Plant Lipids: Metabolism, Mutants, and Membranes

CHRIS SOMERVILLE AND JOHN BROWSE

The mechanisms that regulate plant lipid metabolism determine the dietary and industrial value of storage oils found in economically important species and may control the ability of many plants to survive exposure to temperature extremes. Many of the problems researchers have in defining the pathways, enzymes, and genes involved in plant lipid metabolism appear to be amenable to analysis by genetic approaches. Mutants with alterations in membrane lipid composition have also been used to study the structural and adaptive roles of lipids. The application of genetic engineering methods affords opportunities for researchers to apply knowledge gained about plant lipid metabolism toward enhanced use of plant oils as abundant and renewable sources of reduced carbon.

MANY OF THE QUESTIONS CONCERNING LIPID METABOlism in higher plants are similar or identical to those that orient research on organisms from other orders. In particular, there is broad interest in elucidating the enzymatic pathways involved in lipid synthesis and breakdown, characterizing the relevant enzymes, and defining the mechanisms that regulate membrane and storage lipid compositions. However, there are characteristic themes superimposed on research in plant lipid biochemistry that provide unique dimensions. Because plants are valued for a multitude of uses, few advances in our understanding of plant biology fail to improve the suitability of plants for human purposes. For example, detailed knowledge about plant lipid metabolism can be applied to the development of new or improved plant oils.

In contrast to the rather limited subset of fatty acids found in field crops, there is substantial diversity in the structures of fatty acids and lipids that are accumulated as storage reserves by various wild species (*1*). For every fatty acid with an even number of carbons from 8 to 24, a plant that accumulates it has been identified. These fatty acids may have double or triple bonds at many positions along the chain, and these unsaturations may be accompanied or replaced by various substituents such as cyclopropene, hydroxyl, or epoxy groups (Fig. 1). Many of these fatty acids could be of substantial industrial use if they could be produced with the same efficiency as edible oils and in comparable quantities. The recent development of facile methods for the introduction of cloned genes into higher plants has made possible the production of useful new oils by the transfer of genes for fatty acid–modifying enzymes from undomesticated species to crop species.

A second research theme is the role of membrane lipid composition in the ability of higher plants to survive temperature stress. Many higher plants are subjected to wide seasonal variations in growth temperature and may experience temperature-induced injury at both extremes (*2, 3*). For instance, many plants of tropical origin are injured by exposure to low, nonfreezing temperatures that would not harm plants from temperate zones. Some plants acclimate so that they are able to survive exposure to freezing if they are first given a period of gradual exposure to low temperatures. Similarly, if given a period of growth in progressively warmer conditions, many species acclimate to thermal extremes that injure nonacclimated plants. Recently, evidence from several experimental approaches has implicated membrane lipid composition as a factor in temperature tolerance mechanisms. Because many of the same enzymes participate in both membrane and storage lipid synthesis, research efforts on developing genetic methods to modify the composition of storage oil have joined efforts to test several prominent hypotheses about temperature tolerance.

Many higher plants have properties that facilitate the application of genetic methods to problems in biochemistry and physiology. Diploid species such as barley, maize, or *Arabidopsis thaliana* can be mutagenized at high rates with chemicals or ionizing radiation; it is frequently possible to identify mutants deficient in activity for any inessential gene product by screening only a few thousand individuals (*4*). In addition, the development of methods to isolate genes by the chromosome walking technique (*5*) affords researchers an opportunity to exploit *Arabidopsis* mutants not only for use in comparative physiological analyses but also to clone genes that are not accessible by other approaches. We have undertaken detailed genetic (*6*) and biochemical (*7*) analyses of lipid metabolism in this species. In this article we use the information available about *Arabidopsis* as a framework to discuss recent progress and some central questions and goals in plant lipid metabolism. We have not addressed recent work implicating inositol lipids (*8*) and jasmonic acid (*9*) in signal transduction pathways, the studies of the role of lipid composition in freezing tolerance (*10*), or the large body of genetic and biochemical work concerning the biosynthesis and function of cuticular lipids (*11*).

Glycerolipid Synthesis

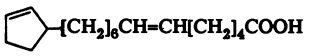
The membrane lipid composition of plants differs from the membrane lipid composition of animals and fungi primarily in the composition of the chloroplast membranes, which are composed almost entirely of glycerolipids containing sugar head groups (galactose or a sulfonated hexose, 6-sulfoquinovose). The lipid compo-

C. Somerville is at Michigan State University, U.S. Department of Energy Plant Research Laboratory, East Lansing, MI 48824. J. Browse is at the Institute for Biological Chemistry, Washington State University, Pullman, WA 99164.

1. $CH_3[CH_2]_4CH{=}CHCH{=}CHOCOCH_2CH{=}C{=}CH[CH_2]_5COOH$

2. $H_3C[CH_2]_4C{\equiv}CCH{=}CH[CH_2]_7COOH$

3. $HC{\equiv}C[CH_2]_7C{=}C[CH_2]_6COOH$ (with CH_2 bridge)

4.  $[CH_2]_6CH{=}CH[CH_2]_4COOH$

5. $CH_3[CH_2]_4CH_2CHCHCH{=}CH[CH_2]_7COOH$ (with O)

6. $CH_3[CH_2]_5CH(OH)CH_2CH{=}CH[CH_2]_7COOH$

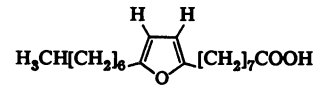
7. 

Fig. 1. Structural formulas for some unusual fatty acids accumulated in seed oils of higher plants: 1, a component of stillingia oil from *Sapium sebiferum* seed oil; 2, crepenynic acid from the seed oil of *Crepis foetida*; 3, sterculynic acid from *Sterculia alata* seed oil; 4, gorlic acid, one of several cyclopentene acids accumulated by members of the family Flacourtiaceae; 5, vernolic acid from the seeds of *Vernonia galamensis*; 6, ricinoleic acid from the seeds of *Ricinus communis*; and 7, a furan-containing fatty acid from the seed oil of *Exocarpus cupressiformis*.

sition of chloroplasts is similar to that of cyanobacterial membranes (*12*), the presumed ancestor of the chloroplast. Fatty acids and lipids are not transported between the cells of higher plants. Thus, each cell is autonomous with respect to lipid synthesis, and the overall lipid and fatty acid composition may vary widely from one cell type to another. Most or all fatty acid synthesis occurs in the plastid (*13*), an organelle that assumes a range of developmental states in different tissues. Elongation of fatty acids to chain lengths of more than 18 carbons occurs later in the cytosol.

Although the overall pathway of fatty acid synthesis in plants is similar to that in other organisms, the plant enzymes differ in several important respects from those in other eukaryotes (*13*). The reactions of the fatty acid synthesis cycle are catalyzed by individual polypeptides, as is the case in *Escherichia coli*, rather than by multifunctional polypeptides, as in animals and fungi. The use of these individual polypeptides permits changes in the stoichiometry of the individually catalyzed reactions by the use of different isozymes in different cell types and for different substrates. For example, evidence indicates the existence of three isozymes of 3-ketoacyl:acyl carrier protein (ACP) synthase that are expressed concurrently in the plastid (*14*). Isozyme III appears to initiate the synthesis of the acyl chain, isozyme I elongates the acyl chain to 16:0-ACP, and isozyme II is specifically required for the elongation of 16:0-ACP to 18:0-ACP [see (*15*) for an explanation of fatty acid abbreviations]. Thus, the plant may vary the ratio of these enzymatic activities to regulate the ratio of C_{16} fatty acids to those with C_{18}.

The 18:0-ACP is rapidly and efficiently converted to 18:1-ACP within the chloroplast by the only known soluble desaturase (*16*). Because this enzyme does not desaturate 16:0-ACP, the regulation of elongation of 16:0-ACP to 18:0-ACP is a key factor in determining the level of saturated fatty acids found in both membrane and storage lipids. The amino acid sequence of this unique desaturase (deduced from complementary DNA sequences) is highly conserved among plants but shows no detectable homology to the corresponding enzymes from yeast or vertebrates, suggesting that the plant enzyme is independently evolved (*17*). No sequence information is available for the corresponding enzyme from bacteria.

In contrast to the 3-ketoacyl:ACP synthase, no clear role has been identified for the multiple isoforms of ACP that are encoded by a gene family (*18*) and are found in all vascular and nonvascular multicellular plants (*19*). The presence of only one form in unicellular algae and cyanobacteria (*19*) suggests that the isoforms in higher plants function in tissue specificity. This idea is supported by the observation that one of the two isoforms in spinach is specific to the leaf, whereas the other is constitutive. The discovery of ACP in plant mitochondria (*20*) provides an explanation for at least one of the genes but raises the more provoking question as to the function of ACP in mitochondria (fatty acid synthesis has not been demonstrated in this organelle). Because ACP can participate as an acyl carrier in reactions other than fatty acid synthesis, one possibility is that the mitochondrial form, and possibly other forms, are involved in secondary acylation reactions.

Because plants are unique in that they acquire reduced carbon by photosynthetic CO_2 fixation, some of the mechanisms that regulate fatty acid synthesis in plants differ from those in organisms that acquire carbon by catabolism. For instance, fatty acid synthesis in leaves is sixfold higher in the light than in the dark. Although the mechanistic basis of this effect has not been established, a recent analysis of the effects of illumination on the composition of the acyl-ACP pool provided in vivo evidence that the rate-limiting step in fatty acid synthesis in leaves was acetyl–coenzyme A (CoA) carboxylase (*21*), which is also thought to be the principal site of regulation in animals and fungi. Thus, although the mechanism regulating acetyl-CoA carboxylase activity is different in plants and animals, the "logic" of metabolic control appears to be similar.

There are two main sites of lipid synthesis, the plastid and the endoplasmic reticulum (Fig. 2). In addition, the recent characterization of several mitochondrial acyltransferases suggests that the mitochondrion is at least partially autonomous with respect to lipid synthesis (*22*). Because of the acyl-group specificity of the acyltransferases in the various organelles, lipids synthesized in the plastid have a C_{16} fatty acid on the *sn*-2 position (*15*), whereas lipids synthesized in the endoplasmic reticulum have a C_{18} fatty acid on the *sn*-2 (*23*). This "molecular pedigree" permits the measurements of the relative flux of fatty acids through the two cycles (Fig. 2). In *Arabidopsis*, about half of the fatty acids remain in the chloroplast and are made into chloroplast-specific lipids by a series of reactions that are collectively termed the prokaryotic pathway (*7, 23*). The other half are exported from the chloroplast and are converted to lipid in the endoplasmic reticulum by a parallel pathway termed the eukaryotic pathway. Curiously, a substantial proportion of the lipid synthesized in the endoplasmic reticulum may be reimported into the chloroplast.

The relative flux through the two pathways varies from species to species (*23*). In plants such as pea and barley, all but a few percent of the fatty acids are incorporated into glycerolipids in the endoplasmic reticulum, so that the chloroplast is almost entirely dependent on imported lipids. By contrast, in many green algae the chloroplast is almost entirely autonomous with respect to membrane lipid synthesis. The presence of this polymorphism was recognized 20 years ago in the observation that some angiosperms (designated "16:3 plants") have substantial quantities of 16:3 fatty acids in their leaf lipids, whereas others ("18:3 plants") do not (*24*). Because lipids containing 16:3 fatty acids are only produced by the prokaryotic pathway, the amount of lipids with 16:3 fatty acids reflects the relative flux through the two pathways. As a general rule, primitive angiosperms and lower plants have the highest amount of flux through the prokaryotic pathway. However, there is no obvious taxonomic grouping of the angiosperm families based on 16:3 plants or 18:3 plants, suggesting that the loss of the plastid pathway has occurred independently several times.

Researchers do not know why some plants use both pathways, whereas other plants have largely dispensed with the prokaryotic pathway. The existence of the two pathways may provide greater metabolic flexibility, which may be of adaptive significance. For instance, it was observed that in the desert shrub *Atriplex lentiformis* the 16:3 fatty acids virtually disappear after acclimation to an elevated growth temperature (*25*). A possible interpretation is that the flux of fatty acids through the prokaryotic pathway is repressed at high temperature. However, it is not yet apparent what advantage this may have for the organism.

Mutant Analysis

By screening for natural or induced variations in crop species, plant breeders have identified many useful genetic variants in the fatty acid compositions of seed oils [reviewed in (*26*)]. A notable example is the elimination of high concentrations of the fatty acid 22:1 (erucic acid) from rapeseed oil in response to concerns that dietary 22:1 fatty acid might be associated with a high incidence of myocarditis in rats (*27*). Oil from the low-erucic cultivars (designated canola in North America) is now considered to be one of the most beneficial sources of dietary lipid (*28*). We have isolated a range of mutations that affect lipid metabolism in the small mustard *Arabidopsis thaliana* by taking small samples of leaf or seed material from randomly chosen plants in a mutagenized population and measuring the fatty acid compositions by gas chromatography (*6*). By screening approximately 10,000 individuals, we have isolated mutations affecting at least 12 of the steps in glycerolipid metabolism; seven classes of the mutants are shown in Fig. 2. None of the mutants can be readily distinguished from the wild type by visual inspection under normal growth conditions.

Most of the mutations cause the loss or reduction in the amount of an unsaturated fatty acid and the corresponding accumulation of a less unsaturated precursor. Thus, it is inferred that the *fadA* (*6*), *fadB* (*29*), *fadC* (*30*), *fadD* (*31*), *fad2*, and *fad3* (*32*) mutants are defective in the desaturation of lipid-linked fatty acids. Because researchers have been unable to detect activity for most of the desaturases in vitro, very little was known about the number of desaturases, the identity of substrates or electron donors, or their cellular localization before analyses of the effects of the mutants. Analyses of the effects of each of the *fad* mutations on the acyl composition of the various lipids confirmed metabolic labeling studies that indicated that, except for the ω*9* double bond in C_{18} fatty acids, double bonds are introduced into fatty acids esterified to lipids, rather than to acyl-CoA or acyl-ACP (*33*). Thus, for instance, the *fadA* mutants are specifically defective in the accumulation of a *trans*-fatty acid at the *sn*-2 position of phosphatidylglycerol (PG) in the plastid (*6*). Similarly, *fadB* affects the desaturation of a 16:0 fatty acid to 16:1 at the *sn*-2 position of monogalactosyldiacylglycerol in the plastid (*29*). By contrast, the other desaturases [for example, those controlled by the *fadC* and *fadD* genes (*30, 31*)] are not specific for the length of the fatty acid, the fatty acid's position on the glycerol backbone, or the nature of the lipid headgroup. Including the stearoyl-ACP desaturase gene for which there is no known mutant, there are at least eight genes that control the activity of specific desaturases in the leaf. All of the *fad* mutations have been genetically mapped and form the basis for attempts to isolate the corresponding genes by the chromosome walking technique from flanking restriction fragment length polymorphisms.

Analysis of the effects of these mutations has provided several insights into the regulation of membrane lipid desaturation. In general, the mutations have metabolic consequences that are similar to simple blocks in a biosynthetic pathway; the precursor accumulates at the expense of the product. However, in the case of the *fadB* mutant, which is deficient in the desaturation of a 16:0 fatty

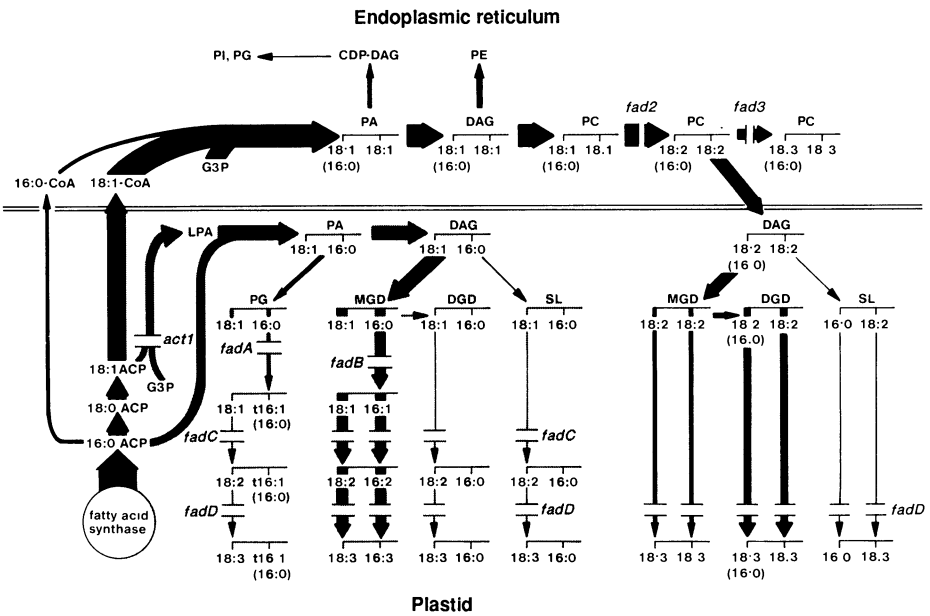


Fig. 2. The major pathways of glycerolipid synthesis in leaf cells of *Arabidopsis*. The width of the arrows indicates the flux through the various steps (*7*). The breaks in the pathway represent the enzymatic deficiencies in various mutants of *Arabidopsis*. The series of reactions within the shaded box is referred to as the prokaryotic pathway. Except for the reactions of fatty acid synthesis, the reactions outside the shaded box are collectively referred to as the eukaryotic pathway. Both pathways are initiated by the synthesis of 16:0-ACP and 18:0-ACP within the plastid. Most of the 18:0-ACP is desaturated to 18:1-ACP by a soluble desaturase. The acyl-ACPs may be used within the plastid for the synthesis of phosphatidic acid (PA) or hydrolyzed to free fatty acids, which move through the plastid envelope and are converted to CoA thioesters in the outer envelope membrane by acyl-CoA synthetase. Further desaturation of fatty acids is catalyzed by enzymes that act on glycerolipids. The chloroplast desaturases use ferredoxin as the electron donor (*64*), whereas the microsomal ω6 desaturase uses cytochrome b_5 (*65*). PA made by the prokaryotic pathway, which has a 16:0 fatty acid at the *sn*-2 position, is used for the synthesis of phosphatidylglycerol (PG), diacylglycerol (DAG), monogalactosyldiacylglycerol (MGD), digalactosyldiacylglycerol (DGD), and sulfolipid (SL). Acyl groups exported from the plastid are used for synthesis of PA in the endoplasmic reticulum. PA made by this pathway, which has a C_{18} fatty acid at the *sn*-2 position, gives rise to phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI), which are characteristic of various extrachloroplast membranes. A substantial proportion of the DAG is transferred by an unknown mechanism to the chloroplast envelope where it contributes to the synthesis of plastid lipids. In many species of higher plants, PG is the only product of the prokaryotic pathway, and the remaining chloroplast lipids are synthesized entirely by the eukaryotic pathway. In other species, such as *Arabidopsis*, both pathways contribute about equally to the synthesis of MGD, DGD, and SL, and the leaf lipids characteristically contain substantial amounts of 16:3 fatty acids, which are found only in MGD and DGD molecules produced by the prokaryotic pathway. The remaining labels are as follows: G3P, glycerol 3-phosphate; CDP-DAG, cytidine diphosphate–DAG; LPA, lysophosphatidic acid.

acid to 16:1, there is also a significant decrease in the proportion of chloroplast lipids synthesized by the prokaryotic pathway (*29*). Because the *fadB* mutation affects only lipids synthesized by the prokaryotic pathway, this decreases the effects of the mutation on the level of membrane lipid unsaturation. The implication is that there is a mechanism that senses some property of the membrane affected by the mutation and partially compensates for the defect by adjusting the flux between the prokaryotic and eukaryotic pathways. A second set of observations about the mechanisms that regulate the desaturases was obtained from an examination of F_1 hybrids from a cross of the wild type with the *fad* mutants. For the *fadA*, *fadD*, and *fad3* mutations, the heterozygotes had fatty acid compositions almost exactly intermediate between those of the wild type and those of the homozygous mutants. By contrast, in the *fadB*, *fadC*, and *fad2* mutants, the heterozygotes more closely resembled the wild type, indicating that a single functional gene resulted in adequate levels of activity. These observations suggest that the activity of the terminal enzyme in each pathway is determined by the amount of gene expression, whereas the activity of the intermediate steps in each pathway appears to be regulated by the activity of the particular enzyme.

In a wide variety of organisms the degree of fatty acid unsaturation increases when the organism is exposed to low temperature. In the leaves of many plants, the effect of cold treatment on fatty acid composition is slight because the membranes are highly unsaturated at normal growth temperatures. However, studies with the *fadD* mutant indicate the presence of a cold-induced desaturase that is not expressed at normal growth temperatures (*34*). The existence of this enzyme, which appears to be an isozyme of *fadD*, became apparent when it was found that three independent *fadD* mutants had wild-type fatty acid compositions at low temperatures but were deficient in trienoic fatty acids (16:3 and 18:3) at growth temperatures above 26°C. The mechanism by which temperature regulates this inducible desaturase activity is not yet apparent. However, cold-induced desaturase activity is pronounced in certain species of cyanobacteria (*12, 35*). The isolation of a desaturase gene from the cyanobacterium *Synechocystis* PCC 6803 (*36*) should permit an analysis of the involvement of the low-temperature regulation of gene expression in this phenomenon (*3*).

A number of *Arabidopsis* mutants were also recovered that have altered fatty acid compositions as a result of defects in fatty acid elongation or in other reactions of lipid synthesis (*32*). The *act1* mutants are deficient in plastid glycerol 3-phosphate (G3P) acyltransferase activity, the first enzyme of the prokaryotic pathway (*37*). As a result of this mutation, all but a small percent of the fatty acids made in the chloroplast are exported to the eukaryotic pathway. Thus, the mutation effectively converts a 16:3 plant into an 18:3 plant. In spite of the increased flux of lipid through the eukaryotic pathway, an equivalent total amount of fatty acid is made. In addition, the same amount of lipid is retained in the endoplasmic reticulum for the biogenesis of extrachloroplast membranes, but approximately three times the normal amount of lipid is transferred back to the plastid so that the amount of accumulated chloroplast membrane lipid remains the same. The ability of the mutant to compensate for the loss of the prokaryotic pathway raises questions about the mechanisms that determine how much of a particular membrane lipid is accumulated. Presumably, in this case, because of the accumulation of protein in the developing chloroplast membranes, these membranes became a "sink" for the additional lipid synthesized by the eukaryotic pathway.

In order to understand the mechanisms by which the *act1* mutants compensate for the loss of the prokaryotic pathway, it is necessary to elucidate the mechanism by which the endoplasmic reticulum directs lipids to the chloroplast. Unfortunately, with the possible exception of the plasma membrane, it is not clear how lipid moves between membranes in any eukaryote (*38*). There is no evidence for vesicular traffic between the endoplasmic reticulum and the chloroplast. Nor is there evidence for lipid transfer by membrane contact between organelles. Therefore, researchers have focused their attention on the role of a class of 9-kD polypeptides that are called lipid transfer proteins because they catalyze the (nonspecific) exchange of lipids between membranes in vitro. These polypeptides are among the most abundant plant proteins and account for as much as 4% of the soluble protein obtained from crude leaf homogenates (*39*). The participation of the lipid transfer proteins in intraorganelle lipid transfer has been questioned by the recent discovery that these polypeptides are cotranslationally inserted into the lumen of the endoplasmic reticulum (*40*). It is therefore unlikely that they could appear in the cytoplasm. The function of these proteins is currently being evaluated in transgenic *Arabidopsis* plants in which the amount of lipid transfer protein has been reduced by the expression of antisense RNA for the corresponding gene (*41*).

The Role of Lipid Unsaturation

Many of the conclusions pertaining to the role of fatty acid unsaturation in membrane structure and function have been inferred from the properties of pure lipid dispersions, the reconstitution of membrane protein activity, the use of inhibitors, and dietary manipulations (*42, 43*). The availability of mutants with specific alterations in membrane lipid fatty acid compositions provides another method to examine the physiological consequences of variation in lipid unsaturation. Because of the availability of sensitive techniques for assaying the function of the photosynthetic electron transport activity of chloroplast membranes, we have initially focused on the analysis of those mutants that affect chloroplast membrane lipid compositions. Chloroplast membranes are highly unsaturated and normally are composed of about 70% trienoic fatty acids. Large changes in the amounts of lipid unsaturation in the *fad* mutants had only minor effects on the rate of the photosynthetic electron transport in any of the conditions that we examined (*6, 29–32, 37*). This contrasts with the results of studies that used inhibitors of fatty acid desaturases, lipase treatments, or other correlative approaches in attempts to understand the roles of lipid composition. However, the results with the mutants are consistent with conclusions drawn from experiments in which chloroplast lipid unsaturation was reduced by catalytic hydrogenation (*43*). In this approach, membranes or whole cells are treated with hydrogen gas in the presence of a water-soluble catalyst. The method is unspecific but does permit an analysis of the results of a major reduction in the amount of unsaturation without allowing the organism to change other components of the membrane in order to compensate for the effects of the mutation. The results of these studies have confirmed that chloroplast membranes can withstand large decreases in the amount of lipid unsaturation without exhibiting significant effects on photosynthetic electron transport.

A pronounced consequence of decreased lipid unsaturation is that several mutants exhibit changes in the ultrastructure of their chloroplast membranes. The cross-sectional area of chloroplasts from *fadD* mutants is only about 50% of the area of chloroplasts in the wild type (*31*). The reduced chloroplast size is accompanied by a corresponding increase in the number of chloroplasts, so that the total amount of chloroplast membrane remains similar in mutant and wild-type plants. This observation implies that the change in unsaturation stimulates the chloroplasts to divide more frequently. Although it is not currently possible to understand how *fadD* mutations exert this effect, the observation highlights the lack of

knowledge about the factors that determine organelle size and shape. The effects of *fadD* mutations are not due to the reduction in the gross level of unsaturation because the greater reduction in chloroplast membrane unsaturation found in *fadC* mutants provokes distinctly different effects on chloroplast ultrastructure (*30*).

A clue to the basis of some of the ultrastructural effects was obtained from studies of the effects of prolonged exposure to low temperature on the mutants' growth. When illuminated at 4°C, the wild type and the *fadA* and *act1* mutant lines remained indistinguishable from one another in growth rates and appearance. In contrast, newly developed tissue of the *fadB* and *fadC* mutants became chlorotic at this temperature, and the plants exhibited a 30 to 40% reduction in growth rate relative to the wild type (*34*). Cytological analysis of the basis for this phenotype indicates that in dividing or expanding cells the chloroplast membranes from the mutants exhibit major ultrastructural abnormalities. In the case of the *fadC* mutant there is only about 25% as much chloroplast membrane as in the wild type. The structure of chloroplast membranes in mutants is relatively normal in cells that were fully expanded before exposure to low temperature.

It appears that the mutants are defective in the biogenesis of new membrane at low temperature because of changes in the physical properties of the lipids caused by the decreased acyl unsaturation. Chloroplast membranes from chlorotic tissues of mutant plants grown at low temperature have substantial changes in polypeptide composition, suggesting that decreased unsaturation impedes protein translocation into chloroplast membranes. Such an effect was previously seen with mutants of *E. coli* defective in fatty acid unsaturation (*44*). Thus, the ultrastructural effects of the mutations at normal growth temperatures may reflect a less dramatic manifestation of a general requirement for a certain degree of lipid unsaturation to accommodate the posttranslational insertion of proteins into chloroplast membranes, rather than a direct effect of lipid unsaturation on ultrastructure.

Although not all *Arabidopsis* mutants have been tested yet, the available evidence indicates that lipid unsaturation is also a component of thermal tolerance. Steady-state fluorescence measurements indicating the temperature at which the chlorophyll a-b binding protein complex dissociated from the photosynthetic reaction centers in the *fadB* and *fadC* mutants indicated significant enhancement of the thermal stability of the mutant chloroplast membranes relative to the wild-type chloroplast membranes (*29, 30*). Also, whole-chain photosynthetic electron transport was less susceptible to thermal denaturation in the mutants than in the wild type. A similar enhancement of thermal stability was observed after catalytic hydrogenation of chloroplast membranes (*43*). There is an inverse correlation between the degree of chlorosis at low temperature caused by the mutations and the degree of thermal tolerance. Thus, it appears that decreased unsaturation has effectively shifted the plant's growth temperature range upward. In short-term growth tests, the *fadB* mutant had a substantially higher growth rate than the wild-type plants did at the highest temperature tested, suggesting that chloroplast membrane thermal stability is a component of thermal tolerance in the whole plant (*29*).

Because of similarities between the composition and function of membranes from cyanobacteria to those of the membranes from chloroplasts of higher plants, cyanobacteria are useful systems for studying the role of lipid composition. Some species of these photosynthetic prokaryotes are cold-sensitive, whereas others are not. Researchers have isolated several mutants of the cold-resistant cyanobacterium *Synechocystis* PCC6803 by screening for mutants unable to grow at low temperature. One of these mutants, designated *fad12*, was found to be defective in an ω6 desaturase, and another was defective in an ω12 desaturase (*35*). Researchers identified the gene for the ω6 desaturase by genetic complementation of the mutation. When the gene was introduced into the cold-sensitive species *Anacystis nidulans*, which does not have polyunsaturated fatty acids, 18:2 fatty acids accumulated in the membrane lipids (*36*). The presence of 18:2 fatty acids made the *A. nidulans* cold-resistant—evidence that lipid unsaturation can mitigate the effects of exposure to low nonfreezing temperatures. The *fad2* and *fadC* mutants of *Arabidopsis* are each deficient in organelle-specific isozymes of the plant desaturase equivalent to the enzyme encoded by the *fad12* gene of *Synechocystis* PCC6803. Preliminary results indicate that the *fad2* mutants, which are defective in desaturation of extrachloroplast membrane lipids, are also cold-sensitive.

Although it appears that large changes in lipid unsaturation can cause cold sensitivity, numerous comparisons of cold-sensitive and cold-resistant plant species have failed to support the idea that gross unsaturation can account for naturally occurring cold sensitivity. Thus, attention has been focused on the possibility that it is the degree of unsaturation of a particular lipid or a particular membrane that is of crucial importance. On the basis of detailed analyses of the molecular species of glycerolipids found in a wide range of plants, researchers have proposed that the amount of PG that contains saturated fatty acids at both the *sn*-1 and *sn*-2 positions affects cold sensitivity (*45*). It appears that variability in the substrate specificity of plastid G3P acyltransferase can account for the species differences in the amount of disaturated PG (*46*). The recent cloning of the gene for this enzyme (*47*) and the availability of *act1* mutants that have low endogenous levels of this enzyme have opened the way for a test of the hypothesis by the production of transgenic plants containing homologous genes from cold-sensitive and cold-resistant species.

Storage Lipids

Many plants accumulate large amounts of storage lipid in their fruits or seeds in the form of triacylglycerols. For instance, in field crops such as peanuts, flax, rapeseed, sunflower, and sesame approximately 45% of the seed weight is composed of oil. Plant oils constitute an important source of renewable fixed carbon and are currently commercially produced in excess of 60 million metric tons annually (*48*). Plant oil is used for human consumption, but a proportion finds use in the manufacturing industries, particularly in the production of detergents, coatings, plastics, and specialty lubricants (*48, 49*). In addition, alcohol esters of some plant oils may find future use as a diesel fuel substitute in urban settings where the desire for reduced emissions of sulfur and aromatic pollutants may offset the increased costs of these oils relative to those of petroleum (*50*). The current estimated break-even price is $1.31 per gallon for rapeseed oil esters (*50*).

In this respect, the oil palm (*Elaeis guineensis* Jacq.) deserves mention. The plant represents an efficient source of biomaterial because it is photosynthetically active throughout the year and diverts a substantial proportion of the photosynthate to storage rather than to growth. Some clones have an annual yield in excess of 7 tons of oil per hectare and may remain productive for up to 100 years, although current practices involve more rapid cycling of the plants (*51*). By contrast, soybeans yield only about 0.4 ton of oil per hectare. Once established, oil palm does not require the kind of energy-intensive input of fertilizer and agrichemicals associated with the production of field crops. Because the breeding cycle of oil palm is about 12 years, acquisition of the knowledge required to permit genetic improvements of oil quality and quantity in this species should be an important goal in the development of renewable resources. For example, a 360,000-square-mile plantation of oil

palm would produce approximately the same amount of oil as the United States imports in petroleum (about 3 billion barrels per year). Because oil of different fatty acid compositions is required for various applications, it is essential both to have a complete understanding of the regulation of triacylglycerol synthesis and to have the genes available that are required to engineer the process.

In principle, triacylglycerol synthesis requires only one enzyme, diacylglycerol (DAG) acyltransferase, in addition to those required for the synthesis of membrane lipids by the eukaryotic pathway (Fig. 3). However, the degree of unsaturation of the fatty acids in triacylglycerols may vary from that of the membrane lipids of the cells that accumulate the oil, and many plants accumulate fatty acids that are only found in triacylglycerols (Fig. 1). Two factors appear to be responsible for regulating the unsaturation of triacylglycerol: The first is that the synthesis of phosphatidylcholine (PC) from DAG by CDP-choline:DAG cholinephosphotransferase is reversible, so that in many oilseeds PC (a substrate for two desaturates) is a direct precursor of the unsaturated species of DAG used in triacylglycerol synthesis. The second is that, in seeds of some species, exchange between the *sn*-2 position of PC and acyl-CoA is catalyzed by an acyl-CoA:lysophosphatidylcholine acyltransferase (*52*). This enriches the acyl-CoA pool with 18:2-CoA and 18:3-CoA, which are then available for synthesis of phosphatidic acid and acylation of DAG to triacylglycerol (Fig. 3).

A central question is the mechanism by which unusual fatty acids are accumulated in triacylglycerols but excluded from membranes (*53*). For instance, in members of the genus *Cuphea*, the most abundant fatty acid in triacylglycerols ranges from C_8 to C_{14}, depending on the species (*54*). The fact that a series of closely related species exhibits such variation suggests that differences in the properties of only one or a few enzymes may be responsible for the biosynthetic diversity. Recent studies of *Umbellularia californica*, which also accumulates fatty acids of medium-chain lengths, suggest that the medium-chain fatty acids are produced in the seed plastids by an additional isozyme of acyl-ACP thioesterase, which preferentially hydrolyses 12:0-ACP thioester (*55*).

A hypothesis as to why these medium-chain fatty acids (or other atypical fatty acids) do not appear in phospholipids is based on the observation that several of the acyltransferases involved in lipid synthesis exhibit striking substrate specificity (*56*). The G3P acyltransferase lacks fatty acid selectivity. By contrast, the lysophosphatidic acid (LPA) acyltransferase appears to esterify medium-chain fatty acids only to LPA that has a medium-chain fatty acid at the *sn*-1 position. The same coupling of substrate preferences is proposed for long-chain fatty acids. In addition, it is proposed that the CDP: choline DAG cholinephosphotransferase and the DAG acyltransferase are relatively specific for DAG molecules with long or medium fatty acyl groups, respectively.

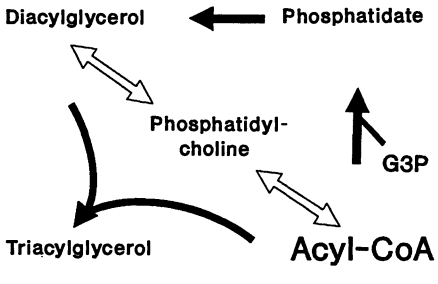


Fig. 3. An abbreviated scheme of the uses of acyl-CoA in triacylglycerol synthesis in typical developing oilseeds. In most species, 16:0-CoA and 18:1-CoA are the major products of plastid fatty acid synthesis. These thioesters are used with G3P for de novo synthesis of PA in the endoplasmic reticulum and with DAG for the synthesis of triacylglycerol. In those oilseeds that accumulate 18:2 and 18:3 fatty acids, a proportion of the DAG is interconvertible with PC. PC is a substrate for an ω6 desaturase that converts the 18:1 fatty acid at the *sn*-2 position to 18:2 and an ω3 desaturase that then converts the 18:2 fatty acid to 18:3. The acyl group at the *sn*-2 position of PC can be exchanged with acyl-CoA by an acyl-CoA:lysophosphatidylcholine acyltransferase (indicated by the lower open arrow). This introduces polyunsaturated fatty acids into the acyl-CoA pool, which can then be utilized for the synthesis of PA or triacylglycerol. The amount of acyl exchange appears to vary among different species (*52*).

An alternate possibility is that there is some as yet unidentified aspect of triacylglycerol biosynthesis that can account for the enzymes' acyl group specificity. For instance, because there is no direct knowledge about the distribution of the lipid enzymes in the microsomal membranes, a possible hypothesis remains that the synthesis of triacylglycerols is spatially segregated from the synthesis of phospholipids. A relevant problem concerns the mechanisms involved in the biogenesis of the oil bodies, the unique organelles in which the lipid is stored (*57*). In the electron microscope these structures appear to be homogeneous oil droplets surrounded by an electron-dense membrane composed of phospholipids and a small family of low molecular mass (about 16 to 34 kD) hydrophobic polypeptides, called oleosins, that may comprise as much as 20% of the cellular protein (*58*). In this respect the plant lipid bodies are morphologically similar to vertebrate serum lipoproteins such as chylomicrons and very low density lipoproteins.

The oil bodies are thought to arise by the accumulation of de novo synthesized triacylglycerols in the interleaflet space of the endoplasmic reticulum. As lipid accumulates, oil droplets develop and eventually break away from the endoplasmic reticulum (*59*). Studies of the developmental timing of oil accumulation, oil body formation, and oleosin synthesis in *Brassica* have led to the conclusion that the cytoplasmic lipid droplets initially lack proteinaceous membranes (*60*). During later stages of seed development, the accretion of a surface coating of the amphipathic oleosins appears to induce fission of the oil bodies, which undergo a reduction to approximately one-thirtieth of their original volumes (*61*). By increasing the surface-to-volume ratio of the oil bodies in way, the oleosins may play a crucial role in germination by ensuring the rapid accessibility of the lipases to the oil bodies.

Lipids and Genetic Engineering

In some cases, such as in the development of canola, researchers achieved a useful change in oil composition by blocking the pathway of fatty acid modification with a simple mutation. However, our present understanding of the biochemistry and genetics of seed lipid synthesis suggests that other desirable alterations will require the use of sophisticated molecular genetic approaches (*48*). For example, for health reasons, 16:0 fatty acids are considered less desirable components of edible oils than are 18:0 or 18:1 fatty acids (*62*). Because, as noted earlier, 3-ketoacyl–ACP synthase II activity may regulate elongation of 16:0 fatty acids to 18:0, a substantial decrease in 16:0 levels might be achieved if the activity of this enzyme could be increased by the overexpression of the corresponding gene in transgenic plants. Although opportunities exist to improve the quality of the food supply, important opportunities also exist in the development of new crops that could produce industrially useful fatty acids. If the fatty acids shown in Fig. 1 could be produced with the same efficiency as are edible fatty acids, these oils could displace petroleum as a feedstock for certain industrial applications that exploit the functional groups of these fatty acids. These new oils would create new markets for the agricultural sector and would be a step toward greater reliance on renewable resources.

There are two strategies that might be used to produce new industrial oil crops—domestication and genetic engineering. Since ongoing attempts to domesticate several plant species during the past 20 years do not yet appear promising, researchers are now interested in using genetic engineering methods. An example of

current efforts focuses on the castor plant (*Ricinus communis* L.), which accumulates three compounds in its seed: toxic lectin (ricin), a potent allergen, and a toxic alkaloid. About 90% of the fatty acid in the seed oil is ricinoleic acid (Fig. 1), known since antiquity for its cathartic effects. Presumably this quality of the oil evolved so that in the unlikely event an organism survived the other challenges associated with eating castor beans, the organism would not gain any nutritional benefits. Because of the presence of the hydroxyl group, ricinoleic acid is among the world's most versatile natural products and has hundreds of industrial uses that include synthesis of nylon-11, lubricants, hydraulic fluids, plastics, cosmetics, and other materials (*49*). Because of the toxic meal that remains after extraction of the oil and because of other agronomic problems that result in relatively poor yields, castor is a minor crop primarily grown in nonindustrialized nations where, in some cases, the seed is hand-harvested from wild plants.

Evidence available about the synthesis mechanisms of ricinoleic acid (*63*) suggests that a single enzyme converts oleic acid to ricinoleic acid. Introduction of a gene for this enzyme into a variety of sunflower with a high oleic acid content could result in the creation of a new crop. Similarly, most of the fatty acids in Fig. 1, as well as other fatty acids, can probably be produced from common precursors by the introduction of a small number of enzymes into a species such as sunflower. Because many of these fatty acids would be more valuable than the fatty acids found in edible oils, the ability to produce these materials in field crops would provide farmers with alternative cash crops. Because of the magnitude of industrial demands for chemical feedstocks, further advances in the knowledge about plant lipid metabolism could lead to significant changes in agricultural practices.

REFERENCES AND NOTES

1. F. D. Gunstone, in *The Lipid Handbook*, F. D. Gunstone, J. L. Harwood, F. B. Harwood, F. B. Padley, Eds. (Chapman & Hall, New York, 1986), pp. 1–25.
2. J. Levitt, *Chilling, Freezing, and High-Temperature Stresses*, vol. 1 of *Responses of Plants to Environmental Stresses* (Academic Press, New York, 1980); A. Sakai and W. Larcher, *Frost Survival of Plants* (Springer-Verlag, Berlin, 1987).
3. C. L. Guy, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41**, 187 (1990).
4. P. McCourt and C. R. Somerville, in *Methodology*, vol. 13 of *The Biochemistry of Plants*, D. D. Davies, Ed. (Academic Press, Orlando, FL, 1987), pp. 34–61.
5. W. Bender, P. Spierer, D. S. Hogness, *J. Mol. Biol.* **168**, 17 (1983); E. M. Meyerowitz, *Cell* **56**, 263 (1989).
6. J. Browse, P. McCourt, C. R. Somerville, *Science* **227**, 763 (1985); P. McCourt, J. Browse, J. Watson, C. J. Arntzen, C. R. Somerville, *Plant Physiol.* **78**, 853 (1985).
7. J. Browse, N. Warwick, C. R. Somerville, C. R. Slack, *Biochem. J.* **235**, 25 (1986).
8. M. R. Blatt, G. Thiel, D. R. Trentham, *Nature* **346**, 766 (1990); S. Gilroy, N. D. Read, A. J. Trewaves, *ibid.*, p. 769; K. J. Einspahr and G. A. Thompson, Jr., *Plant Physiol.* **93**, 361 (1990).
9. E. E. Farmer and C. A. Ryan, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7713 (1990); H. S. Mason and J. E. Mullet, *Plant Cell* **2**, 569 (1990).
10. P. L. Steponkus, D. V. Lynch, M. Uemura, *Philos. Trans. R. Soc. London Ser. B* **326**, 571 (1990).
11. P. E. Kolattukudy, in *Lipids: Structure and Function*, vol. 9 of *The Biochemistry of Plants: A Comprehensive Treatise*, P. K. Stumpf *et al.*, Eds. (Academic Press, Orlando, FL, 1987), pp. 291–314; M. Koornneef, C. J. Hanhart, F. Thiel, *J. Hered.* **80**, 118 (1989).
12. N. Murata and I. Nishida, in *Lipids: Structure and Function*, vol. 9 of *The Biochemistry of Plants: A Comprehensive Treatise*, P. K. Stumpf *et al.*, Eds. (Academic Press, Orlando, FL, 1987), pp. 315–347.
13. J. Ohlrogge, D. Kuhn, P. K. Stumpf, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1194 (1979); J. L. Harwood, *Annu. Rev. Plant Physiol.* **39**, 101 (1988).
14. J. G. Jaworski, R. C. Clough, S. R. Barnum, *Plant Physiol.* **90**, 41 (1989); T. Shimakata and P. K. Stumpf, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5808 (1982); R. W. MacKintosh, D. G. Hardie, A. R. Slabas, *Biochim. Biophys. Acta* **1002**, 114 (1989).
15. The symbols 16:*n* or 18:*n* refer to a C_{16} or C_{18} fatty acid with *n cis* double bonds. For the fatty acids described here, if one bond is present, it is in the ω9 configuration (between carbons 9 and 10 counting from the methyl group). Two double bonds are in the ω6,9 configuration, and three are in the ω3,6,9 configuration. The symbol *sn* refers to the numbering of the carbons in the glycerol backbone of the phosphatidic acid. In the cases described here, the fatty acids are located at the end (*sn*-1) and middle (*sn*-2) carbons of the glycerol moiety.
16. T. A. McKeon and P. K. Stumpf, *J. Biol. Chem.* **257**, 12141 (1982).
17. J. Shanklin and C. R. Somerville, *Proc. Natl. Acad. Sci. U.S.A.*, in press; A. J. Kinney, W. D. Hitz, N. Yadav, in *Plant Lipid Biochemistry, Structure and Utilization*, P. Quinn and J. Harwood, Eds. (Portland Press, Colchester, UK, 1990), pp. 126–128.
18. D. E. Scherer and V. C. Knauf, *Plant Mol. Biol.* **6**, 127 (1987); R. Safford *et al.*, *Eur. J. Biochem.* **174**, 287 (1988); L. Hansen, *Carlsberg Res. Commun.* **52**, 381 (1987); K. M. Schmid and J. B. Ohlrogge, *Plant Mol. Biol.* **15**, 765 (1990).
19. J. F. Battey and J. B. Ohlrogge, *Planta* **180**, 352 (1990).
20. L. Chuman and S. Brody, *Eur. J. Biochem.* **184**, 643 (1989).
21. D. Post-Beittenmiller, J. G. Jaworski, J. B. Ohlrogge, *J. Biol. Chem.* **266**, 1858 (1991).
22. M. Frentzen, M. Neuburger, J. Joyard, R. Douce, *Eur. J. Biochem.* **187**, 395 (1990).
23. E. Heinz and P. G. Roughan, *Plant Physiol.* **72**, 273 (1983); P. G. Roughan and C. R. Slack, *Annu. Rev. Plant Physiol.* **33**, 97 (1982); J. P. Williams and M. U. Khan, *Biochim. Biophys. Acta* **713**, 177 (1982).
24. G. R. Jamieson and E. H. Reid, *Phytochemistry* **10**, 1837 (1971).
25. R. W. Pearcy, *Plant Physiol.* **61**, 484 (1978).
26. J. Ohlrogge, J. Browse, C. R. Somerville, *Biochim. Biophys. Acta*, in press.
27. A. H. Corner, in *High and Low Erucic Acid Rapeseed Oils*, J. K. G. Kramer, F. D. Sauer, W. J. Pigden, Eds. (Academic Press, Toronto, 1983), pp. 293–311; B. R. Stefansson, *ibid.*, pp. 144–158; K. R. Downey, *ibid.*, pp. 1–18.
28. J. Dupont *et al.*, *J. Am. Coll. Nutr.* **8**, 360 (1989).
29. L. Kunst, J. Browse, C. R. Somerville, *Plant Physiol.* **90**, 943 (1989); *ibid.* **91**, 401 (1989).
30. J. Browse, L. Kunst, S. Anderson, C. R. Somerville, *ibid.* **90**, 522 (1989); S. Hugly, L. Kunst, J. Browse, C. R. Somerville, *ibid.*, p. 1134.
31. J. Browse, P. McCourt, C. R. Somerville, *ibid.* **81**, 859 (1986); P. McCourt, L. Kunst, J. Browse, C. R. Somerville, *ibid.* **84**, 353 (1987).
32. B. Lemieux, M. Miquel, C. R. Somerville, J. Browse, *Theor. Appl. Genet.* **80**, 234 (1990); D. W. James and H. K. Dooner, *ibid.*, p. 241.
33. H. A. Norman and J. B. St. John, *Plant Physiol.* **81**, 731 (1986); *ibid.* **85**, 684 (1987).
34. S. Hugly and C. R. Somerville, in preparation.
35. H. Wada and N. Murata, *Plant Cell Physiol.* **30**, 971 (1989); *Plant Physiol.* **92**, 1062 (1990).
36. H. Wada, Z. Gombos, N. Murata, *Nature* **347**, 200 (1990).
37. L. Kunst, J. Browse, C. R. Somerville, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4143 (1988); *Plant Physiol.* **90**, 846 (1989).
38. W. R. Bishop and R. M. Bell, *Annu. Rev. Cell Biol.* **4**, 579 (1988).
39. V. Arondel and J. C. Kader, *Experientia* **46**, 579 (1990).
40. F. Tchang *et al.*, *J. Biol. Chem.* **263**, 16849 (1989); C. Vergnolle *et al.*, *Biochem. Biophys. Res. Commun.* **157**, 37 (1988); W. Bernhard, S. Thoma, J. Botella, C. R. Somerville, *Plant Physiol.* **95**, 164 (1991).
41. S. Thoma, U. Hecht, J. Botella, C. R. Somerville, unpublished results.
42. C. D. Stubbs and A. D. Smith, *Biochim. Biophys. Acta* **779**, 89 (1984).
43. P. J. Quinn and W. P. Williams, *ibid.* **737**, 233 (1983); P. J. Quinn, F. Joo, L. Vigh, *Prog. Biophys. Mol. Biol.* **53**, 71 (1989).
44. J. M. DiRienzo and M. Inouye, *Cell* **17**, 155 (1979).
45. N. Murata, N. Sato, N. Takahashi, Y. Hamazaki, *Plant Cell Physiol.* **23**, 1071 (1982); N. Murata, *ibid* **24**, 81 (1983); _____ and J. Yamaya, *Plant Physiol.* **74**, 1016 (1984); J. Raison and L. C. Wright, *Biochim. Biophys. Acta* **731**, 69 (1983); P. G. Roughan, *Plant Physiol.* **77**, 740 (1985).
46. J. E. Cronan, Jr., and P. G. Roughan, *Plant Physiol.* **83**, 676 (1987).
47. O. Ishizaki, I. Nishida, K. Agata, G. Eguchi, N. Murata, *FEBS Lett.* **238**, 424 (1988).
48. J. F. Battey, K. M. Schmid, J. B. Ohlrogge, *Trends Biotechnol.* **7**, 122 (1989).
49. R. D. Schmid, *J. Am. Oil Chem. Soc.* **64**, 563 (1987); E. H. Pryde and J. A. Rothfus, in *Oil Crops of the World*, G. Röbbelen, R. K. Downey, A. Ashri, Eds. (McGraw-Hill, New York, 1989), pp. 87–117.
50. S. Latta, *Inform (Champaign)* **1**, 434 (1990).
51. J. P. Gascon, J. M. Noiret, J. Meunier, in *Oil Crops of the World*, G. Röbbelen, R. K. Downey, A. Ashri, Eds. (McGraw-Hill, New York, 1989), pp. 475–493; L. H. Jones, in *Proceedings World Conference on Biotechnology for the Fats and Oils Industry*, American Oil Chemists' Society, Hamburg, Germany, 27 September to 2 October 1987, T. H. Applewhite, Ed. (American Oil Chemists' Society, Urbana, IL, 1988), pp. 47–50.
52. G. Griffiths, S. Stymne, K. Stobart, *Planta* **173**, 309 (1988).
53. J. F. Battey and J. B. Ohlrogge, *Plant Physiol.* **90**, 835 (1989).
54. F. Hirsinger and P. F. Knowles, *Econ. Bot.* **38**, 439 (1984); R. B. Wolf, S. A. Graham, R. Kleiman, *J. Am. Oil Chem. Soc.* **60**, 103 (1983).
55. M. R. Pollard, L. Anderson, C. Fan, D. J. Hawkins, H. M. Davies, *Arch. Biochem. Biophys.* **284**, 306 (1991).
56. M. Bafor, L. Jonsson, A. K. Stobart, S. Stymne, *Biochem. J.* **272**, 31 (1990); M. Bafor, A. K. Stobart, S. Stymne, *J. Am. Oil Chem. Soc.* **67**, 217 (1990); K. Oo and A. H. C. Huang, *Plant Physiol.* **91**, 1288 (1989).
57. M. I. Gurr, in *The Biochemistry of Plants*, P. K. Stumpf and E. E. Conn, Eds. (Academic Press, New York, 1980), vol. 4, pp. 205–248.
58. V. B. Vance and A. H. C. Huang, *J. Biol. Chem.* **262**, 11275 (1987); E. M. Herman, *Planta* **172**, 336 (1987); D. E. Fernandez, R. Qu, A. H. C. Huang, L. A. Staehelin, *Plant Physiol.* **86**, 270 (1988); D. J. Murphy *et al.*, *Biochim. Biophys. Acta*, in press.
59. A. K. Stobart, S. Stymne, S. Höglund, *Planta* **169**, 33 (1986).
60. D. J. Murphy, I. Cummins, A. S. Kang, *Biochem. J.* **258**, 285 (1989).
61. I. Cummins and D. J. Murphy, in *Plant Lipid Biochemistry, Structure and Utilization*, P. Quinn and J. Harwood, Eds. (Portland Press, Colchester, UK, 1990), pp.

231–233.
62. A. Bonanome and S. M. Grundy, *N. Engl. J. Med.* **318**, 1244 (1988).
63. R. A. Moreau and P. K. Stumpf, *Plant Physiol.* **67**, 672 (1981).
64. H. Schmidt and E. Heinz, *ibid.* **94**, 214 (1990); *Proc. Natl. Acad. Sci. U.S.A.* **87**, 9477 (1990).
65. M. Smith *et al.*, *Biochem. J.* **272**, 23 (1990); E. Kearns, S. Hugly, C. R. Somerville, *Arch. Biochem. Biophys.* **284**, 431 (1991).

66. We thank W. Thompson, S. Stymne, and P. Steponkus for providing photographs; E. Heinz, S. Stymne, N. Murata, N. Yadav, J. Jaworski, J. Ohlrogge, D. Post-Beintenmiller, D. Murphy, and H. M. Davies for providing unpublished manuscripts. Supported by grants from the U.S. Department of Agriculture–National Science Foundation–Department of Energy Plant Science Center Program, the U.S. Department of Energy (AC02-76ER01338), the National Science Foundation (DCB 8803855), and the U.S. Department of Agriculture (89-37262-4388).

"The bad news is that he's writing a book about the treatment of animals."