REVIEW

Properties and Biotechnological Applications of Acyl-CoA: diacylglycerol Acyltransferase and Phospholipid:diacylglycerol Acyltransferase from Terrestrial Plants and Microalgae

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Abstract Triacylglycerol (TAG) is the major storage lipid in most terrestrial plants and microalgae, and has great nutritional and industrial value. Since the demand for vegetable oil is consistently increasing, numerous studies have been focused on improving the TAG content and modifying the fatty-acid compositions of plant seed oils. In addition, there is a strong research interest in establishing plant vegetative tissues and microalgae as platforms for lipid production. In higher plants and microalgae, TAG biosynthesis occurs via acyl-CoA-dependent or acyl-CoA-independent pathways. Diacylglycerol acyltransferase (DGAT) catalyzes the last and committed step in the acyl-CoA-dependent biosynthesis of TAG, which appears to represent a bottleneck in oil accumulation in some oilseed species. Membrane-bound and soluble forms of DGAT have been identified with very different amino-acid sequences and biochemical properties. Alternatively, TAG can be formed through acyl-CoA-independent pathways via the catalytic action of membrane-bound phospholipid:diacylglycerol acyltransferase (PDAT). As the enzymes catalyzing the terminal steps of TAG formation, DGAT and PDAT play crucial roles in determining the flux of carbon into seed TAG and thus have been considered as the key targets for engineering oil production. Here, we summarize the most recent knowledge on DGAT and PDAT in higher plants and microalgae, with the emphasis on their physiological roles, structural features, and regulation. The development of various metabolic engineering strategies to enhance the TAG content and alter the fatty-acid composition of TAG is also discussed.

Keywords Algae · DGAT · Oil crops · PDAT · Triacylglycerol biosynthesis · Vegetative tissue


Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ABI</td>
<td>ABSCISIC ACID INSENSITIVE</td>
</tr>
<tr>
<td>ACAT</td>
<td>acyl-CoA:cholesterol acyltransferase</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP-activated protein kinase</td>
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<tr>
<td>cDNA</td>
<td>complementary DNA</td>
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<tr>
<td>CRISPR</td>
<td>clustered regularly interspaced short palindromic repeats</td>
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<tr>
<td>DAG</td>
<td>sn-1,2-diacylglycerol</td>
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<tr>
<td>DCR</td>
<td>defective cuticle ridge</td>
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<tr>
<td>DGAT</td>
<td>acyl-CoA:diacylglycerol acyltransferase</td>
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<tr>
<td>EMS</td>
<td>ethyl methanesulfonate</td>
</tr>
<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
</tr>
<tr>
<td>FAD</td>
<td>fatty-acid desaturase</td>
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<tr>
<td>FAH</td>
<td>fatty-acid hydroxylase</td>
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<tr>
<td>G3P</td>
<td>glycerol-3-phosphate</td>
</tr>
<tr>
<td>GPAT</td>
<td>acyl-CoA:sn-glycerol-3-phosphate acyltransferase</td>
</tr>
<tr>
<td>IDR</td>
<td>intrinsically disordered region</td>
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A review dedicated to Professor Randall J. Weselake to celebrate his exceptional career and contributions to the broad field of lipids and AOCS.

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**Introduction**

Triacylglycerol (TAG), which is the major component of vegetable oils, consists of three fatty acids esterified to a glycerol backbone. In terrestrial plants, TAG is mainly stored in seeds functioning as an energy reservoir to facilitate germination and early seedling growth. TAG also provides the precursors for membrane biosynthesis and lipid signaling, which are crucial for normal plant growth and development (Fan et al., 2013b, 2014). In microalgae, TAG serves as a source of energy in response to adverse environmental conditions (for review, see Hu et al., 2008). The primary use of seed oils is for edible applications (food and feed). There is, however, strong interest in using seed oil as renewable feedstock to produce biodiesel, biolubricants, and other bioproducts (Biermann et al., 2011).

The global demand for vegetable oils has been steadily growing over the past 50 years and is expected to further increase due to a rising global population and reliance on vegetable-oil-derived chemicals (Chen et al., 2015). Increasing vegetable oil supply is generally accomplished via two approaches: growing more oil crops and increasing oil content in seeds and/or other oil-forming tissues (e.g. mesocarp of palm [Elaeis spp.] and avocado [Persea Americana]). A direct increase of oil crop-planting area, however, is limited by the finite availability of the arable land (Lu et al., 2011). Recent efforts to meet the rising demands have been focused on increasing the oil content of seeds and vegetative tissues by the implementation of metabolic engineering strategies. In addition, considerable research has focused on exploring the potential of oleaginous microalgae to produce TAG, a process that is not expected to utilize arable land. As the property of vegetable oils is largely affected by fatty-acid composition, research interests have also been directed on the modification of fatty-acid composition to increase the nutritional and industrial values of oils.

In general, TAG biosynthesis in terrestrial higher plants and microalgae is similar. Schematically, TAG assembly can be divided into acyl-CoA-dependent and acyl-CoA-independent pathways. Diacylglycerol acyltransferase (DGAT; EC 2.3.1.20) catalyzes the last and committed step in the acyl-CoA-dependent TAG biosynthesis by transferring an acyl group from acyl-CoA to the (stereospecific numbering) sn-3 position of sn-1,2-diacylglycerol (DAG), which has been the target of numerous studies attempting to engineer the oil content and fatty-acid composition (for review, see Liu et al., 2012). In contrast, phospholipid:diacylglycerol acyltransferase (PDAT; EC 2.3.1.158) catalyzes the acyl-CoA-independent synthesis of TAG using membrane glycerolipids as acyl donors (Dahlqvist et al., 2000). In this review, we begin by providing background information on storage lipid biosynthesis in higher plants and microalgae. Thereafter, the biochemical and physiological properties of DGAT and PDAT are discussed. This, in turn, is followed by discussion on the metabolic engineering of DGAT-catalyzed and PDAT-catalyzed reactions so as to manipulate oil production in terrestrial plants and microalgae.

**Overview of Storage Lipid Biosynthesis in Higher Plants and Microalgae**

TAG formation in oleaginous plants and microalgae involves fatty-acid biosynthesis and TAG assembly (Fig. 1; for reviews, see Chen et al., 2015 and Li-Beisson et al., 2015). Depending on the plant species, TAG formation can also involve a complex interplay with membrane metabolism. For example, seed oils enriched in polyunsaturated fatty acids or unusual fatty acids require processes for routing these fatty acids from the site of their synthesis in membranes into TAG.

**Higher Plants**

In developing seeds of oleaginous plants, *de novo* fatty-acid biosynthesis and TAG assembly occur in different compartments. Fatty-acid biosynthesis operates in the plastid, wherein acetyl-CoAs are converted to fatty acyl chains (for reviews, see Chapman and Ohlrogge, 2012; Ohlrogge and Jaworski, 1997). Acetyl-CoA carboxylase catalyzes the ATP-dependent formation of malonyl-CoA, while the fatty-acid synthase complex uses two carbon fragments derived from malonyl-CoA to form an acyl chain while attached to an acyl carrier protein of the fatty-acid synthase complex. The main source of acetyl-CoA used for fatty-acid synthesis is derived from pyruvate via the catalytic action of the plastidial pyruvate dehydrogenase complex. Pyruvate can be produced from glucose derived from photosyntheate through plastidial and cytosolic glycolysis. Specific transporters are required to move some of the cytosolic intermediates of glycolysis into the plastid (for...
Fig. 1 Generalized scheme for triacylglycerol (TAG) biosynthesis in developing seeds of oleaginous higher plants. ACP, acyl carrier protein; ACCase, acetyl-CoA carboxylase; CoA, coenzyme A; CPT, choline phosphotransferase; DAG, sn-1,2-diacylglycerol; DGAT, diacylglycerol acyltransferase; ER, endoplasmic reticulum; FAD, fatty-acid desaturase; FAE, fatty-acid elongase; FAS, fatty-acid synthase; FAT, acyl-ACP thioesterase; FAX, fatty-acid export; FFA, fatty acid; GPAT, sn-glycerol-3-phosphate acyltransferase; Gro3P, glycerophosphocholine; GPCAT, glycerophosphocholine acyltransferase; Gro3P, sn-glycerol-3-phosphate; Hexose P, hexose phosphate; LACS, long-chain acyl-CoA synthase; lysoPtdOH, lysophosphatidic acid; LPAAT, acyl-CoA:lysophosphatic acid acyltransferase; lysoPtdCho, lysophosphatidylcholine; LPCAT, lysophosphatidylcholine acyltransferase; LPCT, lysophosphatidylcholine transacylase; MCAT, malonyl-CoA:ACP acyltransferase; PAP, phosphatidic acid phosphatase; PDAT, phospholipid:diacylglycerol acyltransferase; PDCT, phosphatidylcholine:diacylglycerol cholinephosphotransferase; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PLA₂, phospholipase A₂; PLC, phospholipase C; PLD, phospholipase D; PPP, pentose phosphate pathway; PtdCho, phosphatidylcholine; PtdOH, phosphatidic acid; Pyr, pyruvate; SAD, stearoyl-ACP desaturase; Triose P, triose phosphate. This figure was developed based on information from reviews and articles on lipid biosynthesis (Bates et al., 2012, 2013; Baud and Lepiniec, 2010; Chapman and Ohlrogge, 2012; Chen et al., 2015; Harwood, 2005; Li et al., 2015a; Ohlrogge and Jaworski, 1997; Rawsthorne, 2002; Shearer et al., 2004). Carriers/transporters on the plastid envelope that are required to move the cytosolic intermediates of glycolysis into the plastid are shown as dark circles.
review, see Rawsthorne, 2002). Fatty acyl chains produced in plastids can extend up to 16 or 18 carbons in length, which can further undergo monounsaturation, before being released from the fatty-acid synthase complex and transported out of the plastid and converted into acyl-CoA.

TAG assembly occurs in the endoplasmic reticulum (ER) and uses sn-glycerol-3-phosphate (Gro3P) derived from glycolysis, and acyl-CoA as an acyl donor. This process is known as the Kennedy pathway and involves the sequential acylation of the glycerol backbone at Gro3P at sn-1, 2, and 3 positions to yield TAG (Weiss and Kennedy, 1956). Three acyl-CoA-dependent acyltransferases, including sn-glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), and DGAT, participate in this process (for review, see Snyder et al., 2009). GPAT catalyzes the first acylation of Gro3P to yield lysophosphatidic acid. Lysophosphatidic acid (lysoPtdOH) is further acylated by the catalytic action of LPAAT to produce phosphatidic acid (PtdOH), which is then converted to sn-1, 2-DAG via the catalytic action of phosphatidic acid phosphatase. DGAT catalyzes the final acylation of the sn-3 position of sn-1, 2-DAG to form TAG, which is the committed step in acyl-CoA-dependent TAG biosynthesis. TAG can also be synthesized through acyl-CoA-independent pathways via the catalytic action of PDAT, which catalyzes the transfer of an acyl moiety from the sn-2 position of phosphatidylcholine (PtdCho) to the sn-3 position of sn-1, 2-DAG to yield TAG (Dahlqvist et al., 2000; Ståhl et al., 2004). Both DGAT and PDAT play crucial roles in determining the flux of carbon into TAG (Aznar-Moreno and Durret, 2017; Harwood et al., 2013; Zhang et al., 2009). They also contribute to the routing of modified fatty acids from PtdCho into TAG in some plant species, such as flax (Linum usitatissimum), castor (Ricinus communis), tung tree (Vernicia fordii), and ironweed (Vernonia galamensis), which produce relatively high levels of polyunsaturated or unusual fatty acids in their seed oils (Kim et al., 2011; Kroon et al., 2006; Li et al., 2010a; Pan et al., 2013; Shockey et al., 2006; van Erp et al., 2011).

The synthesis of long-chain polyunsaturated or unusual fatty acids generally occurs on PtdCho or in the acyl-CoA pool, where the nascent fatty acids derived from the plastid, mainly saturated [16:0 and 18:0] and monounsaturated [18:1n-9c] fatty acids, undergo further modifications, such as elongation and desaturation. The further elongation of the acyl chain is catalyzed by ER-bound fatty-acid elongase with acyl-CoA as substrates (Ghanevati and Jaworski, 2001; Rossak et al., 2001). For fatty-acid desaturation, fatty-acid desaturase (FAD) 2 and FAD3 subsequently introduce double bonds in the cis configuration at positions Δ-12 and Δ-15 of fatty acids on the sn-2 position of PtdCho, respectively (Browse et al., 1993; Vrinten et al., 2005). Other fatty-acid modifications, including hydroxylation, epoxidation and conjugation, also utilize PtdCho as the substrate. The contributing enzymes, such as hydroxylase from castor (van de Loo et al., 1995), conjugase from Momordica charantia (Cahoon et al., 1999), and epoxigenase from V. galamensis (Cahoon and Kinney, 2005), are related to or derived from FAD2.

After being synthesized on PtdCho, the modified fatty acids are moved out of PtdCho and eventually incorporated into TAG via various routes including acyl-editing (Bates et al., 2007; for reviews, see Bates, 2016; Chen et al., 2015). PDAT is capable of directly moving modified fatty acids from their site of synthesis in PtdCho to TAG. Alternatively, PtdCho-derived fatty acids can also be routed into the acyl-CoA or DAG pool. Fatty acids on PtdCho can enter the acyl-CoA pool via the combined action of phospholipase A2 and long-chain acyl-CoA synthetase or via the reverse reaction catalyzed by lysophosphatidylcholine acyltransferase. Phospholipase A2 catalyzes the cleavage of a fatty acid from the sn-2 position of PtdCho, the primary site for acyl modification, and the released fatty acid is further ligated to CoA through the catalytic action of long-chain acyl-CoA synthetase, yielding an acyl-CoA. Alternatively, the reverse action of lysophosphatidylcholine acyltransferase generates acyl-CoA and lysophosphatidylcholine (lysoPtdCho) from PtdCho and free CoA. In turn, the forward action catalyzed by lysophosphatidylcholine acyltransferase can reacylate the vacant sn-2 position of lysophosphatidylcholine generated by the catalytic action of PLA2 and/or the reverse reaction of lysophosphatidylcholine acyltransferase. Furthermore, PtdCho-derived fatty acids can also be incorporated into TAG in the form of DAG. De novo synthesized DAG can be converted into PtdCho for modification via the catalytic action of CDP-choline:sn-1, 2-diacylglycerol cholinephosphotransferase (Slack et al., 1983, 1985). PtdCho can subsequently be converted back to DAG and/or PtdOH via the catalytic action of phospholipase C and/or D, respectively (for reviews, see Bates et al., 2013; Chapman and Ohlrogge, 2012). Alternatively, the PtdCho-DAG conversion could also be achieved via the catalytic action of phosphatidylcholine:diacylglycerol cholinephosphotransferase, which transfers the phosphocholine headgroup of modified-PtdCho to the de novo synthesized DAG in the Kennedy pathway (Lu et al., 2009; Wickramarathna et al., 2015). Although the sn-2 position of PtdCho is the primary site for acyl-editing, a low amount of acyl-editing flux was also observed to go through the sn-1 position (Bates et al., 2007, 2009). The exchange of acyl groups might occur between the sn-1 and sn-2 positions of PtdCho with involvement of the catalytic action of glycerophosphocholine acyltransferase and lysophosphatidylcholine transacylase (Lager et al., 2015).

In developing seeds of oleaginous plants, TAG accumulates between the outer leaflets of the ER. Eventually, lipid

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droplets (oil bodies) ranging from 0.2 to 2 μm in diameter pinch off of the ER and remain surrounded by a monolayer of phospholipid known as a half-unit membrane (for review, see Huang, 1996). In plant seeds, oil-body-membrane-associated proteins are embedded on the outside of the lipid droplets (for reviews, see Huang, 2018; Pyc et al., 2017; Shimada and Hara-Nishimura, 2010). The most abundant lipid droplet proteins are oleosins, which appear to prevent oil droplets from coalescing and function in the formation and turnover of lipid droplets (Pyc et al., 2017). In addition, other lipid droplet proteins, such as caleosins and steroleosins, were identified in seeds according to proteomics analyses of isolated lipid droplets (Pyc et al., 2017). The physiological roles of caleosins and steroleosins in seeds, however, are not entirely clear. Some caleosins appeared to be involved in the plant stress response (Shimada and Hara-Nishimura, 2010).

**Microalgae**

TAG biosynthesis in microalgae, in general, is analogous to higher plants involving both acyl-CoA-dependent and acyl-CoA-independent processes (Fig. 1), but has some different features (for reviews, see Hu et al., 2008; Li-Beisson et al., 2015). In the conventional TAG biosynthetic pathways, TAG is generally assembled in the ER from ER-derived DAG and is deposited mainly in lipid droplets in the cytosol. In the halophytic microalgae Dunaliella bardawil, however, TAG is deposited in plastidial oil droplets—plastoglobuli in addition to cytosolic lipid droplets (Katz et al., 1995). Moreover, the starch-less unicellular green microalga Chlamydomonas reinhardtii has a unique plastidial pathway for TAG biosynthesis using DAG derived almost exclusively from the chloroplast (Fan et al., 2011). Plastidial TAG biosynthesis is largely dependent on de novo fatty-acid biosynthesis, and the resulting TAG is stored in lipid droplets in both the chloroplast and cytosol (Fan et al., 2011). Currently, the chloroplast envelope-based pathway of TAG biosynthesis in microalgae is receiving increasing experimental support (Bagnato et al., 2017; Fan et al., 2011; Goodson et al., 2011; Li et al., 2014; Liu and Benning, 2013); the presence of chloroplast lipid droplets, however, is still debatable. The similar origin and composition of the lipid droplets in cytoplasmic lipid droplets and β-carotene-rich plastoglobuli of D. bardawil suggested the possibility that β-carotene-rich plastoglobuli are formed in part from hydrolysis of chloroplast membrane lipids and in part from fatty acids or TAG derived from cytoplasmic lipid droplets (Davidi et al., 2014). More recently, it was revealed by microscopy that the chloroplast-associated lipid droplets in C. reinhardtii entirely originated in the cytosol and were distinct from the plastoglobuli in the chloroplast stroma, even though some lipid droplets were associated with the outer envelope of the chloroplast without intervention of the ER (Moriyama et al., 2018). Moreover, some microalgae also possess other pathways of TAG biosynthesis that differ from higher plants. For instance, in the microalga C. reinhardtii that lacks PtdCho, the synthesis of TAG involves the participation of the betaine lipid diacylglycerol N,N,N-trimethylhomoserine (for review, see Li-Beisson et al., 2015).

**Acyl-CoA-Dependent Formation of TAG**

To date, the majority of studies on the acyl-CoA-dependent formation of TAG have mainly focused on three DGAT gene families. The first two gene families, including DGAT1 and DGAT2, encode enzymes embedded in the membrane lipid bilayer, whereas DGAT3, the third gene family, encodes a soluble enzyme with DGAT activity (for reviews, see Liu et al., 2012; Lung and Weselake, 2006). DGAT3 is the least investigated of the three DGAT families. In the following several sections, the features of DGAT from each gene family will be discussed along with some discussion of other proteins with DGAT activity.

**Membrane-Bound DGAT**

In 1956, the very first report of DGAT activity was from chicken (Gallus gallus domesticus) liver (Weiss et al., 1960; Weiss and Kennedy, 1956), but over four decades passed before the genes were cloned and characterized (Cases et al., 1998). In 1998, the first DGAT gene, which is a member of the DGAT1 family, was isolated from mouse (Mus musculus) based on its sequence homology to acyl-CoA:cholesterol acyltransferase (ACAT) 1 (Cases et al., 1998). Shortly thereafter, plant DGAT1 were identified in the model plant Arabidopsis thaliana (hereafter referred to as Arabidopsis) (Bouvier-Navé et al., 2000; Hobbs et al., 1999; Routaboul et al., 1999; Zou et al., 1999) and tobacco (Nicotiana tabacum) (Bouvier-Navé et al., 2000). Subsequently, many DGAT1 have been isolated and characterized from various plant species including (but by no means comprehensive) olive (Olea europaea) (Giannoulia et al., 2000), oilseed rape (Brassica napus) (Greer et al., 2015; Nykiforuk et al., 2002), castor bean (R. communis) (He et al., 2004), burning bush (Euonymus alatus) (Milcamps et al., 2005), tung (V. fordii) (Shockey et al., 2006), soybean (Glycine max) (Wang et al., 2006), garden nasturtium (Tropaeolum majus) (Xu et al., 2008), Echium pitardii (Manas-Fernandez et al., 2009), flax (L. usitatissimum) (Pan et al., 2013; Siloto et al., 2009b), sesame (Sesamum indicum) (Wang et al., 2014), and more recently Cuphea avigera var. pulcherrima (Iskandarov et al., 2018).
et al., 2017) and peanut (Arachis hypogaea) (Zheng et al., 2017b).

Following the discovery of DGAT1, DGAT2 was found, which essentially has no amino-acid sequence similarity with DGAT1 and ACAT1. In 2001, the first DGAT2 was isolated from the oleaginous fungus Umbelopsis ramanniana (formerly Mortierella ramanniana) (Lardizabal et al., 2001). In the same study, DGAT2 homologs were isolated from Saccharomyces cerevisiae, Caenorhabditis elegans, and Arabidopsis, but only DGAT2 from the former two species encoded active enzymes. Based on the DGAT2 sequence from U. ramanniana, DGAT2-related genes were rapidly identified from mammals, such as mouse and human (Homo sapiens) (Cases et al., 2001). Although the early attempt to produce active recombinant Arabidopsis DGAT2 (AtDGAT2) was unsuccessful, functional DGAT2 was isolated and characterized from plants, including tung tree, castor bean, ironweed, and flax, on the basis of the putative AtDGAT2 sequence (Kroon et al., 2006; Li et al., 2010a; Pan et al., 2013; Shockey et al., 2006; Xu et al., 2018).

**Physiological Roles of DGAT1 and DGAT2**

DGAT1 is considered to play a critical role in determining the flux of carbon into seed TAG in some species (Harwood et al., 2013). In oilseed crops such as canola-type B. napus and safflower (Carthamus tinctorius), the level of DGAT activity was found to be coordinated with oil accumulation during seed development (Tzen et al., 1993; Weselake et al., 1993). Expression analysis of DGAT1 revealed that this gene is highly expressed in developing embryos in many oilseed crops (Hobbs et al., 1999; Lu et al., 2003) and its expression level is correlated with oil deposition during seed development (Li et al., 2010b). Forward and reverse genetics strategies brought about a more direct piece of evidence that DGAT1 is a major determinant in oil accumulation (Katavic et al., 1995; Zheng et al., 2008; Zou et al., 1999). DGAT1 inactivation resulted in a dramatic decrease in seed oil levels in the Arabidopsis mutant AS11 (Katavic et al., 1995; Zou et al., 1999). Consistently, the activation of DGAT1 [by a phenylalanine insertion in the maize (Zea mays) DGAT1] was responsible for the increased embryo oil content in a high-oil maize line (Zheng et al., 2008). Furthermore, DGAT1 appears to play a role in freezing and/or drought stress responses in Arabidopsis, Boechera stricta, and B. napus. The expression of DGAT1 was found to be highly cold responsive and correlated with the cold tolerance in B. stricta lines (Arisz et al., 2018). Consistently, enhanced DGAT1 expression led to increased freezing tolerance in Arabidopsis (Arisz et al., 2018), whereas Arabidopsis DGAT1 (AtDGAT1) mutant lines were sensitive to freezing (Tan et al., 2018). In addition, overexpression of DGAT1 during seed development in B. napus was shown to decrease the penalty on seed oil content caused by drought (Weselake et al., 2008).

Unlike the substantial contribution of DGAT1 to seed oil accumulation, DGAT2 appears to play a minor role in regulating oil production. The expression of AtDGAT2 did not restore the TAG-synthesizing ability in the S. cerevisiae mutant H1246 (Zhang et al., 2009), and the AtDGAT2 mutants did not show any changes in TAG accumulation (Zhang et al., 2009). Recently, the functionality of AtDGAT2 in TAG biosynthesis was confirmed in N. benthamiana leaves by transient expression of the encoding cDNA (complementary DNA) (Zhou et al., 2013), and S. cerevisiae yeast by heterologous expression using a codon-optimized version of the cDNA (Aymé et al., 2014). Nevertheless, the physiological role of DGAT2 in Arabidopsis remains to be further explored. Interestingly, by characterizing DGAT2 from different plant species accumulating unusual fatty acids, it was revealed that DGAT2 appears to be important for incorporating unusual fatty acids, such as eleostearic acid (18:3trans,11trans,13trans) from tung tree (Shockey et al., 2006), ricinoleic acid from castor (Kroon et al., 2006), and vernolic acid (cis-12-epoxy-octadeca-cis-9-enolic acid) from ironweed (Li et al., 2010a) into storage TAG. Indeed, the expression of DGAT2 during embryo development was found to be at a higher level than DGAT1 expression in the developing seeds from plants accumulating unusual or polyunsaturated fatty acids (Kroon et al., 2006; Li et al., 2010a; Pan et al., 2013; Shockey et al., 2006), whereas DGAT2 transcripts in Arabidopsis and soybean were far below the levels observed for DGAT1 transcripts (Li et al., 2010b). It appears that TAG production by DGAT1 occurs in a distinct ER subdomain than that of DGAT2, since tung tree DGAT1 and DGAT2 were found to localize to different regions of the ER and they differ in substrate preference (Shockey et al., 2006). The physiological roles of DGAT1 and DGAT2 in microalgae also remain largely to be explored. For example, in most of the microalgal species, one DGAT1 and one to multiple DGAT2 genes appear to contribute to the complexity of TAG biosynthesis (Chen and Smith, 2012; Gong et al., 2013; Liu and Benning, 2013; Turchetto-Zolet et al., 2011), and it is still unknown why microalgae need these many redundant copies of DGAT2.

In mammals and yeasts, DGAT2 rather than DGAT1 appears to be the dominant enzyme for TAG synthesis. Knocking out of dgat2 in mouse (Dgat2<sup>−/−</sup>) led to lipopenia, abnormal skin, and early death (Stone et al., 2004), whereas dgat1 defective mouse (Dgat1<sup>−/−</sup>) was viable and capable of TAG synthesis (Smith et al., 2000). While the DGAT2 almost ubiquitously exists in all eukaryotes, DGAT1 is missing in the genome of certain yeasts (S. cerevisiae and Candida albicans) and fungi (Laccaria bicolor, Schizophillum commune, and Agaricus bisporus).
Turchetto-Zolet et al., 2011). DGAT2 is likely the primary TAG-synthesizing enzyme in the yeast *Yarrowia lipolytica*, which also has a *DGAT1* gene (Zhang et al., 2012).

**Structural and Functional Features of DGAT**

DGAT1 and DGAT2 are integral membrane-bound proteins with multiple transmembrane domains (TMD) (for review, see Liu et al., 2012). Currently, there is no three-dimensional structure available for any DGAT or closely homologous enzymes (Liu et al., 2012; Lopes et al., 2015). The recent insights into DGAT structure–function relationships largely rely on the identification of possible functional motifs and the determination of putative membrane topologies. Very recently, the structure of the hydrophilic N-terminal domain of DGAT1 from *B. napus* (BnaDGAT1) was solved, which resulted in a leap forward in gaining insight into the biochemical regulation of this enzyme family (Caldo et al., 2017). Below, the structural and functional features of plant DGAT with relevant reference to the mammalian literature are discussed.

DGAT1 is composed of about 500 amino-acid residues having a large hydrophilic N-terminal region, followed by 8 to 10 predicted TMD (Fig. 2a; Liu et al., 2012). The N-terminal region of DGAT1 is highly variable and is encoded by the first exon, which is separate from the exons

![Fig. 2 Legend on next coloumn.](Image)
encoding the rest of the polypeptide (Greer et al., 2015; Liu et al., 2012). The N-terminal regions of *B. napus* and mouse DGAT1 enzymes were demonstrated to bind acyl-CoA in a sigmoidal fashion, suggesting positive cooperative binding (Siloto et al., 2008; Weselake et al., 2006). In addition, the N-terminal region of *B. napus* DGAT1 formed dimers and tetramers based on crosslinking experiments (Weselake et al., 2006). Consistently, analysis of mouse and *B. napus* DGAT1 showed that the N-terminal region plays a role in self-oligomerization (Caldo et al., 2017; McFie et al., 2010). Furthermore, the hydrophilic N-terminal region of *B. napus* DGAT1 was shown to constitute the enzyme’s regulatory domain, which is not necessary for catalysis (Caldo et al., 2017). This domain is comprised of two distinct segments, specifically an intrinsically disordered region (IDR) and a folded segment (Fig. 2a). The IDR can form interactions that are important for dimerization and may allow it to partially mediate positive co-operativity. Truncation of this IDR resulted in a more active enzyme form, suggesting the IDR encompasses an autoinhibitory motif. This observation agrees with a previous study on mouse DGAT1, wherein removal of N-terminal fragments led to increased normalized enzyme activity (McFie et al., 2010). The solution NMR structure of the folded segment of the N-terminal region of *B. napus* DGAT1 showed that it is composed of an α-helix near the first predicted TMD (Caldo et al., 2017). Loops and coils connected this helix to the IDR. The loop near the α-helix was shown to contain the allosteric site for acyl-CoA and CoA, which serves as a homotropic activator and a feedback inhibitor of the enzyme, respectively (Caldo et al., 2017). The small-angle X-ray scattering structure of this domain showed that the monomer has a highly extended structure, exhibiting various heterogeneous conformations. While the domain is predominantly disordered, it appears to exhibit a small gain in the secondary structure upon binding to acyl-CoA and CoA. Altogether, these studies demonstrated that the hydrophilic N-terminal domain of *B. napus* DGAT1 comprises a regulatory domain that positively and negatively affects enzyme activity.

The remainder of DGAT1 accounting for more than 75% of the enzyme contains the TMD and the catalytic sites. The TMD is expected to form helical bundles in the membrane, which agrees with the circular dichroism profile of purified BnaDGAT1 indicating the predominance of α-helices (Caldo et al., 2017). DGAT1 belongs to a family of enzymes named membrane-bound O-acyltransferases (MBOAT), which were proposed to have highly conserved arginine and histidine residues. Sequence analysis coupled with mutational studies showed a conserved histidine near the C-terminus of mouse DGAT1 represents one of the active site residues (Fig. 2a; McFie et al., 2010). In the same study, mouse DGAT1 was shown to have three TMD, with a cytosolic N-terminus and a C-terminus inside the ER lumen. In contrast, tung tree DGAT1 appeared to have two termini localized in the cytosol, suggesting the presence of even-numbered TMD (Shockey et al., 2006). Furthermore, it was noted that human DGAT1 may have dual topologies as DGAT1 activity was present on both faces of the ER (Wurie et al., 2011). In addition to topological analysis, the membrane-embedded region was also probed for possible substrate binding sites. Two peptide fragments corresponding to the putative binding sites of bovine (*Bos taurus*) DGAT1 were investigated via synchrotron circular dichroism spectroscopy (Lopes et al., 2014). The first peptide spanning the motif, FYxDWWN, was shown to bind the acyl group of acyl-CoA (Fig. 2a). The second peptide having a candidate DAG-binding site (HKWxxRHxYxP), which also exists in protein kinase C and diacylglycerol kinase, interacted with DAG.

DGAT2 is a member of the DGAT2/acyl-CoA:monoacylglycerol acyltransferase family, which also includes acyl-CoA:monoacylglycerol acyltransferases and wax synthases (McFie et al., 2010). It is completely different than DGAT1, being shorter in length and having less TMD (Fig. 2b). The membrane topologies of mouse and *S. cerevisiae* DGAT2 were experimentally determined. Mouse DGAT2 has two TMD with cytosolic N- and C-termini (Stone et al., 2006). On the other hand, *S. cerevisiae* DGAT2 has four TMD, and as in mouse DGAT2, both the N- and C-termini were localized in the cytosol (Liu et al., 2011). The topology of plant DGAT2 has not been determined yet, although preliminary analysis of tung tree DGAT2 showed that both termini are also found in the cytosol (Shockey et al., 2006). The first 30–50 amino-acid residues of mouse and yeast DGAT2 were shown to be not essential for catalysis (Liu et al., 2011; Stone et al., 2006).

No detailed structural analysis of DGAT2 has been reported although various motifs have been proposed to serve as important binding or active sites. In mouse DGAT2, an FLXLXXXn (n = nonpolar amino acid) motif is proposed as a binding site for neutral lipid, and substitution of either of the first two residues in this motif resulted in decreased DGAT activity (Fig. 2b). Furthermore, the substitution of the second leucine residue in mouse DGAT2 resulted in enzyme inactivation (Stone et al., 2006). As for putative active site residues, a conserved HPHG motif has been implicated to play an important function, since substitution of any residue within this motif either led to lower activity or complete enzyme inactivation (Liu et al., 2011; Stone et al., 2006). It should be noted, however, that the topologies of mouse DGAT2 and *S. cerevisiae* DGAT2 (ScDGAT2) did not agree at certain regions. The HPHG motif, for example, is in the cytosol in mouse DGAT2 but is found within the membrane in ScDGAT2. Other conserved motifs with potentially important function identified
in DGAT2 include motifs YFP, RXGFX(K/R)XAXXXGXX(L/V)VPXXXFG(E/Q), and GGXXE (Liu et al., 2012). An ER retrieval motif responsible for the steady state localization of DGAT2 protein in the ER was identified near the C-terminus of tung tree DGAT2 (Shockey et al., 2006). Even though this ER retrieval motif is also present in mouse DGAT2, deletion of this region in mouse DGAT2 did not affect the targeting of the mouse acyltransferase into the ER (McFie et al., 2011).

**Regulation of DGAT**

The activity of enzymes can be regulated at the transcriptional, translational, and post-translational levels. Enzymes can differ in their temporal and spatial expression in plants. The expression profile of DGAT genes has been determined in different plant species particularly in oil crops. In Arabidopsis, DGAT1 was expressed in different plant organs such as leaves, roots, flowers, siliques, seeds, and seedlings. The last two of which exhibited the highest expression levels (Zou et al., 1999). The high expression of AtDGAT1 in developing seeds and pollen correlates with the ability of these organs to accumulate high amounts of TAG (Lu et al., 2003). In addition, DGAT1 was expressed at lower levels in shoots and roots of seedling, which are sites exhibiting active cell division and growth. DGAT1 was suggested to be involved in maintaining a balance of DAG and acyl-CoA for the biosynthesis of membrane lipids and recycling of fatty acids to TAG under conditions where catabolic reactions are halted (Lu et al., 2003). AtDGAT2, however, was expressed at a lower level in seeds compared to other tissues (Li et al., 2010b). Similar to Arabidopsis, the expression level of soybean DGAT1 was much higher relative to DGAT2 throughout seed development (Li et al., 2010b). In contrast, oil crops accumulating unusual fatty acids have higher DGAT2 transcript levels in developing seeds than Arabidopsis and soybean, supporting the possible role of DGAT2 in the accumulation of unusual fatty acids in seed oil (Kroon et al., 2006; Li et al., 2010b; Shockey et al., 2006).

Transcription factors affecting the extent of oil accumulation have been identified including LEAFY COTYLEDON genes (LEC1, LEC2, LIL, and FUS3), ABSCISIC ACID INSENSITIVE (ABI), and WRINKLED1 (WRI) (Santos-Mendoza et al., 2008). These transcription factors have been shown to be involved in the upregulation of genes in late glycolysis and fatty-acid biosynthesis. ABI and WRI were shown to have a direct effect on DGAT1 expression (Fig. 3). ABI4 was found to bind to the AtDGAT1 promoter and activate transcription under stress conditions, such as nitrogen deficiency (Yang et al., 2011) and increased sucrose content (Wind et al., 2013). Similar to ABI4, ABI5 was also shown to synergistically regulate the expression of DGAT1 under stress (Kong et al., 2013). In addition, overexpression of WRI in B. napus increased the expression of DGAT1 together with GPAT9 and LPAT2, although the specific promoter sequence has yet to be identified (Li et al., 2015b). Very recently, the R2R3-type MYB96 transcription factor was shown to regulate TAG biosynthesis by directly activating the expression of DGAT1 and PDAT1 (Fig. 3) (Lee et al., 2018). DGAT1 expression is regulated by MYB96 through binding to the promoter of ABI4, whereas MYB96 regulates PDAT1 expression by directly binding to the PDAT1 promoter (Lee et al., 2018). Transcription factors directly influencing the expression of DGAT2 in plants, however, have not been identified.

After translating a folded and functional enzyme, its activity can be regulated directly through the binding of allosteric effectors and/or post-translational modifications. DGAT1 has been shown to be modulated by its substrate, acyl-CoA, which binds to an allosteric site at the N-terminus (Caldo et al., 2017; Weselake et al., 2006). The presence of this allosteric site for acyl-CoA agrees with kinetic studies of microsomal and purified plant DGAT1 showing that DGAT1 exhibits positive cooperativity with acyl-CoA (Caldo et al., 2017; Roesler et al., 2016; Xu et al., 2017). Interestingly, CoA was identified as a feedback inhibitor of BnaDGAT1 and was shown to bind to the same allosteric site for acyl-CoA. It is thus possible that the hydrophilic N-terminal domain can act as a sensor of the acyl-CoA:CoA ratio, enabling enzyme activity to adjust to the availability of substrates (Fig. 4). Similarly, acyl-CoA and acyl-ACP were identified as feedback inhibitors of the acetyl-CoA carboxylase, the regulatory enzyme in fatty-acid biosynthesis (Andre et al., 2012; Davis and Cronan, 2013).
domain upon activating the enzyme. This activator that can initiate the transition into the more active state possibly by relieving autoinhibition. PtdOH may interact with the N-terminal group, to further inactivate the enzyme (Caldo et al., 2018). An unknown protein phosphatase may be involved in the dephosphorylation process. Finally, phosphatidic acid (PtdOH) serves as a feedforward inactivate the enzyme whereas the binding of acyl-CoA induces the transition into the moderately active state. SnRK1 can add a phosphoryl group, to further inactivate the enzyme (Caldo et al., 2018). An unknown protein phosphatase may be involved in the dephosphorylation process. Finally, phosphatidic acid (PtdOH) serves as a feedforward activator that can initiate the transition into the more active state possibly by relieving autoinhibition. PtdOH may interact with the N-terminal domain upon activating the enzyme. This figure was adapted from Caldo et al., 2018.

2001). Furthermore, PtdOH was identified as a feedforward activator of plant DGAT1 (Caldo et al., 2018). PtdOH was suggested to aid in relieving possible autoinhibition by interacting with the N-terminal regulatory domain spanning the autoinhibitory motif and convert DGAT1 to a more active state that is also less sensitive to substrate inhibition (Fig. 4). The activity of microsomal DGAT activity has been shown to be activated by proteins such as acyl-CoA-binding protein, bovine serum albumin, and human acylation-stimulating protein (Hobbs and Hills, 2000; Little et al., 1994; Weselake et al., 2000; Yurchenko et al., 2014), although specific plant regulatory proteins modulating DGAT1 has yet to be identified. Furthermore, microsomal DGAT activity in Arabidopsis was inhibited by niacin (Hobbs and Hills, 2000); this compound was later shown to noncompetitively inhibit mouse DGAT2 (Ganjii et al., 2004).

DGAT1 is also regulated through phosphorylation/dephosphorylation (Ghiliebert et al., 2011). Initial sequence analysis indicated that mouse DGAT1 contains phosphorylation consensus sequences for tyrosine kinase and protein kinase A as well as protein kinase C (Yen et al., 2008). Later reports, however, demonstrated that a number of these predicted phosphorylation sites had a negligible effect on enzyme activity (Han, 2011; Humphrey et al., 2013). Furthermore, a recent study on mouse DGAT1 identified 24 potential phosphorylation sites and confirmed phosphorylation in several sites through mass spectrometry (Yu et al., 2015). Mutation of three serine phosphorylation sites (S83, S86 and S89) to glutamate to mimic phosphorylation in several sites through mass spectrometry (Yu et al., 2015). Mutation of three serine phosphorylation sites (S83, S86 and S89) to glutamate to mimic phosphorylation also resulted in enzyme variants with higher activity. Previously, a putative sucrose nonfermenting1-related kinase1 (SnRK1) phosphorylation site was also identified in T. majus DGAT1 (Xu et al., 2008). When this residue was mutated to alanine, an increase in enzyme activity was observed. In addition, the overexpression of DGAT1 with the mutated SnRK1 site translated to higher seed TAG levels in Arabidopsis when compared to an unmodified enzyme. A recent study reported that purified BnaDGAT1 can be phosphorylated and inactivated by SnRK1, further confirming the earlier investigation through mutagenesis (Fig. 4; Caldo et al., 2018). In addition, SnRK1 has also been found to act on the WRI transcription factor (Zhai et al., 2017), which subsequently regulates DGAT expression. Similar observations were also noted in studies involving the mammalian AMP-activated protein kinase (AMPK), which corresponds to the SnRK1 enzyme in plants. Elevated AMPK activity reduced the expression levels of transcription factors and lipogenesis-related genes (e.g. DGAT1 and DGAT2), resulting in decreased TAG accumulation in mouse (Yin et al., 2015). It may be possible that mammalian AMPK can also modulate DGAT1 activity directly as observed in plant systems, since AMPK is a member of a kinase family implicated in transcriptional and post-translational regulation (Ghiliebert et al., 2011). Overall, these lines of evidence showed that DGAT1 can be regulated by phosphorylation/dephosphorylation. As for DGAT2, there are no reports discussing the possible regulation of this isoenzyme by phosphorylation/dephosphorylation. The PhosPhat database indicated that there was no phosphorylated site identified yet in AtDGAT2. On the other hand, human DGAT2 was shown to be regulated by ubiquitination via gp78, which is an E3 ligase facilitating ER-associated degradation (Choi et al., 2014).

Soluble DGAT, Diacylglycerol Acetyltransferases, and Other Enzymes with DGAT Activity

The DGAT3 gene family encodes a soluble enzyme localized in the cytosol. The first DGAT3 was isolated from
peanut (*A. hypogea*) through protein purification (Saha et al., 2006). Somewhat later, *DGAT3* was also identified in *Arabidopsis* (Hernández et al., 2012; Peng and Weselake, 2011). Another soluble enzyme with DGAT activity was also identified in *Arabidopsis* and termed defective cuticle ridge (*DCR*) (Rani et al., 2010). The physiological roles of soluble DGAT in plants, however, remain largely to be explored. It has been reported that *Arabidopsis* *DGAT3* appears to be involved in recycling of linoleic acid (18:2Δ9cis, 12cis) and α-linolenic acid (18:3Δ9cis, 12cis, 15cis) into TAG when TAG breakdown was blocked (Hernández et al., 2012), whereas *DCR* is likely to be related to the biosynthesis of cutin rather than seed oil (Rani et al., 2010).

Unlike the puzzling roles of soluble DGAT in plants, several pieces of strong evidence support the involvement of DGAT3 in TAG biosynthesis in microalgae and diatoms. For instance, the involvement of DGAT3 in TAG biosynthesis in the diatom *Phaeodactylum tricornutum* was confirmed by heterologous expression in *S. cerevisiae* mutant H1246 (Cui et al., 2013). Similarly, a novel DGAT exclusive to green microalgae with moderate similarity to plant DGAT3 was found to participate in the chloroplastidial *de novo* synthesis of TAG (Bagnato et al., 2017). Moreover, many other TAG-biosynthetic enzymes, including GPAT, LPAAT, phosphatidic acid phosphatase, and acyl-CoA: monoacylglycerol acyltransferase, have been previously reported to exist in soluble forms (Ghosal et al., 2007; Han et al., 2006; Ichihara et al., 1990; Tumaney et al., 2001; Turnbull et al., 2001). Thus, it is possible that these soluble TAG-biosynthetic enzymes might use different substrate pools and have different physiological roles from the membrane-bound isoforms.

In addition to the aforementioned DGAT, other enzymes with DGAT activity were also identified. One example is the bifunctional wax synthase/DGAT, which predominantly catalyzes the formation of wax esters. This enzyme was first identified in *Acinetobacter* (Kalscheuer et al., 2003) and later characterized in *Arabidopsis* (Li et al., 2008). Another enzyme with putative DGAT activity is chloroplastic phytyl ester synthase. Two *Arabidopsis* chloroplastic phytyl ester synthases were characterized and shown to be involved in fatty-acid phytyl ester synthesis in chloroplasts (Lippold et al., 2012). A special DGAT (diacylglycerol acyltransferase) utilizing acetyl-CoA rather than acyl-CoA as an acyl donor was also identified in *E. alatus*. This unique DGAT catalyzes the formation of 3-acetyl-1, 2-diacyl-sn-glycerol rather than TAG, and the resulting acetyl-TAG has lower viscosity than normal oil (Durrett et al., 2010). The acetyl-CoA utilizing DGAT also belongs to the MBOAT family. Recently, the topology model of diacylglycerol acetyltransferase from *E. alatus* was experimentally determined (Tran et al., 2017). The model shows four TMD with both the N- and C-termini orientated toward the lumen side of the ER (Fig. 2c). In addition, the MBOAT signature region containing the putative histidine active site is embedded in the third TMD in close proximity with the interface between the membrane and the cytosol, and thus may be readily accessible by the cytosolic acetyl-CoA substrate.

**Acyl-CoA-Independent Formation of TAG**

The Kennedy pathway provides a straightforward route toward the formation of TAG using acyl-CoA and Gro3P. As previously discussed, in many species, however, TAG assembly is intricately associated with membrane metabolism (Fig. 1). PDAT catalyzes the transfer of the acyl moiety at the sn-2 position of PtdCho or phosphatidylethanolamine to the sn-3 position of sn-1, 2-DAG, yielding TAG and sn-1-lyso-PtdCho or sn-1-lysophosphatidylethanolamine (Dahlqvist et al., 2000; Ståhl et al., 2004).

The identification of PDAT has historically lagged behind that of DGAT probably because of its exclusive presence in plants, algae, and yeast and its absence in mammals (for review, see Chen et al., 2015). In 2000, PDAT activity was first identified in microsomal preparations of the developing seeds from sunflower (*Helianthus annuus*), castor bean (*R. communis*), and *Crepis palaestina* by Stymne and coworkers (Dahlqvist et al., 2000). In the same study, they also isolated the first PDAT gene (YNR008w, *LR01*) from yeast (*S. cerevisiae*). Yeast PDAT has homology with mammalian lecithin:cholesterol acyltransferase (*LCAT*), which catalyzes the acyl-CoA-independent formation of cholesteryl esters by transferring the acyl group from PtdCho to cholesterol. In addition, *S. cerevisiae* PDAT also displayed low DAG:DAG transacylase activity (Ghosal et al., 2007). In *S. cerevisiae* yeast, PDAT and DGAT2 are the major contributors to TAG biosynthesis and their relative contributions were dependent on the yeast growth stage (Oelkers et al., 2002). PDAT contributed predominantly to yeast TAG accumulation during the exponential growth stage, whereas DGAT2 was involved in the majority of yeast TAG biosynthesis at the stationary growth stage (Oelkers et al., 2002).

Subsequently, two PDAT orthologs, *AtPDAT1* (*At5g13640*) and *AtPDAT2* (*At3g44830*), with 57% amino-acid sequence, were identified in *Arabidopsis* based on sequence homology to yeast PDAT (Ståhl et al., 2004). *AtPDAT1* is expressed generally at higher levels in vegetative tissues than in seeds, whereas *AtPDAT2* is highly expressed in seeds (Pan et al., 2015; Ståhl et al., 2004) (for detailed information on expression pattern, refer to the AtGenExpress database [http://jsp.weigelworld.org/expviz/expviz.jsp; accessed on 10 July 2018] or *Arabidopsis* eFP Browser [http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi; accessed on 10 July 2018]. In contrast with the situation in
yeast where PDAT is a major determinant of TAG biosynthesis at the exponential growth stage, the contribution of PDAT to TAG biosynthesis in Arabidopsis seeds was unclear. Overexpression of AtPDAT1 resulted in no effects on the fatty-acid and lipid composition, despite the fact that increased PDAT activity was observed in microsomes prepared from AtPDAT1 Arabidopsis overexpressor lines (Ståhl et al., 2004). In addition, no change in the fatty-acid content or composition was observed from the seeds of an Arabidopsis knockout line with a T-DNA insertion in the AtPDAT1 locus (Mhaske et al., 2005).

The contribution of PDAT to Arabidopsis seed TAG accumulation was not realized until AtPDAT1 was suppressed by RNA interference (RNAi) in a dgat1 knockout background (Zhang et al., 2009). In a dgat1 knockout background, RNAi silencing of AtPDAT1 resulted in up to 63% further oil content reduction compared with the dgat1 control, whereas neither silencing of AtPDAT2 nor AtDGAT2 showed further reduction in the oil content (Zhang et al., 2009). These results suggested that PDAT1 is a dominant determinant in Arabidopsis seed TAG biosynthesis in the absence of DGAT1 activity. Consistently, AtPDAT1 expression was found to be highly upregulated in the seeds of the AtDGAT1 mutant, whereas the expression of AtPDAT2 and AtDGAT2 was only marginally affected (Xu et al., 2012). Furthermore, the Arabidopsis pdat1 dgat1 double mutant displayed abnormal seed and pollen development (Zhang et al., 2009), indicating that PDAT1 and DGAT1 have overlapping functions in TAG biosynthesis in developing seeds and pollen, which are essential for their normal development.

Recently, PDAT has been suggested to play a crucial role in mediating TAG biosynthesis in leaves. PDAT functions in diverting fatty acids from membrane lipids to TAG before peroxisomal β-oxidation, thereby maintaining leaf membrane homeostasis in Arabidopsis (Fan et al., 2013a, 2013b, 2014). The relative contribution of PDAT1 and DGAT1 to leaf TAG biosynthesis has been examined. Arabidopsis pdat1 mutant displayed a 57% reduction in the TAG content in developing leaves whereas only 31% decrease in the TAG level was observed in the dgat1 mutant (Fan et al., 2013b). In contrast to the sevenfold increase in PDAT1 overexpressing lines, there was only a marginal increase in leaf TAG levels in DGAT1 overexpressing Arabidopsis lines (Fan et al., 2013b). These results suggested that PDAT1 may play a more important role in TAG synthesis in young leaves than DGAT1. Pulse-chase radiolabelling of pdat1 and dgat1 mutants using [14C] 12:0, however, showed that the reduction in TAG accumulation was more severe in the dgat1 mutant rather than the pdat1 mutant, suggesting that DGAT1 is mainly responsible for the TAG biosynthesis in Arabidopsis leaves (Tjellström et al., 2015). Considering medium chain fatty acids such as 12:0 are rarely incorporated into the sn-2 of PtdCho, which is the substrate of PDAT, it is likely that the relative use of PDAT and DGAT in TAG biosynthesis in leaves is dependent on the substrates and acyl flux conditions within the cell (Bates, 2016). Besides Arabidopsis, PDAT genes have also been identified and characterized in various plant and microalgal species, including castor (Kim et al., 2011; van Erp et al., 2011), flax (Pan et al., 2013), Camelina sativa (Aznar-Moreno and Durrett, 2017; Yuan et al., 2017), green algae C. reinhardtii (Yoon et al., 2012), and green algae Myrmeceia incise (Liu et al., 2016b). It should be noted that the PDAT nomenclature in the literature lacks consistency. For example, flax PDAT 2 and 4 are homologs of AtPDAT1 whereas flax PDAT 3 and 6 are AtPDAT2 homologs (Pan et al., 2015).

Although a T-DNA insertion in the AtPDAT1 locus led to no effect on the fatty-acid content or composition in Arabidopsis (Mhaske et al., 2005), Aznar-Moreno and Durrett (2017) introduced mutations in genes encoding PDAT1 in C. sativa (an AtPDAT1 homolog) using the CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein) system and observed reduced seed oil content and altered fatty-acid composition (e.g. decreased linoleic acid content) in many transgenic lines, supporting the contribution of PDAT1 in seed oil biosynthesis. Consistently, microRNA-mediated downregulation of PDAT1 in C. sativa led to a decrease in the linoleic acid content, whereas overexpression of PDAT1 had an opposite effect (Marmor et al., 2017). The changes in fatty-acid composition in these PDAT1 overexpression or downregulation lines, however, were not accompanied by any significant effects on the total oil content. In the same study, the relative importance of C. sativa PDAT and DGAT in oil accumulation in developing seeds and in different embryo sections was examined. DGAT1 appears to dominate the TAG assembly in cotyledons, while PDAT1 may compensate for TAG accumulation in the absence of DGAT1 by specifically routing linoleic acid from PtdCho into TAG. Unlike PDAT1, PDAT2 homologs from Arabidopsis (AtPDAT2), flax (LuPDAT3 and LuPDAT6), and castor bean (RePDAT2) did not show an apparent function in TAG biosynthesis (Kim et al., 2011; Pan et al., 2013; Ståhl et al., 2004; van Erp et al., 2011).

In some plants, PDAT is likely to be a key player in directing modified fatty acids from PtdCho into TAG by displaying unique specificity for the acyl group in PtdCho (Kim et al., 2011; Pan et al., 2013; van Erp et al., 2011). PDAT from castor has been shown to preferentially catalyze the incorporation of ricinoleoyl and vernoloyl groups into TAG in vitro, while C. palaestina PDAT catalyzes the incorporation of vernoloyl groups (Dahlqvist et al., 2000). Consistent with the in vitro results, overexpression of caspore PDAT in Arabidopsis led to large accumulation of
hydroxy fatty acids in the seed oil (Kim et al., 2011; van Erp et al., 2011). Similarly, specialized PDAT for selectively incorporating α-linolenic acid into TAG was also identified in flax (Pan et al., 2013). These PDAT with unique substrate selectivity, including castor PDAT (RcPDAT1A) and flax PDAT (LuPDAT1 and LuPDAT5), have seed-specific expression patterns and are grouped into a single clade that is different from those of PDAT1 and PDAT2 (Pan et al., 2015).

In oilseed crops accumulating high-oleic acid such as B. napus, PDAT may be less important than DGAT in affecting seed oil accumulation, since neither the in vitro enzyme activity nor the transcripts abundance for PDAT or its encoding gene was higher than that of DGAT (Tang et al., 2012; Troncoso-Ponce et al., 2011). This is also supported by a recent detailed lipidomic analysis of developing B. napus seeds, in which the relative contributions of DGAT and PDAT were predicted based on the patterns of their molecular substrates (Woodfield et al., 2018). Furthermore, it has also been recently suggested that PDAT appears to function in stress responses in Arabidopsis (Mueller et al., 2017), C. sativa (Yuan et al., 2017), and green algae (Liu et al., 2016b; Yoon et al., 2012). For instance, PDAT1-mediated TAG accumulation was found to increase the heat resistance of Arabidopsis seedlings (Mueller et al., 2017).

Structure–function studies on PDAT have only been limited to in silico analysis. Phylogenetic analysis showed that plant PDAT can be grouped into four clades, two of which have one putative TMD while the other two are predicted to be entirely soluble (Pan et al., 2015). The majority of PDAT in the database have the single-predicted TMD consisting of a small cytosolic N-terminus and a large C-terminal domain in the ER lumen. The N-terminal region is hydrophilic with arginine clusters similar to those observed in DGAT1 (Liu et al., 2012). The removal of the putative N-terminal TMD in S. cerevisiae PDAT did not affect activity (Ghosal et al., 2007). Similar to LCAT-like proteins, PDAT has the conserved features of human LCAT (Peelman et al., 1999). Sequence alignment of PDAT enzymes identified the highly conserved residues constituting a catalytic triad, specifically a histidine residue and an aspartate residue near the C-terminus and an internal serine residue. Other conserved features include a salt bridge between an aspartate residue and an arginine residue and a lid region with a tryptophan residue, which is proposed to bind released fatty acid for efficient acylation. PDAT exhibits homology to human LCAT (26% identity) and phospholipase A2 (27% identity), the structure of which was recently elucidated (Glukhova et al., 2015; Piper et al., 2015). Using the phospholipase A2 structure, the AtPDAT1 model was determined using PHYRE software with a high confidence level (Fig. 2d; http://www.sbg.bio.ic.ac.uk/phyre2; Kelley et al., 2015), giving a preliminary glance at the possible orientation of the aforementioned functional motifs. In the model, the components of the aforementioned functional motifs are located close to one another, possibly forming contacts through hydrogen bonding. The lid region is composed of loops and a β-sheet on one face of the structure and the salt bridge is in close proximity interacting through electrostatic interactions. About 53% of the polypeptide was modeled using the phospholipase A2 structure. PDAT1 has a long N-terminal sequence that has been predicted to encompass a TMD that is not included in the model (Fig. 2d).

In contrast to DGAT-catalyzed reaction, the equilibrium of which lies far to the right because of the cleavage of a high-energy thioester bond of acyl-CoA and the formation of a carbon–oxygen (ester) bond, PDAT-catalyzed TAG formation uses PtdCho as the acyl donor and is considered thermodynamically neutral. Although the reaction mechanisms of PDAT are still a mystery, some insights might be gained from the work on mammalian LCAT, which shares some homology with PDAT from yeast and plants (Dahlqvist et al., 2000; Pan et al., 2013; Stähli et al., 2004). The recently elucidated structures of human LCAT revealed that the catalytic mechanism of LCAT is similar to that of phospholipase A2, in which the lid-loop can move aside from a tunnel opening allowing lipids to enter the active site and interact with the catalytic triad (serine residue 181, aspartate residue 345, and histidine residue 377) (Glukhova et al., 2015; Piper et al., 2015). During catalysis, serine residue 181 of the catalytic triad functions as a nucleophile residue by attacking the sn-2 acyl-chain of PtdCho and, subsequently, the cleaved acyl chain is covalently bound to the serine residue to form an acyl-enzyme intermediate before transfer to cholesterol to generate a cholesteryl ester. Unlike the acyl-CoA-dependent acyltransferases requiring an activated acyl donor, LCAT uses itself as the donor in acyl transfer and thus it is very likely that the acyl-enzyme intermediate has a high chemical potential (Segrest et al., 2015). In addition, recent molecular simulation results suggested that the acyl-LCAT intermediate can facilitate the entry of cholesterol to the active site by decreasing the free-energy cost (Casteleijn et al., 2018).

Biotechnological Applications of Plant DGAT and PDAT

A rising demand for vegetable oil has been witnessed across the globe due to a ballooning human population and increased consumption of plant oil for food, feed, biofuel, and industrial applications (for reviews, see Durrett et al., 2008; Weselake et al., 2009). As a consequence, various biotechnological strategies have been used to increase the flow of carbon into TAG in oilseeds, vegetative tissues, and microalgae. The importance of DGAT in
governing the flux of substrates into TAG was first reported by Katavic et al. (1995), wherein an Arabidopsis ethyl methanesulphonate (EMS) mutant with inactivated DGAT1 exhibited low TAG levels. PDAT is another dominant determinant in plant seed TAG biosynthesis especially in the absence of DGAT1 activity (Zhang et al., 2009). In some plants, PDAT is also characterized as a key player in contributing to directing modified fatty acids from PtdCho into TAG (Dahlqvist et al., 2000). Thus far, DGAT and PDAT cDNA have been extensively used as biotechnological tools in many studies aimed at increasing the oil content and modifying the fatty-acid composition of oils in seeds of higher plants, vegetative tissues, and microalgae (Table 1).

**Increasing Seed Oil Content**

Jako et al. (2001) initially demonstrated that the overexpression of native DGAT1 in Arabidopsis resulted in increased oil accumulation and seed weight. Increased TAG content was also obtained upon overexpression of Arabidopsis and native DGAT1 in canola-type B. napus under either greenhouse or field conditions (Taylor et al., 2009; Weselake et al., 2008). It was further noted that DGAT1 overexpression improved B. napus oil accumulation when compromised under drought conditions (Weselake et al., 2008). The overexpression of T. majus DGAT1 in Arabidopsis, high-erucic-acid rapeseed, and canola-type B. napus also led to enhanced seed oil content (Xu et al., 2008). Following these aforementioned studies, many groups have further used DGAT1 from different species to boost seed content in various crops such as G. max (Hatanaka et al., 2016; Roesler et al., 2016), B. juncea (Savadi et al., 2015), Z. mays (Alamedin et al., 2017), C. sativa (Kim et al., 2016), and Jatropha curcas (Maravi et al., 2016). Moreover, overexpression of DGAT1 from microalgae, such as Chlorella ellipsoidea and Nanochloropsis oceanica, also led to increased oil content in Arabidopsis and B. napus (Guo et al., 2017; Zienkiewicz et al., 2017). Furthermore, DGAT1 has been used to increase the proportion of unusual fatty acids in seed oil, particularly epoxy fatty acid in G. max (coexpressed with an EPOXYGENASE gene; Li et al., 2010a) and capric acid in C. sativa (in combination with fatty acyl-ACP thioesterase B1 and LPAAT from Cuphea viscosissima; Iskandarov et al., 2017). Similar to DGAT1, seed-specific overexpression of fungal DGAT2 resulted in enhanced seed oil content in G. max (Lardizabal et al., 2008) and Z. mays (Oakes et al., 2011). DGAT2 was also used to increase the proportion of unusual fatty acids in seeds specifically accumulating hydroxy fatty acid in Arabidopsis through coexpression with a cDNA encoding fatty-acid hydroxylase 12 (FAH12; Burgal et al., 2008) and epoxy fatty acids in G. max through coexpression with a cDNA encoding an epoxygenase (Li et al., 2010a). Overexpression of DGAT2 alone in Arabidopsis was also shown to increase the percentage of oleic acid in Arabidopsis seed TAG (Wang et al., 2016; Zhang et al., 2013). PDAT has also successfully been applied in engineering transgenic plants with high levels of unusual fatty acids and polyunsaturated fatty acids, such as hydroxy fatty acid and α-linoleic acid, respectively. Overexpression of flux PDAT in Arabidopsis led to an increase in the accumulation of α-linoleic acid in its seed oil (Pan et al., 2013). Similarly, coexpression of castor FAH12 and PDAT1 in Arabidopsis led to an increase in the accumulation of total hydroxy fatty acid up to 25% (Kim et al., 2011; van Erp et al., 2011). Overexpression of castor DGAT2 in the above coexpression line increased the hydroxy fatty-acid content further to 26.7% (van Erp et al., 2011). As previously suggested by Vanhercke et al. (2013b), the competition between endogenous and transgenically introduced that lipid biosynthetic machinery would limit the full potential of the metabolic engineering intervention. To reduce the endogenous competition from AtDGAT1, van Erp et al. (2015) introduced a mutation in AtDGAT1 in the line expressing castor FAH12, DGAT2, and PDAT and the hydroxy fatty-acid content was further increased to an average of 31.4%.

**Increasing the Oil Content of Vegetative Tissue**

In addition to increasing the seed oil content, it was previously shown that overexpression of DGAT1 could also boost the oil content of tobacco leaves (Bouvier-Navé et al., 2000). This concept of increasing TAG in vegetative tissues has recently gained traction among researchers as a viable alternative to meet the ever-growing demand for plant oil. Tobacco (N. tabacum and N. benthamiana) has served as the most common platform for producing oil in vegetative tissues given its ability to produce high biomass. DGAT1 has been used to boost oil in leaf or/and stem of tobacco through overexpression of DGAT1 alone (Andrianov et al., 2010; Wu et al., 2013) or in combination with one or more cDNA encoding proteins/enzymes such as acyl-CoA:monoacylglycerol acyltransferase, WRI, oleosin, cysteine-oleosin, and thioesterase (Chen et al., 2017; El Tahchy et al., 2017; Kelly et al., 2013; Petrie et al., 2012; Vanhercke et al., 2013a, 2014; Wimichayakul et al., 2013). The latter multigene strategies have proven to be more effective in green tissues for enhancing the carbon flux into TAG at multiple metabolic levels, including upregulation of fatty-acid biosynthesis (“Push”; e.g. overexpression of WRI), enhancing TAG assembly (“Pull”; e.g. overexpression of DGAT1 and introduction of mouse acyl-CoA:monoacylglycerol acyltransferase) and preventing lipid turnover (“Protect”; e.g. overexpression of oleosin) (Vanhercke et al., 2013a).
Table 1 Metabolic engineering interventions targeting DGAT1, DGAT2, or PDAT to increase/modify oil/TAG content in higher plants and microalgae

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<tr>
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<tr>
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<tr>
<td>Arabidopsis</td>
<td>Arabidopsis</td>
<td></td>
<td>Higher seed oil content and seed weight</td>
<td>Jako et al. (2001)</td>
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<tr>
<td>Brassica napus, Arabidopsis</td>
<td>B. napus</td>
<td></td>
<td>Higher seed oil content</td>
<td>Taylor et al. (2009);</td>
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<td>Tropaeolum majus</td>
<td>Arabidopsis, B. napus</td>
<td>Mutated SnRK1 site in DGAT1 (Ser 197 to Ala)</td>
<td>Higher seed oil content</td>
<td>Xu et al. (2008)</td>
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<tr>
<td>Veronica galamensis</td>
<td>Glycine max, Arabidopsis</td>
<td>Stokesia laevis epoxygenase</td>
<td>Higher epoxy fatty acids</td>
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<td>Corylus americana, G. max</td>
<td>G. max, Saccharomyces cerevisiae</td>
<td>Engineered DGAT1 variants</td>
<td>Higher seed oil content, lower soluble carbohydrate, and higher yeast oil content</td>
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<tr>
<td>Sapium sebiferum</td>
<td>B. napus</td>
<td></td>
<td>Higher seed oil content, lower oleic acid, and higher linoleic acid</td>
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<tr>
<td>Camelina sativa</td>
<td>C. sativa</td>
<td></td>
<td>Higher seed oil content</td>
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<tr>
<td>V. galamensis</td>
<td>G. max</td>
<td></td>
<td>Higher seed oil content</td>
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<tr>
<td>Arabidopsis</td>
<td>Jatropha curcas</td>
<td></td>
<td>Higher seed and leaf oil content, higher seed weight</td>
<td>Maravi et al. (2016)</td>
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<tr>
<td>Sesamum indicum</td>
<td>Arabidopsis, G. max</td>
<td></td>
<td>Higher seed oil content and seed weight</td>
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<tr>
<td>Cuphea avigera var. pulcherrima</td>
<td>C. sativa</td>
<td>FatB1 and LPAAT from Cuphea viscosissima</td>
<td>Higher capric acid (10:0) in seed oil</td>
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<td>Chlorella ellipsoidea</td>
<td>Arabidopsis, B. napus var. Westar, S. cerevisiae</td>
<td></td>
<td>Higher seed oil content and seed weight, higher yeast oil content</td>
<td>Guo et al. (2017)</td>
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<td><strong>Overexpression of DGAT1 to increase leaf/microalgae oil content and/or modify oil composition</strong></td>
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<tr>
<td>Arabidopsis</td>
<td>Nicotiana tabacum</td>
<td></td>
<td>Higher leaf oil content</td>
<td>Bouvier-Navé et al. (2000)</td>
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<td>Arabidopsis</td>
<td>N. tabacum</td>
<td>Arabidopsis LEC2</td>
<td>Higher leaf oil content</td>
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<td>Nicotiana benthamiana</td>
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<td>Arabidopsis</td>
<td>Arabidopsis, S. cerevisiae</td>
<td>Cysteine-oleosin (engineered S. indicum oleosin containing up to 13 cysteines)</td>
<td>Higher leaf and root oil content; Higher yeast oil content</td>
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<td>Arabidopsis</td>
<td>N. tabacum</td>
<td></td>
<td>Higher leaf oil content</td>
<td>Wu et al. (2013)</td>
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<tr>
<td>Arabidopsis</td>
<td>Arabidopsis sdp1 (lipase mutant)</td>
<td>Arabidopsis WRI</td>
<td>Higher root, stem and leaf oil content</td>
<td>Kelly et al. (2013)</td>
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<td>Arabidopsis</td>
<td>N. benthamiana</td>
<td>Arabidopsis WRI</td>
<td>Higher leaf oil content</td>
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<tr>
<td>Arabidopsis</td>
<td>N. tabacum</td>
<td>Arabidopsis WRI, S. indicum L. oleosin</td>
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<td>Arabidopsis</td>
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<td>Higher seed and leaf oil content, higher seed weight</td>
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<tr>
<td><em>Zea mays</em></td>
<td><em>Saccharum spp.</em> hybrids</td>
<td>Codon optimized <em>Z. mays</em> DGAT1, Arabidopsis <em>WRI</em> and Arabidopsis <em>oleosin</em> for expression in <em>Saccharum spp.</em> hybrids; RNAi mediated down-regulation of ADP-glucose pyrophosphorylase and peroxisomal ABC transporter1</td>
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<td>Abadopsis</td>
<td><em>Z. mays</em> WRI and <em>oleosin</em> from Arabidopsis</td>
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<td>Higher leaf TAG content</td>
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<tr>
<td>Abadopsis</td>
<td><em>Solanum tuberosum</em> L.</td>
<td>Arabidopsis WRI, <em>S. indicum</em> L. <em>oleosin</em></td>
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<tr>
<td><em>Elaeis guineensis</em></td>
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<td>Medium-chain FAT, <em>GPAT9</em> and <em>LPAAT</em> from <em>Cocos nucifera</em></td>
<td>Higher medium chain fatty acids in leaf oil and Higher leaf oil content</td>
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<tr>
<td>Abadopsis</td>
<td><em>N. benthamiana</em></td>
<td>Arabidopsis WRI, <em>oleosin</em>, and FAT</td>
<td>Higher leaf oil content</td>
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<td><em>B. napus</em></td>
<td><em>S. cerevisiae</em> N. <em>benthamiana</em></td>
<td>Single-site variants</td>
<td>Higher leaf oil content and yeast oil content</td>
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<td>Overexpression of DGAT2 to increase seed/leaf/microalgal oil content and/or modify oil composition</td>
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<td><em>Umboelopsis ramanniana</em></td>
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<td>Higher seed oil content</td>
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<td><em>FAH12</em></td>
<td>Higher hydroxy fatty acids</td>
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<td><em>Z. mays</em></td>
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<td>Oakes et al. (2011)</td>
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<td><em>V. galamensis</em></td>
<td><em>G. max</em></td>
<td><em>Stokesia laevis</em> epoxygenase</td>
<td>Higher epoxy fatty acids</td>
<td>Li et al. (2012)</td>
</tr>
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<td><em>Chlamydomonas reinhardtii</em></td>
<td><em>C. reinhardtii</em></td>
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<td>Higher oil content in microalgae</td>
<td>Deng et al. (2012)</td>
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<td><em>Thraustochytrium aureum</em></td>
<td>Arabidopsis fad3/4ae1 mutant</td>
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<td>Higher oleic acid in seeds</td>
<td>Zhang et al. (2013)</td>
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<td><em>C. reinhardtii</em></td>
<td>Arabidopsis</td>
<td></td>
<td>Higher leaf oil content</td>
<td>Sanjay et al. (2013)</td>
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<td><em>Phaeodactylum tricornutum</em></td>
<td><em>P. tricornutum</em></td>
<td></td>
<td>Higher oil content in microalgae with higher polyunsaturated fatty acid content</td>
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<td><em>B. napus</em></td>
<td><em>C. reinhardtii</em></td>
<td></td>
<td>Higher oil content in microalgae, lower saturated fatty acid content, higher a-linolenic acid content,</td>
<td>Ahmad et al. (2015)</td>
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<tr>
<td><em>Sapium sebiferum</em></td>
<td>Arabidopsis</td>
<td></td>
<td>Higher oleic acid content in seeds</td>
<td>Wang et al. (2016)</td>
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<td><em>Nannochloropsis oceanica</em></td>
<td><em>N. oceanica</em></td>
<td></td>
<td>Higher oil content in microalgae</td>
<td>Li et al. (2016)</td>
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<td><em>Neochloris oleoabundans</em></td>
<td><em>N. oleoabundans</em></td>
<td></td>
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<td><em>S. cerevisiae</em></td>
<td><em>P. tricornutum</em> Arabidopsis <em>oleosin</em></td>
<td></td>
<td>Higher oil content in microalgae</td>
<td>Zulu et al. (2017)</td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td><em>T. chui</em></td>
<td></td>
<td>Higher oil content in microalgae</td>
<td>Úbeda-Mínguez et al. (2017)</td>
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et al., 2014). The combined overexpression of cDNA encoding DGAT1, WRI, and oleosin, respectively, is a very effective approach for increasing leaf TAG, driving oil accumulation to more than 15% dry weight in *N. tabacum* (Vanhercke et al., 2014). Increased incorporation of medium-chain fatty acids into TAG has also been obtained in tobacco leaves through overexpression of DGAT1 together with other genes that can increase the flux of medium-chain fatty acids in the pathway (Reynolds et al., 2015, 2017). Increased oil content in vegetative tissues was also obtained in Arabidopsis (Kelly et al., 2013; Winiwchayakul et al., 2013), *Saccharum* spp. hybrids (Zale et al., 2016) and *J. curcas* (Maravi et al., 2016) through overexpression of DGAT1 alone or in combination with other genes. As for DGAT2, overexpression of a *C. reinhardtii* DGAT2 in Arabidopsis boosted the oil content in leaves (Sanjaya et al., 2013). As mentioned earlier, PDAT1 appears to play a more important role in TAG synthesis in young leaves than DGAT1 (Fan et al., 2013b). The combined overexpression of PDAT1 and OLEOSIN increased leaf TAG accumulation by up to 6.4% and 8.6% of the dry weight in Arabidopsis and the Arabidopsis *trigalactosyldiacylglycerol1–1* mutant, respectively (Fan et al., 2013b).

### Increasing the Oil Content of Microalgae

Microalgae are considered to be one of the most potentially viable sources of storage lipid (TAG) for biofuel production and a future solution to the renewable energy challenge (Hu et al., 2008; Wijffels and Barbosa, 2010). Whereas mostly green algae and eustigmatophytes are regarded as the best suitable oil producers, oil-rich diatoms such as *P. tricornutum* and several red algal species such as *Porphyridium purpureum* and *Cyanidioschyzon merolae* are also getting considerable research attention (Hu et al., 2008; Liu et al., 2017; Sato et al., 2017; Shuba and Kifle, 2018). Microalgal DGAT and PDAT are potential targets to engineer improved oil-rich biomass accumulation (Goncalves et al., 2016). For instance, genetic engineering of *C. reinhardtii* by expressing a truncated DGAT1 cDNA from *B. napus* led to increases in the contents of lipids and polyunsaturated fatty acids (Ahmad et al., 2015). Heterologous expression of DGAT1 from *E. pitardii*, and DGAT2 from *S. cerevisiae* also resulted in higher TAG accumulation in the marine microalgae *Tetraselmis chui* (Úbeda-Mín- guez et al., 2017). In *C. reinhardtii*, however, contrary findings were obtained from *CrDGAT2* overexpressors. Deng et al. (2012) reported an increase in the lipid content

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<td><em>N. oceanica</em></td>
<td><em>N. oceanica</em></td>
<td>Modulate the ratio of DGAT2A:2C:2D transcripts by overexpression and knockdown</td>
<td>Modified fatty-acid composition in microalgae</td>
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<td><em>N. oceanica</em> (CCMP1779)</td>
<td>Arabidopsis, <em>S. cerevisiae</em></td>
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<td>Higher seed and leaf oil content</td>
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<td>Arabidopsis</td>
<td><em>R. communis FAH12</em></td>
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<td><em>R. communis</em></td>
<td>Arabidopsis</td>
<td>FAH12 and DGAT2 from <em>R. communis</em></td>
<td>Higher hydroxy fatty acid in seed oil</td>
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<td><em>R. communis</em></td>
<td>Arabidopsis</td>
<td>FAH12 and DGAT2 from <em>R. communis</em>; silencing of endogenous DGAT1</td>
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<td><em>Linum usitatissimum L.</em></td>
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<td></td>
<td>Higher α-linolenic acid in seed oil</td>
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<tr>
<td>Arabidopsis</td>
<td>Arabidopsis</td>
<td><em>trigalactosyldiacylglycerol1–1</em> mutant</td>
<td>Higher leaf oil content</td>
<td>Fan et al. (2013b)</td>
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FAH, fatty-acid hydroxylase; FAT, fatty acyl-ACP thioesterase; GPAT9, *sn*-glycerol-3-phosphate acyltransferase; LPAAT, lysophosphatidate acyltransferase; LEC, LEAFY COTYLEDON; MGAT, monoacylglycerol acyltransferase; RNAi, RNA interference; SnRK1, sucrose nonfermenting-related protein kinase 1; WRI, WRINKLED1; and TAG, triacylglycerol.
with overexpression of CrDGAT2–1 or CrDGAT2–5 in \textit{C. reinhardtii}, whereas La Russa et al. (2012) reported that overexpression of CrDGAT2 did not increase the lipid content of \textit{C. reinhardtii}. Despite the contrary results in \textit{C. reinhardtii}, overexpression of DGAT2 in \textit{N. oceaniaca} increased the TAG content up to twofold without affecting growth (Li et al., 2016). More recently, increased TAG production with altered fatty-acid composition was also observed in the oleaginous microalg \textit{Neochloris oleoabundans} by the overexpression of DGAT2 (Klaitong et al., 2017). In the diatom \textit{P. tricornutum}, overexpression of DGAT2 resulted in eicosapentaenoic acid (20:5\textsubscript{cis, 8,11,14,17})-rich oil and increased neutral lipid accumulation while sustaining a similar growth rate in the transgenic microalgae (Niu et al., 2013). Recently, Zulu et al. (2017) used heterologous coexpression of yeast DGAT2 and a plant OLEOSIN as an efficient intervention for enhancing TAG accumulation in \textit{P. tricornutum}. Additionally, it has also been shown that DGAT plays a major role in controlling the photosynthetic carbon flux toward TAG in this diatom (Dinamarca et al., 2017). Interestingly, Xin et al. (2017) investigated the acyl-CoA specificity of different isoforms of DGAT2 in the eustigmatophyte \textit{N. oceaniaca} (NoDGAT2) for fatty acids with different unsaturation levels. NoDGAT2A preferred substrates with saturated fatty acids and NoDGAT2D preferred substrates with monounsaturated fatty acids, whereas NoDGAT2C exhibited higher activity toward substrates with polyunsaturated fatty acids. Microalgal transgenic lines were generated with specific saturated fatty acid: monounsaturated fatty acid: polyunsaturated fatty-acid proportions in TAG by modulating the ratio of NoDGAT2A:2C:2D transcripts. The investigators further established a novel strategy to simultaneously improve oil content and quality from microalgae for industrial use.

\textit{Increasing DGAT1 Activity Through Protein Engineering}

Due to the importance of DGAT1 as a molecular tool for increasing oil accumulation, there has been interest in further increasing the activity of the enzyme through protein engineering. Previous efforts to engineer DGAT enzyme performance, however, have largely relied on the identification of natural variation sites and sequence-based site-directed mutagenesis. For instance, a phenylalanine insertion into maize DGAT1 at position 469, which naturally occurs in a high-oil maize line but not a normal-oil maize line, is responsible for the increased DGAT activity and oil content (Zheng et al., 2008). Similarly, analysis of the protein sequences of eight closely related peanut DGAT2 from a collection of peanut varieties revealed that two out of the six identified amino-acid residue substitutions led to increased enzyme activity in DGAT2 variants (Zheng et al., 2017a). Moreover, a previous study on the substitution of a serine residue with an alanine residue in a candidate SnRK1 consensus site in \textit{T. majus} DGAT1 resulted in a variant with higher activity (Xu et al., 2008). Overexpression of the \textit{T. majus} DGAT1 variant in Arabidopsis seeds led to a higher seed oil content than what could be achieved with overexpression of the cDNA encoding the wild-type enzyme. The potential of protein engineering to boost DGAT1 activity was also demonstrated in mouse DGAT1 wherein the mutation of three serine residues to glutamate residues individually in the N-terminal region also resulted in enzyme variants with increased activity (Yu et al., 2015). The aforementioned switch to glutamate residues was argued to mimic the addition of negatively charged phosphate groups, which was hypothesized to be a stimulatory signal in mouse DGAT1. Moreover, production of a recombinant BnaDGAT1 with an N-terminal tag in \textit{S. cerevisiae} also resulted in elevated oil accumulation. The added N-terminal tag resulted in an increase in the level of polypeptide production (Greer et al., 2015).

In contrast to the limited target sites identified from natural variation and sequence-based prediction, directed evolution provides a powerful approach for DGAT engineering, especially in the absence of structural information. Pioneering work on this involved the development of a yeast H1246-based high-throughput system for selection of high-performance enzyme variants (Siloto et al., 2009a). Many improved BnaDGAT1 variants were generated using the aforementioned method and the two most promising ones were used to increase the oil content of tobacco leaves (Chen et al., 2017). Kinetic analysis indicated that one of the BnaDGAT1 variants exhibited apparent decreased substrate inhibition at concentrations of acyl-CoA beyond 5 \textmu M (Xu et al., 2017). The possible role of the ninth and tenth predicted TMD in enzyme regulation was also identified as a considerable number of beneficial mutations were localized near and within this region (Chen et al., 2017). A similar yeast-based high-throughput system also identified \textit{Corylus americana} and \textit{G. max} DGAT1 variants with improved kinetic properties (Roesler et al., 2016). The overexpression of a cDNA encoding a DGAT1 variant with 14 substitutions in soybean resulted in a larger increase in seed TAG when compared to seeds resulting from overexpression of the wild-type enzyme. Furthermore, a truncated BnaDGAT1 was found to increase TAG accumulation in green microalgae (Ahmad et al., 2015).

\textit{Closing Comments}

Ever since the isolation of the first plant DGAT in Arabidopsis (Bouvier-Navé et al., 2000; Hobbs et al., 1999; Routaboul et al., 1999; Zou et al., 1999) and the discovery of
PDAT activity in plant species (Dahlqvist et al., 2000), our understanding in the terminal steps of plant TAG biosynthesis has grown tremendously. Some of the knowledge has been successfully applied in metabolic engineering of oilseed crops to increase seed oil content and modify the fatty-acid composition of seed oil. Meanwhile, the growing interest of using vegetative tissues and microalgae as platforms for industrial oil production has brought them into the spotlight, achieving substantial progress by taking the advantage of the successful applications in oilseed biotechnology. The physiological roles of DGAT and PDAT in regulating plant TAG accumulation underline the potential applicability of overexpression of DGAT or PDAT in transgenic plants and microalgae for increasing oil content, modifying oil quality and improving plant stress tolerance. It should be noted that the relative contributions of DGAT and PDAT to seed TAG accumulation may vary among species (Ramli et al., 2005; Tang et al., 2012; Troncoso-Ponce et al., 2011; Woodfield et al., 2018), and it is therefore, important to choose suitable strategies based on individual plants in manipulating oil production. The recent discovery of alternative splicing variants of peanut DGAT1, as reported by Zheng et al. (2017b), represents another exciting area for further investigation. Thus far, our great progress has been made in probing the properties and regulation of DGAT1 and exploring the biotechnological uses of DGAT1. In addition to overexpression of cDNA encoding the wild-type DGAT1, there have been recent successes in the manipulation of oil production using high-performance enzyme variants generated via directed evolution (Chen et al., 2017; Roesler et al., 2016). Recent advances in genome-editing techniques, such as, CRISPR (Belhaj et al., 2013) and targeting-induced local lesions in genomes (TILLING; Till et al., 2006), open up new perspectives on improving the enzyme action in planta. For instance, the “super DGAT” variants with single amino-acid residue substitutions generated in B. napus DGAT1 provide valuable candidates for genome editing of DGAT1 in different species using CRISPR and TILLING (Chen et al., 2017; Xu et al., 2017). The knowledge obtained from directed evolution, in turn, can provide novel and valuable insights into structure–function relationships of DGAT1 (Chen et al., 2017), especially since no detailed three-dimensional structure is available for the entire DGAT1 enzyme. Recently, the structure of the hydrophilic N-terminal domain of BnaDGAT1 was solved and its self-regulatory function was revealed (Caldo et al., 2017). Since DGAT1 from B. napus has been successfully purified in an active form (Caldo et al., 2015), first steps are made toward obtaining high-resolution structures. The eventual structural elucidation of DGAT and PDAT will uncover the molecular mechanisms of catalysis and provide detailed insights into modes of enzyme regulation thus establishing a basis for rational design of acyltransferases for manipulation of oil production.

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