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REVIEW

Acyl-Trafficking During Plant Oil Accumulation

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Abstract Vegetable oils are an extremely important agricultural commodity. Their use has risen inexorably for the last 50 years and will undoubtedly be even more prevalent in the future. They have a role not only in foodstuffs but also as renewable chemicals. However, our understanding of their metabolism, and particularly its control, is incomplete. In this article we highlight current knowledge and its deficiencies. In particular, we focus on the important role that phosphatidylcholine plays in lipid accumulation and in influencing the quality of the vegetable oils produced.

Keywords Oil crops · Triacylglycerol biosynthesis · Kennedy pathway · Phosphatidylcholine · Metabolic control

Abbreviations

ACCase	Acetyl-CoA carboxylase
ACP	Acyl carrier protein
CDP-choline	Cytidine diphosphate-choline
CPT	Cholinephosphotransferase
DAG	Diacylglycerol
DGAT	Diacylglycerol acyltransferase
ER	Endoplasmic reticulum
FAD	Fatty acid desaturase
FAS	Fatty acid synthase
FAT	Fatty acyl-ACP thioesterase
FAX1	Fatty acid export 1
G3P	*sn*-Glycerol 3-phosphate
GPAT	Glycerol 3-phosphate acyltransferase
GPC	Glycerophosphocholine
GPCAT	Glycerophosphocholine acyltransferase
KAS	β-Ketoacyl-ACP synthase
LACS	Long-chain acyl-CoA synthetase
LPA	Lysophosphatidic acid
LPAAT	Lysophosphatidate acyltransferase
LPC	Lysophosphatidylcholine
LPCAT	Lysophosphatidylcholine acyltransferase
LPCT	Lysophosphatidylcholine transacylase
MGDG	Monogalactosyldiacylglycerol
NAD(P)H	Nicotinamide adenine dinucleotide (phosphate)
NEFA	Nonesterified fatty acid (free fatty acid)
PA	Phosphatidic acid (PtdOH)
PAPase	Phosphatidate phosphohydrolase
PDAT	Phospholipid:diacylglycerol acyltransferase
PDCT	Phosphatidylcholine:diacylglycerol cholinephosphotransferase
PLA	Phospholipase A
PLC	Phospholipase C
PLD	Phospholipase D
PtdCho	Phosphatidylcholine
PtdGro	Phosphatidylglycerol
PtdIns	Phosphatidylinositol
PUFA	Polyunsaturated fatty acid
ROD1	Reduced oleate desaturation 1
TAG	Triacylglycerol

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Introduction

Oil crops are a very important agricultural commodity with current value of about US $120 billion [1]. Usage of these oils has increased steadily at an annual rate of about 5 % over the last 50 years [2]. Although increased sowings and better agricultural productivity have managed to keep pace with demand until now, it is clear that, with limited fertile land, an increasing world population, and limits to productivity improvements, plant oils will soon be in short supply. In addition to the above factors, agricultural fats and oils will be needed increasingly for petrochemical replacements as biofuels or renewable chemicals [3, 4].

The major uses of biological oils are for food, as animal feeds, and as renewable chemicals or precursors thereof (79, 7, and 15 % in 2012, respectively). Four crops dominate world production of commodity oil—oil palm (*Elaeis guineensis*, *E. oleifera*, and *Attalea maripa*), soya bean (*Glycine max*), oilseed rape (*Brassica napus*), and sunflower (*Helianthus annuus*) [5, 6]. Currently these produce about 23, 22, 12, and 9 % of total commodity oils, respectively (Table 1). The rise in palm oil production over the last half century has been particularly marked, but future increases will be much smaller because of its limited growing regions and because of the praiseworthy example of Malaysia in strictly limiting future plantation expansion and the sponsoring of "sustainable palm oil" [7]. It remains to be seen if Indonesia will adopt similarly careful monitoring and regulation of oil palm plantations. The domination of world markets by four crops reduces the range of oils available. Although transgenic varieties of soybean and oilseed rape have been developed, the current public backlash against foods derived through genetic engineering means that the market is limited, especially in the European Union but also in other parts of the world including China and India. For sunflower, there are some varieties available which have altered oil composition such as high oleate (18:1Δ^{9cis}) or high palmitate (16:0)–high oleate varieties [8], but few oil palm cultivars have been developed for oil quality, with efforts instead being focused on crop yield [7].

Many industrial applications require particular types of oil. This has led to the specialized use of many minor crops [6, 9]; for example, coconut (*Cocos nucifera*) oil with its high content of laurate is used for surfactant production [4]. Linseed (flax: *Linum usitatissimum*) enriched in α-linolenate (18:3$\Delta^{9cis,12cis,15cis}$) is used for paints and linoleum, and castor oil (*Ricinus communis*), which has 90 % ricinoleate (12-OH 18:1Δ^{9cis}), is a useful lubricant [2, 4]. Efforts to manipulate high-yielding standard oil crops to produce specialized renewable chemicals have achieved some success. For example, some 20 years ago, Calgene produced genetically engineered oilseed rape that accumulated 60 % laurate [10], but this could not compete on the open market with coconut or, in particular, palm kernel oil. Nevertheless, as discussed below, much effort is focused on the production of specialized oils because it is certain that sooner or later there will be a use for them [4]. The use of genetically engineered oil crops for chemical purposes should attract less public opposition than their use for foods.

Eventually, the genetic manipulation of crops to produce oil more efficiently or to make novel specialized oils for particular purposes should be accepted as a good way to manage the planet's environment. Therefore, scientists need to persevere in their efforts to understand metabolism in oil crops so that they are in a good position to inform future developments in a responsible way. There are two particular aspects that need attention: firstly, to increase total oil yields [11, 12] and, secondly, to understand how to efficiently change the fatty acid composition and channel such acyl chains into triacylglycerol (TAG) [13, 14]. Both of these aspects are discussed in the following sections.

Triacylglycerol Biosynthesis

Almost all plant oils are predominantly composed of TAG, the only significant commercial exception being the wax esters of the desert plant jojoba (*Simmondsia chinensis*). Basically TAG is formed in two phases. First, fatty acids are synthesized de novo in the plastid, from where they can be exported to the cytosol. Second, fatty acid modifications can take place on the endoplasmic reticulum (ER) before they are assembled into TAG end-products via either the acyl-CoA-dependent pathway

Table 1 World edible oil sources: trends, importance, and prices

Crop	Production (million tonnes per annum)			Rank 2014	Price (US$/tonne) 2014
	1976/1980	1996/2000	2013/2014		
Oil palm	3.7	18.7	59.6	1	657
Soya bean	11.2	23.1	44.6	2	712
Oilseed rape	3.0	12.6	26.1	3	820
Sunflower	4.2	9.1	15.6	5	983
Other vegetable oils	13.3	20.1	12.1	6	~740
Animal fats	17.2	21.3	24.4	4	~830
Total	52.6	105.1	165.1		

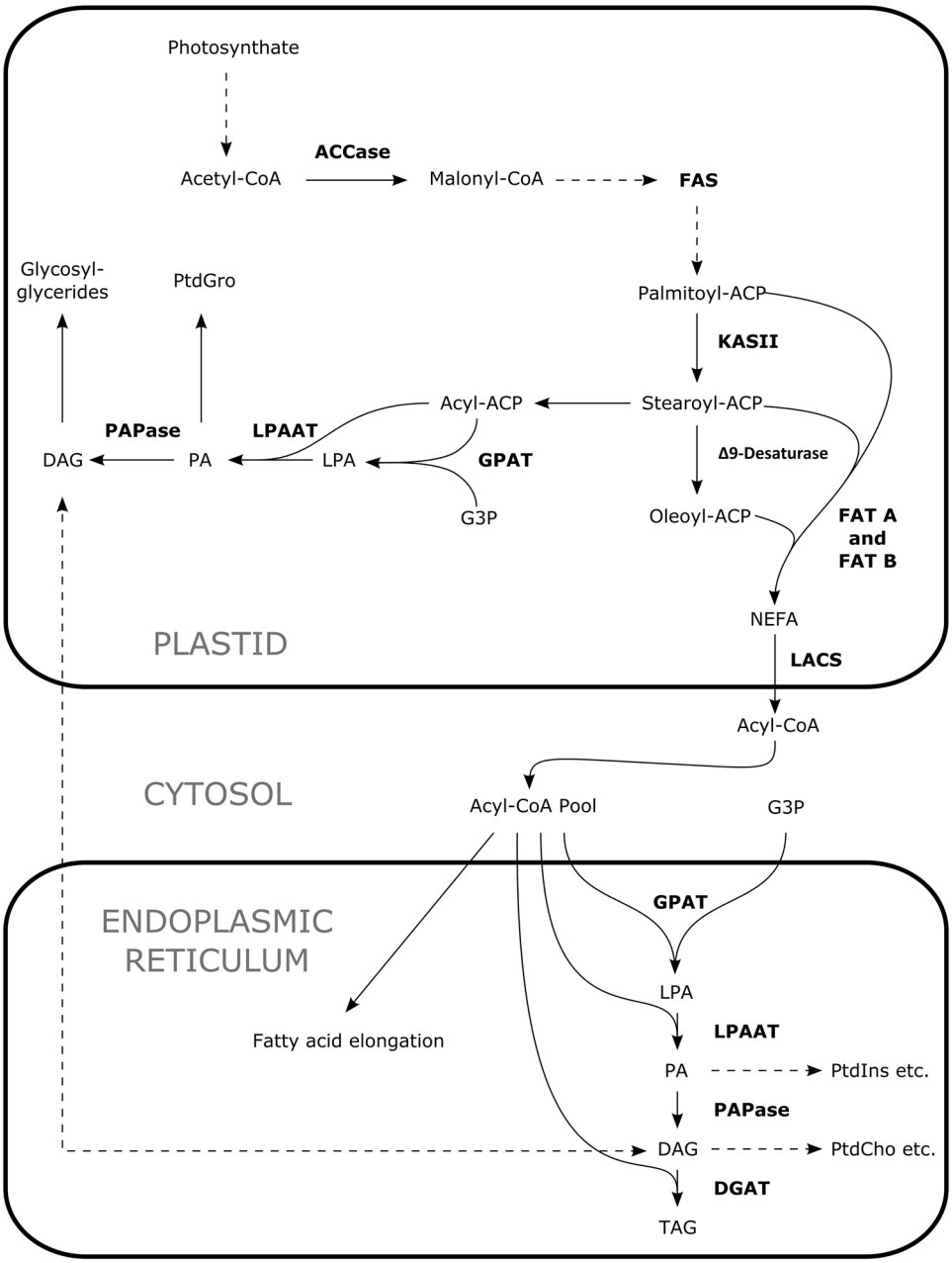

Fig. 1 Triacylglycerol biosynthesis in plants via the Kennedy pathway. Fatty acid biosynthesis occurs in the plastid to generate (mainly) 16 and 18C products. These are then exported outside the plastid where, as acyl-CoAs, they participate in the Kennedy pathway to generate triacylglycerol. Alternative reactions involving phosphatidylcholine are shown in Fig. 2. For abbreviations see list at the front of the article

[also known as the Kennedy pathway (Fig. 1)] or independent pathways (Fig. 2), or both [6, 11, 15, 16].

De novo production of fatty acid uses photosynthate as a source of carbon either directly or via sucrose transport to the seed or fruit. Thus, one possible obvious way to increase oil crop yields is to divert carbon from carbohydrate into oil via the synthesis of acetyl-CoA [17]. The exact location of acetyl-CoA formation is controversial in some tissues [18]. Depending on the tissue, acetyl-CoA can be generated from multiple sources including amino acids, in addition to hexoses. Acetyl-CoA production, catalyzed by pyruvate dehydrogenase, also gives rise to Nicotinamide adenine dinucleotide (phosphate) (NAD(P)H), which is used in turn during fatty acid formation. The whole topic of the interconnection between central pathways of metabolism and generation of important substrates has been the subject of considerable recent research, often using stable isotope techniques (see [19]).

In most plants, acetyl-CoA is used by a multi-subunit plastid form of acetyl-CoA carboxylase (ACCase) which is made up of four proteins–biotin carboxylase, biotin carboxylase carrier protein, and two subunits for the carboxyltransferase [20]. However, the Graminaceae have a multifunctional protein form with a molecular mass of 200–240 kDa [20]. These different forms of ACCase

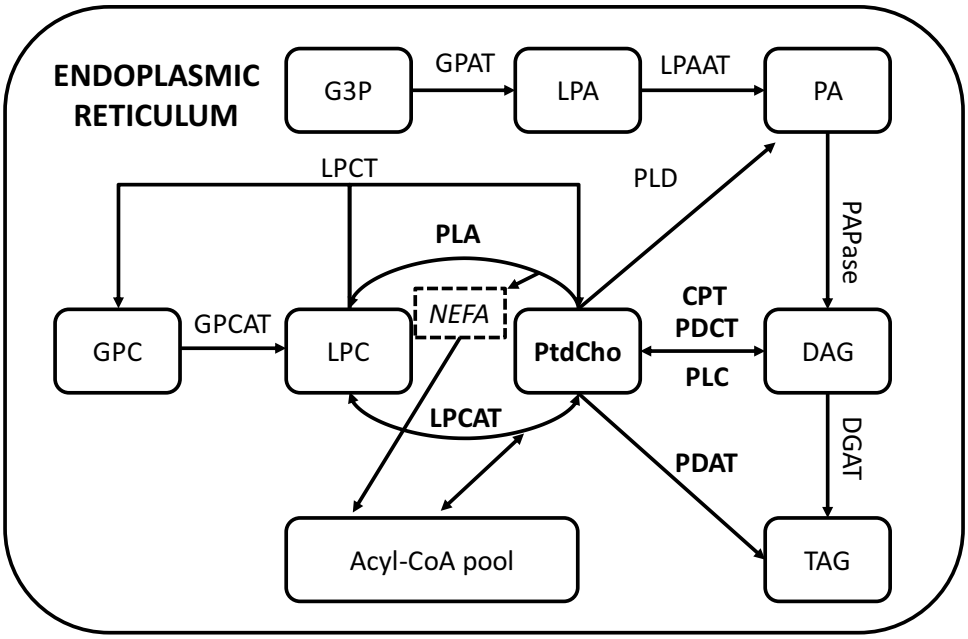

Fig. 2 Schematic of acyl-trafficking via phosphatidylcholine (PtdCho) in plant lipid biosynthesis. PtdCho is a central intermediate in the modification and flux of fatty acid into triacylglycerol (TAG) in developing seeds of oleaginous plants, and important enzymes (in *bold*) are involved in PtdCho-centric acyl-trafficking. For remaining abbreviations see the list at the front of the article

underlie the action of grass-specific herbicides (graminicides—aryloxyphenoxypropionates, cyclohexanediones) which specifically target the Graminaceae chloroplast ACCase [21, 22]. In many organisms, acetyl-CoA carboxylase exerts strong flux control over fatty acid (and lipid) formation. In leaves, ACCase has been shown to be extremely important [23], but the situation is less clear for oilseeds. The coincidence of high ACCase activity with the peak of oil accumulation in *B. napus* led Turnham and Northcote to suggest that ACCase has a major regulatory role in that species [24], but further work questioned this assumption [25]. Indeed, targeting the *Arabidopsis* homomeric ACCase to plastids of rapeseed only produced marginal increases in oil, even when transgenics were grown in growth chambers [26]. However, ACCase can be strongly feedback inhibited when oleate (or oleoyl-CoA/oleoyl-ACP) accumulates in embryonic cultures of *B. napus*, reducing oil yields by 50 % [27]. For oil palm, where most of the control over triacylglycerol synthesis is in fatty acid synthesis, ACCase was important and subject to acetyl-CoA feedback [28]. In vitro experiments in oil palm suggest that acetyl-CoA carboxylase is approximately equally important as fatty acid synthase in controlling overall carbon flux during fatty acid biosynthesis [29]. Finally, in transgenic *Arabidopsis* seeds accumulating hydroxyl-fatty acids, the reduction in oil content seemed to be at the level of ACCase [30]. Thus, it would be fair to conclude that, in many oil crops, ACCase is likely to exert significant flux control. A quantitative measure in vivo for normal TAG accumulation, however, has yet to be carried out.

The malonyl-CoA product of ACCase is used by a type II fatty acid synthase [20]. The sequential addition of two-carbon units to the growing fatty acid chain requires four reactions—condensation, reduction, dehydration, and a second reduction. In plants, there are three condensing enzymes, known as β-ketoacyl-acyl carrier protein (ACP) synthase I, II, and III (KASI, KASII, and KASIII). KASIII initiates the process by catalyzing the condensation of acetyl-CoA with malonyl-ACP. The majority of the other condensations are then catalyzed by KASI up to palmitoyl-ACP. The conversion of palmitoyl-ACP to stearoyl (18:0)-ACP is then catalyzed by KASII. The condensing enzymes can be inhibited (and therefore studied) by different chemicals such as arsenite and cerulenin [20, 31]. In the model plant *Arabidopsis thaliana* (*Arabidopsis*), there are genes encoding two isoforms of the first reductase, two genes for the dehydrase, but only one gene for the second (enoyl-ACP) reductase.

Most plants produce stearate and palmitate (in about a 3:1 ratio) as end-products. However, there are important exceptions; For example, oil palm mesocarp produces a ratio near 1:1, which accounts for the ~44 % palmitate in palm oil. On the other hand, the accumulation of medium-chain fatty acids (e.g., 6:0–12:0 in *Cuphea* species or 10:0 and 12:0 in coconut) is probably due to a distinct thioesterase which was first demonstrated in California bay (*Umbellularia californica*) [10, 32].

The plastid contains a soluble Δ9-acyl-ACP desaturase which usually catalyzes the conversion of stearate to oleate. In most plants, this desaturase also has activity with palmitate to form palmitoleate ($16{:}1\Delta^{9cis}$), a reaction which can be significant in some cases such as in cat's claw (*Doxantha unguis-cati*) and macadamia nut (*Macadamia integrifolia*) [33, 34]. The Δ9-desaturase has been studied by John

Shanklin and his group (e.g., [35]), who were particularly interested in substrate selectivity [36]. The Δ9-desaturase has high activity, therefore significant accumulation of stearate occurs in a limited number of plant species, such as cocoa (*Theobroma cacao*), where levels of approximately 35 % have been reported [2, 37].

Generally, there are two plastid thioesterases in plants, FAT A and FAT B, which catalyze the hydrolysis of newly formed acyl-ACPs, releasing nonesterified (free) fatty acid and ACP. These two thioesterases differ from each other in their acyl-ACP selectivity. FAT A catalyzes the hydrolysis of oleoyl-ACP preferentially, while FAT B also catalyzes the hydrolysis of saturated acyl-ACPs such as palmitoyl-ACP [38]. The plastid free fatty acids are then transported across both inner and outer plastid envelope membranes. This may be catalyzed by fatty acid export 1 (FAX1) [39], although the whole subject has been extensively discussed by Allen et al. [19]. It is generally assumed that the free fatty acids released are then used by a plastid-envelope long-chain acyl-CoA synthetase (LACS) [40–43]. There may be other acyl-CoA synthases found elsewhere in the cell (see [8]). The acyl-CoAs then accumulate in the cytosol, probably bound to acyl-CoA binding protein(s) [44], where they can be used by the acyltransferases of the Kennedy pathway (Fig. 1) [11, 15] or by the fatty acid elongation system [45], or may be partially used by acyl lipid synthesis in plastid [46].

The basic pathway of TAG biosynthesis is widely known as the Kennedy pathway [11, 47]. However, for historical accuracy, it should be noted that the initial two acylations to yield phosphatidate (Fig. 1) were reported first by Kornberg and Pricer [48]. Nevertheless, Eugene Kennedy's seminal contributions included the continuation reactions to yield TAG as well as the various reactions used in the biosynthesis of phosphoglycerides [47, 49]. The Kennedy pathway (Fig. 1) has three acyltransferase reactions in addition to a phosphatidate phosphatase. Each acyltransferase has its own substrate selectivity. For plants producing unusual oils, substrate selectivity of the acyltransferase is pivotal in utilizing the unusual fatty acids that will be incorporated into their TAGs. In cases where genetic manipulation of "standard" agricultural oil crops is attempted, the substrate selectivity of acyltransferases is often an inherent problem which has to be considered seriously during development of the final transgenic crop. Diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA-dependent acylation of DAG to produce TAG, and its activity may have a substantial effect on the flow of carbon into seed oil in some species [11]. In addition to the basic Kennedy pathway, there are a number of important additional enzymes with using phosphatidylcholine (PtdCho) as a central intermediate that may play a significant role in the generation of TAG—depending on the oil crop concerned. These are discussed in the following sections.

Due to an increasing interest in engineering crops to accumulate vegetative oils (particularly in leaves) [50] as a way of boosting their energy content for animal feed and other purposes, the so-called 16:3 and 18:3 plants need some comment. 16:3 crops accumulate hexadecatrienoate in the monogalactosyldiacylglycerol (MGDG) in their plastids, while the MGDG of 18:3 crops is instead dominated by α-linolenate. This difference is due to there being two alternative routes for the assembly of fatty acids onto the glycerol backbone. The first route, known as the eukaryotic pathway, uses acyl-CoA substrates for lipid assembly via the Kennedy pathway in ER, as described above. The second pathway is located entirely in the plastid and is known as the prokaryotic pathway. In this case, acyl-ACPs formed by fatty acid synthase are used directly for assembly onto a glycerol backbone without first undergoing hydrolysis (Fig. 1). Diacylglycerol (DAG) produced by each of these two routes differs in its fatty acid composition. DAG produced by the prokaryotic pathway has a characteristic distribution of fatty acid on the glycerol backbone of 18-carbon and 16-carbon at positions *sn*-1 and *sn*-2, respectively, while DAG originating from the eukaryotic pathway is enriched in 18-carbon fatty acid at both the *sn*-1 and *sn*-2 positions. The difference in plastid membrane lipid composition of 16:3 and 18:3 plants results from preferential use of DAG from the prokaryotic and eukaryotic pathways, respectively, to synthesize the plastid glycosylglycerides and phosphatidylglycerol [51, 52]. These processes underpin leaf TAG accumulation and are therefore important to consider for genetic engineering purposes.

Early Studies on the Role that Phosphatidylcholine Can Play in Fatty Acid Metabolism

Experiments in Tony James' lab at Unilever around 50 years ago implicated PtdCho (and other glycerolipids) in the production of polyunsaturated fatty acids (PUFA) in the green microalga *Chlorella vulgaris* [53]. Further work confirmed that PtdCho was an important intermediate in the conversion of oleate to linoleate ($18{:}2\Delta^{9cis,12cis}$) in *Chlorella vulgaris* [54], and a similar lipid involvement was postulated for the conversion of linoleate to α-linolenate in pumpkin (*Cucurbita pepo*) [55]. The possible use of complex lipids as substrates for fatty acid modifications in algae and plants as proposed originally by Nichols, James, and coworkers had a precedent. Law and colleagues had previously examined cyclopropane synthase from *Clostridium butyricum* in 1964 and showed that the substrate for methyl group addition was attached to phosphatidylethanolanolamine, the major lipid of the organism [56].

After the initial observations at Unilever, the Δ12-desaturation of oleate was studied in a number of systems,

and there was general agreement that PtdCho is the main substrate in most tissues [57–61]. However, there was more controversy for the Δ15-desaturation of linoleate to α-linoleate. Although PtdCho was suggested to be an important substrate for Δ15-desaturation [55], the most definite evidence was initially obtained with photosynthetic tissues where MGDG was the preferred substrate [62–64]. A more detailed discussion is available in Harwood [31].

Experiments with other tissues showed clearly that PtdCho was an effective substrate for fatty acid desaturation. Initially, the best evidence was reported for *Candida lipolytica* [65], but later research included animal systems [66]. The protozoon *Acanthamoeba castellanii* has been studied in considerable detail [67, 68] as well as the desaturase gene involved, which encoded a bifunctional desaturase with both Δ12- and Δ15-desaturase activities [69]. The initial controversy about the relative importance of PtdCho versus MGDG as a substrate for Δ15-desaturation in plants was finally resolved by the identification of the genes encoding the desaturases. Thus, the *FAD3* gene on the ER uses PtdCho, while the *FAD7/8* genes on the plastid thylakoids use MGDG [52]. Clearly, in most seeds accumulating TAG, the enzymes on the ER will be more important than their plastid counterparts and, therefore, expression of *FAD2* (coding the Δ12-desaturase) and *FAD3* will be critical.

The Contribution of Acyl-Trafficking via Phosphatidylcholine to Plant Lipid Biosynthesis

Since the first report of the function of PtdCho in the interrelationship between fatty acid biosynthesis and acyl-lipid synthesis [53], many biochemical and molecular studies have demonstrated that PtdCho is a central intermediate in the modification and flux of fatty acid into TAG in developing seeds of oleaginous plants [11, 15]. In this PtdCho-centric acyl-trafficking, acyl groups can be channeled into PtdCho from the acyl-CoA pool via the activity of lysophosphatidylcholine acyltransferase (LPCAT) or from DAG (Fig. 2) [15, 16]. Acyl groups are then modified on PtdCho in desaturation, epoxidation, conjugation or hydroxylation reactions catalyzed by FAD2/FAD3-like enzymes [3]. The modified acyl groups are subsequently transferred: (1) to the acyl-CoA pool by the combined forward/reverse reactions catalyzed by LPCAT and phospholipase As (PLAs) and LACS, which can be used for lipid biosynthesis on both ER and chloroplast envelope [46, 70–74]; (2) the DAG pool by the catalytic action of phosphatidyl choline:diacylglycerol cholinephosphotransferase (PDCT), phospholipase C (PLC), or the combined activities of phospholipase D (PLD) and phosphatidate phosphohydrolase (PAPase) [15, 75, 76]; or (3) to TAG by the catalytic action of phospholipid:diacylglycerol acyltransferase (PDAT), which uses acyl groups from *sn*-2 position of PtdCho [77]. In addition to the above mechanisms, acyl chains can also be channeled directly from glycerophosphocholine (GPC) to LPC by acyl-CoA:glycerophosphocholine acyltransferase (GPCAT), and LPCAT can be utilized in LPC:LPC transacylation reactions by lysophosphatidylcholine transacylase (LPCT) activities creating PtdCho and GPC [70]. The physiological functions of the GPC-LPC-PtdCho reactions and the enzymes involved are not yet certain. Key enzymes involved in PtdCho-centric acyl-trafficking are discussed in the next section.

Lysophosphatidylcholine Acyltransferase and Phospholipase A

The rapid exchange of fatty acid between PtdCho and the acyl-CoA pool via PtdCho-deacylation and a lysophosphatidylcholine (LPC)-reacylation cycle without net PtdCho synthesis or degradation is a major component of the acyl editing process. The forward reaction catalyzed by LPCAT is involved in the LPC-reacylation. In contrast, PtdCho-deacylation may be catalyzed by both the reverse activity of LPCAT and PLA_2 + LACS action within the Lands cycle [46, 73, 78–81].

In oleaginous plants producing seed oils enriched in PUFA or unusual fatty acids, the majority of fatty acids newly exported from the plastid in the form of acyl-CoA enter PtdCho rather than the Kennedy pathway [19, 82–84]. The forward reaction catalyzed by LPCAT is responsible for this reaction [81, 84]. Microsomes prepared from developing seeds/embryos of different plant species have been used to study the substrate selectivity of LPCATs (see [85]). Recently, recombinant plant LPCATs were produced in yeast, and their biochemical properties were rigorously tested by radioactive labeling experiments [79]. In vitro assay results indicated that LPCAT-mediated acyl editing of PtdCho is a result of the combined forward and reverse reactions catalyzed by the enzyme. In the forward reaction (LPC-reacylation), LPCATs can incorporate acyl groups at both *sn*-1 and *sn*-2 positions with a preference for C18-unsaturated acyl-CoAs but not C16-CoAs or ricinoleoyl-CoA. In the reverse reaction (PtdCho-deacylation), however, all LPCATs prefer ricinoleoyl-PtdCho when an equimolar mixture of ricinoleoyl-PtdCho and oleoyl-PtdCho is used as substrate [79]. In vivo studies with *Arabidopsis lpcat1 lpcat2* double knockout mutants are also strongly supportive of the role of LPCAT in acyl exchange between the acyl-CoA pool and PtdCho [81, 84].

Plants have up to 29 PLAs with diverse structure and substrate preferences [71]. The involvement of PLA_2 in PtdCho deacylation was first proposed in the Lands cycle [86]. Recent in vitro and in vivo analyses suggest that two

PLAs, patatin-like PLA-IIIδ (pPLA-IIIδ) and the small molecular $PLA_2α$ ($sPLA_2α$), may be involved in acyl editing [72, 73, 78]. pPLAIIIδ is a membrane-binding protein with a typical Asp-Gly-Gly catalytic dyad motif. It can catalyze hydrolysis of PtdCho at both the *sn*-1 and *sn*-2 positions with a preference for *sn*-2 position [73]. This enzyme also has thiolesterase activity, as it can catalyze the hydrolysis of acyl-CoA [73]. In *Arabidopsis*, *ppla-IIIδ* T-DNA insertional knockout mutant seeds have significantly lower oil contents, whereas *Arabidopsis* mutants with *pPLA-IIIδ* overexpressed have increased TAG content without any detrimental effect on overall seed yield per plant [73]. In addition, overexpression of *pPLAIIIδ* under the control of a seed-specific promoter boosted seed oil content without compromising plant growth in *Camelina sativa* [72].

The function of castor $sPLA_2α$ in acyl editing has been identified recently [78]. During seed development, castor can accumulate a high level of the hydroxy fatty acid ricinoleate in TAG. *RcsPLA*$_2$*α* has much higher expression in castor endosperm transcriptome compared with that of *Arabidopsis*, and it is the highest among all castor *PLA*s. In vitro assays showed a high selectivity of $RcsPLA_2α$ for ricinoleic acid, superior to that of $AtsPLA_2α$. Coexpression of *RcsPLA*$_2$*α* in *Arabidopsis* with castor hydroxylase *RcFAH12* resulted in a dramatic decrease in hydroxy fatty acid content in both PtdCho and the nonpolar lipid fraction of seeds when compared with *RcFAH12* expression alone, whereas the coexpression of *AtsPLA*$_2$*α* with *RcFAH12* did not. Therefore, $RcsPLA_2α$ functions as a PLA_2 with enhanced selectivity for catalyzing the removal of hydroxy fatty acid from PtdCho. As $sPLA_2α$ generally exists in plants, further studies of this enzyme in transgenic plants accumulating other unusual fatty acids (such as conjugated fatty acids) would improve our understanding of its function in acyl editing.

In summary, the LPC-reacylation cycle is catalyzed by the forward reaction of LPCAT. In contrast, the PtdCho-deacylation cycle may be catalyzed by LPCAT and/or PLA [72, 73, 78, 79, 81]. This conclusion is further supported by a recent in vitro assay of *Arabidopsis* mutants: in the *PAPase lpcat* double knockout mutant, PLA_2s were massively induced as a response to a need for increased PtdCho deacylation in the absence of the LPCAT reverse reaction, whereas none of the *PLA* transcripts showed a significant change in the *PAPase* single mutant where the reverse reaction of LPCAT was enough for PtdCho deacylation [80]. A conclusive in vivo determination of the relative contribution of the reverse reaction of LPCAT versus PLA in PtdCho-deacylation would substantially expand our understanding of PtdCho-centric acyl-trafficking.

Cholinephosphotransferase and Phosphatidylcholine: Diacylglycerol Cholinephosphotransferase

It has been firmly established that PtdCho and DAG can be readily interconverted. Two enzymes, cholinephosphotransferase (CPT) and PDCT, have been proposed to catalyze this interconversion [75, 76]. PLC and/or PLD+PAPase may also be involved in the conversion of PtdCho to DAG [15, 16].

CPT catalyzes the final condensation reaction of DAG with Cytidine diphosphate-choline (CDP-choline) in the CDP-choline pathway for PtdCho synthesis [87, 88]. In 1955, Kennedy and Weiss first reported this enzyme in rat liver, and somewhat later it was shown that CPT is an ER membrane-bound protein and that the CPT reaction is reversible in mammalian cells [89, 90]. In 1971, CPT was characterized from plant tissues (spinach leaf microsomes) for the first time [91]. The results showed that spinach CPT has many similar biochemical properties to animal microsomal CPT, including an optimum pH at about 8.0 and a K_m of 10μM for CDP-choline. Spinach CPT also requires either Mn^{2+} or Mg^{2+} as cofactor, and the saturation concentration for enzymatic activity was 0.3 mM for Mn^{2+} and 13 mM for Mg^{2+}. Further studies of this enzyme were made shortly afterwards by Kates' group [92, 93]. In 1982, Jolliot et al. reported that CPT of potato microsomes was a phospholipid-dependent enzyme, the activity of which could be regulated by the surrounding lipid microenvironment [94]. Based on metabolic labeling experiments, in 1983, Slack et al. demonstrated the reversibility of the CPT reaction in developing linseed cotyledons and proposed that this reaction provides a mechanism for the production of a highly unsaturation DAG pool for synthesis of TAG with enriched 18:2 and 18:3 [95]. The possible synthesis of DAG catalyzed by CPT, however, represents a thermodynamically unfavorable process when compared with the formation of PtdCho. Because CPT is a key enzyme involved in PtdCho synthesis, one attractive hypothesis for this enzyme is that it might have the ability to exclude DAG molecules with unusual fatty acids from being used for membrane lipid biosynthesis [96]. However, CPT from different oilseeds, including safflower [76], *Cuphea*, castor bean, and rapeseed [97], showed little or no preference with a broad range of DAG substrates.

The contribution of PDCT to the dynamic interactions between the PtdCho and DAG pools was demonstrated recently [75, 98]. PDCT is encoded by the *REDUCED OLEATE DESATURASE1* (*ROD1*) gene and was first isolated from *Arabidopsis* [75]. PDCT is closely related to the mammalian sphingomyelin synthease within the large family of lipid phosphatase/phosphotransferase proteins. It transfers the phosphocholine head group from PtdCho to DAG, thereby catalyzing a symmetrical interconversion

between PtdCho and DAG. In contrast to the CPT-catalyzed reaction that leads to a net production of PtdCho from DAG, PDCT action generates new molecular species of PtdCho and DAG, and as a result, there is no net accumulation of PtdCho or DAG [99]. The substrate specificity of PDCT allows oleate flux through PtdCho for further desaturation and movement of linoleate and linolenate back into the DAG pool, thus providing the DAG pool with PtdCho-modified fatty acids as substrates for PUFA-enriched TAG synthesis. A loss-of-function mutation of this gene resulted in significantly reduced linoleate and linolenate and a concomitant increase in oleate in seed TAG. Further analysis of the *rod1*/*lpcat1*/*lpcat2 Arabidopsis* triple mutant indicated that PDCT-mediated PtdCho-DAG interconversion together with LPCAT-mediated acyl editing control the major fluxes of PUFA from PtdCho to TAG (approximately 66 %) in *Arabidopsis* seeds [84]. Recently, overexpression of flax *PDCT* in wild-type *Arabidopsis* was shown to enhance the PUFA content of the seed oil [100]. In addition to the contribution of PDCT in channeling PUFA from PtdCho to DAG, PDCT is also required for efficiently shuffling unusual fatty acids (such as hydroxy fatty acids) from PtdCho to DAG and eventually increasing the amount of hydroxy fatty acids in TAG [98]. Given the potential difficulty associated with CPT-catalyzed formation of DAG, the channeling of PUFA-enriched acyl groups from PtdCho to DAG is probably more associated with PDCT action.

Phospholipid:Diacylglycerol Acyltransferase

In addition to the Kennedy pathway, TAG can also be formed via an acyl-CoA-independent pathway [77, 101]. This pathway is mediated by PDAT, which catalyzes transfer of an acyl group from a phospholipid to DAG, yielding TAG and a lysophospholipid. The *PDAT* gene family is present widely in yeast, microalgae, and plants [102, 103]. It is noteworthy that PDAT activity has also been detected in the bacterium *Streptomyces coelicolor* [104], but this reaction has no counterpart in mammalian cells.

PDAT activity was originally detected in microsomal preparations from three different oilseeds: castor bean, *Crepis palaestina*, and sunflower [77]. In the same study, the first *PDAT* gene was cloned from yeast (*Saccharomyces cerevisiae*) as a homolog of lecithin:cholesterol acyltransferase, which is a soluble enzyme responsible for catalyzing the synthesis of cholesteryl-ester in blood plasma. Knowledge of the yeast *PDAT* sequences enabled the subsequent identification of two *PDAT* orthologs, *AtPDAT1* (At5g13640) and *AtPDAT2* (At3g44830), in *Arabidopsis*. The expression level of *AtPDAT1* was higher in leaves than in seeds [103]. Overexpression or knockout of *AtPDAT1* in *Arabidopsis* resulted in significant changes in oil content and fatty acid composition in leaves but not in seeds [105]. A further study indicated that the AtPDAT1-mediated TAG synthesis is involved in the process of diverting fatty acids from membrane lipids towards peroxisomal β-oxidation and, thereby, is important for maintaining membrane lipid homeostasis in *Arabidopsis* leaves [106]. The role of AtPDAT1 in TAG synthesis has also been studied in the absence of DGAT1 activity. When *PDAT1* was suppressed using RNAi-mediated gene silencing under a *dgat1* knockout background, the oil content was further reduced by 63 % compared with the *dgat1* control [107]. This result suggested that AtPDAT1 is the major contributor to seed oil synthesis when AtDGAT1 activity is compromised. The embryonic lethality in *dgat1*/*pdat1* double mutant suggested that PDAT1 and DGAT1 have overlapping functions for TAG synthesis in pollen and seed and their expression is essential for pollen and seed viability. Studies comparing the relative contribution of AtPDAT1 versus AtDGAT1 to TAG synthesis in leaves have resulted in different conclusions. Fan et al. compared leaf TAG levels of the wild type with that of *dgat1* and *pdat1* mutants as well as with that of 35S-PDAT1 and 35S-DGAT1 overexpression lines [102]. The results suggested that AtPDAT1 plays a more important role than AtDGAT1 in TAG synthesis in young *Arabidopsis* leaves. In contrast, pulse-chase labeling experiments showed that [^{14}C]12:0 was incorporated into TAG by leaves of the *pdat1* mutant at a much higher rate than that of the *dgat1* mutant, indicating that DGAT1 is the predominate enzyme involved in TAG synthesis in young *Arabidopsis* leaves [108]. The relative contribution of AtPDAT1 and AtDGAT1 to lipid metabolism needs to be further explored. The other ortholog, AtPDAT2, has no substantial role in TAG biosynthesis even though the encoding transcript is highly expressed in developing seed [107].

In addition to *Arabidopsis* PDAT, three castor [109, 110], six flax [111], and one green alga (*Chlamydomonas reinhardtii*) [112] PDAT have also been functionally characterized. Overall, these results support the idea that multiple PDAT paralogs arising from the core eudicot-shared ancient genome duplication may have evolved with different TAG-synthesizing abilities and developed divergent expression patterns due to varied selection pressures [103]. It is also important to note that both in vivo and in vitro approaches revealed that PDAT appears to have enhanced preferences for modified fatty acids, including PUFA and unusual fatty acids. Dahlqvist et al. demonstrated that the microsomal PDAT from yeast has a higher preference for ricinoleoyl- or vernoloyl (*cis*-12-epoxyoctadeca-*cis*-9-enoyl)-DAG over dioleoyl-DAG [77]. The PDAT activity in microsomal preparations from leaves from the *AtPDAT1* overexpresser showed that AtPDAT1 has a strong preference for PtdCho containing acyl groups with several double bonds, epoxy, or hydroxyl groups

[113]. Recently, a pair of flax *PDAT* genes were identified, which are preferentially expressed in seeds and encode enzymes with the unique ability to efficiently channel α-linolenic acid into TAG [111]. Similarly, it appears that castor also contains a specialized PDAT for the selective transfer of hydroxy fatty acids into TAG [109, 110]. Together, these results suggest that the contribution of PDAT to TAG synthesis in seeds might be significant in some oilseeds which contain TAG enriched in PUFA or unusual fatty acids.

Closing Comments

Although our understanding of plant oil accumulation has increased substantially over the last two decades, many important questions remain unanswered. There appear to be differences in the relative contributions of fatty acid synthesis versus TAG assembly in different species in the control of the flow of carbon into oil. More detail is required regarding the properties of the enzymes catalyzing the four steps of the Kennedy pathway. The transport of acyl chain and lipid fractions between plastids and the ER needs to be extensively explored. In addition, to inform future genetic manipulation, more in-depth information is required on both the genetic and biochemical regulation of the lipid accumulation process in plants.

PtdCho, as the main membrane lipid in most seeds, has an important role mediating fatty acid desaturation and receiving and donating acyl chains for TAG production. Moreover, for some 30 years this phosphoglyceride has been known to be a key metabolic substrate for desaturation and other fatty acid modifications. Because of this, the passage of fatty acid substrates onto PtdCho has been recognized to be dynamic. In recent years this acyl-editing process has been further detailed with a multitude of ancillary enzymes that can participate. However, delineation of the overall process, its refinement in different plants, and the properties of the individual enzymes involved are in need of further investigation.

Knowledge of these metabolic details will be crucial to understanding plant oil accumulation and in the design of new strategies for biotechnological modification of plant lipids.

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References

1. Oils and fats in the market place—prices of commodity oils. http://lipidlibrary.aocs.org/market/prices.htm. Accessed Mar 2015
2. Gunstone FD, Harwood JL, Dijkstra AJ (eds) (2007) The lipid handbook, 3rd edn. CRC Press, Boca Raton
3. Carlsson AS, Yilmaz JL, Green AG, Stymne S, Hofvander P (2011) Replacing fossil oil with fresh oil—with what and for what? Eur J Lipid Sci Technol 113:812–831
4. Vanhercke T, El Tahchy A, Liu Q, Zhou X-R, Shrestha P, Divi UK, Ral J-P, Mansour MP, Nichols PD, James CN, Horn PJ, Chapman KD, Beaudoin F, Ruiz-López N, Larkin PJ, de Feyter RC, Singh SP, Petrie JR (2014) Metabolic engineering of biomass for high energy density: oilseed-like triacylglycerol yields from plant leaves. Plant Biotech J 12:231–239
5. Murphy DJ (ed) (2005) Plant lipids: biology, utilisation and manipulation. CRC Press, Boca Raton
6. Harwood JL, Ramli US, Tang M, Quant PA, Weselake RJ, Fawcett T, Guschina IA (2013) Regulation and enhancement of lipid accumulation in oil crops: the use of metabolic control analysis for informed genetic manipulation. Eur J Lipid Sci Technol 115:1239–1246
7. Murphy DJ (2014) The future of oil palm as a major global crop: opportunities and challenges. J Oil Palm Res 26:1–24
8. Salas JJ, Martinez-Force E, Harwood JL, Venegas-Caleron M, Aznar-Moreno JA, Moreno-Perez AJ, Ruiz-Lopez N, Serrano-Vega MJ, Graham IA, Mullen RT, Garces R (2014) Biochemistry of high stearic sunflower, a new source of saturated fats. Prog Lipid Res 55:30–42
9. Murphy DJ (ed) (1994) Designer oil crops—breeding, processing and biotechnology. VCH Weinheim, New York
10. Voelker TA, Worrell AC, Anderson L, Bleibaum J, Fan C, Hawkins DJ, Radke SE, Davies HM (1992) Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. Science 257:72–74
11. Weselake RJ, Taylor DC, Rahman MH, Shah S, Laroche A, McVetty PBE, Harwood JL (2009) Increasing the flow of carbon into seed oil. Biotechnol Adv 27:866–878
12. Rahman H, Harwood J, Weselake R (2013) Increasing seed oil content in *Brassica* species through breeding and biotechnology. Lipid Tech 25:182–185
13. Napier JA (2007) The production of unusual fatty acids in transgenic plants. Annu Rev Plant Physiol 58:295–319
14. Haslam RP, Ruiz-Lopez N, Eastmond P, Moloney M, Sayanova O, Napier JA (2013) The modification of plant oil composition via metabolic engineering-better nutrition by design. Plant Biotech J 11:157–168
15. Bates PD, Stymne S, Ohlrogge J (2013) Biochemical pathways in seed oil synthesis. Curr Opin Plant Biol 16:358–364
16. Chapman KD, Ohlrogge JB (2012) Compartmentation of triacylglycerol accumulation in plants. J Biol Chem 287:2288–2294
17. Sanjaya Durrett TP, Weise SE, Benning C (2011) Increasing the energy density of vegetative tissues by diverting carbon from starch to oil biosynthesis in transgenic Arabidopsis. Plant Biotechnol J 9:874–883
18. Liedvogel B (1987) Lipid precursors in plant cells: the problem of acetyl CoA generation for plastid fatty acid synthesis. In: Stumpf P, Mudd JB, Nes WD (eds) The metabolism, structure, and function of plant lipids. Springer, New York
19. Allen DK, Bates PD, Tjellström H (2015) Tracking the metabolic pulse of plant lipid production with isotopic labeling and flux analyses: past, present and future. Prog Lipid Res 58:97–120

20. Harwood J (2005) Fatty acid biosynthesis. In: Murphy DJ (ed) Plant lipids: biology, utilisation and manipulation. CRC Press, Boca Raton
21. Walker A, Ridley S, Lewis T, Harwood J (1989) Action of aryloxy-phenoxy carboxylic acids on lipid metabolism. Rev Weed Sci 4:71–84
22. Harwood J (1991) Herbicides affecting chloroplast lipid synthesis. In: Baker N, Percival M (eds) Topics in photosynthesis, vol 10. Elsevier, Amsterdam
23. Page RA, Okada S, Harwood JL (1994) Acetyl-CoA carboxylase exerts strong flux control over lipid synthesis in plants. Biochim Biophys Acta 1210:369–372
24. Turnham E, Northcote DH (1983) Changes in the activity of acetyl-CoA carboxylase during rape-seed formation. Biochem J 212:223–229
25. Kang F, Ridout C, Morgan C, Rawsthorne S (1994) The activity of acetyl-CoA carboxylase is not correlated with the rate of lipid synthesis during development of oilseed rape (*Brassica napus* L.) embryos. Planta 193:320–325
26. Roesler K, Shintani D, Savage L, Boddupalli S, Ohlrogge J (1997) Targeting of the Arabidopsis homomeric acetyl-coenzyme A carboxylase to plastids of rapeseeds. Plant Physiol 113:75–81
27. Andre C, Haslam RP, Shanklin J (2012) Feedback regulation of plastidic acetyl-CoA carboxylase by 18:1-acyl carrier protein in Brassica napus. Proc Natl Acad Sci USA 109:10107–10112
28. Ramli US, Salas JJ, Quant PA, Harwood JL (2009) Use of metabolic control analysis to give quantitative information on control of lipid biosynthesis in the important oil crop, *Elaeis guineensis* (oilpalm). New Phytol 184:330–339
29. Ramli US. Biochemical studies of lipid biosynthesis in oil palm and olive callus cultures, 1999, PhD thesis, Cardiff University, Wales, UK
30. Bates PD, Johnson SR, Cao X, Li J, Nam JW, Jaworski JG, Ohlrogge JB, Browse J (2014) Fatty acid synthesis is inhibited by inefficient utilization of unusual fatty acids for glycerolipid assembly. Proc Natl Acad Sci USA 111:1204–1209
31. Harwood JL (1988) Fatty acid metabolism. Annu Rev Plant Physiol Plant Mol Biol 39:101–138
32. Pollard MR, Anderson L, Fan C, Hawkins DJ, Davies HM (1991) A specific acyl-ACP thioesterase implicated in medium-chain fatty acid production in immature cotyledons of *Umbellularia californica*. Arch Biochem Biophys 284:306–312
33. Wu Y, Li R, Hildebrand DF (2012) Biosynthesis and metabolic engineering of palmitoleate production, an important contributor to human health and sustainable industry. Prog Lipid Res 51:340–349
34. Cahoon EB, Shah S, Shanklin J, Browse J (1998) A determinant of substrate specificity predicted from the acyl-acyl carrier protein desaturase of developing cat's claw seed. Plant Physiol 117:593–598
35. Lindqvist Y, Huang W, Schneider G, Shanklin J (1996) Crystal structure of delta9 stearoyl-acyl carrier protein desaturase from castor seed and its relationship to other di-iron proteins. EMBO J 15:4081–4092
36. Cahoon EB, Lindqvist Y, Schneider G, Shanklin J (1997) Redesign of soluble fatty acid desaturases from plants for altered substrate specificity and double bond position. Proc Natl Acad Sci USA 94:4872–4877
37. Shukla VKS (1995) Cocoa butter properties and quality. Lipid Tech 7:54–57
38. Ohlrogge JB, Jaworski JG (1997) Regulation of fatty acid synthesis. Annu Rev Plant Physiol Plant Mol Biol 48:109–136
39. Li N, Gügel IL, Giavalisco P, Zeisler V, Schreiber L, Soll J, Philippar K (2015) FAX1, a novel membrane protein mediating plastid fatty acid export. PLoS Biol 13:e1002053
40. Joyard J, Stumpf PK (1981) Synthesis of long-chain acyl-CoA in chloroplast envelope membranes. Plant Physiol 67:250–256
41. Koo AJ, Ohlrogge JB, Pollard M (2004) On the export of fatty acids from the chloroplast. J Biol Chem 279:16101–16110
42. Schnurr JA, Shockey JM, de Boer GJ, Browse JA (2002) Fatty acid export from the chloroplast. Molecular characterization of a major plastidial acyl-coenzyme A synthetase from Arabidopsis. Plant Physiol 129:1700–1709
43. Zhao L, Katavic V, Li F, Haughn GW, Kunst L (2010) Insertional mutant analysis reveals that long-chain acyl-CoA synthetase 1 (LACS1), but not LACS8, functionally overlaps with LACS9 in Arabidopsis seed oil biosynthesis. Plant J 64:1048–1058
44. Xiao S, Chye ML (2011) New roles for acyl-CoA-binding proteins (ACBPs) in plant development, stress responses and lipid metabolism. Prog Lipid Res 50:141–151
45. Cassagne C, Lessire R, Bessoule JJ, Moreau P, Creach A, Schneider F, Sturbois B (1994) Biosynthesis of very long chain fatty acids in higher plants. Prog Lipid Res 33:55–69
46. Jessen D, Roth C, Wiermer M, Fulda M (2015) Two activities of long-chain acyl-coenzyme A synthetase are involved in lipid trafficking between the endoplasmic reticulum and the plastid in Arabidopsis. Plant Physiol 167:351–366
47. Kennedy EP (1961) Biosynthesis of complex lipids. Fed Proc 20:934–940
48. Kornberg A, Pricer WE Jr (1953) Enzymatic esterification of alpha-glycerophosphate by long chain fatty acids. J Biol Chem 204:345–357
49. Weiss SB, Kennedy EP, Kiyasu JY (1960) The enzymatic synthesis of triglycerides. J Biol Chem 235:40–44
50. Chapman KD, Dyer JM, Mullen RT (2013) Commentary: why don't plant leaves get fat? Plant Sci 207:128–134
51. Heinz E, Roughan PG (1983) Similarities and differences in lipid metabolism of chloroplasts isolated from 18:3 and 16:3 plants. Plant Physiol 72:273–279
52. Wallis JG, Browse J (2002) Mutants of Arabidopsis reveal many roles for membrane lipids. Prog Lipid Res 41:254–278
53. Nichols BW, James AT, Breuer J (1967) Interrelationships between fatty acid biosynthesis and acyl-lipid synthesis in *Chlorella vulgaris*. Biochem J 104:486–496
54. Gurr MI, Robinson MP, James AT (1969) The mechanism of formation of polyunsaturated fatty acids by photosynthetic tissue. The tight coupling of oleate desaturation with phospholipid synthesis in *Chlorella vulgaris*. Eur J Biochem 9:70–78
55. Roughan PG (1970) Turnover of the glycerolipids of pumpkin leaves. The importance of phosphatidylcholine. Biochem J 117:1–8
56. Chung AE, Law JH (1964) Cyclopropane fatty acid synthetase: partial purification and properties. Biochemistry (Mosc) 3:967–974
57. Citharel B, Oursel A, Mazliak P (1983) Desaturation of oleoyl and linoleoyl residues linked to phospholipids in growing roots of yellow lupin. FEBS Lett 161:251–256
58. Demandre C, Trémolières A, Justin AM, Mazliak P (1986) Oleate desaturation in six phosphatidylcholine molecular species from potato tuber microsomes. Biochim Biophys Acta 877:380–386
59. Murphy DJ, Woodrow IE, Mukherjee KD (1985) Substrate specificities of the enzymes of the oleate desaturase system from photosynthetic tissue. Biochem J 225:267–270
60. Slack CR, Roughan PG, Browse J (1979) Evidence for an oleoyl phosphatidylcholine desaturase in microsomal preparations from cotyledons of safflower (*Carthamus tinctorius*) seed. Biochem J 179:649–656
61. Stymne S, Stobart AK, Glad G (1983) The role of the acyl-CoA pool in the synthesis of polyunsaturated 18-carbon fatty acids

and triacylglycerol production in the microsomes of developing safflower seeds. Biochim Biophys Acta 752:198–208
62. Jones AV, Harwood JL (1980) Desaturation of linoleic acid from exogenous lipids by isolated chloroplasts. Biochem J 190:851–854
63. Wharfe J, Harwood JL (1978) Fatty acid biosynthesis in the leaves of barley, wheat and pea. Biochem J 174:163–169
64. Ohnishi J, Yamada M (1982) Glycerolipid synthesis in avena leaves during greening of etiolated seedlings III. Synthesis of α-linolenoyl-monogalactosyl diacylglycerol from liposomal linoleoyl-phosphatidylcholine by avena plastids in the presence of phosphatidylcholine-exchange protein. Plant Cell Physiol 23:767–773
65. Pugh EL, Kates M (1973) Desaturation of phosphatidylcholine and phosphatidylethanolamine by a microsomal enzyme system from *Candida lipolytica*. Biochim Biophys Acta 316:305–316
66. Pugh EL, Kates M, Szabo AG (1980) Fluorescence polarization studies of rat liver microsomes with altered phospholipid desaturase activities. Can J Biochem 58:952–958
67. Avery SV, Lloyd D, Harwood JL (1995) Temperature-dependent changes in plasma-membrane lipid order and the phagocytotic activity of the amoeba *Acanthamoeba castellanii* are closely correlated. Biochem J 312:811–816
68. Jones AL, Lloyd D, Harwood JL (1993) Rapid induction of microsomal delta 12 (omega 6)-desaturase activity in chilled *Acanthamoeba castellanii*. Biochem J 296:183–188
69. Sayanova O, Haslam R, Guschina I, Lloyd D, Christie WW, Harwood JL, Napier JA (2006) A bifunctional Delta12, Delta15-desaturase from *Acanthamoeba castellanii* directs the synthesis of highly unusual n-1 series unsaturated fatty acids. J Biol Chem 281:36533–36541
70. Lager I, Glab B, Eriksson L, Chen G, Banas A, Stymne S (2015) Novel reactions in acyl editing of phosphatidylcholine by lysophosphatidylcholine transacylase (LPCT) and acyl-CoA:glycerophosphocholine acyltransferase (GPCAT) activities in microsomal preparations of plant tissues. Planta 241:347–358
71. Chen G, Snyder CL, Greer MS, Weselake RJ (2011) Biology and biochemistry of plant phospholipases. Crit Rev Plant Sci 30:239–258
72. Li M, Wei F, Tawfall A, Tang M, Saettele A, Wang X (2015) Overexpression of patatin-related phospholipase AIIIδ altered plant growth and increased seed oil content in camelina. Plant Biotechnol J 13:766–778
73. Li M, Bahn SC, Fan C, Li J, Phan T, Ortiz M, Roth M, Welti R, Jaworski J, Wang X (2013) Patatin-related phospholipase pPLAIIIδ increases seed oil content with long chain fatty acids in Arabidopsis. Plant Physiol 162:39–51
74. Wang G, Ryu S, Wang X (2012) Plant phospholipases: an overview. Methods Mol Biol 861:123–137
75. Lu CF, Xin ZG, Ren ZH, Miquel M, Browse J (2009) An enzyme regulating triacylglycerol composition is encoded by the *ROD1* gene of Arabidopsis. Proc Natl Acad Sci USA 106:18837–18842
76. Slack CR, Roughan PG, Browse JA, Gardiner SE (1985) Some properties of cholinephosphotransferase from developing safflower cotyledons. Biochim Biophys Acta 833:438–448
77. Dahlqvist A, Stahl U, Lenman M, Banas A, Lee M, Sandager L, Ronne H, Stymne H (2000) Phospholipid:diacylglycerol acyltransferase: an enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. Proc Natl Acad Sci USA 97:6487–6492
78. Bayon S, Chen G, Weselake R, Browse J (2015) A small phospholipase A2-α from castor catalyzes the removal of hydroxy fatty acids from phosphatidylcholine in transgenic Arabidopsis seeds. Plant Physiol 167:1259–1270
79. Lager I, Yilmaz JL, Zhou X-R, Jasieniecka K, Kazachkov M, Wang P, Zou J, Weselake R, Smith MA, Bayon S, Dyer JM, Shockey JM, Heinz E, Green A, Banas A, Stymne S (2013) Plant acyl-CoA:lysophosphatidylcholine acyltransferases (LPCATs) have different specificities in their forward and reverse reactions. J Biol Chem 288:36902–36914
80. Wang L, Kazachkov M, Shen W, Bai M, Wu H, Zou J (2014) Deciphering the roles of Arabidopsis LPCAT and PAH in phosphatidylcholine homeostasis and pathway coordination for chloroplast lipid synthesis. Plant J 80:965–976
81. Wang LP, Shen WY, Kazachkov M, Chen GQ, Chen QL, Carlsson AS, Stymne S, Weselake RJ, Zou JT (2012) Metabolic interactions between the Lands cycle and the Kennedy pathway of glycerolipid synthesis in Arabidopsis developing seeds. Plant Cell 24:4652–4669
82. Williams JP, Imperial V, Khan MU, Hodson JN (2000) The role of phosphatidylcholine in fatty acid exchange and desaturation in *Brassica napus* L. leaves. Biochem J 349:127–133
83. Bates PD, Durrett TP, Ohlrogge JB, Pollard M (2009) Analysis of acyl fluxes through multiple pathways of triacylglycerol synthesis in developing soybean embryos. Plant Physiol 150:55–72
84. Bates PD, Fatihi A, Snapp AR, Carlsson AS, Browse J, Lu CF (2012) Acyl editing and headgroup exchange are the major mechanisms that direct polyunsaturated fatty acid flux into triacylglycerols. Plant Physiol 160:1530–1539
85. Snyder CL, Yurchenko OP, Siloto RMP, Chen X, Liu Q, Mietkiewska E, Weselake RJ (2009) Acyltransferase action in the modification of seed oil biosynthesis. New Biotechnol 26:11–16
86. Lands WEM (1960) Metabolism of glycerolipids: II. The enzymatic acylation of lysolecithin. J Biol Chem 235:2233–2237
87. Kennedy EP, Weiss SB (1955) Cytidine diphosphate choline: a new intermediate in lecithin biosynthesis. J Am Chem Soc 77:250–251
88. Kennedy EP, Weiss SB (1956) The function of cytidine coenzymes in the biosynthesis of phospholipides. J Biol Chem 222:193–214
89. Weiss SB, Smith SW, Kennedy EP (1958) The enzymatic formation of lecithin from cytidine diphosphate choline and D-1,2-diglyceride. J Biol Chem 231:53–64
90. Wilgram GF, Kennedy EP (1963) Intracellular distribution of some enzymes catalyzing reactions in the biosynthesis of complex lipids. J Biol Chem 238:2615–2619
91. Devor KA, Mudd JB (1971) Biosynthesis of phosphatidylcholine by enzyme preparations from spinach leaves. J Lipid Res 12:403–411
92. Marshall MO, Kates M (1972) Biosynthesis of phosphatidylglycerol by cell-free preparations from spinach leaves. Biochim Biophys Acta 260:558–570
93. Marshall MO, Kates M (1974) Biosynthesis of nitrogenous phospholipids in spinach leaves. Can J Biochem 52:469–482
94. Jolliot A, Justin AM, Bimont E, Mazliak P (1982) Regulation by lipids of plant microsomal enzymes: III. Phospholipid dependence of the cytidine-diphospho-choline phosphotransferase of potato microsomes. Plant Physiol 70:206–210
95. Slack CR, Campbell LC, Browse JA, Roughan PG (1983) Some evidence for the reversibility of the cholinephosphotransferasecatalysed reaction in developing linseed cotyledons *in vivo*. Biochim Biophys Acta 754:10–20
96. Bafor M, Jonsson L, Stobart AK, Stymne S (1990) Regulation of triacylglycerol biosynthesis in embryos and microsomal preparations from the developing seeds of *Cuphea lanceolata*. Biochem J 272:31–38
97. Vogel G, Browse J (1996) Cholinephosphotransferase and diacylglycerol acyltransferase: substrate specificities at a key branch point in seed lipid metabolism. Plant Physiol 110:923–931

98. Hu ZH, Ren ZH, Lu CF (2012) The phosphatidylcholine diacylglycerol cholinephosphotransferase is required for efficient hydroxy fatty acid accumulation in transgenic Arabidopsis. Plant Physiol 158:1944–1954
99. Bates PD, Browse J (2012) The significance of different diacylgycerol synthesis pathways on plant oil composition and bioengineering. Front Plant Sci 3:147
100. Wickramarathna AD, Siloto RMP, Mietkiewska E, Singer SD, Pan X, Weselake RJ (2015) Heterologous expression of flax *PHOSPHOLIPID:DIACYLGLYCEROL CHOLINE-PHOSPHOTRANSFERASE* (*PDCT*) increases polyunsaturated fatty acid content in yeast and Arabidopsis seeds. BMC Biotechnol 15:63
101. Oelkers P, Tinkelenberg A, Erdeniz N, Cromley D, Billheimer JT, Sturley SL (2000) A lecithin cholesterol acyltransferase-like gene mediates diacylglycerol esterification in yeast. J Biol Chem 275:15609–15612
102. Fan J, Yan C, Zhang X, Xu C (2013) Dual role for phospholipid:diacylglycerol acyltransferase: enhancing fatty acid synthesis and diverting fatty acids from membrane lipids to triacylglycerol in Arabidopsis leaves. Plant Cell 25:3506–3518
103. Pan X, Peng FY, Weselake R (2015) Genome-wide analysis of *PHOSPHOLIPID:DIACYLGLYCEROL ACYLTRANSFERASE* (*PDAT*) genes in plants reveals the eudicot-wide *PDAT* gene expansion and altered selective pressures acting on the core eudicot PDAT paralogs. Plant Physiol 167:887–904
104. Arabolaza A, Rodriguez E, Altabe S, Alvarez H, Gramajo H (2008) Multiple pathways for triacylglycerol biosynthesis in *Streptomyces coelicolor*. Appl Environ Microbiol 74:2573–2582
105. Mhaske V, Beldjilali K, Ohlrogge J, Pollard M (2005) Isolation and characterization of an *Arabidopsis thaliana* knockout line for phospholipid:diacylglycerol transacylase gene (At5g13640). Plant Physiol Biochem 43:413–417
106. Fan J, Yan C, Roston R, Shanklin J, Xu C (2014) Arabidopsis lipins, PDAT1 acyltransferase, and SDP1 triacylglycerol lipase synergistically direct fatty acids toward β-oxidation, thereby maintaining membrane lipid homeostasis. Plant Cell 26:4119–4134
107. Zhang M, Fan J, Taylor DC, Ohlrogge JB (2009) DGAT1 and PDAT1 acyltransferases have overlapping functions in Arabidopsis triacylglycerol biosynthesis and are essential for normal pollen and seed development. Plant Cell 21:3885–3901
108. Tjellstrom H, Strawsine M, Ohlrogge JB (2015) Tracking synthesis and turnover of triacylglycerol in leaves. J Exp Bot 66:1453–1461
109. Kim HU, Lee KR, Go YS, Jung JH, Suh MC, Kim JB (2011) Endoplasmic reticulum-located PDAT1-2 from castor bean enhances hydroxy fatty acid accumulation in transgenic plants. Plant Cell Physiol 52:983–993
110. van Erp H, Bates PD, Burgal J, Shockey J, Browse J (2011) Castor phospholipid:diacylglycerol acyltransferase facilitates efficient metabolism of hydroxy fatty acids in transgenic Arabidopsis. Plant Physiol 155:683–693
111. Pan X, Siloto RM, Wickramarathna AD, Mietkiewska E, Weselake RJ (2013) Identification of a pair of phospholipid:diacylglycerol acyltransferases from developing flax (*Linum usitatissimum* L.) seed catalyzing the selective production of trilinolenin. J Biol Chem 288:24173–24188
112. Yoon K, Han D, Li Y, Sommerfeld M, Hu Q (2012) Phospholipid:diacylglycerol acyltransferase is a multifunctional enzyme involved in membrane lipid turnover and degradation while synthesizing triacylglycerol in the unicellular green microalga *Chlamydomonas reinhardtii*. Plant Cell 24:3708–3724
113. Stahl U, Carlsson AS, Lenman M, Dahlqvist A, Huang BQ, Banas W, Banas A, Stymne S (2004) Cloning and functional characterization of a phospholipid:diacylglycerol acyltransferase from Arabidopsis. Plant Physiol 135:1324–1335