Chapter 12
Genomics of Soybean Oil Traits

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Introduction

Plant oils including soybean, *Glycine max*, oil are mainly triacylglycerol (TAG) which represents an important edible and industrial resource. TAG also comprises a major part of the value of soybeans and soybeans are the most important source of renewable oil in the US. World-wide production of soybean oil is about the same as that of palm oil. Biodiesel is made from plant TAG and represents an important and growing renewable fuel resource. Surprisingly, the contribution of all the important enzymes to TAG accumulation in plant seeds (or any other plant tissue) even in Arabidopsis is currently unknown. We know even less about TAG biosynthesis in soybeans.

Most plant seeds accumulate storage products during seed development to provide nutrients and energy for seedlings to grow competitively for light and nutrients, especially nitrogen. Soybean seed protein provides the nitrogen and soybean oil provides most of the energy for seedling establishment. Most seeds either accumulate starch or TAG as an energy store for seedling establishment. Many seed crops such as corn, wheat, rice, peas and common beans (*Phaseolus vulgaris*) accumulate starch as the main form of energy storage in the seeds although the embryo or germ portion is often high in oil. Oilseeds such as soybeans, canola (*Brassica napus*), sunflowers, cotton seed, peanuts and many other oilseeds accumulate oil instead of starch. Like many tiny seeds *Arabidopsis thaliana* and tobacco (*Nicotiana tabacum*) seeds accumulate oil as an energy store with Arabidopsis seeds usually being ~42% oil (O'Neill et al., 2003; Zhang et al., 2005). Commercial soybean seeds are about 40% protein and 20% oil on a dry weight basis. Soybean seeds are very high in protein among seed crops but relatively low in oil among oilseeds. Macadamia nut seeds can be as much as 76% oil and palm fruit as much as 79% oil on a dry weight basis (Fig. 12.1). Many oilseeds such as canola, peanuts and sunflower are 40–50%

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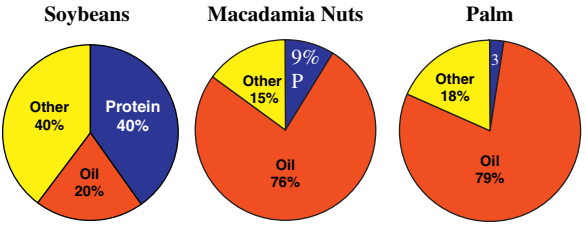

Fig. 12.1 Oil and protein content of soybeans compared with macadamia nuts and palm fruit

oil. The protein + oil content in macadamia nuts is ~85% as opposed to 60% for soybeans. As such soybeans have relatively low oil yields per unit land area among oilseeds with palm being the highest after the tree plantations are well established (Fig. 12.2). Palm oil and olive oil are from the fruit of these trees although palm seeds or kernels also accumulate oil which is different in fatty acid composition from that of the fruit oil.

The genomics of TAG biosynthesis in Arabidopsis has been studied rather well (although much remains to be elucidated) by Ohlrogge and colleagues (Beisson et al., 2003; Ruuska et al., 2004). They maintain an Arabidopsis lipid gene database, http://www.plantbiology.msu.edu/lipids/genesurvey/index.htm, which is regularly updated (e.g. as of January 2007 at the writing of this chapter). To date they have identified more than 620 genes in Arabidopsis involved in acyl-lipid metabolism. This is about 2.4% of the total number of predicted genes in the Arabidopsis genome. They are classified into eight groups plus a miscellaneous class (Table 12.1). Interestingly the largest group in terms of numbers of genes is the lipid signaling group. The groups of greatest importance in the synthesis of seed oil and, therefore, for this chapter are the synthesis of plastid fatty acids, endomembrane lipid synthesis and TAG synthesis and storage.

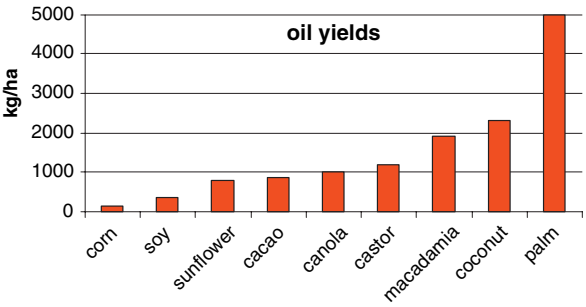

Fig. 12.2 Relative oil yields per unit land area of soybeans compared to some other oil crops

Table 12.1 Genes in Arabidopsis involved in acyl-lipid metabolism (Beisson et al., 2003; Ruuska et al., 2004); http://www.plantbiology.msu.edu/lipids/genesurvey/index.htm

Function	# genes
Synthesis of plastid FA	47
Synthesis of plastid membranes	20
Endomembrane synthesis	59
Mitochondrial acyl-lipid metabolism	29
Oil synthesis and storage	20
Lipid catabolism	43
Lipid signaling	153
Fatty acid elongation, wax & cutin metabolism	75
Miscellaneous	178
Total	624

Fatty Acid Synthesis

As mentioned above the main storage products that accumulate in soybean seeds are protein and oil. Protein mostly accumulates in the form of storage proteins in protein bodies. Oil accumulates as TAG in oil bodies. The amino acids asparagine and glutamine provide the nitrogen and initial carbon skeletons for protein deposition and sucrose provides the energy for protein biosynthesis and the energy and hydrocarbon precursors for TAG accumulation. The key processes and enzymatic steps involved in TAG biosynthesis in seeds such as soybeans are summarized in Fig. 12.3, Table 12.2. This is fueled by sucrose made in the leaves delivered to the apoplasts of the developing seeds. Sucrose is converted into hexose phosphates and then to fructose 1,6 bis- phosphate, which is cleaved into triosphosphates. Triosphosphates, such as dihydroxy acetone phosphate, are reduced to glycerol-3-P that provides the glycerol backbone for membrane lipids and TAGs and oxidized to 3-phosphoglycerate. 3-Phosphoglycerate is isomerized to phosphoenol pyruvate (PEP) and then to pyruvate. Pyruvate and possibly PEP enter the plastids and the

Table 12.2 Enzymes of oil or triacylglycerol (TAG) biosynthesis (Fig. 12.3)

Enzyme abbreviation	Enzyme name
PDH	Pyruvate dehydrogenase
ACC	Acetyl-CoA carboxylase
FAS	Fatty acid synthase complex
KASII	Keto-acyl-ACP synthase II
D9D	Δ-9 desaturase
TE	Thioesterase
ACS	Acyl-CoA synthetase
AT	Acyltransferase
*Fad*2-1	Δ-12 desaturase
*Fad*3	ω-3 desaturase
GPAT	Glycerol-3 phosphate acyltransferase
LPAT	Lysophosphatidic acid acyltransferase
PAP	Phosphatidic acid phosphatase
DGAT	Diacylglycerol acyltransferase

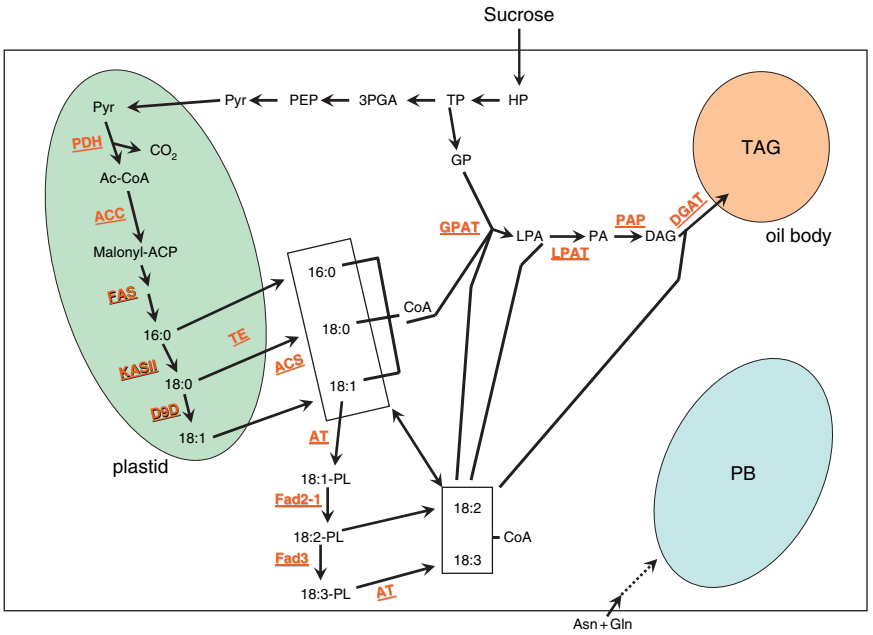

Fig. 12.3 Summary of some of the major steps of oil or triacylglycerol (TAG) biosynthesis in plant cells including developing soybean seeds. PB = protein body. The enzymes discussed are underlined and described in Table 12.2 (*See* also Color Insert)

pyruvate is converted to acetyl-CoA by pyruvate dehydrogenase. Pyruvate synthesis and/or transport to plastids might be a limiting step in fatty acid biosynthesis and accumulation in oil. Acetyl-CoA is a precursor to many molecules in plants and other organisms in multiple organelles. The first committed step of fatty acid biosynthesis is the conversion of acetyl-CoA into malonyl-CoA by acetyl-CoA carboxylase and then to malonyl-ACP by a transacylase. In most tissues of most eukaryotic organisms including plants, malonyl-ACP (malonyl-CoA in eukaryotes without plastids) is elongated in 8 cycles, two carbon units at a time, via the fatty acid synthase complex, to palmitoyl (16:0)-ACP (or -CoA). The fatty acid synthase complex involves four different enzymatic reactions with each cycle starting with a condensation, followed by a reduction, a dehydration and a second reduction (Ohlrogge and Jaworski, 1997). The condensation reactions are catalyzed by enzymes known as **3-ketoacyl-ACP synthases** or KASs. The 1st condensation reaction going from acetyl-CoA to 3-ketobutyrate is catalyzed by KAS III, the reaction from butyryl-ACP (C4) to palmitoyl-ACP (C16) by KAS I and from palmitoyl-ACP to stearoyl-ACP (C18) by KAS II. The reaction stops at C16 and C18 fatty acids not only by virtue of the specificity of the KAS enzymes but also by the action of thioesterases (TEs), which hydrolyze the acyl-S-ACP thioester bonds. Some plants such as coconuts have unusual TEs, known as medium chain TEs, which stop the reaction at C8, C10, C12 or C14 fatty acid chain lengths and these plants can accumulate medium chain fatty acids in their seed oil.

Oil is biosynthesized during the second stage of seed maturation (Harwood and Page, 1994) at which time the relevant biosynthetic enzymes are highly expressed. The major fatty acids of plants (and most other eukaryotic organisms) have a chain length of 16 or 18 carbons and contain from zero to three cis-double bonds. Five fatty acids (18:1, 18:2, 18:3, 16:0 and in some species 16:3) make up over 90% of acyl chains of structural glycerolipids of almost all plant membranes (Ohlrogge and Browse, 1995). The nature of the acyl composition of the TAG is dependent on the availability of the fatty acids from the acyl-CoA substrate pool, as well as the selectivity of the acyltransferases of the Kennedy pathway (Harwood, 1998) and possibly transacylases. These same five fatty acids are the main fatty acids present in soybean oil. The fatty acid composition of the oil of normal soybean cultivars (Fig. 12.4) is:

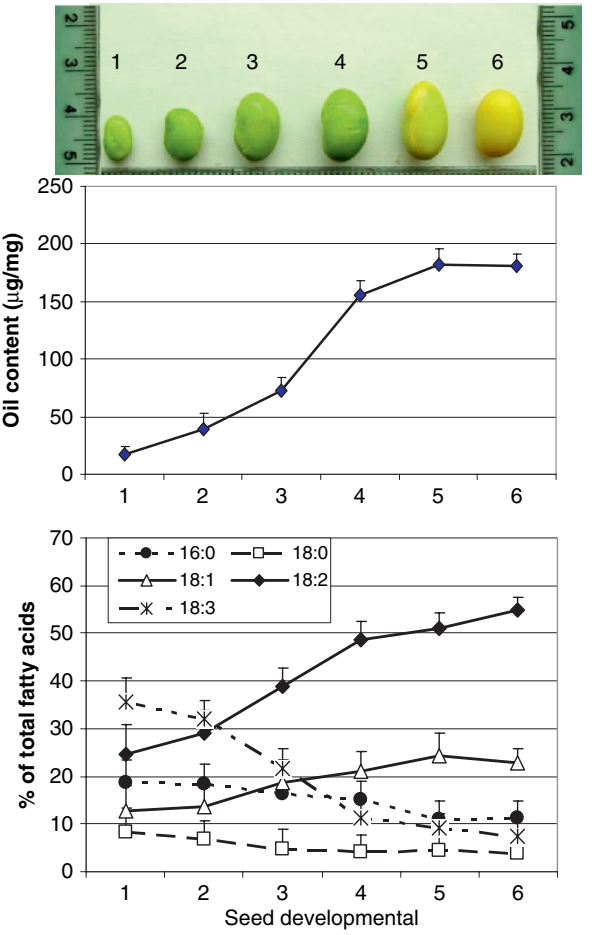

Fig. 12.4 Oil and fatty acid accumulation in soybean seed development (*See* also Color Insert)

Fatty acid	%
16:0	11
18:0	4
18:1	25
18:2	52
18:3	8

Reactions of fatty acid synthesis are terminated by hydrolysis or transfer of the acyl chains from the ACP by ACP-hydrolase or an acyltransferase consecutively. The 'competition' for substrate is thus a competition between the termination of synthesis, a function of thioesterase and transferase activity, and extension, a function of KASI and KASII isoforms (Voelker et al., 1992; Budziszewski et al., 1996). ACP-thioesterases are one of two main types (Klaus et al., 2004). One thioesterase is relatively specific for 18:1 ACP, encoded by Fat A, and a second more specific for saturated acyl-ACPs encoded by Fat B. FA molecules formed in the chloroplast stroma are released from ACPs by thioesterases and cross the membrane by an unknown mechanism. As the FA cross the membrane, they are converted to acyl-CoA esters through the activity of an acyl-CoA synthase (ACS) located on the outer membrane. Plants have multiple ACSs that participate in lipid metabolism (Schnurr et al., 2002; Shockey et al., 2002). ACS enzymes encoded by different genes have differential specificities for particular fatty acids (McKeon et al., 2006).

Fatty Acid Desaturases

In most plant tissues, over 75% of the fatty acids are unsaturated. Two types of desaturases have been identified, one soluble and the other membrane bound, that have different consensus motifs. Database searching for these motifs reveals that these enzymes belong to two distinct multifunctional classes, each of which includes desaturases, hydrolases and epoxygenases that act on FA or other substrates (Shanklin and Cahoon, 1998). Free FA are not thought to be desaturated in vivo, rather they are esterified to acyl carrier protein (ACP) for the soluble plastid desaturase or to coenzyme-A (CoA) or to phospholipids for integral membrane desaturases.

Δ-9 Desaturases

The first double bond in unsaturated FAs is introduced by the soluble enzyme stearoyl-ACP desaturase. This fatty acid desaturase is special to the plant kingdom in that only few other known desaturases are soluble. Soluble Δ-9 stearoyl-ACP desaturases are found in all plant cells and are essential for the biosynthesis of unsaturated membrane lipids (Shanklin and Somerville, 1991; Kaup et al., 2002; Cahoon et al., 1997; Cahoon et al., 1994). Desaturases that convert saturated fatty

acid to mono-unsaturated FA share several common characteristics. They perform stereospecific Δ-9 desaturation of an 18:0/16:0 substrate with the removal of the 9-D and 10-D hydrogens (Bloomfield and Bloch, 1960; Mudd and Stumpf, 1961). Protein crystallographic studies on the purified desaturase from castor bean showed that it contains a diiron cluster (Fox et al., 1993). The protein is active as a homodimer and consists of a single domain of 11 helices. This diiron center is the active site of the desaturase (Lindqvist et al., 1996).

Expression and regulation of Δ-9 desaturase in plants have been studied extensively (Fawcett et al., 1994; Slocombe et al., 1994). The expression of the promoter of the *Brassica napus* stearoyl desaturase gene in tobacco was found to be temporally regulated in developing seed tissues. However, the promoter was also particularly active in other oleogenic tissues such as tapetum and pollen grains, raising the interesting question of whether seed expressed lipid synthesis genes are regulated by separate tissue specific determinants or by a single factor common to all oleogenic tissues (Slocombe et al., 1994). In *Saccharomyces cerevisiae*, addition of saturated fatty acids induced Δ-9 fatty acid desaturase mRNA (Ole1 mRNA) by 1.6-fold, whereas a large family of unsaturated fatty acids repress Ole1 transcription by 60-fold. A 111 bp fatty acid regulation region (FAR), approximately 580 bp upstream of the start codon, was identified that is essential for the transcription activation and unsaturated fatty acid repression (Quittnat et al., 2004). In addition to transcriptional regulation, unsaturated fatty acids mediate changes in the half-life of the Ole1 mRNA (Gonzalez and Martin, 1996).

Currently, industries that manufacture shortening, margarine, and confectionery products use considerable amounts of stearate (18:0) produced mainly from partially hydrogenated plant oils (Facciotti et al., 1999). Hydrogenation not only generates extra cost but also is a generator of significant amounts of trans-fatty acids that were associated with an elevated risk of heart disease (Facciotti et al., 1999; Katan et al., 1995; Nelson, 1998). Industries manufacturing shortenings and confectionery products could benefit from an oil crops capable of accumulating high levels of stearate. However, stearate (18:0) does not naturally accumulate to abundant levels in most cultivated oil crops including soybeans and efforts to produce a high-stearates phenotype through conventional breeding and mutagenesis techniques has had only modest success (Facciotti et al., 1999). Although, stearic acid (18:0) is one of the major saturated fatty acids in most seed oils, its percentages vary among the different oilseed crops from 1.0% in rape seed oil to 3.6% in sesame and corn seed oils and 4.0% in soybean oil with a range from 2.2–7.2% for the genotypes available in the world germplasm collection (Downey and McGregor, 1975; Hymowitz et al., 1972; Rahman et al., 1997; Yasumoto et al., 1993). The fatty acid composition of soybeans has been improved by using selective breeding techniques utilizing natural variants or induced mutagenesis (Graef et al., 1985a,b; Ladd and Knowles, 1970). Hammond and Fehr (1983) were able to increase the amount of stearate (C18:0) produced in the soybean oil to levels up to about 28.1% of the total fatty acid content using mutagenesis. Rahman et al. (2003) reported novel soybean germplasm with high stearic levels. This novel soybean was obtained as a consequence of the combination of the loci

of high palmitic and stearic acids leading to alterations in other fatty acids. As a result, two lines (M25 and HPS) with a 5-fold increase in stearic acid (from 34 to 181 and 171 g kg^{-1}) were developed. This increase in stearic acid was also found to be associated with a change in oleic and linoleic acids content. Furthermore, these authors reported that when both palmitic and stearic acids were considered together in the oil of HPS, this line had a saturated fatty acid content of >380 g kg^{-1}. Thus, such oil might have the potential to increase the utility and also to improve the quality of soybean oil for specific purposes (Rahman et al., 2003).

Vegetable oils rich in monounsaturated fatty acids (MUFA) are not only important in human nutrition but also can be used as renewable sources of industrial chemicals (Cahoon et al., 1997). One particular output trait of current interest is the use of transgenic soybean plans to produce palmitoleic acid fatty acids that have either nutraceutical or pharmaceutical and even industrial properties. Macadamia nut is another source of palmitoleic acid. Its oil is unique in that monounsaturated fatty acids are the predominant component (about 80%) and a considerable portion (17–21%) of this is palmitoleic acid (a component not present in substantial amounts in olive oil; Curb et al., 2000). Grayburn and Hildebrand (1995) and Wang et al. (1996) reported large increases in palmitoleic acid (16:1 Δ-7) after expressing a mammalian or yeast Δ-9 deasturase gene in tobacco or tomato. Since soybeans are an important oil source that is high in linoleic and saturated fatty acids (mostly linoleic and palmitic acid; about 55% and 15%, respectively), conversion of all or part of these saturated fatty acids into palmitoleic acid would be a great benefit, not only for health, since converting much of the remaining PUFAs into palmitoleic acid could have industrial value. Liu et al. (1996) reported converting $\sim$ half of the palmitic acid of soybean somatic embryos into palmitoleic acid with good expression of a Δ9-CoA desaturase. The transformed embryos had 16:1 levels from 0% to over 10% of total fatty acids, while the levels of 16:0 dropped from 25% to approximately 5% of total fatty acids.

A number of studies demonstrated apparently beneficial effects of diets based on high monosaturated fatty acid content primarily derived from olive oil (Curb et al., 2000; Hegsted et al., 1993; Kris-Etherton et al., 1988; Spiller et al., 1992). The health implications of palmitoleic acid were first addressed by Yamori et al. (1986) and Abraham et al. (1989). Curb et al. (2000) compared the effects of a typical American diet (TAD) (diet high in saturated fat '37% energy from fat', the AHA (American Heart Association) 'step 1' diet '30% energy from fat' (half the SFA's, normal amounts of MUFA's and PUFA's, and high levels of carbohydrates), and a macadamia nut-based monounsaturated fat diet (MND) (37% energy from fat). When compared to the typical diet, step 1 and macadamia nut diets both had potentially beneficial effects on cholesterol and LDL cholesterol levels. These results are consistent with previously-reported lipid altering benefits of MUFA-rich diets particularly those involving macadamia nut oil (Ako and Okuda, 1995). Palmitoleic acid was also reported to protect rats from stroke (Yamori et al., 1986), apparently by increasing cell membrane fluidity, clearing lipids from the blood, and altering the activity of important cell membrane transport systems particularly

through inhibition of the Na+, K(+)-ATPase activity within a narrow range (Swarts et al., 1990). In men and women, elevated blood/tissue levels of palmitoleic acid were found to be correlated with protection from ventricular arrhythmias (Abraham et al., 1989) and negatively correlated with markers of artherosclerosis (Theret et al., 1993). Palmitoleic acid was also found to reportedly inhibit mutagenesis in animals (Hayatsu et al., 1988) and was found to be negatively correlated with breast cancer incidence in women (Simonsen et al., 1998).

Many vegetable oils are partially hydrogenated to increase the stability of cooking oils and hydrogenated further for use as margarines and shortenings. The goal of plant geneticists has mainly to develop high stearate oils in order to reduce or eliminate the need for hydrogenation of vegetable oils used for margarines and shortenings. As described in the section above on high stearate oils, many groups were successful in achieving this goal with a variety of vegetable oils using different approaches, including genetic engineering soybeans to a 53% stearic acid content of oil (Knutzon et al., 1992; Kridl, 2002; Martinez-Force et al., 2002). With the advent of genetic engineering, several strategies for increasing stearic acid levels in oilseed crops have been possible and the increase in levels of stearic acid is usually at the expense of oleic (18:1) and linoleic (18:2) acids. Among other strategies, anti-sense suppression or co-suppression to reduce or knock out the activity of stearoyl-ACP desaturase, which is responsible for converting stearoyl-ACP (saturated) to oleoyl-ACP (unsaturated) (Budziszewski et al., 1996) was used routinely. Also, the stearoyl-ACP thioesterase is another possible metabolic target. Thus, up-regulation of this enzyme by sense-oriented reintroduction of the stearoyl-ACP thioesterase was found to increase free stearate release. Kridl (2002) reported transgenic soybeans with stearate levels of as high as approximately 53%, while levels of approximately 4% were observed in non-transformed control plants. This line is low in linoleic and linolenic acids and high in oleic acid in addition to stearic acid. It is important that these large increases in stearate are seed-specific and more so in triacylglycerol than in membrane lipids because high stearate in membranes can reduce membrane fluidity and result in relatively poor germination rates (Kaup et al., 2002; Voelker and Kinney, 2001; Wiberg et al., 2000).

Changes in palmitate levels of soybean oil has been another long time goal of soybean breeders and geneticists and development of genotypes with levels of <4 and $>40\%$ have been achieved (Stoltzfus et al., 2000). Because saturated fatty acids, especially mid-chain saturated fatty acids such as 16:0, are dietary health-risk factors, particularly cardiovascular health (www.americanheart.org), reduced palmitate has been the main goal. Alleles for altered palmitate in soybean oil are known as *fap* alleles. A low palmitate mutant, A22 with 6.8% 16:0, has a single recessive *fap3* allele (Schnebly et al., 1994). A mutant soybean line with elevated 16:0 containing the *fap2* allele had a single base-pair substitution in codon 299 of the *GmKASIIA* gene with TGG $\rightarrow$ TAG converting a tryptophan to a premature stop codon (Aghoram et al., 2006). *KASII* encodes the keto-acyl-ACP synthase that catalyzes the condensation reaction of the FAS complex involved in elongation of 16:0-ACP to 18:0-ACP (Fig. 12.3).

Δ-12 Desaturases

Plant Δ-12 desaturases are plastid membrane-bound or ER membrane-bound enzymes. Arabidopsis plastidal Δ-12 desaturases were isolated using degenerate oligonucleotides, based on amino acid sequences conserved between plant and cyanobacterial desaturases, to screen cDNA libraries (Falcone et al., 1994). The Arabidopsis Δ-12 desaturase was also used to screen rape and soybean cDNA libraries and the homologous sequences isolated (Falcone et al., 1994). These plant chloroplast Δ-12 desaturases all show a high degree of similarity (around 50%) with cyanobacterial Δ-12 desaturases, but less with cyanobacterial and plant ω-3 desaturases.

The Δ-12 desaturase is particularly active in microsomal preparations from developing seed cotyledons of some oilseed species where it is associated with the biosynthesis of triacylglycerols (Stymne and Stobart, 1986; Griffiths et al., 1988). The microsomal Δ-12 desaturase requires NAD(P)H as reductant and molecular oxygen and is inhibited by cyanide but not carbon monoxide, suggesting that cytochrome P450 is not involved in the electron transport chain (Griffiths et al., 1985).

More than 10 plant microsomal and a similar number of plastid Δ-12 cDNAs and genes were isolated and reported to date. Arabidopsis mutants lacking both microsomal Δ-12 (*fad*2) and ω-3 desaturases (*fad*3) were isolated (Browse et al., 1986, 1993). Mutants at the *Fad*2 locus of Arabidopsis that are deficient in the major and, perhaps, only Δ-12 desaturase of the eukaryotic pathway were isolated and characterized. It was shown that the Arabidopsis *fad*2 mutants had similar growth characteristics to wild type at 22 °C but at 12 °C, their growth was greatly impaired and, at 6 °C, the mutants died (Miquel et al., 1993). This experiment showed that Arabidopsis requires polyunsaturated fatty acids for low temperature survival (Tocher et al., 1998). Subsequently, (Okuley et al., 1994) isolated the entire Arabidopsis *fad*2 cDNA sequence with T-DNA tagged line with higher 18:1 content in seeds, roots and leaves than the wild-type line.

After screening soybean libraries with the Arabidopsis *fad*2 cDNA, two different Δ-12 desaturase cDNAs, *FAD*2-1 and *FAD*2-2 were isolated (Heppard et al., 1996). *FAD*2-1 was expressed in developing seeds, whereas *FAD*2-2 was expressed in several tissues (leaves, roots, and stems) in addition to developing seeds.

Comparison of available sequence information reveals that there is a high degree of similarity between the same class of membrane-bound desaturases in different plant species, but much less similarity between different classes of desaturases, even in the same species (Murphy and Piffanelli, 1998). Membrane-bound enzymes most likely contain similar di-iron complexes (Fox et al., 1993). The most strictly conserved feature is the presence of eight histidines in three separate clusters. These clusters are held in position by a different ligation sphere, which may involve the three histidine boxes (Shanklin et al., 1994), which are characteristic for this group of enzymes [HX3-4H, HX2-3HH, (H/Q)X2HH]. This motif was also found in the Δ-12 oleate hydroxylases from castor bean and *Lesquerella fendleri* (van de Loo et al., 1995; Broun et al., 1997), epoxygenase from *Vernonia galamensis* (Hitz, 1998), acetylenase and epoxygenase from *Crepis* spp. (Lee et al., 1998) and the Δ-6 linoleate desaturase from borage (Beremand et al., 1997).

Another major goal of plant breeding has been to develop oils with high oxidative stability without the need for hydrogenation that are liquid at room temperature. Oils high in 18:1 are one way to achieve this. Mutant alleles affecting oleate levels in soybean are given the *ol* designation. An oleate content of >70% has been achieved by conventional breeding/mutagenesis (Alt et al., 2005b). The high oleate mutant, M23, has a deletion in *FAD2-1a* (Alt et al. 2005a). Using sense-mediated PTGS (co-suppression) targeting the Δ 12-desaturase (that converts oleic acid to linoleic acid), Toni Kinney and colleagues at DuPont Co. (Heppard et al., 1996; Kinney, 1998a; Kinney, 1998b) succeeded in producing a soybean with an oxidatively stable oil with a total polyunsaturated content of less than 5% and oleic acid content of 85% by suppressing the *Fad* 2-1 gene, whereas normal soybeans have about 20% oleic acid (18:1). This increase in oleate levels was accompanied by reduced levels of 18:2 from 55% to less than 1% and saturated fatty acids down to 10% (Beisson et al., 2003; Heppard et al., 1996). An oleate content of >90% by seed-specific suppression of *FAD*2-1 was reported by Buhr et al. (2002).

Fatty acid desaturases in all organisms are subject to several different types of regulation, depending on their localization and function. Those desaturases involved in membrane lipid biosynthesis have important 'housekeeping' functions and are therefore constitutively regulated (Murphy and Piffanelli, 1998). A cold-inducible plastidial ω-3 desaturase gene was isolated from Arabidopsis (Gibson et al., 1994) and there are several other reports that are consistent with the presence of cold-inducible ω-3 and Δ-12 desaturase genes in soybeans (Kinney, 1994); (Rennie and Tanner, 1989). However, there are other reports of the isolation of Arabidopsis and soybean Δ-12 desaturase genes that are not regulated by low temperature (Okuley et al., 1994); (Heppard et al., 1996). Since multigene families encode many desaturases, it is possible that some plant species may have both cold-inducible and non-cold-inducible forms of the same class of desaturase enzyme and/or gene (Murphy and Piffanelli, 1998).

ω-3 Desaturases

The Δ-12 and ω-3 desaturases introduce the second and the third double bonds in the biosynthesis of 18:2 and 18:3 fatty acids (which are important constituents of plant membranes). In most species, the fatty acids present in the galactolipids of the chloroplast membrane are ~70–80% trienoic fatty acids. In leaf tissue, there are two distinct pathways for polyunsaturated fatty acid biosynthesis, one located in the microsomes and the other located in the plastid membranes. In non-green tissues and developing seeds, the microsomal pathway predominates. Cytosolic and plastid ω-3 desaturation that result in the production of triene fatty acids are controlled by the *FAD*3, *FAD*7 and *FAD*8 loci in Arabidopsis (Lemieux et al., 1990; Arondel et al., 1992; Yadav et al., 1993; Browse et al., 1986; Lemieux et al., 1990; McConn et al., 1994).

Microsomal ω-3 desaturases are responsible for the production of extraplastidal 18:3. This enzyme accounts for over 80% of the 18:3 in Arabidopsis root tissues.

Arabidopsis *FAD*3 mutants are characterized by reduced levels of 18:3 and correspondingly increased 18:2 levels. However studies with the Arabidopsis *FAD*3 mutants revealed that exchange of lipid between chloroplast and ER allows the chloroplast desaturase to provide highly unsaturated lipid to the extrachloroplast membranes of leaf cells (Browse et al., 1993). Changing 18:3 levels of soybean seed oil has long been a goal of plant breeders and a number of low 18:3 mutants have been generated. Soybean genotypes A5 and A23 have reduced linolenic acid contents when compared with current cultivators. Byrum et al. (1997) reported that the reduced linolenic acid concentration in A5 was at least partially the result of partial or full deletion of a microsomal ω-3 desaturase gene. Alleles for reduced 18:3 in soybeans are designated *fan* alleles with the allele for reduced linolenate in A5 controlled by the *fan1* allele (Byrum et al., 1997). The soybean genome has at least three *FAD*3 ω-3 desaturase genes designated *GmFAD3A, GmFAD3B* and *GmFAD3C* (Bilyeu et al., 2005). Combining mutations *GmFAD3A, GmFAD3B* and *GmFAD3C* into single soybean lines (e.g. A29) can result in linolenate levels ∼1% (Anai et al., 2005; Bilyeu et al., 2006; Sarmiento et al., 1997). Experimental soybean lines with >50% 18:3 were reported by (Cahoon, 2003) by increased expression of a *FAD*3 gene in transgenic soybeans.

Membrane and TAG Synthesis

Both membrane and TAG synthesis begins with the acylation of *sn*-glycerol 3 phosphate producing lysophosphatidic acid, catalyzed by glycerol-3-phosphate acyltransferase (GPAT). A second acylation of lysophosphatidic acid catalyzed by lysophatidic acid acyl transferase (LPAT) produces phosphatidic acid (PA) (Fig. 12.3). The PA formed can be subsequently de-phosphorylated to diacylglycerol (DAG). The DAG then serves as a precursor for TAG. The third acylation step is catalyzed by DAG acyltransferase (DGAT). In oil seeds, phosphatidyl choline (PC) was identified as an intermediate in oil biosynthesis and plays a central role in the production of polyunsaturated fatty acids by serving as a substrate for Δ-6, Δ-9, Δ-12, and Δ-15 desaturases (Jackson et al., 1998). In order to induce large changes in oil composition, LPAT was considered an important target enzyme because of its selective discrimination ability (Śanchez et al., 1998). Rapeseed (*Brassica napus*) and meadowfoam (*Limnanthes*) have 60% and 90% erucic acid in their TAGs. In rapeseed erucic acid is excluded from the *sn*2 position, whereas in meadowfoam it is present in the *sn*2 position of TAGs. This difference was attributed to the substrate specificity of LPAT enzyme in both species (Cao et al., 1990). To alter the stereochemical composition of rapeseed oil, a cDNA encoding *Limnanthes* seed-specific LPAT was expressed in *Brassica napus* plants using a napin expression cassette. In the transgenic plants 22.3% erucic acid was present at the *sn*2 position leading to the production of trierucin. However, alteration of erucic acid at the *sn*2 position did not affect the total erucic acid content. It may be that the meadowfoam LPAT may not increase the erucic acid content of rapeseed (Lassner et al., 1995) because of the limited pool size of the 22:1 coenzyme A in the maturing embryos of *B. napus*. The metabolism of laurate was found to be different in transgenic *Brassica napus*

lines (transformed with a California bay lauroyl-acyl carrier protein thiosesterase cDNA driven by napin promoter) and the natural laurate accumulators coconut, oil palm and *Cuphea wrightii*. When tested at the mid-stage of embryo development, the PC had up to 26 mol % of laurate in the transgenic rapeseed high laurate line, whereas other species it ranged between 1 and 4 mol %. The laurate in the *Brassica* TAG was nearly totally confined to the outer *sn*1 and *sn*3 positions whereas the laurate in coconut and *Cuphea* was highest in the *sn2* position. Very low amounts of laurate were found in the *sn*2 position in DAG and PC of the rapeseed lipids indicating no arrangement of laurate between the outer and *sn*2 positions occurred in any of the lipids. There was an enhanced activity of lauroyl-PC metabolizing enzymes in the laurate producing rapeseed when the embryos were fed with radiolabeled ^{14}C-lauroyl-PC and ^{14}C-palmitoyl-PC. The data indicated that DAG was preferentially utilized from natural laurate accumulators like oil palm, coconut and *Cuphea* (Wiberg et al., 1997). Transgenic rapeseed oil expressing California bay thioesterase produced 60% saturated FA with laurate alone comprising 48%. In these plants laurate was presented at *sn*1 and *sn*3 positions only. When these plants were crossed with transgenic lines expressing coconut LPAT in the resulting hybrids, laurate was present at the *sn*2 position along with *sn*1 and *sn*3 positions. An overall increase in the oil content was also observed.

When the yeast LPAT genes SLC1 and SLC1-1 (mutant form of yeast LPAT) were expressed in *Brassica napus* and Arabidopsis under the CaMV35S promoter both TAG and VLCFA contents were increased by 56% and 80% (Zou et al., 1997). In the transgenic plants seed weight increased indicating at least a partial contribution from enhanced oil content. In the total oil content 60–75% consisted of VLCFAs and 40% that of non-VLCFAs such as palmitate, oleate, linoleate and linolenate. No increase in total oil content was reported in coconut or meadowfoam LPAT transformed rapeseed. This could be due to different regulatory properties of the plant and yeast LPAT enzymes. The plant LPAT genes have 62% amino acid identity among themselves, whereas the yeast gene had 24% homology. In transgenic plants, the high expression of the SLC1-1 gene did not correlate with high oil content indicating that even small levels of expression were sufficient to overcome the PA limitations during TAG biosynthesis. Although SLC1-1 levels were stronger in leaves than in seeds, no significant changes were observed in the fatty acid composition in leaves indicating the pools of available LPA and/or acyl-CoAs may be more tightly regulated in leaves (source) than in seeds (sink). We have preliminary results indicating a 1–2% increase in oil content of soybeans expressing the yeast SLC1 gene.

A DGAT was purified to apparent homogeneity from lipid body fractions of an oleaginous fungi, *Mortierella ramanniana* (Kamisaka et al., 1997). The purified DGAT utilized a broad range of molecular species of both DAG and acyl-coenzyme-A as substrates (Gavilano et al., 2006) and higher plants (Vogel and Browse, 1996). The first plant DGAT was cloned recently from Arabidopsis (Hobbs et al., 1999). The amino acid sequence shared 38% identity and 59% similarity with the mouse DGAT. Analysis revealed 9 membrane spanning helices and also 14 kD hydrophilic domain at the N-terminus. It had no significant sequence homology with plant GPAT and LPAT genes. Studies on expression of the homolog in *Brassica napus*, showed

that the DGAT mRNA was present in the highest concentrations in developing embryos, petals of flowers, and developing flower buds but in very low amounts in leaf and stem tissues.

Another reaction that appears to be involved in TAG accumulation is the reversible conversion of PC into DAG in presence of CDP choline transferase. Slack et al. (1985) gave indirect evidence for the reversibility of PC by labeling studies in vivo with linseed cotyledons and in vitro with safflower cotyledons. When sunflower microsomes were incubated with radiolabeled PC, the radioactivity was progressively incorporated into DAG. When the concentration of the microsomal protein was increased, the activity also increased indicating the reversible reaction of choline transferase in sunflower (Triki et al., 1998). A soybean cDNA encoding an aminoalcoholphosphotransferase (AAPTase) that demonstrates high levels of CDP-choline:*sn*-1,2-diacylglycerol cholinephosphotransferase activity was isolated by Dewey et al. (1994) by complementation of a yeast strain deficient in this function. AAPTases utilize diacylglycerols and cytidine diphosphate (CDP)-aminoalcohols as substrates in the synthesis of the main membrane lipids phosphatidylcholine and phosphatidylethanolamine and can possibly affect DAG pools for TAG synthesis.

Acyl-CoA: diacylglycerol (DAG) acyltransferase (DGAT; EC 2.3.1.20) activity has long been detected in various animal and plant tissues active in TAG synthesis. DGAT catalyzes the reaction:

$$\text{Acyl-CoA} + \text{DAG} \xrightarrow{\textbf{DGAT}} \text{TAG} + \text{CoASH}$$

As expected this enzyme is membrane bound or associated and difficult to work with biochemically. As such, the first DGAT gene was not cloned until 1998. Cases et al. (1998) reported the cloning and functional expression of a DGAT from mice. Hobbs et al. (1999) and Zou et al. (1999) reported the cloning of a DGAT from Arabidopsis. Lardizabal et al. (2001) reported the cloning of a second class of DGAT, DGAT2, from the oleagineous fungus *Mortierella ramanniana*, which had no homology to the earlier identified DGAT sequences now known as DGAT1s. Cases et al. (2001) also cloned a mammalian DGAT2 and its now known that humans have seven DGAT2s (Turkish et al., 2005). Only a single DGAT1 gene (At2g19450) and a single DGAT2 gene (At3g51520) are present in the Arabidopsis genome (Beisson et al., 2003; Mhaske et al., 2005). Soybeans have at least two DGAT1s (see below).

A second mechanism for biosynthesis of TAG in yeast and plants was discovered and reported in 2000 (Dahlqvist et al., 2000; Oelkers et al., 2000) that has homology to lecithin cholesterol acyltransferases (LCATs). This is catalyzed by an enzyme known as phospholipid: diacylglycerol acyltransferase (PDAT, EC 2.3.1.158) that transfers an acyl group (fatty acid) from a phospholipid (PL) to DAG forming TAG and a lysophospholipid (LPL):

$$\text{PL} + \text{DAG} \xrightarrow{\textbf{PDAT}} \text{TAG} + \text{LPL}$$

Arabidopsis has six PDAT/LCAT homologs (Stahl et al., 2004) of which (At5g 13640) is most closely related to the PDAT identified in yeast. Stahl et al. (2004)

demonstrated that this gene is expressed widely in different Arabidopsis tissues and has PDAT activity. In humans, most all TAG is synthesized by DGAT1 and DGAT2 and the only human gene similar to PDAT has phospholipaseA_2 and phospholipid:ceramide transacylase activities (Hiraoka et al., 2002). Mhaske et al. (2005) generated a knockout for At5g13640 and their studies plus those of Stahl et al. (2004) rule out a role for this gene in TAG synthesis in Arabidopsis seeds. A second Arabidopsis PDAT/LCAT homolog most related to t5g13640 (57% identical) is At5g44830. This PDAT/LCAT-like gene was found mainly expressed in developing seeds (Stahl et al., 2004). The authors speculated that it might have a role in seed oil biosynthesis. However this role has not been directly addressed nor has its activity been assessed.

A third TAG biosynthetic activity involving a DAG/DAG transacylase (DGTA) was reported in animals (Lehner and Kuksis, 1993) and plants (Stobart et al., 1997), including Arabidopsis (Stahl et al., 2004). DGTA catalyzes the reaction:

$$\mathrm{DAG} + \mathrm{DAG} \xrightarrow{\mathbf{DGTA}} \mathrm{TAG} + \mathrm{MAG}\,(\text{monoacylglycerol})$$

To date, no DGTA enzyme was biochemically characterized nor the corresponding gene cloned.

A number of mutants with reduced seed oil contents were reported in Arabidopsis and found to be due to defects in DGAT1 (Focks and Benning, 1998; Katavic et al., 1995; Lu and Hills, 2002; Routaboul et al., 1999; Zou et al., 1999) or were impaired in transfer of carbon from sucrose and glucose to TAG, possibly due to impaired hexokinase and pyrophosphate-dependent phosphofructokinase (Focks and Benning, 1998). Arabidopsis DGAT1 mutants have a ~25–50% reduction in seed oil content. DGAT1 is reported to be maximally expressed in developing seeds at a stage of high oil synthesis (Lu et al., 2003). Silencing of DGAT1 in tobacco was also reported to reduce seed oil content (Zhang et al., 2005). Our preliminary data (see below) indicates a role for DGAT1(s) in soybean oil synthesis but this has not been directly addressed. The role of DGAT2 in oil accumulation in Arabidopsis and common oilseeds such as soybeans has not been investigated.

Several reports indicated a role for DGAT in oil accumulation in developing soybean seeds (Kwanyuen and Wilson, 1986; Kwanyuen and Wilson, 1990; Kwanyuen et al., 1988; Settlage et al., 1998). No soybean mutants with large changes in oil levels or defects in DGAT have been reported. It is not yet clear what roles DGAT1, DGAT2 or possible other DGAT play in soybean oil biosynthesis. We detected transcripts for DGAT1, DGAT2 and PDAT in soybean tissues including developing seeds (our unpublished results). Developing soybean seeds accumulate TAG after most cell division has ceased and cotyledons have been formed and cell expansion initiated (Fig. 12.4) (Dahmer et al., 1991). Like most green tissues, linolenate (18:3) is the most abundant fatty acid of soybean oil early in seed development. The 18:3 levels of soybean oil continues to decline throughout seed development with linoleate (18:2) and oleate (18:1) becoming the predominate fatty acids of soybean oil as seeds mature (Dahmer et al., 1991) (Fig. 12.4). DGAT levels correlate with oil accumulation.

Oilseeds including soybeans accumulate TAG in special organelles known as oil bodies. There is strong evidence that oil bodies form with the accumulation of TAG inside the phospholipid bilayer in specialized regions of the ER ballooning out from the accumulating TAG and the remaining phospholipid forming a monolayer surrounding the growing lipid body. Concurrent with this, the oil body-specific protein, oleosin, is co-translationally inserted into the phospholipid monolayer of the oil bodies (Kalinski et al., 1991; Loer and Herman, 1993; Sarmiento et al., 1997; Siloto et al., 2006; Tzen et al., 1990).

Two full-length DGAT1s were cloned from developing soybean cDNA, designated GmDGAT1a (GenBank # AB257589) and GmDGAT1b (GenBank # AB257590). Soybean DGAT1a looks to be the same as GenBank entry # AY496439 (submitted 08 Dec. 2003) from the Institute of Genetics and Developmental Biology, Beijing, China (Wang et al., 2006). They have 99% identity with only two amino acid differences with our clone having a glycine, instead of aspartate, and a histidine, instead of glutamine, both toward the amino terminus and underlined below. This may be due to allelic differences in the genotypes used (GmDGAT1a cDNA is from the Group II cultivar 'Jack' and Wang et al. used cv. '8904'). GmDGAT1b does not match anything previously reported. A comparison of these with another partial soybean DGAT1 reported in GenBank and the Arabidopsis DGAT1 (CLUSTAL W, 1.83) is as follows:

```
soyDGAT1a       -------MAISDEPETVATALNHSSLRRRP---TAAGLFNSPETTTDSSGDDLAKDSGSD
AY496439        -------MAISDEPETVATALNHSSLRRRP---TAAGLFNSPETTTDSSGDDLAKDSGSD
soyDGAT1b       -------MAISDEPESVATALNHSSLRRRPSATSTAGLFNSPETTTDSSGDDLAKDSGSD
AY652765        ------------------------------------------------------------
AtDGAT1         MAILDSAGVTTVTENGGGEFVDLDRLRRRKSRSDSSNGLLLSGSDNNSPSDDVGAPADVR

soyDGAT1a       DSISSDAANSQPQQ---KQDTDFS-----------------VLKFAYRPSVPAHRKVKES
AY496439        DSISSDAANSQPQQ---KQDTDFS-----------------VLKFAYRPSVPAHRKVKES
soyDGAT1b       DSINSDDAAVNSQQQNEKQDTDFS-----------------VLKFAYRPSVPAHRKVKES
AY652765        ------------------------------------------------------------
AtDGAT1         DRIDSVVNDDAQGTANLAGDNNGGGDNNGGGRGGGEGRGNADATFTYRPSVPAHRRARES


soyDGAT1a       PLSSDTIFRQSHAGLFNLCIVVLVAVNSRLIIENLMKYGWLIKSGFWFSSKSLRDWPLFM
AY496439        PLSSDTIFRQSHAGLFNLCIVVLVAVNSRLIIENLMKYGWLIKSGFWFSSKSLRDWPLFM
soyDGAT1b       PLSSDTIFRQSHAGLFNLCIVVLVAVNSRLIIENLMKYGWLIKSGFWFSSKSLRDWPLFM
AY652765        --SSDAIFKQSHAGLFNLCIVVLVAVNSQLIIENLMKYGWSIKYGFWFSSKSLRDWPLFM
AtDGAT1         PLSSDAIFKQSHAGLFNLCVVVLIAVNSRLIIENLMKYGWLIRTDFWFSSRSLRDWPLFM
                  ***:**:**********:***:****:********** *: .*****:*********

soyDGAT1a       CCLSLVVFPFAAFIVEKLAQQKCIPEPVVVVLHIIITSASLFYPVLVILRCDSAFLSGVT
AY496439        CCLSLVVFPFAAFIVEKLAQQKCIPEPVVVVLHIIITSASLFYPVLVILRCDSAFLSGVT
soyDGAT1b       CCLSLVVFPFAAFIVEKLAQRKCIPEPVVVVLHIIITSTSLFYPVLVILRCDSAFVSGVT
AY652765        CCLSLAIFPLAAFVVERLAQQKCISEPVVVLLHLIISTVELCYPVLVILRCDSAFVSGVT
AtDGAT1         CCISLSIFPLAAFTVEKLVLQKYISEPVVIFLHIIITMTEVLYPVYVTLRCDSAFLSGVT
                **:** :**:*** **:*. :* *.****:.**:**: ..: *** * *******:****

soyDGAT1a       LMLFACVVWLKLVSYAHTNYDMRALTKSVEKGEALPDTLNMDYPYNVSFKSLAYFLVAPT
AY496439        LMLFACVVWLKLVSYAHTNYDMRALTKSVEKGEALPDTLNMDYPYNVSFKSLAYFLVAPT
soyDGAT1b       LMLFSCVVWLKLVSYAHTNYDMRALTKLVEKGEALLDTLNMDYPYNVSFKSLAYFLVAPT
AY652765        LMLLTCIVWLKLVSYAHTNYDMRALTVSNEKGETLPNTLIMEYPYTVTFRSLAYFMVAPT
AtDGAT1         LMLLTCIVWLKLVSYAHTSYDIRSLAN-------AADKANPEVSYYVSLKSLAYFMVAPT
                ***::*:***********.**:*:*:*          :.   : .* *:::*****:****
```

```
soyDGAT1a       LCYQPSYPRTPYIRKGWLFRQLVKLIIFTGVMGFIIEQYINPIVQNSQHPLKGNLLYAIE
AY496439        LCYQPSYPRTPYIRKGWLFRQLVKLIIFTGVMGFIIEQYINPIVQNSQHPLKGNLLYAIE
soyDGAT1b       LCYQPSYPRTPYIRKGWLFRQLVKLIIFTGVMGFIIEQYINPIVQNSQHPLKGNLLYATE
AY652765        LCYQTSYPRTPSVRKGWVFRQLVKLIIFTGVMGFIIEQYMNPIVRNSTHPLKGNLLYAIE
AtDGAT1         LCYQPSYPRSACIRKGWVARQFAKLVIFTGFMGFIIEQYINPIVRNSKHPLKGDLLYAIE
                ****.****:. :****: **:.**:****.********:****:** *****:**** *

soyDGAT1a       RVLKLSVPNLYVWLCMFYCFFHLWLNILAELLRFGGREFYQDWWNAKTVEDYWRMWNMPV
AY496439        RVLKLSVPNLYVWLCMFYCFFHLWLNILAELLRFGDREFYQDWWNAKTVEDYWRMWNMPV
soyDGAT1b       RVLKLSVPNLYVWLCMFYCFFHLWLNILAELLRFGDREFYKDWWNAKTVEDYWRMWNMPV
AY652765        RILKLSVPNVYVWLCMFYCFFHLWLNILAELVRFGDREFYKDWWNAKTVEEYWRMWNMPV
AtDGAT1         RVLKLSVPNLYVWLCMFYCFFHLWLNILAELLCFGDREFYKDWWNAKSVGDYWRMWNMPV
                *:*******:*********************: **.****:******:* :*********

soyDGAT1a       HKWMIRHLYFPCLRHGIPKAVALLIAFLVSALFHELCIAVPCHIFKLWAFGGIMFQVPLV
AY496439        HKWMIRHLYFPCLRHGIPKAVALLIAFLVSALFHELCIAVPCHIFKLWAFGGIMFQVPLV
soyDGAT1b       HKWMIRHLYYPCLRHGLPKAAALLIAFLVSALFHELCIAVPCHIFKLWAFGGIMFQVPLV
AY652765        HKWMVRHIYSPCLRRGIPKGAASLIAFLVSAVFHELCIAVPCHMFKLWAFIGIMFQVPLV
AtDGAT1         HKWMVRHIYFPCLRSKIPKTLAIIIAFLVSAVFHELCIAVPCRLFKLWAFLGIMFQVPLV
                ****:**:* ****  :**  * :*******:**********::****** *********

soyDGAT1a       FITNYLQNKFRNSMVGNMIFWFIFSILGHPMCVLLYYHDLMNRKGKLD
AY496439        FITNYLQNKFRNSMVGNMIFWFIFSILGQPMCVLLYYHDLMNRKGKLD
soyDGAT1b       LITNYLQNKFRNSMVGNMIFWFIFSILGQPMCVLLYYHDLMNRKGKLD
AY652765        LITNYLQNKYRNSMVGNMIFWFIFCILGQPMSVLLYYHDLMNRKGEVD
AtDGAT1         FITNYLQERFG-STVGNMIFWFIFCIFGQPMCVLLYYHDLMNRKGSMS
                :******:::  * **********.*:*:**.*************.:.
```

AA sequence comparison of soyDGAT1a and soyDGAT1b

```
soyDGAT1a       MAISDEPE**T**VATALNHSSLRRRP---**TA**AGLFNSPETTTDSSGDDLAKDSGSDDSI**S**SD**A**
soyDGAT1b       MAISDEPE**S**VATALNHSSLRRRPSAT**ST**AGLFNSPETTTDSSGDDLAKDSGSDDSI**N**SD**D**
                         *                  **                                  *  *
soyDGAT1a       A**NSQP**QQ---KQDTDFSVLKFAYRPSVPAHRKVKESPLSSDTIFRQSHAGLFNLCIVVLV
soyDGAT1b       A**AVNS**QQQNEKQDTDFSVLKFAYRPSVPAHRKVKESPLSSDTIFRQSHAGLFNLCIVVLV

                 ****
soyDGAT1a       AVNSRLIIENLMKYGWLIKSGFWFSSKSLRDWPLFMCCLSLVVFPFAAFIVEKLAQ**Q**KCI
soyDGAT1b       AVNSRLIIENLMKYGWLIKSGFWFSSKSLRDWPLFMCCLSLVVFPFAAFIVEKLAQ**R**KCI
                                                                          *
soyDGAT1a       PEPVVVVLHIIITS**A**SLFYPVLVILRCDSAF**L**SGVTLMLF**A**CVVWLKLVSYAHTNYDMRA
soyDGAT1b       PEPVVVVLHIIITS**T**SLFYPVLVILRCDSAF**V**SGVTLMLF**S**CVVWLKLVSYAHTNYDMRA
                           *                    *         *

soyDGAT1a       LTK**S**VEKGEAL**P**DTLNMDYPYNVSFKSLAYFLVAPTLCYQPSYPRTPYIRKGWLFRQLVK
soyDGAT1b       LTK**L**VEKGEAL**L**DTLNMDYPYNVSFKSLAYFLVAPTLCYQPSYPRTPYIRKGWLFRQLVK
                   *       *
soyDGAT1a       LIIFTGVMGFIIEQYINPIVQNSQHPLKGNLLYA**I**ERVLKLSVPNLYVWLCMFYCFFHLW
soyDGAT1b       LIIFTGVMGFIIEQYINPIVQNSQHPLKGNLLYA**T**ERVLKLSVPNLYVWLCMFYCFFHLW
                                                 *
soyDGAT1a       LNILAELLRFG**G**REFY**Q**DWWNAKTVEDYWRMWNMPVHKWMIRHLY**F**PCLRHG**I**PKA**V**ALL
soyDGAT1b       LNILAELLRFG**D**REFY**K**DWWNAKTVEDYWRMWNMPVHKWMIRHLY**Y**PCLRHG**L**PKA**A**ALL
                           *    *                                 *      *   *
soyDGAT1a       IAFLVSALFHELCIAVPCHIFKLWAFGGIMFQVPLV**F**ITNYLQNKFRNSMVGNMIFWFIF
soyDGAT1b       IAFLVSALFHELCIAVPCHIFKLWAFGGIMFQVPLV**L**ITNYLQNKFRNSMVGNMIFWFIF
                                                 *
soyDGAT1a       SILG**H**PMCVLLYYHDLMNRKGKLD
soyDGAT1b       SILG**Q**PMCVLLYYHDLMNRKGKLD
                  *
```

Alignment of soyDGAT1a and soyDGAT1b sequences by using CLUSTALW.

Different residues in two sequences are highlighted with bold letters and indicated with a star (22 aa different; ~ 4%)

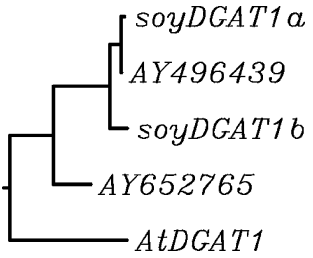

Fig. 12.5 Phylogenetic tree of five DGAT1 cDNA sequences using the CLUSTAL W program (version 1.82) Soy *DGAT1a* (1888 bp) (AB257589); *Soybean DGAT1b* (1960 bp) (AB257590) AY 496439 (accession #): soybean *DGAT1* full sequence (1880 bp) AY 652765 (accession #): soybean DGAT1 partial sequence (1413 bp) At DGAT1: Arabidopsis DGAT1 (1988 bp)

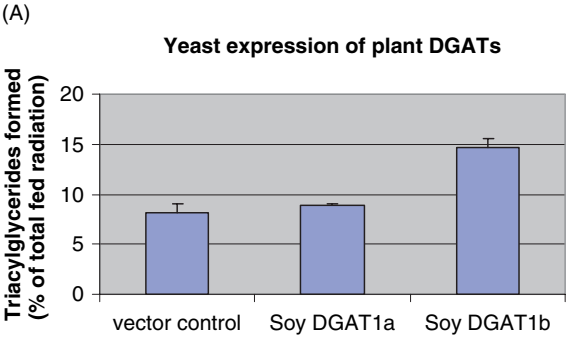

Fig. 12.6A Separately prepared yeast microsomes (50 μg determined by modified Lowry method) fed with 5 μM 18:2-CoA and 200 μM dioleoyl-DAG and incubated at 30 °C for 30 min

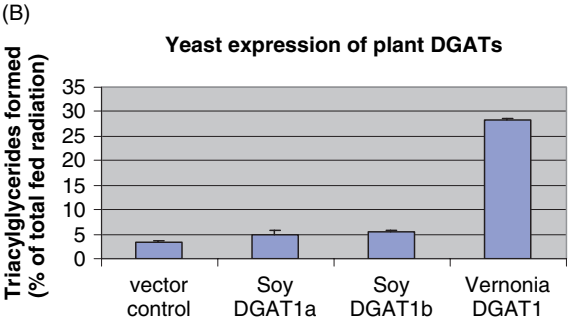

Fig. 12.6B Yeast microsomes assayed as for Fig. 12.6A. The Vernonia galamensis DGAT1 is included for comparison to a high oil accumulator

Interestingly GmDGAT1a and GmDGAT1b show somewhat different expression patterns although both show maximum transcript levels at stages of high TAG biosynthesis. The activity of these soybean DGAT1 cDNAs was analyzed in a yeast expression system. As illustrated in the following figure, we see little increase in DGAT activity with GmDGAT1a compared to the vector control and much greater activity with GmDGAT1b (Fig. 12.6A). Interestingly, we see much higher TAG biosynthetic activity with a DGAT from a much higher oil accumulating plant, *Vernonia galamensis*, than DGAT1s from soybeans (Fig. 12.6B).

The genomic sequence of soybean DGAT1a was recently reported (Wang et al., 2006) and we sequenced the full genomic sequence of DGAT1b. DGAT1a is 7575 bp and DGAT1b is 8164 bp. Both have 14 introns and 15 exons. The 2nd, 6th and 13th introns have the same length with small to large length differences seen with the other introns with most being longer in DGAT1b. Exons 1, 2, 3, 5 and 10 also show length differences between DGAT1a and DGAT1b.

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