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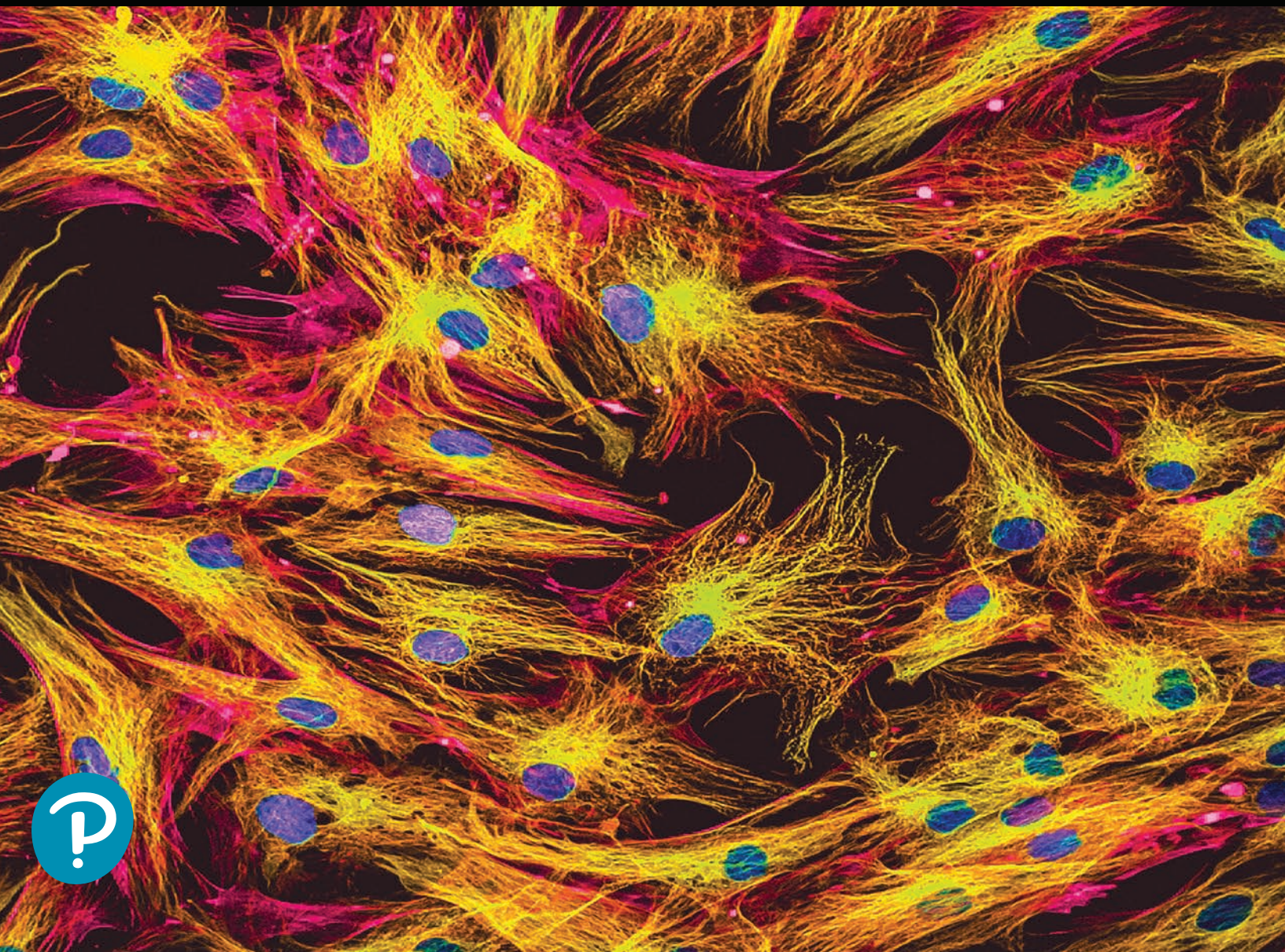

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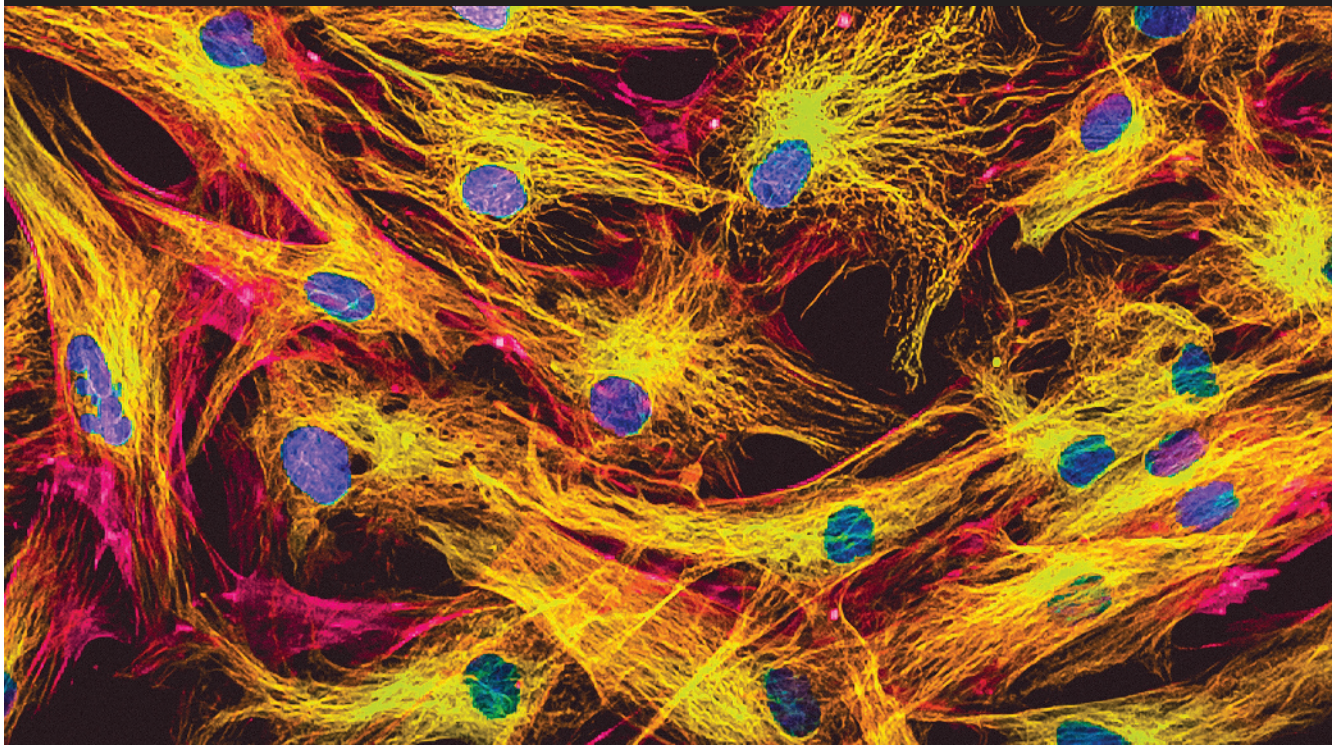

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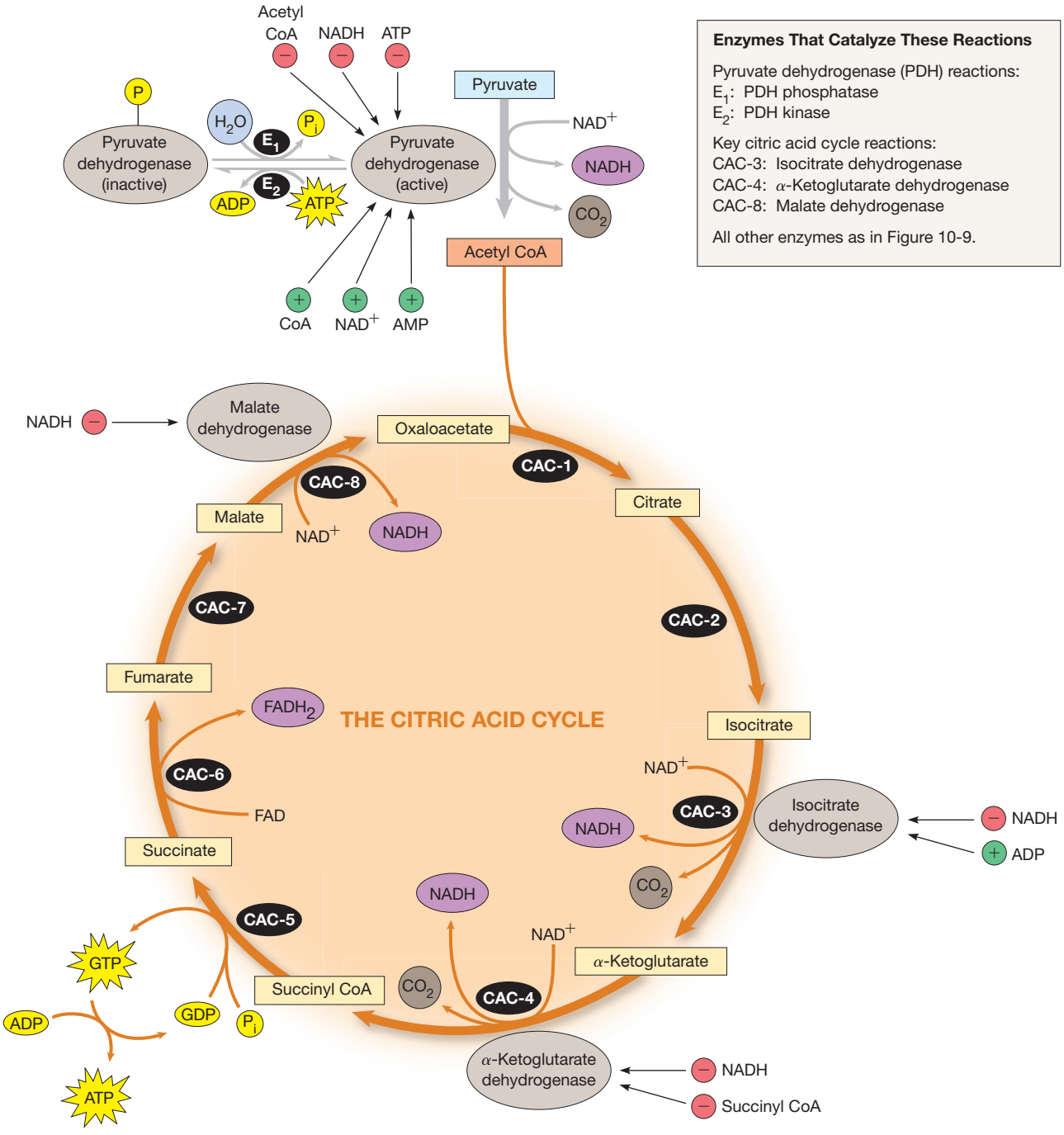

Figure 10-11 Regulation of the Citric Acid Cycle. The pyruvate dehydrogenase reaction and the citric acid cycle are shown here in outline form, with full names given for regulatory enzymes. Major regulatory effects are indicated as either activation (+) or inhibition (–).

dephosphorylation reactions are catalyzed by *PDH kinase* and *PDH phosphatase,* respectively. Not surprisingly, ATP is an activator of the kinase and an inhibitor of the phosphatase. Due to these multiple control mechanisms, generation of acetyl CoA by the PDH complex is sensitive to the [acetyl CoA]/[CoA] and [NADH]/[NAD^+] ratios within the mitochondrion and to mitochondrial ATP status as well.

In addition to being under regulation itself, the citric acid cycle wields feedback control over the glycolytic pathway via the inhibitory effects of citrate and acetyl CoA on phosphofructokinase and pyruvate kinase, respectively (see Figure 9-13).

The Citric Acid Cycle Also Plays a Central Role in the Catabolism of Fats and Proteins

Thus far, we have regarded glucose as the main substrate for cellular respiration. In addition to glucose (and other carbohydrates), we must also note the roles of alternative fuel molecules in cellular energy metabolism and the citric acid cycle, especially fats and proteins, which constitute roughly half of a diet based on recent dietary guidelines (carbohydrates = 50%, fats = 30%, proteins = 20%). Far from being a minor pathway for the catabolism of a single sugar, the citric acid cycle represents the main conduit of aerobic energy metabolism

in a broad spectrum of organisms ranging from microbes to higher plants and animals.

Fat as a Source of Energy. Fats are highly reduced compounds that liberate more energy per gram upon oxidation than do carbohydrates (see Chapter 3). For this reason, fats are an important long-term energy storage form for many organisms. Fat reserves are especially important in hibernating animals and migrating birds. In plant seeds, fats are a common form of energy and carbon storage. Fats are well suited for this storage function because they allow a maximum number of calories to be stored compactly.

Most fat is stored as deposits of **triacylglycerols,** which are neutral triesters of *glycerol* and long-chain *fatty acids,* which we encountered earlier (see Figure 3-27). Catabolism of triacylglycerols begins with their hydrolysis to glycerol and free fatty acids. The glycerol is channeled into the glycolytic pathway by oxidative conversion to dihydroxyacetone phosphate (step E14 in Figure 9-10). The fatty acids are linked to coenzyme A to form fatty acyl CoAs, which are then degraded by **β oxidation,** a catabolic process that generates acetyl CoA and the reduced coenzymes NADH and $FADH_2$.

In bacteria, β oxidation occurs in the cytoplasm; in eukaryotes it occurs both in mitochondria and in peroxisomes. In plants and other eukaryotes that do not depend upon fatty acids as an energy source, β oxidation occurs in the peroxisome and can function as a way to recycle membrane fatty acids. Here we will focus on the process of β oxidation as it occurs in the mitochondrion of animals using saturated fatty acids with an even number of carbons as an energy source.

In animals, most fatty acids derived from dietary fats, like the pyruvate derived from carbohydrates, are oxidatively converted into acetyl CoA in the mitochondrion, which then can be further catabolized by the citric acid cycle. The fatty acids are degraded in a series of repetitive cycles, each of which removes two carbons from the fatty acid until it is completely degraded. This process of fatty acid catabolism to acetyl CoA is called β oxidation because the oxidative events in each cycle occur on the carbon atom in the β position of the fatty acid (that is, the second carbon in from the carboxyl group). Each cycle involves the same four steps—oxidation, hydration, reoxidation, and thiolysis (**Figure 10-12**)—and results in the production of one molecule each of $FADH_2$, NADH, and acetyl CoA as the fatty acid is shortened by two carbons in each cycle.

β oxidation of a fatty acid begins with an energy-requiring activation step in the cytosol. The energy of hydrolysis of ATP drives the attachment of a CoA molecule to the fatty acid (reaction FA-1 in Figure 10-12). This forms a *fatty acyl CoA,* which is transported into the mitochondrion by a specific translocase located in the inner membrane. The next four enzymatic steps are repeated in a series of cycles until the fatty acid is completely degraded, losing two carbons per cycle as acetyl CoA. The first three of these steps closely resemble the oxidation, hydration, and reoxidation steps that convert succinate to oxaloacetate in the citric acid cycle (CAC-6, 7, and 8).

First, an integral membrane protein acting as a *dehydrogenase* oxidizes the fatty acyl CoA, forming a double bond between the α and β carbons (reaction FA-2). The two electrons and two protons removed during formation

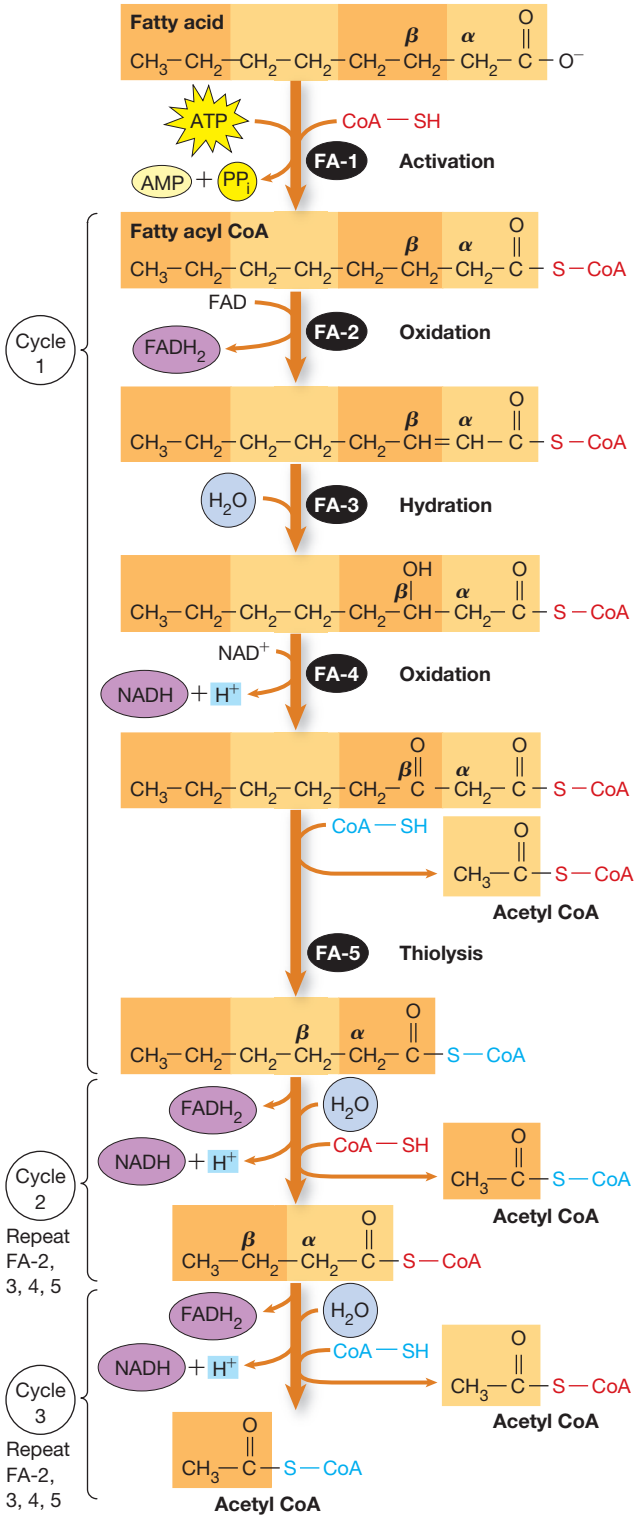

Figure 10-12 The β Oxidation Pathway. Following an ATP-dependent activation step that links a fatty acid to CoA, the fatty acyl CoA derivative is transported into the mitochondrion (or peroxisome) and degraded in a series of repetitive cycles of oxidation, hydration, reoxidation, and thiolysis. Each cycle generates one $FADH_2$ and one NADH in the oxidation steps and releases acetyl CoA in the thiolysis step, shortening the fatty acid by two carbons. The example above is for the eight-carbon fatty acid octanoate, which requires three cycles of β oxidation. (The α and β carbons are shown on the original fatty acid and reaction numbers and chemical details are shown for the first cycle only.)

of the unsaturated derivative are transferred to FAD, forming $FADH_2$. In the next step (reaction FA-3), water is added across the double bond by a *hydratase* so that the α carbon receives a H atom and the β carbon receives a hydroxyl group. Then another dehydrogenase oxidizes the β carbon, converting the hydroxyl group to a keto group (reaction FA-4). The two electrons and one proton removed in this oxidation are used to reduce NAD^+ to NADH. In the fourth step of the cycle (reaction FA-5), the bond between the α and β carbons is broken by a *thiolase.* A second CoA is then attached to the end of the fatty acid, and the original CoA is released along with its attached two-carbon fragment. This results in the production of acetyl CoA and a fatty acyl CoA that is two carbons shorter than the fatty acyl CoA that entered the four-step cycle.

These four steps are repeated using the shortened fatty acyl CoA as a substrate until the original fatty acid is completely degraded. Most dietary fatty acids have an even number of carbons and are completely degraded to acetyl CoA (as shown at the bottom of Figure 10-12), although unsaturated fatty acids require one or two additional enzymes. For unusual fatty acids having an odd number of carbons, the final cycle produces propionyl CoA, which is one carbon longer than acetyl CoA. In a three-step side pathway, a carbon from bicarbonate is added to propionyl CoA to generate succinyl CoA, which then enters the citric acid cycle.

Although glucose is the preferred energy source of most cells, fats provide energy when glucose is limiting (as during starvation), under conditions of very low carbohydrate intake, or following extremely demanding exercise (such as running a marathon). In humans, excessive fat breakdown can deplete free CoA and lead to a condition known as *ketosis.* During ketosis, fats cannot be oxidized completely to CO_2, and partial oxidation products known as *ketone bodies* (acetone, acetoacetate, and β-hydroxybutyrate) are formed. In large quantities, they can lower the pH of the blood, resulting in *ketoacidosis,* a condition often seen in uncontrolled diabetes.

Protein as a Source of Energy and Amino Acids. Although proteins act as enzymes, transport proteins, hormones, and receptors in the cell, they can also be catabolized to generate ATP if necessary, especially during fasting or starvation conditions when carbohydrates and lipid stores are depleted. In plants, catabolism of proteins to free amino acids provides building blocks for protein synthesis during the germination of protein-storing seeds. In addition, when cells degrade proteins and protein-containing structures, the resulting amino acids either can be used to synthesize new proteins or can be degraded oxidatively to yield energy.

Protein catabolism begins with hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain. The process is called **proteolysis,** and the enzymes responsible for it are called *proteases.* The products of proteolytic digestion are small peptides and free amino acids. Further digestion of peptides is catalyzed by *peptidases,* which either hydrolyze internal peptide bonds (*endopeptidases*) or remove successive amino acids from the end of the peptide (*exopeptidases*).

Free amino acids, whether ingested as such or obtained by the digestion of proteins, can be catabolized for energy. Generally, these alternative substrates are converted to intermediates of mainstream catabolism in as few steps as possible. Despite their number and chemical diversity, all of the pathways for amino acid catabolism eventually lead to pyruvate, acetyl CoA, or a few key intermediates in the citric acid cycle, notably α-ketoglutarate, oxaloacetate, fumarate, and succinyl CoA.

Of the 20 amino acids found in proteins, three of them give rise to pyruvate or citric acid cycle intermediates directly: alanine, aspartate, and glutamate can be directly converted to pyruvate, oxaloacetate, and α-ketoglutarate, respectively (**Figure 10-13**). All the other amino acids require more complicated pathways, but ultimately all of them have end-products that are citric acid cycle intermediates.

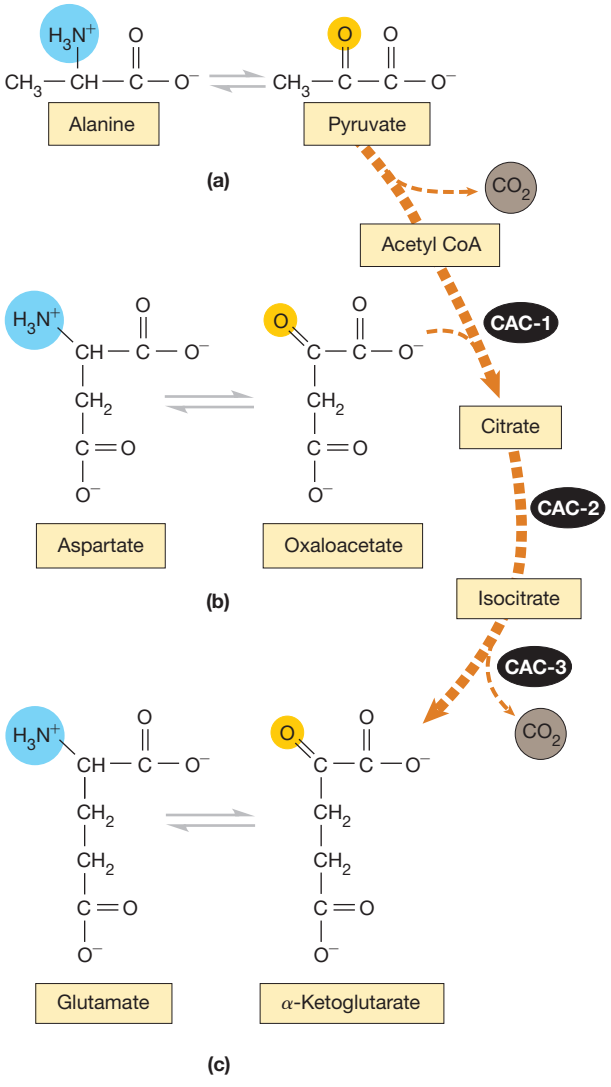

Figure 10-13 Interconversion of Several Amino Acids and Their Corresponding Keto Acids in the Citric Acid Cycle. The amino acids **(a)** alanine, **(b)** aspartate, and **(c)** glutamate can be converted into the corresponding α-keto acids: pyruvate, oxaloacetate, and α-ketoglutarate, respectively. Each of these keto acids is an intermediate in the citric acid cycle, a portion of which is shown to provide the metabolic context for these reactions. In each case, the amino group is shown in blue and the keto group in yellow. These reactions are readily reversible and can occur in either catabolism (oxidizing amino acids to CO_2 and H_2O) or anabolism (producing amino acids for protein synthesis).

The Citric Acid Cycle Serves as a Source of Precursors for Anabolic Pathways

Besides its central role in catabolism, the citric acid cycle is involved in various anabolic processes. For example, the three reactions shown in Figure 10-13 convert α-keto intermediates of the citric acid cycle into the amino acids alanine, aspartate, and glutamate. These amino acids are constituents of proteins, so the citric acid cycle is indirectly involved in protein synthesis by providing several of the amino acids required for the process. Other metabolic precursors provided by the citric acid cycle include succinyl CoA and citrate. Succinyl CoA is the starting point for the biosynthesis of heme, whereas citrate can be transported out of the mitochondrion and used as a source of acetyl CoA for the stepwise synthesis of fatty acids in the cytosol.

There is a considerable flow of four-, five-, and six-carbon intermediates both into and out of the citric acid cycle in most cells. These side reactions can replenish the supply of intermediates in the cycle if needed or use intermediates in the cycle for the synthesis of other compounds in anabolic pathways. Because the citric acid cycle is a central link between catabolic and anabolic pathways, it is often called an **amphibolic pathway** (from the Greek prefix *amphi–*, meaning "both").

The Glyoxylate Cycle Converts Acetyl CoA to Carbohydrates in Plants

Plant species that store carbon and energy reserves in their seeds as fats face a special metabolic challenge when their seeds germinate: they must convert the stored fat to sucrose, the immediate source of carbon and energy for most cells in the seedling. Many plant species are in this category, including such well-known oil-bearing species as soybeans, peanuts, sunflowers, castor beans, and maize. The fat consists mainly of triacylglycerols and is stored in the cell as fat droplets called *lipid bodies.* The electron micrograph in **Figure 10-14** shows the prominence of lipid bodies in the cotyledon (embryonic leaf) of a cucumber seedling.

The advantage of storing fat rather than carbohydrate is that 1 gram of triacylglycerol contains more than twice as much energy as 1 gram of carbohydrate. This difference enables fat-storing species to pack the greatest amount of carbon and calories into the least amount of mass. However, it also means that such species must be able to convert the stored fat into sugar when the seeds germinate.

Whereas many organisms readily convert sugars and other carbohydrate to stored fat (as people do), most eukaryotic organisms, including mammals, cannot carry out the conversion of fat to sugar. For the seedlings of fat-storing plant species, however, the conversion of storage triacylglycerols to sucrose is essential because sucrose is the form in which carbon and energy are transported to the growing shoot and root tips of the developing seedling.

The metabolic pathways that make this conversion possible are β oxidation and the **glyoxylate cycle.** The function of β oxidation is to degrade the stored fat to acetyl CoA. The acetyl CoA then enters the glyoxylate cycle (**Figure 10-15** on page 282), a five-step cyclic pathway that is named for one of its intermediates, the two-carbon keto acid called *glyoxylate.* The glyoxylate cycle is related to the citric acid cycle and uses three of the same reactions. A critical difference, however, is the presence of two glyoxysome-specific enzymes, *isocitrate lyase* and *malate synthase.* Using these enzymes, the glyoxylate cycle bypasses the two decarboxylation reactions of the citric acid cycle in which CO_2 is released.

Instead of degrading a molecule of acetyl CoA to two CO_2 molecules, as in the citric acid cycle, the glyoxylate cycle takes in two molecules of acetyl CoA per turn of the cycle, generating succinate, a four-carbon compound. The succinate is then converted to phosphoenolpyruvate (PEP), from which sugars can be synthesized by gluconeogenesis. Thus, the glyoxylate cycle is anabolic (carbon enters as two two-carbon molecules and leaves as a four-carbon molecule), whereas the citric acid cycle is catabolic (carbon enters as a two-carbon molecule and leaves as two CO_2 molecules).

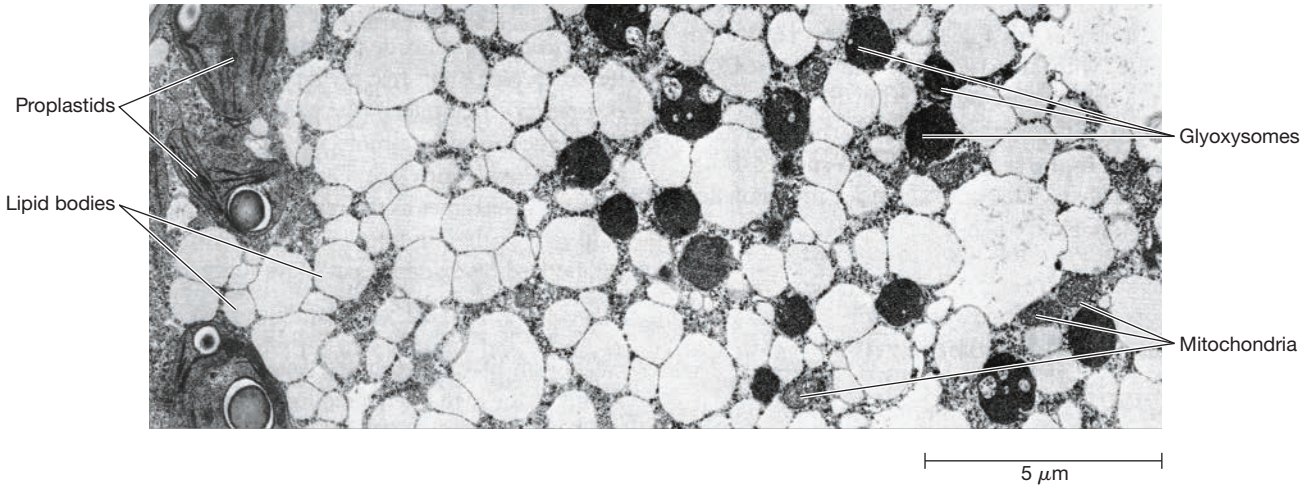

Figure 10-14 The Association of Glyoxysomes and Lipid Bodies in Fat-Storing Seedlings. This micrograph shows a cell from a cucumber cotyledon just after seed germination. Note the abundance of lipid bodies and their association with both glyoxysomes and mitochondria, which are involved in lipid degradation and gluconeogenesis (TEM).

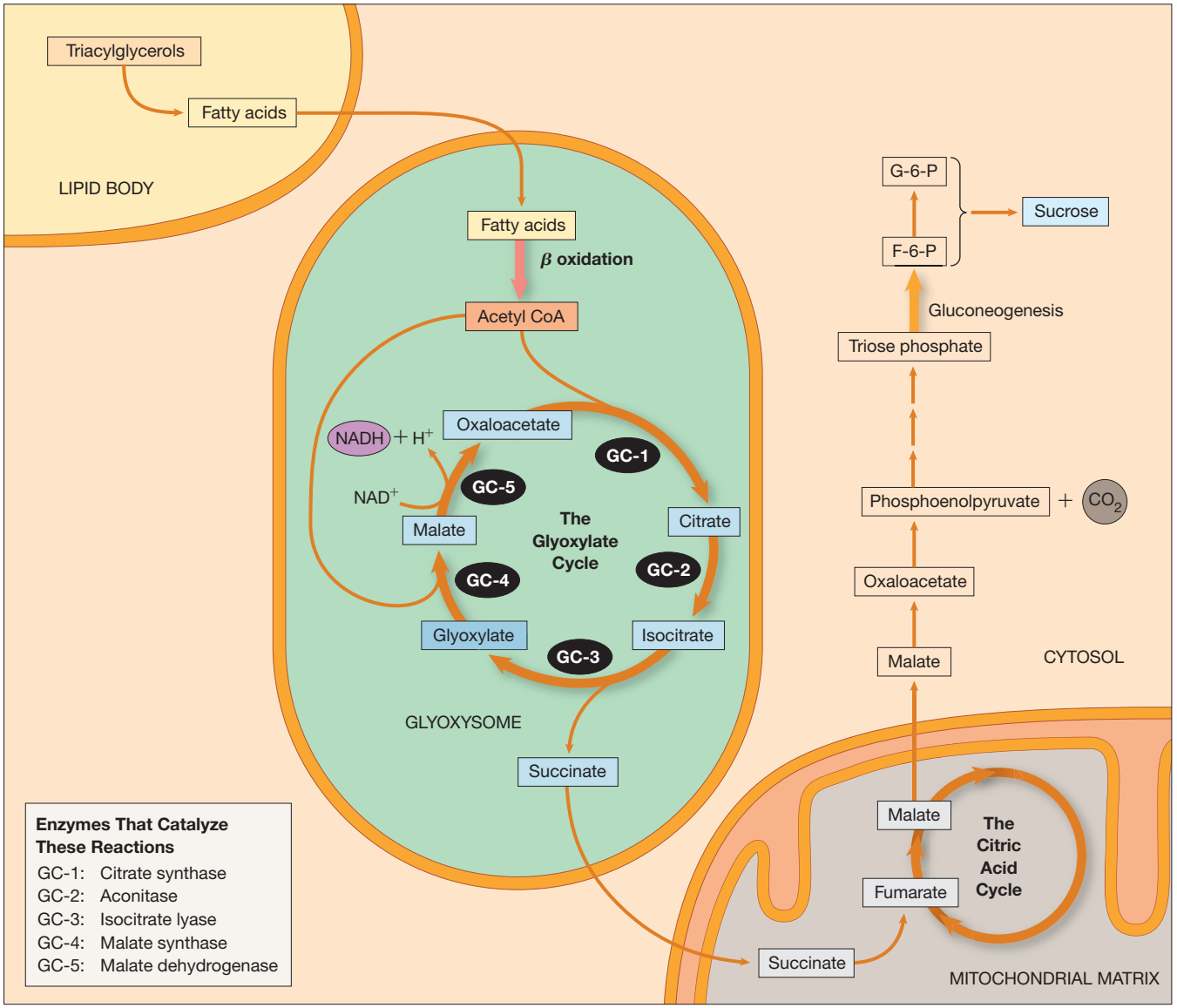

Figure 10-15 The Glyoxylate Cycle and Gluconeogenesis in Fat-Storing Seedlings. Seedlings of fat-storing plant species can convert stored fat into sugar. All the enzymes of β oxidation and the glyoxylate cycle are located in the glyoxysome. Conversion of succinate to malate occurs within the mitochondrion, whereas the further metabolism of malate via phosphoenolpyruvate to hexoses and hence to sucrose takes place in the cytosol.

In the seedlings of fat-storing plant species (and the spores of some fungi), the enzymes of β oxidation and the glyoxylate cycle are localized in a special type of peroxisome called the **glyoxysome** (see Figure 10-14). The intimate association of glyoxysomes with lipid bodies presumably facilitates the transfer of fatty acids from the lipid bodies.

Figure 10-15 shows the relevant metabolism in an intracellular context. Stored triacylglycerols are hydrolyzed in the lipid bodies, releasing fatty acids. The fatty acids are transported into the glyoxysome where they are activated and degraded by β oxidation to acetyl CoA (see Figure 10-12), which is then converted to succinate by the enzymes of the glyoxylate cycle. The succinate moves to the mitochondrion, where it is converted to malate by reactions that are a part of the citric acid cycle. (Notice that mitochondria are adjacent to glyoxysomes in the cucumber cotyledon in Figure 10-14.) Malate goes to the cytosol and is oxidized to oxaloacetate, which is decarboxylated to form PEP. PEP serves as the starting point for gluconeogenesis in the cytosol, ultimately yielding sucrose, the major carbohydrate transported to growing tissues in plants.

The route from stored triacylglycerols to sucrose is obviously quite complex, involving enzymes located in lipid bodies, glyoxysomes, mitochondria, and the cytosol, but it is the metabolic lifeline on which the seedlings of all fat-storing plant species depend.

CONCEPT CHECK 10.3

As pyruvate is completely oxidized to CO_2 in the citric acid cycle, only one ATP molecule is formed. What happens to the rest of the chemical energy in pyruvate that is released when pyruvate is oxidized?

10.4 Electron Transport: Electron Flow from Coenzymes to Oxygen

Having considered the first three stages of aerobic respiration—glycolysis, pyruvate oxidation, and the citric acid cycle—let's pause briefly to ask what has been achieved thus far. As Reaction 10-3 indicates, chemotrophic energy metabolism through the citric acid cycle accounts for the synthesis of four ATP molecules per glucose. Two arise from glycolysis (Chapter 9, page 245), and two from the citric acid cycle (page 278). Complete oxidation of glucose to CO_2 could yield 686 kcal/mol (Chapter 9, page 244), but we have recovered less than 10% of that amount (only four ATP molecules × approximately 10 kcal/mol each, based on the $\Delta G'$ value for ATP synthesis in a typical cell). Where is the rest of the free energy? And when will we get to the substantially greater ATP yield that is characteristic of aerobic respiration?

The answer is straightforward: the free energy is right there in Reaction 10-3, represented by the reduced coenzyme molecules NADH and $FADH_2$. As we will see shortly, large amounts of free energy are released when these reduced coenzymes are reoxidized as their electrons are transferred to molecular oxygen. In fact, about 90% of the potential free energy present in one glucose molecule is conserved in the 12 molecules of NADH and $FADH_2$ that are formed when a molecule of glucose is oxidized to CO_2.

The Electron Transport Chain Conveys Electrons from Reduced Coenzymes to Oxygen

The process of coenzyme reoxidation by the transfer of electrons to oxygen is called **electron transport.** Electron transport is the fourth stage of respiratory metabolism (see Figure 10-1, stage ❹). The accompanying process of ATP synthesis (Figure 10-1, stage ❺) will be discussed later in this chapter. Keep in mind, however, that electron transport and ATP synthesis are not isolated processes. They are both integral parts of cellular respiration, functionally linked to each other by the electrochemical proton gradient that is the result of electron transport as well as the source of the energy that drives ATP synthesis.

Electron Transport and Coenzyme Oxidation. Electron transport involves the highly exergonic oxidation of NADH and $FADH_2$ with O_2 as the *terminal electron acceptor,* so we can write summary reactions as follows:

$$\text{NADH} + \text{H}^+ + \frac{1}{2}\text{O}_2 \rightarrow \text{NAD}^+ + \text{H}_2\text{O}$$
$$\Delta G^{\circ\prime} = -52.4 \text{ kcal/mol} \qquad \textbf{(10-4)}$$

$$\text{FADH}_2 + \frac{1}{2}\text{O}_2 \rightarrow \text{FAD} + \text{H}_2\text{O}$$
$$\Delta G^{\circ\prime} = -45.9 \text{ kcal/mol} \qquad \textbf{(10-5)}$$

Electron transport therefore accounts not only for the reoxidation of coenzymes and the consumption of oxygen but also for the formation of water, which is the reduced form of oxygen and, along with CO_2, one of the two end-products of aerobic energy metabolism.

The Electron Transport Chain. The most important aspect of Reactions 10-4 and 10-5 is the large amount of free energy released upon oxidation of NADH and $FADH_2$ by the transfer of electrons to oxygen. The highly negative $\Delta G^{\circ\prime}$ values for these reactions make it clear that the oxidation of a coenzyme is an extraordinarily exergonic process, enough to synthesize several molecules of ATP. Electron transfer is accomplished as a multistep process that involves an ordered series of reversibly oxidizable electron carriers functioning together in what is called the **electron transport chain (ETC).** The ETC contains a number of integral membrane proteins that are found in the inner mitochondrial membrane of eukaryotes (or the plasma membrane of bacteria).

Our discussion of the electron transport chain will focus on three questions:

1. What are the major *electron carriers* in the ETC?
2. What is the *sequence* of these carriers in the ETC?
3. How are these carriers *organized* in the membrane to ensure that the flow of electrons from reduced coenzymes to oxygen is coupled to the pumping of protons across the membrane, thereby producing the electrochemical proton gradient on which ATP synthesis depends?

The Electron Transport Chain Consists of Five Kinds of Carriers

The carriers that make up the ETC include *flavoproteins, iron-sulfur proteins, cytochromes, copper-containing cytochromes,* and a quinone known as *coenzyme Q*. The flavoproteins and coenzyme Q transport protons along with electrons. Except for coenzyme Q, all the carriers are proteins with specific prosthetic groups capable of being reversibly oxidized and reduced. Almost all the events of electron transport occur within membranes, so it is not surprising that most of these carriers are hydrophobic molecules. In fact, most occur in the membrane as parts of large assemblies of proteins called *respiratory complexes.* We will first look briefly at the chemistry of these electron carriers and then see how they are organized into respiratory complexes and ordered into a sequence that transfers electrons from reduced coenzymes to oxygen.

Flavoproteins. Several membrane-bound **flavoproteins** participate in electron transport, using either *flavin adenine dinucleotide (FAD)* or *flavin mononucleotide (FMN)* as the prosthetic group. FMN is essentially the flavin-containing half of the FAD molecule shown in Figure 10-10. One FMN-containing flavoprotein is *NADH dehydrogenase,* which is part of the protein complex that accepts pairs of electrons from NADH. Another example, already familiar to us from the citric acid cycle, is the enzyme succinate dehydrogenase, which has $FADH_2$ as its prosthetic group and is part of the membrane-bound respiratory complex that accepts pairs of electrons from succinate via $FADH_2$. An important characteristic of the flavoproteins (and of the coenzyme NADH) is that they transfer both electrons and protons as they are reversibly oxidized and reduced.

Iron-Sulfur Proteins. **Iron-sulfur proteins,** also called *nonheme iron proteins,* are a family of proteins, each with an *iron-sulfur (Fe-S) center* that consists of iron and sulfur ions