NAD^+ to NADH and release carboxyl groups that form CO_2 molecules. Alpha-ketoglutarate is the product of step three, and a succinyl group is the product of step four. CoA binds with the succinyl group to form succinyl CoA. The enzyme that catalyzes step four is regulated by feedback inhibition of ATP, succinyl CoA, and NADH.

Step 5. In step five, a phosphate group is substituted for coenzyme A, and a high-energy bond is formed. This energy is used in substrate-level phosphorylation (during the conversion of the succinyl group to succinate) to form either guanine triphosphate (GTP) or ATP. There are two forms of the enzyme, called isoenzymes, for this step, depending upon the type of animal tissue in which they are found. One form is found in tissues that use large amounts of ATP, such as heart and skeletal muscle. This form produces ATP. The second form of the enzyme is found in tissues that have a high number of anabolic pathways, such as liver. This form produces GTP. GTP is energetically equivalent to ATP; however, its use is more restricted. In particular, protein synthesis primarily uses GTP.

Step 6. Step six is a dehydration process that converts succinate into fumarate. Two hydrogen atoms are transferred to FAD, reducing it to $FADH_2$. (Note: the energy contained in the electrons of these hydrogens is insufficient to reduce NAD^+ but adequate to reduce FAD.) Unlike NADH, this carrier remains attached to the enzyme and transfers the electrons to the electron transport chain directly. This process is made possible by the localization of the enzyme catalyzing this step inside the inner membrane of the mitochondrion.

Step 7. Water is added by hydrolysis to fumarate during step seven, and malate is produced. The last step in the citric acid cycle regenerates oxaloacetate by oxidizing malate. Another molecule of NADH is then produced in the process.

LINK TO LEARNING

View an animation of the citric acid cycle here (http://openstax.org/l/krebs_cycle) .

Products of the Citric Acid Cycle

Two carbon atoms come into the citric acid cycle from each acetyl group, representing four out of the six carbons of one glucose molecule. Two carbon dioxide molecules are released on each turn of the cycle; however, these do not necessarily contain the most recently added carbon atoms. The two acetyl carbon atoms will eventually be released on later turns of the cycle; thus, all six carbon atoms from the original glucose molecule are eventually incorporated into carbon dioxide. Each turn of the cycle forms three NADH molecules and one $FADH_2$ molecule. These carriers will connect with the last portion of aerobic respiration, the electron transport chain, to produce ATP molecules. One GTP or ATP is also made in each cycle. Several of the intermediate compounds in the citric acid cycle can be used in synthesizing nonessential amino acids; therefore, the cycle is **amphibolic** (both catabolic and anabolic).

7.4 Oxidative Phosphorylation

By the end of this section, you will be able to do the following:

- Describe how electrons move through the electron transport chain and explain what happens to their energy levels during this process
- Explain how a proton (H^+) gradient is established and maintained by the electron transport chain

You have just read about two pathways in glucose catabolism—glycolysis and the citric acid cycle—that generate ATP. Most of the ATP generated during the aerobic catabolism of glucose, however, is not generated directly from these pathways. Instead, it is derived from a process that begins by moving electrons through a series of electron carriers that undergo redox reactions. This process causes hydrogen ions to accumulate within the intermembranous space. Therefore, a concentration gradient forms in which hydrogen ions diffuse out of the intermembranous space into the mitochondrial matrix by passing through ATP synthase. The current of hydrogen ions powers the catalytic action of ATP synthase, which phosphorylates ADP, producing ATP.

Electron Transport Chain

The electron transport chain (Figure 7.10) is the last component of aerobic respiration and is the only part of glucose metabolism that uses atmospheric oxygen. Oxygen continuously diffuses into plant tissues (typically through stomata), as well as into fungi and bacteria; however, in animals, oxygen enters the body through a variety of respiratory systems. Electron transport is a series of redox reactions that resembles a relay race or bucket brigade in that electrons are passed rapidly from one component to the next, to the endpoint of the chain where the electrons reduce molecular oxygen and, along with associated protons, produces water. There are four complexes composed of proteins, labeled I through IV in Figure 7.10, and the aggregation of these four

complexes, together with associated mobile, accessory electron carriers, is called the **electron transport chain**. The electron transport chain is present with multiple copies in the inner mitochondrial membrane of eukaryotes and within the plasma membrane of prokaryotes.

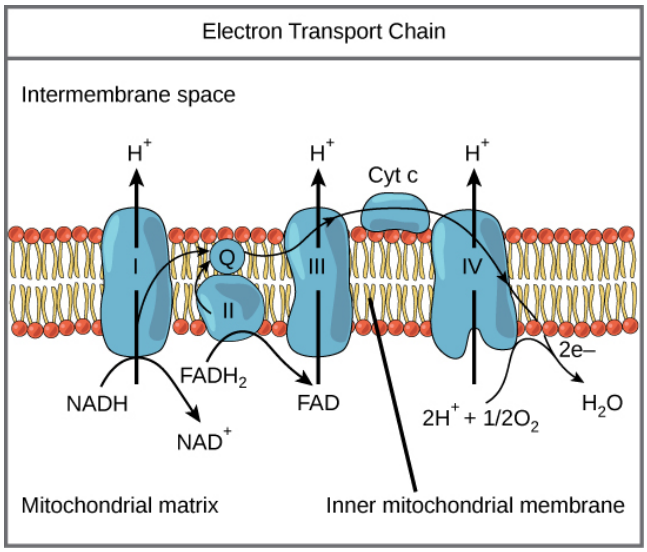

Figure 7.10 The electron transport chain is a series of electron transporters embedded in the inner mitochondrial membrane that shuttles electrons from NADH and $FADH_2$ to molecular oxygen. In the process, protons are pumped from the mitochondrial matrix to the intermembrane space, and oxygen is reduced to form water.

Complex I

First, two electrons are carried to the first complex via NADH. This complex, labeled **I**, is composed of flavin mononucleotide (FMN) and an iron-sulfur (Fe-S)-containing protein. FMN, which is derived from vitamin B_2 (also called riboflavin), is one of several prosthetic groups or cofactors in the electron transport chain. A **prosthetic group** is a nonprotein molecule required for the activity of a protein. Prosthetic groups are organic or inorganic, nonpeptide molecules bound to a protein that facilitate its function. Prosthetic groups include coenzymes, which are the prosthetic groups of enzymes. The enzyme in complex I is NADH dehydrogenase and is a very large protein, containing 45 amino acid chains. Complex I can pump four hydrogen ions across the membrane from the matrix into the intermembrane space, and it is in this way that the hydrogen ion gradient is established and maintained between the two compartments separated by the inner mitochondrial membrane.

Q and Complex II

Complex II directly receives $FADH_2$—which does not pass through complex I. The compound connecting the first and second complexes to the third is **ubiquinone** B. The Q molecule is lipid soluble and freely moves through the hydrophobic core of the membrane. Once it is reduced (QH_2), ubiquinone delivers its electrons to the next complex in the electron transport chain. Q receives the electrons derived from NADH from complex I, and the electrons derived from $FADH_2$ from complex II. This enzyme and $FADH_2$ form a small complex that delivers electrons directly to the electron transport chain, bypassing the first complex. Since these electrons bypass and thus do not energize the proton pump in the first complex, fewer ATP molecules are made from the $FADH_2$ electrons. *The number of ATP molecules ultimately obtained is directly proportional to the number of protons pumped across the inner mitochondrial membrane.*

Complex III

The third complex is composed of cytochrome b—another Fe-S protein, a Rieske center (2Fe-2S center), and cytochrome c proteins. This complex is also called cytochrome oxidoreductase. Cytochrome proteins have a prosthetic group of heme. The heme molecule is similar to the heme in hemoglobin, but it carries electrons, not oxygen. As a result, the iron ion at its core is reduced and oxidized as it passes the electrons, fluctuating between different oxidation states: Fe^{++} (reduced) and Fe^{+++} (oxidized). The heme molecules in the cytochromes have slightly different characteristics due to the effects of the different proteins binding to them, giving slightly different characteristics to each complex. Complex III pumps protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes. (Cytochrome c receives electrons from Q; however, whereas Q carries pairs of electrons, cytochrome c can accept only one at a time.)

Complex IV

The fourth complex is composed of cytochrome proteins c, a, and a_3. This complex contains two heme groups (one in each of the two cytochromes, a, and a_3) and three copper ions (a pair of Cu_A and one Cu_B in cytochrome a_3). The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced by the gain of two electrons. The reduced oxygen then picks up two hydrogen ions from the surrounding medium to make water (H_2O). The removal of the hydrogen ions from the system contributes to the ion gradient that forms the foundation for the process of chemiosmosis.

Chemiosmosis

In chemiosmosis, the free energy from the series of redox reactions just described is used to pump hydrogen ions (protons) across the mitochondrial membrane. The uneven distribution of H^+ ions across the membrane establishes both concentration and electrical gradients (thus, an electrochemical gradient), owing to the hydrogen ions' positive charge and their aggregation on one side of the membrane.

If the membrane were continuously open to simple diffusion by the hydrogen ions, the ions would tend to diffuse back across into the matrix, driven by the concentrations producing their electrochemical gradient. Recall that many ions cannot diffuse through the nonpolar regions of phospholipid membranes without the aid of ion channels. Similarly, hydrogen ions in the matrix space can only pass through the inner mitochondrial membrane by an integral membrane protein called ATP synthase (Figure 7.11). This complex protein acts as a tiny generator, turned by the force of the hydrogen ions diffusing through it, down their electrochemical gradient. The turning of parts of this molecular machine facilitates the addition of a phosphate to ADP, forming ATP, *using the potential energy of the hydrogen ion gradient*.

VISUAL CONNECTION

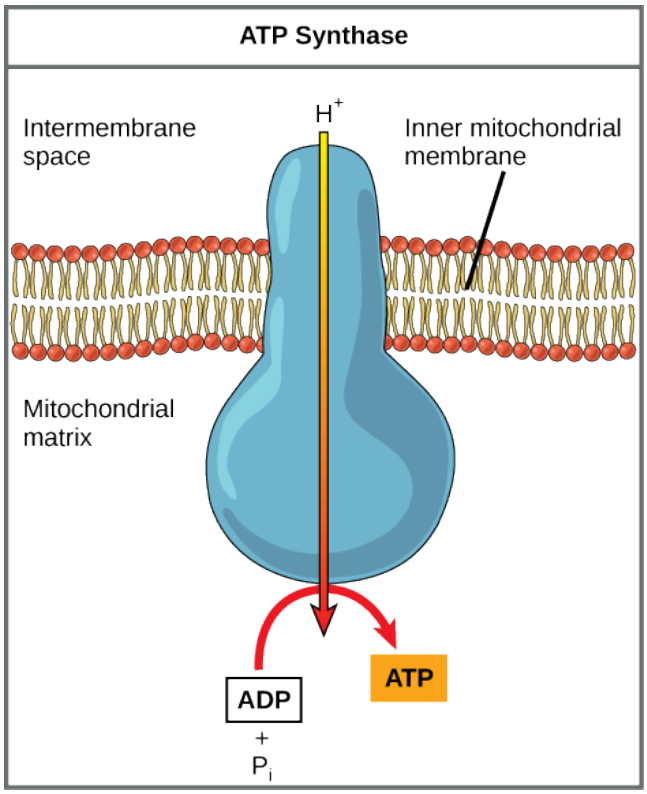

Figure 7.11 ATP synthase is a complex, molecular machine that uses a proton (H^+) gradient to form ATP from ADP and inorganic phosphate (Pi). (Credit: modification of work by Klaus Hoffmeier)

Dinitrophenol (DNP) is an "uncoupler" that makes the inner mitochondrial membrane "leaky" to protons. It was used until 1938 as a weight-loss drug. What effect would you expect DNP to have on the change in pH across the inner mitochondrial membrane? Why do you think this might be an effective weight-loss drug?

Chemiosmosis (Figure 7.12) is used to generate 90 percent of the ATP made during aerobic glucose catabolism; it is also the method used in the light reactions of photosynthesis to harness the energy of sunlight in the process of photophosphorylation. Recall that the production of ATP using the process of chemiosmosis in mitochondria is called oxidative phosphorylation. The overall result of these reactions is the production of ATP from the energy of the electrons removed from hydrogen atoms. These atoms were originally part of a glucose molecule. At the end of the pathway, the electrons are used to reduce an oxygen molecule to oxygen ions. The extra electrons on the oxygen attract hydrogen ions (protons) from the surrounding medium, and water is formed. Thus, oxygen is the final electron acceptor in the electron transport chain.

VISUAL CONNECTION

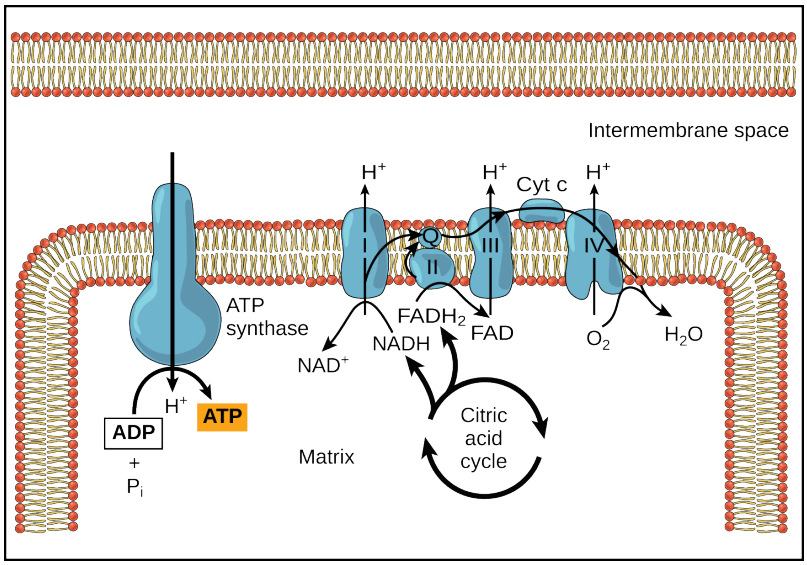

Figure 7.12 In oxidative phosphorylation, the pH gradient formed by the electron transport chain is used by ATP synthase to form ATP.

Cyanide inhibits cytochrome c oxidase, a component of the electron transport chain. If cyanide poisoning occurs, would you expect the pH of the intermembrane space to increase or decrease? What effect would cyanide have on ATP synthesis?

ATP Yield

The number of ATP molecules generated from the catabolism of glucose varies. For example, the number of hydrogen ions that the electron transport chain complexes can pump through the membrane varies between species. Another source of variance stems from the shuttle of electrons across the membranes of the mitochondria. (The NADH generated from glycolysis cannot easily enter mitochondria.) Thus, electrons are picked up on the inside of mitochondria by either NAD^+ or FAD^+. As you have learned earlier, these FAD^+ molecules can transport fewer ions; consequently, fewer ATP molecules are generated when FAD^+ acts as a carrier. NAD^+ is used as the electron transporter in the liver and FAD^+ acts in the brain.

Another factor that affects the yield of ATP molecules generated from glucose is the fact that intermediate compounds in these pathways are also used for other purposes. Glucose catabolism connects with the pathways that build or break down all other biochemical compounds in cells, and the result is somewhat messier than the ideal situations described thus far. For example, sugars other than glucose are fed into the glycolytic pathway for energy extraction. In addition, the five-carbon sugars that form nucleic acids are made from intermediates in glycolysis. Certain nonessential amino acids can be made from intermediates of both glycolysis and the citric acid cycle. Lipids, such as cholesterol and triglycerides, are also made from intermediates in these pathways, and both amino acids and triglycerides are broken down for energy through these pathways. Overall, in living systems, these pathways of glucose catabolism extract about 34 percent of the energy contained in glucose, with the remainder being released as heat.