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CHAPTER 4

Lipid metabolism in plants

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1. Introduction

Plants produce the majority of the world's lipids, and most animals, including humans, depend on these lipids as a major source of calories and essential fatty acids. Like other eukaryotes, plants require lipids for membrane biogenesis, as signal molecules, and as a form of stored carbon and energy. In addition, soft tissues and bark each have distinctive protective lipids that help prevent desiccation and infection. To what extent does the biochemistry of plant lipid metabolism resemble that in other organisms? This chapter mentions a number of similarities, but emphasizes aspects unique to plants. Major differences between lipid metabolism in plants and other organisms are summarized in Table 1.

The presence of chloroplasts and related organelles in plants has a profound effect on both gross lipid composition and the flow of lipid within the cell. Fatty acid

Table 1
Comparison of plant, mammalian, and bacterial lipid metabolism

	Higher plants	Mammals	*E. coli*
Fatty acid synthase			
Structure	Type II (multicomponent)	Type I (multifunctional)	Type II (multicomponent)
Location	Plastids	Cytosol	Cytosol
Acetyl-CoA carboxylase(s)	Multisubunit and multifunctional	Multifunctional	Multisubunit
Primary desaturase substrates			
Δ^9	18 : 0-ACP	18 : 1-CoA	none
ω-6	18 : 1 on glycerolipids	none	none
ω-3	18 : 2 on glycerolipids	none	none
Primary substrate(s) for phosphatidic acid synthesis	acyl-ACP and acyl-CoA	acyl-CoA	acyl-ACP
Prominent bilayer lipids	Galactolipid > phospholipid	Phospholipid	Phospholipid
Main β-oxidation function	Provides acetyl-CoA for glyoxylate cycle	Provides acetyl-CoA for TCA cycle	Provides acetyl-CoA for TCA cycle

synthesis occurs not in the cytosol as in animals and fungi, but in the chloroplast and other plastids. Acyl groups must then be distributed to multiple compartments, and the complex interactions between competing pathways are a major focus of plant lipid biochemists. It is also significant that the lipid bilayers of chloroplasts are largely composed of galactolipids rather than phospholipids. As a result, galactolipids are the predominant acyl lipids in green tissues and probably on earth.

Plant lipids also have a substantial impact on the world economy and human nutrition. More than three-quarters of the edible and industrial oils marketed annually are derived from seed and fruit triacylglycerols. These figures are particularly impressive given that, on a whole organism basis, plants store more carbon as carbohydrate than as lipid. Since plants are not mobile, and since photosynthesis provides fixed carbon on a regular basis, plant requirements for storage lipid as an efficient, light weight energy reserve are less acute than those of animals.

Finally, hundreds of genes required for plant lipid biosynthesis, utilization and turnover have now been cloned. In addition to providing valuable information on enzyme structure and function, these genes are being exploited to design new, more valuable plant oils. The coordination of lipid metabolic genes with each other and with their potential regulators may also become better understood, as DNA microarray and other genomic technologies mature.

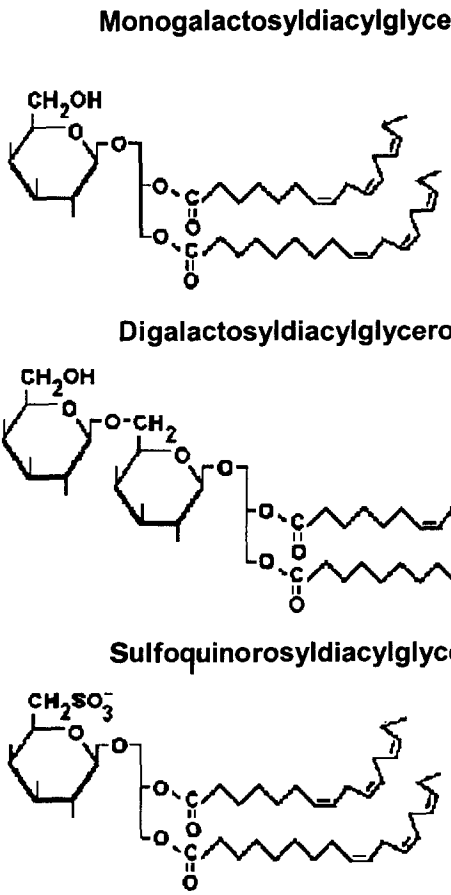
2. *Plant lipid geography*

2.1. Plastids

Although all eukaryotic cells have much in common, the ultrastructure of a plant cell differs from that of the typical mammalian cell in three major ways. The plasma membrane of plant cells is shielded by the cellulosic cell wall, preventing lysis in the naturally hypotonic environment but making preparation of cell fractions more difficult. The nucleus, cytosol and organelles are pressed against the cell wall by the tonoplast, the membrane of the large, central vacuole that can occupy 80% or more of the cell's volume. Finally, all living plant cells contain one or more types of plastid.

The plastids are a family of organelles containing the same genetic material, a circular chromosome present in multiple copies. Young or undifferentiated cells contain tiny proplastids that, depending on the tissue, may differentiate into photosynthetic chloroplasts, carotenoid-rich chromoplasts, or any of several varieties of colorless leucoplasts, including plastids specialized for starch storage [1]. These different types of plastids, which may be interconverted in vivo, have varying amounts of internal membrane but invariably are bounded by two membranes. The internal structure of chloroplasts is dominated by the flattened green membrane sacks known as thylakoids. The thylakoid membranes contain chlorophyll and are the site of the light reactions of photosynthesis.

As noted above, chloroplasts and other plastids are enriched in galactolipids (Fig. 1). They also contain a unique sulfolipid, sulfoquinovosyldiacylglycerol, whose head group is a modified galactose. The phospholipid components of plastids are less



Pea Chloroplasts	%
Thylakoids	56
Inner membrane	64
Outer membrane	1
Daffodil chromoplasts	63
Cauliflower proplastids	31
Potato leucoplasts	14

Pea Chloroplasts	%
Thylakoids	32
Inner membrane	31
Outer membrane	40
Daffodil chromoplasts	18
Cauliflower proplastids	29
Potato leucoplasts	45

Pea Chloroplasts	%
Thylakoids	4
Inner membrane	4
Outer membrane	4
Daffodil chromoplasts	5
Cauliflower proplastids	6
Potato leucoplasts	5

Fig. 1. Composition of plastid membranes. Figures given are percentages (as % of total lipid) of the pictured lipid in the membranes specified. Data from Harwood, J.L. (1980) Plant acyl lipids: structure, distribution and analysis. In: P.K. Stumpf (Ed.) The Biochemistry of Plants, Vol. 4., Academic Press, pp. 2–56 and Sparace, S.A., Kleppinger-Sparace, K.F. (1993) Metabolism in non-photosynthetic, non-oilseed tissues. In: T.S. Moore Jr. (Ed.) Lipid Metabolism in Plants, Boca Raton, FL, CRC Press, pp. 569–589.

abundant. Phosphatidylglycerol is the most prominent phospholipid contributor to the thylakoid membrane system of chloroplasts (but < 10% of the glycerolipids), whereas most of the limited phosphatidylcholine of chloroplasts is associated with their outer membrane.

The synthetic capabilities of plastids and other plant organelles are summarized in Table 2. Representatives of each type of plastid have been isolated and found to incorporate acetate into long-chain fatty acids, to desaturate 18 : 0 to 18 : 1, and to assemble phosphatidic acid and galactolipids. Chloroplasts have also been shown to synthesize phosphatidylglycerol, including molecular species containing the unusual *trans*-3-hexadecenoic acid at the 2-position. In addition to the components normally retained within the plastids, large quantities of fatty acids, particularly 18 : 1 and 16 : 0,

Table 2

Compartmentation of lipids and lipid biosynthesis in plant cells[a]

Membrane or organelle	Activities	Prominent lipids (not listed if <5%)
Plastids	Acetyl-CoA carboxylase, fatty acid synthase, 18:0-ACP desaturase, ω3 and ω6 desaturases, glycerol-3-phosphate acyltransferase, lysophosphatidic acid acyltransferase, phosphatidic acid phosphohydrolase, CTP: phosphatidate cytidylyltransferase, phosphatidylglycerol phosphate synthase and phosphatase, galactolipid and sulfolipid synthesis, diacylglycerol acyltransferase (minor), acyl-CoA synthetase, CTP-dependent lipid kinase	Digalactosyldiacylglycerol, monogalactosyldiacylglycerol, phosphatidylglycerol, sulfoquinovosyldiacylglycerol, phosphatidylcholine
Endoplasmic reticulum	Fatty acid elongase, ω3 and ω6 desaturases, other fatty acid modifying reactions, acyl-CoA thioesterase, glycerol-3-phosphate acyltransferase, lysophosphatidic acid acyltransferase, phosphatidic acid phosphatase, CTP: phosphatidate cytidylyltransferase, phosphatidylglycerol phosphate synthase and phosphatase, CDP-choline: diacylglycerol cholinephosphotransferase, phosphatidylmono- and dimethylethanolamine methyltransferases, CDP-ethanolamine: diacylglycerol ethanolaminephosphotransferase, phosphatidylserine decarboxylase, phosphatidylethanolamine: serine phosphatidyltransferase, CDP-diacylglycerol: serine phosphatidyltransferase, CDP-diacylglycerol: myo-inositol phosphatidyltransferase, diacylglycerol acyltransferase, diacylglycerol: diacylglycerol acyltransferase, some enzymes of sterol and sphingolipid synthesis, *N*-acylphosphatidylethanolamine synthase	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol
Golgi bodies	Glycerol-3-phosphate acyltransferase, lysophosphatidic acid acyltransferase, CDP-choline: diacylglycerol cholinephosphotransferase, CDP-ethanolamine: diacylglycerol ethanolaminephosphotransferase	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine
Lipid bodies	Fatty acid elongase, diacylglycerol acyltransferase, lipase	Triacylglycerol
Mitochondria	Fatty acid synthesis (minor), glycerol-3-phosphate acyltransferase, lysophosphatidic acid acyltransferase, CTP: phosphatidate cytidylyltransferase, phosphatidylglycerol phosphate synthase and phosphatase, phosphatidylglycerol: CDP diacylglycerol phosphatidyltransferase, β-oxidation	Phosphatidylcholine, phosphatidylethanolamine, cardiolipin, phosphatidylinositol
Nuclear membranes	None reported	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol

Plasma membrane	Phosphatidylinositol and phosphatidylinositol phosphate kinases, serine exchange enzyme, sterol glucosyltransferase, glucosylceramide synthase	Phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, sphingolipids, sterols and derivatives
Protein body membranes	CTP : phosphocholine cytidylyltransferase, diacylglycerol kinase, phosphatidylinositol and phosphatidylinositol phosphate kinases	Phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol
Tonoplast	None reported	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, sphingolipids, sterols and derivatives
Glyoxysomes	Lipase, β-oxidation, glyoxylate cycle	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol
Peroxisomes	β-Oxidation, acyl-CoA thioesterase	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol

[a] Information collated primarily from T.S. Moore Jr., (Ed.) (1993) Lipid Metabolism in Plants, CRC Press, Boca Raton, FL; J.L. Harwood (1989) Crit. Rev. Plant Sci. 8, 1–43. Lipids that comprise less than 5% are not listed as prominent lipids. Note that many of the fractions cited were not analyzed for sterols, sterol esters and glycosides, and sphingolipids.

are produced for export to the rest of the cell. An acyl-CoA synthetase identified on the outer membrane of plastids is thought to facilitate release of acyl groups into the cytosol. It should also be noted that, although net lipid traffic is from the plastids, this organelle can likewise be on the receiving end. In addition to small quantities of plastidial phospholipids whose head groups are not known to arise in that compartment, there may be considerable flow of extraplastidially constructed diacylglycerol backbones into the galactolipid synthesis pathway. The quantitative significance of this backflow depends on the plant species, as will be discussed in Section 5.3.

2.2. Endoplasmic reticulum and lipid bodies

The endoplasmic reticulum has traditionally been viewed as the primary source of phospholipids in plant cells. With the exception of cardiolipin, all of the common phospholipids can be produced by microsomal fractions. The endoplasmic reticulum also serves as the major site of fatty acid diversification. Although plastids do have the ability to synthesize polyunsaturated fatty acids, they are formed on acyl lipid substrates and are not typically exported. Thus, the endoplasmic reticulum desaturation pathways are of particular importance for developing seeds that store large quantities of 18 : 2 and 18 : 3. Pathways for the production of unusual fatty acids found primarily in seed oils have likewise been described in microsomes. Not surprisingly, the endoplasmic reticulum also appears to be instrumental in the formation of the triacylglycerols themselves and the lipid bodies in which they are stored (Section 7).

2.3. Mitochondria

Next to plastids and the endoplasmic reticulum, the plant mitochondrion is probably the organelle investigated the most thoroughly with respect to lipid metabolism. Its ability to synthesize phosphatidylglycerol and cardiolipin is well established. Although most fatty acids for mitochondrial membranes are imported from the plastids or the ER, recently mitochondria have been shown to synthesize low levels of fatty acids from malonate. Octanoate is a major product of this pathway and serves as a precursor for the lipoic acid cofactor needed by glycine decarboxylase and pyruvate dehydrogenase [2].

2.4. Glyoxysomes and peroxisomes

A discussion of the compartmentation of lipids and their metabolism would be incomplete without reference to the organelles responsible for fatty acid oxidation. As in mammals, there is evidence for both mitochondrial and peroxisomal β-oxidation systems. In plants, the peroxisomal system appears to be the more significant [3]. Unlike mammals, plants can use the peroxisomal enzymes to catabolize long-chain fatty acids all the way to acetyl-CoA. Under certain conditions, such as oilseed germination, plants also differentiate specialized peroxisomes called glyoxysomes. In addition to the β-oxidation pathway, glyoxysomes contain the enzymes of the glyoxylate cycle, a pathway absent from animals. Plants are able to use the glyoxylate cycle to feed the

acetyl-CoA produced by β-oxidation into carbohydrate synthesis. Since plants cannot transport fatty acids over long distances, only this conversion of acetate to sucrose, which can be transported by the plant's vascular system, makes lipid a practical carbon reserve for the growing shoots and roots of seedlings.

3. Acyl-ACP synthesis in plants

Fatty acid synthases may be classified into two groups. 'Type I' fatty acid synthases are characterized by the large, multifunctional proteins typical of yeast and mammals (Chapter 6), while 'Type II' synthases of most prokaryotes are dissociable into components that catalyze individual reactions (Chapter 3). Plants, while certainly themselves eukaryotic, appear to have inherited a Type II fatty acid synthase from the photosynthetic prokaryotes from which plastids originated.

The ground-breaking studies of Overath and Stumpf in 1964 [4] established not only that the constituents of the avocado fatty acid synthesis system could be dissociated and reconstituted, but also that the heat stable fraction from *E. coli* we now know as acyl carrier protein (ACP) could replace the corresponding fraction from avocado. Plant ACPs share both extensive sequence homology and significant elements of three-dimensional structure with their bacterial counterparts. In plants, this small, acidic protein not only holds the growing acyl chain during fatty acid synthesis, but also is required for synthesis of monounsaturated fatty acids and plastidial glycerolipids.

3.1. Components of plant fatty acid synthase

Fatty acid synthase is generally defined as including all polypeptides required for the conversion of acetyl and malonyl-CoA to the corresponding ACP derivatives, the acyl-ACP elongation cycle diagrammed in Chapter 3, and the cleavage of ACP from completed fatty acids by enzymes termed thioesterases or acyl-ACP hydrolases [5]. All components of fatty acid synthase occur in plastids, although they are encoded in the nuclear genome and synthesized on cytosolic ribosomes. Most of the 8–10 enzymes of the pathway are soluble when isolated from homogenates. Nevertheless, some evidence suggests that at least ACP and some subunits of acetyl-CoA carboxylase may be associated with the plastid membranes.

Despite the presence of acetyl-CoA : ACP acyltransferase activity in plant fatty acid synthase preparations, acetyl-ACP does not appear to play a major role in plant fatty acid synthesis (Jaworski, 1992). Instead, the first condensation takes place between acetyl-CoA and malonyl-ACP. This reaction is catalyzed by β-ketoacyl-ACP synthase III, one of three ketoacyl synthases in plant systems (Fig. 2). The acetoacetyl-ACP product then undergoes the standard reduction–dehydration–reduction sequence to produce 4 : 0-ACP, the initial substrate of ketoacyl-ACP synthase I. KAS I is responsible for the condensations in each elongation cycle up through that producing 16 : 0-ACP. The third ketoacyl synthase, KAS II, is dedicated to the final plastidial elongation, that of 16 : 0-ACP to 18 : 0-ACP.

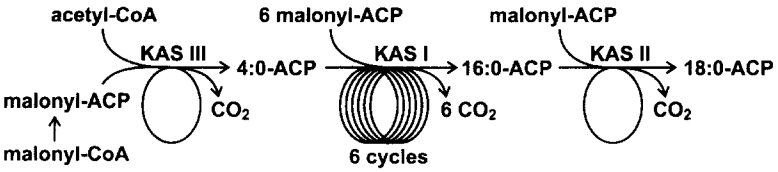


Fig. 2. Contribution of the three ketoacyl synthases (KASI, II and III) to fatty acid elongation. Each circle represents one round of the elongation cycle catalyzed by ketoacyl-ACP synthase, enoyl-ACP reductase, hydroxyacyl-ACP dehydrase, and acyl-ACP reductase.

3.2. Desaturation of acyl-ACPs

The major components of the long-chain acyl-ACP pool in most plant tissues are 16 : 0-ACP, 18 : 0-ACP and 18 : 1-ACP. This finding highlights the importance of stearoyl-ACP desaturase, the plastidial enzyme responsible for Δ^9-desaturation in plants. In contrast to the desaturation system of *Escherichia coli* (Chapter 3), the plant enzyme introduces the double bond directly to the Δ^9-position. Unlike yeast and mammalian Δ^9-desaturases, it is a soluble enzyme and is specific for acyl-ACPs rather than acyl-CoAs (McKeon, 1982). In recent years, work on stearoyl-ACP desaturase has progressed rapidly and is now providing a more detailed understanding of the fundamental mechanisms of oxygenic fatty acid desaturation. Genes for the enzyme have been cloned from a number of species, and the structure of the castor bean Δ^9-desaturase has been determined to 2.4 Å resolution. A combination of the crystal structure and spectroscopic methods has revealed two identical monomers, each with an active site containing a diiron-oxo cluster. Reduction of the iron by ferredoxin leads to its binding of molecular oxygen. The resulting complex ultimately removes electrons at the Δ^9-position, resulting in double bond formation [6].

Although the most common unsaturated fatty acids in plants are derived from oleic acid, a wide range of unusual fatty acids are found in the seed oils of different species. Divergent plastid acyl-ACP desaturases have been shown to account for some of this diversity. For example, *Coriandrum sativum* achieves seed oils rich in Δ^6-18 : 1 (petroselinic acid) by desaturation of 16 : 0 at the Δ^4-position followed by elongation, while *Thunbergia alata* attains a similar oil by direct Δ^6-desaturation of 18 : 0. Several structure–function relationships suggested by unusual desaturases have been tested in the acyl-ACP desaturase system. Shortening the acyl binding pocket of the Δ^9-18 : 0 desaturase by altering a single amino acid as in *Doxantha unguis-cati* shifts substrate specificity in favor of 16 : 0-ACP (Cahoon, 1998). In addition, a set of five specific amino acids suggested by the *Thunbergia* gene transforms the castor bean Δ^9-desaturase to a Δ^6-desaturase, while enzymes with certain subsets of the five amino acids can desaturate at either position [6].

3.3. Acyl-ACP thioesterases

Among prokaryotes, all acyl groups exiting the dissociable fatty acid synthase are transferred directly from ACP to polar lipids. However, plants must also release sufficient fatty acid from ACP to supply the extraplastidial compartments. Since the

typical chloroplast exports primarily 18 : 1 and 16 : 0, the same fatty acids that comprise the greatest fraction of long-chain acyl-ACPs, it might be assumed that a relatively non-specific thioesterase releases 16- and 18-carbon fatty acids from ACP. However, molecular and biochemical analyses of cloned plant thioesterases suggest that plants possess individual thioesterases with specificity either for 18 : 1 or for one or more saturated fatty acids [7]. The most prominent thioesterase in most plants has a strong preference for 18 : 1-ACP, making 18 : 1 the fatty acid most available for extraplastidial glycerolipid synthesis. In contrast, mangosteen, a plant with seed oil particularly high in 18 : 0, contains an 18 : 0-ACP thioesterase gene that has been used to engineer rapeseed with high 18 : 0 content (Hawkins, 1998).

Plants that synthesize certain unusual fatty acids have additional or modified thioesterases. For example, several plant species that produce storage oils containing large amounts of 8- to 14-carbon acyl chains contain thioesterases specific for those chain lengths. By removing acyl groups from ACP prematurely, the medium-chain thioesterases simultaneously prevent their further elongation and release them for triacylglycerol synthesis outside the plastids. In addition, both the standard Δ^9-18 : 1 thioesterase and a Δ^6-18 : 1 thioesterase have been purified from the Δ^6-18 : 1 accumulating coriander plant. Thus plants, by regulating expression of different thioesterases, can both fine tune and radically modify the exported fatty acid pool.

4. Acetyl-CoA carboxylase and control of fatty acid synthesis

4.1. Two forms of acetyl-CoA carboxylase

The malonyl-CoA that supplies all but two carbons per fatty acid is produced from acetyl-CoA and carbon dioxide by acetyl-CoA carboxylase (ACC). In plants, malonyl-CoA for fatty acid synthesis is apparently provided by a plastid ACC, while a cytosolic ACC contributes malonyl units for fatty acid elongation as well as synthesis of flavonoids, polyketides, and other metabolites. As with fatty acid synthase, ACC forms may be categorized either as 'eukaryotic' enzymes, which are dimers of a multifunctional polypeptide (Chapter 6), or 'prokaryotic' enzymes, which are heteromers of biotin carboxyl carrier protein, biotin carboxylase, and two subunits of carboxyltransferase (Chapter 3). In the grass family, both plastids and cytosol house 'eukaryotic' enzymes. However, dicots and monocots other than grasses appear to have both forms, with the 'eukaryotic' form limited primarily to the cytosol, and 'prokaryotic' enzymes dominating in the plastids [8]. Assembly of the 'prokaryotic' form requires participation of both the nuclear genome, which encodes biotin carboxyl carrier protein, biotin carboxylase, and the α-subunit of carboxyltransferase, and the plastid genome, which has retained the gene for the carboxyltransferase β-subunit, perhaps due to a requirement for RNA editing (Sasaki, 2000).

4.2. Acetyl-CoA carboxylase as control point

In other kingdoms, ACC is a major control point for fatty acid biosynthesis. Although the mechanisms acting in plants are incompletely characterized, there is evidence that

plant ACCs are also tightly regulated [9]. For example, both redox regulation via thioredoxin and phosphorylation of the carboxyltransferase have been implicated in up-regulation of the 'prokaryotic' ACC by light. Conversely, feedback inhibition is observed at the level of ACC when tobacco cell cultures are given exogenous fatty acids. Due to its impact on the rate of fatty acid synthesis, ACC is considered a promising target in oilseed improvement programs, and some increases in oil content have been obtained by engineering a cytosolic ACC gene to be expressed in rapeseed plastids.

5. *Phosphatidic acid synthesis: 'prokaryotic' and 'eukaryotic' acyltransferases*

Since phosphatidic acid serves as a precursor of phospholipids, galactolipids and triacylglycerols, it is not surprising that its own synthesis has been reported in four plant compartments: plastids, endoplasmic reticulum, mitochondria, and Golgi bodies. In each case, esterification of the first acyl group to the *sn*-1 position of glycerol-3-phosphate is catalyzed by glycerol-3-phosphate acyltransferase. Lysophosphatidic acid acyltransferase then completes the synthesis by acylating the *sn*-2 position. However, plastidial and extraplastidial acyltransferases show distinct differences in structure and specificity.

5.1. Plastidial acyltransferases

In the plastids, acyltransferases provide a direct route for acyl groups from ACP to enter membrane lipids. Since this is the standard pathway in *E. coli* and cyanobacteria, both the enzymes of phosphatidic acid synthesis in plastids and the glycerolipid backbones they produce are termed 'prokaryotic.' In both chloroplasts and non-green plastids, the glycerol-3-phosphate acyltransferase is a soluble enzyme that, unlike the *E. coli* enzyme, shows preference for 18 : 1-ACP over 16 : 0-ACP. The lysophosphatidic acid acyltransferase, which is a component of the inner envelope of plastids, is extremely selective for 16 : 0-ACP. The presence of a 16-carbon fatty acid at the 2-position is therefore considered diagnostic for lipids synthesized in the plastids.

5.2. Extraplastidial acyltransferases

At least superficially, the mitochondrial and Golgi acyltransferase activities resemble those of the better studied endoplasmic reticulum system. All three compartments have membrane-bound glycerol-3-phosphate and lysophosphatidic acid acyltransferases that utilize acyl-CoA substrates. In the endoplasmic reticulum, which is quantitatively the most significant of the extraplastidial sites for phosphatidic acid synthesis, saturated fatty acids are almost entirely excluded from the *sn*-2 position. The glycerol-3-phosphate acyltransferase is less selective, but, due to substrate availability, more often fills the *sn*-1 position with 18 : 1 than with 16 : 0. It is therefore possible to judge the relative contributions of the prokaryotic and eukaryotic pathways by comparing the proportions of eukaryotic 18/18 or 16/18 glycerolipids with prokaryotic 18/16 or

16/16 glycerolipids [5]. Mitochondrial lysophosphatidic acid acyltransferase, which shows little selectivity for chain length or unsaturation, can usually be ignored in discussions of lipid flow.

5.3. 16 : 3 and 18 : 3 plants

Relative fluxes through the prokaryotic and eukaryotic pathways vary between organisms and among tissues. Plastids have the potential to use phosphatidic acid from the prokaryotic pathway for all of their glycerolipid syntheses. However, not all plants do so; in some cases, the prokaryotic acyl chain arrangement is found only in plastidial phosphatidylglycerol, whereas galactolipids are derived from diacylglycerol imported to the plastids from the ER. As indicated above, the eukaryotic acyltransferases of the endoplasmic reticulum produce substantially more 18/18 than 16/18 lipids, and it is chiefly the 18/18 units that are assembled into galactolipids by plants with a minor prokaryotic pathway. Because galactolipids become highly unsaturated, plants that import diacylglycerol for galactolipids are rich in 18 : 3 and are called 18 : 3 plants. Species in which most galactolipid is derived from the prokaryotic 18/16 or 16/16 diacylglycerol contain substantial 16 : 3 and are known as 16 : 3 plants.

Kunst et al. [10] have demonstrated that a 16 : 3 plant, *Arabidopsis thaliana*, may be converted to a de facto 18 : 3 plant by a single mutation in plastidial glycerol-3-phosphate acyltransferase. Under these conditions, 16 : 3 content is reduced dramatically, and when isolated chloroplasts are labeled with glycerol-3-phosphate, only phosphatidylglycerol is labeled. Nevertheless, the percentage of galactolipids in mutant plants is practically identical to that in wild-type plants, emphasizing the ability of plants to compensate for reduction of the prokaryotic pathway. Other studies in mutants have confirmed that plants have an amazing capacity to adapt to many, but not all, perturbations of lipid metabolism (Section 11).

6. Glycerolipid synthesis pathways

In plants, glycerolipid biosynthesis involves a complex web of reactions distributed among multiple compartments [11,12]. As in mammals, the synthesis of individual glycerolipids is initiated either by the formation of CDP-diacylglycerol from phosphatidic acid and CTP, or by cleavage of phosphate from phosphatidic acid to produce diacylglycerol.

CTP : phosphatidate cytidylyltransferase has been observed in plastids, mitochondria and endoplasmic reticulum. In all three compartments, the CDP-diacylglycerol derived from phosphatidic acid is used in the synthesis of phosphatidylglycerol; in mitochondria, the reaction of phosphatidylglycerol with a second CDP-diacylglycerol then produces cardiolipin. The endoplasmic reticulum can also incorporate CDP-diacylglycerol into phosphatidylinositol and phosphatidylserine.

Phosphatidic acid phosphatase is present in the same three compartments. In the endoplasmic reticulum and mitochondria, diacylglycerol combines with CDP-ethanolamine or CDP-choline to produce phosphatidylethanolamine or phosphatidylcholine,

respectively. Although separate enzymes catalyze ethanolamine and choline transfer in animals and yeast, there are indications that a single aminoalcoholphosphotransferase may be responsible in plants. Flux into phosphatidylcholine is at least partially determined by regulation of the phosphocholine cytidylyltransferase that generates CDP-choline. The production of CDP-ethanolamine from phosphoethanolamine is less well studied, but is also considered a probable regulatory step. In addition, there is clear evidence that phosphothanolamine can be methylated to monomethylphosphoethanolamine, dimethylphosphoethanolamine, and phosphocholine, and that this pathway is inhibited by exogenous choline at the initial methylation step. The methylation of phosphoethanolamine in plants is frequently contrasted with the methylation of phosphatidylethanolamine to phosphatidylcholine in animals and yeast. In general, no significant methylation of phosphatidylethanolamine itself occurs in plants. However, phosphatidylmonomethylethanolamine is synthesized and converted to phosphatidylcholine [11].

The diacylglycerol released in plastids reacts either with UDP-galactose or with UDP-sulfoquinovose to generate sulfolipid or monogalactosyldiacylglycerol. Synthesis of digalactosyldiacylglycerol from the latter may use either UDP-galactose or a second monogalactosyldiacylglycerol as the galactose donor [12] (Kelly, 2002). There is also evidence for some extraplastidial galactolipid synthesis in plants suffering from phospholipid deficiency (Hartel, 2000).

6.1. Glycerolipids as substrates for desaturation

In addition to the soluble acyl-ACP desaturases, plants contain a number of membrane-bound enzymes that desaturate fatty acids while they are esterified within glycerolipids [5,6]. The recent cloning and characterization of these desaturases is of great interest to the scientific community because the products of the membrane-bound systems include $\Delta^{9,12}$-18 : 2 and $\Delta^{9,12,15}$-18 : 3, both of which are essential to the human diet, and thought to play a major role in human health and disease.

Once again, separate pathways occur in plastids and endoplasmic reticulum, although, as should be evident from the discussion above, fatty acids from the endoplasmic reticulum may make their way back to the plastids. Clarification of the number of desaturases involved in plant lipid metabolism and isolation of their genes has been greatly assisted by the isolation of a large number of mutants in *A. thaliana*, a small weed of the mustard family used as a model organism by plant geneticists and molecular biologists. Briefly, three membrane-bound desaturation sequences are evident in *Arabidopsis* [5,6].

(1) In chloroplasts, 16 : 0 at the 2-position of phosphatidylglycerol is desaturated to *trans*-3-16 : 1. This desaturase is most likely encoded by the *FAD4* gene.

(2) Plastids are able to convert 18 : 1 to 18 : 3 and 16 : 0 to 16 : 3 using a combination of three membrane-bound desaturases. One of them, encoded by *FAD5*, is relatively specific for the conversion of 16 : 0 on monogalactosyldiacylglycerol to Δ^{7}-16 : 1. This 16 : 1 and Δ^{9}-18 : 1 may then be given a second and third double bond by the *FAD6* and the *FAD7* or *FAD8* gene products, respectively. The latter two desaturases are less selective in their choice of glycerolipid substrate, and will

accept appropriate fatty acids on phosphatidylglycerol, sulfolipid, or either of the major galactolipids.

(3) In the endoplasmic reticulum, 18 : 1 esterified to phosphatidylcholine or occasionally phosphatidylethanolamine may be desaturated to 18 : 2 by *FAD2* and to 18 : 3 by *FAD3*.

It should be noted that fatty acids entering one of the multistep desaturation pathways listed above are not necessarily committed to completing that set of reactions. It is particularly common for 18 : 2 to be an end product of endoplasmic reticulum desaturation. This 18 : 2 may remain in phospholipid, be incorporated into triacylglycerol, or enter the galactolipid pathway and receive a third double bond in the chloroplast.

7. Lipid storage in plants

A plant stores reserve material in its seeds in order to allow seedling growth of the next generation until photosynthetic capacity can be established. The three major storage materials are oil, protein and carbohydrate, and almost all seeds contain some of each. However, their proportions vary greatly. For example, the amount of oil in different species may range from as little as 1–2% in grasses such as wheat, to as much as 60% of the total dry weight of the castor seed. With the exception of the jojoba plant, which accumulates wax esters in seeds, plants store oil as triacylglycerol (TAG).

7.1. Lipid body structure and biogenesis

In the mature seed, TAG is stored in densely packed lipid bodies, which are roughly spherical in shape with an average diameter of 1 μm (Fig. 3) [13]. This size does not change during seed development, and accumulation of oil is accompanied by an increase in the number of lipid bodies. The very large number of lipid bodies in an oilseed cell (often >1000) contrasts strikingly with animal adipose tissue where oil droplets produced in the cytosol can coalesce into a few or only one droplet. The plant lipid bodies appear to be surrounded by a lipid monolayer in which the polar headgroups face the cytosol, while the non-polar acyl groups are associated with the non-polar TAG within. The membranes of isolated lipid bodies, which comprise less than 5% of a lipid body's weight, contain both phospholipids and characteristic proteins known as caleosins and oleosins. The recently discovered caleosins are calcium binding proteins whose function is still unknown. Oleosins are small (15–26 kDa) proteins that are believed to preserve individual lipid bodies as discrete entities. Desiccated seeds lacking oleosins undergo lipid body fusion and cell disruption when rehydrated (Leprince, 1998). The cDNAs encoding many oleosins have been cloned and each has a sequence encoding a totally hydrophobic domain of 68–74 amino acids which is likely to be the longest hydrophobic sequence found in any organism. Structurally, oleosins are roughly analogous to the animal apolipoproteins which coat the surface of lipid droplets during their transport between tissues.

When a seed germinates, the TAG stored in the lipid bodies becomes the substrate for lipases. In at least some cases, peroxidation of polyunsaturated fatty acids by a lipid

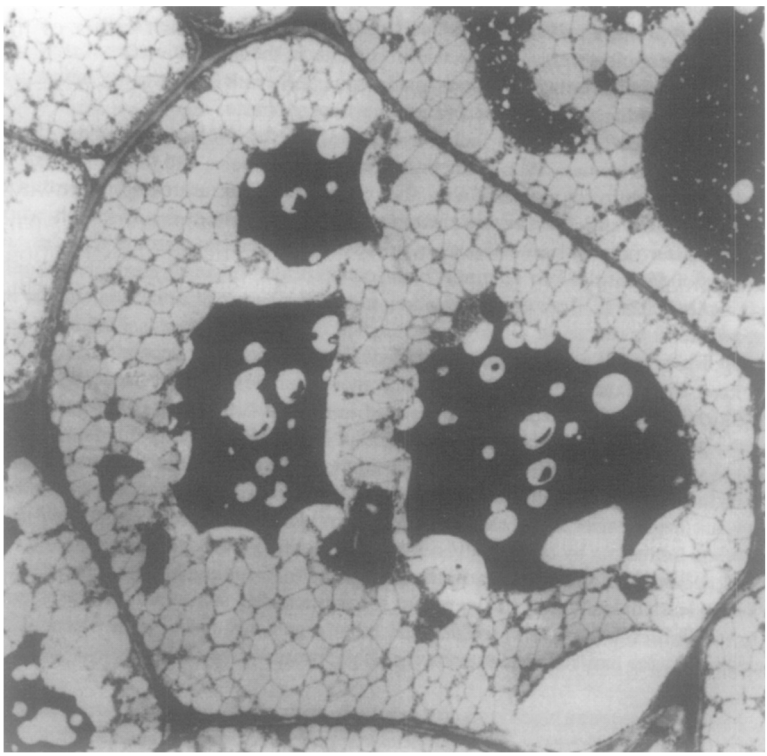


Fig. 3. Thin-sectional view of cells in a cotyledon of a developing cotton embryo harvested 42 days after anthesis. The cells are densely packed with lipid bodies and several large storage protein bodies (dark). Magnification ×9000. Photo courtesy of Richard Trelease, Arizona State University.

body lipoxygenase precedes the release of fatty acids from TAG [14]. Typically lipases and lipid body lipoxygenase are active only after germination is triggered by imbibition and other environmental signals. Fatty acids released by the lipid bodies are further metabolized through the β-oxidation pathway and glyoxylate cycle in the glyoxysomes (Section 2.4).

7.2. Seed triacylglycerols often contain unusual fatty acids

The structural glycerolipids of all plant membranes contain predominantly 5 fatty acids (18 : 1, 18 : 2, 18 : 3, 16 : 0, and in some species, 16 : 3). However, the fatty acid composition of storage oils varies far more than in membrane glycerolipids. Altogether more than 300 different fatty acids are known to occur in seed TAG [15]. Chain length may range from less than 8 to over 22 carbons. The position and number of double bonds may also be unusual, and hydroxy, epoxy or other functional groups can modify the acyl chain. Many of the different fatty acid structures, including hydroxy, epoxy, acetylenic and conjugated varieties, are now known to originate from minor modifications in the amino acid sequence of the oleate desaturase. For example, only

Table 3
Some unusual fatty acids produced in plant seeds

Fatty acid type	Example	Major sources	Major or potential uses	Approx. US market size, 10^6
Medium chain	Lauric acid (12 : 0)	Coconut, palm kernal	Soaps, detergents, surfactants	350
Long chain	Erucic acid (22 : 1)	Rapeseed	Lubricants, anti-slip agents	100
Epoxy	Vernolic acid 18 : 1Δ^9epoxy12,13	Epoxidized soybean oil, *Vernonia*	Plasticizers	70
Hydroxy	Ricinoleic acid 18 : 1Δ^9,12OH	Castor bean	Coatings, lubricants	80
Acetylenic	Crepenynic acid 18 : 2Δ^9,12yne	*Crepis foetida*	Polymers	–
Cyclopropene	Sterculic acid 19 : 1	*Sterculia foetida*	Lubricants, polymers	–
Conjugated	Parinaric 18 : 4Δ^9c11t13t15c	*Impatiens balsamina*	Coatings	–
Trienoic	Linolenic acid (α18 : 3)	Flax	Paints, varnishes, coatings	45
Wax esters	Jojoba oil	Jojoba	Lubricants, cosmetics	10

4 amino acid changes have been shown to convert a desaturase into a hydroxylase [16].

The reason for the great diversity in plant storage oils is unknown. However, the special physical or chemical properties of the 'unusual' plant fatty acids have been exploited for centuries. In fact, approximately one-third of all vegetable oil is used for non-food purposes (Table 3). Reading the ingredients of a soap or shampoo container reveals one of the major end uses of high lauric acid specialty plant oils. Other major applications include the use of erucic acid (22 : 1) derivatives to provide lubricants and as a coating for plastic films. Hydroxy fatty acids from the castor bean have over 100 industrial applications including plastic and lubricant manufacture. As discussed further below, the ability of genetic engineering to transfer genes for some unusual fatty acid production from exotic wild species to high yielding oil crops is now providing the ability to produce new renewable agricultural products and to replace feedstocks derived from petroleum.

7.3. The pathway of triacylglycerol biosynthesis

As in animal tissues, it has been suggested that TAGs are produced by a relatively simple four reaction pathway. According to this model, phosphatidic acid is synthesized by the extraplastidial pathway (Section 5) and dephosphorylated to diacylglycerol. A third fatty acid is then transferred from CoA to the vacant third hydroxyl of the diacylglycerol, producing TAG. This last and single committed step is catalyzed by diacylglycerol acyltransferase (Fig. 4, reaction 6). Although plants possess all of the enzymes for the reactions above, the assembly of three fatty acids onto a glycerol backbone is not always

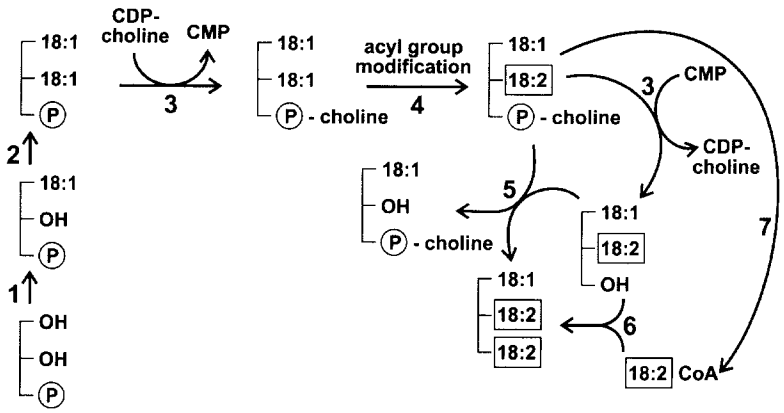


Fig. 4. Pathway depicting how flux through phosphatidylcholine (product of reaction 3) can promote acyl group diversity in plant triacylglycerols. Production of 18 : 2 (boxed) at the *sn*-2 position and its transfer to TAG is used as a sample modification. Other fatty acid alterations may be substituted. Enzymes: 1 = glycerol-3-phosphate : acyl-CoA acyltransferase; 2 = lysophosphatidic acid : acyl-CoA acyltransferase; 3 = CTP : phosphatidate cytidylyltransferase; 4 = T6 18 : 1-desaturase or other fatty acid modifying enzyme; 5 = phospholipid : diacylglycerol acyltransferase; 6 = diacylglycerol acyltransferase; 7 = acyl-CoA : phosphatidylcholine acyltransferase or phospholipase plus acyl-CoA synthetase.

as straightforward as suggested by the above pathway. In many oilseeds, pulse-chase labeling has revealed that fatty acids reach TAG only after passing through phosphatidylcholine, (or phosphatidylethanolamine to a lesser extent). Given the range of desaturation and other modification reactions that can take place on phosphatidylcholine, transit through this phospholipid helps to explain some of the fatty acid diversity in TAG.

Fatty acids from phosphatidylcholine may become available for TAG synthesis in several ways [17]. In some plants, a phospholipid : diacylglycerol acyltransferase produces TAG by direct transfer of a fatty acid from the 2-position of phospholipid to diacylglycerol (Fig. 4, reaction 5) [18]. The second mechanism by which phosphatidylcholine can participate in TAG synthesis is by donation of its entire diacylglycerol unit. In many plants, the synthesis of phosphatidylcholine from diacylglycerol and CDP-choline appears to be rapidly reversible. As shown in Fig. 4 (reaction 3), this activity of the choline phosphotransferase can allow diacylglycerol moieties modified on phosphatidylcholine to be incorporated into TAG. Finally, a fatty acid removed from phosphatidylcholine may subsequently be used for TAG synthesis. Such an 'acyl exchange' may provide acyl-CoA either by the combined reverse and forward reactions of an acyl-CoA : phosphatidylcholine acyltransferase, or by a combination of phospholipase and acyl-CoA synthase.

7.4. Challenges in understanding triacylglycerol synthesis

Although the basic reactions of TAG biosynthesis have been determined, several fundamental and potentially related questions persist. As highlighted above, TAG and membrane lipids frequently have radically different fatty acid compositions. How do

plants control which fatty acids are stored in TAG as opposed to which fatty acids are restricted to membranes? Are unusual fatty acids excluded from membranes because their physical and chemical idiosyncracies would perturb membrane fluidity or other physical characteristics? Is TAG synthesis spatially distinct from the synthesis of membrane lipids, or do enzyme specificities dictate the partitioning of fatty acid species among glycerolipids? Although all of these factors may be significant, selectivity by enzymes such as phospholipid : diacylglycerol acyltransferase for unusual fatty acids, and editing of unusual fatty acids from phospholipids, are currently the best documented [17,18].

8. Protective lipids

In plants, tissues are protected against desiccation and pathogens by both a cuticle and epicuticular wax. The cuticle itself contains some wax, but is anchored to the plant cell wall by cutin, a complex polyester of fatty acid derivatives with a wide range of oxygen-containing functional groups (Fig. 5). 16- and 18-carbon dicarboxylic acids with one or more hydroxyl groups are particularly common [19]. *Arabidopsis* transformed with cutinase from a fungal plant pathogen not only develops a leaky cuticle, but also suffers from fusions between leaves and flower parts (Sieber, 2000).

Surface waxes are complex mixtures including a range of very long-chain alkanes, aldehydes and ketones as well as wax esters and their building blocks. Although only one mutation in cutin formation has been identified (Wellesen, 2001), visual screening of plant surfaces has allowed isolation of several wax mutants blocked in malonyl-CoA-dependent elongation of fatty acids, decarbonylation and reduction (Post-Beittanmiller, 1996). The cDNA of an elongase required for extension of wax acyl units beyond 24 carbons has been cloned, and resembles the condensing enzymes involved in synthesis of erucic acid in seed oil and wax ester precursors in jojoba seeds (Millar, 1999). A cDNA encoding the wax synthase of jojoba seeds has been cloned and successfully expressed in *Arabidopsis* (Lardizabal, 2000), and may provide clues to the corresponding genes for surface waxes.

Bark, wound callus, and specialized tissues such as the endodermis that controls entry into the root vascular system, have walls lined with suberin. Suberin, like cutin, is a polyester incorporating fatty acids enriched in carboxyl and hydroxyl groups. In addition to placement on the inner surface of cell walls rather than outside, the tough, waterproof suberin differs from cutin in its preference for longer fatty acids and in its incorporation of large amounts of phenylpropanoids [19].

9. Sterol, isoprenoid and sphingolipid biosynthesis

In the plant kingdom, isoprenoids represent the most diverse range of natural products with over 25,000 lipophilic structures known, ranging from small, volatile compounds to rubber. Quantitatively, the photosynthetic apparatus is probably the primary consumer of isoprenoids, since carotenoids, plastoquinone and the phytol tail of chlorophyll

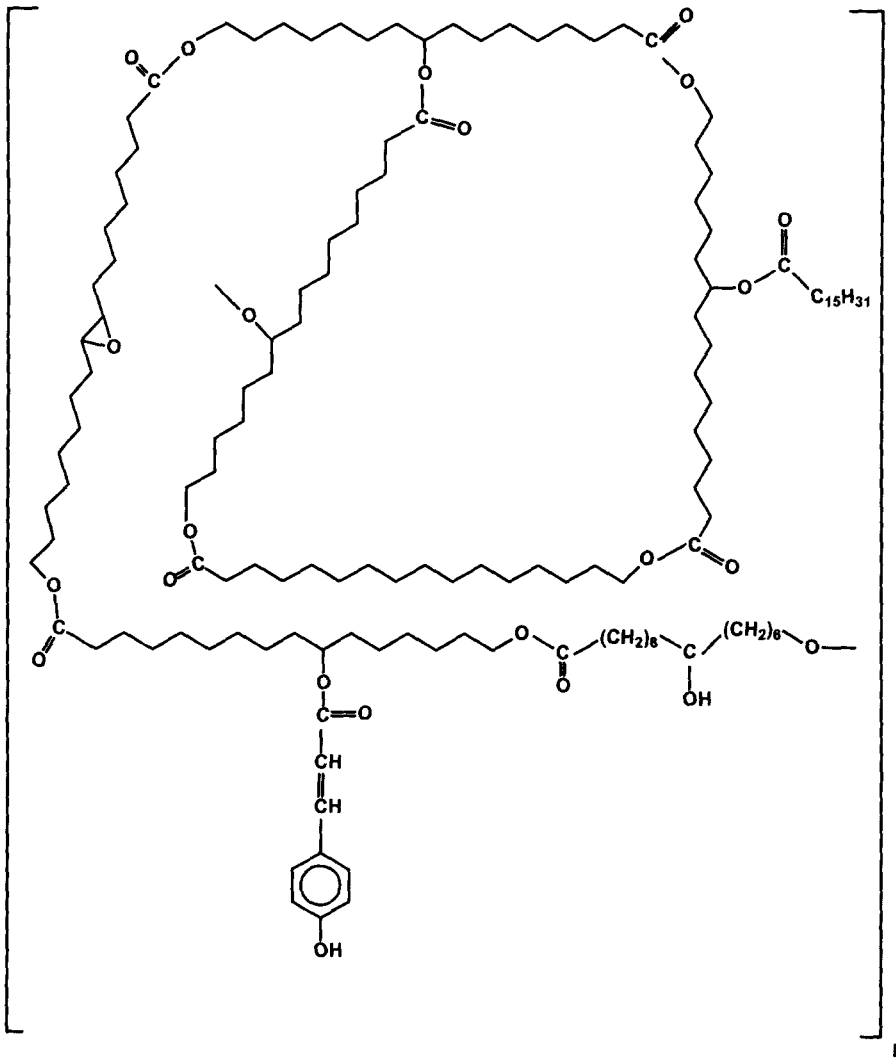


Fig. 5. Model showing some of the linkages in cutin, after Kolattukudy [19]. Note the cross-linking made possible by mono- and dihydroxy-fatty acids.

all belong to this group. Given that vital plant hormones such as gibberellin and abscisic acid, plus many defensive compounds, are isoprenoids, the early steps of this pathway have been studied intensely. However, surprisingly, it was not until the late 1990s that researchers realized that plants have two very different pathways for production of isopentenyl pyrophosphate, the five-carbon central precursor of all isoprenoids [20]. For several decades it was known that, as in other organisms, plants join three molecules of acetyl-CoA to form hydroxymethylglutaryl-CoA followed by the highly regulated reduction of that compound to mevalonic acid. Furthermore, plants contain multiple well-studied hydroxymethylglutaryl-CoA reductase genes that

are differentially expressed during development and in response to such stimuli as light, wounding and infection. It was incorrectly suspected that this 'mevalonate' pathway was localized in both cytosol and plastids and produced all classes of isoprenoids. The story has now been clarified [20] with the discovery that plastids produce isopentenyl pyrophosphate by a 'non-mevalonate' pathway that begins with the condensation of pyruvate with glyceraldehyde-3 phosphate to produce 1-deoxy-D-xylulose-5-P. At least three additional enzymes are required to produce isopentenyl pyrophosphate in the plastids. In parallel to work in plants, this pathway has also been demonstrated in bacteria and algae. The non-mevalonate pathway in plastids is responsible for production of the classic plant photosynthetic isoprenoids such as phytols and carotenoids, as well as mono- and diterpenes.

The mevalonate pathway in the cytosol is responsible for biosynthesis of sterols, sesquiterpenes and triterpenoids. After conversion of mevalonic acid to isopentenyl pyrophosphate, three C5 units can be joined head to tail to produce a C15 compound, farnesyl pyrophosphate. Two farnesyl pyrophosphates are then united head to head to form squalene, the progenitor of the C30 isoprenoids from which sterols are derived. The plant squalene synthetase, like its mammalian homologue, is found in the endoplasmic reticulum and the reaction proceeds via a presqualene pyrophosphate intermediate. In the last step prior to cyclization, squalene is converted to squalene 2,3-epoxide.

It is also in the cyclization step that photosynthetic and non-photosynthetic organisms diverge. Whereas animals and fungi produce lanosterol, organisms with a photosynthetic heritage produce cycloartenol. Despite the differences in the cyclization product, there is substantial conservation between the enzymes responsible, with 34% identity between an *Arabidopsis* cycloartenol synthase and lanosterol synthase.

A complex series of reactions including opening of the cyclopropane ring, double bond formation and isomerization, demethylation of ring carbons, and methylation of the side chain result in formation of a number of different plant sterols. Sitosterol is the most common plant sterol (Fig. 6); however, plants normally contain mixtures of sterols whose proportions differ from tissue to tissue. In addition, sterol esters, sterol glycosides, and acylated sterol glycosides are common plant constituents whose physiological significance is under scrutiny. Both cold adaptation and pathogenesis drastically alter free and derivatized sterol pools. Plants also produce a steroid hormone, brassinolide, required for both light-induced development and fertility. Interestingly, the gene for a 5α-reductase in the brassinolide pathway can complement the corresponding reductase in the testosterone pathway [21].

Sphingolipids are usually considered minor constituents of plant lipids, accounting for 5% or less of most lipid extracts. This fact, and the more complex methods needed for their identification and characterization have resulted in a comparative lack of information on plant sphingolipid biosynthesis and function. Nevertheless, sphingolipids make up a substantial proportion (25% or more) of the composition of plasma and tonoplast membranes, with the glucosylceramides constituting the largest fraction. In addition, difficulties in extraction and analysis may have led to underestimates of sphingolipid contents. As in animals (Chapter 14), sphingolipid biosynthesis begins with condensation of palmitoyl-CoA with serine to form 3-keto-sphinganine, with the enzyme from *A. thaliana* showing 68% similarity to the human homologue (Tamura,

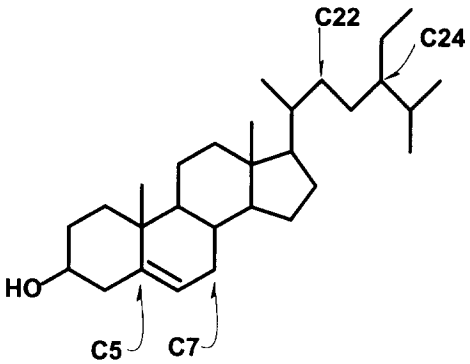


Fig. 6. Sitosterol (24α-ethylcholesterol), shown here, is the most common plant sterol, but plants generally contain complex mixtures of sterols. Other prominent phytosterols differ from sitosterol as follows. Campesterol, 24α-methyl; stigmasterol, C22 double bond; dihydrospinasterol, move double bond from C5 to C7; spinasterol, move C5 double bond to C7, add C22 double bond; dihydrobrassicasterol, 24β-methyl; brassicasterol, 24β-methyl, add C22 double bond.

2000). Reduction of 3-keto-sphinganine by an NADPH-dependent reaction yields sphinganine, and cDNAs encoding enzymes for the subsequent C4-hydroxylation and Δ^8-desaturation of sphinganine have recently been cloned [22]. Less information is available on the synthesis of ceramides and glucosylceramides, although there is evidence that either sterol glucoside or UDP-glucose could serve as the glucose donor for the latter [23]. The finding that sphingolipids serve as signal molecules in plants, particularly in the defensive apoptosis induced by some plant pathogens, has begun to stimulate interest in this relatively neglected area of plant lipid biochemistry.

10. Oxylipins as plant hormones

Jasmonate is one of several lipid-derived plant growth regulators referred to as oxylipins [24]. The structure and biosynthesis of jasmonate have intrigued plant biologists because of the parallels to some eicosanoids (Chapter 13), which are central to inflammatory responses and other physiological processes in mammals. In plants, jasmonate derives from α-linolenic, presumably released from membrane lipids by a phospholipase A. The linolenic acid is oxidized by lipoxygenase and the resulting products, 9-hydroperoxylinolenic acid or 13-hydroperoxylinolenic acid may be further metabolized by one of three routes to produce a wide variety of oxylipin (Fig. 7). The pathways by which 13-hydroperoxylinoleic acid may be metabolized include hydroperoxide lyase catalyzed scission of the *trans*-11,12-double bond to produce a C6 aldehyde, *cis*-3-hexenal, and the C12 compound, 12-oxo-*cis*-9-dodecenoic acid. The acid is subsequently metabolized to 12-oxo-*trans*-10-dodecenoic acid, the wound hormone traumatin. The enzyme hydroperoxide dehydratase (allene oxide synthase) catalyzes the dehydration of hydroperoxides to unstable allene oxides that readily decompose to form a 9,12-ketol or a 12,13-ketol. The allene oxide of 13-hydroperoxylinolenic acid may also be converted by allene oxide cyclase to 12-oxo-phytodienoic acid which can

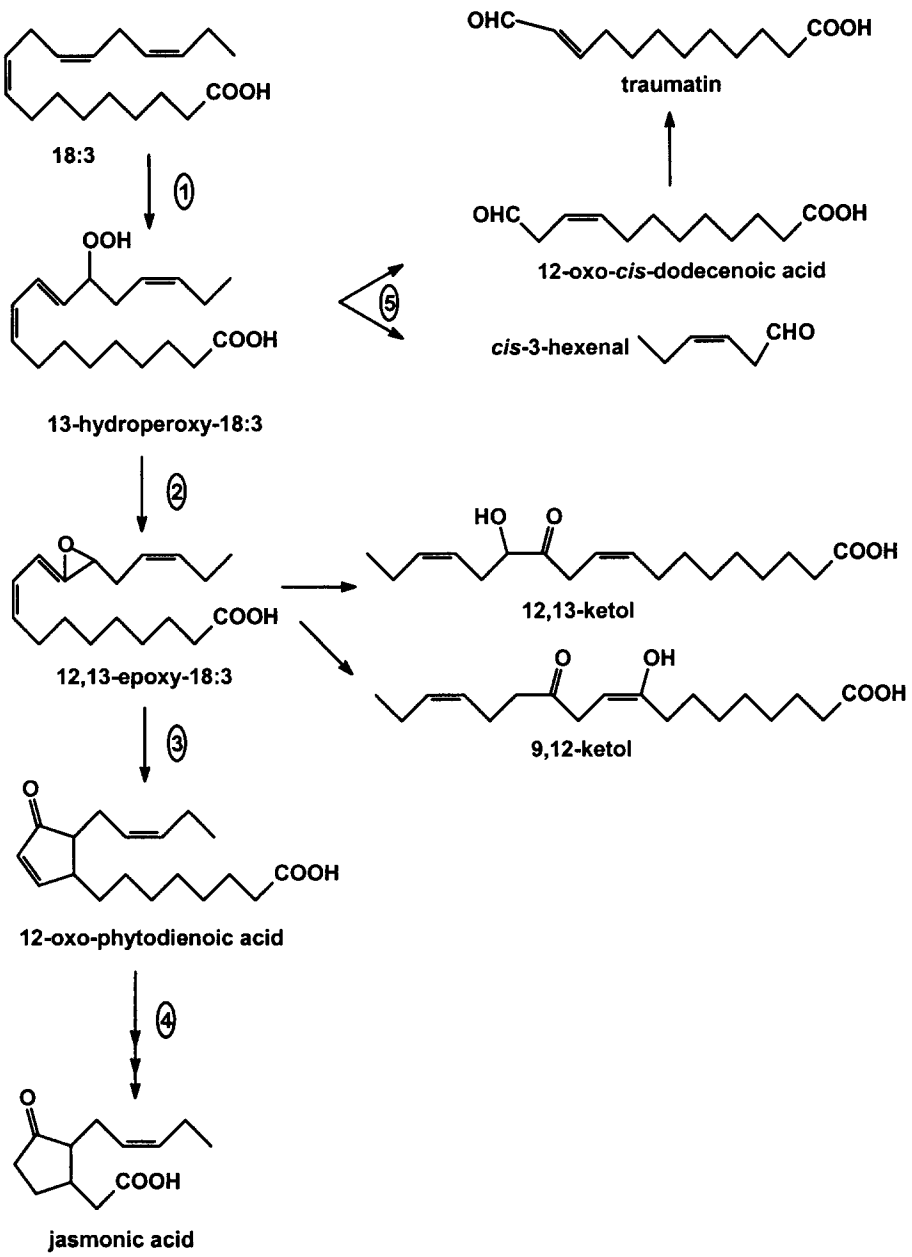


Fig. 7. Metabolism of 18 : 3 to oxylipins. 1, lipoxygenase; 2, allenoxide synthase; 3, allene oxide cyclase; 4, 12-oxo-phytodienoic acid reductase, β-oxidation; 5, hydroperoxide lyase.

be further metabolized to 7-iso-jasmonic acid. 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 leads to the induction of protease inhibitors and other defense compounds. Furthermore, mutants of tomato and *Arabidopsis* that are deficient in jasmonate synthesis are much more susceptible to insect damage. In addition to jasmonate, a number of the other oxylipins 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, C6–C10 alkenals act as volatile elicitors of a defense response in cotton.

11. Progress in plant lipid research: the value of mutants

Biochemical approaches toward understanding plant lipid biosynthesis and function provided much of the information summarized above. However, in recent years, the isolation of mutants in plant lipid metabolism has been extremely fruitful in providing new insights and new methods for gene isolation. Much of the progress in the genetic dissection of plant lipid metabolism has come from the extensive studies of Somerville, Browse and coworkers with *A. thaliana*, which has one of the smallest genomes (113 megabases) of higher plants. By using gas chromatography to screen several thousand randomly selected plants from a mutagenized population, Somerville and Browse were able to obtain an extensive collection of mutants showing altered leaf or seed fatty acid compositions. As described above, these mutants included isolates instrumental in confirming the relationships between prokaryotic and eukaryotic phosphatidic acid synthesis and in the analysis and cloning of membrane-bound desaturases.

11.1. Mutants in lipid metabolism have helped link lipid structure and function

Two other major benefits have been derived from the *Arabidopsis* lipid mutants. First, the physiological effects of the mutations have provided the opportunity to evaluate the relationships between lipid structure and function. There has been a long-term assumption, based on the strong association of high levels of polyunsaturated fatty acids with photosynthetic membranes and the conservation of this property among higher and lower plant species, that these fatty acids must be essential to photosynthesis. However, many attempts to understand the relationships between membrane fatty acid composition and cell physiology or photosynthesis have led to equivocal results. The isolation of mutants totally lacking certain unsaturated fatty acids has now provided much more convincing evaluations of their function and indeed, the results have forced re-evaluation of several previous hypotheses. For example, Δ^3-*trans*-hexadecenoate is an unusual plant fatty acid which is associated with phosphatidylglycerol of chloroplast membranes, is evolutionarily conserved, and is synthesized in coordination with the assembly of the photosynthetic apparatus. These observations led to the suggestion that Δ^3-*trans*-hexadecenoate is a highly essential component of photosynthesis. However, mutants which contain no detectable Δ^3-*trans*-hexadecenoate grow as well as wild type plants, and all photosynthetic parameters examined appear normal (Browse, 1985). A minor difference in stability of some components of the photosystem can be detected by polyacrylamide

gel electrophoresis. It has been concluded from such analyses that, although Δ^3-*trans*-hexadecenoate may facilitate assembly of the light harvesting complex into thylakoids, a more obvious phenotype could be restricted to certain unusual environmental conditions.

As mentioned above, a number of mutants blocked in the production of polyunsaturated fatty acid biosynthesis have also been isolated (Table 4). Because leaves have desaturases both in chloroplasts and in the endoplasmic reticulum, single mutations lead only to partial reduction of polyunsaturated fatty acid levels. Again, these mutants grow normally under most conditions and have normal photosynthetic parameters. However, several alterations in physiology are observed including changes in chloroplast ultrastructure, a reduction in the cross-sectional area of chloroplasts, and increased stability to thermal disruption of photosynthesis. Moreover, whereas wild-type *Arabidopsis* plants are chilling resistant and can reproduce normally at temperatures as low as 6°C, the mutants blocked in plastidial Δ^7 (*fad5*) and ω6 (*fad6*) desaturation become chlorotic at 6°C and show a 20–30% reduction in growth rate relative to the wild-type plant. The *fad2* mutants, in which the endoplasmic reticulum ω-6 desaturase is blocked, are even more sensitive to 6°C and die if left at this temperature for several days. These results demonstrate that polyunsaturated fatty acids are essential for maintaining cellular function and plant viability at low temperatures.

While most mutants which are reduced only in polyunsaturated fatty acid synthesis grow and develop normally at 22°C, a high-stearate mutant with 14% 18 : 0 is strikingly abnormal (Fig. 8) [26]. Many cell types fail to expand, resulting in mutant plants growing to less than one-tenth the size of wild-type. At higher growth temperatures (36°C), the effects are less dramatic, suggesting that the physical properties or fluidity of highly saturated membranes are less impaired under these conditions.

Other large scale alterations in membrane fatty acid composition and phenotypes have been obtained by creation of multiple-mutant lines. When mutants defective in endoplasmic reticulum ω6-desaturase were crossed with plants defective in the plastid ω6-desaturase, double mutants could be recovered only on sucrose-supplemented media. The sucrose grown plants, which contained less than 6% polyunsaturated fatty acids, were chlorotic and unable to carry out photosynthesis but otherwise remarkably normal. These results, while confirming the significance of polyunsaturated fatty acids to photosynthesis, indicate that the vast majority of membrane functions can proceed despite drastically reduced levels of polyunsaturates [27].

Triunsaturated fatty acids normally dominate chloroplast membranes and thus are the most abundant fatty acids on the planet. By constructing a triple mutant of *fad3*, *fad7* and *fad8*, it has been possible to eliminate triunsaturated fatty acids from *Arabidopsis* without affecting 16 : 2 and 18 : 2 production (McConn, 1996). Surprisingly, these plants are able to grow, photosynthesize, and even flower. However, they are male sterile and therefore cannot produce seeds. This observation led to the discovery of a very different role for jasmonate. This mutant cannot synthesize jasmonate because it lacks the 18 : 3 precursor, and 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. This result is a dramatic example of a change in fatty acid composition having a very specific effect on an essential developmental and tissue specific reproductive process.

Table 4
Biochemical and physiological responses of selected *Arabidopsis* lipid mutants [a]

Mutant	Enzyme blocked [a]	Fatty acid or lipid phenotype	Physiological response
fab1	3-Ketoacyl-ACP synthase II	16 : 0↑	Death of plants after prolonged exposure to 2°C
fab2	18 : 0-ACP Δ^9-desaturase	18 : 0↑	Dwarf at 22°C
fad4	tΔ^3, 16 : 0 desaturase?	tΔ^3, 16 : 1↓	Altered stability of photosystem?
fad5	16 : 0 Δ^7-desaturase	16 : 0↑; 16 : 3↓	Enhanced growth rate at high temperatures. Leaf chlorosis, reduced growth rate and impaired chloroplast development at low temperature.
fad6	Plastid ω-6 desaturase	16 : 1↑; 16 : 3↓	Leaf chlorosis, reduced growth rate and impaired chloroplast development at low temperature. Enhanced thermotolerance of photosynthetic electron transport at high temperatures.
fad7	Plastid ω-3 desaturase	16 : 2↑; 18 : 2↑; 16 : 3↓; 18 : 3↓	Reduced chloroplast size and altered chloroplast ultrastructure.
fad2	Cytosolic ω-6 desaturase	18 : 1↑; 18 : 2↓	Greatly reduced stem elongation at 12°C. Death at 6°C.
fad2/fad6	Plastid and cytosolic ω-6 desaturase	<6% Polyunsaturated	Loss of photosynthesis
fad3/fad7/fad8	Plastid and cytosolic ω-3 desaturase	<1% Trienoic	Male sterile, insect resistance decreased
dgd1	Digalactosyldiacylglycerol synthase	Digalactosyldiacylglycerol↓	Dwarfism, abnormal chloroplast size
act1	Plastid acyl-ACP : G3P acyltransferase	Phosphatidylglycerol↓, 16 : 3↓	Altered chloroplast structure
mgd1	Diacylglycerol glycosyltransferase	Monogalactosyldiacylglycerol↓	Abnormal chloroplast development
AS11	Diacylglycerol acyltransferase	50% Reduction in seed TAG	Slow germination
wri1	Unknown; glycolysis impaired	80% Reduction in seed TAG	Slow germination

[a] In some cases, the enzyme defect in the mutation is not known and this table lists the most likely possibility.

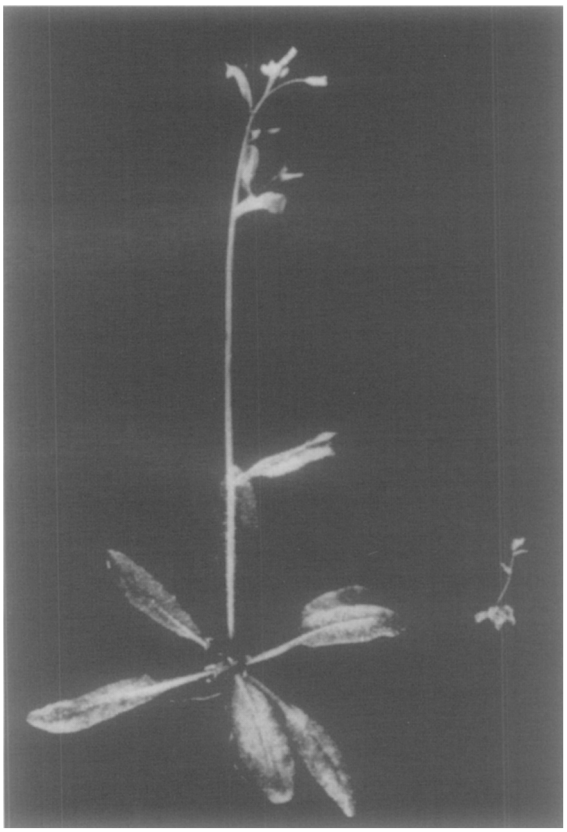


Fig. 8. Increased stearic acid causes severe dwarfing of *Arabidopsis*. A wild-type *Arabidopsis* plant (left) is compared to the *fab2* mutant (right) in which leaf stearic acid content has increased from 1 to 14%. Photo courtesy of John Browse, Washington State University.

11.2. Arabidopsis mutants have allowed cloning of desaturases and elongases

A. thaliana mutants have also provided a means of cloning genes difficult to isolate by other methods. As in other kingdoms, the membrane bound enzymes of plants have been notoriously difficult to purify and characterize. However, cDNAs or genes encoding a number of these enzymes have now been isolated using molecular genetic strategies based on mutations. Several approaches have been successful. A cDNA encoding the ω-3 desaturase which converts linoleic to linolenic acid was isolated in 1992 by Arondel et al. [28] after a mutation leading to the loss of function was genetically mapped and a yeast artificial clone corresponding to this region was selected. The genomic region was then used to screen a cDNA library, and some of the clones which hybridized to the yeast artificial chromosome had sequence similarity to cyanobacterial desaturases. These clones subsequently were shown to complement the loss of 18 : 3.

Gene 'tagging' strategies have also proved enormously valuable in identifying clones of membrane bound enzymes. *Arabidopsis* genes can be 'tagged' by insertional

inactivation when T-DNA from *Agrobacterium tumefaciens* inserts randomly in the genome. When a promising phenotype is observed, the inactivated gene can be identified by probing with the T-DNA sequence. This method was used to identify the ω-6 desaturase required for the 18 : 1 to 18 : 2 conversion in endoplasmic reticulum [25]. In addition, transposon tagging led to the cloning of a gene which controls elongation of oleic acid to 20 : 1 and 22 : 1 in developing *Arabidopsis* seeds [29]. Since no membrane-bound fatty acid elongase had ever been completely purified or cloned from a eukaryotic organism, the finding that this gene encodes a 60-kDa condensing enzyme provided the first direct evidence that membrane fatty acid elongation is not catalyzed by a type I multifunctional fatty acid synthase.

12. Design of new plant oils

In recent years, tremendous progress has occurred not only in the isolation of many plant lipid biosynthetic genes, but also in the use of these genes to manipulate plant oil composition. As shown in Table 5, both substantial changes in seed oil composition and introduction of unusual fatty acids to heterologous species have been achieved. Progress in this area has been accelerated by several industrial laboratories whose goal has been the production of higher value oilseeds.

Table 5
Some examples of genetic engineering of plant lipid metabolism

Modification achieved	Enzyme engineered	Source of gene	Plant transformed
Lauric acid production	Acyl-ACP thioesterase	California Bay	Rapeseed
Increased stearic acid	Antisense of stearoyl-ACP desaturase	Rapeseed	Rapeseed
Reduced saturated fatty acids	Stearoyl-CoA desaturase	Rat, yeast	Tobacco
	Acyl-ACP thioesterase	Soybean	Soybean
Reduced 18 : 3	ω-3 Desaturase	Soybean, canola	Soybean, rapeseed
Altered lauric acid distribution in TAG	1-Acyl-glycerol-3-phosphate acyltransferase	Coconut	Rapeseed
Altered cold tolerance	Acyl-ACP : glycerol-3-phosphate acyltransferase	*E. coli*, squash, *Arabidopsis*	Tobacco, *Arabidopsis*
Petroselinic acid production	Acyl-ACP desaturase	Coriander	Tobacco
Increased linolenic acid	ω-3 Desaturase	*Arabidopsis*	*Arabidopsis*
Cyclopropane fatty acid production	Cyclopropane synthase	*E. coli*, *Sterculia*	Tobacco
γ-Linolenic acid production	Linolenic Δ^6-desaturase	*Synechocystis*	Tobacco
Increased long-chain fatty acids	Fatty acid elongase	Jojoba	Rapeseed
Wax ester synthesis	Wax ester synthase, fatty acid reductase, fatty acid elongase	Jojoba	*Arabidopsis*

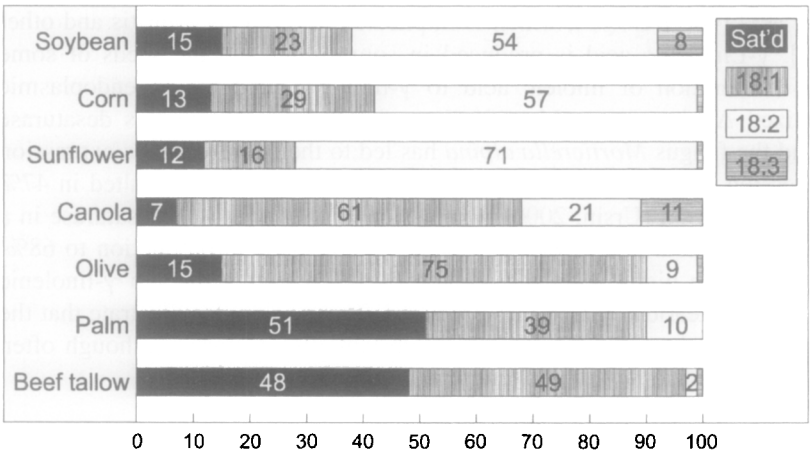


Fig. 9. Fatty acid composition of dietary vegetable oils and beef tallow. The values shown represent typical compositions of varieties grown commercially. Lines modified substantially through breeding or genetic engineering are available for soybean, canola, corn and sunflower.

12.1. Design of new edible oils

12.1.1. Improvements in nutritional value and stability of vegetable oils

Vegetable oils have gradually replaced animal fats as the major source of lipids in human diets and now constitute 15–25% of total caloric intake by industrialized nations. As shown in Fig. 9, vegetable oils display a wide range in the relative proportions of saturated and unsaturated fatty acid acids although in the United States, up to 80% of vegetable oil consumed is soybean oil making it the single largest source of calories in American diets. Most nutritionists recommend a reduction in saturated fat content in diets, and genetic engineering of plant oils can substantially help achieve this goal. Most of the saturated fatty acid in common plant oils is palmitic acid, and its occurrence is largely related to the action of a palmitoyl-ACP thioesterase. Reduction of the expression of this activity in transgenic soybean using co-suppression has led to decreases of the palmitic acid content from 15 to 6%.

A very high stability liquid soybean oil with low saturated fatty acids was also produced in soybean by suppression of the oleoyl-desaturase (Kinney, 1996). Oleic acid content was increased up to 89%, 18 : 2 content reduced from 57% to less than 3%, and saturated fatty acids reduced to less than 7%. This oil has been produced commercially and is extremely stable for high-temperature frying applications. In addition, its stability matches that of mineral oil derived lubricants, and therefore such non-food uses as biodegradable lubricants are underway. One added consumer benefit to wide future use of the engineered high-oleic acid oils may be reduction in the pathologies associated with high ω-6 consumption.

Besides reducing the amounts of *trans* and ω-6 unsaturated fatty acids in vegetable oils, efforts directed at increasing the amount of health beneficial fatty acids in vegetable oils appear to be promising. Two potential health-promoting fatty acids are stearidonic, ($18:4\text{-}\Delta^{6,9,12,15}$), an ω-3 fatty acid precursor of the ω-3 structures found in fish oils,

and γ-linolenic acid (18 : 3-$\Delta^{6,9,12}$), which is implicated in relieving arthritis and other conditions [30]. γ-Linolenic acid is produced in some fungi and the seeds of some plants by the desaturation of linoleic acid to γ-linolenic acid by an endoplasmic reticulum-localized Δ^6-desaturase. Identification of cDNAs encoding this desaturase from borage and the fungus *Mortierella alpina* has led to their heterologous expression in plants. Expression of the *Mortierella* gene in *Brassica napus* seed resulted in 47% γ-linolenic acid production (Ursin, 2000). Expression of the fungal Δ^6-desaturase in a low α-linolenic acid canola line further enhanced γ-linolenic acid production to 68%. When canola lines with high α-linolenic content were crossed with high γ-linolenic acid lines, up to 17% stearidonic acid was produced. These examples illustrate that the level of desired end-product can sometimes be substantially increased, although often unpredictably, by the choice of host and/or enzyme source.

12.1.2. Alternatives to hydrogenated margarines and shortenings

Since most vegetable oils are liquids at room temperature, the production of margarines and shortenings from such oils requires alteration of their physical properties. This is most frequently achieved by catalytic hydrogenation of the oil, a process which reduces the double bonds and thereby increases the melting point of the oil. However, hydrogenation substantially increases the saturated fat content of the oil. An additional side reaction which occurs during hydrogenation is the conversion of much of the naturally occurring *cis* double bonds to the *trans* configuration. Typically, hydrogenated oils used in margarine or shortening contain up to 25–40% saturated or *trans* fatty acids. Although convincing evidence for a deleterious effect of *trans*-isomers in the diet is lacking, some nutritionists consider reduction of *trans*-double bonds in the diet advantageous. An added disadvantage to hydrogenation is the 2–3 cents per pound cost which it adds to the price of the oil. Thus, for several reasons, an alternative to vegetable oil hydrogenation is desirable for manufacture of margarines and shortenings.

Further progress toward reducing the need for hydrogenation has been made using a molecular genetic approach to increase the melting point of rapeseed oil. As described in Section 3.2, the introduction of the first double bond into plant fatty acids occurs by the action of stearoyl-ACP desaturase. An obvious route to alter the activity of this enzyme in oilseeds is the use of antisense RNA. This objective was achieved in *Brassica napus* and other plants, by suppression of stearoyl-ACP desaturase messenger RNA, which reduced enzyme activity and desaturase protein to barely detectable levels (Knutzon, 1992). As a result, the content of stearic acid in the seed oil was increased from 2 to 40%. Due to its high saturated fatty acid content, the oil from these plants has a high melting point and may be directly suitable for margarine or shortening manufacture without added hydrogenation.

An alternative fatty acid modification that might simultaneously increase unsaturation in diets and reduce the need for hydrogenation is the production of petroselinic acid rich vegetable oils. Petroselinic acid is an isomer of oleic acid which has a *cis* double bond at the 6th carbon from the carboxyl end of the molecule rather than the 9th carbon. This minor modification of the structure alters the melting point of the fatty acid such that petroselinic acid melts at 33°C whereas oleic acid melts at 5°C. Thus petroselinic acid might provide the means to produce an unsaturated vegetable oil which

is also a solid at room temperatures and therefore ideal for margarine and shortening manufacture. Although petroselinic acid is a major component of seed oils in species such as coriander and carrot, these crops have a low yield of oil per hectare. It is therefore hoped that an oilseed crop can be engineered to become a commercially viable source of petroselinic acid. When a cDNA encoding the acyl-ACP desaturase involved in petroselinic acid synthesis was cloned from coriander and used to transform other plants, petroselinic acid was produced but only at low levels. Therefore, to achieve economic production of petroselinic acid, it may be necessary to introduce additional genes. It is now known that coriander and its relatives possess several proteins devoted to production of this special fatty acid, including ACP isoforms, a modified condensing enzyme (3-ketoacyl-ACP synthase) specific for the elongation of *cis*-4-16 : 1-ACP to *cis*-6-18 : 1-ACP and a novel acyl-ACP thioesterase specific for petroselinoyl-ACP.

12.2. Design of new industrial oils

As described above, plants have evolved the ability to produce a diverse range of fatty acid structures. A number of specialty fatty acids have already been extensively exploited for industrial uses such as lubricants, plasticizers and surfactants (Table 3). In fact, approximately one-third of all vegetable oil is now used for non-food products, and this figure is expected to increase as petroleum reserves are depleted. Thus, in addition to providing food, oilseed crops can be considered efficient, low polluting chemical factories which are able to harness energy from sunlight and transform it into a variety of valuable chemical structures with a multitude of non-food uses.

12.2.1. High laurate and caprate oils

One of the major non-food uses of vegetable oils, consuming approximately 500 million pounds of oil per annum in the US, is the production of soaps, detergents and other surfactants. The solubility and other physical properties of medium-chain fatty acids and their derivatives make them especially suited for surfactant manufacture. Coconut and palm kernel oils, which contain 40–60% lauric acid (12 : 0), are the current major feedstocks for the surfactant industry. The mechanism for synthesis of lauric and other medium-chain fatty acids in plants involves the action of a medium-chain acyl-ACP thioesterase which terminates fatty acid synthesis after a 10 or 12 carbon chain has been assembled (Pollard, 1991). A cDNA encoding such a thioesterase was isolated from seeds of the California Bay tree and transformed into rapeseed. As shown in Fig. 10, the introduction of this specialized thioesterase resulted in transgenic seeds that produce up to 60 mol% lauric acid. The plants grow normally and oil yields are very similar to those of the untransformed cultivars. Commercial production of high lauric rapeseed oil began in 1995, and this crop has the potential to provide a new, non-tropical source of lauric oils for the surfactant industry.

Since current oil crops do not produce significant amounts of caprate (10 : 0) and caprylate (8 : 0), the cloning of thioesterases from plants accumulating these species raised hopes for development of commercial sources of 8 : 0 and 10 : 0. Surprisingly, medium-chain thioesterase from elm, a 10 : 0 accumulator, recognized either 10 : 0 or 16 : 0, and a thioesterase from a C_{8-10}-rich *Cuphea* species, although showing strong

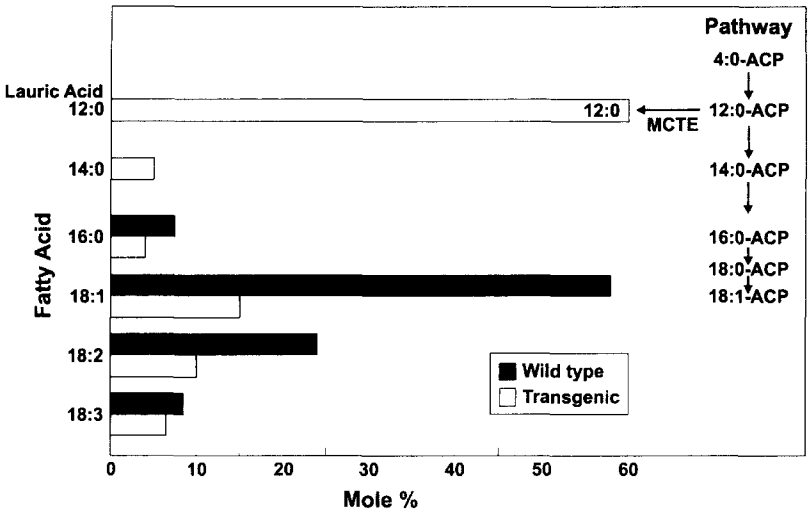


Fig. 10. Genetic engineering of rapeseed oil to produce lauric acid. mol% of major fatty acids in a typical canola cultivar are compared to the composition achieved through genetic engineering using a California Bay medium chain acyl-ACP thioesterase (MCTE) under control of a napin promoter.

selectivity for medium-chain fatty acids, gave disappointing results in transformed plants. Part of this discrepancy was resolved with the discovery that medium-chain production in *Cuphea* also involves a specific keto-acyl synthase. Cotransformation with the medium-chain thioesterase and the keto-acyl synthase IV give plants with substantial caprate and caprylate [31], and further improvements may be possible with addition of acyltransferases better able to introduce 8 : 0 and 10 : 0 to underrepresented positions in triacylglycerol [32].

12.2.2. Production of waxes

Long-chain wax esters were once harvested from sperm whales and were a major ingredient of industrial lubricants and transmission fluids. Banning of whale harvests led to searches for alternative biological sources of such structures. Jojoba, a desert shrub found in the American southwest, is the only plant species known to accumulate waxes (up to 60% dry weight) rather than TAG as a seed storage reserve. These waxes are mostly derived from C20–C24 monounsaturated fatty acids and alcohols and are synthesized by the elongation of oleate followed by reduction to alcohols by a fatty acid reductase. The wax storage lipid is formed by a fatty acyl-CoA: fatty alcohol acyltransferase, also referred to as wax synthase. The reductase and acyltransferase were purified from jojoba and the cognate cDNAs cloned. Coordinated expression of three genes, a *Lunaria annua* long-chain acyl-CoA elongase, and the jojoba reductase and acyltransferase in *Arabidopsis*, resulted in wax production at up to 70% of the oil present in mature seeds (Lardizabal, 2000). The high levels of accumulation indicated that all the genes necessary for this trait were identified. If this trait can be successfully transferred to commercial crops this would represent a large potential source of waxes used for a variety of applications including cosmetics and industrial lubricants.

12.2.3. Other industrial oils

Many of the unusual fatty acids produced by plants would have substantial value as industrial feedstocks if they were available in sufficient quantity at low prices. Examples in this category include fatty acids with hydroxy, epoxy, cyclopropane or branched chains. These specialty fatty acids are often produced in wild species which have not been optimized for high agronomic and oil yields, and therefore such specialty oils are expensive to produce. An alternative to the long-term effort required for domestication of such plants is the introduction of genes relevant to unusual fatty acid production into existing high-yielding oil crops. A step in this direction was made by the isolation of a cDNA for a fatty acid hydroxylase from the castor oil plant. When this gene was introduced into transgenic plants, approximately 20% hydroxy fatty acids were produced (Broun, 1997). Genes for specialty fatty acid production need not be isolated only from plants. As mentioned above, the stearoyl-CoA desaturases from animals and yeast are active in plants. Furthermore, the cyclopropane synthase of *E. coli* (Schmid, 1995) and desaturases from cyanobacteria and fungi have been successfully expressed in transgenic plants. Thus, in principle there are no fundamental barriers to producing a wide range of oil compositions using genes borrowed from diverse organisms. Furthermore, protein engineering offers even more possibilities to tailor the substrates and products of plant enzymes to produce 'designer oil crops' for specific end uses [6].

13. Future prospects

It is now possible to say that the biosynthetic pathways have been determined for all major plant lipids and most of the genes identified for enzymes in these pathways have been cloned. Clearly there has been great progress, although the biosynthetic pathways of sphingolipids, *trans*-Δ^3-16 : 1 and some details of several pathways remain elusive. In addition, the enzymes involved in the production of many unusual fatty acids found in seed oils are largely unexplored.

One area of expanding interest is the production of lipid hormones and signal molecules. Several lipids including phosphatidylinositol phosphates, diacylglycerol and *N*-acylphosphatidylethanolamine have been implicated in signal transduction in plants. Another intriguing similarity between plants and animals is their use of oxygenated fatty acids in response to wounding. As mentioned in Section 10, jasmonate, a plant growth regulator derived from 18 : 3, is able at femtomolar concentrations to induce proteinase inhibitors and other plant defense genes. Like leukotriene synthesis in animals, jasmonate biosynthesis begins with the generation of a hydroperoxide by lipoxygenase. Jasmonate itself contains a cyclopentane ring comparable to those of prostaglandins. The common roles and origins of oxygenated fatty acids in plants and animals suggest a very early common ancestor for these pathways.

Application of molecular genetics and genomics to problems in lipid biochemistry should continue to expand. Particularly stimulating has been the complete sequencing of the *Arabidopsis* genome and the availability of over 1 million expressed sequence tag (EST) sequences from a variety of plants. A recent survey of *Arabidopsis* genes involved in plant acyl lipid metabolism identified over 450 genes

Table 6
Internet resources related to plant lipid metabolism

Organization	Web site content	URL
National Plant Lipid Consortium (NPLC)	Directory of scientists involved in plant lipid research. E-mail newsgroup for information on plant lipids. Abstracts of NPLC meetings	http://www.msu.edu/user/ohlrogge/
Michigan State University	Survey and catalog of genes for plant lipid metabolism.	http://www.canr.msu.edu/lgc/index.html http://www.plantbiology.msu.edu/gene_survey/front_page.htm
	Gene expression profiles based on plant lipid ESTs and microarrays	http://www.bpp.msu.edu/Seed/SeedArray.htm
Kathy Schmid, Butler University	Links to many oilseed research laboratories and web sites	http://blue.butler.edu/~kschmid/lipids.html
Benning and Ohlrogge Laboratories	Database and analysis of >10,000 ESTs from developing *Arabidopsis* seeds	http://benningnt.bch.msu.edu
USDA Oilseed Database	Data on fatty acid composition of seeds of thousands of species	www.ncaur.usda.gov/nc/ncdb/search.html-ssi

(http://www.plantbiology.msu.edu/gene_survey/front_page). Although most of these could be assigned a tentative function based on sequence similarity to previously characterized genes, only a handful have been examined individually at an experimental level, and therefore the precise function of hundreds of genes awaits further work. For example, there are 8 genes which are similar to acyl-CoA desaturases whose function has not yet been identified and there are 19 genes for lipid transfer proteins and 51 additional lipid transfer protein-like genes. The physiological reasons underlying the existence of large gene families for lipid transfer proteins, plastid ACPs, and stearoyl desaturases, but only one gene for keto acyl-ACP synthase III and for most fatty acid synthase and ACC subunits, etc. remain to be elucidated. The availability of large collections of T-DNA insertion mutants means that the impact of gene disruptions can be tested. However, because 60% of *Arabidopsis* genes are present as duplicates, such gene disruptions must be supplemented by other strategies of functional genomics. Some websites related to these efforts in the plant lipid field are presented in Table 6.

Much of past lipid research has focused on a reductionist approach in which cells are taken apart and their pieces analyzed. The overall success of this approach and the wealth of new clones and sequence information have given us an unprecedented knowledge of the pieces of the puzzle which represent lipid metabolism. However, as in any puzzle, it is not just complete knowledge of the pieces, but an understanding

of how (and when) they fit together that defines the challenge. Microarrays that permit simultaneous monitoring of expression of many genes have begun to provide a more global overview of how genes work together to control seed metabolism (Girke, 2000). Together with the ability to rapidly over- and under-express genes in transgenic plants and the strengths of classical biochemistry, such recent advances in analytical techniques should allow us to enter a new stage of lipid research emphasizing the interplay between metabolic compartments and the control of lipid synthesis during the plant life cycle.

Abbreviations

ACC	acetyl-CoA carboxylase
ACP	acyl carrier protein
EST	expressed sequence tag
CoA	coenzyme A
TAG	triacylglycerol

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