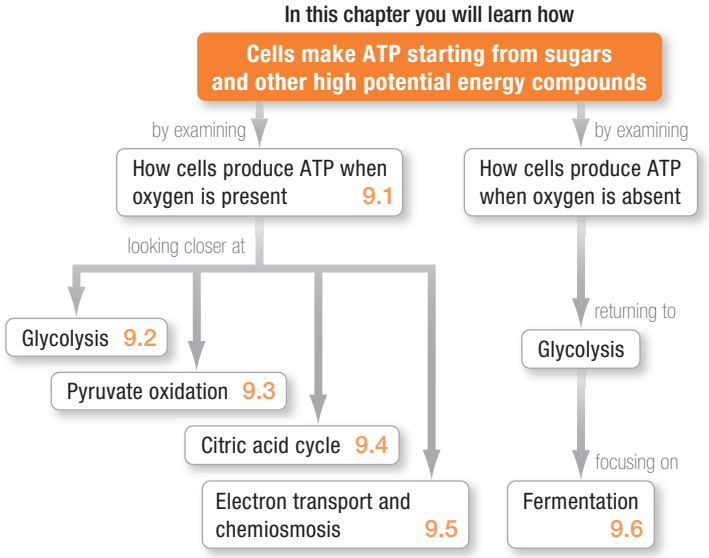
9 Cellular Respiration and Fermentation

This hydroelectric dam on the Duero, a river between Spain and Portugal, uses pumps to move water from the lower reservoir to the upper reservoir. During periods of high energy demand, the potential energy stored as a result of this activity is used to generate electricity. Cells use a similar process to produce ATP during cellular respiration.



BIG PICTURE

This chapter is part of the Big Picture. See how on pages 232–233.

Life requires energy. From the very start, chemical evolution was driven by energy from chemicals, radiation, heat, or other sources (see Chapter 2). Harnessing energy and controlling its flow has been the single most important step in the evolution of life.

What fuels life in cells? The answer is the nucleotide adenosine triphosphate (ATP). ATP has high potential energy and allows cells to overcome life's energy barriers (see Chapter 8).

This chapter investigates how cells make ATP, starting with an introduction to the metabolic pathways that harvest energy from high-energy molecules like the sugar **glucose**—the most common source of chemical energy used by organisms. As cells process sugar, the energy that is released is used to transfer

a phosphate group to adenosine diphosphate (ADP), generating ATP. (You can see the Big Picture of how the production of glucose in photosynthesis is related to its catabolism in cellular respiration on pages 232–233.)

9.1 An Overview of Cellular Respiration

In general, a cell contains only enough ATP to sustain from 30 seconds to a few minutes of normal activity. Because it has such high potential energy, ATP is unstable and is not stored. As a result, most cells are making ATP all the time.

Much of the ATP your cells produce is made using the chemical energy from glucose. How do cells obtain glucose? Photosynthetic organisms can produce glucose from the products of photosynthesis, where the energy in sunlight is used to reduce carbon dioxide (CO_2). These organisms will either use the glucose to make ATP or store it in other energy-rich molecules like starch. When photosynthetic organisms are eaten or decompose, their glucose molecules are obtained by animals, fungi, and many bacteria and archaea.

Storage carbohydrates, such as starch and glycogen, act like savings accounts for chemical energy (see Chapter 5). ATP, in contrast, is like cash. To withdraw chemical energy from the accounts to get cash, storage carbohydrates are first hydrolyzed into their glucose monomers. The glucose is then used to produce ATP through one of two general processes: cellular respiration or fermentation (**Figure 9.1**). The primary difference between these two processes lies in the degree to which glucose is oxidized.

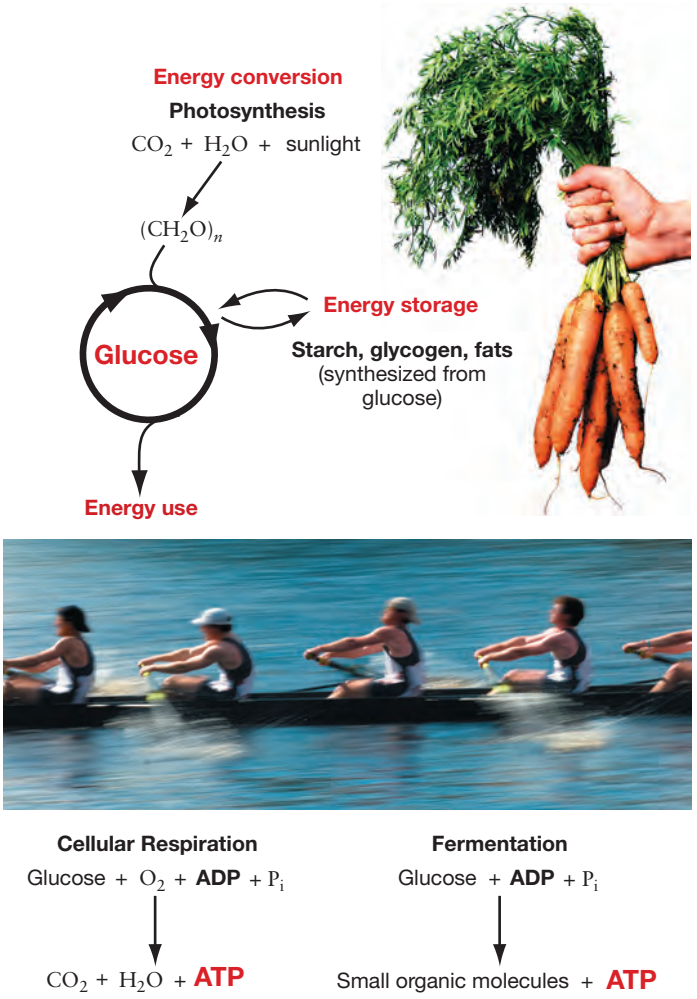


Figure 9.1 Glucose Is the Hub of Energy Processing in Cells. Glucose is a product of photosynthesis. Both plants and animals store glucose and oxidize it to provide chemical energy in the form of ATP.

What Happens When Glucose Is Oxidized?

When glucose undergoes the uncontrolled oxidation reaction called burning, some of the potential energy stored in its chemical bonds is converted to kinetic energy in the form of heat and light:

$$\underset{\text{glucose}}{C_6H_{12}O_6} + 6\,\underset{\text{oxygen}}{O_2} \longrightarrow \underset{\text{carbon dioxide}}{6\,CO_2} + \underset{\text{water}}{6\,H_2O} + \underset{\text{energy}}{\text{Heat and light}}$$

More specifically, a total of about 685 kilocalories (kcal) of heat is released when one mole of glucose is oxidized. To put this in perspective, if you burned one mole of glucose (~180 grams), it would give off enough heat to bring almost 2.5 gallons of room-temperature water to a boil.

Glucose does not burn in cells, however. Instead, it is oxidized through a long series of carefully controlled redox reactions (see Chapter 8). These reactions are occurring, millions of times per minute, in your cells right now. Instead of releasing all of this energy as heat, the released free energy is used to synthesize ATP from ADP and P_i. You use this ATP to read, think, move, and stay alive.

Fermentation is another process that oxidizes glucose. So how does fermentation differ from cellular respiration? Cellular respiration, like burning, results in the complete oxidation of glucose into CO_2 and water. Fermentation, on the other hand, does not fully oxidize glucose. Instead, small, reduced organic molecules are produced as waste. As a result, cellular respiration releases more energy from glucose than fermentation.

You can think of the complete oxidation of glucose via cellular respiration as a set of four interconnected processes that together convert the chemical energy in glucose to chemical energy in ATP. Each of the four processes consists of a distinctive starting molecule, a series of chemical reactions, and a characteristic set of products.

1. ***Glycolysis*** During **glycolysis,** one six-carbon molecule of glucose is broken into two molecules of the three-carbon compound pyruvate. During this process, ATP is produced from ADP and P_i, and nicotinamide adenine dinucleotide (NAD^+) is reduced to form NADH.
2. ***Pyruvate processing*** Each pyruvate is processed to release one molecule of CO_2, and the remaining two carbons are used to form the compound acetyl CoA. The oxidation of pyruvate results in more NAD^+ being reduced to NADH.

3. ***Citric acid cycle*** Each acetyl CoA is oxidized to two molecules of CO_2. During this sequence of reactions, more ATP and NADH are produced, and flavin adenine dinucleotide (FAD) is reduced to form $FADH_2$.

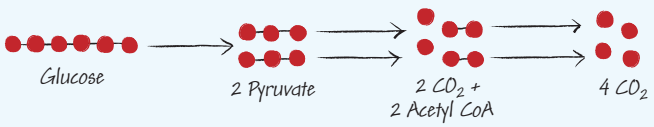
4. ***Electron transport and oxidative phosphorylation*** Electrons from NADH and $FADH_2$ move through a series of proteins that together are called an electron transport chain (ETC). The energy released in this chain of redox reactions is used to create a proton gradient across a membrane; the ensuing flow of protons back across the membrane is used to make ATP. Because this mode of ATP production links the phosphorylation of ADP with the oxidation of NADH and $FADH_2$, it is called **oxidative phosphorylation.**

Figure 9.2 summarizes the four processes in cellular respiration. Formally, **cellular respiration** is defined as any set of reactions that uses electrons harvested from high-energy molecules to produce ATP via an electron transport chain. Making Models 9.1 provides some tips for how you can use models like the one shown in Figure 9.2 as references to draw your own models of cellular respiration. Such models are essential in biology to distill complex topics into understandable narratives.

The enzymes, products, and intermediates involved in cellular respiration do not exist in isolation. Instead, they are part of a huge and dynamic inventory of chemicals inside the cell.

Making Models 9.1 Tips on Drawing Flow Charts

Cellular respiration is complex. By drawing your own simple models, you can practice keeping track of the main events. The details you choose to include depend on the focus of your model. For example, the flow chart in Figure 9.2 summarizes the main In's and Out's of the four processes of cellular respiration. The flow chart below uses "balls" to represent carbons to track the fate of carbon during cellular respiration.



MODEL Where do all the carbons of glucose end up when glucose is completely oxidized? Add detail to the model by labeling the processes represented by the arrows.

To see this model in action, go to https://goo.gl/8T54kd

This complexity can be boiled down to a simple essence, however. Two of the most fundamental requirements of a cell are energy and carbon. They need a source of energy for generating ATP and a source of carbon that can be used as raw material to synthesize DNA, RNA, proteins, fatty acids, and other

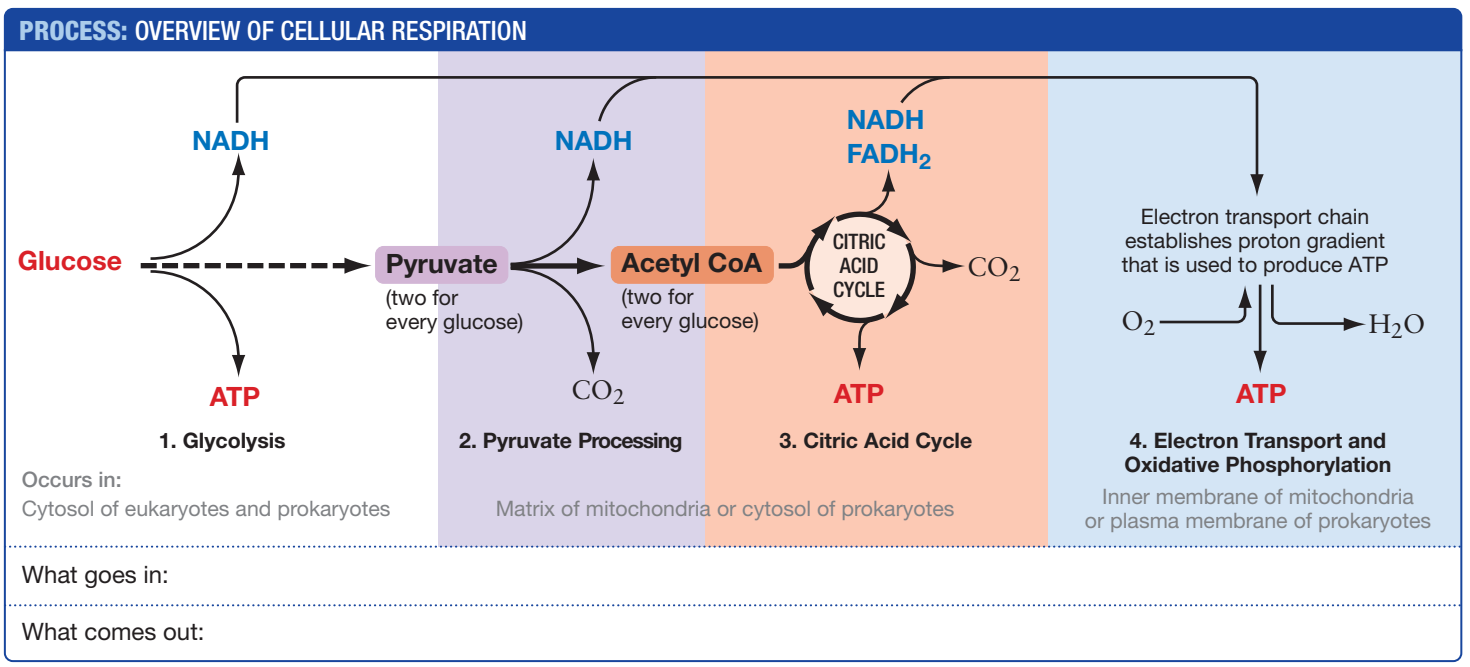


Figure 9.2 Cellular Respiration Oxidizes Glucose to Make ATP. Cells produce ATP from glucose via a series of processes: (1) glycolysis, (2) pyruvate processing, (3) the citric acid cycle, and (4) electron transport and oxidative phosphorylation. Each process produces high-energy molecules in the form of nucleotides (ATP) and/or electron carriers (NADH or $FADH_2$). Because the four processes are connected, cellular respiration is an integrated metabolic pathway. The first three processes oxidize glucose to produce NADH and $FADH_2$, which then feed the electron transport chain.

✔ Use what you have learned in the text to fill in the chart along the bottom of the figure.

molecules. With these requirements in mind, let's take a closer look at the central role cellular respiration plays in cellular metabolism.

Cellular Respiration Plays a Central Role in Metabolism

Recall that sets of reactions that break down molecules are called catabolic pathways (Chapter 8). These reactions often harvest stored chemical energy to produce ATP. Anabolic pathways, on the other hand, are sets of reactions that synthesize larger molecules from smaller components. Anabolic reactions often use energy in the form of ATP.

Does the process of cellular respiration interact with other catabolic and anabolic pathways? The answer is most definitely yes! Let's first consider how other catabolic pathways feed into cellular respiration, then examine how the intermediates and products of glycolysis, pyruvate processing, and the citric acid cycle feed into anabolic pathways.

Catabolic Pathways Break Down a Variety of Molecules Most organisms ingest, absorb, or synthesize many different carbohydrates—not just glucose. These molecules range from sucrose, maltose, and other simple sugars to large polymers such as glycogen and starch (see Chapter 5). Using enzyme-catalyzed reactions, cells can break down and transform these other carbohydrates to produce glucose or intermediates in cellular respiration.

Carbohydrates are not the only important source of carbon compounds used in catabolic pathways, however. Fats are highly reduced macromolecules consisting of glycerol bonded to chains of fatty acids (see Chapter 6). In cells, enzymes routinely break down fats to release the glycerol and convert the fatty acids into acetyl CoA molecules. Glycerol can be further processed and enter glycolysis. Acetyl CoA enters the citric acid cycle.

Proteins can also be catabolized, meaning that they can be broken down and used to produce ATP. Once they are hydrolyzed to their constituent amino acids, enzyme-catalyzed reactions remove the amino ($-NH_2$) groups. The amino groups are excreted in urine as waste, and the remaining carbon compounds are converted to pyruvate, acetyl CoA, or other intermediates in glycolysis and the citric acid cycle.

The top half of **Figure 9.3** summarizes the catabolic pathways of carbohydrates, fats, and proteins and shows how their breakdown products feed an array of steps in cellular respiration. When all three types of molecules are available in the cell to generate ATP, carbohydrates are used up first, then fats, and finally proteins.

Catabolic Intermediates Are Used in Anabolic Pathways Where do cells get the precursor molecules required to synthesize amino acids, RNA, DNA, phospholipids, and other cell components? Not surprisingly, the answer often involves intermediates in cellular respiration. For example,

- In humans, about half the required amino acids can be synthesized from molecules siphoned from the citric acid cycle.
- Acetyl CoA is the starting point for anabolic pathways that result in the synthesis of fatty acids. Fatty acids can then be used to build phospholipids and fats.
- Intermediates in glycolysis can be used in the synthesis of ribonucleotides and deoxyribonucleotides. Nucleotides, in turn, are building blocks used in RNA and DNA synthesis.
- If ATP is abundant, pyruvate and lactate (from fermentation) can be used in the synthesis of glucose. Excess glucose may be converted to glycogen or starch and stored.

The bottom half of Figure 9.3 summarizes how intermediates in carbohydrate metabolism are drawn off to synthesize macromolecules. The take-home message is that the same molecule can serve many different functions in the cell. As a result, catabolic

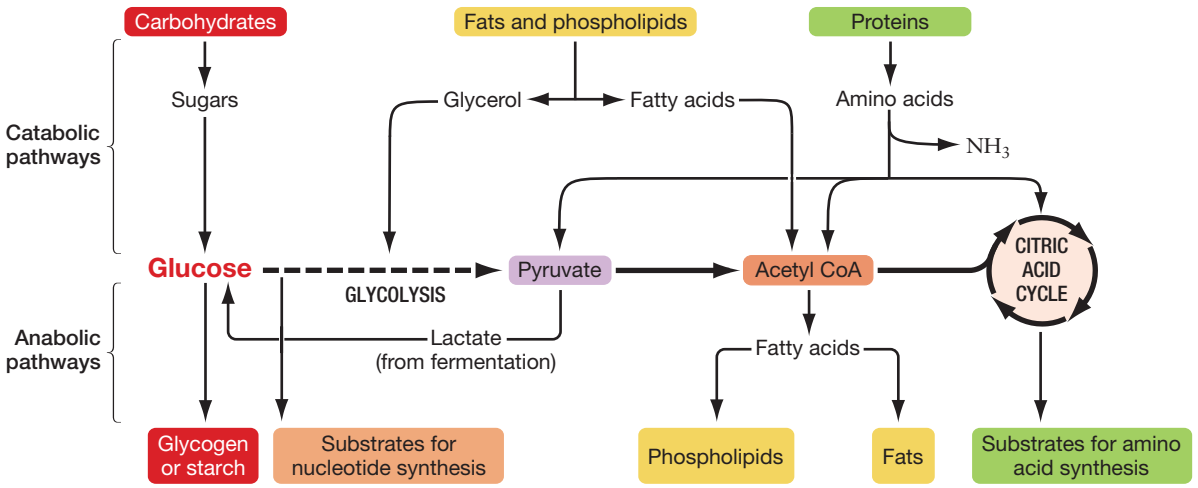


Figure 9.3 Cellular Respiration Interacts with Other Catabolic and Anabolic Pathways. A variety of high-energy compounds from carbohydrates, fats, or proteins can be broken down in catabolic reactions and used by cellular respiration for ATP production. Several of the intermediates in cellular respiration serve as precursor molecules in anabolic reactions leading to the synthesis of carbohydrates, nucleotides, lipids, and amino acids.

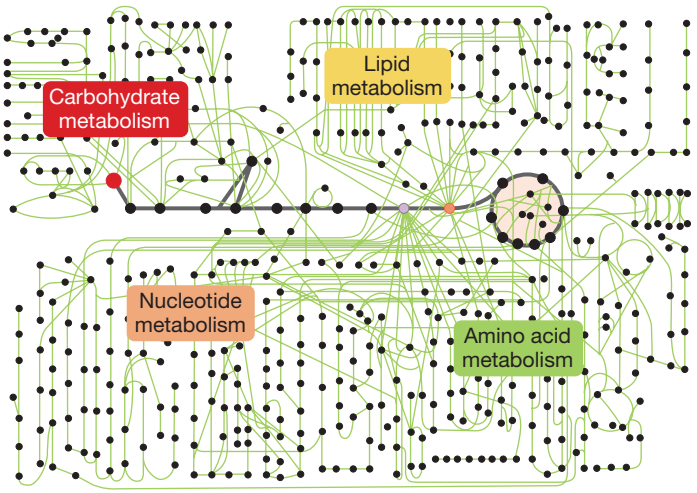


Figure 9.4 Cellular Respiration Plays a Central Role in the Metabolic Activity of Cells. Cellular respiration is connected to a multitude of different chemical reactions. In this schematic diagram, dots represent a few of the many thousands of molecules involved in metabolism, and green lines represent enzyme-catalyzed reactions. At the center of all this, the first three metabolic pathways involved in cellular respiration (see Figure 9.3) are emphasized by bold dots along a thick black line. For reference, the bold dots representing glucose, pyruvate, and acetyl CoA are identified by the same distinctive colors used in Figure 9.3.

and anabolic pathways are closely intertwined. ✓ **CAUTION** **If you understand this relationship, you should be able to explain why many different molecules—including lipids, amino acids, and CO_2—end up as radiolabeled when cells are fed glucose with radioactive carbons (^{14}C).**

Metabolism comprises thousands of different chemical reactions, yet the amounts and identities of molecules inside cells are relatively constant. By regulating key reactions involved in catabolic and anabolic pathways, the cell is able to maintain its internal environment even under different environmental conditions—a condition referred to as **homeostasis.** While the ATP generated by cellular respiration and fermentation are crucial for survival, the intermediates in these pathways also are central parts of a highly integrated metabolism (Figure 9.4).

Once you've filled in the chart at the bottom of Figure 9.2, you'll be ready to analyze each of the four steps of cellular respiration in detail. As you delve in, keep asking yourself the same key questions: What goes in and what comes out? What happens to the energy that is released? Where does each step occur, and how is it regulated? Then take a look in the mirror. All these processes are occurring right now, in virtually all your cells.

9.2 Glycolysis: Oxidizing Glucose to Pyruvate

Because the enzymes responsible for glycolysis have been observed in nearly every prokaryote and eukaryote, it is logical to infer that the ancestor of all organisms living today made ATP by glycolysis. It's ironic, then, that the process was discovered by accident.

In the 1890s Hans and Edward Buchner were working out techniques for breaking open baker's yeast cells and extracting the contents for commercial and medicinal use. (Yeast extracts are still added to some foods as a flavor enhancer or nutritional supplement.) In one set of experiments, the Buchners added sucrose to their extracts. At the time, sucrose was commonly used as a preservative—a substance used to prevent food from decaying.

Instead of preserving the yeast extracts, though, the sucrose was quickly broken down and alcohol appeared as a by-product. This was a key finding: It showed that metabolic pathways could be studied in vitro—outside the organism. Until then, researchers thought that metabolism could take place only in intact organisms.

When researchers studied how the sugar was being processed, they found that the reactions could go on much longer than normal if inorganic phosphate were added to the mixture. This result implied that some of the compounds involved were being phosphorylated. Soon after, a molecule called fructose bisphosphate was isolated. (The prefix *bis–* means that the phosphate groups are attached to the fructose molecule at two different locations.) Subsequent work showed that all but the starting and ending molecules in glycolysis—glucose and pyruvate—are phosphorylated.

In 1905 researchers found that the processing of sugar by yeast extracts stopped if they boiled the reaction mix. Because it was known that enzymes could be inactivated by heat, this discovery suggested that enzymes were involved in at least some of the processing steps. Years later, investigators realized that each step in glycolysis is catalyzed by a different enzyme. Eventually, each of the 10 reactions and enzymes involved was worked out.

Glycolysis Is a Sequence of 10 Reactions

In both eukaryotes and prokaryotes, all 10 reactions of glycolysis occur in the cytosol (see Figure 9.5 on page 194). Note three key points about this reaction sequence:

1. Glycolysis starts by *using* ATP, not producing it. In the initial step, glucose is phosphorylated to form glucose-6-phosphate. After the second reaction rearranges the sugar to form fructose-6-phosphate, the third reaction adds a second phosphate group, forming the compound fructose-1,6-bisphosphate observed by early researchers. Thus, in reactions 1–5, two ATP molecules are used up before any ATP is produced. This part of glycolysis is referred to as the energy-investment phase.

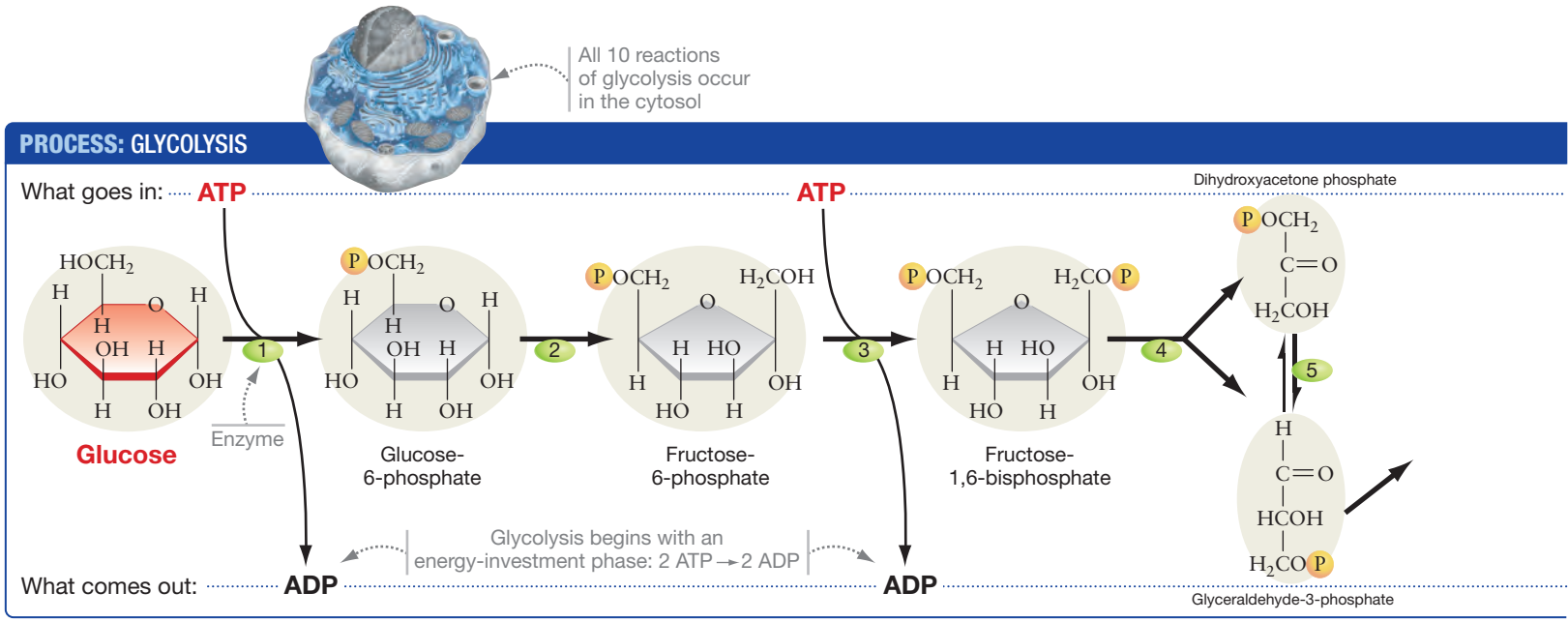


Figure 9.5 Glycolysis Pathway. This sequence of 10 reactions oxidizes glucose to pyruvate. Each reaction is catalyzed by a different enzyme to produce two net ATP (4 ATP are produced, but 2 are invested), two molecules of NADH, and two molecules of pyruvate. In step 4, fructose-1,6-bisphosphate is divided into two products that both proceed through steps 6–10. The amounts for "What goes in" and "What goes out" are the combined totals for both molecules.

2. The energy-payoff phase of glycolysis occurs in reactions 6–10 of Figure 9.5. The first high-energy molecules are produced in the sixth reaction, where two molecules of NAD^+ are reduced to form two NADH. In reactions 7 and 10, enzymes catalyze the transfer of a phosphate group from a phosphorylated substrate to ADP, forming ATP. Enzyme-catalyzed reactions that result in ATP production are termed **substrate-level phosphorylation** (Figure 9.6).
3. For each molecule of glucose processed by glycolysis, the net yield is two molecules of NADH, two of ATP, and two of pyruvate.

The discovery and elucidation of the glycolytic pathway ranks as one of the great achievements in the history of biochemistry. For more detail about the enzymes that catalyze each step, see Table 9.1. While the catabolism of glucose can occur via other pathways, this set of reactions is among the most ancient and fundamental of all life processes.

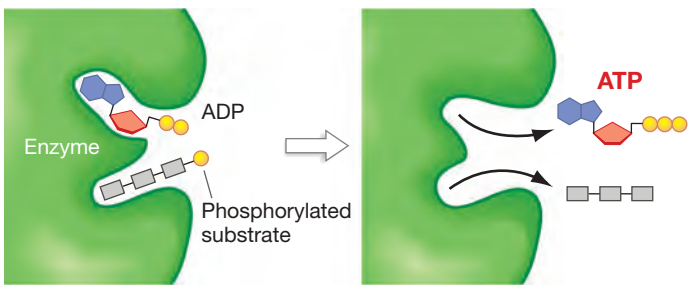


Figure 9.6 Substrate-Level Phosphorylation Involves an Enzyme and a Phosphorylated Substrate. Substrate-level phosphorylation occurs when an enzyme catalyzes the transfer of a phosphate group from a phosphorylated substrate to ADP, forming ATP.

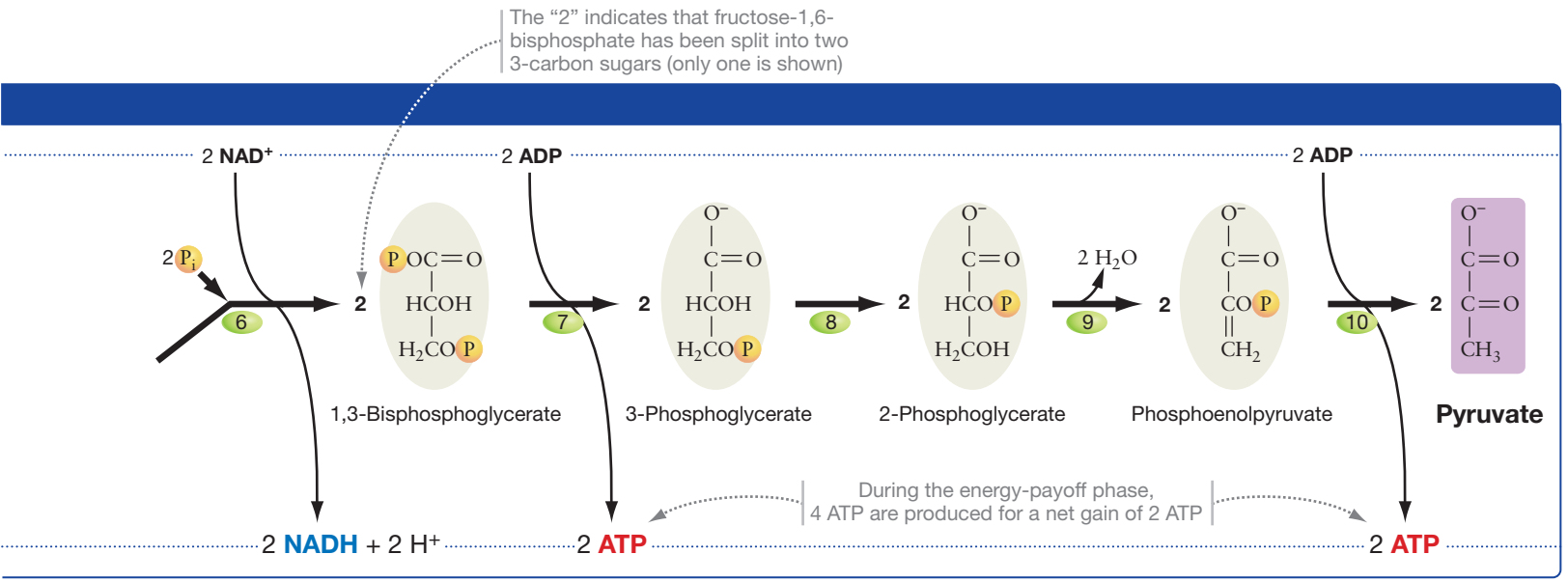
How Is Glycolysis Regulated?

An important advance in understanding how glycolysis is regulated occurred when biologists observed that high levels of ATP inhibit a key glycolytic enzyme called phosphofructokinase. **Phosphofructokinase** catalyzes reaction 3 in Figure 9.5—the synthesis of fructose-1,6-bisphosphate from fructose-6-phosphate. This is a key step in the sequence.

The products of reactions 1 and 2 can be easily converted back to glucose by an array of enzymes. Before reaction 3, then, the sequence is not committed to glycolysis and glucose can be used in other pathways. But once fructose-1,6-bisphosphate is synthesized, it will not be converted back to glucose. Based on these observations, it makes sense that the pathway is regulated at the first committed step—reaction 3. How do cells do it?

As shown in Figure 9.5, ATP serves as a substrate for the addition of a phosphate to fructose-6-phosphate. In the vast majority of cases, increasing the concentration of a substrate would *speed* the rate of a chemical reaction, but in this case, it inhibits it. Why would ATP—a substrate that is required for the reaction—also serve as an inhibitor of the reaction? The answer lies in knowing that ATP is also the end product of the overall catabolic pathway.

Recall that when an enzyme in a pathway is inhibited by the product of the reaction sequence, feedback inhibition occurs (see Chapter 8). When the product molecule is abundant, it can inhibit its own production by interfering with one of the reactions used to create it. Cells that are able to stop glycolytic



reactions when ATP is abundant can conserve their stores of glucose for times when ATP is scarce. As a result, homeostasis is maintained via feedback inhibition.

How do high levels of the substrate inhibit the enzyme? As Figure 9.7 on page 196 shows, phosphofructokinase has two distinct binding sites for ATP. ATP can bind at the enzyme's active site, where it is used to phosphorylate fructose-6-phosphate, or at a regulatory site, where it turns off the enzyme's activity.

The key to feedback inhibition lies in the ability of the two sites to bind to ATP. When concentrations are low, ATP binds only to the active site, which has a greater affinity for ATP than does the regulatory site. As ATP concentrations increase, however, it also binds at the regulatory site on phosphofructokinase. When ATP binds at this second location, the enzyme's conformation changes in a way that dramatically lowers the reaction rate at the active site. In phosphofructokinase, ATP acts as an allosteric regulator (see Chapter 8). ✔ **QUANTITATIVE** **If you understand how ATP regulates glycolysis, you should be able to draw a graph showing the rate of ATP production as a function of ATP concentration. Predict how the rate would change if the regulatory site in phosphofructokinase had higher affinity for ATP than the active site did.**

SUMMARY **Table 9.1 The Reactions of Glycolysis**

Step	Enzyme	Reaction
1	Hexokinase	Uses **ATP** to phosphorylate glucose, increasing its potential energy.
2	Phosphoglucose isomerase	Converts glucose-6-phosphate to fructose-6-phosphate; referred to as an isomer of glucose-6-phosphate.
3	Phosphofructokinase	Uses **ATP** to phosphorylate the opposite end of fructose-6-phosphate, increasing its potential energy.
4	Fructose-bis-phosphate aldolase	Cleaves fructose-1,6-bisphosphate into two different three-carbon sugars.
5	Triose phosphate isomerase	Converts dihydroxyacetone phosphate (DAP) to glyceraldehyde-3-phosphate (G3P). Although the reaction is fully reversible, the DAP-to-G3P reaction is favored because G3P is immediately used as a substrate for step 6.
6	Glyceraldehyde-3-phosphate dehydrogenase	A two-step reaction that first oxidizes G3P using the **NAD^+** coenzyme to produce **NADH**. Energy from this reaction is used to attach a P_i to the oxidized product to form 1,3-bisphosphoglycerate.
7	Phosphoglycerate kinase	Transfers a phosphate from 1,3-bisphosphoglycerate to **ADP** to make 3-phosphoglycerate and **ATP**.
8	Phosphoglycerate mutase	Rearranges the phosphate in 3-phosphoglycerate to make 2-phosphoglycerate.
9	Enolase	Removes a water molecule from 2-phosphoglycerate to form a C=C double bond and produce phosphoenolpyruvate.
10	Pyruvate kinase	Transfers a phosphate from phosphoenolpyruvate to **ADP** to make pyruvate and **ATP**.

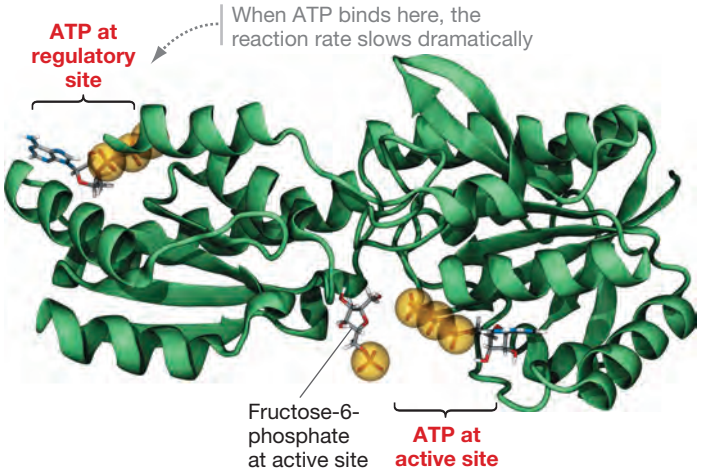


Figure 9.7 Phosphofructokinase Has Two Binding Sites for ATP. A model of one of the four identical subunits of phosphofructokinase. In the active site, ATP is used as a substrate to transfer one of its phosphate groups to fructose-6-phosphate. In the regulatory site, ATP binding inhibits the reaction by changing the shape of the enzyme.

To summarize, glycolysis starts with one 6-carbon glucose molecule and ends with two 3-carbon pyruvate molecules. The reactions occur in the cytosol, and the energy that is released is used to produce a net total of two ATP and two NADH. Now the question is, what happens to the pyruvate?

9.3 Processing Pyruvate to Acetyl CoA

In eukaryotes, the pyruvate produced by glycolysis is transported from the cytosol to mitochondria. Mitochondria are organelles found in virtually all eukaryotes (see Chapter 7).

As shown in Figure 9.8, mitochondria have two membranes, called the outer membrane and inner membrane. Portions of the inner membrane fill the interior of the organelle with sac-like structures called **cristae.** Short tubes connect the cristae to the rest of the inner membrane. The regions between the outer and inner membranes and within the cristae make up the intermembrane space. The region enclosed within the inner membrane is the **mitochondrial matrix.**

Pyruvate moves across the mitochondrial outer membrane through small pores and is transported into the matrix through a carrier protein in the inner membrane. Once it is inside the matrix, a sequence of reactions occurs inside an enormous and intricate enzyme complex called **pyruvate dehydrogenase.** In eukaryotes, this complex is located in the mitochondrial matrix. In bacteria and archaea, pyruvate dehydrogenase is located in the cytosol.

As pyruvate is being processed, one of its carbons is oxidized to CO_2 and NAD^+ is reduced to NADH. The remaining two-carbon acetyl unit ($-COCH_3$) reacts with a compound called **coenzyme A (CoA).** Coenzyme A is sometimes abbreviated as CoA-SH to call attention to its key sulfhydryl functional group. The acetyl is transferred to CoA to produce acetyl CoA (Figure 9.9). In this and many other reactions, CoA acts as a coenzyme by accepting and then later transferring an acetyl group to another substrate ("A" stands for acetylation).

Acetyl CoA is the final product of the pyruvate-processing step in glucose oxidation. Pyruvate, NAD^+, and CoA go in; CO_2, NADH, and acetyl CoA come out.

Like glycolysis, pyruvate processing is regulated by feedback inhibition. When the products of glycolysis and pyruvate processing are in abundant supply, the process shuts down. Pyruvate processing stops when the pyruvate dehydrogenase complex becomes phosphorylated and changes shape. The rate

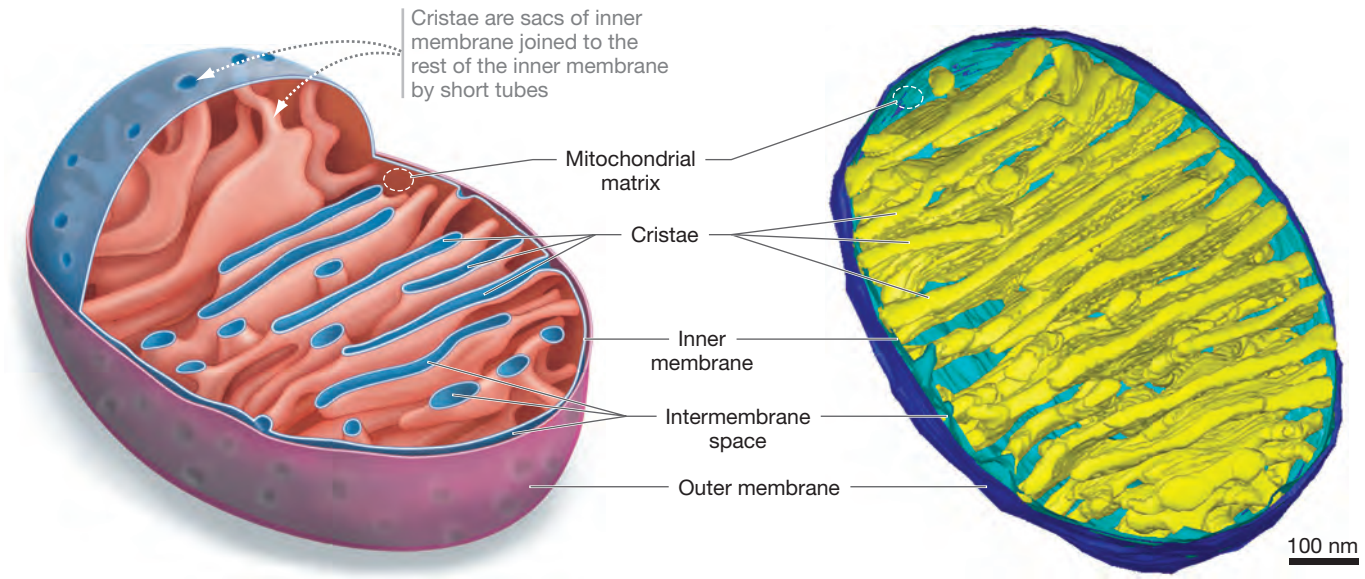


Figure 9.8 The Structure of the Mitochondrion. Mitochondria have outer and inner membranes that define the intermembrane space and matrix. Pyruvate processing occurs within the mitochondrial matrix. Recent research using cryo-electron tomography (the colorized image on the right) shows that the sac-like cristae are expansions of short tubes formed from the inner membrane.

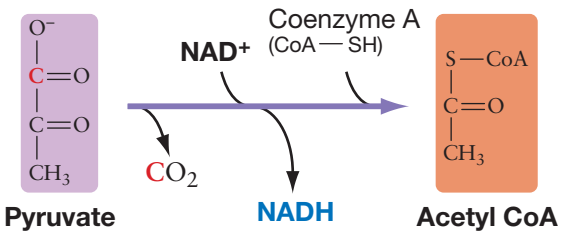


Figure 9.9 Pyruvate Is Oxidized to Acetyl CoA. The reaction shown here is catalyzed by pyruvate dehydrogenase. In the process, one carbon (red in the diagram) is fully oxidized to CO_2 and released.

of phosphorylation increases when one or more of the products are at high concentration.

In contrast, a high concentration of reaction substrates—which indicates low ATP supplies—results in more dephosphorylated and active forms of the pyruvate dehydrogenase complex.

Pyruvate processing is thus under both positive and negative regulation. Large supplies of products inhibit the enzyme complex; large supplies of reactants and low supplies of products stimulate it. ✔ **If you understand positive and negative regulation, you should be able to list three molecules whose presence speeds up the reaction shown in Figure 9.9. Label them "Positive regulation." Then list three molecules whose presence slows down the reaction. Label them "Negative regulation by feedback inhibition."**

To summarize, pyruvate processing starts with a three-carbon pyruvate molecule and ends with one carbon released as CO_2 and the remaining two carbons in the form of acetyl CoA. The reactions occur in the mitochondrial matrix, and the released free energy is used to produce one NADH for each pyruvate that is processed. Now the question is, what happens to the acetyl CoA?

9.4 The Citric Acid Cycle: Oxidizing Acetyl CoA to CO_2

While researchers were working out the sequence of reactions in glycolysis, biologists in other laboratories were focusing on redox reactions that oxidize small organic acids called **carboxylic acids.** Note that carboxylic acids all have carboxyl functional groups (R-COOH), hence the name.

Early researchers identified eight small carboxylic acids that are rapidly oxidized in sequence, from most reduced to most oxidized. Redox reactions that involve carboxylic acids often produce carbon dioxide, which is the endpoint of glucose oxidation via cellular respiration. When they added one of the eight carboxylic acids to cells, the rate of glucose oxidation increased, suggesting that the reactions are somehow connected to pathways involved in glucose catabolism. What they found next was puzzling. Whichever carboxylic acid they added, it did not appear to be used up. Instead, virtually all the acids could be recovered later. How is this possible?

Hans Krebs solved the mystery when he proposed that the reaction sequence occurs in a cycle instead of a linear pathway. Krebs had another crucial insight when he suggested that the reaction sequence was directly tied to the processing of pyruvate.

To test these hypotheses, Krebs and a colleague set out to determine if adding pyruvate could link the two ends of the sequence of eight carboxylic acids. If pyruvate is the key link in forming a cycle, it would need to be involved in the conversion of oxaloacetate, the most oxidized of the eight carboxylic acids, to citrate, the most reduced carboxylic acid. When Krebs added pyruvate, the series of redox reactions occurred. The conclusion? The sequence of eight carboxylic acids is indeed arranged in a cycle (see Figure 9.10 on page 198).

Many biologists now refer to the cycle as the **citric acid cycle** because it starts with citrate, which is the salt of citric acid after the protons are released. The citric acid cycle is also known as the tricarboxylic acid (TCA) cycle, because citrate has three carboxyl groups, or as the Krebs cycle, after its discoverer.

In each cycle, the energy released by the oxidation of one molecule of acetyl CoA is used to produce three molecules of NADH, one of $FADH_2$, and one of ATP or guanosine triphosphate (GTP), through substrate-level phosphorylation. Whether ATP or GTP is produced depends on the version of the enzyme used in the fifth reaction.[1] For example, the enzyme used in muscle cells of mammals produces ATP, while the enzyme used in liver cells produces GTP. For simplicity, ATP has been used as the product of the citric acid cycle throughout this chapter.

In prokaryotes (bacteria and archaea), the enzymes responsible for the citric acid cycle are located in the cytosol. In eukaryotes, most of the enzymes responsible for the citric acid cycle are located in the mitochondrial matrix. Because glycolysis produces two molecules of pyruvate, the cycle turns twice for each molecule of glucose processed in cellular respiration.

How Is the Citric Acid Cycle Regulated?

By now, it shouldn't surprise you to learn that the citric acid cycle is also carefully regulated. The citric acid cycle can be turned off at multiple points, via several different mechanisms of feedback inhibition. Reaction rates are high when ATP and NADH are scarce; the rates are low when ATP or NADH is abundant.

Figure 9.11 on page 198 highlights the major control points. In step 1, the enzyme that combines acetyl CoA and oxaloacetate to form citrate is shut down when ATP binds at an allosteric regulatory site. In step 3, NADH interferes with the reaction by binding to the enzyme's active site. This is an example of competitive inhibition (see Chapter 8). In step 4, ATP again functions as an allosteric regulator.

To summarize, the citric acid cycle starts with the two-carbon acetyl molecule in the form of acetyl CoA and ends with the release of two CO_2. For more detail concerning the enzymes that

[1] Traditionally it was thought that the citric acid cycle produced GTP, which was later converted to ATP in the same cell. Recent work suggests that ATP is produced directly in some cell types, while GTP is produced in other cells. See J. D. Johnson et al., Genetic evidence for the expression of ATP- and GTP-specific succinyl-CoA synthetases in multicellular eukaryotes. *Journal of Biological Chemistry* 42 (1998): 27580–27586.

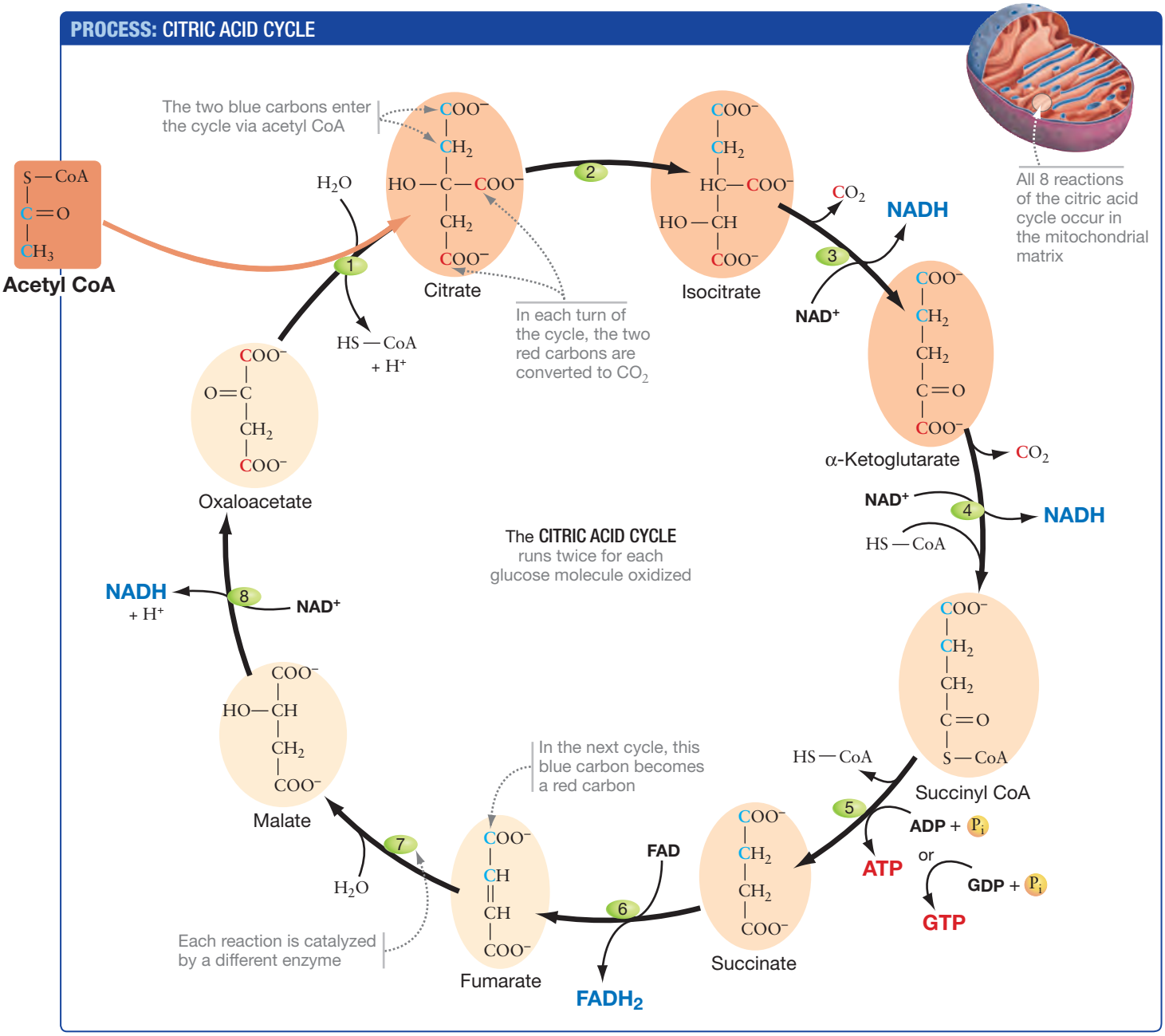


Figure 9.10 The Citric Acid Cycle Completes the Oxidation of Glucose. Acetyl CoA goes into the citric acid cycle, and carbon dioxide, NADH, $FADH_2$, and ATP or GTP come out. ATP or GTP is produced by substrate-level phosphorylation. If you follow individual carbon atoms around the cycle several times, you'll come to an important conclusion: Each of the carbons in the cycle is eventually a "red carbon" that is released as CO_2.

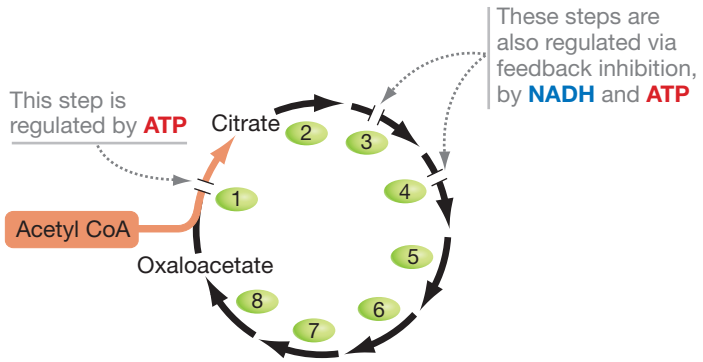


Figure 9.11 The Citric Acid Cycle Is Regulated by Feedback Inhibition. The citric acid cycle slows down when ATP and NADH are plentiful. ATP acts as an allosteric regulator, while NADH acts as a competitive inhibitor.

SUMMARY **Table 9.2** The Reactions of the Citric Acid Cycle

Step	Enzyme	Reaction
1	Citrate synthase	Transfers the 2-carbon acetyl group from acetyl CoA to the 4-carbon oxaloacetate to produce the 6-carbon citrate.
2	Aconitase	Converts citrate to isocitrate by the removal of one water molecule and the addition of another water molecule.
3	Isocitrate dehydrogenase	Oxidizes isocitrate using the **NAD^+** coenzyme to produce **NADH** and release one CO_2, resulting in the formation of the five-carbon molecule α-ketoglutarate.
4	α-Ketoglutarate dehydrogenase	Oxidizes α-ketoglutarate using the **NAD^+** coenzyme to produce **NADH** and release one CO_2. The remaining four-carbon molecule is added to coenzyme A (CoA) to form succinyl CoA.
5	Succinyl-CoA synthetase	CoA is removed, converting succinyl CoA to succinate. The energy released is used to transfer P_i to ADP to form **ATP**, or to GDP to form **GTP**, depending on the enzyme used.
6	Succinate dehydrogenase	Oxidizes succinate by transferring two hydrogens to the coenzyme **FAD** to produce **$FADH_2$**, resulting in the formation of fumarate.
7	Fumarase	Converts fumarate to malate by the addition of one water molecule.
8	Malate dehydrogenase	Oxidizes malate by using the **NAD^+** coenzyme to produce **NADH**, resulting in the regeneration of the oxaloacetate that will be used in step 1 of the cycle.

catalyze each step, see **Table 9.2**. All of these reactions occur in the mitochondrial matrix, and the released free energy is used to produce three NADH, one $FADH_2$, and one ATP for each acetyl oxidized. But a major question remains.

What Happens to the NADH and $FADH_2$?

Figure 9.12 reviews the relationships of glycolysis, pyruvate processing, and the citric acid cycle and identifies where each process takes place in eukaryotic cells. As you study this figure, note that for each molecule of glucose that is fully oxidized to 6 carbon dioxide molecules, the cell produces 10 molecules of NADH, 2 of $FADH_2$, and 4 of ATP. The ATP molecules are produced by substrate-level phosphorylation and can be used to drive endergonic reactions. The CO_2 molecules are a gas that is disposed of as you exhale.

What happens to the NADH and $FADH_2$ produced by glycolysis, pyruvate processing, and the citric acid cycle? Recall that the overall reaction for glucose oxidation is

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O + \text{Energy}$$

These three steps account for the glucose, the CO_2, and—because ATP is produced—some of the chemical energy that results from the overall reaction. But the O_2 and the H_2O are

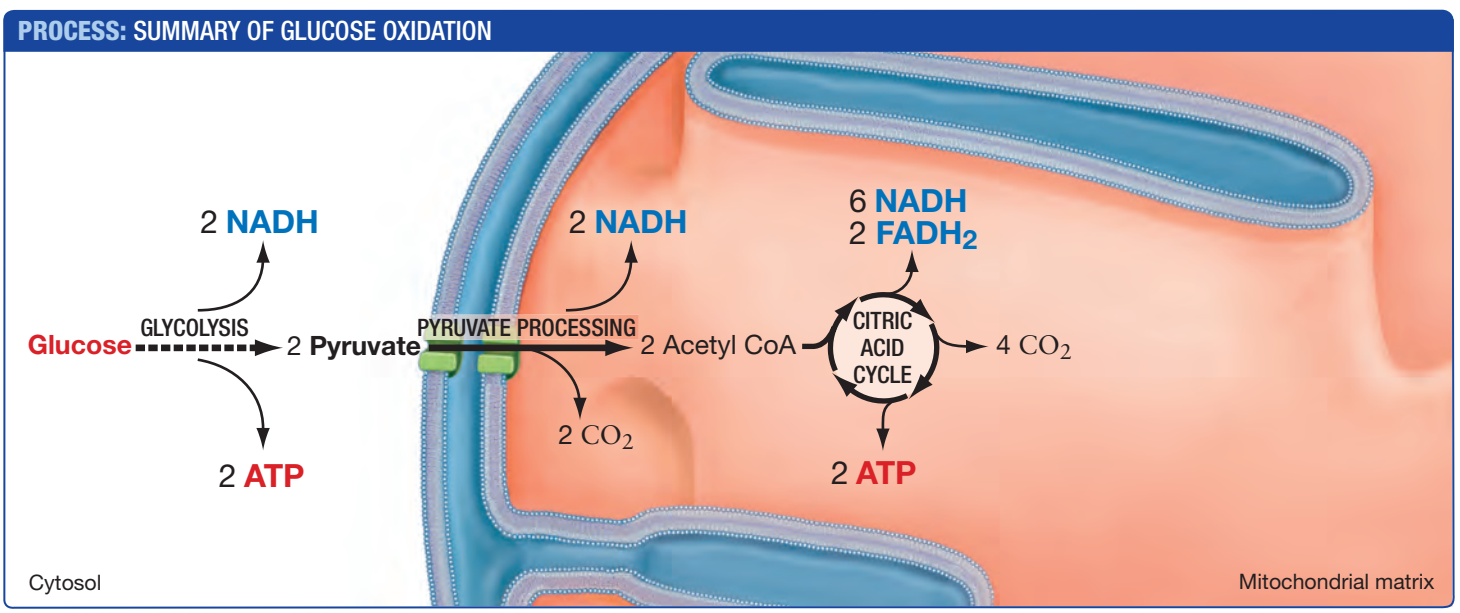


Figure 9.12 Glucose Oxidation Produces ATP, NADH, $FADH_2$, and CO_2. Glucose is completely oxidized to carbon dioxide via glycolysis, pyruvate processing, and the citric acid cycle. In eukaryotes, glycolysis occurs in the cytosol; pyruvate oxidation and the citric acid cycle take place in the mitochondrial matrix.

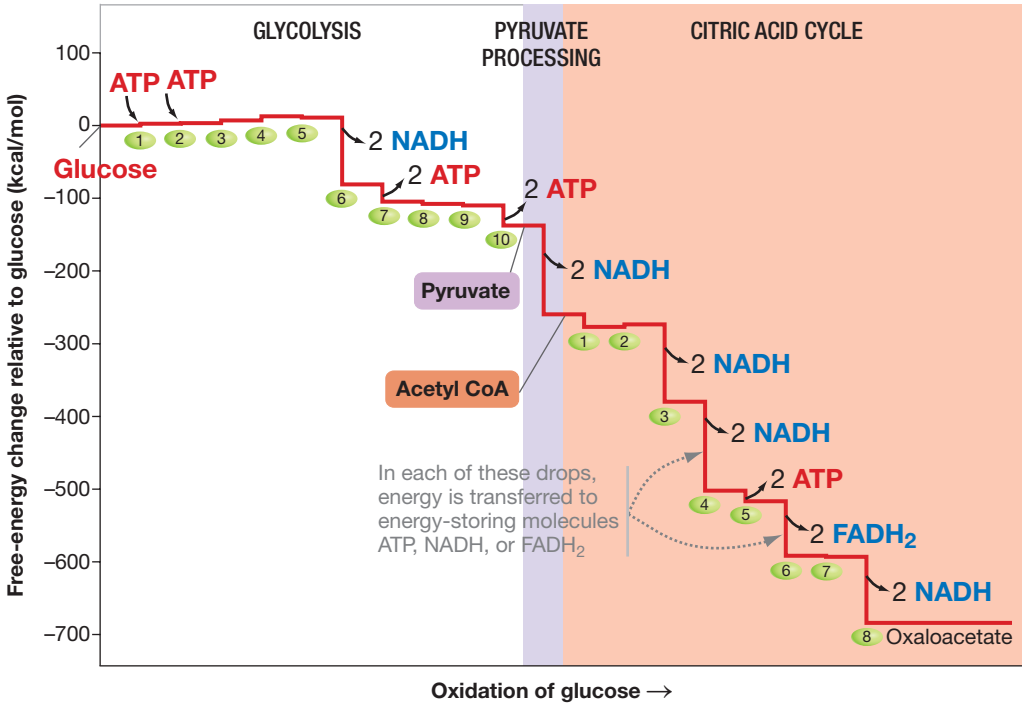


Figure 9.13 Free Energy Changes as Glucose Is Oxidized. If you read the vertical axis of this graph carefully, it should convince you that about 685 kcal/mol of free energy is released from the oxidation of glucose. Much of the energy is harnessed in the form of ATP, NADH, and $FADH_2$. The numbered green ovals identify the reaction steps in glycolysis and the citric acid cycle (see Tables 9.1 and 9.2).

DATA: Li, X., R. K. Dash, R. K. Pradhan, et al. 2010. *Journal of Physical Chemistry B.* 114: 16068–16082.

✔ **QUANTITATIVE** Based on the data in this graph, which one of the three high-energy molecules produced during glucose oxidation would you expect to carry the highest amount of chemical energy? Justify your answer.

still unaccounted for. As it turns out, so is much of the chemical energy. The relative changes in free energy that occur as the carbons in glucose are oxidized are shown in Figure 9.13.

In glycolysis, pyruvate processing, and the citric acid cycle, redox reactions transfer electrons to NAD^+ and FAD to form NADH and $FADH_2$. At this point, the reaction that has yet to occur is

$$10\,NADH + 2\,FADH_2 + 6\,O_2 + 10\,H^+ \longrightarrow 10\,NAD^+ + 2\,FAD + 12\,H_2O + Energy$$

In the above reaction, those electrons are transferred from NADH and $FADH_2$ to oxygen. Recall that when NADH is formed, it takes on two electrons, but only one proton (Chapter 8). The second proton for each NADH is represented by the 10 H^+ in the equation. As NADH and $FADH_2$ are oxidized to NAD^+ and FAD, oxygen is reduced to form water.

Now, all the components of the overall reaction for glucose oxidation are accounted for, except for the energy. What happens to the energy that is released as electrons are transferred from NADH and $FADH_2$ to the highly electronegative oxygen atoms? Specifically, how is the energy that is released from these reactions used to make ATP? In the 1960s—decades after the details of glycolysis and the citric acid cycle had been worked out—an unexpected answer to this question emerged.

CHECK YOUR UNDERSTANDING

If you understand that …

- During glycolysis, glucose is oxidized to pyruvate in the cytosol.
- During pyruvate processing, pyruvate is oxidized to acetyl CoA in the mitochondrial matrix.
- In the citric acid cycle, the acetyl from acetyl CoA is oxidized to carbon dioxide (CO_2) in the mitochondrial matrix.
- Glycolysis, pyruvate processing, and the citric acid cycle are all regulated processes. The cell produces ATP only when ATP is needed.

✔ You should be able to …

1. **MODEL** The flow chart in Making Models 9.1 tracks the fate of carbons as glucose is oxidized to CO_2. Now draw a flow chart to track the flow of electrons from glucose to NADH or $FADH_2$ as glucose is oxidized to CO_2. (Hint: Rather than showing balls for carbons, use triangles to represent pairs of electrons, starting with 12 triangles for glucose. One pair should go to each NADH or $FADH_2$ formed.)
2. Which processes involved in cellular respiration are *negatively* regulated? For each glucose oxidized, determine the number of CO_2, ATP, NADH, and $FADH_2$ molecules that could be produced up to each regulation point. (If a process has multiple points of negative regulation, assume you are calculating based on the first that would occur in the pathway.)

Answers are available in Appendix A.

9.5 Electron Transport and Chemiosmosis: Building a Proton Gradient to Produce ATP

The answer to one fundamental question about the oxidation of NADH and $FADH_2$ turned out to be relatively straightforward. By isolating different parts of mitochondria, researchers determined that NADH is oxidized when combined with the inner membrane of the mitochondria, including the cristae. In prokaryotes, the oxidation of NADH occurs in the plasma membrane. These membranes were then hypothesized to contain components responsible for oxidizing NADH and $FADH_2$.

Biologists made a key discovery when they isolated the membrane components after exposing them to NADH and $FADH_2$—the components were found to cycle between oxidized and reduced states. What are these molecules, and how do they work?

The Electron Transport Chain

Collectively, the molecules responsible for the oxidation of NADH and $FADH_2$ are designated the **electron transport chain (ETC).** Several points are fundamental to understanding how the ETC works:

- Most of the molecules are proteins that contain distinctive cofactors and prosthetic groups where the redox events take place (see Chapter 8). They include iron–sulfur complexes, ring-containing structures called flavins, or iron-containing heme groups called cytochromes. Each of these groups is readily reduced or oxidized.
- The inner membrane of the mitochondrion also contains a molecule called **ubiquinone,** which is not a protein. Ubiquinone got its name because it is nearly ubiquitous in organisms and belongs to a family of compounds called quinones. Also called **coenzyme Q,** or simply Q, ubiquinone is lipid soluble and moves efficiently throughout the hydrophobic interior of the inner mitochondrial membrane.
- The molecules involved in processing NADH and $FADH_2$ differ in their ability to accept electrons in a redox reaction, referred to as the **redox potential** of the electron acceptors. In addition, some of the molecules pick up a proton with each electron, forming hydrogen atoms, while others obtain only electrons.

Because Q and the ETC proteins differ in redox potential, investigators realized that it should be possible to arrange them into a logical sequence. The idea was that electrons would pass from a molecule with a lower redox potential to one with a higher redox potential, via a redox reaction.

As electrons moved through the chain, they would be held more and more tightly. As a result, a small amount of energy would be released in each reaction, and the potential energy in each successive bond would lessen.

Organization of the Electron Transport Chain Researchers worked out the sequence of the redox reactions in the ETC by experimenting with poisons that inhibit particular proteins in the inner membrane. It was expected that if part of the chain were inhibited, then the components upstream of the block would become reduced and those downstream would remain oxidized.

Experiments with various poisons showed that NADH donates an electron to a flavin-containing protein (FMN) at the top of the chain, while $FADH_2$ donates electrons to an iron- and sulfur-containing protein (Fe·S) that then passes them directly to Q. After passing through each of the remaining components in the chain, the electrons are finally accepted by oxygen.

Figure 9.14 shows how the potential energy in shared electrons steps down from the electron carriers NADH and $FADH_2$ to O_2. The *x*-axis plots the sequence of redox reactions in the ETC; the *y*-axis plots the free-energy changes that occur. ✔ **If you understand how electrons are transferred in the electron transport chain, you should be able to use Figure 9.14 to identify the ETC electron acceptor with the highest redox potential and the acceptor with the lowest redox potential.**

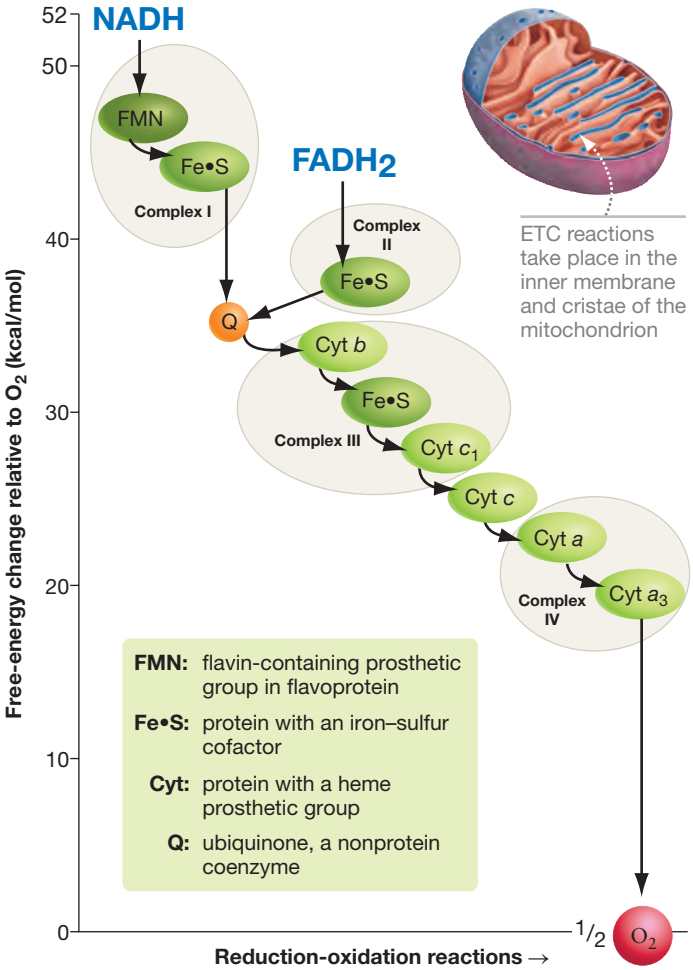


Figure 9.14 A Series of Reduction–Oxidation Reactions Occur in an Electron Transport Chain. The potential energy in shared electrons steps down from the electron carriers NADH and $FADH_2$ through an electron transport chain to a final electron acceptor. In this electron transport chain, oxygen is the final electron acceptor and it forms water as a by-product. The overall free-energy change of 52 kcal/mol (from NADH to oxygen) is broken into small steps.

DATA: Wilson, D. F., M. Erecinska, and P. L. Dutton. 1974. *Annual Review of Biophysics and Bioengineering* 3: 203–230. Also Sled, V. D., N. I. Rudnitzky, Y. Hatefi, et al. 1994. *Biochemistry* 33: 10069–10075.

The components of the electron transport chain are organized into four large complexes of proteins, often referred to as simply complexes I–IV. Q and the protein **cytochrome *c*** act as shuttles that transfer electrons between these complexes. Once the electrons at the bottom of the ETC are accepted by oxygen to form water, the oxidation of glucose is complete. Details on the names of the complexes and their role in the electron transport chain are provided in **Table 9.3** on page 202.

Under controlled conditions in the laboratory, the total potential energy difference from NADH to oxygen is a whopping 52 kilocalories/mole (kcal/mol). Oxidation of the 10 molecules of NADH produced from each glucose therefore accounts for almost 80 percent of the total energy released from the sugar. What does the ETC do with all this energy?

SUMMARY **Table 9.3** The Reactions of the Electron Transport Chain

ETC Component	Descriptive Name	Reaction
Complex I	NADH dehydrogenase	Oxidizes **NADH** and transfers the two electrons through proteins containing FMN prosthetic groups and Fe·S cofactors to reduce an oxidized form of ubiquinone (Q). Four H^+ are pumped out of the matrix to the intermembrane space.
Complex II	Succinate dehydrogenase	Oxidizes **$FADH_2$** and transfers the two electrons through proteins containing Fe·S cofactors to reduce an oxidized form of Q. This complex is also used in step 6 of the citric acid cycle.
Q	Ubiquinone	Reduced by complexes I and II and moves throughout the hydrophobic interior of the ETC membrane, where it is oxidized by complex III.
Complex III	Cytochrome *c* reductase	Oxidizes Q and transfers one electron at a time through proteins containing heme prosthetic groups and Fe·S cofactors to reduce an oxidized form of cytochrome *c* (cyt *c*). A total of four H^+ for each pair of electrons is transported from the matrix to the intermembrane space.
Cyt *c*	Cytochrome *c*	Reduced by accepting a single electron from complex III and moves along the surface of ETC membrane, where it is oxidized by complex IV.
Complex IV	Cytochrome *c* oxidase	Oxidizes cyt *c* and transfers each electron through proteins containing heme prosthetic groups to reduce oxygen gas (O_2), which picks up two H^+ from the matrix to produce water. Two additional H^+ are pumped out of the matrix to the intermembrane space.

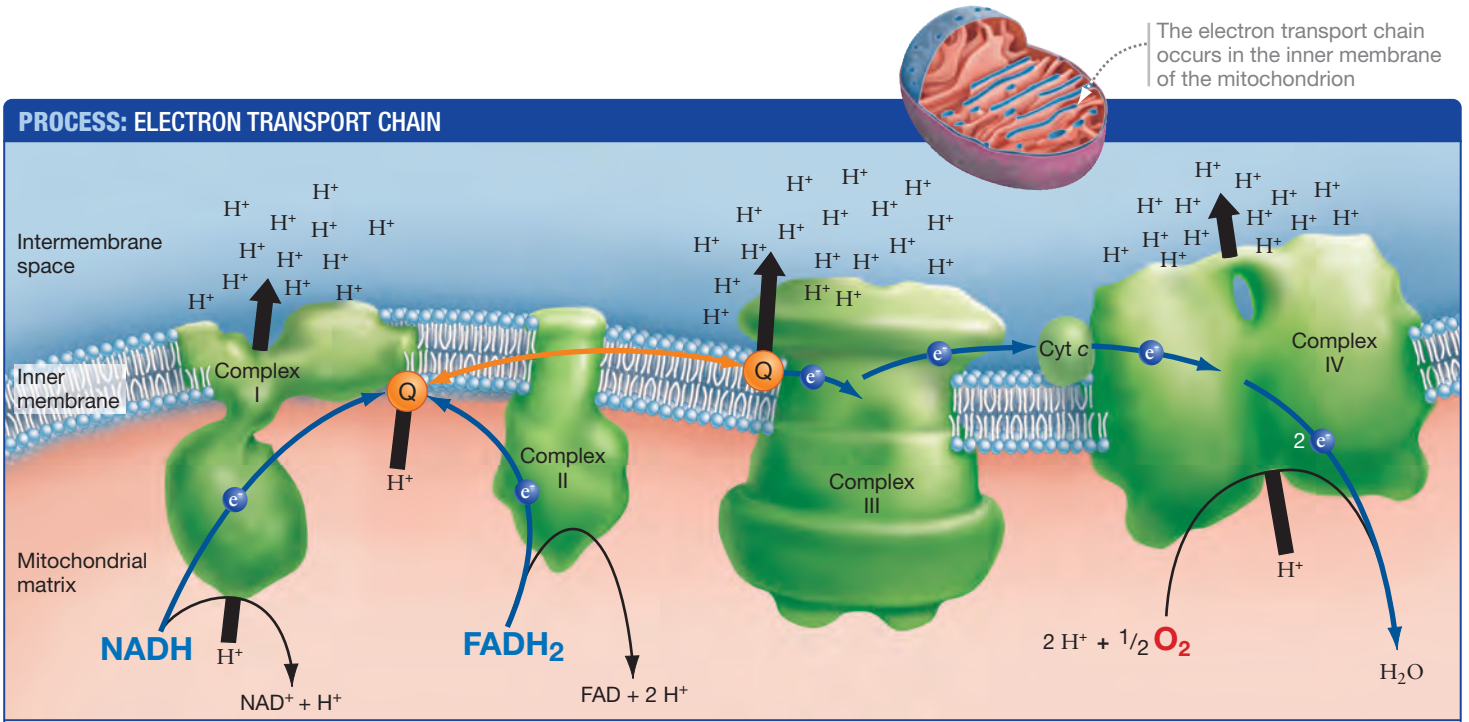


Figure 9.15 **How Does the Electron Transport Chain Work?** The individual components of the electron transport chain diagrammed in Figure 9.14 are found in the inner membrane of mitochondria. Electrons are carried from one complex to another by Q and by cytochrome *c;* Q also shuttles protons across the membrane. The orange arrow indicates Q moving back and forth. Complexes I and IV use the energy released by the redox reactions to pump protons from the mitochondrial matrix to the intermembrane space.

✔ Draw an arrow across the membrane from low to high proton concentration and label it "Proton gradient." In the boxes at the bottom, list "What goes in" and "What comes out" for each complex.

Role of the Electron Transport Chain Throughout the 1950s most biologists working on cellular respiration assumed that electron transport chains include enzymes that catalyze substrate-level phosphorylation. Recall that when substrate-level phosphorylation occurs, a phosphate group is transferred from a phosphorylated substrate to ADP, forming ATP. Despite intense efforts, however, no one was able to find an enzyme among the components of the ETC that would catalyze the phosphorylation of ADP to produce ATP.

But researchers did find that the energy released from the redox reactions is used to actively transport protons across the inner membrane from the matrix into the intermembrane space (see **Figure 9.15**). The exact route and mechanism used to pump protons is still being worked out. In some cases, it is not clear how the complex uses redox reactions to transport protons.

The best-understood interaction between electron transport and proton transport takes place in complex III. Research has shown that when Q accepts electrons from complex I or complex II, it picks up protons from the matrix side of the inner membrane. The reduced form of Q then diffuses through the inner membrane, where its electrons are used to reduce a component of complex III near the intermembrane space. The protons held by Q are then released to the intermembrane space.

In this way, through redox reactions alone, Q shuttles electrons and protons from one side of the membrane to the other. The electrons proceed down the transport chain, and the transported protons contribute to an electrochemical gradient.

Once the nature of the electron transport chain became clear, biologists understood the fate of the electrons and the energy carried by NADH and $FADH_2$. Much of the chemical energy that was originally present in glucose is now accounted for in the proton electrochemical gradient. This is satisfying, except for a key question: If electron transport doesn't make ATP, what does?

The Discovery of ATP Synthase

In 1960 Efraim Racker made several key observations about how ATP is synthesized in mitochondria. When he used mitochondrial membranes to make vesicles, Racker noticed that some vesicles formed with their membrane inside out. Electron microscopy revealed that the inside-out membranes had many large proteins studded along their surfaces. Each protein appeared to have a base in the membrane, from which a lollipop-shaped stalk and a knob projected (**Figure 9.16**). If the solution was vibrated or treated with a compound called urea, the stalks and knobs fell off.

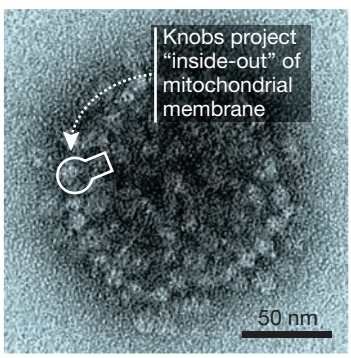


Figure 9.16 The Discovery of ATP Synthase. When patches of mitochondrial membrane turn inside out and form vesicles, the lollipop-shaped stalk-and-knob structures of ATP synthase proteins face outward. Normally, the stalk and knob face inward, toward the mitochondrial matrix.

Racker seized on this technique to isolate the stalks and knobs and do experiments with them. For example, he found that these isolated structures could hydrolyze ATP, forming ADP and inorganic phosphate. The vesicles that contained just the base component, without the stalks and knobs, could not process ATP. The base components were, however, capable of transporting protons across the membrane.

Based on these observations, Racker proposed that the stalk-and-knob component of the protein was an enzyme that both hydrolyzes and synthesizes ATP. To test his idea, Racker added the stalk-and-knob components back to vesicles that had been stripped of them and confirmed that the vesicles regained the ability to synthesize ATP. The entire complex is known as **ATP synthase.** Follow-up work also confirmed his hypothesis that the membrane-bound base component of ATP synthase is a proton channel. Is there a connection between proton transport and ATP synthesis?

The Chemiosmosis Hypothesis

In 1961 Peter Mitchell broke with the prevailing ideas that electron transport produces ATP via substrate phosphorylation. Instead, he proposed something completely new—an indirect connection between electron transport and ATP production. Mitchell proposed that the real job of the electron transport chain is to pump protons across the inner membrane of mitochondria from the matrix to the intermembrane space. After a proton gradient is established, an enzyme in the inner membrane, like Racker's ATP synthase, would synthesize ATP from ADP and P_i.

Mitchell introduced the term **chemiosmosis** to describe the use of a proton gradient to drive energy-requiring processes, like the production of ATP. Similar to osmosis, chemiosmosis involves diffusion across a membrane, but in this case, protons are diffusing along its gradient rather than water. Although proponents of a direct link between electron transport and substrate-level phosphorylation objected vigorously to Mitchell's idea, several key experiments supported it.

Figure 9.17 on page 204 illustrates how the existence of a key element in Mitchell's hypothesis was confirmed: A proton gradient alone can be used to synthesize ATP via ATP synthase. The researchers made vesicles from artificial membranes that contained Racker's ATP synthase from mitochondria along with ADP and P_i. To generate a proton gradient across the membrane, they also included bacteriorhodopsin, a well-studied membrane protein that acts as a light-activated proton pump.

When light strikes bacteriorhodopsin, it absorbs some of the light energy and changes conformation in a way that pumps protons from the interior of a membrane to the exterior. As a result, the experimental vesicles established a strong electrochemical gradient favoring proton movement to the interior. When the vesicles were illuminated to initiate proton pumping, ATP began to be produced from ADP and P_i inside the vesicles.

Mitchell's prediction was correct: In this situation, ATP production depended solely on the existence of a **proton-motive force,** which is based on a proton electrochemical gradient. It could occur in the *absence* of an electron transport chain. This result, along with many others, has provided strong support for the hypothesis of chemiosmosis. Most of the ATP produced by cellular respiration is made by a flow of protons.

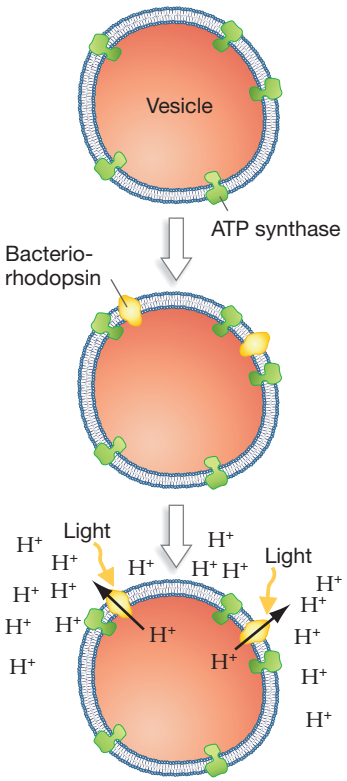
RESEARCH

QUESTION: How are the electron transport chain and ATP production linked?

CHEMIOSMOTIC HYPOTHESIS: The linkage is indirect. The ETC creates a proton gradient and ATP synthase uses the gradient to synthesize ATP.

ALTERNATIVE HYPOTHESIS: The linkage is direct. Specific ETC proteins are required for ATP synthesis by ATP synthase.

EXPERIMENTAL SETUP:



1. Produce vesicles from artificial membranes; add ATP synthase, an enzyme found in mitochondria.

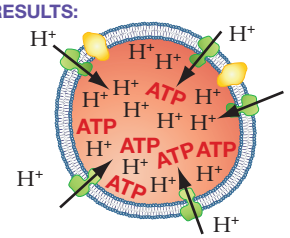
2. Add bacteriorhodopsin, a protein that acts as a light-activated proton pump.

3. Illuminate vesicle so that bacteriorhodopsin pumps protons out of vesicle, creating a proton gradient.

PREDICTION OF CHEMIOSMOTIC HYPOTHESIS: ATP will be produced within the vesicle.

PREDICTION OF ALTERNATIVE HYPOTHESIS: No ATP will be produced without the ETC.

RESULTS:



ATP is produced within the vesicle, in the absence of the electron transport chain.

CONCLUSION: The linkage between electron transport and ATP production by ATP synthase is indirect; the synthesis of ATP only requires a proton gradient.

Figure 9.17 Evidence for the Chemiosmotic Hypothesis.

SOURCE: Racker, E., and W. Stoeckenius. 1974. Reconstitution of purple membrane vesicles catalyzing light-driven proton uptake and adenosine triphosphate formation. *Journal of Biological Chemistry* 249: 662–663.

✔ **PROCESS OF SCIENCE** If bacteriorhodopsin were not available, how else could the researchers have generated a proton gradient?

✔ **If you understand chemiosmosis, you should be able to explain the relationships among glucose oxidation, the proton gradient, and ATP synthase.**

Organisms throughout the tree of life use electron transport chains and ATP synthases. These processes are humming away in your cells now and produce most of the ATP that keeps you alive. Let's look in more detail at how they function.

The Proton-Motive Force Couples Electron Transport to ATP Synthesis

As **Figure 9.18** shows, the structure of ATP synthase is now well understood. The ATP synthase "knob" component is called the F_1 unit; the membrane-bound, proton-transporting base component is the F_o unit. The F_1 and F_o units are connected by a shaft, as well as by a stator, which holds the two units in place.

The F_o unit serves as a rotor, whose turning is conveyed to the F_1 unit via the shaft. A flow of protons through the F_o unit causes the rotor and shaft to spin. By attaching long actin filaments to the shaft and examining them with a videomicroscope, researchers have been able to see the rotation, which can reach speeds of 350 revolutions per second. As the shaft spins within the F_1 unit, it is thought to change the conformation of the F_1 subunits in a way that catalyzes the phosphorylation of ADP to ATP.

Chemiosmosis resembles the process of generating electricity in a hydroelectric dam like the one pictured on the first page of this chapter. The ETC pumps protons across the inner membrane, similar to the way a series of gigantic pumps force

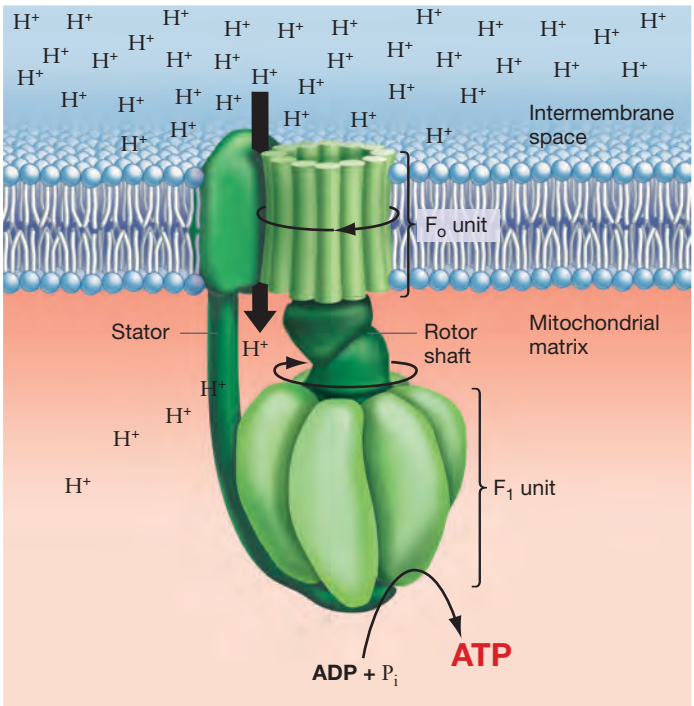


Figure 9.18 Oxidative Phosphorylation Involves the ATP Synthase Motor and a Proton Gradient. ATP synthase has two major components, designated F_o and F_1, connected by a shaft. The F_o unit spins as protons pass through. The shaft transmits the rotation to the F_1 unit, causing it to make ATP from ADP and P_i.

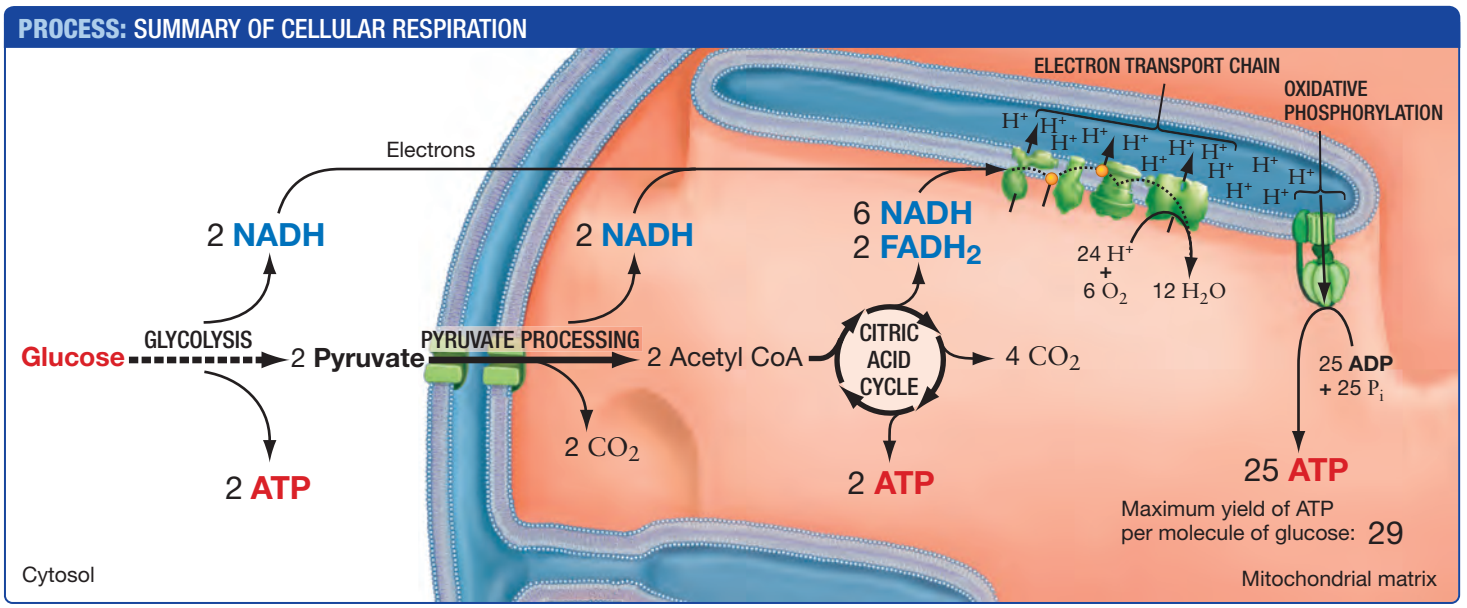


Figure 9.19 **ATP Yield during Cellular Respiration.** The actual yield of ATP per glucose (29 ATP) is lower than the theoretical calculation (38 ATP) because the proton motive force is used to drive other mitochondrial activities, such as the active transport of P_i into the mitochondrial matrix.

water up and behind a dam. When protons pass through the ATP synthase, it spins and releases energy used to synthesize ATP. This is analogous to how water passing through the turbines of hydroelectric dams causes them to spin and generate electricity.

The key idea to note here is that the energy to produce ATP in oxidative phosphorylation comes from an established proton gradient, not phosphorylated substrates as used in substrate-level phosphorylation.

It has been determined that the ETC transports enough protons to produce approximately three ATP for each NADH and two for each $FADH_2$, depending on the type of ATP synthase used. These yields, however, are not observed in cells, since the proton-motive force is also used to drive other processes, such as the import of phosphates into the mitochondrial matrix.

ATP synthase also can hydrolyze ATP and reverse the direction of its spin. If the proton gradient dissipates, this ATP-powered reversal is used to pump protons from the matrix to the intermembrane space. Understanding how these reactions occur is currently the focus of intense research.

The Proton-Motive Force and Chemical Evolution

Since chemiosmosis is responsible for most of the ATP produced by cells throughout the tree of life, it likely arose early in evolution. But how could a complex electron transport chain evolve to produce the proton-motive force without a proton-motive force to supply the energy?

This apparent conundrum left many of the chemical evolution theorists perplexed until a key discovery was made deep in the ocean along the Mid-Atlantic Ridge—the Lost City hydrothermal vents (see Chapter 2). Researchers propose that the alkaline fluid (low proton concentration) released from these vents in the acidic oceans (high proton concentration) of early Earth may have provided such a gradient.

While the debate continues over the role hydrothermal vents may have played in chemical evolution, their discovery has generated much excitement. By harnessing the natural electrochemical gradient deep in the early oceans, oxidative phosphorylation may have evolved before an electron transport chain existed. If this were the case, the subsequent evolution of a proton-pumping electron transport chain would have been necessary to establish an environment that would mimic the natural gradients present in the vent environment.

Organisms Use a Diversity of Electron Acceptors

Figure 9.19 summarizes glucose oxidation and cellular respiration by tracing the fate of the carbon atoms and electrons in glucose. Notice that electrons from glucose are transferred to NADH and $FADH_2$, passed through the electron transport chain, and accepted by oxygen. Proton pumping during electron transport creates the proton-motive force that drives ATP synthesis.

The diagram also indicates the approximate yield of ATP from each component of the process. Recent research shows that about 29 ATP molecules are produced from each molecule of glucose.[2] Of these, 25 ATP molecules are produced by ATP synthase. What is the fundamental message here? The vast majority of the "payoff" from the oxidation of glucose occurs via oxidative phosphorylation.

The chemical equation that represents the overall process involved in cellular respiration is

$$C_6H_{12}O_6 + 6\,H_2O + 6\,O_2 + 29\,ADP + 29\,P_i \longrightarrow 6\,CO_2 + 12\,H_2O + 29\,ATP$$

[2] Traditionally, biologists thought that up to 38 ATP would be synthesized for every molecule of glucose oxidized in cells. More recent work has shown that actual yield is only about 29 ATP [see P. R. Rich, The molecular machinery of Keilin's respiratory chain. *Biochemical Society Transactions* 6 (2003): 1095–1105]. Also, it's important to note that yield varies with conditions in the cell.

The reactants include six water molecules that are used in glycolysis and the citric acid cycle (some of these are depicted in Figure 9.10). For simplicity, the NADH and $FADH_2$ electron carriers are not shown.

Aerobic versus Anaerobic Respiration During cellular respiration, oxygen is the electron acceptor used by all eukaryotes and a wide diversity of prokaryotes. Species that depend on oxygen as an electron acceptor for the ETC use **aerobic** respiration and are called aerobic organisms. (The Latin root *aero* means "air.")

It is important to recognize, though, that cellular respiration can occur without oxygen. Many thousands of bacterial and archaeal species rely on electron acceptors other than oxygen, and electron donors other than glucose. For example, nitrate (NO_3^-) and sulfate (SO_4^{2-}) are particularly common electron acceptors in species that live in oxygen-poor environments (see Chapter 26). In addition, many bacteria and archaea use H_2, H_2S, CH_4, or other inorganic compounds as electron donors—not glucose.

Cells that depend on electron transport chains with electron acceptors other than oxygen are said to use **anaerobic** ("no air") respiration. Even though the starting and ending points of cellular respiration differ, aerobic and anaerobic respiring cells still use an ETC to create a proton-motive force that drives the synthesis of ATP. In bacteria and archaea, the ETC and ATP synthase are located in the plasma membrane.

Aerobic Respiration Is Most Efficient Even though an array of compounds can serve as the final electron acceptor in cellular respiration, oxygen provides the greatest energy yield. Because oxygen is so highly electronegative, the potential energy in bonds between an oxygen atom and a non-oxygenic atom, such as hydrogen, is low. As a result, there is a large difference between the potential energy of reduced electron donors, like NADH, and reduced forms of oxygen, like water (see Figure 9.14). This large differential in potential energy means that the electron transport chain can generate a large proton-motive force.

Cells that do not use oxygen as an electron acceptor cannot generate such a large potential energy difference. As a result, they make less ATP per electron donor, such as glucose, than cells that use aerobic respiration. This finding is important: If cells that use anaerobic respiration compete with cells using aerobic respiration, those that use oxygen as an electron acceptor almost always grow faster and reproduce more.

What happens when the electron acceptors in an ETC get used up? When there is no terminal electron acceptor, the electrons in each of the complexes of the electron transport chain have no place to go and the electron transport chain stops. Without an oxidized complex I, NADH remains reduced. The concentration of NAD^+ drops rapidly as cells continue to convert NAD^+ to NADH.

This situation is life threatening. When there is no longer any NAD^+ to drive glycolysis, pyruvate processing, and the citric acid cycle, then no ATP can be produced. If NAD^+ cannot be regenerated somehow, the cell will die.

How do cells cope?

CHECK YOUR UNDERSTANDING

If you understand that …

- As electrons from NADH and $FADH_2$ move through the electron transport chain, protons are pumped into the intermembrane space of mitochondria.
- The electrochemical gradient across the inner mitochondrial membrane drives protons through ATP synthase, resulting in the production of ATP from ADP.

✓ You should be able to …

MODEL Draw a model of a segment of inner mitochondrial membrane, using a rectangle to represent the electron transport chain and an oval to represent ATP synthase. Label the membrane, the intermembrane space, and the mitochondrial matrix. Now add triangles to represent the number of electron pairs delivered by NADH and $FADH_2$ and show where these electrons end up. Also, add arrows to show the movement of protons across the membrane and the ultimate process that is driven by this movement.

Answers are available in Appendix A.

9.6 Fermentation

Fermentation is a metabolic pathway that regenerates NAD^+ by oxidizing stockpiles of NADH. The electrons removed from NADH are transferred to pyruvate, or a molecule derived from pyruvate, instead of an electron transport chain (**Figure 9.20**).

In respiring cells, fermentation serves as an emergency backup to produce ATP even when the ETC and oxidative phosphorylation is shut down. If the ETC is not available to oxidize NADH, then the concentration of NAD^+ rapidly drops and glycolysis, pyruvate processing, and the citric acid cycle will halt. Fermentation may allow the cell to survive in the absence of an active electron transport chain by regenerating NAD^+ in the cytosol, where glycolysis can continue to produce ATP. How does fermentation regenerate NAD^+, and what is being reduced in this redox reaction?

Many Different Fermentation Pathways Exist

When you run up a long flight of stairs, your muscles begin metabolizing glucose so fast that the supply of oxygen is rapidly used up by their mitochondria. When oxygen runs out, the electron transport chains shut down and NADH cannot donate its electrons there. When fermentation takes place in your cells, the pyruvate produced by glycolysis then begins to accept electrons from NADH. This process, called **lactic acid fermentation,** regenerates NAD^+ by forming lactate: a deprotonated form of lactic acid (**Figure 9.21a**).

When your muscles are deprived of oxygen, your body reacts by making you breathe faster and increasing your heart rate. By getting more oxygen to your muscle cells, the electron transport chain is revived. The lactic acid produced by fermentation can be converted back to pyruvate and used as a source of energy to drive cellular respiration when oxygen is present.

In many cases, however, the cell cannot use the molecule that is formed when pyruvate (or another electron acceptor) accepts electrons from NADH. This by-product may even be toxic and excreted from the cell as waste even though it has not been fully oxidized.

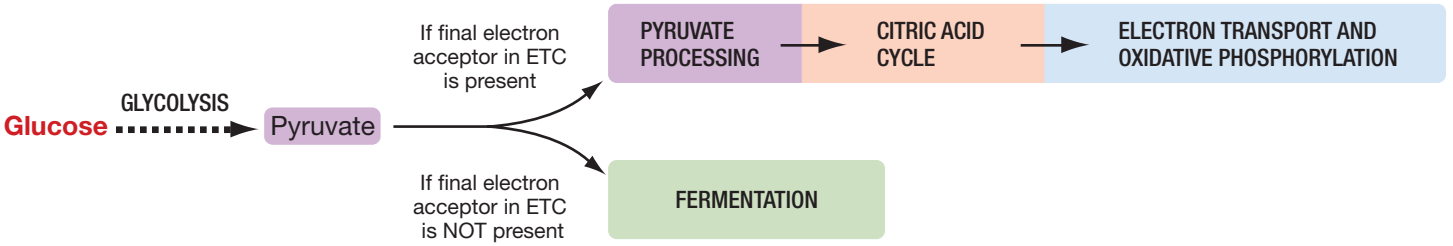
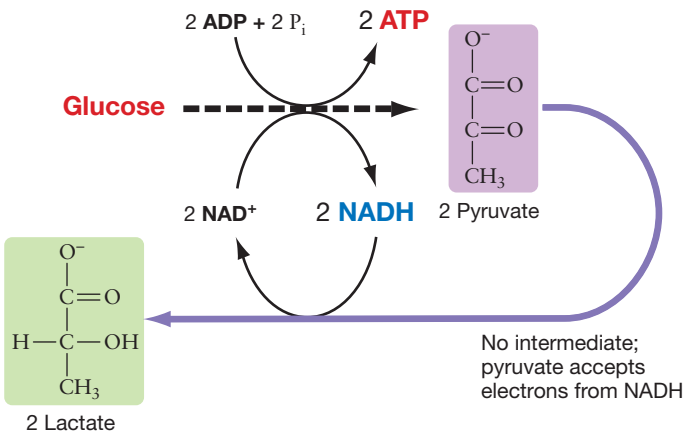


Figure 9.20 Cellular Respiration and Fermentation Are Alternative Pathways for Producing ATP. When oxygen or another final electron acceptor used by the ETC is present in a cell, the pyruvate produced by glycolysis enters the citric acid cycle and the electron transport system is active. But if no electron acceptor is available to keep the ETC running, then pyruvate undergoes reactions known as fermentation.

Figure 9.21b illustrates **alcohol fermentation,** which occurs in the eukaryote *Saccharomyces cerevisiae*, strains of which are used to make baker's and brewer's yeast. When yeast cells grow in bread dough or a bottle of grape juice, they quickly use up all the available oxygen. Instead of using NADH to reduce pyruvate, yeast first convert pyruvate to the two-carbon compound acetaldehyde.

(a) Lactic acid fermentation occurs in humans.



(b) Alcohol fermentation occurs in yeast.

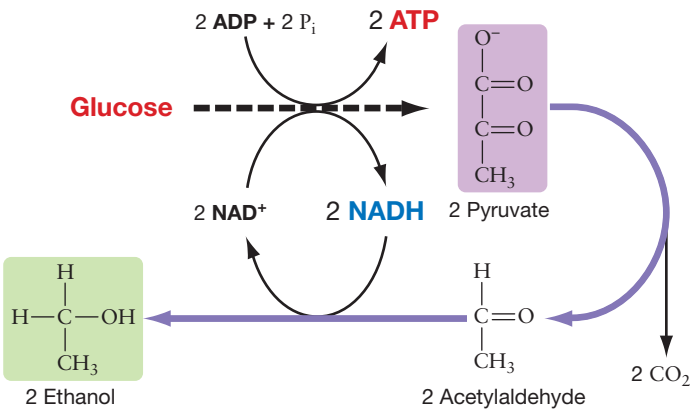


Figure 9.21 Fermentation Regenerates NAD^+ So That Glycolysis Can Continue. These are just two examples of the many types of fermentation that occur in prokaryotes and eukaryotes.

This reaction gives off carbon dioxide, which causes bread to rise and produces the bubbles in champagne and beer.

Acetaldehyde then accepts electrons from NADH, forming the NAD^+ required to keep glycolysis going. The addition of electrons to acetaldehyde forms ethanol as a waste product. The yeast cells excrete ethanol as waste. In essence, the active ingredient in alcoholic beverages is like yeast urine.

Cells that employ other types of fermentation are used commercially in the production of soy sauce, tofu, yogurt, cheese, vinegar, and other products. The products of these reactions are responsible for many of the complex flavors in these foods.

Bacteria and archaea that rely exclusively on fermentation are called obligate anaerobes. These organisms are present in phenomenal numbers in your intestines and in the first compartment of a cow's stomach, called the rumen. The rumen is a specialized digestive organ that contains over 10^{10} (10 billion) bacterial and archaeal cells per *milliliter* of fluid. The fermentations that occur in these cells produce an array of high-energy products, like fatty acids. Cattle don't actually live off grass directly—they eat it to feed their bacteria and archaea and then use the fermentation by-products for energy.

Fermentation as an Alternative to Cellular Respiration

Even though fermentation is a widespread type of metabolism, it is extremely inefficient compared with cellular respiration. Fermentation produces just 2 molecules of ATP per molecule of glucose metabolized, while aerobic cellular respiration produces about 29—almost 15 times more ATP per glucose

CHECK YOUR UNDERSTANDING

If you understand that …

- Fermentation most often occurs in the absence of an electron acceptor at the end of an ETC. It consists of reactions that oxidize NADH to regenerate the NAD^+ required for glycolysis.

✓ You should be able to …

Compare and contrast the roles of lactic acid fermentation and the electron transport chain. Why would fermentation alone not be sufficient to keep your cells alive?

Answers are available in Appendix A.

molecule than fermentation. The reason for the disparity is that the fermentation reactions that follow glycolysis are not used to generate ATP.

Organisms that can switch between fermentation and aerobic cellular respiration are called **facultative anaerobes.** The adjective "facultative" reflects the ability to use aerobic cellular respiration when oxygen is present and anaerobic fermentation when it is absent. Many of your cells can function as facultative anaerobes to a certain extent; however, you cannot survive for long without oxygen. To make this point clear, try holding your breath—it should take only a minute for you to realize how important electron transport is to your cells.

CHAPTER 9 REVIEW

For media, go to MasteringBiology

9.1 An Overview of Cellular Respiration

- Cellular respiration is based on redox reactions that oxidize a compound with high potential energy, such as glucose, and produce molecules with low potential energy, such as CO_2 and water.
- In eukaryotes, cellular respiration consists of four steps: glycolysis, pyruvate processing, the citric acid cycle, and electron transport coupled to oxidative phosphorylation.
- Glycolysis, pyruvate processing, and the citric acid cycle are central to the metabolism of most cells. Other catabolic pathways feed into them, and the intermediates of the central pathways are used in the synthesis of many key molecules.

9.2 Glycolysis: Oxidizing Glucose to Pyruvate

- The glycolytic pathway is a 10-step reaction sequence in which glucose is broken down into two molecules of pyruvate. It takes place in the cytosol and produces ATP and NADH.
- Glycolysis slows when ATP binds to a regulatory site in phosphofructokinase.

9.3 Processing Pyruvate to Acetyl CoA

- Pyruvate processing is a series of reactions that convert pyruvate to acetyl CoA in the mitochondrial matrix in eukaryotes and the cytosol of prokaryotes. NADH and CO_2 are produced.
- The pyruvate dehydrogenase complex is inhibited when it is phosphorylated by ATP. It speeds up in the presence of reactants and slows down in the presence of products.

9.4 The Citric Acid Cycle: Oxidizing Acetyl CoA to CO_2

- The citric acid cycle is an eight-step reaction cycle in the matrix of mitochondria or cytosol of prokaryotes. It begins with acetyl CoA and produces $FADH_2$, NADH, and ATP or GTP. By the end of the citric acid cycle, all of the carbons from glucose are completely oxidized to CO_2.
- Certain enzymes in the citric acid cycle are inhibited when NADH or ATP binds to them.

9.5 Electron Transport and Chemiosmosis: Building a Proton Gradient to Produce ATP

- The electron transport chain resides in the inner membrane of mitochondria and consists of a series of electron acceptors that vary in their redox potential. It begins with the oxidation of NADH and $FADH_2$ and ends with the reduction of a terminal electron acceptor, like O_2.
- The energy released from redox reactions in the electron transport chain is used to transport protons across the inner mitochondrial membrane, creating an electrochemical gradient.
- ATP production is coupled to the ETC by oxidative phosphorylation. The potential energy stored in the proton gradient is used to spin components of the ATP synthase to produce ATP. This process is responsible for most of the ATP made by cellular respiration.

9.6 Fermentation

- Fermentation occurs in the cytosol of many cells when an electron transport chain is not present or it is inactive due to an insufficient amount of the final electron acceptor. It begins by reducing pyruvate, or a molecule derived from pyruvate, to regenerate NAD^+ from NADH.
- Production of NAD^+ enables glycolysis to continue producing ATP, albeit significantly less ATP than produced by cellular respiration. Depending on the molecule that acts as an electron acceptor, fermentation pathways produce lactate, ethanol, or other reduced organic compounds as a by-product.

Answers are available in Appendix A

✓ TEST YOUR KNOWLEDGE

1. Where does the citric acid cycle occur in eukaryotes?
   a. in the cytosol of cells
   b. in the intermembrane space of mitochondria
   c. in the inner membrane of mitochondria
   d. in the matrix of mitochondria
2. What does the chemiosmotic hypothesis claim?
   a. ATP is generated using phosphates taken from intermediates in the electron transport chain.
   b. ATP is generated using a phosphate gradient produced by glycolysis and the citric acid cycle.
   c. ATP is generated using a proton-motive force that is produced by the electron transport chain.
   d. Water is generated using electrons taken from NADH and $FADH_2$ and transported through the electron transport chain.
3. After glucose is fully oxidized by glycolysis, pyruvate processing, and the citric acid cycle, where is most of its energy stored?

4. What is the primary function of the reactions that follow glycolysis in a fermentation pathway?
   a. to regenerate NAD^+ from NADH, so glycolysis can continue
   b. to synthesize pyruvate from lactate
   c. to regenerate NADH from NAD^+, so electrons can be donated to the electron transport chain
   d. to synthesize electron acceptors, so that cellular respiration can continue

✓ TEST YOUR UNDERSTANDING

5. Compare and contrast substrate-level phosphorylation and oxidative phosphorylation.
6. If you were to expose cells that are undergoing aerobic cellular respiration to a radioactive oxygen isotope in the form of O_2, which of the following molecules would you expect to be radiolabeled?
   a. pyruvate **b.** water **c.** NADH **d.** CO_2
7. In step 3 of the citric acid cycle, the enzyme isocitrate dehydrogenase is regulated by NADH. Compare and contrast the regulation of this enzyme with the regulation of phosphofructokinase in glycolysis.
8. Explain the relationship between electron transport and oxidative phosphorylation. What does ATP synthase look like, and how does it work?

✓ TEST YOUR PROBLEM-SOLVING SKILLS

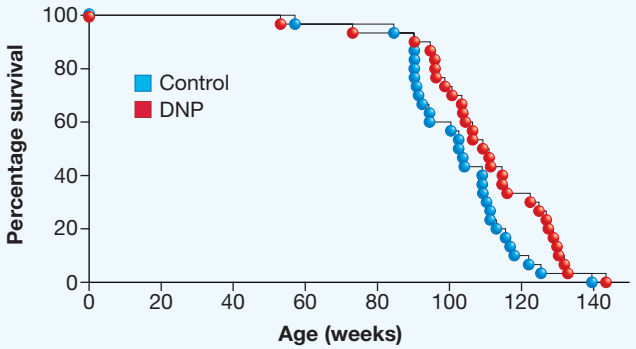
9. Cyanide ($C{\equiv}N^-$) blocks complex IV of the electron transport chain. Suggest a hypothesis for what happens to the ETC when complex IV stops working. Your hypothesis should explain why cyanide poisoning in humans is fatal.
10. **QUANTITATIVE** Early estimates suggested that the oxidation of glucose via aerobic respiration would produce 38 ATP. Based on what you know of the theoretical yields of ATP from each step, show how this total was determined. Why do biologists now think this amount of ATP/glucose is not achieved in cells?

✓ PUT IT ALL TOGETHER: Case Study

Does the Fountain of Youth spring from the mitochondrial proton gradient?

For thousands of years, explorers have sought mythical waters that promote a long life. In modern times, the quest for extending the human life span continues. Current research points to changes in the mitochondrial electron transport chain and the proton gradient as a cause of aging. How is the ETC involved in aging? Can it be manipulated to increase longevity?

11. Research has shown that cellular damage associated with aging occurs via the formation of oxygen radicals called reactive oxygen species (ROS). These radicals are produced when an excessive proton gradient across the inner mitochondrial membrane slows down the rate of electron transport in the ETC. Propose a hypothesis to explain how a proton gradient can reduce the rate of electron transport.
12. **QUANTITATIVE** The production of ROS can be reduced using drugs that allow protons to freely pass through the inner membrane. The effect of a drug called DNP on the life span of mice is shown in the graph below. Each point represents the death of a single mouse. At what age is the difference in survival between the DNP-treated and the untreated control the greatest?



Source: C. C. Caldeira da Silva et al. 2008. *Aging Cell* 7: 552–560.

13. **QUANTITATIVE** In the above study, the investigators determined that a *low* concentration of DNP increased the average life span from 719 days (Control) to 770 days (DNP). If the U.S. population has an average life span of 79 years, then how many years would be added if the same percentage increase were observed?
14. **PROCESS OF SCIENCE** How could you determine if the mitochondrial ETC is affected in DNP-treated mice? Propose an experiment to determine if there is a correlation between life span and ETC activity in mitochondria isolated from mice used in the experiment above.
15. In addition to an increased life span, mice treated with *low* concentrations of DNP also showed a significantly lower weight gain compared to the control group despite no difference in the amount or type of food ingested. Propose an explanation for why DNP would have this effect.
16. **SOCIETY** In the 1930s, DNP was introduced as a diet drug until it was banned from human use because of adverse side effects when *high* concentrations of the drug were used. These included increased respiration and even death. Propose an explanation for the side effects based on the effect DNP has on the proton gradient.