CHAPTER

11

Respiration and Lipid Metabolism

Photosynthesis provides the organic building blocks that plants (and nearly all other organisms) depend on. Respiration, with its associated carbon metabolism, releases the energy stored in carbon compounds in a controlled manner for cellular use. At the same time it generates many carbon precursors for biosynthesis.

We will begin this chapter by reviewing respiration in its metabolic context, emphasizing the interconnections among the processes involved and the special features that are peculiar to plants. We will also relate respiration to recent developments in our understanding of the biochemistry and molecular biology of plant mitochondria. Then we will describe the pathways of lipid biosynthesis that lead to the accumulation of fats and oils, which many plants use for energy and carbon storage. We will also examine lipid synthesis and the influence of lipids on membrane properties. Finally, we will discuss the catabolic pathways involved in the breakdown of lipids and the conversion of their degradation products into sugars that occurs during the germination of fat-storing seeds.

Overview of Plant Respiration

Aerobic (oxygen-requiring) respiration is common to nearly all eukaryotic organisms, and in its broad outlines, the respiratory process in plants is similar to that found in animals and lower eukaryotes. However, some specific aspects of plant respiration distinguish it from its animal counterpart. **Aerobic respiration** is

the biological process by which reduced organic compounds are mobilized and subsequently oxidized in a controlled manner. During respiration, energy is released and transiently stored in a compound, **adenosine triphosphate** (**ATP**), that is readily utilized by the cell for maintenance and development.

Glucose is most commonly cited as the substrate for respiration. In a functioning plant cell, however, reduced carbon is derived mainly from sources such as the disaccharide sucrose, triose phosphates from photosynthesis, fructose-containing polymers (fructans), and other sugars, as well as from lipids (primarily triacylglycerols), organic acids, and on occasion, proteins (FIGURE 11.1).

From a chemical standpoint, plant respiration can be expressed as the oxidation of the 12-carbon molecule sucrose and the reduction of 12 molecules of O_2:

$$C_{12}H_{22}O_{11} + 13\ H_2O \rightarrow 12\ CO_2 + 48\ H^+ + 48\ e^-$$

$$12\ O_2 + 48\ H^+ + 48\ e^- \rightarrow 24\ H_2O$$

giving the following net reaction:

$$C_{12}H_{22}O_{11} + 12\ O_2 \rightarrow 12\ CO_2 + 11\ H_2O$$

This reaction is the reversal of the photosynthetic process; it represents a coupled redox reaction in which sucrose is completely oxidized to CO_2 while oxygen serves as the ultimate electron acceptor and is reduced to water in the process. The change in standard **Gibbs free energy** ($\Delta G^{0\prime}$) for the net reaction is –5760 kJ per mole (342 g) of sucrose oxidized. This large negative value is a consequence of the equilibrium point being strongly shifted to the right, and energy is therefore released by sucrose degradation. The controlled release of this free energy, along with its coupling to the synthesis of ATP, is the primary, although by no means the only, role of respiratory metabolism.

To prevent damage (incineration) of cellular structures, the cell mobilizes the large amount of free energy released in the oxidation of sucrose in a series of step-by-step reactions. These reactions can be grouped into four major processes: glycolysis, the oxidative pentose phosphate pathway, the citric acid cycle, and oxidative phosphorylation. These pathways do not function in isolation, but exchange metabolites at several levels. The substrates of respiration enter the respiratory process at different points in the pathways, as summarized in Figure 11.1:

- **Glycolysis** involves a series of reactions catalyzed by enzymes located in both the cytosol and the plastids. A sugar—for example, sucrose—is partly oxidized via six-carbon sugar phosphates (hexose phosphates) and three-carbon sugar phosphates (triose phosphates) to produce an organic acid—

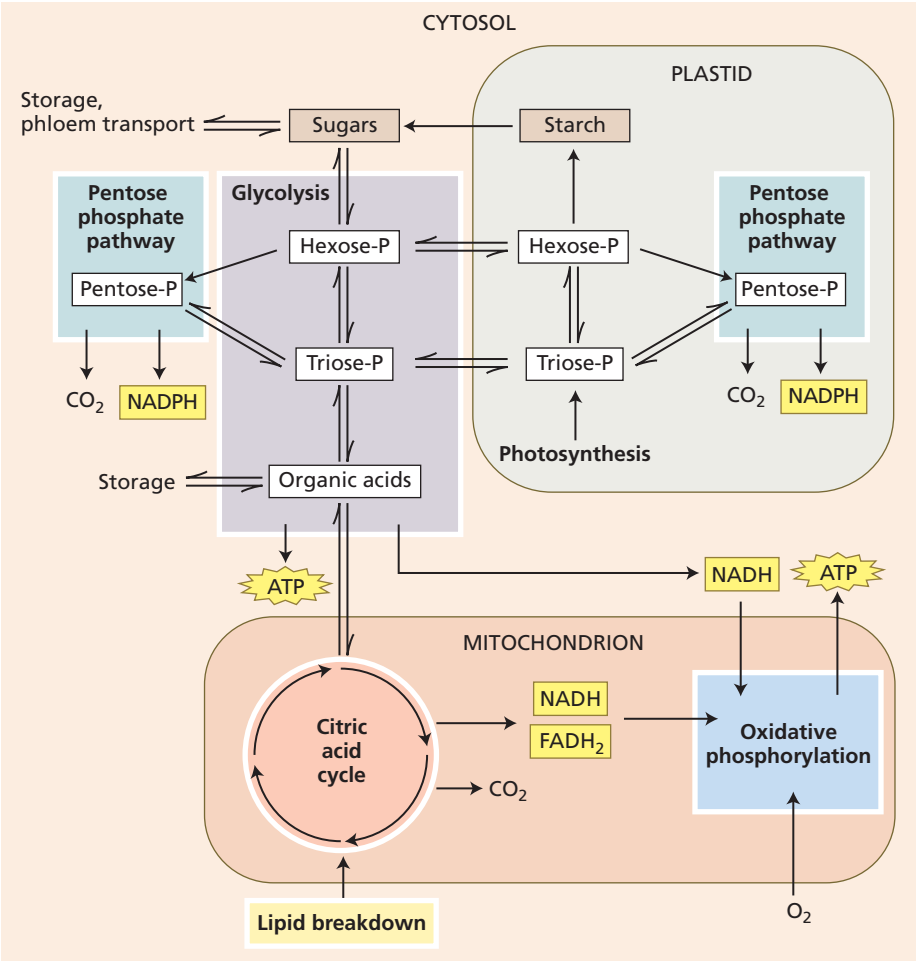


FIGURE 11.1 Overview of respiration. Substrates for respiration are generated by other cellular processes and enter the respiratory pathways. Glycolysis and the oxidative pentose phosphate pathways in the cytosol and plastids convert sugars into organic acids such as pyruvate, via hexose phosphates and triose phosphates, generating NADH or NADPH, and ATP. The organic acids are oxidized in the mitochondrial citric acid cycle, and the NADH and $FADH_2$ produced provide the energy for ATP synthesis by the electron transport chain and ATP synthase in oxidative phosphorylation. In gluconeogenesis, carbon from lipid breakdown is broken down in the glyoxysomes, metabolized in the citric acid cycle, and then used to synthesize sugars in the cytosol by reverse glycolysis.

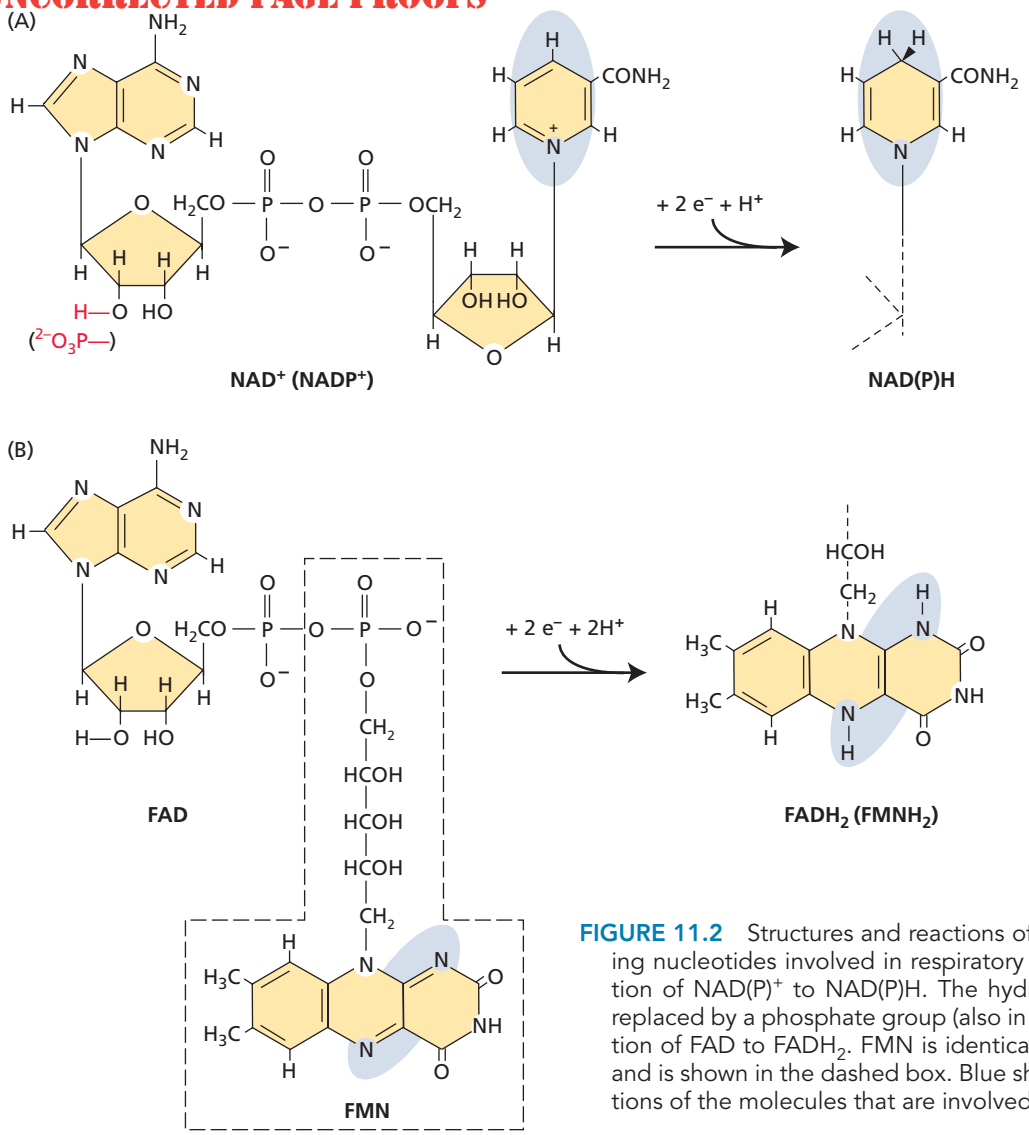


FIGURE 11.2 Structures and reactions of the major electron-carrying nucleotides involved in respiratory bioenergetics. (A) Reduction of $NAD(P)^+$ to NAD(P)H. The hydrogen (in red) in NAD^+ is replaced by a phosphate group (also in red) in $NADP^+$. (B) Reduction of FAD to $FADH_2$. FMN is identical to the flavin part of FAD and is shown in the dashed box. Blue shaded areas show the portions of the molecules that are involved in the redox reaction.

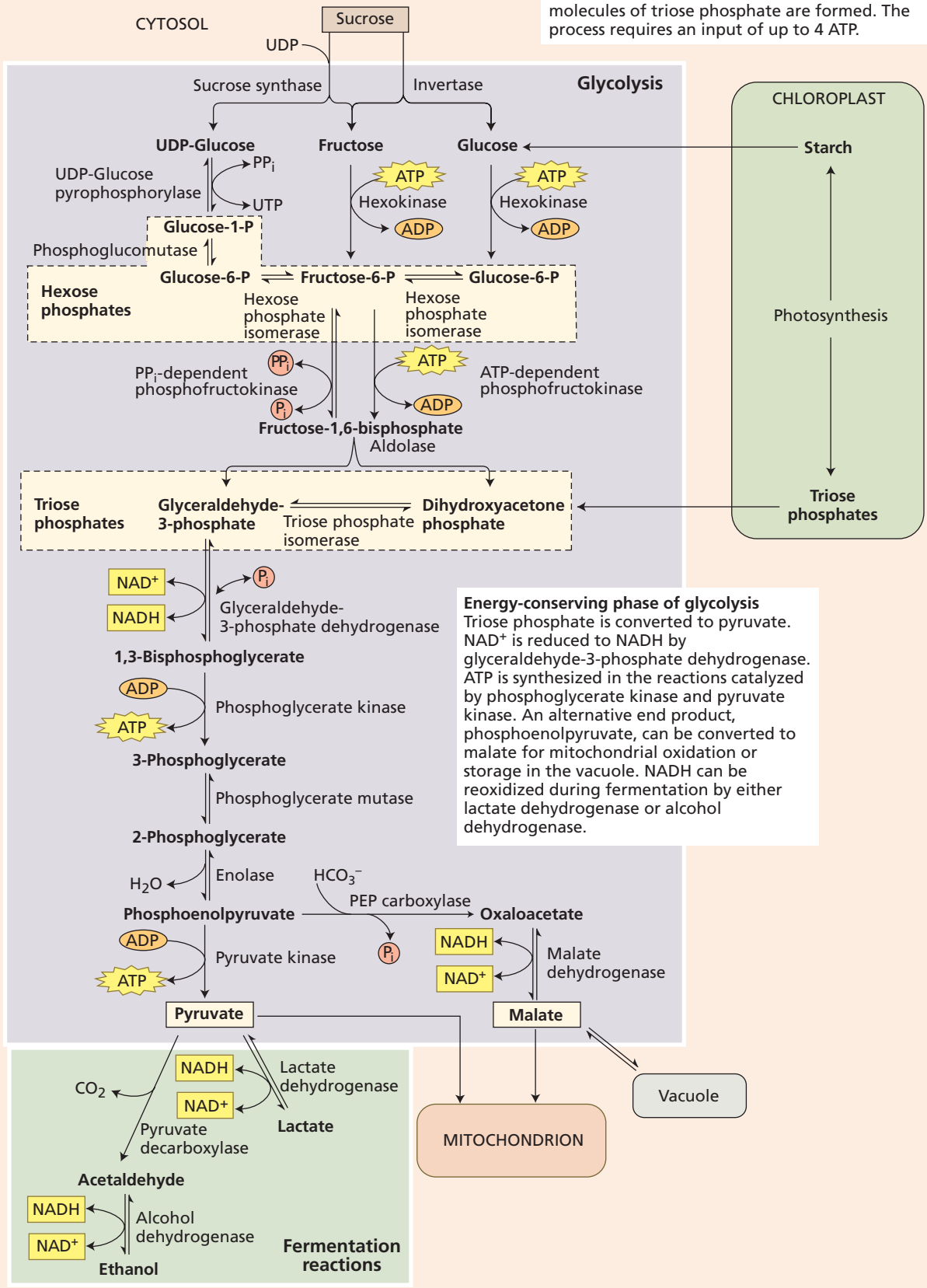
for example, pyruvate. The process yields a small amount of energy as ATP and reducing power in the form of a reduced nicotinamide nucleotide, NADH.

- In the **oxidative pentose phosphate pathway**, also located in both the cytosol and the plastids, the six-carbon glucose-6-phosphate is initially oxidized to the five-carbon ribulose-5-phosphate. Carbon is lost as CO_2, and reducing power is conserved in the form of two molecules of another reduced nicotinamide nucleotide, NADPH. In subsequent near-equilibrium reactions of the pentose phosphate pathway, ribulose-5-phosphate is converted into sugars containing three to seven carbon atoms.
- In the **citric acid cycle**, pyruvate is oxidized completely to CO_2. This process generates the major amount of reducing power (16 NADH + 4 $FADH_2$ per sucrose) from the breakdown of sucrose. With one exception (succinate dehydrogenase), these reactions are carried out by enzymes located in the internal aqueous compartment, or matrix, of the mitochondrion. As we will discuss later, succinate dehydrogenase is localized in the inner of the two mitochondrial membranes.
- In **oxidative phosphorylation**, electrons are transferred along an electron transport chain consisting of a collection of electron transport proteins bound to the inner of the two mitochondrial membranes. This system transfers electrons from NADH (and related species)—produced by glycolysis, the oxidative pentose phosphate pathway, and the citric acid cycle—to oxygen. This electron transfer releases a large amount of free energy, much of which is conserved through the synthesis of ATP from ADP and P_i (inorganic phosphate), catalyzed by the enzyme ATP synthase. Collectively, the redox reactions of the electron transport chain and the synthesis of ATP are called oxidative phosphorylation.

Nicotinamide adenine dinucleotide (NAD^+/NADH) is an organic cofactor (coenzyme) associated with many enzymes that catalyze cellular redox reactions. NAD^+ is the oxidized form of the cofactor, which undergoes a reversible two-electron reaction that yields NADH (FIGURE 11.2):

$$NAD^+ + 2\,e^- + H^+ \rightarrow NADH$$

(A)



Initial phase of glycolysis Substrates from different sources are channeled into triose phosphate. For each molecule of sucrose that is metabolized, four molecules of triose phosphate are formed. The process requires an input of up to 4 ATP.

Energy-conserving phase of glycolysis Triose phosphate is converted to pyruvate. NAD^+ is reduced to NADH by glyceraldehyde-3-phosphate dehydrogenase. ATP is synthesized in the reactions catalyzed by phosphoglycerate kinase and pyruvate kinase. An alternative end product, phosphoenolpyruvate, can be converted to malate for mitochondrial oxidation or storage in the vacuole. NADH can be reoxidized during fermentation by either lactate dehydrogenase or alcohol dehydrogenase.

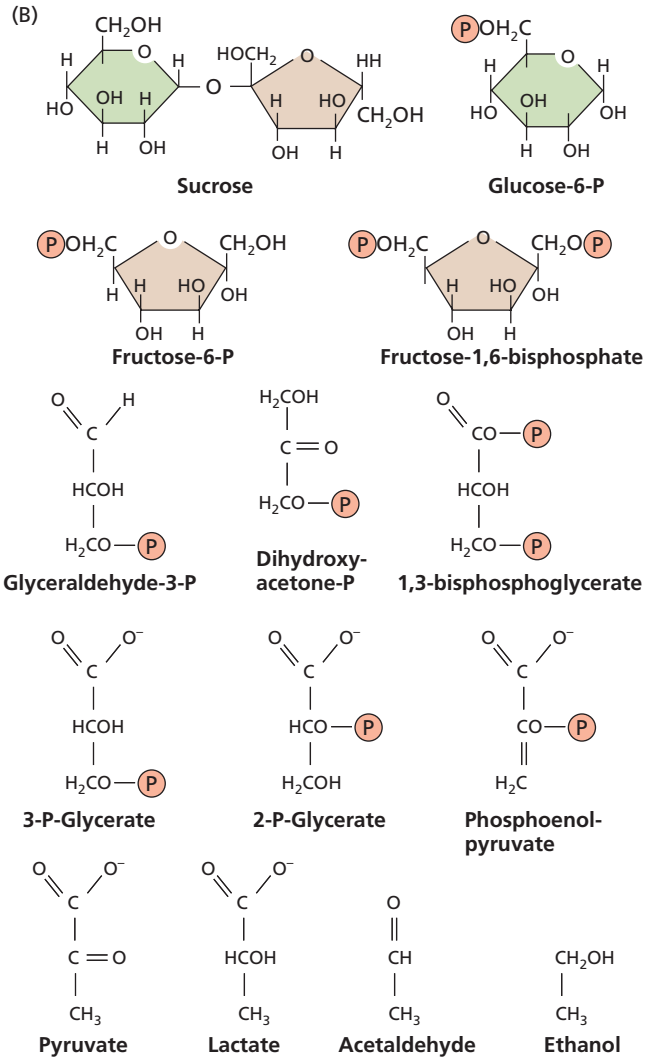


FIGURE 11.3 Reactions of plant glycolysis and fermentation. (A) In the main glycolytic pathway, sucrose is oxidized via hexose phosphates and triose phosphates to the organic acid pyruvate, but plants also carry out alternative reactions. All the enzymes included in this figure have been measured at levels sufficient to support the respiration rates observed in intact plant tissues, and flux through the pathway has been observed in vivo. The double arrows denote reversible reactions; the single arrows, essentially irreversible reactions. (B) The structures of the carbon intermediates. P, phosphate group.

The standard reduction potential for this redox couple is about –320 mV, which makes it a relatively strong reductant (i.e., electron donor). NADH is thus a good molecule in which to conserve the free energy carried by the electrons released during the stepwise oxidations of glycolysis and the citric acid cycle. A related compound, nicotinamide adenine dinucleotide phosphate ($NADP^+$/NADPH), functions in the redox reactions of photosynthesis (see Chapters 7 and 8) and of the oxidative pentose phosphate pathway; it also takes part in mitochondrial metabolism (Møller and Rasmusson 1998). These roles will be discussed later in the chapter.

The oxidation of NADH by oxygen via the electron transport chain releases free energy (220 kJ mol^{-1}) that drives the synthesis of approximately 60 ATP (as we will see later). We can formulate a more complete picture of respiration as related to its role in cellular energy metabolism by coupling the following two reactions:

$$C_{12}H_{22}O_{11} + 12\ O_2 \rightarrow 12\ CO_2 + 11\ H_2O$$

$$60\ ADP + 60\ P_i \rightarrow 60\ ATP + 60\ H_2O$$

Keep in mind that not all the carbon that enters the respiratory pathway ends up as CO_2. Many respiratory intermediates are the starting points for pathways that assimilate nitrogen into organic form, pathways that synthesize nucleotides and lipids, and many others.

Glycolysis

In the early steps of glycolysis (from the Greek words *glykos*, "sugar," and *lysis*, "splitting"), carbohydrates are converted into hexose phosphates, each of which is then split into two triose phosphates. In a subsequent energy-conserving phase, each triose phosphate is oxidized and rearranged to yield one molecule of pyruvate, an organic acid. Besides preparing the substrate for oxidation in the citric acid cycle, glycolysis yields a small amount of chemical energy in the form of ATP and NADH.

When molecular oxygen is unavailable—for example, in plant roots in flooded soils—glycolysis can be the main source of energy for cells. For this to work, the *fermentative pathways*, which are carried out in the cytosol, must reduce pyruvate to recycle the NADH produced by glycolysis. In this section we will describe the basic glycolytic and fermentative pathways, emphasizing features that are specific to plant cells. In the following section we will discuss the pentose phosphate pathway, another pathway for sugar oxidation in plants.

Glycolysis metabolizes carbohydrates from several sources

Glycolysis occurs in all living organisms (prokaryotes and eukaryotes). The principal reactions associated with the classic glycolytic pathway in plants are almost identical to those in animal cells (**FIGURE 11.3**). However, plant glycolysis has unique regulatory features, alternative enzymatic routes for several steps, and a parallel partial glycolytic pathway in plastids.

In animals, the substrate of glycolysis is glucose, and the end product is pyruvate. Because sucrose is the major translocated sugar in most plants, and is therefore the form of carbon that most nonphotosynthetic tissues import, sucrose (not glucose) can be argued to be the true sugar

substrate for plant respiration. The end products of plant glycolysis include another organic acid, malate.

In the early steps of glycolysis, sucrose is split into its two monosaccharide units—glucose and fructose—which can readily enter the glycolytic pathway. Two pathways for the splitting of sucrose are known in plants, both of which also take part in the unloading of sucrose from the phloem (see Chapter 10): the invertase pathway and the sucrose synthase pathway.

Invertases present in the cell wall, vacuole, or cytosol hydrolyze sucrose into its two component hexoses (glucose and fructose). The hexoses are then phosphorylated by a hexokinase that uses ATP to form **hexose phosphates**. Alternatively, *sucrose synthase*, located in the cytosol, combines sucrose with UDP to produce fructose and UDP-glucose. UDP-glucose pyrophosphorylase then converts UDP-glucose and pyrophosphate (PP_i) into UTP and glucose-6-phosphate (see Figure 11.3). While the sucrose synthase reaction is close to equilibrium, the invertase reaction is essentially irreversible, driving the flux in the forward direction. In general, invertase predominates in tissues where carbohydrates are mainly catabolized for respiration, whereas sucrose synthase predominates in conversions providing monosaccharides for synthesis of carbohydrate polymers.

In plastids, a partial glycolysis occurs that produces metabolites for biosynthetic reactions there, but can also supply substrates for glycolysis in the cytoplasm. Starch is both synthesized and catabolized only in plastids, and carbon obtained from starch degradation (for example, in a chloroplast at night) enters the glycolytic pathway in the cytosol primarily as glucose (see Chapter 8). In the light, photosynthetic products can also enter the glycolytic pathway directly as triose phosphate (Hoefnagel et al. 1998). So glycolysis works like a funnel with an initial phase collecting carbon from different cellular sources, depending on physiological conditions.

In the initial phase of glycolysis, each hexose unit is phosphorylated twice and then split, eventually producing two molecules of **triose phosphate**. This series of reactions consumes two to four molecules of ATP per sucrose unit, depending on whether the sucrose is split by sucrose synthase or invertase. These reactions also include two of the three essentially irreversible reactions of the glycolytic pathway, which are catalyzed by hexokinase and phosphofructokinase (see Figure 11.3). As we will see later, the phosphofructokinase reaction is one of the control points of glycolysis in both plants and animals.

The energy-conserving phase of glycolysis extracts usable energy

The reactions discussed thus far transfer carbon from the various substrate pools to triose phosphates. Once *glyceraldehyde-3-phosphate* is formed, the glycolytic pathway can begin to extract usable energy in the energy-conserving phase. The enzyme *glyceraldehyde-3-phosphate dehydrogenase* catalyzes the oxidation of the aldehyde to a carboxylic acid, reducing NAD^+ to NADH. This reaction releases sufficient free energy to allow the phosphorylation (using inorganic phosphate) of glyceraldehyde-3-phosphate to produce 1,3-bisphosphoglycerate. The phosphorylated carboxylic acid on carbon 1 of 1,3-bisphosphoglycerate (see Figure 11.3) has a large standard free-energy change ($\Delta G^{0\prime}$) of hydrolysis (–49.3 kJ mol^{-1}). Thus 1,3-bisphosphoglycerate is a strong donor of phosphate groups.

In the next step of glycolysis, catalyzed by *phosphoglycerate kinase*, the phosphate on carbon 1 is transferred to a molecule of ADP, yielding ATP and 3-phosphoglycerate. For each sucrose entering the pathway, four ATPs are generated by this reaction—one for each molecule of 1,3-bisphosphoglycerate.

This type of ATP synthesis, traditionally referred to as **substrate-level phosphorylation**, involves the direct transfer of a phosphate group from a substrate molecule to ADP to form ATP. ATP synthesis by substrate-level phosphorylation is mechanistically distinct from ATP synthesis by the ATP synthases involved in oxidative phosphorylation in mitochondria (which will be described later in this chapter) or photophosphorylation in chloroplasts (see Chapter 7).

In the subsequent two reactions, the phosphate on 3-phosphoglycerate is transferred to carbon 2, and then a molecule of water is removed, yielding the compound *phosphoenolpyruvate* (*PEP*). The phosphate group on PEP has a high $\Delta G^{0\prime}$ of hydrolysis (–61.9 kJ mol^{-1}), which makes PEP an extremely good phosphate donor for ATP formation. Using PEP as substrate, the enzyme *pyruvate kinase* catalyzes a second substrate-level phosphorylation to yield ATP and pyruvate. This final step, which is the third essentially irreversible step in glycolysis, yields four additional molecules of ATP for each sucrose molecule that enters the pathway.

Plants have alternative glycolytic reactions

The sequence of reactions leading to the formation of pyruvate from glucose occurs in all organisms that carry out glycolysis. In addition, organisms can operate this pathway in the opposite direction to synthesize sugars from organic acids. This process is known as **gluconeogenesis**.

Gluconeogenesis is particularly important in the seeds of plants (such as the castor oil plant *Ricinus communis* and sunflower) that store a significant quantity of their carbon reserves in the form of oils (triacylglycerols). After such a seed germinates, much of the oil is converted by gluconeogenesis into sucrose, which is then used to support the growing seedling. In the initial phase of glycolysis, gluconeogenesis overlaps with the pathway for synthesis of sucrose from photosynthetic triose phosphate described in Chapter 8, which is typical of plants.

Because the glycolytic reaction catalyzed by *ATP-dependent phosphofructokinase* is essentially irreversible (see Figure 11.3), an additional enzyme, *fructose-1,6-bisphosphate phosphatase*, converts fructose-1,6-bisphosphate

into fructose-6-phosphate and P_i during gluconeogenesis. ATP-dependent phosphofructokinase and fructose-1,6-bisphosphate phosphatase represent a major control point of carbon flux through the glycolytic/gluconeogenic pathways of both plants and animals as well as in sucrose synthesis in plants (see Chapter 8).

In plants, the interconversion of fructose-6-phosphate and fructose-1,6-bisphosphate is made more complex by the presence of an additional (cytosolic) enzyme, *PP_i-dependent phosphofructokinase* (pyrophosphate:fructose-6-phosphate 1-phosphotransferase), which catalyzes the following reversible reaction (see Figure 11.3):

$$\text{Fructose-6-P} + PP_i \leftrightarrow \text{fructose-1,6-bisphosphate} + P_i$$

where -P represents bound phosphate. PP_i-dependent phosphofructokinase is found in the cytosol of most plant tissues at levels that are considerably higher than those of ATP-dependent phosphofructokinase (Kruger 1997). Suppression of PP_i-dependent phosphofructokinase in transgenic potato plants has shown that it contributes to glycolytic flux, but that it is not essential for plant survival, indicating that other enzymes can take over its function. The existence of different pathways that serve a similar function and can therefore replace each other without a clear loss in function is called **metabolic redundancy**; it is a common feature in plant metabolism.

The reaction catalyzed by PP_i-dependent phosphofructokinase is readily reversible, but it is unlikely to operate in sucrose synthesis (Dennis and Blakely 2000). Like ATP-dependent phosphofructokinase and fructose bisphosphate phosphatase, this enzyme appears to be regulated by fluctuations in cell metabolism (discussed later in the chapter), suggesting that under some circumstances operation of the glycolytic pathway in plants has some unique characteristics (see **WEB ESSAY 11.1**).

At the end of the glycolytic process, plants have alternative pathways for metabolizing PEP. In one pathway PEP is carboxylated by the ubiquitous cytosolic enzyme **PEP carboxylase** to form the organic acid oxaloacetate. The oxaloacetate is then reduced to malate by the action of *malate dehydrogenase*, which uses NADH as a source of electrons, and thus has a similar effect as the dehydrogenases during fermentation (see Figure 11.3). The resulting malate can be stored by export to the vacuole or transported to the mitochondrion, where it can enter the citric acid cycle. Thus the action of pyruvate kinase and PEP carboxylase can produce pyruvate or malate for mitochondrial respiration, although pyruvate dominates in most tissues.

In the absence of oxygen, fermentation regenerates the NAD^+ needed for glycolysis

Oxidative phosphorylation does not function in the absence of oxygen. Glycolysis thus cannot continue to operate because the cell's supply of NAD^+ is limited and once all the NAD^+ becomes tied up in the reduced state (NADH), the catalytic activity of glyvceraldehyde-3-phosphate dehydrogenase comes to a halt. To overcome this limitation, plants and other organisms can further metabolize pyruvate by carrying out one or more forms of **fermentation** (see Figure 11.3).

Alcoholic fermentation is common in plants, although more widely known from brewer's yeast. Two enzymes, pyruvate decarboxylase and alcohol dehydrogenase, act on pyruvate, ultimately producing ethanol and CO_2 and oxidizing NADH in the process. In lactic acid fermentation (common in mammalian muscle, but also found in plants), the enzyme lactate dehydrogenase uses NADH to reduce pyruvate to lactate, thus regenerating NAD^+.

Plant tissues may be subjected to low (hypoxic) or zero (anoxic) concentrations of ambient oxygen. These conditions force the tissues to carry out fermentative metabolism. The best-studied example involves flooded or waterlogged soils in which the diffusion of oxygen is sufficiently reduced to cause root tissues to become hypoxic.

In corn, the initial response to low oxygen concentrations is lactic acid fermentation, but the subsequent response is alcoholic fermentation. Ethanol is thought to be a less toxic end product of fermentation because it can diffuse out of the cell, whereas lactate accumulates and promotes acidification of the cytosol. In numerous other cases plants function under near-anoxic conditions by carrying out some form of fermentation.

It is important to consider the efficiency of fermentation. *Efficiency* is defined here as the energy conserved as ATP relative to the energy potentially available in a molecule of sucrose. The standard free-energy change ($\Delta G^{0\prime}$) for the complete oxidation of sucrose to CO_2 is –5760 kJ mol^{-1}. The $\Delta G^{0\prime}$ for the synthesis of ATP is 32 kJ mol^{-1}. However, under the nonstandard conditions that normally exist in both mammalian and plant cells, the synthesis of ATP requires an input of free energy of approximately 50 kJ mol^{-1}.

Normal glycolysis leads to a net synthesis of four ATP molecules for each sucrose molecule converted into pyruvate. With ethanol or lactate as the final product, the efficiency of fermentation is only about 4%. Most of the energy available in sucrose remains in the ethanol or lactate. Changes in the glycolytic pathway under oxygen deficiency can increase the ATP yield. This is the case when sucrose is degraded via sucrose synthase instead of invertase, avoiding ATP consumption by the hexokinase in the initial phase of glycolysis. Such modifications emphasize the importance of energetic efficiency for plant survival in the absence of oxygen (see **WEB ESSAY 11.1**).

Because of the low energy recovery of fermentation, an increased rate of carbohydrate breakdown is needed to sustain the ATP production necessary for cell survival. Glycolysis is up-regulated by changes in metabolite levels and by the induction of genes encoding the enzymes of glycolysis and fermentation. The increased glycolytic rate is called the *Pasteur effect* after the French microbiologist

Louis Pasteur, who first noted it when yeast switched from aerobic respiration to fermentation.

In contrast to the products of fermentation, the pyruvate produced by glycolysis during aerobic respiration is further oxidized by mitochondria, resulting in a much more efficient utilization of the free energy available in sucrose.

Plant glycolysis is controlled by its products

In vivo, glycolysis appears to be regulated at the level of fructose-6-phosphate phosphorylation and PEP turnover. In contrast to animals, AMP and ATP are not major effectors of plant phosphofructokinase and pyruvate kinase. A more important regulator of plant glycolysis is the cytosolic concentration of PEP, which is a potent inhibitor of the plant ATP-dependent phosphofructokinase.

This inhibitory effect of PEP on phosphofructokinase is strongly decreased by inorganic phosphate, making the cytosolic ratio of PEP to P_i a critical factor in the control of plant glycolytic activity. Pyruvate kinase and PEP carboxylase, the enzymes that metabolize PEP in the last steps of glycolysis (see Figure 11.3), are in turn sensitive to feedback inhibition by citric acid cycle intermediates and their derivatives, including malate, citrate, 2-oxoglutarate, and glutamate.

In plants, therefore, the control of glycolysis comes from the "bottom up" (as discussed later in the chapter), with primary regulation at the level of PEP metabolism by pyruvate kinase and PEP carboxylase. Secondary regulation is exerted by PEP at the conversion of fructose-6-phosphate into fructose-1,6-bisphosphate (see Figure 11.3). In contrast, regulation in animals operates from "top down," with primary activation occurring at the phosphofructokinase and secondary activation at the pyruvate kinase.

One possible benefit of bottom-up control of glycolysis is that it permits plants to regulate net glycolytic flux to pyruvate independently of related metabolic processes such as the Calvin cycle and sucrose–triose phosphate–starch interconversion (Plaxton 1996). Another benefit of this control mechanism is that glycolysis can adjust to the demand for biosynthetic precursors.

A consequence of bottom-up control of glycolysis is that its rate can influence cellular concentrations of sugars, in combination with sugar-supplying processes such as phloem transport. Glucose and sucrose are potent signaling molecules that make the plant adjust its growth and development to its sugar status. The glycolytic enzyme hexokinase not only functions as an enzyme in the cytosol, but also as a glucose receptor in the nucleus, where it modulates gene expression responses to several plant hormones (Rolland et al. 2006).

The presence of more than one enzyme metabolizing PEP in plant cells—pyruvate kinase and PEP carboxylase—may have consequences for the control of glycolysis. Although the two enzymes are inhibited by similar metabolites, PEP carboxylase can, under some conditions, catalyze a reaction that bypasses pyruvate kinase. The resulting malate can then enter the mitochondrial citric acid cycle.

Experimental support for multiple pathways of PEP metabolism comes from the study of transgenic tobacco plants with less than 5% of the normal level of cytosolic pyruvate kinase in their leaves (Plaxton 1996). In these plants, neither rates of leaf respiration nor rates of photosynthesis differed from those in controls with wild-type levels of pyruvate kinase. However, reduced root growth in the transgenic plants indicated that the pyruvate kinase reaction could not be circumvented without some detrimental effects.

Fructose-2,6-bisphosphate also affects the phosphofructokinase reaction, but unlike PEP, it affects the reaction in both the forward and reverse direction (see Chapter 8 for a detailed discussion). Therefore, fructose-2,6-bisphosphate mediates control of the partitioning of sugars between respiration and biosynthesis.

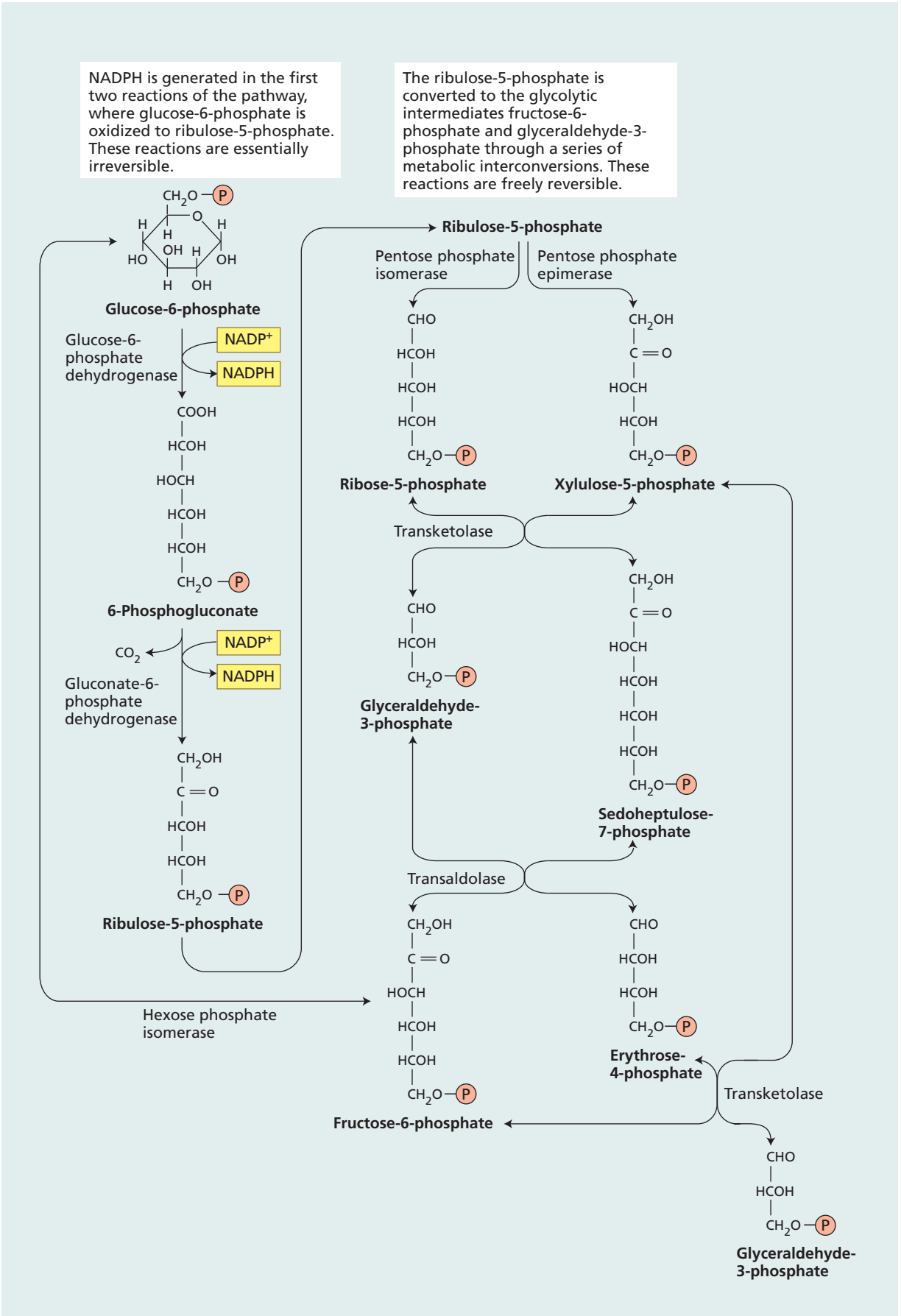
Another level of regulation may ensue from changes in the location of the glycolytic enzymes. These enzymes were believed to be dissolved in the cytosol; however, it is now clear that under high respiratory demand, there is a substantial pool of glycolytic enzymes bound to the mitochondrial outer surface. This positioning allows direct movement of intermediates from one enzyme to the next (called *substrate channeling*), which separates mitochondrially bound glycolysis from glycolysis in the cytosol. The latter can then contribute carbon intermediates for other processes without interfering with pyruvate production (Graham et al. 2007).

Understanding of the regulation of glycolysis requires the study of temporal changes in metabolite levels. Rapid extraction, separation, and analysis of many metabolites can be achieved by an approach called *metabolic profiling* (see **WEB ESSAY 11.2**).

The Oxidative Pentose Phosphate Pathway

The glycolytic pathway is not the only route available for the oxidation of sugars in plant cells. The oxidative pentose phosphate pathway (also known as the *hexose monophosphate shunt*) can also accomplish this task (**FIGURE 11.4**). The reactions are carried out by soluble enzymes present in the cytosol and in plastids. Under most con-

FIGURE 11.4 Reactions of the oxidative pentose phosphate pathway in plants. The first two reactions—which are oxidizing reactions—are essentially irreversible. They supply NADPH to the cytoplasm and to plastids in the absence of photosynthesis. The downstream part of the pathway is reversible (as denoted by double-headed arrows), so it can supply five-carbon substrates for biosynthesis even when the oxidizing reactions are inhibited; for example, in chloroplasts in the light. ▶



ditions, the pathway in plastids predominates over that in the cytosol (Dennis et al. 1997).

The first two reactions of this pathway involve the oxidative events that convert the six-carbon molecule glucose-6-phosphate into the five-carbon unit **ribulose-5-phosphate**, with loss of a CO_2 molecule and generation of two molecules of NADPH (not NADH). The remaining reactions of the pathway convert ribulose-5-phosphate into the glycolytic intermediates glyceraldehyde-3-phosphate and fructose-6-phosphate. These products can be further metabolized by glycolysis to yield pyruvate. Alternatively, glucose-6-phosphate can be regenerated from glyceraldehyde-3-phosphate and fructose-6-phosphate by glycolytic enzymes. For six turns of this cycle, we can write the reaction as follows:

$$6 \text{ Glucose-6-P} + 12 \text{ NADP}^+ + 7 \text{ H}_2\text{O} \rightarrow$$
$$5 \text{ glucose-6-P} + 6 \text{ CO}_2 + \text{P}_i + 12 \text{ NADPH} + 12 \text{ H}^+$$

The net result is the complete oxidation of one glucose-6-phosphate molecule to CO_2 (five molecules are regenerated) with the concomitant synthesis of 12 NADPH molecules.

Studies of the release of CO_2 from isotopically labeled glucose indicate that the pentose phosphate pathway accounts for 10–25% of the glucose breakdown, with the rest occurring mainly via glycolysis. As we will see, the contribution of the pentose phosphate pathway changes during development and with changes in growth conditions (Kruger and von Schaewen 2003) as the plant's requirements for specific products vary.

The oxidative pentose phosphate pathway produces NADPH and biosynthetic intermediates

The oxidative pentose phosphate pathway plays several roles in plant metabolism:

- *NADPH supply in the cytosol.* The product of the two oxidative steps is NADPH. This NADPH drives reductive steps associated with biosynthetic and defensive reactions that occur in the cytosol and is a substrate for reactions that remove reactive oxygen species (ROS). Because plant mitochondria possess an NADPH dehydrogenase located on the external surface of the inner membrane, the reducing power generated by the pentose phosphate pathway can be balanced by mitochondrial NADPH oxidation. The pentose phosphate pathway may therefore also contribute to cellular energy metabolism; that is, electrons from NADPH may end up reducing O_2 and generating ATP through oxidative phosphorylation.
- *NADPH supply in plastids.* In nongreen plastids, such as amyloplasts in the root, and in chloroplasts functioning in the dark, the pentose phosphate pathway is a major supplier of NADPH. The NADPH is used for biosynthetic reactions such as lipid synthesis and nitrogen assimilation. The formation of NADPH by glucose-6-phosphate oxidation in amyloplasts may also signal sugar status to the thioredoxin system for control of starch synthesis (Schürmann and Buchanan 2008).
- *Supply of substrates for biosynthetic processes.* In most organisms, the pentose phosphate pathway produces ribose-5-phosphate, which is a precursor of the ribose and deoxyribose needed in the synthesis of nucleic acids. In plants, however, ribose appears to be synthesized by another, as yet unknown, pathway (Sharples and Fry 2007). Another intermediate in the pentose phosphate pathway, the four-carbon *erythrose-4-phosphate*, combines with PEP in the initial reaction that produces plant phenolic compounds, including aromatic amino acids and the precursors of lignin, flavonoids, and phytoalexins (see Chapter 13). This role of the pentose phosphate pathway is supported by the observation that its enzymes are induced by stress conditions such as wounding, under which biosynthesis of aromatic compounds is needed for reinforcing and protecting the tissue.

The oxidative pentose phosphate pathway is redox-regulated

Each enzymatic step in the oxidative pentose phosphate pathway is catalyzed by a group of isoenzymes that vary in their abundance and regulatory properties among plant organs. The initial reaction of the pathway, catalyzed by **glucose-6-phosphate dehydrogenase**, is in many cases inhibited by a high ratio of NADPH to $NADP^+$.

In the light, little operation of the pentose phosphate pathway occurs in chloroplasts. Glucose-6-phosphate dehydrogenase is inhibited by a reductive inactivation involving the *ferredoxin–thioredoxin system* (see Chapter 8) and by the NADPH to $NADP^+$ ratio. Moreover, the end products of the pathway, fructose-6-phosphate and glyceraldehyde-3-phosphate, are being synthesized by the Calvin cycle. Thus mass action will drive the nonoxidative reactions of the pathway in the reverse direction. In this way, synthesis of erythrose-4-phosphate can be maintained in the light. In nongreen plastids, the glucose-6-phosphate dehydrogenase is less sensitive to inactivation by reduced thioredoxin and NADPH, and can therefore reduce $NADP^+$ to maintain a high reduction of plastid components in the absence of photosynthesis (Kruger and von Schaewen 2003).

The Citric Acid Cycle

During the nineteenth century, biologists discovered that in the absence of air, cells produce ethanol or lactic acid, whereas in the presence of air, cells consume O_2 and produce CO_2 and H_2O. In 1937 the German-born British biochemist Hans A. Krebs reported the discovery of the citric acid cycle—also called the *tricarboxylic acid cycle* or *Krebs cycle*. The elucidation of the citric acid cycle not only explained how pyruvate is broken down into CO_2 and H_2O, but also highlighted the key concept of cycles in metabolic pathways. For his discovery, Hans Krebs was awarded the Nobel Prize in physiology or medicine in 1953.

Because the citric acid cycle occurs in the mitochondrial matrix, we will begin with a general description of mitochondrial structure and function, the knowledge of which was obtained mainly through experiments on isolated mitochondria (see WEB TOPIC 11.1). We will then review the steps of the citric acid cycle, emphasizing the features that are specific to plants and how they affect respiratory function.

Mitochondria are semiautonomous organelles

The breakdown of sucrose into pyruvate releases less than 25% of the total energy in sucrose; the remaining energy is stored in the four molecules of pyruvate. The next two stages of respiration (the citric acid cycle and oxidative phosphorylation) take place within an organelle enclosed by a double membrane, the **mitochondrion** (plural *mitochondria*).

In electron micrographs, plant mitochondria usually look spherical or rodlike (FIGURE 11.5). They range from 0.5 to 1.0 µm in diameter and up to 3 µm in length (Douce 1985). With some exceptions, plant cells have substantially fewer mitochondria than are found in a typical animal cell. The number of mitochondria per plant cell varies; it is usually directly related to the metabolic activity of the tissue, reflecting the mitochondrial role in energy metabolism. Guard cells, for example, are unusually rich in mitochondria.

The ultrastructural features of plant mitochondria are similar to those of mitochondria in other organisms (see Figure 11.5). Plant mitochondria have two membranes: a smooth **outer membrane** completely surrounds a highly invaginated **inner membrane**. The invaginations of the inner membrane are known as **cristae** (singular *crista*). As a consequence of its greatly enlarged surface area, the inner membrane can contain more than 50% of the total mitochondrial protein. The region between the two mitochondrial membranes is known as the **intermembrane space**. The compartment enclosed by the inner membrane is referred to as the mitochondrial **matrix**. It has a very high content of macromolecules, approximately 50% by weight. Because there is little water in the matrix, mobility is restricted, and it is likely that matrix proteins are organized into multienzyme complexes to facilitate substrate channeling.

Intact mitochondria are osmotically active; that is, they take up water and swell when placed in a hypo-osmotic medium. Most inorganic ions and charged organic molecules are not able to diffuse freely into the matrix. The inner membrane is the osmotic barrier; the outer membrane is permeable to solutes that have a molecular mass of less than approximately 10,000 Da—that is, most cellular metabolites and ions, but not proteins. The lipid fraction of

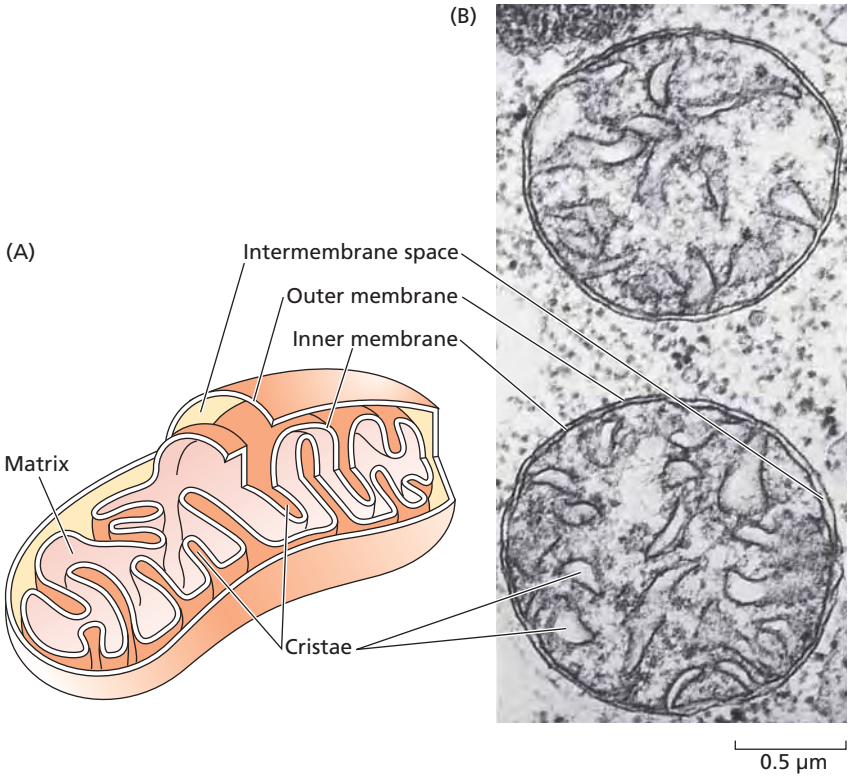


FIGURE 11.5 Structure of plant mitochondria. (A) Three-dimensional representation of a mitochondrion, showing the invaginations of the inner membrane, called cristae, as well as the locations of the matrix and intermembrane space (see also Figure 11.9). (B) Electron micrograph of mitochondria in a mesophyll cell of broad bean (*Vicia faba*). Typically, individual mitochondria are 1 to 3 µm long in plant cells, which means that they are substantially smaller than nuclei and plastids. (B from Gunning and Steer 1996.)

both membranes is primarily made up of phospholipids, 80% of which are either phosphatidylcholine or phosphatidylethanolamine. About 15% is diphosphatidylglycerol (also called cardiolipin), which occurs in cells only in the inner mitochondrial membrane.

Like chloroplasts, mitochondria are semiautonomous organelles because they contain ribosomes, RNA, and DNA, which encodes a limited number of mitochondrial proteins. Plant mitochondria are thus able to carry out the various steps of protein synthesis and to transmit their genetic information. The number of mitochondria in a cell can vary dynamically due to divisions and fusions (see WEB ESSAY 11.3) while keeping up with cell division. In most plants, mitochondria are maternally inherited during sexual reproduction.

Pyruvate enters the mitochondrion and is oxidized via the citric acid cycle

The citric acid cycle is also known as the *tricarboxylic acid cycle* because of the importance of the tricarboxylic acids citric acid (citrate) and isocitric acid (isocitrate) as early intermediates (FIGURE 11.6). This cycle constitutes the second stage in respiration and takes place in the mitochondrial

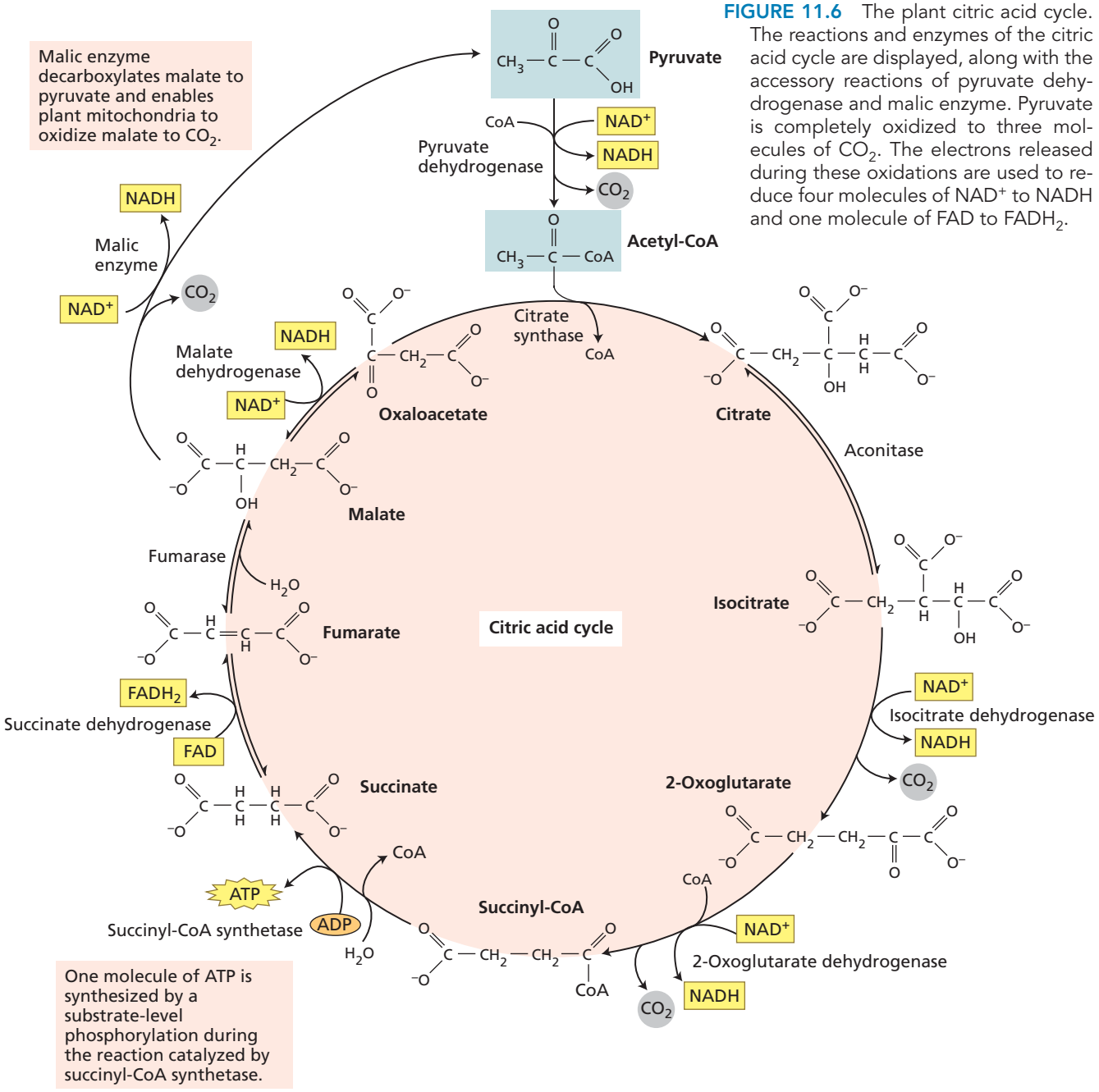


FIGURE 11.6 The plant citric acid cycle. The reactions and enzymes of the citric acid cycle are displayed, along with the accessory reactions of pyruvate dehydrogenase and malic enzyme. Pyruvate is completely oxidized to three molecules of CO_2. The electrons released during these oxidations are used to reduce four molecules of NAD^+ to NADH and one molecule of FAD to $FADH_2$.

matrix. Its operation requires that the pyruvate generated in the cytosol during glycolysis be transported through the impermeable inner mitochondrial membrane via a specific transport protein (as will be described shortly).

Once inside the mitochondrial matrix, pyruvate is decarboxylated in an oxidation reaction catalyzed by **pyruvate dehydrogenase**, a large complex containing several enzymes. The products are NADH, CO_2, and acetyl-CoA, in which the acetyl group derived from pyruvate is linked by a thioester bond to a cofactor, coenzyme A (CoA) (see Figure 11.6).

In the next reaction, the enzyme citrate synthase, formally the first enzyme in the citric acid cycle, combines the acetyl group of acetyl-CoA with a four-carbon dicarboxylic acid (*oxaloacetate*) to give a six-carbon tricarboxylic acid (citrate). Citrate is then isomerized to isocitrate by the enzyme aconitase.

The following two reactions are successive oxidative decarboxylations, each of which produces one NADH and releases one molecule of CO_2, yielding a four-carbon product bound to CoA, succinyl-CoA. At this point, three molecules of CO_2 have been produced for each pyruvate that entered the mitochondrion, or 12 CO_2 for each molecule of sucrose oxidized.

In the remainder of the citric acid cycle, succinyl-CoA is oxidized to oxaloacetate, allowing the continued operation of the cycle. Initially the large amount of free energy available in the thioester bond of succinyl-CoA is conserved through the synthesis of ATP from ADP and P_i via a substrate-level phosphorylation catalyzed by *succinyl-CoA synthetase*. (Recall that the free energy available in the thioester bond of acetyl-CoA was used to form a carbon–carbon bond in the step catalyzed by citrate synthase.) The resulting succinate is oxidized to fumarate by *succinate dehydrogenase*, which is the only membrane-associated enzyme of the citric acid cycle and also part of the electron transport chain.

The electrons and protons removed from succinate end up not on NAD^+, but on another cofactor involved in redox reactions: **flavin adenine dinucleotide** (**FAD**). FAD is covalently bound to the active site of succinate dehydrogenase and undergoes a reversible two-electron reduction to produce $FADH_2$ (see Figure 11.2B).

In the final two reactions of the citric acid cycle, fumarate is hydrated to produce malate, which is subsequently oxidized by *malate dehydrogenase* to regenerate oxaloacetate and produce another molecule of NADH. The oxaloacetate produced is now able to react with another acetyl-CoA and continue the cycling.

The stepwise oxidation of one molecule of pyruvate in the mitochondrion gives rise to three molecules of CO_2, and much of the free energy released during these oxidations is conserved in the form of four NADH and one $FADH_2$. In addition, one molecule of ATP is produced by a substrate-level phosphorylation.

The citric acid cycle of plants has unique features

The citric acid cycle reactions outlined in Figure 11.6 are not all identical to those carried out by animal mitochondria. For example, the step catalyzed by succinyl-CoA synthetase produces ATP in plants and GTP in animals. These nucleotides are energetically equivalent.

A feature of the plant citric acid cycle that is absent in many other organisms is the presence of **malic enzyme** in the mitochondrial matrix of plants. This enzyme catalyzes the oxidative decarboxylation of malate:

$$\text{Malate} + NAD^+ \rightarrow \text{pyruvate} + CO_2 + \text{NADH}$$

The activity of malic enzyme enables plant mitochondria to operate alternative pathways for the metabolism of PEP derived from glycolysis (see **WEB ESSAY 11.1**). As already described, malate can be synthesized from PEP in the cytosol via the enzymes PEP carboxylase and malate dehydrogenase (see Figure 11.3). For degradation, malate is transported into the mitochondrial matrix, where malic enzyme can oxidize it to pyruvate. This reaction makes possible the complete net oxidation of citric acid cycle intermediates such as malate (**FIGURE 11.7A**) or citrate (**FIGURE 11.7B**) (Oliver and McIntosh 1995). Many plant tissues, not only those that carry out crassulacean acid metabolism (see Chapter 8), store significant amounts of malate or other organic acids in their vacuoles. Degradation of malate via mitochondrial malic enzyme is important for regulating levels of organic acids in cells—for example, during fruit ripening.

Instead of being degraded, the malate produced via PEP carboxylase can replace citric acid cycle intermediates used in biosynthesis. Reactions that replenish intermediates in a metabolic cycle are known as *anaplerotic*. For example, export of 2-oxoglutarate for nitrogen assimilation in the chloroplast causes a shortage of malate for the citrate synthase reaction. This malate can be replaced through the PEP carboxylase pathway (**FIGURE 11.7C**).

Gamma-aminobutyric acid (GABA) is an amino acid that accumulates under several biotic and abiotic stress conditions in plants. GABA is synthesized from 2-oxoglutarate and degraded into succinate by a reaction that bypasses the citric acid cycle, called the **GABA shunt** (Bouché and Fromm 2004). The functional relationship between GABA accumulation and stress remains poorly understood.

Mitochondrial Electron Transport and ATP Synthesis

ATP is the energy carrier used by cells to drive life processes, so chemical energy conserved during the citric acid cycle in the form of NADH and $FADH_2$ must be converted into ATP to perform useful work in the cell. This O_2-dependent process, called oxidative phosphorylation, occurs in the inner mitochondrial membrane.

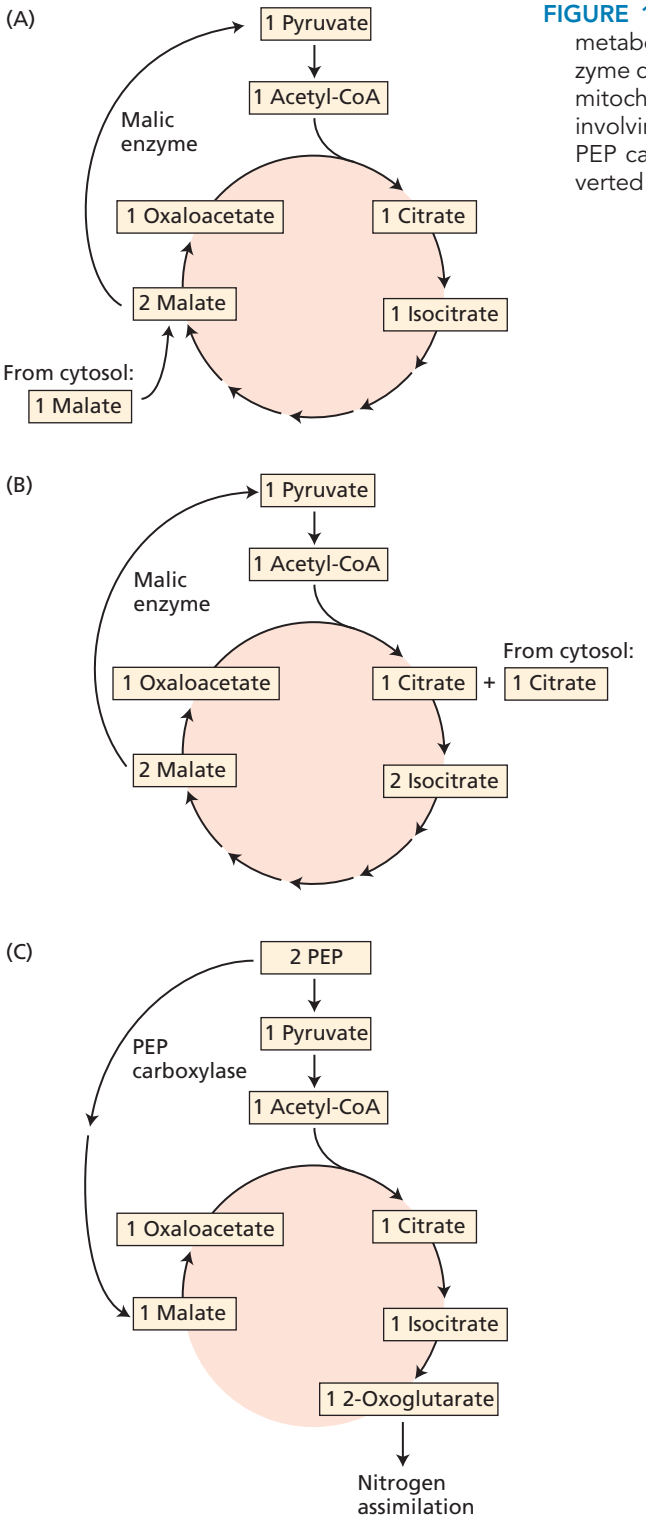


FIGURE 11.7 Malic enzyme and PEP carboxylase provide plants with metabolic flexibility for the metabolism of PEP and pyruvate. Malic enzyme converts malate into pyruvate and thus makes it possible for plant mitochondria to oxidize both malate (A) and citrate (B) to CO_2 without involving pyruvate delivered by glycolysis. With the added action of PEP carboxylase to the standard pathway, glycolytic PEP can be converted into 2-oxoglutarate, which is used for nitrogen assimilation (C).

In this section we will describe the process by which the energy level of the electrons from NADH and $FADH_2$ is lowered in a stepwise fashion and conserved in the form of an electrochemical proton gradient across the inner mitochondrial membrane. Although fundamentally similar in all aerobic cells, the electron transport chain of plants (and fungi) contains multiple NAD(P)H dehydrogenases and an alternative oxidase not found in mammalian mitochondria.

We will also examine the enzyme that uses the energy of the proton gradient to synthesize ATP: the F_oF_1-ATP synthase. After examining the various stages in the production of ATP, we will summarize the energy conservation steps at each stage, as well as the regulatory mechanisms that coordinate the different pathways.

The electron transport chain catalyzes a flow of electrons from NADH to O_2

For each molecule of sucrose oxidized through glycolysis and the citric acid cycle, four molecules of NADH are generated in the cytosol, and sixteen molecules of NADH plus four molecules of $FADH_2$ (associated with succinate dehydrogenase) are generated in the mitochondrial matrix. These reduced compounds must be reoxidized, or the entire respiratory process will come to a halt.

The electron transport chain catalyzes a transfer of two electrons from NADH (or $FADH_2$) to oxygen, the final electron acceptor of the respiratory process. For the oxidation of NADH, the reaction can be written as

$$NADH + H^+ + \tfrac{1}{2}\,O_2 \rightarrow NAD^+ + H_2O$$

From the reduction potentials for the NADH–NAD^+ pair (–320 mV) and the H_2O–½ O_2 pair (+810 mV), it can be calculated that the standard free energy released during this overall reaction ($-nF\Delta E0'$) is about 220 kJ per mole of NADH. Because the succinate–fumarate reduction potential is higher (+30 mV), only 152 kJ per mole of succinate is released. The role of the electron transport chain is to bring about the oxidation of NADH (and $FADH_2$) and, in the process, utilize some of the free energy released to generate an electrochemical proton gradient, $\Delta\tilde{\mu}_{H^+}$, across the inner mitochondrial membrane.

The electron transport chain of plants contains the same set of electron carriers found in the mitochondria of other organisms (**FIGURE 11.8**) (Siedow and Umbach 1995). The individual electron transport proteins are organized into four transmembrane multiprotein complexes (identified by roman numerals I through IV), all of which are localized in the inner mitochondrial membrane. Three of these complexes are engaged in proton pumping (I, III, and IV).

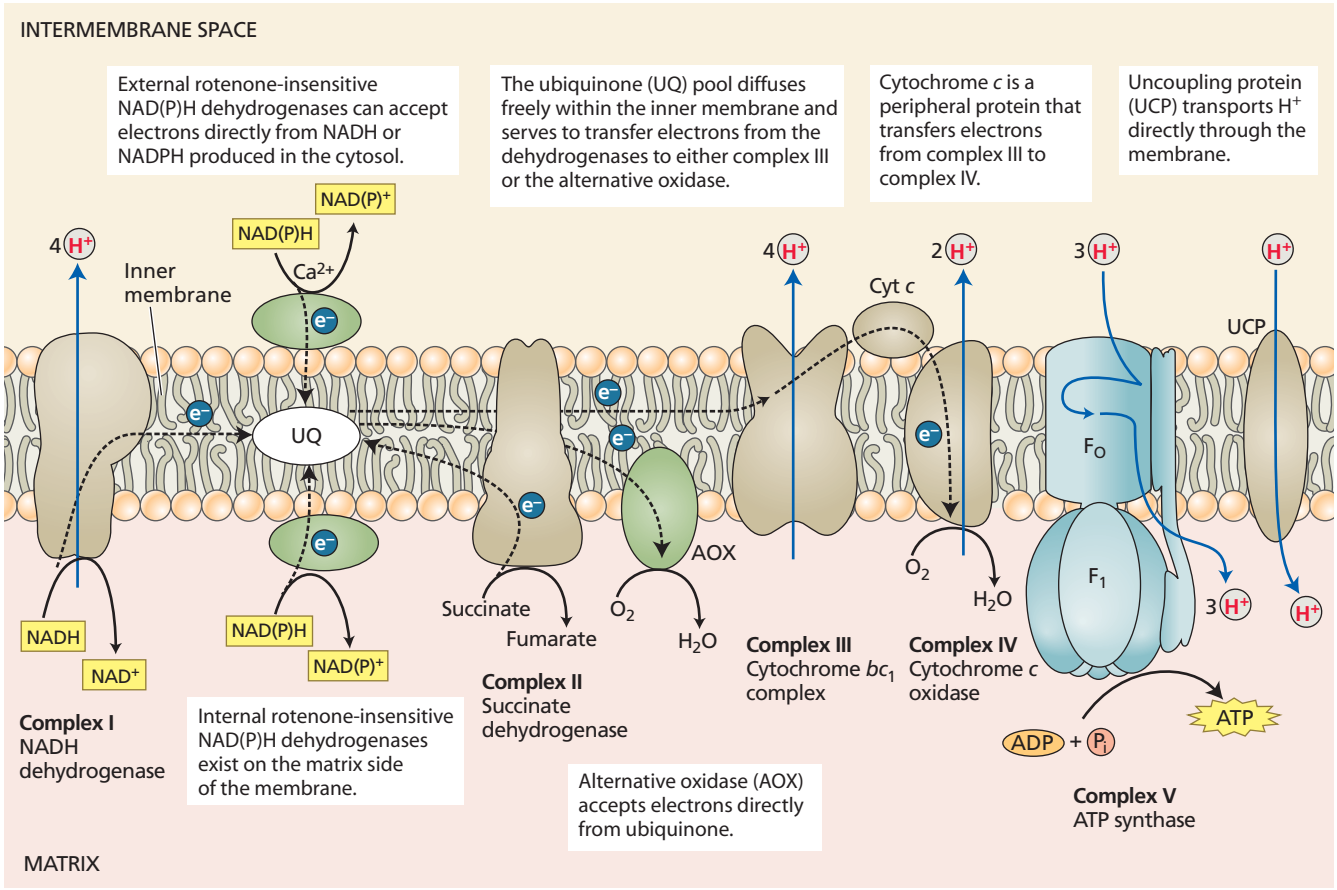


FIGURE 11.8 Organization of the electron transport chain and ATP synthesis in the inner membrane of the plant mitochondrion. Mitochondria from nearly all eukaryotes contain the four standard protein complexes: I, II, III, and IV. The structures of most of these complexes have been determined, but they are shown here as simplified shapes. The electron transport chain of the plant mitochondrion contains additional enzymes (depicted in green) that do not pump protons. Additionally, uncoupling proteins directly bypass the ATP synthase by allowing passive proton influx. This multiplicity of bypasses in plants, whereas animals have only the uncoupling protein, gives a greater flexibility to plant energy coupling (see **WEB TOPIC 11.3**).

COMPLEX I (NADH DEHYDROGENASE) Electrons from NADH generated in the mitochondrial matrix during the citric acid cycle are oxidized by complex I (an **NADH dehydrogenase**). The electron carriers in complex I include a tightly bound cofactor (**flavin mononucleotide**, or **FMN**, which is chemically similar to FAD; see Figure 11.2B) and several iron–sulfur centers. Complex I then transfers these electrons to ubiquinone. Four protons are pumped from the matrix into the intermembrane space for every electron pair passing through the complex.

Ubiquinone, a small lipid-soluble electron and proton carrier, is localized within the inner membrane. It is not tightly associated with any protein, and it can diffuse within the hydrophobic core of the membrane bilayer.

COMPLEX II (SUCCINATE DEHYDROGENASE) Oxidation of succinate in the citric acid cycle is catalyzed by this complex, and the reducing equivalents are transferred via the $FADH_2$ and a group of iron–sulfur centers to ubiquinone. Complex II does not pump protons.

COMPLEX III (CYTOCHROME bc_1 COMPLEX) Complex III oxidizes reduced ubiquinone (ubiquinol) and transfers the electrons via an iron–sulfur center, two *b*-type cytochromes (b_{565} and b_{560}), and a membrane-bound cytochrome c_1 to cytochrome *c*. Four protons per electron pair are pumped out of the matrix by complex III using a mechanism called the **Q-cycle** (see **WEB TOPIC 11.2**).

Cytochrome *c* is a small protein loosely attached to the outer surface of the inner membrane and serves as a mobile carrier to transfer electrons between complexes III and IV.

COMPLEX IV (CYTOCHROME *c* OXIDASE) Complex IV contains two copper centers (Cu_A and Cu_B) and cytochromes *a* and a_3. This complex is the terminal oxidase and brings

about the four-electron reduction of O_2 to two molecules of H_2O. Two protons are pumped out of the matrix per electron pair (see Figure 11.8).

Both structurally and functionally, ubiquinone and the cytochrome bc_1 complex are very similar to plastoquinone and the cytochrome b_6f complex, respectively, in the photosynthetic electron transport chain (see Chapter 7).

Reality may be more complex than the description above implies. Plant respiratory complexes contain a number of plant-specific subunits whose function is still unknown. Several of the complexes contain subunits that participate in functions other than electron transport, such as protein import. Finally, several of the complexes appear to be present in supercomplexes, instead of freely mobile in the membrane, although the functional significance of these supercomplexes is not clear (Millar et al. 2005).

The electron transport chain has supplementary branches

In addition to the set of protein complexes described in the previous section, the plant electron transport chain contains components not found in mammalian mitochondria (see Figure 11.8 and **WEB TOPIC 11.3**). These additional enzymes are bound to the surfaces of the inner membrane and do not pump protons, so energy conservation is lower whenever they are used.

- **NAD(P)H dehydrogenases**, mostly Ca^{2+}-dependent, are attached to the outer surface of the inner membrane facing the intermembrane space. They oxidize either NADH or NADPH from the cytosol. Electrons from these external NAD(P)H dehydrogenases—ND_{ex}(NADH) and ND_{ex}(NADPH)—enter the main electron transport chain at the level of the ubiquinone pool (Rasmusson et al. 2008).
- Plant mitochondria have two pathways for oxidizing matrix NADH. Electron flow through complex I, described in the previous section, is sensitive to inhibition by several compounds, including rotenone and piericidin. In addition, plant mitochondria have a rotenone-insensitive dehydrogenase, ND_{in}(NADH), on the matrix surface of the inner mitochondrial membrane. This enzyme oxidizes NADH derived from the citric acid cycle, and may also be a bypass engaged when complex I is overloaded, such as under photorespiratory conditions, as we will see shortly. An NADPH dehydrogenase, ND_{in}(NADPH), is also present on the matrix surface, but very little is known about this enzyme (Rasmusson et al. 2004).
- Most, if not all, plants have an "alternative" respiratory pathway for the oxidation of ubiquinol and reduction of oxygen. This pathway involves the so-called **alternative oxidase**, which, unlike cytochrome *c* oxidase, is insensitive to inhibition by cyanide, carbon monoxide, or the signal molecule nitric oxide (see **WEB ESSAY 11.4**).

The nature and physiological significance of these supplementary electron transport enzymes will be considered more fully later in the chapter. Some additional electron transport chain dehydrogenases present in plant mitochondria directly perform important carbon conversions (Rasmusson et al. 2008). A *proline dehydrogenase* oxidizes the amino acid proline. Proline accumulates during osmotic stress (see Chapter 26), and it is degraded by this mitochondrial pathway when water status returns to normal. An electron transfer flavoprotein:quinone oxidoreductase mediates the degradation of several amino acids that are used by plants as a reserve under carbon starvation conditions induced by light deprivation (Ishizaki et al. 2005). Finally, a galactono-gamma-lactone dehydrogenase, specific to plants, performs the last step in the major pathway for synthesis of the antioxidant *ascorbic acid* (also known as vitamin C). The enzyme uses cytochrome *c* as its electron acceptor, in competition with normal respiration (Millar et al. 2003).

ATP synthesis in the mitochondrion is coupled to electron transport

In oxidative phosphorylation, the transfer of electrons to oxygen via complexes I, III, and IV is coupled to the synthesis of ATP from ADP and P_i via the F_oF_1-ATP synthase (complex V). The number of ATPs synthesized depends on the nature of the electron donor.

In experiments conducted on isolated mitochondria, electrons derived from matrix NADH (e.g., generated by malate oxidation) give ADP:O ratios (the number of ATPs synthesized per two electrons transferred to oxygen) of 2.4 to 2.7 (**TABLE 11.1**). Succinate and externally added NADH each give values in the range of 1.6 to 1.8, while ascorbate, which serves as an artificial electron donor to cytochrome *c*, gives values of 0.8 to 0.9. Results such as these (for both plant and animal mitochondria) have led to the general concept that there are three sites of energy conservation along the electron transport chain, at complexes I, III, and IV.

The experimental ADP:O ratios agree quite well with the values calculated on the basis of the number of H^+ pumped by complexes I, III, and IV and the cost of 4 H^+ for synthesizing one ATP (see next section and Table 11.1). For instance, electrons from external NADH pass only complexes III and IV, so a total of 6 H^+ are pumped, giving 1.5 ATP (when the alternative oxidase pathway is not used).

The mechanism of mitochondrial ATP synthesis is based on the **chemiosmotic hypothesis**, described in Chapter 7, which was first proposed in 1961 by Nobel laureate Peter

TABLE 11.1
Theoretical and experimental ADP:O ratios in isolated plant mitochondria

Substrate	ADP:0 ratio	
	Theoretical[a]	Experimental
Malate	2.5	2.4–2.7
Succinate	1.5	1.6–1.8
NADH (external)	1.5	1.6–1.8
Ascorbate	1.0[b]	0.8–0.9

[a]It is assumed that complexes I, III, and IV pump 4, 4, and 2 H^+ per 2 electrons, respectively; that the cost of synthesizing one ATP and exporting it to the cytosol is 4 H^+ (Brand 1994); and that the nonphosphorylating pathways are not active.

[b]Cytochrome *c* oxidase pumps only two protons when it is measured with ascorbate as electron donor. However, two electrons move from the outer surface of the inner membrane (where the electrons are donated) across the inner membrane to the inner, matrix side. As a result, 2 H^+ are consumed on the matrix side. This means that the net movement of H^+ and charges is equivalent to the movement of a total of 4 H^+, giving an ADP:O ratio of 1.0.

Mitchell as a general mechanism of energy conservation across biological membranes (Nicholls and Ferguson 2002). According to the chemiosmotic hypothesis, the orientation of electron carriers within the inner mitochondrial membrane allows for the transfer of protons across the inner membrane during electron flow (see Figure 11.8).

Because the inner mitochondrial membrane is highly impermeable to protons, an **electrochemical proton gradient** can build up. As discussed in Chapters 6 and 7, the free energy associated with the formation of an electrochemical proton gradient ($\Delta\tilde{\mu}_{H^+}$, also referred to as a *proton motive force*, Δp, when expressed in units of volts) is made up of an electrical transmembrane potential component (ΔE) and a chemical-potential component (ΔpH) according to the following equation:

$$\Delta p = \Delta E - 59\Delta\text{pH (at 25°C)}$$

where

$$\Delta E = E_{\text{inside}} - E_{\text{outside}}$$

and

$$\Delta\text{pH} = \text{pH}_{\text{inside}} - \text{pH}_{\text{outside}}$$

ΔE results from the asymmetric distribution of a charged species (H^+) across the membrane, and ΔpH is due to the proton concentration difference across the membrane. Because protons are translocated from the mitochondrial matrix to the intermembrane space, the resulting ΔE across the inner mitochondrial membrane has a negative value.

As this equation shows, both ΔE and ΔpH contribute to the proton motive force in plant mitochondria, although ΔE is consistently found to be of greater magnitude, probably because of the large buffering capacity of both cytosol and matrix, which prevents large pH changes. This situation contrasts with that in the chloroplast, where almost all of the proton motive force across the thylakoid membrane is due to ΔpH (see Chapter 7).

The free-energy input required to generate $\Delta\tilde{\mu}_{H^+}$ comes from the free energy released during electron transport. How electron transport is coupled to proton translocation is not well understood in all cases. Because of the low permeability (conductance) of the inner membrane to protons, the proton electrochemical gradient can be utilized to carry out chemical work (ATP synthesis). The $\Delta\tilde{\mu}_{H^+}$ is coupled to the synthesis of ATP by an additional protein complex associated with the inner membrane, the F_oF_1-ATP synthase.

The **F_oF_1-ATP synthase** (also called *complex V*) consists of two major components, F_o and F_1 (see Figure 11.8). **F_o** (subscript "o" for oligomycin-sensitive) is an integral membrane protein complex of at least three different polypeptides. They form the channel through which protons cross the inner membrane. The other component, **F_1**, is a peripheral membrane protein complex that is composed of at least five different subunits and contains the catalytic site for converting ADP and P_i into ATP. This complex is attached to the matrix side of F_o.

The passage of protons through the channel is coupled to the catalytic cycle of the F_1 component of the ATP synthase, allowing the ongoing synthesis of ATP and the simultaneous utilization of the $\Delta\tilde{\mu}_{H^+}$. For each ATP synthesized, 3 H^+ pass through the F_o component from the intermembrane space to the matrix, down the electrochemical proton gradient.

A high-resolution structure for the F_1 component of the mammalian ATP synthase provided evidence for a model in which a part of F_o rotates relative to F_1 to couple H^+ transport to ATP synthesis (Abrahams et al. 1994) (see WEB TOPIC 11.4). The structure and function of the mitochondrial ATP synthase is similar to that of the CF_o–CF_1 ATP synthase in chloroplasts (see Chapter 7).

The operation of a chemiosmotic mechanism of ATP synthesis has several implications. First, the true site of ATP formation on the inner mitochondrial membrane is the ATP synthase, not complex I, III, or IV. These complexes serve as sites of energy conservation whereby electron transport is coupled to the generation of a $\Delta\tilde{\mu}_{H^+}$. The synthesis of ATP decreases the $\Delta\tilde{\mu}_{H^+}$ and as a consequence, its restriction on the electron transport complexes. Electron transport is therefore stimulated by a large supply of ADP.

The chemiosmotic hypothesis also explains the action mechanism of **uncouplers**. These are a wide range of chemically unrelated, artificial compounds (including 2,4-dinitrophenol and *p*-trifluoromethoxycarbonylcyanide phenylhydrazone [FCCP]) that decrease mitochondrial

ATP synthesis but stimulate the rate of electron transport (see WEB TOPIC 11.5). All of these uncoupling compounds make the inner membrane leaky to protons, which prevents the buildup of a sufficiently large $\Delta\tilde{\mu}_{H^+}$ to drive ATP synthesis or restrict electron transport.

Transporters exchange substrates and products

The electrochemical proton gradient also plays a role in the movement of the organic acids of the citric acid cycle, and of the substrates and products of ATP synthesis, into and out of mitochondria. Although ATP is synthesized in the mitochondrial matrix, most of it is used outside the mitochondrion, so an efficient mechanism is needed for moving ADP into and ATP out of the organelle.

The ADP/ATP (adenine nucleotide) transporter performs the active exchange of ADP and ATP across the inner membrane (FIGURE 11.9). The movement of the more negatively charged ATP^{4-} out of the mitochondrion in exchange for ADP^{3-}—that is, one net negative charge out—is driven by the electrical-potential gradient (ΔE, positive outside) generated by proton pumping.

The uptake of inorganic phosphate (P_i) involves an active phosphate transporter protein that uses the chemical-potential component (ΔpH) of the proton motive force to drive the electroneutral exchange of P_i^- (in) for OH^- (out). As long as a ΔpH is maintained across the inner membrane, the P_i content within the matrix remains high. Similar reasoning applies to the uptake of pyruvate, which is driven by the electroneutral exchange of pyruvate for OH^-, leading to continued uptake of pyruvate from the cytosol (see Figure 11.9).

The total energetic cost of taking up one phosphate and one ADP into the matrix and exporting one ATP is the movement of one H^+ from the intermembrane space into the matrix:

- Moving one OH^- out in exchange for P_i^- is equivalent to 1 H^+ in, so this electroneutral exchange consumes the chemical potential, but not the electrical potential.
- Moving one negative charge out (ADP^{3-} entering the matrix in exchange for ATP^{4-} leaving), is the same as moving one positive charge in, so this transport lowers only the electrical potential.

This proton, which drives the exchange of ATP for ADP and P_i, should also be included in our calculation of the cost of synthesizing one ATP. Thus the total cost is 3 H^+ used by the ATP synthase plus 1 H^+ for the exchange across the membrane, or a total of 4 H^+.

The inner membrane also contains transporters for dicarboxylic acids (malate or succinate) exchanged for P_i^{2-} and for the tricarboxylic acid citrate exchanged for dicarboxylic acids (see Figure 11.9 and WEB TOPIC 11.5).

FIGURE 11.9 Transmembrane transport in plant mitochondria. An electrochemical proton gradient, $\Delta\tilde{\mu}_{H^+}$, consisting of an electrical potential component (ΔE, –200 mV, negative inside) and a chemical potential component (ΔpH, alkaline inside), is established across the inner mitochondrial membrane during electron transport, as outlined in the text. Specific metabolites are moved across the inner membrane by specialized proteins called transporters or carriers. (After Douce 1985.)

Aerobic respiration yields about 60 molecules of ATP per molecule of sucrose

The complete oxidation of a sucrose molecule leads to the net formation of

- Eight molecules of ATP by substrate-level phosphorylation (four from glycolysis and four from the citric acid cycle)
- Four molecules of NADH in the cytosol
- Sixteen molecules of NADH plus four molecules of $FADH_2$ (via succinate dehydrogenase) in the mitochondrial matrix

On the basis of theoretical ADP:O values (see Table 11.1), we can estimate that 52 molecules of ATP will be generated per molecule of sucrose by oxidative phosphorylation. The complete aerobic oxidation of sucrose (including substrate-level phosphorylation) results in a total of about 60 ATPs synthesized per sucrose molecule (TABLE 11.2).

Using 50 kJ mol^{-1} as the actual free energy of formation of ATP in vivo, we find that about 3010 kJ mol^{-1} of free energy is conserved in the form of ATP per mole of sucrose oxidized during aerobic respiration. This amount

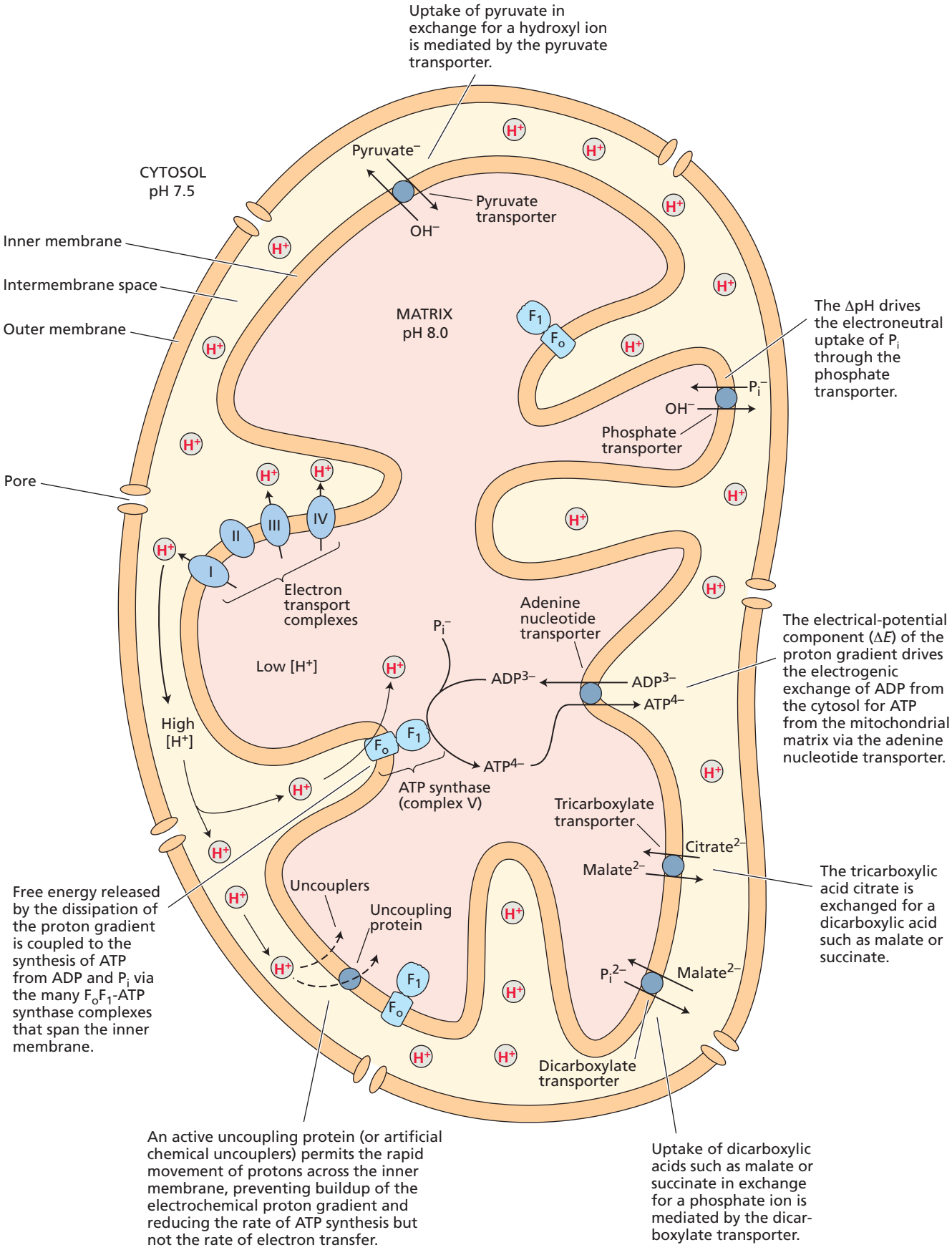
TABLE 11.2
The maximum yield of cytosolic ATP from the complete oxidation of sucrose to CO_2 via aerobic glycolysis and the citric acid cycle

Part reaction	ATP per sucrose[a]
Glycolysis	
4 substrate-level phosphorylations	4
4 NADH	$4 \times 1.5 = 6$
Citric acid cycle	
4 substrate-level phosphorylations	4
4 $FADH_2$	$4 \times 1.5 = 6$
16 NADH	$16 \times 2.5 = 40$
Total	60

Source: Adapted from Brand 1994.

Note: Cytosolic NADH is assumed oxidized by the external NADH dehydrogenase. The nonphosphorylating pathways are assumed not to be engaged.

[a]Calculated using the theoretical ADP/O values from Table 11.1



represents about 52% of the standard free energy available from the complete oxidation of sucrose; the rest is lost as heat. It also represents a vast improvement over fermentative metabolism, in which only 4% of the energy available in sucrose is converted into ATP.

Several subunits of respiratory complexes are encoded by the mitochondrial genome

The genetic system of the plant mitochondrion differs not only from that of the nucleus and the chloroplast, but also from those found in the mitochondria of animals, protists, or fungi. Most notably, processes involving RNA differ between plant mitochondria and mitochondria from most other organisms (see WEB TOPIC 11.6). Major differences are found in

- RNA splicing (for example, special introns are present)
- RNA editing (in which the nucleotide sequence is changed)
- Signals regulating RNA stability
- Translation (plant mitochondria use the universal genetic code, whereas mitochondria in other eukaryotes have deviant codons)

The size of the plant mitochondrial genome varies substantially even between closely related plant species, but at 180 to almost 3000 kilobase pairs (kbp), it is always much larger than the compact and uniform 16 kbp genome found in mammalian mitochondria. The size differences are due mainly to the presence of noncoding DNA including numerous introns, in plant **mitochondrial DNA** (**mtDNA**). Mammalian mtDNA encodes only 13 proteins, in contrast to the 35 known proteins encoded by Arabidopsis mtDNA (Marienfeld et al. 1999). Both plant and mammalian mitochondria contain genes for rRNAs and tRNAs.

Plant mtDNA encodes several subunits of respiratory complexes I–V as well as proteins that take part in cytochrome biogenesis. The mitochondrially encoded subunits are essential for the activity of the respiratory complexes.

Except for the proteins encoded by mtDNA, all mitochondrial proteins (possibly more than 2000) are encoded by nuclear DNA—including all the proteins in the citric acid cycle (Millar et al. 2005). These nuclear-encoded mitochondrial proteins are synthesized by cytosolic ribosomes and imported via translocators in the outer and inner mitochondrial membranes. Therefore, oxidative phosphorylation is dependent on expression of genes located in two separate genomes. Any change in expression of these nuclear and mitochondrial genes must therefore be coordinated.

Whereas the expression of nuclear genes for mitochondrial proteins is regulated like that of other nuclear genes, less is known about the expression of mitochondrial genes. Genes can be down-regulated by a decreased copy number for the segment of mtDNA that contains the gene (Leon et al. 1998). Also, gene promoters in mtDNA are of several kinds and show different transcriptional activities. However, the biogenesis of respiratory complexes appears to be controlled by changes in the expression of the nuclear-encoded subunits; coordination with the mitochondrial genome takes place posttranslationally (Giegé et al. 2005).

The mitochondrial genome is important for pollen development. Naturally occurring rearrangements of genes in the mtDNA lead to so called *cytoplasmic male sterility* (cms). This trait leads to perturbed pollen development by inducing a premature **programmed cell death** (see WEB ESSAY 11.5), on otherwise unaffected plants. The cms traits are used in breeding of several crop plants for making hybrid seed stocks.

Plants have several mechanisms that lower the ATP yield

As we have seen, a complex machinery is required for conserving energy in oxidative phosphorylation. So it is perhaps surprising that plant mitochondria have several functional proteins that reduce this efficiency (see WEB TOPIC 11.3). Plants are probably less limited by energy supply (sunlight) than by other factors in the environment (e.g., access to water and nutrients). As a consequence, metabolic flexibility may be more important to them than energetic efficiency.

In the following subsections we will discuss the role of three nonphosphorylating mechanisms and their possible usefulness in the life of the plant: the alternative oxidase, the uncoupling protein, and the rotenone-insensitive NADH dehydrogenases.

THE ALTERNATIVE OXIDASE If cyanide in the m*M* range is added to actively respiring animal cells, cytochrome *c* oxidase is completely inhibited, and the respiration rate quickly drops to less than 1% of its initial level. However, most plants display a capacity for *cyanide-resistant respiration* that is comparable to the capacity of the cytochrome *c* oxidase pathway. The enzyme responsible for this cyanide-resistant oxygen uptake has been identified as a ubiquinol oxidase called the **alternative oxidase** (Vanlerberghe and McIntosh 1997) (see Figure 11.8 and WEB TOPIC 11.3).

Electrons feed off the main electron transport chain into this alternative pathway at the level of the ubiquinone pool (see Figure 11.8). The alternative oxidase, the only component of the alternative pathway, catalyzes a four-electron reduction of oxygen to water and is specifically inhibited by several compounds, most notably salicylhydroxamic acid (SHAM).

When electrons pass to the alternative pathway from the ubiquinone pool, two sites of proton pumping (at complexes III and IV) are bypassed. Because there is no energy conservation site in the alternative pathway between

ubiquinone and oxygen, the free energy that would normally be conserved as ATP is lost as heat when electrons are shunted through this pathway.

How can a process as seemingly energetically wasteful as the alternative pathway contribute to plant metabolism? One example of the functional usefulness of the alternative oxidase is its activity during floral development in certain members of the Araceae (the arum family)—for example, the voodoo lily (*Sauromatum guttatum*). Just before pollination, parts of the inflorescence exhibit a dramatic increase in the rate of respiration via the alternative pathway. As a result, the temperature of the upper appendix increases by as much as 25°C over the ambient temperature. During this extraordinary burst of heat production, certain amines, indoles, and terpenes are volatilized, and the plant therefore gives off a putrid odor that attracts insect pollinators (see **WEB ESSAY 11.6**). Salicylic acid has been identified as the signal initiating this thermogenic event in the voodoo lily (Raskin et al. 1989) and was later found also to be involved in plant pathogen defense (see Chapter 13).

In most plants, the respiratory rates are too low to generate sufficient heat to raise the temperature significantly. What other role(s) does the alternative pathway play? To answer that question, we need to consider the regulation of the alternative oxidase: Its transcription is often specifically induced, for example, by various types of abiotic and biotic stress. The activity of the alternative oxidase, which functions as a dimer, is regulated by reversible oxidation–reduction of an intermolecular sulfhydryl bridge, by the reduction level of the ubiquinone pool, and by pyruvate. The first two factors ensure that the enzyme is most active under reducing conditions, while the latter factor ensures that the enzyme has high activity when there is plenty of substrate for the citric acid cycle (see **WEB TOPIC 11.3**).

If the respiration rate exceeds the cell's demand for ATP (i.e., if ADP levels are very low), the reduction level in the mitochondrion will be high, and the alternative oxidase will be activated. Thus the alternative oxidase makes it possible for the mitochondrion to adjust the relative rates of ATP production and synthesis of carbon skeletons for use in biosynthetic reactions.

Another possible function of the alternative pathway is in the response of plants to a variety of stresses (phosphate deficiency, chilling, drought, osmotic stress, and so on), many of which can inhibit mitochondrial respiration (see Chapter 26). In response to stress, the electron transport chain generates increased amounts of reactive oxygen species, which act as a signal for the activation of alternative oxidase expression. By draining off electrons from the ubiquinone pool (see Figure 11.8), the alternative pathway prevents overreduction, which, if left unchecked, can lead to the generation of destructive reactive oxygen species such as hydroxyl radicals. In this way, the alternative pathway may lessen the detrimental effects of stress on respiration (Rhoads and Subbaiah 2007; Møller 2001) (see **WEB ESSAY 11.7**). This is an example of *retrograde regulation*, in which nuclear gene expression responds to changes in organellar status (**FIGURE 11.10**).

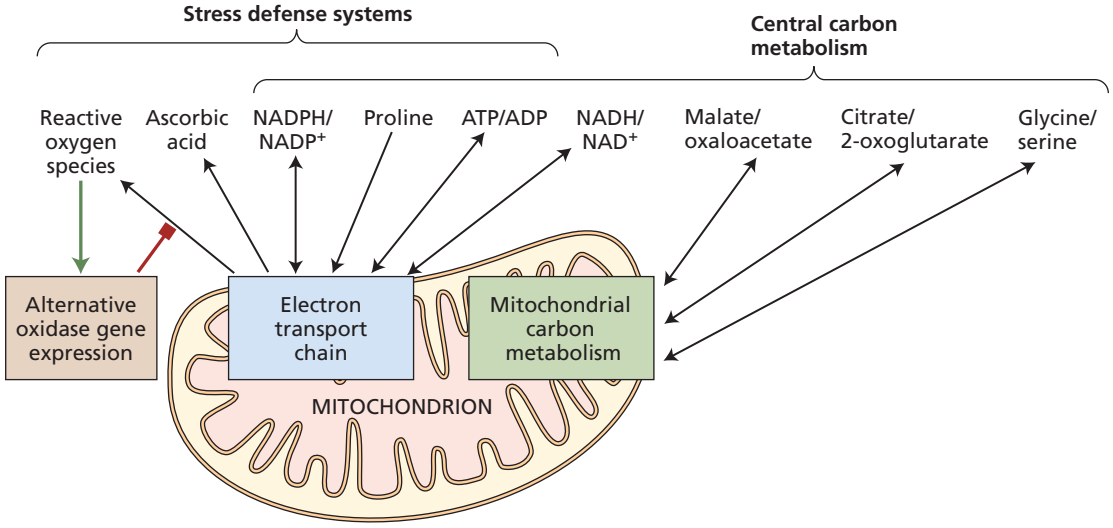


FIGURE 11.10 Metabolic interactions between mitochondria and cytosol. Mitochondrial activities can influence the cytosolic levels of redox and energy molecules involved in stress defense and in central carbon metabolism (such as growth processes and photosynthesis). An exact distinction between stress defense and carbon metabolism cannot be made because they have components in common. Arrows denote influences caused by changes in mitochondrial synthesis (e.g., reactive oxygen species [ROS], ATP, or ascorbic acid) or degradation (e.g., NAD[P]H, proline, or glycine). The ROS-mediated activation of expression of nuclear genes for the alternative oxidase is an example of retrograde regulation.

THE UNCOUPLING PROTEIN A protein found in the inner membrane of mammalian mitochondria, the **uncoupling protein**, can dramatically increase the proton permeability of the membrane and thus act as an uncoupler. As a result, less ATP and more heat are generated. Heat production appears to be one of the uncoupling protein's main functions in mammalian cells.

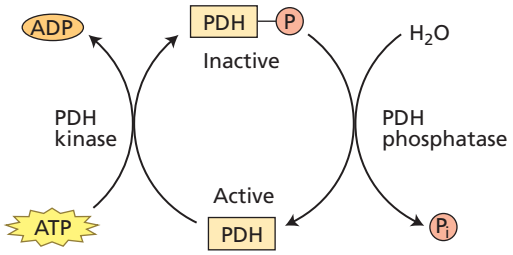
It had long been thought that the alternative oxidase in plants and the uncoupling protein in mammals were simply two different means of achieving the same end. It was therefore surprising when a protein similar to the uncoupling protein was discovered in plant mitochondria (Vercesi et al. 1995; Laloi et al. 1997). This protein is induced by stress and, like the alternative oxidase, may function to prevent overreduction of the electron transport chain and formation of reactive oxygen species (see **WEB TOPIC 11.3** and **WEB ESSAY 11.7**). It remains unclear, however, why plant mitochondria require both mechanisms.

ROTENONE-INSENSITIVE NADH DEHYDROGENASES The rotenone-insensitive NADH dehydrogenases are among the multiple NAD(P)H dehydrogenases found in plant mitochondria (see Figure 11.8 and **WEB TOPIC 11.3**). The internal, rotenone-insensitive NADH dehydrogenase (ND_{in}[NADH]) may work as a non-proton-pumping bypass when complex I is overloaded. Complex I has a higher affinity (ten times lower K_m) for NADH than ND_{in}(NADH). At lower NADH levels in the matrix, typically when ADP is available, complex I dominates, whereas when ADP is rate-limiting, NADH levels increase, and ND_{in}(NADH) is more active. For example, photorespiration leads to a massive generation of NADH from glycine oxidation in the matrix (see Chapter 8). ND_{in}(NADH) and the alternative oxidase probably recycle the NADH into NAD^+ to maintain pathway activity. Since reducing power can be shuttled from the matrix to the cytosol by the exchange of different organic acids, external NADH dehydrogenases can have bypass functions similar to those of ND_{in}(NADH). Taken together, these NADH dehydrogenases and the NADPH dehydrogenases are likely to make plant respiration more flexible and aid in the control of cellular redox homeostasis (see Figure 11.10).

Short-term control of mitochondrial respiration occurs at different levels

The substrates of ATP synthesis—ADP and P_i—appear to be key short-term regulators of the rates of glycolysis in the cytosol and of the citric acid cycle and oxidative phosphorylation in the mitochondria. Control points exist at all three stages of respiration; here we will give just a brief overview of some major features of respiratory control.

The best-characterized site of posttranslational regulation of mitochondrial respiratory metabolism is the pyruvate dehydrogenase complex, which is phosphorylated by a *regulatory kinase* and dephosphorylated by a *phosphatase*.



Pyruvate + CoA + NAD^+ ⟶ Acetyl-CoA + CO_2 + NADH

Effect on PDH activity	Mechanism
Activating	
Pyruvate	Inhibits kinase
ADP	Inhibits kinase
Mg^{2+} (or Mn^{2+})	Stimulates phosphatase
Inactivating	
NADH	Inhibits PDH Stimulates kinase
Acetyl CoA	Inhibits PDH Stimulates kinase
NH_4^+	Inhibits PDH Stimulates kinase

FIGURE 11.11 Metabolic regulation of pyruvate dehydrogenase (PDH) activity, directly and by reversible phosphorylation. Upstream and downstream metabolites regulate PDH activity by direct actions on the enzyme itself and/or by regulating its kinase or phosphatase.

Pyruvate dehydrogenase is inactive in the phosphorylated state, and the regulatory kinase is inhibited by pyruvate, allowing the enzyme to be active when substrate is available (**FIGURE 11.11**). Pyruvate dehydrogenase forms the entry point to the citric acid cycle, so this regulation adjusts the activity of the cycle to the cellular demand.

Thioredoxins control many enzymes by reversible redox dimerization of cysteine residues (see Chapter 8). Numerous mitochondrial enzymes, representing virtually all pathways, are modified by thioredoxins (Buchanan and Balmer 2005). Although the detailed mechanisms have not been worked out yet, it is likely that mitochondrial redox status exerts an important control on respiratory processes.

The citric acid cycle oxidations, and subsequently respiration, are dynamically controlled by the cellular level of adenine nucleotides. As the cell's demand for ATP in the cytosol decreases relative to the rate of synthesis of ATP in the mitochondria, less ADP is available, and the electron transport chain operates at a reduced rate (see Figure 11.9). This slowdown could be signaled to citric acid cycle enzymes through an increase in matrix NADH, which inhibits the activity of several citric acid cycle dehydrogenases (Oliver and McIntosh 1995).

The buildup of citric acid cycle intermediates (such as citrate) and their derivates (such as glutamate) inhibits the action of cytosolic pyruvate kinase, increasing the cytosolic PEP concentration, which in turn reduces the rate of conversion of fructose-6-phosphate into fructose-1,6-bisphosphate, thus inhibiting glycolysis.

In summary, plant respiratory rates are *allosterically controlled* from the "bottom up" by the cellular level of ADP (**FIGURE 11.12**). ADP initially regulates the rate of electron transfer and ATP synthesis, which in turn regulates citric acid cycle activity, which, finally, regulates the rates of the glycolytic reactions. This bottom-up control allows the respiratory carbon pathways to adjust to the demand for biosynthetic building blocks, thereby increasing respiratory flexibility.

Respiration is tightly coupled to other pathways

Glycolysis, the oxidative pentose phosphate pathway, and the citric acid cycle are linked to several other important metabolic pathways, some of which will be covered in greater detail in Chapter 12. The respiratory pathways produce the central building blocks for synthesis of a wide variety of plant metabolites, including amino acids, lipids and related compounds, isoprenoids, and porphyrins (**FIGURE 11.13**). Indeed, much of the reduced carbon that is metabolized by glycolysis and the citric acid cycle is diverted to biosynthetic purposes and not oxidized to CO_2.

Mitochondria are also integrated into the cellular redox network. Variations in consumption or production of redox and energy-carrying compounds such as NAD(P)H and organic acids are likely to affect metabolic pathways in the cytosol and in plastids. Of special importance is the synthesis of *ascorbic acid*, a central redox and stress defense molecule in plants, by the electron transport chain (see Figure 11.10) (Noctor et al. 2007). Mitochondria also carry out steps in the biosynthesis of *coenzymes* necessary for many metabolic enzymes in other cell compartments (see **WEB ESSAY 11.8**).

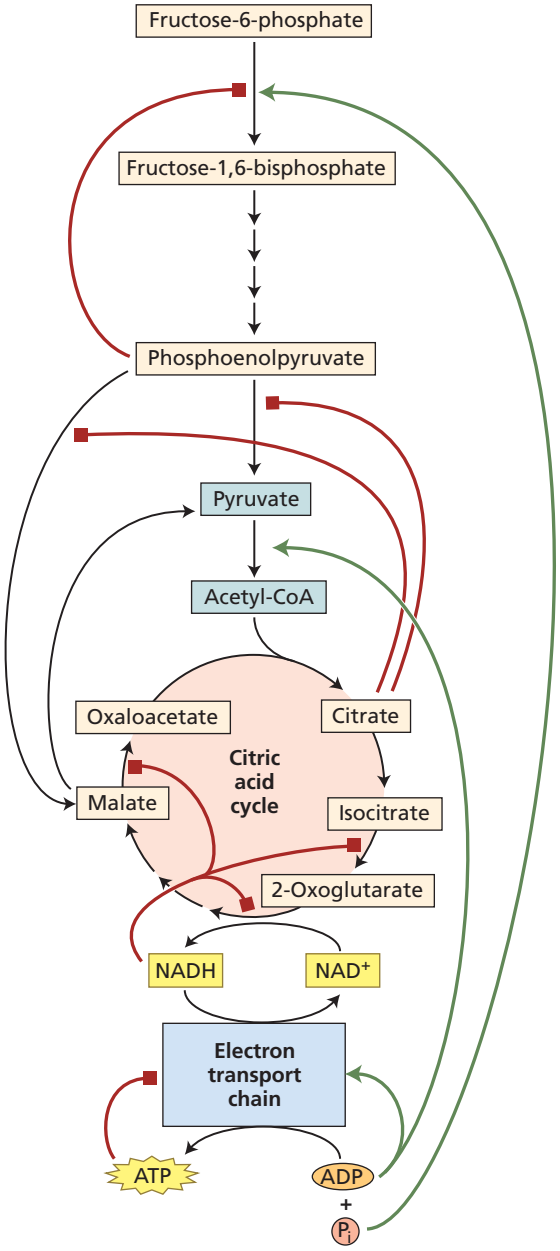


FIGURE 11.12 Model of bottom-up regulation of plant respiration. Several substrates for respiration (e.g., ADP) stimulate enzymes in early steps of the pathways (green arrows). In contrast, accumulation of products (e.g., ATP) inhibits upstream reactions (red lines and squares) in a stepwise fashion. For instance, ATP inhibits the electron transport chain, leading to an accumulation of NADH. NADH inhibits citric acid cycle enzymes such as isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase. Citric acid cycle intermediates such as citrate inhibit the PEP-metabolizing enzymes in the cytosol. Finally, PEP inhibits the conversion of fructose-6-phosphate into fructose-1,6-bisphosphate and restricts carbon flow into glycolysis. In this way, respiration can be up- or down-regulated in response to changing demands for either of its products: ATP and organic acids.

Respiration in Intact Plants and Tissues

Many rewarding studies of plant respiration and its regulation have been carried out on isolated organelles and on cell-free extracts of plant tissues. But how does this knowledge relate to the function of the whole plant in a natural or agricultural setting?

In this section we will examine respiration and mitochondrial function in the context of the whole plant under a variety of conditions. First we will explore what happens when green organs are exposed to light: Respiration and photosynthesis operate simultaneously and are functionally integrated in the cell. Next we will discuss rates of respiration in different tissues, which may be under devel-

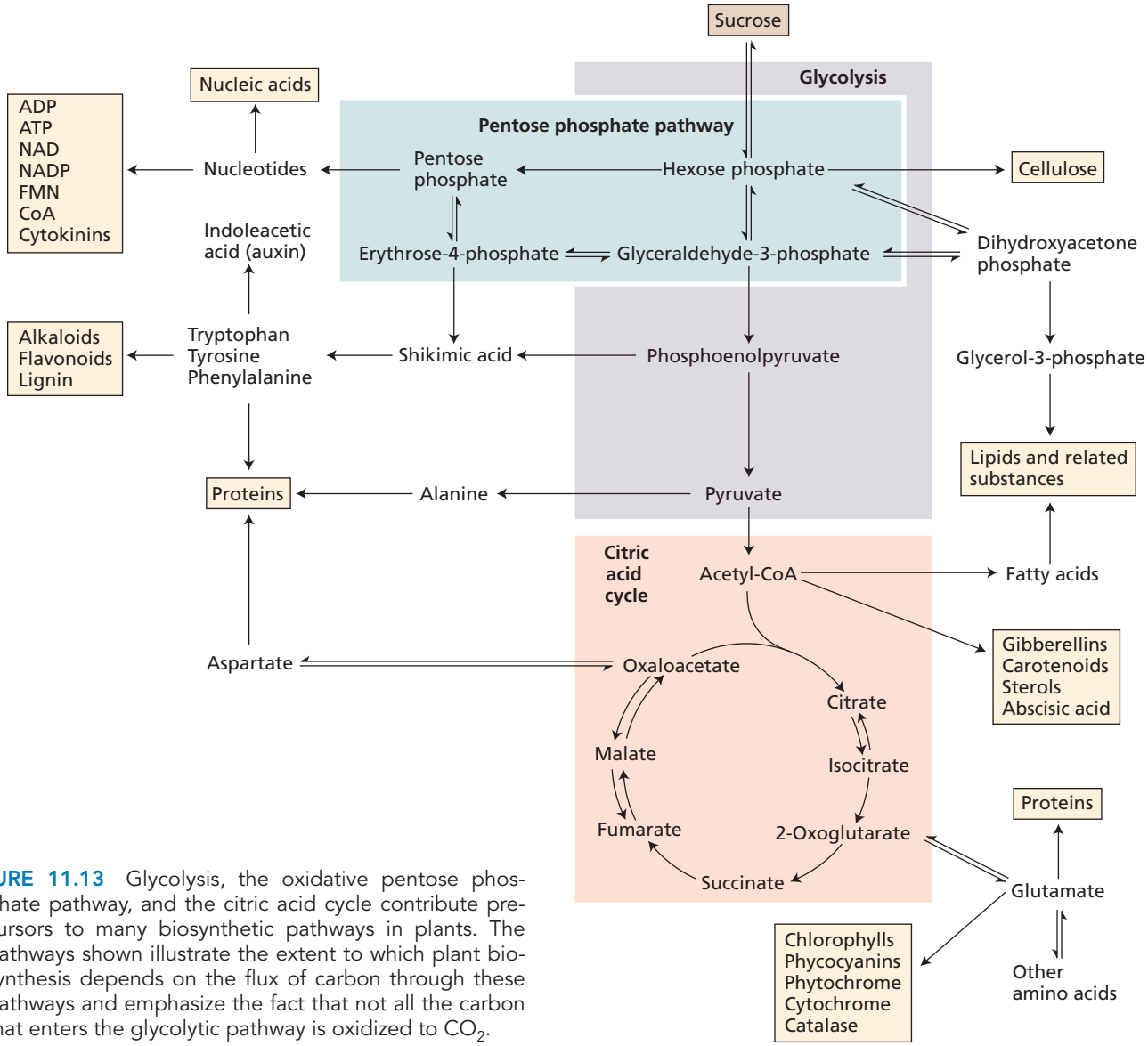


FIGURE 11.13 Glycolysis, the oxidative pentose phosphate pathway, and the citric acid cycle contribute precursors to many biosynthetic pathways in plants. The pathways shown illustrate the extent to which plant biosynthesis depends on the flux of carbon through these pathways and emphasize the fact that not all the carbon that enters the glycolytic pathway is oxidized to CO_2.

opmental control. Finally, we will look at the influence of various environmental factors on respiration rates.

Plants respire roughly half of the daily photosynthetic yield

Many factors can affect the respiration rate of an intact plant or of its individual organs. Relevant factors include the species and growth habit of the plant, the type and age of the specific organ, and environmental variables such as light, external O_2 and CO_2 concentrations, temperature, and nutrient and water supply (see Chapter 26). By measuring different oxygen isotopes, it is possible to measure the in vivo activities of the alternative oxidase and cytochrome *c* oxidase simultaneously. Therefore we know that a significant part of respiration in most tissues takes place via the "energy-wasting" alternative pathway (see **WEB ESSAY 11.9**).

Whole-plant respiration rates, particularly when considered on a fresh-weight basis, are generally lower than respiration rates reported for animal tissues. This difference is mainly due to the presence in plant cells of a large vacuole and a cell wall, neither of which contains mitochondria. Nonetheless, respiration rates in some plant tissues are as high as those observed in actively respiring animal tissues, so the plant respiratory process is not inherently slower than in animals. In fact, isolated plant mitochondria respire as fast as or faster than mammalian mitochondria.

The contribution of respiration to the overall carbon economy of the plant can be substantial. Whereas only green tissues photosynthesize, all tissues respire, and they do so 24 hours a day. Even in photosynthetically active tissues, respiration, if integrated over the entire day, utilizes a substantial fraction of gross photosynthesis. A survey of several herbaceous species indicated that 30 to 60% of the daily gain in photosynthetic carbon is lost to res-

piration, although these values tend to decrease in older plants (Lambers 1985). Trees respire a similar fraction of their photosynthetic production, but their respiratory loss increases with age as the ratio of photosynthetic to nonphotosynthetic tissue decreases.

Respiration operates during photosynthesis

Mitochondria are involved in the metabolism of photosynthesizing leaves in several ways. The glycine generated by photorespiration is oxidized to serine in the mitochondrion in a reaction involving mitochondrial oxygen consumption (see Chapter 8). At the same time, mitochondria in photosynthesizing tissue carry out normal mitochondrial respiration (i.e., via the citric acid cycle). Relative to the maximum rate of photosynthesis, rates of mitochondrial respiration measured in green tissues in the light are far slower, generally by a factor of 6- to 20-fold. Given that rates of photorespiration can often reach 20 to 40% of the gross photosynthetic rate, daytime photorespiration is a larger provider of NADH for the respiratory chain than the normal respiratory pathways.

The activity of pyruvate dehydrogenase, one of the ports of entry into the citric acid cycle, decreases in the light to 25% of its activity in darkness (Budde and Randall 1990). Consistently, the overall rate of mitochondrial respiration decreases in the light, but the extent of the decrease remains uncertain at present. It is clear, however, that the mitochondrion is a major supplier of ATP to the cytosol (e.g., for driving biosynthetic pathways) even in illuminated leaves (Krömer 1995).

Another role of mitochondrial respiration during photosynthesis is to supply precursors for biosynthetic reactions, such as the 2-oxoglutarate needed for nitrogen assimilation (see Figures 11.7C and 11.13). The formation of 2-oxoglutarate also produces NADH in the matrix, linking the process to oxidative phosphorylation or to nonphosphorylating respiratory chain activities (Hoefnagel et al. 1998; Noctor and Foyer 1998).

Additional evidence for the involvement of mitochondrial respiration in photosynthesis has been obtained in studies with mitochondrial mutants defective in respiratory complexes. Compared with the wild type, these plants have slower leaf development and photosynthesis because changes in levels of redox-active metabolites are communicated between mitochondria and chloroplasts, negatively affecting photosynthetic function (Noctor et al. 2007).

Different tissues and organs respire at different rates

Respiration is often considered to have two components of comparable magnitude. **Maintenance respiration** is needed to support the function and turnover of the tissues already present. **Growth respiration** provides the energy needed for converting sugars into the building blocks that make up new tissues. A useful rule of thumb is that the greater the overall metabolic activity of a given tissue, the higher its respiration rate. Developing buds usually show very high rates of respiration, and respiration rates of vegetative organs usually decrease from the point of growth (e.g., the leaf tip in dicotyledons and the leaf base in monocotyledons) to more differentiated regions. A well-studied example is the growing barley leaf (Thompson et al. 1998).

In mature vegetative organs, stems generally have the lowest respiration rates, whereas leaf and root respiration varies with the plant species and the conditions under which the plants are growing. Low availability of soil nutrients, for example, increases the demand for respiratory ATP production in the root. This increase reflects increased active ion uptake and root growth in search of nutrients. (See **WEB TOPIC 11.7** for a discussion of how crop yield is affected by changes in respiration rates.)

When a plant organ has reached maturity, its respiration rate either remains roughly constant or decreases slowly as the tissue ages and ultimately senesces. An exception to this pattern is the marked rise in respiration, known as the *climacteric*, that accompanies the onset of ripening in many fruits (e.g., avocado, apple, and banana) and senescence in detached leaves and flowers. Both ripening and the climacteric respiratory rise are triggered by the endogenous production of ethylene, or may be induced by an exogenous application of ethylene (see Chapter 22). In general, *ethylene-induced respiration* is associated with the cyanide-resistant alternative pathway, but the role of this pathway in ripening is not clear (Tucker 1993).

Different tissues can use different substrates for respiration. Sugars dominate overall, but in specific organs other compounds, such as organic acids in maturing apples or lemons and lipids in germinating sunflower or canola seedlings, may provide the carbon for respiration. These compounds are built with different ratios of carbon to oxygen atoms. Therefore, the ratio of CO_2 release to O_2 consumption, which is called the **respiratory quotient**, or **RQ**, varies with the substrate oxidized. Lipids, sugars, and organic acids represent a series of rising RQ because lipids contain little oxygen per carbon and organic acids much. Alcoholic fermentation releases CO_2 without consuming O_2, so a high RQ is also a marker for fermentation. Since RQ can be determined in the field, it is an important parameter in analyses of carbon metabolism on a larger scale.

Environmental factors alter respiration rates

Many environmental factors can alter the operation of metabolic pathways and change respiratory rates. Here we will examine the roles of environmental oxygen (O_2), temperature, and carbon dioxide (CO_2).

OXYGEN Oxygen can affect plant respiration because of its role as a substrate in the overall respiratory process. At 25°C, the equilibrium concentration of O_2 in an air-saturated (21%

O_2) aqueous solution is about 250 μ*M*. The K_m value for oxygen in the reaction catalyzed by cytochrome *c* oxidase is well below 1 μ*M*, so there should be no apparent dependence of the respiration rate on external O_2 concentrations. However, respiration rates decrease if the atmospheric oxygen concentration is below 5% for whole organs or below 2 to 3% for tissue slices. These findings show that oxygen supply can impose a limitation on plant respiration.

Oxygen diffuses slowly in aqueous solutions, so the network of intercellular air spaces (*aerenchyma*) found in plant tissues is needed to supply oxygen to the mitochondria. If this gaseous diffusion pathway throughout the plant did not exist, the respiration rates of many plants would be limited by an insufficient oxygen supply. Compact organs such as seeds and potato tubers have a noticeable O_2 concentration gradient from the surface to the center, which restricts the ATP/ADP ratio. Diffusion limitation is even more significant in seeds with a thick seed coat or in plant organs submerged in water. When plants are grown hydroponically, the solutions must be aerated to keep oxygen levels high in the vicinity of the roots (see Chapter 5). The problem of oxygen supply also arises with plants growing in very wet or flooded soils (see Chapter 26).

Some plants, particularly trees, have a restricted geographic distribution because of the need to maintain a supply of oxygen to their roots. For instance, dogwood (*Cornus florida*) and tulip tree poplar (*Liriodendron tulipifera*) can survive only in well-drained, aerated soils. On the other hand, many plant species are adapted to grow in flooded soils. For example, rice and sunflower rely on a network of aerenchyma running from the leaves to the roots to provide a continuous gaseous pathway for the movement of oxygen to the flooded roots.

Limitation in oxygen supply can be more severe for trees with very deep roots that grow in wet soils. Such roots must survive on anaerobic (fermentative) metabolism or develop structures that facilitate the movement of oxygen to the roots. Examples of such structures are outgrowths of the roots, called *pneumatophores*, that protrude out of the water and provide a gaseous pathway for oxygen diffusion into the roots. Pneumatophores are found in *Avicennia* and *Rhizophora*, both trees that grow in mangrove swamps under continuously flooded conditions.

TEMPERATURE Respiration operates over a wide temperature range (see **WEB ESSAYs 11.4 and 11.6**). It typically increases with temperatures between 0 and 30°C and reaches a plateau at 40 to 50°C. At higher temperatures, it again decreases because of inactivation of the respiratory machinery. The increase in respiration rate for every 10°C increase in temperature is commonly called the **temperature coefficient**, Q_{10}. This coefficient describes how respiration responds to short-term temperature changes, and it varies with plant development and external factors. On a longer time scale, plants acclimate to low temperatures by increasing their respiratory capacity so that ATP production can be continued (Atkin and Tjoelker 2003).

Low temperatures are utilized to retard postharvest respiration during the storage of fruits and vegetables, but those temperatures must be adjusted with care. For instance, when potato tubers are stored at temperatures above 10°C, respiration and ancillary metabolic activities are sufficient to allow sprouting. Below 5°C, respiration rates and sprouting are reduced, but the breakdown of stored starch and its conversion into sucrose impart an unwanted sweetness to the tubers. Therefore, potatoes are best stored at 7 to 9°C, which prevents the breakdown of starch while minimizing respiration and germination.

CARBON DIOXIDE It is common practice in commercial storage of fruits to take advantage of the effects of oxygen concentration and temperature on respiration by storing fruits at low temperatures under 2 to 3% O_2 and 3 to 5% CO_2 concentrations. The reduced temperature lowers the respiration rate, as does the reduced O_2 level. Low levels of oxygen, instead of anoxic conditions, are used to avoid lowering tissue oxygen tensions to the point at which fermentative metabolism sets in. Carbon dioxide has a limited direct inhibitory effect on respiration at the artificially high concentration of 3 to 5%.

The atmospheric CO_2 concentration is normally 360 ppm, but it is increasing as a result of human activities, and it is projected to double, to 700 ppm, before the end of the twenty-first century (see Chapter 9). The flux of CO_2 between plants and the atmosphere by photosynthesis and respiration is much larger than the flux of CO_2 to the atmosphere caused by the burning of fossil fuels. Therefore, the effects of elevated CO_2 concentrations on plant respiration will strongly influence future global atmospheric changes. Laboratory studies have shown that 700 ppm CO_2 does not directly inhibit plant respiration, but measurements on whole ecosystems indicate that respiration per biomass unit may decrease with increased CO_2 concentrations. The mechanism behind the latter effect is not yet clear, and it is at present not possible to fully predict the potential importance of plants as a sink for anthropogenic CO_2 (Gonzales-Meler et al. 2004).

Lipid Metabolism

Whereas animals use fats for energy storage, plants use them for both energy and carbon storage. Fats and oils are important storage forms of reduced carbon in many seeds, including those of agriculturally important species such as soybean, sunflower, canola, peanut, and cotton. Oils serve a major storage function in many nondomesticated plants that produce small seeds. Some fruits, such as olives and avocados, also store fats and oils.

In this final part of the chapter we will describe the biosynthesis of two types of glycerolipids: the *triacylglycerols*

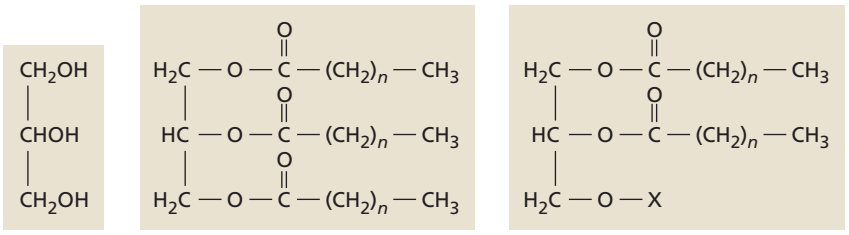


FIGURE 11.14 Structural features of triacylglycerols and polar glycerolipids in higher plants. The carbon chain lengths of the fatty acids, which always have an even number of carbons, range from 12 to 20, but are typically 16 or 18. Thus the value of *n* is usually 14 or 16.

X = H	Diacylglycerol (DAG)
X = HPO_3^-	Phosphatidic acid
X = $PO_3^- — CH_2 — CH_2 — \overset{+}{N}(CH_3)_3$	Phosphatidylcholine
X = $PO_3^- — CH_2 — CH_2 — NH_2$	Phosphatidylethanolamine
X = galactose	Galactolipids

(the fats and oils stored in seeds) and the *polar glycerolipids* (which form the lipid bilayers of cellular membranes) (**FIGURE 11.14**). We will see that the biosynthesis of triacylglycerols and polar glycerolipids requires the cooperation of two organelles: the plastids and the endoplasmic reticulum. We will also examine the complex process by which germinating seeds obtain carbon skeletons and metabolic energy from the oxidation of fats and oils.

Fats and oils store large amounts of energy

Fats and oils belong to the general class termed *lipids*, a structurally diverse group of hydrophobic compounds that are soluble in organic solvents and highly insoluble in water. Lipids represent a more reduced form of carbon than carbohydrates, so the complete oxidation of 1 g of fat or oil (which contains about 40 kJ of energy) can produce considerably more ATP than the oxidation of 1 g of starch (about 15.9 kJ). Conversely, the biosynthesis of lipids requires a correspondingly large investment of metabolic energy.

Other lipids are important for plant structure and function but are not used for energy storage. These lipids include the phospholipids that make up plant membranes, as well as sphingolipids, which are also important membrane components; waxes, which make up the protective cuticle that reduces water loss from exposed plant tissues, and terpenoids (also known as isoprenoids), which include carotenoids involved in photosynthesis and sterols present in many plant membranes (see Chapter 13).

Triacylglycerols are stored in oil bodies

Fats and oils exist mainly in the form of **triacylglycerols** (*acyl* refers to the fatty acid portion), in which fatty acid molecules are linked by ester bonds to the three hydroxyl groups of glycerol (see Figure 11.14).

The fatty acids in plants are usually straight-chain carboxylic acids having an even number of carbon atoms. The carbon chains can be as short as 12 units and as long as 30 or more, but most commonly are 16 or 18 carbons long. *Oils* are liquid at room temperature, primarily because of the presence of unsaturated bonds in their component fatty acids; *fats*, which have a higher proportion of saturated fatty acids, are solid at room temperature. The major fatty acids in plant lipids are shown in **TABLE 11.3**.

TABLE 11.3
Common fatty acids in higher plant tissues

Name[a]	Structure
Saturated fatty acids	
Lauric acid (12:0)	$CH_3(CH_2)_{10}CO_2H$
Myristic acid (14:0)	$CH_3(CH_2)_{12}CO_2H$
Palmitic acid (16:0)	$CH_3(CH_2)_{14}CO_2H$
Stearic acid (18:0)	$CH_3(CH_2)_{16}CO_2H$
Unsaturated fatty acids	
Oleic acid (18:1)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$
Linoleic acid (18:2)	$CH_3(CH_2)_4CH{=}CH{—}CH_2{—}CH{=}CH(CH_2)_7CO_2H$
Linolenic acid (18:3)	$CH_3CH_2CH{=}CH{—}CH_2{—}CH{=}CH{—}CH_2{—}CH{=}CH{—}(CH_2)_7CO_2H$

[a]Each fatty acid has a numerical abbreviation. The number before the colon represents the total number of carbons; the number after the colon is the number of double bonds.

The proportions of fatty acids in plant lipids vary with the plant species. For example, peanut oil is about 9% palmitic acid, 59% oleic acid, and 21% linoleic acid, and cottonseed oil is 25% palmitic acid, 15% oleic acid, and 55% linoleic acid. The biosynthesis of these fatty acids will be discussed shortly.

In most seeds, triacylglycerols are stored in the cytoplasm of either cotyledon or endosperm cells in organelles known as **oil bodies** (also called *spherosomes* or *oleosomes*) (see Chapter 1). The oil-body membrane is a single layer of phospholipids (i.e., a half-bilayer) with the hydrophilic ends of the phospholipids exposed to the cytosol and the hydrophobic acyl hydrocarbon chains facing the triacylglycerol interior (see Chapter 1). The oil body is stabilized by the presence of specific proteins, called *oleosins*, that coat its outer surface and prevent the phospholipids of adjacent oil bodies from coming in contact and fusing with it.

The unique membrane structure of oil bodies results from the pattern of triacylglycerol biosynthesis. Triacylglycerol synthesis is completed by enzymes located in the membranes of the endoplasmic reticulum (ER), and the resulting fats accumulate between the two monolayers of the ER membrane bilayer. The bilayer swells apart as more fats are added to the growing structure, and ultimately a mature oil body buds off from the ER (Napier et al. 1996).

Polar glycerolipids are the main structural lipids in membranes

As outlined in Chapter 1, each membrane in the cell is a bilayer of *amphipathic* (i.e., having both hydrophilic and hydrophobic regions) lipid molecules in which a polar head group interacts with the aqueous environment while hydrophobic fatty acid chains form the core of the membrane. This hydrophobic core prevents random diffusion of solutes between cell compartments and thereby allows the biochemistry of the cell to be organized.

FIGURE 11.15 Major polar glycerolipid classes found in plant membranes: glyceroglycolipids and a sphingolipid (A) and glycerophospholipids (B). At least six different fatty acids may be attached to the glycerol backbone. One of the more common molecular species is shown for each glycerolipid class. The numbers given below each name refer to the number of carbons (number before the colon) and the number of double bonds (number after the colon).

The main structural lipids in membranes are the **polar glycerolipids** (see Figure 11.14), in which the hydrophobic portion consists of two 16-carbon or 18-carbon fatty acid chains esterified to positions 1 and 2 of a glycerol backbone. The polar head group is attached to position 3 of the glycerol. There are two categories of polar glycerolipids:

1. **Glyceroglycolipids**, in which sugars form the head group (FIGURE 11.15A)
2. **Glycerophospholipids**, in which the head group contains phosphate (FIGURE 11.15B)

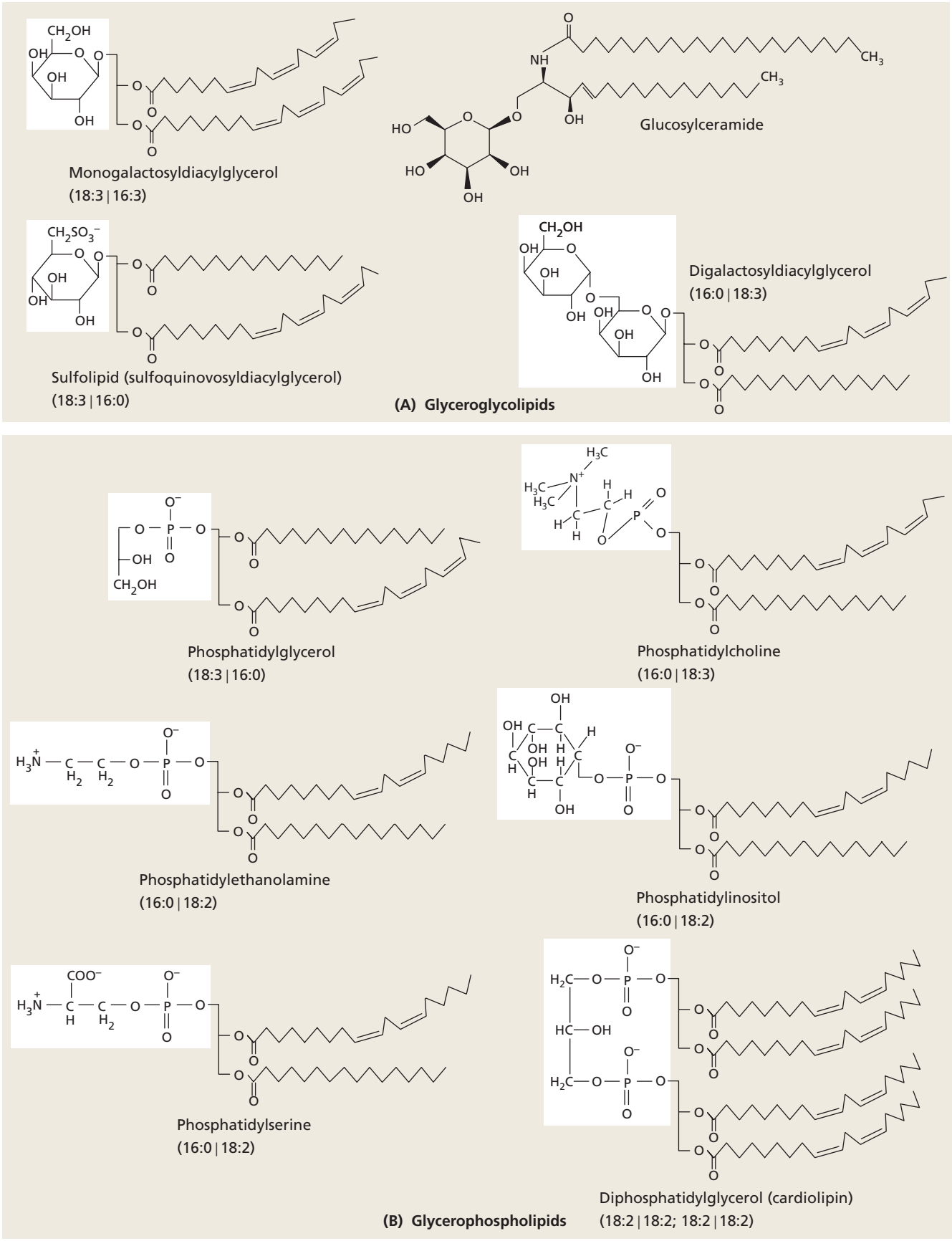
Plant membranes have additional structural lipids, including sphingolipids and sterols (see Chapter 13), but these are minor components. Other lipids perform specific roles in photosynthesis and other processes. Included among these lipids are chlorophylls, plastoquinone, carotenoids, and tocopherols, which together account for about one-third of the lipids in plant leaves.

Figure 11.15 shows the nine major glycerolipid classes in plants, each of which can be associated with many different fatty acid combinations. The structures shown in Figure 11.15 illustrate some of the more common molecular species.

Chloroplast membranes, which account for 70% of the membrane lipids in photosynthetic tissues, are dominated by glyceroglycolipids; other membranes of the cell contain glycerophospholipids (TABLE 11.4). In nonphotosynthetic tissues, glycerophospholipids are the major membrane glycerolipids.

TABLE 11.4
Glycerolipid components of cellular membranes

	Lipid composition (percentage of total)		
Lipid	Chloroplast	Endoplasmic reticulum	Mitochondrion
Phosphatidylcholine	4	47	43
Phosphatidylethanolamine	—	34	35
Phosphatidylinositol	1	17	6
Phosphatidylglycerol	7	2	3
Diphosphatidylglycerol	—	—	13
Monogalactosyldiacylglycerol	55	—	—
Digalactosyldiacylglycerol	24	—	—
Sulfolipid	8	—	—



Fatty acid biosynthesis consists of cycles of two-carbon addition

Fatty acid biosynthesis involves the cyclic condensation of two-carbon units derived from acetyl-CoA. In plants, fatty acids are synthesized exclusively in the plastids; in animals, fatty acids are synthesized primarily in the cytosol.

The enzymes of the biosynthesis pathway are thought to be held together in a complex that is collectively referred to as *fatty acid synthase*. The complex probably allows the series of reactions to occur more efficiently than it would if the enzymes were physically separated from one another. In addition, the growing acyl chains are covalently bound to a low-molecular-weight, acidic protein called the **acyl carrier protein** (**ACP**). When conjugated to the acyl carrier protein, an acyl chain is referred to as **acyl-ACP**.

The first committed step in the pathway (i.e., the first step unique to the synthesis of fatty acids) is the synthesis of malonyl-CoA from acetyl-CoA and CO_2 by the enzyme *acetyl-CoA carboxylase* (FIGURE 11.16) (Sasaki et al. 1995). The tight regulation of acetyl-CoA carboxylase appears to control the overall rate of fatty acid synthesis (Ohlrogge and Jaworski 1997). The malonyl-CoA then reacts with ACP to yield malonyl-ACP in the following four steps:

1. In the first cycle of fatty acid synthesis, the acetate group from acetyl-CoA is transferred to a specific cysteine of *condensing enzyme* (3-ketoacyl-ACP synthase) and then combined with malonyl-ACP to form acetoacetyl-ACP.

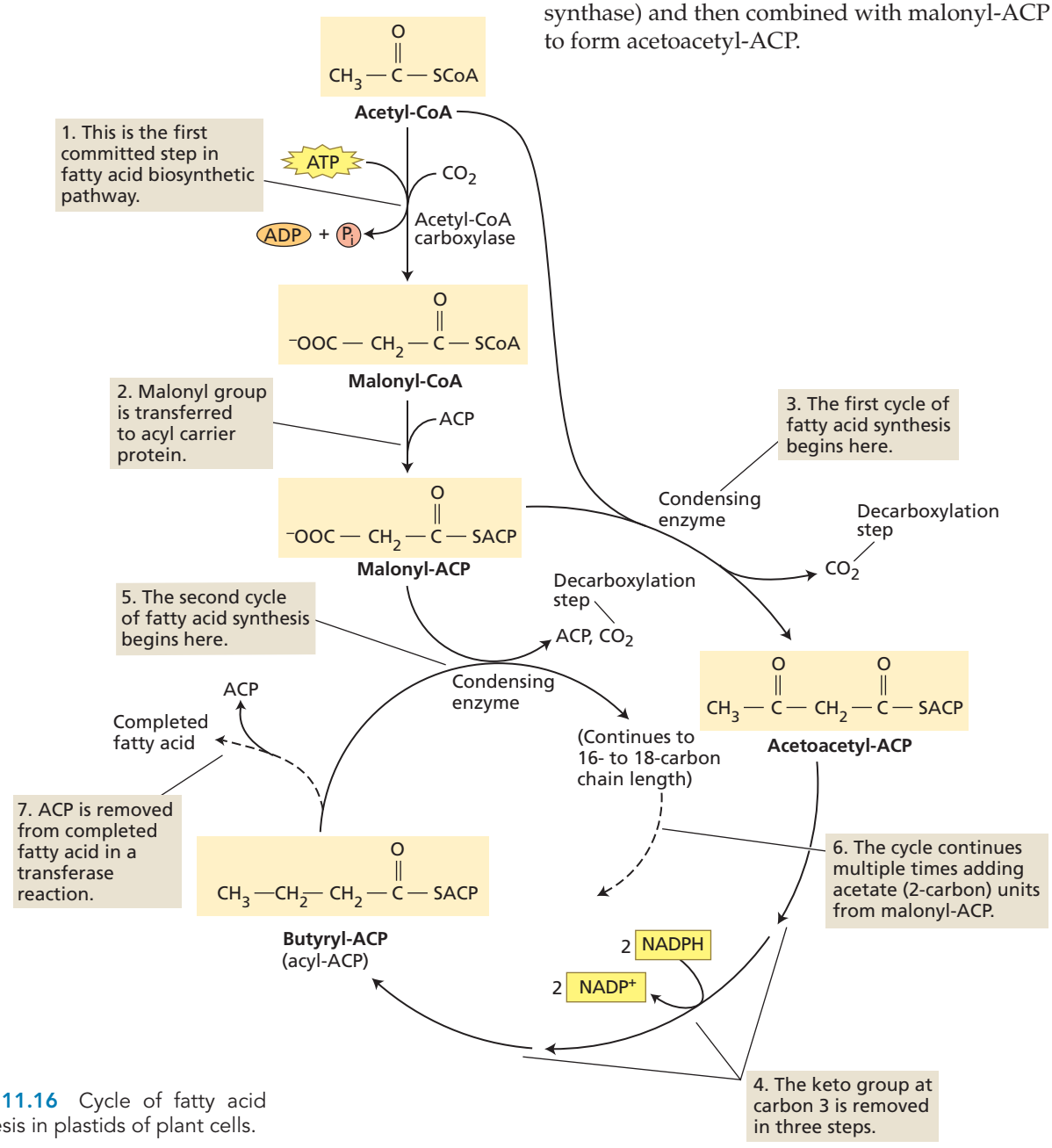


FIGURE 11.16 Cycle of fatty acid synthesis in plastids of plant cells.

2. Next the keto group at carbon 3 is removed (reduced) by the action of three enzymes to form a new acyl chain (butyryl-ACP), which is now four carbons long (see Figure 11.16).
3. The four-carbon fatty acid and another molecule of malonyl-ACP then become the new substrates for condensing enzyme, resulting in the addition of another two-carbon unit to the growing chain. The cycle continues until 16 or 18 carbons have been added.
4. Some 16:0-ACP is released from the fatty acid synthase machinery, but most molecules that are elongated to 18:0-ACP are efficiently converted into 18:1-ACP by a desaturase enzyme. Thus 16:0-ACP and 18:1-ACP are the major products of fatty acid synthesis in plastids (FIGURE 11.17).

Fatty acids may undergo further modification after they are linked with glycerol to form glycerolipids. Additional double bonds are placed in the 16:0 and 18:1 fatty acids by a series of desaturase isozymes. *Desaturase isozymes* are integral membrane proteins found in the chloroplast and the endoplasmic reticulum (ER). Each desaturase inserts a double bond at a specific position in the fatty acid chain, and the enzymes act sequentially to produce the final 18:3 and 16:3 products (Ohlrogge and Browse 1995).

Glycerolipids are synthesized in the plastids and the ER

The fatty acids synthesized in the chloroplast are next used to make the glycerolipids of membranes and oil bodies. The first steps of glycerolipid synthesis are two acylation reactions that transfer fatty acids from acyl-ACP or acyl-CoA to glycerol-3-phosphate to form **phosphatidic acid**.

The action of a specific phosphatase produces **diacylglycerol (DAG)** from phosphatidic acid. Phosphatidic acid can also be converted directly into phosphatidylinositol or phosphatidylglycerol; DAG can give rise to phosphatidylethanolamine or phosphatidylcholine (see Figure 11.17).

The localization of the enzymes of glycerolipid synthesis reveals a complex and highly regulated interaction between the chloroplast, where fatty acids are synthesized, and other membrane systems of the cell. In simple terms, the biochemistry involves two pathways referred to as the prokaryotic (or chloroplast) pathway and the eukaryotic (or ER) pathway (Ohlrogge and Browse 1995):

1. In chloroplasts, the **prokaryotic pathway** utilizes the 16:0-ACP and 18:1-ACP products of chloroplast fatty acid synthesis to synthesize phosphatidic acid and its derivatives. Alternatively, the fatty acids may be exported to the cytoplasm as CoA esters.
2. In the cytoplasm, the **eukaryotic pathway** uses a separate set of acyltransferases in the ER to incorporate the fatty acids into phosphatidic acid and its derivatives.

A simplified version of this two-pathway model is depicted in Figure 11.17.

In some higher plants, including Arabidopsis and spinach, the two pathways contribute almost equally to chloroplast lipid synthesis. In many other angiosperms, however, phosphatidylglycerol is the only product of the prokaryotic pathway, and the remaining chloroplast lipids are synthesized entirely by the eukaryotic pathway.

The biochemistry of *triacylglycerol synthesis* in oilseeds is generally the same as described for the glycerolipids. 16:0-ACP and 18:1-ACP are synthesized in the plastids of the cell and exported as CoA thioesters for incorporation into DAG in the endoplasmic reticulum (see Figure 11.17).

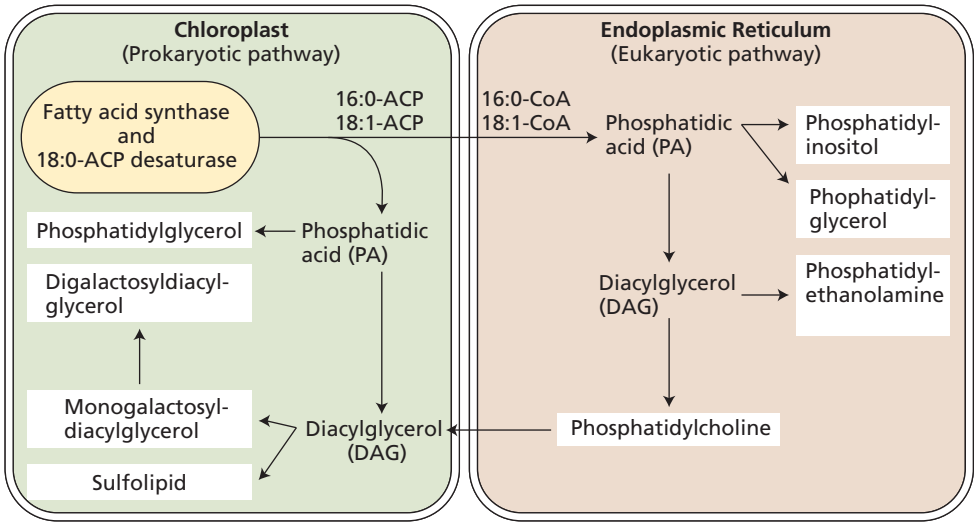


FIGURE 11.17 The two pathways for glycerolipid synthesis in the chloroplast and endoplasmic reticulum of Arabidopsis leaf cells. The major membrane components are shown in boxes. Glycerolipid desaturases in the chloroplast, and enzymes in the ER convert 16:0 and 18:1 fatty acids into the more highly unsaturated fatty acids shown in Figure 11.15.

The key enzymes in oilseed metabolism (not shown in Figure 11.17) are *acyl-CoA:DAG acyltransferase* and *PC:DAG acyltransferase*, which catalyze triacylglycerol synthesis (Dahlqvist et al. 2000). As noted earlier, triacylglycerol molecules accumulate in specialized subcellular structures—the oil bodies—from which they can be mobilized during germination and converted into sugars.

Lipid composition influences membrane function

A central question in membrane biology is the functional reason behind lipid diversity. Each membrane system of the cell has a characteristic and distinct complement of lipid types, and within a single membrane, each class of lipids has a distinct fatty acid composition (see Table 11.4).

Our understanding of a membrane is one in which lipids make up the fluid, semipermeable bilayer that is the matrix for the functional membrane proteins. Since this bulk lipid role could be satisfied by a single unsaturated species of phosphatidylcholine, such a simple model is obviously unsatisfactory. Why is lipid diversity needed? One aspect of membrane biology that might offer answers to this central question is the relationship between lipid composition and the ability of organisms to adjust to temperature changes (Iba 2002). For example, chill-sensitive plants experience sharp reductions in growth rate and development at temperatures between 0 and 12°C (see Chapter 26). Many economically important crops, such as cotton, soybean, maize, rice, and many tropical and subtropical fruits, are classified as chill-sensitive. In contrast, most plants that originate from temperate regions are able to grow and develop at chilling temperatures and are classified as chill-resistant plants.

It has been suggested that, because of the decrease in lipid fluidity at lower temperatures, the primary event of chilling injury is a *transition from a liquid-crystalline phase to a gel phase* in cellular membranes. According to this hypothesis, such a transition would result in alterations in the metabolism of chilled cells and would lead to injury and death of the chill-sensitive plants. The degree of unsaturation of the fatty acids would determine the temperature at which such damage occurred.

Recent research, however, suggests that the relationship between membrane unsaturation and plant responses to temperature is more subtle and complex (see **WEB TOPIC 11.8**). The responses of Arabidopsis mutants with increased saturation of fatty acids to low temperatures are not what is predicted by the chill-sensitivity hypothesis, suggesting that normal chilling injury may not be strictly related to the level of unsaturation of membrane lipids.

On the other hand, experiments with transgenic tobacco plants that are chill-sensitive show opposite results. The transgenic expression of exogenous genes in tobacco has been used specifically to decrease the level of saturated phosphatidylglycerol or to bring about a general increase in membrane unsaturation. In each case, damage caused by chilling was alleviated to some extent.

These new findings make it clear that either the extent of membrane unsaturation or the presence of particular lipids, such as desaturated phosphatidylglycerol, can affect the responses of plants to low temperatures. As discussed in **WEB TOPIC 11.8**, more work is required to fully understand the relationship between lipid composition and membrane function.

Membrane lipids are precursors of important signaling compounds

Plants, animals, and microbes all use membrane lipids as precursors for compounds that are used for intracellular or long-range signaling. For example, jasmonate—derived from linolenic acid (18:3)—activates plant defenses against insects and many fungal pathogens (see Chapter 13). In addition, jasmonate regulates other aspects of plant growth, including the development of anthers and pollen (Browse 2009).

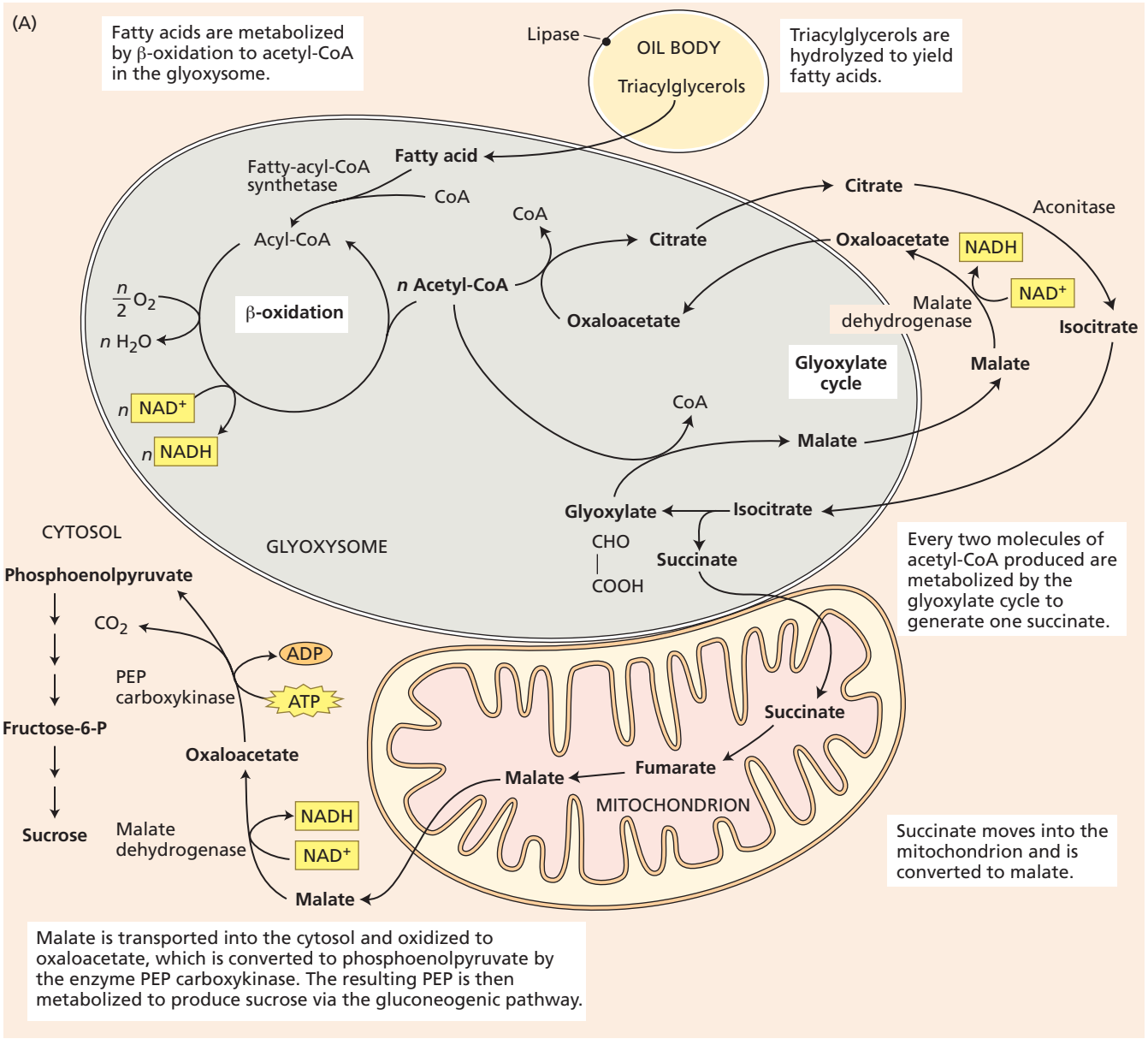
Phosphatidylinositol-4,5-bisphosphate (PIP_2) is the most important of several phosphorylated derivatives of phosphatidylinositol known as *phosphoinositides*. In animals, receptor-mediated activation of phospholipase C leads to the hydrolysis of PIP_2 into inositol trisphosphate ($InsP_3$) and diacylglycerol, both of which act as intracellular secondary messengers.

The action of $InsP_3$ in releasing Ca^{2+} into the cytoplasm (through Ca^{2+}-sensitive channels in the tonoplast and other membranes), and thereby regulating cellular processes, has been demonstrated in several plant systems, including the stomatal guard cells (Schroeder et al. 2001). Information about other types of lipid signaling in plants is becoming available through biochemical and molecular genetic studies of phospholipases (Wang 2001) and other enzymes involved in the generation of these signals.

Storage lipids are converted into carbohydrates in germinating seeds

After germinating, oil-containing seeds metabolize stored triacylglycerols by converting them into sucrose (Graham 2008). Plants are not able to transport fats from the cotyledons to other tissues of the germinating seedling, so they must convert stored lipids into a more mobile form of carbon, generally sucrose. This process involves several steps that are located in different cellular compartments: oil bodies, glyoxysomes, mitochondria, and the cytosol.

OVERVIEW: LIPIDS TO SUCROSE In oilseeds, the conversion of lipids into sucrose is triggered by germination. It begins with the hydrolysis of triacylglycerols stored in oil bodies into free fatty acids, followed by oxidation of those fatty acids to produce acetyl-CoA (**FIGURE 11.18**). The



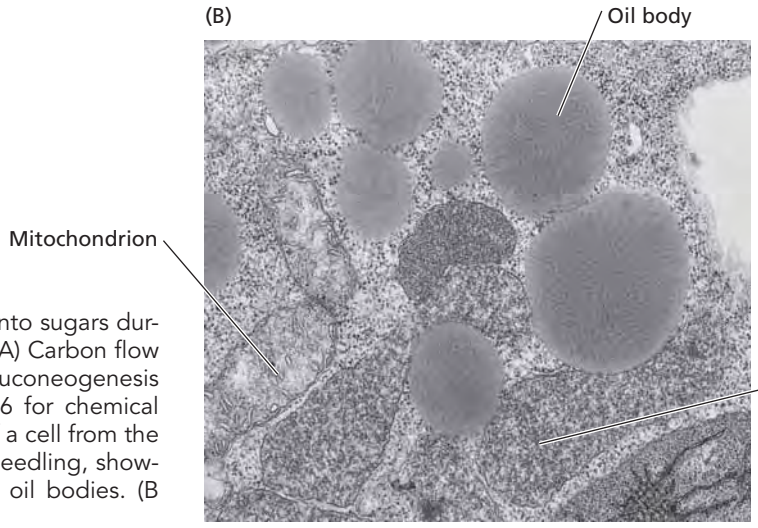


FIGURE 11.18 The conversion of fats into sugars during germination in oil-storing seeds. (A) Carbon flow during fatty acid breakdown and gluconeogenesis (refer to Figures 11.2, 11.3, and 11.6 for chemical structures). (B) Electron micrograph of a cell from the oil-storing cotyledon of a cucumber seedling, showing glyoxysomes, mitochondria, and oil bodies. (B courtesy of R. N. Trelease.)

fatty acids are oxidized in a type of peroxisome called a **glyoxysome**, an organelle enclosed by a single membrane bilayer that is found in the oil-rich storage tissues of seeds. Acetyl-CoA is metabolized in the glyoxysome and cytoplasm (see Figure 11.18A) to produce succinate, which is transported from the glyoxysome to the mitochondrion, where it is converted first into fumarate and then into malate. The process ends in the cytosol with the conversion of malate into glucose via gluconeogenesis, and then into sucrose. In most oilseeds, approximately 30% of the acetyl-CoA is used for energy production via respiration, and the rest is converted into sucrose.

LIPASE-MEDIATED HYDROLYSIS The initial step in the conversion of lipids into carbohydrates is the breakdown of triacylglycerols stored in oil bodies by the enzyme lipase, which hydrolyzes triacylglycerols into three fatty acid molecules and one molecule of glycerol. During the breakdown of lipids, oil bodies and glyoxysomes are generally in close physical association (see Figure 11.18B).

β–OXIDATION OF FATTY ACIDS The fatty acid molecules enter the glyoxysome, where they are activated by conversion into fatty-acyl-CoA by the enzyme *fatty-acyl-CoA synthetase*. Fatty-acyl-CoA is the initial substrate for the **β-oxidation** series of reactions, in which C_n fatty acids (fatty acids composed of n carbons) are sequentially broken down into $n/2$ molecules of acetyl-CoA (see Figure 11.18A). This reaction sequence involves the reduction of ½ O_2 to H_2O and the formation of one NADH for each acetyl-CoA produced.

In mammalian tissues, the four enzymes associated with β-oxidation are present in the mitochondrion. In plant seed storage tissues, they are located exclusively in the glyoxysome or the equivalent organelle in vegetative tissues, the peroxisome (see Chapter 1).

THE GLYOXYLATE CYCLE The function of the **glyoxylate cycle** is to convert two molecules of acetyl-CoA into succinate. The acetyl-CoA produced by β-oxidation is further metabolized in the glyoxysome through a series of reactions that make up the glyoxylate cycle (see Figure 11.18A). Initially, the acetyl-CoA reacts with oxaloacetate to give citrate, which is then transferred to the cytoplasm for isomerization to isocitrate by aconitase. Isocitrate is reimported into the glyoxysome and converted into malate by two reactions that are unique to the glyoxylate cycle:

1. First, isocitrate (C_6) is cleaved by the enzyme isocitrate lyase to give succinate (C_4) and glyoxylate (C_2). The succinate is exported to the mitochondria.
2. Next, malate synthase combines a second molecule of acetyl-CoA with glyoxylate to produce malate.

Malate is then transferred to the cytoplasm and converted into oxaloacetate by the cytoplasmic isozyme of malate dehydrogenase. Oxaloacetate is reimported into the glyoxysome and combines with another acetyl-CoA to continue the cycle (see Figure 11.18A). The glyoxylate produced keeps the cycle operating, but the succinate is exported to the mitochondria for further processing.

THE MITOCHONDRIAL ROLE Moving from the glyoxysomes to the mitochondria, the succinate is converted into malate by the normal citric acid cycle reactions. The resulting malate can be exported from the mitochondria in exchange for succinate via the dicarboxylate transporter located in the inner mitochondrial membrane. Malate is then oxidized to oxaloacetate by malate dehydrogenase in the cytosol, and the resulting oxaloacetate is converted into carbohydrates by the reversal of glycolysis (gluconeogenesis). This conversion requires circumventing the irreversibility of the pyruvate kinase reaction (see Figure 11.3) and is facilitated by the enzyme PEP carboxykinase, which utilizes the phosphorylating ability of ATP to convert oxaloacetate into PEP and CO_2 (see Figure 11.18A).

From PEP, gluconeogenesis can proceed to the production of glucose, as described earlier. Sucrose is the final product of this process, and is the primary form of reduced carbon translocated from the cotyledons to the growing seedling tissues. Not all seeds quantitatively convert fat into sugar, however (see **WEB TOPIC 11.9**).

SUMMARY

Using the building blocks provided by photosynthesis, respiration releases the energy stored in carbon compounds in a controlled manner for cellular use. At the same time it generates many carbon precursors for biosynthesis and cellular functions.

Overview of Plant Respiration

- In plant respiration, reduced cellular carbon generated by photosynthesis is oxidized to CO_2 and water, and this oxidation is coupled to the synthesis of ATP.
- Respiration takes place by four main processes: glycolysis, the oxidative pentose phosphate pathway, the citric acid cycle, and oxidative phosphorylation (the electron transport chain and ATP synthesis) (**Figure 11.1**).

SUMMARY continued

Glycolysis

- In glycolysis, carbohydrates are converted into pyruvate in the cytosol, and a small amount of ATP is synthesized via substrate-level phosphorylation. NADH is also produced (**Figure 11.3**).
- Plant glycolysis has alternative enzymes for several steps. These allow differences in substrates used, products made, and the direction of the pathway.
- When O_2 is not available, fermentation regenerates NAD^+ for glycolysis. Only a minor fraction of the energy available in sugars is conserved by fermentation (**Figure 11.3**).
- Plant glycolysis is regulated from the "bottom up" by its products.

The Oxidative Pentose Phosphate Pathway

- Carbohydrates can be oxidized via the oxidative pentose phosphate pathway, which provides biosynthetic building blocks and reducing power as NADPH (**Figure 11.4**).

The Citric Acid Cycle

- Pyruvate is oxidized to CO_2 within the mitochondrial matrix through the citric acid cycle, generating a large number of reducing equivalents in the form of NADH and $FADH_2$ (**Figures 11.5, 11.6**).
- A unique feature of the plant citric acid cycle is malic enzyme, which participates in alternative pathways for the metabolism of malate derived from glycolysis (**Figures 11.6, 11.7**).

Mitochondrial Electron Transport and ATP Synthesis

- Electron transport from NADH and $FADH_2$ to oxygen is coupled by enzyme complexes to proton transport across the inner mitochondrial membrane. This generates an electrochemical proton gradient used for powering synthesis and export of ATP (**Figure 11.8, 11.9**).
- During aerobic respiration, up to 60 molecules of ATP are produced per molecule of sucrose (**Table 11.2**).
- Typical for plant respiration is the presence of several proteins (alternative oxidase, NAD(P)H dehydrogenases, and uncoupling protein) that lower the energy recovery (**Figure 11.8**).
- The main products of the respiratory process are ATP and metabolic intermediates used in biosynthesis. The cellular demand for these compounds regulates respiration via control points in the electron transport chain, the citric acid cycle, and glycolysis (**Figures 11.10–11.13**).

Respiration in Intact Plants and Tissues

- More than 50% of the daily photosynthetic yield may be respired by a plant.
- Many factors can affect the respiration rate observed at the whole-plant level. These factors include the nature and age of the plant tissue and environmental factors such as light, temperature, nutrient and water supply, and O_2 and CO_2 concentrations.

Lipid Metabolism

- Triacylglycerols (fats and oils) are an efficient form for storage of reduced carbon, particularly in seeds. Polar glycerolipids are the primary structural components of membranes (**Figures 11.14, 11.15; Tables 11.3, 11.4**).
- Triacylglycerols are synthesized in the ER and accumulate within the phospholipid bilayer, forming oil bodies.
- Fatty acids are synthesized in plastids using acetyl-CoA, in cycles of two-carbon addition. Fatty acids from the plastids can be transported to the ER, where they are further modified (**Figures 11.16, 11.17**).
- The function of a membrane may be influenced by its lipid composition. The degree of unsaturation of the fatty acids influences the sensitivity of plants to cold, but does not seem to be involved in normal chilling injury.
- Certain membrane lipid breakdown products, such as jasmonic acid, can act as signaling agents in plant cells.
- During germination in oil-storing seeds, the stored lipids are metabolized to carbohydrates in a series of reactions that include the glyoxylate cycle. The glyoxylate cycle takes place in glyoxysomes, and subsequent steps occur in the mitochondria (**Figure 11.18**).
- The reduced carbon generated during lipid breakdown in the glyoxysomes is ultimately converted into carbohydrates in the cytosol by gluconeogenesis (**Figure 11.18**).

WEB MATERIAL

Web Topics

11.1 Isolation of Mitochondria
Intact, functional mitochondria can be purified for analysis in vitro.

11.2 The Q-Cycle Explains How Complex III Pumps Protons across the Inner Mitochondrial Membrane
A cyclic process allows for a higher proton-to-electron stoichiometry.

11.3 Multiple Energy Conservation Bypasses in Oxidative Phosphorylation of Plant Mitochondria
With molecular characterization, the physiological roles of the enigmatic "energy-wasting" pathways of respiration are being uncovered.

11.4 F_oF_1-ATP Synthases: The World's Smallest Rotary Motors
Rotation of the γ subunit brings about the conformational changes that couple proton flux to ATP synthesis.

11.5 Transport Into and Out of Plant Mitochondria
Plant mitochondria transport metabolites, coenzymes, and macromolecules.

11.6 The Genetic System in Plant Mitochondria Has Several Special Features
The mitochondrial genome encodes about 40 mitochondrial proteins.

11.7 Does Respiration Reduce Crop Yields?
Crop yield is correlated with low respiration rates in a way that is not understood.

11.8 The Lipid Composition of Membranes Affects the Cell Biology and Physiology of Plants
Lipid mutants are expanding our understanding of the ability of organisms to adapt to temperature changes.

11.9 Utilization of Oil Reserves in Cotyledons
In some species, only part of the stored lipid in the cotyledons is exported as carbohydrate.

Web Essay

11.1 Metabolic Flexibility Helps Plants to Survive Stress
The ability of plants to carry out a metabolic step in different ways increases plant survival under stress.

11.2 Metabolic Profiling of Plant Cells
Metabolic profiling complements genomics and proteomics.

11.3 Mitochondrial Dynamics: When Form Meets Function
New microscopy methods have shown that mitochondria dynamically change shape in vivo.

11.4 Seed Mitochondria and Stress Tolerance
Seeds experience a large range of stresses and are dependent on respiration for germination.

11.5 Balancing Life and Death: The Role of the Mitochondrion in Programmed Cell Death
Programmed cell death is an integral part of the life cycle of plants, often directly involving mitochondria.

11.6 Respiration by Thermogenic Flowers
The temperature of thermogenic flowers, such as the *Arum* lilies, can increase up to 35°C above their surroundings.

11.7 Reactive Oxygen Species (ROS) and Plant Respiration
The production of reactive oxygen species is an unavoidable consequence of aerobic respiration.

11.8 Coenzyme Synthesis in Plant Mitochondria
Pathways for synthesis of coenzymes are often split between organelles.

11.9 In Vivo Measurement of Plant Respiration
The activities of the alternative oxidase and cytochrome *c* oxidase can be simultaneously measured.

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