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Biochemical pathways in seed oil synthesis

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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.

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Introduction

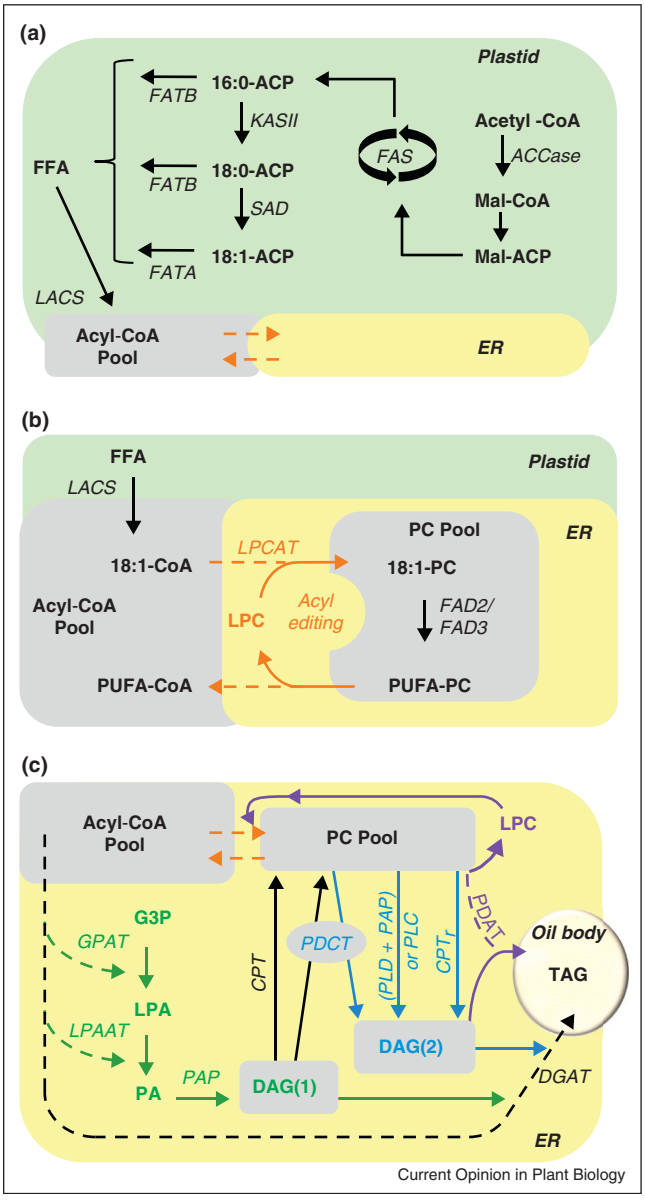
Oils are the most energy-dense plant reserves, supplying humans with much of the calories and essential fatty acids required in our diet. Because they are composed of long chain hydrocarbons, plant oils can also replace petroleum in many applications, including as feedstocks for the chemical industry 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)