Chapter

11

Respiration and Lipid Metabolism

PHOTOSYNTHESIS PROVIDES the organic building blocks that plants (and nearly all other life) 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. In the first part of this chapter we will review respiration in its metabolic context, emphasizing the interconnections 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.

In the second part of the chapter we will describe the pathways of lipid biosynthesis that lead to the accumulation of fats and oils, which many plants use for 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 the degradation products to sugars that occurs during seed germination.

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, free energy is released and transiently stored in a compound, ATP, which can be readily utilized for the maintenance and development of the plant.

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

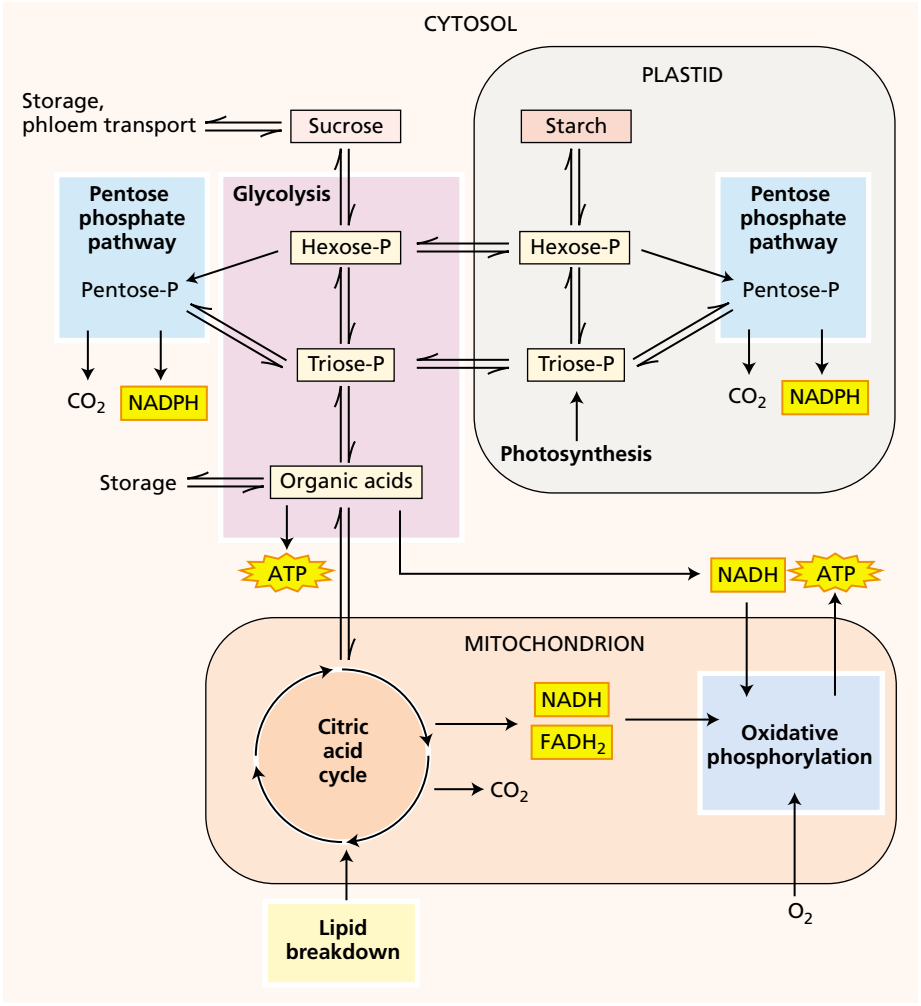


FIGURE 11.1 Overview of respiration. Substrates for respiration are generated by other cellular processes and enter the respiratory pathways. Glycolysis and the pentose phosphate pathways in the cytosol and plastid convert sugars to organic acids, via hexose phosphates and triose phosphates, generating 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.

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, being reduced to water. The standard free-energy decrease for the reaction as written is 5760 kJ (1380 kcal) per mole (342 g) of sucrose oxidized. The controlled release of this free energy, along with its coupling to the synthesis of ATP, is the primary, though by no means 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 citric acid cycle, the reactions of the pentose phosphate pathway, and oxidative phosphorylation. 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 carried out by a group of soluble enzymes located in both the cytosol and the plastid. 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—for example, pyruvate. The process yields a small amount of energy as ATP, and reducing power in the form of a reduced pyridine nucleotide, NADH.
- In the **pentose phosphate pathway**, also located both in the cytosol and the plastid, the six-carbon glucose-6-phosphate is initially oxidized to the five-carbon ribulose-5-phosphate. The carbon is lost as CO_2, and reducing power is conserved in the form of two molecules of another reduced pyridine nucleotide, NADPH. In the following near-equilibrium reactions, ribulose-5-phosphate is converted into three- to seven-carbon sugars.

- In the **citric acid cycle**, pyruvate is oxidized completely to CO_2, and a considerable amount of reducing power (16 NADH + 4 $FADH_2$ equivalents per sucrose) is generated in the process. With one exception (succinate dehydrogenase), these reactions involve a series of enzymes located in the internal aqueous compartment, or matrix, of the mitochondrion (see Figure 11.5). 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 during glycolysis, the 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**. This final stage completes the oxidation of sucrose.

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, and it undergoes a reversible two-electron reaction that yields NADH (Figure 11.2):

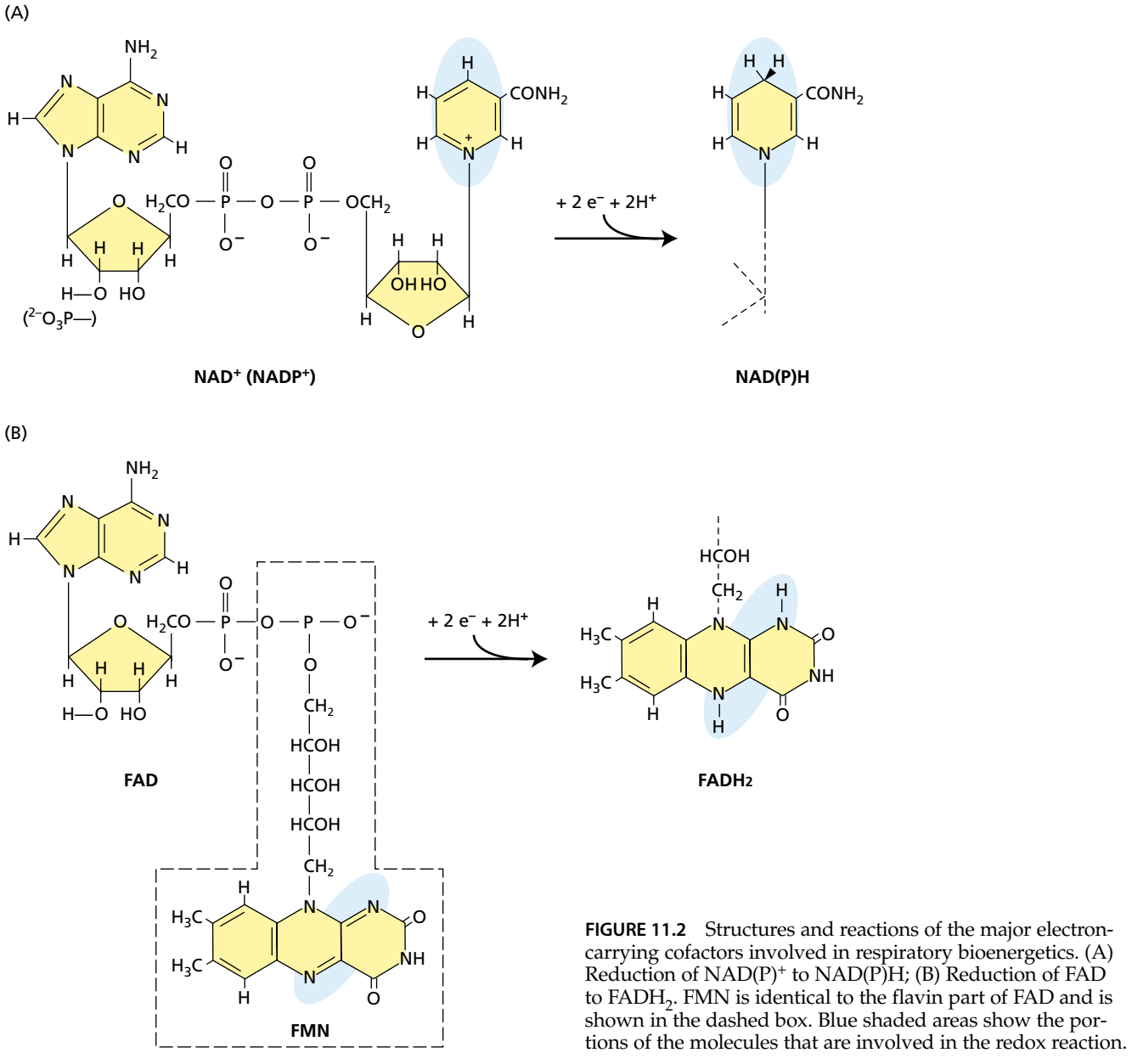


FIGURE 11.2 Structures and reactions of the major electron-carrying cofactors involved in respiratory bioenergetics. (A) Reduction of $NAD(P)^+$ to NAD(P)H; (B) Reduction of FAD to $FADH_2$. FMN is identical to the flavin part of FAD and is shown in the dashed box. Blue shaded areas show the portions of the molecules that are involved in the redox reaction.

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

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 electrons released during the stepwise oxidations of glycolysis and the citric acid cycle. A related compound, nicotinamide adenine dinucleotide phosphate ($NADP^+$/NADPH), functions in redox reactions of photosynthesis (see Chapter 8) and of the oxidative pentose phosphate pathway; it also takes part in mitochondrial metabolism (Møller and Rasmusson 1998). This 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}, or 52 kcal mol^{-1}) that drives the synthesis of ATP. We can now 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 (see Figure 11.13).

GLYCOLYSIS: A CYTOSOLIC AND PLASTIDIC PROCESS

In the early steps of glycolysis (from the Greek words *glykos*, "sugar," and *lysis*, "splitting"), carbohydrates are converted to hexose phosphates, which are then split into two triose phosphates. In a subsequent energy-conserving phase, the triose phosphates are oxidized and rearranged to yield two molecules 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 **fermentation pathways**, which are localized in the cytosol, 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 for plant cells. We will end by discussing the pentose phosphate pathway.

Glycolysis Converts Carbohydrates into Pyruvate, Producing NADH and ATP

Glycolysis occurs in all living organisms (prokaryotes and eukaryotes). The principal reactions associated with the classic glycolytic and fermentative pathways in plants are almost identical with those of animal cells (Figure 11.3). However, plant glycolysis has unique regulatory features, as well as a parallel partial glycolytic pathway in plastids and alternative enzymatic routes for several cytosolic steps. In animals the substrate of glycolysis is glucose and the end product 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 broken down into the two monosaccharides—glucose and fructose—which can readily enter the glycolytic pathway. Two pathways for the degradation of sucrose are known in plants, both of which also take part in the unloading of sucrose from the phloem (see Chapter 10).

In most plant tissues sucrose synthase, localized in the cytosol, is used to degrade sucrose by combining 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). In some tissues, invertases present in the cell wall, vacuole, or cytosol hydrolyze sucrose to its two component hexoses (glucose and fructose). The hexoses are then phosphorylated in a reaction that uses ATP. Whereas the sucrose synthase reaction is close to equilibrium, the invertase reaction releases sufficient energy to be essentially irreversible.

Plastids such as chloroplasts or amyloplasts (see Chapter 1) can also supply substrates for glycolysis. Starch is synthesized and catabolized only in plastids (see Chapter 8), and carbon obtained from starch degradation enters the glycolytic pathway in the cytosol primarily as hexose phosphate (which is translocated out of amyloplasts) or triose phosphate (which is translocated out of chloroplasts). Photosynthetic products can also directly enter the glycolytic pathway as triose phosphate (Hoefnagel et al. 1998).

Plastids convert starch into triose phosphates using a separate set of glycolytic isozymes that convert hexose phosphates to triose phosphates. All the enzymes shown in Figure 11.3 have been measured at levels sufficient to support the respiration rates observed in intact plant tissues.

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 that are catalyzed by hexokinase and phosphofructokinase (see Figure 11.3). The phosphofructokinase reaction is one of the control points of glycolysis in both plants and animals.

The energy-conserving phase of glycolysis. The reactions discussed thus far transfer carbon from the various substrate pools into 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 of hydrolysis (–49.3 kJ mol^{-1}, or –11.8 kcal 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. As we will see, ATP synthesis by substrate-level phosphorylation is mechanistically distinct from ATP synthesis by ATP synthases involved in the oxidative phosphorylation in mitochondria (which will be described later in this chapter) or photophosphorylation in chloroplasts (see Chapter 7).

In the following reaction, the phosphate on 3-phosphoglycerate is transferred to carbon 2 and a molecule of water is removed, yielding the compound phosphoenylpyruvate (PEP). The phosphate group on PEP has a high standard free energy of hydrolysis (–61.9 kJ mol^{-1}, or –14.8 kcal 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 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 sugar from organic acids. This process is known as **gluconeogenesis**.

Gluconeogenesis is not common in plants, but it does operate in the seeds of some plants, such as castor bean and sunflower, that store a significant quantity of their carbon reserves in the form of oils (triacylglycerols). After the seed germinates, much of the oil is converted by gluconeogenesis to 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 for plants.

Because the glycolytic reaction catalyzed by ATP-dependent phosphofructokinase is essentially irreversible (see Figure 11.3), an additional enzyme, fructose-1,6-bisphosphatase, converts fructose-1,6-bisphosphate to fructose-6-phosphate and P_i during gluconeogenesis. ATP-dependent phosphofructokinase and fructose-1,6-bisphosphatase represent a major control point of carbon flux through the glycolytic/gluconeogenic pathways in both plants and animals, as well as in sucrose synthesis in plants (see Chapter 8).

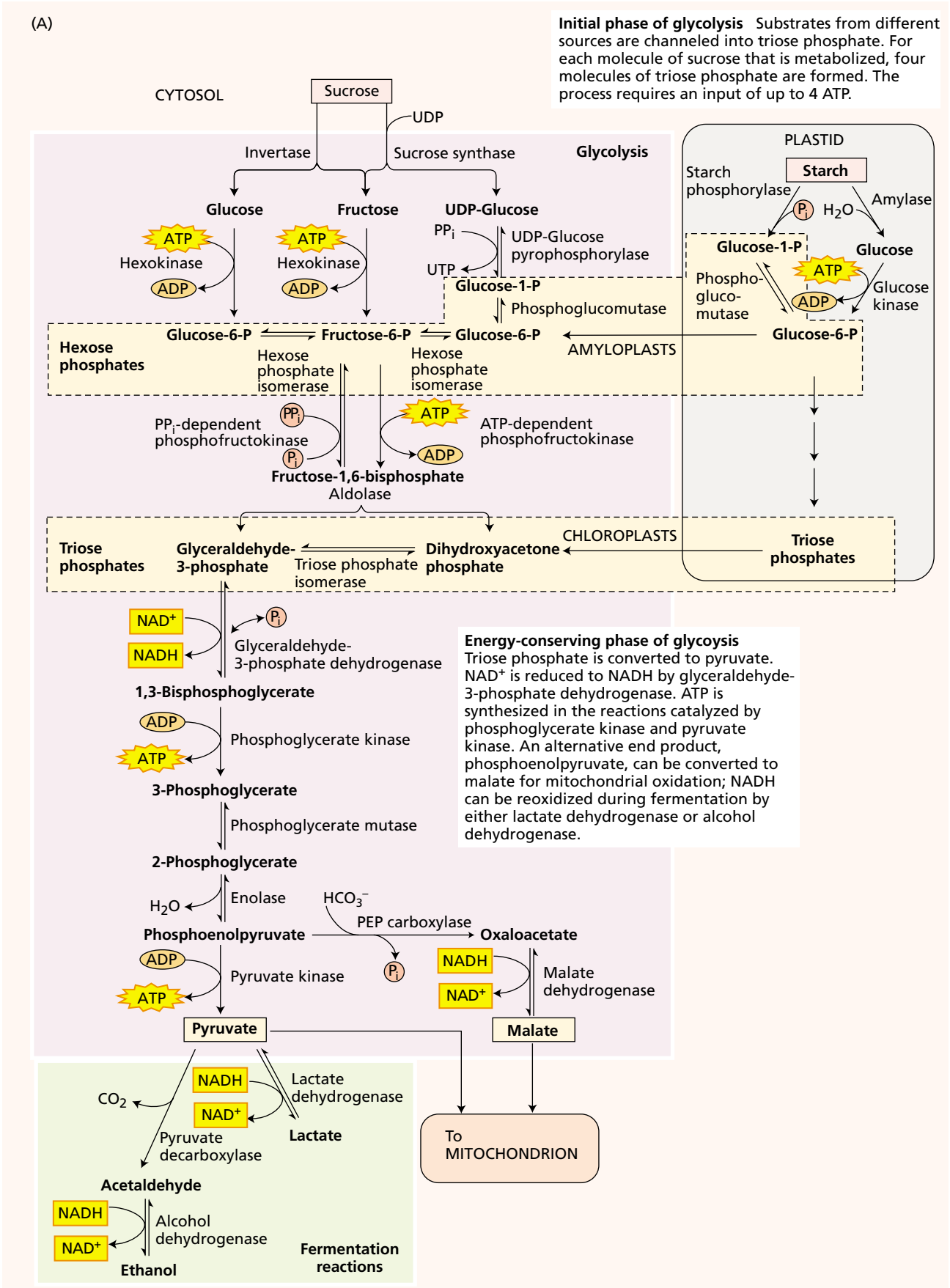
In plants, the interconversion of fructose-6-phosphate and fructose-1,6-bisphosphate is made more complex by the presence of an additional (cytosolic) enzyme, a PP_i-dependent phosphofructokinase (pyrophosphate:fructose-6-phosphate 1-phosphotransferase), which catalyzes the following reversible reaction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

$$\text{Fructose-6-P} + PP_i \leftrightarrow \text{fructose-1,6-}P_2 + P_i$$

where P represents phosphate and P_2 bisphosphate. PP_i-dependent phosphofructokinase is found in the cytosol of most plant tissues at levels that are considerably higher than those of the ATP-dependent phosphofructokinase (Kruger 1997). Suppression of the PP_i-dependent phosphofructokinase in transgenic potato has indicated 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 reaction catalyzed by the 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 bisphosphatase, 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 differs from that in many other organisms.

At the end of the glycolytic sequence, 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 (OAA). The OAA is then reduced to malate by the action of malate dehydrogenase, which uses NADH as the source of electrons, and this performs a role similar to that of the dehydrogenases during fermentative metabolism (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 operation of pyruvate kinase and PEP carboxylase can produce alternative organic acids—pyruvate or malate—for mitochondrial respiration, though pyruvate dominates in most tissues.


(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.
CYTOSOL
Sucrose
UDP
Invertase
Sucrose synthase
Glycolysis
PLASTID
Starch
Starch phosphorylase
Amylase
Glucose
Fructose
UDP-Glucose
ATP
Hexokinase
ADP
PPi
UTP
UDP-Glucose pyrophosphorylase
Glucose-1-P
Phosphoglucomutase
Phospho-gluco-mutase
Glucose kinase
Hexose phosphates
Glucose-6-P
Fructose-6-P
AMYLOPLASTS
Hexose phosphate isomerase
PPi-dependent phosphofructokinase
ATP-dependent phosphofructokinase
Fructose-1,6-bisphosphate
Aldolase
CHLOROPLASTS
Triose phosphates
Glyceraldehyde-3-phosphate
Dihydroxyacetone phosphate
Triose phosphate isomerase
NAD+
NADH
Glyceraldehyde-3-phosphate dehydrogenase
1,3-Bisphosphoglycerate
Phosphoglycerate kinase
3-Phosphoglycerate
Phosphoglycerate mutase
2-Phosphoglycerate
Enolase
H2O
HCO3−
PEP carboxylase
Phosphoenolpyruvate
Oxaloacetate
Malate dehydrogenase
Pyruvate kinase
Pyruvate
Malate
Energy-conserving phase of glycoysis 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; NADH can be reoxidized during fermentation by either lactate dehydrogenase or alcohol dehydrogenase.
CO2
Lactate dehydrogenase
Lactate
Pyruvate decarboxylase
Acetaldehyde
Alcohol dehydrogenase
Ethanol
Fermentation reactions
To MITOCHONDRION

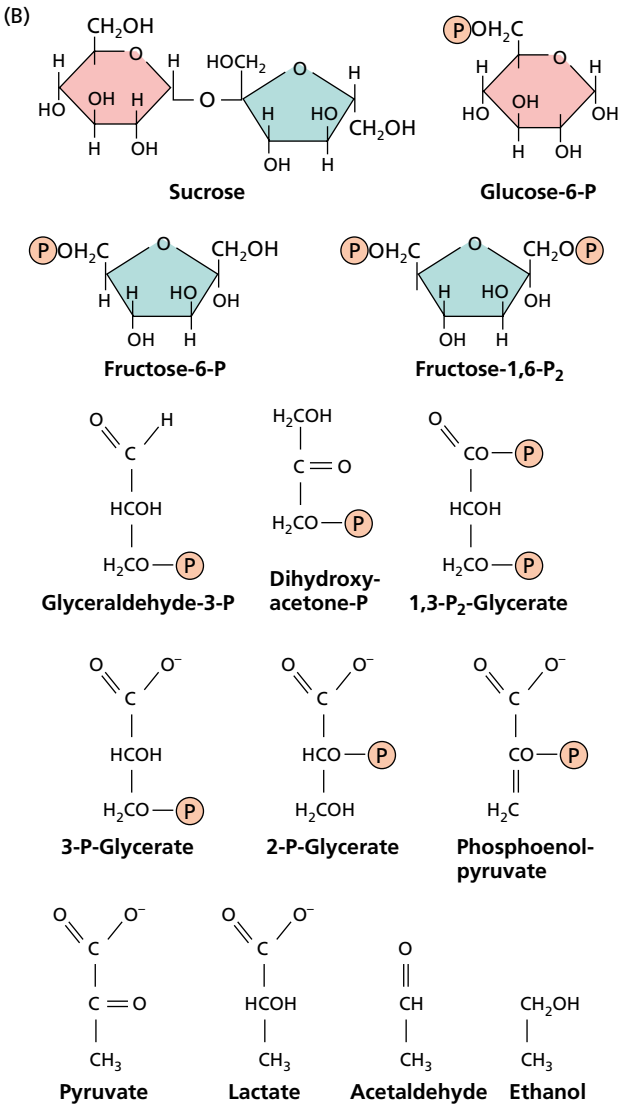


FIGURE 11.3 Reactions of plant glycolysis and fermentation. (A) In the main pathway, sucrose is oxidized to the organic acid pyruvate. The double arrows denote reversible reactions; the single arrows, essentially irreversible reactions. (B) The structures of the intermediates. P, phosphate; P_2, bisphosphate.

In the Absence of O_2, Fermentation Regenerates the NAD^+ Needed for Glycolysis

In the absence of oxygen, the citric acid cycle and oxidative phosphorylation cannot function. 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 reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase cannot take place. To overcome this problem, plants and other organisms can further metabolize pyruvate by carrying out one or more forms of **fermentative metabolism** (see Figure 11.3).

In alcoholic fermentation (common in plants, but more widely known from brewer's yeast), the 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 to mammalian muscle but also found in plants), the enzyme lactate dehydrogenase uses NADH to reduce pyruvate to lactate, thus regenerating NAD^+.

Under some circumstances, plant tissues may be subjected to low (hypoxic) or zero (anoxic) concentrations of ambient oxygen, forcing them 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 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-anaerobic conditions by carrying out some form of fermentation.

Fermentation Does Not Liberate All the Energy Available in Each Sugar Molecule

Before we leave the topic of glycolysis, we need 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 is –5760 kJ mol^{-1} (1380 kcal mol^{-1}). The value of $\Delta G^{0\prime}$ for the synthesis of ATP is 32 kJ mol^{-1} (7.7 kcal 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} (12 kcal mol^{-1}). (For a discussion of free energy, see Chapter 2 on the web site.)

Given the net synthesis of four molecules of ATP for each sucrose molecule that is converted to ethanol (or lactate), the efficiency of anaerobic fermentation is only about 4%. Most of the energy available in sucrose remains in the reduced by-product of fermentation: lactate or ethanol. During aerobic respiration, the pyruvate produced by glycolysis is transported into mitochondria, where it is further oxidized, resulting in a much more efficient conversion of the free energy originally available in the sucrose.

Because of the low efficiency of energy conservation under fermentation, an increased rate of glycolysis is needed to sustain the ATP production necessary for cell survival. This is called the *Pasteur effect* after the French microbiologist Louis Pasteur, who first noted it when yeast switched from aerobic respiration to anaerobic alcoholic fermentation. The higher rates of glycolysis result from changes in glycolytic metabolite levels, as well as from increased expression of genes encoding enzymes of glycolysis and fermentation (Sachs et al. 1996).

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 (see **Web Essay 11.1**). In contrast to animals, AMP and ATP are not major effectors of plant phosphofructokinase and pyruvate kinase. The cytosolic concentration of PEP, which is a potent inhibitor of the plant ATP-dependent phosphofructokinase, is a more important regulator of plant glycolysis.

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" (see Figure 11.12), with primary regulation at the level of PEP metabolism by pyruvate kinase and PEP carboxylase and secondary regulation exerted by PEP at the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate (see Figure 11.3). In animals, the primary control operates at the phosphofructokinase, and secondary control at the pyruvate kinase.

One conceivable benefit of bottom-up control of glycolysis is that it permits plants to control 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 may adjust to the demand for biosynthetic precursors.

The presence of two enzymes metabolizing PEP in plant cells—pyruvate kinase and PEP carboxylase—has consequences for the control of glycolysis that are not quite clear. Though the two enzymes are inhibited by similar metabolites, the PEP carboxylase can under some conditions perform a bypass reaction around the pyruvate kinase. The resulting malate can then enter the mitochondrial citric acid cycle. Hence, the bottom-up regulation enables a high flexibility in the control of plant glycolysis.

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, rates of both leaf respiration and photosynthesis were unaffected relative to controls having 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.

The regulation of the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate is also complex. Fructose-2,6-bisphosphate, another hexose bisphosphate, is present at varying levels in the cytosol (see Chapter 8). It markedly inhibits the activity of cytosolic fructose-1,6-bisphosphatase but stimulates the activity of PP_i-dependent phosphofructokinase. These observations suggest that fructose-2,6-bisphosphate plays a central role in partitioning flux between ATP-dependent and PP_i-dependent pathways of fructose phosphate metabolism at the crossing point between sucrose synthesis and glycolysis.

Understanding of the fine levels of glycolysis regulation requires the study of temporal changes in metabolite levels (Givan 1999). Methods are now available by rapid extraction and simultaneous analyses of many metabolites—for example, by mass spectrometry—an approach called *metabolic profiling* (see **Web Essay 11.2**).

The Pentose Phosphate Pathway Produces NADPH and Biosynthetic Intermediates

The glycolytic pathway is not the only route available for the oxidation of sugars in plant cells. Sharing common metabolites, 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. Generally, the pathway in plastids predominates over the cytosolic pathway (Dennis et al. 1997).

The first two reactions of this pathway involve the oxidative events that convert the six-carbon glucose-6-phosphate to a five-carbon sugar, 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 to the glycolytic intermediates glyceraldehyde-3-phosphate and fructose-6-phosphate. Because glucose-6-phosphate can be regenerated from glyceraldehyde-3-phosphate and fructose-6-phosphate by glycolytic enzymes, for six turns of the 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 with the concomitant synthesis of 12 NADPH molecules.

Studies of the release of $^{14}CO_2$ from isotopically labeled glucose indicate that glycolysis is the more dominant breakdown pathway, accounting for 80 to 95% of the total carbon flux in most plant tissues. However, the pentose phosphate pathway does contribute to the flux, and developmental studies indicate that its contribution increases as plant cells develop from a meristematic to a more differentiated state (Ap Rees 1980). The oxidative pentose phosphate pathway plays several roles in plant metabolism:

- The product of the two oxidative steps is NADPH, and this NADPH is thought to drive reductive steps associated with various biosynthetic reactions that occur in the cytosol. In nongreen plastids, such as amyloplasts, and in chloroplasts functioning in the

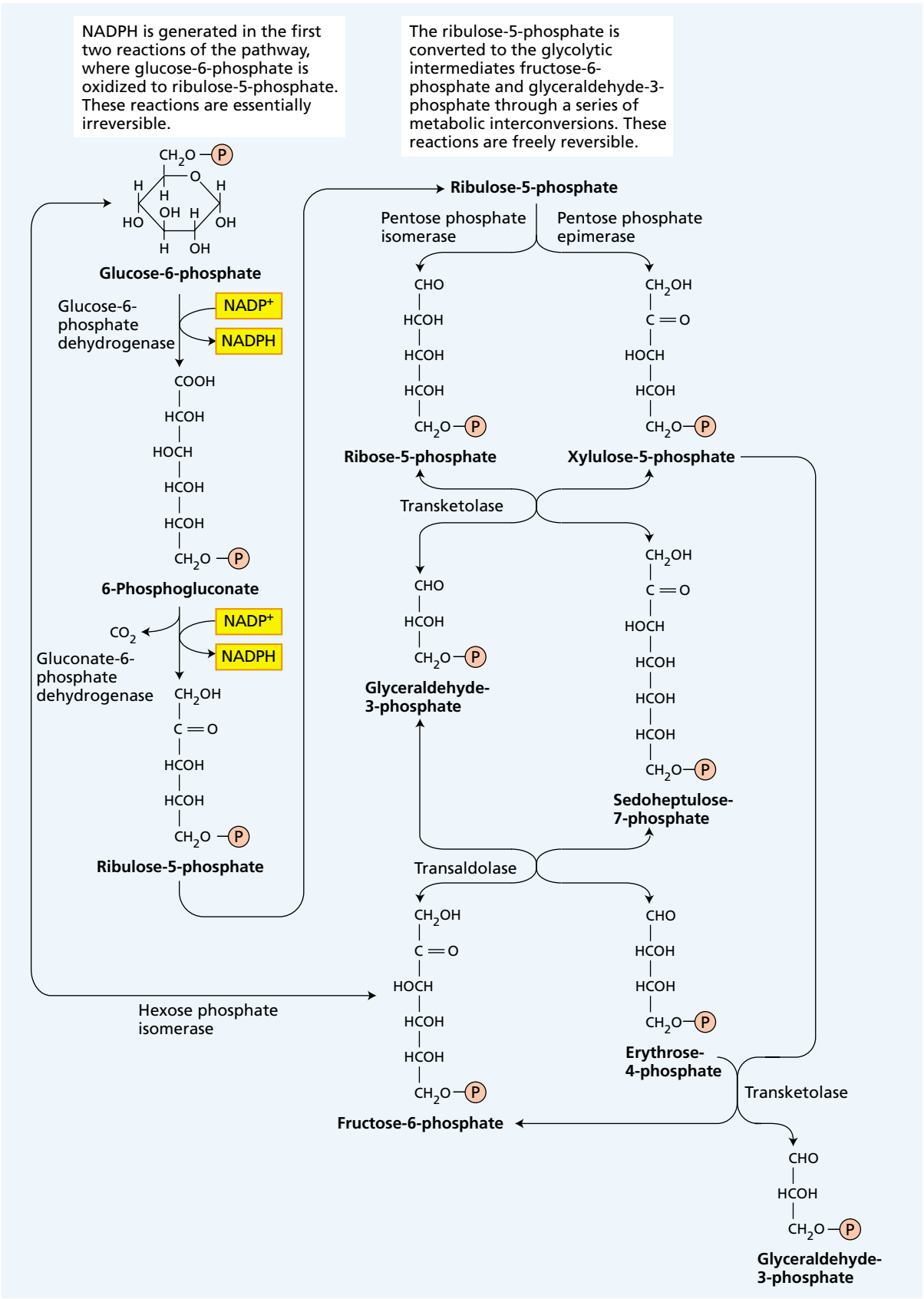


FIGURE 11.4 Reactions of the oxidative pentose phosphate pathway in higher plants. P, phosphate.

dark, the pathway may also supply NADPH for biosynthetic reactions such as lipid biosynthesis and nitrogen assimilation.

- Because plant mitochondria are able to oxidize cytosolic NADPH via an NADPH dehydrogenase localized on the external surface of the inner membrane, some of the reducing power generated by this pathway may contribute to cellular energy metabolism; that is, electrons from NADPH may end up reducing O_2 and generating ATP.
- The pathway produces ribose-5-phosphate, a precursor of the ribose and deoxyribose needed in the synthesis of RNA and DNA, respectively.
- Another intermediate in this pathway, the four-carbon erythrose-4-phosphate, combines with PEP in the initial reaction that produces plant phenolic compounds, including the aromatic amino acids and the precursors of lignin, flavonoids, and phytoalexins (see Chapter 13).
- During the early stages of greening, before leaf tissues become fully photoautotrophic, the oxidative pentose phosphate pathway is thought to be involved in generating Calvin cycle intermediates.

Control of the oxidative pathway. The oxidative pentose phosphate pathway is controlled by the initial reaction of the pathway catalyzed by glucose-6-phosphate dehydrogenase, the activity of which is markedly inhibited by a high ratio of NADPH to $NADP^+$.

In the light, however, little operation of the oxidative pathway is likely to occur in the chloroplast because 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 interconversions of the pathway in the direction of pentose synthesis. Moreover, glucose-6-phosphate dehydrogenase will be inhibited during photosynthesis by the high ratio of NADPH to $NADP^+$ in the chloroplast, as well as by a reductive inactivation involving the ferredoxin–thioredoxin system (see Chapter 8).

THE CITRIC ACID CYCLE: A MITOCHONDRIAL MATRIX PROCESS

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 to CO_2 and H_2O; it also highlighted the key concept of cycles in metabolic pathways. For his discovery, Hans Krebs was awarded the Nobel Prize in physiology and medicine in 1953.

Because the citric acid cycle is localized in the matrix of mitochondria, we will begin with a general description of mitochondrial structure and function, knowledge 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. For all plant-specific properties, we will consider how they affect respiratory function.

Mitochondria Are Semiautonomous Organelles

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

In electron micrographs, plant mitochondria—whether in situ or in vitro—usually look spherical or rodlike (Figure 11.5), ranging from 0.5 to 1.0 μm in diameter and up to 3 μm in length (Douce 1985). With some exceptions, plant cells have a substantially lower number of mitochondria than that found in a typical animal cell. The number of mitochondria per plant cell varies, and 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 nonplant tissues (see Figure 11.5). Plant mitochondria have two membranes: a smooth **outer membrane** that completely surrounds a highly invaginated **inner membrane**. The invaginations of the inner membrane are known as **cristae** (singular *crista*). As a consequence of the greatly enlarged surface area, the inner membrane can contain more than 50% of the total mitochondrial protein. The aqueous phase contained within the inner membrane is referred to as the mitochondrial **matrix** (plural *matrices*), and the region between the two mitochondrial membranes is known as the **intermembrane space**.

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 space. 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 (i.e., most cellular metabolites and ions, but not proteins). The lipid fraction of both membranes is primarily made up of phospholipids, 80% of which are either phosphatidylcholine or phosphatidylethanolamine.

Like chloroplasts, mitochondria are semiautonomous organelles because they contain ribosomes, RNA, and

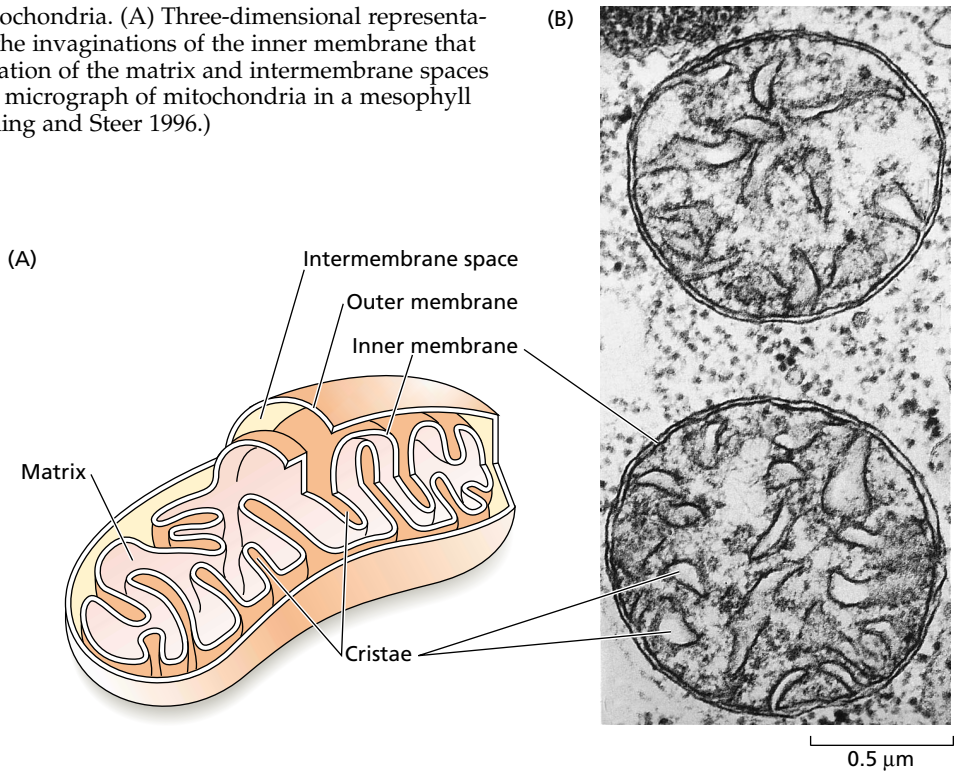


FIGURE 11.5 Structure of plant mitochondria. (A) Three-dimensional representation of a mitochondrion, showing the invaginations of the inner membrane that are called cristae, as well as the location of the matrix and intermembrane spaces (see also Figure 11.10). (B) Electron micrograph of mitochondria in a mesophyll cell of *Vicia faba*. (Photo from Gunning and Steer 1996.)

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. Mitochondria proliferate through the division by fission of preexisting mitochondria and not through de novo biogenesis of the organelle.

Pyruvate Enters the Mitochondrion and Is Oxidized via the Citric Acid Cycle

As already noted, 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 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 by the enzyme pyruvate dehydrogenase. The products are NADH (from NAD^+), CO_2, and acetic acid in the form of acetyl-CoA, in which a thioester bond links the acetic acid to a sulfur-containing cofactor, coenzyme A (CoA) (see Figure 11.6). Pyruvate dehydrogenase exists as a large complex of several enzymes that catalyze the overall reaction in a three-step process: decarboxylation, oxidation, and conjugation to CoA.

In the next reaction the enzyme citrate synthase combines the acetyl group of acetyl-CoA with a four-carbon dicarboxylic acid (oxaloacetate, OAA) 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 molecule, 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.

During the remainder of the citric acid cycle, succinyl-CoA is oxidized to OAA, 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 (which is the next major topic to be discussed in this chapter).

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

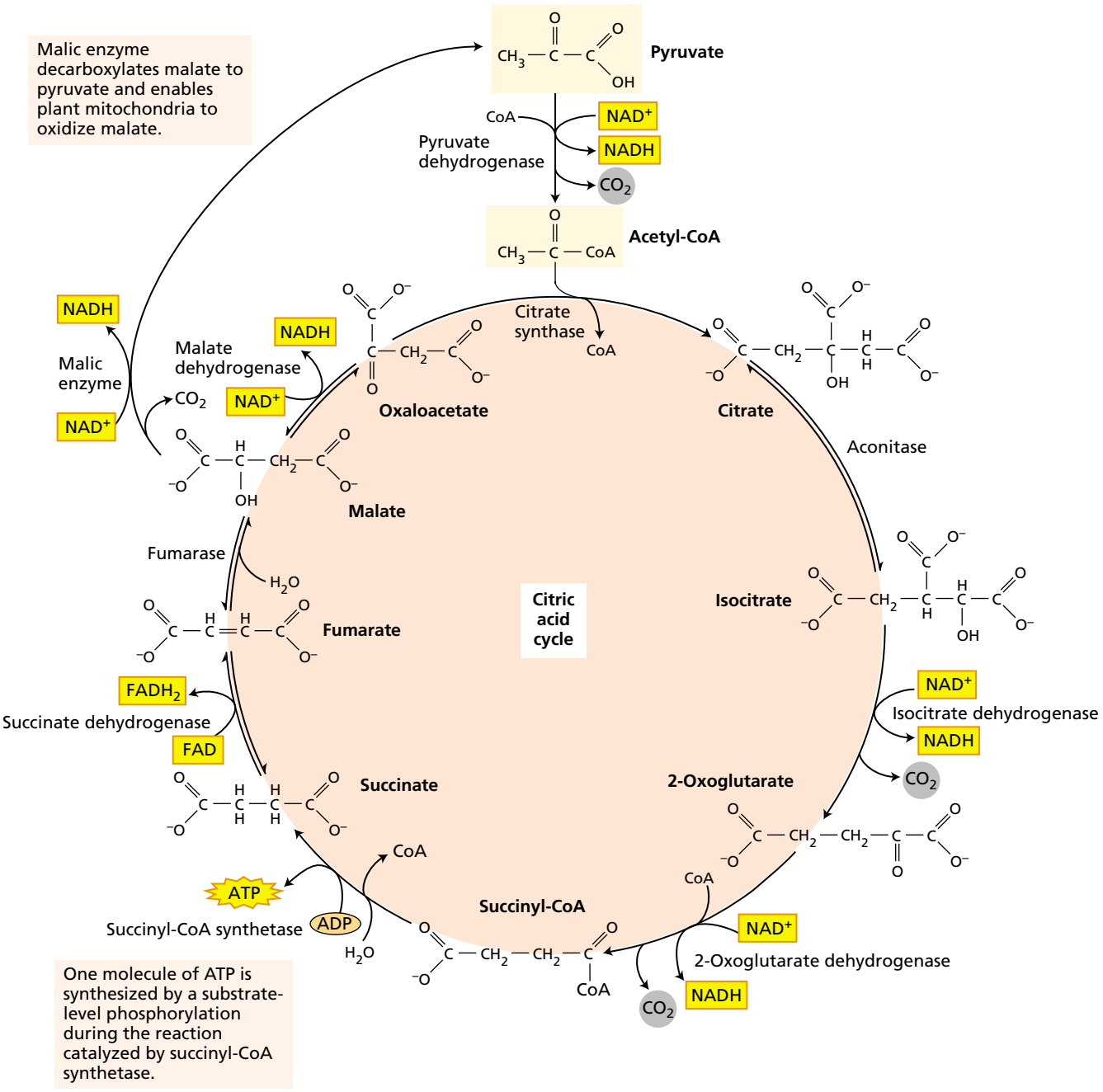


FIGURE 11.6 Reactions and enzymes of the plant citric acid cycle. Pyruvate is completely oxidized to three molecules of CO_2. The electrons released during these oxidations are used to reduce four molecules of NAD^+ to NADH and one molecule of FAD to $FADH_2$.

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 OAA and produce another molecule of NADH. The OAA 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 during the citric acid cycle.

All the enzymes associated with the citric acid cycle are found in plant mitochondria. Some of them may be associated in multienzyme complexes, which would facilitate movement of metabolites between the enzymes.

The Citric Acid Cycle of Plants Has Unique Features

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

A feature of the plant citric acid cycle that is absent in many other organisms is the significant activity of NAD^+ malic enzyme, which has been found in the matrix of all plant mitochondria analyzed to date. This enzyme catalyzes the oxidative decarboxylation of malate:

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

The presence of NAD^+ malic enzyme enables plant mitochondria to operate alternative pathways for the metabolism of PEP derived from glycolysis. As already described, malate can be synthesized from PEP in the cytosol via the enzymes PEP carboxylase and malate dehydrogenase (see Figure 11.3). Malate is then transported into the mitochondrial matrix, where NAD^+ 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).

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

The presence of an alternative pathway for the oxidation of malate is consistent with the observation that many plants, in addition to those that carry out crassulacean acid metabolism (see Chapter 8), store significant levels of malate in their central vacuole.

ELECTRON TRANSPORT AND ATP SYNTHESIS AT THE INNER MITOCHONDRIAL MEMBRANE

ATP is the energy carrier used by cells to drive living processes, and chemical energy conserved during the citric acid cycle in the form of NADH and $FADH_2$ (redox equivalents with high-energy electrons) must be converted to ATP to perform useful work in the cell. This O_2-dependent process, called **oxidative phosphorylation**, occurs in the inner mitochondrial membrane.

In this section we will describe the process by which the energy level of the electrons 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 mul-

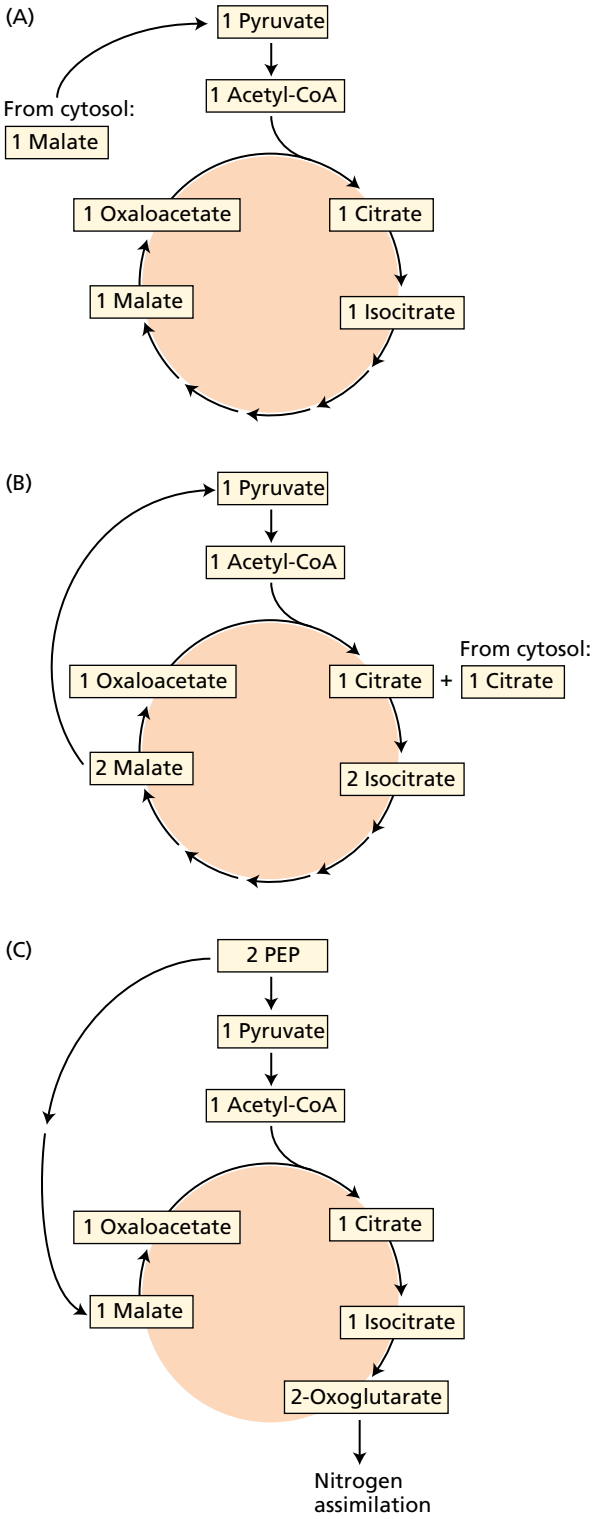


FIGURE 11.7 Malic enzyme and PEP carboxylase provide plants with metabolic flexibility for the metabolism of phosphoenolpyruvate. Malic enzyme makes it possible for plant mitochondria to oxidize both malate (A) and citrate (B) to CO_2 without involving pyruvate delivered by glycolysis. The joint action of PEP carboxylase and pyruvate kinase can convert glycolytic PEP to 2-oxoglutarate, which is used for nitrogen assimilation (C).

tiple 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 pathways, 4 molecules of NADH are generated in the cytosol and 16 molecules of NADH plus 4 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 an electron flow from NADH (and $FADH_2$) to oxygen, the final electron acceptor of the respiratory process. For the oxidation of NADH, the overall two-electron transfer can be written as follows:

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

From the reduction potentials for the NADH–NAD^+ pair (–320 mV) and the H_2O–$\frac{1}{2}O_2$ pair (+810 mV), it can be calculated that the standard free energy released during this overall reaction ($-nF\Delta E^{0\prime}$) is about 220 kJ mol^{-1} (52 kcal mol^{-1}) per two electrons (for a detailed discussion on standard free energy see Chapter 2 on the web site). Because the succinate–fumarate reduction potential is higher (+30 mV), only 152 kJ mol^{-1} (36 kcal mol^{-1}) of energy is released for each two electrons generated during the oxidation of succinate. 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 mitochondria from other organisms (Figure 11.8) (Siedow 1995; Siedow and Umbach 1995). The individual electron transport proteins are organized into four multiprotein complexes (identified by Roman numerals I through IV), all of which are localized in the inner mitochondrial membrane:

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 [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 to the intermembrane space for every electron pair passing through the complex.

Ubiquinone, a small lipid-soluble electron and proton carrier, is located 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 proteins into the ubiquinone pool. This complex does not pump protons.

Complex III (cytochrome bc_1 complex). This complex 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 by complex III.

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). This complex contains two copper centers (Cu_A and Cu_B) and cytochromes *a* and a_3. Complex IV is the terminal oxidase and brings about the four-electron reduction of O_2 to two molecules of H_2O. Two protons are pumped per electon 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).

Some Electron Transport Enzymes Are Unique to Plant Mitochondria

In addition to the set of electron carriers described in the previous section, plant mitochondria contain some components not found in mammalian mitochondria (see Figure 11.8). Note that none of these additional enzymes pump protons and that energy conservation is therefore lower whenever they are used:

- Two NAD(P)H dehydrogenases, both Ca^{2+}-dependent, attached to the outer surface of the inner membrane facing the intermembrane space can oxidize cytosolic NADH and NADPH. 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 (see **Web Topic 11.2**) (Møller 2001).
- 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-resistant dehydrogenase, ND_{in}(NADH), for

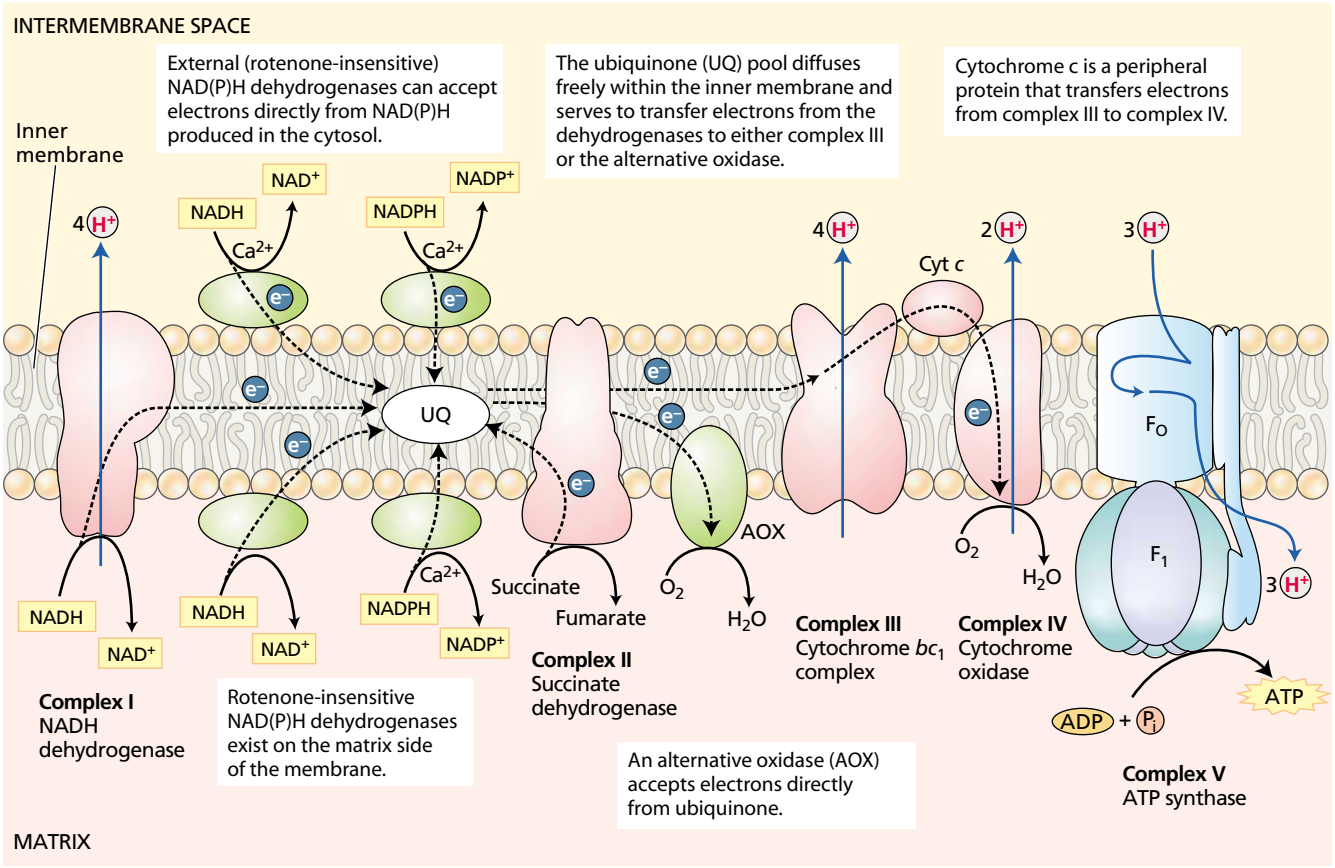


FIGURE 11.8 Organization of the electron transport chain and ATP synthesis in the inner membrane of plant mitochondria. In addition to the five standard protein complexes found in nearly all other mitochondria, the electron transport chain of plant mitochondria contains five additional enzymes marked in green. None of these additional enzymes pumps protons. Specific inhibitors, rotenone for complex I, antimycin for complex III, cyanide for complex IV, and salicylhydroxamic acid (SHAM) for the alternative oxidase, are important tools to investigate the electron transport chain of plant mitochondria.

the oxidation of NADH derived from citric acid cycle substrates. The role of this pathway may well be as a bypass being engaged when complex I is overloaded (Møller and Rasmusson 1998; Møller 2001), such as under photorespiratory conditions, as we will see shortly (see also **Web Topic 11.2**).

- An NADPH dehydrogenase, ND_{in}(NADPH), is present on the matrix surface. Very little is known about this enzyme.
- Most, if not all, plants have an "alternative" respiratory pathway for the reduction of oxygen. This pathway involves the so-called alternative oxidase that, unlike cytochrome *c* oxidase, is insensitive to inhibition by cyanide, azide, or carbon monoxide (see **Web Topic 11.3**).

The nature and physiological significance of these plant-specific enzymes will be considered more fully later in the chapter.

ATP Synthesis in the Mitochondrion Is Coupled to Electron Transport

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

In experiments conducted with the use of isolated mitochondria, electrons derived from internal (matrix) NADH 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.

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

Substrate	ADP:O 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 non-phosphorylating 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.

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 **Web Topic 6.3** and Chapter 7, which was first proposed in 1961 by Nobel laureate Peter Mitchell as a general mechanism of energy conservation across biological membranes (Nicholls and Ferguson 2002). According to the chemiosmotic theory, the orientation of electron carriers within the mitochondrial inner membrane allows for the transfer of protons (H^+) across the inner membrane during electron flow. Numerous studies have confirmed that mitochondrial electron transport is associated with a net transfer of protons from the mitochondrial matrix to the intermembrane space (see Figure 11.8) (Whitehouse and Moore 1995).

Because the inner mitochondrial membrane is impermeable to H^+, 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 electric transmembrane potential component (ΔE) and a chemical-potential component (ΔpH) according to the following equation:

$$\Delta p = \Delta E - 59\Delta\text{pH}$$

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 is negative.

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 prevent large pH changes. This situation contrasts to that in the chloroplast, where almost all of the proton motive force across the thylakoid membrane is made up by a proton gradient (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 is reasonably stable, once generated, and the free energy $\Delta\tilde{\mu}_{H^+}$ 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_1 and F_o (see Figure 11.8). **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 to ATP. This complex is attached to the matrix side of the inner membrane. **F_o** is an integral membrane protein complex that consists of at least three different polypeptides that form the channel through which protons cross the inner membrane.

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 from the intermembrane space to the matrix down the electrochemical proton gradient.

A high-resolution X-ray structure of most of the F_1 complex of the mammalian mitochondrial ATP synthase supports a "rotational model" for the catalytic mechanism of ATP synthesis (see **Web Topic 11.4**) (Abrahams et al. 1994). The structure and function of the mitochondrial ATP synthase is similar to that of the CF_o–CF_1 ATP synthase in photophosphorylation (see Chapter 7).

The operation of a chemiosmotic mechanism of ATP synthesis has several implications. First, the true site of ATP formation on the mitochondrial inner 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 $\Delta\tilde{\mu}_{H^+}$.

Second, the chemiosmotic theory explains the action mechanism of uncouplers, a wide range of chemically

unrelated compounds (including 2,4-dinitrophenol and FCCP [*p*-trifluoromethoxycarbonylcyanide phenylhydrazone]) that decreases mitochondrial ATP synthesis but often stimulates the rate of electron transport (see **Web Topic 11.5**). All of these compounds make the inner membrane leaky to protons, which prevents the buildup of a sufficiently large $\Delta\tilde{\mu}_{H^+}$ to drive ATP synthesis.

In experiments on isolated mitochondria, higher rates of electron flow (measured as the rate of oxygen uptake in the presence of a substrate such as succinate) are observed upon addition of ADP (referred to as *state 3*) than in its absence (Figure 11.9). ADP provides a substrate that stimulates dissipation of the $\Delta\tilde{\mu}_{H^+}$ through the F_oF_1-ATP synthase during ATP synthesis. Once all the ADP has been converted to ATP, the $\Delta\tilde{\mu}_{H^+}$ builds up again and reduces the rate of electron flow (*state 4*). The ratio of the rates with and without ADP (state 3:state 4) is referred to as the *respiratory control ratio*.

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 substrates and products of ATP synthesis in 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 in and ATP out of the organelle.

Adenylate transport involves another inner-membrane protein, the ADP/ATP (adenine nucleotide) transporter, which catalyzes an exchange of ADP and ATP across the inner membrane (Figure 11.10). The movement of the more negatively charged ATP^{4-} out of the mitochondria in exchange for ADP^{3-}—that is, one net negative charge out—is driven by the electric-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 proton gradient 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 will remain 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 11.10).

The total cost of taking up a phosphate (1 OH^- out, which is the same as 1 H^+ in) and exchanging ADP for ATP (one negative charge out, which is the same as one positive charge in) is 1 H^+. This proton should also be included in 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 malate (see Figure 11.10 and **Web Topic 11.5**).

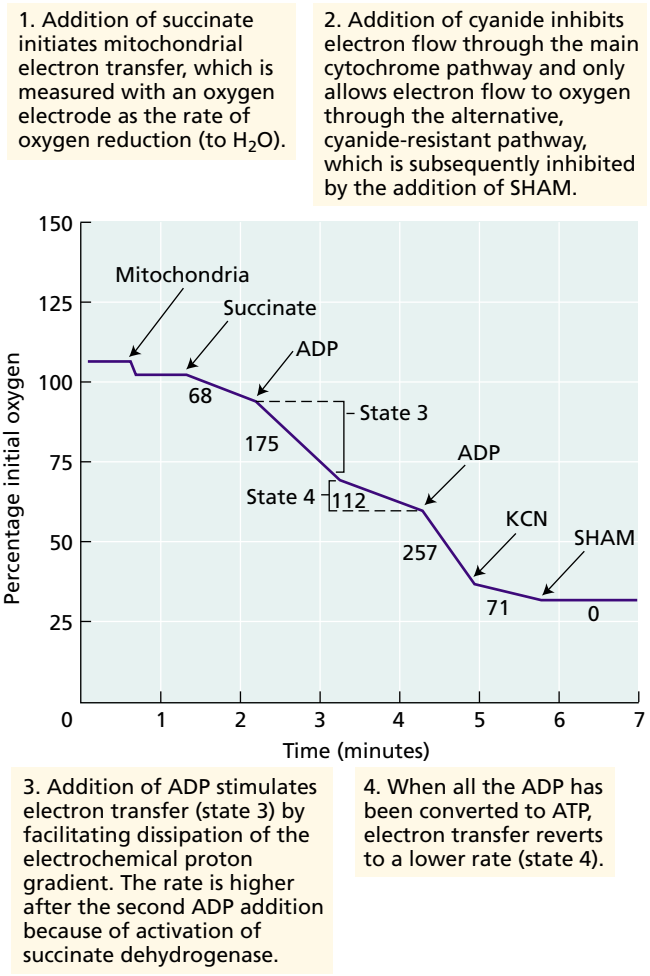


FIGURE 11.9 Regulation of respiratory rate by ADP during succinate oxidation in isolated mitochondria from mung bean (*Vigna radiata*). The numbers below the traces are the rates of oxygen uptake expressed as O_2 consumed (nmol min^{-1} mg $protein^{-1}$). (Data courtesy of Steven J. Stegink.)

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

- 8 molecules of ATP by substrate-level phosphorylation (4 during glycolysis and 4 in the citric acid cycle)
- 4 molecules of NADH in the cytosol
- 16 molecules of NADH plus 4 molecules of $FADH_2$ (via succinate dehydrogenase) in the mitochondrial matrix

On the basis of theoretical ADP:O values (see Table 11.1), a total of approximately 52 molecules of ATP will be generated

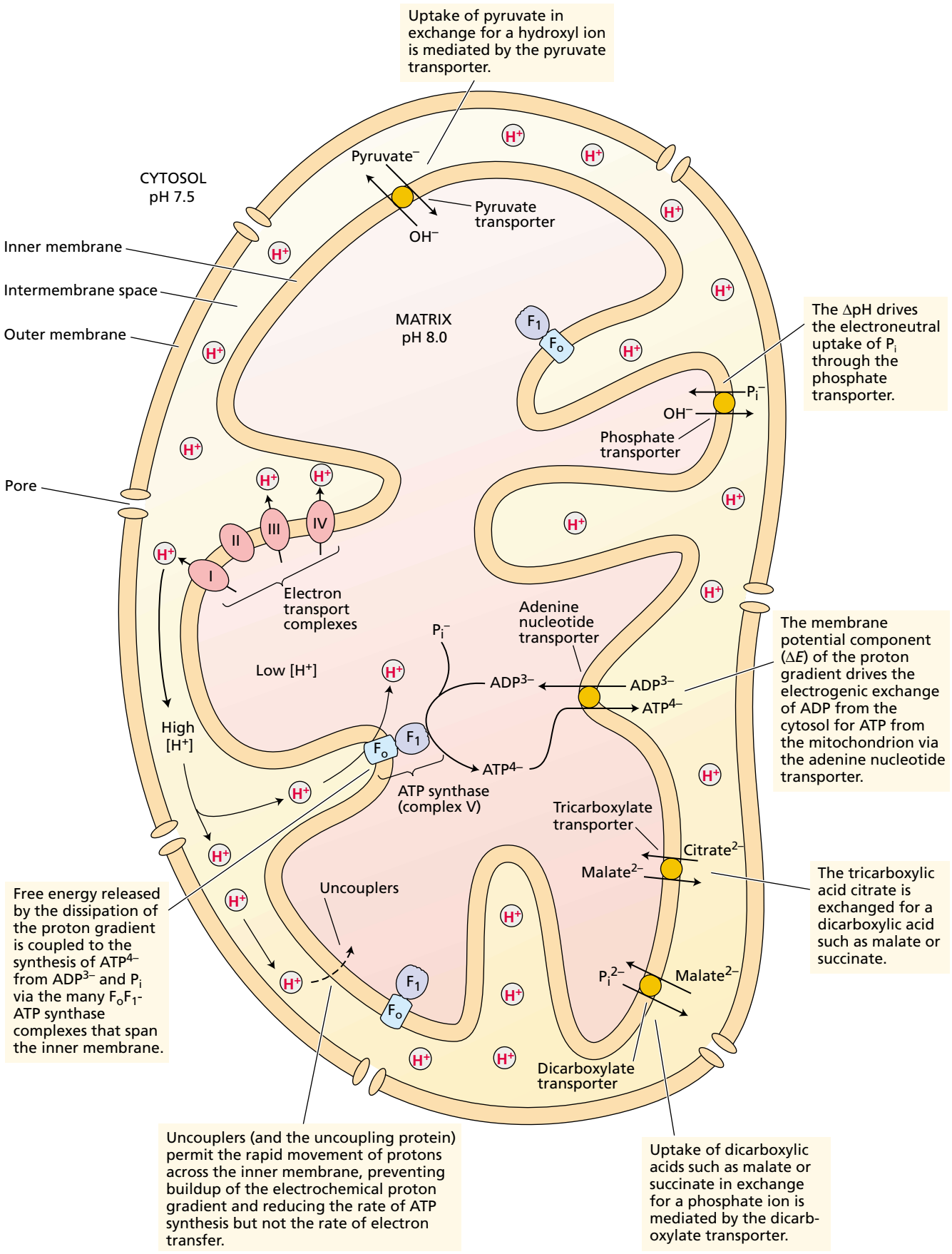

Uptake of pyruvate in exchange for a hydroxyl ion is mediated by the pyruvate transporter.
CYTOSOL
pH 7.5
Pyruvate⁻
Pyruvate transporter
OH⁻
Inner membrane
Intermembrane space
Outer membrane
MATRIX
pH 8.0
F1
Fo
The ΔpH drives the electroneutral uptake of Pi through the phosphate transporter.
Pi⁻
OH⁻
Phosphate transporter
Pore
H+
II
III
IV
I
Electron transport complexes
Low [H+]
High [H+]
Adenine nucleotide transporter
Pi⁻
ADP3–
ADP3–
ATP4–
ATP4–
The membrane potential component (ΔE) of the proton gradient drives the electrogenic exchange of ADP from the cytosol for ATP from the mitochondrion via the adenine nucleotide transporter.
ATP synthase (complex V)
Tricarboxylate transporter
Citrate2–
Malate2–
The tricarboxylic acid citrate is exchanged for a dicarboxylic acid such as malate or succinate.
Free energy released by the dissipation of the proton gradient is coupled to the synthesis of ATP4– from ADP3– and Pi via the many FoF1-ATP synthase complexes that span the inner membrane.
Uncouplers
Pi2–
Malate2–
Dicarboxylate transporter
Uncouplers (and the uncoupling protein) permit the rapid movement of protons across the inner membrane, preventing buildup of the electrochemical proton gradient and reducing the rate of ATP synthesis but not the rate of electron transfer.
Uptake of dicarboxylic acids such as malate or succinate in exchange for a phosphate ion is mediated by the dicarb-oxylate transporter.

FIGURE 11.10 Transmembrane transport in plant mitochondria. An electrochemical proton gradient ($\Delta\tilde{\mu}_{H^+}$) consisting of a membrane potential (ΔE, –200mV, negative inside) and a 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).

per sucrose by oxidative phosphorylation. The result is a total of about 60 ATPs synthesized per sucrose (Table 11.2).

Using 50 kJ mol^{-1} (12 kcal mol^{-1}) as the actual free energy of formation of ATP in vivo, we find that about 3010 kJ mol^{-1} (720 kcal mol^{-1}) of free energy is conserved in the form of ATP per mole of sucrose oxidized during aerobic respiration. This amount represents about 52% of the standard free energy available from the complete oxidation of sucrose; the rest is lost as heat. This is a vast improvement over the conversion of only 4% of the energy available in sucrose to ATP that is associated with fermentative metabolism.

Several Subunits of Respiratory Complexes Are Encoded by the Mitochondrial Genome

With the first complete sequencing of plant mitochondrial DNA (mtDNA) in *Arabidopsis thaliana* (Marienfeld et al. 1999), our knowledge about the mitochondrial genome has taken a great leap forward.

Some characteristics of the plant mitochondrial genetic system are not generally found in the mitochondria of animals, protozoans, or even fungi. Most notably, RNA processing differs between plant mitochondria and mitochondria from most other organisms. Several plant mitochondrial genes contain introns, and some genes are even split between separate transcript molecules, which must be joined by splicing. Plant mtDNA also lacks strict complementarity to translated mRNA (see **Web Topic 11.6**). Another characteristic feature of the plant mitochondrial genetic system is that it strictly observes the universal genetic code, showing none of the deviations found in mtDNA in all other kingdoms.

Plant mitochondrial genomes are generally much larger than those of animals. The plant mtDNA is in the range of 200 to 2400 kilobase pairs (kb) in size, with large variations even between closely related plant species. This size compares with the compact and uniform 16 kb genome found in mammalian mitochondria. The size differences are due mainly to the presence of much noncoding sequence, including numerous introns, in plant mtDNA. Mammalian mtDNA encodes only 13 proteins, in contrast to the 35 known proteins encoded by the *Arabidopsis* mtDNA. Both plant and mammalian mitochondria encode rRNAs and tRNAs.

The genes of the mtDNA can be divided into two main groups: those needed for expression of mitochondrial genes (tRNA, rRNA, and ribosome proteins) and those for oxidative phosphorylation complexes. Plant mtDNA encodes nine subunits for complex I, one for complex III, three for complex IV, three for ATP synthase, and five proteins for biogenesis of cytochromes (Marienfeld et al. 1999). The mitochondrially encoded subunits are essential for the activity of the respiratory complexes, a feature also evident in the sequence conservation to their bacterial homologs. The nuclear genome encodes all proteins not encoded in mtDNA, and the nuclear-encoded proteins are the large majority—for example, all proteins in the citric acid cycle. The nuclear-encoded mitochondrial proteins are synthesized by cytosolic ribosomes and imported via translocators in the outer and inner mitochondrial membrane. Therefore, oxidative phosphorylation is dependent on expression of genes located in two separate genomes. Any change in expression in response to a stimulus or for developmental reasons must be coordinated.

Whereas the expression of nuclear genes for mitochondrial proteins appears to be regulated as other nuclear genes, much less is known about the expression of mitochondrial genes. The master circle of plant mtDNA is normally split into several smaller subgenomic segments, and genes can be down-regulated by decreased copy number for a segment of the mtDNA (Leon et al. 1998). The gene promoters in mtDNA are of several kinds and show different transcriptional activity. However, a main control of mitochondrial gene expression appears to take place at the posttranslational level, by degradation of excess polypeptides (McCabe et al. 2000).

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 ×1.5	6
Citric acid cycle		
4 substrate level phosphorylations		4
4 $FADH_2$	4 × 1.5	6
16 NADH	16 × 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 values from Table 11.1

Plants Have Several Mechanisms That Lower the ATP Yield

As we have seen, a complex machinery is required for a high efficiency of energy conservation in oxidative phosphorylation. So it is perhaps surprising that plant mitochondria have several functional proteins that reduce this efficiency. Probably plants are less limited by the energy supply (sunlight) than by other factors in the environment (e.g., access to nitrogen or phosphate). As a consequence, adaptational flexibility may be more important than energetic efficiency.

In the following subsections we will discuss the role of the nonphosphorylating mechanisms and their possible usefulness in the life of the plant.

The alternative oxidase. If cyanide (1 m*M*) is added to actively respiring animal tissues, cytochrome *c* oxidase is inhibited and the respiration rate quickly drops to less than 1% of its initial level. However, most plant tissues display a level of cyanide-resistant respiration that can represent 10 to 25%, and in some tissues up to 100%, of the uninhibited control rate. The enzyme responsible for this oxygen uptake has been identified as a cyanide-resistant oxidase component of the plant mitochondrial electron transport chain called the **alternative oxidase** (see Figure 11.8 and **Web Topic 11.3**) (Vanlerberghe and McIntosh 1997).

Electrons feed off the main electron transport chain into the 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 the alternative 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, tissues of the clublike inflorescence, called the *appendix*, which bears male and female flowers, 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 for a period of about 7 hours.

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. Salicylic acid, a phenolic compound related to aspirin (see Chapter 13), has been identified as the chemical signal responsible for initiating this thermogenic event in the voodoo lily (Raskin et al. 1989) (see **Web Essay 11.3**). In most plants, however, both the respiratory rates and the rate of cyanide-resistant respiration are too low to generate sufficient heat to raise the temperature significantly, so what other role(s) does the alternative pathway play?

It has been suggested that the alternative pathway can function as an "energy overflow" pathway, oxidizing respiratory substrates that accumulate in excess of those needed for growth, storage, or ATP synthesis (Lambers 1985). This view suggests that electrons flow through the alternative pathway only when the activity of the main pathway is saturated. Such saturation is reached in the test tube in state 4 (see Figure 11.9); in vivo, saturation may occur if the respiration rate exceeds the cell's demand for ATP (i.e., if ADP levels are very low). However, it is now clear that the alternative oxidase can be active before the cytochrome pathway is saturated. 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 25 and **Web Essay 11.1**) (Wagner and Krab 1995).

By draining off electrons from the electron transport chain, the alternative pathway prevents a potential overreduction of the ubiquinone pool (see Figure 11.8), which, if left unchecked, can lead to the generation of destructive reactive oxygen species such as superoxide anions and hydroxyl radicals. In this way the alternative pathway may lessen the detrimental effects of stress on respiration (see **Web Essay 11.4**) (Wagner and Krab 1995; Møller 2001).

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 is generated. Heat production appears to be one of the uncoupling protein's main functions in mammalian cells.

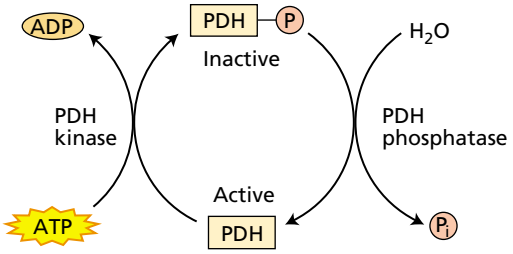
It has long been thought that the alternative oxidase in plants and the uncoupling protein in mammals were simply two different means of achieving the same end. It was therefore surprising when a protein similar to the uncoupling protein was discovered in plant mitochondria (Vercesi et al. 1995; Laloi et al. 1997). This protein is stress induced and, like the alternative oxidase, may function to prevent overreduction of the electron transport chain (see **Web Topic 11.3** and **Web Essay 11.4**). It remains unclear, however, why plant mitochondria require both mechanisms.

The internal, rotenone-insensitive NADH dehydrogenase, ND_{in}(NADH). This is one of the multiple NAD(P)H dehydrogenases found in plant mitochondria (see Figure 11.8). It has been suggested to work as a non-proton-pumping bypass when complex I is overloaded. Complex I has a higher affinity for NADH (ten times lower K_m), than ND_{in}(NADH). At lower NADH levels in the matrix, typically when ADP is available (state 3), complex I will dominate, whereas when ADP is rate limiting (state 4), NADH levels will increase and ND_{in}(NADH) will be more active. The physiological importance of this enzyme is, however, still unclear.

Mitochondrial Respiration Is Controlled by Key Metabolites

The substrates of ATP synthesis—ADP and P_i—appear to be key regulators of the rates of glycolysis in the cytosol, as well as 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.

The best-characterized site of regulation of the citric acid cycle is at the pyruvate dehydrogenase complex, which is reversibly phosphorylated by a regulatory kinase and a 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). In addition, several citric acid cycle enzymes, including pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase, are directly inhibited by NADH.



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 Regulation of pyruvate dehydrogenase (PDH) activity by reversible phosphorylation and by other metabolites.

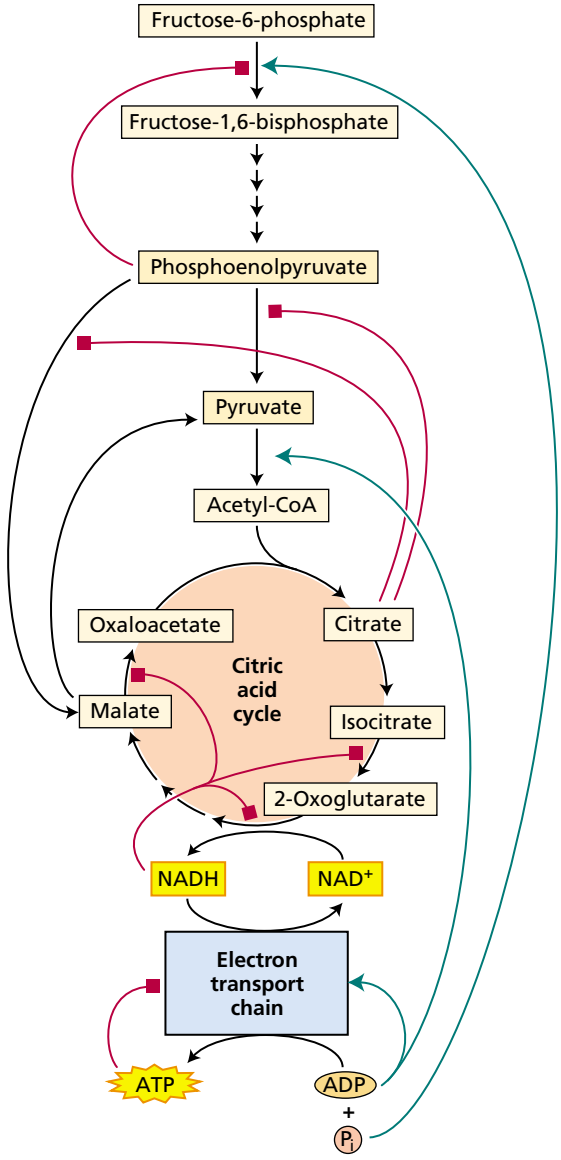


FIGURE 11.12 Concept of bottom-up regulation of plant respiration. Several substrates for respiration (e.g., ADP) stimulate enzymes in early steps of the pathways (green arrows . In contrast, accumulation of products (e.g., ATP) inhibits (red squares) earlier reactions in a stepwise fashion. For instance, ATP inhibits the electron transport chain leading to an accumulation of NADH. NADH inhibits citric acid enzymes such as isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase. Then, citric acid cycle intermediates like citrate inhibit the PEP-metabolizing enzymes in the cytosol. Finally, PEP inhibits the conversion of fructose-6-phosphate to fructose-1,6-biphosphate and restricts carbon feeding into glycolysis.

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 will be available, and the electron transport chain will operate at a reduced rate (see Figure 11.10). This slowdown could be signaled to citric acid cycle enzymes through an increase in matrix NADH, inhibiting the activity of several citric acid cycle dehydrogenases (Oliver and McIntosh 1995).

The buildup of citric acid cycle intermediates and their derivates, such as citrate and 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 to fructose-1,6-bisphosphate, thus inhibiting glycolysis.

In summary, plant respiratory rates are 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 rate of the glycolytic reactions.

Respiration Is Tightly Coupled to Other Pathways

Glycolysis, the 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 13. The respiratory pathways are central to the production 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.

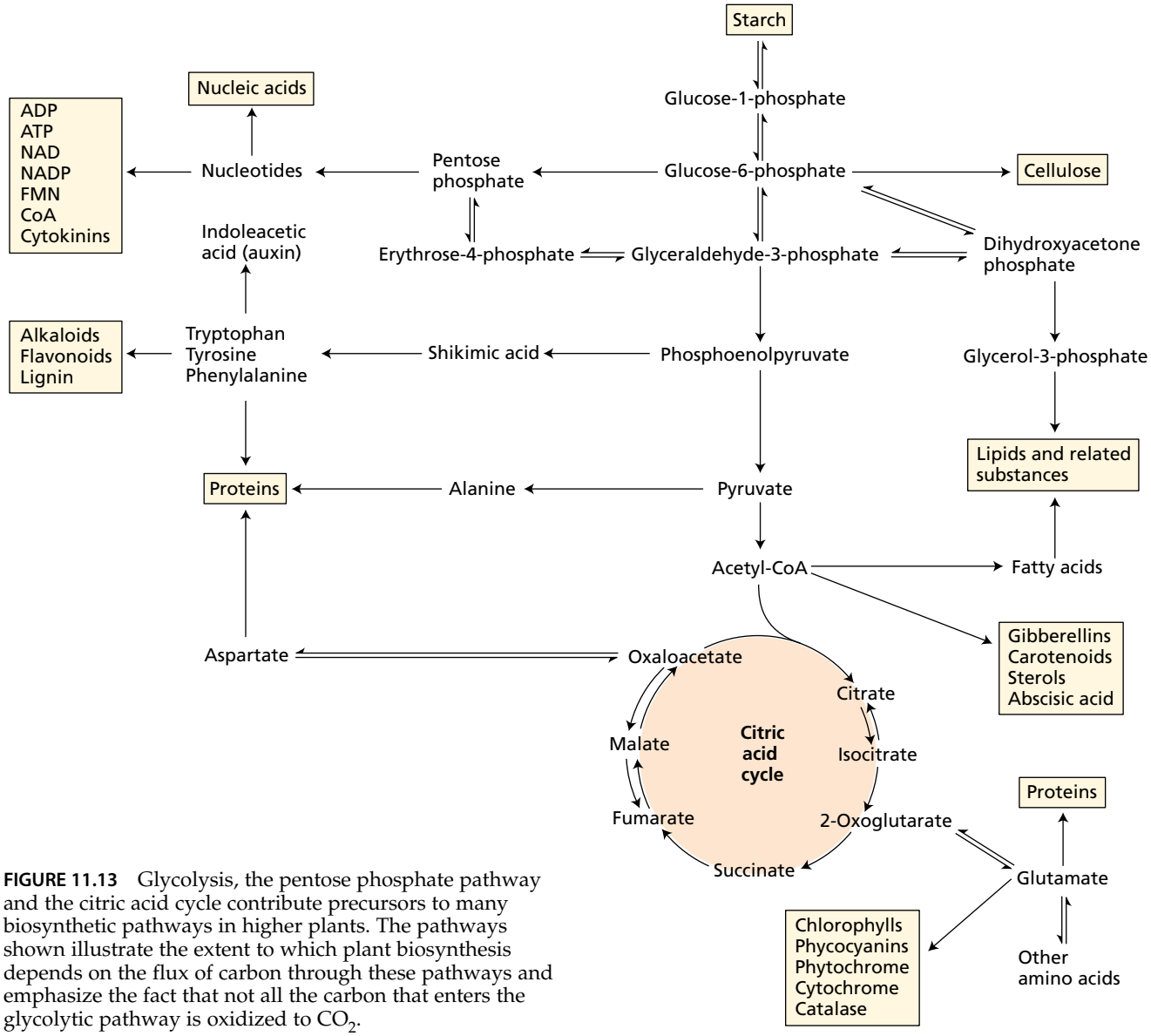


FIGURE 11.13 Glycolysis, the pentose phosphate pathway and the citric acid cycle contribute precursors to many biosynthetic pathways in higher plants. The pathways shown illustrate the extent to which plant biosynthesis depends on the flux of carbon through these pathways and emphasize the fact that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

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'll examine respiration and mitochondrial function in the context of the whole plant under a variety of conditions. First, when green tissues are exposed to light, respiration and photosynthesis operate simultaneously and interact in complex ways. Next we will discuss different rates of tissue respiration, which may be under developmental control, as well as the very interesting case of cytoplasmic male sterility. 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 the external oxygen concentration, temperature, and nutrient and water supply (see Chapter 25, **Web Topic 11.7**, and **Web Essay 11.5**).

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 due in large part to the presence, in plant cells, of a large central vacuole and cell wall compartments, 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 respiration in animals. In fact, isolated plant mitochondria respire faster than mammalian mitochondria, when expressed on a per mg protein basis.

Even though plants generally have low respiration rates, the contribution of respiration to the overall carbon economy of the plant can be substantial (see **Web Topic 11.7**). 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, can represent a substantial fraction of gross photosynthesis. A survey of several herbaceous species indicated that 30 to 60% of the daily gain in photosynthetic carbon was lost to respiration, although these values tended to decrease in older plants (Lambers 1985).

Young trees lose roughly a third of their daily photosynthate as respiration, and this loss can double in older trees as the ratio of photosynthetic to nonphotosynthetic tissue decreases. In tropical areas, 70 to 80% of the daily photosynthetic gain can be lost to respiration because of the high dark respiration rates associated with elevated night temperatures.

Respiration Operates during Photosynthesis

Mitochondria are involved in the metabolism of photosynthesizing leaves. The glycine generated by photorespiration is oxidized to serine in the mitochondrion (see Chapter 8). At the same time, mitochondria in photosynthesizing tissue also carry out respiration via the citric acid cycle (often called *dark respiration* because it does not require light). Relative to the maximum rate of photosynthesis, dark respiration rates measured in green tissues are far slower, generally by a factor ranging from 6- to 20-fold. Given that rates of photorespiration can often reach 20 to 40% of the gross photosynthetic rate, citric acid cycle-mediated mitochondrial respiration operates at rates also well below the rate of photorespiration.

A question that has not been adequately answered is how much mitochondrial respiration (apart from the involvement of mitochondria in the photorespiratory carbon oxidation cycle) operates simultaneously with photosynthesis in illuminated green tissues. The activity of pyruvate dehydrogenase, one of the ports of entry into the citric acid cycle, decreases in the light to 25% of the dark activity (Budde and Randall 1990). The overall rate of 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 even in illuminated leaves (Krömer 1995).

Another role of mitochondrial respiration during photosynthesis is to supply carbon metabolites for biosynthetic reactions—for example, by formation of 2-oxoglutarate needed for nitrogen assimilation. Leaf mitochondria typically have high capacities of nonphosphorylating pathways in the electron transport chain. By oxidizing NADH with lower ATP yield, mitochondria can maintain a higher 2-oxoglutarate production by the respiratory pathways without being restricted by the cytosolic demand for ATP (see Figures 11.7C and 11.12) (Hoefnagel et al. 1998; Noctor and Foyer 1998).

Additional evidence for the involvement of mitochondrial respiration in photosynthesizing leaves has been obtained in studies with mitochondrial mutants defective in respiratory complexes, showing that leaf development and photosynthesis are negatively affected (Vedel et al. 1999).

Different Tissues and Organs Respire at Different Rates

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 (on a dry-weight basis), and respiration rates of vegetative tissues 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 tissues, stems generally have the lowest respiration rates, and leaf and root respiration varies with the plant species and the conditions under which the plants are growing.

When a plant tissue has reached maturity, its respiration rate will either remain roughly constant or decrease 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 (avocado, apple, banana) and senescence in detached leaves and flowers. Both ripening and the climacteric respiratory rise are triggered by the endogenous production of ethylene, as well as by an exogenous application of ethylene (see Chapter 22). In general, ethylene-induced respiration is associated with an active cyanide-resistant alternative pathway, but the role of this pathway in ripening is not clear (Tucker 1993).

Mitochondrial Function Is Crucial during Pollen Development

A physiological feature directly linked to the plant mitochondrial genome is a phenomenon known as **cytoplasmic male sterility**, or ***cms***. Plant lines that display *cms* do not form viable pollen—hence the designation *male sterility*. The term *cytoplasmic* here refers to the fact that this trait is transmitted in a non-Mendelian fashion; the *cms* genotype is always maternally inherited with the mitochondrial genome. *cms* is a very important trait in plant breeding because a stable male sterile line can facilitate the production of hybrid seed stock. For this use, *cms* traits that produce no major effects throughout the plant's life cycle, except for male sterility, have been found for many species.

All plants carrying the *cms* trait that have been characterized at the molecular level show the presence of distinct rearrangements in their mtDNA, relative to wild-type plants. These rearrangements create novel open reading frames and have been strongly correlated with *cms* phenotypes in various systems. Nuclear restorer genes can overcome the effects of the mtDNA rearrangements and restore fertility to plants with the *cms* genotype. Such restorer genes are essential for the commercial utilization of *cms* if seeds are the harvested product.

An interesting consequence of the use of the *cms* gene occurred in the late 1960s, at which time 85% of the hybrid feed corn grown in the United States was derived from the use of a *cms* line of maize called *cms*-T (Texas). In *cms*-T maize, the mtDNA rearrangements give rise to a unique 13 kDa protein, URF13 (Levings and Siedow 1992). How the URF13 protein acts to bring about male sterility is not known, but in the late 1960s a disease appeared, caused by a race of the fungus *Bipolaris maydis* (also called *Cochliobolus heterostrophus*). This specific race synthesizes a compound (HmT-toxin) that specifically interacts with the URF13 protein to produce pores in the inner mitochondrial membrane, with the result that selective permeability is lost.

The interaction between HmT-toxin and URF13 made *Bipolaris maydis* race T a particularly virulent pathogen on *cms*-T maize and led to an epidemic in the corn-growing regions of the United States that was known as southern corn leaf blight. As a result of this epidemic, the use of *cms*-T in the production of hybrid maize was discontinued. No other *cms* maize has been found to be a suitable replacement, so current production of hybrid corn seed has reverted to manual detasseling that prevents self-pollination.

As compared to other organs, the amount of mitochondria per cell and the expression of respiratory proteins are very high in developing anthers, where pollen development is an energy-demanding process (Huang et al. 1994). Male sterility is a common phenotype of mutations in mitochondrial genes for subunits of the complexes of oxidative phosphorylation (Vedel et al. 1999). Such mutants can be viable because of the existence of the alternative nonphosphorylating respiratory pathways.

Programmed cell death (**PCD**) is part of normal anther development. There are now indications that mitochondria are involved in plant PCD and that PCD is premature in anthers of *cms* sunflower (see **Web Essay 11.6**).

Environmental Factors Alter Respiration Rates

Many environmental factors can alter the operation of metabolic pathways and 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 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 (see Chapter 2 on the web site for a discussion of K_m). However, respiration rates decrease if the atmospheric oxygen concentration is below 5% for whole tissues or below 2 to 3% for tissue slices. These findings show that oxygen diffusion through the aqueous phase in the tissue imposes a limitation on plant respiration.

The diffusion limitation imposed by an aqueous phase emphasizes the importance of the intercellular air spaces found in plant tissues for oxygen availability in the mitochondria. If there were no gaseous diffusion pathway throughout the plant, the cellular respiration rates of many plants would be limited by an insufficient oxygen supply (see **Web Essay 11.3**).

Water saturation/low O_2. Diffusion limitation is even more significant when plant organs are growing in an

aqueous medium. When plants are grown hydroponically, the solutions must be aerated vigorously to keep oxygen levels high in the vicinity of the roots. The problem of oxygen supply also arises with plants growing in very wet or flooded soils (see Chapter 25).

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 and tulip tree poplar can survive only in well-drained, aerated soils because their roots cannot tolerate more than a limited exposure to a flooded condition. On the other hand, many plant species are adapted to grow in flooded soils. Herbaceous species such as rice and sunflower often rely on a network of intercellular air spaces (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 having 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*, trees that grow in mangrove swamps under continuously flooded conditions.

Temperature. Respiration typically increases with temperature (see, however, **Web Essay 11.3**). Between 0 and 30°C, the increase in respiration rate for every 10°C increase in ambient temperature (commonly referred to as the dimensionless, temperature coefficient, Q_{10}) is about 2. Above 30°C the respiration rate often increases more slowly, reaches a plateau at 40 to 50°C and decreases at even higher temperatures. High night temperatures are thought to account for the high respiratory rates of tropical plants.

Low temperatures are utilized to retard postharvest respiration rates during the storage of fruits and vegetables. However, complications may arise from such storage. 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 in most tissues, but the breakdown of stored starch and its conversion to sucrose impart an unwanted sweetness to the tubers. As a compromise, potatoes are stored at 7 to 9°C, which prevents the breakdown of starch while minimizing respiration and germination.

CO_2 concentration. It is common practice in the commercial storage of fruits to take advantage of the effects of atmospheric oxygen and temperature on respiration, and to store fruits at low temperatures under 2 to 3% oxygen and 3 to 5% CO_2. The reduced temperature lowers the respiration rate, as does the reduced oxygen. Low levels of oxygen are used instead of anoxic conditions to avoid lowering tissue oxygen tensions to the point that stimulates fermentative metabolism.

Carbon dioxide has a limited direct inhibitory effect on the respiration rate at a concentration of 3 to 5%, which is well in excess of the 0.036% (360 ppm) normally found in the atmosphere. The atmospheric CO_2 concentration is increasing rapidly 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).

Compared to plants grown at 350 ppm CO_2, plants grown at 700 ppm CO_2 have been reported to have a 15 to 20% slower dark respiration rate (on a dry-weight basis) (Drake et al. 1999), but this has been questioned (Jahnke 2001; Bruhn et al. 2002). The number of mitochondria per unit cell area actually doubles in the high CO_2 environment. These data imply that the respiratory activity in the light instead may increase at higher ambient CO_2 concentrations (Griffin et al. 2001). Thus it is presently a matter of debate how plants growing at an increased CO_2 concentration will contribute to the global carbon cycle.

LIPID METABOLISM

Whereas animals use fats for energy storage, plants use them mainly for 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, peanut, and cotton. Oils often serve a major storage function in 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 describe the biosynthesis of two types of glycerolipids: the *triacylglycerols* (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. Plants can also use fats and oils for energy production. We will thus examine the complex process by which germinating seeds obtain 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 *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, or 9.3 kcal, of energy) can produce considerably more ATP than the oxidation of 1 g of starch (about 15.9 kJ, or 3.8 kcal). Conversely, the biosynthesis of

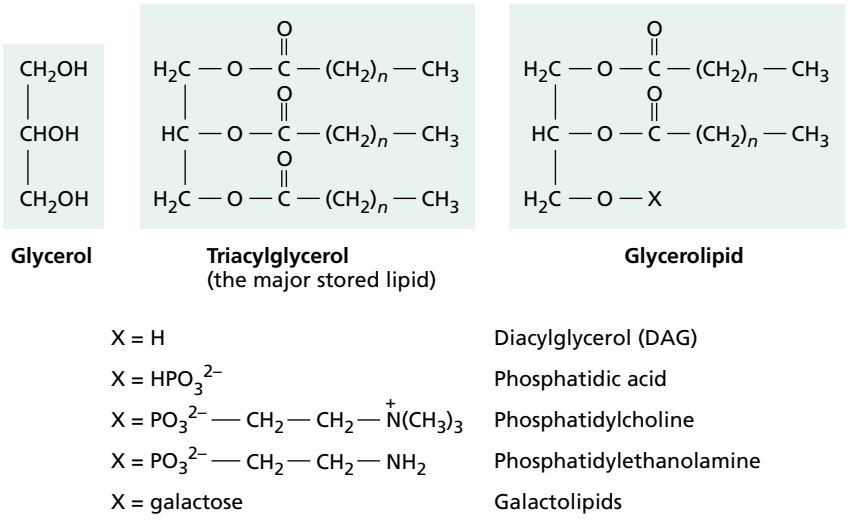


FIGURE 11.14 Structural features of triacylglycerols and polar glycerolipids in higher plants. The carbon chain lengths of the fatty acids, which always have an even number of carbons, range from 12 to 20 but are typically 16 or 18. Thus, the value of n is usually 14 or 16.

fats, oils, and related molecules, such as the phospholipids of membranes, 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 include 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 Oleosomes

Fats and oils exist mainly in the form of triacylglycerols (*acyl* refers to the fatty acid portion), or triglycerides, 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 20, but more commonly they 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.

The composition of fatty acids in plant lipids varies with the species. For example, peanut oil is about 9% palmitic acid, 59% oleic acid, and 21% linoleic acid, and cottonseed oil is 20% palmitic acid, 30% oleic acid, and 45% linoleic acid. The biosynthesis of these fatty acids will be discussed shortly.

Triacylglycerols in most seeds are stored in the cytoplasm of either cotyledon or endosperm cells in organelles known as **oleosomes** (also called *spherosomes* or *oil bodies*) (see Chapter 1). Oleosomes have an unusual membrane barrier that separates the triglycerides from the aqueous cytoplasm. A single layer of phospholipids (i.e., a half-bilayer) surrounds the oil body 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 oleosome is stabilized

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.

by the presence of specific proteins, called *oleosins*, that coat the surface and prevent the phospholipids of adjacent oil bodies from coming in contact and fusing.

This unique membrane structure for oleosomes 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 phase while hydrophobic fatty acid chains form the center 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.

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, phospholipids are the major membrane glycerolipids.

Fatty Acid Biosynthesis Consists of Cycles of Two-Carbon Addition

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

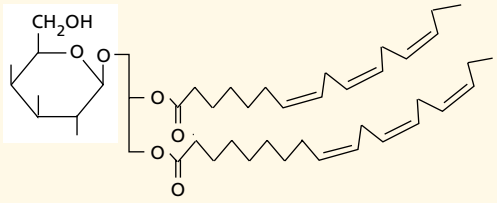
The enzymes of the pathway are thought to be held together in a complex that is collectively referred to as *fatty acid synthase*. The complex probably allows the series of reactions to occur more efficiently than it would if the enzymes were physically separated from each other. In addition, the growing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

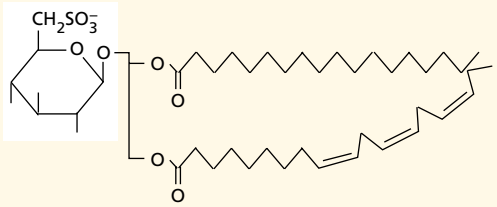
1. In the first cycle of fatty acid synthesis, the acetate group from acetyl-CoA is transferred to a specific cys-

TABLE 11.4
Glycerolipid components of cellular membranes

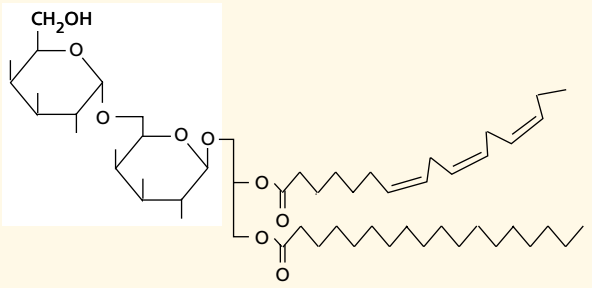
	Lipid composition (percentage of total)		
	Chloroplast	Endoplasmic reticulum	Mitochondrion
Phosphatidylcholine	4	47	43
Phosphatidylethanolamine	—	34	35
Phosphatidylinositol	1	17	6
Phosphatidylglycerol	7	2	3
Diphosphatidylglycerol	—	—	13
Monogalactosyldiacylglycerol	55	—	—
Digalactosyldiacylglycerol	24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



Monogalactosyldiacylglycerol
(18:3 | 16:3)

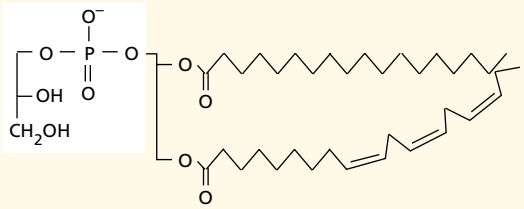


Sulfolipid (sulfoquinovosyldiacylglycerol)
(18:3 | 16:0)

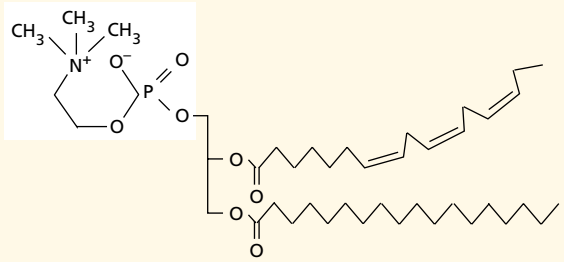


Digalactosyldiacylglycerol
(16:0 | 18:3)

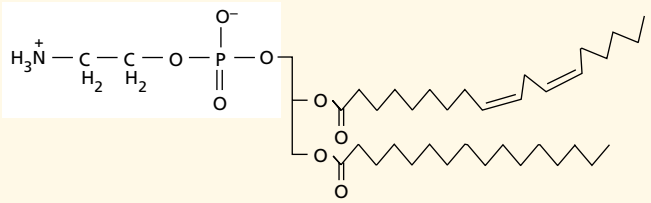
(A) Glyceroglycolipids



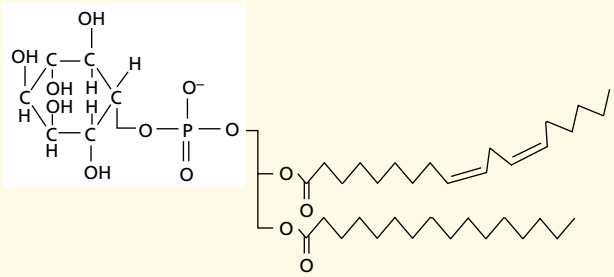
Phosphatidylglycerol
(18:3 | 16:0)



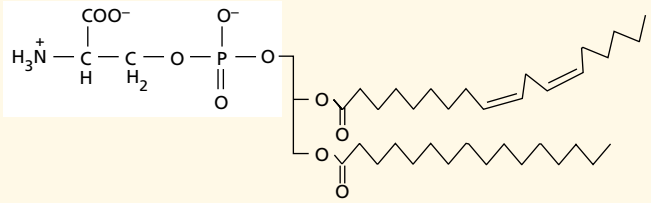
Phosphatidylcholine
(16:0 | 18:3)



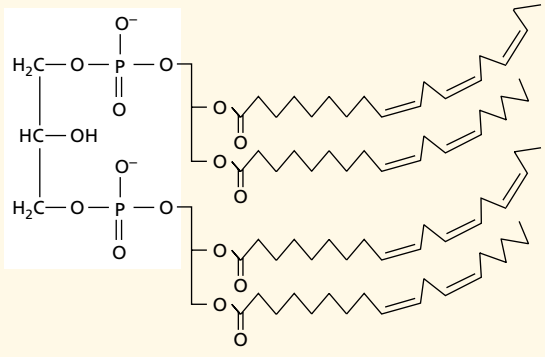
Phosphatidylethanolamine
(16:0 | 18:2)



Phosphatidylinositol
(16:0 | 18:2)



Phosphatidylserine
(16:0 | 18:2)



Diphosphatidylglycerol (cardiolipin)
(18:2 | 18:2)

(B) Glycerophospholipids

FIGURE 11.15 Major polar lipids of plant membranes: (A) glyceroglycolipids and (B) glycerophospholipids. At least six different fatty acids may be attached to the glycerol backbone. One of the more common molecular species is shown for each lipid. 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).

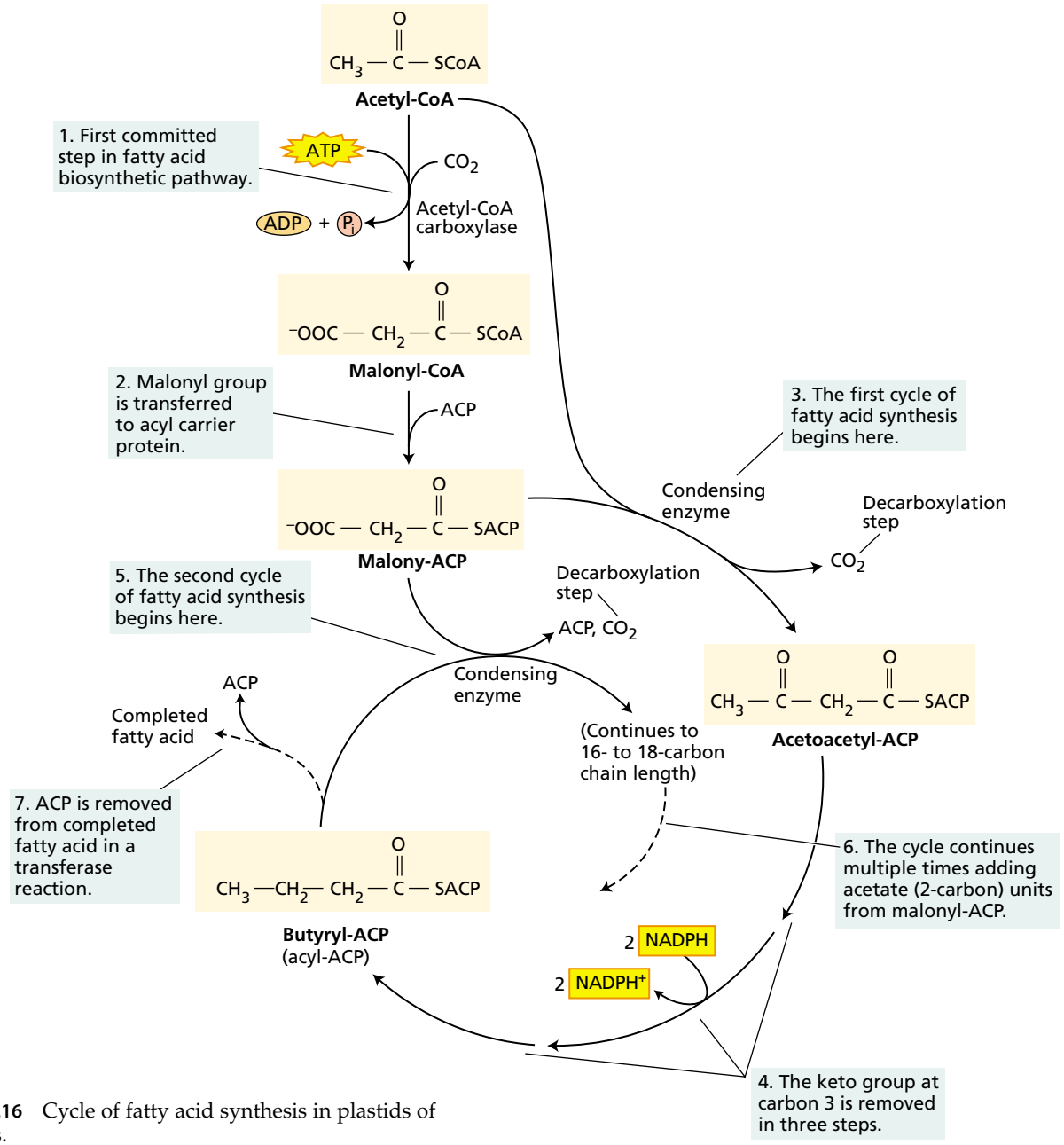


FIGURE 11.16 Cycle of fatty acid synthesis in plastids of plant cells.

teine of *condensing enzyme* (3-ketoacyl-ACP synthase) and then combined with malonyl-ACP to form acetoacetyl-ACP.

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 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, and 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 to 18:1-ACP by a desaturase enzyme. The repetition of this sequence of events makes 16:0-ACP and 18:1-ACP 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 plastid are next used to make the glycerolipids of membranes and oleosomes. 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 to 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.

1. In chloroplasts, the **prokaryotic pathway** utilizes the 16:0- 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 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.

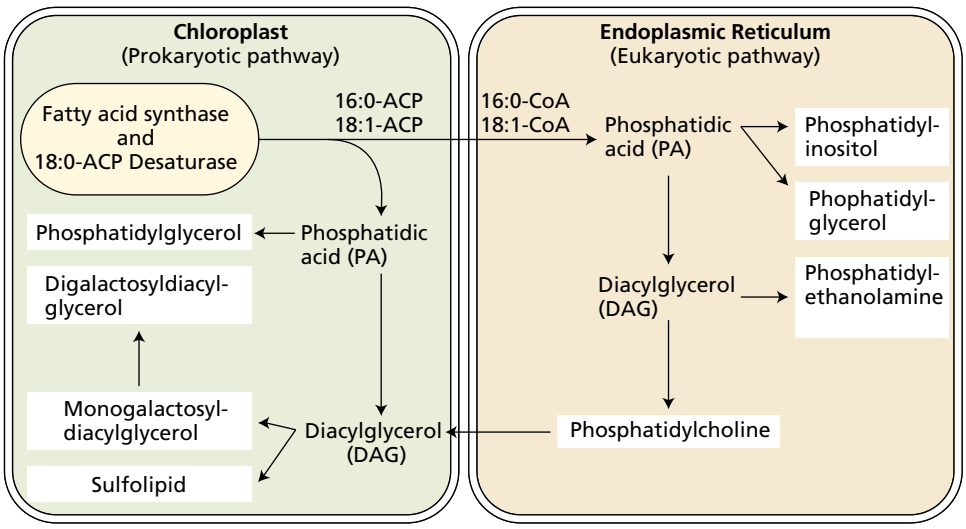


FIGURE 11.17 The two pathways for glycerolipid synthesis in the chloroplast and ER of *Arabidopsis* leaf cells. The major membrane components are shown in boxes. Glycerolipid desaturates in the chloroplast, and enzymes in the endoplasmic reticulum convert 16:0 and 18:1 fatty acids to the more highly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

16:0- 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).

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 oleosomes—from which they can be mobilized during germination and converted to sugar.

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. 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, obviously such a simple model is 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 (Wolter et al. 1992). For example, chill-sensitive plants experience sharp reductions in growth rate and development at temperatures between 0 and 12°C (see Chapter 25). 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 the cellular membranes. According to this proposal, this transition would result in alterations in the metabolism of chilled cells and 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 temperature appear quite distinct from what is predicted by the chilling 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 the extent of membrane unsaturation or the presence of particular lipids, such as disaturated phosphatidylglycerol, can affect the responses of plants to low temperature. 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

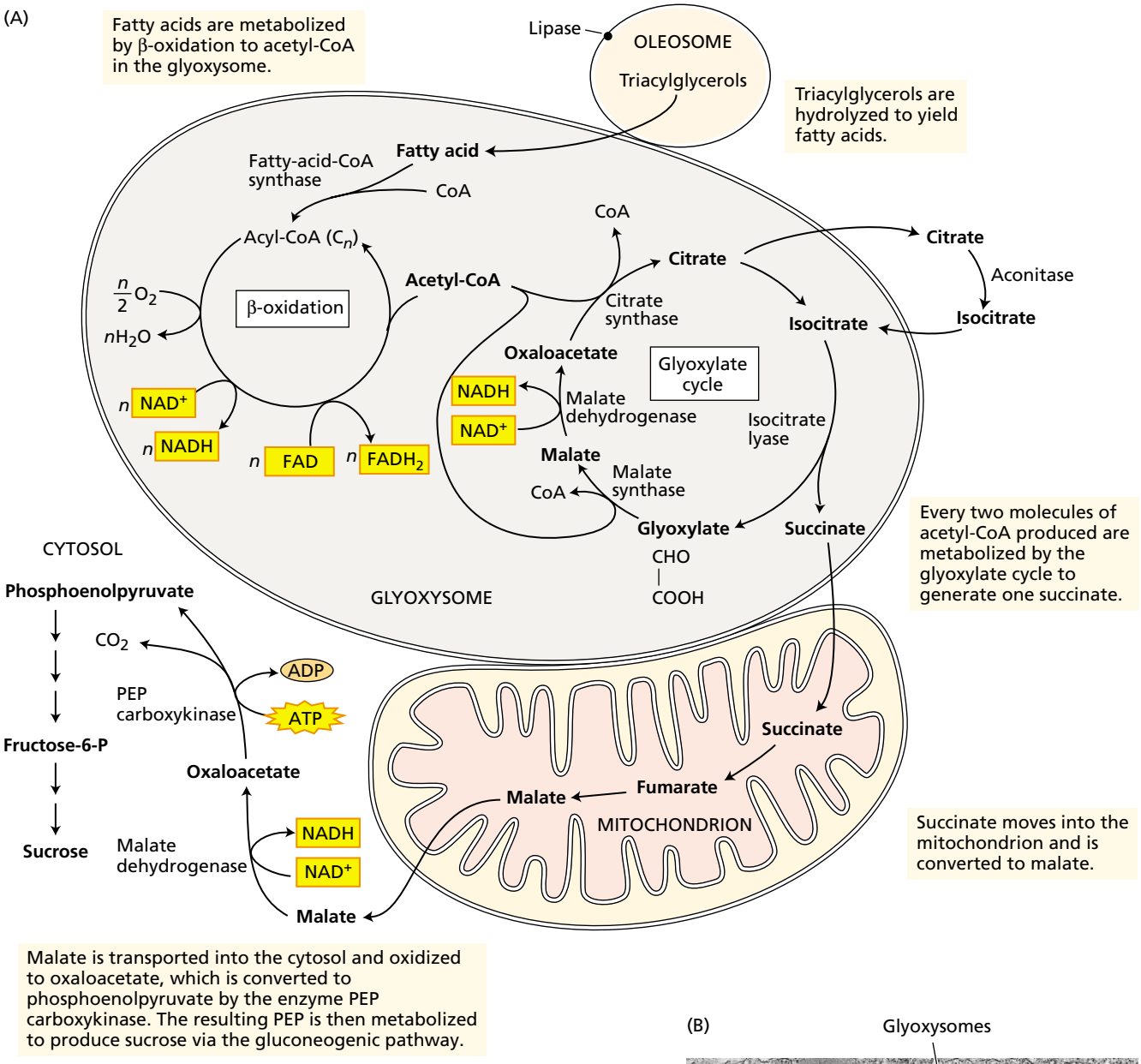
Plants, animals, and microbes all use membrane lipids as precursors for compounds that are used for intracellular or long-range signaling. For example, jasmonate derived from linolenic acid (18:3) activates plant defenses against insects and many fungal pathogens. In addition, jasmonate regulates other aspects of plant growth, including the development of anthers and pollen (Stintzi and Browse 2000). **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 to inositol trisphosphate (IP_3) and diacylglycerol, which both act as intracellular secondary messengers.

The action of IP_3 in releasing Ca^{2+} into the cytoplasm (through calcium-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 lipids to sucrose. Plants are not able to transport fats from the endosperm to the root and shoot tissues of the germinating seedling, so they must convert stored lipids to a more mobile form of carbon, generally sucrose. This process involves several steps that are located in different cellular compartments: oleosomes, glyoxysomes, mitochondria, and cytosol.

Overview: Lipids to sucrose. The conversion of lipids to sucrose in oilseeds is triggered by germination and begins with the hydrolysis of triacylglycerols stored in the oil bodies to free fatty acids, followed by oxidation of the fatty acids to produce acetyl-CoA (Figure 11.18). The fatty



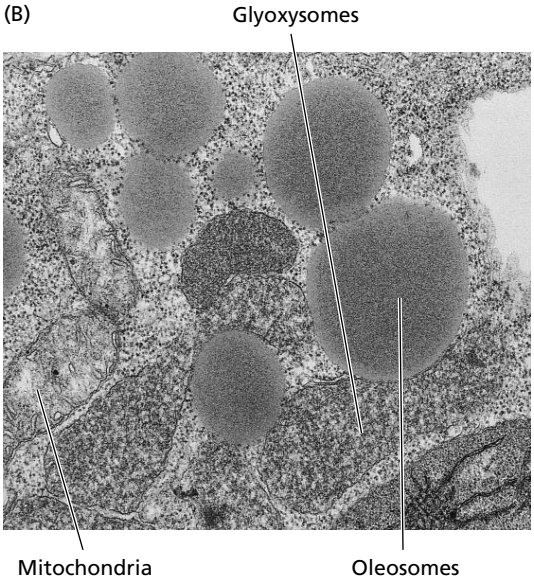


FIGURE 11.18 The conversion of fats to sugars during germination in oil-storing seeds. (A) Carbon flow during fatty acid breakdown and gluconeogenesis (refer to Figures 11.2, 11.3, and 11.6 for structures). (B) Electron micrograph of a cell from the oil-storing cotyledon of a cucumber seedling, showing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

acids are oxidized in a type of peroxisome called a **glyoxysome,** an organelle enclosed by a single bilayer membrane that is found in the oil-rich storage tissues of seeds. Acetyl-CoA is metabolized in the glyoxysome (see Figure 11.18A) to produce succinate, which is transported from the glyoxysome to the mitochondrion, where it is converted first to oxaloacetate and then to malate. The process ends in the cytosol with the conversion of malate to glucose via gluconeogenesis, and then to sucrose.

Although some of this fatty acid–derived carbon is diverted to other metabolic reactions in certain oilseeds, in castor bean (*Ricinus communis*) the process is so efficient that each gram of lipid metabolized results in the formation of 1 g of carbohydrate, which is equivalent to a 40% recovery of free energy in the form of carbon bonds ([15.9 kJ/40 kJ] × 100 = 40%).

Lipase hydrolysis. The initial step in the conversion of lipids to carbohydrate is the breakdown of triglycerides stored in the oil bodies by the enzyme lipase, which, at least in castor bean endosperm, is located on the half-membrane that serves as the outer boundary of the oil body. The lipase hydrolyzes triacylglycerols to three molecules of fatty acid and glycerol. Corn and cotton also contain a lipase activity in the oil body, but peanut, soybean, and cucumber show lipase activity in the glyoxysome instead. During the breakdown of lipids, oil bodies and glyoxysomes are generally in close physical association (see Figure 11.18B).

β-Oxidation of fatty acids. After hydrolysis of the triacylglycerols, the resulting fatty acids enter the glyoxysome, where they are activated by conversion to fatty-acyl-CoA by the enzyme fatty-acyl-CoA synthase. 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 number of carbons) are sequentially broken down to $n/2$ 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 1 NADH and 1 $FADH_2$ 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 localized exclusively in the glyoxysome. Interestingly, in plant vegetative tissues (e.g., mung bean hypocotyl and potato tuber), the β-oxidation reactions are localized in a related organelle, the peroxisome (see Chapter 1).

The glyoxylate cycle. The function of the glyoxylate cycle is to convert two molecules of acetyl-CoA to 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 peroxisome and converted to malate by two reactions that are unique to the glyoxylate pathway.

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

Malate is then oxidized by malate dehydrogenase to oxaloacetate, which can combine with another acetyl-CoA to continue the cycle (see Figure 11.18A). The glyoxylate produced keeps the cycle operating in the glyoxysome, 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 to 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 to carbohydrate.

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 to 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 the primary form of reduced carbon translocated from the cotyledons to the growing seedling tissues. Not all seeds quantitatively convert fat to sugar (see **Web Topic 11.9**).

SUMMARY

In plant respiration, reduced cellular carbon generated during photosynthesis is oxidized to CO_2 and water, and this oxidation is coupled to the synthesis of ATP. Respiration takes place in three main stages: glycolysis, the citric acid cycle, and oxidative phosphorylation. The latter comprises the electron transport chain and ATP synthesis.

In glycolysis, carbohydrate is converted in the cytosol to pyruvate, and a small amount of ATP is synthesized via substrate-level phosphorylation. Pyruvate is subsequently oxidized within the mitochondrial matrix through the citric acid cycle, generating a large number of reducing equivalents in the form of NADH and $FADH_2$.

In the third stage, oxidative phosphorylation, electrons from NADH and $FADH_2$ pass through the electron transport chain in the inner mitochondrial membrane to reduce oxygen. The chemical energy is conserved in the form of an electrochemical proton gradient, which is created by the

coupling of electron flow to proton pumping from the matrix to the intermembrane space. This energy is then converted into chemical energy in the form of ATP by the F_oF_1-ATP synthase, also located in the inner membrane, which couples ATP synthesis from ADP and P_i to the flow of protons back into the matrix down their electrochemical gradient.

Aerobic respiration in plants has several unique features, including the presence of a cyanide-resistant alternative oxidase and multiple NAD(P)H dehydrogenases, none of which pumps protons. Substrate oxidation during respiration is regulated at control points in glycolysis, the citric acid cycle, and the electron transport chain, but ultimately substrate oxidation is controlled by the level of cellular ADP. Carbohydrates can also be oxidized via the oxidative pentose phosphate pathway, in which the reducing power is produced in the form of NADPH mainly for biosynthetic purposes. Numerous glycolytic and citric acid cycle intermediates also provide the starting material for a multitude of biosynthetic pathways.

More than 50% of the daily photosynthetic yield can be respired by a plant, but many factors can affect the respiration rate observed at the whole-plant level. These factors include the nature and age of the plant tissue, as well as environmental factors such as light, oxygen concentration, temperature, and CO_2 concentration.

Lipids play a major role in plants: Amphipathic lipids serve as the primary nonprotein components of plant membranes; fats and oils are an efficient storage form of reduced carbon, particularly in seeds. Glycerolipids play important roles as structural components of membranes. Fatty acids are synthesized in plastids using acetyl-CoA. Fatty acids from the plastid can be transported to the ER, where they are further modified.

Membrane function may be influenced by the 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. On the other hand, certain membrane lipid breakdown products, such as jasmonic acid, can act as signaling agents in plant cells.

Triacylglycerol is synthesized in the ER and accumulates within the phospholipid bilayer, forming oil bodies. During germination in oil-storing seeds, the stored lipids are metabolized to carbohydrate in a series of reactions that involve a metabolic sequence known as the glyoxylate cycle. This cycle takes place in glyoxysomes, and subsequent steps occur in the mitochondria. The reduced carbon generated during lipid breakdown in the glyoxysomes is ultimately converted to carbohydrate in the cytosol by gluconeogenesis.

Web Material

Web Topics

11.1 Isolation of Mitochondria

Methods for the isolation of intact, functional mitochondria have been developed.

11.2 The Electron Transport Chain of Plant Mitochondria Contains Multiple NAD(P)H Dehydrogenases

Mitochondrial NAD(P)H dehydrogenases oxidize NADH or NADPH and pass the electrons to ubiquinone.

11.3 The Alternative Oxidase

The alternative oxidase is an oxidoreductase localized at the inner membrane of plant mitochondria.

11.4 F_oF_1-ATP Synthases: The World's Smallest Rotary Motors

Rotation of the γ subunit brings about the conformational changes that allow the release of ATP from the enzyme.

11.5 Transport In and Out of Plant Mitochondria

Plant mitochondria operate different transport mechanisms.

11.6 The Genetic System of Plant Mitochondria Has Some Special Features

The mitochondrial genome encodes about 40 mitochondrial proteins.

11.7 Does Respiration Reduce Crop Yields?

Empirical relations between plant respiration rates and crop yield have been established.

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 Essays

11.1 Metabolic Flexibility Helps Plants 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 Temperature Regulation by Thermogenic Flowers

In thermogenic flowers, such as the Arum lilies, temperature can increase up to 20°C above their surroundings.

11.4 Reactive Oxygen Species (ROS) and Plant Mitochondria

The production of damaging reactive oxygen species is an unavoidable consequence of aerobic respiration.

11.5 The Role of Respiration in Desiccation Tolerance

Respiration has both positive and negative effects on the survival of plant cells under water stress.

11.6 Balancing Life and Death; The Role of Mitochondria in Programmed Cell Death

Programmed cell death is an integral part of the life cycle of plants, directly involving mitochondria.

Chapter References

Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994) Structure at 2.8 Å resolution of F_1-ATPase from bovine heart mitochondria. *Nature* 370: 621–628.

Ap Rees, T. (1980) Assessment of the contributions of metabolic pathways to plant respiration. In *The Biochemistry of Plants*, Vol. 2, D. D. Davies, ed., Academic Press, New York, pp. 1–29.

Brand, M. D. (1994) The stoichiometry of proton pumping and ATP synthesis in mitochondria. *Biochemist* 16(4): 20–24.

Bruhn, D., Mikkelsen, T. N., and Atkin, O. K. (2002) Does the direct effect of atmospheric CO_2 concentration on leaf respiration vary with temperature? Responses in two species of *Plantago* that differ in relative growth rate. *Physiol. Plant.* 114: 57–64.

Budde, R. J. A., and Randall, D. D. (1990) Pea leaf mitochondrial pyruvate dehydrogenase complex is inactivated *in vivo* in a light-dependent manner. *Proc. Natl. Acad. Sci. USA* 87: 673–676.

Dahlqvist, A., Stahl, U., Lenman, M., Banas, A., Lee, M., Sandager, L., Ronne, H., and Stymne, S. (2000) Phospholipid:diacylglycerol acyltransferase: An enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. *Proc. Natl. Acad. Sci. USA* 97: 6487–6492.

Dennis, D. T., and Blakely, S. D. (2000) Carbohydrate metabolism. In *Biochemistry & Molecular Biology of Plants*, B. Buchanan, W. Gruissem, and R. Jones, eds., American Society of Plant Physiologists, Rockville, MD, pp. 630–674.

Dennis, D. T., Huang, Y., and Negm, F. B. (1997) Glycolysis, the pentose phosphate pathway and anaerobic respiration. In *Plant Metabolism*, 2nd ed., D. T. Dennis, D. H. Turpin, D. D. Lefebvre, and D. B. Layzell, eds., Longman, Singapore, pp. 105–123.

Douce, R. (1985) *Mitochondria in Higher Plants: Structure, Function, and Biogenesis*. Academic Press, Orlando, FL.

Drake, B. G., Azcon-Bieto, J., Berry, J., Bunce, J., Dijkstra, P., Farrar, J., Gifford, R. M., Gonzalez-Meler, M. A., Koch, G., Lambers, H., Siedow, J., and Wullschleger, S. (1999) Does elevated atmospheric CO_2 concentration inhibit mitochondrial respiration in green plants? *Plant Cell Environ.* 22: 649–657.

Givan, C. V. (1999) Evolving concepts in plant glycolysis: Two centuries of progress. *Biol. Rev.* 74: 277–309.

Griffin, K. L., Anderson, O. R., Gastrich, M. D., Lewis, J. D., Lin, G., Schuster, W., Seemann, J. R., Tissue, D. T., Turnbull, M. H., and Whitehead, D. (2001) Plant growth in elevated CO_2 alters mitochondrial number and chloroplast fine structure. *Proc. Natl. Acad. Sci. USA* 98: 2473–2478.

Gunning, B. E. S., and Steer, M. W. (1996) *Plant Cell Biology: Structure and Function of Plant Cells*. Jones and Bartlett, Boston.

Hoefnagel, M. H. N., Atkin, O. K., and Wiskich, J. T. (1998) Interdependence between chloroplasts and mitochondria in the light and the dark. *Biochim. Biophys. Acta* 1366: 235–255.

Huang, J., Struck, F., Matzinger, D. F., and Levings, C. S. (1994) Flower-enhanced expression of a nuclear-encoded mitochondrial respiratory protein is associated with changes in mitochondrion number. *Plant Cell* 6: 439–448.

Jahnke, S. (2001) Atmospheric CO_2 concentration does not directly affect leaf respiration in bean or poplar. *Plant Cell Environ.* 24: 1139–1151.

Krömer, S. (1995) Respiration during photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46: 45–70.

Kruger, N. J. (1997) Carbohydrate synthesis and degradation. In *Plant Metabolism*, 2nd ed., D. T. Dennis, D. H. Turpin, D. D. Lefebvre, and D. B. Layzell, eds., Longman, Singapore, pp. 83–104.

Laloi, M., Klein, M., Riesmeier, J. W., Müller-Röber, B., Fleury, C., Bouillaud, F., and Ricquier, D. (1997) A plant cold-induced uncoupling protein. *Nature* 389: 135–136.

Lambers, H. (1985) Respiration in intact plants and tissues. Its regulation and dependence on environmental factors, metabolism and invaded organisms. In *Higher Plant Cell Respiration* (Encyclopedia of Plant Physiology, New Series, Vol. 18), R. Douce and D. A. Day, eds., Springer, Berlin, pp. 418–473.

Leon, P., Arroyo, A., and Mackenzie, S. (1998) Nuclear control of plastid and mitochondrial development in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49: 453–480.

Levings, C. S., III, and Siedow, J. N. (1992) Molecular basis of disease susceptibility in the Texas cytoplasm of maize. *Plant Mol. Biol.* 19: 135–147.

Marienfeld, J., Unseld, M., and Brennicke, A. (1999) The mitochondrial genome of *Arabidopsis* is composed of both native and immigrant information. *Trends Plant Sci.* 4: 495–502.

McCabe, T. C., Daley, D., and Whelan, J. (2000) Regulatory, developmental and tissue aspects of mitochondrial biogenesis in plants. *Plant Biol.* 2: 121–135.

Møller, I. M. (2001) Plant mitochondria and oxidative stress. Electron transport, NADPH turnover and metabolism of reactive oxygen species. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52: 561–591.

Møller, I. M., and Rasmusson, A. G. (1998) The role of NADP in the mitochondrial matrix. *Trends Plant Sci.* 3: 21–27.

Napier, J. A., Stobart, A. K., and Shewry, P. R. (1996) The structure and biogenesis of plant oil bodies: The role of the ER membrane and the oleosin class of proteins. *Plant Mol. Biol.* 31: 945–956.

Nicholls, D. G., and Ferguson, S. J. (2002) *Bioenergetics 3*, 3rd ed. Academic Press, San Diego, CA.

Noctor, G., and Foyer, C. H. (1998) A re-evaluation of the ATP:NADPH budget during C3 photosynthesis: A contribution

from nitrate assimilation and its associated respiratory activity? *J. Exp. Bot.* 49: 1895–1908.

Ohlrogge, J. B., and Browse, J. A. (1995) Lipid biosynthesis. *Plant Cell* 7: 957–970.

Ohlrogge, J. B., and Jaworski, J. G. (1997) Regulation of fatty acid synthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48: 109–136.

Oliver, D. J., and McIntosh, C. A. (1995) The biochemistry of the mitochondrial matrix. In *The Molecular Biology of Plant Mitochondria*, C. S. Levings III and I. Vasil, eds., Kluwer, Dordrecht, Netherlands, pp. 237–280.

Plaxton, W. C. (1996) The organization and regulation of plant glycolysis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 185–214.

Raskin, I., Turner, I. M., and Melander, W. R. (1989) Regulation of heat production in the inflorescences of an *Arum* lily by endogenous salicylic acid. *Proc. Natl. Acad. Sci. USA* 86: 2214–2218.

Sachs, M. M., Subbaiah, C. C., and Saab, I. N. (1996) Anaerobic gene expression and flooding tolerance in maize. *J. Exp. Bot.* 47: 1–15.

Sasaki, Y., Konishi, T., and Nagano, Y. (1995) The compartmentation of acetyl-coenzyme A carboxylase in plants. *Plant Physiol.* 108: 445–449.

Schroeder, J. I., Allen, G. J., Hugouvieux, V., Kwak, J. M., and Waner, D. (2001) Guard cell signal transduction. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52: 627–658.

Siedow, J. N. (1995) Bioenergetics: The plant mitochondrial electron transfer chain. In *The Molecular Biology of Plant Mitochondria*, C. S. Levings III and I. Vasil, eds., Kluwer, Dordrecht, Netherlands, pp. 281–312.

Siedow, J. N., and Umbach, A. L. (1995) Plant mitochondrial electron transfer and molecular biology. *Plant Cell* 7: 821–831.

Stintzi, A., and Browse, J. (2000) The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. *Proc. Natl. Acad. Sci. USA* 97: 10625–10630.

Thompson, P., Bowsher, C. G., and Tobin, A. K. (1998) Heterogeneity of mitochondrial protein biogenesis during primary leaf development in barley. *Plant Physiol.* 118: 1089–1099.

Tucker, G. A. (1993) Introduction. In *Biochemistry of Fruit Ripening*, G. Seymour, J. Taylor, and G. Tucker, eds., Chapman & Hall, London, pp. 1–51.

Vanlerberghe, G. C., and McIntosh, L. (1997) Alternative oxidase: From gene to function. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48: 703–734.

Vedel, F., Lalanne, É., Sabar, M., Chétrit, P., and De Paepe, R. (1999) The mitochondrial respiratory chain and ATP synthase complexes: Composition, structure and mutational studies. *Plant Physiol. Biochem.* 37: 629–643.

Vercesi, A. E., Martins I. S., Silva, M. P., and Leite, H. M. F. (1995) PUMPing plants. *Nature* 375: 24.

Wagner, A. M., and Krab, K. (1995) The alternative respiration pathway in plants: Role and regulation. *Physiol. Plant.* 95: 318–325.

Wang, X. (2001) Plant phospholipases. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52: 211–231.

Whitehouse, D. G., and Moore, A. L. (1995) Regulation of oxidative phosphorylation in plant mitochondria. In *The Molecular Biology of Plant Mitochondria*, C. S. Levings III and I. K. Vasil, eds., Kluwer, Dordrecht, Netherlands, pp. 313–344.

Wolter, F. P., Schmidt, R., and Heinz, E. (1992) Chilling sensitivity of *Arabidopsis thaliana* with genetically engineered membrane lipids. *EMBO J.* 11: 4685–4692.