Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Plant Biology

Biochemical pathways in seed oil synthesis

Philip D Bates[1,4], Sten Stymne[2] and John Ohlrogge[3]

Oil produced in plant seeds is utilized as a major source of calories for human nutrition, as feedstocks for non-food uses such as soaps and polymers, and can serve as a high-energy biofuel. The biochemical pathways leading to oil (triacylglycerol) synthesis in seeds involve multiple subcellular organelles, requiring extensive lipid trafficking. Phosphatidylcholine plays a central role in these pathways as a substrate for acyl modifications and likely as a carrier for the trafficking of acyl groups between organelles and membrane subdomains. Although much has been clarified regarding the enzymes and pathways responsible for acyl-group flux, there are still major gaps in our understanding. These include the identity of several key enzymes, how flux between alternative pathways is controlled and the specialized cell biology leading to biogenesis of oil bodies that store up to 80% of carbon in seeds.

Addresses
[1] Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA
[2] Department of Plant Breeding, Swedish University of Agricultural Sciences, S 230 53 Alnarp, Sweden
[3] Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA

Corresponding author: Bates, Philip D (phil_bates@wsu.edu)

[4] Current address: Department of Chemistry and Biochemistry, The University of Southern Mississippi, Hattiesburg, MS 39406, USA.

Current Opinion in Plant Biology 2013, **16**:358–364

This review comes from a themed issue on **Physiology and metabolism**

Edited by **John Browse** and **Edward Farmer**

For a complete overview see the Issue and the Editorial

Available online 23rd March 2013

1369-5266

http://dx.doi.org/10.1016/j.pbi.2013.02.015

Introduction

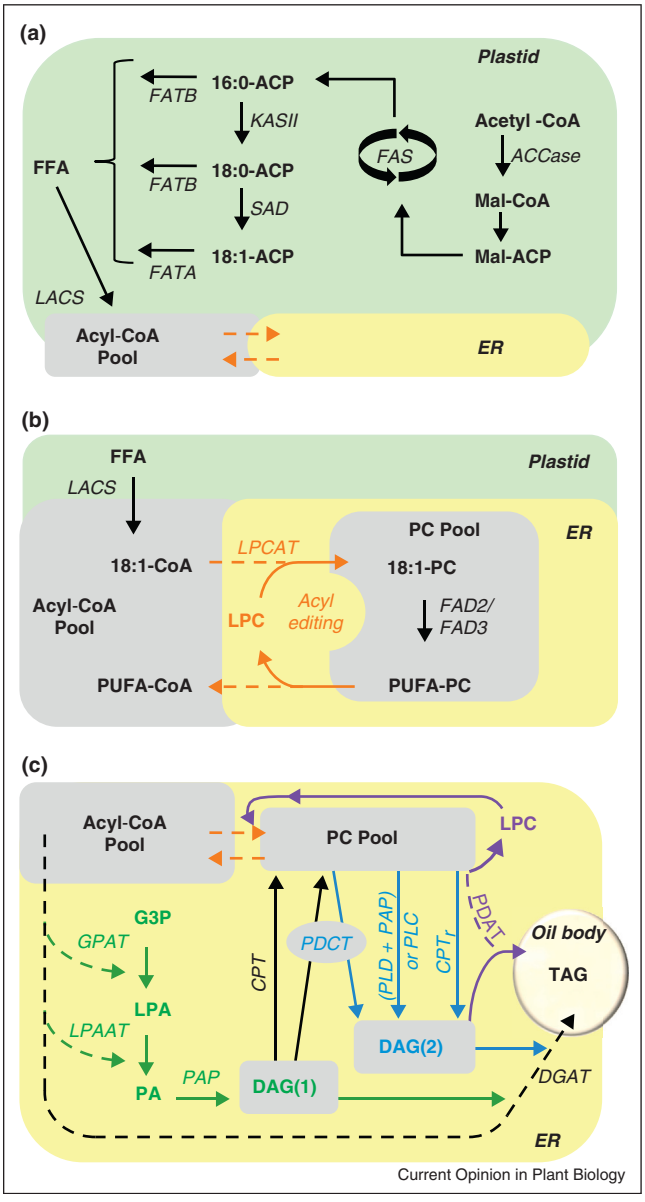
Oils are the most energy-dense plant reserves, supplying humans with much of the calories and essential fatty acids required in our diet. Because they are composed of long chain hydrocarbons, plant oils can also replace petroleum in many applications, including as feedstocks for the chemical industry and as biofuels. The majority of the plant oils we consume are accumulated in seeds. World production from oilseed crops was approximately 100 billion kg of oil in 2011 [1] with a value near US$120 billion [2]. Vegetable oil consumption is expected to double by 2040 [3]. The important uses, high value and growing demand are a major reason why oil biosynthesis in seeds has been extensively studied. A recent review [4••] and website (aralip.plantbiology.msu.edu) provide details on the very large number of genes involved in Arabidopsis oil synthesis and lipid metabolism.

Seed oil biosynthesis synthesis begins in the plastid

Fatty acid (FA) synthesis is localized to plastids (Figure 1a), whereas assembly of the TAG molecule occurs outside the plastid and may be associated with both the endoplasmic reticulum (ER) and the oil body (Figure 1b,c) [5–7]. In most seeds, carbon is delivered to FA synthesis via glycolysis with hexose and/or triose as the predominant carbohydrate entering the plastid. However, green seeds can also use light to supply NADPH and ATP, which allows a 'bypass' of glycolysis via ribulose-1,5-bisphosphate carboxylase activity and pentose phosphate enzymes. This alternative pathway is more carbon efficient, resulting in 20% more acetyl-CoA available for oil synthesis, and also does not require reductant supply from the oxidative pentose phosphate pathway [8•]. The plastid FA synthesis pathway determines the chain length (up to 18 carbons) and the level of saturated FAs in seed oils. The first committed enzyme in the pathway is acetyl-CoA carboxylase (ACCase). As in yeast, animals and bacteria, plant ACCase is highly regulated and is a key control point over the flux of carbon into FAs [9]. In addition to control by phosphorylation, redox status and PII interactions [10•], feedback on ACCase by 18:1-ACP has recently been described [11••]. Assembly of FAs occurs on acyl carrier protein (ACP) via a cycle of 4 reactions that elongate the acyl chain by 2 carbons each cycle. After 7 cycles, the saturated 16 carbon acyl-ACP can either be hydrolyzed by the FATB acyl-ACP thioesterase or further elongated by KASII to 18:0-ACP, which is then desaturated to 18:1-ACP and hydrolyzed by the FATA thioesterase [4••]. The resulting 16:0 and 18:1 free acids are the main products of plastid FA synthesis, and their relative proportions are determined by the activities of FATA, FATB, 18:0-ACP desaturase (SAD) and KASII (Figure 1a). Transgenics and mutants have demonstrated that seed FA chain length and saturation can be altered by manipulation of any of these four enzymes [12]. For example, a dramatic demonstration of the control of chain length is the production of 60% lauric acid (12:0) in transgenic *B. napus* that expresses a FATB with specificity for this FA [13].

The transcription factor WRI1 [14] controls the expression of at least 15 enzymes including pyruvate dehydrogenase, ACCase and members of the FA synthesis and glycolytic pathways [15••]. Thus, WRI1 expression is

Figure 1



Overview of major reactions involved in fatty acid and triacylglycerol synthesis. **(a)** Plastid fatty acid synthesis; **(b)** Acyl editing and **(c)** TAG synthesis. Acyl transfer reactions are dashed lines. Green lines are de novo TAG synthesis, blue lines are PC-derived DAG synthesis, orange lines are acyl editing, and purple represents phospholipid:diacylglycerol acyltransferase (PDAT). DAG(1) is de novo synthesized DAG and DAG(2) is PC-derived DAG. *Abbreviations*: substrates are in bold: **ACP**, acyl carrier protein; **DAG**, diacylglycerol; **FFA**, free fatty acid; **G3P**, glycerol-3-phosphate; **LPA**, lyso-phosphatidic acid; **LPC**, lyso-phosphatidylcholine; **Mal**, malonate; **PA**, phosphatidic acid; **PC**, phosphatidylcholine; **PUFA**, polyunsaturated fatty acids; **TAG**, triacylglycerol. Enzymatic reactions are in italics: *ACCase*, acetyl-CoA carboxylase; *CPT*, CDP-choline:DAG cholinephosphotransferase; *DGAT*, acyl-CoA:DAG acyltransferase; *FAD*, fatty acid desaturase; *FAS*, fatty acid synthase; *FATA*, acyl-ACP thioesterase A; *FATB*, acyl-ACP thioesterase B; *GPAT*, acyl-CoA:G3P acyltransferase; *KASII*, ketoacyl-ACP synthase II; *LACS*, long chain acyl-CoA synthetase; *LPAAT*, acyl-CoA:LPA acyltransferase; *LPCAT*, acyl-CoA:LPC acyltransferase; *PAP*, PA phosphatase; *PDCT*, PC:DAG cholinephosphotransferase; *PLC*, phospholipase C; *PLD*, phospholipase D; *SAD*, Stearoyl-ACP desaturase.

pivotal in directing the carbon flux that enters the seed toward the synthesis of FAs. Oil content is reduced to the extent of 80% in *wri1* mutants, and overexpression of WRI1 increases maize embryo oil content by over 30% and oil yield per hectare by over 20% [16•]. So far there is no evidence that WRI1 controls expression of any of the acyltransferases or other 'downstream' enzymes involved in TAG assembly [81]. An intriguing observation is that the temporal patterns of gene expression for the enzymes of FA synthesis and for TAG assembly are very different during seed development. There is a general lack of understanding of the regulation of gene expression and enzyme activity for these later steps in oil biosynthesis [17•,18•].

How do acyl chains move from the plastid to the ER?

After FA synthesis, the free FA products of the FATA/B thioesterases are exported from the plastid. Although there is evidence for a channeled pathway through the plastid envelope [19] a major unknown is whether there are specific transporters for this process. After export, it is presumed that long-chain acyl-CoA synthetase (LACS) on the outer plastid envelope forms the acyl-CoA that is the substrate for glycerolipid assembly (Figure 1a,b). However, it is puzzling that mutants in LACS9, the major plastid LACS, have no seed oil phenotype [20] suggesting that other members of the LACS family may be able to compensate [21]. Without more evidence, it cannot be ruled out that another type of enzyme, or reaction may activate FAs after their export from plastids. Regardless, it is likely that esterification of newly synthesized FAs to PC via the acyl-editing cycle can occur at the plastid envelope via acyl-CoA:lysophosphatidylcholine acyltransferase (LPCAT) [22] (Figure 1b). In this scenario we speculate that PC may act as a carrier of FAs from plastids to the ER, perhaps via direct connections between these organelles [23]. Inter-membrane transport of lipids can occur much more rapidly than diffusion through the cytosol via soluble carriers, and thus may provide an efficient plastid-ER acyl transfer mechanism. Recently, an ABC transporter has been proposed to be involved in the delivery of FA to the ER for oil synthesis [24]. However, both microarray and RNAseq data indicate that this transporter's expression in seeds is much lower than other lipid-related ABC transporters, and the radiolabeling evidence presented is uncertain because incubations were performed in the dark and without a supply of carbon. Thus, how this transporter modifies seed oil biosynthesis remains to be determined.

Simple and complex pathways of TAG assembly

The de novo assembly of TAG from glycerol-3-phosphate and acyl-CoAs (also known as the Kennedy pathway)

involves only four enzymatic steps: first, two acylations of G3P by *sn*-1 glycerol-3-phosphate acyltransferase (GPAT) and lysophosphatidic acid acyltransferase (LPAAT), followed by phosphatidic acid phosphatase (PAP), and a third acylation by diacylglycerol acyltransferase (DGAT) (Figure 1c, green arrows). The pathway was first characterized in animals over 50 years ago [25,26] and soon after in plants [27]. Since that time biochemical, molecular genetic and metabolic flux analyses of plants have defined an additional more complex pathway in which the membrane lipid phosphatidylcholine (PC) is a central intermediate in the flux of FAs (Figure 1b) or diacylglycerol (Figure 1c), or both substrates into TAG [28]. The FA esterified to the *sn*-2 position of PC is the major site of ER localized FA modification (e.g. desaturation, hydroxylation, etc. [29,30]). Therefore, acyl fluxes into and out of PC are crucial for the production of TAG containing high levels of PC-modified FAs, such as the polyunsaturated FAs (PUFA) that are essential for human health [31]. The pathway of TAG synthesis appears to differ between plant species ranging from just a simple Kennedy pathway to a pathway where >90% of the FAs within the seed fluxes through PC before incorporation into TAG [28].

Genes involved in *de novo* triacylglycerol assembly (Kennedy pathway)

Surprisingly, the *sn*-1 GPAT that initiates TAG assembly is not yet certain, but it may be the ER localized 'GPAT9' in Arabidopsis [32]. *GPAT9* is a homolog of mammalian and yeast GPATs involved in TAG production [33,34]. Arabidopsis also expresses an eight-member GPAT family (*GPAT1-8*) that is only found in land plants; however at least five of these encode *sn-2* acyltransferases that are involved in synthesis of extracellular lipids (e.g. cutin, suberin) and almost certainly do not participate in membrane lipid or TAG synthesis [35]. Genes that encode LPAAT, the second step in *de novo* TAG assembly have been identified from several plants [36,37]. However, the isozyme(s) of PAP responsible for the majority of de novo synthesized diacylglycerol (DAG) (Figure 1c, DAG(1)) synthesis have yet to be identified. Arabidopsis contains at least 11 genes annotated as potential PAPs (aralip.plantbiology.msu.edu). A double knockout of two genes (*PAH1* and *PAH2*) only resulted in a 15% reduction in seed FA levels [38] indicating other PAP isozymes must also be contributing to TAG synthesis. For the last step of the Kennedy pathway, plants possess multiple unrelated DGAT enzymes, which appear to have differential involvement during TAG accumulation in oil producing tissues of different plants [7]. DGAT1 and DGAT2 are related to DGATs involved in TAG synthesis in animals and fungi [7,39,40]. Genetic manipulation of DGAT1 has demonstrated it to be the major enzyme catalyzing TAG production in Arabidopsis [41–43]. The function of DGAT2 in Arabidopsis remains unclear [43] but in some plants such as *Ricinus communis* and *Vernicia fordii*, DGAT2 is more highly expressed than DGAT1 during seed maturation and DGAT2 appears to be the enzyme responsible for most TAG synthesis [44,45]. A distinct DGAT enzyme from *Euonymus alatus* is related to wax synthases, and utilizes acetyl-CoA rather than long chain acyl-CoAs to produce uniquely structured TAGs with an acetyl-group at the *sn*-3 hydroxyl of TAG [46]. Other enzymes with DGAT activity include a soluble DGAT3 first identified in developing peanut cotyledons [47] and that may have a role in TAG recycling within germinating Arabidopsis seedlings [48]. Two other Arabidopsis enzymes with DGAT activity have recently been identified but also do not appear to be involved in seed oil synthesis [49,50].

The central role of phosphatidylcholine in TAG synthesis

Three mechanisms allow the flux of FA through PC for eventual TAG synthesis. First, ''Acyl editing'' (Figure 1b, orange arrows) [51–53]. Second, direct transfer of a FA from PC to DAG producing TAG by the phospholipid:-diacylglycerol acyltransferase (PDAT; Figure 1c, purple arrows) [54]. Third, utilization of a PC-derived DAG as the substrate for TAG synthesis (Figure 1c, DAG(2), blue arrows) [28].

Acyl editing mechanisms

Acyl editing is a PC-deacylation and lysophosphatidylcholine (LPC)-reacylation cycle which exchanges the FA on PC with the acyl-CoA pool without net PC synthesis or degradation (Figure 1b, orange arrows). The acyl editing cycle allows: first, nascent FA from the plastid to be incorporated into PC while second, FAs that have been desaturated or otherwise modified on PC are released into the acyl-CoA pool where they can participate in the Kennedy pathway or other reactions. *In vitro* and *in vivo* metabolic analyses of acyl fluxes in/out of PC in multiple plant tissues [28,53] suggest that acyl editing may involve both the forward and reverse actions of LPCAT [55]. This mechanism is supported *in planta* by the inability of the Arabidopsis *lpcat1 lpcat2* double mutant to incorporate newly synthesized FA into PC through acyl editing in seeds [56••]. These results indicate that LPCATs are involved in at least the LPC-reacylation half of the acyl editing cycle. Other broad specificity lysophospholipid acyltransferases, such as lyso-phosphatidylethanolamine acyltransferases [57], cannot compensate for the loss of LPCAT acyl editing in Arabidopsis seeds [56••]. The molecular identity of the enzyme(s) responsible for the PC-deacylation half of the acyl editing cycle is a key unknown, and may involve the reverse activity of LPCAT [55,56••] and/or a phospholipase A [58].

A second recent analysis of the *lpcat1 lpcat2* double mutant indicated increased total PC synthesis and degradation without a change in steady-state PC levels but with an increase in steady state levels of LPC [59]. The acyl

editing cycle does not require net PC synthesis, thus the increase in PC synthesis-degradation in the *lpcat1 lpcat2* double mutant was unexpected. However, the accumulation of LPC due to the lack of LPCAT activity (for acyl editing (Figure 1b) or PDAT activity (Figure 1c)) may induce LPC turnover due to its inherent detergent properties on membranes, and thus require synthesis of new PC in a futile cycle in these mutant plants.

The involvement of PDAT in TAG synthesis

The relative flux of acyl chains onto the *sn-3* position of TAG through either the DGAT or PDAT activities is unclear in most oilseed plants. The *dgat1* mutant of Arabidopsis only reduces oil content by 20–30% [41,42] and the *pdat1* mutant has no impact on TAG accumulation [60]. These results can be explained by overlapping functions of DGAT and PDAT that allow PDAT to partially compensate for a DGAT knockout, and DGAT to fully compensate for a lack of PDAT. A double knockout is pollen lethal and seed-specific RNAi of PDAT in the *dgat1* mutant reduced oil 80% [43]. Within the Arabidopsis *dgat1* mutant background PDAT expression is upregulated [61], perhaps to achieve ~80% of wild-type TAG levels [41–43]. It is possible that in some oilseeds PDAT has a primary role in maintaining membrane homeostasis, as in yeast [62], whereas in other species PDAT can play a more major role in TAG production [82]. PDATs from Arabidopsis and other plants have been shown to have high activity with PC containing unusual FA [63–65], suggesting that PDAT may be involved in removal of damaged or unusual FA from the membrane and sequestering them in TAG.

PC-derived DAG production

In addition to the above mechanisms, acyl chains on PC can also be incorporated into the *sn*-1 and *sn-2* positions of TAG by remaining esterified to the glycerol backbone of PC until DAG is derived from PC for TAG synthesis. Three alternative enzymatic routes can generate DAG from PC (Figure 1c, blue arrows). First, phosphatidylcholine:diacylglycerol cholinephosphotransferase (PDCT) which exchanges phosphocholine between PC and DAG [66]; second, the reverse reaction of CDP-choline:-diacylglycerol cholinephosphotransferase (CPT) [67,68]; or third, a lipase-based mechanism utilizing phospholipase C, or phospholipase D plus PAP.

Acyl fluxes through PC may or may not determine TAG FA composition

Different plants likely utilize different combinations of the above pathways of acyl flux through PC to accumulate PC-modified FA in TAG. For instance, characterization of *pdct1* [66] and the *pdct1 lpcat1 lpcat2* triple mutant indicates that LPCAT-based acyl editing and DAG produced by PDCT together are responsible for at least 2/3 of the flux of PUFA from PC to TAG in wild-type Arabidopsis seeds [56••]. A residual PC-derived DAG flux utilizing phospholipase D, may be involved in the remaining flux of PUFA from PC to substrates for TAG synthesis in Arabidopsis [56••] and possibly higher levels in other plants such as that in soybean [69•]. However, not all FA's that flux through PC are further modified. High flux of acyl chains into PC via acyl editing is likely to occur in oilseeds that modify very little FA in TAG (e.g. coriander [70]; and Thunbergia seeds [71]). Analysis of acyl fluxes in developing soybean embryos estimated that ~90% of all FA in TAG fluxed through PC either by acyl editing or by the predominant use of PC-derived DAG for TAG synthesis (regardless of whether the FA was further desaturated or not) [51]. Similar conclusions have been made in Arabidopsis which also contains high rates of PC acyl editing [22,56••] and predominantly utilizes PC-derived DAG during TAG synthesis [72]. High rates of acyl editing without accompanying FA modification may imply the importance of acyl editing as a mechanism for the export of FA from the plastid [22] and PC-mediated 'FA trafficking' to specific sites of the ER for TAG biosynthesis, or for additional cellular processes such as maintenance of membrane homeostasis [52].

Multiple substrate pools and channeling

In vivo metabolic flux analysis of soybeans strongly suggests that de novo DAG (Figure 1c, DAG(1)) and PC-derived DAG (Figure 1c, DAG(2)) are distinct pools that do not intermix [51]. Additionally, many plants can produce unusual modified FA on PC, but these FAs do not accumulate in membrane lipids yet can accumulate to over 90% of the FA in TAG [73]. Together, these results suggest that plants may generate specific lipid substrate pools that can be channeled into TAG and kept separate from membrane lipid synthesis. How such channeling is achieved is unknown but may involve enzymes specific for unusual FAs [63,65,74–76] as well as separation of lipid biosynthetic activities to different subdomains of the ER membrane [44,77]. If there is spatial separation of Kennedy pathway de novo DAG synthesis from PC-derived DAG synthesis in different microdomains of the ER, then PC may serve as a 'DAG trafficking' molecule through the ER membrane.

What determines seed oil content?

Increasing oil production in seeds has been a target of plant breeders and metabolic engineers for decades. Even a few % increase in oil yield per ha in a crop such as soybean can add more than US$1 billion to the crop's annual world value. How can this be achieved? In general, flux into metabolic pathways can be controlled either by increasing the supply of upstream substrates (source control) or by increasing 'demand' or 'sink' strength in the last steps of a pathway. Increased seed oil accumulation has been engineered at both stages. An increase in maize embyo oil by overexpressing the WRI1 transcription factor [16•] and increased soybean and *B. napus* oil by expression of DGAT [78,79], represent successes at

source and sink levels, respectively. Although there are dozens of other reports of gene manipulations that increase seed oil [80], so far there are only a few examples where such increases have been demonstrated in multiple field trials. Further research is required to determine whether PC acyl flux reactions can also be utilized to modify oil content.

Questions and unknowns

There is no shortage of questions that must be answered to better understand oil synthesis in plant seeds. These include identifying which genes are involved in: first, FA export from plastids; second, the GPAT and the PA to DAG reactions of the Kennedy pathway; third, PC deacylation during acyl editing. In addition, there is a fundamental lack of clarity about the cell biology of TAG assembly. Unknowns include: first, is PC a universal FA/DAG carrier between organelles? Second, to what extent are substrate channeling and distinct subcellular pools of DAG, PC, acyl-CoA (and other intermediates) involved in TAG biosynthesis? Third, do membrane and TAG synthesis require different locations of their biosynthetic enzymes and are oilbody:ER connections involved? Fourth, how are unusual FA excluded from membranes and channeled to TAG? Fifth, to what extent is PC involved in acyl fluxes of saturated or other FA that are not modified in the ER? Finally, how are levels of transcripts, proteins and activities of DGAT and other downstream enzymes of TAG assembly controlled?

Acknowledgements

Our work was supported by the U.S. National Science Foundation (DBI-0701919) (PDB), by VINNOVA and FORMAS (SS), and by the Department of Energy – Great Lakes Bioenergy Research Center Cooperative Agreement DE-FC02-07ER6449 (JBO).

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. World Vegetable Oil Consumption: http://www.soystats.com/2012/page_35.htm; 2011

2. Prices of Commodity Oils: http://lipidlibrary.aocs.org/market/prices.htm

3. World Agriculture: Towards 2015/2030: *An FAO Perspective*. http://www.fao.org/docrep/005/y4252e/y4252e05c.htm

4. •• Li-Beisson Y, Shorrosh B, Beisson F, Andersson M, Arondel V, Bates P, Baud S, Bird D, DeBono A, Durrett T *et al.*: **Acyl lipid metabolism.The Arabidopsis Book**. 2013: e0161.
This review provides an in-depth analysis of metabolism of acyl chains in Arabidopsis, including in seeds, and is accompanied by a website (ARALIP.plantbiology.msu.edu) with details on over 600 genes.

5. Chapman KD, Ohlrogge JB: **Compartmentation of triacylglycerol accumulation in plants**. *J Biol Chem* 2012, **287**:2288-2294.

6. Chapman KD, Dyer JM, Mullen RT: **Biogenesis and functions of lipid droplets in plants: thematic review series: lipid droplet synthesis and metabolism: from yeast to man**. *J Lipid Res* 2012, **53**:215-226.

7. Liu Q, Siloto RMP, Lehner R, Stone SJ, Weselake RJ: **Acyl-CoA: diacylglycerol acyltransferase: molecular biology, biochemistry and biotechnology**. *Prog Lipid Res* 2012, **51**:350-377.

8. • Hay J, Schwender J: **Computational analysis of storage synthesis in developing *Brassica napus* L. (oilseed rape) embryos: flux variability analysis in relation to (1)(3)C metabolic flux analysis**. *Plant J* 2011, **67**:513-525.
Demonstration that in green oilseeds light can increase the carbon efficiency of oil synthesis through the production of energy and reductant, as well as a 'RuBisCo bypass' to recapture respired CO_2 and channel it into FA synthesis.

9. Nikolau BJ, Ohlrogge JB, Wurtele ES: **Plant biotin-containing carboxylases**. *Arch Biochem Biophys* 2003, **414**:211-222.

10. • Bourrellier ABF, Valot B, Guillot A, Ambard-Bretteville F, Vidal J, Hodges M: **Chloroplast acetyl-CoA carboxylase activity is 2-oxoglutarate-regulated by interaction of PII with the biotin carboxyl carrier subunit**. *Proc Natl Acad Sci U S A* 2010, **107**:502-507.
The identification of new proteins involved in the regulation of FA synthesis may suggest additional targets for oilseed engineering.

11. •• Andre C, Haslam RP, Shanklin J: **Feedback regulation of plastidic acetyl-CoA carboxylase by 18:1-acyl carrier protein in *Brassica napus***. *Proc Natl Acad Sci U S A* 2012, **109**:10107-10112.
ACCase is a key regulatory step of fatty acid synthesis in all organisms and this study demonstrates that an endproduct of the plastid pathway, 18:1-ACP, can act on ACCase as a feedback regulator. Different forms of ACCase without this feedback may offer a method to increase oil production.

12. Cahoon E, Clemente T, Damude H, Kinney A: **Modifying vegetable oils for food and non-food purposes**. In *Oil Crops*. Edited by Vollmann J, Rajcan I. New York: Springer; 2010:31-56. Handbook of Plant Breeding, vol 4.

13. Voelker TA, Hayes TR, Cranmer AM, Turner JC, Davies HM: **Genetic engineering of a quantitative trait: metabolic and genetic parameters influencing the accumulation of laurate in rapeseed**. *Plant J* 1996, **9**:229-241.

14. Cernac A, Benning C: **WRINKLED1 encodes an AP2/EREB domain protein involved in the control of storage compound biosynthesis in Arabidopsis**. *Plant J* 2004, **40**:575-585.

15. •• Baud S, Lepiniec L: **Physiological and developmental regulation of seed oil production**. *Prog Lipid Res* 2010, **49**:235-249.
The authors provide a superb review of seed oil biosynthesis and its integration within the architecture of seed physiology and gene network controls.

16. • Shen B, Allen WB, Zheng PZ, Li CJ, Glassman K, Ranch J, Nubel D, Tarczynski MC: **Expression of ZmLEC1 and ZmWRI1 Increases Seed Oil Production in Maize**. *Plant Physiol* 2010, **153**:980-987.
Overexpression of maize WRI1 led to major increases in embryo and total seed oil, but had no impact on endosperm; raising the question of what else is needed to produce oil in high starch seed tissues.

17. • Troncoso-Ponce MA, Kilaru A, Cao X, Durrett TP, Fan J, Jensen JK, Thrower NA, Pauly M, Wilkerson C, Ohlrogge JB: **Comparative deep transcriptional profiling of four developing oilseeds**. *Plant J* 2011, **68**:1014-1027.
Comparison of transcript levels in diverse oilseeds indicated that glycolytic and FA synthesis enzymes were highly regulated with similar stoichiometry in all cases but that downstream TAG assembly steps changed much less and not in coordination with upstream enzymes.

18. • Bourgis F, Kilaru A, Cao X, Ngando-Ebongue GF, Drira N, Ohlrogge JB, Arondel V: **Comparative transcriptome and metabolite analysis of oil palm and date palm mesocarp that differ dramatically in carbon partitioning**. *Proc Natl Acad Sci U S A* 2011, **108**:12527-12532.
The mesocarp that surrounds seeds of oil palm produces the highest oil content found in nature. As in seeds, WRI1 is a key determinant that controls oil synthesis. Intriguingly, oil and date palm differed only slightly in transcript levels for TAG assembly enzymes.

19. Koo AJK, Ohlrogge JB, Pollard M: **On the export of fatty acids from the chloroplast**. *J Biol Chem* 2004, **279**:16101-16110.

20. Schnurr JA, Shockey JM, de Boer GJ, Browse JA: **Fatty acid export from the chloroplast. Molecular characterization of a major plastidial acyl-coenzyme A synthetase from Arabidopsis**. *Plant Physiol* 2002, **129**:1700-1709.

21. Zhao L, Katavic V, Li F, Haughn GW, Kunst L: **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* 2010, **64**:1048-1058.

22. Tjellstrom H, Yang Z, Allen DK, Ohlrogge JB: **Rapid kinetic labeling of Arabidopsis cell suspension cultures: implications for models of lipid export from plastids**. *Plant Physiol* 2012, **158**:601-611.

23. Andersson MX, Goksor M, Sandelius AS: **Optical manipulation reveals strong attracting forces at membrane contact sites between endoplasmic reticulum and chloroplasts**. *J Biol Chem* 2007, **282**:1170-1174.

24. Kim S, Yamaoka Y, Ono H, Kim H, Shim D, Maeshima M, Martinoia E, Cahoon EB, Nishida I, Lee Y: **AtABCA9 transporter supplies fatty acids for lipid synthesis to the endoplasmic reticulum**. *Proceedings of the National Academy of Sciences* 2013, **110**:773-778.

25. Kennedy EP: **Biosynthesis of complex lipids**. *Fed Proc* 1961:20.

26. Weiss SB, Kennedy EP, Kiyasu JY: **Enzymatic synthesis of triglycerides**. *J Biol Chem* 1960, **235**:40-44.

27. Barron EJ, Stumpf PK: **Fat metabolism in higher plants, XIX. The biosynthesis of triglycerides by avocado-mesocarp enzymes**. *Biochim Biophys Acta* 1962, **60**:329-337.

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

29. Sperling P, Linscheid M, Stocker S, Muhlbach HP, Heinz E: **In vivo desaturation of cis-delta-9-monounsaturated to cis-delta-9, 12-diunsaturated alkenylether glycerolipids**. *J Biol Chem* 1993, **268**:26935-26940.

30. Vandeloo FJ, Broun P, Turner S, Somerville C: **An oleate 12-hydroxylase from *Ricinus communis* L. is a fatty acyl desaturase homolog**. *Proc Natl Acad Sci U S A* 1995, **92**:6743-6747.

31. Wallis JG, Watts JL, Browse J: **Polyunsaturated fatty acid synthesis: what will they think of next?** *Trends Biochem Sci* 2002, **27**:467-473.

32. Gidda SK, Shockey JM, Rothstein SJ, Dyer JM, Mullen RT: **Arabidopsis thaliana GPAT8 and GPAT9 are localized to the ER and possess distinct ER retrieval signals: functional divergence of the dilysine ER retrieval motif in plant cells**. *Plant Physiol Biochem* 2009, **47**:867-879.

33. Chen X, Snyder CL, Truksa M, Shah S, Weselake RJ: **sn-Glycerol-3-phosphate acyltransferases in plants**. *Plant Signal Behav* 2011, **6**:1695-1699.

34. Wendel AA, Lewin TM, Coleman RA: **Glycerol-3-phosphate acyltransferases: rate limiting enzymes of triacylglycerol biosynthesis**. *Biochim Biophys Acta: Mol Cell Biol Lipids* 2009, **1791**:501-506.

35. Yang W, Simpson JP, Li-Beisson Y, Beisson F, Pollard M, Ohlrogge JB: **A land-plant-specific glycerol-3-phosphate acyltransferase family in Arabidopsis: substrate specificity, sn-2 preference, and evolution**. *Plant Physiol* 2012, **160**:638-652.

36. Maisonneuve S, Bessoule J-J, Lessire R, Delseny M, Roscoe TJ: **Expression of rapeseed microsomal lysophosphatidic acid acyltransferase isozymes enhances seed oil content in Arabidopsis**. *Plant Physiol* 2010, **152**:670-684.

37. Snyder CL, Yurchenko OP, Siloto RMP, Chen X, Liu Q, Mietkiewska E, Weselake RJ: **Acyltransferase action in the modification of seed oil biosynthesis**. *New Biotechnol* 2009, **26**:11-16.

38. Eastmond PJ, Quettier A-L, Kroon JTM, Craddock C, Adams N, Slabas AR: **PHOSPHATIDIC ACID PHOSPHOHYDROLASE1 and 2 regulate phospholipid synthesis at the endoplasmic reticulum in Arabidopsis**. *Plant Cell Online* 2010, **22**:2796-2811.

39. Lung SC, Weselake RJ: **Diacylglycerol acyltransferase: a key mediator of plant triacylglycerol synthesis**. *Lipids* 2006, **41**:1073-1088.

40. Turchetto-Zolet A, Maraschin F, de Morais G, Cagliari A, Andrade C, Margis-Pinheiro M, Margis R: **Evolutionary view of acyl-CoA diacylglycerol acyltransferase (DGAT), a key enzyme in neutral lipid biosynthesis**. *BMC Evol Biol* 2011, **11**:263.

41. Katavic V, Reed DW, Taylor DC, Giblin EM, Barton DL, Zou JT, Mackenzie SL, Covello PS, Kunst L: **Alteration of seed fatty-acid composition by an ethyl methanesulfonate-induced mutation in Arabidopsis-thaliana affecting diacylglycerol acyltransferase activity**. *Plant Physiol* 1995, **108**:399-409.

42. Zou JT, Wei YD, Jako C, Kumar A, Selvaraj G, Taylor DC: **The *Arabidopsis thaliana* TAG1 mutant has a mutation in a diacylglycerol acyltransferase gene**. *Plant J* 1999, **19**:645-653.

43. Zhang M, Fan J, Taylor DC, Ohlrogge JB: **DGAT1 and PDAT1 Acyltransferases have overlapping functions in Arabidopsis triacylglycerol biosynthesis and are essential for normal pollen and seed development**. *Plant Cell* 2009, **21**:3885-3901.

44. Shockey J, Gidda S, Chapital D, Kuan J, Dhanoa P, Bland J, Rothstein S, Mullen R, Dyer J: **Tung tree DGAT1 and DGAT2 have nonredundant functions in triacylglycerol biosynthesis and are localized to different subdomains of the endoplasmic reticulum**. *Plant Cell* 2006, **18**:2294-2313.

45. Kroon JTM, Wei W, Simon WJ, Slabas AR: **Identification and functional expression of a type 2 acyl-CoA:diacylglycerol acyltransferase (DGAT2) in developing castor bean seeds which has high homology to the major triglyceride biosynthetic enzyme of fungi and animals**. *Phytochemistry* 2006, **67**:2541-2549.

46. Durrett T, McClosky D, Tumaney A, Elzinga D, Ohlrogge J, Pollard M: **A distinct DGAT with sn-3 acetyltransferase activity that synthesizes unusual, reduced-viscosity oils in Euonymus and transgenic seeds**. *Proc Natl Acad Sci U S A* 2010, **107**:9464-9469.

47. Saha S, Enugutti B, Rajakumari S, Rajasekharan R: **Cytosolic triacylglycerol biosynthetic pathway in oilseeds. Molecular cloning and expression of peanut cytosolic diacylglycerol acyltransferase**. *Plant Physiol* 2006, **141**:1533-1543.

48. Hernández ML, Whitehead L, He Z, Gazda V, Gilday A, Kozhevnikova E, Vaistij FE, Larson TR, Graham IA: **A cytosolic acyltransferase contributes to triacylglycerol synthesis in sucrose-rescued Arabidopsis seed oil catabolism mutants**. *Plant Physiol* 2012, **160**:215-225.

49. Rani SH, Krishna THA, Saha S, Negi AS, Rajasekharan R: **Defective in Cuticular Ridges (DCR) of *Arabidopsis thaliana*, a gene associated with surface cutin formation encodes a soluble diacylglycerol acyltransferase**. *J Biol Chem* 2010, **285**:38337-38347.

50. Lippold F, vom Dorp K, Abraham M, Hölzl G, Wewer V, Yilmaz JL, Lager I, Montandon C, Besagni C, Kessler F *et al.*: **Fatty acid phytyl ester synthesis in chloroplasts of Arabidopsis**. *Plant Cell Online* 2012, **24**:2001-2014.

51. Bates PD, Durrett TP, Ohlrogge JB, Pollard M: **Analysis of Acyl fluxes through multiple pathways of triacylglycerol synthesis in developing soybean embryos**. *Plant Physiol* 2009, **150**:55-72.

52. Bates PD, Ohlrogge JB, Pollard M: **Incorporation of newly synthesized fatty acids into cytosolic glycerolipids in pea leaves occurs via acyl editing**. *J Biol Chem* 2007, **282**:31206-31216.

53. Stymne S, Stobart A (Eds): *Triacylglycerol Biosynthesis*. New York: Academic Press; 1987.

54. Dahlqvist A, Stahl U, Lenman M, Banas A, Lee M, Sandager L, Ronne H, Stymne H: **Phospholipid: diacylglycerol acyltransferase: An enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants**. *Proc Natl Acad Sci U S A* 2000, **97**:6487-6492.

55. Stymne S, Stobart AK: **Evidence for the reversibility of the acyl-coA-lysophosphatidylcholine acyltransferase in microsomal preparations from developing safflower (*Carthamus-tinctorius* L.) cotyledons and rat-liver**. *Biochem J* 1984, **223**:305-314.

56. •• Bates PD, Fatihi A, Snapp AR, Carlsson AS, Browse J, Lu C: **Acyl editing and headgroup exchange are the major mechanisms that direct polyunsaturated fatty acid flux into triacylglycerols**. *Plant Physiol* 2012, **160**:1530-1539.
Demonstration that Arabidopsis LPCAT1 and LPCAT2 are essential for acyl editing in seeds, and together with PDCT provide 2/3 of the flux of PUFA from PC into TAG.

57. Stalberg K, Stahl U, Stymne S, Ohlrogge J: **Characterization of two *Arabidopsis thaliana* acyltransferases with preference for lysophosphatidylethanolamine**. *BMC Plant Biol* 2009:9.

58. Chen G, Snyder CL, Greer MS, Weselake RJ: **Biology and biochemistry of plant phospholipases**. *Crit Rev Plant Sci* 2011, **30**:239-258.

59. Wang L, Shen W, Kazachkov M, Chen G, Chen Q, Carlsson AS, Stymne S, Weselake RJ, Zou J: **Metabolic interactions between the lands cycle and the Kennedy pathway of glycerolipid synthesis in Arabidopsis developing seeds**. *Plant Cell Online* 2012.

60. Mhaske V, Beldjilali K, Ohlrogge J, Pollard M: **Isolation and characterization of an Arabidopsis thaliana knockout line for phospholipid: diacylglycerol transacylase gene (At5g13640)**. *Plant Physiol Biochem* 2005, **43**:413-417.

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

62. Mora G, Scharnewski M, Fulda M: **Neutral lipid metabolism influences phospholipid synthesis and deacylation in *Saccharomyces cerevisiae***. *PLoS One* 2012, **7**:e49269.

63. van Erp H, Bates PD, Burgal J, Shockey J, Browse J: **Castor phospholipid: diacylglycerol acyltransferase facilitates efficient metabolism of hydroxy fatty acids in transgenic Arabidopsis**. *Plant Physiol* 2011, **155**:683-693.

64. Stahl U, Carlsson AS, Lenman M, Dahlqvist A, Huang BQ, Banas W, Banas A, Stymne S: **Cloning and functional characterization of a phospholipid: diacylglycerol acyltransferase from Arabidopsis**. *Plant Physiol* 2004, **135**:1324-1335.

65. Kim HU, Lee K-R, Go YS, Jung JH, Suh M-C, Kim JB: **Endoplasmic reticulum-located PDAT1-2 from castor bean enhances hydroxy fatty acid accumulation in transgenic plants**. *Plant Cell Physiol* 2011, **52**:983-993.

66. Lu C, Xin Z, Ren Z, Miquel M, Browse J: **An enzyme regulating triacylglycerol composition is encoded by the ROD1 gene of Arabidopsis**. *Proc Natl Acad Sci U S A* 2009, **106**:18837-18842.

67. Slack CR, Roughan PG, Browse JA, Gardiner SE: **Some properties of cholinephosphotransferase from developing safflower cotyledons**. *Biochim Biophys Acta* 1985, **833**:438-448.

68. Slack CR, Campbell LC, Browse JA, Roughan PG: **Some evidence for the reversibility of the cholinephosphotransferase-catalysed reaction in developing linseed cotyledons in vivo**. *Biochim Biophys Acta* 1983, **754**:10-20.

69. • Lee J, Welti R, Schapaugh WT, Trick HN: **Phospholipid and triacylglycerol profiles modified by PLD suppression in soybean seed**. *Plant Biotechnol J* 2011, **9**:359-372.
Demonstration that PLD may be involved in PC-derived DAG synthesis in soybeans

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

71. Schultz DJ, Ohlrogge JB: **Biosynthesis of triacylglycerol in Thunbergia alata: additional evidence for involvement of phosphatidylcholine in unusual monoenoic oil production**. *Plant Physiol Biochem* 2000, **38**:169-175.

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

73. Millar AA, Smith MA, Kunst L: **All fatty acids are not equal: discrimination in plant membrane lipids**. *Trends Plant Sci* 2000, **5**:95-101.

74. Li R, Yu K, Hatanaka T, Hildebrand DF: **Vernonia DGATs increase accumulation of epoxy fatty acids in oil**. *Plant Biotechnol J* 2010, **8**:184-195.

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

76. Hu Z, Ren Z, Lu C: **The phosphatidylcholine diacylglycerol cholinephosphotransferase is required for efficient hydroxy fatty acid accumulation in transgenic Arabidopsis**. *Plant Physiol* 2012, **158**:1944-1954.

77. Gidda SK, Shockey JM, Falcone M, Kim PK, Rothstein SJ, Andrews DW, Dyer JM, Mullen RT: **Hydrophobic-domain-dependent protein–protein interactions mediate the localization of GPAT enzymes to ER subdomains**. *Traffic* 2011, **12**:452-472.

78. Lardizabal K, Effertz R, Levering C, Mai J, Pedroso MC, Jury T, Aasen E, Gruys K, Bennett K: **Expression of Umbelopsis ramanniana DGAT2A in seed increases oil in soybean**. *Plant Physiol* 2008, **148**:89-96.

79. Taylor DC, Zhang Y, Kumar A, Francis T, Giblin EM, Barton DL, Ferrie JR, Laroche A, Shah S, Zhu W *et al.*: **Molecular modification of triacylglycerol accumulation by over-expression of DGAT1 to produce canola with increased seed oil content under field conditions**. *Botany* Botanique2009, **87**:533-543.

80. Weselake RJ, Taylor DC, Rahman MH, Shah S, Laroche A, McVetty PBE, Harwood JL: **Increasing the flow of carbon into seed oil**. *Biotechnol Adv* 2009, **27**:866-878.

81. To A, Joubès J, Barthole G, Lécureuil A, Scagnelli A, Jasinski S, Lepiniec L, Baud S: **WRINKLED transcription factors orchestrate tissue-specific regulation of fatty acid biosynthesis in Arabidopsis**. *The Plant Cell Online* 2012, **24**:5007-5023.

82. Banas W, Garcia AS, Banas A, Stymne S: **Activities of acyl-CoA:diacylglycerol acyltransferase (DGAT) and phospholipid:diacylglycerol acyltransferase (PDAT) in microsomal preparations of developing sunflower and safflower seeds**. *Planta* 2013, In press.