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REVIEW

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ER–plastid contact sites as molecular crossroads for plastid lipid biosynthesis

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Abstract

Membrane contact sites are specialized regions where organelle membranes are in close proximity, enabling lipid transfer while preserving membrane identity. In plants, ER–chloroplast contact sites are critical for maintaining glycerolipid homeostasis. This review examines the lipid-modifying and lipid-transfer proteins/complexes involved in these processes. Key proteins at these sites, including components of the TGD and VAP27–ORP2A complexes, as well as Sec14 proteins, facilitate lipid exchange. Additionally, the roles of lipid-modifying proteins at these contact sites are discussed. Despite significant progress, further research is needed to identify additional proteins, investigate ER–chloroplast dynamics under stress and explore ER contact sites in non-chloroplast plastids.

Keywords Contact sites, Plastid, Chloroplast, Endoplasmic reticulum, Glycerolipid, Lipid transfer, TGD4, SFH5, ORP, NTMC2 T5

Introduction

Membrane contact sites

Membrane contact sites are specialized regions where the membranes of different organelles come into close proximity, typically 10–80 nm apart. In some cases, certain proteins can span larger distances, reaching up to 300 nm [1], acting as physical bridges that connect opposing organelle membranes and mediate shared cellular functions, such as lipid transfer or stress responses. Despite their proximity, opposing membranes at membrane contact sites do not fuse, allowing each organelle to maintain its distinct identity while facilitating the communication and exchange of molecules. The key features of membrane contact sites include (1) the absence of membrane fusion between the interacting organelles, (2) the presence of tethering forces resulting from protein–protein or protein–lipid interactions, (3) the accumulation of specific proteins and lipids at these junctions and (4) the facilitation of essential functions, such as the bidirectional transport of molecules (e.g. lipids), signal transduction and positioning of certain enzymes for cross-membrane activity [2]. Common examples of membrane contact sites are those formed between the endoplasmic reticulum (ER) and the plasma membrane, mitochondria, the Golgi apparatus or chloroplasts.

In plants, membrane contact sites play crucial roles in development and stress responses, often mediating calcium ion exchange between organelles. Under abiotic and biotic stress conditions, membrane contact sites are known to modulate signalling pathways and facilitate efficient metabolite and lipid exchange between organelles, enabling plants to adjust their metabolic processes in response to external stimuli. Recent reviews have summarized our understanding of membrane contact sites [2–6]; however, these reviews are largely focused on animal systems and do not address ER–plastid contact sites, which is the focus of this review. A growing body of research highlights the importance of ER–plastid interactions in regulating lipid metabolism in plants,

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underscoring the need for further studies to fully elucidate the functional significance of these contact sites in plant biology.

The endoplasmic reticulum as a hub for organelle connectivity

The ER is an organelle with a vast membrane surface [7], and its structure and functions have been extensively reviewed [8–12]. The ER consists of distinct structural domains that are interconnected and continuous, including the outer nuclear envelope, sheet-like cisternae and a polygonal array of tubules connected by three-way junctions. Peripheral ER sheets are typically studded with ribosomes and are traditionally classified as "rough" ER, which is associated with protein biosynthesis. In contrast, the tubules are composed of high-degree curved surfaces, spread throughout the cytosol and contain fewer ribosomes and are thus classified as "smooth" ER. Smooth ER interacts with other cellular organelles [7, 13, 14] and is usually associated with lipid synthesis. However, rearrangements in ER morphology are highly dynamic, with cisternae having the ability to rapidly convert into tubules and *vice versa* [12, 15, 16].

Specialized, high-curvature discrete ER regions form membrane contact sites with other organelles, including the plasma membrane, the Golgi apparatus, endosomes, mitochondria and chloroplasts [11]. Membrane curvature is a key physical property that influences both the formation and function of these contact sites. High curvature promotes organelle proximity and directly impacts biochemical processes at membrane contact sites. For example, curved membranes can facilitate the extraction or insertion of lipid molecules into the bilayer, making them particularly suitable for lipid transfer functions. Differences in membrane curvature between membrane contact sites and general ER regions have been previously reviewed [17]. The ratio of sheets to high-curvature tubules in the ER is regulated by curvature-inducing and curvature-stabilizing proteins, such as Reticulon, Lunapark and Atlastin family members [8, 9, 18]. These proteins shape the ER through distinct structural features and mechanisms. Reticulons, for example, contain a conserved reticulon homology domain (RHD) composed of two transmembrane helices flanking a hydrophilic loop, creating a wedge-like topology within the membrane. This configuration, along with their ability to oligomerize, promotes local membrane curvature. The generation, sensing and maintenance of high local ER membrane curvature is an active, tightly regulated process that is essential for the formation and function of many membrane contact sites and involves a diverse array of proteins and mechanisms [8, 10, 11, 17].

Plastid structure and functional diversity

Plastids are dynamic organelles surrounded by two envelope membranes that contain their own DNA. They can differentiate into various types, such as chloroplasts, chromoplasts or leucoplasts, each serving distinct functions in processes such as photosynthesis, pigment storage and starch synthesis. Among these, chloroplasts are the most studied owing to their critical role in photosynthesis, converting light energy into chemical energy [19], and in acting as hubs for signalling and metabolic pathways. Chloroplasts are essential for the biosynthesis of many important compounds, such as starch [20], aromatic amino acids [21], phytohormones [22], isoprenoids [23] and lipids such as fatty acids and galactolipids [24].

Structurally, chloroplasts are composed of three membrane systems [25]: the outer envelope membrane (OEM), the inner envelope membrane (IEM) and the thylakoid membranes, where the components of the photosynthetic electron transport chain reside [26]. Thylakoids are organized into stacked structures known as grana, which are interconnected by stroma lamellae [27], whereas the space surrounding the thylakoids is referred to as the stroma.

Plastids can also form stromules (stroma-filled tubules), which are thin, dynamic, tubular extensions that are typically less than 1 μm in diameter and are found in various plastid types, including chloroplasts. Stromules are enclosed by the OEM and the IEM [28], and they were proposed to form due to changes in the protein-to-lipid ratio in the OEM [29]. Although their exact functions remain under study, stromules are thought to increase plastid surface area, potentially facilitating interorganelle communication and acting as conduits for molecular exchange. Moreover, stromules play roles in cellular stress responses, pathogen defence and the autophagic degradation of plastid components [30–33].

ER–plastid contacts are now emerging as important yet poorly characterized structures in plant cells. In this review, we highlight recent advances in the study of ER–plastid contact sites, particularly in the context of lipid metabolism, and discuss their potential roles in plant development or stress adaptation.

Discovery of ER–chloroplast contact sites: from early observations to modern insights

The relationship between the ER and chloroplasts was first described in the 1960 s. The evidence of this interaction is scattered throughout the literature, where it was initially referred to as an "association", "connection" or "continuity", and was reviewed by Sarah P. Gibbs in 1981 [34]. Here, we highlight key studies and provide a historical overview of how ER–chloroplast contact sites have been investigated.

The first observation of a "unique" relationship between the ER and chloroplasts was made by Gibbs [35], who used electron microscopy to study several algal species from the *Cryptophyceae* class. In the following years, independent studies consistently revealed that the ER closely envelops plastids in various species, including the yellow–green alga *Xanthophyceae* [36], and in plants such as *Acer and Pinus* [37]. In 1965, G Benjamin Bouck coined the term "chloroplast ER" [38], establishing the connection between the plastid, the peripheral ER and the nuclear envelope [39]. These findings were further supported by other observations in *Dryopteris borreri* [40], *Pteris* [41], phloem cells of *Tozzia alpina* scale leaves [42], green algae [43] and embryonic pea cells [44].

Although the association between chloroplasts and the ER was previously reported, membrane continuity between these organelles was first demonstrated by Diers (1966) through microdissections of developing eggs of the liverwort *Sphaerocarpos donnellii* [45]. Subsequent studies confirmed the close association of these two organelles using rapid freezing and freeze-fracturing techniques in the green alga *Chara globularis*, the lower vascular plant *Equisetum telmateia* [43] and the angiosperm *Phaseolus vulgaris* [46].

Nearly a decade later, the first confocal images showing the ER surrounding chloroplasts in tobacco leaf cells were published, although this relationship was not explicitly highlighted [47]. A significant advancement came when Andersson et al. [48] employed laser optical manipulation combined with confocal microscopy and biochemical studies to isolate chloroplasts. They reported that the ER remained attached to chloroplasts post-isolation and named these ER membranes "plastid-associated membranes" (PLAMs). They reported that these membranes could not be detached from chloroplasts using forces up to 400 pN, suggesting the involvement of protein–protein interactions. Nevertheless, this association could be disrupted by lowering the pH or treating with trypsin. The lipid and protein compositions of the PLAMs were found to be distinct from those of the OEM and the ER, as the PLAMs were characterized by a low galactolipid content, the presence of phosphatidylethanolamine (PE) and reduced sterol and glycosylceramide levels, distinguishing PLAMs from the lipid composition of the ER. Later studies using laser stimulation techniques further demonstrated the presence of membrane contact sites at what the author termed the "chloroplast/endoplasmic reticulum nexus" [49]. Recently, bimolecular fluorescence complementation coupled with confocal microscopy has been used to successfully visualize ER–chloroplast contact sites [50, 51].

Additional evidence for ER–plastid associations came from studies on plastid stromules and their alignment with ER tubules. Stromules and ER tubules have also been observed to move in a coordinated manner [31, 52, 53]. In a recent study, the interplay between plastid behaviour and ER dynamics was examined, with a particular focus on the role of membrane contact sites linking these organelles [54]. Time-lapse imaging and fluorescence-tagged proteins suggested that transient membrane contact sites between the plastid envelope and the ER facilitate this coordinated movement [54]. However, because both ER and stromule dynamics and morphology are influenced by the cytoskeleton, further investigations, including investigations of this third player, are essential to fully understand the nature and function of these ER–chloroplast contact sites.

Finally, evidence of metabolic continuity between chloroplasts and the ER was first demonstrated through transorganellar complementation studies. In these studies, mutations in plastid-resident enzymes involved in tocopherol and carotenoid biosynthesis were complemented by ER-targeted versions of these enzymes, demonstrating the functionality of ER–chloroplast associations [55, 56]. Notably, both chloroplasts and the ER contribute to the synthesis of key lipids through biosynthetic pathways that encompass distinct, non-overlapping steps in each organelle [57], and several proteins have been identified at ER–plastid contact sites, including some involved in lipid metabolism, which are summarized in the next section.

In addition to chloroplasts, plant cells can contain other types of plastids, including proplastids, amyloplasts and chromoplasts. The unique characteristics of each plastid type are closely linked to their specific functions [58]. To date, membrane contact sites between the ER and other plastids have not been studied. However, given the dynamic nature of plastids and their capacity to interconvert in response to developmental and environmental cues, it is reasonable to hypothesize the existence of such structures in plastids beyond chloroplasts, with future research likely providing further insights.

Lipid and protein composition of chloroplast membranes

The protein and lipid compositions of chloroplast membranes are unique and share similarities with those of cyanobacteria [59]. Under normal physiological conditions, chloroplast membranes are typically enriched in galactolipids and low in phospholipids, whereas other lipid types, such as sphingolipids and sterols, are either present in minimal amounts or nearly absent in some chloroplastic membranes. Galactolipids are a type of glycerolipid characterized by the presence of one or more

Table 1 Major glycerolipid classes (in mol. %) of intact chloroplasts envelope membranes and thylakoids

Plant membrane	MGDG	DGDG	SQDG	PC	PG	PI	PE	Ref
Intact chloroplast								
Pea seedlings	46	32	7	7	6	1	ND	[68]
A. thaliana	54	21	4	12	10	ND	ND	[69]
Total envelope								
Vicia faba leaves	29	32	ND	30	9	ND	0	[70]
Daffodil flowers	24	26	5	23	9	3	0	[71]
Wheat leaves	22	43	11	14	10	-	-	[72]
Helianthus annus leaves	31	26	1	29	5	1	2	[73]
Zea mays leaves	34	24	< 0.5	30	4	1	1	[73]
Spinach leaves	16	27	7	20	11	2	0	[74]
Spinach leaves	38	30	7	14	9	2	0	[75]
Spinach leaves	36	29	6	18	9	2	0	[76]
Cauliflower buds	31	27	6	20	9	5	1	[77]
Outer envelope membrane								
Pea seedlings	6	33	3	44	6	5	1	[78]
Spinach leaves	17	29	6	32	10	5	ND	[76]
Inner envelope membrane								
Spinach leaves	49	30	5	6	8	1	ND	[76]
Thylakoid								
Spinach leaves	45	26	12	-	17	-	-	[79]
Vicia faba leaves	65	26	ND	3	6	ND	0	[70]
Wheat leaves (lamellar)	42	37	9	2	10	-	-	[72]
Wheat leaves (grana)	47	36	7	1	9	-	-	[72]
Pea seedlings	45	31	2	10	7	2	1	[78]
Spinach leaves	52	26	7	5	10	2	0	[76]
Pea seedlings	46	31	7	ND	11	ND	ND	[80]
A. hippocastanum leaves	43	31	5	ND	15	ND	ND	[80]
Pea seedlings	51	33	9	2	5	< 0.5	ND	[68]

DGDG digalactosyldiacylglycerol, *MGDG* monogalactosyldiacylglycerol, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *SQDG* sulfoquinovosyldiacylglycerol, *ND* not detected

galactose molecules linked to diacylglycerol (DAG) by a glycosidic bond. Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) can constitute up to 75% of total chloroplast membrane lipids [60]. Additionally, chloroplast membranes contain sulfoquinovosyldiacylglycerol (SQDG), phosphatidylglycerol (PG) and phosphatidylcholine (PC), albeit in relatively small amounts (Table 1). However, lipid composition varies among different chloroplast membranes. The OEM is particularly enriched in DGDG and PC, whereas MGDG is predominant in the IEM and thylakoids. The IEM and thylakoid membranes share similar lipid profiles and are composed primarily of MGDG, DGDG, SQDG and PG, with PG being the only phospholipid (Table 1).

Similarly, each glycerolipid exists in multiple molecular species whose fatty acid composition differs at the sn-1 and sn-2 positions on glycerol, reflecting the complexity of lipid biosynthetic pathways within the cell. Glycerolipids are classified into two main types based on their fatty acid composition: plastidial-type (also referred to as "prokaryotic"), which contains C16 fatty acids at the sn-2 position, and eukaryotic-type, which contains C18 fatty acids at this position [61, 62].

Chloroplastic MGDG and DGDG can originate from both eukaryotic and plastidial pathways [63], and their distributions vary among plant species. In many species of higher plants, including pea (*Pisum sativum*), olive tree (*Olea europaea*) and corn (*Zea mays*), galactolipids are synthesized via the eukaryotic pathway. These species, termed 18:3 plants, produce galactolipids, which predominantly include C18:3 (α-linolenic acid, C18:3$^{\Delta 9cis,12cis,15cis}$) [64–66]. In contrast, species such as *Arabidopsis thaliana*, spinach (*Spinacia oleracea*) or potato (*Solanum tuberosum*) utilize both pathways to synthetize MGDG, DGDG and SQDG. The leaf lipids characteristically contain significant amounts of C16:3 (7cis,10cis,13cis-hexadecatrienoic acid, C16:3$^{\Delta 7cis,10cis,13cis}$) within MGDG and DGDG molecules

derived from the prokaryotic pathway. These species are referred to as 16:3 plants to distinguish them from 18:3 plants. Finally, chloroplastic PG species are almost exclusively of the plastidial type, with some containing a C16:1 *trans* fatty acid (3-*trans*-hexadecenoic acid, C16:1$^{\Delta 3\ trans}$) at the *sn-2* position [61, 67].

Compared with thylakoid membranes, chloroplast envelope membranes are characterized by a notably greater lipid-to-protein ratio. Among plant cell membranes, the OEM exhibits the highest lipid-to-protein ratio, ranging from 2.5 to 3 mg of lipids per mg of protein [76], and contains functionally specialized regions. Fluorescent protein tagging in the alga *Chlamydomonas reinhardtii* has shown that many of the analysed OEM proteins display heterogeneous localization patterns, forming distinct patches and puncta [81]. As the OEM is thought to host proteins involved in ER–chloroplast contact sites, it is likely that some of these discrete regions are important for such interactions. Many of these proteins are conserved across land plants, suggesting that certain structures are likely conserved as well, although this needs to be demonstrated.

Glycerolipid synthesis depends on ER–chloroplast lipid trafficking

Glycerolipid biosynthesis in plants is a complex, multistep process involving numerous enzymatic reactions within both the ER and chloroplasts. This section summarizes key aspects of chloroplast glycerolipid synthesis, as numerous comprehensive reviews have extensively covered all facets of the topic [24, 63, 82–87].

Fatty acid synthesis begins in the chloroplast stroma and is catalysed by the fatty acid synthase (FAS) multienzyme complex [88], which primarily produces C16:0-ACP (acyl carrier protein) and C18:0-ACP. However, a significant portion of C18:0-ACP is converted into C18:1$^{\Delta 9cis}$-ACP by stearoyl-ACP Δ^9-desaturase (SAD) [89, 90]. Some of these acyl-ACPs are subsequently incorporated into lipid precursors, such as phosphatidic acid (PA) and DAG, within the chloroplast [63] through the prokaryotic pathway. Additionally, thioesterases, such as FATA and FATB, release fatty acids from their acyl carrier proteins [91, 92], allowing their export from the chloroplast via FAX1 [93] proteins. Once exported, these fatty acids are activated by conversion into acyl-CoAs via long-chain acyl-CoA synthetases (LACSs) and subsequently incorporated into phospholipids through the eukaryotic pathway within the ER, contributing to the formation of essential cellular membrane components (Fig. 1).

In both the chloroplast and the ER, the assembly of fatty acids into PA occurs through two consecutive acylation reactions of glycerol-3-phosphate (Fig. 1). First, glycerol-3-phosphate acyltransferase (GPAT) catalyses the formation of lysophosphatidic acid (LPA), which is subsequently converted into PA by a lysophosphatidic acid acyltransferase (LPAT). The GPAT and LPAT enzymes in the chloroplast and ER exhibit different substrate specificities, leading to the production of distinct PA species in each organelle [63, 94]. Additionally, plants can generate PA through the activity of phospholipases. Phospholipase D (PLD) hydrolyses PC to generate PA and choline, whereas phospholipase C (PLC) hydrolyses PC to produce DAG, which is subsequently phosphorylated by diacylglycerol kinase (DGK) to synthetize PA [95, 96]. Radiolabelling studies have identified three metabolically distinct PC pools in plant cells: (1) PC synthesized de novo through the eukaryotic pathway, which serves as a desaturation substrate and provides PC for ER-based distribution; (2) a PC pool used for MGDG synthesis; and (3) a more recently identified PC pool linked to chloroplastic lysophosphatidylcholine acyltransferases (LPCAT1 and LPCAT2). This latter PC pool, potentially located at ER–chloroplast membrane contact sites, may facilitate acyl editing [97], as LPCAT enzymes catalyse the acylation of lysophosphatidylcholine (Lyso-PC) to produce PC.

Chloroplast lipids—MGDG, DGDG and SQDG—are synthesized within the chloroplast from DAG, which is mostly generated from the dephosphorylation of PA. Both DAG and PA are derived from de novo synthesis in the chloroplast or from ER-derived glycerolipids that are transported back to the plastid for this purpose [63, 98–100]. Although the precise mechanisms and lipid classes exchanged between the ER and chloroplasts at membrane contact sites remain unclear, four lipids are the strongest candidates to shuttle between these organelles: PC, DAG, PA and Lyso-PC [101] (Fig. 1). In the chloroplast, PA is dephosphorylated by PA phosphatases (PAPs), generating DAG, the main substrate for chloroplast galactolipid synthesis. PAP enzymes are classified into two types: soluble phosphatidate phosphohydrolases (PAHs) and membrane-integrated lipid phosphate phosphatases (LPPs). The loss of the plastidial (prokaryotic) lipid biosynthetic pathway in certain plant lineages (so-called 18:3 plants) correlates with the disappearance of PA phosphatases in multiple clades [102, 103]. In Arabidopsis, nine LPPs have been identified, although most are not directly involved in lipid metabolism [104, 105]. Among them, LPPα2 (ER-localized) and LPPϵ1 (plastid-localized) are implicated in lipid metabolism [106], and plastidial LPPγ and LPPϵ1 exhibit redundant activity at the OEM, contributing to plant development. However, none of these enzymes appear to play a major role in plastidial galactolipid biosynthesis (Fig. 2) [107]. Conversely, cytosolic PAH1

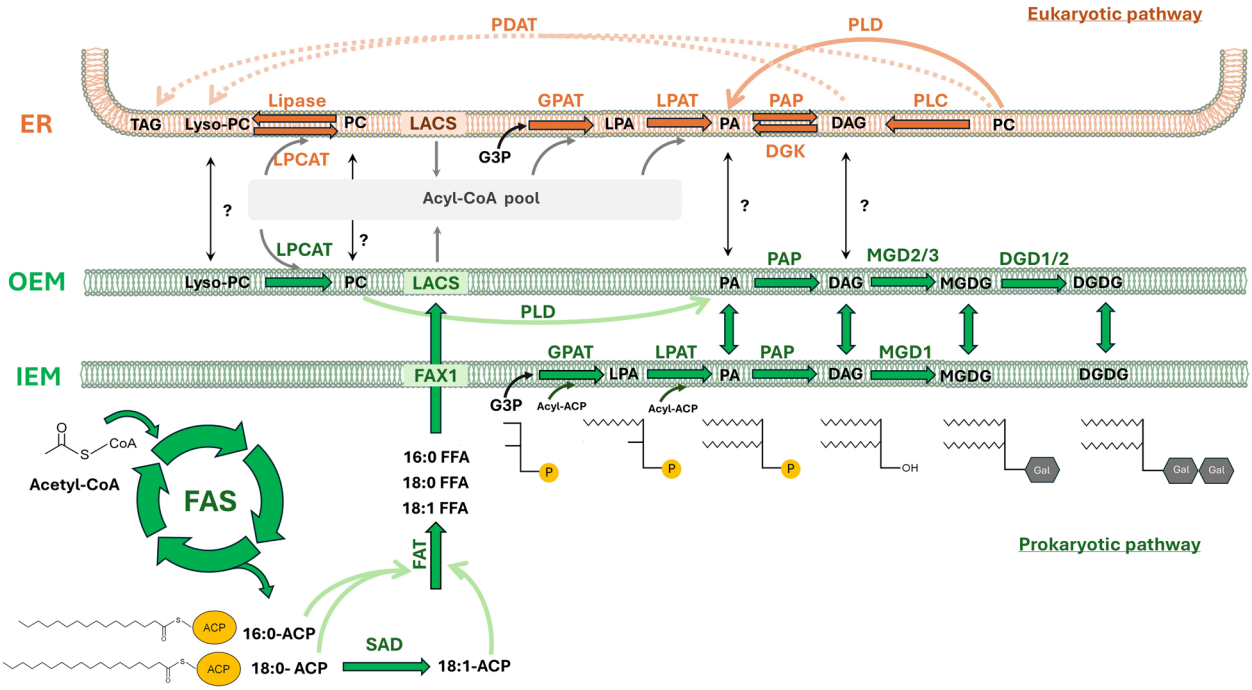

Fig. 1 Schematic representation of key reactions involved in glycerolipid synthesis in plants. Fatty acids are synthesized in the chloroplast stroma by the fatty acid synthase (FAS) complex, which uses acetyl-CoA to produce fatty acids conjugated with ACP. The 18:0-ACP molecules can be desaturated to 18:1-ACP by SAD. Acyl-ACPs (16:0, 18:0 and 18:1) are either incorporated into the prokaryotic lipid synthesis pathway within the chloroplast or exported for eukaryotic lipid synthesis at the ER. To participate in ER glycerolipid synthesis, these acyl-ACPs must first be converted into acyl-CoAs and transported to the cytosol through the sequential actions of the FAT, FAX1 and LACS enzymes. The acyl group is then transferred to G3P to produce LPA, which then enters the eukaryotic lipid synthesis pathway. Subsequent reactions catalysed by LPAT and PAP generate PA and DAG, respectively. Within the chloroplast, similar reactions occur, leading to the production of analogous lipids. Additionally, in the ER, glycerolipids undergo *lipid remodelling*, modifying their head groups, acyl chain lengths and degrees of unsaturation. This process involves enzymes such as LPCAT, PDAT, PLD and PLC, which generate various species of Lyso-PC, PC, PA and DAG. Some of these lipids can be transferred back to the chloroplast, although most of the proteins and complexes involved in this transport remain unidentified. In the chloroplast, DAG serves as the precursor for galactolipid synthesis, which is catalysed by MGD synthases (MGDG1/2/3) and DGD synthases (DGD1/2), generating DGDG. Chloroplast enzymes are shown in green; ER enzymes are shown in orange. Question marks ("?") indicate proteins that remain to be identified

Abbreviations: ACP, acyl carrier protein; CoA, coenzyme A; DAG, diacylglycerol; DGK, diacylglycerol kinase; DGD, digalactosyldiacylglycerol synthases; DGDG, digalactosyldiacylglycerol; ER, endoplasmic reticulum; FAD, fatty acid desaturase; FAS, fatty acid synthase; FATA/B, fatty acyl-ACP thioesterase A/B; FAX1, fatty acid exporter 1; FFA, free fatty acid; G3P, glycerol 3-phopshate; Gal, galactose; GPAT, glycerol-3-phosphate acyltransferase; IEM, inner envelope membrane; LACS, long-chain acyl-CoA synthetase; LPA, lysophosphatidic acid; LPAT, lysophosphatidic acid acyltransferase; LPCAT, lysophosphatidylcholine acyltransferase; Lyso-PC, lysophosphatidylcholine; MGD, monogalactosyldiacylglycerol synthases; MGDG, monogalactosyldiacylglycerol; OEM, outer envelope membrane; P, phosphate, PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PC, phosphatidylcholine; PDAT, phospholipid: diacylglycerol acyltransferase 1; PLC/D, phospholipase C/D; SAD, stearoyl-acyl carrier protein Δ9 desaturase; TAG, triacylglycerol; UDP-Gal, uridine diphosphate-galactose

and PAH2, soluble Mg^{2+}-dependent PA phosphatases, redundantly contribute to galactolipid synthesis and lipid remodelling, particularly under conditions of phosphate starvation [108, 109] or nitrogen depletion [110]. Finally, emerging evidence suggests that the RHOMBOID LIKE10 (RBL10) protein may aid in converting PA to DAG precursors for MGDG biosynthesis. These findings indicate that PA dephosphorylation in the plastidial pathway may partially depend on RBL10, although its precise biochemical activity remains unclear [111]. Thus, the identities of the plastidial PA phosphatases responsible for producing DAG within the chloroplast and directly involved in galactolipid biosynthesis remain unidentified, and it is plausible that a different enzyme class is responsible for plastid PA dephosphorylation.

Finally, MGDG synthase (MGD) transfers a galactose molecule from uridine diphosphate-galactose (UDP-Gal) to DAG, forming MGDG [112, 113]. MGDG can undergo the addition of a second galactose molecule via DGDG synthase (DGD), which uses MGDG and UDP-Gal as substrates to produce DGDG [114] (Fig. 1). In *A.*

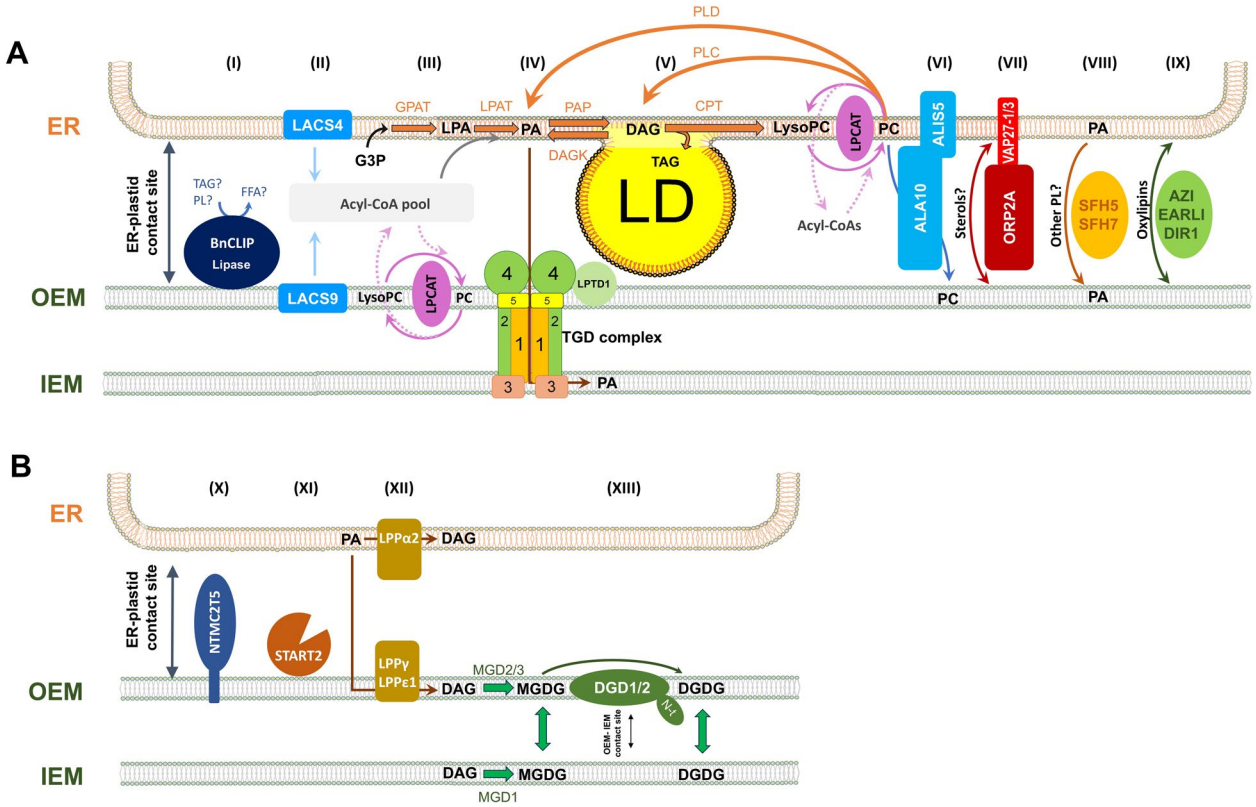

Fig. 2 **A** Proteins involved in lipid metabolism at ER–plastid contact sites. (I) BnCLIP is a putative lipase and was the first protein to be identified at ER–chloroplast contact sites. (II) LACS proteins activate fatty acids into acyl-CoAs. (III) LPCATs are involved in lipid remodelling, catalysing the reacylation of lyso-PC into PC by incorporating an acyl group from the acyl-CoA pool while also facilitating the reverse reaction, generating lyso-PC from PC. (IV) The trigalactosyldiacylglycerol (TGD) complex is essential for PA transport between the OEM and IEM of the chloroplast and may also mediate DAG transport. LPTD1 has been proposed to work in conjunction with TGD4. (V) Lipid droplets originate from the ER and primarily store TAG. Growing evidence suggests an interplay between the ER and chloroplasts in lipid droplet development. (VI) ALA10 is a lipid flippase anchored to the ER. When associated with the chaperone ALIS5, ALA10 localizes near chloroplasts, where it may facilitate the transfer of PC to chloroplasts by creating a localized enrichment of PC on the cytosolic leaflet of the ER membrane. (VII) The VAP27-ORP2A complex may be involved in regulating sterol transport at ER–chloroplast contact sites. (VIII) SFH5 and SFH7 are soluble proteins localized to both the ER and chloroplasts. They transfer PA—and potentially other phospholipids—from the ER to chloroplasts, regulating chloroplast lipid composition and thylakoid development. (IX) AZI1 (azelaic acid-induced 1), EARL1 (early Arabidopsis response to low light 1) and DIR1 (defective in induced resistance 1) are lipid transfer soluble proteins localized at the ER and chloroplast. They transfer oxylipins between membranes and are involved in systemic acquired resistance. **B** Proteins to be explored. (X) NTMC2 T5 proteins contain a lipid transfer domain, are anchored to the OEM and may be involved in lipid transfer at ER–plastid contact sites. (XI) The START2 protein contains a START domain and has been suggested to play a role as a lipid transporter from the ER to the chloroplast. (XII) LPPε1, LPPγ localized at the chloroplast OEM, facilitates MGDG biosynthesis from ER-derived phospholipids. LPPα2, an ER-localized protein, along with LPPε1, has a collaborative role in lipid metabolism. (XIII) DGD1 localizes to the OEM and is responsible for the synthesis of DGDG. It facilitates the transfer of lipids from the OEM to the IEM. However, the direct interaction between DGD1 and ER-chloroplast contact sites requires further investigation. Question marks ("?") indicate that these molecules remain to be unambiguously demonstrated

Abbreviations: ALA10, aminophospholipid-ATPase 10; ALIS5, ALA-interacting subunit 5; AZI1, azelaic acid-induced 1; BnCLIP1, Brassica napus chloroplast-localized lipase 1; CoA, coenzyme A; CPT, diacylglycerol-choline phosphotransferase; DAG, diacylglycerol; DAGK, diacylglycerol kinase; DGD, digalactosyldiacylglycerol synthases; DGDG, digalactosyldiacylglycerol; DIR1, defective in induced resistance 1; EARLI1, early Arabidopsis aluminium-induced 1; ER, endoplasmic reticulum; FAX1, fatty acid exporter 1; FFA, free fatty acid; G3P, glycerol 3-phosphate; GPAT, glycerol 3-phosphate acyltransferase; IEM, inner envelope membrane; LACS, long-chain acyl-CoA synthetase; LD, lipid droplet; LPA, lysophosphatidic acid; LPCAT, lysophosphatidylcholine acyltransferases; LPP, lipid phosphate phosphatase; LPTD1: Lipoprotein transporter D1; Lyso-PC, lysophosphatidylcholine; MGD, monogalactosyldiacylglycerol synthases; MGDG, monogalactosyldiacylglycerol; NTMC2T5, N-terminal-transmembrane-C2 domain type 5; OEM, outer envelope membrane; ORP2A, oxysterol-binding protein-related protein 2A; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PL, phospholipid; PLC/D, phospholipase C/D; SFH5/7, Sec14 homology proteins 5/7; STAR2, steroidogenic acute regulatory protein-related lipid transfer 2; TAG, triacylglycerol; TGD, trigalactosyldiacylglycerol complex; VAP27-1/3, vesicle-associated membrane protein (VAMP)-associated proteins

thaliana, MGD1 and DGD1 are the primary enzymes involved in the synthesis of galactolipids in photosynthetic tissues. Additionally, other MGDG synthases (MGD2 and MGD3) and DGDG synthase 2 (DGD2) are involved in galactolipid synthesis during phosphate starvation. MGD1 localizes to the IEM, whereas MGD2 and 3 and DGD1 and 2 are found on the OEM [113, 114]. The acyl compositions of the resulting chloroplastic MGDG and DGDG molecules differ due to several factors, including the fatty acids synthesized, the specificity and subcellular localization of galactolipid biosynthetic enzymes, the composition and availability of the DAG pool, and the lipid remodelling processes within the cell.

Arabidopsis contains several lipases, including plastid lipases (PLIPs), which also play a role in lipid metabolism. PLIP2 and PLIP3 release fatty acids, particularly C18:3, from MGDG and PG in the chloroplast. The free C18:3 generated by PLIP2 or PLIP3 can be converted into jasmonic acid precursors, leading to jasmonate accumulation [115]. In contrast, PLIP1 is a PG-specific phospholipase A1 that preferentially hydrolyses PG containing *sn*-2 C16:1$^{\Delta 3\ trans}$, releasing C18:2 and C18:3. These fatty acids are subsequently exported to the cytosol, where they are incorporated into PC and TAG [116]. PLIP1 facilitates the transfer of acyl groups from plastids to the ER for TAG synthesis, establishing a link between plastidial lipid metabolism and TAG production in the ER, which is crucial for seed oil biosynthesis [116, 117].

Initially, glycerolipids are synthesized using mainly C16:0, C18:0 and C18:1 acyl groups. However, these lipids can be desaturated into more unsaturated forms by membrane-bound fatty acid desaturases (FADs) located in the chloroplast and the ER. In *Arabidopsis* chloroplasts, FAD5 catalyses the desaturation of C16:0 to C16:1 (C16:1$^{\Delta 7cis}$) [118, 119], specifically at the *sn-2* position of galactolipids, an activity characteristic of 16:3 plants. Chloroplast galactolipids can also be modified by FAD6 [120, 121], which acts on both C18:1 and C16:1. Subsequently, FAD7 [64, 122] and FAD8 [123, 124] convert these intermediates into polyunsaturated forms, yielding C18:3 and C16:3. Additionally, plastidial FAD4 can introduce a Δ3*trans* double bond in C16:0, specifically at the *sn*−2 position in PG [125, 126]. Conversely, in the ER, a key desaturation step is catalysed by FAD2 [127], which converts C18:1 (oleic acid, C18:1Δ^{9cis}) into C18:2 (linoleic acid, C18:2$^{\Delta 9cis,12cis}$). This can be followed by FAD3 [128], which introduces a third double bond at the Δ15 position, producing α-linolenic acid. Both FAD2 and FAD3 act mostly on acyl groups esterified to PC [97].

In addition to their roles in galactolipid synthesis, increasing evidence over the past decade has suggested an interplay between the ER and chloroplasts in lipid droplet development. Lipid droplets store neutral lipids for energy production, membrane synthesis and stress responses [129]. They originate from the ER, and most remain attached to it until degradation occurs [130]. Lipid droplets have been shown to reshape their ER interfaces throughout their life cycle, suggesting the existence of specialized contact sites with distinct protein and lipid compositions that may serve diverse functions [131]. In the green eukaryotic alga *Chlamydomonas reinhardtii*, lipid droplets have occasionally been observed in close association with both the ER and the OEM of the chloroplast, particularly under high light or nitrogen deprivation conditions or in a starchless mutant [132–134]. Furthermore, a metabolic connection between chloroplasts and lipid droplets has been suggested in *Chlamydomonas* during nitrogen depletion, as evidenced by the chloroplast localization of key enzymes involved in TAG assembly, as well as the incorporation of typical chloroplast lipids such as DGDG and MGDG into the lipid droplet coat [133–135]. Additionally, the *Chlamydomonas* mutant *tgd2*, which carries a mutation in a component of the chloroplast lipid transporter TRIGALACTOSYLDIACYLGLYCEROL (TGD), exhibits increased lipid droplet formation and enhanced conversion of MGDG to TAG, which accumulates in lipid droplets. These findings suggest an indirect link between lipid trafficking among the ER, chloroplasts and lipid droplets [136]. However, direct evidence for the existence of lipid droplet–ER–chloroplast contact sites remains lacking, and lipid trafficking at these interfaces in plants remains poorly understood.

Proteins involved in lipid metabolism at ER–plastid contact sites

Unlike the well-characterized vesicular transport systems between the ER and other organelles, such as the Golgi apparatus or plasma membrane, vesicular trafficking between the ER and chloroplasts remains poorly understood. Chloroplasts are unique in their biogenesis and lipid transport mechanisms, as they are not part of the classical endomembrane system. Instead, lipid exchange between the ER and chloroplasts is thought to occur through mechanisms independent of vesicular transport. Several mechanisms have been proposed to explain lipid movement between these two organelles. One model suggests direct lipid transfer through membrane fusion or "hemifusion", allowing lipids to diffuse across without the need for vesicles or lipid-binding proteins [56]. Another hypothesis, known as "vectorial acylation", posits that lipids are transported as acyl intermediates, such as acyl-CoAs, which are shuttled between the ER and chloroplasts by specific lipid-binding proteins [85, 87]. Recent studies suggest that complex lipids can be

transferred via specialized lipid-binding proteins located at these membrane contact sites. These various mechanisms may cooperate in lipid trafficking at ER–plastid contact sites, with specific pathways depending on the types of lipids and proteins involved. In this section, we summarize the lipid transporters and modifying proteins that have been described as being localized at ER–plastid contact sites (Fig. 2).

BnCLIP1

An important discovery in the field of lipid trafficking at ER–chloroplast contact sites was the identification of a *Brassica napus* lipase, named chloroplast-localized lipase 1 (BnCLIP1) [137]. Phylogenetic analysis, which is based on the similarity of the conserved domain sequence between BnCLIP1 and several lipases with different targets, suggested that BnCLIP may be a multisubstrate lipase [137]. Transient expression of BnCLIP1-eGFP in tobacco leaf tissue revealed a distinct punctate localization on the plastid envelope, with ER membranes positioned closely to the BnCLIP1-eGFP puncta [137]. BnCLIP1-GFP is enriched on the OEM, where plastid and ER membranes become linked (Fig. 2). This dynamic linkage influences plastid movement, positioning and stromule extension and retraction [54]. Furthermore, heterologous expression of BnCLIP1 in *Saccharomyces cerevisiae* resulted in a reduction in total fatty acid content, with a substrate preference for C16:0 lipids, demonstrating its lipolytic activity. The transcription of BnCLIP1 is upregulated during seed senescence (when the chlorophyll content in developing seeds begins to decline), suggesting a role in maintaining chloroplast integrity and mobilizing lipids [137]. However, the specific function of BnCLIP1 in degrading triacylglycerols or other glycerolipids within the OEM remains unclear. Further research is needed to fully elucidate its function in lipid metabolism at ER–chloroplast contact sites.

Long-chain acyl-CoA synthetases (LACSs)

LACS proteins play essential roles in lipid synthesis, fatty acid catabolism and the transport of fatty acids between subcellular compartments. They catalyse the conversion of fatty acyl chains into fatty acyl-CoAs (Fig. 1). In Arabidopsis, LACS9 primarily localizes to the chloroplast envelope (Fig. 2), where it activates fatty acids for export from plastids [138]. However, *lacs9* knockout mutants do not exhibit major phenotypic changes, suggesting that other LACSs compensate for this activity [138–140]. In contrast, LACS4 is localized in the ER, yet the *lacs4* mutant is phenotypically indistinguishable from the wild type. However, *lacs4 lacs9* double mutants exhibit defects in glycolipid biosynthesis, resulting in a significant reduction in C18:2 at the *sn-2* position of MGDG and a reduced fatty acid content per seed [141], indicating impaired lipid precursor transfer between the ER and plastids [140, 141]. Unlike *tgd* mutant lines (see below), *lacs4 lacs9* mutants do not accumulate trigalactosyldiacylglcyerol (TGDG) or TAG in leaves. The subcellular localization of LACS4 and LACS9 suggests that they might function at ER–plastid contact sites, generating a local pool of acyl-CoA at these sites. Despite their localization in distinct subcellular compartments, genetic and biochemical evidence indicates that LACS4 and LACS9 have overlapping functions, supporting their roles in ER–plastid lipid trafficking.

Lysophosphatidylcholine acyltransferases (LPCATs)

Lysophosphatidylcholine acyltransferases are enzymes that catalyse the reacylation of lyso-PC into PC by transferring an acyl group from acyl-CoA. This activity plays a crucial role in phospholipid acyl remodelling, allowing for the modification of the fatty acid composition of PC [97]. Notably, LPCATs can catalyse both forwards and reverse reactions, further contributing to lipid homeostasis [142]. Lyso-PC can be spontaneously transferred between membranes, which suggests that it may serve as a lipid precursor for chloroplast membranes, and this transport is believed to occur at ER–chloroplast contact sites. LPCAT activity has been detected in the chloroplast envelopes of several plant species [141, 143], indicating that it may participate in PC remodelling and lipid transfer at these contact sites. Experimental evidence supports this idea, as pulse-chase experiments in leek (*Allium porrum*) seedlings suggest that LACS generates an acyl-CoA pool that could be used by LPCAT in PC synthesis at ER–chloroplast contact sites [144]. However, more recent lipid pulse-chase labelling experiments in an Arabidopsis mutant lacking the prokaryotic lipid synthesis pathway and of the two major LPCAT enzymes (*act1 lpcat1 lpcat2*) revealed that LPCAT activity is not required for MGDG production. Instead, LPCAT appears to facilitate the incorporation of fatty acids exported from chloroplasts into ER phospholipids via PC acyl editing [97]. This study also suggests the existence of a distinct PC pool in chloroplasts, where LPCAT1 and LPCAT2 play a role in acyl editing for fatty acid export to the ER at ER–chloroplast contact sites. Despite these findings, the precise functions of LPCAT in lipid trafficking between the ER and chloroplasts remain unclear. Further research is needed to fully elucidate its role, confirm its localization at ER–chloroplast contact sites and determine the extent to which it contributes to lipid transport and remodelling in plant cells.

TGD complex

The TGD complex functions as a non-classical ABC transporter system composed of TGD1, TGD2 and TGD3, which are in the IEM of the chloroplast. TGD4, which is found in the OEM, is thought to interact with the ER and TGD5 (Fig. 2). The name "TGD" (trigalactosyldiacylglycerol) reflects the accumulation of oligogalactolipids in mutants lacking these proteins. Together, these proteins mediate the transfer of glycerolipid precursors, such as PA and DAG, from the ER to the chloroplast envelope membranes [57, 145].

TGD1 is similar to the permease component of bacterial ABC transporters. The Arabidopsis *tgd1* mutant presents a complex lipid phenotype, including the accumulation of oligogalactolipids and triacylglycerols, impaired assembly of galactolipids from ER-derived precursors and elevated PA levels [146, 147]. The accumulation of PA and reduced incorporation of labelled PA into galactoglycerolipids in *tgd1* chloroplasts suggest that TGD1 is involved in transporting PA across the IEM [146, 147]. TGD2 is similar to membrane-tethered substrate-binding proteins in bacterial ABC transporters [148]. Mutants of *tgd2* present a lipid phenotype nearly identical to that of *tgd1*. Additionally, the recombinant TGD2 protein lacking its single membrane-spanning domain has been shown to specifically bind PA [148]. In 2007, TDG3 was identified as a small ATPase within this translocator complex, localizing beyond the IEM [149]. These three proteins form the "core" of a complex similar in size to standard ABC transporters, with approximately 8–12 copies of the substrate-binding protein TGD2 found per functional transporter [150].

Later, TGD4 was identified in a mutant screen for lipid phenotypes [57]. The *tgd4* mutant presents a pale green phenotype due to impaired chloroplast function and reduced chlorophyll content, which correlates with defects in thylakoid membrane synthesis. Lipid analyses of *tgd4* revealed the accumulation of triacylglycerols and oligogalactoglycerolipids, similar to other TGD mutants. Despite these lipid abnormalities, the mutant retains some capacity for chloroplast development, although it is significantly compromised. Localization studies confirmed that TGD4 associates with the OEM, with its N-terminal region exposed to the cytosol [151]. Interestingly, TGD4 appears to function differently from the TGD1-3 complex, potentially playing a more relevant role at the OEM, where it may interact with the ER. Studies of double mutants involving *tgd1* or *tgd4* in various genetic backgrounds, such as *fad2* (ER desaturase) or *fad6* (plastid desaturase), suggest that the role of TGD proteins is primarily involved in lipid import into plastids and does seem to extend to lipid export to extraplastidic membranes [152]. In protein–lipid binding assays, TGD4 was shown to specifically bind PA but not other phospholipids, highlighting its selectivity for this lipid class [151]. This binding is crucial for lipid trafficking, as PA accumulates in extraplastidic membranes in *tgd4* plants, suggesting that TGD4 could be involved in the transfer of lipids from the ER to the chloroplast. Further research revealed that TGD4 is a dimeric barrel protein that binds PA at its N-terminus and contains dimerization domains at its C-terminus. Specifically, amino acids 1–80 and 110–145 are required and sufficient for PA binding [145]. However, although TGD4 is associated with PA binding, direct evidence for PA transport via TGD4 is lacking, and PA may play a regulatory role in TGD complex activity. It remains to be demonstrated whether PA or other lipids are transported between the ER and the OEM at these contact sites, and additional research is needed to clarify the exact molecular dynamics involved.

In 2015, TGD5, also known as green less stomata 1 (GLES1), was identified as a small glycine-rich protein (27.5% glycine) localized to the IEM but absent from thylakoids. TGD5 interacts with other TGD proteins (TGD1, TGD2, TGD3 and TGD4) [153]. Disruption of TGD5 results in a significant reduction in ER-derived thylakoid lipids and the accumulation of oligogalactolipids and triacylglycerol, although these effects are less severe than those in *tgd1* mutants. The *gles1* mutation impairs chloroplast biogenesis in guard cells, leading to defective stomatal regulation, with abnormal CO_2-induced closure and light-induced opening [154]. TGD5 also plays a critical role in maintaining plastid morphology, particularly in non-mesophyll tissues [155]. Another *tgd5* mutant (*suba1*) displays excessive stromule formation in non-mesophyll cells, such as the leaf epidermis, whereas mesophyll chloroplasts remain unaffected. This mutation disrupts normal plastid morphogenesis, leading to the accumulation of lipid droplets and autophagic engulfment by vacuoles, suggesting a distinct mechanism regulating plastid morphology in non-mesophyll cells, possibly involving differential contributions of the plastidial and ER pathways in lipid metabolism [155].

These studies highlight the fundamental importance of the TGD protein complex and lipid transfer pathways in plastid function, emphasizing their roles in thylakoid biogenesis, plastid morphogenesis in non-mesophyll tissues and stomatal response mechanisms. Research has shown that disruptions in this lipid pathway lead to a wide range of phenotypic effects, from altered plastid shapes and lipid accumulation to impaired environmental responses, offering valuable insights into the complexity of lipid metabolism and its broader implications in plant biology. Further research is needed to elucidate the exact molecular interactions between these proteins and to determine

whether they work in concert with other proteins to mediate lipid transfer between the ER and chloroplasts.

atLPTD1

In the *Arabidopsis thaliana* proteome, there are only two orthologues of the bacterial and plant-conserved OEM β-barrel-shaped lipid-A transporter (LptD) proteins. One of these is TGD4 [57], and the other has been identified as LptdD1 [156]. Studies have revealed that LPTD1 works in conjunction with TGD4 to coordinate lipid transport between the ER and chloroplasts, particularly under phosphate stress [156]. Like TGD4, LPTD1 functions as a cation-selective channel and may also facilitate lipid transfer across membranes. Under phosphate starvation, the LPTD1 protein content increases, suggesting a stress-responsive role, and RNAi lines targeting LPTD1 exhibit growth defects, especially in the *tgd4-1* mutant background, which further increases sensitivity to light and phosphate stress. Additionally, the increases in DGDG and SQDG observed under phosphate limitation in WT plants were compromised in these RNAi mutants [156]. However, it is still unclear whether LptD1 functions as an additional component of the TDG complex or is part of a different lipid-transfer complex at ER–chloroplast contact sites.

ALA10

PC is located primarily at the outer OEM monolayer in chloroplasts, with none detected at the internal OEM monolayer and minimal amounts in other plastid membranes [157] (Table 1). Although PC synthesis does not occur within chloroplasts, recent research has suggested that the P4-type ATPase aminophospholipid ATPase 10 (ALA10), a member of the phospholipid flippase family, may contribute to the presence of PC on the chloroplast surface. The Arabidopsis *ala10* knockout mutant displays deficiencies in phosphate starvation responses, as well as perturbations in chloroplast lipid homeostasis [158, 159]. ALA10 localizes to the ER, but when it is coexpressed with its chaperone ALA-interacting subunit 5 (ALIS5), it associates near chloroplasts. These findings suggest that the ALA10–ALIS5 complex may function at ER-chloroplast contact sites (Fig. 2). In Arabidopsis, ALA10 overexpression leads to increased C18:2 and reduced C18:3 levels in chloroplast PC, suggesting that ALA10 facilitates the translocation of C18:2-rich PC from the lumen-facing to the cytosolic side of the ER membrane [159] and that the ALA10–ALIS5 complex facilitates the efficient transport of C18:2-PC to the chloroplast. Yeast-based fluorescent lipid uptake assays have shown that ALA10 preferentially translocates PC but also exhibits activity towards other lipids, including PA [160, 161]. Additionally, large-scale plant analysis indicated that the MGDG–PC ratio is significantly influenced by ALA10 expression and that ALA10 enhances MGDG synthesis at the expense of PC [159]. However, definitive evidence confirming ALA10 localization at ER–chloroplast contact sites and direct evidence of ALA10–ALIS5-mediated lipid translocation at these sites are still lacking.

VAP27-1 VAP27-3 with ORP2A

A recent study by Renna et al. (2024) revealed significant advances in elucidating a new mechanism behind lipid transport at ER–chloroplast contact sites by identifying a protein complex involving VAP27-1/VAP27-3 and ORP2A in *Arabidopsis thaliana* (Fig. 2). Oxysterol-binding protein-related proteins (ORPs) are a conserved family of lipid transfer proteins found in eukaryotic organisms and are known to be lipid exchangers, as they mediate the counterdirectional transport of two different lipid ligands, such as cholesterol and phospholipids, at membrane contact sites [162, 163]. Alternatively, VAP27 proteins are plant homologues of the vesicle-associated membrane protein (VAMP)-associated proteins (VAPs) found in animal cells. In plants, VAP27 proteins are localized primarily to the ER, where they play key roles in mediating interactions between the ER and other organelles, including the plasma membrane or Golgi apparatus [164]. One of the key findings of this study was the localization of VAP27-1 and VAP27-3 to specific ER subdomains that are close to chloroplasts. This localization was demonstrated using live-cell confocal microscopy, which revealed that VAP27 proteins accumulated at sites with reduced mobility compared with other regions of the ER, as confirmed by fluorescence recovery after photobleaching (FRAP) experiments. This reduced mobility suggested that the proteins were stabilized at these membrane contact sites, likely due to their interaction with chloroplasts. Further investigation into the roles of these proteins revealed that VAP27-1 and VAP27-3 physically interact with ORP2A. Additionally, ORP2A was shown to bind to specific chloroplast lipids, particularly MGDG, as well as phytosterols, such as β-sitosterol and campesterol, via protein–lipid overlay assays. Lipidomic analyses of *vap27-1/vap27-3/orp2a-1* mutants revealed subtle yet important changes in the acyl composition of key chloroplast lipids, particularly MGDG and PG, as well as increased levels of sterols in chloroplast membranes, suggesting that the VAP27-ORP2A complex is involved in regulating sterol transport and homeostasis at ER–chloroplast contact sites.

Sec14 proteins (SFH5 and SFH7)

A recent study elucidated a new mechanism by which PA is transported from the ER to chloroplasts [165]. The Sec14 family is characterized by a conserved Sec14

domain, known for its ability to transfer a variety of lipids and hydrophobic molecules [166]. Confocal microscopy revealed that the Arabidopsis proteins SFH5 and SFH7 are localized in both the ER and chloroplasts, with the nodulin domain at their C-terminus being essential for this subcellular localization. This dual localization suggests that these proteins mediate lipid transport between the two organelles (Fig. 2). Double Arabidopsis *sfh5 sfh7* mutants presented chloroplast structural defects and reduced chlorophyll content, underscoring the critical roles of these proteins in chloroplast development [165]. Crystallographic studies further demonstrated that SFH5 preferentially binds PA but can also interact with PC. Additionally, lipidomic analyses revealed that PA, PC and PG levels were significantly reduced in the chloroplasts of *sfh5 sfh7* mutants, leading to impaired synthesis of essential chloroplast lipids, including MGDG and DGDG. Notably, the reduction in MGDG levels triggered increased expression of DGD1, suggesting a compensatory response to the disruption of lipid homeostasis. This study provides critical insights into the roles of SFH5 and SFH7 in lipid transport in plants, although the exact lipids they transport and their specific localization at ER–chloroplast contact sites remain to be confirmed.

AZI1, EARLI and DIR1

The precise subcellular localization of defence factors is essential for an effective plant immune response. To trigger systemic acquired resistance (SAR), plants generate signals that can travel to activate defence mechanisms in distant tissues. Among these signals are various hydrophobic or lipid-related molecules, including azelaic acid (AZA), a C9 oxylipin derived from unsaturated C18 fatty acids. Azelaic acid-induced 1 (AZI1) and early Arabidopsis aluminium-induced 1 (EARLI1) are two soluble lipid transfer proteins that are key components of the AZA signalling pathway [167–169]. Both AZI1 and EARLI1 are essential proteins for AZA-induced priming and for establishing systemic acquired resistance. In leaves, AZI1 and EARLI1 localize to the ER, plasma membrane, plasmodesmata [170] and the plastid OEM where AZA is produced (Fig. 2). This lipid transfer protein family is suggested to reside at ER–chloroplast contact sites, facilitating the transport of AZA molecules [167]. The screening of T-DNA *Arabidopsis thaliana* lines for mutants defective in systemic acquired resistance led to the characterization of the *dir1-1* (defective in induced resistance 1) mutant. DIR1 is crucial for systemic acquired resistance and functions as a carrier for lipid-based signals, which reside at ER–chloroplast contact sites and play roles in immune responses [170, 171]. Collectively, AZI1, EARLI1 and DIR1 illustrate the dynamic interplay between lipid metabolism at ER–chloroplast contact sites and defence signalling, enabling plants to respond effectively to environmental stress [167].

Proteins to be explored

To explore potential membrane contact site proteins in plants, a list of candidate Arabidopsis proteins was generated based on homologous membrane contact site proteins and lipid-binding domains from other systems [101]. These candidates were evaluated for their chloroplast localization and expression in photosynthetic tissues. Among them, the N-terminal-transmembrane-C2 domain type 5 SMP proteins (NTMC2T5.1 and NTMC2T5.2) were suggested as candidates, and a recent preprint [172] using confocal microscopy in *N. benthamiana* confirmed their localization at chloroplasts and their attachment to the ER (Fig. 2). SMP proteins, an evolutionarily conserved family of proteins in eukaryotes, tether membrane contact sites by interacting with other proteins and membrane lipids. Biochemical studies have demonstrated that SMP domain proteins can transport a diverse range of lipid species owing to the unique hydrophobic cavity within the SMP domain. For example, the Arabidopsis SYT1 and SYT3 proteins transfer DAG at ER–plasma membrane contact sites [173], whereas SYT5 has distinct biological functions [174]. As a result, these NTMC2T5 proteins are strong candidates for mediating lipid transport at ER–chloroplast contact sites, although further research is needed to confirm this hypothesis.

In the review by Labrant et al. (2018), several OSBP proteins, including ORP1A (AT2G31020), ORP1D (AT1G13170), ORP3A (AT5G02100), ORP3B (AT3G09300) and ORP3C (AT5G59420), were identified as potential candidates for lipid transport at ER–chloroplast membrane contact sites. Recent research has shown that the ER proteins VAP27-1 and VAP27-3 interact with ORP2A, which is associated with the chloroplast OEM, and that disruption of this complex alters chloroplast sterol levels [51]. These findings suggest that other OSBP proteins, such as those proposed, may also be involved in lipid trafficking between the ER and chloroplast. Additionally, a few other proteins have been proposed as potential chloroplast-targeted membrane contact site components, including two putative pleckstrin homology (PH) domain-containing proteins (AT4G11790 and AT4G23895) and three proteins with calcium-dependent lipid-binding domains: CAR5 (AT1G48590), SYNAPTOTAGMIN 4 (AT5G11100) and AT5G55530. However, their localization at ER–chloroplast contact sites and their involvement in lipid trafficking have yet to be confirmed.

Moreover, the START2 protein from *Marchantia polymorpha*, an emerging model plant with low genetic redundancy, contains the steroidogenic acute regulatory

protein-related lipid transfer (START) domain. This protein belongs to an evolutionarily conserved superfamily of lipid transfer proteins that are widely distributed across the tree of life [175]. Recent studies have shown that START2 localizes to the OEM (Fig. 2) in a punctate pattern and is necessary for increasing the number of C20 fatty acids synthesized in the ER in chloroplast glycolipids under phosphate deprivation [176], suggesting that START2 functions as a lipid transporter, facilitating lipid transfer from the ER to the chloroplast. Most START domain-containing proteins in plants are plant-specific and evolutionarily distant from the lipid transfer proteins found in animals [177]. Some plant-specific START proteins function as transcription factors, whereas others contain domains of unknown function. The plant START minimal proteins, which contain only one START domain, are homologous to the animal lipid transfer proteins [177] and thus may be involved in lipid transfer in plant cells. However, the function and subcellular localization of these proteins remain unexplored. Further studies are needed to determine whether these homologues play a role in ER–plastid lipid trafficking in plants.

Recent studies of lipids on LPPs (lipid phosphate phosphatases) have demonstrated that LPPϵ1 and LPPγ are localized at the OEM of chloroplasts. However, LPPϵ1 is also found in proximity to the ER, and LPPα2 is localized to the ER. Although no mutant phenotype was observed in single knockout mutants of *lppα2* and *lppε1*, these proteins are essential for maintaining regular lipid metabolism. Suppressing both enzymes disrupts pollen development and ER phospholipid biosynthesis in mature siliques and seeds [106]. The Arabidopsis double mutant *lppγ lppε1* exhibits reduced flux through the ER pathway of galactolipid synthesis, suggesting that these activities facilitate MGDG biosynthesis from ER-derived phospholipids [107]. However, evidence that these proteins are localized at ER–chloroplast contact sites is still lacking, and the specific physiological roles of LPPγ, LPPϵ1 and LPPϵ2 remain to be fully understood. Additionally, the identity of the phosphatidic acid phosphatase involved in plastid galactolipid biosynthesis remains unknown.

DGD1 (digalactosyldiacylglycerol synthase 1) and DGD2 are proteins localized to the OEM of the chloroplast, with their catalytic domains facing the cytosol. These enzymes convert MGDG into DGDG. DGD1 is primarily responsible for synthesizing the majority of DGDG in chloroplasts under normal growth conditions. A unique feature of DGD1 is its N-terminal extension, which is essential for its integration into the OEM and facilitates lipid transfer between the OEM and IEM [178]. This N-terminal sequence mediates binding to PA, induces membrane fusion in vitro and suggests a potential role in lipid translocation between envelope membranes. The *dgd1* mutant exhibits significantly reduced DGDG content, leading to impaired photosynthesis, altered chloroplast morphology, and severe phenotypic effects such as extremely short inflorescence stems due to the overproduction of jasmonic acid. Additionally, *dgd1* mutants present defective membrane structures in etioplasts during dark growth [114, 179–181]. In contrast, DGD2 plays a key role in galactoglycerolipid synthesis under phosphate starvation conditions and in non-green tissues [114, 180], although DGD1 also contributes to DGDG production under these conditions. During phosphate deprivation, galactolipids are exported to extraplastidic membranes to maintain cellular integrity while prioritizing phosphate allocation for DNA/RNA protein synthesis [182]. However, because no direct transporters for galactolipids have been identified thus far and DGD1 has been suggested to facilitate lipid transfer between the OEM and IEM, DGD1 and DGD2 may not only be involved in lipid synthesis and envelope membrane transport but could also play roles in ER–chloroplast interactions, similar to their proposed functions in bridging the IEM and OEM. Nevertheless, their potential interaction with the ER and involvement in lipid trafficking at ER–plastid contact sites remain speculative, as no direct evidence currently supports this hypothesis.

Finally, a superfamily of conserved lipid transfer proteins with long hydrophobic grooves, composed of multiple repeating β-groove (RBG) domains, has emerged as a promising group of candidates for lipid transfer at various contact sites [183]. Within this superfamily, vacuolar protein sorting-associated protein 13 (VPS13) proteins are evolutionarily conserved across eukaryotes and have been shown to mediate lipid transport at membrane contact sites in yeast and mammals [184]. A phylogenetic study of VPS13 proteins in Archaeplastida highlighted the complex evolutionary history of this protein family and its role in lipid transport [185]. In *Arabidopsis*, four paralogues have been identified, with AtVPS13S localized in the endomembrane system and playing roles in regulating root growth, cell patterning and reproduction [186]. More recently, AtVPS13M1 has been shown to localize to mitochondria and participate in lipid remodelling under low-phosphate conditions [187]. Similarly, studies on bridge-like lipid transport protein 2 (BLTP2) orthologues, including sabre, kinky pollen and aberrant pollen transmission 1 (APT1) in plants, suggest that these proteins fold into rod-like structures with internal hydrophobic grooves, similar to VPS13. These proteins have been implicated in root growth and pollen germination [188, 189]. Although their localization at plastid-associated membrane contact sites remains unconfirmed, their conserved functions in lipid transport make them

candidates for future investigations into ER–plastid lipid trafficking.

Conclusion and perspectives

Membrane contact sites linking the ER and chloroplasts are fundamental for maintaining cellular functions in plants, particularly by facilitating lipid exchange, ion transfer and adaptive responses to environmental stresses. This review highlights the specialized roles of key proteins at ER–chloroplast contact sites, such as the TGD complex, Sec14 family proteins and VAP27-ORP2A complex, which play distinct roles in lipid transport, chloroplast membrane composition stability and chloroplast functionality. For example, the TGD complex is essential for transporting phosphatidic acid from the ER and across chloroplast membranes, a process critical for chloroplast lipid homeostasis and overall functionality. Similarly, Sec14 family proteins selectively transport PA, supporting chloroplast development, whereas the VAP27-ORP2A complex is putatively involved in sterol transport. Disruptions in these proteins frequently lead to chloroplast defects, diminished photosynthetic efficiency and compromised stress response capacities.

Despite these advances, several key questions remain unanswered. One of the major challenges is to elucidate the precise lipid species transported by these proteins and their impact on plastidial/ER membrane composition. The current lack of detailed lipidomic data for OEM, IEM and thylakoids in Arabidopsis highlights a critical knowledge gap. Addressing this issue will require advanced lipidomic approaches combined with high-resolution imaging techniques to visualize lipid dynamics in vivo.

To date, only a limited number of proteins, mainly those involved in lipid transport, have been definitively identified at ER–chloroplast contact sites, with a few additional candidates for these locations. The mechanisms by which these proteins attach to both membranes and facilitate lipid transport remain largely unknown. Therefore, a key challenge moving forwards will be to identify the proteins responsible for physically establishing the ER–plastid contact sites (i.e. the tethering proteins), as well as other proteins that may be localized at these junctions. Another major challenge ahead is determining whether different membrane contact site proteins exhibit functional redundancy or specificity. However, we are only beginning to uncover the full scope of these interactions, suggesting that more proteins involved in these processes may be identified in the future.

It is reasonable to speculate that, as with other membrane contact sites, ER–chloroplast contact sites may exhibit dynamic protein compositions; there may even be multiple types of ER–chloroplast contact sites with distinct functional roles. The regulation of these protein interactions in response to environmental stresses remains largely unexplored. The role of lipid transfer proteins in stress adaptation is an emerging field that requires further investigation. Future research should focus on how stress conditions such as drought, high salinity or pathogen attack influence membrane contact site formation and lipid flux, potentially revealing new targets for crop improvement. Additionally, investigating membrane contact sites in non-photosynthetic plastids, such as proplastids, amyloplasts or chromoplasts, may reveal additional mechanisms for lipid transport and metabolic adaptation across diverse organelles. Unravelling the full repertoire of proteins involved, elucidating the specific lipids transported and deciphering the spatiotemporal dynamics of these processes remain critical frontiers in this field.

From a biotechnological perspective, deciphering the molecular mechanisms that govern ER–chloroplast contact sites opens new avenues for crop improvement and metabolic engineering. These membrane contact sites are crucial for lipid transport, stress signalling and metabolic coordination, all of which are vital for plant growth and adaptation. By identifying key proteins and regulatory pathways involved in ER–chloroplast communication, researchers could develop strategies to increase photosynthetic efficiency, optimize lipid metabolism and improve stress resistance in crops. Moreover, engineering these contact sites could facilitate the production of high-value metabolites, such as biofuels or nutritionally enhanced lipids, by optimizing chloroplast metabolic functions. Given the increasing demand for sustainable agricultural solutions, understanding and manipulating ER–chloroplast interactions represent promising frontiers for developing climate-resilient crops and enhancing plant-based bioproduction systems.

In summary, the findings summarized in this review represent a step forwards in unravelling the complexity of lipid exchange at ER–plastid contact sites. However, significant challenges remain in characterizing lipid specificity, functional redundancy, stress regulation and biotechnological applications. Addressing these gaps will require interdisciplinary approaches that combine cell biology, biochemistry and omics technologies. Future research in this area has the potential to not only advance fundamental plant biology but also hold potential implications for agricultural innovation.

Abbreviations

ACP	Acyl carrier protein
Acyl-CoA	Acyl-Coenzyme A
ALA	Aminophospholipid ATPases
ALIS5	ALA interacting subunit 5
APT1	Aberrant pollen transmission 1
AZA	Azelaic acid

AZI1	Azelaic acid induced 1
BLTP2	Bridge-Like Lipid Transport Proteins 2
CLIP1	Chloroplast-localized lipase 1
DAG	Diacylglycerol
DGD	Digalactosyldiacylglycerol synthase
DGDG	Digalactosyldiacylglycerol
DGK	Diacylglycerol kinase
DIR1	Defective induced resistance 1
EARLI1	Early Arabidopsis aluminium induced 1
ER	Endoplasmic reticulum
FAD	Fatty acid desaturase
FAS	Fatty acid synthase
FATA/B	Fatty acyl-ACP thioesterase A/B
FAX1	Fatty acid exporter 1
FFA	Free fatty acid
GLES1	Green less stomata 1
GPAT Acyl-ACP	glycerol-3-phosphate acyltransferase
IEM	Inner envelope membrane
LACS	Long-chain acyl-CoA synthetases
LD	Lipid droplet
LPA	Lysophosphatidic acid
LPAT	Acyl-ACP: LPA acyltransferase
LPC	Lysophosphatidylcholine
LPCAT	Lysophosphatidylcholine acyltransferase
LPP	Lipid phosphate phosphatase
LPTD	Lipoprotein transporter D
MGD	Monogalactosyldiacylglycerol synthase
MGDG	Monogalactosyldiacylglycerol
NTMC2T5	N-terminal-transmembrane-C2 domain type 5 protein
OEM	Outer envelope membrane
ORP	Oxysterol-binding protein-related protein
PA	Phosphatidic acid
PAH	Phosphatidate phosphohydrolases
PAP	Phosphatidic acid phosphatase
PC	Phosphatidylcholine
PDAT1	Phospholipid: diacylglycerol acyltransferase 1
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PLAMs	Plastid-associated membranes
PLIP	Plastid lipases
PLC	Phospholipase C
PLD	Phospholipase D
RBG	Repeating β-groove
RBL10	Rhomboid like 10
RHD	Reticulon homology domain
SAD	Stearoyl-ACP Δ9-desaturase
SFH	Sec14 homology protein
SQDG	Sulfoquinovosyldiacylglycerol
START	Steroidogenic acute regulatory protein-related lipid transfer
TAG	Triacylglycerol
TGD	Trigalactosyldiacylglycerol complex
TGDG	Trigalactosyldiacylglycerol
UDP-Gal	Uridine diphosphate-galactose
VAP	Vesicle-associated membrane protein (VAMP)-associated protein
VPS13	Vacuolar protein sorting-associated protein 13

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Data and resource declaration
This manuscript summarizes other studies for which data has been made available.

Authors' contributions
C.H and M.MB are co-first authors as they contributed equally to this work. Conceptualisation, N.RL; writing, C.H, M.MB, V.SV and N.RL; Figures, O.C; All authors have contributed to reviwing and editing. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

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N/A.

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References

1. Ping HA, Kraft LM, Chen WT, Nilles AE, Lackner LL. Num1 anchors mitochondria to the plasma membrane via two domains with different lipid binding specificities. J Cell Biol. 2016;213:513–24.
2. Scorrano L, De Matteis MA, Emr S, Giordano F, Hajnóczky G, Kornmann B, et al. Coming together to define membrane contact sites. Nat Commun. 2019;10:1287.
3. Michaud M, Jouhet J. Lipid Trafficking at Membrane Contact Sites During Plant Development and Stress Response. Front Plant Sci. 2019;10:2. https://doi.org/10.3389/fpls.2019.00002.
4. Leterme S, Michaud M. Non-vesicular glycerolipids transport in plant cells. In: Advances in Botanical Research. Academic Press Inc.; 2022. p. 121–89.
5. Wu H, Carvalho P, Voeltz GK. Here, there, and everywhere: The importance of ER membrane contact sites. Science. 1979;2018:361.
6. Voeltz GK, Sawyer EM, Hajnóczky G, Prinz WA. Making the connection: How membrane contact sites have changed our view of organelle biology. Cell. 2024;187:257–70.
7. Stefano G, Brandizzi F. Advances in plant ER architecture and dynamics. Plant Physiol. 2018;176:178–86.
8. McMahon HT, Boucrot E. Membrane curvature at a glance. J Cell Sci. 2015;128:1065–70.
9. Kriechbaumer V, Brandizzi F. The plant endoplasmic reticulum: an organized chaos of tubules and sheets with multiple functions. J Microsc. 2020;280:122–33.
10. Pain C, Kriechbaumer V, Kittelmann M, Hawes C, Fricker M. Quantitative analysis of plant ER architecture and dynamics. Nature Communications 2019 10:1. 2019;10:1–15.
11. Friedman JR, Voeltz GK. The ER in 3D: a multifunctional dynamic membrane network. Trends Cell Biol. 2011;21:709–17.
12. Brandizzi F. Maintaining the structural and functional homeostasis of the plant endoplasmic reticulum. Dev Cell. 2021;56:919–32.
13. Stefano G, Hawes C, Brandizzi F. ER – the key to the highway. Curr Opin Plant Biol. 2014;22:30–8.
14. Westrate LM, Lee JE, Prinz WA, Voeltz GK. Form Follows Function: The Importance of Endoplasmic Reticulum Shape. Annu Rev Biochem. 2015;84:791–811.

15. Ridge RW, Uozumi Y, Plazinski J, Hurley UA, Williamson RE. Developmental Transitions and Dynamics of the Cortical ER of Arabidopsis Cells Seen with Green Fluorescent Protein. Plant Cell Physiol. 1999;40:1253–61.
16. Sparkes I, Runions J, Hawes C, Griffing L. Movement and Remodeling of the Endoplasmic Reticulum in Nondividing Cells of Tobacco Leaves. Plant Cell. 2009;21:3937.
17. Khaddaj R, Kukulski W. Piecing together the structural organisation of lipid exchange at membrane contact sites. Curr Opin Cell Biol. 2023;83:102212.
18. Shibata Y, Voeltz GK, Rapoport TA. Rough Sheets and Smooth Tubules. Cell. 2006;126:435–9.
19. Zhang S, Zou B, Cao P, Su X, Xie F, Pan X, et al. Structural insights into photosynthetic cyclic electron transport. Mol Plant. 2023;16:187–205.
20. Pfister B, Zeeman SC. Formation of starch in plant cells. Cell Mol Life Sci. 2016;73:2781–807.
21. Hildebrandt TM, Nunes Nesi A, Araújo WL, Braun H-P. Amino Acid Catabolism in Plants. Mol Plant. 2015;8:1563–79.
22. Bittner A, Cieśla A, Gruden K, Lukan T, Mahmud S, Teige M, et al. Organelles and phytohormones: a network of interactions in plant stress responses. J Exp Bot. 2022;73:7165–81.
23. Lange BM, Rujan T, Martin W, Croteau R. Isoprenoid biosynthesis: The evolution of two ancient and distinct pathways across genomes. Proc Natl Acad Sci U S A. 2000;97:13172–7.
24. Hölzl G, Dörmann P. Chloroplast lipids and their biosynthesis. Annu Rev Plant Biol. 2019;70:51–81.
25. Staehelin LA. Chloroplast Structure and Supramolecular Organization of Photosynthetic Membranes. Photosynthesis. 1986;III:1–84.
26. Eberhard S, Finazzi G, Wollman F-A. The Dynamics of Photosynthesis. Annu Rev Genet. 2008;42:463–515.
27. Kirchhoff H. Chloroplast ultrastructure in plants. New Phytol. 2019;223:565–74.
28. Gray JC, Sullivan JA, Hibberd JM, Hansen MR. Stromules: Mobile Protrusions and Interconnections Between Plastids. Plant Biol. 2001;3:223–33.
29. Breuers FKH, Bräutigam A, Geimer S, Welzel UY, Stefano G, Renna L, et al. Dynamic remodeling of the plastid envelope membranes - A tool for chloroplast envelope in vivo localizations. Front Plant Sci. 2012;3 JAN:17957.
30. Spitzer C, Li F, Buono R, Roschzttardtz H, Chung T, Zhang M, et al. The endosomal protein CHARGED MULTIVESICULAR BODY PROTEIN1 regulates the autophagic turnover of plastids in Arabidopsis. Plant Cell. 2015;27:391–402.
31. Kumar AS, Park E, Nedo A, Alqarni A, Ren L, Hoban K, et al. Stromule extension along microtubules coordinated with actin-mediated anchoring guides perinuclear chloroplast movement during innate immunity. Elife. 2018;7:e23625. https://doi.org/10.7554/eLife.23625.
32. Caplan JL, Kumar AS, Park E, Padmanabhan MS, Hoban K, Modla S, et al. Chloroplast stromules function during innate immunity. Dev Cell. 2015;34:45–57.
33. Hanson MR, Conklin PL. Stromules, functional extensions of plastids within the plant cell. Curr Opin Plant Biol. 2020;58:25–32.
34. Gibbs SP. The Chloroplast Endoplasmic Reticulum: Structure, Function, and Evolutionary Significance. Int Rev Cytol. 1981;72:49–99.
35. Gibbs SP. Nuclear envelope-chloroplast relationships in algae. J Cell Biol. 1962;14:433–44.
36. Descomps S. Contribution à l'étude infrastructurale des Vaucheries (Xanthophycées, Chromophytes). Compte rendu hebdomadaire des séances de l'Académie des sciences. 1963;256:1333–5.
37. Wooding FBP, Nokthcote DH. Association of the endoplasmic reticulum and the plastids in Acer and Pinus. Am J Bot. 1965;52:526–31.
38. Bouck GB. Fine structure and organelle associations in brown algae. J Cell Biol. 1965;26:523–37.
39. Bouck GB. Extracellular microtubules. The origin, structure, and attachment of flagellar hairs in Fucus and Ascophyllum antherozoids. J Cell Biol. 1969;40:446–60.
40. Cran DG, Dyer AF. Membrane continuity and associations in the fern Dryopteris borreri. Protoplasma. 1973;76:103–8.
41. Crotty WJ, Ledbetter MC. Membrane Continuities Involving Chloroplasts and Other Organelles in Plant Cells. Science. 1979;1973(182):839–41.
42. Renaudin S, Capdepon M. Association of the endoplasmic reticulum and the plastids in Tozzia aplina L. scale leaves. J Ultrastruct Res. 1977;61:303–8.
43. McLean B, Whatley JM, Juniper BE. Continuity of chloroplast and endoplasmic reticulum membranes in Chara and Equisetum. New Phytol. 1988;109:59–65.
44. Kaneko Y, Keegstra K. Plastid biogenesis in embryonic pea leaf cells during early germination. Protoplasma. 1996;195:59–67.
45. Diers L. On the plastids, mitochondria and other cell constituents during oögenesis of a plant. J Cell Biol. 1966;28:527–43.
46. Whatley JM, McLean B, Juniper BE. Continuity of chloroplast and endoplasmic reticulum membranes in Phaseolus vulgaris. New Phytol. 1991;117:209–17.
47. Köhler RH, Hanson MR. Plastid tubules of higher plants are tissue-specific and developmentally regulated. J Cell Sci. 2000;113:81–9.
48. Andersson MX, Goksör M, Sandelius AS. Optical manipulation reveals strong attracting forces at membrane contact sites between endoplasmic reticulum and chloroplasts. J Biol Chem. 2007;282:1170–4.
49. Griffing LR. Laser stimulation of the chloroplast/endoplasmic reticulum nexus in tobacco transiently produces protein aggregates (Boluses) within the endoplasmic reticulum and stimulates local ER remodeling. Mol Plant. 2011;4:886–95.
50. Li T, Xiao Z, Li H, Liu C, Shen W, Gao C. A combinatorial reporter set to visualize the membrane contact sites between endoplasmic reticulum and other organelles in plant cell. Front Plant Sci. 2020;11:1280.
51. Renna L, Stefano G, Puggioni MP, Kim S-J, Lavell A, Froehlich JE, et al. ER-associated VAP27-1 and VAP27-3 proteins functionally link the lipid-binding ORP2A at the ER-chloroplast contact sites. Nat Commun. 2024;15:6008.
52. Schattat M, Barton K, Baudisch B, Klösgen RB, Mathur J. Plastid Stromule Branching Coincides with Contiguous Endoplasmic Reticulum Dynamics. Plant Physiol. 2011;155:1667–77.
53. Mathur J, Kroeker OF, Lobbezoo M, Mathur N. The ER Is a Common Mediator for the Behavior and Interactions of Other Organelles. Front Plant Sci. 2022;13: 846970.
54. Mathur J, Kunjumon TK, Mammone A, Mathur N. Membrane contacts with the endoplasmic reticulum modulate plastid morphology and behaviour. Front Plant Sci. 2023;14:1293906.
55. Mehrshahi P, Johnny C, DellaPenna D. Redefining the metabolic continuity of chloroplasts and ER. Trends Plant Sci. 2014;19:501–7.
56. Mehrshahi P, Stefano G, Andaloro JM, Brandizzi F, Froehlich JE, DellaPenna D. Transorganellar complementation redefines the biochemical continuity of endoplasmic reticulum and chloroplasts. Proc Natl Acad Sci. 2013;110:12126–31.
57. Xu C, Fan J, Cornish AJ, Benning C. Lipid trafficking between the endoplasmic reticulum and the plastid in Arabidopsis requires the extraplastidic TGD4 protein. Plant Cell. 2008;20:2190–204.
58. Choi H, Yi T, Ha S-H. Diversity of Plastid Types and Their Interconversions. Front Plant Sci. 2021;12:692024. https://doi.org/10.3389/fpls.2021.692024.
59. Sato N. Are cyanobacteria an ancestor of chloroplasts or just one of the gene donors for plants and algae? Genes (Basel). 2021;12(6):823. https://doi.org/10.3390/genes12060823.
60. Douce R. Site of Biosynthesis of Galactolipids in Spinach Chloroplasts. Science. 1979;1974(183):852–3.
61. Siebertz HP, Heinz E, Linscheid M, Joyard J, Douce R. Characterization of lipids from chloroplast envelopes. Eur J Biochem. 1979;101:429–38.
62. Heinz E, Harwood JL. Incorporation of carbon dioxide, acetate and sulphate into the glycerolipids of Vicia faba leaves. Hoppe Seylers Z Physiol Chem. 1977;358:897–908.
63. Ohlrogge J, Browse J. Lipid biosynthesis. Plant Cell. 1995;7:957–70.
64. Browse J, McCourt P, Somerville C. A mutant of Arabidopsis deficient in c(18:3) and c(16:3) leaf lipids. Plant Physiol. 1986;81:859–64.
65. Browse J, Warwick N, Somerville CR, Slack CR. Fluxes through the prokaryotic and eukaryotic pathways of lipid synthesis in the "16:3" plant Arabidopsis thaliana. Biochemical Journal. 1986;235:25.
66. Mongrand S, Bessoule JJ, Cabantous F, Cassagne C. The C16:3\C18:3 fatty acid balance in photosynthetic tissues from 468 plant species. Phytochemistry. 1998;49:1049–64.

67. Fritz M, Lokstein H, Hackenberg D, Welti R, Roth M, Zähringer U, et al. Channeling of eukaryotic diacylglycerol into the biosynthesis of plastidial phosphatidylglycerol. J Biol Chem. 2007;282:4613–25.
68. Andersson MX, Kjellberg JM, Sandelius AS. Chloroplast biogenesis. Regulation of lipid transport to the thylakoid in chloroplasts isolated from expanding and fully expanded leaves of pea. Plant Physiol. 2001;127:184–93.
69. Browse J, Somerville CR. Glycerolipids Cold Spring Harbor Monograph Archive. 1994;27:881–912.
70. Mackender RO, Leech RM. The Galactolipid, Phospholipid, and Fatty Acid Composition of the Chloroplast Envelope Membranes of *Vicia faba*. L Plant Physiol. 1974;53:496–502.
71. Liedvogel B, Kleinig H. Galactolipid synthesis in chromoplast internal membranes of the daffodil. Planta. 1976;129:19–21.
72. Bahl J, Francke B, Monéger R. Lipid composition of envelopes, prolamellar bodies and other plastid membranes in etiolated, green and greening wheat leaves. Planta. 1976;129:193–201.
73. Poincelot RP. Lipid and Fatty Acid Composition of Chloroplast Envelope Membranes from Species with Differing Net Photosynthesis. Plant Physiol. 1976;58:595–8.
74. Douce R, Joyard J. Chloroplast envelope lipids: Detection and biosynthesis. Methods Enzymol. 1980;69:290–301.
75. Dorne AJ, Block MA, Joyard J, Douce R. The galactolipid:galactolipid galactosyltransferase is located on the outer surface of the outer membrane of the chloroplast envelope. FEBS Lett. 1982;145:30–4.
76. Block MA, Dorne AJ, Joyard J, Douce R. Preparation and characterization of membrane fractions enriched in outer and inner envelope membranes from spinach chloroplasts. II. Biochemical characterization. J Biol Chem. 1983;258:13281–6.
77. Alban C, Joyard J, Douce R. Preparation and Characterization of Envelope Membranes from Nongreen Plastids. Plant Physiol. 1988;88:709–17.
78. Cline K, Andrews J, Mersey B, Newcomb EH, Keegstra K. Separation and characterization of inner and outer envelope membranes of pea chloroplasts. Proc Natl Acad Sci. 1981;78:3595–9.
79. Allen CF, Good P, Trosper T, Park RB. Chlorophyll, glycerolipid and protein ratios in spinach chloroplast grana and stroma lamellae. Biochem Biophys Res Commun. 1972;48:907–13.
80. Chapman DJ, De Felice J, Barber J. Polar lipid composition of chloroplast thylakoids isolated from leaves grown under different lighting conditions. Photosynth Res. 1986;8:257–65.
81. Wang L, Patena W, Van Baalen KA, Xie Y, Singer ER, Gavrilenko S, et al. A chloroplast protein atlas reveals punctate structures and spatial organization of biosynthetic pathways. Cell. 2023;186:3499-3518.e14.
82. Moreau P, Bessoule JJ, Mongrand S, Testet E, Vincent P, Cassagne C. Lipid trafficking in plant cells. Prog Lipid Res. 1998;37:371–91.
83. Wang Z, Benning C. Chloroplast lipid synthesis and lipid trafficking through ER–plastid membrane contact sites. Biochem Soc Trans. 2012;40:457–63.
84. Koenig AM, Benning C, Hoffmann-Benning S. Lipid trafficking and signaling in plants. Lipid Signaling and Metabolism. Elsevier; 2020. pp. 23–44. https://doi.org/10.1016/B978-0-12-819404-1.00002-6.
85. Li-Beisson Y, Shorrosh B, Beisson F, Andersson MX, Arondel V, Bates PD, et al. Acyl-Lipid Metabolism Arabidopsis Book. 2013;11: e0161.
86. Bates PD. Understanding the control of acyl flux through the lipid metabolic network of plant oil biosynthesis. Biochimica et Biophysica Acta (BBA) - Molecular Cell Biol Lipids. 2016;1861:1214–25.
87. Li N, Xu C, Li-Beisson Y, Philippar K. Fatty Acid and Lipid Transport in Plant Cells. Trends Plant Sci. 2016;21(2):145–58.
88. Ohlrogge JB, Kuhn DN, Stumpf PK. Subcellular localization of acyl carrier protein in leaf protoplasts of Spinacia oleracea. Proc Natl Acad Sci. 1979;76:1194–8.
89. Schmidt H, Heinz E. Direct desaturation of intact galactolipids by a desaturase solubilized from spinach (Spinacia oleracea) chloroplast envelopes. Biochem J. 1993;289 (Pt 3) Pt 3:777–82.
90. Thompson GA, Scherer DE, Foxall-Van Aken S, Kenny JW, Young HL, Shintani DK, et al. Primary structures of the precursor and mature forms of stearoyl-acyl carrier protein desaturase from safflower embryos and requirement of ferredoxin for enzyme activity. Proc Natl Acad Sci. 1991;88:2578–82.
91. Voelker TA, Worrell AC, Anderson L, Bleibaum J, Fan C, Hawkins DJ, et al. Fatty Acid Biosynthesis Redirected to Medium Chains in Transgenic Oilseed Plants. Science. 1979;1992(257):72–4.
92. Kalinger RS, Pulsifer IP, Hepworth SR, Rowland O. Fatty Acyl Synthetases and Thioesterases in Plant Lipid Metabolism: Diverse Functions and Biotechnological Applications. Lipids. 2020;55:435–55.
93. Li N, Gügel IL, Giavalisco P, Zeisler V, Schreiber L, Soll J, et al. FAX1, a Novel Membrane Protein Mediating Plastid Fatty Acid Export. PLoS Biol. 2015;13: e1002053.
94. Frentzen M, Heinz E, McKeon TA, Stumpf PK. Specificities and selectivities of glycerol-3-phosphate acyltransferase and monoacylglycerol-3-phosphate acyltransferase from pea and spinach chloroplasts. Eur J Biochem. 1983;129:629–36.
95. Dubots E, Botté C, Boudière L, Yamaryo-Botté Y, Jouhet J, Maréchal E, et al. Role of phosphatidic acid in plant galactolipid synthesis. Biochimie. 2012;94:86–93.
96. Pokotylo I, Kravets V, Martinec J, Ruelland E. The phosphatidic acid paradox: Too many actions for one molecule class? Lessons from plants Prog Lipid Res. 2018;71:43–53.
97. Karki N, Johnson BS, Bates PD. Metabolically distinct pools of phosphatidylcholine are involved in trafficking of fatty acids out of and into the chloroplast for membrane production. Plant Cell. 2019;:tpc.00121.2019.
98. Allen DK. Assessing compartmentalized flux in lipid metabolism with isotopes. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2016;1861:1226–42.
99. Xu C, Fan J, Shanklin J. Metabolic and functional connections between cytoplasmic and chloroplast triacylglycerol storage. Prog Lipid Res. 2020;80: 101069.
100. Lavell AA, Benning C. Cellular Organization and Regulation of Plant Glycerolipid Metabolism. Plant Cell Physiol. 2019;60:1176–83.
101. LaBrant E, Barnes AC, Roston RL. Lipid transport required to make lipids of photosynthetic membranes. Photosynthesis Research 2018 138:3. 2018;138:345–60.
102. Petroutsos D, Amiar S, Abida H, Dolch LJ, Bastien O, Rébeillé F, et al. Evolution of galactoglycerolipid biosynthetic pathways – From cyanobacteria to primary plastids and from primary to secondary plastids. Prog Lipid Res. 2014;54:68–85.
103. Heinz E, Roughan PG. Similarities and differences in lipid metabolism of chloroplasts isolated from 18:3 and 16:3 plants. Plant Physiol. 1983;72:273–9.
104. Nakamura Y, Tsuchiya M, Ohta H. Plastidic phosphatidic acid phosphatases identified in a distinct subfamily of lipid phosphate phosphatases with prokaryotic origin. J Biol Chem. 2007;282:29013–21.
105. Sato N, Awai K. "Prokaryotic Pathway" Is Not Prokaryotic: Noncyanobacterial Origin of the Chloroplast Lipid Biosynthetic Pathway Revealed by Comprehensive Phylogenomic Analysis. Genome Biol Evol. 2017;9:3162–78.
106. Nguyen VC, Nakamura Y. Distinctly localized lipid phosphate phosphatases mediate endoplasmic reticulum glycerolipid metabolism in Arabidopsis. Plant Cell. 2023;35:1548–71.
107. Cook R, Froehlich JE, Yang Y, Korkmaz I, Kramer DM, Benning C. Chloroplast phosphatases LPPγ and LPPϵ1 facilitate conversion of extraplastidic phospholipids to galactolipids. Plant Physiol. 2024;195:1506–20.
108. Nakamura Y, Koizumi R, Shui G, Shimojima M, Wenk MR, Ito T, et al. Arabidopsis lipins mediate eukaryotic pathway of lipid metabolism and cope critically with phosphate starvation. Proc Natl Acad Sci U S A. 2009;106:20978–83.
109. Eastmond PJ, Quettier AL, Kroon JTM, Craddock C, Adams N, Slabas AR. Phosphatidic acid phosphohydrolase 1 and 2 regulate phospholipid synthesis at the endoplasmic reticulum in Arabidopsis. Plant Cell. 2010;22:2796–811.
110. Yoshitake Y, Sato R, Madoka Y, Ikeda K, Murakawa M, Suruga K, et al. Arabidopsis phosphatidic acid phosphohydrolases are essential for growth under nitrogen-depleted conditions. Front Plant Sci. 2017;8:1847.
111. Xu Y, Kambhampati S, Morley SA, Cook R, Froehlich J, Allen DK, et al. Arabidopsis ACYL CARRIER PROTEIN4 and RHOMBOID LIKE10 act independently in chloroplast phosphatidate synthesis. Plant Physiol. 2023;193:2661–76.
112. Dubots E, Audry M, Yamaryo Y, Bastien O, Ohta H, Breton C, et al. Activation of the Chloroplast Monogalactosyldiacylglycerol Synthase

MGD1 by Phosphatidic Acid and Phosphatidylglycerol. J Biol Chem. 2010;285:6003–11.
113. Awai K, Maréchal E, Block MA, Brun D, Masuda T, Shimada H, et al. Two types of MGDG synthase genes, found widely in both 16:3 and 18:3 plants, differentially mediate galactolipid syntheses in photosynthetic and nonphotosynthetic tissues in Arabidopsis thaliana. Proc Natl Acad Sci U S A. 2001;98:10960–5.
114. Kelly AA, Froehlich JE, Dörmann P. Disruption of the Two Digalactosyldiacylglycerol Synthase Genes DGD1 and DGD2 in Arabidopsis Reveals the Existence of an Additional Enzyme of Galactolipid Synthesis. Plant Cell. 2003;15:2694–706.
115. Wang K, Guo Q, Froehlich JE, Hersh HL, Zienkiewicz A, Howe GA, et al. Two Abscisic Acid-Responsive Plastid Lipase Genes Involved in Jasmonic Acid Biosynthesis in Arabidopsis thaliana. Plant Cell. 2018;30:1006–22.
116. Wang K, Froehlich JE, Zienkiewicz A, Hersh HL, Benning C. A Plastid Phosphatidylglycerol Lipase Contributes to the Export of Acyl Groups from Plastids for Seed Oil Biosynthesis. Plant Cell. 2017;29:1678–96.
117. Aulakh K, Durrett TP. The Plastid Lipase PLIP1 Is Critical for Seed Viability in diacylglycerol acyltransferase1 Mutant Seed. Plant Physiol. 2019;180:1962–74.
118. Kunst L, Browse J, Somerville C. A mutant of Arabidopsis deficient in desaturation of Palmitic àcid in leaf lipids. Plant Physiol. 1989;90:943–7.
119. Heilmann I, Mekhedov S, King B, Browse J, Shanklin J. Identification of the Arabidopsis Palmitoyl-Monogalactosyldiacylglycerol Δ7-Desaturase Gene FAD5, and Effects of Plastidial Retargeting of Arabidopsis Desaturases on the fad5 Mutant Phenotype. Plant Physiol. 2004;136:4237.
120. Falcone DL, Gibson S, Lemieux B, Somerville C. Identification of a gene that complements an Arabidopsis mutant deficient in chloroplast omega 6 desaturase activity. Plant Physiol. 1994;106:1453–9.
121. Browse J, Kunst L, Anderson S, Hugly S, Somerville C. A mutant of Arabidopsis deficient in the chloroplast 16:1/18:1 desaturase. Plant Physiol. 1989;90:522–9.
122. Iba K, Gibson S, Nishiuchi T, Fuse T, Nishimura M, Arondel V, et al. A gene encoding a chloroplast omega-3 fatty acid desaturase complements alterations in fatty acid desaturation and chloroplast copy number of the fad7 mutant of Arabidopsis thaliana. J Biol Chem. 1993;268:24099–105.
123. Gibson S, Arondel V, Iba K, Somerville C. Cloning of a Temperature-Regulated Gene Encoding a Chloroplast [omega]-3 Desaturase from Arabidopsis thaliana. Plant Physiol. 1994;106:1615–21.
124. Le MM, Hugly S, Browse J, Somerville C. A Mutation at the fad8 Locus of Arabidopsis Identifies a Second Chloroplast [omega]-3 Desaturase. Plant Physiol. 1994;106:1609–14.
125. Gao J, Ajjawi I, Manoli A, Sawin A, Xu C, Froehlich JE, et al. FATTY ACID DESATURASE4 of Arabidopsis encodes a protein distinct from characterized fatty acid desaturases. Plant J. 2009;60:832–9.
126. Browse J, Mccourt P, Somerville CR. A Mutant of Arabidopsis Lacking a Chloroplast-Specific Lipid. Science. 1979;1985(227):763–5.
127. Okuley J, Lightner J, Feldmann K, Yadav N, Lark E, Browse J. Arabidopsis FAD2 Gene Encodes the Enzyme That Is Essential for Polyunsaturated Lipid Synthesis. Plant Cell. 2007;6:147.
128. Browse J, McConn M, James D, Miquel M. Mutants of Arabidopsis deficient in the synthesis of alpha-linolenate. Biochemical and genetic characterization of the endoplasmic reticulum linoleoyl desaturase. J Biol Chem. 1993;268:16345–51.
129. Walther TC, Chung J, Farese RV. Lipid Droplet Biogenesis. Annu Rev Cell Dev Biol. 2017;33:491–510.
130. Valm AM, Cohen S, Legant WR, Melunis J, Hershberg U, Wait E, et al. Applying systems-level spectral imaging and analysis to reveal the organelle interactome. Nature. 2017;546:162–7.
131. Hugenroth M, Bohnert M. Come a little bit closer! Lipid droplet-ER contact sites are getting crowded. Biochimica et Biophysica Acta (BBA) - Molecular Cell Res. 2020;1867:118603.
132. Moriyama T, Toyoshima M, Saito M, Wada H, Sato N. Revisiting the algal "chloroplast lipid droplet": The absence of an entity that is unlikely to exist. Plant Physiol. 2018;176:1519–30.
133. Goold H, Beisson F, Peltier G, Li-Beisson Y. Microalgal lipid droplets: composition, diversity, biogenesis and functions. Plant Cell Rep. 2015;34:545–55.
134. Fan J, Andre C, Xu C. A chloroplast pathway for the de novo biosynthesis of triacylglycerol in Chlamydomonas reinhardtii. FEBS Lett. 2011;585:1985–91.
135. Tsai CH, Zienkiewicz K, Amstutz CL, Brink BG, Warakanont J, Roston R, et al. Dynamics of protein and polar lipid recruitment during lipid droplet assembly in Chlamydomonas reinhardtii. Plant J. 2015;83:650–60.
136. Warakanont J, Tsai CH, Michel EJS, Murphy GR, Hsueh PY, Roston RL, et al. Chloroplast lipid transfer processes in Chlamydomonas reinhardtii involving a TRIGALACTOSYLDIACYLGLYCEROL 2 (TGD2) orthologue. Plant J. 2015;84:1005–20.
137. Tan X, Wang Q, Tian B, Zhang H, Lu D, Zhou J. A Brassica napus lipase locates at the membrane contact sites involved in chloroplast development. PLoS ONE. 2011;6: e26831.
138. Schnurr JA, Shockey JM, De Boer GJ, Browse JA. Fatty Acid Export from the Chloroplast. Molecular Characterization of a Major Plastidial Acyl-Coenzyme A Synthetase from Arabidopsis. Plant Physiol. 2002;129:1700.
139. Zhao L, Katavic V, Li F, Haughn GW, Kunst L. Insertional mutant analysis reveals that long-chain acyl-CoA synthetase 1 (LACS1), but not LACS8, functionally overlaps with LACS9 in Arabidopsis seed oil biosynthesis. Plant J. 2010;64:1048–58.
140. Zhao L, Haslam TM, Sonntag A, Molina I, Kunst L. Functional Overlap of Long-Chain Acyl-CoA Synthetases in Arabidopsis. Plant Cell Physiol. 2019;60:1041–54.
141. Jessen D, Roth C, Wiermer M, Fulda M. Two Activities of Long-Chain Acyl-Coenzyme A Synthetase Are Involved in Lipid Trafficking between the Endoplasmic Reticulum and the Plastid in Arabidopsis. Plant Physiol. 2015;167:351–66.
142. Lager I, Yilmaz JL, Zhou XR, Jasieniecka K, Kazachkov M, Wang P, et al. Plant Acyl-CoA:lysophosphatidylcholine acyltransferases (LPCATs) have different specificities in their forward and reverse reactions. J Biol Chem. 2013;288:36902–14.
143. Bessoule J-J, Testet E, Cassagne C. Synthesis of Phosphatidylcholine in the Chloroplast Envelope after Import of Lysophosphatidylcholine from Endoplasmic Reticulum Membranes. Eur J Biochem. 1995;228:490–7.
144. Mongrand S, Cassagne C, Bessoule JJ. Import of Lyso-Phosphatidylcholine into Chloroplasts Likely at the Origin of Eukaryotic Plastidial Lipids. Plant Physiol. 2000;122:845–52.
145. Wang Z, Anderson NS, Benning C. The phosphatidic acid binding site of the arabidopsis trigalactosyldiacylglycerol 4 (TGD4) protein required for lipid import into chloroplasts. J Biol Chem. 2013;288:4763–71.
146. Xu C, Fan J, Froehlich JE, Awai K, Benning C. Mutation of the TGD1 Chloroplast Envelope Protein Affects Phosphatidate Metabolism in Arabidopsis. Plant Cell. 2005;17:3094–110.
147. Xu C, Fan J, Riekhof W, Froehlich JE, Benning C. A permease-like protein involved in ER to thylakoid lipid transfer in Arabidopsis. EMBO J. 2003;22:2370–9.
148. Awai K, Xu C, Tamot B, Benning C. A phosphatidic acid-binding protein of the chloroplast inner envelope membrane involved in lipid trafficking. Proc Natl Acad Sci. 2006;103:10817–22.
149. Lu B, Xu C, Awai K, Jones AD, Benning C. A small ATPase protein of Arabidopsis, TGD3, involved in chloroplast lipid import. J Biol Chem. 2007;282:35945–53.
150. Roston RL, Gao J, Murcha MW, Whelan J, Benning C. TGD1, -2, and -3 Proteins Involved in Lipid Trafficking Form ATP-binding Cassette (ABC) Transporter with Multiple Substrate-binding Proteins. J Biol Chem. 2012;287:21406–15.
151. Wang Z, Xu C, Benning C. TGD4 involved in endoplasmic reticulum-to-chloroplast lipid trafficking is a phosphatidic acid binding protein. Plant J. 2012;70:614–23.
152. Xu C, Moellering ER, Muthan B, Fan J, Benning C. Lipid transport mediated by arabidopsis TGD proteins is unidirectional from the endoplasmic reticulum to the plastid. Plant Cell Physiol. 2010;51:1019–28.
153. Fan J, Zhai Z, Yan C, Xu C. Arabidopsis TRIGALACTOSYLDIACYLGLYCEROL5 Interacts with TGD1, TGD2, and TGD4 to Facilitate Lipid Transfer from the Endoplasmic Reticulum to Plastids. Plant Cell. 2015;27:tpc.15.00394.
154. Negi J, Munemasa S, Song B, Tadakuma R, Fujita M, Azoulay-Shemer T, et al. Eukaryotic lipid metabolic pathway is essential for functional

chloroplasts and CO2 and light responses in Arabidopsis guard cells. Proc Natl Acad Sci U S A. 2018;115:9038–43.
155. Itoh RD, Nakajima KP, Sasaki S, Ishikawa H, Kazama Y, Abe T, et al. TGD5 is required for normal morphogenesis of non-mesophyll plastids, but not mesophyll chloroplasts, in *Arabidopsis*. Plant J. 2021;107:237–55.
156. Hsueh YC, Ehmann C, Flinner N, Ladig R, Schleiff E. The plastid outer membrane localized LPTD1 is important for glycerolipid remodelling under phosphate starvation. Plant Cell Environ. 2017;40:1643–57.
157. Dorne AJ, Joyard J, Douce R. Do thylakoids really contain phosphatidylcholine? Proc Natl Acad Sci U S A. 1990;87:71–4.
158. Poulsen LR, López-Marqués RL, Pedas PR, McDowell SC, Brown E, Kunze R, et al. A phospholipid uptake system in the model plant Arabidopsis thaliana. Nat Commun. 2015;6:7649. https://doi.org/10.1038/ncomms8649.
159. Botella C, Sautron E, Boudiere L, Michaud M, Dubots E, Yamaryo-Botté Y, et al. ALA10, a phospholipid flippase, controls FAD2/FAD3 desaturation of phosphatidylcholine in the ER and affects chloroplast lipid composition in Arabidopsis thaliana. Plant Physiol. 2016;170:1300–14.
160. Jensen MS, Costa SR, Duelli AS, Andersen PA, Poulsen LR, Stanchev LD, et al. Phospholipid flipping involves a central cavity in P4 ATPases. Sci Rep. 2017;7:1–13.
161. Davis JA, Poulsen LR, Kjeldgaard B, Moog MW, Brown E, Palmgren M, et al. Deficiencies in cluster-2 ALA lipid flippases result in salicylic acid-dependent growth reductions. Physiol Plant. 2024;176(2):e14228. https://doi.org/10.1111/ppl.14228.
162. Pietrangelo A, Ridgway ND. Bridging the molecular and biological functions of the oxysterol-binding protein family. Cell Mol Life Sci. 2018;75:3079–98.
163. Olkkonen VM, Ikonen E. Getting to Grips with the Oxysterol-Binding Protein Family – a Forty Year Perspective. Contact (Thousand Oaks). 2024;7:25152564241273598. https://doi.org/10.1177/25152564241273598.
164. Wang P, Richardson C, Hawkins TJ, Sparkes I, Hawes C, Hussey PJ. Plant VAP27 proteins: domain characterization, intracellular localization and role in plant development. New Phytol. 2016;210:1311–26.
165. Yao H-Y, Lu Y-Q, Yang X-L, Wang X-Q, Luo Z, Lin D-L, et al. Arabidopsis Sec14 proteins (SFH5 and SFH7) mediate interorganelle transport of phosphatidic acid and regulate chloroplast development. Proc Natl Acad Sci U S A. 2023;120(6):e2221637120. https://doi.org/10.1073/pnas.2221637120.
166. Bankaitis VA, Mousley CJ, Schaaf G. The Sec14-superfamily and mechanisms for crosstalk between lipid metabolism and lipid signaling. Trends Biochem Sci. 2010;35:150.
167. Cecchini NM, Steffes K, Schlappi MR, Gifford AN, Greenberg JT. Arabidopsis AZI1 family proteins mediate signal mobilization for systemic defence priming. Nat Commun. 2015 6:1. 2015;6:1–12.
168. Cecchini NM, Roychoudhry S, Speed DQJ, Steffes K, Tambe A, Zodrow K, et al. Underground azelaic acid–conferred resistance to pseudomonas syringae in arabidopsis. Mol Plant Microbe Interact. 2019;32:86–94.
169. Jung HW, Tschaplinski TJ, Wang L, Glazebrook J, Greenberg JT. Priming in systemic plant immunity. Science. 1979;2009(324):89–91.
170. Yu K, Soares JM, Mandal MK, Wang C, Chanda B, Gifford AN, et al. A Feedback Regulatory Loop between G3P and Lipid Transfer Proteins DIR1 and AZI1 Mediates Azelaic-Acid-Induced Systemic Immunity. Cell Rep. 2013;3:1266–78.
171. Lascombe M, Bakan B, Buhot N, Marion D, Blein J, Larue V, et al. The structure of "defective in induced resistance" protein of Arabidopsis thaliana, DIR1, reveals a new type of lipid transfer protein. Protein Sci. 2008;17:1522–30.
172. Huercano C, Percio F, Sanchez-Vera V, Morello-López J, Botella MA, Ruiz-Lopez N. Identification of plant exclusive lipid transfer SMP proteins at membrane contact sites in Arabidopsis and Tomato. BioRxiv. 2022;12(14)520452. https://doi.org/10.1101/2022.12.14.520452.
173. Ruiz-Lopez N, Pérez-Sancho J, del Valle AE, Haslam RP, Vanneste S, Catalá R, et al. Synaptotagmins at the endoplasmic reticulum–plasma membrane contact sites maintain diacylglycerol homeostasis during abiotic stress. Plant Cell. 2021;33:2431–53.
174. García-Hernández S, Rubio L, Rivera-Moreno M, Pérez-Sancho J, Morello-López J, Esteban del Valle A, et al. Functional and Structural Analysis Reveals Distinct Biological Roles of Plant Synaptotagmins in Response to Environmental Stress. Plant Cell Environ. 2025;48:260–71.
175. Mahtha SK, Kumari K, Gaur V, Yadav G. Cavity architecture based modulation of ligand binding tunnels in plant START domains. Comput Struct Biotechnol J. 2023;21:3946–63.
176. Hirashima T, Jimbo H, Kobayashi K, Wada H. A START domain-containing protein is involved in the incorporation of ER-derived fatty acids into chloroplast glycolipids in Marchantia polymorpha. Biochem Biophys Res Commun. 2021;534:436–41.
177. Schrick K, Nguyen D, Karlowski WM, Mayer KF. START lipid/sterol-binding domains are amplified in plants and are predominantly associated with homeodomain transcription factors. Genome Biol. 2004;5:1–16.
178. Kelly AA, Kalisch B, Hölzl G, Schulze S, Thiele J, Melzer M, et al. Synthesis and transfer of galactolipids in the chloroplast envelope membranes of Arabidopsis thaliana. Proc Natl Acad Sci U S A. 2016;113:10714–9.
179. Lin YT, Chen LJ, Herrfurth C, Feussner I, Li HM. Reduced Biosynthesis of Digalactosyldiacylglycerol, a Major Chloroplast Membrane Lipid, Leads to Oxylipin Overproduction and Phloem Cap Lignification in Arabidopsis. Plant Cell. 2015;28:219.
180. Froehlich JE, Benning C, Dörmann P. The Digalactosyldiacylglycerol (DGDG) Synthase DGD1 is inserted into the outer envelope membrane of chloroplasts in a manner independent of the general import pathway and does not depend on direct interaction with Monogalactosyldiacylglycerol Synthase for DGDG biosynthesis. J Biol Chem. 2001;276:31806–12.
181. Fujii S, Kobayashi K, Nagata N, Masuda T, Wada H. Digalactosyldiacylglycerol Is Essential for Organization of the Membrane Structure in Etioplasts. Plant Physiol. 2018;177:1487–97.
182. Moellering ER, Benning C. Galactoglycerolipid metabolism under stress: A time for remodeling. Trends Plant Sci. 2011;16:98–107.
183. Levine TP. Sequence Analysis and Structural Predictions of Lipid Transfer Bridges in the Repeating Beta Groove (RBG) Superfamily Reveal Past and Present Domain Variations Affecting Form, Function and Interactions of VPS13, ATG2, SHIP164. Hobbit and Tweek Contact. 2022;5:251525642211343.
184. Adlakha J, Hong Z, Li P, Reinisch KM. Structural and biochemical insights into lipid transport by VPS13 proteins. J Cell Biol. 2022;221(5):e202202030. https://doi.org/10.1083/jcb.202202030.
185. Leterme S, Bastien O, Aiese Cigliano R, Amato A, Michaud M. Phylogenetic and Structural Analyses of VPS13 Proteins in Archaeplastida Reveal Their Complex Evolutionary History in Viridiplantae. Contact (Thousand Oaks). 2023;6:25152564231211976. https://doi.org/10.1177/25152564231211976.
186. Tangpranomkorn S, Kimura Y, Igarashi M, Ishizuna F, Kato Y, Suzuki T, et al. A land plant-specific VPS13 mediates polarized vesicle trafficking in germinating pollen. New Phytol. 2025;245:1072–89.
187. Leterme S, Albrieux C, Brugière S, Couté Y, Dellinger J, Gillet B, et al. AtVPS13M1 is involved in lipid remodeling in low phosphate and is located at the mitochondria surface in plants. BioRxiv. 2024;05(22)594332. https://doi.org/10.1101/2024.05.22.594332.
188. Xu Z, Dooner HK. The Maize aberrant pollen transmission 1 Gene Is a SABRE / KIP Homolog Required for Pollen Tube Growth. Genetics. 2006;172:1251–61.
189. Procissi A, Guyon A, Pierson ES, Giritch A, Knuiman B, Grandjean O, et al. KINKY POLLEN encodes a SABRE-like protein required for tip growth in Arabidopsis and conserved among eukaryotes. Plant J. 2003;36:894–904.

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