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?*

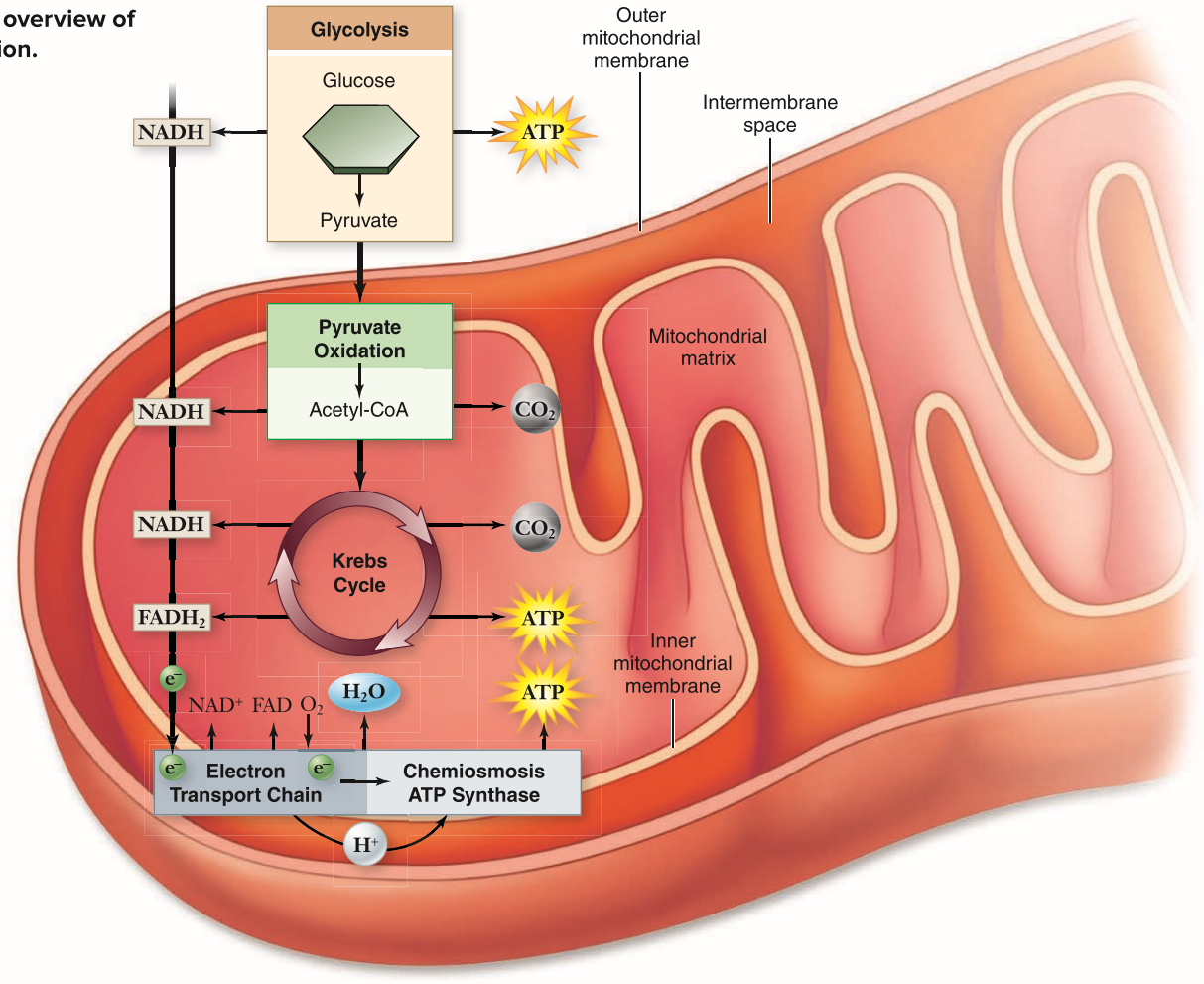


Figure 7.5 An overview of aerobic respiration.

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)

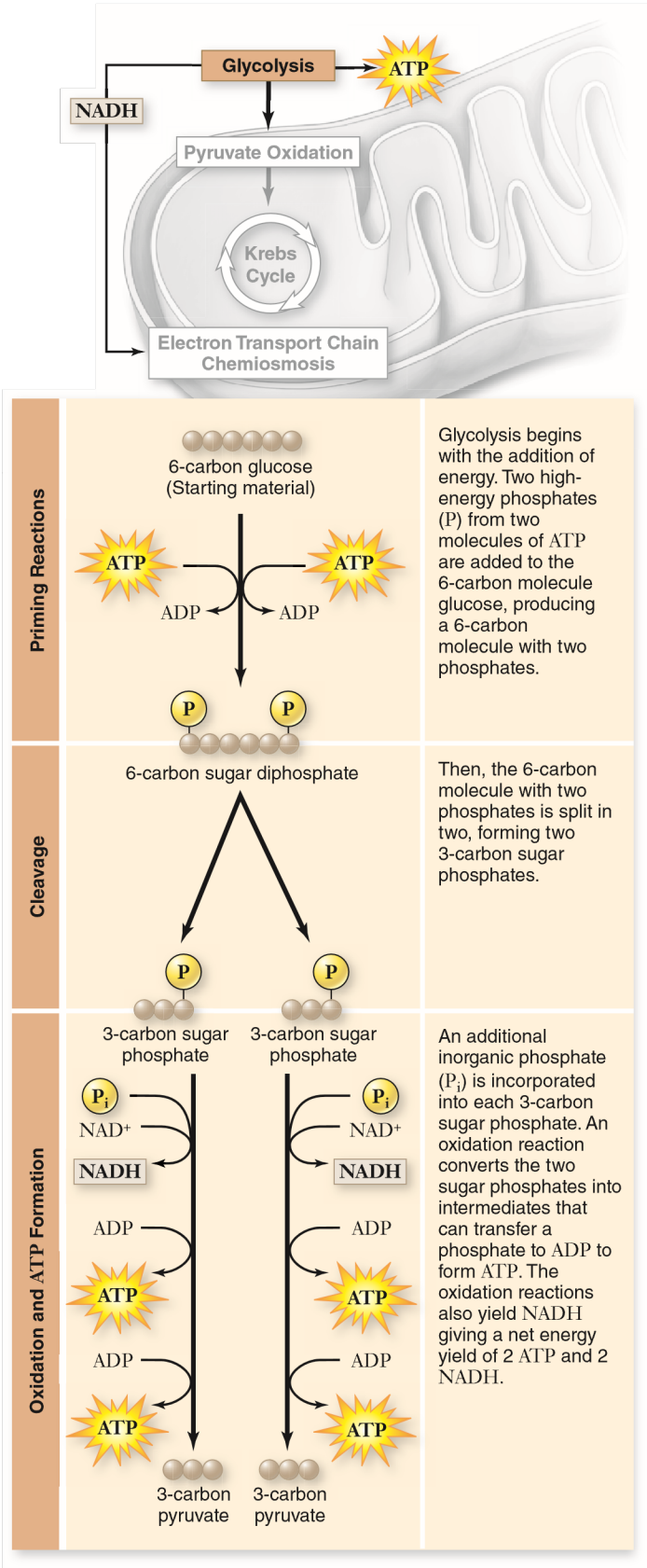
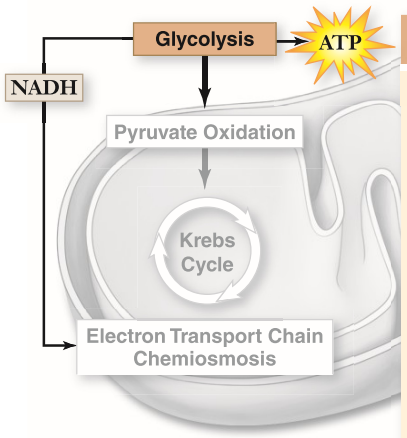


Figure 7.6 An overview of glycolysis.



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.

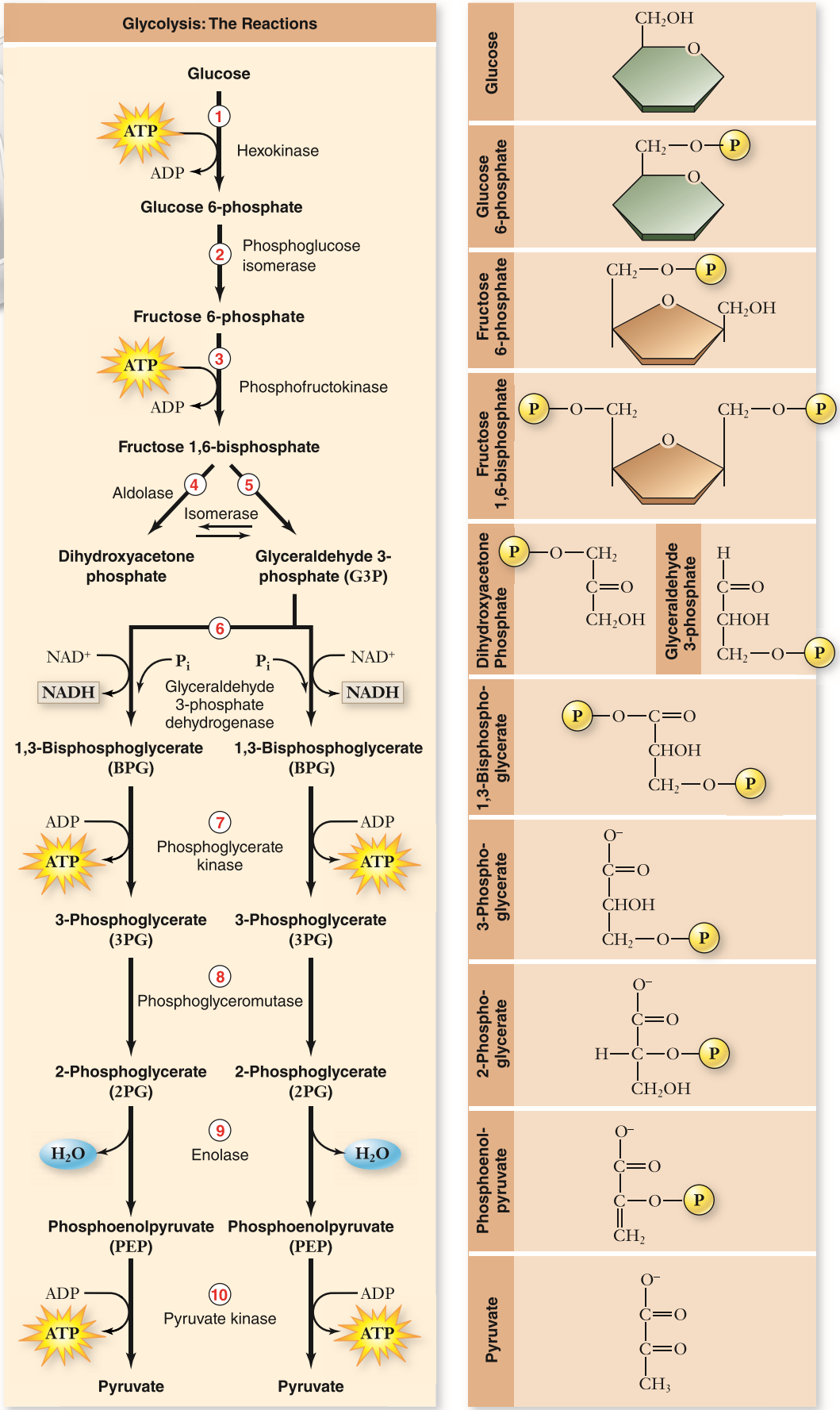
6. Oxidation followed by phosphorylation produces two NADH molecules and two molecules of BPG, each with one high-energy phosphate bond.

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:

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

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

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

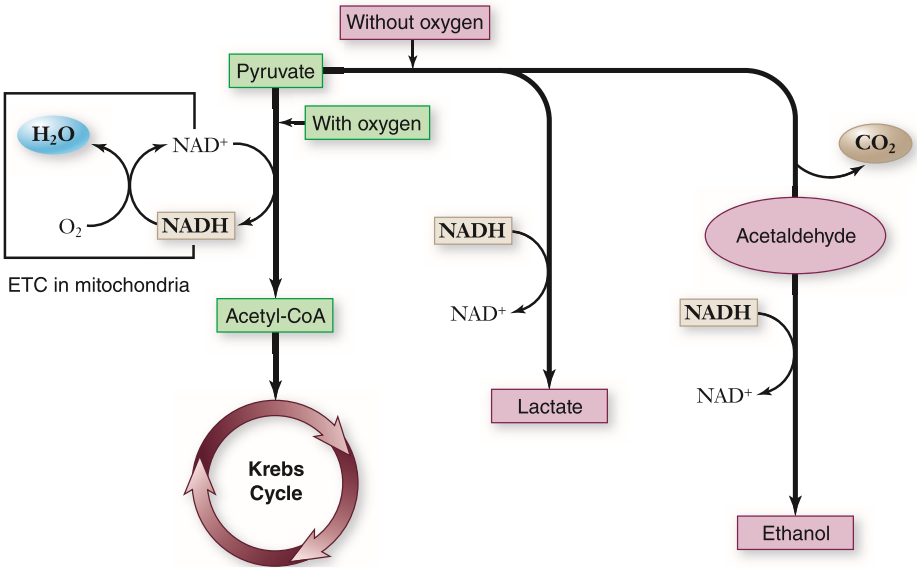


Figure 7.8 The fate of pyruvate and NADH produced by glycolysis. In the presence of oxygen, NADH is oxidized by the electron transport chain (ETC) in mitochondria using oxygen as the final electron acceptor. This regenerates NAD^+, allowing glycolysis to continue. The pyruvate produced by glycolysis 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

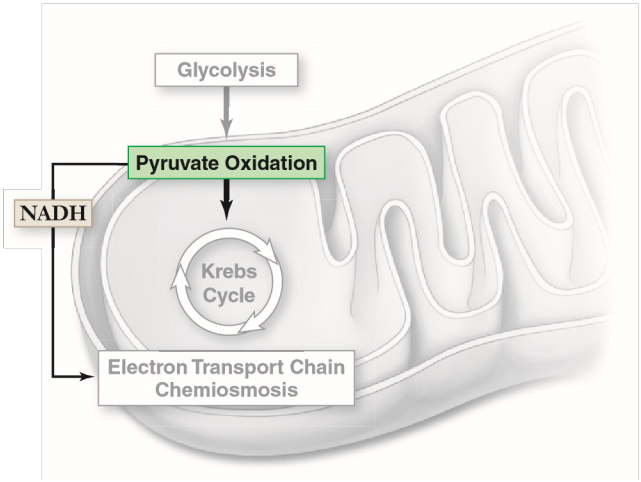
1. *Diagram how the oxidation of pyruvate links glycolysis with the Krebs cycle.*

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



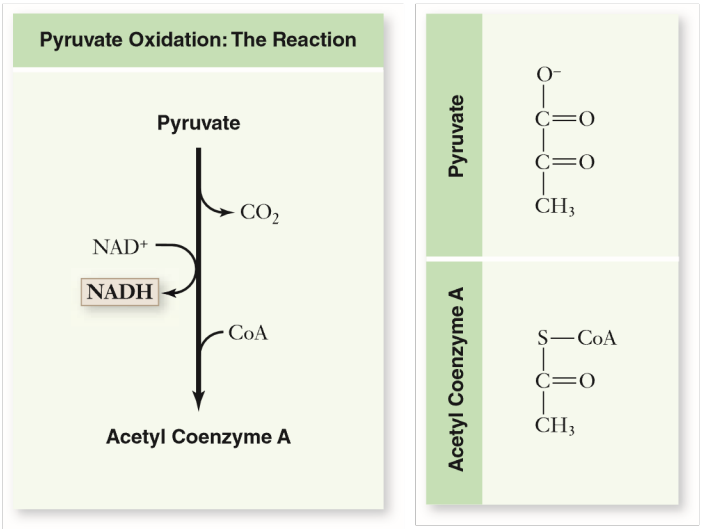


Figure 7.9 The oxidation of pyruvate. This complex reaction uses NAD^+ to accept electrons, reducing it to NADH. The product, acetyl coenzyme A (acetyl-CoA), feeds the acetyl unit into the Krebs cycle, and the CoA is recycled for another oxidation of pyruvate. NADH provides energetic electrons for the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

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

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?*