecule is cleaved into two 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 in section 7.1, 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.

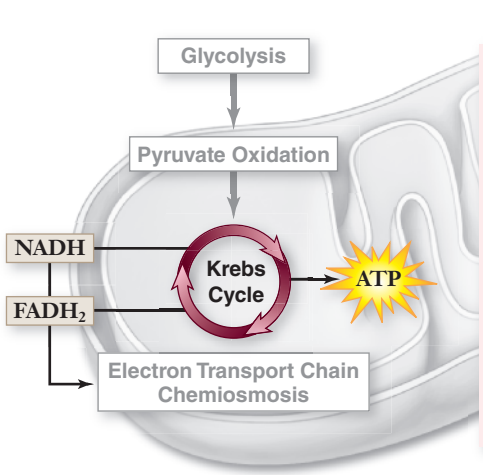
NADH delivers electrons to the beginning of the electron transport chain, and oxygen captures them at the end. The oxygen then joins with hydrogen ions to form water. At each step in the chain, the electrons move to a slightly more electronegative carrier, and their positions shift slightly. Thus, the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

Learning Outcomes Review 7.4

The Krebs cycle completes the oxidation of glucose begun with glycolysis. In the first segment, acetyl-CoA is added to oxaloacetate to produce citrate. In the next segment, five reactions produce succinate, two NADH from NAD^+, and one ATP. Finally, succinate undergoes three more reactions to regenerate oxaloacetate, producing one more NADH and one $FADH_2$ from FAD.

- ***What happens to the electrons removed from glucose at this point?***



1. Reaction 1: Condensation

2–3. Reactions 2 and 3: Isomerization

4. Reaction 4: The first oxidation

5. Reaction 5: The second oxidation

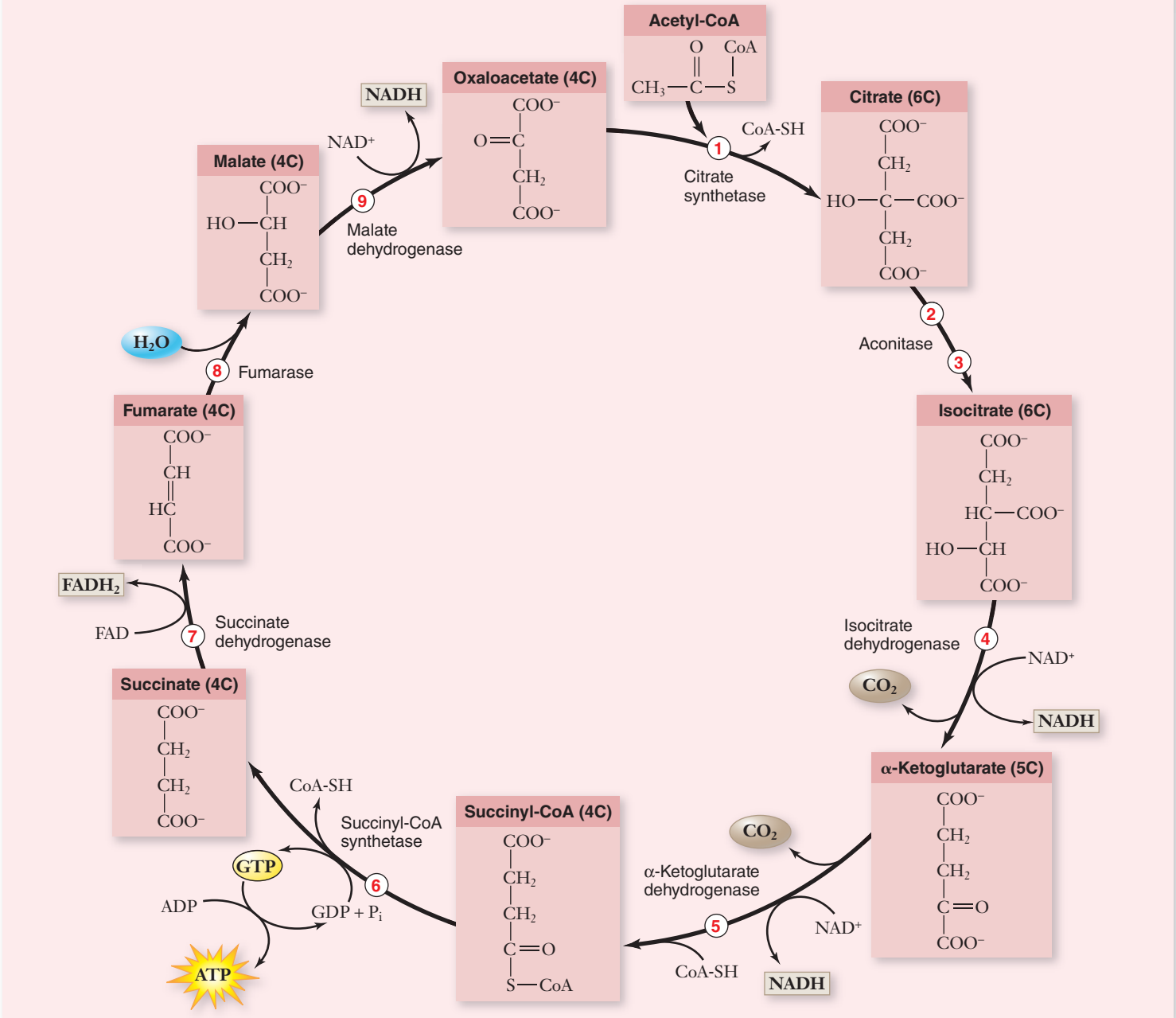
6. Reaction 6: Substrate-level phosphorylation

7. Reaction 7: The third oxidation

8–9. Reactions 8 and 9: Regeneration of oxaloacetate and the fourth oxidation

Figure 7.11 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.

Krebs Cycle: The Reactions



7.5 The Electron Transport Chain and Chemiosmosis

Learning Outcome

1. *Describe the structure and function of the electron transport chain.*
2. *Diagram how the proton gradient connects electron transport with ATP synthesis.*

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 and $FADH_2$ 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.*

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 7.12*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:

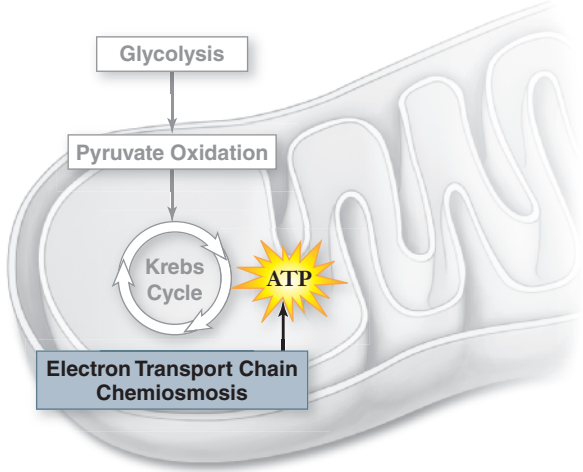
$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$$

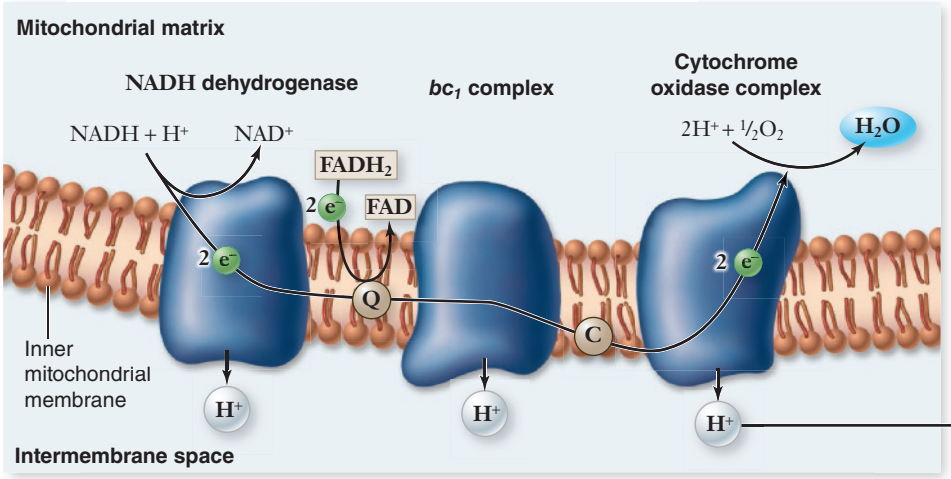
In contrast to NADH, which contributes its electrons to NADH dehydrogenase, $FADH_2$, which is located in the inner mitochondrial membrane, feeds its electrons to ubiquinone, which is also in the membrane. Electrons from $FADH_2$ thus "skip" the first step in the electron transport chain.

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.

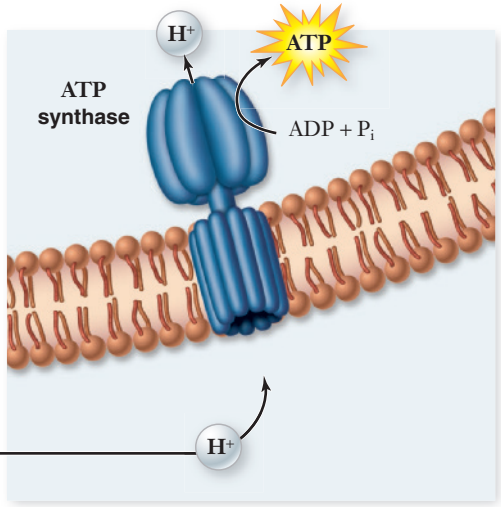
Electron transport powers proton pumps in the inner membrane

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 in section 7.1, protons (H^+) are produced





a. The electron transport chain



b. Chemiosmosis

Figure 7.12 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.

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 (figure 7.12*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

Because the mitochondrial matrix is negative compared with the intermembrane space, positively charged protons are attracted to 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 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 7.12*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. This chemiosmotic mechanism for the coupling of electron transport and ATP synthesis was controversial when it was proposed. Over the years, experimental evidence accumulated to support this hypothesis (figure 7.13).

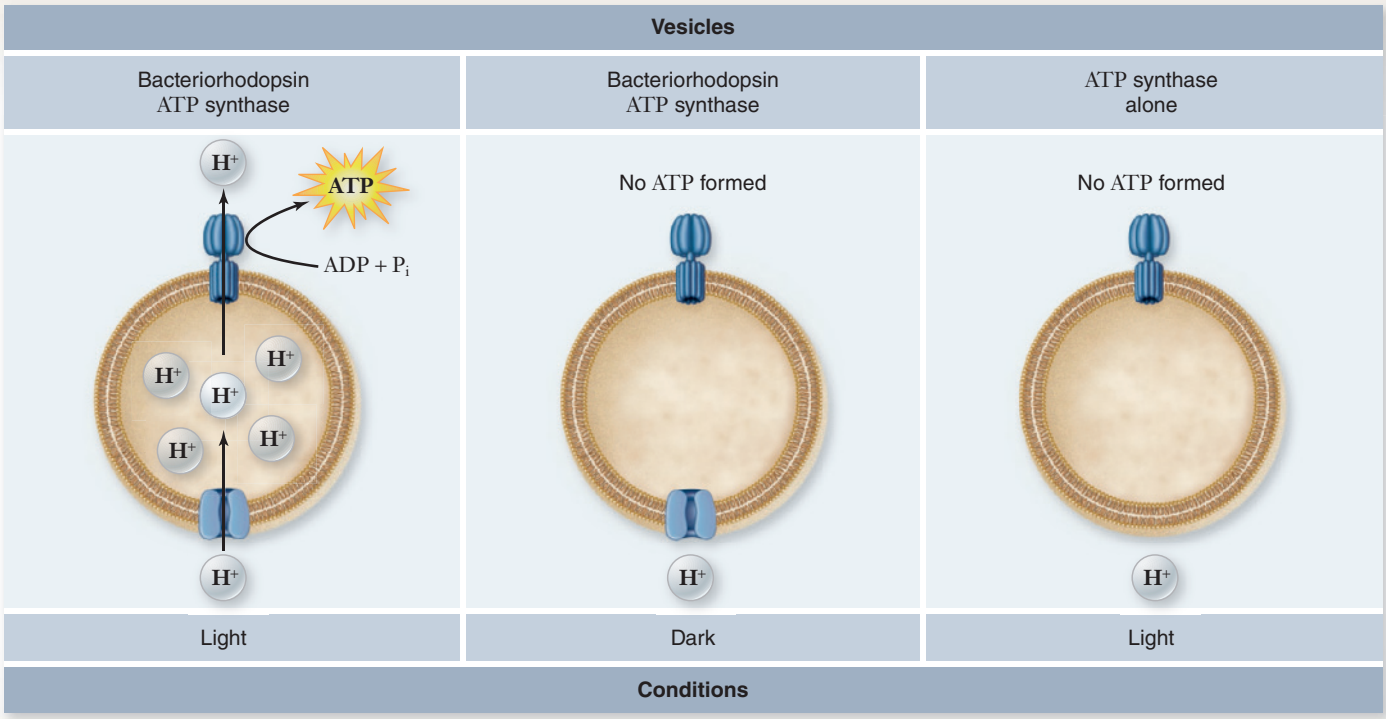
The energy released by the reactions of cellular respiration ultimately drives the proton pumps that produce the proton gradient. The proton gradient provides the energy required for the synthesis of ATP. Figure 7.14 summarizes the overall process.

SCIENTIFIC THINKING

Hypothesis: *ATP synthase enzyme uses a proton gradient to provide energy for phosphorylation reaction.*

Prediction: *The source of the proton gradient should not matter. A proton gradient formed by the light-driven pump bacteriorhodopsin should power phosphorylation in the light but not in the dark.*

Test: *Artificial vesicles are made with bacteriorhodopsin and ATP synthase, and ATP synthase alone. These are illuminated with light and assessed for ATP production.*



Result: *The vesicle with both bacteriorhodopsin and ATP synthase can form ATP in the light but not in the dark. The vesicle with ATP synthase alone cannot form ATP in the light.*

Conclusion: *ATP synthase is able to utilize a proton gradient for energy to form ATP.*

Further Experiments: *What other controls would be appropriate for this type of experiment? Explain why this experiment is a more direct test of the chemiosmotic hypothesis than the Jagendorf acid bath experiment (see figure 8.16).*

Figure 7.13 **Evidence for the chemiosmotic synthesis of ATP by ATP synthase.**

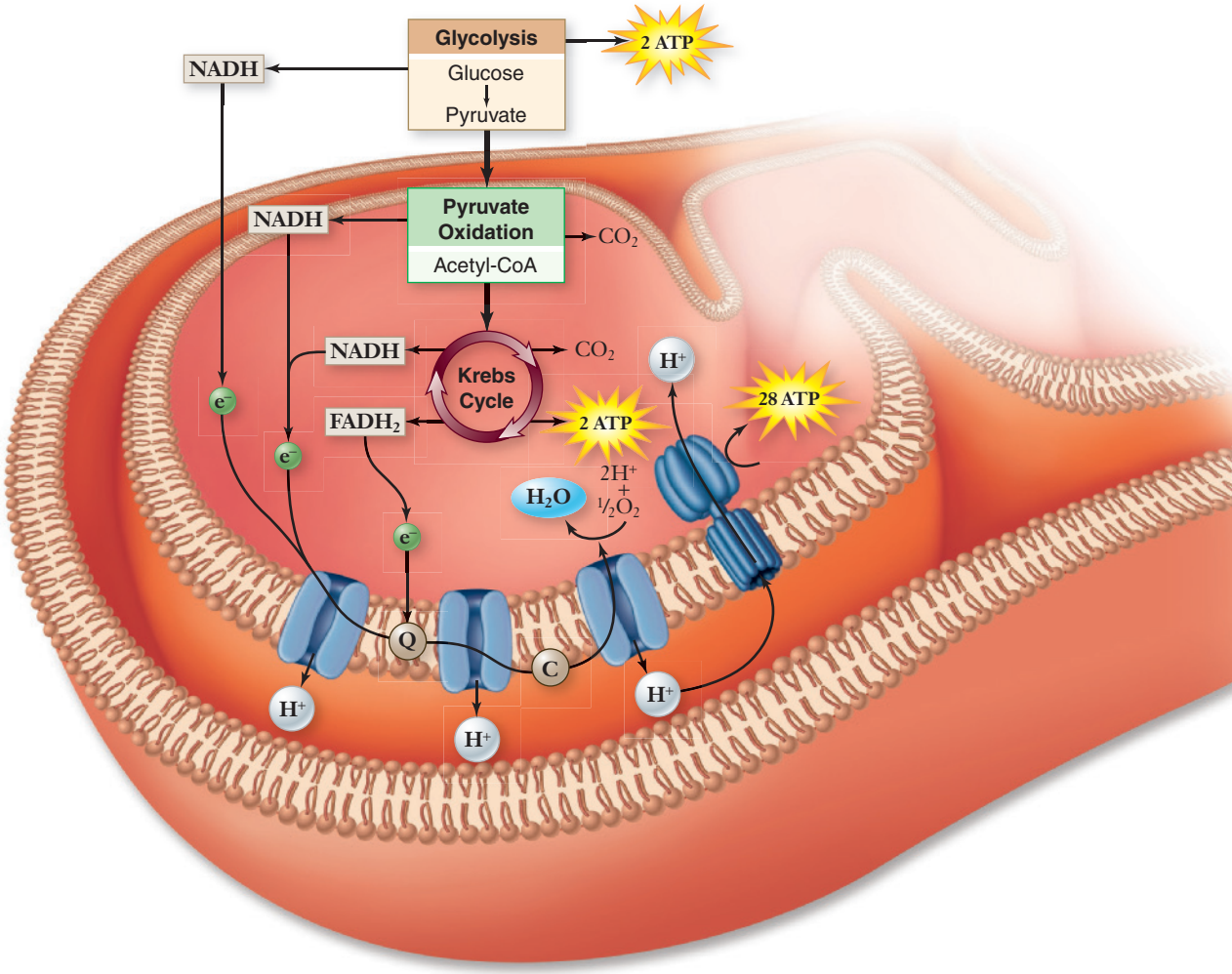


Figure 7.14 Aerobic respiration in the mitochondria. The entire process of aerobic respiration is shown in cellular context. Glycolysis occurs in the cytoplasm with the pyruvate and NADH produced entering the mitochondria. Here, pyruvate is oxidized and fed into the Krebs cycle to complete the oxidation 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.

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 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 flow of protons is like that of water in a hydroelectric power plant. Like the flow of water driven by gravity causes a turbine to rotate and generate electrical current, the proton gradient produces the energy that drives the rotation of the ATP synthase generator.

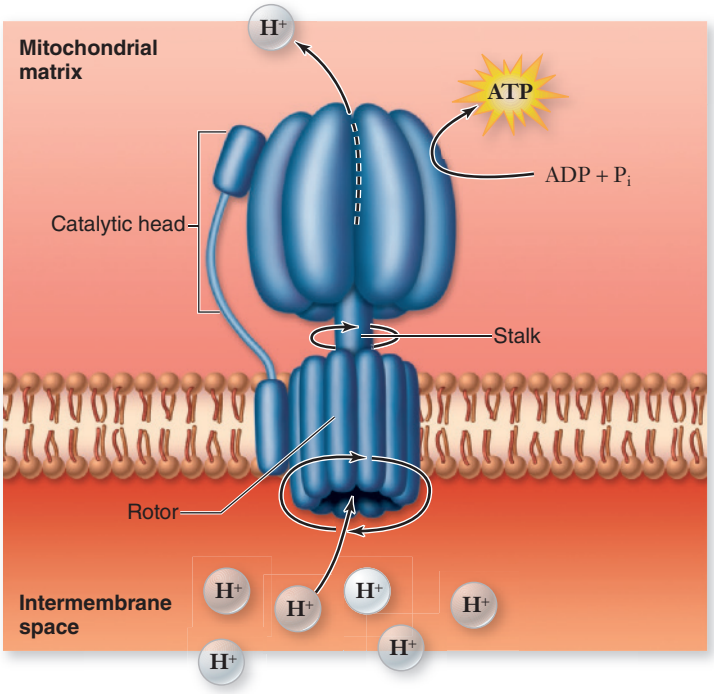


Figure 7.15 The ATP rotary engine. Protons move across the membrane down their concentration gradient. The energy released causes the rotor and stalk structures to rotate. This mechanical energy alters the conformation of the ATP synthase enzyme to catalyze the formation of ATP.

Learning Outcomes Review 7.5

The electron transport chain receives electrons from NADH and $FADH_2$ and passes them down the chain to oxygen. The protein complexes of the electron transport chain, in the inner membrane of mitochondria, use the energy from electron transfer to pump protons across the membrane, creating an electrochemical gradient. The enzyme ATP synthase uses this gradient to drive the endergonic reaction of phosphorylating ADP to ATP.

- *How would poking a small hole in the outer membrane affect ATP synthesis?*

7.6 Energy Yield of Aerobic Respiration

Learning Outcome

1. *Calculate the number of ATP molecules produced by aerobic respiration.*

How much metabolic energy (in the form of ATP) does a cell gain from aerobic breakdown of glucose? This simple question has actually been a source of some controversy in biochemistry.

The theoretical yield for eukaryotes is 30 molecules of ATP per glucose molecule

The number of molecules of ATP produced by ATP synthase per molecules of glucose depends on the number of protons transported across the inner membrane, and the number of protons needed per ATP synthesized. The number of protons transported per NADH and $FADH_2$ is 10 and 6 H^+, respectively. Each ATP synthesized requires 4 H^+, leading to 10/4 = 2.5 ATP/NADH, and 6/4 = 1.5 ATP/$FADH_2$.

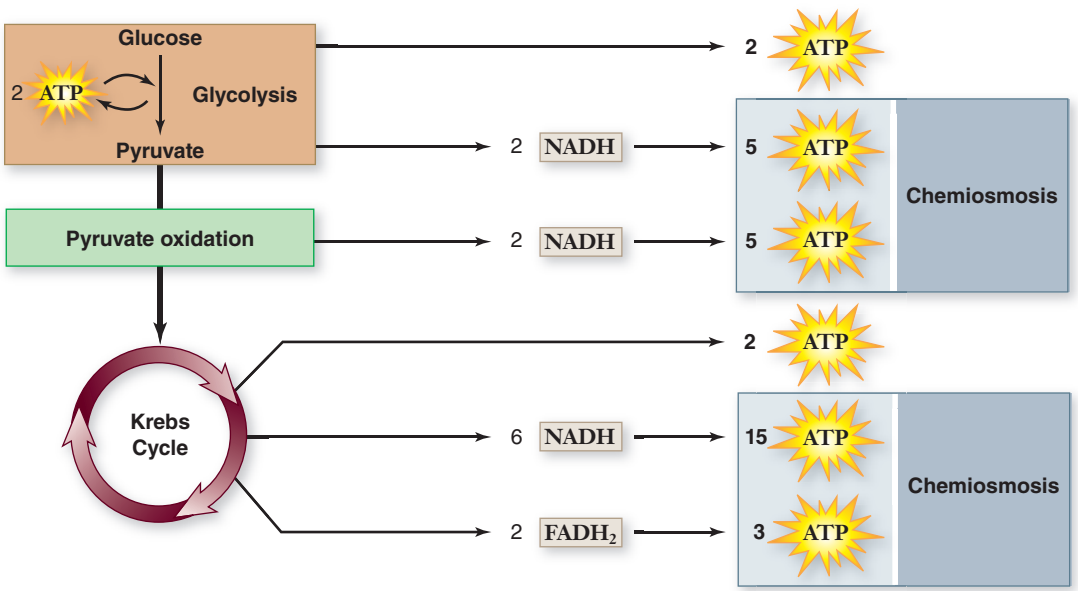
To finish the bookkeeping: oxidizing glucose to pyruvate via glycolysis yields 2 ATP directly, and 2 × 2.5 = 5 ATP from NADH. The oxidation of pyruvate to acetyl-CoA yields another 2 × 2.5 = 5 ATP from NADH. Lastly, the Krebs cycle produces 2 ATP directly, 6 × 2.5 = 15 ATP from NADH, and 2 × 1.5 = 3 ATP from $FADH_2$. Summing all of these leads to 32 ATP for respiration (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 one ATP per NADH transported. This reduces the predicted yield for eukaryotes to 30 ATP.

Calculation of P/O ratios has changed over time

The value for the amount of ATP synthesized per O_2 molecule reduced is called the phosphate-to-oxygen ratio (P/O ratio). Both theoretical calculations, and direct measurement of this value, have been contentious issues. When theoretical calculations were first made, we lacked detailed knowledge of the respiratory chain, and the mechanism for coupling electron transport to ATP synthesis. Since redox reactions occur at three sites for NADH and two sites for $FADH_2$, it was assumed that three molecules of ATP were produced per NADH and two per $FADH_2$. We now know that assumption was overly simplistic.

Understanding that a proton gradient is the link between electron transport and ATP synthesis changed the nature of the calculations. We need to know the number of protons pumped during electron transport: 10 H^+ per NADH, and 6 H^+ per $FADH_2$. Then we need to know the number of protons needed per ATP. Since ATP synthase is a rotary motor, this calculation depends on the number of binding sites for ATP, and the number of protons required for rotation. We know that ATP synthase has three binding sites for ATP. If 12 protons are used per rotation, you get the value of 4 H^+ per ATP



Total net ATP yield = 32
(30 in eukaryotes)

Figure 7.16 Theoretical ATP yield. The theoretical yield of ATP harvested from glucose by aerobic respiration totals 32 molecules. In eukaryotes this is reduced to 30 because it takes 1 ATP to transport each molecule of NADH that is generated by glycolysis in the cytoplasm into the mitochondria.

used in the previous calculation. Actual measurements of the P/O ratio have been problematic, but now appear to be at most 2.5.

We can also calculate how efficiently respiration captures the free energy released by the oxidation of glucose in the form of ATP. The amount of free energy released by the oxidation of glucose is 686 kcal/mol, and the free energy stored in each ATP is 7.3 kcal/mol. Therefore, a eukaryotic cell harvests about $(7.3 \times 30)/686 = 32\%$ 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 energy 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 became more effective at using respiration to extract energy from molecules derived from other organisms. As long as some organisms captured energy by photosynthesis, others could exist solely by feeding on them.

Learning Outcome Review 7.6

Passage of electrons down the electron transport chain produces roughly 2.5 molecules of ATP per molecule of NADH (1.5 ATP per $FADH_2$). This process plus the ATP from substrate-level phosphorylation can yield a maximum of 32 ATP for the complete oxidation of glucose. NADH generated in the cytoplasm of eukaryotes yields only two ATP/NADH due to the cost of transport into the mitochondria, lowering the yield to 30 ATP.

- ***How does chemiosmosis allow for noninteger numbers of ATP/NADH?***

7.7 Regulation of Aerobic Respiration

Learning Outcome

1. *Understand the control points for cellular 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 (see chapter 6) of phosphofructokinase, as is the Krebs cycle intermediate citrate. High levels of both ATP and citrate inhibit phosphofructokinase. Thus, under conditions when

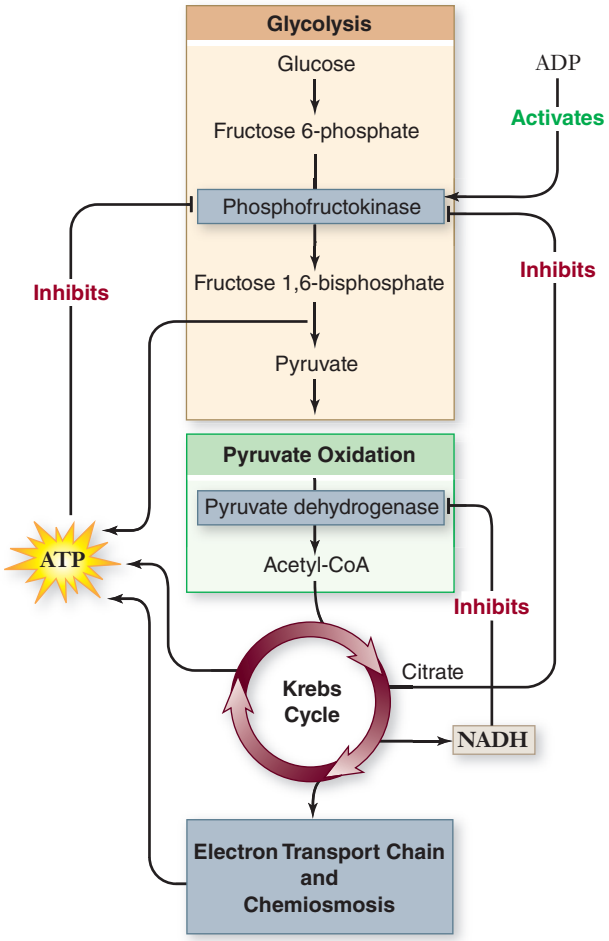


Figure 7.17 Control of glucose catabolism. The relative levels of ADP and ATP and key intermediates NADH and citrate control the catabolic pathway at two key points: the committing reactions of glycolysis and the Krebs cycle.

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 phosphofructo-kinase, pyruvate dehydrogenase, and two other Krebs cycle enzymes), slowing down the entire catabolic pathway.

Learning Outcome Review 7.7

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, which is inhibited by ATP or citrate (or both). The main control point in oxidation of pyruvate is the enzyme pyruvate dehydrogenase, inhibited by NADH.

- ***How does feedback inhibition ensure economic production of ATP?***

7.8 Oxidation Without O_2

Learning Outcomes

1. Compare anaerobic and aerobic respiration.
2. Distinguish between fermentation and anaerobic respiration.

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. The amount of ATP produced is less, but the process is still respiration and not fermentation.

Methanogens use carbon dioxide

Among the heterotrophs that practice anaerobic respiration are 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 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 discussed 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

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.

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^+:

$$\text{organic molecule} + \text{NADH} \longrightarrow \text{reduced organic molecule} + \text{NAD}^+$$

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. 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 (figure 7.19). It therefore closes the metabolic circle, allowing glycolysis to continue as long as glucoseis 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.

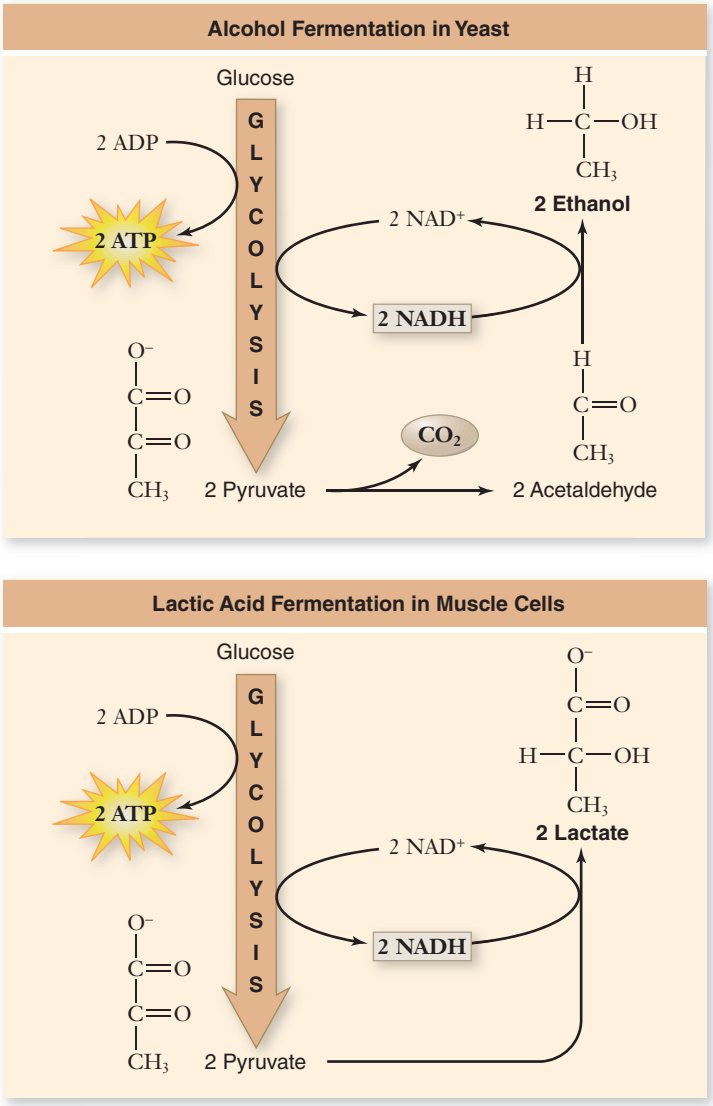


Figure 7.19 Fermentation. Yeasts carry out the conversion of pyruvate to ethanol. Muscle cells convert pyruvate into lactate, which is less toxic than ethanol. In each case, the reduction of a metabolite of glucose has oxidized NADH back to NAD^+ to allow glycolysis to continue under anaerobic conditions.

Learning Outcomes Review 7.8

Nitrate, sulfur, and CO_2 are all used as terminal electron acceptors in anaerobic respiration of different organisms. Organic molecules can also accept electrons in fermentation reactions that regenerate NAD^+. Fermentation reactions produce a variety of compounds, including ethanol in yeast and lactic acid in humans.

- *In what kinds of ecosystems would you expect to find anaerobic respiration?*

7.9 Catabolism of Proteins and Fats

Learning Outcomes

1. *Identify the entry points for proteins and fats in energy metabolism.*
2. *Recognize the importance of key intermediates in metabolism.*

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,

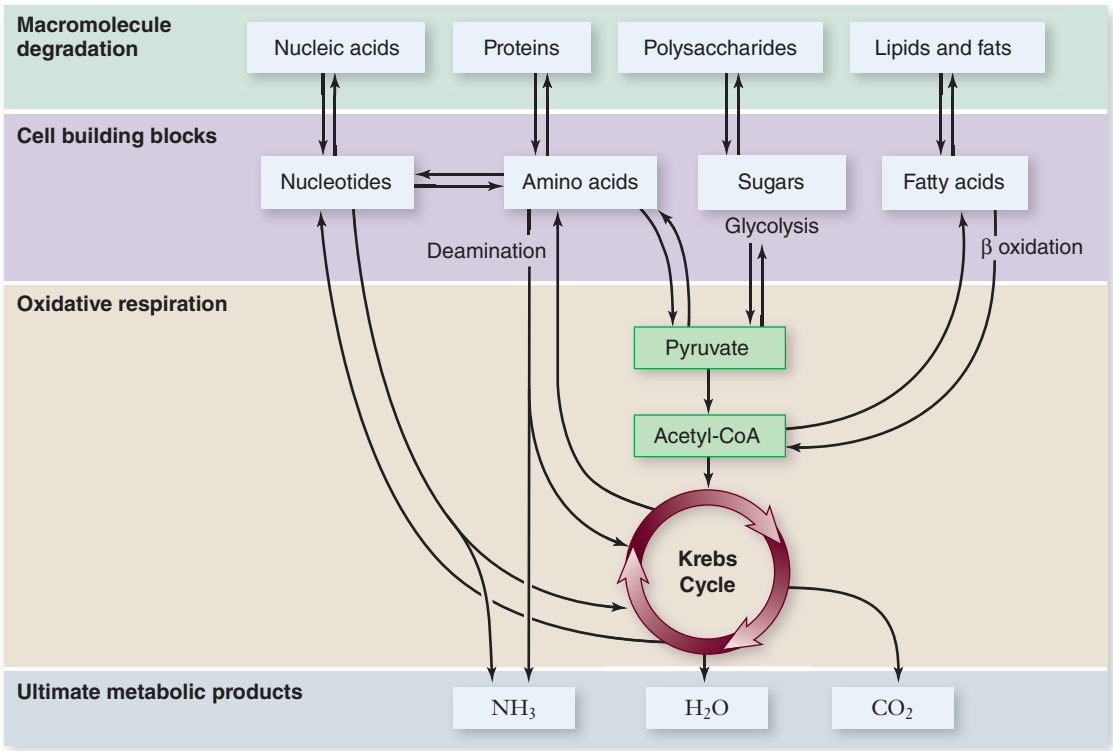


Figure 7.20 How cells extract chemical energy. All eukaryotes and many prokaryotes extract energy from organic molecules by oxidizing them. The first stage of this process, breaking down macromolecules into their constituent parts, yields little energy. The second stage, oxidative or aerobic respiration, 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.

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 converts 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.

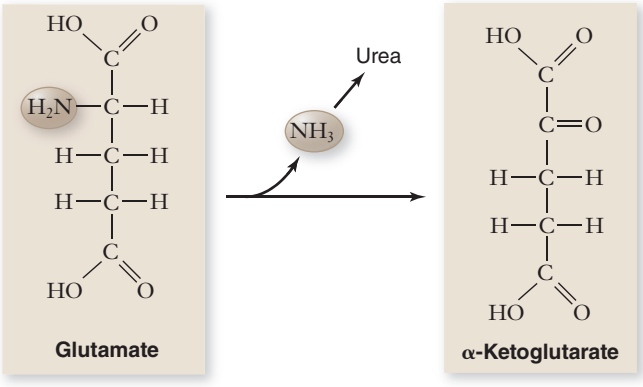


Figure 7.21 Deamination. After proteins are broken down into their amino acid constituents, the amino groups are removed from the amino acids to form molecules that participate in glycolysis and the Krebs cycle. For example, the amino acid glutamate becomes α-ketoglutarate, a Krebs cycle intermediate, when it loses its amino group.

Catabolism of fatty acids produces acetyl groups for the Krebs cycle

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 6-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 ATP per NADH, and 1.5 ATP 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 is 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

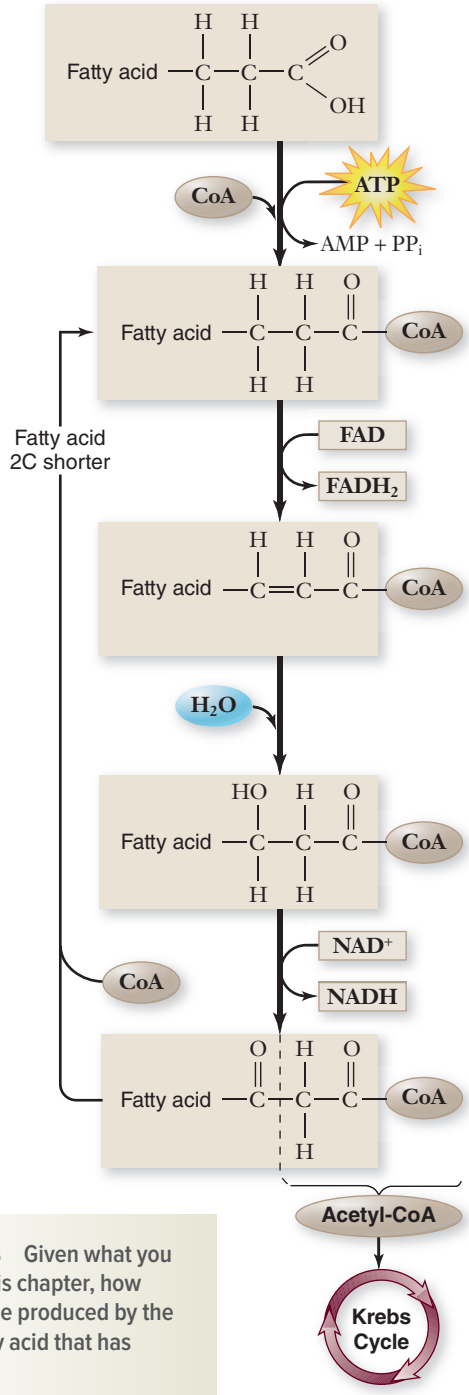


Figure 7.22
β oxidation.
Through a series of reactions known as β oxidation, the last two carbons in a fatty acid combine with coenzyme A to form acetyl-CoA, which enters the Krebs cycle. The fatty acid, now two carbons shorter, enters the pathway again and keeps reentering until all its carbons have 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.

Data analysis 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?

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.

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. 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.

Learning Outcomes Review 7.9

Proteins can be broken into their constituent amino acids, which are then deaminated and can enter metabolism at glycolysis or different steps of the Krebs cycle. Fats can be broken into units of acetyl-CoA by β oxidation and then fed into the Krebs cycle. Many metabolic processes can be used reversibly, to either build up (anabolism) or break down (catabolism) the major biological macromolecules. Key intermediates, such as pyruvate and acetyl-CoA, connect these processes.

- *Can fats be oxidized in the absence of O_2?*

7.10 Evolution of Metabolism

Learning Outcome

1. *Describe one possible hypothesis for the 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 more than 2 billion years.

Anoxygenic photosynthesis allowed the capture of light energy

The third major event in the evolution of metabolism was anoxygenic 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

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. Chapter 8 explores photosynthesis in detail.

Learning Outcome Review 7.10

Major milestones in the evolution of metabolism include the evolution of pathways to extract energy from organic compounds, the pathways of photosynthesis, and those of nitrogen fixation. Photosynthesis began as an anoxygenic process that later evolved to produce free oxygen, thus allowing the evolution of aerobic metabolism.

- ***What evidence can you cite for this hypothesis of the evolution of metabolism?***

AP Chapter Review

Chapter 7 How Cells Harvest Energy

The following summaries are a snapshot of the material covered in the chapter. Sections 7.1 - 7.5 and 7.7 - 7.9 correlate to Essential Knowledge in the AP Biology curriculum. However, the information contained in the other sections is important to your understanding of biology and the AP Curriculum.

7.1 Overview of Respiration

Cellular respiration is the complete oxidation of glucose. Aerobic respiration uses oxygen as the final electron acceptor for redox reactions. Anaerobic respiration uses inorganic molecules as electron acceptors, and fermentation uses organic molecules. Electron carriers play a critical role in energy metabolism. Electron carriers can be reversibly oxidized and reduced. For example, NAD^+ is reduced to NADH by acquiring two electrons, and NADH supplies these electrons to other molecules to reduce them. Mitochondria of eukaryotic cells move electrons in steps via the electron transport chain to capture energy efficiently. The ultimate goal of cellular respiration is synthesis of ATP, which is used to power most of the cell's activities. Substrate-level phosphorylation transfers a phosphate directly to ADP. Oxidative phosphorylation generates ATP via the enzyme ATP synthase. **1.B.1**, **2.A.1**, **2.A.2**

7.2 Glycolysis: Splitting Glucose

Glycolysis is a several-step process which converts glucose into two pyruvate molecules, forming two ATP and two NADH in the process. In the presence of oxygen, pyruvate is oxidized to acetyl-CoA, which can be oxidized by the Krebs cycle. This process leads to a large amount of ATP. In the absence of oxygen, a fermentation reaction uses pyruvate to oxidize NADH. **2.A.1**, **2.A.2**

7.3 The Oxidation of Pyruvate Produces Acetyl-CoA

In the presence of oxygen, pyruvate is oxidized to acetyl-CoA. This reaction yields one molecule of CO_2, one NADH, and one acetyl-CoA. Acetyl-CoA enters the Krebs cycle. **2.A.2**

7.4 The Krebs Cycle

As a glucose molecule is broken down to CO_2, some of its energy is preserved in four ATP, 10 NADH, and two $FADH_2$. The first reaction of the Krebs cycle is an irreversible condensation that produces citrate; it is inhibited when ATP is plentiful. The second and third reactions rearrange citrate to isocitrate. The fourth and fifth parts of the reactions are oxidations; in each, one NAD^+ is reduced to NADH. The sixth reaction is a substrate-level phosphorylation producing GTP, and from that ATP. The seventh reaction is another oxidation that reduces FAD to $FADH_2$. Reactions eight and nine regenerate oxaloacetate, including one final oxidation that reduces NAD^+ to NADH. **2.A.1**, **2.A.2**

7.5 The Electron Transport Chain and Chemiosmosis

The electron transport chain produces a proton gradient. In the inner mitochondrial membrane, NADH is oxidized to NAD^+ by NADH dehydrogenase. This results in three protons being pumped into the intermembrane space. A similar reaction involving $FADH_2$ results in two protons pumped into the intermembrane space. Electron transport powers proton pumps in the inner membrane where electrons move to cytochrome oxidase, and join with H^+ and O_2 to form H_2O. Chemiosmosis is a little different and utilizes the electrochemical gradient to produce ATP. Protons diffuse back into the mitochondrial matrix via the ATP synthase channel. ATP synthase is a molecular rotary motor to synthesize ATP. **2.A.2**, **4.A.2**

7.6 Energy Yield of Aerobic Respiration

The theoretical yield for eukaryotes is 30 molecules of ATP per glucose molecule. Calculation of P/O ratios has changed over time.

7.7 Regulation of Aerobic Respiration

Glucose catabolism is controlled by the concentration of ATP molecules, which act as allosteric enzymes and intermediates in the Krebs cycle. **4.B.1**

7.8 Oxidation Without O_2

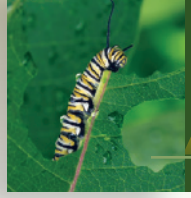
In the absence of oxygen, other final electron acceptors can be used for respiration. Fermentation uses organic compounds as electron acceptors. Fermentation is the regeneration of NAD^+ by oxidation of NADH and reduction of an organic molecule. In yeast, pyruvate is decarboxylated then reduced to ethanol. In animals, pyruvate is reduced directly to lactate and stored in the muscles. **2.A.2**

7.9 Catabolism of Proteins and Fats

Catabolism of proteins removes amino groups (NH_2). Catabolism of fatty acids produces acetyl groups for the Krebs cycle. Fatty acids are converted to acetyl groups by successive oxidations. These acetyl groups feed into the Krebs cycle to be oxidized and generate NADH for electron transport and ATP production. **2.A.2**

7.10 Evolution of Metabolism

Major milestones are recognized in the evolution of metabolism; the order of events is hypothetical. The earliest life-forms degraded carbon-based molecules present in the environment. The evolution of glycolysis also occurred early. **1.D.2**

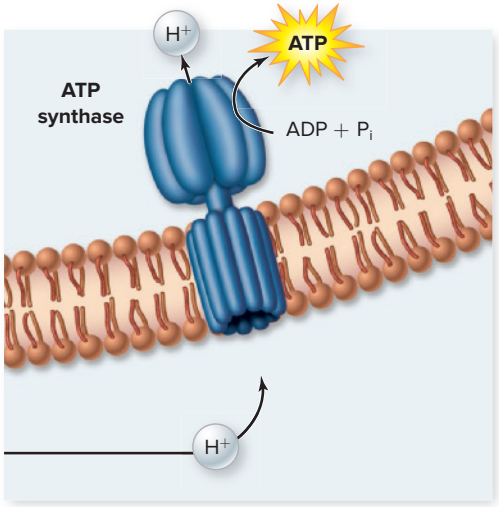


AP Review Questions

AP MULTIPLE CHOICE

1. The ultimate goal of cellular respiration is to produce ATP molecules. Cells use two different mechanisms to do this: substrate-level phosphorylation and oxidative phosphorylation. Oxidative phosphorylation produces the majority of ATP in eukaryotes. Which process listed below does NOT occur during oxidative phosphorylation?
   a. A proton gradient is developed through chemiosmosis
   b. A phosphate group is transferred from a substrate to ADP
   c. ATP synthase transfers a phosphate group to ADP
   d. Electrons are transferred between electron acceptors in the mitochondrial membrane
2. In the process of glycolysis, glucose is split and put through a series of reactions that ultimately produce two molecules of pyruvate. This series of steps produces four molecules of ATP. However, when totaling up the number of ATPs made in respiration, only two ATPs are listed as coming from glycolysis. Why?
   a. Four molecules of ATP are made, but two of them are unstable and break down.
   b. Four molecules of ATP are made, but two of them are immediately released out of the cell.
   c. In order to begin the reaction, two molecules of NADH consume two ATPs.
   d. In order to begin the reaction, two molecules of ATP are split to provide activation energy.
3. It is common knowledge that humans need to breathe in oxygen, and that without it humans die. What is the direct cause of death when deprived of oxygen?
   a. Oxygen is required as the final electron acceptor in the electron transport chain of oxidative phosphorylation; without it, the process stops.
   b. Oxygen molecules attract electrons from water molecules, releasing hydrogen; as hydrogen builds up, cells become too acidic.
   c. Without oxygen, glycolysis will not occur, and no pyruvate is created for the Krebs cycle.
   d. Without oxygen, coenzyme A cannot be oxidized and will not release the acetyl group into the Krebs cycle.
4. Members of the domain Archaea such as methanogens are currently found in anoxic environments such as marsh soils and the digestive systems of ruminants. There they practice anaerobic respiration, using CO_2 as an electron acceptor, reducing it to the methane for which they are named. In times past—3 billion years ago—they were much more abundant. What is the most likely explanation for why methanogens were common then but restricted today?
   a. Methanogens were wiped out by the evolution of multicellular organisms.
   b. Early humans actively destroyed the marshes where methanogens thrived.
   c. The early Earth atmosphere was deficient in carbon dioxide.
   d. As photosynthesis had not evolved 3 bya, oxygen was not present to inhibit their anaerobic processes.
5. While we think of cellular respiration as involving the breakdown of glucose, other monomers can have energy extracted from them. For example, amino acids may be deaminated and then enter the Krebs cycle. What happens when amino acids are deaminated?
   a. The molecule is killed
   b. The molecule is frozen in one shape
   c. An amino group is added to the molecule
   d. An amino group is removed from the molecule
6. Aerobic respiration occurs in the mitochondria of eukaryotes. Aerobic prokaryotes do not have mitochondria, but can perform aerobic respiration by utilizing infoldings of their plasma membranes. What advantage is provided by both mitochondrial membranes and bacterial membranes?
   a. Increased surface area, providing space for more reactions
   b. Increased protection for the substrates of respiration
   c. Both provide holding areas for all of the enzymes needed for Krebs cycle
   d. Both divide the cell into very dry areas suitable for hydrophobic molecules

AP FREE RESPONSE

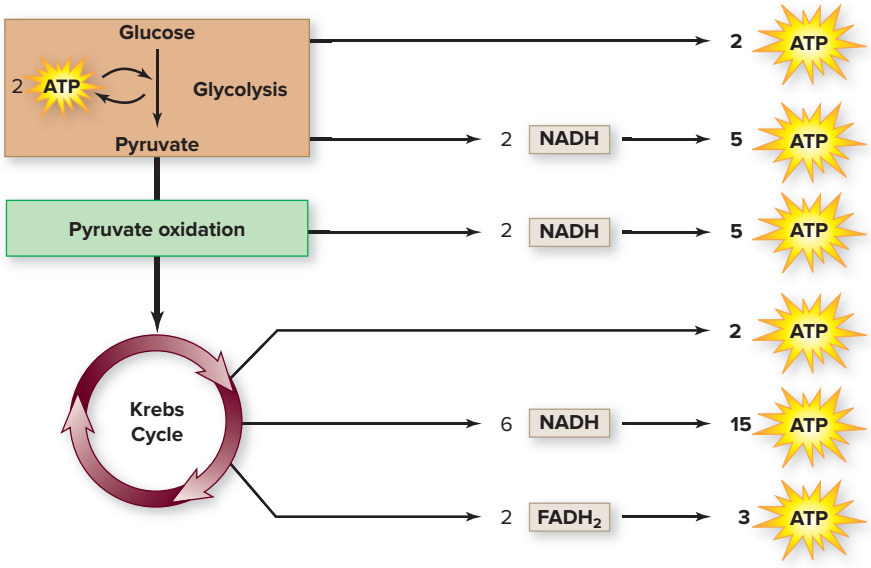


1. Chemiosmosis was considered quite a radical idea when first proposed by Peter Mitchell in the 1950's. Chemists were not used to the idea that movement could be a vital part of a chemical process. Using the diagram of ATP synthase above, **identify** two forms of movement associated with chemiosmosis. **Explain** how this movement results in the formation of ATP.

2. Consider these two processes: glycolysis and cellular respiration. **Describe** how glucose, pyruvate, oxygen, ATP, and CO_2 function in these systems to produce and maintain energy.

3. 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. **Propose** an explanation for the role of brown fat in thermogenesis of animals.

AP GRID-IN



Total net ATP yield = 32
(30 in eukaryotes)

The figure above shows the theoretical amounts of ATP that can be produced from one molecule of glucose going through aerobic respiration.

Calculate the total number of ATP that are produced in processes that do not involve chemiosmosis.

FOCUS REVIEW GUIDE

Complete the activities in Chapter 7 of your *Focus Review Guide* to review content essential for your AP exam.