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Mechanisms and functions of membrane lipid remodeling in plants

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SUMMARY

Lipid remodeling, defined herein as post-synthetic structural modifications of membrane lipids, play crucial roles in regulating the physicochemical properties of cellular membranes and hence their many functions. Processes affected by lipid remodeling include lipid metabolism, membrane repair, cellular homeostasis, fatty acid trafficking, cellular signaling and stress tolerance. Glycerolipids are the major structural components of cellular membranes and their composition can be adjusted by modifying their head groups, their acyl chain lengths and the number and position of double bonds. This review summarizes recent advances in our understanding of mechanisms of membrane lipid remodeling with emphasis on the lipases and acyltransferases involved in the modification of phosphatidylcholine and monogalactosyldiacylglycerol, the major membrane lipids of extraplastidic and photosynthetic membranes, respectively. We also discuss the role of triacylglycerol metabolism in membrane acyl chain remodeling. Finally, we discuss emerging data concerning the functional roles of glycerolipid remodeling in plant stress responses. Illustrating the molecular basis of lipid remodeling may lead to novel strategies for crop improvement and other biotechnological applications such as bioenergy production.

Keywords: membrane lipid, remodeling, lipase; acyltransferase, triacylglycerol, abiotic stress

INTRODUCTION

Biological membranes are essential components of living systems. They form a boundary between the cell and its environment, mediate intracellular signaling transduction and cell-to-cell communications and establish a selective permeable boundary that only allows certain molecules to enter or leave the cell. In eukaryotic organisms, membranes divide the cell into discrete subcellular compartments that segregate vital but, in many cases, incompatible metabolic reactions. The fundamental structure of cellular membranes is the bilayer comprising two sheets of lipid molecules, into which proteins with important functions such as enzymes in energy transducing systems, receptors and transporters are either partially or fully embedded. According to the fluid mosaic model (Singer and Nicolson, 1972), a critical property of biological membranes is that they are present in a fluid state in which lipids and proteins are loosely bound to one another via chemical interactions and individual molecules are generally able to rotate and move laterally. Such fluidity is important for membrane-associated functions such as transport, synthesis of biomolecules, energy transduction and cell signaling, and it is influenced by both temperature and lipid composition (Los and Murata, 2004, van Meer *et al.*, 2008, Ernst *et al.*, 2016).

In addition to their structural role, membrane lipids regulate the localization, structure and function of membrane proteins by lipid-lipid and lipid-protein interactions and by physical effects (van Meer *et al.*, 2008, Quinn, 2012, Nyholm, 2015, Harayama and Riezman, 2018). Some lipids can define membrane microdomains that serve as sorting platforms and hubs for cell signal transduction machinery for a wide range of metabolic processes (Sezgin *et al.*, 2017, Levental *et al.*, 2020). Lipids also play crucial roles in membrane fusion events critical for cell division, organelle proliferation and membrane trafficking (van Meer *et al.*, 2008, Harayama and Riezman, 2018). Further, some lipids are known to function directly in cell signal pathways as messengers or regulators (Sunshine and Iruela-Arispe, 2017).

Membrane lipids can be grouped into four major classes: phospholipids, glycolipids, sterols and sphingolipids (Figure 1) (Harayama and Riezman, 2018). Phospholipids and glycolipids are glycerol-based lipids consisting of two hydrophobic fatty acids attached to the *sn-1* and *sn-2* positions and a phosphate group or a sugar moiety to the *sn-3* position of a glycerol backbone, respectively. The phosphate group of phospholipids can be modified by a polar alcohol such as choline, ethanolamine, glycerol, inositol and serine, which gives this class of lipids their names phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylserine (PS), respectively. The fatty acids of phospholipids and glycolipids vary in chain length, the degree of saturation and double bond position. Phospholipids are the most abundant membrane lipids in both yeast and mammals. In photosynthetic tissues of plants, however, glycolipids including galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) and the sulfolipid sulfoquinovosyldiacylglycerol (SQDG) are far more abundant that phospholipids. Sterols are a subgroup of steroids with a characteristic structure consisting of four rings of carbon atoms, while sphingolipids are defined by the presence of a sphingoid-base covalently linked to a fatty acid via an amide bond. In addition to the structural diversity, the different classes of

membrane lipids are not distributed equally among tissues, organelles or even between two leaflets of the same membrane, but rather have specific locations, and the collective action of their bulky lipids defines the identity and function of different organelles (van Meer *et al.*, 2008, Harayama and Riezman, 2018). For example, in plants, galactolipids are located exclusively in chloroplasts, while sterols and sphingolipids are enriched in lipid microdomains of the plasma membrane. Galactolipids play a key role in the biogenesis of photosynthetic membranes and are important for the optimal function of embedded photosynthetic pigment-protein complexes in higher plants (Kobayashi, 2016).

Lipids are the major determinants of the physicochemical properties of cellular membranes that in turn are crucial for membrane functions (Ernst *et al.*, 2016, Harayama and Riezman, 2018). Both the nature of the glycerolipid head group and the length and degree of saturation of their acyl chains influence the membrane's physical properties such as fluidity, permeability, bilayer thickness, charge and intrinsic curvature of membranes. In this context, glycerolipids with a relatively large head group such as PC and DGDG approximate a cylindrical molecular shape and tend to form bilayer lipid phases with no curvature strain. In contrast, the shapes of PE and MGDG are more conical, due to the presence of relatively small head groups. They impose negative curvature stress on membranes and are prone to form non-bilayer lipid structures in membranes. Anionic lipids PG, SQDG, PI and PS are key determinants of membrane surface charge and hence play crucial roles in mediating lipid-protein interactions (Jouhet, 2013, Harayama and Riezman, 2018) (Figure 1). Sterols interact more favorably with saturated than with unsaturated acyl chains of phospholipids (Nystrom *et al.*, 2010, Nyholm *et al.*, 2019). These interactions regulate membrane fluidity, lipid bilayer stability and membrane microdomain formation. In addition to lipid class composition, membrane physical properties and function are also dependent on the fatty acid composition of lipid molecules. In general, lipids with saturated fatty acids decrease membrane fluidity due to the tight packing of straight saturated acyl tails and stronger interactions of saturated acyl chains with sterols. The packing of unsaturated lipids, on the other hand, increases membrane fluidity because *cis* double bonds create a rigid bend preventing tight packing of their fatty acids (Munro, 2003, Harayama and Riezman, 2018). In addition to the degree of fatty acid desaturation, their acyl chain length and their positional distribution on the glycerol backbone affect organization and dynamics of membranes.

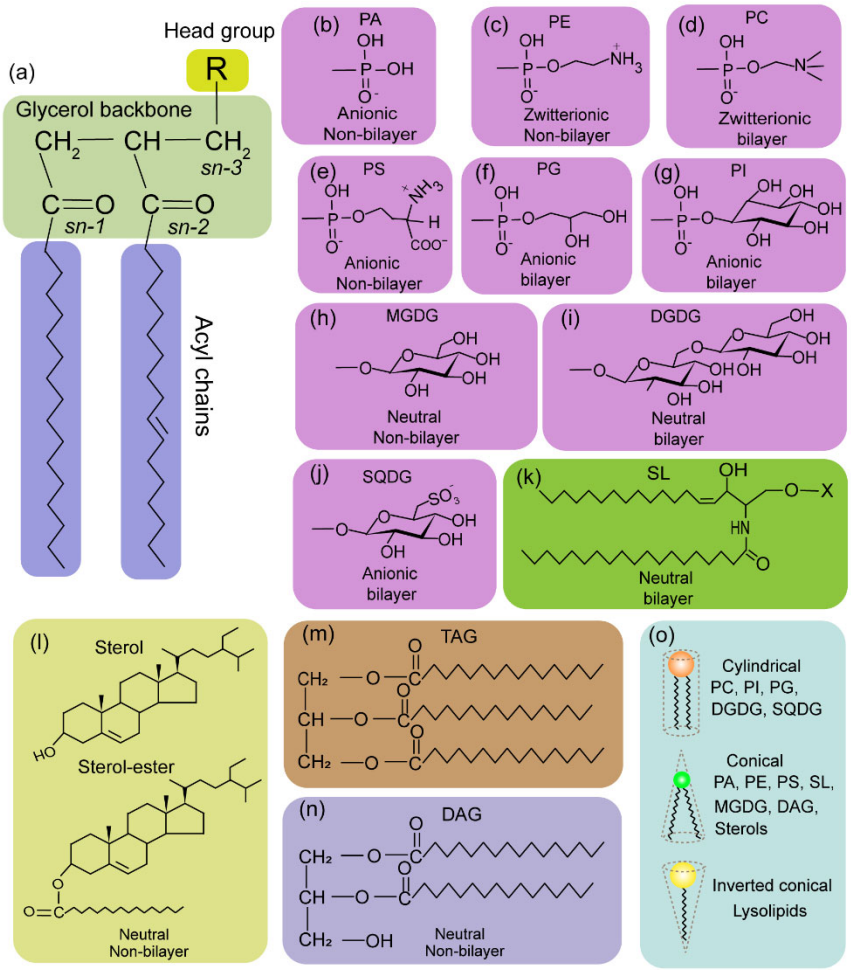

Figure 1. Schematic representation of the chemical structures of membrane lipids and lipid molecular shapes. (a-n) Structures of membrane lipids. Membrane lipids are subdivided in four major categories: phospholipids (b-g), glycolipids (h-j), sphingolipids (a) and sterols (l). Triacylglycerol (TAG) is a storage glycerolipid (m). Classes of phospholipids are defined by the hydrophilic head groups (R) attached to the *sn-3* position of the glycerol backbone. Sphingolipids constitute a large category of lipids with diverse acyl chains and headgroups (X). (o) Schematic representation of lipid molecular shapes.

Membrane lipid compositions are determined by a range of metabolic processes including lipid biosynthesis, transport, turnover, remodeling and degradation. Glycerolipids are major structural components of cellular membranes. The enzymatic steps and pathways involved in glycerolipid biosynthesis are well defined and the mechanisms of lipid transport well studied. However, much less is known about the molecular processes underlying lipid modifications after their synthesis. This review will summarize our current knowledge about post-synthetic modifications of fatty acids and head groups, with a focus on the candidate enzymes involved in remodeling of acyl chains and head groups of glycerolipids. In addition, we will discuss the functional role of TAG metabolism in lipid remodeling. Finally, new information about the functions of membrane lipid remodeling will be summarized.

OVERVIEW OF GLYCEROLIPID BIOSYNTHESIS

Two parallel pathways compartmentalized in the plastid or the ER contribute to glycerolipid biosynthesis in plants (Figure 2). Fatty acids, the main component of glycerolipids, are almost exclusively synthesized in plastids. The end products of plastid fatty acid synthesis in order of abundance are mainly 18:1, 16:0 and 18:0 (the number of carbon atoms in the fatty acid chain: the number of double bonds). These fatty acids can be used inside the plastid to produce lysophosphatidic acid (LPA) and phosphatidic acid (PA) by the glycerol-3-phosphate acyltransferase (GPAT) and lysophosphatidic acid acyltransferase (LPAAT), respectively. The resulting PA can be used to synthesize PG or be dephosphorylated by phosphatidic acid phosphatase (PAP) to produce diacylglycerol (DAG). DAG is as a precursor for glycolipid synthesis via the plastid pathway. Alternatively, fatty acids can be exported to the ER and used to synthesize LPA, PA and DAG by ER-resident GPAT, LPAAT and phosphatidic acid phosphohydrolase (PAH), respectively via the Kennedy pathway (Kennedy and Weiss, 1956). However, radiotracer labeling studies showed that most of nascent fatty acids exported from the plastid are first incorporated into PC through acyl remodeling (or editing) that allows the desaturation of PC-bound monosaturated fatty acids into polyunsaturated fatty acids (PUFAs) by ER-resident fatty acid desaturase 2 (FAD2) and FAD3 and the subsequent release of PUFAs for the synthesis of PA by ER-resident GPATS and LPAATs (Bates *et al.*, 2007, Bates *et al.*, 2009). PA and its dephosphorylated product DAG can serve as precursors for the synthesis of phospholipids in the ER. In addition, phospholipids assembled in the ER can return to the plastid to provide DAG moieties for the synthesis of galactolipids and SQDG in the plastid. Because the substrate specificity of plastid and ER LPAATs differs, glycerolipids assembled by the plastid and ER pathway contain fatty acids with 16 and 18 carbon chain lengths (C16 and C18) at the *sn-2* position of the glycerol backbone, respectively. Another important feature of acyltransferases involved in PA synthesis is that GPATs prefer saturated fatty acids and fatty acids with one double bond. Therefore, the substrate specificity of acyltransferases, PC acyl remodeling and the balance between the plastid and ER pathways of thylakoid lipid synthesis influence the composition of glycerolipids in plant cell membranes.

DAG formed in the ER is also an immediate precursor for the acyl-CoA-dependent acylation by diacylglycerol acyltransferase (DGAT) at the *sn-3* position of the glycerol backbone to produce triacylglycerol (TAG) (Routaboul *et al.*, 1999, Zou *et al.*, 1999). TAG can also be produced by phospholipid:diacylglycerol acyltransferase (PDAT), which catalyzes the transfer of acyl groups from the *sn-2* position of PC to DAG to form TAG and lysophosphatidylcholine (LPC) (Dahlqvist *et al.*, 2000). Non-seed tissues such as leaves do not accumulate TAG to significant levels (Xu and Shanklin, 2016, Xu *et al.*, 2020), though they contain high TAG synthetic activities (Dahlqvist *et al.*, 2000).

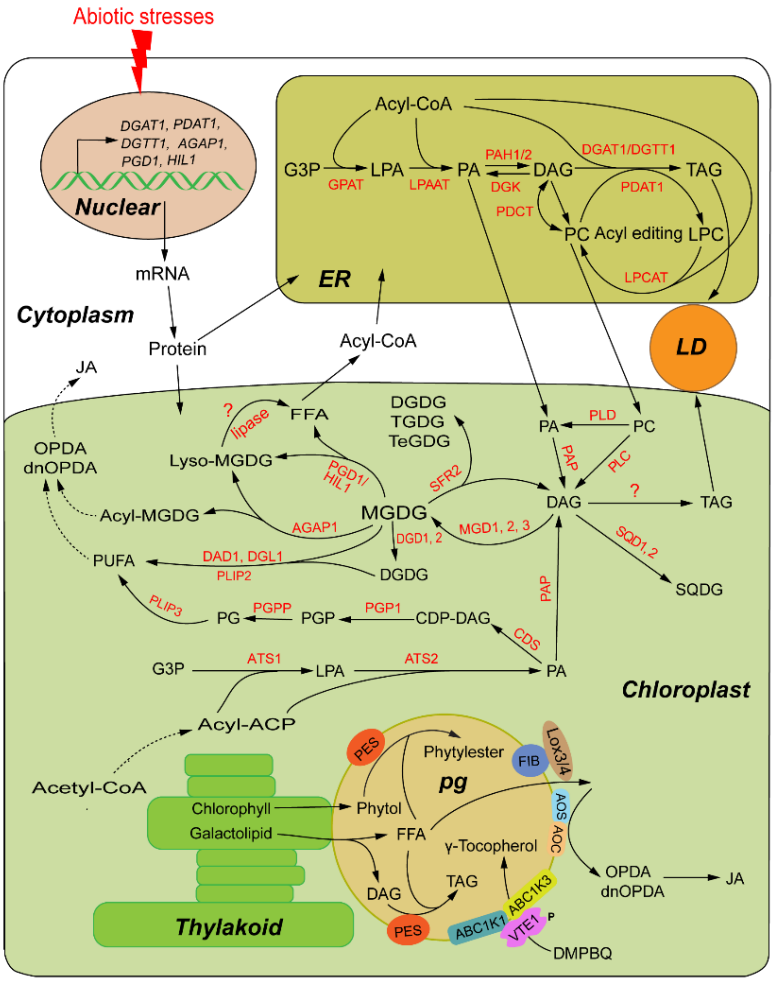

Figure 2 Membrane lipid remodeling during abiotic stresses.

Multiple abiotic stresses (cold, heat, drought or salt) induce the expression of genes encoding many enzymes, such as DGAT1, PDAT1, DGTT1, AGAP1, PGD1 and HIL1 in plants and algae. These transcripts are translated, and the corresponding proteins are translocated to the ER and chloroplasts, where they modify lipid composition to fine-tune plant responses to environmental cues. In the ER, glycerol lipids are synthesized through the Kennedy pathway and TAG are assembled by DGAT1, DGTT1 and PDAT1. PC can be used to generate DAG via PDCT. Acyl-CoA is incorporated into PC by acyl-editing reactions presumably via LPCATs. Stresses trigger the synthesis of PA through activating the hydrolysis of PC by PLD and/or phosphorylation of DAG by DGKs. PC and PA are imported from ER to chloroplast, where they are converted to DAG. MGDG is produced via the transfer of a galactose from UDP-galactose onto DAG by MGD enzymes. MGDG is subsequently converted to DGDG by DGD enzymes. Abiotic stresses activate enzymes such as SFR2, PGD1, HIL1, AGAP1, PLIP2, DAD1 and DGL1, which are involved in mobilization of unsaturation fatty acids from MGDG/DGDG for lipid remodeling and TAG and JA biosynthesis under stress conditions. FAs are synthesized in the plastid stroma from acetyl-CoA and incorporated to phospholipids through the plastid pathway. Abiotic stresses also trigger plastoglobule (pg) accumulation in chloroplasts. Stresses can cause thylakoid breakdown, leading to the release of FA, DAG and free phytol. Plastoglobule-localized PES1 and -2 convert phytol and FA into phytylesters as well as FA and DAG into TAG. Plastoglobules recruit enzymes of the jasmonate biosynthesis pathway, which redirects fatty acids from thylakoid lipids to JA production. ABC1K1 and ABC1K3 phosphorylate VTE1 to promote tocopherol production under high light. Question marks indicate the enzymes involved in the reactions remain to be determined.

CANDIDATE ENZYMES FOR MEMBRANE LIPID REMODELING

Glycerolipid remodeling can be achieved through modifications of acyl chains or head groups. Glycerolipid acyl remodeling is a process in which one or both fatty acids are exchanged, creating an intermediate lysophospholipid (LPL). Phospholipid acyl remodeling in animal systems is known as the Lands cycle (Lands, 1960), in which fatty acids attached to the *sn-2* position of phospholipids are liberated by phospholipases. The resultant LPLs can be reacylated by lysophospholipid acyltransferases to generate phospholipids with different fatty acids. Alternatively, processes of acyl remodeling can include an exchange of both acyl groups with a glycerophosphodiester or DAG intermediate. In addition, acyl exchange in transacylation reactions in which an acyl group is transferred from a phospholipid or a LPL donor to an LPL acceptor have also been reported (Yamashita *et al.*, 2014).

Lysophosphatidylcholine acyltransferase

So far, most of the knowledge regarding the mechanism of membrane lipid acyl remodeling is derived from studies on PC because of its central role in glycerolipid metabolism. First, PC is a site of fatty acid desaturation (Sperling and Heinz, 1993). Second, PC acyl remodeling serves as a dominant entry point for acyl groups exported from the plastid into the ER pathway (Bates *et al.*, 2007, Bates *et al.*, 2009). Third, PC remodeling provides a main source of fatty acids and DAG for TAG biosynthesis in developing seeds (Bates *et al.*, 2009, Lu *et al.*, 2009). Finally, DAG moieties derived from PC are important precursors for the synthesis of thylakoid lipids (Ohlrogge and Browse, 1995). In Arabidopsis, two lysophosphatidylcholine acyltransferases, namely LPCAT1 and LPCAT2, have been shown to play central roles in PC acyl remodeling in developing seeds (Wang *et al.*, 2012) and leaves (Karki *et al.*, 2019). These LPCAT enzymes catalyze the reacylation of LPC preferentially at the *sn-2* position using fatty acids exported from the plastid (Bates *et al.*, 2007, Bates *et al.*, 2009). In vitro enzymatic assays, however, showed that LPCAT1 and LPCAT2 can catalyze the acylation of fatty acids at the *sn-1* position of PC at a rate ranging from 15 to 70% of that of the *sn-2* position (Lager *et al.*, 2013). In addition, acyl substrate specificity analysis showed that LPCATs from five different plant species have a strong preference for C18-unsaturated acyl chains over 16:0 or unusual fatty acids (Lager *et al.*, 2013).

The lipases responsible for removing fatty acids from PC to generate LPC substrate for LPCAT-catalyzed acylation in plants remain elusive. There are more than 200 genes annotated as lipases (Troncoso-Ponce *et al.*, 2013), but most of their functional roles remain uncertain. Two studies showed that *Fatty Acid Reducer* genes, which encode GDSL lipases, are involved in seed oil accumulation in Arabidopsis and *Brassica napus* (Chen *et al.*, 2012, Karunarathna *et al.*, 2020) Arabidopsis LPCAT enzymes have been shown to be able to catalyze the direct transfer of acyl groups of PC to free CoA in in vitro assays, leading to the generation of LPC and acyl-CoA (Lager *et al.*, 2013), but the functional significance of this observation requires further investigation. An alternative hypothesis is that PDAT is involved in the provision of LPC for LPCAT-catalyzed acylation (Lager *et al.*, 2013). In support of this possibility, LPC acylation reactions catalyzed by LPCAT1 and LPCAT2 have been shown to be critical for maintaining the supply of PC as an acyl donor in PDAT1-mediated TAG synthesis in developing seeds (Xu *et al.*, 2012).

Phospholipase

Phospholipases can be classified into A, B, C and D families based on the bond they hydrolyze within a phospholipid molecule (Hong *et al.*, 2016). Phospholipase A enzymes release the acyl chain at the *sn-1* or *sn-2* position to generate LPL. Phospholipase Bs (PLBs) hydrolyze acyl ester bonds at both the *sn-1* or *sn-2* position, whereas phospholipase C (PLC) and D (PLD) cleave the phosphodiester bond linked to the glycerol backbone and the polar head group to generate DAG and PA, respectively. Among the four phospholipase families, multiple forms of PLD, C, and A have been extensively characterized in plants and they have been implicated in the remodeling of membrane phospholipids and/or in the generation of signal lipids (Wang, 2001, Welti *et al.*, 2002, Nakamura, 2013, Hong *et al.*, 2016). Both transcript levels and protein abundance of non-specific PLCs (NPCs), namely NPC4 and NPC5, have been shown to be enhanced in plants starved for inorganic phosphate (Pi) (Andersson *et al.*, 2005, Nakamura *et al.*, 2005, Gaude *et al.*, 2008). In addition, transcript analysis has shown that, among 12 isoforms of PLDs, *PLDζ1* expression is increased in response to Pi starvation (Cruz-Ramirez *et al.*, 2006).

PG is the only phospholipid present in chloroplast photosynthetic membranes. Radiotracer pulse-chase labeling (Hellgren and Sandelius, 2001) and in vivo lipid 'tag and track' methods (Hurlock *et al.*, 2018) indicate that chloroplast PG undergoes extensive post-synthetic acyl remodeling, possibly due to its tight association with highly oxidative photosynthetic protein complexes (Hellgren and Sandelius, 2001). A plastid lipase1 (PLIP1) has recently been shown to be involved in acyl remodeling of PG, contributing to the export of acyl groups from plastids to TAG synthesis in developing seeds (Wang *et al.*, 2017, Aulakh and Durrett, 2019). PLIP1 is a phospholipase that specifically hydrolyzes polyunsaturated fatty acids from the *sn-1* position of plastid PG.

Galactolipase

Several acylhydrolases capable of releasing fatty acids from galactolipids have recently been identified in microalgae and higher plants. Among them, plastid galactolipid degradation1 (PGD1) was first reported in the green algae Chlamydomonas (Li *et al.*, 2012, Du *et al.*, 2018). The enzyme specifically hydrolyzes 18:1-containing MGDG but not MGDG containing polyunsaturated fatty acids. Disruption of PGD1 alters galactolipid content and acyl composition, suggesting a role of this lipase in acyl remodeling of de novo-synthesized chloroplast membrane lipids (Du *et al.*, 2018). Another chloroplast MGDG lipase in Arabidopsis, heat inducible lipase1 (HIL1), has been implicated in removing polyunsaturated acyl groups (18:3) from 34:6-MGDGs (Higashi *et al.*, 2018). Additionally, two closely related chloroplast-targeted lipases, DEFECTIVE IN ANTHER DEHISCENCE1 (DAD1) and DONGLE1 (DGL1), have been reported to be involved in jasmonic acid (JA) biosynthesis in respond to wounding. DAD1 has weak galactolipase and strong PLA1 activities, while DGL1 has strong galactolipase and weak PLA1 activities (Ishiguro *et al.*, 2001, Hyun *et al.*, 2008, Ellinger *et al.*, 2010).

Glycerophosphocholine acyltransferase

Glycerophosphocholine acyltransferase (GPCAT) catalyzes the acylation of glycerol-3-phosphocholine (GPC), the product of complete PC deacylation, with acyl-CoA as acyl donor to generate LPC. Reacylation of LPC by LPCATs can lead to the formation of PC with new fatty acids. GPCAT activity has been demonstrated in cell-free extracts and microsomal fractions of yeast and plants (Stalberg *et al.*, 2008, Lager *et al.*, 2015). The yeast gene encoding GPCAT was identified by screening enzyme activities of extracts from a yeast knock-out library (Glab *et al.*, 2016). Loss of GPCAT affects PC fatty acid profiles, suggesting a role of this enzyme in post-synthetic acyl remodeling of PC (Anaokar *et al.*, 2019). The plant GPCAT has been shown to exhibit broad substrate specificity for acyl donors (Glab *et al.*, 2016). The plant GPCAT can also catalyze transacylation from LPC and lysophosphatidylethanolamine (LPE) to GPC, suggesting a role of this enzyme in acyl group exchange between PC and PE. In yeast, the B type of phospholipases (PLBs) deacylate PC to form free fatty acids and GPC (Lee *et al.*, 1994). The pathways and enzymes involved in GPC formation and the exact functional role of GPCAT remain to be established in plants.

Fatty acid desaturase

The desaturation of acyl chains of glycerolipids to polyunsaturated forms typical of cellular membranes occurs in both the plastids and ER by membrane-bound fatty acid desaturates (FADs) (Browse and Somerville, 1991, Shanklin and Cahoon, 1998). In the plastid, FAD4 and FAD5 are specifically responsible for the conversion of 16:0 of PG to *trans*-palmitoleic acid (*t*16:1) and 16:0 of MGDG to 16:1, palmitoleic acid, respectively (Browse *et al.*, 1985, Kunst *et al.*, 1989b). The generation of fatty acids with two double bonds in all plastidic glycerolipids is catalyzed by FAD6 (Browse *et al.*, 1989) and further desaturation of 16:2 and 18:2 to 16:3 and 18:3 is catalyzed by either FAD7 or FAD8 isozymes (Iba *et al.*, 1993, McConn *et al.*, 1994). In extraplastidic membranes, two ER-desaturases FAD2 and FAD3 are responsible for converting 18:1 in glycerolipid substrates to 18:2 and 18:3, respectively (Miquel and Browse, 1992, Browse *et al.*, 1993).

Phosphatidylcholine:diacylglycerol cholinephosphotransferase

Phosphatidylcholine:diacylglycerol cholinephosphotransferase (PDCT) catalyzes the headgroup exchange between PC and DAG (Lu *et al.*, 2009). Genetic evidence suggest that the main function of this enzyme is to facilitate further desaturation of the 18:1-containing DAG produced by the Kennedy pathway on PC and the subsequent release of polyunsaturated DAG for TAG synthesis in developing seeds (Lu *et al.*, 2009, Bates *et al.*, 2012). Consequently, disruption of PDCT results in a 40% reduction in polyunsaturated seed fatty acid content (Lu *et al.*, 2009) and combined disruption of LPCAT1, LPCAT2 and PDCT reduces the levels of polyunsaturated fatty acids by 66% (Bates *et al.*, 2012). The *PDCT* gene is expressed in both seeds and non-seed tissues, but a nonsense mutation in PDCT (also known as ROD1) has no obvious effects on lipid and fatty acid composition of leaves (Lu *et al.*, 2009), hinting at a possible role of PDCT in the remodeling of membrane lipids in vegetative tissues under stress conditions (Lu *et al.*, 2009).

Sensitive to freezing2

The *sensitive to freezing2* (*SFR2*) gene was discovered in a genetic screen for Arabidopsis mutants sensitive to freezing (Warren *et al.*, 1996). The SFR2 protein was originally described as a family I glycosyl-hydrolase (Thorlby *et al.*, 2004). Later on, Moellering et al. (Moellering *et al.*, 2010) found that SFR2 is a galactolipid:galactolipid galactosyl transferase originally reported in isolated chloroplasts (Heemskerk *et al.*, 1987, Heemskerk *et al.*, 1988), that is capable of processively transferring galactosyl residues from MGDG to a second galactolipid acceptor, forming DGDG and oligogalactolipids with DAG as a by-product (Moellering *et al.*, 2010) (Figure 2). SFR2 is a membrane protein associated with the chloroplast outer envelope (Roston *et al.*, 2014) and its activity increases in response to freezing and ozone fumigation (Sakaki *et al.*, 1990a, Moellering *et al.*, 2010) and in Arabidopsis mutants defective in ER-to-plastid lipid trafficking (Xu *et al.*, 2003, Awai *et al.*, 2006, Lu *et al.*, 2007, Xu *et al.*, 2008, Fan *et al.*, 2015).

Acylated galactolipid-associated phospholipase

Acylated galactolipid-associated phospholipase1 (AGAP1) transfers a fatty acid from one MGDG molecule to the galactose residue in another MGDG, thereby producing acyl-MGDG and lyso-MGDG (Nilsson *et al.*, 2015). Acyl-MGDGs, predominantly 18:3-MGDG, were found to accumulate in plant tissues in response to abiotic or biotic stress (Vu *et al.*, 2014b, Nilsson *et al.*, 2015). In Arabidopsis, acyl-MGDGs frequently contains oxidized fatty acids in the form of the JA precursor 12-oxo-phytodienoic acid (OPDA) (Kourtchenko *et al.*, 2007). In contrast to acyl-MGDGs, lyso-MGDGs do not accumulate, indicating they are either rapidly hydrolyzed to release fatty acids that could potentially end up in TAGs via PC remodeling, or reacylated to form MGDGs (Mueller *et al.*, 2017) (Figure 2). Like SFR2, the Arabidopsis AGAP1 is localized to the chloroplast envelope membranes (Nilsson *et al.*, 2015).

ROLE OF TRIACYLGLYCEROL METABOLISM IN MEMBRANE LIPID REMODELING

Both TAG and phospholipid synthesis occur in the ER where they share the same acyl-CoA pools derived from acyl remodeling of PC and possibly the same DAG pool also. Therefore, it is reasonable to expect that the rate of TAG synthesis and the activity of the TAG assembly enzymes may affect membrane lipid synthesis via their effects on acyl-CoA and DAG pools.

In plants, both the acyl-CoA-dependent reactions catalyzed by DGATs and the acyl-CoA-independent processes catalyzed by PDATs contribute to TAG synthesis (Zhang *et al.*, 2009b). At least four types of DGATs, namely integral membrane proteins DGAT1 and DGAT2, soluble DGAT3 and multifunctional acyltransferases have been implicated in TAG synthesis in plants (Xu *et al.*, 2020). Among these enzymes, DGAT1 prefers saturated and very long-chain acyl groups over polyunsaturated acyl chains (Katavic *et al.*, 1995), DGAT2 prefers unsaturated acyl species over saturated ones (Zhou *et al.*, 2013, Ayme *et al.*, 2014) and DGAT3 has higher preference for polyunsaturated fatty acids (Hernandez *et al.*, 2012). Therefore, the relative contributions of DGAT1, DGAT2 and DGAT3 to TAG synthesis may affect the composition of acyl-CoA pools and thus the fatty acid composition of membrane lipids. The rate of PDAT1-mediated TAG synthesis has been shown to be highest towards phospholipids containing polyunsaturated fatty acids or oxygenated acyl groups (Stahl *et al.*, 2004). Thus, increasing PDAT1 activity may be expected to cause decreases in membrane lipid content and the polyunsaturated fatty acids of

membrane lipids. Indeed, overexpression of PDAT1 in Arabidopsis has been shown to cause a decrease of 18:3 in major membrane lipids such as PC, PE, DGDG and MGDG (Fan *et al.*, 2013a, Fan *et al.*, 2013b).

In plants and other eukaryotes, TAGs synthesized in the ER are packaged into dynamic subcellular structures named lipid droplets (LD) for subsequent use as a source of fatty acids for energy production or for membrane biogenesis (Chapman *et al.*, 2012, Xu *et al.*, 2020). The metabolic breakdown of TAG stored in LDs for energy production can proceed via cytosolic lipolysis and lipophagy (Fan *et al.*, 2019, Xu *et al.*, 2020). During cytosolic lipolysis, TAG lipases such as SUGAR-DEPNDENT1 (SDP1) hydrolyze TAGs to release free fatty acids in the cytoplasm (Eastmond, 2006) and the resulting fatty acids are imported into peroxisomes by PEROXISOMAL ABC TRANSPORTER1 (PXA1) to enter the β-oxidation pathway (Zolman *et al.*, 2001). Alternatively, LDs can be delivered by autophagy into vacuoles, where resident lipases degrade TAGs in LDs into free fatty acids (Fan *et al.*, 2019). The released fatty acids can be exported into the cytosol to be used for TAG synthesis or as substrates for peroxisomal β-oxidation (Zechner *et al.*, 2017). In addition to TAG, membrane lipids can serve as a source of fatty acids for energy production via β-oxidation in peroxisomes under stress conditions such as dark-induced starvation (Kunz *et al*., 2009; Fan *et al*., 2017). In this scenario, fatty acids released from membrane lipids are first incorporated into TAGs and stored in LDs. TAGs in LDs are then hydrolyzed by SDP1 and the released fatty acids are imported into peroxisomes by PXA1 prior to being used for energy production via β-oxidation (Fan *et al.*, 2014, Yu *et al.*, 2018). Since activation of free fatty acids into acyl-CoA esters by cytosolic acyl-CoA synthases is a prerequisite for entry of fatty acids into peroxisomes (De Marcos Lousa *et al.*, 2013), activities of cytosolic acyl-CoA synthases, SDP1, PXA1 and lipophagy may impact the composition and content of cytosolic acyl-CoA pools, thereby effecting membrane lipid synthesis. In support of this possibility, Arabidopsis mutants lacking PXA1 accumulate acyl-CoAs (Footitt *et al.*, 2002) and blocking the β-oxidation pathway results in changes in membrane lipid content and its fatty acid composition (Fan *et al.*, 2014, Yu *et al.*, 2018).

TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL REGULATION OF LIPID REMODELING

The transcript abundance of several enzymes involved in lipid remodeling varies across different tissues and cell types, and in response to developmental and environmental cues (Kargiotidou *et al*., 2008, Fan *et al.*, 2014, Higashi *et al.*, 2015, Dar *et al*., 2017, Yuan *et al.*, 2017, Arisz *et al.*, 2018). These results hint at the possible involvement of transcriptional networks in regulating membrane lipid remodeling. In support of this possibility, a transcription factor MYB96 has been suggested to mediate abscisic acid (ABA)-dependent TAG accumulation in vegetative tissues under drought stress through the transcriptional regulation of DGAT1 and PDAT1 (Lee *et al.*, 2019). In addition, ABSCISIC ACID INSENSITVE 4 (ABI4), a transcription factor in ABA signaling, has been implicated in regulating the *DGAT1* transcript abundance under stress conditions (Yang *et al.*, 2011, Kong *et al.*, 2013). Further, both *cis*-regulatory elements and transcription factors are involved in the regulation of *FAD2* and *FAD3* expression (Dar *et al*., 2017, He *et al.*, 2020).

Mammalian LPCATs can be regulated by posttranslational modifications such as phosphorylation (Morimoto *et al.*, 2010), but no information is available regarding the regulation of plant LPCATs at either the transcriptional or posttranscriptional level. On the other hand, posttranscriptional mechanisms have also been suggested to be involved in regulating the activities of FAD2 (Dar *et al*., 2017), DGAT1 (Caldo *et al.*, 2018) and phospholipases (Singh *et al.*, 2015). Similarly, the *SFR2* gene is constitutively expressed in various plant tissues and both SFR2 mRNA and protein levels remain constant during development and in response to abiotic stress (Thorlby *et al.*, 2004, Wang *et al.*, 2016). Various factors, including cytoplasmic acidification (Barnes *et al.*, 2016), $MgCl_2$ (Heemskerk *et al.*, 1987, Barnes *et al.*, 2016) and free fatty acids (Sakaki *et al.*, 1990b, Fan *et al.*, 2015), have been implicated in the regulation of SFR2 activity.

CONSEQUENCES OF MEMBRANE LIPID REMODELING

The ability to remodel membrane lipid composition in response to environmental and developmental cues is important for development, biomass production and plant survival. One of key biological roles for membrane lipid remodeling is to adjust membrane physiochemical properties to optimize its functions with respect to a new set of conditions (Moellering and Banning, 2011, Patton-Vogt and de Kroon, 2020). In addition, emerging evidence suggests that glycerolipid acyl remodeling plays important roles in removing oxidized or damaged acyl chains, sequestering cytotoxic fatty acids, releasing signaling lipids and in stress responses (Hermansson *et al.*, 2011, Nakamura, 2013, Vu *et al.*, 2014a, Renne *et al.*, 2015, Patton-Vogt and de Kroon, 2020). In plants, PC remodeling has been implicated in fatty acid desaturation and the subsequent release of desaturated fatty acids and DAG for membrane lipid and TAG synthesis (Bates *et al.*, 2013, Li-Beisson *et al.*, 2013). For more detailed information on membrane lipid remodeling and its function under abiotic stresses, the reader is directed to several recent reviews (Nakamura, 2013, Yang and Benning, 2018, Guo *et al.*, 2019, Lu *et al.*, 2020).

Modifications of glycerolipid desaturation

One of the most intensively studied mechanisms of lipid remodeling involves modifications of the acyl chain profile, particularly the degree of desaturation of glycerolipids in response to changes in temperature and many other environmental challenges. In general, organisms increase acyl desaturation at lower temperatures while decreasing it at higher temperatures to counteract effects of temperature variations on membrane fluidity (Ernst *et al.*, 2016). This phenomenon has been demonstrated in many studies (Hugly *et al.*, 1989, Kunst *et al.*, 1989a, Wada *et al*., 1990, Hugly and Somerville, 1992, Welti *et al.*, 2002, Falcone *et al.*, 2004, Chen *et al.*, 2006, Qin *et al.*, 2020). A similar link between the degree of acyl desaturation and plant stress tolerance has been reported for plants under drought and salt conditions (Gigon *et al.*, 2004, Zhang *et al.*, 2005, Liu *et al.*, 2013, Li *et al.*, 2014, Sui and Han, 2014, Sui *et al.*, 2018). A series of desaturase mutants in Arabidopsis, including *fad2* (Miquel *et al.*, 1993), *acyl-lipid desaturase2* (*ads2*) (Chen and Thelen, 2013), *fad5* (Hugly and Somerville, 1992), *fad6* (Hugly *et al.*, 1989) and *fad8* (Matsuda *et al.*, 2005) were found to be sensitive to low temperature. In addition, overexpression of the *FAD3* gene led to an increase in 18:3 fatty acid levels and an improvement in chilling tolerance in tomato (Yu *et al.*, 2009). *Arabidopsis* mutants deficient in desaturation of fatty acids, such as *fad6*

(*formerly referred to as fadcC*) (Hugly *et al.*, 1989), *fabB* (Kunst *et al.*, 1989a) and *fad7fad8* (Murakami *et al.*, 2000) have been shown to be more tolerant to high temperature. All these studies illustrate a key role of FADs in adaptation to temperature stresses. Moreover, FADs are important in plant responses to other environmental stresses. For examples, Arabidopsis FAD2 and FAD6 are required for salt tolerance (Zhang *et al.*, 2009a, Zhang *et al.*, 2012), and stearoyl-acyl carrier protein $\triangle^9$-desaturase6 (SAD6) and FAD3 are involved in drought and hypoxia response in Arabidopsis crown galls (Klinkenberg *et al.*, 2014). Ectopic expression *Brassica napus FAD3* or *Arabidopsis FAD8* improved drought tolerance in tobacco (Zhang *et al.*, 2005), whereas antisense expression of an Arabidopsis *FAD7* compromised drought and salt tolerance in transgenic tobacco plants (Im *et al.*, 2002).

An alternative way to adjust membrane fatty acid composition and lipid content is to alter the balance between the parallel plastid and ER pathways of glycerolipid biosynthesis (Yu L, 2020). Reports from Li et al. (2015, 2016) provides good examples of how the two glycerolipid biosynthesis pathways cooperate to regulate the fatty acid composition in plant response to temperature stress. They found that low temperature enhanced the plastid pathway of galactolipid biosynthesis and conversely, high temperature enhanced the ER pathway. Consistent with these observations, a recent study found that the synthesis of glycerolipids via the plastid pathway was severely compromised, whereas lipid assembly via the ER pathway was slightly enhanced during moderate heat stress (Qin *et al.*, 2020). Moreover, higher temperature caused an increase in the transport of DAG moieties with C16/C18 from the ER to the chloroplast for MGDG and DGDG biosynthesis at the expense of DAG moieties with C18/C18, while lower temperature resulted in the opposite (Li *et al.*, 2015).

Modifications of phospholipid composition

An alternative route for lipid remodeling is the head group exchange among different lipid classes. Remodeling of head groups might lead to substantial changes in the proportion of different lipid classes, consequently altering the biochemical and physical properties of membranes. It may play important roles in plant adaptation to adverse environmental conditions by preventing the phase transition from a liquid-crystalline phase to a non-bilayer phase or hexagonal II (HII) phase. Large negative curvature favoring lipids such as PA, PE, PS and MGDG tend to form a HII phase or cubic phase, whereas small curvature favoring lipids such as PC, PG, PI, DGDG and SQDG tend to form bilayers (Jouhet, 2013). Extensive membrane lipid profiling analysis in different plant species showed dynamic lipid compositional changes under different stress conditions (Zheng *et al.*, 2011, Degenkolbe *et al.*, 2012, Li *et al.*, 2015, Legeret *et al.*, 2016, Narayanan *et al.*, 2016, Marla *et al.*, 2017, Djanaguiraman *et al.*, 2018, Kenchanmane Raju *et al.*, 2018, Guo *et al.*, 2019). For example, PC and PE levels were found to decrease in response to low temperature in Arabidopsis (Kenchanmane Raju *et al.*, 2018) and in response water stress in wheat seedlings (Wang *et al.*, 2020), whereas increases in relative abundance of these lipids were found in most natural Arabidopsis accessions during cold acclimation (Degenkolbe *et al.*, 2012) and in wheat leaf at 4 ℃(Li *et al.*, 2015). Under high temperature stress, Narayanan *et al*. (2018) found that PC and PE generally decreased in wheat while Li *et al*. (2015) found that PC increased with no change in PE levels in leaves of *Atriplex lentiformis*. A significant increase in PC:PE ratio was observed

in wheat leaf and pollen at high temperatures (Narayanan *et al.*, 2016, 2018). Higher PC:PE ratios may reduce the propensity of membrane to form non-bilayer phases (de Vries *et al.*, 2004). However, changes of major phospholipids PC and PE are not very consistent under different stresses, probably due to dynamic turnover of these two lipids and differences in plant sources and growth conditions. PG levels were found to decrease consistently in Arabidopsis under low temperature with exception of one report, in which no significant changes were identified (Kenchanmane Raju *et al.*, 2018). Increases in SQDG and PG levels were observed in grasses during drought stress (Perlikowski *et al.*, 2016). Low temperature has been shown to activate phospholipase D (PLD) and diacylglycerol kinase (DGK) in Arabidopsis, leading to an increase in PA levels (Welti *et al.*, 2002, Tan *et al.*, 2018). Moreover, other environmental signals, including heat, drought, salinity, wounding, and pathogen attack, also can trigger a rapid synthesis of PA through activation of either PLD, the PLC/DGK pathway, or both. PA is not only a non-bilayer-forming lipid but also a key signal molecule in stress responses (Mishkind *et al.*, 2009, Testerink and Munnik, 2011, Hong *et al.*, 2016, Yao and Xue, 2018).

Modifications of galactolipid composition

The most pronounced effect of temperature stress on lipid composition is changes in levels of MGDG and DGDG. As a non-bilayer lipid, MGDG was mostly found to decrease under cold, heat or drought stresses in different organisms such as Arabidopsis (Li *et al.*, 2015, Arisz *et al.*, 2018, Higashi *et al.*, 2018), sorghum (Marla *et al.*, 2017), wheat (Li *et al.*, 2015), tomato (Spicher *et al.*, 2016), Chlamydomonas (Legeret *et al.*, 2016), cowpea (Torres-Franklin *et al.*, 2007) and *Craterostigma plantagineum* (Gasulla *et al.*, 2013), while the bilayer-forming lipid DGDG was found to be increased. As a result, the ratio of DGDG to MGDG increased under these stress conditions. The DGDG to MGDG ratio is important for correct protein folding and insertion, chloroplast shape, JA production and intracellular protein trafficking in the chloroplast (Bruce, 1998, Lin *et al.*, 2016, Yu *et al.*, 2020). Higher DGDG to MGDG ratios have been suggested to enhance the stability of the thylakoid membrane in response to abiotic stresses (Suss and Yordanov, 1986, Torres-Franklin *et al.*, 2007, Wang *et al.*, 2014, Zhang *et al.*, 2019). Mutations in the DGDG synthase1 (DGD1) reduce DGDG level and the ratio of DGDG to MGDG, leading to impaired plant growth and photosynthetic efficiency (Dörmann *et al.*, 1995) as well as decreased basal and acquired thermotolerance (Chen *et al.*, 2006). In contrast, overexpressing a rice MGDG synthase1 (MGD1) in tobacco resulted in significantly higher MGDG and DGDG contents, higher DGDG-MGDG ratios and enhanced salt tolerance (Wang *et al.*, 2014b).

Several proteins including SFR2, PGD1, HIL1 and AGAP1 have been suggested to be involved in MGDG remodeling under various environmental stresses. The chloroplast localized SFR2 converts MGDG to oligogalactolipids and DGDGs (Moellering et al., 2010). This mechanism of remodeling stabilizes chloroplast membranes by increasing the ratio of bilayer-forming to nonbilayer-forming galactolipids and by removing extra membrane lipids as the cytoplasm and chloroplast shrinks due to dehydration during freezing and salt stress (Moellering *et al.*, 2010, Wang *et al.*, 2016). Chlamydomonas PGD1 is involved in adjusting thylakoid membrane lipid levels, in particular the ratio of DGDG/MGDG in response to various environmental stresses (Du *et al.*, 2018). Another chloroplastic MGDG lipase in Arabidopsis, HIL1, has been implicated in

enhancing thylakoid membrane stability in the response to heat, osmotic and high light stresses (Higashi *et al.*, 2018). More importantly, galactolipid remodeling are closely linked to the biosynthesis of oxylipins, such as JA, 12-oxo-phytodienoic acid (OPDA) and dinor-OPDA (dnOPDA), which are important signaling compounds involved in various abiotic and biotic stresses (Wasternack and Hause, 2013, Kazan, 2015, Dolan, 2020, Monte *et al.*, 2020). It is generally accepted that PUFAs (18:3, 16:3) released from galactolipids by acyl-hydrolyzing enzymes are the precursors of the JA and oxylipin pathway (Scherer *et al.*, 2010, Deboever *et al.*, 2020). Two Arabidopsis PLA1 lipases, PLIP2 and PLIP3, were found to be capable of releasing PUFAs from the chloroplast membrane lipids (primary MGDG and PG, respectively) for JA production. Transcripts of PLIP2 and PLIP3 were induced by ABA and possibly by abiotic stresses, such as cold and drought, thus providing a mechanistic link between ABA-mediated abiotic stress responses and oxylipin signaling (Wang *et al.*, 2018).

Replacement of phospholipids with non-phosphorus lipids

Under Pi starvation, membrane phospholipids are replaced by non-phosphorus lipids, typically DGDG and SQDG, to conserve Pi (Essigmann et al., 1998, Härtel et al., 2000, Yu et al., 2002). This is achieved via degradation of phospholipids such as PC and plastidic PG by phospholipases to release DAG and Pi, recycling of DAG for glycolipid synthesis and transfer of DGDG from the plastid envelope to extraplastidic membranes including the plasma membrane, the tonoplast and mitochondrial membranes (Jouhet *et al.*, 2003, Andersson *et al.*, 2005, Nakamura *et al.*, 2005, Cruz-Ramirez *et al.*, 2006, Li *et al.*, 2006, Nakamura *et al.*, 2009), likely via intraorganellar membrane contact sites (Michaud and Jouhet, 2019).

The phospholipases involved in Pi starvation-induced phospholipid degradation include NPC4, NPC5, PLDζ1 and PLDζ2 (Nakamura *et al.*, 2005, Cruz-Ramirez *et al.*, 2006, Li *et al.*, 2006, Gaude *et al.*, 2008). PA, the product of the PLD-mediated phospholipid hydrolysis, can be further metabolized to generate DAG for DGDG biosynthesis by phosphatidic acid phosphatases (PAPs) including soluble phosphatide phosphohydrolases (PAHs) and membrane-bound PAP2 (Nakamura, 2013). The Arabidopsis genome harbors two *PAH* genes, *PAH1* and *PAH2*. Knockout of both genes resulted in reduced galactolipid level, increased PC content and impaired seedling growth under Pi starvation, suggesting a role of PAHs in Pi homeostasis (Nakamura *et al.*, 2009). An alternative pathway involves PLB and glycerophosphodiester phosphodiesterase (GDPD) (Nakamura, 2013). GDPD removes alcohols from glycerophosphodiester (GPD) to generate G3P, which can enter the G3P stepwise acylation pathway to produce DAG and subsequently DGDG (Figure 2).

Three isoforms, namely MGD1, 2 and 3, are responsible for MGDG synthesis in Arabidopsis (Awai *et al.*, 2001). Among them, the ubiquitously expressed *MGD1* is responsible for the bulk of MGDG synthesis in photosynthetic membrane under normal growth conditions. *MGD2* and *MGD3*, on the other hand, are expressed mainly in flowers and roots. Both *MGD2* and *MGD3* are induced by Pi starvation (Awai *et al.*, 2001) and knockout of both genes almost eliminates the ability of Arabidopsis roots to synthesize DGDG under Pi starvation, suggesting a major role of these two isoforms in membrane lipid remodeling (Kobayashi *et al.*, 2009).

DGDG is synthesized by the enzyme DGDG synthase, which catalyzes a galactose from UDP-galactose onto MGDG to produce DGDG (Dörmann *et al.*, 1999). The Arabidopsis genome harbors two genes for DGDG synthesis, *DGD1* and *DGD2*. Inactivation of DGD1 causes a 90% reduction in DGDG content, suggesting that this enzyme is dominant in DGDG synthesis under normal growth conditions (Dörmann *et al.*, 1995). Both *DGD1* and *DGD2* transcript levels are upregulated under Pi-limiting conditions and gene knockout studies showed that both enzymes contribute to the synthesis of Pi starvation-induced DGDG synthesis at the outer envelope of chloroplasts (Härtel *et al.*, 2000, Kelly and Dormann, 2002).

Under Pi starvation conditions, a decrease in the chloroplast PG level was found to be accompanied by an increase in the amount of the sulfolipid SQDG (Essigmann *et al.*, 1998, Yu *et al.*, 2002). Although the metabolic basis underlying PG to SQDG conversion remains largely unknown, it has been reported that the expression of *SQD1*, which encodes a UDP-sulfoquinovose synthase catalyzing the limiting step in SQDG synthesis, is induced under Pi starvation (Essigmann *et al.*, 1998). Disruption of the sulfolipid synthase2 (SQD2), which catalyzes the transfer of sulfoquinovose from UDP-sulfoquinovose onto DAG, results in reduced growth under Pi-limiting conditions, suggesting an important role for PG to SQDG conversion in plant acclimation to Pi starvation (Yu *et al.*, 2002). Interestingly, a recent study has shown that the conversion of PG to glycolipids also occurs in photosynthetic cells grown under low carbon dioxide (Jimbo *et al.*, 2021).

Protection against lipotoxicity during stress-induced lipid remodeling

Membrane lipid remodeling often results in the formation of cytotoxic lipid intermediates such as free fatty acids, DAG and other hydrolytic products as byproducts, and the accumulation of which can cause membrane disruption, oxidative stress and even cell death in a process collectively known as lipotoxicity (Garbarino *et al.*, 2009, Petschnigg *et al.*, 2009, Fan *et al.*, 2013a, Fan *et al.*, 2017, Lu *et al.*, 2020). During evolution, plants have acquired a series of adaptive mechanisms to combat lipotoxic stress, including sequestration of toxic lipids as TAG inside LDs and avoiding excessive exposure of the cytoplasm to free fatty acids by facilitating their trafficking through membrane contacts and by LD-peroxisome connections (Xu *et al.*, 2020).

Many environmental factors can boost TAG accumulation in leaves (Lu *et al.*, 2020), likely through regulating the expression of several key TAG biosynthesis genes (Figure 2). In line with this possibility, the *DGAT1* transcript has been shown to be induced by ABA, high salinity, hyperosmotic stress, nitrogen deprivation, heat and cold (Yang *et al.*, 2011, Kong *et al.*, 2013, Higashi *et al.*, 2015, Mueller *et al.*, 2017, Arisz *et al.*, 2018). *PDAT1* expression was significantly induced by heat (Higashi *et al.*, 2015), salt stress and drought (Yuan *et al.*, 2017).

As previously discussed, several enzymes, such as AGAP1 (Nilsson *et al.*, 2015), MGD1 (Du *et al.*, 2018) and HIL1 (Higashi *et al.*, 2018) are involved in the mobilization of unsaturation fatty acids from membrane lipids for TAG biosynthesis under stress conditions. SFR2 can generate a highly unsaturated pool of DAGs for TAG production by remodeling galactolipids in chloroplasts in response to freezing, drought and salt stress (Moellering *et al.*, 2010, Wang *et al.*, 2016). DGTT1 is induced by heat stress and plays roles in the conversion of MGDG to TAG in heat-stressed cells

(Legeret *et al.*, 2016). Deficiency in TAG synthesis leads to premature cell death when fatty acids are produced in excess of demand for membrane lipid synthesis (Fan *et al.*, 2013a). Blocking TAG hydrolysis by disrupting SDP1 impairs fatty acid β-oxidation, alters membrane lipid homeostasis in Arabidopsis, increases TAG accumulation in LDs and thus significantly enhancing plant tolerance to extended darkness (Fan *et al.*, 2014, Fan *et al.*, 2017). Disruption of DGAT1- and PDAT1-mediated TAG synthesis impairs plant freezing and heat tolerance, respectively (Mueller *et al.*, 2017, Arisz *et al.*, 2018, Tan *et al.*, 2018). Two *dgat1* mutants have been shown to be more susceptible to drought stress than wild-type plants, suggesting that TAG accumulation in vegetative tissues is required for drought tolerance (Lee *et al.*, 2019). Moreover, LD accumulation under salt stress exerts a positive impact on salt stress tolerance, likely through their involvement in providing fatty acids and enzymes to facilitate membrane reconstruction (You *et al.*, 2019). However, artificially boosting TAG accumulation by overexpression of PDAT1 in Arabidopsis resulted in an increase in sensitivity to salt stress and extended darkness, probably because high TAG accumulation resulted in an increase in the flux of fatty acids into the β-oxidation pathway, thereby enhancing ROS production (Yu *et al.*, 2019).

LDs can accumulate in chloroplasts as well as the cytoplasm under several stress conditions (Xu *et al.*, 2020). Chloroplast LDs are often referred to as plastoglobules. They mainly consist of fatty acid phytyl esters, TAGs, carotenoids, tocopherols and quinones. By analogy with cytosolic lipid bodies, plastoglobules may act as a buffering reservoir for fatty acids during thylakoid membrane lipid remodeling (van Wijk and Kessler, 2017). Two plastoglobuli-localized proteins, phytyl ester synthases, PES1 and PES2, were found to contribute to the deposition of free phytol and fatty acids in the form of phytyl esters and TAGs, thus playing a role in maintaining the integrity of the photosynthetic membrane during senescence and abiotic stress (Lippold *et al.*, 2012). There are seven plastoglobule-associated fibrillin (FIB) proteins found in Arabidopsis (Singh and McNellis, 2011). Loss-of-function and transcriptional analysis of FIB1a, -1b, -2 suggested they are involved in plant stress responses (Singh and McNellis, 2011). Study of another plastoglobule protein FIB4 revealed that deregulated plastoglobule accumulation results in broad stress sensitivity and altered photosynthetic activity (Singh *et al.*, 2010). Plastoglobules are sites of initiation of JA biosynthesis, particularly during stress, by recycling of fatty acids from thylakoid lipids (van Wijk and Kessler, 2017). FIB1a, -1b, and -2 condition JA production during low-temperature induced photooxidative stress (Youssef *et al.*, 2010). Plastoglobule-localized kinases ABC1 domain containing kinase1 (ABC1K1) and ABC1K3 are involved in adaptation of Arabidopsis to stresses and in thylakoid remodeling during growth (Lundquist *et al.*, 2013).

CONCLUSIONS AND FUTURE DIRECTIONS

The biochemical characterization of acyltransferases, desaturases and lipases involved in membrane lipid remodeling and genetic analysis of mutants defective in various steps in lipid modification pathways provide the mechanistic basis to further understand the processes and functions of membrane lipid remodeling during development and in response to stress. It is likely that the combined action of these enzymes regulates the physicochemical properties of membranes, including fluidity, thickness, phase behavior, permeability, lipid-protein interactions and stability of cellular membranes. These factors ultimately affect membrane-associated functions and ultimately physiological processes such as stress tolerance. However, details of how changes in membrane lipid composition and content affect plant physiology remain largely unknown. In addition, although significant progress has been made towards understanding the enzymes involved in PC and MGDG modifications, we are only beginning to understand the molecular machinery involved in remodeling of other membrane glycerolipids such as PE, PG, PI, PS and cardiolipin. Changes in membrane lipid composition and content have been reported under a wide variety of developmental and environmental conditions, but how plant cells sense their membrane lipid compositions and how such signals regulate the transcript levels and activities of enzymes involved in membrane remodeling remains to be explored. In this context, additional studies are needed to dissect the transcriptional and posttranslational mechanisms underlying the regulation of lipid modification enzymes such as LPCATs and SFR2. Furthermore, fatty acids form the building blocks of membrane lipids, storage TAG and plant surface lipids, but whether changes in levels of membrane lipids and TAG during lipid remodeling impact surface lipid metabolism and accumulation is presently unknown. Other issues requiring further investigation include 1) the molecular identity of lipases involved in PC diacylation; 2) the relative contribution of the LPCAT back reaction and PDAT1 to PC remodeling; 3) the physiological relevance of thylakoid membrane lipid remodeling and the roles of GPCAT, PDCAT and AGAP1 in lipid remodeling in photosynthetic cells; and 4) the contribution of intracellular lipid transport in membrane lipid remodeling and organellar-specific changes in membrane lipid composition. In addition to glycerolipids, our knowledge regarding how abiotic stresses affect sterol and sphingolipid content and composition, roles of sterols and sphingolipids in membrane microdomain formation and their functional relevance in stress tolerance is very limited. Addressing these issues will reveal novel aspects of lipid metabolism, lipid homeostasis and cellular mechanisms of stress tolerance, and will likely inform novel

Box1: Summary

- Plant cells remodel their membrane lipid composition to maintain normal physicochemical properties and hence their many functions.
- Membrane lipid remodeling can be achieved by modifying lipid head groups and/or their acyl chain length and degree of fatty acid unsaturation.
- Acyltransferases, lipases and desaturases are key players in membrane remodeling.
- Triacylglycerol metabolism plays an important role in membrane lipid remodeling by affecting acyl-CoA and DAG pools.
- Membrane lipid remodeling plays a critical role in many aspects of lipid metabolism and stress tolerance.

Box2: Open questions

- How do changes in membrane lipid composition affect membrane physiological properties?
- How do plant cells sense membrane lipid composition and relay the signals to regulate the activity of enzymes and the transcript levels of genes involved in lipid remodeling?
- What are the molecular identities of phospholipases involved in PC acyl remodeling?
- What are the roles of sterols and sphingolipids in membrane remodeling under stress?
- What is the role of intracellular lipid transport in membrane lipid remodeling?
- What are the mechanisms of PE, PI, PS and cardiolipin remodeling?

strategies for crop improvement and other biotechnological applications such as enhancing TAG production in seeds and the vegetative tissues of plants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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