Engineering oilseeds for sustainable production of industrial and nutritional feedstocks: solving bottlenecks in fatty acid flux

Edgar B Cahoon[1,*], Jay M Shockey[2,*], Charles R Dietrich[1], Satinder K Gidda[3], Robert T Mullen[3] and John M Dyer[2]

Oilseeds provide a unique platform for the production of high-value fatty acids that can replace non-sustainable petroleum and oceanic sources of specialty chemicals and aquaculture feed. However, recent efforts to engineer the seeds of crop and model plant species to produce new types of fatty acids, including hydroxy and conjugated fatty acids for industrial uses and long-chain omega-3 polyunsaturated fatty acids for farmed fish feed, have met with only modest success. The collective results from these studies point to metabolic 'bottlenecks' in the engineered plant seeds that substantially limit the efficient or selective flux of unusual fatty acids between different substrate pools and ultimately into storage triacylglycerol. Evidence is emerging that diacylglycerol acyltransferase 2, which catalyzes the final step in triacylglycerol assembly, is an important contributor to the synthesis of unusual fatty acid-containing oils, and is likely to be a key target for future oilseed metabolic engineering efforts.

Addresses
[1] US Department of Agriculture-Agricultural Research Service Plant Genetics Research Unit, Donald Danforth Plant Science Center, 975 North Warson Road, Saint Louis, Missouri 63132, USA
[2] US Department of Agriculture-Agricultural Research Service, Commodity Utilization Research Unit, Southern Regional Research Center, New Orleans, Louisiana 70124, USA
[3] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Corresponding author: Cahoon, Edgar B (ecahoon@danforthcenter.org)
[*] These authors contributed equally to this article.

Current Opinion in Plant Biology 2007, **10**:236–244

This review comes from a themed issue on
Physiology and metabolism
Edited by Clint Chapple and Malcolm M Campbell

Available online 16th April 2007

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2007.04.005

Introduction

Vegetable oils are composed almost entirely of triacylglycerol molecules, which consist of three fatty acids bound to a glycerol backbone, and represent a renewable source of raw materials that can be easily and economically extracted from seeds. Although vegetable oils are currently used primarily for edible applications (e.g. cooking oils and margarine), they hold considerable potential for a wide range of uses depending upon the physicochemical properties conferred by their constituent fatty acids. As crude oil supplies decline, vegetable oils are gaining increasing interest as substitutes for petroleum-derived materials in fuels, lubricants, and specialty chemicals [1]. For example, conventional vegetable oils, such as those derived from soybean seeds, are currently used for biodiesel production [2]. Oxidatively stable vegetable oils that have high and medium oleic acid contents are finding increasing use as high temperature, biodegradable lubricants [3]. Through metabolic engineering of their fatty acid compositions, it is possible to expand the potential uses of vegetable oils as renewable substitutes for petroleum-derived chemical feedstocks. Indeed, the extensive structural diversity of fatty acids found in nature, especially within the plant kingdom, reflects a deep reservoir of genes that could be used to produce novel or unusual fatty acids in existing oilseed crops [4]. By introducing these genes into crop plants, vegetable oils that have new physical and chemical properties could be produced to match the needs of different specialty chemical markets, such as plasticizers, coatings, and nylons.

Metabolic engineering of oilseeds also holds promise for providing a sustainable source of nutritional oil for aquaculture feed. This is especially important for Atlantic salmon farming, which exceeds 1.2 million tonnes of world-wide production [5]. As carnivores, Atlantic salmon have diets that are derived primarily from wild fish and other marine sources. The current feed regimen is, however, unsustainable because every kg of farmed salmon produced requires more than 3 kg of wild fish for feed [6]. In addition, about 25% of the diet of Atlantic salmon is fish oil, which is required to generate heart-healthy long-chain omega-3 polyunsaturated fatty acids (LCPUFAs) in the salmon meat in sufficient amounts to meet the nutritional expectations of consumers [6]. Because LCPUFAs are obtained primarily from dietary sources, attempts to replace fish oil in salmon feed stocks completely with conventional vegetable oils, such as linseed oil and palm oil, have failed to yield the desired amounts of LCPUFAs [7,8]. Consequently, to improve the feed quality of vegetable oils and to address the unsustainable nature of salmon farming, considerable attention has been directed in recent years toward the metabolic engineering of LCPUFA pathways in oilseed crops using genes isolated from diverse organisms that produce these fatty acids

[9,10,11••]. Notably, engineering of LCPUFA pathways in oilseeds will also improve the nutritional value of vegetable oils in food products.

Recent attempts to engineer industrial fatty acid and LCPUFA pathways in the seeds of crop and model species have met with some degree of success, but metabolic 'bottlenecks' appear to limit the full potential of oilseed crops to accumulate economically sufficient amounts of these novel fatty acids. These developments and their shortcomings are discussed, as are new insights into the role of specialized fatty acid acyltransferases in mediating the flux of unusual fatty acids in engineered seeds.

Metabolic engineering to produce improved vegetable oils for industrial usage or aquaculture feed

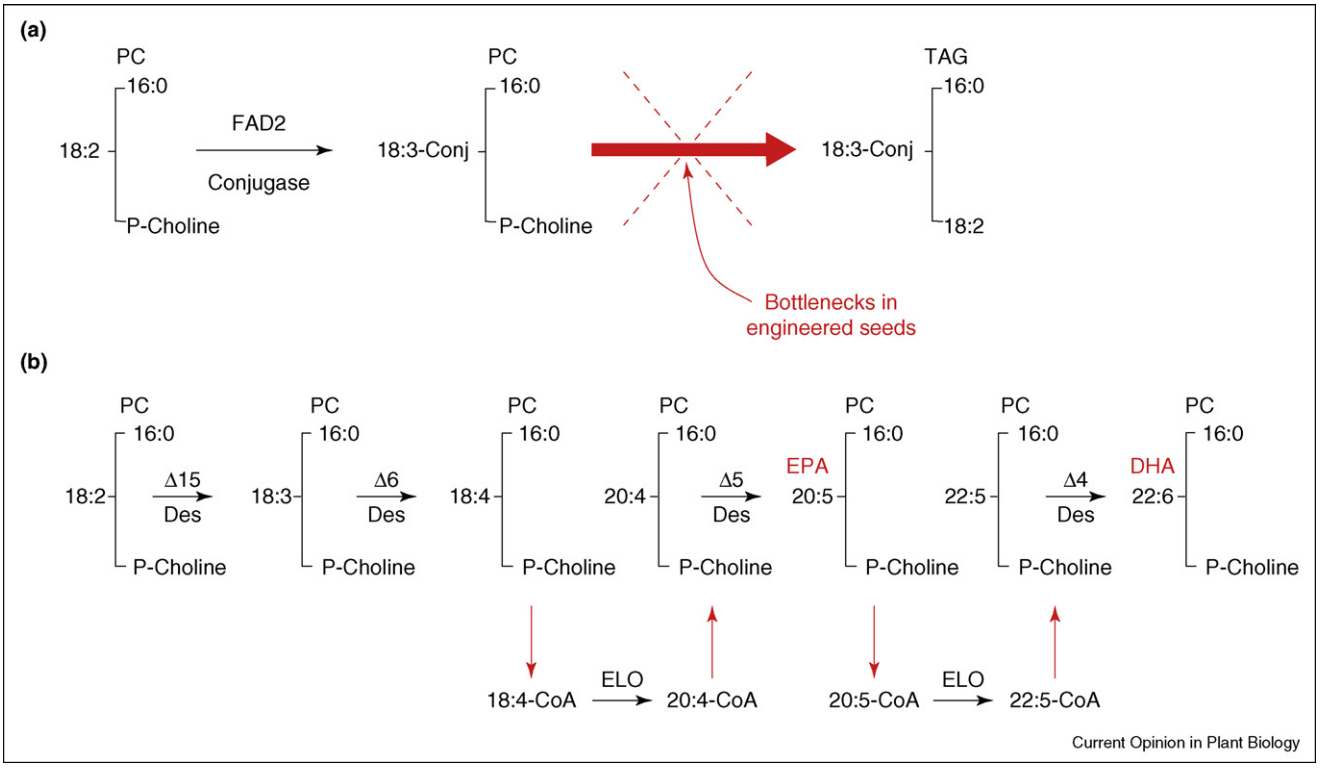
Recent efforts to produce unusual fatty acids in engineered seeds for novel industrial oils have focused largely on divergent forms of the Δ^{12}-oleic acid desaturase (FAD2) [4]. The typical FAD2 catalyzes the introduction of the *cis*-Δ^{12} double bond in oleic acid to produce linoleic acid. However, divergent forms of FAD2 have been identified in seeds from several non-agronomic species that catalyze a remarkably wide range of fatty acid modifications, including hydroxylation, epoxygenation, and double bond conjugation [4]. Many of these modified fatty acids have potential industrial significance. Castor bean seeds, for example, produce high levels of hydroxy fatty acids from the activity of a hydroxylase, which introduces a hydroxyl group at the Δ^{12} position of oleic acid to produce ricinoleic acid [12]. Vegetable oils that are rich in ricinoleic acid have properties that are desirable in nylon production and for lubricants, soaps and resins. Transgenic expression of the castor hydroxylase in *Arabidopsis* seeds has yielded oils that contain nearly 20% ricinoleic acid [13•,14]. This level has been achieved largely by use of *Arabidopsis* mutant backgrounds that provide more optimal fatty acid substrate pools for the hydroxylase [14]. Another recent focus has been the use of divergent FAD2 enzymes termed 'conjugases', which catalyze the formation of conjugated double bonds, to generate seed oils with improved drying properties for paint, ink and other coating applications (Figure 1a). The transfer of a FAD2 conjugase from pot marigold (*Calendula officinalis*) to soybean, for example, produced seed oils that contain 20% of the unusual conjugated fatty acid calendic acid (18:3$\Delta^{8trans,10trans,12cis}$) [15•].

The production of LCPUFAs in oilseed crops has been an even greater challenge because of the apparent complexity of the biosynthetic pathways for these fatty acids. The principle targets of this research have been the production of eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) (Figure 1b). Oils from most crop species are, however, enriched in linoleic acid, which contains only 18 carbon atoms and two double bonds. As a result, the production of EPA requires the introduction of at least four genes that encode Δ^{15}-, Δ^{6}-, and Δ^{5}-desaturases and a fatty acid elongating enzyme (ELO) [11••]. DHA production requires an additional Δ^{4} desaturase and a second ELO for elongation of the C_{20} fatty acid precursor (Figure 1b; [11••]). To date, the genes for these enzymes have been isolated from several marine algal, thraustochytrid, mammalian, and fungal sources [9,10,11••], and engineering of these pathways into *Brassica juncea* seeds, for instance, has yielded EPA and DHA levels of 8% and 0.2% of the total fatty acids, respectively [11••]. Similar experiments conducted in *Arabidopsis* generated seed oils that contained 2.4% EPA and 0.5% DHA [10].

'Bottlenecks' in fatty acid flux

Despite the successes of recent efforts to produce novel industrial oils in engineered plants by use of divergent FAD2 enzymes, the amounts of unusual fatty acids that accumulate are considerably less than those found in seeds from the natural sources of these fatty acids. For example, ricinoleic acid accumulates to more than 90% of the total fatty acids in castor seeds, which greatly contrasts with the 20% that has been engineered in *Arabidopsis* seeds [13•,14]. Similarly, calendic acid accounts for about 55% of the oil of pot marigold seeds, but only about 20% of the fatty acids in soybean seeds engineered to express the pot marigold FAD2 conjugase [15•]. One likely limitation to achieving high levels of divergent FAD2-derived unusual fatty acids is the inability of transgenic seeds to catalyze the efficient and selective flux of these fatty acids from phosphatidylcholine (PC) to storage in triacylglycerols (TAG) (Figure 1a). In this regard, fatty acids that are bound to PC serve as substrates for divergent FAD2s and, in order for the unusual fatty acid products to be sequestered in TAG, they must transit in some form from PC [12]. In a recent study, it was observed that soybean and *Arabidopsis* seeds that were engineered to produce conjugated fatty acids accumulate these fatty acids in PC-bound forms in relative amounts that are at least equal to those sequestered in TAG [15•]. By contrast, in seeds from five different species that naturally produce conjugated fatty acids, the accumulation of PC-bound conjugated fatty acids was limited to less than 1.5% of total fatty acids. The conjugated fatty acids instead only accumulated to high amounts in TAG. These results strongly indicate that mechanisms have not only evolved to synthesize conjugated fatty acids but have also evolved to selectively channel these fatty acids out of PC following their synthesis on this lipid. Such mechanisms for the maintenance of selective and efficient flux of conjugated fatty acids are apparently lacking in the engineered *Arabidopsis* and soybean seeds. Efficient flux of hydroxy fatty acids from their synthesis in PC to storage in TAG might also be limiting in the seeds of transgenic plants. For example, *Arabidopsis* seeds that

Figure 1



Routes for the biosynthesis of **(a)** conjugated fatty acids and **(b)** long-chain omega-3 polyunsaturated fatty acids (LCPUFA). Conjugated fatty acids (18:3-Conj), such as calendic acid (18:3$\Delta^{8trans,10trans,12cis}$) and eleostearic acid (18:3$\Delta^{9cis,11trans,13trans}$), are synthesized by the activity of FAD2-related enzymes termed fatty acid 'conjugases', which convert an existing double bond of linoleic acid (18:2$\Delta^{9cis,12cis}$) into two conjugated double bonds. As shown in (a), this reaction uses fatty acid substrates that are bound to phosphatidylcholine (PC), which is typical of divergent FAD2s including hydroxylases and epoxygenases. The resulting product is then sequestered into storage TAG in seeds, potentially through one or more of the reactions shown in Figure 2. Engineered seeds from soybean and *Arabidopsis* appear to have metabolic 'bottlenecks' that limit (as indicated by the red X) the efficient and selective flux of the 18:3-Conj product into triacylglycerol (TAG). This results in the accumulation of 18:3-Conj in PC and other phospholipids. By contrast, nearly all of the 18:3-Conj in seeds from plants that naturally produce conjugated fatty acids is sequestered in TAG. As shown in (b), the synthesis of LCPUFAs, such as eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), requires the shuttling of fatty acids between PC, the substrate for various desaturases (Des), and acyl-CoA pools for fatty acid elongation (ELO) (as indicated by the red arrows). In the case of DHA synthesis from linoleic acid, four different fatty acid desaturases modify the fatty acid substrates and two of these desaturation steps are interrupted by elongation steps. The efficiency of fatty acid transfer between the PC and acyl-CoA substrate pools probably limits the amounts of EPA and DHA produced by these pathways. Flux between these pools might be mediated by enzymes such as phospholipases and lysophosphatidylcholine acyltransferase. LCPUFAs that are produced at any of the steps shown can be sequestered into TAG, thereby removing the biosynthetic intermediates from the metabolite pools and further limiting the production of EPA and DHA.

express the castor hydroxylase display futile cycling of ricinoleic acid through degradative pathways (i.e. β-oxidation) in peroxisomes [16]. This is possibly triggered by the build-up of ricinoleoyl-CoA caused by a metabolic bottleneck in engineered seeds that restricts the flux of this fatty acid into TAG [16].

Fatty acid flux between different substrate pools, and competition for fatty acid intermediates between biosynthetic and storage pathways, appears to limit the production and accumulation of LCPUFAs in engineered oilseeds. In LCPUFA biosynthetic pathways, fatty acid desaturation reactions typically use PC as a substrate, whereas the elongation reactions use acyl-CoA substrates ([9,17]; Figure 1b). As a result, fatty acid precursors that are destined for conversion to EPA and DHA transit between PC and acyl-CoA pools. This presents a challenge especially for attempts to engineer DHA synthesis, in which desaturation reactions are interrupted by two fatty acid elongation steps. In addition, evidence from detailed analyses of lipids in tobacco and flax seeds engineered to produce EPA suggests that a portion of the product of Δ6 desaturation is shunted out of the LCPUFA biosynthetic pathway for use in TAG synthesis [9]. This effectively decreases the amount of fatty acid substrates that are available for ultimate conversion to EPA and DHA. Hence, bottlenecks in the flux of LCPUFA intermediates between PC and acyl-CoA substrate pools, and the channeling of

fatty acid intermediates out of the LCPUFA biosynthetic pathway for TAG synthesis, may account for the relatively low accumulations of DHA (~0.5%) that have been reported in the seed oils of transgenic *Brassica juncea* and *Arabidopsis* [10,11••], which contrasts with the 5% DHA that is typically found in fish oil [8]. One solution that has been proposed for increasing the accumulation of EPA and DHA in engineered oilseeds is the introduction of enzymes that mediate the rapid and specific flux of fatty acid intermediates, especially the Δ6 desaturation product, between PC and acyl-CoA pools [9].

Improving fatty acid flux through knowledge-based gene discovery and application

Figure 2 summarizes the enzymatic activities that are currently known or suspected to play roles in the flux of fatty acids between the PC, acyl-CoA and TAG pools. The example shown illustrates the possible pathways that can account for the metabolism of conjugated fatty acids; however, one or more of these reactions is probably involved in the transit of other fatty acids, including LCPUFAs and hydroxy fatty acids, into TAG. This model of TAG biosynthesis has been developed on the basis of studies of seeds that produce common C_{16} and C_{18} fatty acids. It is envisioned, however, that plants that accumulate unusual fatty acids have evolved divergent forms of one or more of the enzymes in Figure 2 that are capable of directing flux of the unusual fatty acid through the different pathways shown, resulting in their eventual accumulation in TAG. For unusual fatty acids such as conjugated and hydroxy fatty acids, it appears that specialized metabolic enzymes have also evolved to prevent the build-up of the unusual fatty acids in PC and other membrane phospholipids. For example, radiolabeling studies with castor bean have suggested that a specific phospholipase A_2 removes ricinoleic acid from PC (Figure 2, reaction b) following its synthesis on this lipid, and that the released ricinoleic acid is then activated to an acyl-CoA (reaction c) before its incorporation into TAG (reactions f, g, or i) [18]. Plants are known to contain very large gene families for phospholipases [19] and acyl-CoA synthetases [20,21], but no isoforms for these enzymes have been demonstrated to play a specific role in the channeling of unusual fatty acids into TAG. In LCPUFA synthesis, phospholipases and acyl-CoA synthetases might play a role in the flux of fatty acids from PC and into the bulk acyl-CoA pool for elongation (Figure 1b).

In vitro evidence also suggests that unusual fatty acids can be channeled into TAG by direct transfer from PC to diacylglycerol (DAG), forming TAG via an acyl CoA-independent reaction that is catalyzed by phospholipid:-diacylglycerol acyltransferase (PDAT; Figure 2, reaction e) [22]. Genes for PDAT have been identified in plants [22,23], but little evidence has been found to date to suggest that these enzymes play a major quantitative or qualitative role in seed TAG metabolism [24].

Flux of unusual fatty acids out of the phospholipid pool might also be controlled in part by choline phosphotransferase (CPT), an enzyme that interconverts PC and DAG (reaction d), and/or by acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT), a reversible acyltransferase that interconverts acyl-CoA and lysophosphatidylcholine with PC (reaction a) [25]. Notably, LPCAT activity has been suggested to be a limiting factor in the flux of Δ6 desaturation products between phospholipid and acyl-CoA pools in LCPUFA synthesis in the seeds of engineered plants [9]. Determining the general roles of these enzymes in mediating fatty acid flux in developing seeds has, however, been hampered by a lack of molecular analysis of cloned plant LPCAT genes.

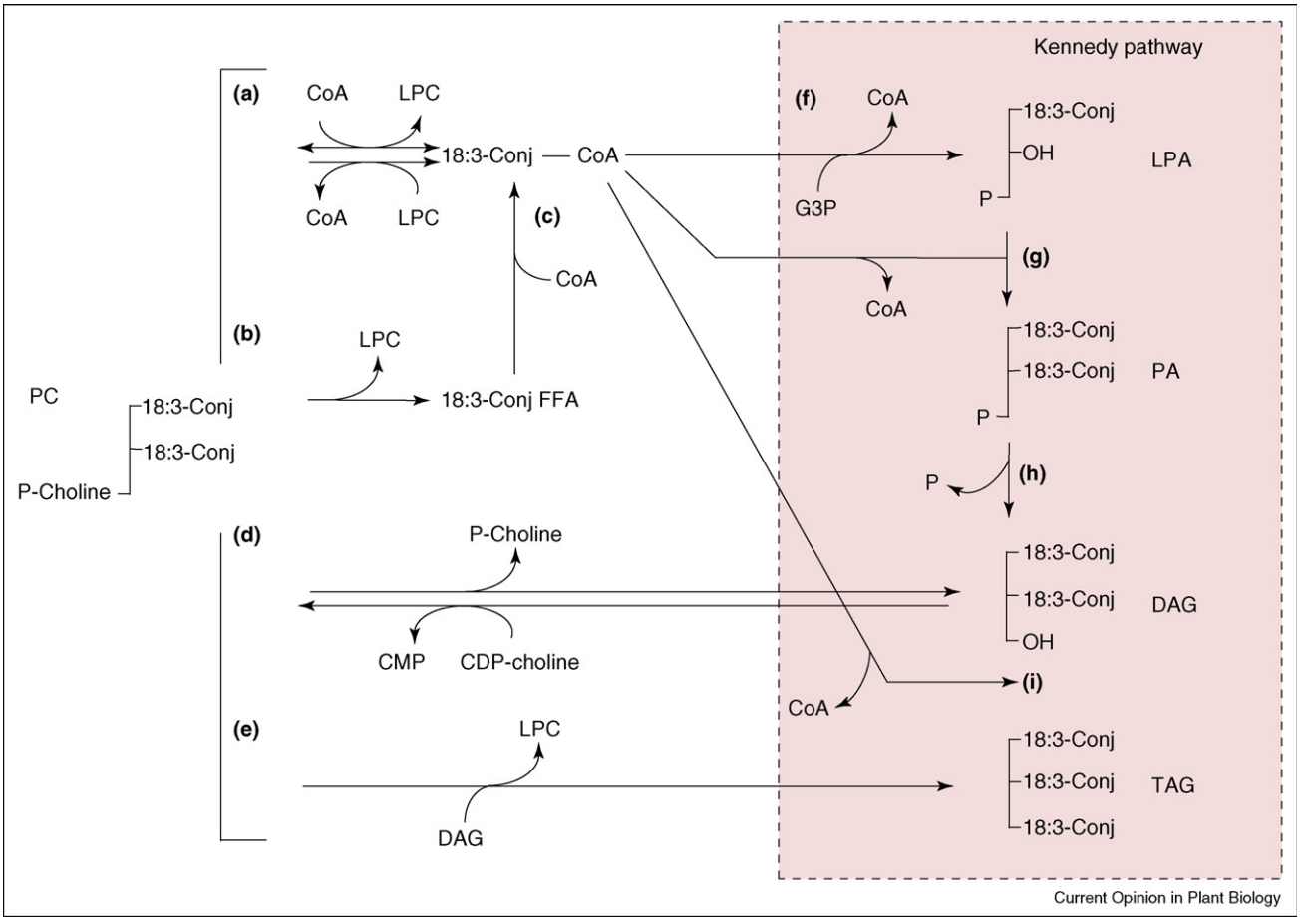
More attention has focused on the characterization of the three enzymes that carry out the successive acylations of the glycerol backbone to produce TAG: glycerol-3-phosphate acyltransferase (GPAT, reaction 6) [26], lysophosphatidic acid acyltransferase (LPAT, reaction g) [27], and diacylglycerol acyltransferase (DGAT) (reaction i) [28–30]. Collectively known as the Kennedy pathway (red box in Figure 2), these three enzyme classes play important roles in determining the fatty acid content of TAG in oilseed crops. *Arabidopsis* contains at least eight GPAT genes, most of which are probably targeted to the endoplasmic reticulum (ER) [26]. To date, only the AtGPAT1 and AtGPAT5 isoforms have been studied in detail, and neither has been shown to play a clear role in seed TAG biosynthesis [27,31]. LPAT is encoded by perhaps as many as five genes in *Arabidopsis* [27], and LPAT genes from various other species have been cloned and the corresponding enzymes shown to display selective substrate specificities [32–34]. LPAT genes have proven to be useful for increasing the accumulation of target fatty acids in TAG in transgenic crops [34,35], and specific isoforms are likely to be important for increasing levels of high-value unusual fatty acids in the seeds of engineered plants.

Emerging roles for DGAT enzymes in selective fatty acid flux

The interplay between the many acyltransferases (Figure 2) is not well understood, but there is general agreement that DGAT, the last enzyme in the Kennedy pathway, exerts a strong influence on the amount and composition of TAG synthesized in developing seeds.

DGAT catalyzes the committed step of oil biosynthesis by transferring a fatty acyl group from acyl-CoA to a diacylglycerol substrate to form TAG (Figure 2, reaction i). The biochemical activity of DGAT has been known for many years, but the genes that encode these enzymes have been discovered only recently. Surprisingly, at least

Figure 2



Multiple routes exist for the transfer of conjugated fatty acids from phospholipids to triacylglycerol (TAG). After the synthesis of conjugated (18:3-Conj) fatty acids (or many other types of unusual fatty acids) on phosphatidylcholine (PC) (structure shown on the left), several different enzymes contribute to the channeling of these fatty acids into TAG. **(a)** Reversible lysophosphatidylcholine acyltransferase (LPCAT) transfers a fatty acid from PC to CoA, yielding a fatty acyl-CoA (18:3-Conj-CoA) and lysophatidylcholine (LPC). **(b)** Phospholipase A_2 (PLA_2) cleaves a fatty acid from PC, yielding a 'free' (non-esterified) fatty acid (18:3-Conj FFA) and LPC. **(c)** Long-chain acyl-CoA synthetase (LACS) ligates a FFA to CoA, yielding a fatty acyl-CoA (18:3-Conj-CoA). **(d)** Reversible choline phosphotransferase (CPT) catalyzes the release of the PC head group (P-Choline) to produce to diacylglycerol (DAG). **(e)** Phospholipid:diacylglycerol acyltransferase (PDAT) transfers a fatty acid from PC to DAG to produce TAG and LPC. Fatty acids in the form of acyl-CoA (18:3-Conj-CoA) can be incorporated into TAG by the enzymes of the Kennedy pathway (reactions highlighted on the right in the red box). **(f)** Glycerol-3-phosphate acyltransferase (GPAT) transfers a fatty acid from fatty acyl-CoA (18:3-Conj-CoA) to glycerol-3-phosphate (G3P), yielding CoA and lysophosphatidic acid (LPA). **(g)** Lysophosphatidic acid acyltransferase (LPAT) transfers a fatty acid from the fatty acyl-CoA (18:3-Conj-CoA) pool to LPA, forming CoA and phosphatidic acid (PA). **(h)** Phosphatidic acid phosphatase (PAP) cleaves the phosphate head group from PA, yielding phosphate and DAG. **(i)** Diacylglycerol acyltransferase (DGAT) transfers a fatty acid from the fatty acyl-CoA (18:3-Conj-CoA) pool to DAG, yielding CoA and TAG. Recent findings implicate particular isoforms of DGAT as key regulators of the overall flux of specific fatty acids in TAG.

three different, structurally unrelated enzymes catalyze DGAT activity. The first two enzymes to be identified were DGAT1 and DGAT2, both of which are ER-localized, containing ten and two predicted membrane-spanning domains, respectively [28–30,36•,37••]. The third enzyme is a soluble DGAT (DGAT3), which was only recently identified in peanut and other plant species [38•].

DGATs appear to be crucial for mediating quantitative and qualitative aspects of seed oil synthesis in transgenic plants. Indeed, overexpression of *DGAT1* has been shown to increase oil content modestly in *Arabidopsis* seeds [39], whereas suppression of DGAT activity reduced oil content [29,40]. DGAT activity is also likely to be important for the channeling of unusual fatty acids into seed storage oils. For example, microsomal preparations from *Vernonia* and *Stokesia* seeds contain DGAT enzyme activities that prefer substrates containing the unusual epoxy fatty acid vernolic acid, which accumulates in the seeds of these species [41]. Furthermore, the recent cloning and comparative analysis of DGAT1 and DGAT2 from various

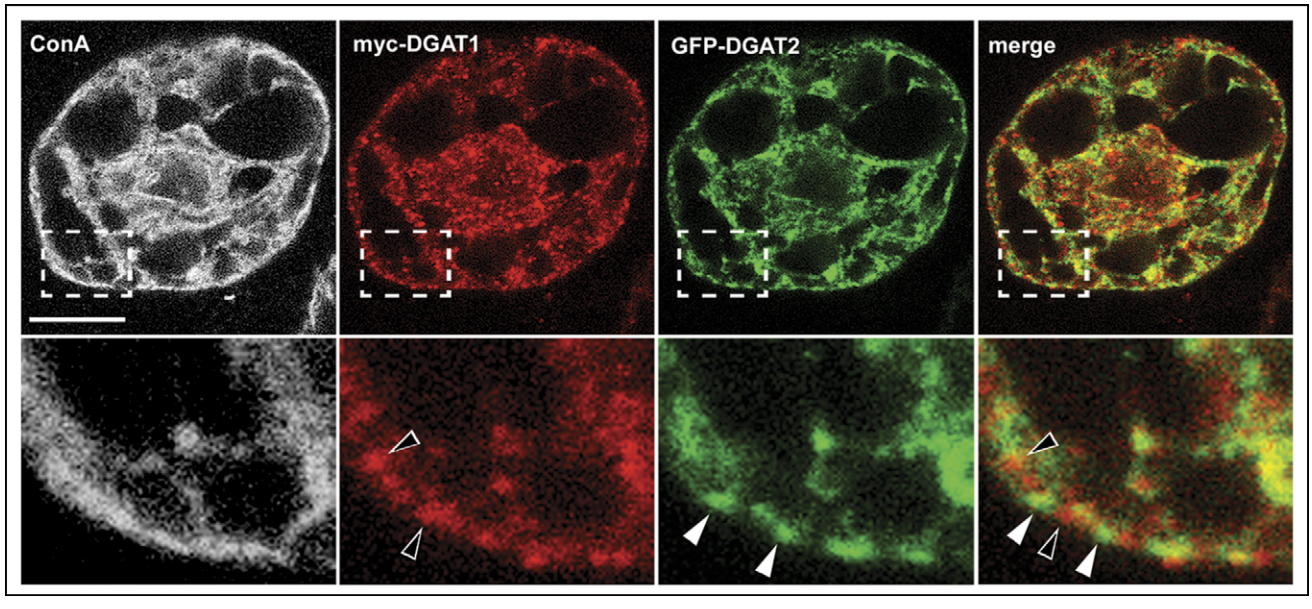
plant species has begun to suggest that DGAT2 enzymes are predominantly involved in the transfer of unusual fatty acids into storage oil [36•,37••]. For example, expression of *DGAT2* from tung (whose seeds accumulate more than 80% of their fatty acids as the conjugated fatty acid eleostearic acid) in yeast cells resulted in elevated synthesis of TAG molecules containing eleostearoyl groups relative to those produced by expression of tung *DGAT1* [37••]. The tung *DGAT2* gene is more highly expressed than tung *DGAT1* in developing tung seeds, and the timing of *DGAT2* gene expression coincides closely with the onset of eleostearic acid biosynthesis and total oil accumulation in tung seed [37••]. Similarly, in castor bean seeds, *DGAT2* gene expression predominates over *DGAT1*, showing an 18-fold enhancement in developing seeds when compared to leaves, whereas *DGAT1* is expressed at nearly equal levels in seeds and leaves [36•,42]. However, castor *DGAT1* might undergo post-translational regulation, as DGAT1 protein levels closely parallel DGAT activity but do not correlate with *DGAT1* mRNA levels during the development of castor seeds [43]. Both castor DGAT1 and DGAT2 use substrates that contain ricinoleic acid [38•,43]; however, neither enzyme has yet undergone thorough biochemical analysis with respect to its ability to preferentially synthesize triricinolein. Collectively, these data suggest that additional studies will be required to substantiate the roles of DGAT1 and DGAT2 in plants.

The role of the soluble DGAT3 in storage oil production has yet to be clarified, but it is likely that this enzyme, together with DGAT1 and DGAT2, has a non-redundant function in plant cells. Thus, co-expression of enzymes for unusual fatty acid synthesis, along with an appropriate DGAT enzyme from the source plant species (particularly a DGAT2 activity), is likely to increase the accumulation of unusual fatty acids in the seeds of transgenic plants.

Elucidating cellular aspects of storage oil formation

A better understanding of the molecular and cellular details of storage oil production will undoubtedly increase our ability to engineer plants rationally to produce oils that have tailor-made fatty acid compositions. Toward this end, one of the long-standing questions in plant lipid metabolism is whether there are specific regions of the ER that are dedicated to storage oil biosynthesis. Recent evidence from both plants and animals indicates that this is likely to be the case, and furthermore, that the correct assembly of these 'oil domains' might be essential for the channeling of fatty acids into TAG. For example, recent

Figure 3



Localization of DGAT1 and DGAT2 to distinct subdomains in the endoplasmic reticulum. Reproduced from [37••] (copyright American Society of Plant Biologists). Myc-epitope-tagged versions of tung DGAT1 and green fluorescent protein (GFP)–tagged tung DGAT2 were co-expressed (via particle bombardment) in tobacco BY-2 suspension-cultured cells and (immuno)fluorescence was detected by confocal laser scanning microscopy. The micrographs show a representative triple-stained cell, illustrating the fluorescence attributable to the general ER (ConA), DGAT1 (myc-DGAT1) and DGAT2 (GFP–DGAT2) at low (top panels) and high (bottom panels) magnification. Hatched boxes represent the portion of the cell shown at higher magnification in the panels below. The general ER (ConA) exhibits a more uniform staining pattern, whereas the fluorescence attributable to DGAT1 and DGAT2 is concentrated at specific regions (subdomains) of the ER, giving rise to the appearance of distinct red or green spots, respectively (refer to black and white arrowheads). Interestingly, the regions of the ER occupied by DGAT1 and DGAT2 are not shared, as evidenced by the non-overlapping red and green spots in the merged image. The scale bar represents 10 μm.

studies with tung DGAT1 and DGAT2 have demonstrated that these enzymes are located in distinct regions of the ER in plant cells ([37••]; Figure 3). In addition, freeze-fracture immunolocalization of mammalian cells has shown enrichment of the protein adipophilin in specific ER regions that are closely apposed to nascent oil bodies [44•]. On the basis of these observations, the general picture that emerges is that oil production, like many other cellular processes that involve multiple metabolic steps, is specifically compartmentalized to ensure the proper channeling and flux of metabolites and the accumulation of appropriate products. Notably, it has been demonstrated recently that stearoyl-CoA desaturase (a FAD2-like enzyme) is physically associated with DGAT2 in mammalian cells, and that this close physical relationship is essential for the channeling of oleic acid into storage oil [45••].

Conclusions and perspectives

Recent efforts to engineer the seeds of crop and model plant species to produce high-value fatty acids, including conjugated and hydroxy fatty acids and LCPUFAs, for industrial and nutritional uses have met with modest success. The production of vegetable oils that contain economically relevant amounts of these fatty acids hinges on an increased understanding of factors that mediate fatty acid flux between the PC, acyl-CoA and TAG pools. Of the fatty acid metabolic enzymes characterized to date, DGAT2 appears to be an important determinant in the incorporation of unusual fatty acids that are produced by FAD2-type enzymes, including conjugated and hydroxy fatty acids, into TAG. Subcellular localization studies of DGAT2, as well as DGAT1, also point to the possibility that discrete subdomains within the ER of seeds are associated with TAG biosynthesis. Ultimately, through the identification of enzymes that are specialized for the metabolism of unusual fatty acids and by determining the subcellular organization of these enzymes, it should be possible to design the next generation of oilseed crops more rationally for the sustainable production of high value fatty acids.

Acknowledgements

We thank Dr Ernst Heinz for critical reading of the manuscript.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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proteins known to be involved in oil body formation, and that the ER membrane forms a 'cup' within these regions in which the developing oil body appears to reside. These results suggest that there are spatially distinct pools of lipid metabolites and enzymes that are involved in regular 'housekeeping' ER lipid metabolism and storage oil formation.

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