Towards rational control of seed oil composition: dissecting cellular organization and flux control of lipid metabolism

Philip D. Bates[1*] and Jay Shockey[2]

[1]Institute of Biological Chemistry, Washington State University, Pullman, WA, USA

[2]United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Allen Toussaint Blvd, New Orleans, LA, USA

*Corresponding Author:

Philip D. Bates

100 Dairy Road, PO Box 647411

Pullman, WA 99164-7411

phil_bates@wsu.edu

Running title: Towards rational control of seed oil composition

Advances Box

- The network of possible pathways to produce seed oils continues to grow with the identification of triacylglycerol remodeling in *Physaria fenderi*
- Seed oil fatty acid composition is highly influenced by diacylglycerol acyltransferase selectivity for acyl-CoA and diacylglycerol molecular species, and the availability of *sn*-1,2 or *sn*-2,3 enantiomers of diacylglycerol
- Lipid metabolism may be organized into different metabolons within the endoplasmic reticulum that separate membrane lipid and oil biosynthesis, and control oil production from different pools of diacylglycerol

Abstract:

Plant lipids represent a fascinating field of scientific study, in part due to a stark dichotomy in the limited fatty acid (FA) composition of cellular membrane lipids versus the huge diversity of FAs that can accumulate in triacylglycerols (TAGs), the main component of seed storage oils. With few exceptions, the strict chemical, structural, and biophysical roles imposed on membrane lipids since the dawn of life has constrained their FA composition to predominantly lengths of 16-18 carbons and containing 0-3 methylene-interrupted carbon-carbon double bonds in cis-configuration. However, over 450 “unusual” FA structures can be found in seed oils of different plants (Ohlrogge et al., 2018), and we are just beginning to understand the metabolic mechanisms required to produce and maintain this dichotomy. Here we review the current state of plant lipid research, specifically addressing the knowledge gaps in membrane

and storage lipid synthesis from three angles: pathway fluxes including newly discovered TAG remodeling, key acyltransferase substrate selectivities, and the possible roles of “metabolons”.

For many plants, including a large majority of oilseed crops, the storage triacylglycerol (TAG) fatty acid (FA) composition mirrors that of membrane lipids containing common FAs (Fig. 1 A-D). In most of these oils, each specific FA constitutes no more than ~30-35% of the total. Most well-known seed oils, such as corn, soybean, peanut, and canola, fall into this category, and provide a major proportion of global human and animal caloric intake. Decades of biochemical and genetic studies indicate that the assembly of different plant oils occurs through multiple different metabolic pathways and enzyme classes that can overlap with essential membrane lipid synthesis (Ohlrogge and Browse, 1995; Gunstone et al., 2007; Bates and Browse, 2012; Bates et al., 2013; Li-Beisson et al., 2013; Napier et al., 2014; Chen et al., 2015; Lee et al., 2015; Bates, 2016; Zhu et al., 2016; Aznar-Moreno and Durrett, 2017; Bates, 2022; Busta et al., 2022). However, many of the ‘cartographic’ details of the metabolic network structure and nuances of pathway regulation necessary to produce specific molecular species of both membrane and storage lipids remain to be determined.

This foundational knowledge void is amplified even further when considering the second category of plant oils, that contain ‘unusual FAs’ (Fig. 1 E-F) (Ohlrogge et al., 2018). Some of these are ‘industrial oils’ and serve as lucrative feedstocks for production of biofuels and dozens of other chemical compounds such as foams, plastics, adhesives, nylons, and coatings (Gunstone et al., 2007; Carlsson et al., 2011; Chen, 2017). This bifurcation primarily derives from fundamental differences in seed oil FA composition. While membrane lipid FA composition in these species is typically very similar to that of all plants, oils with high chemical utility typically contain common FAs and one or more unusual FAs (Ohlrogge et al., 2018). These include acyl groups with novel chain lengths and desaturation states, ranging in length from ~C8-C24 and including methylene-interrupted and conjugated arrangements of zero to six C-C cis- and trans-double bonds, and C-C triple bonds. This diversity is also apparent in the array of characterized side-chain functionalities including hydroxy, epoxy, furan, and carbocyclic groups. And unlike the relatively balanced FA composition found in common oilseeds, some unusual FAs (such as ricinoleic acid in castor bean and α-eleostearic acid in tung tree, Fig. 1F, I) can constitute up to ~70-90% of TAG FA.

A thorough catalog of known or suspected enzyme activities that contribute to the various branches of plant lipid metabolism has been previously described and encompasses hundreds of genes (Li-Beisson et al., 2013; McGlew et al., 2015). However, putting this suite of gene products into a coherent biochemical context that explains the enzymology and subcellular compartmentalization of common and unusual FA biosynthesis and their utilization in both membrane and storage lipid production remains a challenging goal.

Here we review the current state of plant lipid research, specifically addressing the knowledge gaps in membrane and storage lipid synthesis from three angles: pathway fluxes including newly discovered TAG remodeling, key acyltransferase substrate selectivities, and the possible roles of “metabolons”.

Ever-expanding “pathways” of triacylglycerol synthesis

A metabolic pathway may be thought of as a series of consecutive enzymatic reactions that lead to the synthesis or degradation of a particular metabolite. However, within lipid metabolism *de novo* FA synthesis, FA elongation to ≥20 carbons, FA desaturation, or other modifications (e.g. hydroxylation, epoxidation, etc.) all occur within different substrate pools, and in different cellular locations, with multiple different possible pathways of acyl flux to assemble a given lipid class thus representing a network of possible pathways (Fig. 2). Additionally, the acyltransferases and lipases involved in lipid assembly and turnover can have selectivity for different molecular species of the substrate lipid class. Therefore, the FA composition of seed oils is dependent on both the capacity to produce different FAs, and the path of substrate flux through the lipid metabolic network for different FA modification and lipid assembly reactions.

Production of FA substrates for TAG biosynthesis

De novo FA synthesis (FAS) in the plastid builds FAs on acyl carrier proteins (ACP) up to 18 carbons. Desaturation of 18:0-ACP produces oleic acid (18:1), the major product of FAS in most plant tissues (Li-Beisson et al., 2013; Bates, 2022) (Fig. 2). In some plant seeds (e.g. coconut, California bay, *Cuphea* spp.) (Ohlrogge et al., 2018), *de novo* FAS also produces significant amounts of saturated FAs less than 16 carbons that accumulate exclusively in TAG. Acyl-ACP thioesterases (FAT) hydrolyze FAs from ACP (Kalinger and Rowland, 2023), thus determining the FA length and initiating free FA export from the plastid (Koo et al., 2004; Li et al., 2015; Tian et al., 2019). Exported free FAs are activated to acyl-CoA by long chain acyl-CoA synthetase (LACS9) on the outer chloroplast envelope (Schnurr et al., 2002). Acyl-CoA may be further elongated to ≥20 carbons through the endoplasmic reticulum (ER)-localized FA elongation complex (Haslam and Kunst, 2013), or the FAs may be further desaturated within the membrane lipid pool. The ER localized Fatty Acid Desaturases (FAD) FAD2 and FAD3 primarily act on phosphatidylcholine (PC) to desaturate 18:1 to linoleic acid (18:2) and linolenic acid (18:3), respectively (Fig. 1). Membrane lipid-based FA desaturation also occurs on galactolipids in the plastid by FAD6/7/8 (Shanklin et al., 2009; Li-Beisson et al., 2013). While ER localized production of polyunsaturated FAs (PUFA) on PC is the major source for ER TAG biosynthesis, PUFAs released from galactolipids can be incorporated into TAG under some circumstances such as abiotic stress or metabolic adaptations to mutations (Neumann et al.; Moellering et al., 2010; Aulakh and Durrett, 2019; Bhattacharya, 2022). In some species, PC is also the substrate for other FA modifications by FAD2 enzyme variants (Shanklin et al., 2009) that produce a diverse range of structures, including but not limited to hydroxyl, epoxy, or cyclopropyl functional groups, or uncommon double bond locations in seed oil FAs (Ohlrogge et al., 2018) (Fig. 1).

Acyl editing to generate diverse acyl substrate pools for TAG biosynthesis

Acyl editing is a cycle of PC de-acylation and lyso-PC re-acylation that exchanges FAs between PC and the acyl-CoA pool (Fig. 2). *In vivo* isotopic tracing of lipid metabolism indicated that 18:1 exported from the plastid is incorporated into glycerolipids predominantly through *sn*-2 PC acyl editing in leaf and seed tissues in species such as *Arabidopsis thaliana*, *Brassica napus*, *Camelina sativa*, *Glycine max*, *Nicotiana tabacum*, *Physaria fendleri*, and *Pisum sativum* (Williams et al., 2000; Bates et al., 2007; Bates et al., 2009; Bates et al., 2012; Wang et al., 2012; Yang et al., 2017; Karki et al., 2019; Zhou et al., 2020; Bhandari and Bates, 2021). PC de-acylation may proceed by either phospholipase A_2 (PLA_2, as in the Lands cycle (Lands, 1965)) or the reverse action of acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT). Free FAs produced by Lands Cycle PLA_2 activity are activated to acyl-CoA for further use likely by ER localized LACS (Shockey and Browse, 2011). The lyso-PC re-acylation proceeds through the LPCAT forward reaction. Plant

LPCATs can have different acyl specificities in their forward and reverse reactions (Lager et al., 2013; Pan et al., 2015; Jasieniecka-Gazarkiewicz et al., 2016), when combined with acyl modification on PC, leads to diverse acyl-CoA and PC molecular species pools. The Arabidopsis *lpcat1/lpcat2* mutant shifts the flux of nascent FA to *de novo* glycerolipid synthesis rather than acyl editing (Stahl et al., 2008; Bates et al., 2012; Wang et al., 2012; Karki et al., 2019) and reduces accumulation of PUFAs in TAG (Bates et al., 2012; Wang et al., 2012), demonstrating the important contribution of acyl editing to the final TAG FA composition. Interestingly, both lipid composition analysis and isotopic tracing in the *lpcat1/lpcat2* mutant background suggested some acyl flux through PC for transfer of PUFAs to other lipids still occurs (Bates et al., 2012; Wang et al., 2012; Karki et al., 2019), possibly through *de novo* PC synthesis and various PC turnover/exchange mechanisms including phosphatidylcholine:diacylglycerol cholinephosphotransferase (PDCT) or phospholipase D (Lee et al., 2011; Bates et al., 2012; Yang et al., 2017). An alternative non-LPCAT based acyl editing mechanism may also contribute to acyl flux through PC in the *lpcat1/lpcat2* mutant. Lyso-PC produced by PLA_2 action may be converted back to PC by a lyso-PC:lyso-PC transacylase (LPCT) which co-produces glycerophosphocholine. Glycerophosphocholine acyltransferase (GPCAT) can regenerate lyso-PC to maintain the LPCT reaction (Lager et al., 2015). As of yet, the molecular identity of only the GPCAT, but not LPCT, has been identified (Gląb et al., 2016). Still unknown is the relative contribution of forward/reverse LPCAT, PLA_2, LPCT, and GPCAT activities to overall acyl flux through PC and their contribution to seed TAG FA composition in wild-type and engineered plants that accumulate novel FA compositions (Bates, 2016; Correa et al., 2020).

Production of *de novo sn*-1,2-diacylglycerol for TAG biosynthesis

Two major pathways have been identified that produce the *sn*-1,2-diacylglycerol (*sn*-1,2-DAG) substrate for TAG biosynthesis. First, *de novo* glycerolipid assembly (also known as the Kennedy pathway (Weiss et al., 1960)) involves the consecutive reactions of acyl-CoA dependent glycerol-3-phosphate acyltransferase (GPAT) and lysophosphatidic acid acyltransferase (LPAT) to produce lysophosphatidic acid (LPA, 1-acyl-sn-glycerol 3-phosphate) and phosphatidic acid (PA, 1,2-diacyl-sn-glycerol-3-phosphate), respectively. Subsequent PA dephosphorylation by PA phosphatase/hydrolase produces *de novo sn*-1,2-DAG (Fig. 2, DAG(1)). Thus, GPAT and LPAT acyl selectivities control *de novo* DAG composition. In *Arabidopsis* there are 10 GPATs (Yang et al., 2012), but only GPAT9 has a confirmed role in ER-localized membrane lipid and TAG production (Shockey et al., 2016; Singer et al., 2016). Similarly, there are five LPATs in *Arabidopsis*. LPAT2 is the major isoform involved in ER TAG and membrane lipid production in most plants studied to date (Kim et al., 2005; Maisonneuve et al., 2010; Barroga and Nakamura, 2022), but *RcLPAT3B* and *BnLPAT5* may also contribute to seed TAG accumulation in *Ricinus communis* and *B. napus*, respectively (Kim et al., 2020; Zhang et al., 2022).

In general, LPATs have more rigid acyl selectivities than GPATs, which contributes to the strict limitation of saturated FAs at the *sn*-2 position of ER membrane lipids and TAG from most species (Ohlrogge and Browse, 1995; Brown et al., 2002). Both *in vitro* and *in vivo* analyses have shown that some GPAT and LPAT enzymes can have enhanced selectivity for unusual FAs specific to that species, leading to production of TAG with diverse and/or unusual FA compositions. For example, the GPAT and LPAT activities from *R. communis* or *Cuphea lanceolata* seed tissue possess selectivity for ricinoleoyl- or decanoyl-CoAs, respectively (Bafor et al., 1990; Bafor et al., 1991). Likewise, co-expression of the *R. communis, Cuphea viscosissima*, or *Vernicia fordii* GPAT and/or LPAT with the respective FA hydroxylase, thioesterase, or desaturase that produces the species-specific unusual FAs led to enhanced accumulation of ricinoleate, decanoate, or α-eleostearate, respectively, (Fig. 1 E, F, I) in TAG of transgenic plants (Kim et al., 2015; Lunn

et al., 2019; Shockey et al., 2019). These results indicate that GPAT/LPAT acyl selectivity can co-evolve with unusual FA biosynthesis to produce TAG molecular species enriched in unusual FAs. However, GPAT acyl selectivity does not always coincide with TAG *sn*-1 FA composition. For example, *Crambe abyssinica* accumulates TAG with ~60% erucic acid (22:1, Fig. 1K) which is localized to the *sn*-1 and *sn*-3 positions. However, GPAT assays with developing seed extracts indicated 22:1-CoA was only about 3% as effective as 18:1-CoA (Guan et al., 2014), making it unclear how the *sn*-1 position of *C. abyssinica* TAG is composed almost entirely of 22:1. Multiple possibilities may account for the apparent discrepancy between GPAT selectivity and TAG composition. First, the molecular species specificity of LPAT and/or DGAT may be high enough for 22:1-containing substrates to specifically produce these TAG molecular species from diverse LPA and DAG substrate pools, respectively. Alternatively, TAG remodeling may change the *sn*-1 FA composition after initial TAG biosynthesis (discussed more below) (Bhandari and Bates, 2021; Parchuri et al., 2024). Thus, *de novo* DAG and subsequent TAG biosynthesis with molecular species selective acyl transferases can produce unique TAG composition. Examples of species where biochemical evidence suggests TAG synthesis from *de novo* DAG(1) (Fig. 2) is the major pathway for TAG accumulation include *R. communis*, *Cuphea lanceolata, Persea americana, Coriandrum sativum,* and *Theobroma cacao* (Griffiths et al., 1988; Bafor et al., 1990; Bafor et al., 1991; Griffiths and Harwood, 1991; Cahoon and Ohlrogge, 1994).

Production of PC-derived *sn*-1,2-diacylglycerol for TAG biosynthesis

In many different oilseed species including Arabidopsis, *B. napus*, *C. sativa*, *Carthamus tinctorius*, *Linum usitatissimum*, *G. max*, and *P. fendleri in vivo* isotopic tracing has indicated that DAG derived from PC (Fig. 2, DAG(2)), rather than *de novo* DAG, can contribute to or be the predominant source of *sn*-1,2-DAG utilized for TAG biosynthesis (Slack et al., 1978; Griffiths et al., 1988; Bates et al., 2009; Bates and Browse, 2011; Guan et al., 2014; Yang et al., 2017; Bhandari and Bates, 2021; Pollard and Shachar-Hill, 2022). Because PC is the site for acyl editing and FA modification, the molecular species of PC-derived DAG(2) can be distinct from *de novo* DAG(1). There are multiple mechanisms to produce PC-derived DAG(2) including PC synthesis from *de novo* DAG(1) and CDP-choline via cholinephosphotranserase (CPT; also known as aminoalcoholphosphotransferase, AAPT) and subsequent PC turnover by the reverse action of CPT, phospholipase C (PLC) hydrolysis, or phospholipase D (PLD) hydrolysis and subsequent dephosphorylation by PA phosphatase (Bates, 2016; Ali et al., 2022; Bates, 2022). Gene knockdown, knockout, and overexpression studies support the roles for nonspecific phospholipase C 6 (AtNPC6), AtPLDζ, and GmPLDα in PC-derived DAG production in *Arabidopsis* and *G. max*, respectively (Lee et al., 2011; Yang et al., 2017; Cai et al., 2020). The changes in TAG amounts and/or FA composition (or measured lipid flux) for each was consistent with at least a partial role in PC-derived DAG production, however it is unclear if these PC turnover enzymes are selective for specific PC molecular species that ultimately affect the TAG FA composition.

A major alternative to *de novo* PC synthesis and turnover is phosphocholine exchange between PC and DAG producing new molecules of DAG and PC, a reaction catalyzed by phosphatidylcholine:diacylglycerol cholinephosphotransferase (PDCT) (Lu et al., 2009). PDCT activity can incorporate DAG into PC for further acyl modification (e.g. desaturation), and concomitant conversion of modified PC molecular species back into DAG for TAG biosynthesis. Reduced PUFA content of the Arabidopsis, *B. napus*, *G. max,* and *Thlaspi arvense* PDCT mutants is consistent with this role (Lu et al., 2009; Bai et al., 2020; Jarvis et al., 2021; Li et al., 2023). Additionally, *in vitro* assays of *C. sativa* PDCT demonstrated strong activity with DAG and PC substrates containing 18:1, 18:2, 18:3, or 22:1 FAs indicating a possible role of equilibrating molecular species of DAG and PC (Demski et al., 2021). However, PDCT can also be highly selective for or against

unusual FA-containing DAG molecular species. *R. communis* predominantly accumulates TAG containing three ricinoleates; *in vitro* assays indicated *RcPDCT* was selective for DAG containing one ricinoleate and against DAG containing two ricinoletates. However, PDCT from *C. sativa* (that does not naturally produce ricinoleic acid) would not use DAG containing ricinoleate (Lager et al., 2020; Demski et al., 2022). This result is similar to *in vivo* isotopic tracing of transgenic ricinoleate producing Arabidopsis, where the flux of ricinoleate-containing DAG into PC was inhibited (Bates and Browse, 2011), which in turn limited ricinoleate-containing *de novo* DAG flux into PC then TAG. However, *RcPDCT* expression in the same transgenic Arabidopsis background enhanced the accumulation of ricinoleate in TAG (Hu et al., 2012), and similar overexpression studies with *PDCT* genes from various plants have correlated PDCT activity with enrichment of PC modified FAs in TAG (Wickramarathna et al., 2015; Yu et al., 2019; Wang et al., 2021). Thus, the PDCT DAG molecular species selectivity can influence multiple outcomes, including limiting the flux of some molecular species into PC or enhancing the flux of select DAG molecular species into PC. Both actions would enrich the DAG pool in certain DAG molecular species for TAG biosynthesis. Once in PC, the FAs can either be further modified, shuttled into the acyl-CoA pool through acyl editing, or directly utilized for TAG biosynthesis by phospholipid:diacylglycerol acyltransferase (PDAT). Collectively, these findings suggest that PDCT primarily serves to edit the DAG molecular species pool utilized for TAG biosynthesis.

Diverse molecular species produced by TAG biosynthetic enzymes

The final step to synthesize TAG from DAG is catalyzed by an acyl-CoA:diacylglycerol acyltransferase (DGAT), or by direct FA transfer from PC by PDAT. Plant DGATs and PDATs have been extensively studied and recently expertly reviewed in detail (Xu et al., 2018; Chen et al., 2022; Sah et al., 2024). Here we focus on the key aspects pertaining to their control of TAG molecular species compositions. Plants contain three unrelated DGAT enzymes. In many cases, the ER-localized DGAT1 and/or DGAT2 are the major seed oil producing acyltransferases, and the acyl-CoA selectivity of the dominant DGATs typically is consistent with the FA composition of the seed oil, as demonstrated by either *in vitro* assays or transgenic expression experiments (Xu et al., 2018; Correa et al., 2020; Chen et al., 2022). However, even within a species, different isoforms of DGAT1 and/or DGAT2 can have different acyl-CoA selectivities (Demski et al., 2019; Chen et al., 2022; Parchuri et al., 2024). The role of the soluble DGAT3 in lipid metabolism is less clear. The relatively scant experimental evidence suggests localization to the chloroplast (Aymé et al., 2018; Carro et al., 2022) and a role in lipid homeostasis in Arabidopsis vegetative tissues (Hernández et al., 2012), but results suggest a contribution of DGAT3 to *C. sativa* seed oil accumulation (Lee et al., 2022). DGAT1 and DGAT2 typically have distinct expression patterns, do not interact or co-localize in the ER, and can utilize metabolically distinct pools of DAG (Fig. 2 DAG(1-4)) (Shockey et al., 2006; Bourgis et al., 2011; Troncoso-Ponce et al., 2011; Horn et al., 2016; Regmi et al., 2020). Recently, assays of DGAT1 and DGAT2 from *C. sativa*, *Crambe hispanica*, and *P. fendleri* indicate that DGAT1 and DGAT2 can have very distinct and complementary DAG molecular species selectivities which contribute to the final oil composition (Jeppson et al., 2019; Lager et al., 2020; Parchuri et al., 2024). Figure 2 indicates where evidence suggests species-specific utilization of select DAG pools by DGAT1 or DGAT2 including: the use of *de novo* DAG(1) by RcDGAT2 in *R. communis* endosperm (Bafor et al., 1991; Burgal et al., 2008; Troncoso-Ponce et al., 2011); the use of PC-derived DAG(2) by AtDGAT1 or PfeDGAT1 (Regmi et al., 2020; Parchuri et al., 2024); or the use of *sn*-2,3-DAG(4) produced through TAG remodeling by PfeDGAT2 (Parchuri et al., 2024). Interestingly, when expressed transgenically, the DGAT may not utilize the same DAG pool that is exploited in the host species. For example, RcDGAT2 expressed in the Arabidopsis *dgat1-1* mutant did not utilize *de novo* DAG(1) or the initially produced PC-derived DAG(2), but utilized a more slowly turned over PC-derived DAG(3) pool

that was also utilized by AtPDAT1 (Regmi et al., 2020). Therefore, the control of DAG pool utilization may not reside with the DGAT itself, but how that specific DGAT incorporates into the host plant lipid metabolic network.

In addition to DGATs, PDATs also contribute to seed oil accumulation and FA composition. *Arabidopsis* contains two *PDAT* genes (Dahlqvist et al., 2000; Stahl et al., 2004). Many plants contain one or more homeologs of *AtPDAT1* and/or *AtPDAT2*, and some plants have additional *PDAT* isoforms (Sah et al., 2024). *In vitro*, PDATs can utilize both PC and phosphatidylethanolamine (PE) as an acyl donor, but *in vivo* results indicate that PDATs predominantly enhance the transfer of PC-modified FAs from *sn*-2 PC to TAG. The lyso-PC coproduct is converted back to PC by LPCAT, thus acyl editing and PDAT can work together to channel 18:1 into PC for desaturation (or other modification) and subsequent transfer into TAG. Various TAG FA composition engineering studies support this role for PDAT1 (van Erp et al., 2011; Xu et al., 2012; Pan et al., 2013; Marmon et al., 2017; Lunn et al., 2020; Park and Kim, 2024). PDAT1s can also have high selectivity for the acyl donor and DAG molecular species (Lager et al., 2020) ultimately affecting the TAG acyl composition.

In plants, PDAT1 appears to be secondary to DGAT1 or DGAT2 for seed oil synthesis. The *Arabidopsis pdat1* mutant has no seed oil phenotype, however AtPDAT1 enzymatic activity provides ~80% of wild-type TAG biosynthetic capacity in the *dgat1-1* mutant background (Zhang et al., 2009). Interestingly, *in vivo* flux analysis in *dgat1-1* mutant demonstrated AtPDAT1 utilizes a metabolically distinct pool of PC-derived DAG (Fig. 2, DAG(3)) from that of AtDGAT1 (Regmi et al., 2020), indicating AtPDAT1 does not just replace AtDGAT1 in TAG biosynthesis. Instead lipid metabolism adapts to the loss of AtDGAT1 to utilize AtPDAT1, including altered acyl flux through chloroplast lipids (Aulakh and Durrett, 2019). Additionally, in *C. sativa* mRNA knockdowns or gene mutations of *PDAT1* altered the oil content, although to a lesser extent than the corresponding *DGAT1* mRNA knockdowns or mutants, which also suggests PDAT1 as accessory to DGATs for seed oil biosynthesis (Aznar-Moreno and Durrett, 2017; Marmon et al., 2017). Genome-wide analysis of the *PDAT* family in four *Gossypium* species indicated none of the *PDAT* genes correlated with quantitative trait loci for oil content, despite expression in developing seeds, further suggesting a secondary role to DGATs for oil quantity and composition (Zang et al., 2019). The endogenous role of *PDAT2* has been less studied, and *AtPDAT2* cannot complement for the loss of *AtDGAT1* and *AtPDAT1* in Arabidopsis (Zhang et al., 2009). However, overexpression of various plant *PDAT2*s in yeast or plant tissue can increase TAG production and alter the FA content, indicating they are functional TAG synthesizing enzymes (Pan et al., 2013; Yuan et al., 2017; Parchuri et al., 2022). PDATs have also been highly associated with changes in membrane and TAG content during plant stresses in various tissues (Sah et al., 2024; Shomo et al., 2024). Considering that PDAT activity directly connects the PC and TAG pools it is tempting to speculate that the role of PDATs during seed oil filling may be related to membrane editing during high rates of FA synthesis and FA modification. The contribution to TAG biosynthesis may be a byproduct of maintaining membrane lipid homeostasis, especially when unusual FAs may disrupt the membrane structure/function. A major unknown in most plant seeds is the relative contribution of DGAT1, DGAT2, DGAT3, PDAT1, or PDAT2 to the final oil amount and molecular species composition.

Initial TAG biosynthesis in not a metabolic end point in seed oil accumulation

Seed oil is predominantly a storage product until germination where it is broken down to provide carbon and energy for seedling establishment. During seed maturation in many species ~10% of TAG is broken down due to expression of the SDP1 TAG lipase in preparation for germination (Eastmond, 2006; Kelly et

al., 2013; Kim et al., 2014; Kanai et al., 2019; Azeez et al., 2022; Aznar-Moreno et al., 2022). Yet, during the oil accumulation phase of seed development TAG production has been historically considered a metabolic end point. However, recently the remodeling of TAG molecular species was discovered through *in vivo* metabolic tracing of *P. fendleri* developing seeds (Bhandari and Bates, 2021). TAG remodeling is defined as a cycle of partial TAG degradation (through lipase removal of the *sn*-1 or *sn*-3 FA producing *sn*-1,2-DAG or *sn*-2,3-DAG), and subsequent TAG resynthesis with different acyl-CoA species producing new molecular species of TAG. *P. fendleri* accumulates high levels of lesquerolic acid, a 20-carbon hydroxylated FA (HFA), (Fig. 1J), at the *sn*-1 and *sn*-3 positions of TAG, but not in membrane lipids (Hayes and Kleiman, 1996; Chen et al., 2011; Bhandari and Bates, 2021). TAG synthesis starts with PC-derived *sn*-1,2-DAG (not containing HFA) and an *sn*-3 HFA is added by PfeDGAT1 that is selective for the *sn*-1,2-DAG enantiomer structure and molecular species not containing HFA. The initially produced TAG containing a single HFA is then remodeled to contain a second HFA by a TAG lipase (PfeTAGL1) that interacts with PfeDGAT1 and removes the *sn*-1 common FA. PfeDGAT2 is selective for HFA-containing *sn*-2,3-DAG enantiomer and lesqueroyl-CoA, and produces the final TAG species containing HFA at both *sn*-1 and *sn*-3 (Bhandari and Bates, 2021; Parchuri et al., 2024). Consequently, TAG remodeling in *P. fenderli* utilizes two DGATs with differential selectivity for the *sn*-1,2 or *sn*-2,3 enantiomers of DAG. Thus, *P. fendleri* changes the TAG FA composition after initial synthesis and may be a way to utilize PC-derived DAG but also incorporate FAs into TAG that are incompatible with membrane lipid intermediates.

It is likely that TAG remodeling also occurs in other plants. Homologs of *PfeTAGL1* are found throughout the plant kingdom but their role in metabolism has yet to be characterized, although the Arabidopsis homolog (*At1g23330*) was identified associated with lipid droplets by proteomics (Kretzschmar et al., 2020). *De novo* DAG produced from PA or PC-derived DAG are both *sn*-1,2 DAG enantiomers due to their original synthesis from glycerol-3-phosphate. However, TAG lipases can remove FAs from either the *sn*-1 or *sn*-3 position producing both the *sn*-1,2 or *sn*-2,3 enantiomers of DAG. The utilization of these TAG-derived DAGs to produce different molecular species of TAG constitutes TAG remodeling but requires an acyltransferase that can utilize the *sn*-2,3-DAG. The DAG enantiomer selectivity of DGATs has not been assayed in species other than *P. fendleri* (Parchuri et al., 2024), however if additional plant DGATs demonstrate selectivity for specific DAG enantiomers it may suggest that TAG remodeling occurs in other species and may help explain the differential roles of DGAT1, DGAT2, or DGAT3 in plant TAG metabolism. Additionally, TAG remodeling may help to explain discrepancies between seed TAG FA composition and observed acyltransferase selectivities. For example, erucic acid (22:1) is highly enriched in the *sn*-1 position of *Crambe abyssinica* TAG, but the microsomal GPAT does not efficiently utilize 22:1-CoA (Guan et al., 2014). TAG remodeling could be a mechanism to incorporate 22:1 into *sn*-1 TAG. It is also possible that TAG remodeling may be induced by stress conditions during seed development and could help explain the effect of cold on increasing PUFA content in *Helianthus annuus* seeds (Garces et al., 1994; Sarmiento et al., 1998).

The control of lipid metabolic flux may be dependent on cellular organization of lipid metabolism

How acyl flux through the complex lipid metabolic network for TAG assembly (Fig. 2) is controlled to produce specific TAG molecular species, while maintaining distinct acyl compositions within membrane lipids, is still an unsolved metabolic mystery. Additionally, one of the major unknowns regarding the cellular control of metabolism (including but not limited to lipid biosynthesis) is the “blueprint” used for subcellular and suborganellar compartmentalization of reactions. The ER membrane contains at least 16 morphologically (and likely physiologically) distinct domains (Staehelin, 1997; Levine and Rabouille, 2005).

Some of these domains likely serve as specialized sites for either membrane or TAG biosynthesis (Fernandez and Staehelin, 1987; Vogel and Browse, 1996). 'Metabolons' provide the conditions necessary for tightly regulated, highly coordinated transfer of intermediates directly from one enzyme to the next (Dastmalchi and Facchini, 2016; Bassard and Halkier, 2018; Coleman, 2019). Metabolons contain all the necessary enzymes, scaffolding proteins, and other cofactors required for efficient metabolite production; unique ER lipid composition and interaction with cellular scaffolding may also contribute to protein associations within the metabolon. Sequestration of substrates in metabolons serves to increase their local concentrations, thus enhancing reaction rates. Substrate channeling helps to prevent the release and potential degradation of pathway intermediates. Metabolon protein complexes can be homomeric or heteromeric and exist in either permanent or transient states. Various properties of permanent and transient complexes differ, especially including the binding affinities between the different protein subunits and may affect metabolon function (Dahmani et al., 2023). Because metabolons can be dissociable, determining what is a metabolon for substrate channeling vs just interacting proteins can be difficult.

Plant terpenoid biosynthesis is strongly suspected to be regulated, at least in part, by metabolon formation. Terpenoids are a large category of lipophilic chemicals that serve important general roles as electron carriers, hormones, and pigments, while specialized terpenoids are used by certain plant families to mediate both helpful and harmful biotic interactions. Even when ignoring the biochemistry necessary to create the fully elaborated profile of dozens or hundreds of specific terpenoids found in each species or tissue, the biosynthesis of the foundational terpenoid building blocks is itself very complex. Two primary biosynthetic pathways operate in parallel for the early biosynthetic reactions. These pathways occupy at least three subcellular compartments and draw upon the same isopentenyl diphosphate and dimethylallyl diphosphate precursors. Efficient channeling of metabolites through these pathways strongly suggests that plant cells can contain and channel certain plant terpenoid intermediates through the appropriate branches of the respective biosynthetic networks, as expertly reviewed recently (Gutensohn et al., 2022). Additionally, different transgenic engineering approaches have been used to enhance terpenoid accumulation via metabolic channeling using non-traditional approaches such as protein fusions between successive enzymes and the use of different types of protein scaffolds (Brodelius et al., 2002; Dueber et al., 2009; Han et al., 2016; Sadre et al., 2019; Gutensohn et al., 2022).

Within plant membrane lipid and TAG synthesis, multiple studies have provided enticing suggestions for existence of lipid biosynthetic metabolons. All the enzymes and cofactors necessary for FA synthesis are integrated within spinach chloroplasts, leading to highly efficient channeling of carbon into FAs and other complex lipids, even when chloroplasts were disrupted and even though the chloroplastic FA synthase complex is made up of several soluble enzymes that could reasonably be expected to leak from lysed organelles *in vitro* (Roughan and Ohlrogge, 1996). Other findings demonstrated that ER membrane lipid metabolic domains themselves might be further separated into additional categories, as evidenced by non-overlapping targeting of two different DGAT enzymes from tung tree (*Vernicia fordii*) (Shockey et al., 2006). Combined with the contrasting expression profiles and transgenic product profiles produced by the two tung enzymes, it seems likely that they co-localize with different protein partners and serve substantially different roles *in vivo*. The strongest evidence for possible metabolons in lipid metabolism comes from the *in vivo* metabolic tracing discussed above, where plants can control the flux of Kennedy pathway produced *de novo* DAG into PC rather than directly into TAG and eventually utilize PC-derived DAG for TAG synthesis (Bates et al., 2009; Bates and Browse, 2011; Yang et al., 2017; Regmi et al., 2020;

Bhandari and Bates, 2021), despite all known enzymes involved in *de novo* DAG, PC, PC-derived DAG, and TAG synthesis localizing to the ER membrane. What is lacking is evidence of the organization of metabolon protein components that may control this metabolism, but supportive results are starting to accumulate (Xu et al., 2023).

Our focus lies mostly with plant lipids, yet recent work in *Saccharomyces cerevisiae* (Greenwood et al., 2023) is especially relevant to plant TAG production. Extensive interactions between all three Kennedy pathway acyltransferases (GPAT, LPAT, and DGAT) was reported. Additionally, a 'supercomplex' containing the Δ9 FA desaturase Ole1 and the latter two Kennedy pathway enzymes LPAT (Slc1) and DGAT (Dga1) was described. Dubbed as a 'desaturasome' (Greenwood et al., 2023), this complex likely helps to channel unsaturated FAs into phospholipids during rapid cell division, thereby helping to maintain proper membrane fluidity, and may also help to initiate lipid droplet formation by supplying unsaturated FAs for DAG and TAG (Zoni et al., 2021).

Yeast two-hybrid (Y2H) was used to demonstrate the interaction of Arabidopsis DGAT1 with PDCT and LPCAT2, suggesting possible delivery of PC-derived DAG and acyl-CoA for TAG synthesis. Notably, the yeast two-hybrid system used in this study (and others like it) utilizes a split-ubiquitin approach that allows for assessment of soluble proteins, membrane-bound proteins, or combinations thereof (Gidda et al., 2011). Additionally, both Y2H and biomolecular fluorescence complementation (BiFC) demonstrated the interaction of AtDGAT1 and AtPDAT1 (Lee and Seo, 2019; Regmi et al., 2020), consistent with accumulation of heart-healthy oleic acid in TAG in transgenic systems expressing avocado PaDGAT1 and PaPDAT1 (Behera et al., 2022). However, *in vivo* metabolic tracing in developing seeds of Arabidopsis wild-type and *dgat1-1* mutant (where AtPDAT1 synthesizes TAG) suggested the use of different PC-derived DAG pools for TAG biosynthesis by AtDGAT1 and AtPDAT1. Thus, more information is needed to understand how potential metabolon components control substrate flux into specific TAG molecular species. Flax (*Linum usitatissimum*) seed oil contains very high levels of the omega-3 FA α-linolenic acid (18:3, Fig. 1D). The efficient accumulation of 18:3 may be explained in part by substrate channeling/TAG metabolon formation. Y2H and BiFC approaches demonstrated physical interactions between flax DGATs and other lipid biosynthetic enzymes, including LuLPCAT2 and LuPDCT (Xu et al., 2019). Notably, in contrast to the *Arabidopsis* results cited above, these authors did not observe interactions between LuDGAT1 and LuPDAT1, further indicating that more information is needed to understand the coordinated TAG biosynthesis between DGAT and PDAT in different species. Caution must be used in interpreting Y2H and BiFC data, because positive interactions in these systems do not necessarily imply substrate channeling or metabolon formation, but are nonetheless compelling evidence that points in those directions.

Another example of potential metabolons in plant lipid metabolism is the growing evidence for lineage specific coordination of proteins to produce specific TAG molecular species (Busta et al., 2022). For example, Castor (*Ricinus communis*) accumulates ~90% of the HFA ricinoleic acid (Fig. 1I) in its seed oils, but little to no ricinoleic acid in membrane lipids. This dichotomy suggests evolutionary optimization of HFA biosynthesis, acyltransferase substrate selectivity, and substrate channeling, that could be accomplished by metabolon formation. While the primary castor oleate hydroxylase enzyme is capable of modest ricinoleate production in transgenic systems, these lines often suffered from oil yield penalties, poor germination, and other problems. Stacking of multiple castor acyltransferases and TAG metabolic genes into the hydroxylase Arabidopsis lines progressively overcame these physiological defects while also resulting in large increases in the total amounts of ricinoleate produced in seed oils (Lu et al., 2006; Burgal et al., 2008; van Erp et al., 2011; Lunn et al., 2019; Shockey et al., 2019). The enhanced efficiency seen in

these lines suggests formation of protein:protein complexes among the castor enzymes, creating more efficient HFA flux into TAG. Similarly, *in vivo* metabolic tracing, protein:protein interactions, and transgenic expression support a potential metabolon for the accumulation of the HFA lesquerolic acid (Fig. 1J) through TAG remodeling in *P. fenderli* (Bhandari and Bates, 2021; Parchuri et al., 2024). Thus, metabolons may be one mechanism to separate unusual FA metabolism for TAG biosynthesis from common FA metabolism required for membrane function.

While all these studies suggest some of the contextual possibilities for intersection of metabolon formation and lipid biosynthesis, our research has raised another question (additionally, see Outstanding Questions). Aside from the possible protein composition of lipid metabolons, how might metabolons play a role in the spatial segregation of the various routes of TAG biosynthesis? Could the sorting of key enzymes into distinct metabolons control the diversity of metabolic pathways used to produce TAG from *de novo* DAG, PC-derived DAG, or through TAG remodeling? One possibility is that lipid metabolism is organized into metabolons that separate *de novo* membrane lipid biosynthesis, from that of TAG biosynthesis, such that intermediates like PC act as effective "DAG transport molecules" that shuttle between ER domains (Shockey et al., 2016; Regmi et al., 2020; Bates, 2022). Further characterization of the protein components of different lipid biosynthetic metabolons and how each effects substrate flux will be essential for understanding the potential roles of metabolons in lipid metabolism.

Concluding Remarks

As our knowledge of plant TAG biosynthesis increases, so does our appreciation of the complexity of the pathways and mechanisms required to produce different TAG molecular species. The advent of rapid and inexpensive genome and transcriptome sequencing has allowed us to quickly identify homologs of many TAG biosynthesis-related enzymes that are expressed in oil accumulating tissues. However, as we presented here the same types of TAG biosynthetic enzymes may be utilized within different metabolic pathways and have different selectivities for substrate molecular species or even stereochemical structures. Therefore, to thoroughly comprehend oil biosynthesis in any plant species will require understanding the biochemical activities of the enzymes involved and how they cooperate to control the overall carbon flux through lipid metabolism (see Outstanding Questions). Additionally, despite the deceptive simplicity of drawing "metabolic pathways", it is important to remember that metabolism occurs within the multi-compartmental and three-dimensional confines of a dynamic living cell. Therefore, additional insights to the cellular organization of lipid metabolism will be essential to understand how the overlapping pathways of membrane lipid and storage oil biosynthesis are organized and controlled. Finally, as the recent discovery of TAG remodeling demonstrates (Bhandari and Bates, 2021; Parchuri et al., 2024), there are likely additional unanticipated metabolic pathways (or network branches) involved in TAG accumulation, a least in some plant species. Understanding the species-specific differences and organization of TAG metabolism will be key for rational engineering of designer oils for the benefit of humankind.

Outstanding Questions box

- What controls acyl flux through different branches of the lipid metabolic network?
- Does TAG remodeling occur in species that do not accumulate hydroxylated fatty acids?

- Do DGATs from species other than *P. fendleri* have selectivity for *sn*-1,2- or *sn*-2,3-DAG enantiomers? If so, what role does DAG enantiomer selectivity or lack thereof have within lipid metabolism?
- What is the relative contribution of different acyltransferase isoforms to TAG biosynthesis in different species?
- Do the protein:protein interactions identified within plant TAG synthesis actually function to channel substrates into TAG as a functional metabolon?
- Could the sorting of key enzymes into distinct metabolons control the diversity of metabolic pathways used to produce TAG from *de novo* DAG, PC-derived DAG, or through TAG remodeling?
- What controls the localization of different enzymes and/or distinct metabolite pools to distinct metabolons to produce different TAG molecular species?
- Can engineering artificial metabolons allow us to more efficiently tailor seed oil fatty acid composition without disturbing essential membrane lipid compositions?
- Considering the huge diversity of fatty acid structures in nature, are there additional uncharacterized metabolic pathways of TAG assembly that are key to controlling seed oil fatty acid compositions?

Acknowledgments

This material is based upon work supported by the National Science Foundation under grant Nos. 1829365 and 2242822; the United States Department of Agriculture National Institute of Food and Agriculture grant Nos. 2020-67013-30899 and 2023-67013-39022, Hatch Project No. 1015621 and Multi-State Project No. 1013013; and the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, under award No. DE-SC0023142. This work was supported in part by the U.S. Department of Agriculture, Agricultural Research Service. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by USDA. USDA is an equal opportunity provider and employer.

FIGURE LEGENDS

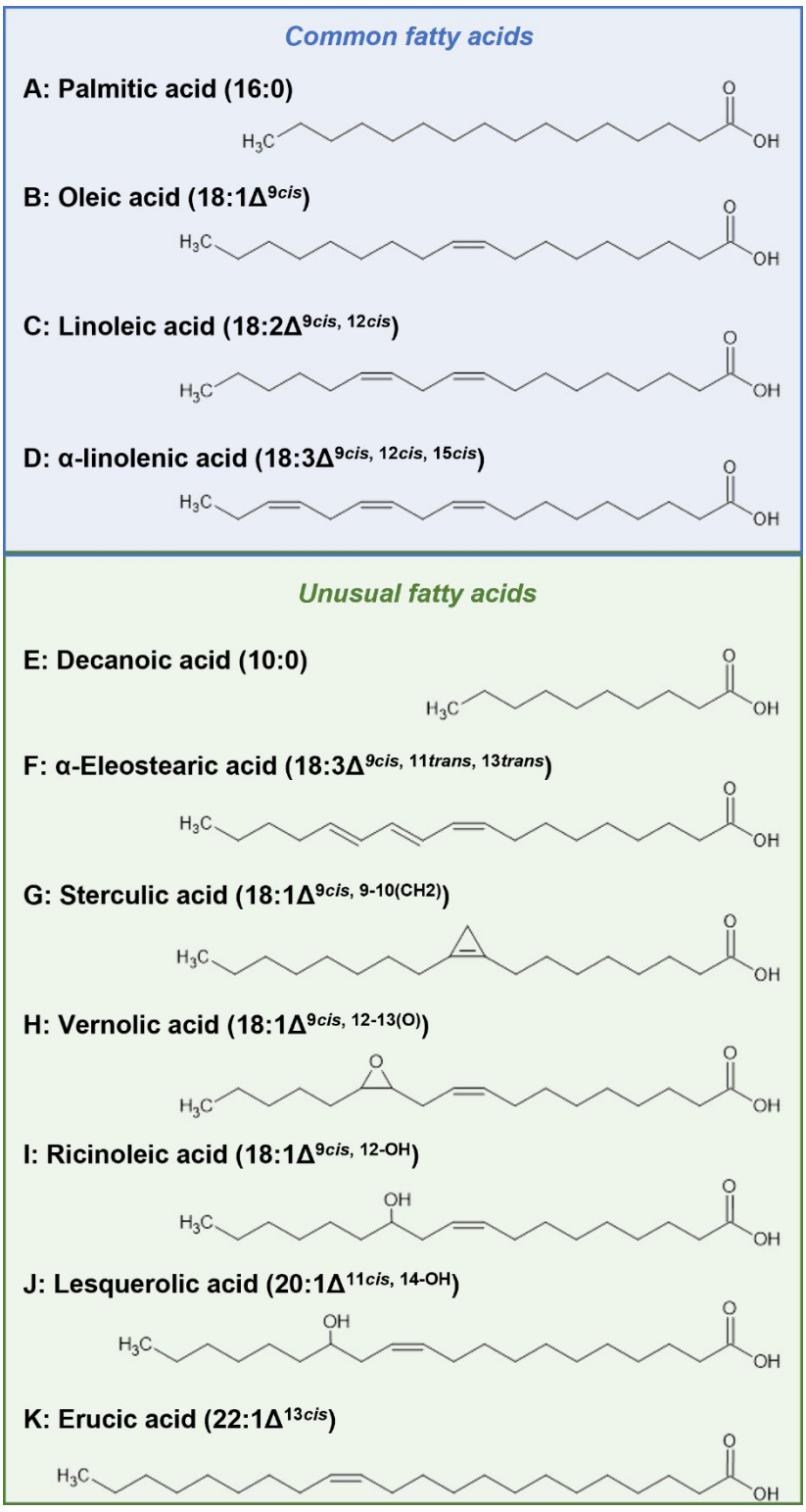

Figure 1. Examples of common and unusual fatty acids.

The fatty acid common names, and where appropriate common abbreviations, are in the figure. The IUPAC names are: A, hexadecanoic acid; B, (Z)-octadec-9-enoic acid; C, (9Z,12Z)-octadeca-9,12-dienoic acid; D, (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid; E, decanolic acid; F, (9Z,11E,13E)-octadeca-9,11,13-trienoic

acid; G, 8-(2-octylcyclopropen-1-yl)octanoic acid; H, (Z)-11-[(2S,3R)-3-pentyloxiran-2-yl]undec-9-enoic acid; I, (Z,12R)-12-hydroxyoctadec-9-enoic acid; J, (Z,14R)-14-hydroxyicos-11-enoic acid; K, (Z)-docos-13-enoic acid.

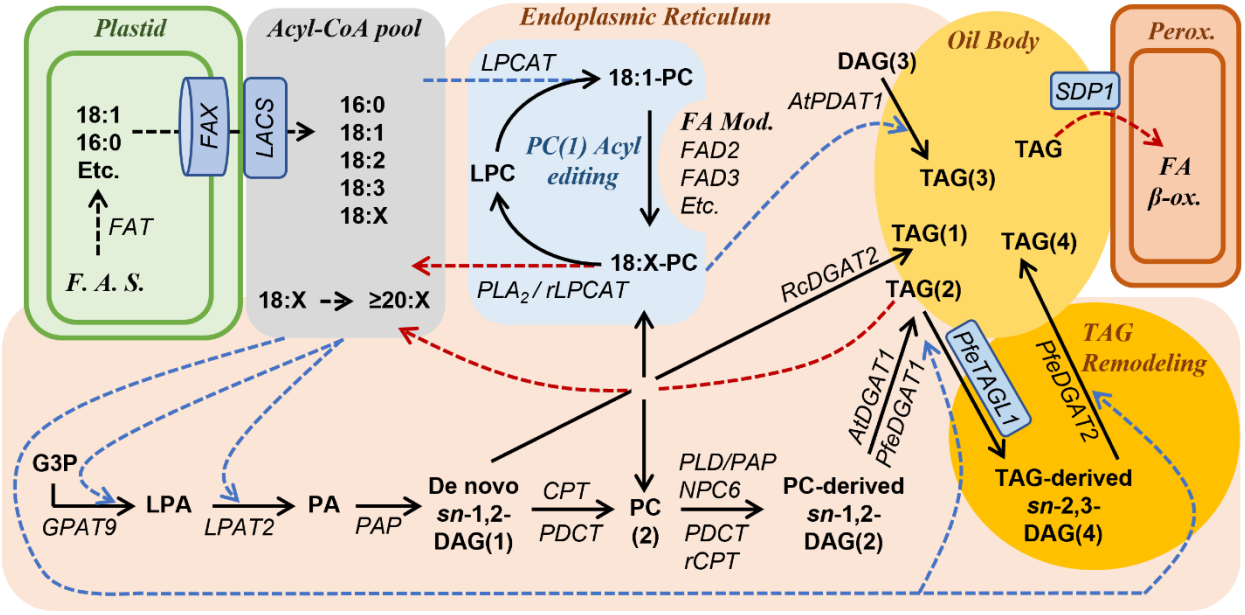

Figure 2. Expanding diversity of metabolic reactions controlling TAG molecular species accumulation.

Solid black lines involve glycerol backbone flux. Dashed arrows are acyl transfers, blue are glycerolipid assembly, red are fatty acid removal from glycerolipids. Substrates are in bold, where appropriate numbered substrates represent different metabolic pools as identified from isotopic tracing studies. Enzymes next to arrows are in italics, where appropriate specific genes isoforms are indicated. For TAG synthesizing enzymes that utilize different DAG pools, examples of species genes are indicated where results suggest which DAG pool each utilizes in that species. Enzymes in boxes represent reactions at the interphase of two cellular compartments. Cellular locations or description of a multi-step metabolic activity is Times New Roman font in italics, abbreviations: Perox., peroxisome; β-ox., beta-oxidation; FA Mod., fatty acid modification; F. A. S., fatty acid synthesis. Substrate abbreviations in bold: DAG, diacylglycerol; G3P, glycerol-3-phosphate; LPA, lyso-phosphatidic acid; LPC, lysophosphatidylcholine; PA, phosphatidic acid; PC, phosphatidylcholine; TAG, triacylglycerol. Enzyme abbreviations in italics: CPT, CDP-choline:DAG cholinephosphotransferase; DGAT, acyl-CoA:DAG acyltransferase; FAD, fatty acid desaturase; FAT, fatty acid thioesterase; FAX, fatty acid exporter; GPAT9, acyl-CoA:G3P acyltransferase 9; LACS, long chain acyl-CoA synthetase; LPAT2, acyl-CoA:LPA acyltransferase 2; LPCAT, acyl-CoA:LPC acyltransferase; NPC6, non-specific phospholipase C 6; PAP, PA phosphatase; PDCT, PC:DAG cholinephosphotransferase; PDAT, phospholipid:DAG acyltransferase; PLA, phospholipase A; PLD, phospholipase D. Lowercase r before enzyme indicates reverse reaction.

REFERENCES

Ali U, Lu S, Fadlalla T, Iqbal S, Yue H, Yang B, Hong Y, Wang X, Guo L (2022) The functions of phospholipases and their hydrolysis products in plant growth, development and stress responses. Progress in Lipid Research **86:** 101158

Aulakh K, Durrett TP (2019) The Plastid Lipase PLIP1 Is Critical for Seed Viability in diacylglycerol acyltransferase1 Mutant Seed. Plant Physiol **180:** 1962-1974

Aymé L, Arragain S, Canonge M, Baud S, Touati N, Bimai O, Jagic F, Louis-Mondésir C, Briozzo P, Fontecave M, Chardot T (2018) Arabidopsis thaliana DGAT3 is a [2Fe-2S] protein involved in TAG biosynthesis. Scientific Reports **8:** 17254

Azeez A, Parchuri P, Bates PD (2022) Suppression of Physaria fendleri SDP1 Increased Seed Oil and Hydroxy Fatty Acid Content While Maintaining Oil Biosynthesis Through Triacylglycerol Remodeling. Front Plant Sci **13:** 931310

Aznar-Moreno JA, Durrett TP (2017) Review: Metabolic engineering of unusual lipids in the synthetic biology era. Plant Sci **263:** 126-131

Aznar-Moreno JA, Durrett TP (2017) Simultaneous Targeting of Multiple Gene Homeologs to Alter Seed Oil Production in Camelina sativa. Plant Cell Physiol **58:** 1260-1267

Aznar-Moreno JA, Mukherjee T, Morley SA, Duressa D, Kambhampati S, Chu KL, Koley S, Allen DK, Durrett TP (2022) Suppression of SDP1 Improves Soybean Seed Composition by Increasing Oil and Reducing Undigestible Oligosaccharides. Frontiers in Plant Science **13**

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. Biochemical Journal **272:** 31-38

Bafor M, Smith MA, Jonsson L, Stobart K, Stymne S (1991) Ricinoleic acid biosynthesis and triacylglycerol assembly in microsomal preparations from developing castor-bean (Ricinus-communis) endosperm. Biochemical Journal **280:** 507-514

Bai S, Wallis JG, Denolf P, Engelen S, Bengtsson JD, Van Thournout M, Dierickx J, Haesendonckx B, Browse J (2020) The biochemistry of headgroup exchange during triacylglycerol synthesis in canola. The Plant Journal **103:** 83-94

Barroga NAM, Nakamura Y (2022) LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE 2 (LPAT2) is required for de novo glycerolipid biosynthesis, growth, and development in vegetative and reproductive tissues of Arabidopsis. Plant J **112:** 709-721

Bassard J-E, Halkier BA (2018) How to prove the existence of metabolons? Phytochemistry Reviews **17:** 211-227

Bates PD (2016) Understanding the control of acyl flux through the lipid metabolic network of plant oil biosynthesis. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids **1861:** 1214-1225

Bates PD (2022) Chapter Six - The plant lipid metabolic network for assembly of diverse triacylglycerol molecular species. *In* F Rébeillé, E Maréchal, eds, Advances in Botanical Research, Vol 101. Academic Press, pp 225-252

Bates PD, Browse J (2011) The pathway of triacylglycerol synthesis through phosphatidylcholine in Arabidopsis produces a bottleneck for the accumulation of unusual fatty acids in transgenic seeds. Plant Journal **68:** 387-399

Bates PD, Browse J (2012) The significance of different diacylgycerol synthesis pathways on plant oil composition and bioengineering. Front Plant Sci **3:** 147

Bates PD, Durrett TP, Ohlrogge JB, Pollard M (2009) Analysis of Acyl Fluxes through Multiple Pathways of Triacylglycerol Synthesis in Developing Soybean Embryos. Plant Physiology **150:** 55-72

Bates PD, Fatihi A, Snapp AR, Carlsson AS, Browse J, Lu C (2012) Acyl editing and headgroup exchange are the major mechanisms that direct polyunsaturated fatty acid flux into triacylglycerols. Plant Physiology **160:** 1530-1539

Bates PD, Ohlrogge JB, Pollard M (2007) Incorporation of Newly Synthesized Fatty Acids into Cytosolic Glycerolipids in Pea Leaves Occurs via Acyl Editing. Journal of Biological Chemistry **282:** 31206-31216

Bates PD, Stymne S, Ohlrogge J (2013) Biochemical pathways in seed oil synthesis. Current Opinion in Plant Biology **16:** 358-364

Behera J, Rahman MM, Shockey J, Kilaru A (2022) Acyl-CoA-dependent and acyl-CoA-independent avocado acyltransferases positively influence oleic acid content in nonseed triacylglycerols. Front Plant Sci **13:** 1056582

Bhandari S, Bates PD (2021) Triacylglycerol remodeling in Physaria fendleri indicates oil accumulation is dynamic and not a metabolic endpoint. Plant Physiol **187:** 799-815

Bhattacharya A (2022) Lipid Metabolism in Plants Under Low-Temperature Stress: A Review. *In* Physiological Processes in Plants Under Low Temperature Stress. Springer Singapore, Singapore, pp 409-516

Bourgis F, Kilaru A, Cao X, Ngando-Ebongue G-F, Drira N, Ohlrogge JB, Arondel V (2011) Comparative transcriptome and metabolite analysis of oil palm and date palm mesocarp that differ dramatically in carbon partitioning. Proceedings of the National Academy of Sciences **108:** 12527-12532

Brodelius M, Lundgren A, Mercke P, Brodelius PE (2002) Fusion of farnesyldiphosphate synthase and epi-aristolochene synthase, a sesquiterpene cyclase involved in capsidiol biosynthesis in Nicotiana tabacum. European journal of biochemistry **269:** 3570-3577

Brown AP, Slabas AR, Denton H (2002) Substrate selectivity of plant and microbial lysophosphatidic acid acyltransferases. Phytochemistry **61:** 493-501

Burgal J, Shockey J, Lu C, Dyer J, Larson T, Graham I, Browse J (2008) Metabolic engineering of hydroxy fatty acid production in plants: RcDGAT2 drives dramatic increases in ricinoleate levels in seed oil. Plant Biotechnol J **6:** 819-831

Busta L, Chapman KD, Cahoon EB (2022) Better together: Protein partnerships for lineage-specific oil accumulation. Current Opinion in Plant Biology **66:** 102191

Cahoon EB, Ohlrogge JB (1994) Apparent role of phosphatidylcholine in the metabolism of petroselinic acid in developing umbelliferae endosperm. Plant Physiology **104:** 845-855

Cai G, Fan C, Liu S, Yang Q, Liu D, Wu J, Li J, Zhou Y, Guo L, Wang X (2020) Nonspecific phospholipase C6 increases seed oil production in oilseed Brassicaceae plants. New Phytol **226:** 1055-1073

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

Carro MLM, Gonorazky G, Soto D, Mamone L, Bagnato C, Pagnussat LA, Beligni MV (2022) Expression of Chlamydomonas reinhardtii chloroplast diacylglycerol acyltransferase 3 is induced by light in concert with triacylglycerol accumulation. Plant J **110:** 262-276

Chen G, Harwood JL, Lemieux MJ, Stone SJ, Weselake RJ (2022) Acyl-CoA:diacylglycerol acyltransferase: Properties, physiological roles, metabolic engineering and intentional control. Progress in Lipid Research **88:** 101181

Chen G, Woodfield H, Pan X, Harwood J, Weselake R (2015) Acyl-Trafficking During Plant Oil Accumulation. Lipids **50:** 1057-1068

Chen GQ (2017) Castor and Lesquerella Oils: Production, Composition and Uses. Nova Science Publishers, Inc.

Chen GQ, Lin J-T, Lu C (2011) Hydroxy fatty acid synthesis and lipid gene expression during seed development in Lesquerella fendleri. Industrial Crops and Products **34:** 1286-1292

Coleman RA (2019) It takes a village: channeling fatty acid metabolism and triacylglycerol formation via protein interactomes. Journal of Lipid Research **60:** 490-497

Correa SM, Fernie AR, Nikoloski Z, Brotman Y (2020) Towards model-driven characterization and manipulation of plant lipid metabolism. Prog Lipid Res **80:** 101051

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. Proceedings of the National Academy of Sciences of the United States of America **97:** 6487-6492

Dahmani I, Qin K, Zhang Y, Fernie AR (2023) The formation and function of plant metabolons. The Plant Journal **114:** 1080-1092

Dastmalchi M, Facchini PJ (2016) Plant metabolons assembled on demand. Science **354:** 829-830

Demski K, Jeppson S, Lager I, Misztak A, Jasieniecka-Gazarkiewicz K, Waleron M, Stymne S, Banas A (2019) Isoforms of Acyl-CoA:Diacylglycerol Acyltransferase-2 Differ Substantially in Their Specificities Towards Erucic Acid. Plant Physiology**:** pp.01129.02019

Demski K, Jeppson S, Stymne S, Lager I (2021) Camelina sativa phosphatidylcholine:diacylglycerol cholinephosphotransferase-catalyzed interconversion does not discriminate between substrates. Lipids **56:** 591-602

Demski K, Jeppson S, Stymne S, Lager I (2022) Phosphatidylcholine:diacylglycerol cholinephosphotransferase's unique regulation of castor bean oil quality. Plant Physiol **189:** 2001-2014

Dueber JE, Wu GC, Malmirchegini GR, Moon TS, Petzold CJ, Ullal AV, Prather KL, Keasling JD (2009) Synthetic protein scaffolds provide modular control over metabolic flux. Nature biotechnology **27:** 753-759

Eastmond PJ (2006) SUGAR-DEPENDENT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating Arabidopsis seeds. Plant Cell **18:** 665-675

Fernandez DE, Staehelin LA (1987) Does gibberellic acid induce the transfer of lipase from protein bodies to lipid bodies in barley aleurone cells? Plant Physiology **85:** 487-496

Garces R, Sarmiento C, Mancha M (1994) Oleate from triacylglycerols is desaturated in cold-induced developing sunflower (Helianthus annuus L.) seeds. Planta **193:** 473-477

Gidda SK, Shockey JM, Falcone M, Kim PK, Rothstein SJ, Andrews DW, Dyer JM, Mullen RT (2011) Hydrophobic-Domain-Dependent Protein–Protein Interactions Mediate the Localization of GPAT Enzymes to ER Subdomains. Traffic **12:** 452-472

Gląb B, Beganovic M, Anaokar S, Hao M-S, Rasmusson A, Patton-Vogt J, Banaś A, Stymne S, Lager I (2016) Cloning of glycerophosphocholine acyltransferase (GPCAT) from fungi and plants; a novel enzyme in phosphatidylcholine synthesis. Journal of Biological Chemistry **291:** 25066-25076

Greenwood BL, Luo Z, Ahmed T, Huang D, Stuart DT (2023) Saccharomyces cerevisiae Δ9-desaturase Ole1 forms a supercomplex with Slc1 and Dga1. Journal of Biological Chemistry **299**

Griffiths G, Harwood JL (1991) The regulation of triacylglycerol biosynthesis in cocoa (Theobroma-cacao) L. Planta **184:** 279-284

Griffiths G, Stymne S, Stobart AK (1988) Phosphatidylcholine and its relationship to triacylglycerol biosynthesis in oil-tissues. Phytochemistry **27:** 2089-2093

Guan R, Lager I, Li X, Stymne S, Zhu L-H (2014) Bottlenecks in erucic acid accumulation in genetically engineered ultrahigh erucic acid Crambe abyssinica. Plant Biotechnology Journal **12:** 193-203

Gunstone FD, Harwood JL, Dijkstra AJ (2007) The lipid handbook with CD-ROM. CRC Press

Gutensohn M, Hartzell E, Dudareva N (2022) Another level of complex-ity: The role of metabolic channeling and metabolons in plant terpenoid metabolism. Frontiers in Plant Science **13:** 954083

Han J, Wang H, Kanagarajan S, Hao M, Lundgren A, Brodelius PE (2016) Promoting artemisinin biosynthesis in Artemisia annua plants by substrate channeling. Molecular Plant **9:** 946-948

Haslam TM, Kunst L (2013) Extending the story of very-long-chain fatty acid elongation. Plant Science **210:** 93-107

Hayes DG, Kleiman R (1996) A detailed triglyceride analysis ofLesquerella fendleri oil: Column chromatographic fractionation followed by supercritical fluid chromatography. Journal of the American Oil Chemists' Society **73:** 267-269

Hernández ML, Whitehead L, He Z, Gazda V, Gilday A, Kozhevnikova E, Vaistij FE, Larson TR, Graham IA (2012) A Cytosolic Acyltransferase Contributes to Triacylglycerol Synthesis in Sucrose-Rescued Arabidopsis Seed Oil Catabolism Mutants. Plant Physiology **160:** 215-225

Horn PJ, Liu J, Cocuron JC, McGlew K, Thrower NA, Larson M, Lu C, Alonso AP, Ohlrogge J (2016) Identification of multiple lipid genes with modifications in expression and sequence associated with the evolution of hydroxy fatty acid accumulation in Physaria fendleri. Plant J **86:** 322-348

Hu Z, Ren Z, Lu C (2012) The Phosphatidylcholine Diacylglycerol Cholinephosphotransferase Is Required for Efficient Hydroxy Fatty Acid Accumulation in Transgenic Arabidopsis. Plant Physiology **158:** 1944-1954

Jarvis BA, Romsdahl TB, McGinn MG, Nazarenus TJ, Cahoon EB, Chapman KD, Sedbrook JC (2021) CRISPR/Cas9-Induced fad2 and rod1 Mutations Stacked With fae1 Confer High Oleic Acid Seed Oil in Pennycress (Thlaspi arvense L.). Frontiers in Plant Science **12:** 652319

Jasieniecka-Gazarkiewicz K, Demski K, Lager I, Stymne S, Banas A (2016) Possible Role of Different Yeast and Plant Lysophospholipid:Acyl-CoA Acyltransferases (LPLATs) in Acyl Remodelling of Phospholipids. Lipids **51:** 15-23

Jeppson S, Demski K, Carlsson AS, Zhu L-H, Banaś A, Stymne S, Lager I (2019) Crambe hispanica Subsp. abyssinica Diacylglycerol Acyltransferase Specificities Towards Diacylglycerols and Acyl-CoA Reveal Combinatorial Effects That Greatly Affect Enzymatic Activity and Specificity. Frontiers in Plant Science **10:** 1442

Kalinger RS, Rowland O (2023) Determinants of substrate specificity in a catalytically diverse family of acyl-ACP thioesterases from plants. BMC Plant Biology **23:** 1

Kanai M, Yamada T, Hayashi M, Mano S, Nishimura M (2019) Soybean (Glycine max L.) triacylglycerol lipase GmSDP1 regulates the quality and quantity of seed oil. Scientific Reports **9:** 8924

Karki N, Johnson BS, Bates PD (2019) Metabolically Distinct Pools of Phosphatidylcholine Are Involved in Trafficking of Fatty Acids out of and into the Chloroplast for Membrane Production. Plant Cell **31:** 2768-2788

Kelly AA, Shaw E, Powers SJ, Kurup S, Eastmond PJ (2013) Suppression of the SUGAR-DEPENDENT1 triacylglycerol lipase family during seed development enhances oil yield in oilseed rape (Brassica napus L.). Plant Biotechnology Journal **11:** 355-361

Kim HJ, Silva JE, Iskandarov U, Andersson M, Cahoon RE, Mockaitis K, Cahoon EB (2015) Structurally divergent lysophosphatidic acid acyltransferases with high selectivity for saturated medium chain fatty acids from Cuphea seeds. Plant J **84:** 1021-1033

Kim HU, Li YB, Huang AHC (2005) Ubiquitous and endoplasmic reticulum-located lysophosphatidyl acyltransferase, LPAT2, is essential for female but not male gametophyte development in Arabidopsis. Plant Cell **17:** 1073-1089

Kim HU, Park M-E, Lee K-R, Suh MC, Chen GQ (2020) Variant castor lysophosphatidic acid acyltransferases acylate ricinoleic acid in seed oil. Industrial Crops and Products **150:** 112245

Kim MJ, Yang SW, Mao HZ, Veena SP, Yin JL, Chua NH (2014) Gene silencing of Sugar-dependent 1 (JcSDP1), encoding a patatin-domain triacylglycerol lipase, enhances seed oil accumulation in Jatropha curcas. Biotechnol Biofuels **7:** 36

Koo AJK, Ohlrogge JB, Pollard M (2004) On the export of fatty acids from the chloroplast. Journal of Biological Chemistry **279:** 16101-16110

Kretzschmar FK, Doner NM, Krawczyk HE, Scholz P, Schmitt K, Valerius O, Braus GH, Mullen RT, Ischebeck T (2020) Identification of Low-Abundance Lipid Droplet Proteins in Seeds and Seedlings. Plant Physiology **182:** 1326-1345

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

Lager I, Jeppson S, Gippert AL, Feussner I, Stymne S, Marmon S (2020) Acyltransferases Regulate Oil Quality in Camelina sativa Through Both Acyl Donor and Acyl Acceptor Specificities. Front Plant Sci **11:** 1144

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. Journal of Biological Chemistry **288:** 36902-36914

Lands WEM (1965) Lipid metabolism. Annual Review of Biochemistry **34:** 313-&

Lee HG, Seo PJ (2019) Interaction of DGAT1 and PDAT1 to enhance TAG assembly in Arabidopsis. Plant Signaling & Behavior **14:** 1554467

Lee J, Welti R, Schapaugh WT, Trick HN (2011) Phospholipid and triacylglycerol profiles modified by PLD suppression in soybean seed. Plant Biotechnology Journal **9:** 359-372

Lee K-R, Chen G, Kim H (2015) Current progress towards the metabolic engineering of plant seed oil for hydroxy fatty acids production. Plant Cell Reports**:** 1-13

Lee K-R, Kim YM, Yeo Y, Kim S, Suh MC (2022) Camelina cytosol-localized diacylglycerol acyltransferase 3 contributes to the accumulation of seed storage oils. Industrial Crops and Products **189:** 115808

Levine T, Rabouille C (2005) Endoplasmic reticulum: one continuous network compartmentalized by extrinsic cues. Current opinion in cell biology **17:** 362-368

Li-Beisson Y, Shorrosh B, Beisson F, Andersson MX, Arondel V, Bates PD, Baud S, Bird D, Debono A, Durrett TP, Franke RB, Graham IA, Katayama K, Kelly AA, Larson T, Markham JE, Miquel M, Molina I, Nishida I, Rowland O, Samuels L, Schmid KM, Wada H, Welti R, Xu C, Zallot R, Ohlrogge J (2013) Acyl-lipid metabolism. Arabidopsis Book **11:** e0161

Li H, Zhou R, Liu P, Yang M, Xin D, Liu C, Zhang Z, Wu X, Chen Q, Zhao Y (2023) Design of high-monounsaturated fatty acid soybean seed oil using GmPDCTs knockout via a CRISPR-Cas9 system. Plant Biotechnol J **21:** 1317-1319

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

Lu C, Xin Z, Ren Z, Miquel M, Browse J (2009) An enzyme regulating triacylglycerol composition is encoded by the ROD1 gene of Arabidopsis. Proceedings of the National Academy of Sciences **106:** 18837-18842

Lu CF, Fulda M, Wallis JG, Browse J (2006) A high-throughput screen for genes from castor that boost hydroxy fatty acid accumulation in seed oils of transgenic Arabidopsis. The Plant Journal **45:** 847-856

Lunn D, Le A, Wallis JG, Browse J (2020) Castor LPCAT and PDAT1A Act in Concert to Promote Transacylation of Hydroxy-Fatty Acid onto Triacylglycerol. Plant Physiol **184:** 709-719

Lunn D, Wallis JG, Browse J (2019) Tri-Hydroxy-Triacylglycerol Is Efficiently Produced by Position-Specific Castor Acyltransferases. Plant Physiology **179:** 1050-1063

Maisonneuve S, Bessoule JJ, Lessire R, Delseny M, Roscoe TJ (2010) Expression of rapeseed microsomal lysophosphatidic acid acyltransferase isozymes enhances seed oil content in Arabidopsis. Plant Physiol **152**

Marmon S, Sturtevant D, Herrfurth C, Chapman K, Stymne S, Feussner I (2017) Two Acyltransferases Contribute Differently to Linolenic Acid Levels in Seed Oil. Plant Physiol **173:** 2081-2095

McGlew K, Shaw V, Zhang M, Kim R, Yang W, Shorrosh B, Suh M, Ohlrogge J (2015) An annotated database of Arabidopsis mutants of acyl lipid metabolism. Plant Cell Reports **34:** 519-532

Moellering ER, Muthan B, Benning C (2010) Freezing Tolerance in Plants Requires Lipid Remodeling at the Outer Chloroplast Membrane. Science **330:** 226-228

Napier JA, Haslam RP, Beaudoin F, Cahoon EB (2014) Understanding and manipulating plant lipid composition: Metabolic engineering leads the way. Current Opinion in Plant Biology **19:** 68-75

Neumann N, Harman M, Kuhlman A, Durrett TP Arabidopsis diacylglycerol acyltransferase1 mutants require fatty acid desaturation for normal seed development. The Plant Journal **n/a**

Ohlrogge J, Browse J (1995) Lipid Biosynthesis. Plant Cell **7:** 957-970

Ohlrogge J, Thrower N, Mhaske V, Stymne S, Baxter M, Yang W, Liu J, Shaw K, Shorrosh B, Zhang M, Wilkerson C, Matthaus B (2018) PlantFAdb: a resource for exploring hundreds of plant fatty acid structures synthesized by thousands of plants and their phylogenetic relationships. Plant J **96:** 1299-1308

Pan X, Chen G, Kazachkov M, Greer MS, Caldo KM, Zou J, Weselake RJ (2015) In Vivo and in Vitro Evidence for Biochemical Coupling of Reactions Catalyzed by Lysophosphatidylcholine Acyltransferase and Diacylglycerol Acyltransferase. J Biol Chem **290:** 18068-18078

Pan X, Siloto RMP, 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. Journal of Biological Chemistry **288:** 24173-24188

Parchuri P, Bhandari S, Azeez A, Chen G, Johnson K, Shockey J, Smertenko A, Bates PD (2024) Identification of triacylglycerol remodeling mechanism to synthesize unusual fatty acid containing oils. Nature Communications **15:** 3547

Parchuri P, Pappanoor A, Naeem A, Durrett TP, Welti R, R.V S (2022) Lipidome analysis and characterization of Buglossoides arvensis acyltransferases that incorporate polyunsaturated fatty acids into triacylglycerols. Plant Science **324:** 111445

Park ME, Kim HU (2024) PDAT1 genome editing reduces hydroxy fatty acid production in transgenic Arabidopsis. BMB Rep **57:** 86-91

Pollard M, Shachar-Hill Y (2022) Kinetic complexities of triacylglycerol accumulation in developing embryos from Camelina sativa provide evidence for multiple biosynthetic systems. J Biol Chem **298:** 101396

Regmi A, Shockey J, Kotapati HK, Bates PD (2020) Oil-Producing Metabolons Containing DGAT1 Use Separate Substrate Pools from those Containing DGAT2 or PDAT. Plant Physiology **184:** 720-737

Roughan PG, Ohlrogge JB (1996) Evidence that isolated chloroplasts contain an integrated lipid-synthesizing assembly that channels acetate into long-chain fatty acids. Plant physiology **110:** 1239-1247

Sadre R, Kuo P, Chen J, Yang Y, Banerjee A, Benning C, Hamberger B (2019) Cytosolic lipid droplets as engineered organelles for production and accumulation of terpenoid biomaterials in leaves. Nature Communications **10:** 853

Sah SK, Fan J, Blanford J, Shanklin J, Xu C (2024) Physiological Functions of Phospholipid:Diacylglycerol Acyltransferases. Plant Cell Physiol **65:** 863-871

Sarmiento C, Garces R, Mancha M (1998) Oleate desaturation and acyl turnover in sunflower (Helianthus annuus L.) seed lipids during rapid temperature adaptation. Planta **205:** 595-600

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 Physiology **129:** 1700-1709

Shanklin J, Guy JE, Mishra G, Lindqvist Y (2009) Desaturases: Emerging Models for Understanding Functional Diversification of Diiron-containing Enzymes *. Journal of Biological Chemistry **284:** 18559-18563

Shockey J, Browse J (2011) Genome-level and biochemical diversity of the acyl-activating enzyme superfamily in plants. The Plant Journal **66:** 143-160

Shockey J, Lager I, Stymne S, Kotapati HK, Sheffield J, Mason C, Bates PD (2019) Specialized lysophosphatidic acid acyltransferases contribute to unusual fatty acid accumulation in exotic Euphorbiaceae seed oils. Planta **249:** 1285-1299

Shockey J, Regmi A, Cotton K, Adhikari N, Browse J, Bates PD (2016) Identification of Arabidopsis GPAT9 (At5g60620) as an Essential Gene Involved in Triacylglycerol Biosynthesis. Plant Physiology **170:** 163-179

Shockey JM, Gidda SK, Chapital DC, Kuan JC, Dhanoa PK, Bland JM, Rothstein SJ, Mullen RT, Dyer JM (2006) Tung tree DGAT1 and DGAT2 have nonredundant functions in triacylglycerol biosynthesis and are localized to different subdomains of the endoplasmic reticulum. Plant Cell **18:** 2294-2313

Shomo ZD, Mahboub S, Vanviratikul H, McCormick M, Tulyananda T, Roston RL, Warakanont J (2024) All members of the Arabidopsis DGAT and PDAT acyltransferase families operate during high and low temperatures. Plant Physiology **195:** 685-697

Singer SD, Chen G, Mietkiewska E, Tomasi P, Jayawardhane K, Dyer JM, Weselake RJ (2016) Arabidopsis GPAT9 contributes to synthesis of intracellular glycerolipids but not surface lipids. J Exp Bot **67:** 4627-4638

Slack CR, Roughan PG, Balasingham N (1978) Labeling of glycerolipids in cotyledons of developing oilseeds by [1-C-14]acetate and [2-H-3]glycerol. Biochemical Journal **170:** 421-433

Staehelin LA (1997) The plant ER: a dynamic organelle composed of a large number of discrete functional domains. The Plant Journal **11:** 1151-1165

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 Physiology **135:** 1324-1335

Stahl U, Stalberg K, Stymne S, Ronne H (2008) A family of eukaryotic lysophospholipid acyltransferases with broad specificity. FEBS Lett **582:** 305-309

Tian Y, Lv X, Xie G, Wang L, Dai T, Qin X, Chen F, Xu Y (2019) FAX2 Mediates Fatty Acid Export from Plastids in Developing Arabidopsis Seeds. Plant Cell Physiol **60:** 2231-2242

Troncoso-Ponce MA, Kilaru A, Cao X, Durrett TP, Fan J, Jensen JK, Thrower NA, Pauly M, Wilkerson C, Ohlrogge JB (2011) Comparative deep transcriptional profiling of four developing oilseeds. The Plant Journal **68:** 1014-1027

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 Physiology **155:** 683-693

Vogel G, Browse J (1996) Cholinephosphotransferase and diacylglycerol acyltransferase - Substrate specificities at a key branch point in seed lipid metabolism. Plant Physiology **110:** 923-931

Wang J, Xu Y, Holic R, Yu X, Singer SD, Chen G (2021) Improving the Production of Punicic Acid in Baker's Yeast by Engineering Genes in Acyl Channeling Processes and Adjusting Precursor Supply. Journal of Agricultural and Food Chemistry **69:** 9616-9624

Wang L, Shen W, Kazachkov M, Chen G, Chen Q, Carlsson AS, Stymne S, Weselake RJ, Zou J (2012) Metabolic Interactions between the Lands Cycle and the Kennedy Pathway of Glycerolipid Synthesis in Arabidopsis Developing Seeds. The Plant Cell Online **24:** 4652-4669

Weiss SB, Kennedy EP, Kiyasu JY (1960) Enzymatic synthesis of triglycerides. Journal of Biological Chemistry **235:** 40-44

Wickramarathna AD, Siloto RM, Mietkiewska E, Singer SD, Pan X, Weselake RJ (2015) Heterologous expression of flax PHOSPHOLIPID:DIACYLGLYCEROL CHOLINEPHOSPHOTRANSFERASE (PDCT) increases polyunsaturated fatty acid content in yeast and Arabidopsis seeds. BMC Biotechnol **15:** 63

Williams JP, Imperial V, Khan MU, Hodson JN (2000) The role of phosphatidylcholine in fatty acid exchange and desaturation in Brassica napus L. leaves. Biochemical Journal **349:** 127-133

Xu J, Carlsson A, Francis T, Zhang M, Hoffmann T, Giblin M, Taylor D (2012) Triacylglycerol synthesis by PDAT1 in the absence of DGAT1 activity is dependent on re-acylation of LPC by LPCAT2. Bmc Plant Biology **12:** 4

Xu Y, Caldo KMP, Jayawardhane K, Ozga JA, Weselake RJ, Chen GQ (2019) A transferase interactome that may facilitate channeling of polyunsaturated fatty acid moieties from phosphatidylcholine to triacylglycerol. Journal of Biological Chemistry **294:** 14838-14844

Xu Y, Caldo KMP, Pal-Nath D, Ozga J, Lemieux MJ, Weselake RJ, Chen G (2018) Properties and Biotechnological Applications of Acyl-CoA:diacylglycerol Acyltransferase and Phospholipid:diacylglycerol Acyltransferase from Terrestrial Plants and Microalgae. Lipids **53:** 663-688

Xu Y, Singer SD, Chen G (2023) Protein interactomes for plant lipid biosynthesis and their biotechnological applications. Plant Biotechnology Journal **21:** 1734-1744

Yang W, Simpson JP, Li-Beisson Y, Beisson F, Pollard M, Ohlrogge JB (2012) A Land-Plant-Specific Glycerol-3-Phosphate Acyltransferase Family in Arabidopsis: Substrate Specificity, sn-2 Preference, and Evolution. Plant Physiology **160:** 638-652

Yang W, Wang G, Li J, Bates PD, Wang X, Allen DK (2017) Phospholipase Dzeta Enhances Diacylglycerol Flux into Triacylglycerol. Plant Physiol **174:** 110-123

Yu X-H, Cai Y, Chai J, Schwender J, Shanklin J (2019) Expression of a Lychee PHOSPHATIDYLCHOLINE: DIACYLGLYCEROL CHOLINEPHOSPHOTRANSFERASE with an Escherichia coli CYCLOPROPANE SYNTHASE enhances cyclopropane fatty acid accumulation in Camelina Seeds. Plant physiology **180:** 1351-1361

Yuan L, Mao X, Zhao K, Ji X, Ji C, Xue J, Li R (2017) Characterisation of phospholipid: diacylglycerol acyltransferases (PDATs) from Camelina sativa and their roles in stress responses. Biology Open **6:** 1024-1034

Zang X, Geng X, Ma L, Wang N, Pei W, Wu M, Zhang J, Yu J (2019) A genome-wide analysis of the phospholipid: diacylglycerol acyltransferase gene family in Gossypium. BMC Genomics **20:** 402

Zhang K, He J, Yin Y, Chen K, Deng X, Yu P, Li H, Zhao W, Yan S, Li M (2022) Lysophosphatidic acid acyltransferase 2 and 5 commonly, but differently, promote seed oil accumulation in Brassica napus. Biotechnology for Biofuels and Bioproducts **15:** 83

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

Zhou XR, Bhandari S, Johnson BS, Kotapati HK, Allen DK, Vanhercke T, Bates PD (2020) Reorganization of Acyl Flux through the Lipid Metabolic Network in Oil-Accumulating Tobacco Leaves. Plant Physiol **182:** 739-755

Zhu L-H, Krens F, Smith MA, Li X, Qi W, van Loo EN, Iven T, Feussner I, Nazarenus TJ, Huai D, Taylor DC, Zhou X-R, Green AG, Shockey J, Klasson KT, Mullen RT, Huang B, Dyer JM, Cahoon EB (2016) Dedicated Industrial Oilseed Crops as Metabolic Engineering Platforms for Sustainable Industrial Feedstock Production. Scientific Reports **6:** 22181

Zoni V, Khaddaj R, Campomanes P, Thiam AR, Schneiter R, Vanni S (2021) Pre-existing bilayer stresses modulate triglyceride accumulation in the ER versus lipid droplets. Elife **10:** e62886