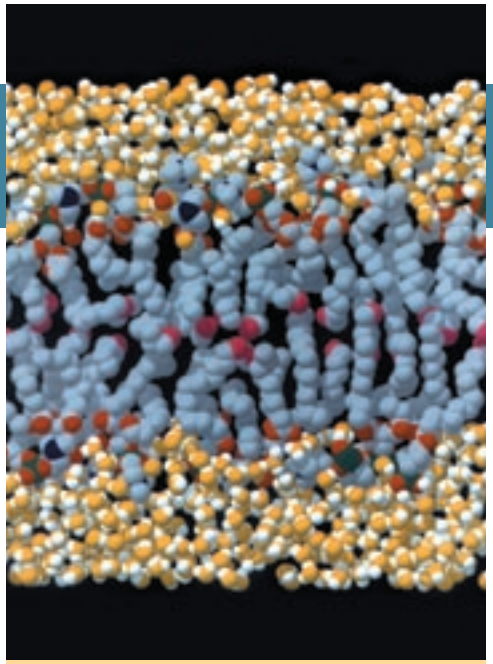
Biochemistry & Molecular Biology of Plants, B. Buchanan, W. Gruissem, R. Jones, Eds.



CHAPTER 10

Lipids

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CHAPTER OUTLINE

Introduction

The term **lipid** refers to a structurally diverse group of molecules that are preferentially soluble in a nonaqueous solvent such as chloroform. Lipids include a wide variety of fatty acid–derived compounds, as well as many pigments and secondary compounds that are metabolically unrelated to fatty acid metabolism. Although we will limit our discussion of lipids to those compounds with origins in fatty acid synthesis, this limitation still provides a broad group of compounds to explore, many of which are vital to the normal functioning of a cell. Each plant cell contains a diverse range of lipids, often located in specific structures. Furthermore, different plant species may contain different lipids.

Although the metabolism of fatty acids and lipids in plants has many features in common with other organisms, the lipid pathways in plants are complex and not well understood. The complexity arises primarily from cellular compartmentalization of the pathways and the extensive intermixing of lipid pools between these compartments (Fig. 10.1). In addition, higher plants collectively accumulate more than 200 different fatty acids, so there are many open questions about the nature of the enzymes involved in the synthesis of these compounds. Among the many challenges facing plant biochemists today is the complete elucidation of these pathways and the mechanisms that regulate them.

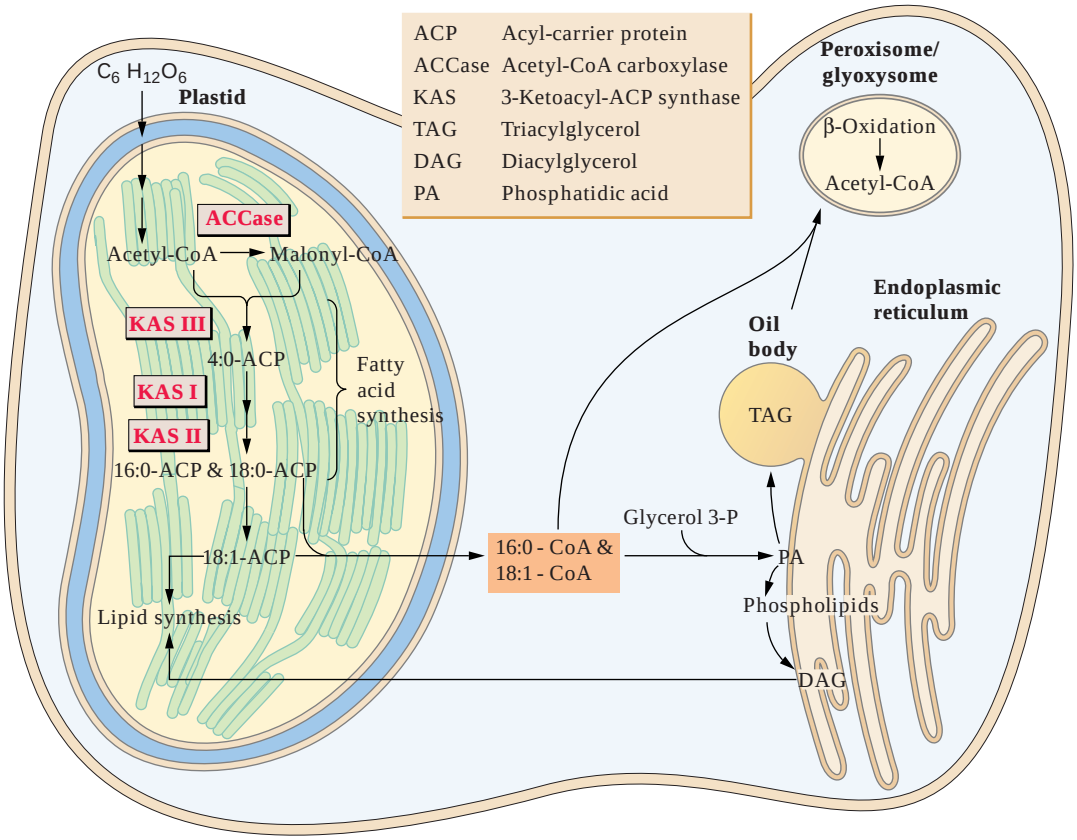


Figure 10.1
Lipid synthesis and metabolism take place in various organelles and in some cases involve movement of lipids from one cellular compartment to another.

10.1 Structure and function of lipids

10.1.1 Lipids have diverse roles in plants.

Lipids serve many functions in plants (Table 10.1). As the major components of biological membranes, they form a hydrophobic barrier that is critical to life (see Chapter 1). Membranes not only separate cells from their surroundings; they also separate the contents of organelles, such as chloroplasts and mitochondria, from the cytoplasm. Cellular compartmentalization depends on polar lipids forming a bilayer that prevents free diffusion of hydrophilic molecules between the cellular organelles and prevents diffusion in and out of the cells. The membranes of chloroplasts, in which the light reactions of photosynthesis take place, primarily contain **galactolipids.** Membranes external to plastids are composed mainly of mixtures of **phospholipids.** Although a single gram of leaf tissue may contain as much as 1 m^2 of membrane, lipids make up a relatively small proportion of the total mass of plant tissue (Fig. 10.2).

Lipids also represent a substantial chemical reserve of free energy. Because fatty acids are substantially more reduced organic molecules than carbohydrates, fatty acid oxidation has a higher potential for producing energy. Furthermore, **triacylglycerols** are largely hydrophobic and exist in an essentially anhydrous environment. Carbohydrates, however, are hydrophilic, and the water of hydration adds substantially to their mass. Thus, on a mass basis, the ATP yield from catabolism to CO_2 and H_2O is

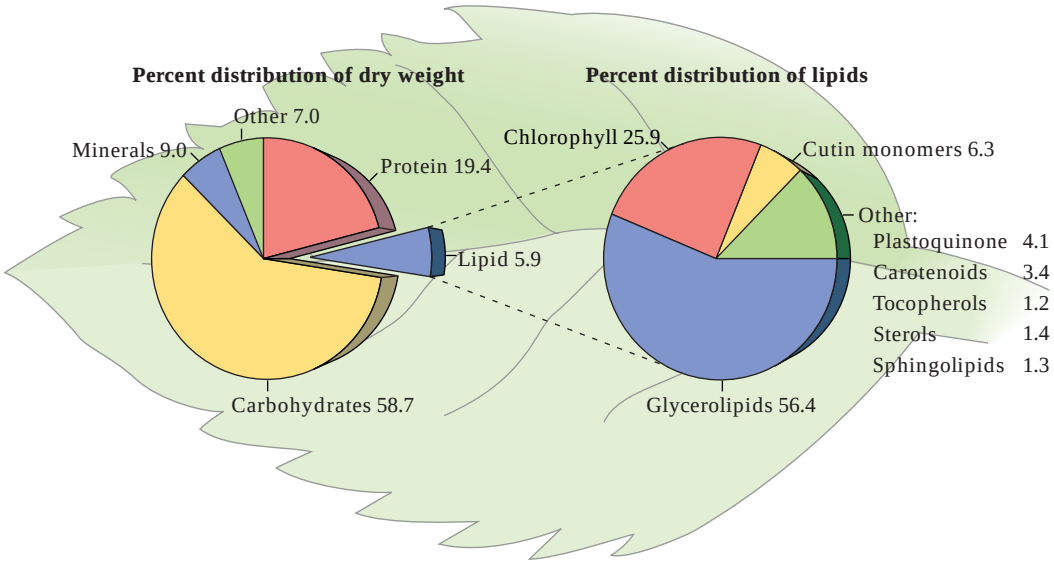
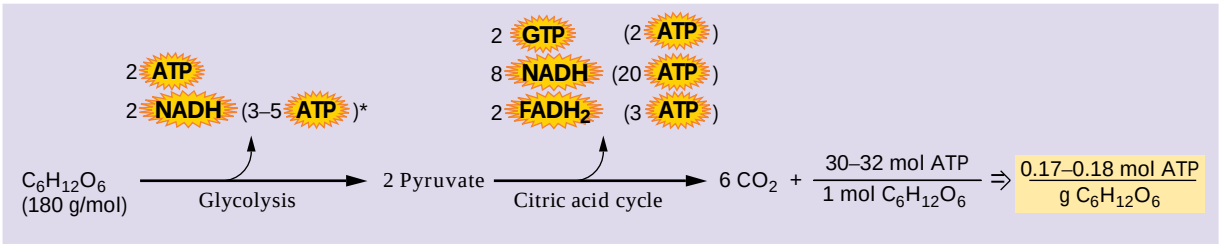


Figure 10.2
Approximate distributions of cellular constituents (as a percentage of total dry weight) and lipid types (as a percentage of total lipids by weight) in leaf tissues of *Arabidopsis*. Some of the values were extrapolated from results obtained with other species.

approximately twice as high for triacylglycerols as for carbohydrates (Fig. 10.3). In cases where a compact seed mass is advantageous for facilitating dispersal or other processes, the carbon and energy required for seed germination are often stored in the form of triacylglycerols rather than as starch.

Fatty acids are also the precursors for other significant components of plant metabolism. The **waxes** that coat and protect plants from the environment are complex mixtures of long-chain hydrocarbons, aldehydes, alcohols, acids, and esters derived almost entirely from fatty acids. The **cutin** and **suberin** layers of epidermal cells also are composed of oxygenated fatty acids esterified with one another to produce a tough, polyester skin. Thus, in epidermal cells of

ATP/g from sugar



ATP/g from fatty acid

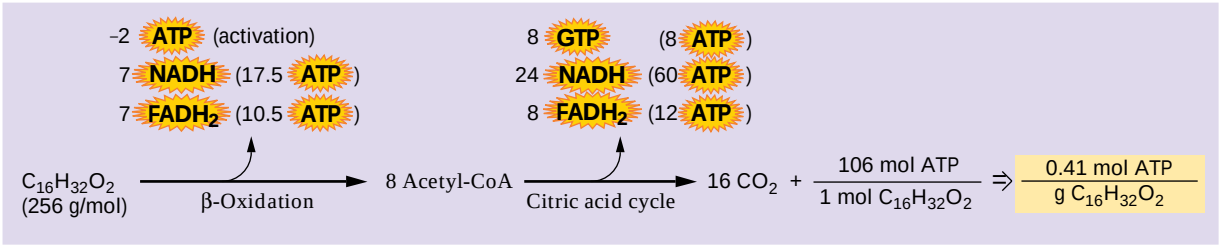


Figure 10.3
Comparison of energy yield in animals from metabolism of fatty acids and carbohydrates to CO_2 and H_2O. Metabolism of fatty acids produces 0.41 mol of ATP per gram of fatty acid, whereas metabolism of carbohydrate yields 0.17 to 0.18 mol / g. Oxidation of one NADH by the mitochondrial electron transport chain is assumed to yield 2.5 ATP, whereas oxidation of one $FADH_2$ is assumed to yield 1.5 ATP. *The number of ATPs derived from one NADH oxidized in the cytosol or peroxisome varies according to the mechanism by which the reducing equivalents (electrons) are transferred into the mitochondrion (see Chapter 14).

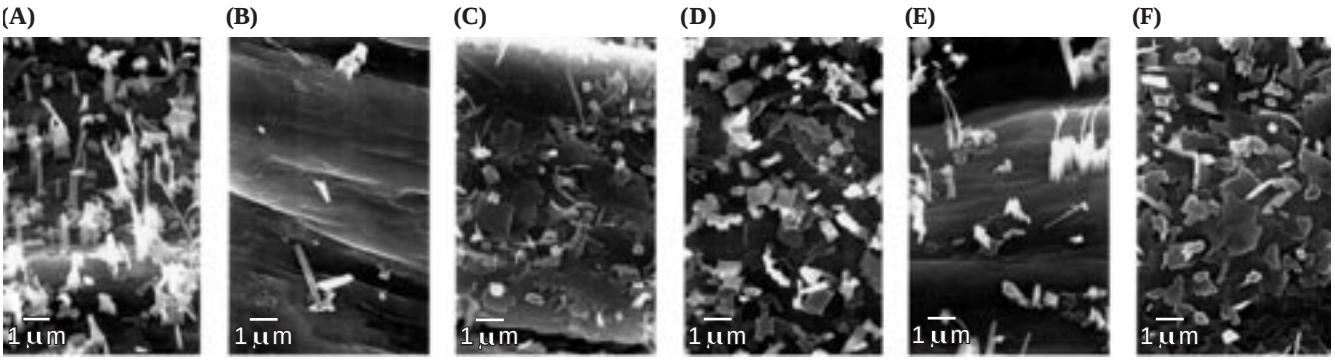


Figure 10.4
Scanning electron micrographs of wild-type and mutant *Arabidopsis* stems (A–C) and siliques (D–F). Wild-type surfaces (A,D) are covered with various tubes and lobed plates of wax. Surfaces of the *cer7* mutant (B,E) have both fewer wax crystals and altered crystalline structures. Surfaces of *cer17* mutants (C,F) have fewer tubes than the wild-type plants.

aerial organs, the bulk of fatty acid synthesis is devoted to production of wax and cutin for protection (Fig. 10.4).

Some fatty acids may play major roles in certain signal transduction pathways. The synthesis of the growth regulator **jasmonic acid** from linolenic acid and the activities of jasmonates as plant hormones and second messengers are widely studied (see Section 10.8.5 and Chapters 17 and 21). Similarly, phosphatidylinositol and its derivatives may be messengers with regulatory roles, similar to messenger compounds found in other eukaryotes, which play important roles in signal transduction pathways (see Chapter 18). Fatty acids may also be involved in regulating various cellular processes via acylation of proteins.

Table 10.1 Functions of lipid molecules in higher plants

Function	Lipid types involved[a]
Membrane structural components	Glycerolipids Sphingolipids Sterols
Storage compounds	Triacylglycerols Waxes
Compounds active in electron transfer reactions	Chlorophyll and other pigments Ubiquinone, plastoquinone
Photoprotection	Carotenoids (xanthophyll cycle)
Protection of membranes against damage from free radicals	Tocopherols
Waterproofing and surface protection	Long-chain and very-long-chain fatty acids and their derivatives (cutin, suberin, surface waxes) Triterpenes
Protein modification	
Addition of membrane anchors	
Acylation	Mainly 14:0 and 16:0 fatty acids
Prenylation	Farnesyl and geranylgeranyl pyrophosphate
Other membrane anchor components	Phosphatidylinositol, ceramide
Glycosylation	Dolichol
Signaling	
Internal	Abscisic acid, gibberellins, brassinosteroids 18:3 Fatty acid precursors of jasmonate Inositol phosphates Diacylglycerols
External	Jasmonate Volatile insect attractants
Defense and antifeeding compounds	Essential oils Latex components (rubber, etc.) Resin components (terpenes)

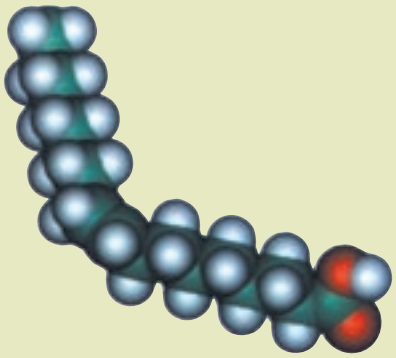
[a] The isoprenoids and related lipids are described in Chapter 24.

Box 10.1 Abbreviations make lipid nomenclature more manageable.

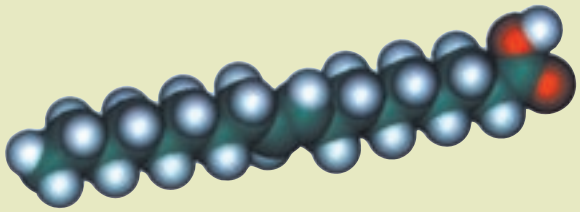
A simple shorthand notation based on molecule length and the number and position of double bonds has been developed to designate fatty acids. For example, the saturated C_{16} fatty acid, palmitic acid (hexadecanoic acid), is designated 16:0. The first value, 16, represents the number of carbon atoms. The second value, 0, indicates the number of double bonds. The monounsaturated 18-carbon fatty acid, oleic acid (*cis*-9-octadecenoic acid), is designated $18{:}1^{\Delta 9}$. The Δ9 superscript designates the position of the single double bond, counting the carboxyl group as carbon atom number 1. Because the double bonds in fatty acids are almost exclusively cis isomers, no designation for the configuration of double bond is used unless it is a trans isomer, as in $16{:}1^{\Delta 3t}$. As shown in the illustration of oleic and elaidic acids, introduction of a cis unsaturation creates a bend in the acyl chain, whereas a trans unsaturation does not.

Some authors also designate the position of the double bonds relative to the terminal methyl group (the ω carbon). Thus, an ω3, or *n*-3, fatty acid contains a double bond three carbons from the methyl end of the fatty acid (e.g., the polyunsaturated α-linolenic acid, $18{:}3^{\Delta 9,12,15}$ is an ω3 fatty acid). A limitation of this nomenclature is that $18{:}1^{\Delta 15}$ would also be referred to as an ω3 or *n*-3 fatty acid.

Abbreviations are also used to designate the position at which a fatty acid is esterified to the glycerol backbone of glycerolipids. *sn*-3 (stereospecific nomenclature-3) denotes the terminal hydroxyl that is phosphorylated in glycerol 3-phosphate, *sn*-2 refers to the central hydroxyl, and *sn*-1 is the terminal hydroxyl that is not phosphorylated.



Oleic acid ($18{:}1^{\Delta 9}$)



Elaidic acid ($18{:}1^{\Delta 9t}$)

10.1.2 Most, but not all, lipids contain fatty acids esterified to glycerol.

Fatty acids are carboxylic acids of highly reduced hydrocarbon chains. The typical fatty acids found in the membranes of plants contain 16 or 18 carbons and are listed in Table 10.2, along with some unusual fatty acids that typically accumulate only in the storage triacylglycerols of seeds. Some of the nomenclature used in abbreviations for fatty acids and lipids is described in Box 10.1.

A major fraction of the fatty acids in plants are the polyunsaturated fatty acids linoleic acid ($18{:}2^{\Delta 9,12}$) and α-linolenic acid ($18{:}3^{\Delta 9,12,15}$). Only a few plants accumulate fatty acids with double bonds closer to the carboxyl group than the Δ9 position. In addition to the C_{16} and C_{18} common fatty acids, some plants also produce fatty acids of 8 to 32 carbons in length. These are usually accumulated in storage lipids or epicuticular wax. The fatty acid composition of lipids can be determined by using gas chromatography to separate the methylated derivatives of the fatty acids (Box 10.2).

Glycerolipids consist of fatty acids esterified to derivatives of glycerol. Four principal types are found in plants: triacylglycerols, phospholipids, galactolipids, and a sulfolipid. In addition, plants contain small amounts of sphingolipids. In most cases, purification of a particular type of lipid from a plant extract yields a complex mixture. For example, seven different classes of phospholipid (Table 10.3) are defined by the structure of the head group, and each class is composed of distinct molecular species defined by the fatty acids attached to the *sn*-1 and *sn*-2 positions of the glycerol backbone. Thus, the phosphatidylcholine molecule depicted in Figure 10.5A has a saturated fatty acid esterified to the *sn*-1 position and a diunsaturated fatty acid esterified to the *sn*-2 position. Some of the factors that control the fatty acid composition of lipids are discussed in Sections 10.5.3, 10.7.1, and 10.7.4.

Lipids are usually stored as triacylglycerols, three fatty acids esterified to glycerol (Fig. 10.5B). Triacylglycerols are frequently referred to as neutral lipids because of their nonpolar nature. Found primarily in seeds

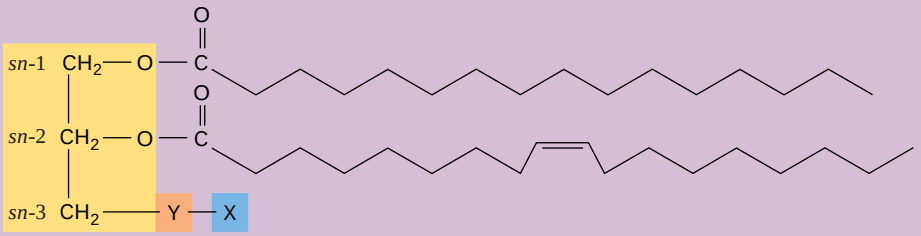
Table 10.2 Selected fatty acids present in plants

Common name	Systematic name	Structure	Abbreviation[a]
Saturated fatty acids			
Lauric acid	*n*-Tetradecanoic acid	$CH_3(CH_2)_{10}COOH$	12 : 0
Palmitic acid[b]	*n*-Hexadecanoic acid	$CH_3(CH_2)_{12}CH_2CH_2COOH$	16 : 0
Stearic acid[b]	*n*-Octadecanoic acid	$CH_3(CH_2)_{12}CH_2CH_2CH_2CH_2COOH$	18 : 0
Arachidic acid	*n*-Eicosanoic acid	$CH_3(CH_2)_{12}CH_2CH_2CH_2CH_2CH_2CH_2COOH$	20 : 0
Behenic acid	*n*-Docosanoic acid	$CH_3(CH_2)_{12}CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$	22 : 0
Lignoceric acid	*n*-Tetracosanoic acid	$CH_3(CH_2)_{12}CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$	24 : 0
Unsaturated fatty acids			
Oleic acid[b]	*cis*-9-Octadecenoic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	$18 : 1^{\Delta 9}$
Petroselenic acid	*cis*-6-Octadecenoic acid	$CH_3(CH_2)_{10}CH{=}CH(CH_2)_4COOH$	$18 : 1^{\Delta 6}$
Linolenic acid[b]	*cis,cis*-9,12-Octadecatrienoic acid	$CH_3(CH_2)_4CH{=}CH{-}CH_2{-}CH{=}CH(CH_2)_7COOH$	$18 : 2^{\Delta 9,12}$
α-Linoleic acid[b]	*all-cis*-9,12,15-Octadecatrienoic acid	$CH_3CH_2CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}CH{=}CH(CH_2)_7COOH$	$18 : 3^{\Delta 9,12,15}$
γ-Linoleic acid	*all-cis*-6,9,12-Octadecatrienoic acid	$CH_3(CH_2)_4CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}CH{=}CH(CH_2)_4COOH$	$18 : 3^{\Delta 6,9,12}$
Roughanic acid	*all-cis*-7,10,13-Hexadecatrienoic acid	$CH_3CH_2CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}CH{=}CH(CH_2)_5COOH$	$16 : 3^{\Delta 7,10,13}$
Erucic acid	*cis*-13-Eicosenoic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$	$22 : 1^{\Delta 13}$
Some unusual fatty acids			
Ricinoleic acid	12-Hydroxyoctadeca-9-enoic acid	$CH_3(CH_2)_5CH(OH){-}CH_2{-}CH{=}CH(CH_2)_7COOH$	$12\text{-OH-}18 : 1^{\Delta 9}$
Vernolic acid	12,13-Epoxyoctadeca-9-enoic acid	$CH_3(CH_2)_4CH(O)CH{-}CH_2{-}CH{=}CH(CH_2)_7COOH$	

[a] See Box 10.1 for an explanation of abbreviation nomenclature.
[b] These five fatty acids are commonly found as the principal constituents of membrane lipids. The others are found principally in storage lipids.

Table 10.3 Major classes of membrane lipids

Glycerophospholipid (general structure)



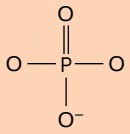
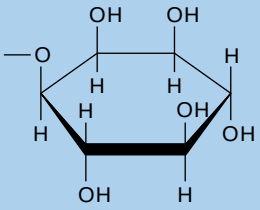
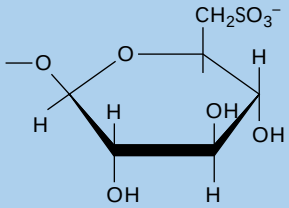
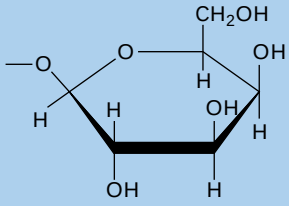
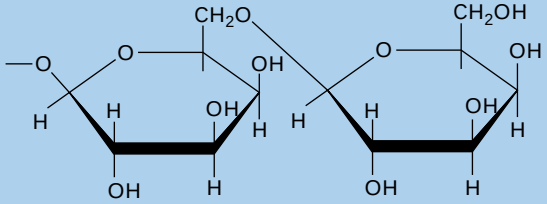
Substituent			Name of lipid
Formula of Y	**Name of X—OH**	**Formula of X**	
	Water	—H	Phosphatidic acid (PA)
	Choline	—$CH_2CH_2\overset{+}{N}(CH_3)_3$	Phosphatidylcholine (PC)
	Ethanolamine	—$CH_2CH_2\overset{+}{N}H_3$	Phosphatidylethanolamine (PE)
	Serine	—CH_2—CH($\overset{+}{N}H_3$)—COO^-	Phosphatidylserine (PS)
	Glycerol	—$CH_2CH(OH)CH_2OH$	Phosphatidylglycerol (PG)
	Phosphatidylglycerol	—$CH_2CH(OH)$—CH_2—O—P(=O)(O^-)—O—CH_2—CH(O—C(=O)—CH_2OCR)—CH_2—O—C(=O)—RCOCH	Diphosphatidylglycerol (cardiolipin)
	myo-Inositol		Phosphatidylinositol (PI)
None	Sulfoquinovose		Sulfoquinovosyldiacylglycerol (SQD), also called sulfolipid (SL)
	Galactose		Monogalactosyldiacylglycerol (MGD)
	Digalactose		Digalactosyldiacylglycerol (DGD)

Table 10.3 *(Facing page)*
Major classes of membrane lipids. The basic structure of a glycerolipid is shown at the top. The C_3 backbone (highlighted in dark yellow) is usually esterified to two fatty acids at the carbons labeled *sn*-1 and *sn*-2. The modifications of the *sn*-3 carbon can be described by the substituents *X* and *Y*, which correspond to the compounds shown in the lower part of the table. *sn* numbers refer to the stereochemical nomenclature system, which is, by convention, based on the structures of D- and L-glyceraldehyde. The convention with respect to glycerol is that, in a Fisher projection of L-glycerol (not shown), the central hydroxyl is shown to the left. By definition, the carbon above the *sn*-2 carbon is the *sn*-1 position and the position below is the *sn*-3 position.

and pollen, triacylglycerols serve as energy and carbon stores. Because neutral lipids are not soluble in the aqueous phase of cells, they do not contribute to the osmotic potential of the cell. This is important to their role as storage materials because they accumulate in amounts that would otherwise disrupt the maintenance of normal cellular osmolality.

Phospholipids are synthesized by esterification of fatty acids to the two hydroxyl groups of *sn*-glycerol 3-phosphate to produce phosphatidic acid. All other phospholipids are derived from phosphatidic acid by esterification of a polar "head group" to the phosphoryl group (Table 10.3). Phospholipids are **amphipathic,** containing both hydrophobic (noncharged, nonpolar) fatty acids and a hydrophilic (charged, polar) head group. This property allows phospholipids—and other amphipathic glyerolipids—to form a bilayer in which the hydrophilic heads are in contact with an aqueous environment such as the cytosol, while the hydrophobic tails remain in contact with other hydrophobic tails (Fig. 10.6; see also Chapter 1).

Galactolipids, another major class of glycerolipids, are localized in the plastid membranes. These lipids have a galactosyl or sulfoquinovosyl group replacing the phosphoryl head group of the phospholipids (Table 10.3). The three major lipids belonging to this class of lipids are the galactolipids monogalactosyldiacylglycerol and digalactosyldiacylglycerol and the plant sulfolipid sulfoquinovosyldiacylglycerol. These **glycolipids** contain high concentrations

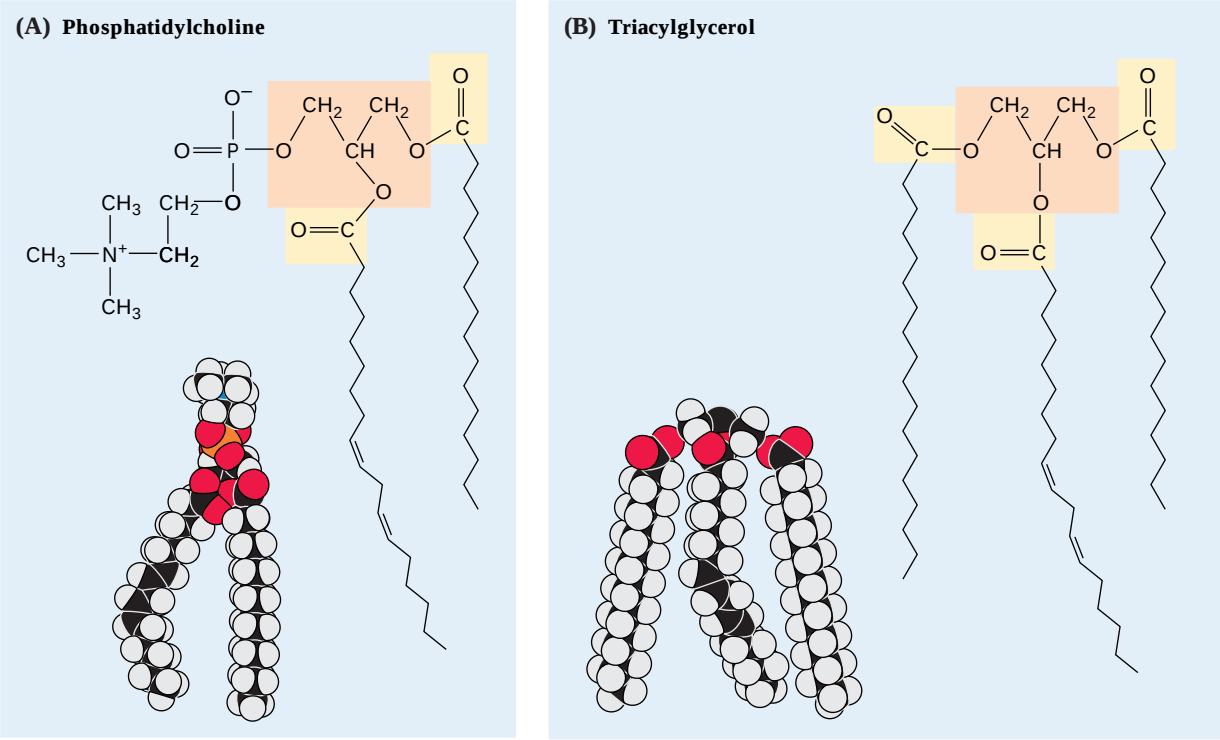


Figure 10.5
Space-filling and conformational models of (A) the phospholipid phosphatidylcholine and (B) triacylglycerol. The ester linkages are highlighted in yellow, and the glycerol backbone is in orange.

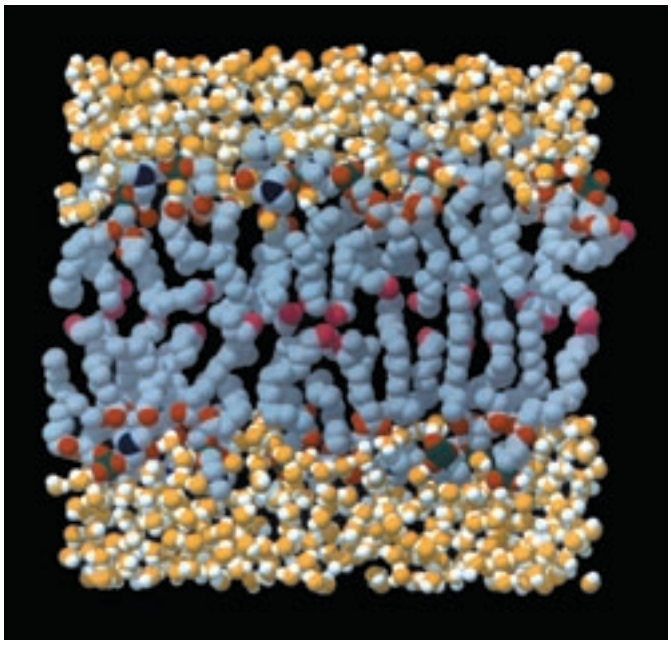


Figure 10.6
Computer simulation of a cross-section of a membrane. The coloring is as follows: phosphorus, dark green; nitrogen, dark blue; lipid oxygens, red; terminal chain methyl groups, magenta; other carbons, gray; water oxygens, yellow; water hydrogens, white. For clarity, the radii of heavy atoms are reduced slightly from their van der Waals values, the radii of water hydrogens are increased, and carbon-bound hydrogens are omitted.

of polyunsaturated fatty acids. In the photosynthetic tissue of some plants, α-linolenic acid ($18{:}3^{\Delta 9,12,15}$) can constitute as much as 90% of the fatty acids in glycolipids. Some plants, such as peas, in which glycolipids contain exclusively C_{18} polyunsaturated fatty acids, are sometimes referred to as "18:3 plants." Others, such as spinach, contain glycolipids having appreciable amounts of a C_{16} polyunsaturated fatty acid, $16{:}3^{\Delta 7,10,13}$, localized exclusively in the *sn*-2 position, and are called "16:3 plants." The underlying difference between 18:3 plants and 16:3 plants is now understood: 18:3 plants synthesize most or all of their lipids in the endoplasmic reticulum (ER), whereas 16:3 plants utilize biosynthetic pathways in the plastid as well (see Sections 10.7.2 and 10.7.3).

Sphingolipids (Fig. 10.7), which represent less than 5% of the total plant lipids, are concentrated in the plasma membrane, where they may make up as much as 26% of the mass of plasma membrane lipids. The sphingolipid bases are generally toxic and are present in very low concentrations so that only the ceramides and glycosylceramides accumulate to any extent. Sphingolipids are unusual in that they are not esters of glycerol, but rather consist of a long-chain amino alcohol that forms an amide linkage to a fatty acid; the acyl group is often longer than C_{18}. Complex sphingolipids, such as glucosylceramide, form from a simple sphingolipid (e.g., ceramide) by the addition of phosphocholine or one or more sugars. The synthesis of these lipids is discussed in Section 10.7.10.

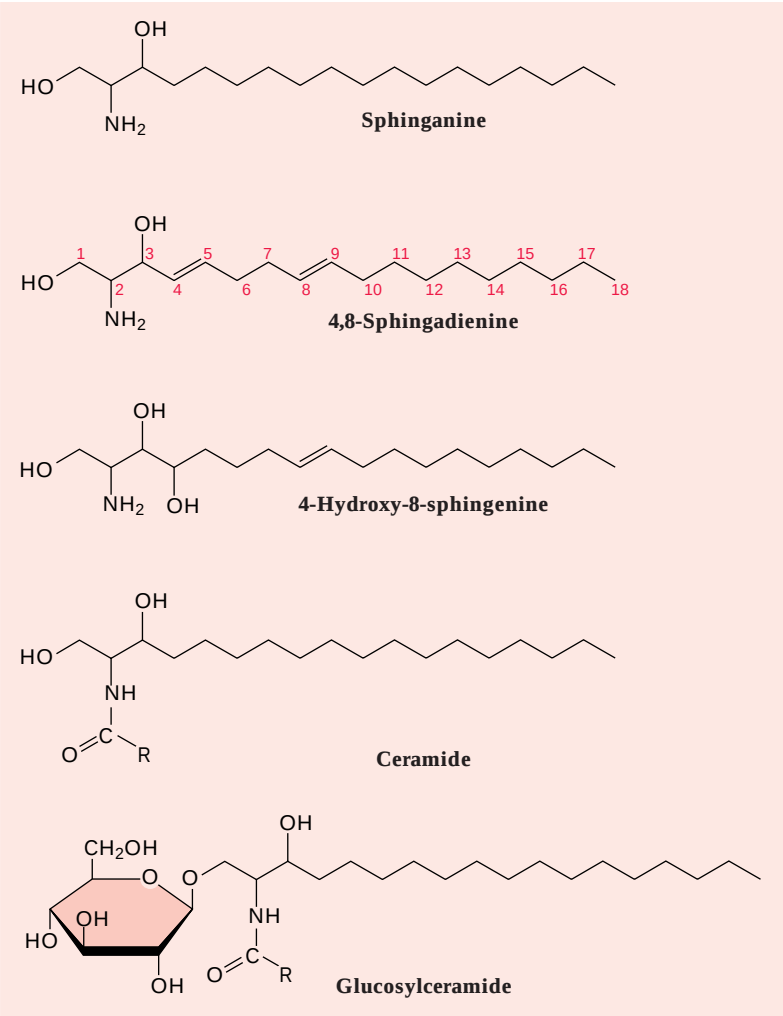


Figure 10.7
Structures of selected plant sphingolipids. Sphinganine, 4,8-sphingadienine, and 4-hydroxy-8-sphingenine are called "sphingolipid bases." The carbons are numbered from the primary hydroxyl. The numbers in the names refer to the position of double bonds or of other functional groups as indicated. The fatty acids (R) are usually saturated or diunsaturated C_{16}–C_{24} hydroxy fatty acids.

Box 10.2 Methyl esters of fatty acids can be quantified by gas chromatography.

The fatty acid composition of samples is commonly measured by gas chromatography of the methyl esters. The esterification renders the fatty acids more volatile so they can be analyzed at temperatures that do not alter the chemical structure of most fatty acids. The esters are typically formed by Fisher ester synthesis: The fatty acid is protonated by a strong acid such as HCl, which facilitates its reaction with methanol:

$$RCOOH + CH_3OH \overset{H^+}{\rightleftharpoons} RCOOCH_3 + H_2O$$

10.2 Fatty acid biosynthesis

10.2.1 Fatty acid biosynthesis in plants is similar to that in bacteria.

Fatty acid biosynthesis in plants takes place within plastids, organelles widely thought to have originated from a photosynthetic bacterial symbiont. Thus it is perhaps not surprising that fatty acid metabolism in plants closely resembles that of bacteria.

During fatty acid biosynthesis, a repeated series of reactions incorporates acetyl moieties of acetyl-CoA into an acyl group 16 or 18 carbons long. The enzymes involved in this synthesis are acetyl-CoA carboxylase (ACCase) and fatty acid synthase (FAS) (Fig. 10.8). The name fatty acid synthase refers to a complex of several individual enzymes that catalyze the conversion of acetyl-CoA and malonyl-CoA to 16:0 and 18:0 fatty acids (see Section 10.4). **Acyl-carrier protein** (ACP), an essential protein cofactor, is generally considered a component of FAS.

Fatty acid biosynthesis is initiated by the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA. The malonyl group is transferred next to ACP. Subsequent decarboxylation of the malonyl moiety acts to drive a condensation reaction, in which a carbon–carbon bond forms between C-1 of an acetate "primer" and C-2 of the malonyl group on ACP. This two-carbon chain length extension results initially in the formation of acetoacetyl-ACP. Subsequently, a sequence of three reactions—reduction, dehydration, and reduction again—leads to the formation of the fully reduced acyl-ACP. This sequence, progressing in three steps from a 3-ketoacyl group to a saturated acyl group, is a common reaction series found in biochemical pathways. For example, both β-oxidation and the citric acid cycle use the same series of reactions, but in reverse order.

Fatty acyl chains and their derivatives are among the most reduced molecules found in cells. Producing these molecules from their more-oxidized precursors requires a large investment of reducing power. As indicated above, each cycle of two-carbon addition involves two reduction steps. Thus, for a typical C_{18} fatty acid, 16 molecules of NAD(P)H are consumed. In illuminated chloroplasts, abundant reducing power is available from Photosystem I. In the dark and in tissues lacking chloroplasts, the oxidative pentose phosphate pathway is the most likely origin of reduced NADPH (Chapter 13).

10.2.2 Carbon precursors for fatty acid synthesis can be provided by reactions inside or outside the plastids.

Acetyl-CoA is the initial substrate for synthesis of the carbon backbone of all fatty acids. Also a central intermediate in many aspects of cellular metabolism, it is produced and consumed by dozens of reactions in the cell. Until recently, there has been some uncertainty as to which of these reactions produce the acetyl-CoA used in fatty acid biosynthesis. Most likely, acetyl-CoA from more than one reaction finds its way into fatty acid synthesis (Fig. 10.9). Contributions of individual reactions depend on developmental and other factors.

Experimental results have shown that within plastids, pyruvate dehydrogenase can directly produce acetyl-CoA from pyruvate generated during glycolysis (see Chapter 13). The activity of pyruvate dehydrogenase in isolated oilseed plastids is sufficient to

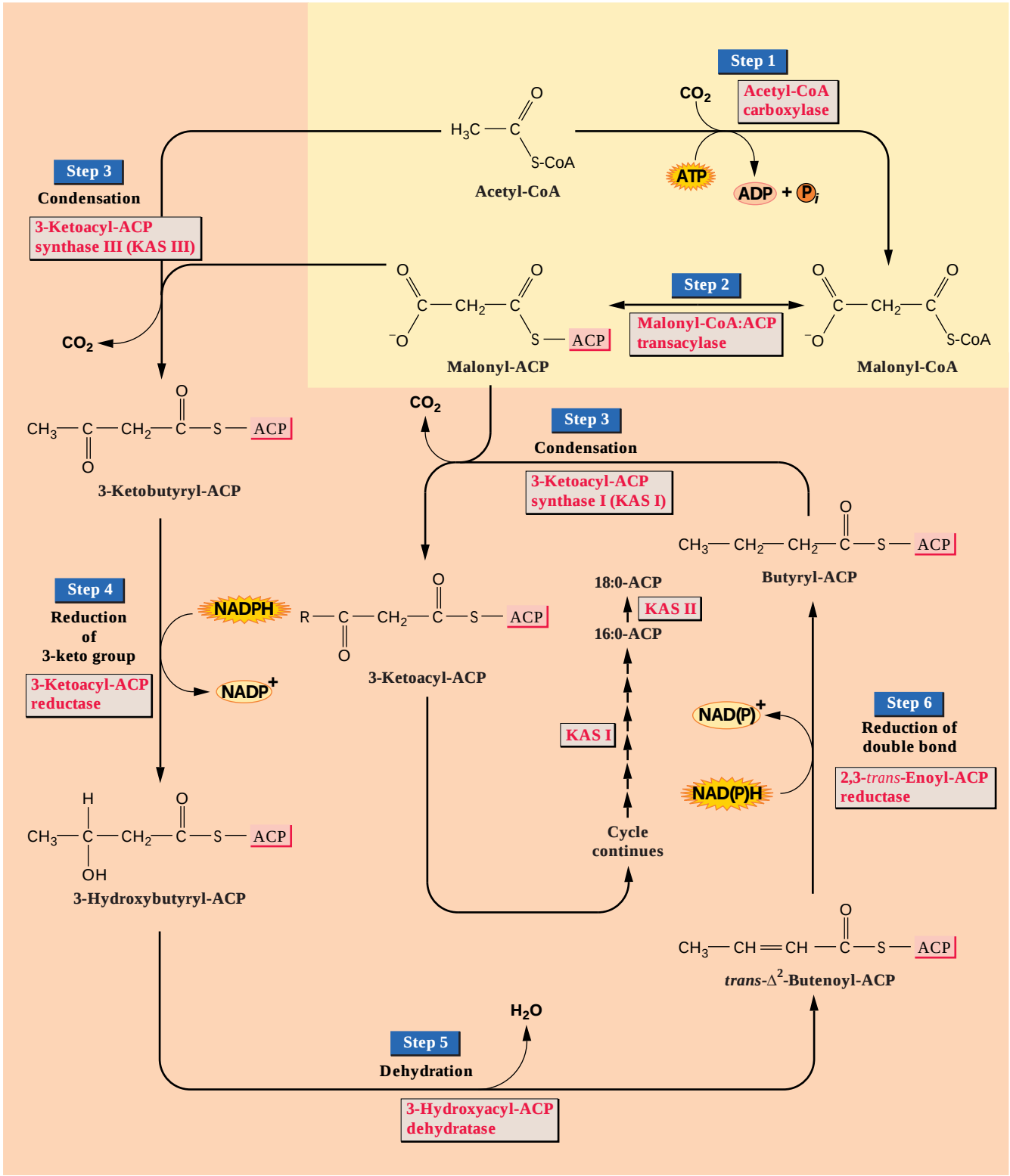


Figure 10.8
Overview of fatty acid synthesis. Fatty acids grow by addition of two-carbon (C_2) units. The reactions highlighted in yellow show how malonyl-CoA enters the cycle; those highlighted in orange represent the cyclic reactions. Synthesis of a C_{16} fatty acid requires that the cycle be repeated seven times. During the first turn of the cycle, the condensation reaction (step 3) is catalyzed by ketoacyl-ACP synthase (KAS) III. For the next six turns of the cycle, the condensation reaction is catalyzed by isoform I of KAS. Finally, KAS II is used during the conversion of 16:0 to 18:0.

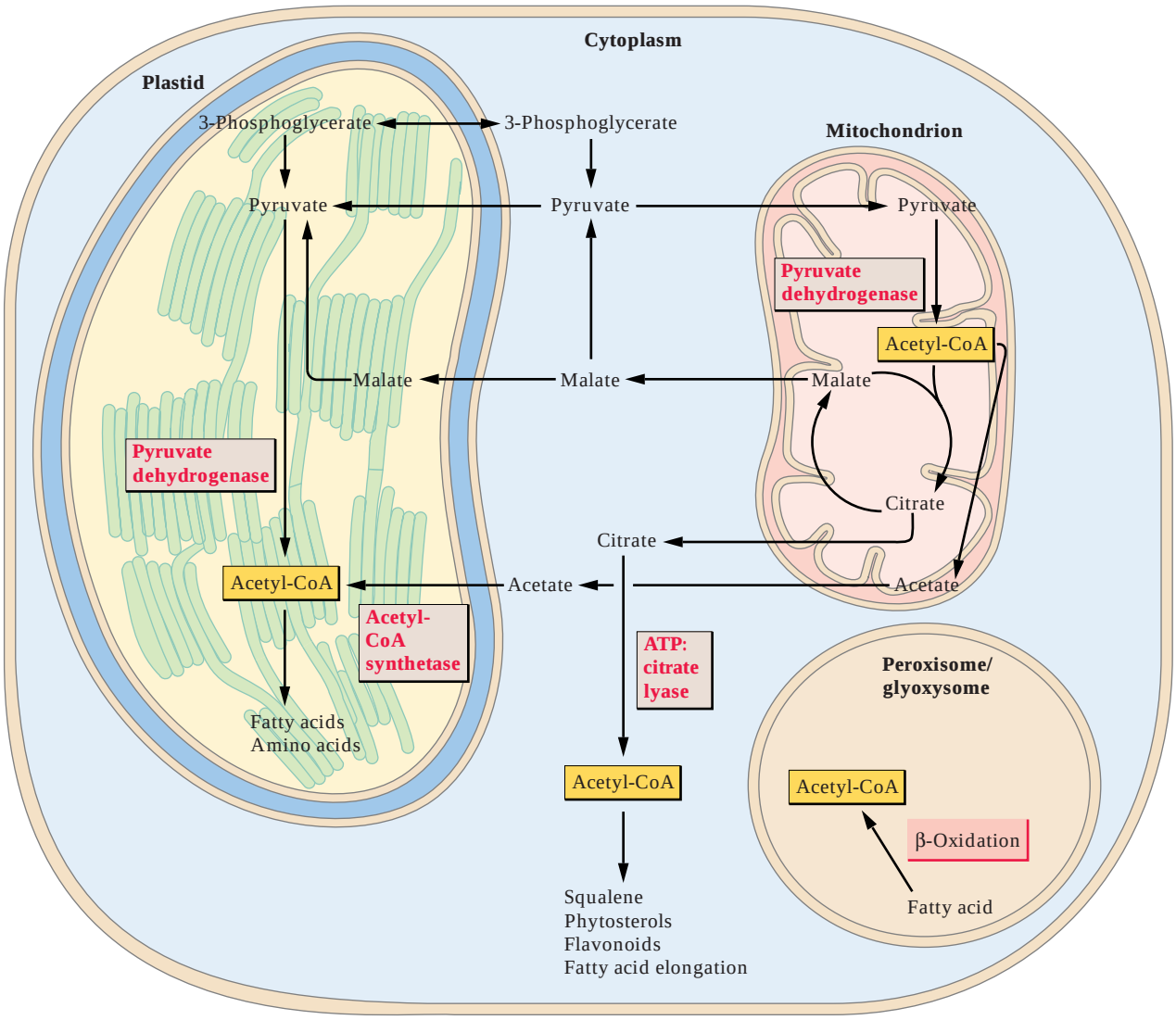


Figure 10.9
The central role of acetyl-CoA in metabolism. Acetyl-CoA may be the most central intermediate in cellular metabolism, providing a link between many pathways. The major pathways involved in its production include glycolysis (via pyruvate dehydrogenase) and fatty acid oxidation. Acetyl-CoA is the starting material for biosynthesis of fatty acids, several amino acids, flavonoids (via chalcone synthase), sterols, and many isoprenoid derivatives synthesized in the cytosol. During respiration, acetyl-CoA is the source of carbon input into the citric acid cycle in the mitochondria. Despite this central role, acetyl-CoA is not believed to cross membranes and must be produced in the compartment in which it is utilized.

account for in vivo rates of fatty acid synthesis. Furthermore, pyruvate is one of the most efficient precursors for synthesis of fatty acids by isolated oilseed plastids. However, some chloroplasts with low pyruvate dehydrogenase activity can sustain in vivo rates of fatty acid synthesis when supplied with exogenous free acetate but not with exogenous pyruvate. These results suggest that the acetate for chloroplast fatty acid synthesis might be generated outside the plastid. Candidate reactions include mitochondrial pyruvate dehydrogenase and cytosolic ATP citrate-lyase, which converts citrate, ATP, and coenzyme A (CoASH) to acetyl-CoA, oxaloacetate, ADP, and inorganic phosphate. Free acetate is probably taken into plastids and activated by acetyl-CoA synthetase in the stroma (Fig. 10.9) because acetyl-CoA does not cross membranes by diffusion and no transporters are known in plants.

10.3 Acetyl-CoA carboxylase

10.3.1 Malonyl-CoA formation is catalyzed in a two-step reaction by acetyl-CoA carboxylase.

Long-chain fatty acids are assembled two carbons at a time from acetyl-CoA. However, formation of the carbon–carbon bond between successive acetate units is an energy-consuming process. To provide an energetically favorable leaving group for the subsequent condensation reaction, cells first carboxylate acetyl-CoA. This ACCase reaction occurs in two steps. First, a biotin prosthetic group is carboxylated in an ATP-dependent process; then, acetyl-CoA reacts with carboxybiotin to produce malonyl-CoA (Fig. 10.10).

10.3.2 Plants contain both homomeric and heteromeric forms of ACCase.

ACCase catalyzes the initial step in fatty acid synthesis; indeed, in most plant cells, the major pathway consuming malonyl-CoA is plastid-localized fatty acid synthesis. However, plants also require malonyl-CoA

Reaction 10.1: Acetyl-CoA carboxylase

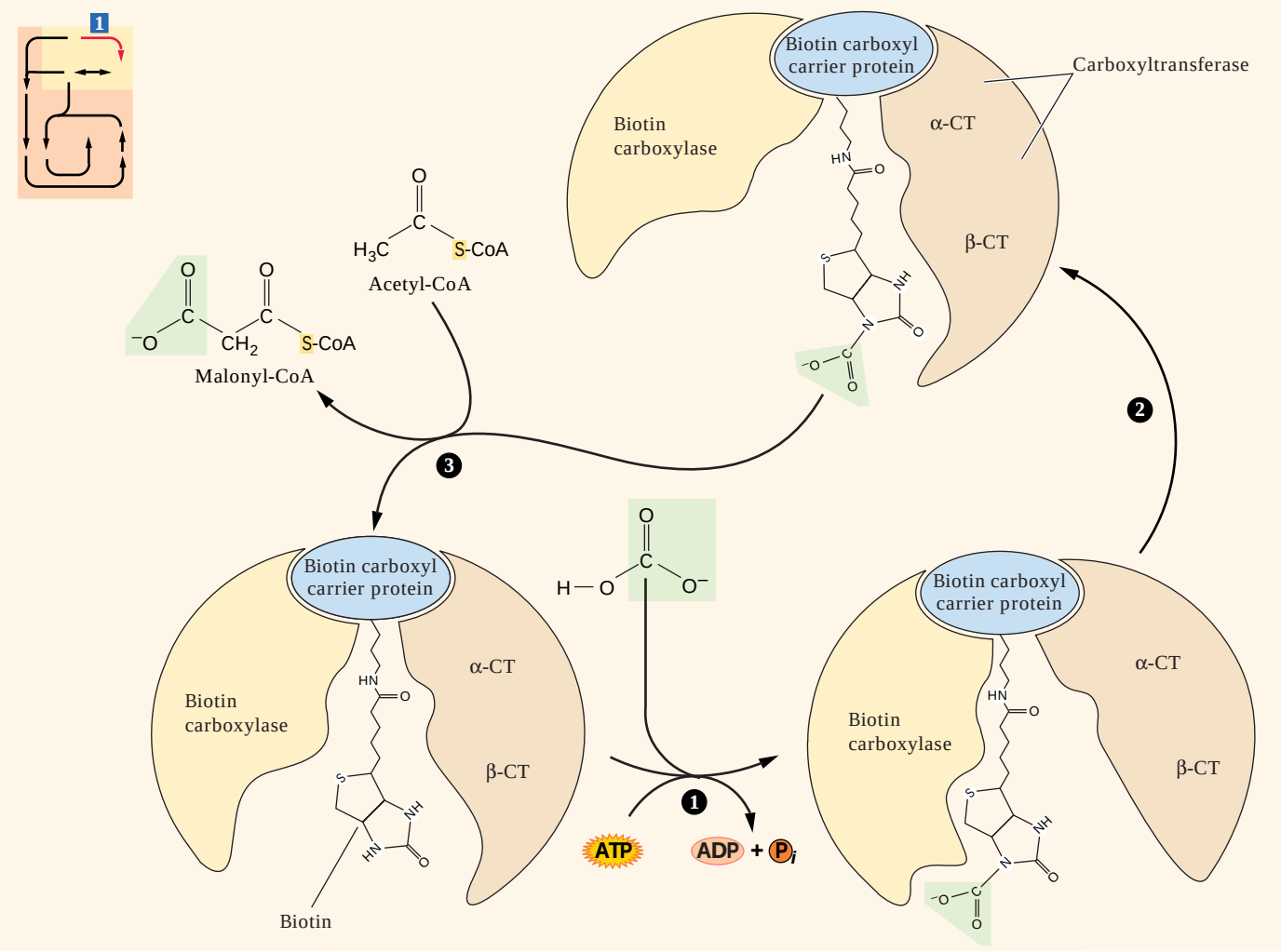


Figure 10.10
Schematic diagram of the acetyl-CoA carboxylase (ACCase) reaction. ACCase catalyzes step 1 in the reaction sequence shown in Figure 10.8. ACCase has three functional components that form malonyl-CoA from acetyl-CoA. (1) In an ATP-dependent reaction, biotin carboxylase activates CO_2 (as HCO_3^-) by attaching it to a nitrogen in the biotin ring of biotin carboxyl carrier protein (BCCP). (2) The flexible biotin arm of BCCP carries the activated CO_2 from the biotin carboxylase active site to the carboxyltransferase site (α-CT and β-CT). (3) The transcarboxylase transfers activated CO_2 from biotin to acetyl-CoA, producing malonyl-CoA.

outside the plastid, where it serves as a substrate for the flavonoid biosynthetic pathway, for fatty acid elongation reactions at the ER, for malonylation of some amino acids, and for the ethylene precursor, aminocyclopropanecarboxylic acid (Fig. 10.11). Because ACCase activity is believed to be highly regulated and a major determinant of the overall rate of fatty acid synthesis, many research groups have concentrated efforts toward understanding this enzyme. Until 1994, however, there was much confusion about its structure because different research groups could not agree on whether plant ACCase had a structure similar to that described for bacteria, with four separate subunits, or to that of fungi and animals, with a single polypeptide. It is now established that most plants utilize a prokaryotic-type ACCase in plastids and a multifunctional ACCase in the cytosol (Box 10.3).

The plastid form of ACCase has four subunits: biotin carboxyl carrier protein (BCCP), biotin carboxylase (BC), and the α- and β-subunits of carboxyltransferase (CT). These four subunits form a heteromeric complex of more than 650 kDa, which may be membrane-associated (Fig. 10.12). Three of the subunits are encoded by distinct nuclear genes, whereas the fourth (β-CT) is encoded in the chloroplast genome.

The cytosolic form of ACCase is a single large (greater than 500 kDa) homodimeric protein. The four subunits described above are integrated into domains of a single polypeptide, two of which associate to make up

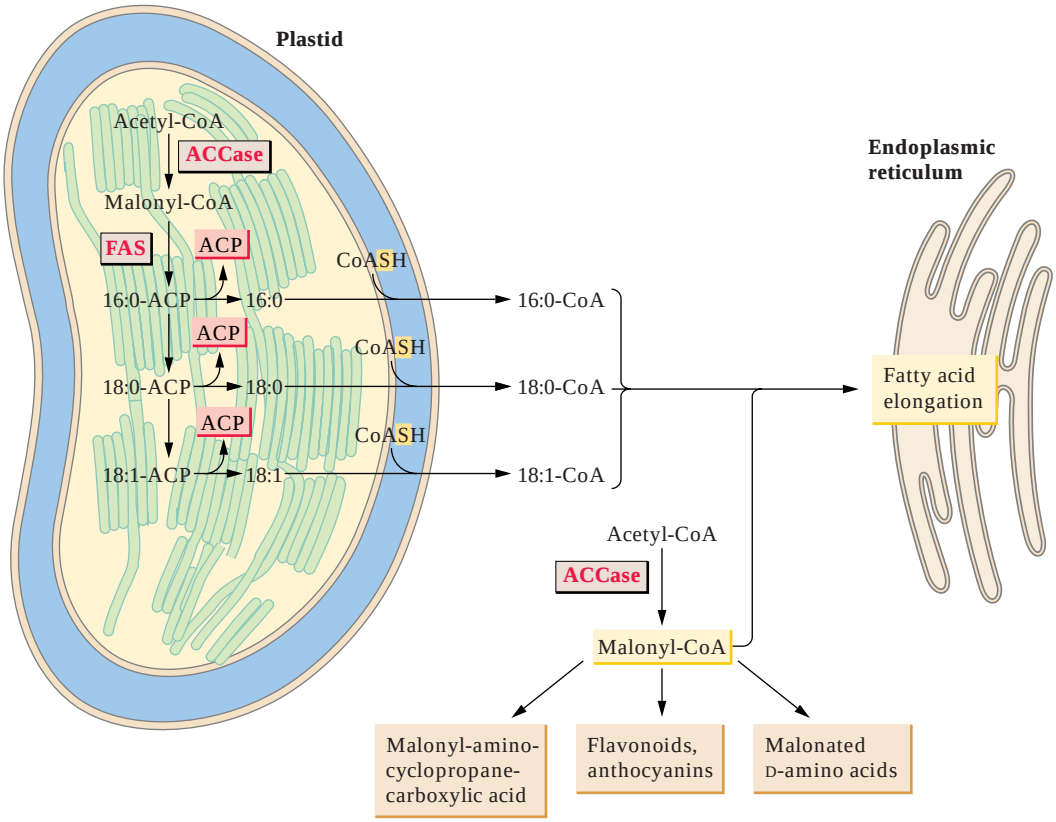


Figure 10.11
Multiple fates of malonyl-CoA. Malonyl-CoA produced by the ACCase reaction enters several pathways in plants. Within the plastid, malonyl-CoA is used exclusively for the production of fatty acids. In the cytosol, malonyl-CoA is the carbon donor for fatty acid elongation, producing the precursors for surface waxes and certain seed lipids. Condensation of three molecules of malonyl-CoA produces a diverse range of flavonoids and their derivatives (see Chapter 24). In many plant tissues, the major form of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid is the inactive malonylated derivative, produced by reaction with malonyl-CoA. Finally, many D-amino acids and other secondary metabolites react with malonyl-CoA to form malonylated derivatives.

Box 10.3 Understanding the structure of ACCase arose from large-scale DNA sequencing projects.

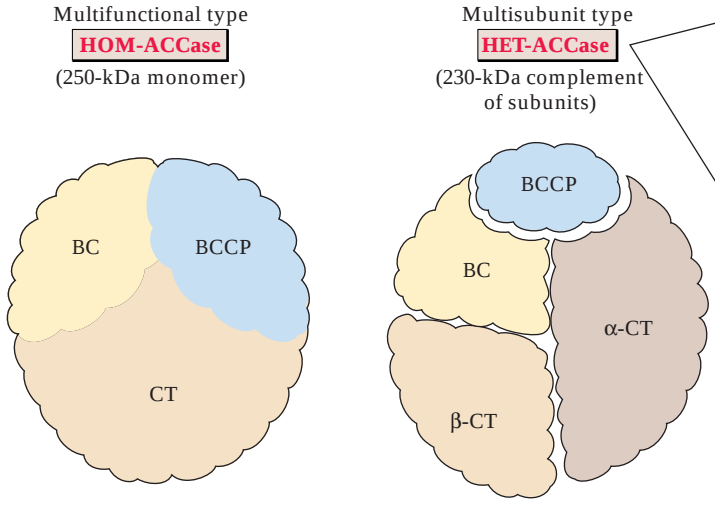
In 1972, Gamini Kannangara and Paul Stumpf reported that spinach chloroplasts have a prokaryotic type ACCase with four separate subunits for biotin carboxyl carrier protein (BCCP), biotin carboxylase (BC), and carboxyltransferase (CT). Although some later work also supported this interpretation, for the next 20 years almost all efforts to purify the enzyme or to obtain clones came to a different conclusion. Several laboratories purified ACCase and obtained a structure similar to that of the animal enzyme. Furthermore, the first cDNA clones for ACCase also clearly encoded a single, large, multifunctional polypeptide with high similarity to fungal and animal sequences. In light of these results, many early experiments that had suggested multiple subunits for ACCase were incorrectly reinterpreted as reflecting proteolytic breakdown of a larger polypeptide.

A clearer understanding of ACCase emerged when Yukiko Sasaki and coworkers in Japan followed up on information originating from the sequencing of the chloroplast genome. One open reading frame of unknown function had sequence similarity to carboxylases, including acetyl-CoA and propionyl-CoA carboxylases. Sasaki raised antibodies to a part of this protein and found that the antibodies both inhibited ACCase activity from chloroplasts and coprecipitated a biotin-containing protein. Recent results from several other laboratories have now shown that both prokaryotic and eukaryotic ACCase enzymes exist in plants; recognition of this fact has explained much of the apparently contradictory results.

After Sasaki's characterization of the β-CT subunit, identifying the remaining components of the multisubunit structure might have proceeded by purification of the chloroplast enzyme. However, the prokaryotic-type ACCase complex readily dissociates and loses activity, making conventional approaches to protein purification difficult. Fortunately, the large amount of DNA sequence data available for plants has led to identification of three additional subunits, based on their similarity to bacterial ACCase gene sequences. The BC and BCCP were identified as a result of projects that sequenced large numbers of anonymous cDNA clones. The α-CT subunit was first identified as a clone encoding a chloroplast inner membrane protein and was only later shown to be part of ACCase. All four subunits known to participate in the bacterial enzyme have now been characterized in plants (see Fig. 10.12).

the homodimer (Fig. 10.12). This structure is similar to the ACCase found in animals and fungi. Indeed, there is as much as 50% amino acid identity between the sequences of the enzymes from organisms in these kindoms. In animals and fungi, however, the active form of ACCase is a highly polymerized filament. In these systems, the enzyme is deactivated by phosphorylation, which also causes the polymerized form to dissociate into monomers.

Almost all monocot and dicot plants so far examined have the two types of ACCase described above—with the heteromeric, multisubunit form in the plastid and the homodimeric form in the cytosol. The grass family (Poaceae) is an exception, with similar homodimeric forms being found in both plastids and cytosol. Furthermore, the β-CT gene is partially or completely missing from the chloroplast genome, and none of the subunits of the heteromeric ACCase can be



Name	Abbreviation	Size
Biotin carboxylase	BC	50 kDa
Biotin carboxyl carrier protein	BCCP	21 kDa
α-Carboxyl-transferase	α-CT	91 kDa
β-Carboxyl-transferase	β-CT	67 kDa

Figure 10.12
Two different forms of ACCase occur in plants. A homodimeric form (HOM-ACCase) has three functional domains encoded in a single polypeptide of about 250 kDa. A heteromeric form (HET-ACCase) consists of four subunits that together form a plastid-localized complex of 650 to 700 kDa.

detected. Although the reason for the different ACCase organization in grasses remains unknown, it has substantial practical significance in agriculture. Several widely used grass-specific herbicides are now known to kill grasses by specifically inhibiting the homodimeric form of ACCase in the plastid, thereby blocking plastid fatty acid biosynthesis.

10.3.3 Malonyl-CoA formation is the first committed step in fatty acid synthesis.

In many primary metabolic pathways, biochemical regulation occurs at the first committed step. Although malonyl-CoA is used in several pathways in the cell, fatty acid biosynthesis is the only known fate for malonyl-CoA within the plastid. Therefore, the ACCase reaction in plastids is the first committed step for fatty acid synthesis. Several lines of evidence indicate that plastid ACCase activity is tightly regulated and that this regulation determines, in large part, the overall rate of fatty acid synthesis. First, the concentration of malonyl-CoA in chloroplasts changes quickly during light–dark transitions and remains proportional to the rate of fatty acid synthesis. Second, for herbicides that specifically target the plastid ACCase, the same concentrations inhibit foliar fatty acid synthesis both in vivo and in vitro. Finally, addition of exogenous lipids to suspension cultures slows production of new fatty acids. Analysis of the substrates and products of plastid fatty acid metabolism indicates this regulation occurs at the ACCase reaction. Thus, plastid-localized ACCase appears to be a highly regulated enzyme subject to feedback and other biochemical controls. Recently, modification of ACCase by thioredoxin and by phosphorylation has been discovered.

10.4 Fatty acid synthase

10.4.1 Different types of FAS exist in different kingdoms.

FAS refers to all enzyme activities in fatty acid biosynthesis except ACCase. Although the reactions catalyzed by FAS are essentially the same for all organisms, two distinctly different types of FAS are found in nature. Animals and yeast use a Type I FAS, a single multifunctional enzyme complex characterized by large subunits (250 kDa). Each subunit is capable of catalyzing several different reactions. By contrast, plants and most bacteria have a Type II FAS, in which each enzyme activity resides on an individual protein that can be readily separated from the other activities participating in fatty acid synthesis. Type II FAS also includes ACP. The Type II FAS functions much like a metabolic pathway, whereas Type I FAS functions like a large protein complex (e.g., pyruvate dehydrogenase).

The assembly of a C_{18} fatty acid from acetyl-CoA in Type II fatty acid synthesis requires 48 reactions involving at least 12 different proteins. How is this complex pathway organized? Although to date no direct evidence has been established, some type of supramolecular organization seems very likely. The estimated concentrations of many acyl-ACP intermediates of the pathway are in the nanomolar range, far below the values predicted by kinetic analyses of the enzymes involved. Calculations suggest the enzyme activities available at these low substrate concentrations are not sufficient to support observed in vivo rates of fatty acid synthesis. Accordingly, some form of substrate channeling seems essential. Furthermore, in osmotically lysed chloroplasts, neither acetyl-CoA nor malonyl-CoA competes with radiolabeled free acetate for incorporation into fatty acids, suggesting that acetate is channeled directly into fatty acid synthesis. Thus, Type II fatty acid synthesis is catalyzed by separate enzymes that appear to be complexed in a tightly coupled pathway.

10.4.2 ACP transports intermediates of fatty acid synthesis through the pathway.

After the formation of malonyl-CoA in the ACCase reaction, the assembly of fatty acids involves a central cofactor, ACP (Fig. 10.13). This small protein is about 80 amino acids long and contains a phosphopantetheine prosthetic group covalently linked to a serine residue near the middle of the polypeptide chain. The phosphopantetheine group, also found in CoASH, contains a terminal

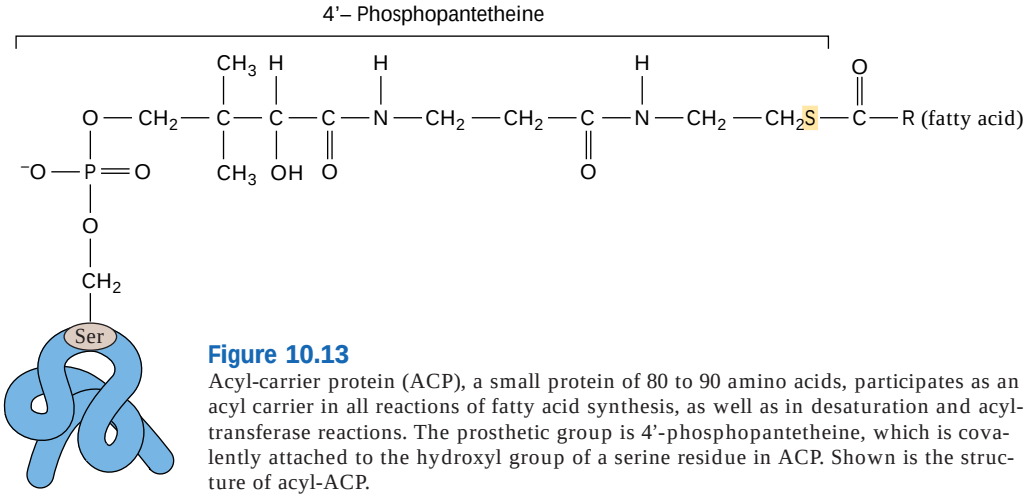


Figure 10.13
Acyl-carrier protein (ACP), a small protein of 80 to 90 amino acids, participates as an acyl carrier in all reactions of fatty acid synthesis, as well as in desaturation and acyl-transferase reactions. The prosthetic group is 4'-phosphopantetheine, which is covalently attached to the hydroxyl group of a serine residue in ACP. Shown is the structure of acyl-ACP.

sulfhydryl. The thioester linkage that forms between a fatty acid and this sulfur is a high-energy bond with a free energy of hydrolysis similar to that for ATP.

10.4.3 Malonyl-CoA:ACP transacylase transfers a malonyl moiety from CoASH to ACP.

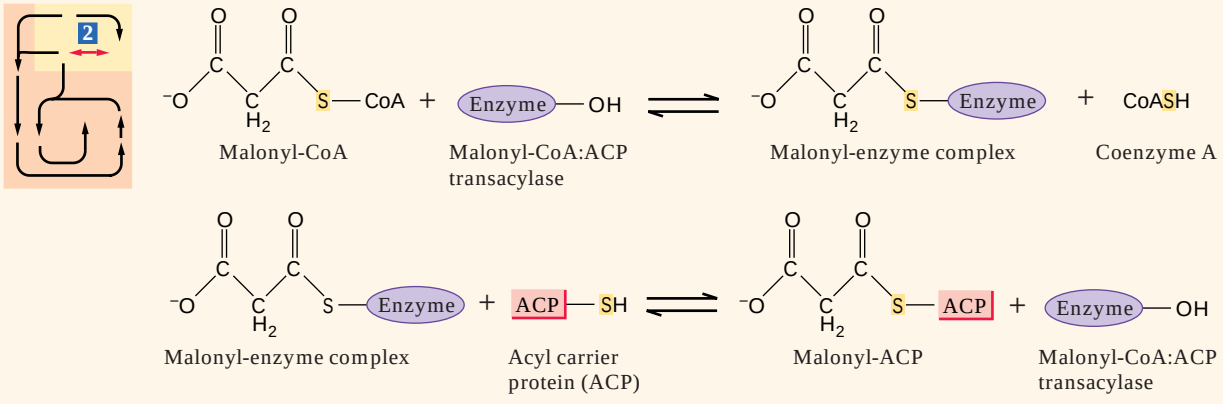
ACP first becomes involved in the fatty acid synthesis pathway when the malonyl group produced by ACCase is transferred from CoASH to the sulfhydryl of ACP by the reactions catalyzed by malonyl-CoA:ACP transacylase (Rx. 10.2; see also step 2, Fig. 10.8).

The reaction mechanism involves a covalent malonyl–enzyme intermediate (Fig. 10.14). Analysis of the malonyl transacylase (note: the terms transacylase and acyltransferase are equivalent) from *E. coli* has demonstrated that this intermediate is a serine ester. Two isoforms of this enzyme have been found in both soybean and leek, although no functional difference between the two forms is known. After the malonyl-transacylation, all subsequent reactions of fatty acid synthesis involve ACP.

10.4.4 The three plant isoforms of 3-ketoacyl-ACP synthase demonstrate different substrate specificities.

The defining reaction of fatty acid synthesis is the elongation of a "primer" acyl chain by two carbons donated from malonyl-ACP (step 3, Fig. 10.8). The condensation reaction to form a new carbon–carbon bond is catalyzed by 3-ketoacyl-ACP synthase (KAS), commonly called condensing enzyme (Fig. 10.15). All plants examined to date contain

Reaction 10.2: Malonyl-CoA:ACP transacylase



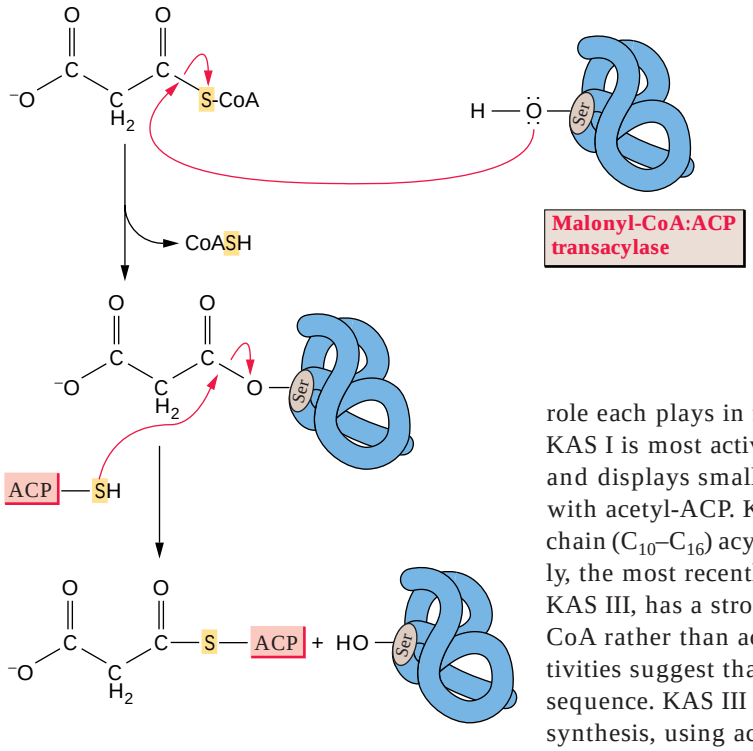
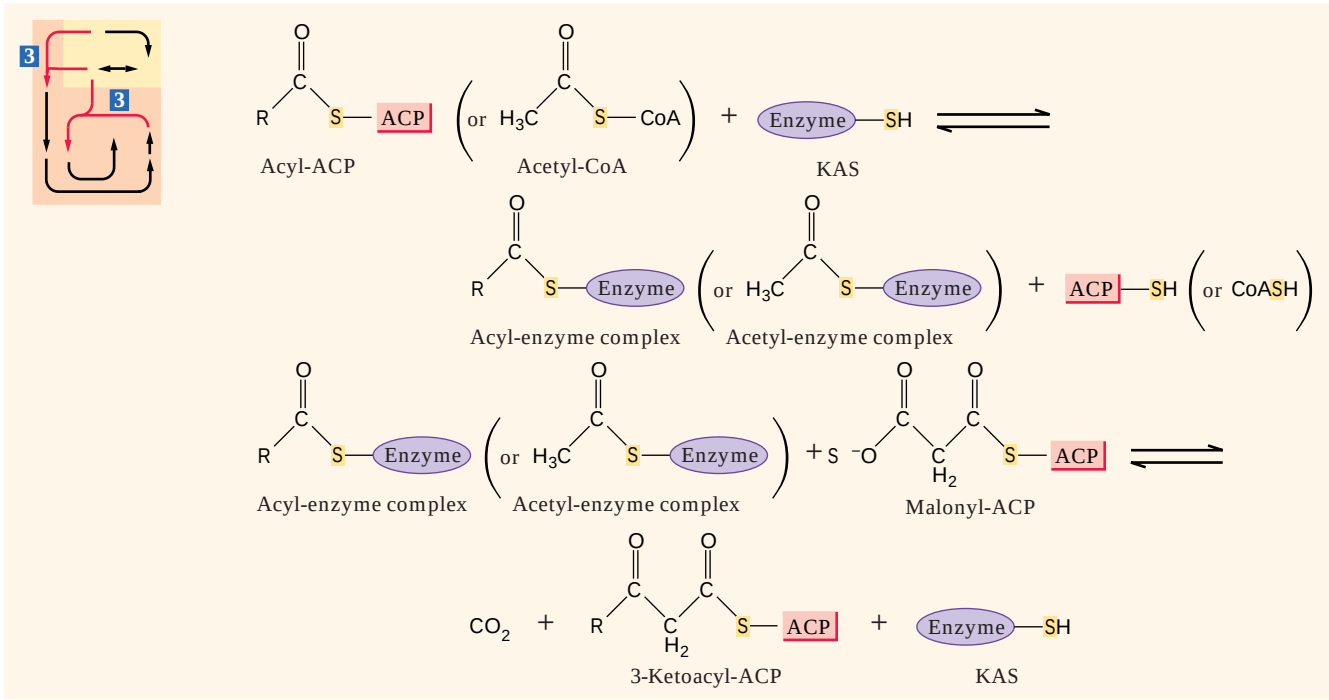


Figure 10.14
Mechanism of the malonyl-CoA:ACP transacylase reaction. In the first step of the reaction, the malonyl group is transferred to a serine residue on the enzyme and CoASH is released. In the second step, the acyl group is transferred to the phosphopantetheine sulfhydryl group of ACP to form the malonyl-ACP thioester.

three KAS isoenzymes (I, II, and III), each distinguished by its substrate specificity. The general reaction for KAS occurs in two steps (Rx. 10.3). The in vitro substrate specificity of each of the KAS isoenzymes suggests the role each plays in fatty acid biosynthesis. KAS I is most active with C_4–C_{14} acyl-ACPs and displays small but significant activity with acetyl-ACP. KAS II accepts only longer-chain (C_{10}–C_{16}) acyl-ACPs as substrates. Finally, the most recently discovered isoenzyme, KAS III, has a strong preference for acetyl-CoA rather than acyl-ACP. These in vitro activities suggest that KAS I isoenzymes act in sequence. KAS III initiates fatty acid biosynthesis, using acetyl-CoA as a primer. KAS I then extends the acyl chain to C_{12}–C_{16}. Finally, KAS II completes the synthesis to C_{18}.

The characterization of KAS III raises the question of the role of acetyl-ACP in fatty acid biosynthesis. Acetyl-ACP had long been considered the first substrate in bacterial

Reaction 10.3: 3-Ketoacyl-ACP synthase (KAS)



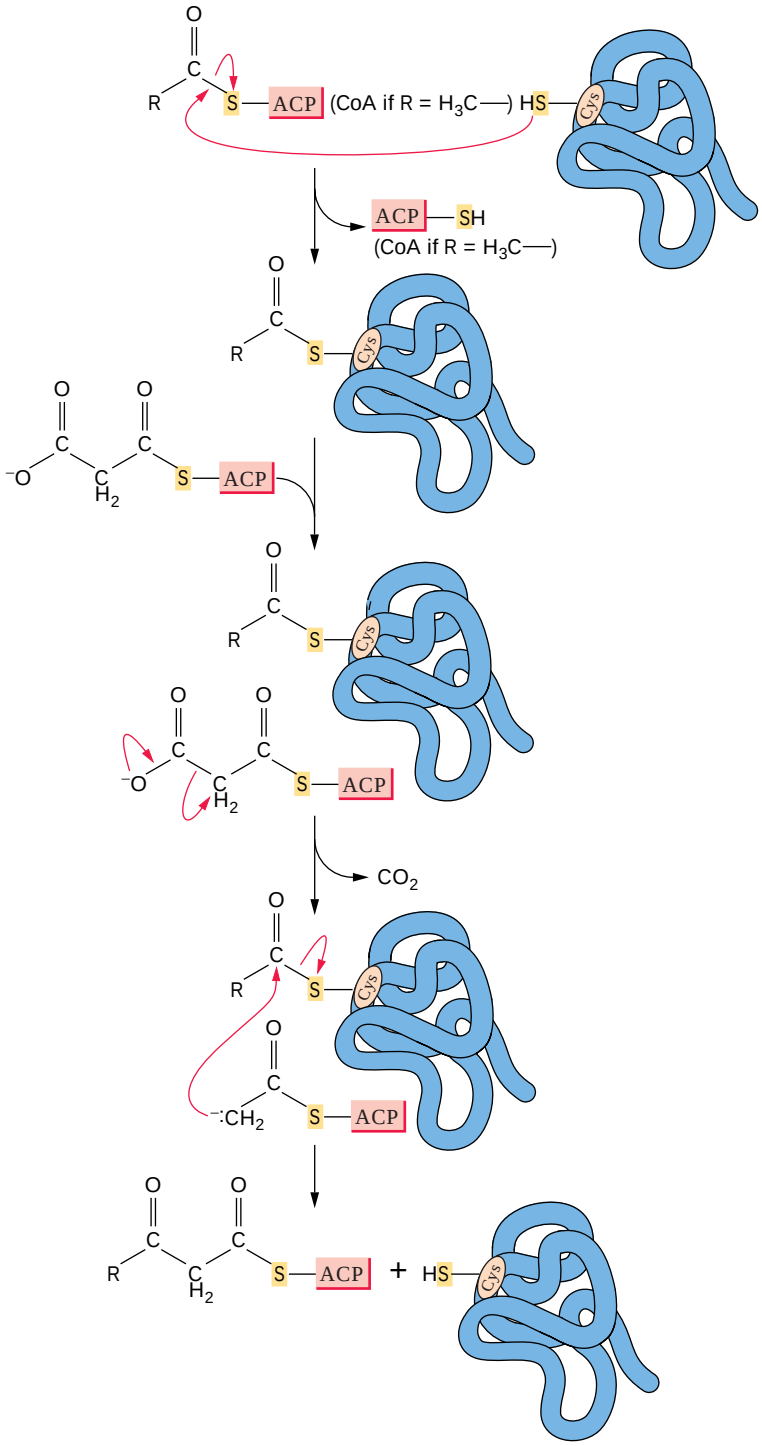


Figure 10.15
3-Ketoacyl-ACP synthases catalyze Claisen condensations. This sequential reaction involves, first, the acyl transfer from an ACP or CoA thioester to an active-site cysteine, followed by the entry of malonyl-ACP and decarboxylation of the malonate. The resulting carbanion then condenses with the acyl group to form a new C–C bond before release of the acyl group from the cysteine.

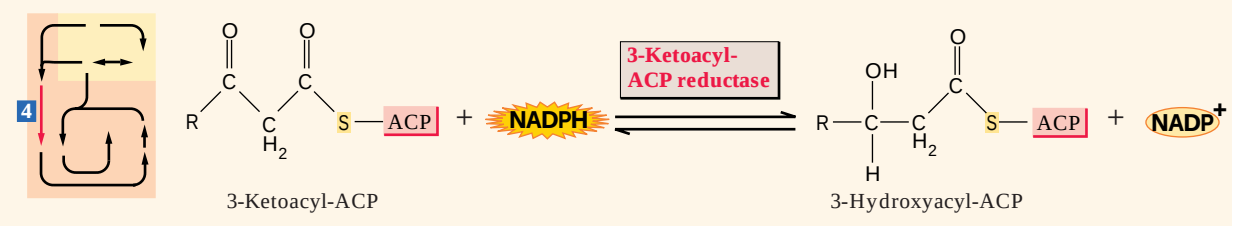
and plant fatty acid synthesis. However, the favored route of fatty acid synthesis apparently bypasses acetyl-ACP: KAS III can use acetyl-CoA directly at a rate several times greater than that with acetyl-ACP. Direct evidence for this favored route was obtained from experiments with isolated spinach chloroplasts.

10.4.5 The last three steps of the fatty acid synthesis cycle reduce a 3-ketoacyl substrate to form a fully saturated acyl chain.

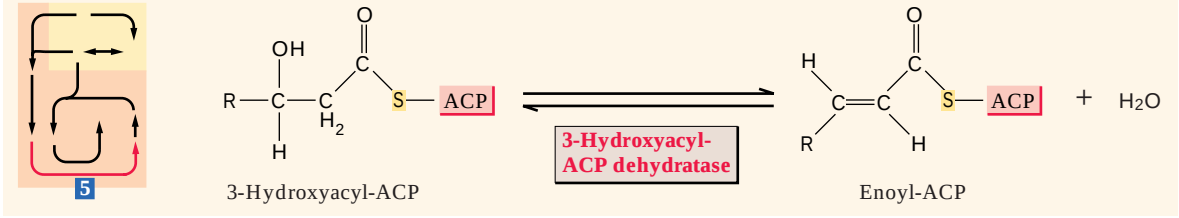
The initial reductive step of fatty acid biosynthesis is the conversion of a 3-ketoacyl-ACP to a 3-hydroxyacyl-ACP (Rx. 10.4). Native 3-ketoacyl-ACP reductase from avocado has a molecular mass of 130 kDa and a subunit mass of 28 kDa, suggesting that it is a tetramer. At least two isoforms of this reductase occur in avocado, an NADPH-dependent form and an NADH-dependent form. The predominant form is NADPH-dependent and can account for all the required 3-ketoacyl-ACP reductase activity for fatty acid synthesis. The metabolic role of the NADH-dependent form has not been elucidated.

The removal of water from the 3-hydroxyacyl-ACP to form the 2,3-*trans*-enoyl-ACP is catalyzed by 3-hydroxyacyl-ACP dehydrase (Rx 10.5). The purified dehydrase from spinach has a molecular mass of 85 kDa and a subunit size of 19 kDa.

Reaction 10.4: 3-Ketoacyl-ACP reductase



Reaction 10.5: 3-Ketoacyl-ACP dehydratase



Thus it appears to be a homotetramer. Its substrate specificity is very broad, with high activity demonstrated for acyl groups ranging from C_4 to C_{16}.

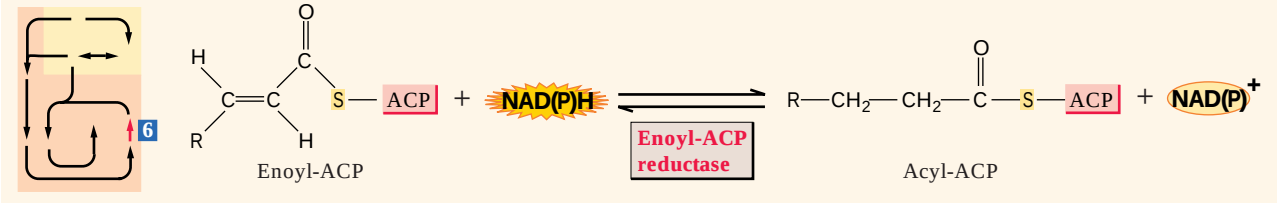
In the final reduction step, enoyl-ACP reductase converts the 2,3-*trans*-enoyl-ACP to the corresponding saturated acyl-ACP (Rx. 10.6). Enoyl-ACP reductase exists in two isoforms. The major form is specific for NADH; a homotetramer with a native molecular mass of 115 to 140 kDa and a subunit molecular mass of 32.5 to 34.8 kDa, this isomer has been purified from several plants. A second isoform uses either NADPH or NADH and is specific for longer-chain (C_{10}) enoyl-ACPs.

10.4.6 Thioesterase reactions terminate the fatty acid biosynthesis cycle.

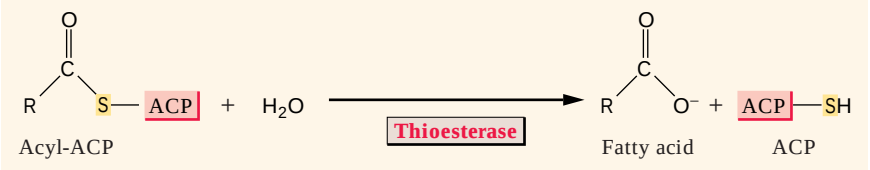
Each cycle of fatty acid synthesis adds two carbons to the acyl chain. Typically, fatty acid synthesis ends at 16:0 or 18:0, when one of several reactions stops the process. The most common reactions are hydrolysis of the acyl moiety from ACP by a thioesterase, transfer of the acyl moiety from ACP directly onto a glycerolipid by an acyl transferase, or double-bond formation on the acyl moiety by an acyl-ACP desaturase. The thioesterase reaction yields a sulfhydryl ACP (Rx. 10.7).

Two principal types of acyl-ACP thioesterases occur in plants (Fig. 10.16). The major class, designated FatA, is most active with 18:1$^{\Delta 9}$-ACP. A second class (FatB), typified by 16:0-ACP thioesterase, is most active with shorter-chain, saturated acyl-ACPs. In both cases, however, the metabolic consequence of these reactions is the same. The cleavage of the acyl moiety from ACP prevents extension and targets the acyl group for export out of the plastid by an unknown mechanism. Thioesterases play an important role in plants that have unusually short fatty acids, such as coconut, many species of *Cuphea*, and California bay *(Umbellularia californica)*. These plants have thioesterases that are especially active with C_{10} to C_{12} acyl-ACPs; by prematurely

Reaction 10.6: Enoyl-ACP reductase



Reaction 10.7: Thioesterase



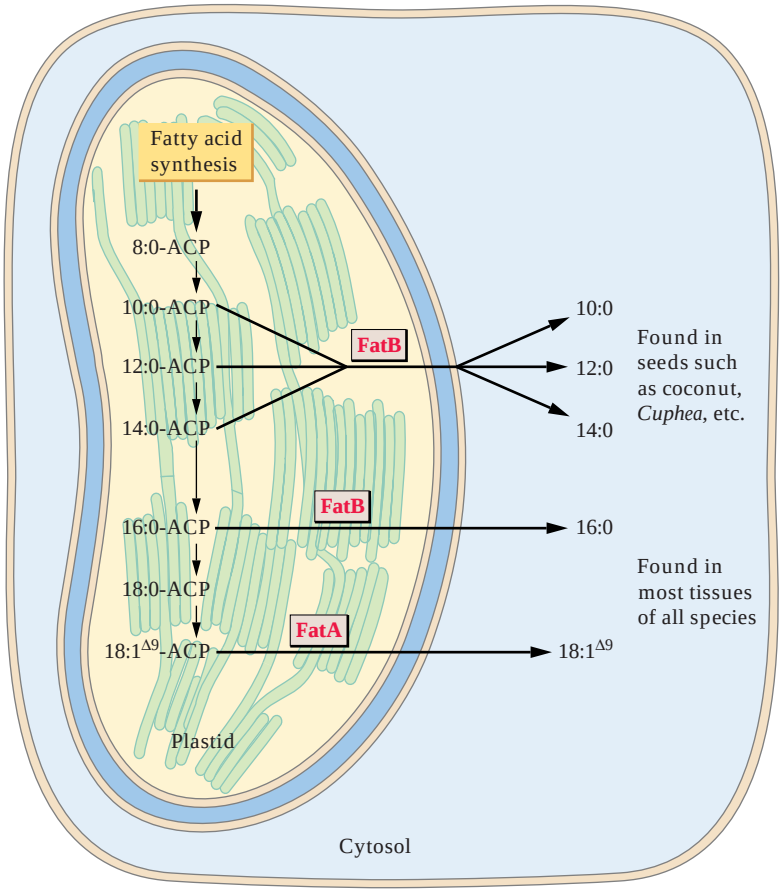


Figure 10.16
Principal types of acyl-ACP thioesterases in plants. The FatA class is most active with $18:1^{\Delta 9}$ and the FatB class is most active with saturated acyl-ACPs. The FatA $18:1^{\Delta 9}$ thioesterase and the FatB 16:0-ACP thioesterase are found in all plant tissues. Some FatB thioesterases, especially those most active on acyl-ACP acyl groups smaller than C_{16}, are species-specific and are found only in seed tissue.

terminating fatty acid biosynthesis, thioesterase activity results in the accumulation of 10:0 and 12:0 in the seed triacylglycerols.

10.5 Desaturation and elongation of C_{16} and C_{18} fatty acids

If membranes contained only saturated or trans-unsaturated fatty acids, the hydrophobic lipid tails would form a semicrystalline gel, impairing the permeability barrier and interfering with the mobility of membrane components. By contrast, cis-double bonds, which introduce "kinks" into a fatty acid chain, can enhance membrane fluidity by dramatically lowering the temperature at which these ordered matrices melt. For example, the desaturation step that converts stearic acid (18:0) to oleic acid ($18:1^{\Delta 9}$) lowers the melting point of the fatty acid from 69°C to 13.4°C. Double bonds at other positions in the chain also exert large effects on the melting temperature of fatty acids and of the lipids that contain them (Fig. 10.17; see also Chapter 1, Table 1.2). Formation of such double bonds is catalyzed by various desaturase enzymes, which generate a diverse array of unsaturated lipids found in membranes, storage reserves, and extracellular waxes.

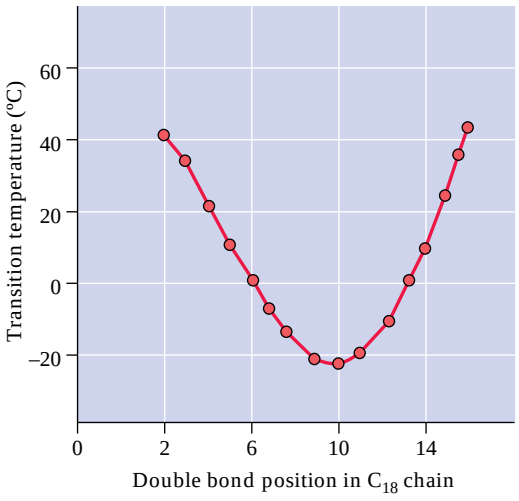


Figure 10.17
The transition temperature, or melting temperature, of a lipid is strongly influenced by the presence and position of double bonds in the acyl groups. In this example, the transition temperature has been determined for several molecular species of phosphatidylcholine in which the two acyl groups are C_{18} fatty acids that contain double bonds at various positions along the chains. Thus, for instance, when the two acyl groups have double bonds between C-2 and C-3, the transition temperature is approximately 40°C, whereas for molecules with double bonds near the center of the acyl groups, the melting temperature is decreased by about 60°C. Thus, by controlling the position of the fatty acyl unsaturations, organisms can exert control over the physical properties of lipids.

10.5.1 Plants contain a soluble, plastid-localized stearoyl-ACP desaturase.

Unsaturated C_{18} acyl chains found in membrane lipids throughout the plant cell are downstream products of a soluble chloroplast enzyme, stearoyl-ACP Δ9-desaturase (Fig. 10.18). All eukaryotes and some prokaryotes have desaturases that catalyze

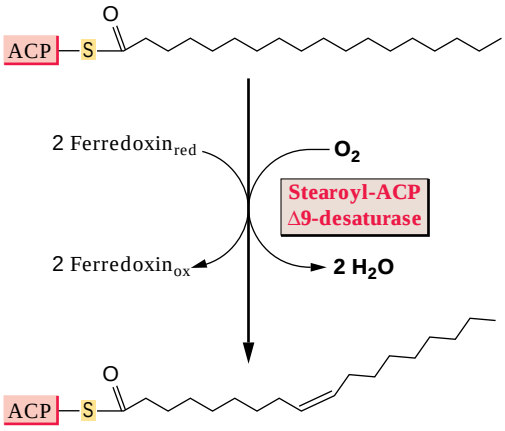


Figure 10.18
Desaturation of stearic acid is catalyzed by stearoyl-ACP Δ9-desaturase.

similar reactions, but in most cases these enzymes utilize acyl-CoA substrates and are integral membrane proteins. Some bacteria such as *Escherichia coli* lack such desaturases and instead introduce double bonds during the synthesis of the fatty acids. Recently, stearoyl-ACP Δ9-desaturase has come to be recognized as the prototype of a distinct family of structurally similar enzymes that introduce double bonds at various locations along the acyl chain. Species-specific genes for two isoforms, which catalyze desaturations of palmitic acid at the Δ4 or Δ6 position, have recently been isolated from coriander *(Coriandrum sativum)* and black-eyed susan *(Thunbergia alata)*, respectively.

Unlike the stearoyl-CoA desaturases of fungi and animals, which are located in the ER membranes, the members of the stearoyl-ACP desaturase enzyme family are soluble, a fact that has greatly facilitated structural and mechanistic studies. The tertiary structure of castor bean *(Ricinus communis)* stearoyl-ACP Δ9-desaturase has been solved by X-ray crystallography (Fig. 10.19A). As the crystal structure shows, the protein contains a cavity that can bind the 18:0 substrate in the correct orientation with respect to the active site (Fig. 10.19B). The amino acid sequences of known soluble desaturases from several plants are sufficiently homologous that we can deduce their active sites by mapping the sequences of the Δ4- and Δ6-desaturases onto the structure of the Δ9-desaturase. When the gene encoding the castor bean enzyme was expressed in *E. coli*, it produced a functional enzyme that could be purified in large amounts. Mössbauer spectrometry of the recombinant castor bean enzyme from cultures of *E. coli*

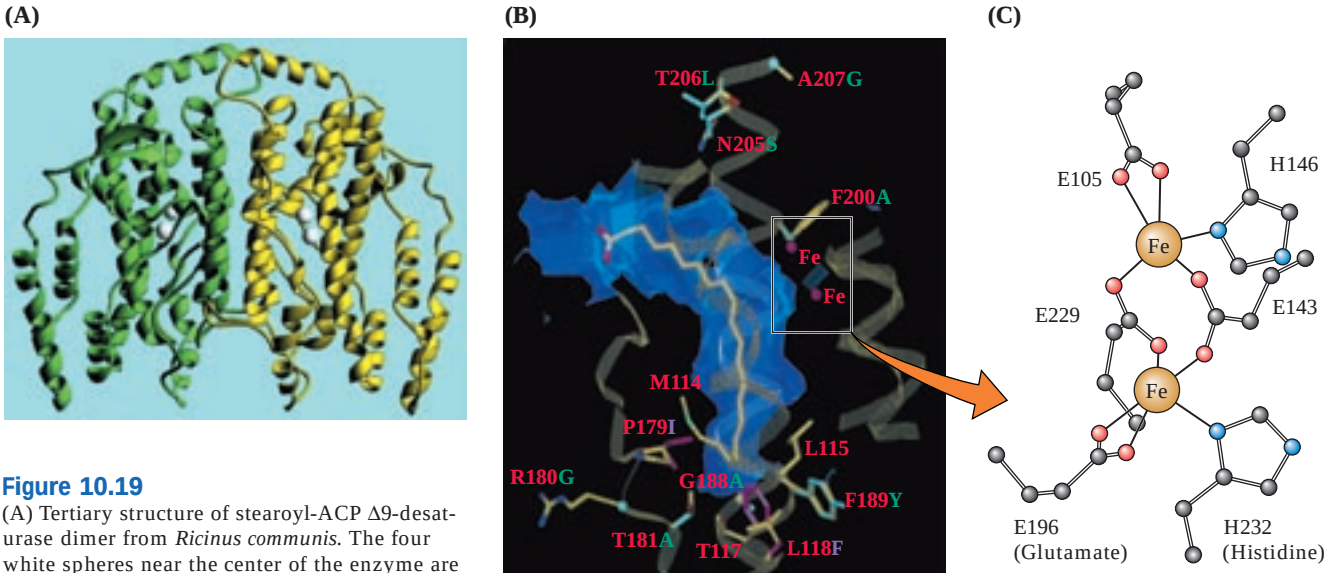


Figure 10.19
(A) Tertiary structure of stearoyl-ACP Δ9-desaturase dimer from *Ricinus communis*. The four white spheres near the center of the enzyme are two pairs of iron ions that catalyze the desaturation reaction. (B) Schematic view of the substrate channel of a stearoyl-ACP Δ9-desaturase monomer. A model of the stearoyl substrate moiety is fitted in the binding pocket of the desaturase. (C) Details of the residues that coordinate the diiron-oxo group at the active site.

grown in ^{57}Fe revealed the presence of a non-heme iron center in the desaturases—a structure now known to be an Fe-O-Fe (diiron) center of the type found in bacterial methane monooxygenase (Fig. 10.19C). This provides an explanation for many properties of the desaturases, such as the observation that the overall desaturation reaction requires transfer of two electrons from a donor such as ferredoxin or cytochrome b_5 (Fig. 10.20).

10.5.2 Most fatty acyl desaturases are membrane-localized proteins.

Except for the soluble acyl-ACP desaturase family, all other fatty acid desaturases from animals, yeast, cyanobacteria, and plants are integral membrane proteins. The plant and cyanobacterial enzymes desaturate fatty acids of glycerolipids, whereas some yeast and animal desaturases act on acyl-CoAs.

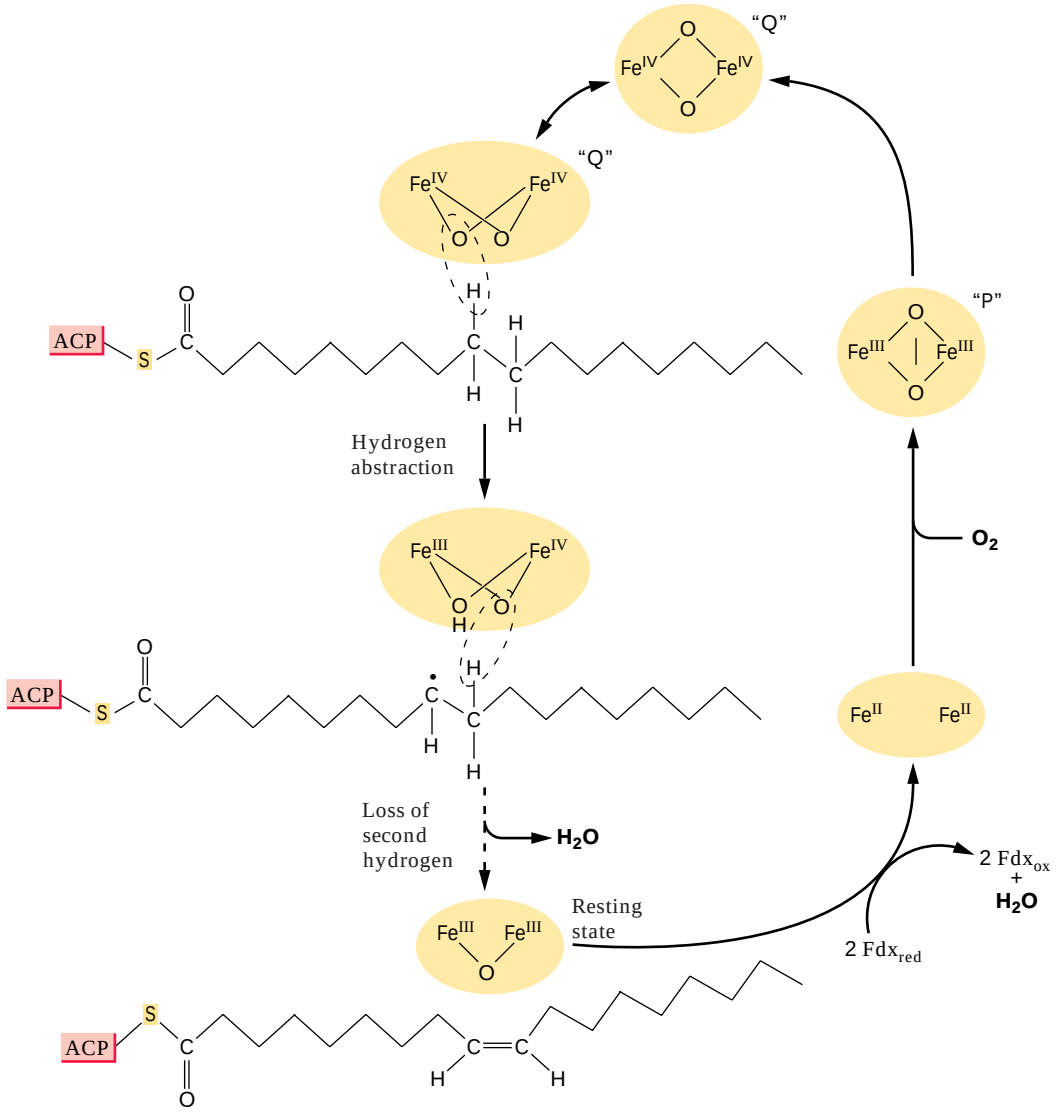


Figure 10.20
Proposed catalytic mechanism for fatty acid desaturation. In the resting state, the diiron center is in the oxidized (diferric, or Fe^{III}–Fe^{III}) form with a μ-oxo bridge. Reduction of both iron ions by electron transfer from two ferredoxins (Fdx) results in the reduced (diferrous, or Fe^{II}–Fe^{II}) form. The reduced enzyme binds molecular oxygen, resulting in the formation of a peroxo intermediate, "P." Scission of the O–O bond results in the formation of an activated form of the diiron center, "Q" (diferryl, or Fe^{IV}–Fe^{IV}). By analogy with the methane monooxygenase reaction, Q has been proposed to perform an energy-demanding hydrogen abstraction from the methylene group of the unactivated fatty acid to yield a radical intermediate. Loss of the second hydrogen results in formation of the double bond along with the loss of H_2O and regeneration of the oxidized active site and the μ-oxo bridge.

Solubilizing and purifying the plant enzymes have proven very difficult, limiting investigation by traditional biochemical techniques. Genetic analysis in *Arabidopsis* has provided an alternative approach to biochemical methods for assessing the function of the enzymes.

The number and properties of different desaturases in plants are known from the isolation of a comprehensive collection of *Arabidopsis* mutants with defects in each of eight desaturase genes. The enzymes encoded by these genes differ in substrate specificity, subcellular location, mode of regulation, or some combination of these (Table 10.4). The mutants were identified by analyzing lipid samples from leaves or seeds of individual plants from heavily mutagenized populations of plants. The biochemical defect of each class of mutants is shown by breaks in the pathway in Figure 10.21. The mutations that disrupt the activity of specific fatty acid desaturases are designated *fab2* and *fad2* through *fad8*. Two of the desaturases are ER-localized enzymes, oleate desaturase (FAD2) and linoleate desaturase (FAD3). Three structurally related enzymes are located in the plastids: one oleate desaturase (FAD6) and two functionally similar linoleate desaturases (FAD7 and FAD8). The identities of these enzymes have been confirmed by cloning the corresponding genes and using the cloned genes to complement the corresponding mutations in transgenic *Arabidopsis* plants, thereby restoring enzyme activity.

The ER-localized enzymes act on fatty acids esterified to phosphatidylcholine, and possibly other phospholipids, and utilize cytochrome b_5 as an intermediate electron donor. Cytochrome b_5 is reduced by another membrane protein, cytochrome b_5 reductase. Thus, three proteins are required for the overall desaturation reaction catalyzed by the ER-localized desaturases. In contrast, the membrane-localized chloroplast enzymes use soluble ferredoxin as an electron donor and act on fatty acids esterified to galactolipids, sulfolipids, and phosphatidylglycerol. Analysis of the mutants suggests the FAD4 enzyme is completely specific for phosphatidylglycerol, and the FAD5 enzyme appears to be specific for monogalactosyldiacylglycerol (Fig. 10.21). Despite differences in the glycerolipid substrates and electron donors used, and the position in which they insert the double bond, all of the membrane-localized plant desaturases cloned thus far are structurally related to each other and to the membrane-localized desaturases from animals and yeast. They contain a pair of histidine-rich sequences (HXXHH) that have been proposed to bind the two iron ions required for catalysis (see Fig. 10.19). Although the tertiary structure of the membrane-bound desaturases is not known, the iron-binding site may resemble that found in the diiron protein hemerythrin (Fig. 10.22).

From analysis of the effects of the mutations on glycerolipid fatty acid composition,

Table 10.4 Fatty acid desaturases of *Arabidopsis*

Name	Subcellular location	Fatty acid substrates	Site of double-bond insertion	Notes
FAD2	ER	$18{:}1^{\Delta 9}$	Δ12	Preferred substrate is phosphatidylcholine
FAD3	ER	$18{:}2^{\Delta 9,12}$	ω3	Preferred substrate is phosphatidylcholine
FAD4	Chloroplast	16:0	Δ3	Produces 16:1-*trans* at *sn*-2 of phosphatidylglycerol
FAD5	Chloroplast	16:0	Δ7	Desaturates 16:0 at *sn*-2 of monogalactosyldiacylglycerol
FAD6	Chloroplast	$16{:}1^{\Delta 7}$ $18{:}1^{\Delta 9}$	ω6	Acts on all chloroplast glycerolipids
FAD7	Chloroplast	$16{:}2^{\Delta 7,11}$ $18{:}2^{\Delta 9,12}$	ω3	Acts on all chloroplast glycerolipids
FAD8	Chloroplast	$16{:}2^{\Delta 7,11}$ $18{:}2^{\Delta 9,12}$	ω3	Isoenzyme of FAD7 induced by low temperature
FAB2	Chloroplast	18:0	Δ9	Stromal stearoyl-ACP desaturase

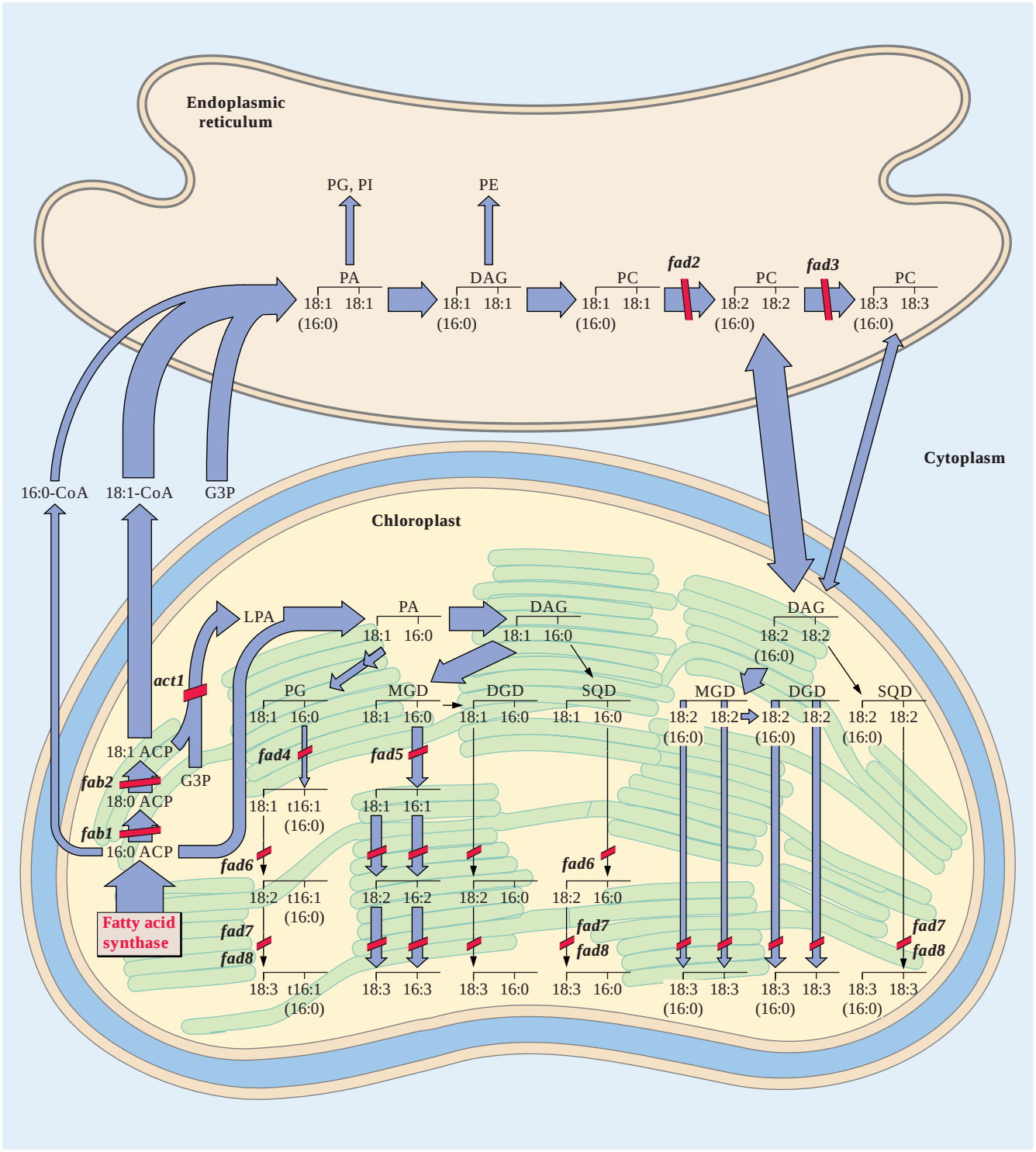


Figure 10.21
Abbreviated scheme for lipid synthesis in leaves of *Arabidopsis*. The set of reactions occurring solely within the chloroplast are termed the prokaryotic pathway; those that involve glycerolipid synthesis in the ER and subsequent transfer to the chloroplast constitute the eukaryotic pathway. The widths of the arrows represent the relative fluxes through the various steps of the pathways. The breaks in the pathway (red) represent some of the sites at which mutations have been obtained in *Arabidopsis* (see Table 10.4). DAG, diacylglycerol; DGD, digalactosyldiacylglycerol; G3P, glycerol 3-phosphate; LPA, lysophosphatidic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; MGD, monogalactosyldiacylglycerol; SQD, sulfoquinovosyldiacylglycerol.

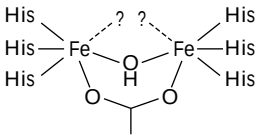


Figure 10.22
Proposed ligation sphere of the integral membrane desaturases and related enzymes possessing two HXXHH motifs, in which all of the histidine residues have been shown to be essential for catalysis. The model is based on the structure of hemerythrin and shows a hydroxo bridge and a single carboxylate ligand that has yet to be defined. Question marks indicate the presence of two undefined ligands.

a loss-of-function mutant appears to be available for every desaturase in *Arabidopsis* except stearoyl-ACP Δ9-desaturase, which is encoded by a family of genes. Thus, the *fab2* mutation, thought to inactivate one of the stearoyl-ACP desaturase genes, causes a significant increase in stearate concentrations but does not completely eliminate unsaturated fatty acids.

10.5.3 What factors determine glycerolipid desaturation?

A central question concerning the mechanisms that control desaturation is, What factors determine the extent of desaturation of a specific glycerolipid in a particular membrane? In cyanobacteria, the steady-state concentration of an oleate Δ12-desaturase mRNA is inversely correlated with temperature (Fig. 10.23). The stimulatory effect of low temperature on desaturase gene expression can be mimicked by using nonlethal catalytic hydrogenation to reduce the number of double bonds in membranes of living cells, thereby decreasing the fluidity of the membranes. Thus, cyanobacteria appear to have a mechanism that senses the fluidity of the membrane and regulates the level of desaturase mRNA accordingly.

Most plants also increase the extent of membrane glycerolipid desaturation when grown at low temperature. However, with one exception, the amount of mRNA for the desaturases genes in *Arabidopsis* does not appear to change significantly in response to changes in growth temperature. The one exception is the *FAD8* gene, which exhibits a strong increase in mRNA abundance at low temperature. If a regulatory mechanism controlled desaturase gene expression in response to changes in the physical properties of membranes, we would expect this mechanism to detect large changes in membrane composition and to signal an increase in desaturase gene expression. However, expression of the cold-inducible *fad8* gene is not altered in *fad7 fad8* double mutants, which are highly deficient in $16{:}3^{\Delta 7,10,13}$ and $18{:}3^{\Delta 9,12,15}$ fatty acids. Indeed, none of the loss-of-function *fad* mutations alters *FAD* gene expression, indicating that if such a sensing mechanism exists in plants, it must act at the posttranscriptional level. Presumably, the complexities associated with regulating the composition of many intracellular membranes in plants may require posttranscriptional mechanisms that can adjust the composition of each membrane independently.

10.5.4 Specialized elongase systems produce long-chain fatty acids.

Fatty acid synthesis generally results in C_{16} and C_{18} fatty acids, yet plants have numerous requirements for longer fatty acids. All plants produce waxes, usually derived from C_{26} to C_{32} fatty acids. Often, sphingolipids contain C_{22} and C_{24} fatty acids. And, in some plants, triacylglycerols contain large

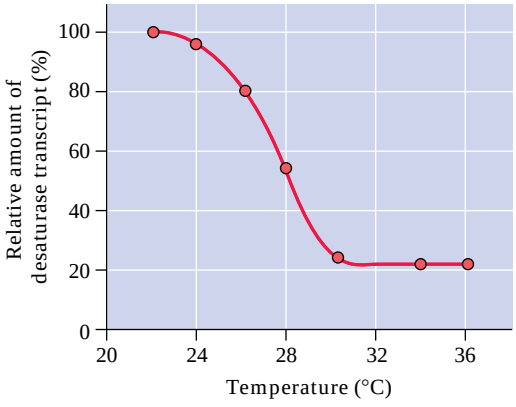


Figure 10.23
In cyanobacteria, the amount of desaturase mRNA is regulated by growth temperature.

amounts of C_{20} and C_{22} fatty acids. Where and how are these very-long-chain fatty acids synthesized?

Plants and most other eukaryotic organisms have a specialized elongase system for extension of fatty acids beyond C_{18}. These elongase reactions have several important features in common with FAS reactions (see Fig. 10.8). Each uses a reaction series that condenses two carbons at a time from malonyl-CoA to an acyl primer, followed by reduction, dehydration, and a final reduction. The result is that the same acyl intermediates are used for the two processes. Although the enzymology of the elongase is not well understood, several important differences between the two systems are known (Fig. 10.24):

- Fatty acid elongases are localized in the cytosol and are membrane-bound.
- ACP is not involved in this process.
- The elongase 3-ketoacyl-CoA synthase (elongase KCS) catalyzes the condensation of malonyl-CoA with an acyl primer.

Recent cloning of elongase KCS genes from *Arabidopsis* and jojoba (*Simmondsia chinensis*) has revealed that these 60-kDa enzymes bear very little sequence similarity to any other condensing enzymes. The identification of additional clones suggests that the elongase KCSs in *Arabidopsis* belong to a

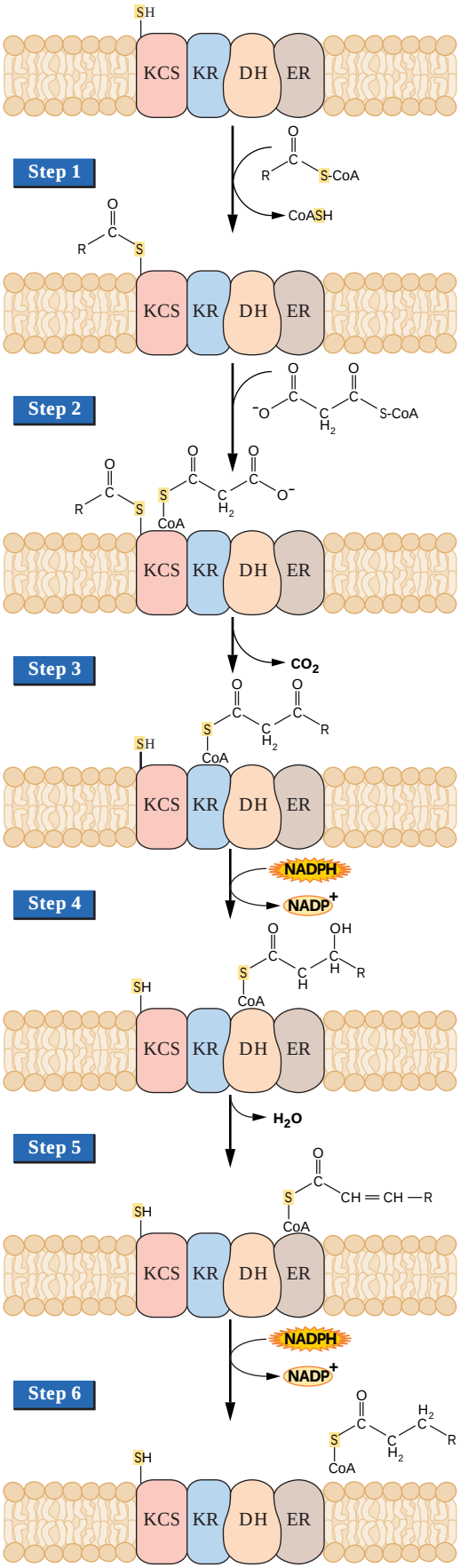


Figure 10.24
Sequence of events during a single cycle of fatty acid elongation by the membrane-bound fatty acid elongase system, which consists of at least four components: 3-ketoacyl-CoA synthase (KCS), 3-ketoacyl-CoA reductase (KR), 3-hydroxyacyl-CoA dehydrase (DH), and enoyl-CoA reductase (ER). The initial cycle of elongation starts with a C_{18} acyl-CoA, either stearoyl-CoA or oleoyl-CoA. Steps 1–3 are catalyzed by KCS. First, the acyl-CoA acylates an active-site cysteine. Next, the malonyl-CoA binds to the active site. Finally, a concerted reaction occurs in which the malonyl moiety is decarboxylated and a Claisen condensation with the acyl group results. Then Steps 4–6, a reduction, a dehydration, and a second reduction, occur sequentially on the remaining components of the elongase system and result in the release of an acyl-CoA that is two carbons longer than the starting acyl-CoA. This acyl-CoA may then undergo additional cycles of elongation or be used in other pathways of lipid metabolism.

complex gene family with at least 15 members and possibly more than 25. The requirement for multiple genes may be related to the relatively narrow acyl substrate specificity of elongase KCSs, so that several different elongase KCSs would be required to synthesize a C_{30} fatty acid. Elongase KCSs may also be specific for a particular physiological function, such as wax biosynthesis or sphingolipid biosynthesis.

The additional components of the elongase system remain largely uncharacterized. The reductase and dehydrase activities are membrane-localized, and the reductases prefer NADPH. To date, none has been purified or characterized. However, *glossy8*, a gene in maize that is required for normal wax composition, has been cloned and may encode a 3-ketoacyl reductase.

10.6 Synthesis of unusual fatty acids

10.6.1 More than 200 fatty acids occur in plants.

Extensive surveys of the fatty acid composition of seed oils from different plants species have resulted in the identification of more than 200 naturally occurring fatty acids, which can be broadly classified into 18 structural classes. The classes are defined by the number and arrangement of double or triple bonds and various functional groups, such as hydroxyls, epoxys, cyclopentenyl or cyclopropyl groups, or furans (Fig. 10.25).

The most common fatty acids, which often occur in both membrane and storage lipids, belong to a small family of C_{16} and C_{18} fatty acids that may contain as few as zero, or as many as three, cis-double bonds. All members of the family are descended from the fully saturated species as the result of a series of sequential desaturations that begin at C-9 and progress in the direction of the methyl carbon (Fig. 10.26). Fatty acids that cannot be described by this simple algorithm are generally considered "unusual" because each is found almost exclusively in the seed oil of only a few plant species. Several, however, such as lauric (12:0), erucic ($20{:}1^{\Delta 14}$), and ricinoleic (12-OH, $18{:}1^{\Delta 9}$) acids, are of substantial commercial importance (see Section 10.11.4).

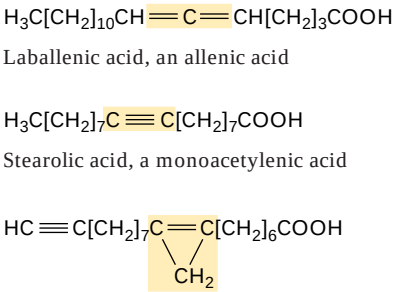


Figure 10.25
Some of the functional groups found in unusual plant fatty acids.

10.6.2 Some enzymes that synthesize unusual fatty acids resemble enzymes involved in the biosynthesis of common fatty acids.

Much of the research concerning unusual plant fatty acids has been focused on the identification of new structures or cataloging the composition of fatty acids found in various plant species. Less is known about the mechanisms responsible for the synthesis and accumulation of unusual fatty acids, or of their significance to the fitness of the plants that accumulate them. Recently, however, a gene for the oleate Δ12-hydroxylase that catalyzes the synthesis of ricinoleic and other hydroxylated fatty acids was cloned from castor bean and *Lesquerella fenderli*. This enzyme exhibits about 70% amino acid sequence

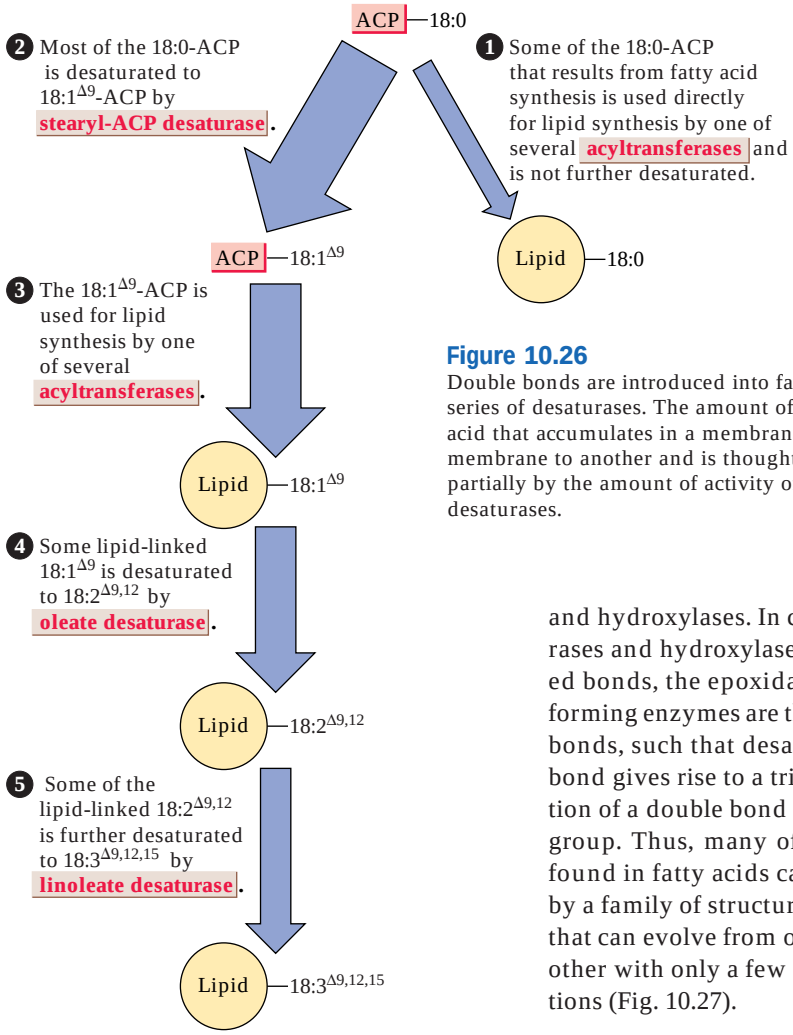


Figure 10.26
Double bonds are introduced into fatty acids by a series of desaturases. The amount of a particular fatty acid that accumulates in a membrane varies from one membrane to another and is thought to be controlled partially by the amount of activity of the various desaturases.

similarity to the microsomal oleate Δ12-desaturase from the same species. To identify the amino acid differences responsible for causing the enzyme to be a hydroxylase instead of a desaturase, investigators have used site-directed mutagenesis to test the importance of the amino acids conserved in one class of enzymes but not in the other. On the basis of these studies, it appears that the hydroxylase can be converted to a desaturase by as few as seven amino acid substitutions that alter the geometry of the active site. The fact that such a small number of amino acid substitutions can alter the outcome of the enzymatic reaction indicates that the diiron center used by these enzymes is capable of catalyzing different reactions, depending on the precise geometry of the active site. Indeed, recent evidence also indicates that the enzymes that introduce epoxy groups and triple bonds are also structurally similar to the desaturases and hydroxylases. In contrast to the desaturases and hydroxylases, which act on saturated bonds, the epoxidases and acetylene-forming enzymes are thought to act on double bonds, such that desaturation of a double bond gives rise to a triple bond, and oxidation of a double bond gives rise to an epoxy group. Thus, many of the modifications found in fatty acids can be accounted for by a family of structurally related enzymes that can evolve from one specificity to another with only a few amino acid substitutions (Fig. 10.27).

10.6.3 Taxonomic relationships between plants having similar or identical kinds of unusual fatty acids are not predictable.

In some cases, particular fatty acids occur mostly or solely in related taxa. For example, the cyclopentenyl fatty acids (e.g., chaulmoogric acid, Fig. 10.25) have been found only in the family Flacourtiaceae, although the presence of cyclopentenylglycine, the biosynthetic precursor of the cyclopentenyl fatty acids, in the Passifloraceae (passionflower family) and Turneraceae (e.g., yellow alder) suggests that these fatty acids may also be found in these other families of the order Violales. Petroselinic acid is most commonly found in the related families Apiaceae (carrot and parsley family), Araliaceae (ginseng family), and Garryaceae (silk tassel family), but it has also been observed in more divergent families.

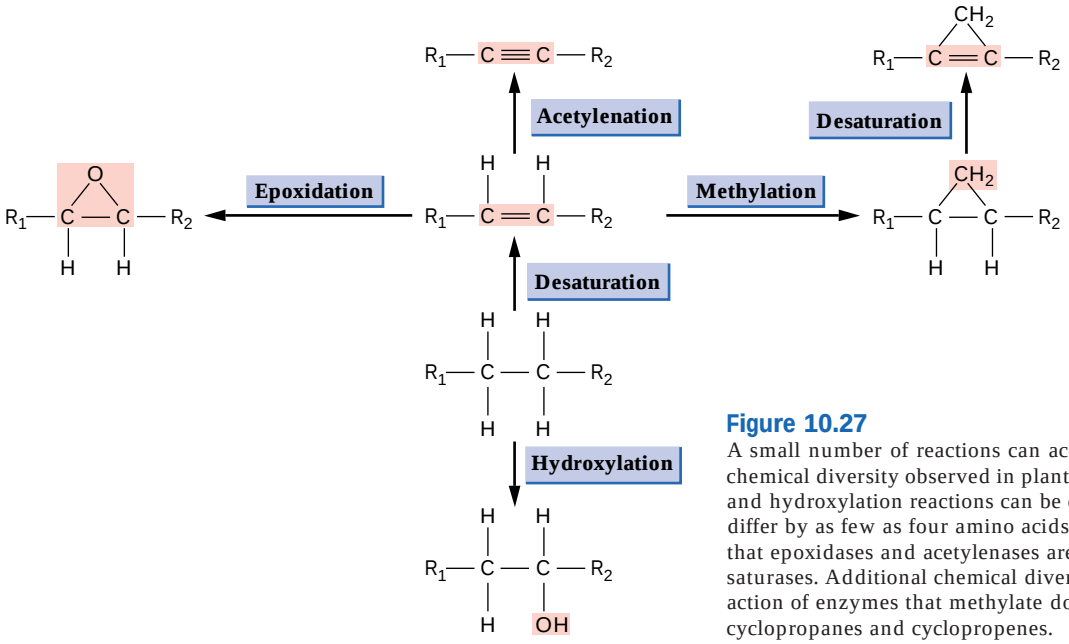


Figure 10.27
A small number of reactions can account for most of the chemical diversity observed in plant fatty acids. Desaturation and hydroxylation reactions can be catalyzed by enzymes that differ by as few as four amino acids. Recent evidence suggests that epoxidases and acetylenases are also closely related to desaturases. Additional chemical diversity may arise through the action of enzymes that methylate double bonds to produce cyclopropanes and cyclopropenes.

In other cases there does not appear to be a direct link between taxonomic relationships and the occurrence of unusual fatty acids. For example, lauric acid is prominent in two unrelated families, the Lauraceae (laurel family) and Arecaceae (palm family). Similarly, ricinoleic acid (see Fig. 10.25) has now been identified in 12 genera from 10 families. If any conclusion can be drawn from the taxonomic distribution of unusual fatty acids, it would be that the ability to synthesize some unusual fatty acids appears to have evolved several times independently, whereas for others it may have evolved only once. The studies of the desaturases and related enzymes described above provide an insight into how this may have happened, in at least some cases, by the accumulation of a small number of amino acid substitutions.

10.6.4 Unusual fatty acids occur almost exclusively in seed oils and may serve a defense function.

Given that the ability to synthesize various unusual fatty acids must have evolved independently, the common confinement of triacylglycerols to seed indicates some selective constraint or functional significance. One possible function of unusual fatty acids is that by being toxic or indigestible they protect the seed against herbivory. Some unusual fatty acids may be inherently toxic, such as the acetylenic fatty acids or some of their metabolites. Other unusual fatty acids are toxic upon catabolism by the herbivore, such as the 4-fluoro fatty acids of *Dichapetalum toxicarium*. Cyclopentenyl fatty acids were long used in the treatment of leprosy, and the activity of hydnocarpic acid against many *Mycobacterium* species has been demonstrated. The cyclopropenoid fatty acids also appear to have biological activities, possibly because of the accumulation in animal tissues of partial catabolites containing the cyclopropene ring, which inhibits β-oxidation of fatty acids. Malvalic and sterculic acids, produced by cotton plants, inhibit the growth of seed-eating lepidopteran larvae and may be part of a defense mechanism against these insects. These fatty acids may also be effective antifungal agents, inhibiting the growth of some plant pathogenic fungi at concentrations that appear biologically relevant.

From a dietary viewpoint, the most intensely studied of the unusual fatty acids is erucic acid (see Table 10.2), because of fears that the consumption of rapeseed oil might be detrimental to human health. Chronic feeding of erucic acid to experimental animals has a range of deleterious effects (Fig. 10.28), but whether these are sufficiently severe to propose a herbivore-defense role for erucic acid in seeds remains questionable.

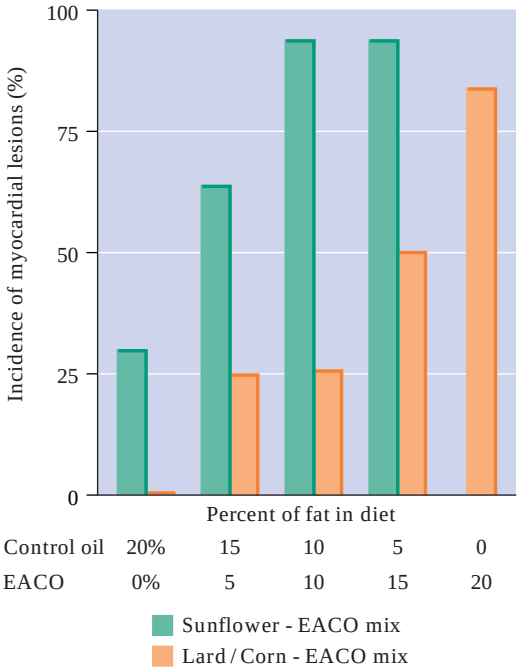


Figure 10.28
Relationship between the incidence of myocardial lesions in rats and the percentage of dietary fat derived from high erucic acid–containing oil (EACO). Shown are the ratios of EACO to control oil (either sunflower oil or a 3:1 [by wt.] lard:corn oil mixture). In both experiments, myocardial lesions increase dramatically with the proportion of erucic acid in the diet.

10.7 Synthesis of membrane lipids

In 1979, Grattan Roughan and Roger Slack first proposed that there are two distinct pathways for membrane synthesis in higher plants and named these the "prokaryotic pathway" and the "eukaryotic pathway." An abbreviated schematic diagram of the two pathways of glycerolipid synthesis in *Arabidopsis* leaves is shown in Figure 10.29. The prokaryotic pathway refers to the synthesis of lipids within the plastid. The eukaryotic pathway refers to the sequence of reactions involved in synthesis of lipids in the ER, transfer of some lipids between the ER and the plastid, and further modification of the lipids within the plastid.

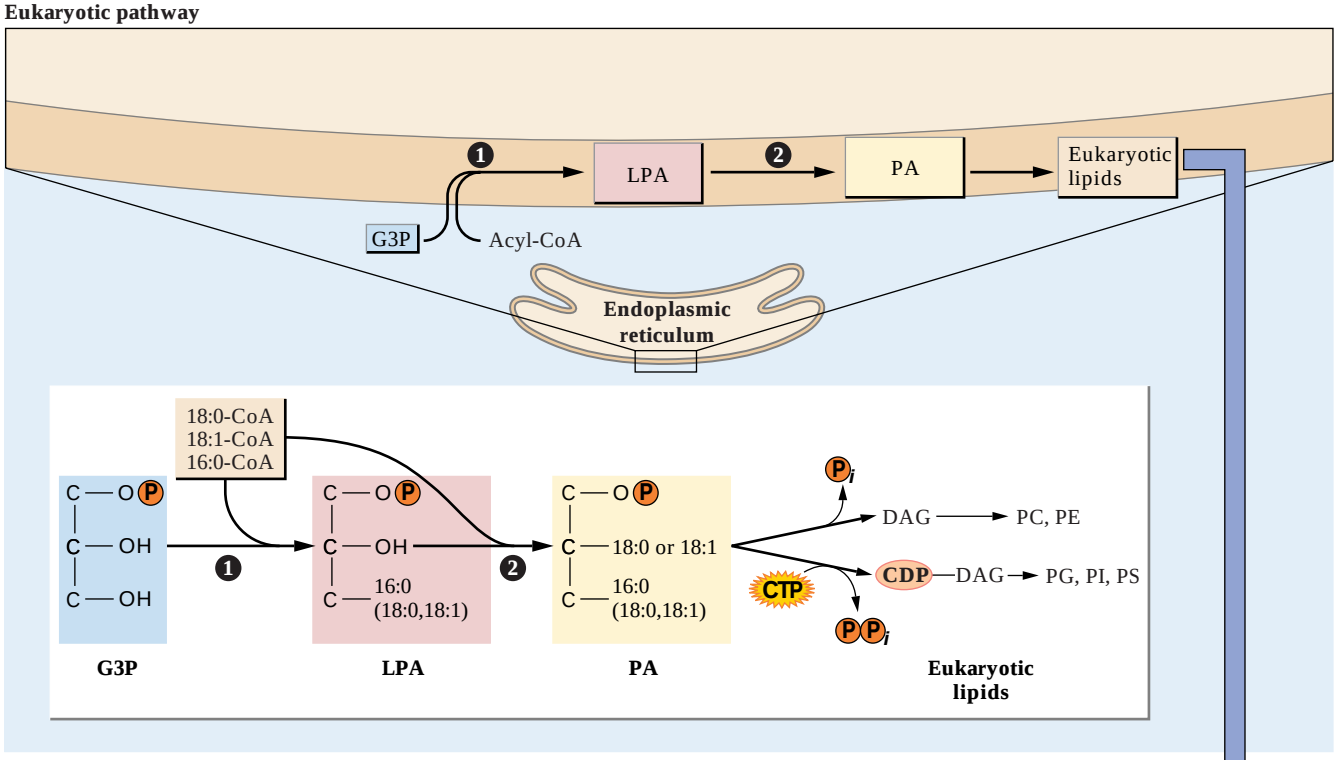
Superimposed on the main pathways for glycerolipid synthesis are several additional pathways for the synthesis of other lipids, such as waxes, cutin, sphingolipids, and sterols. Relatively little is known about the cellular localization of these pathways. Sphingolipid synthesis is likely to take place in the ER. Similarly, elongation of fatty acids for wax synthesis and production of oxygenated fatty acids for cutin synthesis are also thought to take place in the ER.

10.7.1 Phosphatidic acids formed in the plastids via the "prokaryotic pathway" and in the ER via the "eukaryotic pathway" differ in fatty acyl composition and position.

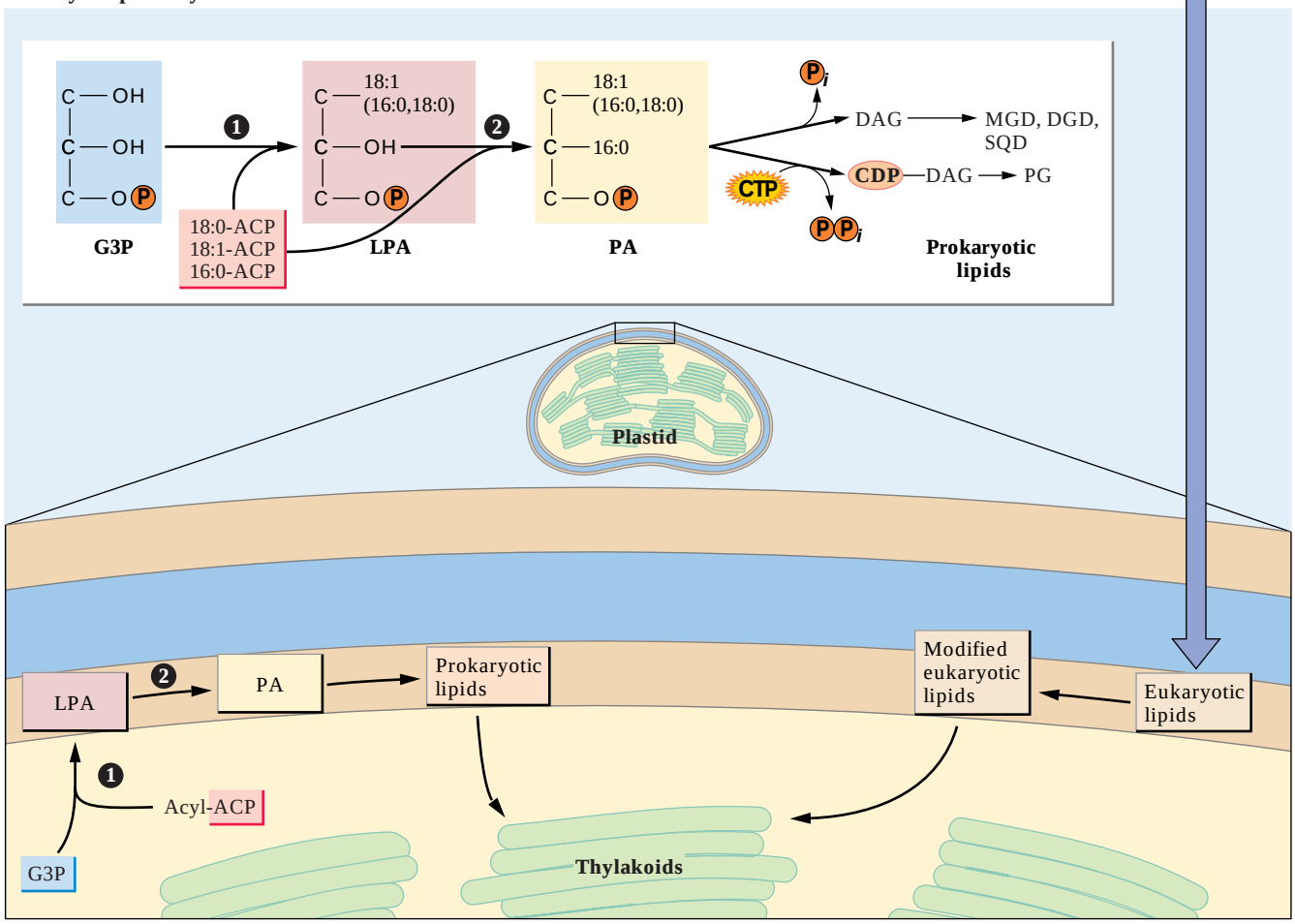
Glycerolipid synthesis involves a complex and highly regulated interaction between the chloroplast, where fatty acids are synthesized, and other membrane systems of the cell. The 16:0-, 18:0-, and $18:1^{\Delta 9}$-ACP products of plastid fatty acid synthesis may be either incorporated directly into chloroplast lipids by the plastid-localized prokaryotic pathway or exported to the cytoplasm as CoA esters, which are then incorporated into ER lipids by an independent set of eukaryotic pathway acyltransferases (see Fig. 10.29).

Figure 10.29 ***(Facing page)***
Prokaryotic and eukaryotic pathways of glycerolipid synthesis. The prokaryotic pathway takes place in plastids and predominantly esterifies palmitate to the *sn*-2 position of lysophosphatidate (LPA). The eukaryotic pathway occurs outside the plastid, primarily in the ER, and results in C_{18} fatty acids being esterified to the *sn*-2 position of glycerolipids. In the prokaryotic pathway, acyl-ACP is condensed with glycerol 3-phosphate (G3P) by a soluble enzyme, G3P acyltransferase (1). The product, LPA, rapidly partitions into the membranes, where it is converted to phosphatidate (PA) by a membrane-localized LPA acyltransferase (2). PA is then converted to the other lipids found in chloroplasts. Most of the reactions of lipid synthesis by the prokaryotic pathway are thought to take place in the inner envelope of the plastids. The initial reactions of the eukaryotic pathway are similar except that acyl-CoA substrates are used and the G3P acyltransferase is thought to be associated with the ER. Lipids move from the ER to the other organelles, including the outer envelope of plastids. Eukaryotic lipids in the outer envelope are transferred into the inner membranes by an unknown mechanism and are there modified by the replacement of head groups and the action of desaturases. CDP-DAG, cytidine diphosphate-diacylglycerol; DAG, diacylglycerol; DGD, digalactosyldiacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; MDG, monogalactosyldiacylglycerol; SQD, sulfoquinovosyldiacylglycerol.

Eukaryotic pathway



Prokaryotic pathway



The first steps of glycerolipid synthesis are two acylation reactions that transfer fatty acids from acyl-ACP or acyl-CoA to glycerol 3-phosphate, forming phosphatidic acid (see Fig. 10.30). Because of the substrate specificities of the plastid acyltransferases, the phosphatidic acid made by the prokaryotic pathway has 16:0 at the *sn*-2 position and, in most cases, $18{:}1^{\Delta 9}$ at the *sn*-1 position (see Fig. 10.29). In contrast to the plastid isoenzymes, the acyl-transferases of the ER produce phosphatidic acid that is highly enriched with C_{18} fatty acids at the *sn*-2 position; 16:0, when present, is confined to the *sn*-1 position (see Fig. 10.29).

10.7.2 Membrane lipid synthesis requires a complex collaboration between cell compartments.

Both the prokaryotic and eukaryotic pathways were formulated initially on the basis of labeling studies and have subsequently been tested by genetic studies in *Arabidopsis*. Both are initiated by the synthesis of phosphatidic acid. In the chloroplast pathway, phosphatidic acid is used for the synthesis of phosphatidylglycerol or is converted to diacylglycerol by a phosphatidic acid–phosphatase located in the inner plastid envelope. The diacylglycerol pool can act as a precursor for the synthesis of the other major chloroplast lipids: monogalactosyldiacylglycerol; digalactosyldiacylglycerol; and sulfoquinovosyldiacylglycerol (SQD), also called sulfolipid (see Fig. 10.29). In contrast to most other eukaryotic membranes, phospholipids (mostly phosphatidylglycerol) constitute 16% or less of the glycerolipids in chloroplast membranes (Fig. 10.31); the remainder is primarily galactolipid.

In the eukaryotic pathway, phosphatidic acid is synthesized in the ER from fatty acids exported from the chloroplast. ER-derived phosphatidic acid gives rise to the phospholipids, such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine, which are characteristic of the various membranes external to the chloroplast. However, the diacylglycerol moiety of phosphatidylcholine is returned to the chloroplast envelope, where it enters the diacylglycerol pool and contributes to the synthesis of plastid lipids (see Fig. 10.21). This lipid exchange between the ER and the

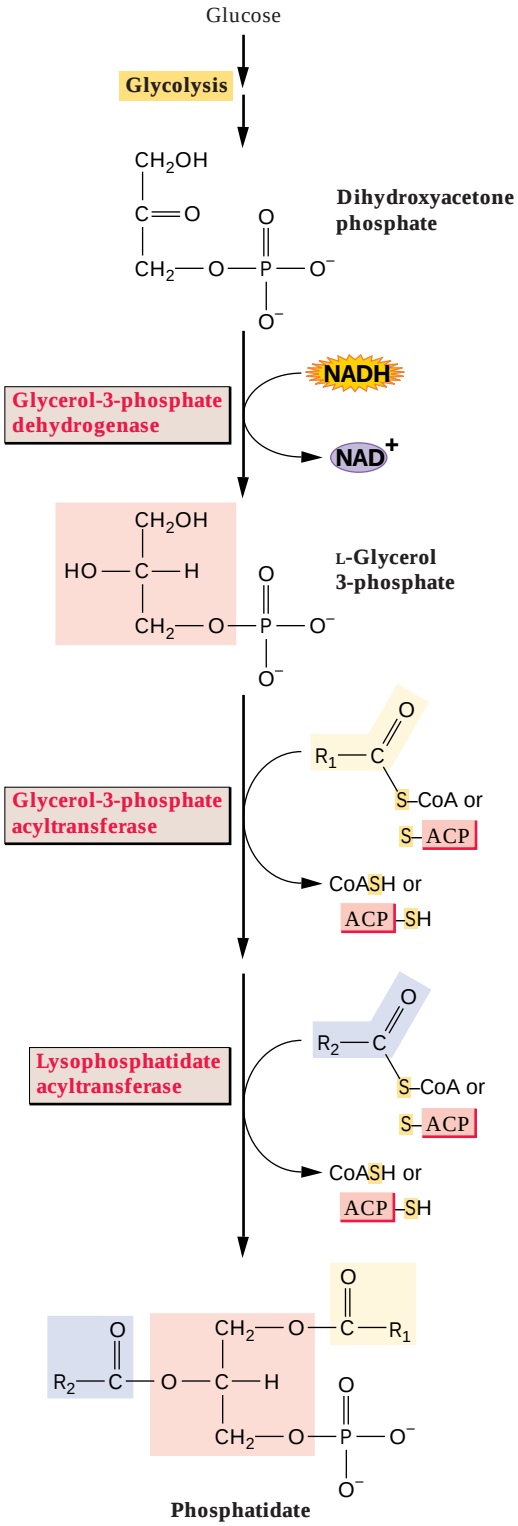


Figure 10.30
The biosynthetic pathway to phosphatidate. Fatty acids activated by esterification to CoASH or ACP are transferred by acyltransferases to the hydroxyl groups of glycerol 3-phosphate. The plastid glycerol-3-phosphate acyltransferase is a soluble enzyme. All other known acyltransferases are thought to be membrane-associated.

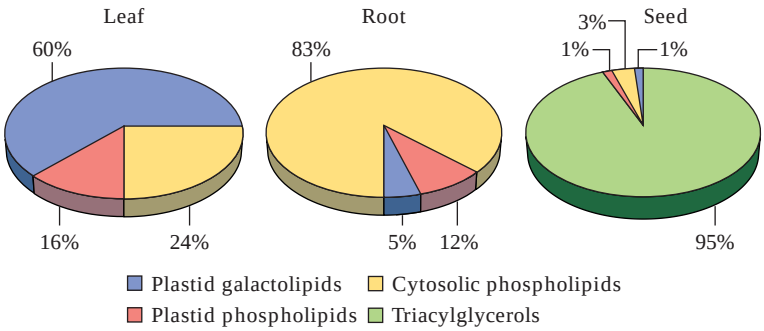


Figure 10.31
Glycerolipid compositions differ in different cell types. In leaves, where the most abundant membranes are chloroplast lamellae, galactolipids are the most abundant class. In roots, by contrast, most of the membranes are present in extraplastidic systems such as the endoplasmic reticulum and Golgi apparatus. Seeds of oilseed species, such as *Arabidopsis*, contain mostly triacylglycerols.

chloroplast is reversible to some extent (see Section 10.7.4). The mechanism by which lipids move between the ER and chloroplast is not known. Some steps that have been hypothesized are shown in Figure 10.32. The eukaryotic pathway is the principal route of glycerolipid synthesis in all nonphotosynthetic tissues as well as in the photosynthetic tissues of many higher plants. Among the angiosperms, only so-called 16:3 plants, such as spinach and *Arabidopsis*, produce more than 10% of their glycerolipids by the prokaryotic pathway (see Section 10.1.2 and below).

10.7.3 The fatty acid composition of lipids can reveal their pathway of origin.

The relative amount of glycerolipid synthesized in plastids and the ER may vary in

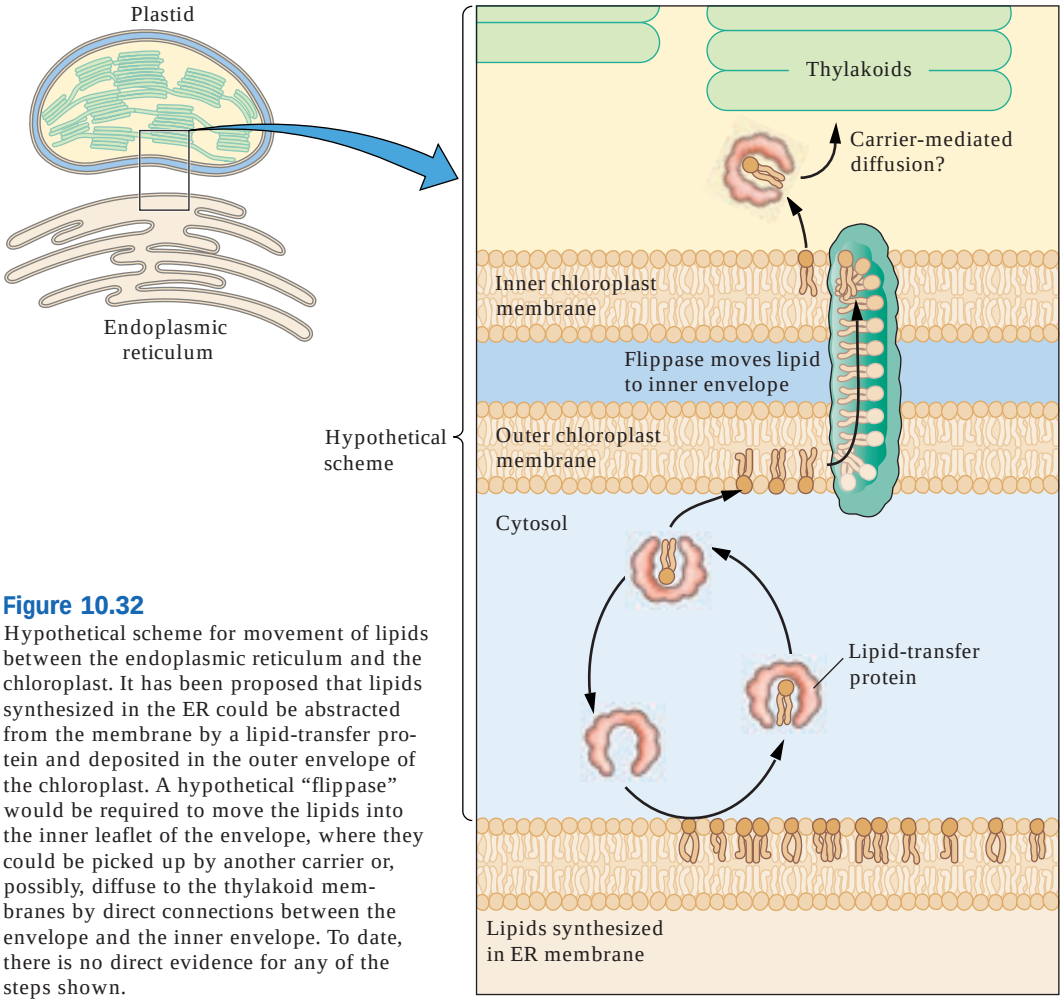


Figure 10.32
Hypothetical scheme for movement of lipids between the endoplasmic reticulum and the chloroplast. It has been proposed that lipids synthesized in the ER could be abstracted from the membrane by a lipid-transfer protein and deposited in the outer envelope of the chloroplast. A hypothetical "flippase" would be required to move the lipids into the inner leaflet of the envelope, where they could be picked up by another carrier or, possibly, diffuse to the thylakoid membranes by direct connections between the envelope and the inner envelope. To date, there is no direct evidence for any of the steps shown.

different tissues or in different plant species. In species such as pea and barley, phosphatidylglycerol is the only product of the prokaryotic pathway, and the remaining chloroplast lipids are synthesized entirely by the eukaryotic pathway. By contrast, in leaves of 16:3 plants as much as 40% of cellular glycerolipid in leaf cells is synthesized within the chloroplasts. Because $16{:}3^{\Delta 7,10,13}$ is a major product of the pathway of glycerolipid synthesis in the chloroplast (see Fig. 10.21), the relative flux through the chloroplast prokaryotic pathway can be determined readily by the presence of this fatty acid (hence the name, 16:3 plants). Plants with very small amounts of $16{:}3^{\Delta 7,10,13}$ are called 18:3 plants. However, this terminology has probably outlived its usefulness because at least some plants appear to vary the relative flux between the two pathways, depending on the growth temperature. The contribution of the eukaryotic pathway to monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and sulfolipid synthesis is diminished in nonvascular plants, and in many green algae the chloroplast is almost entirely autonomous with respect to membrane lipid synthesis.

10.7.4 Large quantities of lipid appear to move between the ER and chloroplasts.

Each plant cell is autonomous with respect to glycerolipid synthesis, and there is no extensive transport of fatty acids or glycerolipids between cells. However, membrane biogenesis in plants involves a massive movement of fatty acids or lipids from one organelle to another (see Fig. 10.21). The quantitatively most significant movement of lipids is between the ER and the chloroplasts, which contain as much as 80% of leaf-cell glycerolipids. In epidermal cells, there is also substantial movement of wax and cutin monomers from the ER to the extracellular space. The mechanisms by which lipids move between the ER and the chloroplasts, or from the ER to the outside of the cell, are not known.

Several lines of evidence indicate the existence of regulatory mechanisms that coordinate the activity of the two pathways for glycerolipid synthesis in plants. The *act1* mutants of *Arabidopsis* are deficient in activity of chloroplast acyl-ACP:*sn*-glycerol-3-phosphate acyltransferase, the first enzyme of the prokaryotic pathway (see Fig. 10.21). This deficiency severely diminishes the amount of movement through the prokaryotic pathway but is compensated for by increased synthesis of chloroplast glycerolipids via the eukaryotic pathway (Fig. 10.33). These and related results indicate that even in the face of a major disruption of one of the pathways of glycerolipid synthesis, mechanisms exist to ensure the synthesis and transfer of enough glycerolipids to support normal rates of membrane biogenesis. This striking example raises many unanswered questions. In particular, how is the demand for increased glycerolipid synthesis communicated to the lipid biosynthetic pathways during membrane expansion?

A partial answer to this question lies in the recognition that, at least for the ER and the plastid, lipid moves in both directions between the membranes. Detailed analysis of the effects of various *Arabidopsis fad* mutations on the fatty acid composition of membranes indicates that even though each enzyme is located exclusively in one compartment or the other, most of the mutations affect the composition of both chloroplast and extrachloroplast membranes (see Fig. 10.21). Thus, lipids must travel to and from chloroplast membranes. Because most of the chloroplast glycerolipids are not found in other membranes, these lipids must be converted to some other form (e.g., phosphatidate, diacylglycerol, or phosphatidylcholine) before or immediately after transfer. Similarly, phosphatidylcholine, the principal glycerolipid in the extrachloroplast membranes, occurs only in the outer leaflet of the chloroplast envelope. Furthermore, chloroplasts completely lack phosphatidylethanolamine, the other major component of the ER. Thus, phosphatidylcholine or another lipid is probably converted to one of the common chloroplast lipids in the chloroplast envelope before being transferred to the inner membranes of the chloroplast.

A confounding problem is that no conclusive evidence exists for vesicular traffic between the ER and the plastids. Thus, there has been sustained interest in the possible role of lipid-transfer proteins as carriers of lipids between the various membranes (see Fig. 10.32). These small proteins are defined by their ability to catalyze the in vitro exchange of lipids between membranes.

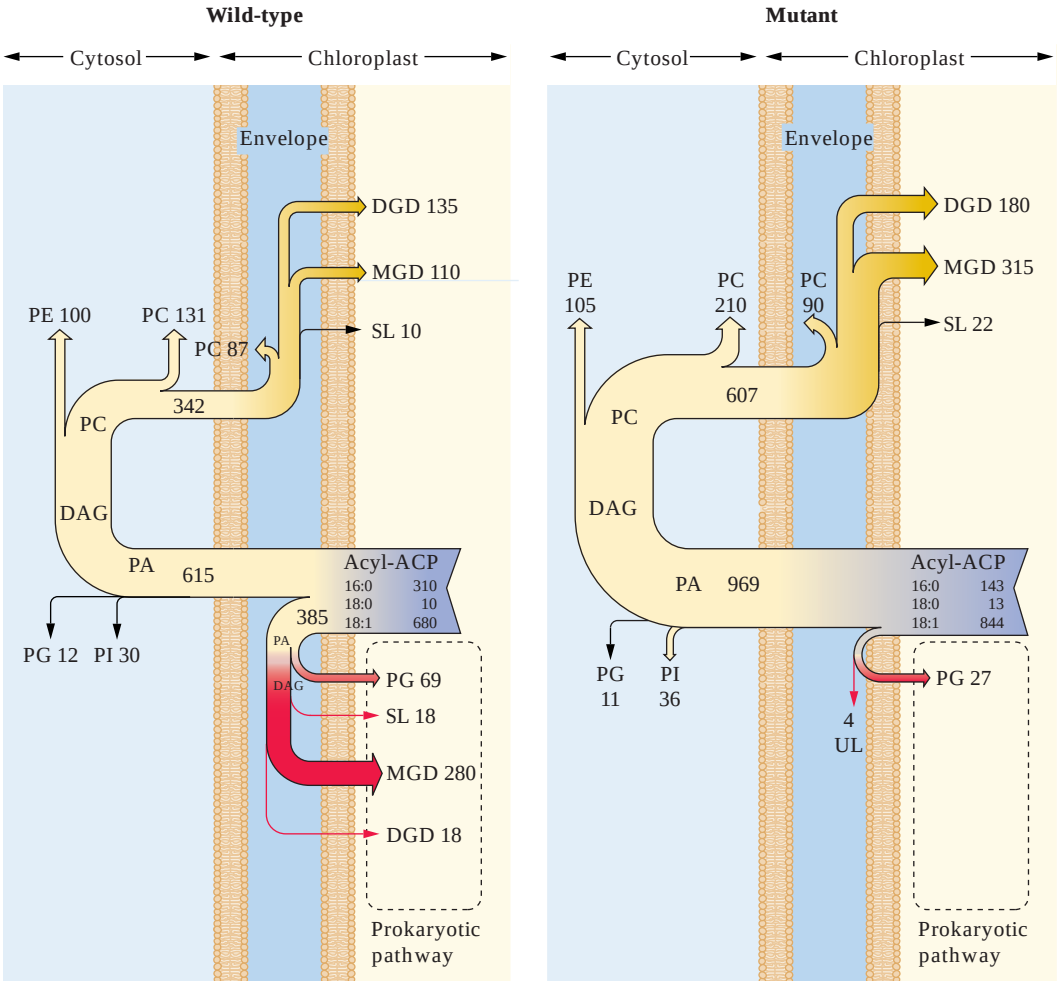


Figure 10.33
Flux diagrams for wild-type *Arabidopsis* and *act1* mutant, showing how fatty acids synthesized in chloroplasts are directed to different lipids in the chloroplasts and extraplastidic membranes. The diagram shows the average fate of 1000 molecules of fatty acid. In wild-type *Arabidopsis* approximately 39% of the fatty acids are used for lipid synthesis within the chloroplast. Of the remaining fatty acids, about 27% are used for synthesis of lipids for extrachloroplast membranes such as the ER, Golgi, and plasma membrane, and roughly 34% are transferred to the chloroplast for lipid synthesis. In a mutant of *Arabidopsis* deficient in plastid glycerol-3-phosphate acyltransferase activity, most of the fatty acids are exported to the cytoplasm and then approximately 61% are reimported into the chloroplast. This suggests a mechanism that adjusts the flow of lipid between the membranes when necessary. SL, sulfolipid; UL, unknown lipid; other abbreviations as in Figure 10.29.

However, the plant lipid-transfer proteins that were originally identified by in vitro assays apparently are located outside the plasma membrane in the cell wall space of epidermal cells. The function of these proteins is not known but, based on their location, they may play a role in transferring wax and cutin monomers from the plasma membrane to the cell surface (Fig. 10.34).

10.7.5 During de novo glycerolipid synthesis, the change in free energy that drives attachment of the polar head group is provided by nucleotide activation of either diacylglycerol or the head group itself.

A general reaction common to all phospholipid biosynthesis pathways involves the attachment of the phospholipid head group. In one case, a hydroxyl located on the head group carries out a nucleophilic attack on the β-phosphate group of CDP-diacylglycerol. In the other, the *sn*-3 hydroxyl of diacylglycerol attacks the β-phosphate of a CDP-activated

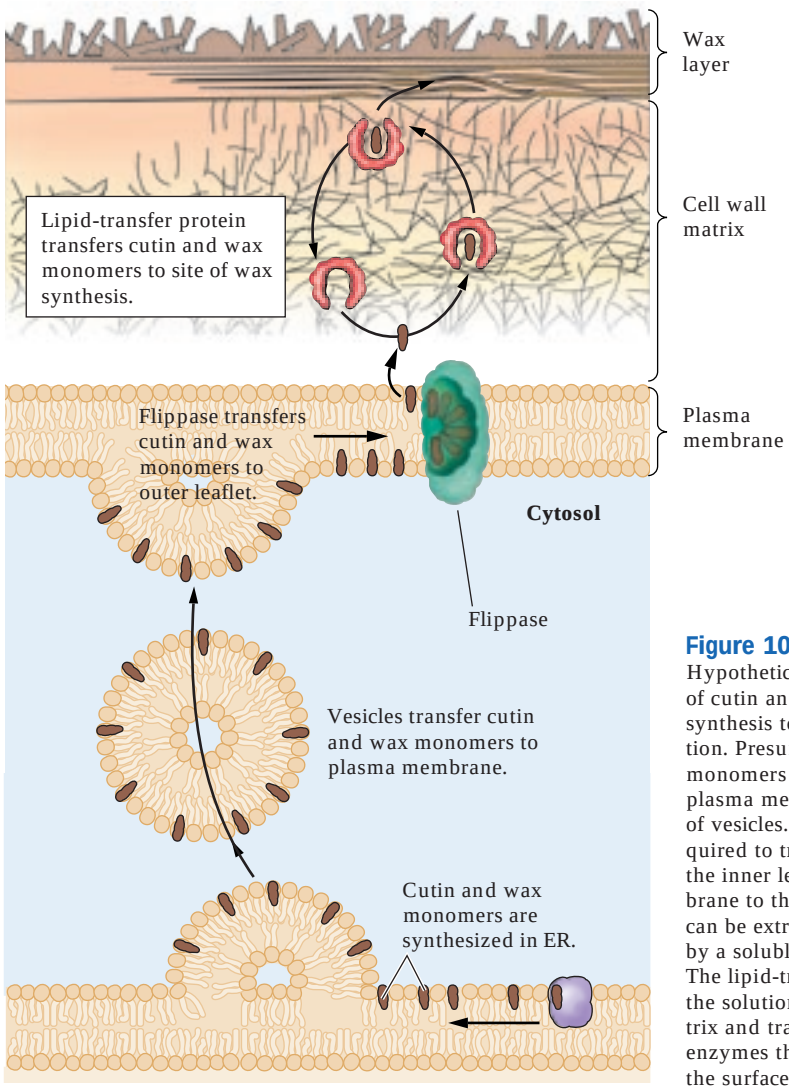


Figure 10.34
Hypothetical scheme for the transport of cutin and wax monomers for cutin synthesis to the site of polymerization. Presumably, cutin and wax monomers move from the ER to the plasma membrane in the membranes of vesicles. A "flippase" may be required to transfer the monomers from the inner leaflet of the plasma membrane to the outer leaflet, where they can be extracted from the membrane by a soluble lipid-transfer protein. The lipid-transfer protein diffuses in the solution phase of the cell wall matrix and transfers the monomers to enzymes that polymerize them near the surface of epidermal cells.

head group (Fig. 10.35). Thus, phospholipid biosynthesis can be divided into two general types of pathways, referred to as the CDP-diacylglycerol and the diacylglycerol pathways, distinguished by whether the activated substrate is CDP-diacylglycerol or a CDP-head group.

Unlike prokaryotes, in which only the CDP-diacylglycerol pathway is present, plants, yeast, and animals synthesize phospholipids by using both the CDP-diacylglycerol and the diacylglycerol pathways, but the contributions of each pathway vary. For example, yeasts can synthesize all their phospholipids by using the CDP-diacylglycerol pathways, but under some conditions, they can repress a CDP-diacylglycerol pathway and use diacylglycerol instead. Animals make their major phospholipids—phosphatidylcholine and phosphatidylethanolamine—by using diacylglycerol pathways. The details of the synthesis of phospholipids in plants are not well characterized, and much of what is known has been learned by analogy to other organisms. Many of the pathways present in animals and yeast, for example, also are present in plants, although the extent to which each pathway contributes to the final phospholipid components of a cell may vary among organisms.

10.7.6 Phosphatidate is a substrate for both the CDP-diacylglycerol and the diacylglycerol pathways.

CDP-diacylglycerol is synthesized from phosphatidate and CTP by CDP:diacylglycerol

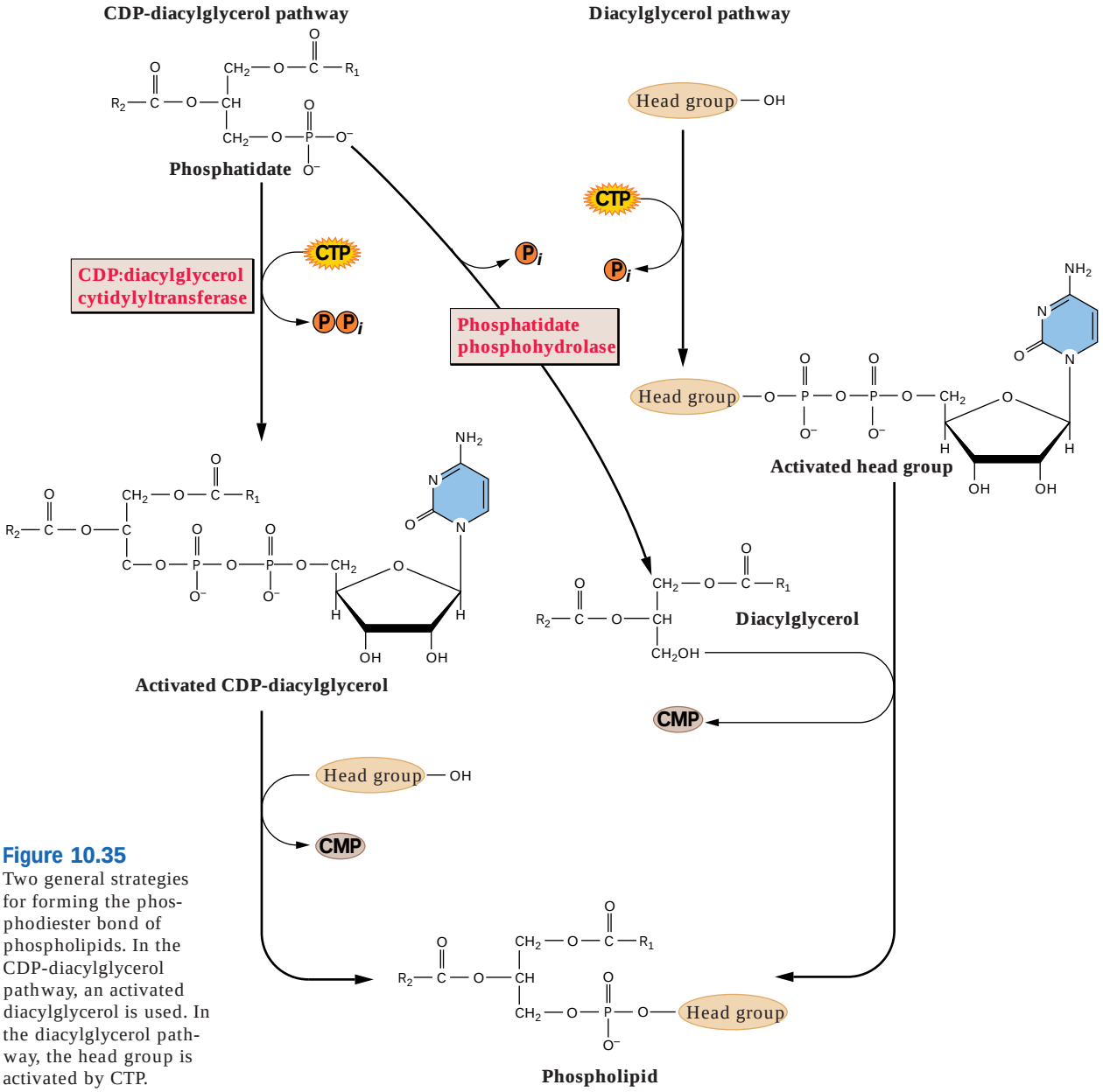


Figure 10.35
Two general strategies for forming the phosphodiester bond of phospholipids. In the CDP-diacylglycerol pathway, an activated diacylglycerol is used. In the diacylglycerol pathway, the head group is activated by CTP.

cytidylyltransferase (Fig. 10.35). In plants, this membrane-bound enzyme is associated with several different organelles, being localized in the inner chloroplast envelope and also found in the inner mitochondrial envelope. Two CDP: diacylglycerol cytidylyltransferases occur in castor bean endosperm: one localized in the mitochondria, the other in a microsomal fraction. Diacylglycerol is produced by the dephosphorylation of phosphatidate by phosphatidate phosphohydrolase, an enzyme in the inner chloroplast envelope membranes, microsomes, and soluble fractions. Analogy to yeast phospholipid synthesis makes it likely that a mitochondrial form of this enzyme also exists, although it has not yet been detected in plants.

10.7.7 CDP-diacylglycerol and diacylglycerol pathways generate distinct types of lipids.

Phospholipids derived from CDP-diacylglycerol include phosphatidylglycerol, the only phospholipid found in chloroplast thylakoids, and diphosphatidylglycerol (cardiolipin), which occurs exclusively in the inner

mitochondrial membrane. As in *E. coli,* phosphatidylglycerol is synthesized in two steps in plants. Reaction of CDP-diacylglycerol with glycerol 3-phosphate produces phosphatidylglycerol phosphate, which is then dephosphorylated to yield phosphatidylglycerol (Fig. 10.36). This reaction also can take place in the ER. In mitochondria, diphosphatidylglycerol is synthesized by the reaction of phosphatidylglycerol with a second molecule of CDP-diacylglycerol. This contrasts with *E. coli,* in which diphosphatidylglycerol is synthesized from two molecules of phosphatidylglycerol.

Other phospholipids synthesized from CDP-diacylglycerol include phosphatidylinositol and phosphatidylserine (Fig. 10.36). Phosphatidylinositol is synthesized from free inositol in a reaction catalyzed by phosphatidylinositol synthase. Phosphatidylserine synthesis in most plant tissues uses serine and is catalyzed by phosphatidylserine synthase in a reaction similar to that found in *E. coli* and yeast. This contrasts with phosphatidylserine synthesis in animals, in which phosphatidylserine is a product of the diacylglycerol pathway, arising from phosphatidylethanolamine by exchanging ethanolamine with a serine (see next section).

The diacylglycerol pathway is used primarily for the synthesis of phosphatidylethanolamine and phosphatidylcholine in both plants and animals. In each case, diacylglycerol displaces a CMP from CDP-ethanolamine or CDP-choline to produce phosphatidylethanolamine or phosphatidylcholine, respectively (Fig. 10.37). The synthesis of the two CDP-alcohols parallels the synthesis of CDP-diacylglycerol. For example, choline is first phosphorylated to form phosphocholine before reacting with CTP to produce CDP-choline.

10.7.8 Pools of CDP-diacylglycerol–derived phospholipids and diacylglycerol-derived phospholipids interact in plants and in animals.

Tracing the origin of specific lipids is often more difficult than the preceding section might suggest. For example, phosphatidylethanolamine, a phosphatidylserine precursor in animals, is a product of the diacylglycerol pathway in both animals and plants. In germinating castor bean endosperm, where one might expect the concentrations of diacylglycerol to be high, phosphatidylserine appears to be made exclusively from diacylglycerol by the phosphatidylethanolamine exchange reaction (Fig. 10.38). Likewise, phosphatidylethanolamine can be generated from phosphatidylserine by decarboxylation. The net effect of this cycle is to produce ethanolamine from serine. Indeed, this may be the primary metabolic source of ethanolamine. Direct conversion of phosphatidylethanolamine to phosphatidylcholine occurs in animals and yeast by the sequential methylation of phosphatidylethanolamine by *S*-adenosylmethionine. In plants, however, this appears to be only a minor pathway, phosphatidylcholine being synthesized almost entirely from CDP-choline and diacylglycerol. A nonlipid product, phosphocholine, is synthesized by the methylation of phosphoethanolamine, with *S*-adenosylmethionine acting as the methyl donor. Thus in plants, choline originates mainly from ethanolamine, but phosphatidylcholine does not typically originate from phosphatidylethanolamine.

10.7.9 Galactolipids and sulfolipids are synthesized from diacylglycerol.

Diacylglycerol produced from phosphatidic acid by a specific phosphatase in the plastid is the substrate for galactolipid and sulfolipid synthesis. Cleavage of uridine diphosphate (UDP) esters provides energy for several of the reactions. UDP-galactose and UDP-sulfoquinovose are substrates for monogalactosyldiacylglycerol and sulfolipid synthesis, respectively. The synthesis of digalactosyldiacylglycerol involves the transfer of the galactose head group from one monogalactosyldiacylglycerol molecule to a second by the action of a monogalactosyldiacylglycerol dismutase or galactolipid galactosyltransferase (Fig. 10.39).

Sulfolipid is of interest because relatively little is known about how the carbon–sulfur bond forms. From in vitro assays and from analyzing mutants of the photosynthetic purple bacterium *Rhodobacter sphaeroides,* the final step in the synthesis

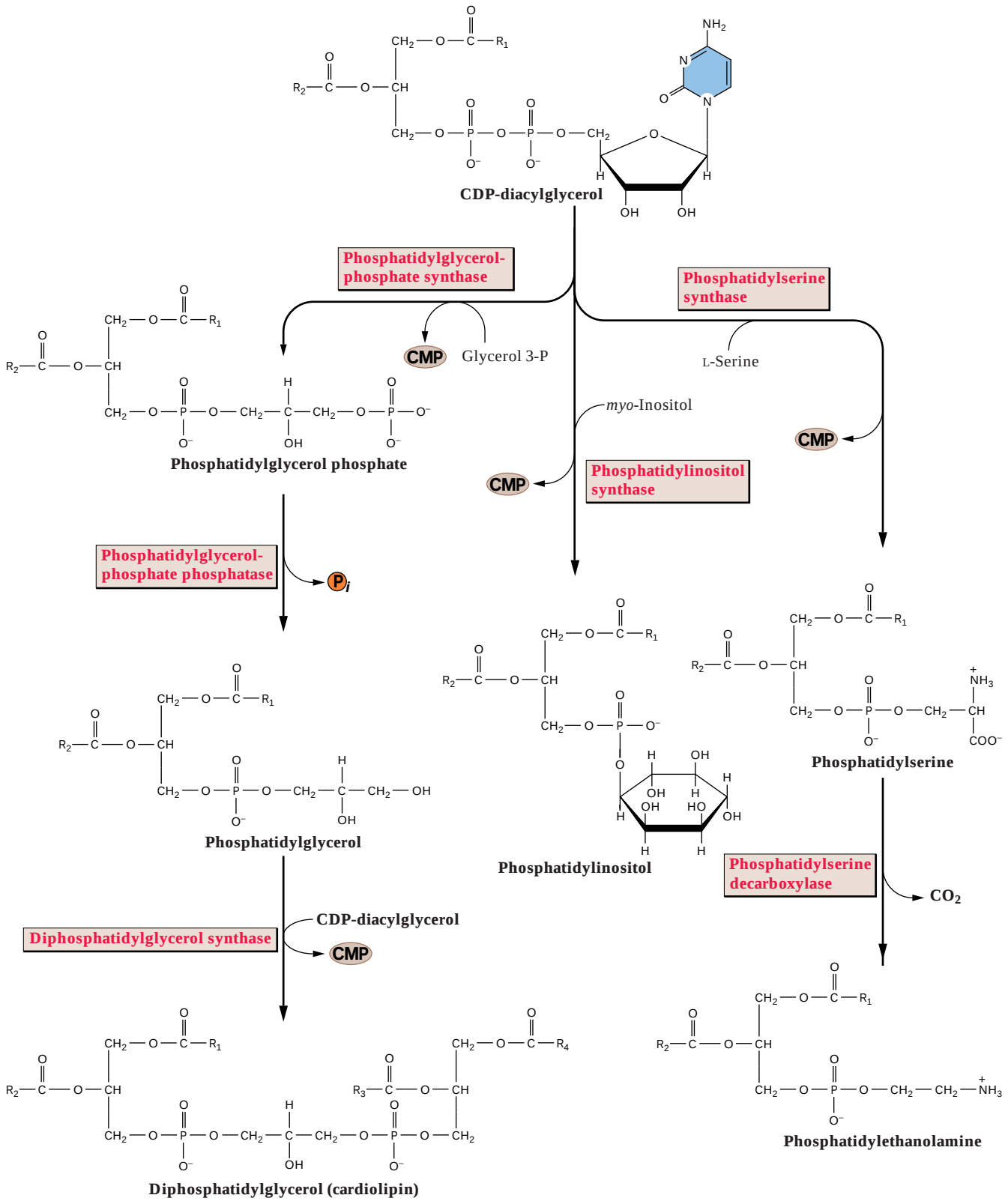


Figure 10.36
Synthesis of phospholipids by the CDP-diacylglycerol pathway. Phosphatidylserine, phosphatidylinositol, and phosphatidylglycerol phosphate are synthesized when the head group displaces CMP from CDP-diacylglycerol. Phosphatidylglycerol phosphate is dephosphorylated to phosphatidylglycerol. Diphosphatidylglycerol (cardiolipin) is formed from two molecules of phosphatidylglycerol.

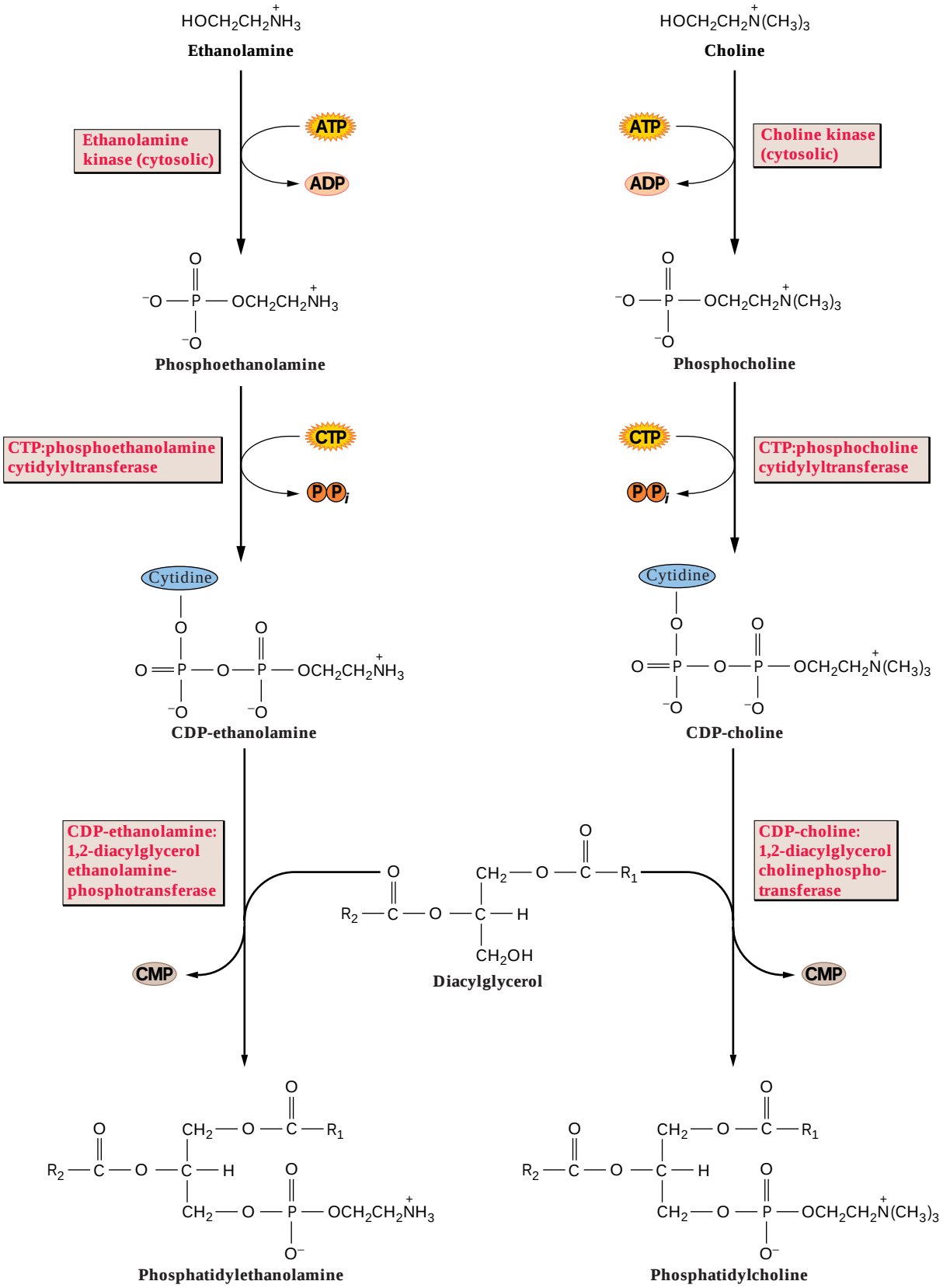


Figure 10.37
The diacylglycerol pathway is the principal route for synthesis of phosphatidylcholine and phosphatidylethanolamine in plants.

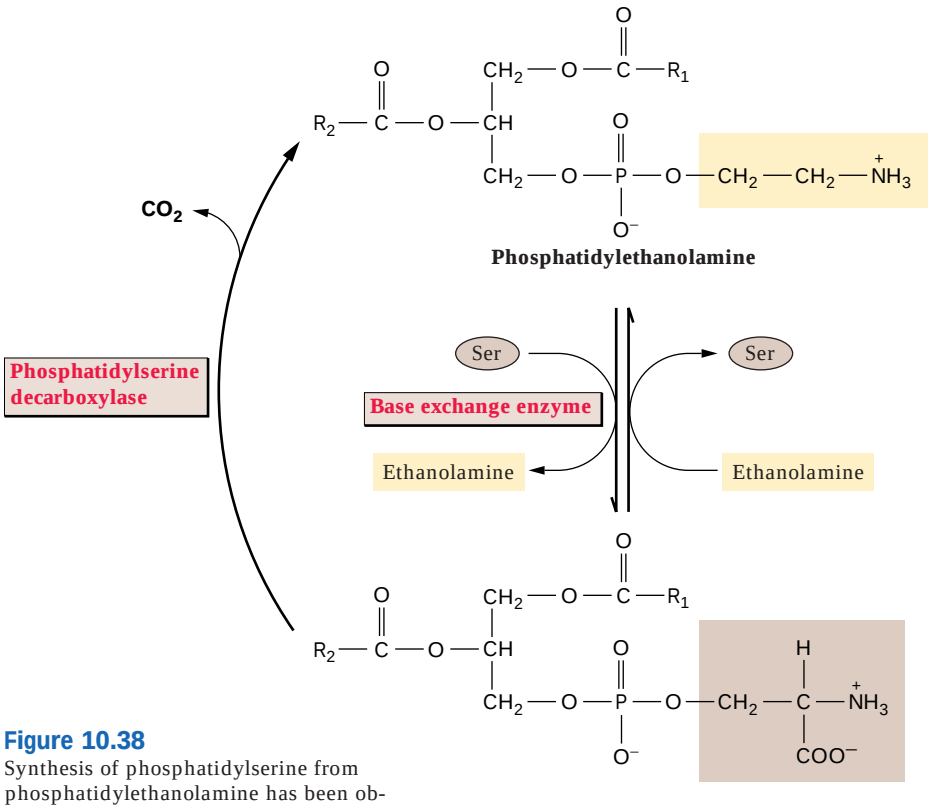


Figure 10.38
Synthesis of phosphatidylserine from phosphatidylethanolamine has been observed in some plant tissues.

of sulfoquinovosyldiacylglycerol, the plant sulfolipid, is the transfer of the sulfoquinovosyl group from UDP-sulfoquinovose to diacylglycerol (Fig. 10.40). UDP-sulfoquinovose could be formed by addition of sulfite to either UDP-4-ketoglucose-5-ene or UDP-glucose-5-ene, followed by reduction. These intermediates can, in principle, be formed by enzymes such as deoxythymidine diphosphate–glucose-4,6-dehydratase. Four genes required for sulfolipid synthesis in *R. sphaeroides* have been cloned and should be useful in testing hypotheses about the early steps of the pathway. Recently, sequence information about the *R. sphaeroides* genes has been used to identify homologs in cyanobacteria and higher plants. Thus, the pathway is probably similar or identical in bacteria and higher plants.

All organisms that carry out oxygenic photosynthesis contain sulfolipid as a major component of their photosynthetic lamellae—a fact stimulating sustained interest in the possibility that this lipid plays some essential role in supporting the light reactions of photosynthesis. However, mutants of *R. sphaeroides* or cyanobacteria that are unable to synthesize sulfolipid have normal rates of photosynthesis. Apparently, the main reason photosynthetic organisms have sulfolipid is to minimize the phosphate required for synthesizing the large amounts of membrane required to support high rates of light capture (Box 10.4).

10.7.10 The limited information on sphingolipid biosynthesis in plants is derived mostly by analogy to animal systems.

Just as diacylglycerol is the basic component of all glycerolipids, ceramide is the basic component of all sphingolipids. Complex sphingolipids, such as the glucosylsphingolipids that predominate in plants, are formed by modification of ceramide.

The initial phase of ceramide synthesis involves first condensation of a palmitoyl-CoA with serine, resulting in a 3-ketosphinganine (Fig. 10.41). This reaction is analogous to the condensation reactions catalyzed by FASs and

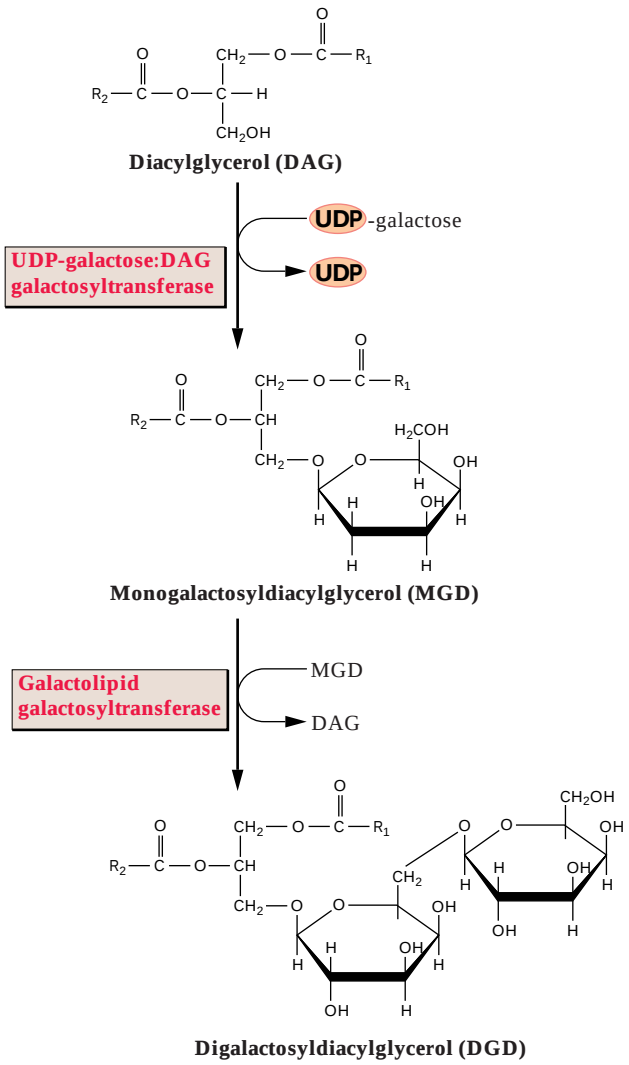


Figure 10.39
Pathway of synthesis of galactolipids. The synthesis of monogalactosyldiacylglycerol is catalyzed by what appears to be a typical glycosyltransferase that utilized a nucleotide sugar-activated substrate. In contrast, the synthesis of digalactosyldiacylglycerol is thought to utilize a dismutation reaction in which the galactosyl moiety of one monogalactosyldiacylglycerol is transferred to another.

elongases. Subsequently, the 3-keto group is reduced to the alcohol to produce sphinganine. In the second phase, ceramide is synthesized when either a long-chain acyl-CoA or a fatty acid reacts to form an amide linkage with the sphinganine. Both acyl-CoA–dependent and acyl-CoA–independent pathways for ceramide synthesis occur in plants. The fatty acids attached to the sphinganine can range from C_{16} to C_{26} in different species. Often, the fatty acid is hydroxylated at C-2 subsequent to formation of the ceramide.

The structures of the most commonly occurring long-chain bases—ceramide and glucosylceramide—are shown in Figure 10.7. Although glucosylceramide is a major complex sphingolipid in plants, the details of its synthesis have not been elucidated. In animals, glucosylceramide is synthesized from the reaction of ceramide with UDP-glucose. Attempts to demonstrate a similar reaction in plants have not been successful, possibly indicating that a glucose donor other than UDP-glucose is used.

10.8 Function of membrane lipids

10.8.1 Membrane lipid composition affects plant form and function.

Each membrane in a plant cell has a characteristic and distinct complement of lipid types (Fig. 10.42), and within a single membrane each class of lipids has a distinct fatty

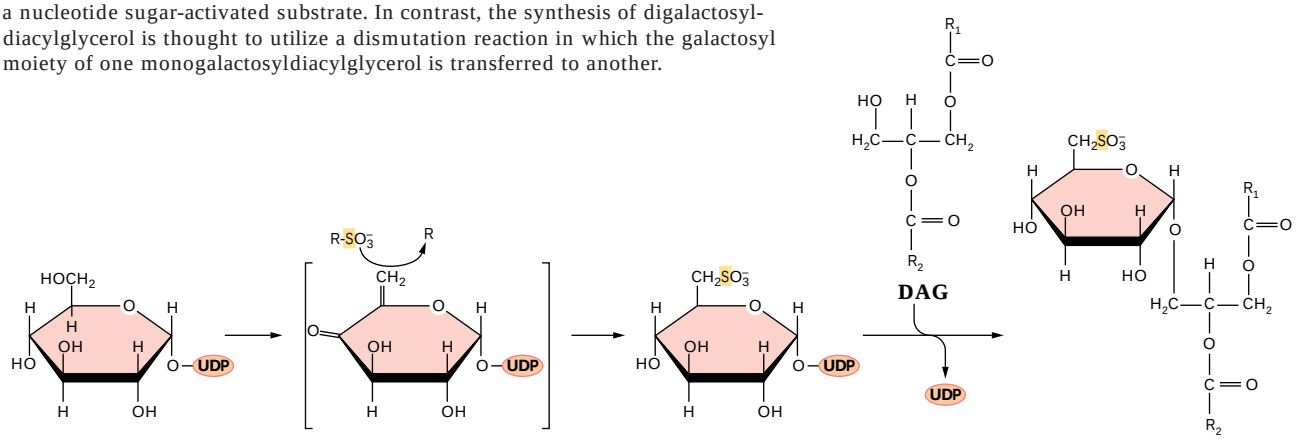
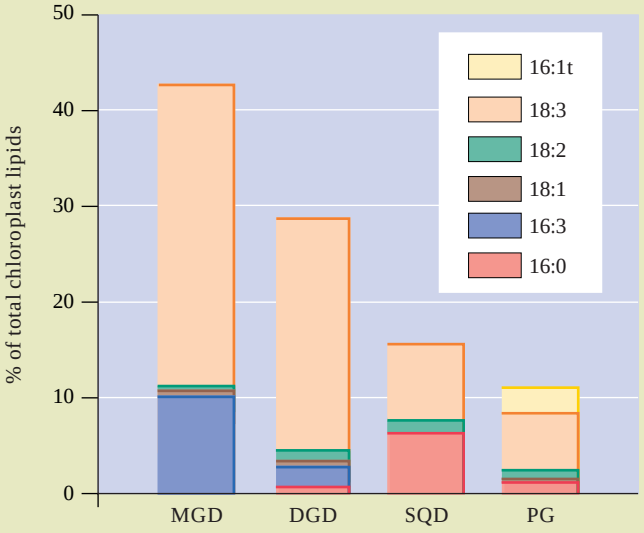


Figure 10.40
Hypothetical scheme for the synthesis of sulfoquinovosyldiacylglycerol (SQD). The identity of the sulfur donor is not known. Only the final step of the pathway, the condensation of UDP-sulfoquinovose (UDP-SQ) with DAG, is known with certainty.

Box 10.4 Plants conserve phosphate by using sulfolipids and galactolipids for chloroplast membrane synthesis.

Each gram of leaf material contains approximately 1 m^2 of chloroplast membrane. If this membrane were composed primarily of phospholipids, as the membranes from animals and fungi are, plants would require much more phosphate for growth than they currently do. Given that phosphate is a limiting nutrient in many natural ecosystems, it is advantageous to plants to minimize the need for phosphate in membrane synthesis. In what appears to be an evolutionary adaptation to the problem of phosphate limitation, plants and other photosynthetic organisms use monogalactosyldiacylglycerol (MGD), digalactosyldiacylglycerol (DGD), and sulfoquinovosyldiacylglycerol (SQD, or sulfolipid) as substitutes for phospholipids such as phosphatidylglycerol (PG). For example, in the chloroplast membranes of spinach (a 16:3 plant), less than 15% of the bilayer lipids are phospholipids (see graph).

Evidence in support of this concept was obtained by the isolation of sulfolipid-lacking mutants of the photosynthetic purple bacterium *Rhodobacter sphaeroides* and the cyanobacterium *Synechococcus* PCC7942. When the mutants were grown in the presence of high concentrations of phosphate, their growth rate and photosynthetic characteristics were indistinguishable from those of the wild type. Under conditions of limiting phosphate, however, the growth of the mutants was inhibited. In addition, as the amount of phosphate available for membrane synthesis became limiting, several novel glycolipids accumulated to high concentrations.



acid composition. Until recently, we knew almost nothing about the reasons for this remarkable diversity. Our knowledge remains limited, but the examples discussed in this section demonstrate some of the important developmental processes and responses to environmental factors that are affected by membrane lipid composition.

One of the most visually dramatic phenotypes produced by a change in lipid composition is that of the *fab2* mutant of *Arabidopsis*. The *fab2* plants are miniatures, a result of the accumulation of 18:0 in membrane lipids (Fig. 10.43A). The decrease in leaf size results from decreased size of several specific cell types. The failure of mesophyll and epidermal cells to enlarge produces a dramatic "brick-wall" appearance to the cross-section of a mutant leaf, contrasting with the characteristic less-compact leaf anatomy evident in the wild type (Fig. 10.43B,C). We do not know the mechanism by which increased 18:0 produces the miniature phenotype, but biophysical principles lead to the expectation that increased temperature would ameliorate the effects of increased saturation on the bilayer. Accordingly, the morphology of *fab2* more closely resembles that of the wild type when plants are grown at 35°C (Fig. 10.43D,E). Furthermore, at this temperature, *fab2* leaves develop typical palisade and spongy mesophyll layers.

10.8.2 Photosynthesis is impaired in plants that lack polyunsaturated membrane lipids.

Highly unsaturated $18:3^{\Delta 9,12,15}$ and $16:3^{\Delta 7,10,13}$ fatty acids account for approximately 70% of all the thylakoid membrane fatty acids in plants and more than 90% of the fatty acids in monogalactosyldiacylglycerol, the most abundant chloroplast lipid (see Fig. 10.42). These very high amounts are noteworthy because free radical byproducts of the photosynthetic light reactions stimulate oxidation

of polyunsaturated fatty acids. If plants maintain high levels of unsaturation in thylakoids despite the risk of oxidation, one might reason that photosynthesis is critically dependent on membrane unsaturation. Surprisingly, an *Arabidopsis* triple mutant *(fad3 fad7 fad8)*, completely lacking 18:3$^{\Delta 9,12,15}$ and 16:3$^{\Delta 7,10,13}$, exhibits normal rates of vegetative growth and photosynthesis at 22°C (Fig. 10.44). These results clearly demonstrate that 18:3$^{\Delta 9,12,15}$ and 16:3$^{\Delta 7,10,13}$ are not essential for photosynthesis in plants. These two fatty acids are not irrelevant, however: Conservation of thylakoid composition attests to their importance, but their role is more subtle than expected. In fact, the loss of 18:3$^{\Delta 9,12,15}$ and 16:3$^{\Delta 7,10,13}$ has noticeable effects on photosynthesis only at low (below 10°C) and high (above 30°C) temperatures.

Although eliminating triunsaturated 18:3$^{\Delta 9,12,15}$ and 16:3$^{\Delta 7,10,13}$ has only minor consequences for photosynthesis, the process is greatly affected in an *Arabidopsis fad2 fad6* mutant lacking the diunsaturated fatty acids 18:2$^{\Delta 9,12}$ and 16:2$^{\Delta 7,10}$ and their downstream triunsaturated derivatives 18:3$^{\Delta 9,12,15}$ and 16:3$^{\Delta 7,10,13}$. These mutants lose nearly all photosynthetic capacity and cannot grow autotrophically. Nevertheless, *fad2 fad6* plants grow on sucrose media, under which conditions growth and organ development are remarkably normal (Fig. 10.44). These observations indicate that the vast majority of receptor-mediated and transport-related membrane functions required to sustain

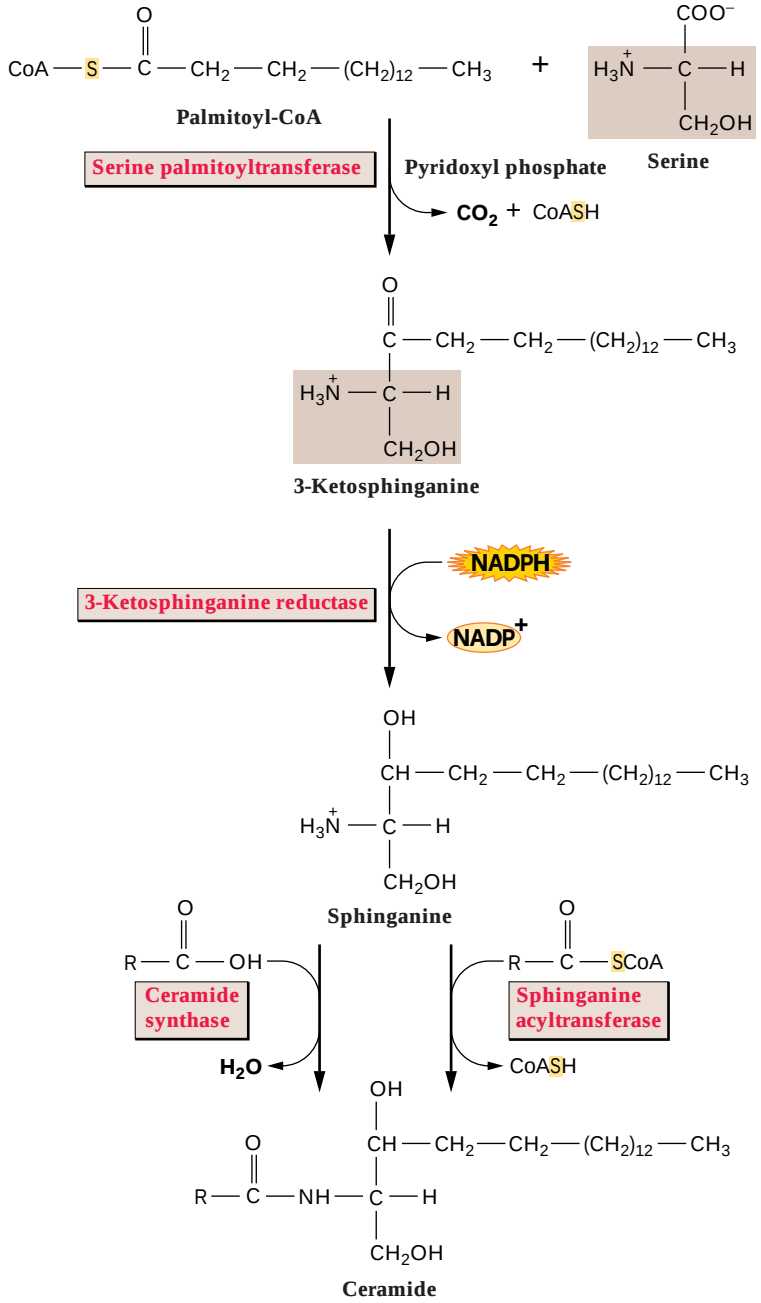


Figure 10.41
Tentative pathway for ceramide biosynthesis in plants, including the two mechanisms for amide bond formation found in plant membrane preparations. The acyl chain (designated R in the figure) may contain a hydroxyl group at the C-2 and the sphingosine base may contain hydroxyl groups or double bonds at C-4 and C-8.

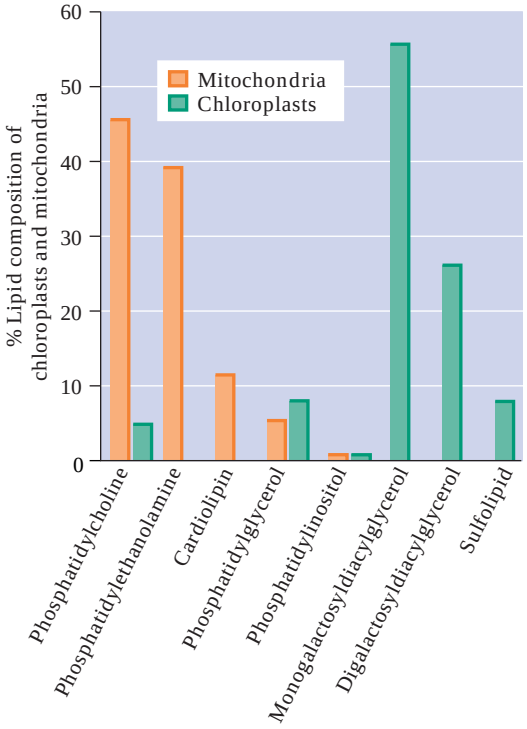


Figure 10.42
Comparison of the lipid compositions of chloroplasts and mitochondria.

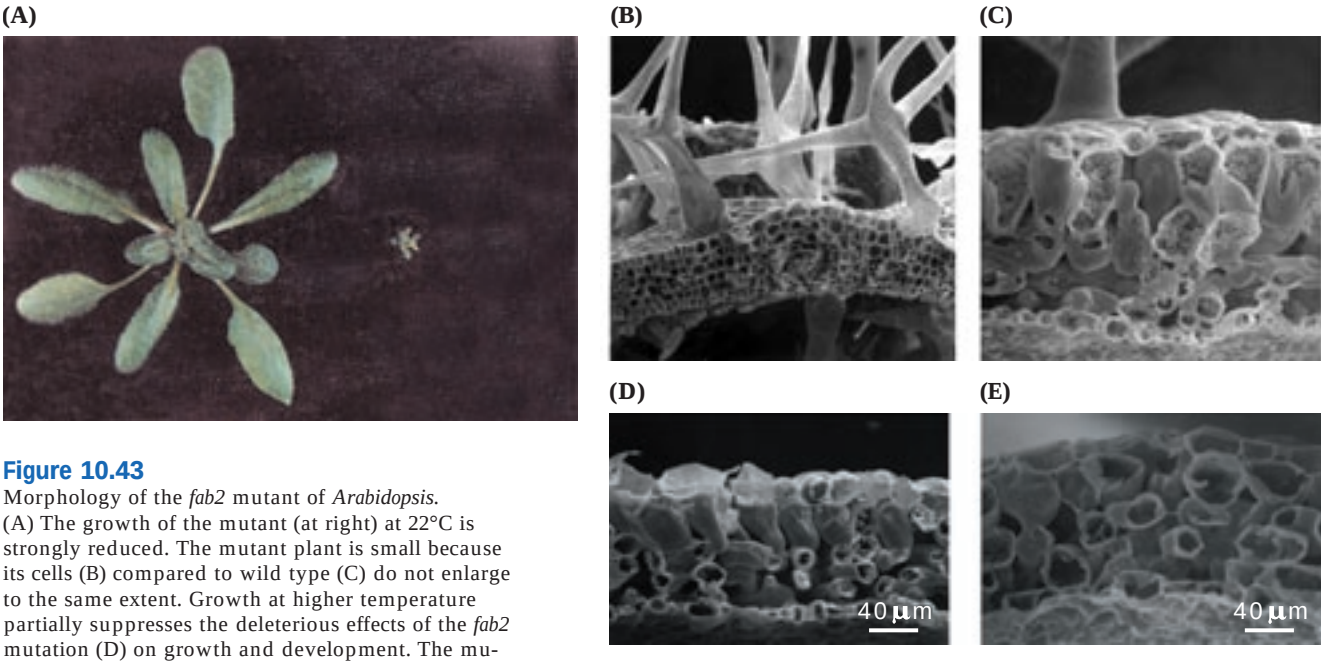


Figure 10.43
Morphology of the *fab2* mutant of *Arabidopsis*. (A) The growth of the mutant (at right) at 22°C is strongly reduced. The mutant plant is small because its cells (B) compared to wild type (C) do not enlarge to the same extent. Growth at higher temperature partially suppresses the deleterious effects of the *fab2* mutation (D) on growth and development. The mutant grown at 36°C more closely resembles the wild-type control (E).

the organism and to induce proper development are well supported in this double mutant, which contains almost no polyunsaturated lipids. Apparently, therefore, photosynthesis is the only vegetative cell function that requires high levels of membrane polyunsaturation.

The work with *Arabidopsis* mutants indicates that plants require polyunsaturated lipids to maintain the photosynthetic machinery, but this is not true of cyanobacteria. Mutants of *Synechocystis* PCC6803 that lack polyunsaturated fatty acids can photosynthesize normally except at low temperature.

10.8.3 Does lipid composition affect chilling sensitivity?

One of the most extensively studied issues in membrane biology is the relationship between lipid composition and the ability of organisms to adjust to temperature changes. Chilling-sensitive plants undergo sharp reductions in growth rate and development at temperatures between 0°C and 12°C (Fig. 10.45). **Chilling injury** includes physical and physiological changes induced by low temperatures as well as subsequent symptoms of stress. Many economically important crops are

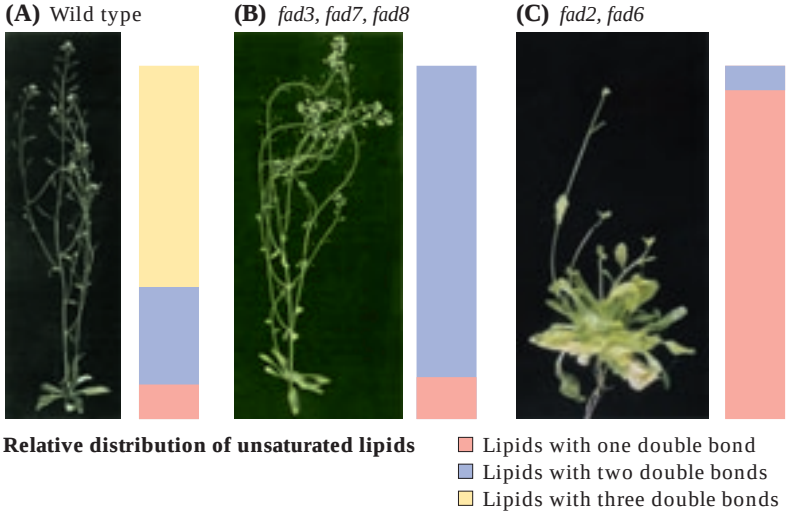


Figure 10.44
Arabidopsis mutants reveal the roles of polyunsaturated fatty acids. Compared with a wild-type plant shown in (A), a mutant lacking $18{:}3^{\Delta 9,12,15}$ and $16{:}3^{\Delta 7,10,13}$ fatty acids (B) grows normally at 22°C. However, this mutant is male-sterile because jasmonate derived from $18{:}3^{\Delta 9,12,15}$ is required for pollen maturation and release. A mutant deficient in all polyunsaturated fatty acids cannot grow autotrophically. When grown in sucrose-enriched medium (C), this mutant is robust, indicating that photosynthesis is the only process that absolutely requires a polyunsaturated membrane.

sensitive to cold, including cotton, soybean, maize, rice, and many tropical and subtropical fruits. In contrast, most plants of temperate origin, which continue to grow and develop at low temperatures, are classified as chilling-resistant plants, including *Arabidopsis.*

In attempts to link the biochemical and physiological changes that characterize chilling injury with a single "trigger" or site of damage, investigators have suggested that the primary event of chilling injury is a phase transition from the liquid crystalline state to the gel state in cellular membranes (Fig. 10.46). According to this proposal, the phase transition from liquid crystalline to gel results in alterations in the metabolism of chilled cells and leads to injury and death of the chilling-sensitive plants. Because desaturation of membrane lipids favors membrane fluidity (see Section 10.5), researchers have sought to define the relationship between membrane composition and chilling sensitivity. A related hypothesis specific to chloroplast membranes has been proposed, in which molecular species of chloroplast phosphatidylglycerol containing a combination of saturated fatty acid (16:0 and 18:0) at the *sn*-1 position of the glycerol backbone and either a saturated fatty acid or $16{:}1^{\Delta 3}$-*trans* fatty acid at the *sn*-2 position are suggested to confer chilling sensitivity on plants. Because the trans-double bond leaves the $16{:}1^{\Delta 3}$-*trans* fatty acid with a structure similar to 16:0 (see illustration in Box 10.1), these molecules are termed disaturated phosphatidylglycerol. The presence of significant quantities of disaturated phosphatidylglycerol in the chloroplast membranes would presumably promote the change from liquid crystalline to gel phase at chilling temperature, and the phase separation within the membranes would cause chilling sensitivity.

As shown in studies with five different *Arabidopsis* mutants, diminished unsaturation resulted in plants that grew well at 22°C but were less robust than wild type when grown at 2°C to 5°C. These results were observed even though the lipid changes in most of the mutants were insufficient to cause a lipid-phase transition. In addition, the low-temperature symptoms that developed in these lines appeared to be quite distinct from classic chilling sensitivity (Fig. 10.47A,B). Increased concentrations of disaturated phosphatidylglycerol (to as much as 60% of total phosphatidylglycerol) were obtained in transgenic *Arabidopsis,* and in these plants the damaging effects of low temperature became evident more quickly.

A complementary series of experiments was carried out in tobacco, a chilling-sensitive plant. Transgenic expression of exogenous genes was used to specifically decrease the concentrations of disaturated phosphatidylglycerol or to bring about a general increase in membrane unsaturation, and the damage caused by chilling was alleviated to some extent. These findings indicate that the extent of membrane unsaturation or the presence of particular lipids such as disaturated phosphatidylglycerol can affect the low-temperature responses of plants.

However, some recent research results indicate that the relationship between membrane unsaturation and plant temperature responses is subtle and complex. In one mutant, *fab1,* disaturated molecular species of phosphatidylglycerol accounted for 43% of the total leaf phosphatidylglycerol—a higher percentage than is found in many chilling-sensitive plants. Nevertheless, the mutant was completely unaffected (when compared with wild-type controls) by a range of low-temperature treatments that quickly led to the death of cucumber and other chilling-sensitive plants. Growth of *fab1* plants slowed, relative to the wild type, only after more than two weeks' exposure to 2°C (Fig. 10.47C,D).

Figure 10.45
Exposure of chilling-sensitive plants such as cucumber *(Cucumis sativus)* to 2°C for one day causes severe injury. The plant on the right was kept at 25°C.

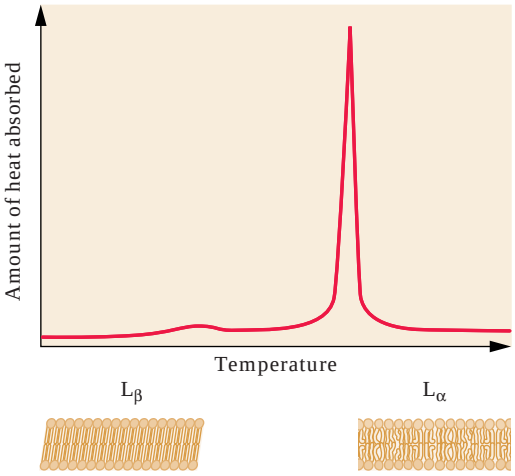


Figure 10.46
Thermal transition from the gel phase (L_β) to liquid crystalline phase (L_α) in a pure phosphatidylcholine bilayer (lower panel). At low temperatures, motion of the fatty acid chains is limited by Van der Waals forces. As the temperature is raised through the phase transition, heat is absorbed (upper panel) and Van der Waals forces are disrupted to form a bilayer with melted fatty acid chains (L_α).

10.8.4 Membrane lipid composition can influence plant cell responses to freezing.

Freezing stress in plants differs from chilling stress, and tolerance of freezing requires a specialized set of biological mechanisms. During initial freezing of plant tissue (down to –5°C), ice forms outside the plasma membrane. Because solutes are excluded from the ice, the solute concentration in the remaining extracellular aqueous phase increases, forcing water out through the plasma membrane by osmosis, and plasmolyzing the cell. When the temperature later increases and the ice melts, cellular damage may result.

If plants are allowed to acclimate to cold by growth at low, but not freezing, temperatures for a few days before exposure to freezing, many can withstand freezing to temperatures that would otherwise cause extensive damage or death (Fig. 10.48). Protoplasts from rye leaves have been used as a model system to investigate the molecular basis for this acclimation response (Fig. 10.49).

When nonacclimated protoplasts are placed in a hyperosmotic medium, the plasma membrane buds off endocytotic vesicles, as shown in Figure 10.49. However, if the protoplasts are first preincubated with monounsaturated or diunsaturated species of phosphatidylcholine, so that the phospholipid is incorporated into the plasma membrane, hyperosomotic treatment results in the formation of exocytotic extrusions. Disaturated species of phosphatidylcholine do not induce this change. These differences in plasma membrane behavior correlate with protoplast survival during freezing: Preincubation of nonacclimated protoplasts with monounsaturated or diunsaturated phosphatidylcholine is as effective as cold acclimation in promoting protoplast survival, whereas preincubation with disaturated phosphatidylcholine has no beneficial effect. These observations suggest that one

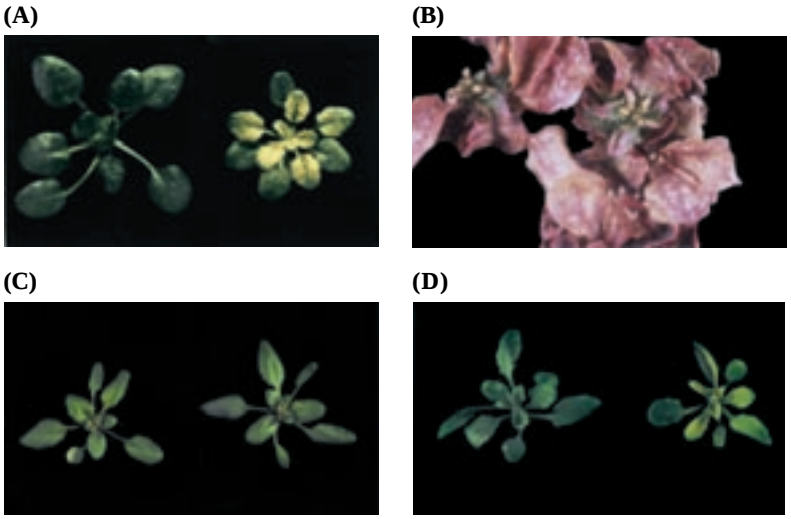


Figure 10.47
Three different chilling responses in lipid mutants of *Arabidopsis*. (A) Compared with wild-type plants (left), the *Arabidopsis fad6* mutant (right) becomes chlorotic after three weeks at 5°C. (B) *fad2 Arabidopsis* plants die after seven weeks at 6°C. (C) Compared with wild-type plants (left), the *fab1* mutant (right) is unaffected by up to one week of exposure to chilling at 2°C. (D) After four weeks at 2°C, however, *fab1* plants (right) show clear symptoms of chlorosis and reduced growth.

aspect of cold acclimation is an increase in unsaturated phospholipids in the plasma membrane. Physicochemical considerations indicate that such changes could mediate the shift from formation of endocytotic vesicles to formation of exocytotic extrusions.

10.8.5 Membrane lipids function in signaling and in defensive processes.

Plants, animals, and microbes all use membrane lipids as precursors for the synthesis of compounds that have intracellular or long-range signaling activities. Phosphatidylinositols, frequently referred to as phosphoinositides, have important functions in signal transduction pathways. Their role in regulation of metabolic pathways has been most extensively studied in animal systems, but recent studies suggest that they may serve a similar function in plant systems (see Chapter 18). In the principal active molecule, the phosphatidylinositol 4,5-bisphosphate (PIP_2), the inositol moiety is phosphorylated at C-4 and C-5. In animals, phosphoinositides serve as second messengers to extracellular signals. The signal activates a phosphatidylinositol-specific phospholipase C, which cleaves PIP_2 to produce inositol 1,4,5-triphosphate (IP_3) and diacylglycerol, each of which acts as a second messenger (Fig. 10.50).

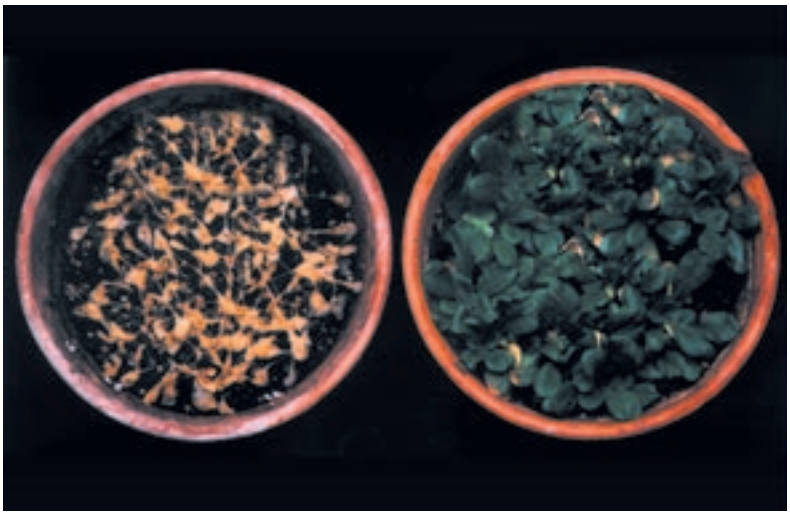


Figure 10.48
Cold acclimation allows plants to survive freezing. The *Arabidopsis* plants on the right were incubated at 4°C for 4 days to acclimate the plants, after which both pots were kept at –5°C for 4 days, and then transferred to growth conditions at 23°C for 10 days.

Jasmonate is another of several lipid-derived plant growth regulators (see Chapter 17). The structure and biosynthesis of jasmonate have intrigued plant biologists because of the parallels to some eicosanoids, which are central to inflammatory responses and other physiological processes in mammals. In plants, jasmonate derives from

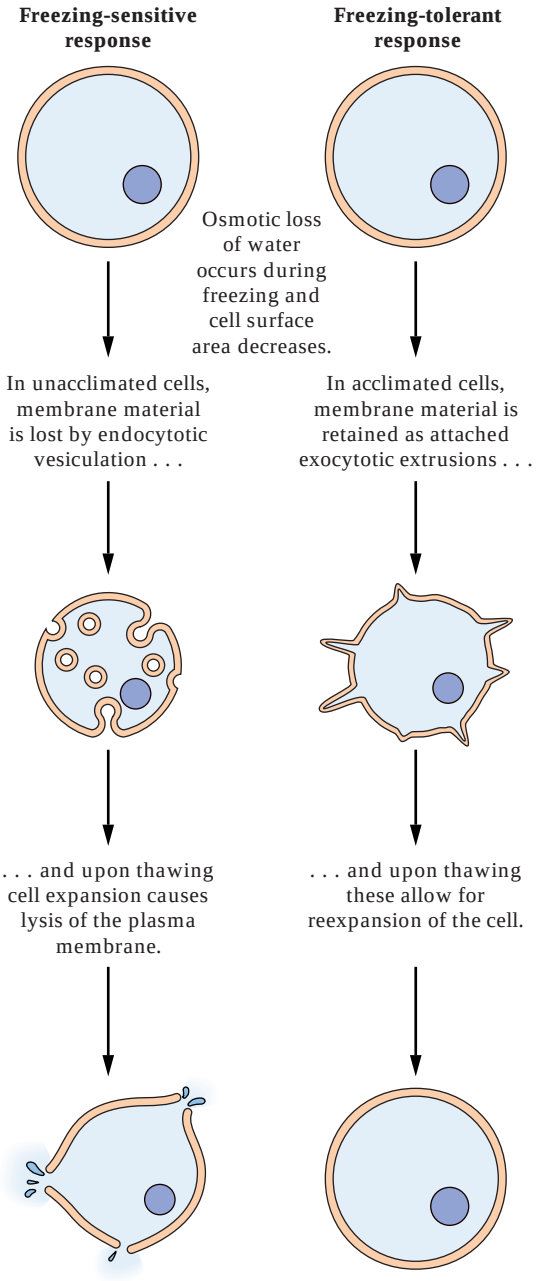


Figure 10.49
During freezing stress, changes in plasma membrane morphology determine death or survival of the cell.

18:3$\Delta^{9,12,15}$, presumably released from membrane lipids by phospholipase A_2. The linolenic acid is oxidized by lipoxygenase, and the resulting product, 9-hydroperoxylinolenic acid or 13-hydroperoxylinolenic acid, may be further metabolized by one of three routes to produce a wide variety of oxylipins, a diverse group of fatty acid derivatives that includes jasmonate (Fig. 10.51).

The pathways by which 13-hydroperoxylinoleic acid may be metabolized are shown in Figure 10.52. The enzyme hydroperoxide lyase catalyzes α-scission of the trans-11,12-double bond to produce a C_6 aldehyde, *cis*-3-hexenal, and a C_{12} compound, 12-oxo-*cis*-9-dodecenoic acid. The acid is subsequently metabolized to 12-oxo-*trans*-10-dodecenoic acid, also known as the wound hormone traumatin. The enzyme hydroperoxide dehydratase (allene-oxide synthase) catalyzes the dehydration of hydroperoxides to unstable allene oxides, which readily decompose to form 9,12-ketols or 12,13-ketols. The allene oxide of 13-hydroperoxylinolenic acid may also be converted by allene-oxide cyclase to 12-oxo-phytodienoic acid, which can be further metabolized to 7-isojasmonic acid. Recently, 16:3$^{\Delta7,10,13}$ also has been found to metabolize, presumably by a similar route, to a biologically active compound called dinoroxo-phytodienoic acid.

The actions of jasmonate are dramatic and wide-ranging. In the last few years, it has become clear that jasmonate is a key component of a wound-signaling pathway that allows plants to protect themselves against insect attack. When experimentally applied to plants at low concentrations, jasmonate induces expression of genes that lead to the production of proteinase inhibitors and other defense compounds. Furthermore, mutants of tomato and *Arabidopsis* that are deficient in jasmonate synthesis are much more susceptible to insect damage (Fig. 10.53). More recently, a very different role for jasmonate was revealed by the *fad3 fad7 fad8* triple mutant of *Arabidopsis*, which cannot synthesize jasmonate because it lacks the precursor 18:3$^{\Delta9,12,15}$. The plants are male-sterile because pollen does not mature properly and is not released from the anthers. Application of jasmonate or linolenic acid to the anthers restores fertility, demonstrating that jasmonate is a key signal in pollen development. The same mutant has

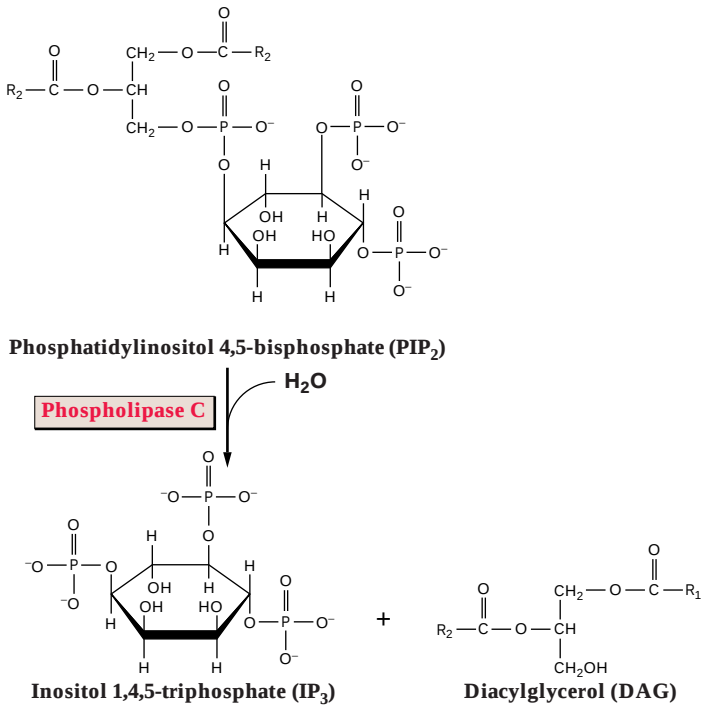


Figure 10.50
Phospholipase C hydrolyzes phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-triphosphate and diacylglycerol.

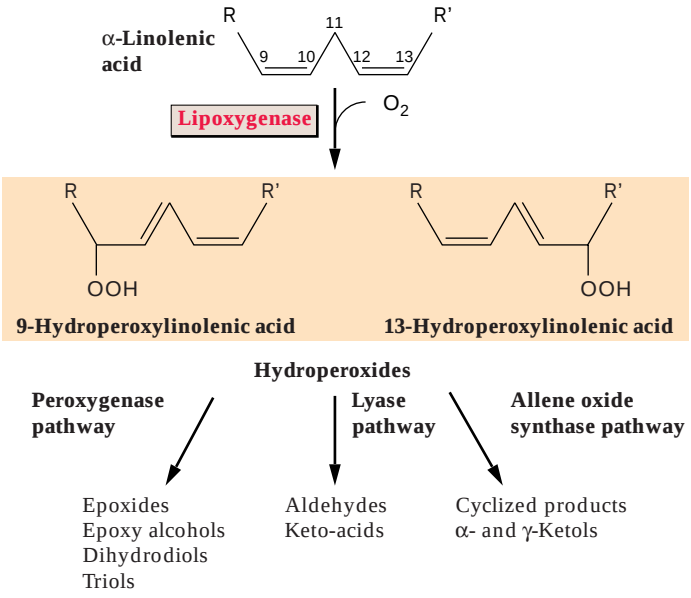


Figure 10.51
The lipoxygenase pathway. In the dioxygenase reaction catalyzed by lipoxygenase, there is no net oxidation or reduction. A cis–trans conjugated diene, 13-hydroperoxylinolenic acid, forms in the reaction. The hydroperoxy acid can be metabolized by three separate pathways to a wide variety of products, including jasmonate. The pathway starts with the action of lipoxygenase on 18:3$^{\Delta9,12,15}$ to produce 9-hydroperoxylinolenic acid or 13-hydroperoxylinolenic acid, depending on the source of the enzyme.

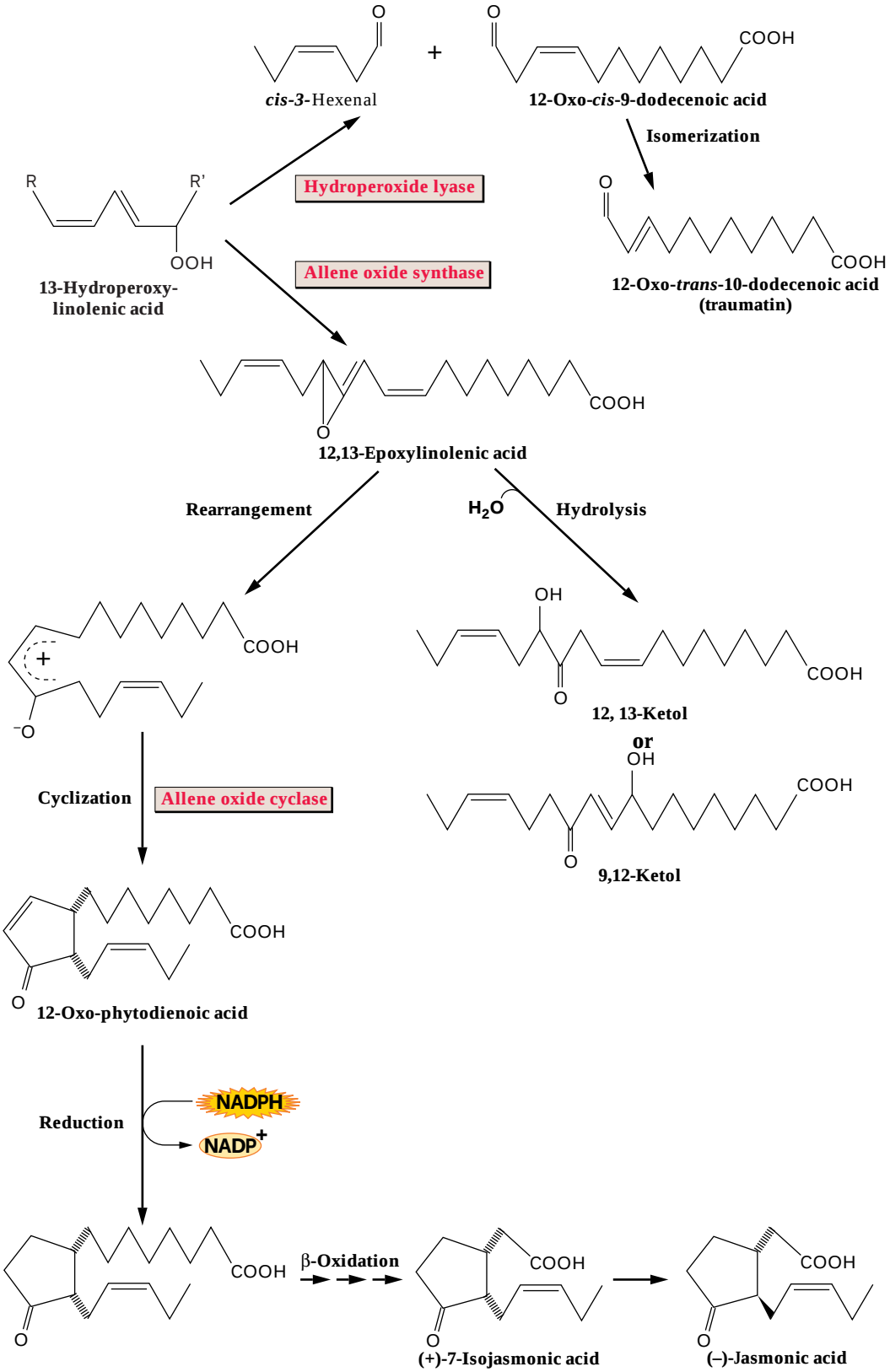


Figure 10.52
Metabolism of 13-hydroperoxylinolenic acid. Additional reactions not shown include conversion of 11,12-epoxylinolenic acid to 13-hydroxylinolenic acid by peroxygenase and rearrangement of the 13-hydroxylinolenic acid to form 15,16-epoxy-13-hydroxyoctadecenoic acid. Also *cis*-3-hexenal and its corresponding alcohol are thought to give rise to several isomers.

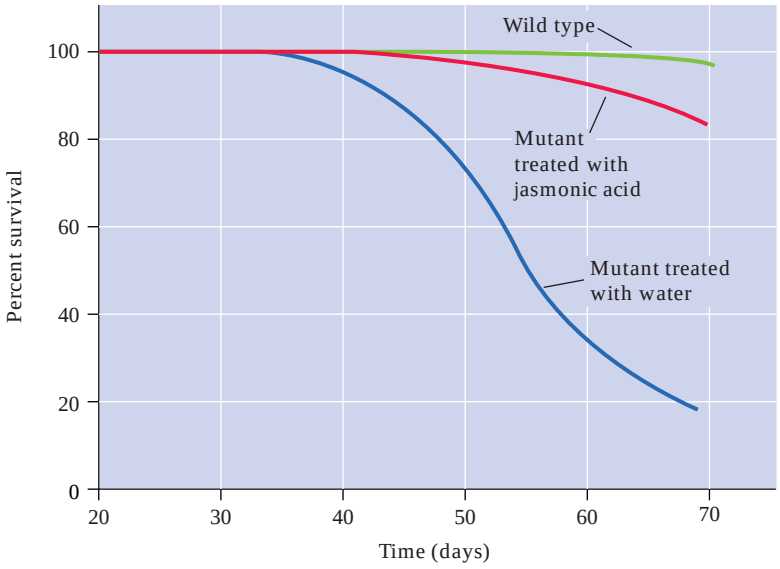


Figure 10.53
Jasmonic acid signaling protects plants from insect predation. Wild-type *Arabidopsis* and a *fad3 fad7 fad8* mutant were enclosed with adult flies of the fungal gnat *Bradysia impatiens*. The mutant plants, which were unable to synthesize jasmonic acid because of the *fad* mutations, were sprayed with either water or low concentrations of jasmonic acid.

been used to demonstrate that jasmonate (along with ethylene) is an important chemical signal in nonhost resistance against fungal pathogens.

In addition to jasmonate, several of the other oxylipins also have been reported to function as signal molecules. In particular, the oxylipin traumatin has been suggested to trigger cell division at the site of wounds, leading to the development of a protective callus. The lipoxygenase product 13-hydroxylinolenic acid triggers phytoalexin production. Similarly, C_6–C_{10} alkenals act as volatile elicitors of a defense response in cotton.

10.9 Synthesis and function of structural lipids

10.9.1 Cutin and suberin provide an epidermal barrier to water loss and pathogen infection.

Most epidermal cells of the aerial parts of vascular plants and some bryophytes are covered by a film of soluble and polymerized lipids, collectively called the cuticle (Fig. 10.54). The primary role of this layer is to provide a permeability barrier against water loss; it also provides some degree of resistance to pathogens and insects. Plant roots contain a group of cells called the endodermis to control the entry of materials and organisms from the soil. The walls of these cells contain a highly polymerized lipophilic material called suberin.

The fine structure of the cuticle is not completely understood. In part, this is because the microscopic appearance and chemical composition may vary among different species, or within one species at different stages of development (Fig. 10.55). In spite of the apparent differences, however, the underlying processes that give rise to the cuticle and the general elements of its overall structure are thought to be similar among higher plants.

A major component of the cuticle is a lipid polyester called cutin, which in turn is covered by surface waxes (Fig. 10.55). Cutin is a polymeric network of oxygenated C_{16} and C_{18} fatty acids cross-linked by ester bonds, such that the carboxyl group of one fatty acid is linked to a primary or secondary hydroxyl group of another (Fig. 10.56). Thus, the interesterified acyl chains form a highly cross-linked and relatively inelastic meshwork with a strongly hydrophobic character. However, because of the relatively large "pore size" of the cutin network, it is unlikely that cutin provides a significant barrier to water loss. Rather, it may act as a relatively inelastic outer skin that assists in providing rigidity to turgid plant tissues. Because of its physical strength, cutin may provide some defense against penetration of pathogens. To penetrate the cutin layer, pathogens probably need to secrete cutinases, enzymes that hydrolyze

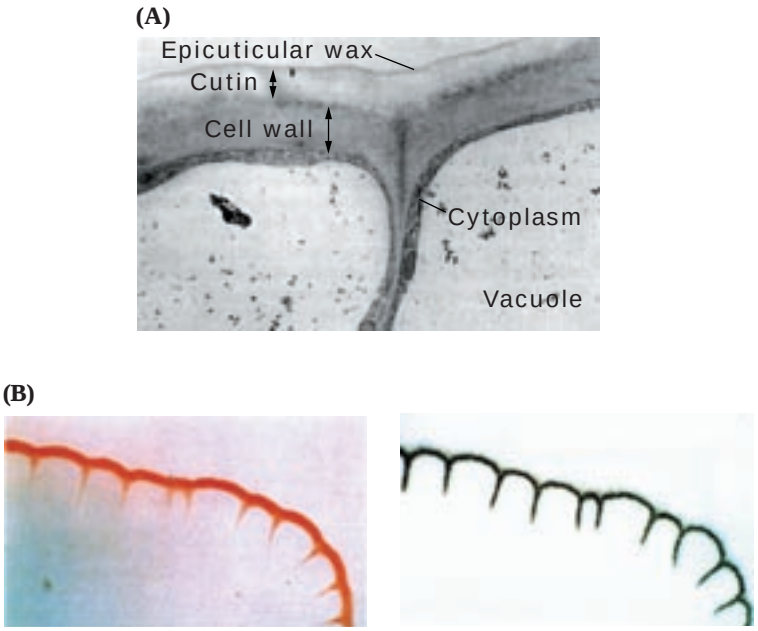


Figure 10.54
(A) Transmission electron micrograph of a transverse section through the cuticle of a leaf of *Clivia miniata.* (B) Cutin is deposited on the outer surface of epidermal cells. In the upper panel, a section of a leaf has been stained with Sudan III, which stains lipophilic materials. The lower panel is an autoradiograph of a leaf after incubation with radioactive fatty acids for 24 h. Subsequent extraction with methanol and methanol:chloroform (1:1 by vol.) removed the soluble fatty acids, leaving only cutin in place.

the ester linkages. No mutants deficient in cutin have been identified in any plant, and no genes involved in cutin biosynthesis are known.

The monomers that give rise to cutin have largely been deduced by analyzing the composition of chemically depolymerized cutin. The principal constituents are monohydroxylated, polyhydroxylated, and epoxidated fatty acids (Fig. 10.57). Cutin monomers are synthesized from fatty acyl-CoAs by oxidases in the ER. Apparently, the monomers are transported to the plasma membrane and secreted into the cell wall space. How the cutin monomers are polymerized or how they are transported to the site of cutin deposition is not well understood. Presumably, acyl-CoA derivatives of the various monomers are secreted into the cell wall space (see Fig. 10.34), where acyltransferases catalyze synthesis of the esters.

Epidermal cells of root tissues do not appear to be covered with cutin to the same extent as the aerial tissues. This may reflect the fact that root epidermal tissues function to take up water and solutes and must therefore maintain hydrophilic contact with soil moisture. However, roots do have an internal hydrophobic layer composed of suberin,

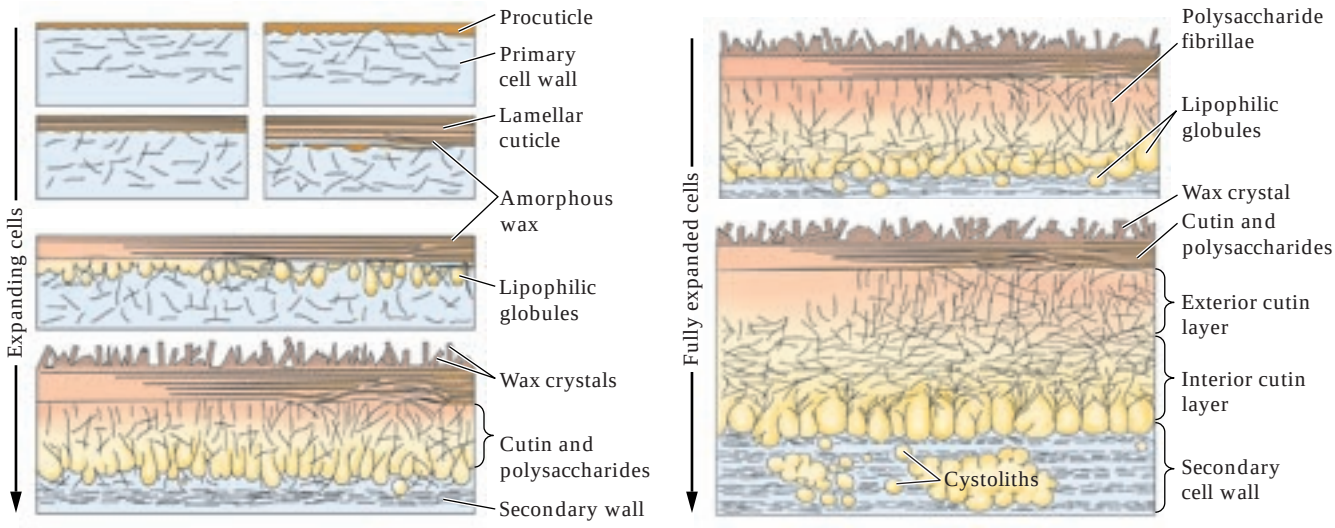


Figure 10.55
Stages in the development of a plant cuticle. At early stages, the primary cell wall is covered by a thin amorphous layer of wax. As the leaf expands, the amount of wax increases by agglomeration of secreted globules. Near the time that leaf expansion stops, wax crystals start to appear on the surface and the deposition of cutin begins. After leaf expansion, cutin deposition increases and secondary cell wall deposition starts. The cutin layer may take on a fibrillar appearance, thought to reflect codeposition of cutin and secondary cell wall materials such as hemicellulose. In the fully expanded mature leaf, distinct zones—the exterior cutin layer and the interior cutin layer—may be visible. Some studies indicate that these layers differ in chemical composition. In some species, lipophilic globules called cystoliths are observed at late stages of cuticle development and are thought to contain cutin precursors that have been secreted from the epidermal cell.

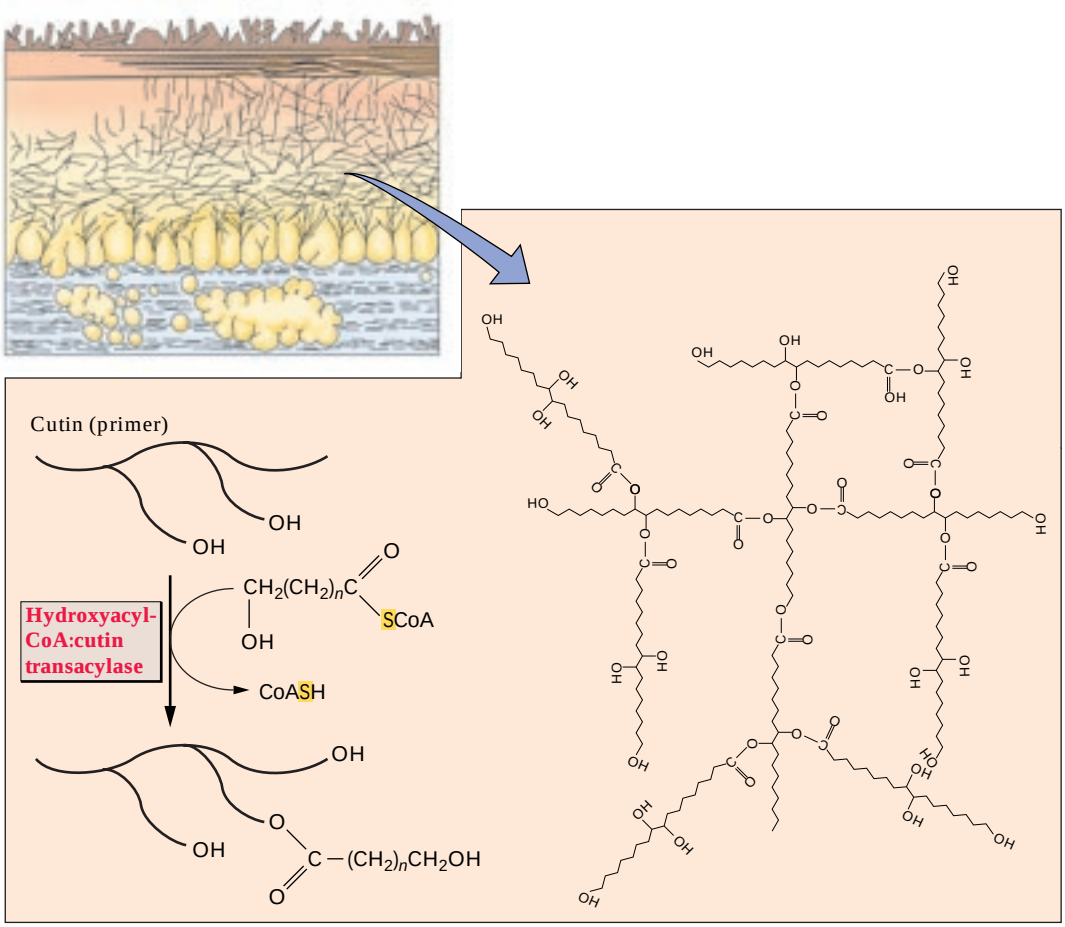


Figure 10.56
Hypothetical structure of a region of a cutin network. For the sake of clarity, this example was constructed by randomly linking carboxyl groups of each of 11 identical cutin monomers (9,10,18-trihydroxyoctadecanoic acid) to the hydroxyl group of the other 10 fatty acids. In a leaf, the structure would reflect the availability of fatty acids produced by the plant. For instance, in leaves of *Clivia*, the substrates are approximately 23% 9,16- or 10,16-dihydroxyhexanoic acid, 47% 9,10-epoxy-18-hydroxyoctadecanoic acid, 18% 18-hydroxy-9-octadecenoic acid, and 7% 9,10,18-trihydroxyoctadecanoic acid, the remainder being composed of roughly equal amounts of 23 other fatty acids. The esters are thought to be synthesized by the condensation of a fatty acyl-CoA and an alcohol group of another cutin precursor or of partially esterified cutin by the enzyme hydroxyacyl-CoA:cutin transacylase.

the components of which are similar to those of cutin. The principal constituents are very-long-chain fatty acids, fatty alcohols, diacids, and phenolics such as *p*-coumaric acid (Fig. 10.58). The main differences between cutin and suberin are thought to be that the fatty acids in suberin do not have secondary alcohols or epoxy groups and are usually longer than 18 carbons. In addition, suberin has a relatively high phenolic content (Chapter 24). These components are thought to be interesterified in much the same way that cutin is. However, because of the relatively low content of hydrophilic groups, suberin is more hydrophobic than cutin; as a result, the endodermis appears highly impermeable to aqueous solutions. Thus, materials must move across the endodermis and into the vascular tissues by symplasmic transport. Suberin is also found surrounding bundle sheath cells in C_4 species. The function in this case may be to prevent CO_2 produced by decarboxylation reactions from diffusing out of the bundle sheath cells.

10.9.2 Epicuticular wax reduces water loss.

The aerial surfaces of plants are covered with a layer of chloroform-soluble nonvolatile

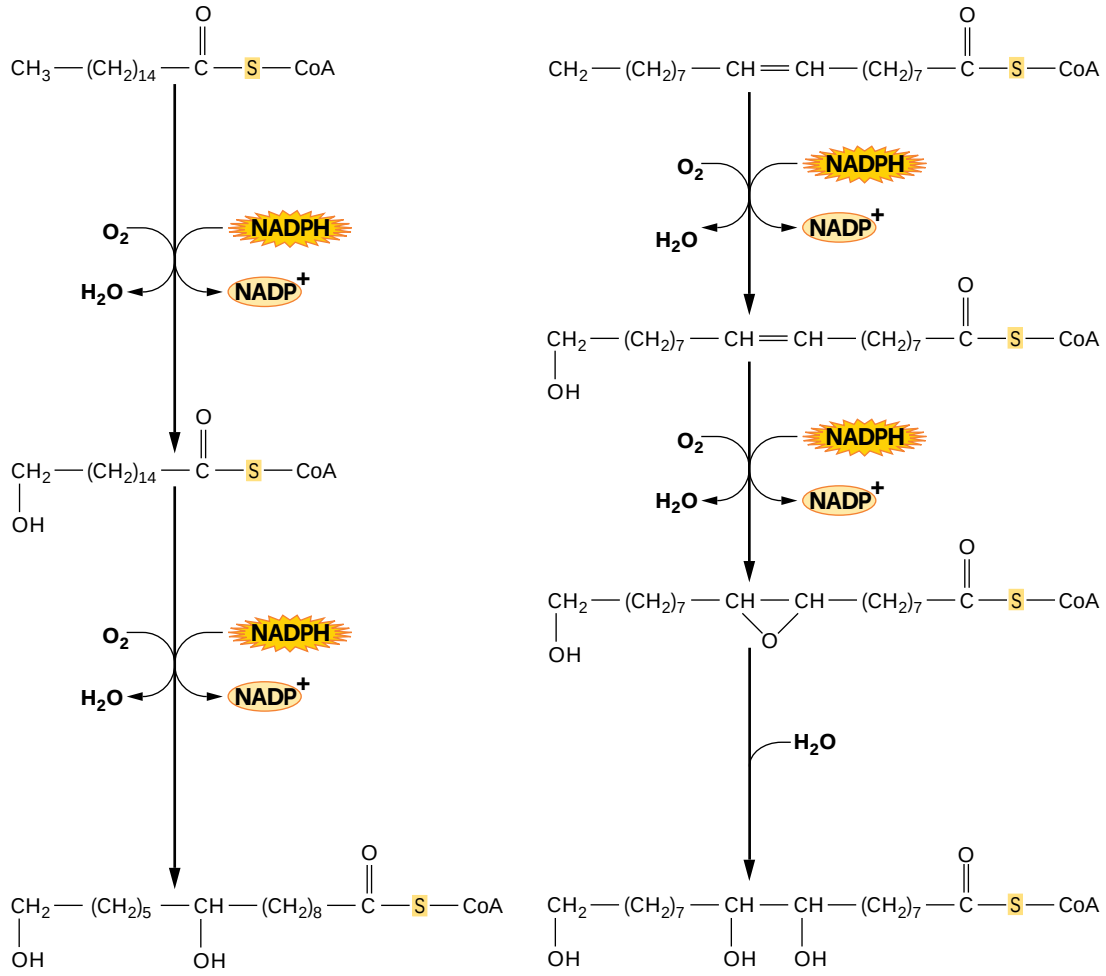


Figure 10.57
The principal components of cutin are synthesized from 16:0 and 18:1 fatty acyl-CoAs by oxidations thought to be catalyzed by cytochrome P450 enzymes located in the endoplasmic reticulum. In most cases the enzymes have not been purified or characterized in detail and the reactions shown here are inferred from assays using crude extracts. All of the fatty acid moieties of the acyl-CoAs shown here are found as constituents of cutin but the relative amounts of each may vary from one plant species to another. It is not known if the order in which the hydroxylations take place only occurs as shown or whether the sequence of reactions can vary.

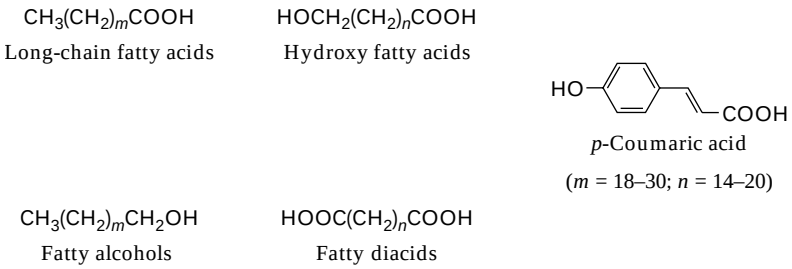


Figure 10.58
Principal components of suberin. The first four compounds are derived from fatty acid metabolism.

lipids, collectively called wax (see Fig. 10.54). The wax layer reduces water loss by orders of magnitude, thereby making terrestrial plant life possible. The amounts and composition of wax deposited are controlled by the plant in response to environmental factors such as relative humidity, soil moisture, and light intensity (Fig. 10.59). Wax composition varies from one plant species to another, but wax generally contains a mixture of long-chain hydrocarbons, acids, alcohols, ketones, aldehydes, and esters. The functional significance of interspecies differences in wax composition is not known. However, from the study of mutants with altered wax

composition, the wax composition appears to affect the structure of the wax crystals. Some plants produce filaments, others produce plates or tubes or spiral-shaped forms (Fig. 10.60). The mechanisms responsible for the shape of the crystals are not known. Different crystal structures may vary in their ability to reflect light—a property that may be useful in adaptation to growth in different light intensities. More important, perhaps, some pathogens and herbivorous insects are attracted to or repelled by specific wax compositions. Thus, the wax composition of particular plant species may reflect the balance of selective pressures exerted on a particular plant species.

Wax monomers are thought to be synthesized from C_{16} and C_{18} fatty acids by an elongase complex located in the ER (Fig. 10.61). Although the reactions involved in elongation, reduction, decarbonylation, and oxidation have been demonstrated in cell extracts, relatively little is known about the specific enzymes involved. As with cutin biosynthesis, it is not well understood how the wax monomers move from the site of synthesis to the surface of epidermal cells. Because the principal components are completely insoluble in aqueous solutions, the monomers are likely transported by lipid-transfer proteins (see Fig. 10.34).

10.9.3 Wax is required for productive pollen–pistil interactions.

Pollen grains are covered with a waxy layer called the exine, which is composed of a complex polymer called sporopollenin. Embedded in the exine is a layer of lipophilic material called tryphine or pollenkitt (Fig. 10.62; see also Chapter 19). In *Arabidopsis*, the tryphine layer includes small lipid bodies that appear to contain primarily C_{28} and C_{30} wax monomers. Probably an important function of the tryphine layer is to reduce water loss from the pollen grain. However, experiments using *Arabidopsis cer* mutants with altered wax composition hint at a more complex role for wax. Some *cer* mutants are male-sterile. However, the fertility of *cer* pollen can be rescued by mixing irradiation-killed wild-type pollen with *cer* pollen; accordingly, some factor in the wild-type pollen appears to complement the defect in the *cer* pollen. These experiments suggest that the normal wax coat may play a signaling role in pollen–pistil interactions (see Chapter 19).

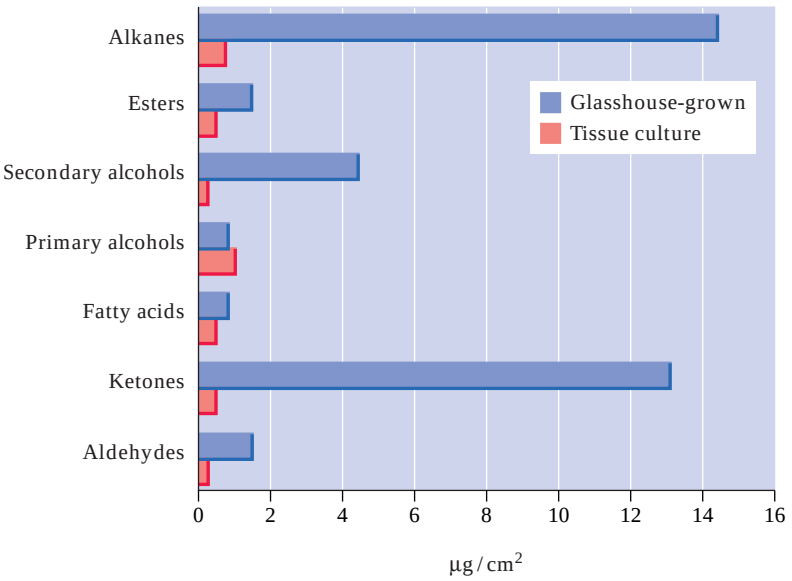


Figure 10.59
Wax composition of *Brassica oleracea* plant grown in high-humidity conditions in tissue culture or in low-humidity conditions in a glasshouse.

10.10 Synthesis and catabolism of storage lipids

Lipids in the form of triacylglycerols are widely found as a major carbon and chemical energy reserve in seeds, fruits, and pollen grains (Fig. 10.63). One of the few plants that stores lipids in a form other

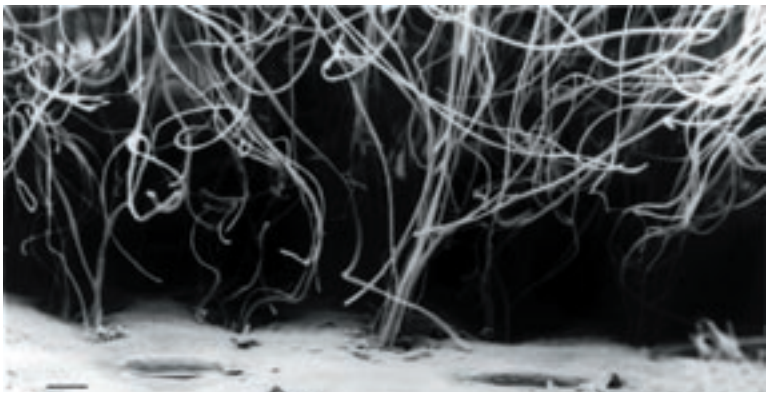


Figure 10.60
Scanning electron micrograph of the surface of a sorghum leaf sheath. In this species, the wax crystallizes as filaments that appear to emerge from defined regions of the cuticle. Scale bar (lower left) = 10 µm.

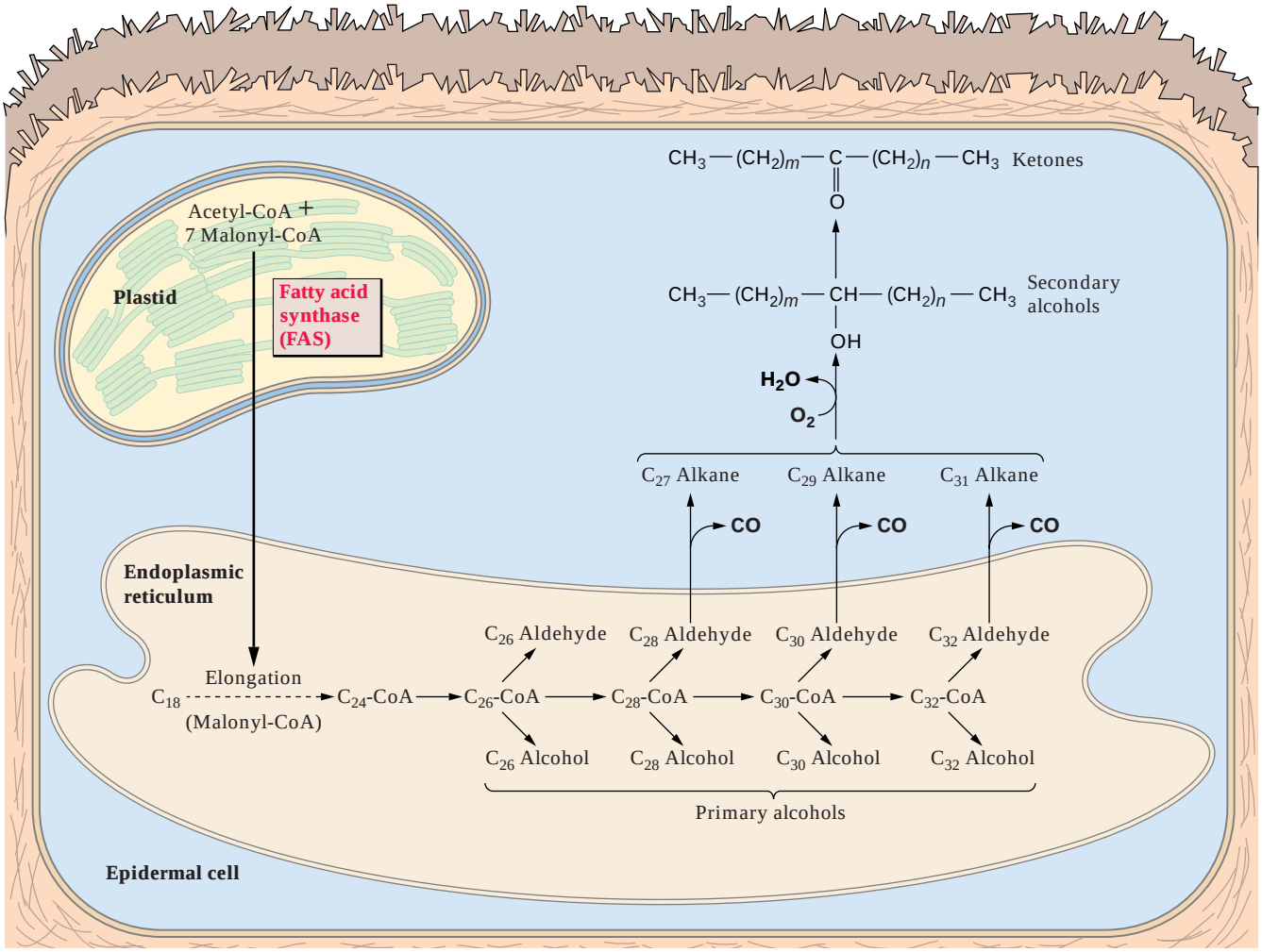


Figure 10.61
Proposed pathway for synthesis of the principal components of wax. C_{16} or C_{18} precursors for wax are produced by the de novo fatty acid synthesis pathway in the plastids. After export from the plastid, these acyl chains are elongated to C_{26} to C_{32} fatty acyl chains in the ER. The fatty acids (attached to coenzyme A) can be reduced to alcohols or to aldehydes, and the aldehydes can be decarbonylated to form alkanes with an odd number of carbons. The reactions forming secondary alcohols and ketones are not yet well understood. Together, the numerous long-chain substrates, including the primary alcohols, aldehydes, alkanes, secondary alcohols, and ketones diagrammed here, contribute to wax formation.

than triacylglycerols is jojoba, a perennial shrub that stores fatty acids as wax esters in seeds. Plant storage lipids are also an important source of dietary fats for humans and other animals. Approximately 40% of the daily energy requirement of humans in industrialized countries is supplied by dietary triacylglycerols, of which about half is from plant sources. Furthermore, triacylglycerols find use in manufacturing industries, particularly in the production of detergents, coatings, plastics, and specialty lubricants. For both food and industrial applications, the fatty acid composition of the oil determines its usefulness and, therefore, its commercial value.

10.10.1 Triacylglycerol synthesis involves acyltransferase and acyl-exchange reactions that move fatty acids between pools of membrane and storage lipids.

Many of the biochemical reactions and some aspects of subcellular compartmentalization of triacylglycerol synthesis in developing oilseeds are the same as for membrane lipids. However, because of interest in the factors that control the precise fatty acid composition of triacylglycerols, it is useful to consider seed lipid biosynthesis in terms of a scheme that emphasizes the acyltransferase and acyl-exchange reactions (Fig. 10.64). This

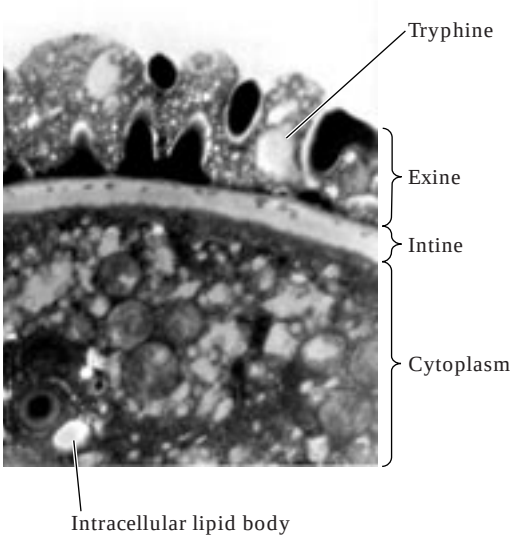


Figure 10.62
Transmission electron micrograph through an *Arabidopsis* pollen grain.

scheme was developed from the results from many oilseed species but is drawn to describe the metabolism of developing *Arabidopsis* seeds. *Arabidopsis* seed lipids contain substantial proportions of both unsaturated C_{18} fatty acids (30% $18{:}2^{\Delta 9,12}$, 20% $18{:}3^{\Delta 9,12,15}$) and long-chain fatty acids (22% $20{:}1^{\Delta 11}$) derived from $18{:}1^{\Delta 9}$. As a result, *Arabidopsis* is a good model for the biochemistry of both $18{:}2^{\Delta 9,12}$ / $18{:}3^{\Delta 9,12,15}$-rich oilseeds and those species containing longer fatty acids.

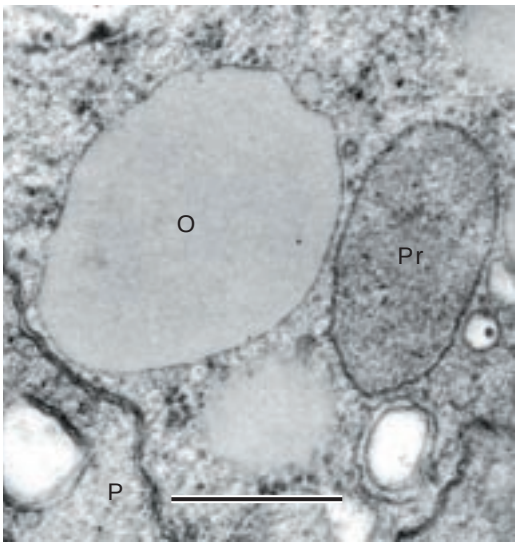


Figure 10.63
Electron micrograph of a thin section of an oil body (O), peroxisome / glyoxysome (Pr), and a small portion of a plastid (P) in the subapical zone of a one-day-old shoot apex of maize seedling. The bar represents 0.5 µm.

As in other tissues, 16:0-ACP and $18{:}1^{\Delta 9}$-ACP are usually the major products of plastid fatty acid synthesis and 18:0-ACP desaturase activity in oilseeds. These products either are utilized for lipid synthesis within the plastid or are hydrolyzed to free fatty acids by thioesterases in the plastid stroma and transferred through the plastid envelope by an unknown mechanism. The free fatty acids are converted to acyl-CoAs in the outer plastid envelope, forming the substrates for subsequent reactions in other cellular compartments. Newly produced $18{:}1^{\Delta 9}$-CoA, 18:0-CoA, and 16:0-CoA can be used for the synthesis of phosphatidylcholine, as shown in Figure 10.64. Phosphatidylcholine is the main substrate for the sequential desaturation of $18{:}1^{\Delta 9}$ to $18{:}2^{\Delta 9,12}$ and $18{:}3^{\Delta 9,12,15}$. Cholinephosphotransferase is freely reversible, so in many oilseeds phosphatidylcholine is a direct precursor of highly unsaturated species of diacylglycerol used for triacylglycerol synthesis. However, the acyl-CoA pool does not contain only 16:0 and $18{:}1^{\Delta 9}$: Exchange of $18{:}1^{\Delta 9}$from $18{:}1^{\Delta 9}$-CoA with the fatty acid at position *sn*-2 of phosphatidylcholine provides inputs of $18{:}2^{\Delta 9,12}$and $18{:}3^{\Delta 9,12,15}$ back into the cellular acyl-CoA pool. In some oilseeds, including *Arabidopsis* and rapeseed, $18{:}1^{\Delta 9}$-CoA can be modified by elongation to $20{:}1^{\Delta 11}$-CoA and $22{:}1^{\Delta 13}$-CoA. Synthesis of diacylglycerol may also involve these components of the acyl-CoA pool, as does the final acylation of diacylglycerol to form triacylglycerol by the enzyme acyl-CoA:1,2-diacylglycerol *O*-acyltransferase.

10.10.2 Triacylglycerols accumulate in discrete subcellular organelles called oil bodies.

Electron microscopic studies show that in oilseeds, oil bodies are surrounded by what appears to be a "half-unit" membrane instead of the more usual bilayer. The oil body is thus a droplet of triacylglycerol surrounded by a monolayer of phospholipids, with the hydrophobic acyl moieties of the phospholipids interacting with the triacylglycerols and the hydrophilic head groups

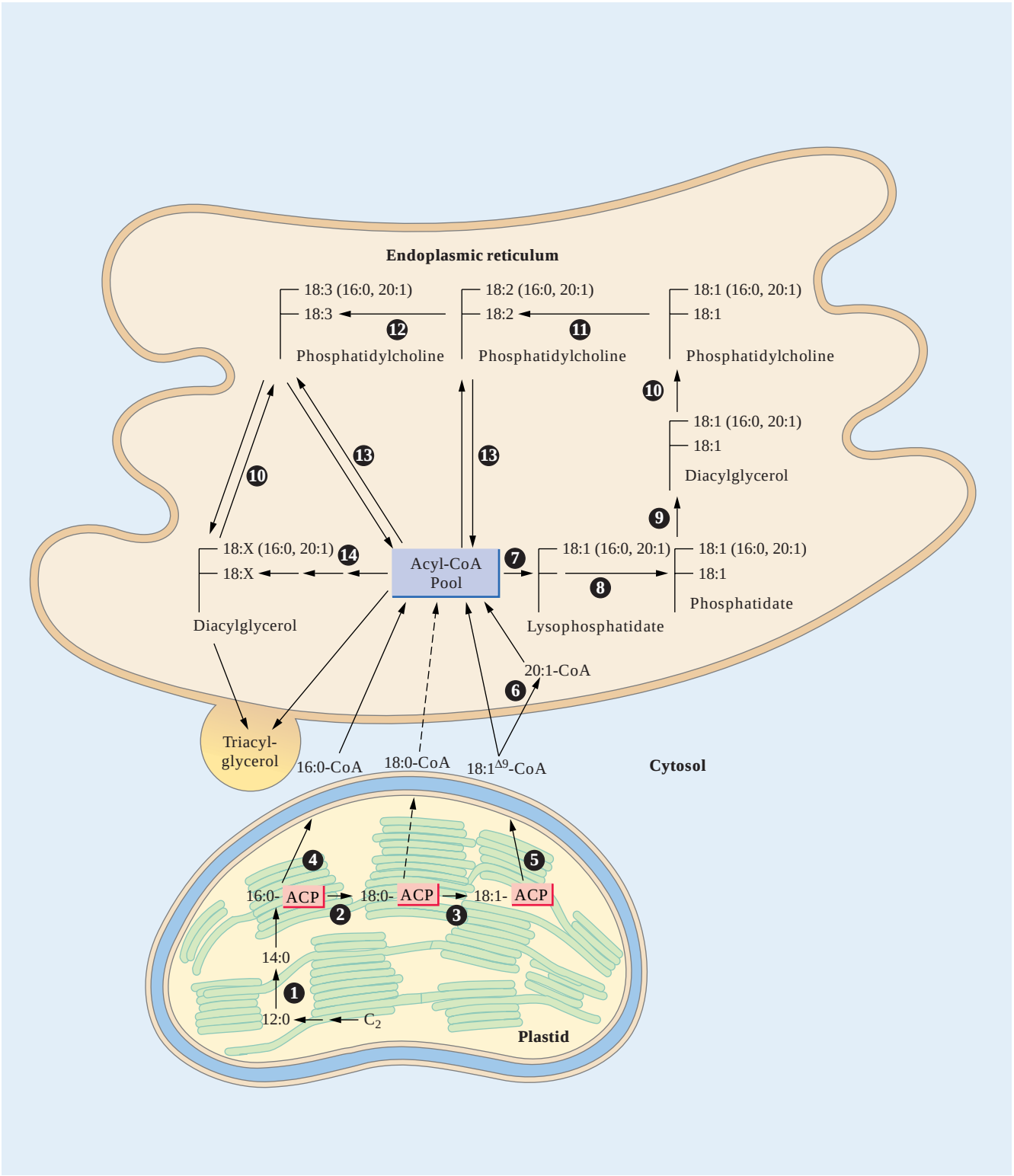


Figure 10.64
Abbreviated scheme for the reactions of triacylglycerol synthesis in seeds of *Arabidopsis* and other oilseeds. The enzyme-catalyzed steps are indicated by numbers and involve the following enzymes: (1) KAS I– and KAS III–dependent FAS; (2) KAS II–dependent FAS; (3) stearoyl-ACP desaturase; (4) palmitoyl-ACP thioesterase; (5) oleoyl-ACP thioesterase; (6) oleate elongase; (7) acyl-CoA:glycerol-3-phosphate acyltransferase; (8) acyl-CoA:lysophosphatidate acyltransferase; (9) phosphatidate phosphatase; (10) CDP-choline: diacylglycerol cholinephosphotransferase; (11) oleate desaturase, *FAD2;* (12) linoleate desaturase, *FAD3;* (13) acyl-CoA: *sn*-1 acyllysophosphatidylcholine acyltransferase; (14) same as in (7), (8), and (9), but any fatty acids used are from the acyl-CoA pool.

facing the cytosol. Oil bodies also contain major protein components called oleosins, which are not found in significant amounts in any other cellular location (Fig. 10.65). Oleosins are low-molecular-mass proteins (15 to 25 kDa), of which the defining feature is a sequence of 70 to 80 hydrophobic amino acids toward the middle of the protein. The sequence of this hydrophobic domain is conserved in oleosins from different plant species, but these proteins are not found in animals, bacteria, or fungi. Although some question of the protein secondary structure in the hydrophobic domain (β-strand or β-sheet) remains, there is general agreement that it protrudes into the triacylglycerol core of the oil body. Very possibly, the more hydrophilic N- and C-terminal domains form amphipathic helices at the oil body surface.

Although it is tempting to suppose that oleosins help stabilize the interface between the oil body and the aqueous cytoplasm, this is probably not their function. Oil bodies in fruit tissues (such as those of avocado and olive) do not contain oleosin homologs. Oleosins are found only in oil bodies of seeds and pollen, both of which undergo dehydration during maturation. Thus, oleosins may function to stabilize oil bodies at low water potential when hydration of the surface phospholipids is not sufficient to prevent the oil bodies from coalescing and fusing. Oleosins also may regulate the size of oil bodies by imparting a defined curvature to the surface—which could be important for regulating the surface-to-volume ratio to facilitate rapid breakdown of oil bodies during germination. In this respect, the mesocarp lipids in avocado and olive are not thought to contribute to the germination or growth of the seedling but are probably made by the plant to facilitate seed dispersal by animals.

The ontogeny of oil bodies is not absolutely clear, but one popular view suggests that they arise by deposition of triacylglycerols between the two leaflets of the ER and then develop into discrete organelles that may or may not remain attached to the ER membranes (Fig. 10.66). This model accounts for the half-unit membrane of oil bodies and is consistent with biochemical results that demonstrate high rates of triacylglycerol synthesis by microsomal membrane preparations. The exact nature of the relationship between the oil bodies and ER takes on extra significance because diacylglycerols are precursors for the synthesis of both triacylglycerols and the major membrane phospholipids. Because many oilseeds contain high amounts of unusual fatty acids that are largely excluded from the membrane phospholipids, it follows that diacylglycerols must occupy a critical branch point in oilseed lipid metabolism.

10.10.3 Membrane and storage lipids often have distinct compositions.

Unusual fatty acids (see Table 10.2 and Fig. 10.25) often constitute 90% or more of all fatty

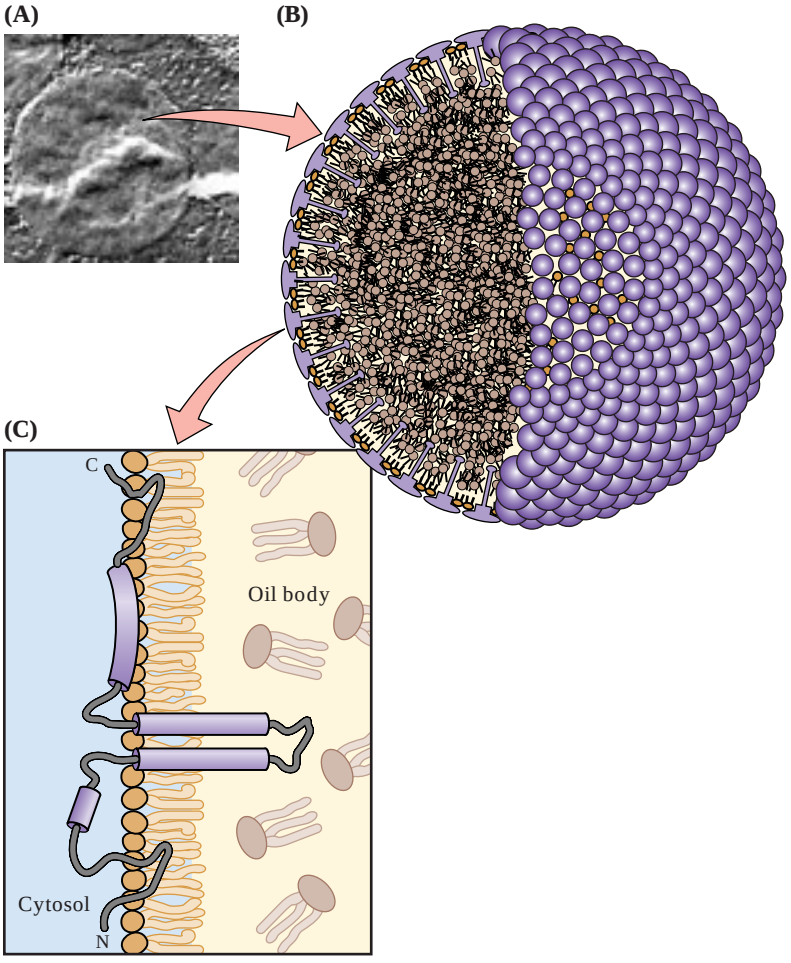


Figure 10.65
(A) Transmission electron micrograph close-up of oil body. (B) Scale-model of a maize oil body. The oleosin molecule is depicted in the shape of an 11-nm-long hydrophobic stalk attached to an amphipathic and hydrophilic globular structure that forms the outer surface of the oil body. (C) Proposed model of the conformation of a maize 18-kDa oleosin. The cylinders depict helices.

acids produced in the seed, but in almost all cases these fatty acids are present only in triacylglycerols and are excluded from membrane lipids. This probably occurs because fatty acids with unusual structures could create undesirable physical or chemical properties in the membrane or perturb membrane fluidity. Therefore, plants that produce such unusual fatty acids must also have mechanisms to prevent the accumulation of these compounds in membranes. Furthermore, hydroxy-fatty acids, epoxy-fatty acids, and some other unusual fatty acids are first synthesized on phosphatidylcholine, a major membrane lipid. What mechanisms ensure first the removal of these modified fatty acids from phospholipids and then their targeting to storage oils? At present, no clear and complete answer is available. However, developing seeds from species that produce unusual fatty acids often have specific phospholipases that can remove the fatty acids from polar lipids. Furthermore, these seeds usually have a suite of specialized acyltransferases and other enzymes specific for metabolizing unusual fatty acids.

One possible method for keeping membrane and storage fatty acids distinct may be subcellular compartmentation. Although

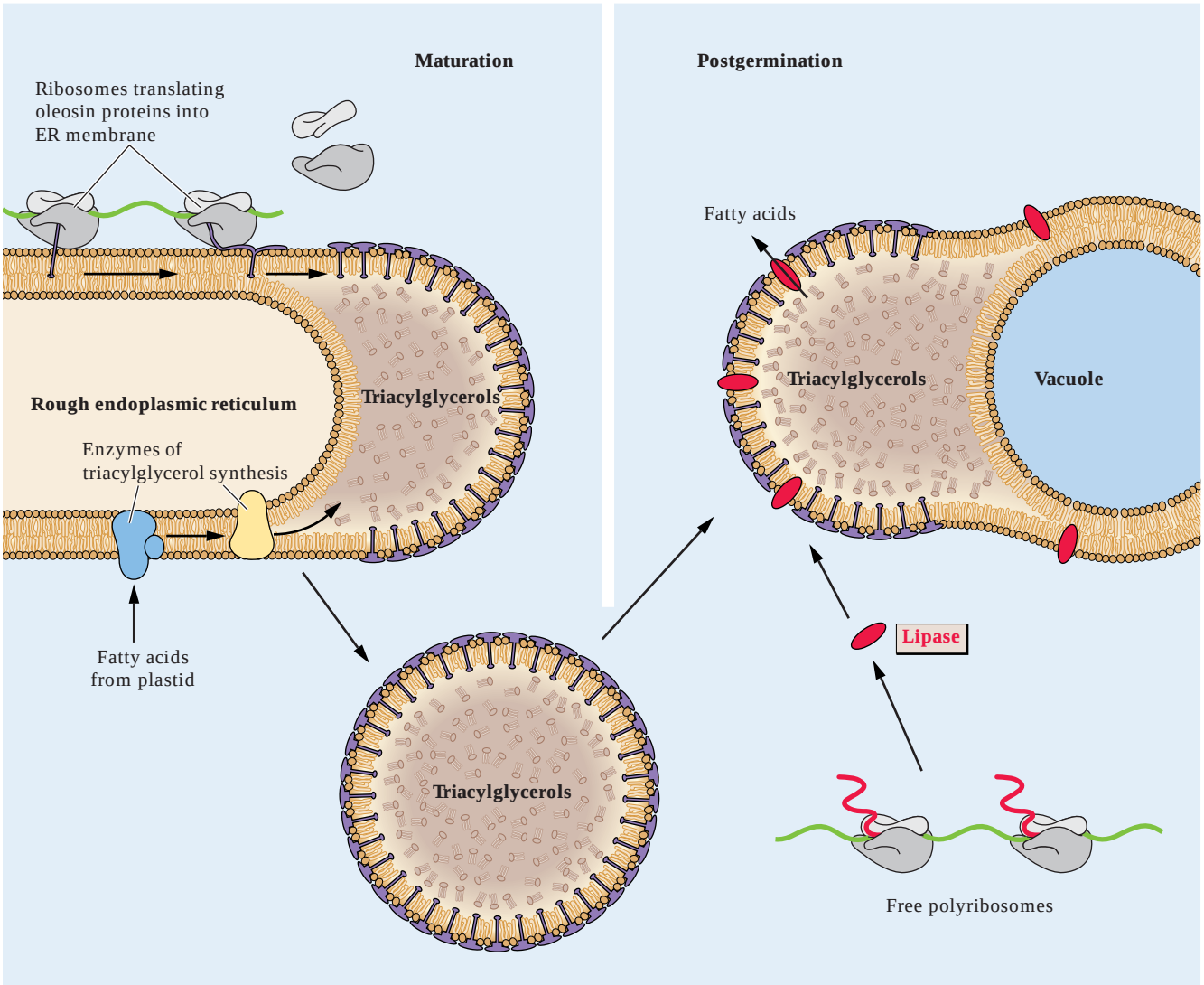


Figure 10.66
Model of the synthesis and degradation of an oil body in a maize embryo during seed maturation and postgermination.

membrane and storage lipid synthesis proceed by similar pathways and share common chemical intermediates (phosphatidate, diacylglycerol, and phosphatidylcholine; see Fig. 10.64), the specialized enzymes involved in storage oil biosynthesis may have subcellular locations distinct from enzymes that process the "common" fatty acids for incorporation into membranes.

10.10.4 Mobilization of storage lipids provides carbon and chemical energy for germination and pollination.

Triacylglycerols function as an efficient source of carbon and energy for germination of seeds and pollen because lipids are a far more compact form of storage than is carbohydrate or protein (see Fig. 10.3). Relatively few species accumulate significant quantities of storage lipids in roots, tubers, or other storage organs where compact size is not important. Because of their hydrophobicity and insolubility in water, triacylglycerols are segregated into lipid droplets, which do not raise the osmolarity of the cytosol. Furthermore, unlike polysaccharides, triacylglycerols do not contain extra weight as water of solvation. The relative chemical inertness of triacylglycerols allows their intracellular storage in large quantity without risking undesired chemical reactions with other cellular constituents.

The same properties that make triacylglycerols good storage compounds present problems in their utilization. Because of their insolubility in water, triacylglycerols must be hydrolyzed to fatty acids before they are available for metabolism. The relative stability of the C–C bonds in the alkyl moiety of a fatty acid is overcome by activation of the fatty acid carboxyl group at C-1 by attachment to CoASH, followed by oxidative attack at the C-3 position. This latter carbon atom is also called the β-carbon in common nomenclature, from which the oxidation of fatty acids gets its common name: β-oxidation. The oxidation of long-chain fatty acids to acetyl-CoA by a four-step cycle is a central energy-yielding pathway during the germination of seeds with high lipid contents and during the germination and growth of pollen tubes during fertilization. The acetyl-CoA formed during β-oxidation may be converted, via the glyoxylate cycle and gluconeogenesis, to carbohydrate (Fig. 10.67; see also Chapters 1 and 14).

Although the biological role of fatty acid oxidation differs from organism to organism, the enzyme reactions are essentially the same in plants and animals. Details of the enzymatic mechanisms can be found in biochemistry textbooks that focus primarily on animal metabolism. Therefore, the discussion below highlights those features of the pathway that differ between plants and animals.

10.10.5 β-Oxidation takes place in peroxisomes and glyoxysomes.

The major site of fatty acid oxidation in animal cells is the mitochondrial matrix. In contrast, fatty acid oxidation in plants occurs primarily in the peroxisomes of leaf tissue and the glyoxysomes of germinating seeds (Fig. 10.67). Plant peroxisomes and glyoxysomes are similar in structure and function. Glyoxysomes may be considered specialized peroxisomes (see Chapter 1).

Unlike in animals, the β-oxidation pathway is not used solely for production of metabolic energy in plants. The biological role of β-oxidation in peroxisomes and glyoxysomes is to provide biosynthetic precursors from stored lipids. During germination, triacylglycerols stored in seeds are converted into glucose and a wide variety of essential metabolites. Fatty acids released from triacylglycerols are activated to form their CoA derivatives and are oxidized in glyoxysomes by the same four-step process that occurs in peroxisomes. The acetyl-CoA produced is converted by way of the glyoxylate cycle to C_4 precursors for gluconeogenesis (Fig. 10.67; see also Chapter 14, Fig. 14.41). Glyoxysomes, like peroxisomes, contain high concentrations of catalase, which converts the H_2O_2 produced by β-oxidation to H_2O and O_2 (see Chapter 1).

In animal mitochondria, each of the four enzymes of β-oxidation is a separate, soluble protein, similar in structure to the analogous enzyme in Gram-positive bacteria. In contrast, the enzymes of peroxisomes and glyoxysomes form a complex of proteins. As in the oxidation of fatty acids in mitochondria,

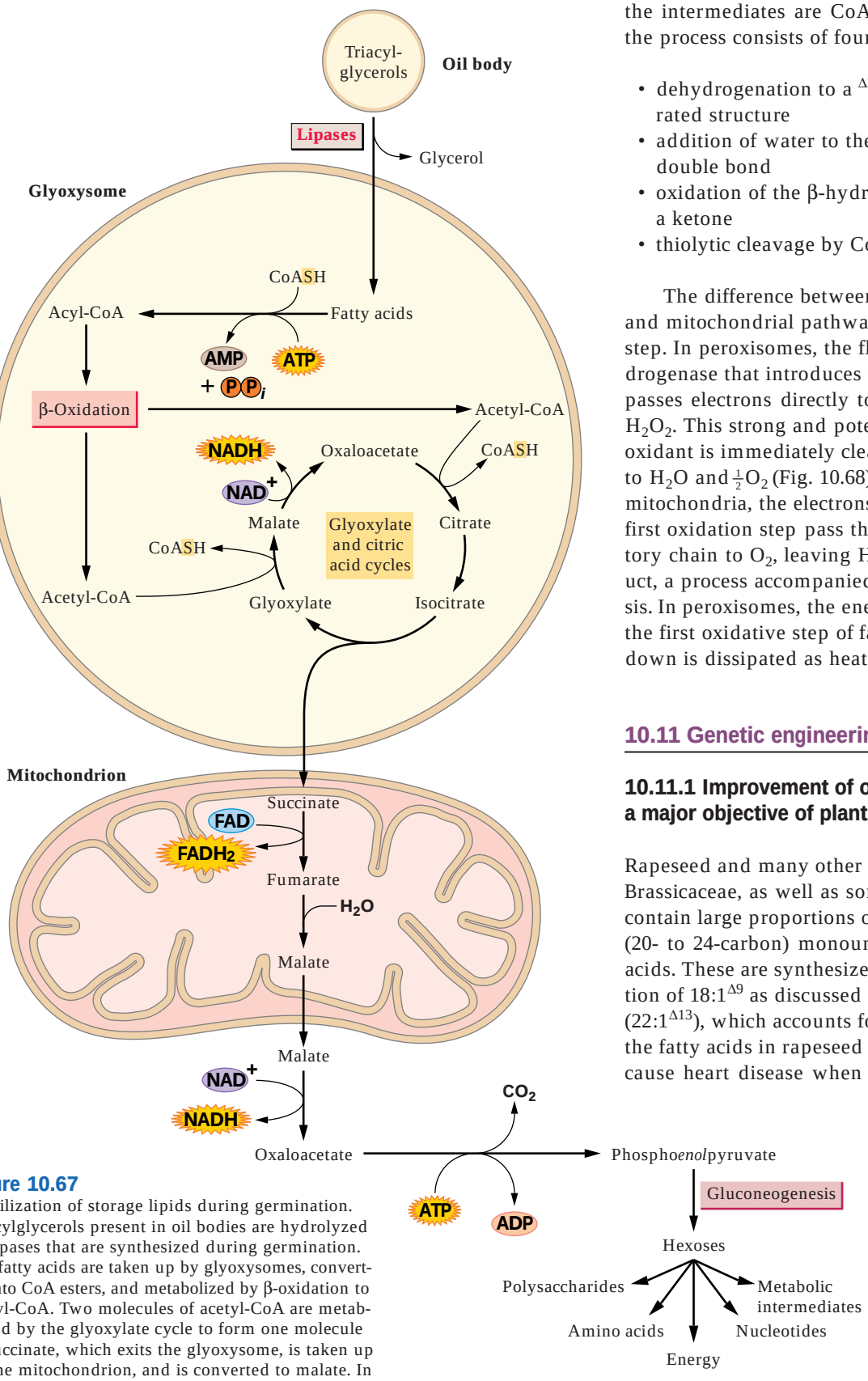


Figure 10.67
Mobilization of storage lipids during germination. Triacylglycerols present in oil bodies are hydrolyzed by lipases that are synthesized during germination. The fatty acids are taken up by glyoxysomes, converted into CoA esters, and metabolized by β-oxidation to acetyl-CoA. Two molecules of acetyl-CoA are metabolized by the glyoxylate cycle to form one molecule of succinate, which exits the glyoxysome, is taken up by the mitochondrion, and is converted to malate. In the cytosol, malate is oxidized and the resulting oxaloacetate is converted into hexose by gluconeogenesis.

the intermediates are CoA derivatives, and the process consists of four steps (Fig. 10.68):

- dehydrogenation to a $^{\Delta 2}$-*trans* unsaturated structure
- addition of water to the resulting double bond
- oxidation of the β-hydroxyacyl-CoA to a ketone
- thiolytic cleavage by CoASH

The difference between the peroxisomal and mitochondrial pathways lies in the first step. In peroxisomes, the flavoprotein dehydrogenase that introduces the double bond passes electrons directly to O_2, producing H_2O_2. This strong and potentially damaging oxidant is immediately cleaved by catalase to H_2O and $\frac{1}{2}O_2$ (Fig. 10.68). In contrast, in mitochondria, the electrons removed in the first oxidation step pass through the respiratory chain to O_2, leaving H_2O as the product, a process accompanied by ATP synthesis. In peroxisomes, the energy released in the first oxidative step of fatty acid breakdown is dissipated as heat.

10.11 Genetic engineering of lipids

10.11.1 Improvement of oil quality is a major objective of plant breeders.

Rapeseed and many other members of the Brassicaceae, as well as some other species, contain large proportions of very-long-chain (20- to 24-carbon) monounsaturated fatty acids. These are synthesized by chain elongation of $18{:}1^{\Delta 9}$ as discussed earlier. Erucic acid ($22{:}1^{\Delta 13}$), which accounts for about 50% of the fatty acids in rapeseed oil, was shown to cause heart disease when included in the

diet of laboratory animals (see Fig. 10.28). Although the high erucic acid content made rapeseed oil useful for certain industrial applications, it prevented the widespread use of rapeseed *(Brassica napus)* as an edible oil crop. In the 1950s, an extensive search was made for varieties of *B. napus* with low contents of erucic acid and glucosinolates, another antinutritional component of oil from *Brassica* species (Fig. 10.69; see also Chapter 16). During a period of about 20 years, several natural isolates containing reduced amounts of erucic acid were identified, and the two loci responsible for the phenotype were introgressed into cultivars of *B. napus* by many rounds of back-crossing. To distinguish the cultivars that produced oil with low erucic acid (LEAR) from those with high erucic acid (HEAR) content, the LEAR cultivars are now called Canola (Fig. 10.69). Expression of a jojoba β-ketoacyl-CoA synthase in transgenic Canola plants restored the high erucic acid trait. Thus, the two genes introgressed into *B. napus* to produce Canola apparently are naturally occurring mutations in two genes for β-ketoacyl-CoA synthase. The presence of two functionally homologous genes in Canola is probably related to the fact that the plant is a tetraploid. This is one of the few instances in which the biochemical basis for an important agronomic trait has been discovered.

10.11.2 Edible oils can be improved by metabolic engineering.

Approximately 20% of the calories consumed in developed countries are derived from plant oils. The fatty acid composition of dietary oils, particularly the saturated fatty acid content, is believed to influence the etiology of major diseases such as atherosclerosis and cancer. Many attempts have been made to alter the fatty acid compositions of food oils and reduce the proportions of 16:0, 18:0, $18{:}2^{\Delta 9,12}$, and $18{:}3^{\Delta 9,12,15}$ in favor of $18{:}1^{\Delta 9}$. The move to develop monounsaturated oils is based on epidemiological data and laboratory experiments that suggest that these oils may reduce atherosclerosis (and associated heart attacks and strokes) by increasing the ratio of high-density lipoproteins to low-density lipoproteins in the blood. Furthermore, oils low in $18{:}2^{\Delta 9,12}$ and $18{:}3^{\Delta 9,12,15}$ are

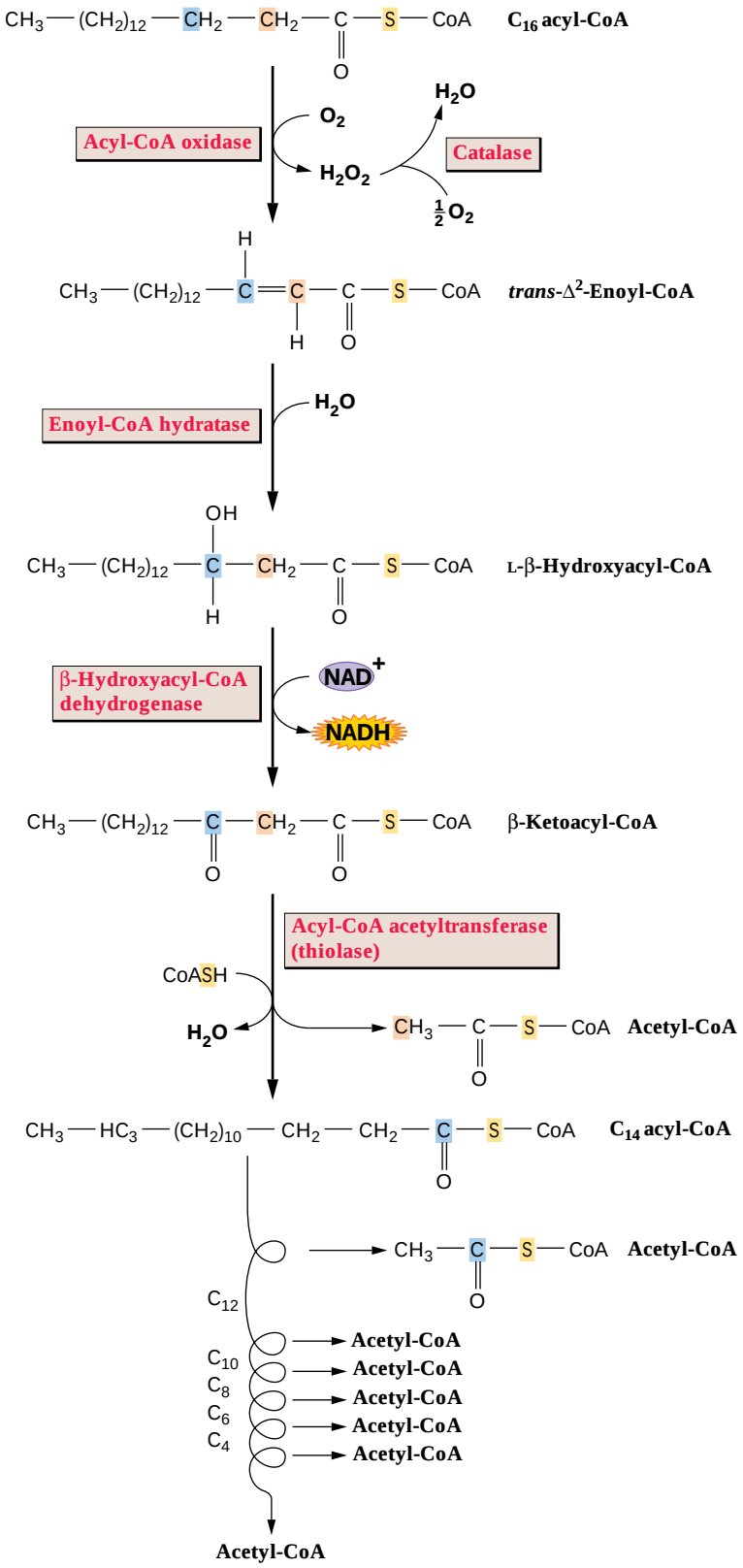


Figure 10.68
β-Oxidation pathway in peroxisomes. In each pass through the sequence, one acetyl residue is removed in the form of acetyl-CoA from the carboxyl end of an acyl-CoA. Seven passes through the cycle are required to oxidize a C_{16} fatty acid to eight molecules of acetyl-CoA.

much more stable, particular in high-temperature food frying.

The targets for reducing polyunsaturates are the $18:1^{\Delta 9}$- and $18:2^{\Delta 9,12}$ desaturases of the ER, which, in *Arabidopsis*, are encoded by the *FAD2* and *FAD3* genes. Isolation of these genes by gene-tagging *(FAD2)* and map-based cloning *(FAD3)* in *Arabidopsis* has led quickly to identifying analogous genes in soybean, Canola, and other crops. This has now allowed genetic engineering of edible oils with improved nutritional properties. One particular success involves cosuppression of the oleoyl desaturase in soybean; as a result, oleic acid has increased from less than 10% to more than 85% of the total fatty acids, and at the same time, saturated fatty acids have been reduced from more than 15% to less than 5%. Oil from these soybean varieties is expected to have both improved health benefits and improved stability.

Production of margarine and shortenings currently involves catalytic hydrogenation of unsaturated oils to provide a semisolid fat. The cost of this processing and the associated production of trans fatty acids has provided incentives to produce oils with increased contents of saturated fatty acids or a high-melting point monounsaturated fatty acid, such as petroselinic acid ($18:1^{\Delta 6}$). High stearate (18:0) Canola lines have been designed by antisense RNA suppression of the expression of the gene for stearoyl-ACP desaturase. These plants produce oils with 30% to 40% saturated fatty acids, a content that causes the oil to be semisolid at room temperature and thus suitable for margarine or shortening manufacture. Because petroselinic acid is a cis-unsaturated fatty acid but has a melting point above room temperature, oils containing this fatty acid could provide physical properties suitable for margarine manufacture but without the high saturated fatty acid content associated with health problems. A novel acyl-ACP desaturase responsible for petroselinic acid biosynthesis has been cloned from coriander and can function in transgenic plants. However, at least two other genes are required for high-level petroselinic acid biosynthesis, and efforts are underway to combine these in oilseed crops such as Canola.

10.11.3 Molecular genetic approaches have been used to increase oil yields.

In addition to efforts to modify the types of fatty acids produced in plant seeds, there is considerable interest in increasing the yield of oil obtainable from oilseed crops. But a major unanswered question in plant lipid metabolism remains: What determines the quantity of oil stored in a seed? In addition to its interest to basic researchers, this question is of considerable practical importance if chemicals derived from oilseeds are to compete economically with petrochemical alternatives.

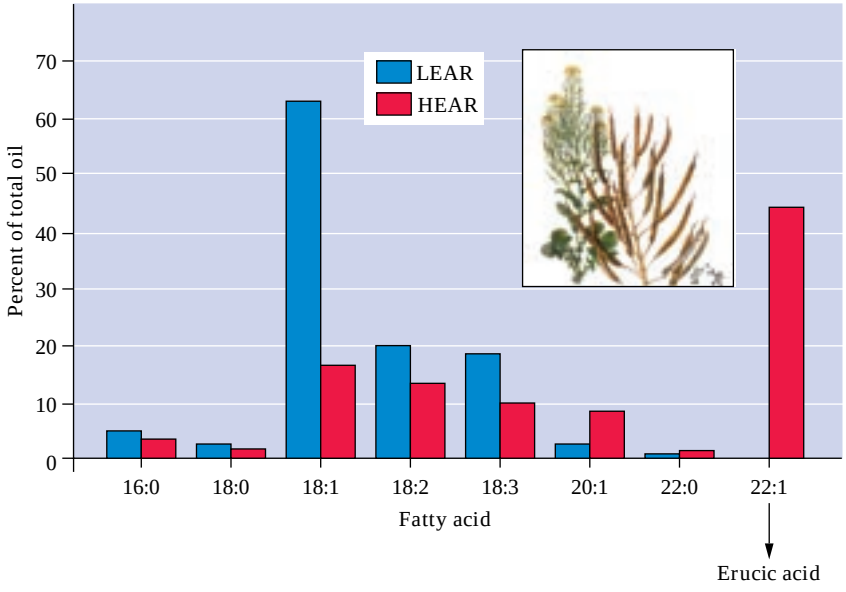


Figure 10.69
Fatty acid composition of seed oil from high- and low-erucic acid rapeseed (HEAR and LEAR, respectively) varieties. Additional improvements by plant breeding have reduced the 18:3 content of the most recent cultivars to a small percentage of the total fatty acids, with corresponding increases in the 18:1 content. Rapeseed is the major oilseed crop of Europe, Canada, and many countries with short growing seasons. After soybean and oil palm, it is the world's third largest source of vegetable oils. Varieties that have been bred to contain low amounts of erucic acid are referred to as Canola. Because of the high oil content (45% of seed weight) and the relative ease of transformation, Canola has become the first crop genetically engineered to produce new oils.

More than 30 reactions are required to convert acetyl-CoA to triacylglycerol, so many genes could control the yield of the end-product storage oil. Recently, two approaches have resulted in increased oil content in seeds. In the first, the gene for the cytosolic ACCase of *Arabidopsis* was fused to a plastid transit peptide sequence and the promoter of the gene for the *B. napus* seed storage protein napin. This chimeric gene was used to transform rapeseed. Because plastid ACCase is a highly regulated enzyme, its overexpression might result in its down-regulation and therefore might fail to increase net movement through the pathway. By targeting the cytosolic enzyme to the plastid, researchers hoped to circumvent feedback or other mechanisms that control the plastid enzyme. This approach was at least partially successful: The resulting plants had seed oil contents 5% higher than control transformants (Fig. 10.70). The second attempt to modify oil yields required manipulation near the end of the triacylglycerol biosynthesis pathway. Expression of a yeast *sn*-2 acyltransferase in *Arabidopsis* and rapeseed increased seed oil content by more than 25%. Both the ACCase and acyltransferase results have so far been demonstrated only in growth chambers and must be confirmed in field trials to establish whether increased oil yields per hectare can be achieved.

10.11.4 Fatty acids have numerous industrial applications.

Soybean oil accounts for approximately 68% of the US production of vegetable oil, with approximately 6.5 million tons of oil produced annually. Most plant-derived oil is currently used for food. However, nonfood industrial uses of plant lipids include the manufacture of soaps and detergents, paints, varnishes, lubricants, adhesives, and plastics (Table 10.5). The largest nonfood use, by volume, is lauric acid (C_{12}) from coconut and palm kernel for the production of detergents. Another large use is the production of ricinoleic acid (12-hydroxyoctadecenoic acid) for the production of a wide variety of compounds. Ricinoleic acid can be pyrolyzed to sebacic acid, which is used to produce certain types of nylon. The lithium salts of sebacic acid are also used as high-temperature greases for jet engines. Erucic acid is used to make erucamide, a slip agent in the plastics industry that makes plastic films and other products easier to handle.

At present, genetic engineering of oilseeds is largely concerned with altering the quality of oil produced in temperate crops. An immediate goal is to expand the range of fatty acids available from crop species so that the uses of plant fatty acids can be expanded. In the long term, perhaps plant

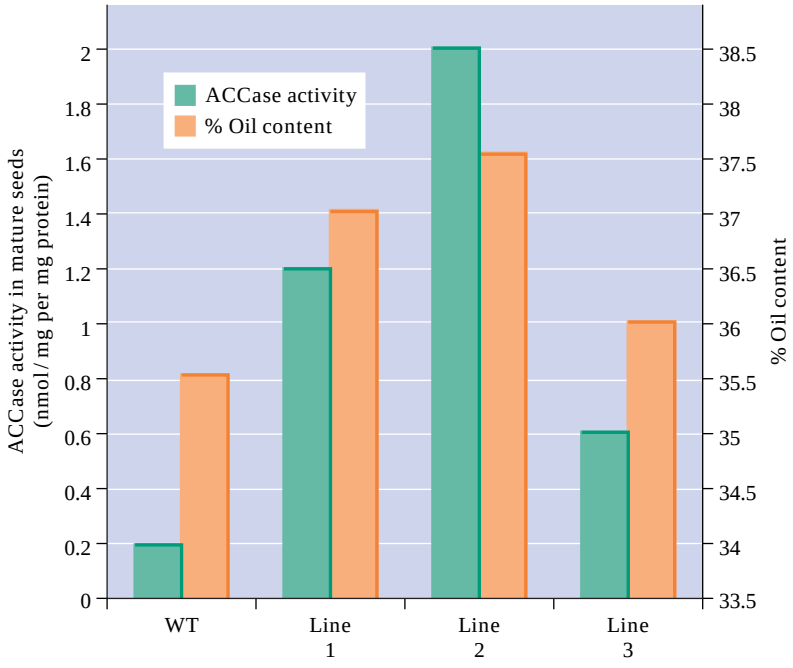


Figure 10.70
Expression of a cDNA that encodes cytosolic ACCase in *Brassica* plastids results in increased oil content of seeds. WT, wild type.

lipids will also be used as a source of fuel such as "biodiesel." Methyl esters of plant fatty acids have virtually the same performance characteristics in diesel engines as petroleum-derived diesel fuel but lack the pollutants produced by burning diesel fuel. Perennial tropical species such as oil palm are capable of producing as much as 100 barrels of oil per hectare per year with very low inputs of agrochemicals. Thus, when it becomes possible to genetically engineer oil palm, we may be able to produce a wide range of chemicals from a renewable source at a cost that is competitive with petroleum.

10.11.5 High-lauric-content rapeseed: a case study in successful oilseed engineering.

The acyl-ACP thioesterases have become the first enzymes of lipid biosynthesis for which genes were engineered and introduced into transgenic plants to produce a commercial product. A major ingredient of soaps, shampoos, detergents, and related products is the surfactants derived from medium-chain fatty acids and obtained from coconut or palm kernel oils grown in the tropics. Worldwide, approximately $1 billion worth of such oils are used for production of surfactants. In part because of periodic price instability in these raw material supplies, a long-term goal of the surfactant industry has been to establish a temperate crop that could produce medium-chain fatty acids. Such a crop could provide alternative supplies and thereby lower or stabilize prices.

In the early 1980s, the advent of plant gene transfer techniques raised the question of whether temperate oilseed crops could be engineered to produce medium-chain fatty acids and other novel fatty acid compositions with the use of foreign genes. For example, it was unclear whether addition of new, unusual fatty acids might disrupt lipid metabolism or some other process in oilseed cells. More specifically, would a new fatty acid such as lauric acid be correctly targeted to triacylglycerol and excluded from membranes? This question arose because oilseeds usually segregate unusual fatty acids into the storage triacylglycerols and exclude them from membrane glycerolipids.

Work at the biotechnology company Calgene led first to biochemical demonstration of the existence of medium-chain acyl-ACP thioesterases and then to the cloning of 12:0-ACP thioesterase from the California bay *(U. californica).* Introduction of a single gene encoding the medium-chain acyl-ACP thioesterase into transgenic plants dramatically altered the chain length of fatty acids stored in the seed oils. In 1995, the first commercial production of a genetically engineered oil was obtained when about 1 million pounds of oil was extracted from rapeseed plants engineered to produce 40% to 50% lauric acid (Fig. 10.71).

In addition to the laurate-specific acyl-ACP thioesterase from California bay, thioesterases with specificity for other chain lengths have been identified from a variety of plants, including *Cuphea.* In several cases, transgenic plants that produce oils enriched in other medium-chain fatty acids, such as myristic (14:0) and decanoic (10:0), are becoming available. This type of research may soon make available a range of specialty rapeseed varieties with oil compositions tailored to meet specific commercial applications.

Table 10.5 Some nonfood uses of plant fatty acids

Lipid type	Example	Major sources	Major uses	Approximate U.S. market 1989 ($ 10^6)
Medium-chain	Lauric acid (12:0)	Coconut, palm kernel	Soaps, detergents, surfactants	350
Long-chain	Erucic acid (22:1)	Rapeseed	Lubricants, slip agents	100
Epoxy	Vernolic acid	Epoxidized soybean oil, *Vernonia*	Plasticizers, coatings, paints	70
Hydroxy	Ricinoleic acid	Castor bean	Coatings, lubricants, polymers	50
Trienoic	Linolenic acid (18:3)	Flax	Paints, varnishes, coatings	45
Wax esters	Jojoba oil	Jojoba	Lubricants, cosmetics	10

10.11.6 Expression of a glycerolipid hydroxylase from castor bean can drive synthesis of ricinoleic acid in tobacco.

Many of the unusual fatty acids found in nature have important industrial uses. However, the plants producing them are often poorly

suited for high-production agriculture. As an alternative, the isolation of key genes directing the synthesis of a particular fatty acid can provide the means to genetically engineer agronomically suitable oilseed crops to produce the desired oil more easily and cheaply. For example, ricinoleic acid (12-OH-18:1$^{\Delta 9}$) produced by castor bean is an extremely versatile natural product with industrial applications that include the synthesis of nylon-11, lubricants, hydraulic fluids, plastics, cosmetics, and other materials. However, castor bean contains ricin, an extremely toxic lectin (see Chapter 9, Box 9.3), as well as other poisons and allergens. In addition, agronomic problems result in poorer yields than other crops. As a result of these and other factors, castor bean is a minor crop grown mainly in nonindustrialized countries.

The successful cloning of a gene that encodes the hydroxylase responsible for 12-OH-18:1$^{\Delta 9}$ synthesis from 18:1$^{\Delta 9}$ relied on a detailed understanding of the biochemistry involved. For some time, the conversion of 18:1$^{\Delta 9}$ to 12-OH-18:1$^{\Delta 9}$ was thought probably to involve a single enzyme inserting a hydroxyl group at the same position as the double bond introduced by FAD2 desaturase (Fig. 10.72). Consideration of the probable reaction cycle of the desaturases suggested that 12-OH-18:1$^{\Delta 9}$ might be produced by a stalled desaturase. Partial sequencing of randomly chosen cDNAs from a library derived from developing castor bean endosperm identified a relatively abundant cDNA that encodes a protein with homology to the glycerolipid desaturases of other oilseeds. Expression of the castor bean cDNA in transgenic tobacco plants led to the synthesis of 12-OH-18:1$^{\Delta 9}$ in seed tissues.

10.11.7 A Δ6-desaturase from borage, identified by homology to sequences of conserved membrane-bound desaturases, can catalyze synthesis of γ-linolenic acid in transgenic plants.

The successful use of random sequencing projects to clone genes for enzymes involved in the synthesis of unusual fatty acids does not require high levels of sequence homology. Because all the membrane-bound desaturases described from animals, microbes, and plants contain three highly conserved histidine-rich sequences, new members of the gene family can be identified easily, even when overall homology is low. For example, production of γ-linolenate (18:3$^{\Delta 6,9,12}$) in seeds of borage, black currant, and evening primrose is known to involve a Δ6-desaturase active on 18:2$^{\Delta 9,12}$ at the *sn*-2 position of phosphatidylcholine. To clone the glycerolipid Δ6-desaturase gene, researchers partially sequenced 600 cDNAs from a subtracted library prepared from developing borage seeds. One class of cDNAs showed low overall homology to other desaturases—about 30% identity at the amino acid

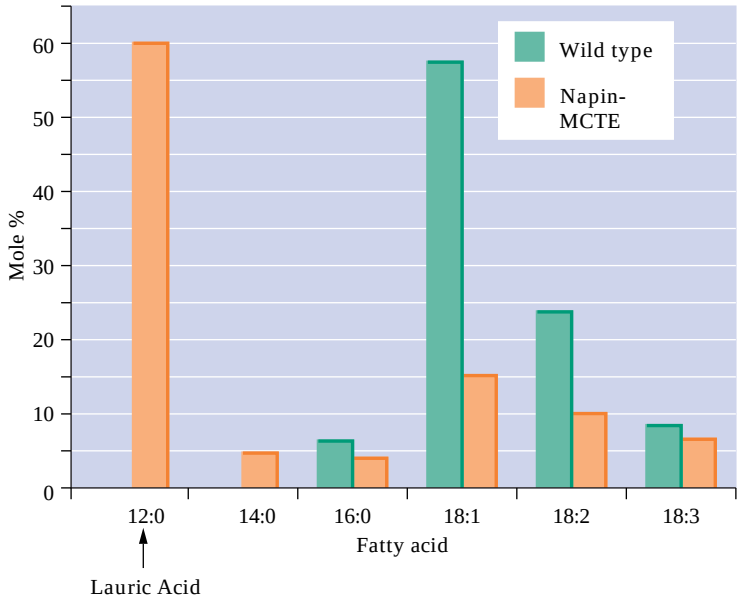


Figure 10.71
Expression of a cDNA from California bay that encodes a medium-chain thioesterase (MCTE) under control of the seed-specific napin promoter diverts carbon into medium-chain fatty acids to produce an oil with high laurate content.

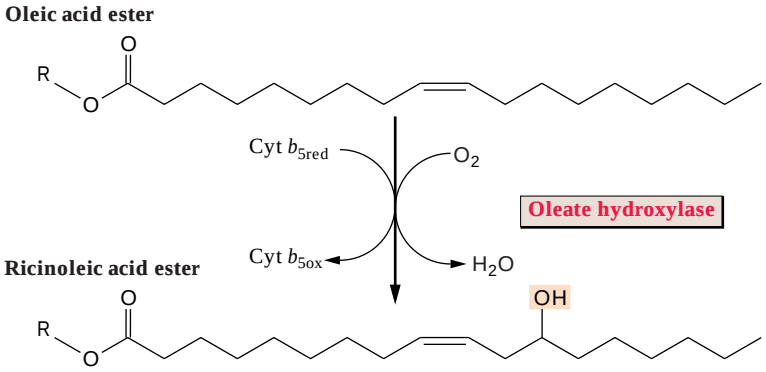


Figure 10.72
Reaction catalyzed by oleate hydroxylase. The R group is thought to be a lysolipid such as lysophosphatidylcholine.

level—but did contain characteristic histidine boxes with spacings similar to those in the Δ6-desaturase of *Synechocystis*. Expression of the borage cDNA in transgenic tobacco and carrot suspension culture protoplasts led to the production of γ-linolenate and 18:4$^{\Delta 6,9,12,15}$.

10.11.8 Biodegradable plastics can be produced in plants.

Many species of bacteria synthesize and accumulate granules of biodegradable plastics called polyhydroxyalkanoates (PHAs). The majority of PHAs are composed of *R*-(–)-3-hydroxyalkanoic acid monomers ranging from 3 to 14 carbons long. More than 40 PHAs have been characterized, with some polymers containing unsaturated bonds or other functional groups. One such PHA produced commercially by fermentation of the bacterium *Alcaligenes eutrophus* is Biopol, a random copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate units (Fig. 10.73). The copolymer combines biodegradability with water resistance and good physical properties, making it a suitable polymer for a wide variety of uses. The major drawback of Biopol and other bacterial PHAs is their high production cost, which makes them substantially more expensive than synthetic plastics and thereby restricts their large-scale use in consumer products.

Polyhydroxybutyrate (PHB), a PHA that is a linear polyester of 3-hydroxybutyrate, is synthesized from acetyl-CoA by the sequential action of three enzymes (Fig. 10.74). The first enzyme of the pathway, 3-ketothiolase, catalyzes the reversible condensation of two acetyl-CoA moieties to form acetoacetyl-CoA. Acetoacetyl-CoA reductase subsequently reduces acetoacetyl-CoA to *R*-(–)-3-hydroxybutyryl-CoA, which is then polymerized by the action of PHA synthase to form PHB. PHB is typically produced as a polymer of 10^3 to 10^4 monomers and accumulates as inclusions of 0.2 to 0.5 μm in diameter. In *A. eutrophus*,

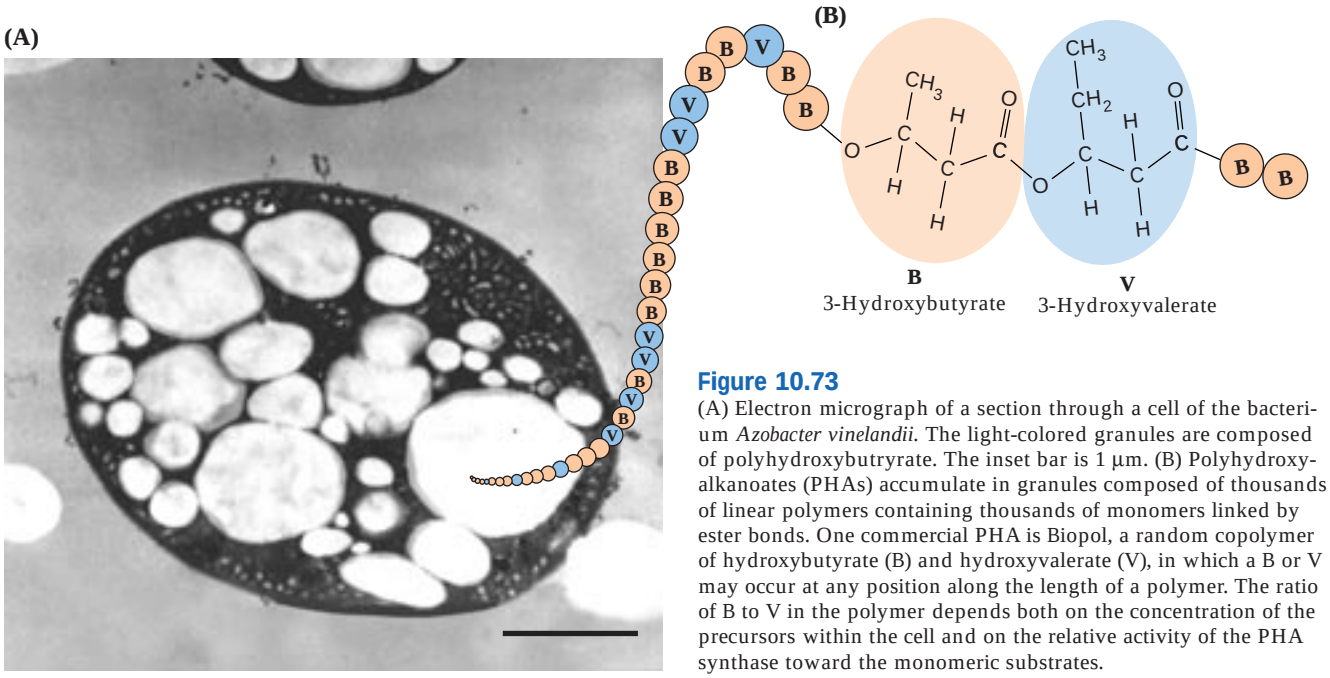


Figure 10.73
(A) Electron micrograph of a section through a cell of the bacterium *Azobacter vinelandii*. The light-colored granules are composed of polyhydroxybutyrate. The inset bar is 1 μm. (B) Polyhydroxyalkanoates (PHAs) accumulate in granules composed of thousands of linear polymers containing thousands of monomers linked by ester bonds. One commercial PHA is Biopol, a random copolymer of hydroxybutyrate (B) and hydroxyvalerate (V), in which a B or V may occur at any position along the length of a polymer. The ratio of B to V in the polymer depends both on the concentration of the precursors within the cell and on the relative activity of the PHA synthase toward the monomeric substrates.

PHB inclusions can typically accumulate to 80% of the dry weight of bacteria grown in media containing excess carbon, such as glucose, but limited in one essential nutrient, such as nitrogen or phosphate. Under these conditions, PHB synthesis acts as a carbon reserve and an electron sink. When growth-limiting conditions are alleviated (by addition of phosphorus or nitrogen), PHB is catabolized to acetyl-CoA. PHAs are biodegradable because PHB and related copolymers are readily degraded by enzymes (PHA depolymerases) secreted by bacteria in a wide range of environments, including anaerobic sewage, compost, and landfill.

Recently, the possibility of producing PHA in plants has been investigated by expressing the PHB biosynthetic genes of *A. eutrophus* in *Arabidopsis*. Because the major movement of acetyl-CoA occurs in plastids, the pathway was targeted to this organelle. Plastids are also the site of starch accumulation (starch, like PHB, is synthesized as osmotically inert inclusions in plastids without disruption of organelle function). The *A. eutrophus* enzymes 3-ketothiolase, acetoacetyl-CoA reductase, and PHB synthase were targeted to the plastid by modifying the genes to encode the transit peptide of the small subunit of ribulose bisphosphate carboxylase at the N termini of the proteins. Genes for the modified bacterial enzymes were expressed in *Arabidopsis* under the control of a constitutive promoter. Transgenic plants expressing the genes produced PHB inclusions exclusively in the plastids (Fig. 10.75). The maximal amount of PHB detected in fully expanded leaves of transgenic plants was 10 mg / g fresh weight (about 14% dry weight). No significant deleterious effect on growth or seed yield was detected in plants that accumulated high amounts of PHB in plastids. These results demonstrated the possibility of producing PHA in plants to industrially significant amounts without detrimental effects on plant growth and viability.

Oilseed crops are regarded as the most amenable targets for seed-specific PHA production. Because both oil and PHB are derived from acetyl-CoA, metabolic engineering of plants for the diversion of acetyl-CoA toward PHB accumulation can be more directly achieved in the seeds of crops having a naturally high movement of carbon through acetyl-CoA.

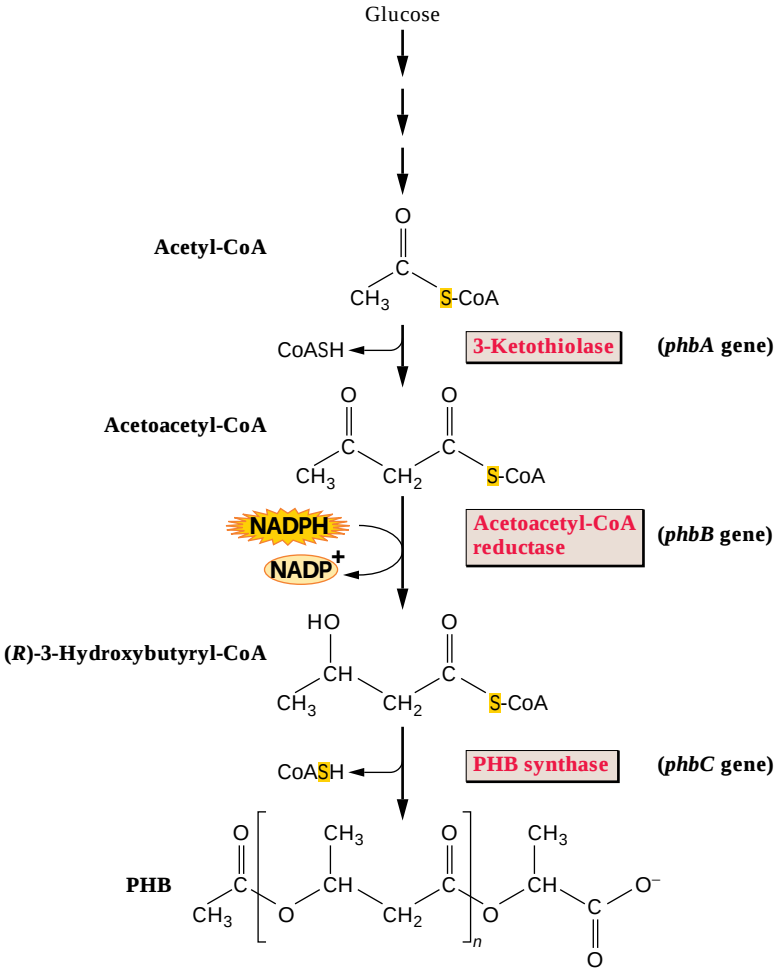


Figure 10.74
Pathway of polyhydroxybutyrate (PHB) synthesis in *Alcaligenes eutrophus*.

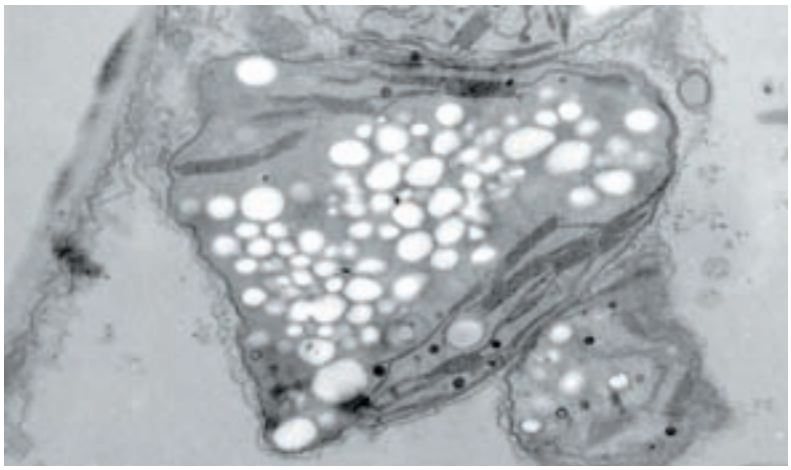


Figure 10.75
Accumulation of PHA inclusions in a chloroplast of a transgenic *Arabidopsis* plant. The oval inclusions are PHB grains. The normal morphology of the chloroplast has been distorted by the accumulation of the granules.

Summary

Lipids have diverse and essential roles in plants. As the hydrophobic barrier of membranes, they are essential for integrity of cells and organelles. In addition, they are a major form of chemical energy storage in seeds and are now recognized as a key component of some signal transduction pathways. Most lipids, but not all, contain fatty acids esterified to glycerol, and consideration of this area of metabolism involves, first, the synthesis of the fatty acid and, second, the synthesis of lipid after esterification of the fatty acid to form phosphatidic acid.

Fatty acid biosynthesis in plants is very similar to that in bacteria and is carried out in the plastid. Fatty acid synthesis begins with the carboxylation of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase. This is the first committed step of fatty acid synthesis and is a likely site for regulation of the whole pathway. Acetyl-CoA and malonyl-CoA are subsequently converted into fatty acids by a series of reactions that add two carbons at a time to a growing chain. Acyl-carrier protein is a 9-kDa protein that transports the intermediates of fatty acid synthesis through the pathway. In plants, each reaction is catalyzed by a separate gene product—in contrast to fatty acid synthesis in animals, which depends on a multifunctional protein.

Fatty acid biosynthesis can be terminated by several different reactions, including hydrolysis of the thioester bond, transfer of the acyl group to a glycerolipid, and acyl desaturation. Plants contain an unusual stearoyl-ACP desaturase that is soluble and plastid-localized. Most fatty acyl desaturases are membrane proteins localized in the endoplasmic reticulum or the plastid.

Plants are capable of synthesizing unusual fatty acids, and more than 200 different fatty acids having been found in plants. Some of the enzymes that synthesize unusual fatty acids bear close resemblances to common fatty acid enzymes, such as membrane-bound desaturases. These unusual fatty acids are found almost exclusively in seed oils, and it is thought that they may serve a defense function.

There are two distinct pathways for the synthesis of membrane glycerolipids. The prokaryotic pathway, located in the chloroplast inner envelope, uses 18:1-ACP and 16:0-ACP for the sequential acylation of glycerol 3-phosphate and synthesis of glycerolipid components of the chloroplast membranes. The eukaryotic pathway involves *(a)* export of 16:0 and 18:1 fatty acids from the chloroplast to the endoplasmic reticulum as acyl-CoAs and *(b)* their incorporation into phosphatidylcholine and other phospholipids that are the principal structural lipids of all the membranes of the cell except the chloroplast. In addition, the diacylglycerol moiety of phosphatidylcholine can be returned to the chloroplast envelope and used as a second source of precursors for the synthesis of chloroplast lipids.

Membrane lipids serve as a hydrophobic barrier, delimiting the cell and dividing it into functional compartments. The membrane lipid composition also affects plant form as well as many cellular functions. For example, photosynthesis is impaired in plants lacking polyunsaturated membrane lipids, and lipid composition can affect chilling sensitivity and influence plant cell responses to freezing. In addition, membrane lipids function in signal transduction pathways and defensive processes.

Storage lipids play a distinctly different role from membrane lipids. Storage lipids are almost exclusively triacylglycerols and accumulate in discrete subcellular organelles called oil bodies. The mobilization and catabolism of triacylglycerols provides energy for germination and pollination. The catabolism of the released fatty acids occurs via β-oxidation in peroxisomes and glyoxysomes.

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