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Review

Glycerolipids in photosynthesis: Composition, synthesis and trafficking ☆

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ABSTRACT

Glycerolipids constituting the matrix of photosynthetic membranes, from cyanobacteria to chloroplasts of eukaryotic cells, comprise monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulfoquinovosyldiacylglycerol and phosphatidylglycerol. This review covers our current knowledge on the structural and functional features of these lipids in various cellular models, from prokaryotes to eukaryotes. Their relative proportions in thylakoid membranes result from highly regulated and compartmentalized metabolic pathways, with a cooperation, in the case of eukaryotes, of non-plastidic compartments. This review also focuses on the role of each of these thylakoid glycerolipids in stabilizing protein complexes of the photosynthetic machinery, which might be one of the reasons for their fascinating conservation in the course of evolution. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.

1. Introduction

The glycerolipid composition of photosynthetic membranes (thylakoid lipidome) has been remarkably conserved in the course of evolution, from cyanobacteria to chloroplast-containing eukaryotes [1–3]. Glycerolipids are a category of lipids having a 3-carbon glycerol scaffold (each carbon is numbered following the stereospecific numbering nomenclature *sn*-1, *sn*-2, *sn*-cp3), harboring one or two acyl chains esterified at positions *sn*-1 and *sn*-2, and a polar head at position *sn*-3 (Fig. 1). Table 1 shows that primary chloroplasts (i.e. photosynthetic plastids deriving from a primary endosymbiosis), analyzed from green algae to vascular plants [4–6], have a unique glycerolipid composition compared to other subcellular compartments: they are characterized by a very low content in phosphoglycerolipids, mostly PG (phosphatidylglycerol), PC (phosphatidylcholine), and PI (phosphatidylinositol), and a very high content in glycoglycerolipids that contain no phosphorus, mainly MGDG (monogalactosyldiacylglycerol), DGDG (digalactosyldiacylglycerol) and SQDG (sulfoquinovosyldiacylglycerol). This table also shows that MGlcDG (monoglucosyldiacylglycerol) is uniquely detected in cyanobacteria and is not found in chloroplasts. These very basic observations highlight the conservation of a quartet of lipids, i.e. MGDG, DGDG, SQDG and PG, from cyanobacteria to primary chloroplasts and raise questions regarding (*i*) the roles that these lipids may have in photosynthesis and (*ii*) the molecular processes establishing and regulating this unique lipid profile. Here, we summarize our current knowledge on the generic and specific roles of thylakoid lipid classes, focusing on their relation with photosynthesis, based on converging evidence from biochemical, biophysical, physiological, genetic and chemical genetic studies. We then detail our current understanding on the dynamic processes that establish and control this very peculiar and essential lipid composition in different biological models, in various physiological contexts and in response to environmental factors.

2. Classes of glycerolipid found in photosynthetic membranes

2.1. Neutral glycolipids: galactoglycerolipids (monogalactosyldiacylglycerol and digalactosyldiacylglycerol)

Galactoglycerolipids are the most abundant lipids in photosynthetic membranes. They comprise two major structures, conserved from cyanobacteria to primary chloroplasts, based on the number of galactose residues in their polar head, i.e. MGDG (1,2-diacyl-3-O-(β-D-galactopyranosyl)-*sn*-glycerol) and DGDG (1,2-diacyl-3-O-(α-

Abbreviations: ATS1, sn-glycerol-3-phosphate acyltransferase; ATS2, 1-acylglycerol-phosphate acyltransferase; DAG, diacylglycerol; DGD1 and DGD2, digalactosyldiacylglycerol synthase 1 and 2; DGDG, digalactosyldiacylglycerol; IEM, inner envelope membrane of the chloroplast; LHCII, light harvesting complex II; MGlcDG, monoglucosyldiacylglycerol; MGD1, MGD2 and MGD3, monogalactosyldiacylglycerol synthase 1, 2 and 3; MGDG, monogalactosyldiacylglycerol; OEC, oxygen evolving complex; OEM, outer envelope membrane of the chloroplast; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; Psb, lumenal extrinsic proteins of the oxygen evolving complex, interacting with photosystem II (includes PsbO, PsbU, PsbV, PsbQ and PsbP); PSI, photosystem I; PSII, photosystem II; Q_A, primary quinone acceptor; Q_B, diffusing quinone; SQDG, sulfoquinovosyldiacylglycerol; WT, wild type

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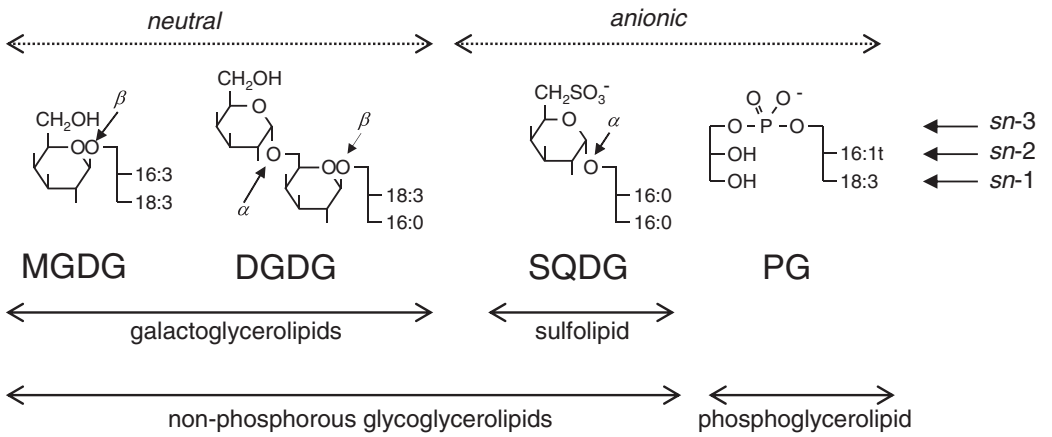

Fig. 1. Main glycerolipid classes conserved in photosynthetic membranes from cyanobacteria to primary chloroplasts of algae and plants. In this illustration of representative lipids from thylakoids of *Arabidopsis* chloroplasts, positions *sn*-1 and *sn*-2 of the glycerol backbone are esterified to fatty acids with 16 or 18 carbon atoms and position *sn*-3 harbors the polar head. MGDG, monogalactosyldiacylglycerol; DGDG, diagalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PG, phosphatidylglycerol.

D-galactopyranosyl-(1 → 6)-O-β-D-galactopyranosyl)-*sn*-glycerol) [7,8] (Fig. 1). Due to the extensive surface of thylakoids in cyanobacteria, algae and plant cells, MGDG and DGDG constitute the most profuse lipid class on earth [9].

Galactoglycerolipids, and mostly MGDG, are characterized by their high content of polyunsaturated fatty acids. In angiosperms, MGDG contains mostly 16- and 18-carbon ω-3 trienoic acids, i.e. *cis*-7,10,13-hexadecatrienoic acid (16:3) and *cis*-9,12,15-octadecatrienoic acid (18:3) respectively [10]. In algae, MGDG often contains very long chain polyunsaturated acids, with more than 20 carbon atoms and more than 3 double bonds such as eicosapentaenoic acid [11,12]. When present, 16:3 is exclusively at position *sn*-2 of MGDG. In angiosperms, DGDG is mainly 18:3-rich; however, by contrast with MGDG, when a 16-carbon fatty acid is present, DGDG contains mostly the saturated form, palmitic acid (16:0) at position *sn*-1 of the glycerol backbone.

The MGDG/DGDG and galactoglycerolipid/phosphoglycerolipid ratios appear stable, at least in angiosperms, when plants grow under favorable controlled conditions, i.e. when fed with sufficient nutrient sources. This observation supports the existence of a controlled steady state. This steady state was shown to respond to environmental changes. On the one hand, (*i*) environmental stresses that could be detrimental for membrane integrity, like freezing, drought or exposure to ozone, induce the accumulation of additional galactoglycerolipids, i.e. a specific DGDG with a β-galactopyranosyl-(1 → 6)-β-galactopyranosyl polar head instead of the α-galactopyranosyl-(1 → 6)-β-galactopyranosyl polar head and tri- and tetra-galactoglycerolipids in chloroplasts of angiosperms [13–17]. This phenomenon seems to be a recent invention in the evolution of land plants, but will not be discussed in more details in this review. On the other hand, (*ii*) a shortage of phosphate in the environment was shown to trigger a significant increase of the galactoglycerolipid/phosphoglycerolipid ratio, from algae [18] to land

Table 1
Representative glycerolipid compositions of membranes from cyanobacteria and chloroplasts from green algae and angiosperms. The distribution of main glycerolipids is given in percents of total lipid mass. Detected phosphoglycerolipids include PC (phosphatidylcholine), PE (phosphatidylethanolamine), PI (phosphatidylinositol) and PG (phosphatidylglycerol). DPG (diphosphatidylglycerol or cardiolipin), a marker of the contamination by mitochondrial membranes, was below the detection threshold. Conserved glycoglycerolipids consist of MGDG (monogalactosyldiacylglycerol), DGDG (digalactosyldiacylglycerol) and SQDG (sulfoquinovosyldiacylglycerol). MGlcDG (monoglucosyldiacylglycerol) is uniquely detected in cyanobacteria.

	Phosphoglycerolipids					Non-phosphorous glycoglycerolipids			
	PC	PE	PI	DPG	PG	MGlcDG	MGDG	DGDG	SQDG
Membranes from cyanobacteria (*Synechocystis* PCC 6308)[a]									
Envelope and thylakoids	–	–	–	–	11.4	1.7	52	11.7	22
Chloroplasts from green algae (*Chlamydomonas reinhardtii*)[b]									
Envelope membranes	*	4.6	4.1	–	3.5	–	27	31	8
Thylakoids	*	–	2.7	–	6	–	55	20	13
Chloroplasts from angiosperm leaves (*Spinacia oleracea*)[c]									
Envelope membranes									
Total	20	–	4	–	9	–	32	30	6
OEM	32	–	5	–	10	–	17	29	6
IEM	6		1		8	–	49	30	5
Thylakoids	4.5	–	1.5	–	9.5	–	52	26	6.5
Etioplasts from angiosperm (*Pisum sativum*)[d]									
Envelope membranes	17	–	4	–	5	–	34	31	6
Prolamellar body	9	–	2	–	5	–	42	35	6

[a] Present article.
[b] [4].
[c] [5].
[d] [6].
* 21% and 5% of an additional lipid was observed in *Chlamydomonas* in the envelope and thylakoids respectively, initially identified as PC; this phosphoglycerolipid being absent in *Chlamydomonas*, other lipids like DGTS (diacylglyceryltrimethylhomoserine) might be contaminants of envelope preparations.

plants [19]. Galactoglycerolipids have long been considered to be restricted to plastids, however a relocation of DGDG to non-plastid membranes following phosphate shortage was demonstrated a decade ago, at least in angiosperms [19] and is now considered as a major process of the cell membrane lipid homeostasis. In brief, DGDG is exported to various extraplastidial membranes [20–22], where it can substitute phosphoglycerolipids [1,20,23,24]. We still do not know if this export also occurs in algae: experimental evidence should be provided in the future to assess whether DGDG transfer is a universal phenomenon or if it is restricted to vascular plants. Chloroplast being an organelle with a very rapid phosphate turn over, it is possible that its high proportion of galactoglycerolipids helps saving phosphate. Thus, the ability to replace phosphoglycerolipids by DGDG might be the basis for a positive selection pressure, adding to the fitness of plants, and sustaining the conservation of galactoglycerolipids in the course of evolution.

In algae and plants exposed to various abiotic or biotic stresses, polyunsaturated fatty acids can be released from galactoglycerolipids [25] and converted into oxylipins, including jasmonic acids (JAs) [26]. Phosphoglycerolipids can also provide the polyunsaturated fatty acids required for the generation of oxylipins, and in the red alga *Gracilaria*, both galactoglycerolipids and phosphoglycerolipids are utilized [27]. Interestingly, in *Arabidopsis*, MGDG and DGDG can also directly harbor oxo-phytodienoic acyls [26] and other oxygenated fatty acids [28,29], forming a species-specific class of galactoglycerolipids, called the 'arabidopsides' that accumulate in chloroplast membranes under certain conditions like pathogen attacks. The presence of arabidopsides seems restricted to a very small number of species analyzed to date and their importance in angiosperms is currently debated.

2.2. Anionic glycolipid: sulfoquinovosyldiacylglycerol

SQDG is a unique glycerolipid, characterized by a sulfur-containing anionic polar head (1,2-diacyl-3-O-(6-sulfo-6-deoxy-α-D-glucosyl)-*sn*-glycerol) [30–33] (Fig. 1). The proportion of the sulfolipid in photosynthetic membranes is the lowest in the quartet of lipids conserved from cyanobacteria to chloroplasts. Its proportion in algae and plants ranges from about 2 to 20% with recorded peaks at 50% [34]. A series of in vitro analyses have shown that this lipid interacts with photosynthetic proteins, the import machinery and some annexins [34] (see below). Genetic disruption in *Arabidopsis* leading to a complete loss of SQDG [35] indicated that, in vivo and under normal growth conditions, SQDG was nevertheless dispensable. This observation suggested that SQDG function might be compensated by another lipid. Based on the initial observation that SQDG synthetic genes were specifically induced upon phosphate starvation, and following important physiological and genetic analyses [36–42], it was shown that the SQDG level was linked to that of PG, with a SQDG/PG ratio tuned in response to phosphate availability, a phenomenon again conserved from cyanobacteria to vascular plants [34,41].

2.3. Anionic phospholipid: phosphatidylglycerol

Among the quartet of lipids, PG is the only phosphoglycerolipid that has been conserved in photosynthetic membranes from prokaryotes to eukaryotes [43]. In angiosperms and numerous algae analyzed to date, thylakoid PG is characterized by the presence of a unique fatty acid molecular species, *trans*-3-hexadecenoic acid (C16:1t), located exclusively at position *sn*-2 of the glycerol backbone (Fig. 1). This fatty acid is important for structural interaction with proteins (see below). The phospho-glycerol polar head of PG is negatively charged (Fig. 1) like that of SQDG, and as mentioned above, PG levels are inversely correlated with those of SQDG. Upon phosphate shortage, the level of PG in photosynthetic membranes decreases significantly, being compensated by an increase of the sulfolipid level [34,40–42]. Attempts to genetically impair PG synthesis in *Arabidopsis* have shown that a complete abolition of PG in chloroplast was lethal [44]. After disruption of the PGP1 gene in the *Arabidopsis* genome, plants could only grow in presence of sucrose and chloroplast development was impaired [45]. By contrast, although the PGP1 gene encodes isoforms that can be targeted to both the chloroplast and the mitochondrion, the respiratory organelle was not altered by the PGP1 knock out, showing that mitochondria could alternatively import PG from the ER for the biosynthesis of the cardiolipin [46]. An *Arabidopsis* mutant with low PG levels in chloroplasts exhibits an impairment of growth and reduced photosynthesis, a phenotype that is more severe when combined with a genetic disruption of SQDG synthesis [42]. PG is also involved in the tolerance to chilling [43]. In summary, PG is therefore a vital lipid, mainly for its role as a cofactor of photosystems (see below), and SQDG appears as a sort of stand-in actor, playing parts of PG functions when phosphate is limited.

2.4. Other glycerolipids

Presence of other glycerolipids in chloroplasts of eukaryotic cells, besides the MGDG/DGDG/PG/SQDG quartet, has been proposed based on analyses of subcellular and suborganellar membrane fractions. PC has thus been detected in various proportions in the envelope membranes and in the thylakoids prepared from spinach leaves [5,6] (see Table 1). Could PC be a contaminant from other subcellular membranes in the process of chloroplast purification? Following incubation of intact chloroplasts with a non-specific phospholipase C (PLC, an enzyme cleaving the polar head of phospholipids and releasing DAG), it was shown that PC was mainly located in the outer envelope membrane of chloroplasts, more precisely in the cytosolic leaflet [47]. At the level of photosynthetic membranes, PC is undetectable in thylakoids purified from PLC-treated chloroplasts of *Spinacia* [47] but measurable in thylakoids from chloroplasts of *Pisum* [48]. It is not clear whether this latter observation is due to an actual presence of PC in thylakoids or a contamination. PE was also detected in thylakoids of chloroplasts from *Chlamydomonas* [5], but the question of a possible contamination by non-plastidic PE is, to our knowledge, unresolved, especially considering that it is difficult to get rid of the 10–15% contamination by mitochondria when purifying chloroplasts from *Chlamydomonas* [49]. Since mitochondrial membranes contain up to 50% PE this could explain the detection of this lipid in purified membrane fractions from *Chlamydomonas*. Presence of PI is less controversial and has been reported in all thylakoids isolated so far from algae or plant chloroplasts, with proportions ranging from 1 to 5% (see Table 1). However, no clear function has been proposed for this very interesting lipid class in the context of photosynthesis.

In addition to membrane polar glycerolipids, chloroplasts can also contain substantial amounts of triacylglycerol (TAG). In green algae like *Chlamydomonas*, TAG can be synthesized within chloroplasts following nutrient starvations [50,51], subsequently forming oil droplets in the stroma. In angiosperms, various stresses including nutrient shortage can trigger the accumulation of lipid droplets within chloroplasts, called plastoglobuli, containing TAG together with plasto-hydroquinone, and α-tocopherol [52–54]. As mentioned above, freezing, drought stresses or exposure to ozone, trigger the production of tri- and tetragalactoglycerolipids from preexisting galactoglycerolipids, following the activation of a galactolipid:galactolipid galactosyltransferase, thus leading to the accumulation of diacylglycerol (DAG) that serves as substrate for the production of TAG [13–17]. TAG can thus be generated in chloroplasts following a broad range of stresses, using a DAG substrate generated by the hydrolysis of galactoglycerolipids. The biogenesis of chloroplast TAG droplets and their structural relationship with thylakoid membranes are still poorly understood.

2.5. Major features of thylakoid lipid homeostasis

In summary, thylakoid membranes are composed of lipid classes that have been conserved over evolution, i.e. MGDG, DGDG, SQDG and PG. Their relative proportions are stable, indicating the presence of

mechanisms establishing and controlling a steady state (homeostasis) of the membrane lipidome in thylakoids, in given environmental and physiological contexts. The thylakoid lipidome results from the activities of biosynthetic enzymes that generate each lipid, the trafficking of lipid intermediates, the catabolic pathways and the regulatory processes that ensure that appropriate proportions are reached [55]. This composition is adjusted in response to environmental changes. Main features of this lipid homeostasis consist in a control of the MGDG/DGDG and the SQDG/PG ratios within the thylakoids of cyanobacteria and chloroplasts, and the galactoglycerolipid/phosphoglycerolipid (or DGDG/PC) ratio at the whole cell level in chloroplast-containing eukaryotes. It is therefore possible to dissect the structural and functional roles of these lipid classes in the local context of photosynthetic membranes and, in a more global context, in the complex dialog between the chloroplast and other subcellular membrane compartments.

3. Structural and functional roles of thylakoid lipids

3.1. Glycerolipids make the bulk of photosynthetic membranes

The first and most obvious basic role of polar glycerolipids in thylakoids is to constitute the lipophilic matrix in which photosystems are embedded. The cumulative surface of photosynthetic membranes is extraordinarily amplified by the organization as flat cisternae, which can form piles within the stroma, reaching, for example in angiosperms, a total area of hundreds of square meters of photosynthetic membranes per square meter of green tissues [55]. The role played by each lipid class in the 'membrane mosaic' relies on their physicochemical properties, including:

- the ability of MGDG to form reverse micelles (Hexagonal II) [56], a property which might be beneficial in highly curved membrane domains or at the vicinity of some large protein complexes;
- vice versa the ability of DGDG to stabilize membrane bilayers (bilayer-forming lipids) [56];
- the presence of 'rigid' sugars (i.e. hexose residues in cyclic conformations) in the polar head of glycoglycerolipids and of a 'flexible' phospho-glycerol (linear chain of three carbons) in PG;
- the absence of charges in MGDG and DGDG and the presence of a negative charge in PG and SQDG.

The integrity of photosynthetic membranes is thus likely to rely on an appropriate proportion of membrane-stabilizing lipids, mainly DGDG, of Hexagonal II MGDG that might enable the presence of highly curved regions (although this remains to be demonstrated), and on a smaller proportion of charged polar heads, mainly from PG and SQDG, as their excess might result in inappropriate repulsive forces between neighboring membranes (to be demonstrated as well) [57].

This matrix should allow the lateral diffusion of the photosystems, the diffusion of plastoquinone between protein complexes, and some highly dynamic remodeling processes like photosynthetic state transitions [58–61]. The lipid/protein ratio in the thylakoids is 0.6–0.8 mg mg protein^{-1}, whereas this ratio is close to 1.0 in the inner envelope membrane (IEM) and 2.5–3.0 in the outer envelope membrane (OEM) [2,3]. Photosynthetic membranes are therefore crowded by protein complexes and the lateral mobility might be relatively limited. A segregation of membrane proteins in domains with very low lipid content is observed at least in non oxygenic photosynthesis [62]. Membrane crowding with proteins could play a role in essential processes in photosynthesis, like light harvesting by the antenna complexes and the repair of the photosynthetic machinery upon photodamage ([63–66], see also below). The presence of multiple double bonds in fatty acids of galactoglycerolipids and the abundance of the Hexagonal II-MGDG might ease the lateral dynamics in this packed environment. The low lipid/protein ratio in thylakoids also highlights that in addition to their role in the lipid mixture, each lipid class might be also important at the level of individual molecules, by interacting with protein complexes.

3.2. Glycerolipids are critical for the structural stabilization and function of photosystems

MGDG, DGDG, SQDG and PG associate specifically with protein complexes including photosystems [67,68] and the plastid protein import machinery [69]. SQDG at the outer surface of the OEM is also likely to specifically bind to an annexin [70], thus giving a molecular basis for a possible dialog (signaling, coordination) between chloroplast lipids and cytosolic partners.

Lipids have been identified as possible 'cofactors' for complexes involved in photosynthesis (i.e. bound to dedicated binding pockets at the surface of protein subunits) based on (*i*) X-ray crystallographic studies, mostly performed in cyanobacteria, combined with (*ii*) biochemical characterizations of lipids extracted from purified protein fractions [67,68]. Most analyses summarized below suggest that lipid interaction with proteins is more important with photosystem II (PSII) (Fig. 2).

The X-ray crystallographic study of PSII from *Thermosynechococcus elongatus* has thus shown the presence of 6 to 11 MGDG, 4 to 7 DGDG, 3 to 5 SQDG and 1 to 2 PG molecules per PSII complex [71,72] (Fig. 2). In the most recent crystallographic study of *T. elongatus* PSII, 6 MGDG, 5 DGDG, 4 SQDG and 5 PG molecules were present per monomer, together with 3 additional unassigned lipids and 15 single alkyl chains, among which some may be lipids [73]. Consistently, the analysis of lipids extracted from the purified PSII of *Thermosynechococcus vulcanus* indicated the presence of 8 MGDG, 6 DGDG, 6 SQDG and 8 PG per PSII complex [74]. Lipids extracted from the PSII of *Synechocystis* PCC 6803 also showed the association with 6 MGDG, 3 DGDG, 5 SQDG and 6 PG [74].

By contrast with this strong association of lipids to PSII, the X-ray study of the light harvesting complex II (LHCII) from *Spinacia oleracea* showed only 1 DGDG and 1 PG per LHCII complex [75] and the structure of PSI of *Thermosynechococcus elongatus* supported the association with only 1 MGDG and 3 PG per PSI complex [76]. The X-ray analysis of the cytochrome *b6f* complex (Cyt b_6f, plastoquinol-plastocyanin reductase) from *Mastigocladus laminosus* indicated the possible interaction with 1 PC [77], whereas the resolution of the Cyt b_6f of *Chlamydomonas reinhardtii* supported the likely interaction with only 2 MGDG and 1 SQDG per complex [78]. Based on the unique detection of monomers following delipidation of Cyt b_6f from *Chlamydomonas*, it was suggested that lipids could maintain the dimeric structure of the Cyt b_6f complex [79].

The observation of glycerolipids associated to a protein complex is not sufficient to assess that they are critical for the function of the complex. Clues regarding the role of these lipids as possible functional cofactors could be deduced from genetic studies.

- MGDG: The analysis of the role of MGDG in photosynthetic organisms cannot be achieved by a complete abolition of the synthesis of this lipid, which leads to a lethal phenotype regarding its role as precursor of the main lipid class of thylakoids. Mutants of *Arabidopsis* containing significantly less MGDG could nevertheless be obtained by *knocking down* (KD) or *knocking out* (KO) *MGD1*, one of the three genes coding for MGDG synthases mostly involved in thylakoid-MGDG production [80–83]. The synthesis of MGDG could also be tuned down chemically using a specific inhibitor of MGDG synthases called galvestine-1 [55,84]. The *mgd1-1* KD mutant exhibited a deficiency in chlorophyll content, a defect in chloroplast development and a severe growth phenotype [80]. The *mgd1-2* KO did not contain any chlorophyll in standard conditions and could only grow on sucrose-supplemented media [85]. Galvestine-1-treated *Arabidopsis* also showed this strong chlorotic phenotype, a defect in thylakoid development and an impairment of growth [55,84]. MGDG reduction has therefore a drastic impact on the complete structure of the thylakoid membranes, an impact that one would obviously expect for such an abundant lipid. In the *mgd1-1*

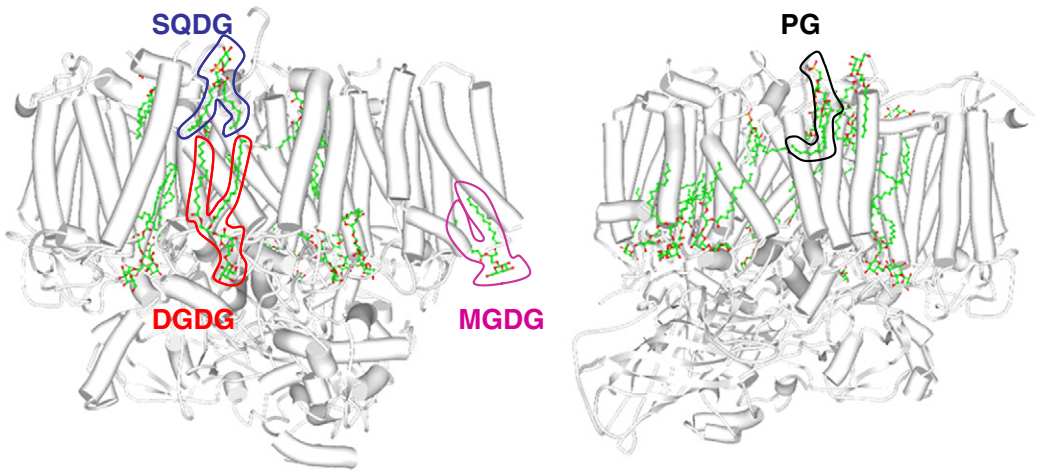

Fig. 2. Thylakoid lipids associated to the monomeric form of cyanobacterial Photosystem II. These two lateral views of the PSII structure of *Thermosynechococcus elongatus* solved by Guskov et al. in 2009 were obtained from the Protein Data Bank (PDB ID: 3KZ1) and vizualized using Protein Workshop so as to show chloroplast lipid ligands. Examples of phosphatidylglycerol (PG), sulfoquinovosyldiacylglycerol (SQDG), mono and digalactosyldiacylglycerol (MGDG, DGDG) are circled.

mutant, the linear electron flux capacity was diminished by 25–30% when calculated per leaf area, but was comparable to the WT when normalized to chlorophyll content. Functional analysis of the PSII performances in these mutants showed no major effect on PSII, suggesting that the remaining MGDG (40% of WT level) might be sufficient to maintain the structure and function of this complex [86]. Nevertheless, after high-light exposure, the *mgd1-1* mutant suffered from increased PSII photoinhibition as a result of inefficient non-photochemical quenching (NPQ; thermal dissipation of excess light energy in the PSII antenna bed during short-term high-light stress) [87,88].

- DGDG: A mutant of *Synechocystis* totally lacking DGDG could be obtained by *knocking out* the dgdA gene (coding for the cyanobacterial DGDG synthase), allowing an investigation of the role of this lipid in cyanobacteria [89–91]. Growth of *dgdA*$^{-}$ mutants was not affected in low light, and photosynthesis appeared identical to WT. Under high light intensity or high temperature, growth was altered and a phenotype similar to that of mutants altered at the level of the subunits of the oxygen evolving complex (OEC), was observed [89,90]. In photosynthetic organisms, the extrinsic domain of PSII is responsible for water splitting. In the current understanding, the cyanobacterial OEC is composed of PsbO, PsbU, PsbV, PsbQ and PsbP, whereas in green algae and plants, subunits U and V have been lost [92,93]. In the *dgdA*$^{-}$ mutant, PsbU is dissociated from PSII and DGDG appears therefore as a critical lipid to shape the final structure of PSII through the binding of extrinsic proteins stabilizing the OEC. Mutants of *Arabidopsis* containing significantly less DGDG could also be obtained by knocking out two genes encoding enzymes generating this lipid in *Arabidopsis*, i.e. *DGD1* and *DGD2*. The *dgd1* mutant contained markedly less DGDG compared to WT, was pale green and had a strongly altered chloroplast structure [94]. The *dgd1* mutant showed a decreased PSII/PSI ratio. Analyses of the low-temperature chlorophyll fluorescence of thylakoid fractions suggested a strongly modified energetic interaction between the antenna and the reaction centers in both reaction centers [95]. The *dgd1* mutant also shows an uncoupling of the PSI antenna, based on the data presented in the *dgd1* mutant analysis [95]. Quantification of pigments and pigment-binding apoproteins showed an impaired stoichiometry of pigment-protein complexes. In addition, an increase in the amount of peripheral LHCII subunits relative to the inner antenna (Lhcb4 and Lhcb5) and PSII core (D1 and α-subunit of Cyt b_{559}) complexes was observed [95]. The *dgd1* × *dgd2* mutant contained only traces of DGDG, generated by a distinct pathway (possibly via the action of a galactolipid:galactolipid galactosyltransferase, see below) and had a more severe phenotype compared to *dgd1* [96]. Functional analyses of photosynthesis in the *mgd1-1*, *dgd1* and *dgd1* × *dgd2* mutants, done by measuring transient fluorescence upon excitation with an actinic laser flash, were consistent with the idea that the small fraction of DGDG molecules left in the thylakoid membranes of the mutant was specifically bound to PSII and essential for its molecular function [86]. This fraction of DGDG is likely critical for the structure and function of PSII at its donor side. Altogether, mutant analyses show a critical role of DGDG in the structure and function of PSII from cyanobacteria to angiosperms.
- SQDG: A mutant of *Synechocystis* lacking SQDG could be obtained by *knocking out* the sqdB gene [97]. The *sqdB*$^{-}$ mutant showed a strong damage of PSII, but could grow photoautotrophically on SQDG-supplemented medium. This phenotype was not observed in a mutant of *sqdB* obtained in *Synechococcus* [37,97], where SQDG appeared dispensable for PSII. In a SQDG-deficient mutant of the green alga *Chlamydomonas*, PSI activity was intact whereas that of PSII was impaired, a phenotype that is rescued by addition of SQDG in the medium [98]. SQDG deficiency was deleterious at both acceptor and donor sites of PSII, and led to a higher sensitivity to the herbicide DCMU, suggesting that this lipid affects the equilibrium constant between the primary quinone acceptor Q_A and the diffusing quinone Q_B [67,98]. The higher sensitivity to DCMU was also reported in the *Synechocystis* mutant lacking SQDG [97]. In *Chlamydomonas*, SQDG was also shown to contribute to the stability of the interaction of PSII with extrinsic proteins, like in the case of DGDG (see above) [67]. In *Arabidopsis* mutants completely lacking SQDG, following the KO of *SQD1* or *SQD2*, no effect could be observed on photosynthesis, supporting that, in angiosperms, SQDG is dispensable for the structure and function of the photosynthetic machinery [34,38,67].
- PG: Mutants of *Synechocystis* lacking PG have been obtained by *knocking out* the cdsA (presumably encoding a cytidine 5′-diphosphate-diacylglycerol synthase) [36] or pgsA (encoding a phosphatidylglycerol 3-phosphate synthase) [99] genes. These mutants need a supply of PG in the medium for photoautotrophic growth. PSII activity appeared specifically altered [36,99], with phenotypic traits similar to those observed following a deficiency of SQDG: a defect in the electron transport from Q_A to Q_B [98], at the acceptor site, but also a dysfunction at the donor site, with a disassembly of extrinsic proteins from PSII [100]. In the green alga *Chlamydomonas*, a KO of the desaturase that catalyzes the synthesis of the 16:1t fatty acid, which is specifically and uniquely found in chloroplastic PG, was sufficient to prevent the assembly of the LHCII trimer [101], but a similar mutation in *Arabidopsis* did not induce

the same defect in the PSII antennas [102]. In angiosperms, the role of PG in photosynthetic membranes was initially studied by depleting PG from thylakoids isolated from *Pisum stativum* by an enzymatic treatment with a phospholipase A2 [103] or a phospholipase C [104]. In both cases, PSI appeared intact whereas the function of PSII was strongly altered. Recent work has however partially challenged this conclusion, showing that PG plays a role in protecting PSI from photodamage in tobacco plants subjected to cold stress [105].

As mentioned above, a deficiency of PG in thylakoids could not be obtained by mutating genes involved in its synthesis in *Arabidopsis*, without a very severe phenotype [44], which is consistent with a vital role of this specific lipid for the function of PSII. Consistently with their importance for chloroplast biogenesis and for the structure and function of the photosynthetic machinery, particularly at the level of PSII, it is not possible to genetically abolish the syntheses of MGDG [81] or PG [44] from cyanobacteria to angiosperms. Although DGDG null mutants could be generated in cyanobacteria [89–91], a complete deficiency of DGDG could not be obtained in angiosperms [96]. SQDG seems vital in some cyanobacteria species, but in most cases it is dispensable, playing a role under specific environmental conditions, in particular as surrogate for PG upon phosphate starvation, saving PG for its core function as a cofactor of PSII.

4. The biosynthesis of thylakoid lipids implies the cooperation of the stroma, the chloroplast envelope membranes and the endoplasmic reticulum

The demand of lipids for the expansion of photosynthetic membranes is very high. The relative proportions of MGDG, DGDG, SQDG and PG in thylakoids is close to that of the IEM [5], as shown in Table 1, highlighting the role of the IEM in the biogenesis of thylakoid membranes, using very active and coordinated lipid-synthetic machineries. From cyanobacteria to algae and angiosperms, the stroma and the envelope membranes cooperate for the biosynthesis of thylakoid lipids (for recent reviews [55,106]). The endoplasmic reticulum (ER) is also involved in eukaryotes. We shall give here an overview of this system in the chloroplast of angiosperms, and point, when necessary, differences that could occur in cyanobacteria. The identification and characterization of each individual enzyme involved in glycerolipid biosynthetic pathways in the chloroplast have relied on genetic screens, protein purifications and biochemical analyses. Our understanding of the sub-organellar compartmentalization of these pathways has benefited of the proteomic determination of each of the membrane sub-fractions using highly sensitive mass spectrometry techniques [106,107].

4.1. Origin of the building blocks for thylakoid lipids

4.1.1. In situ syntheses and import of precursors for the diacylglyceryl moiety: fatty acids, glycerol-3-phosphate, phosphatidic acid and diacylglycerol

The biosynthesis of fatty acids, thio-esterified to acyl carrier protein (in angiosperms: 16:0-ACP and C18:0-ACP), is achieved by two enzymatic systems found in the stroma: an acetyl-CoA carboxylase complex and a dissociable fatty acid synthase of type II (FASII). Following the release of acyl-ACP by FASII, a stromal delta-9 acyl/stearoyl-ACP desaturase (FAB2) catalyzes the synthesis of 18:1-ACP from 18:0-ACP. In some photosynthetic eukaryotes, this enzyme catalyzes the desaturation of 16:0-ACP into 16:1-ACP. Fresh fatty acids can be either used in the stroma or exported to the cytosol where they are activated by CoA, thus feeding the cytosolic pool of acyl-CoAs [1,107–110].

The synthesis of glycerol-3-phophate (G3P) can occur in the stroma by the action of a glycerol-3-phosphate dehydrogenase, using dihydroxyacetone-phosphate as a substrate. The location of dihydroxyacetone-phosphate is thus important for the latter production of glycerolipid, possibly explaining the presence of multiple isoforms of fructose-bisphosphate aldolases in different sites of the chloroplast [107]. The proportion of G3P utilized for the biosynthesis of glycerolipids in plastids also depends on concurrent consumption for other metabolic purposes and possible shuttling via the phosphate/triose-phosphate translocator [106].

Using acyl-ACP and G3P as initial substrates, the envelope is the site of biosynthesis of phosphatidic acid (PA) and DAG, which are precursors for MGDG, DGDG, SQDG and PG [1], by the stepwise action of two acyltransferases (ATS1 in the stroma and ATS2 in the IEM), and a phosphatidic acid phosphatase (PAP in the IEM) [44] (Fig. 3, enzymes 1, 2, 3). The specificity of ATS1 and ATS2 in angiosperms leads to the production of PA and DAG with 18:1 and 16:0 at positions *sn*-1 and *sn*-2 respectively. The glycerolipids thus assembled in the plastid, with 16-carbon fatty acids at *sn*-2 position, harbor a diacyl structure known as 'prokaryotic', similar to that observed in cyanobacteria glycerolipids [1,14,24,107].

In eukaryotes, the ER is also mobilized to fulfill the very high demand of precursors for chloroplasts, in particular for galactoglycerolipids. Acyl-CoAs and G3P are thus used for the stepwise synthesis of PA within the ER, with 16:0, 18:0 or 18:1 at *sn*-1 and 18:0 or 18:1 at *sn*-2 positions. PA can then be dephosphorylated into DAG. Both PA and DAG are at the origin of all phosphoglycerolipids synthesized in the ER. Membrane glycerolipids generated in the ER, with 18-carbon acyls at *sn*-2 position, harbor a diacyl structure known as 'eukaryotic' [1,14,24,107]. Import of ER precursors in the chloroplast is reflected by the high proportion of this eukaryotic signature in galactoglycerolipids. Although this import has been known for long [111], only recent advances combining the analysis of the response of *Arabidopsis* to environmental stresses, transcriptomic studies and characterization of genetic mutants, have pinpointed some proteins involved in this import (see below).

4.1.2. In situ synthesis and import of precursors for the polar heads: UDP-galactose, UDP-sulfoquinovose and glycerol-3-phosphate

The assembly of thylakoid glycerolipids requires the availability of polar head precursors: G3P and cytidine-triphosphate (CTP) for the synthesis of PG, uridine-triphosphate (UTP) for the syntheses of uridine-diphospho-galactose (UDP-Gal) and -sulfoquinovose (UDP-Sq) required for MGDG, DGDG and SQDG.

- The synthesis of G3P in the stroma has been detailed above [107].
- The import of CTP and UTP could possibly operate through a nucleoside-triphosphate transporter [106].
- The precursor for UDP-sugars is UDP-glucose (UDP-Glc), which synthesis in the stroma has been demonstrated [112]. UDP-Sq is clearly generated in the stroma, from UDP-Glc [113], allowing the accumulation of this substrate for the biosynthesis of SQDG. By contrast, cytosolic UDP-Gal can be provided to the intermembrane space separating the IEM and the OEM, or directly at the surface of the OEM, since enzymes assembling MGDG and DGDG are either in the OEM or in the IEM [114–117]. An epimerase converting UDP-Glc into UDP-Gal in the stroma of rice chloroplasts was nevertheless shown essential for the biosynthesis of galactoglycerolipids [118], suggesting that the polar head of galactoglycerolipids could be synthesized using either UDP-Gal from the stroma, deriving from UDP-Glc, or cytosolic UDP-Gal.

4.2. Assembly of thylakoid lipids in the chloroplast envelope

4.2.1. Biosynthetic machineries in the chloroplast envelope

In the envelope membranes, surrounding chloroplasts of angiosperms, PA and DAG are available for the biosynthesis of lipids. They are either neosynthesized in the IEM, with a 'prokaryotic' signature (18:1/16:0), or imported from the ER and harboring a 'eukaryotic' signature (with 18-carbon acyls at the *sn*-2 position and structures containing 16:0 at the *sn*-1 position). PG can be synthesized from PA, via

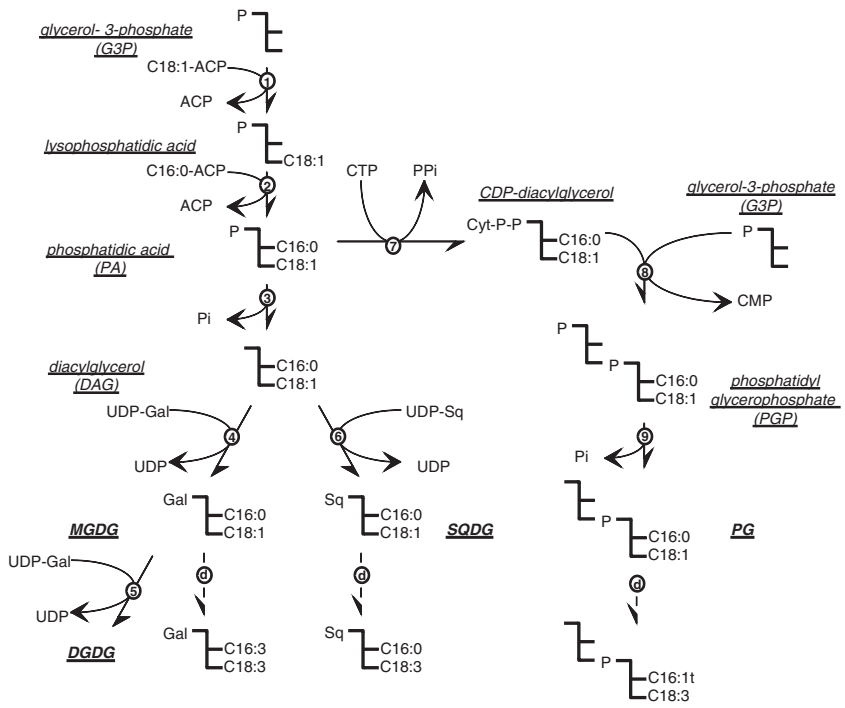

Fig. 3. Biosynthesis of thylakoid lipids in the chloroplast envelope of angiosperms. (1) *sn*-Glycerol-3-phosphate acyltransferase or ATS1, (2) 1-acylglycerol-phosphate acyltransferase or ATS2, (3) phosphatidate phosphatase, (4) MGDG synthase, (5) DGDG synthase, (6) SQDG synthase, (7) CTP:phosphatidate cytosine transferase, also called CDP-DAG synthase or CDS, (8) glycerol-3-phosphate:CDP-diacylglycerol phosphatidyltransferase, also called phosphatidylglycerophosphate synthase, (9) phosphatidylglycerol synthase or phosphatidylglycerol phosphate phosphatase, (d) desaturases. In cyanobacteria, a major difference occurs in the synthesis of galactolipids, by a two-step process involving a synthesis of MGlcDG followed by an epimerisation of the glucose polar head into galactose, thus forming MGDG (see text).

a CDP-DAG pathway and MGDG, DGDG and SQDG can be assembled from DAG. Nevertheless, the diacyl-moiety of each of these lipids indicates that the enzymes do not collect their substrates indistinctly in the original PA/DAG pool. A sorting of substrates is operated, leading to distinct acyl profiles in each of the lipid classes.

Fig. 3 shows the different reactions occurring in the envelope of the chloroplast, at least in angiosperms.

PG is considered as the sole phosphoglycerolipid that can be synthesized in the chloroplast envelope. In *Arabidopsis*, thylakoid PG is derived from 'prokaryotic' PA, regardless of the massive import of 'eukaryotic' precursors that might occur [119]. In at least five *Arabidopsis* allelic mutants of *ATS1*, thought to be null, a remaining leaky activity allowed the production of prokaryotic PA that was mainly used for the production of PG [44]. In the *ats1-1* leaky mutant, a RNAi repression of *ATS2* led to an interruption of the biosynthesis of PG and a very severe phenotype confirming that PG relied on the prokaryotic pathway [44]. Prokaryotic PA is thus mostly used for the stepwise biosynthesis of CDP-DAG (catalyzed by a CDP-DAG synthase or CDS), phosphatidylglycerophosphate or PGP (catalyzed by a PGP synthase) and eventually PG (catalyzed by a PGP phosphatase, or PGP-P) (see Fig. 3; enzymes 7, 8 and 9). PG is then substrate for a desaturase that specifically adds a double bond in *trans* conformation to 16:0 at the *sn*-2 position, leading to the presence of 16:1t [120] thought to be structurally important for the role of chloroplast PG at least in *Chlamydomonas* (see above).

Why is PG mostly synthesized from prokaryotic PA? Is the position of 16:1t essential at the *sn*-2 position? Is one of the enzymes involved in the biosynthesis of PG from PA strictly specific of a 'prokaryotic' substrate? By expressing a diacylglycerol kinase in chloroplasts of *Nicotiana tabacum*, an accumulation of eukaryotic PA, after phosphorylation of eukaryotic DAG, could be artificially promoted, and led to the accumulation of PG with eukaryotic signatures in chloroplasts [121]. In the newly formed PG, the Δ3-*trans* double bond was also found in *cis*-unsaturated 18-carbon fatty acids at position *sn*-2. Minor effects could be observed on the function of the photosynthetic apparatus, but a significant reduction of growth suggested that PG played more roles than those initially dissected at the level of photosynthetic membranes. Indeed, PG also acts as a positive activator of MGDG synthesis (see below) and the overexpression of a diacylglycerol kinase is likely to disturb the overall homeostasis of chloroplast lipids, leading to additional and unsuspected indirect effects. The synthesis of chloroplast PG in the diacylglycerol kinase over-expressor also indicates that the lack of eukaryotic structures in PG in the WT is not strictly due to the specificity of the enzyme catalyzing the synthesis of CDP-DAG, but rather on a channeling from PA to PG, which remains to be elucidated.

The synthesis of galactoglycerolipids is initiated by the production of MGDG in the envelope. In *Arabidopsis*, a multigenic family of 3 MGDG synthases (MGD1, MGD2, MGD3) [115], catalyzes the transfer of a galactosyl residue from UDP-Gal to a DAG acceptor (Fig. 3, enzyme 4). By contrast with PG, MGDG can be synthesized from prokaryotic or eukaryotic precursors. MGD enzymes are therefore at a key position to play a role in the coupling of extra-plastidial lipid metabolism with chloroplast lipid metabolism. Based on in vitro enzymatic studies, on analyses of subcellular fractions of chloroplast membranes and using GFP-fusion localization by epifluorescent microscopy [114,115,122–125], MGD1 was shown to be the most abundant and most active MGDG synthase, localized in the IEM, and being essential for the massive expansion of thylakoids. A KO of *MGD1* (*mgd1-2*) [85], is lethal in the absence of an external source of carbon substrate [81]. MGD2 and MGD3 are localized in the OEM and operate under Pi shortage [115]. Genetic disruption of *MGD2* and *MGD3* genes and double *mgd2* x *mgd3* KO mutants have no striking phenotype in normal growth conditions [126], as these enzymes seem to act mostly in specific contexts like in response to phosphate starvation or in specific cell types like in elongating pollen tubes [84].

It is important to note that in cyanobacteria, MGDG is not synthesized by a homolog of MGDG synthases, but after a two-step process [127]. In cyanobacteria, MGlcDG is synthesized by transfer of a glucosyl residue from a UDP-Glc donor to a DAG acceptor [128]. The glucosyl

polar head is then massively converted into galactose by the action of an epimerase. MGDG is thus produced by very distinct enzymatic machineries in cyanobacteria and chloroplast-containing eukaryotes, raising the questions of the importance of conserving MGDG in photosynthetic membranes and of the loss of the MGlcDG synthase and evolutionary origin of MGDG synthases in eukaryotes following the primary endosymbiosis. Molecular phylogeny reconstructions support the lateral transfer of a MGDG synthase ancestral gene from a chlorobacterium ancestor [127].

In chloroplasts, part of MGDG, with a eukaryotic structure, is converted into DGDG by the action of DGDG synthases (DGD1 and DGD2 in *Arabidopsis*) [24] (Fig. 3, enzyme 5). In angiosperms, the fatty acid composition of MGDG and DGDG are different, MGDG being 16:3-rich whereas DGDG is 16:0-rich. This observation suggests that (*i*) FAD5 catalyzes the very rapid desaturation of 16:0 into 16:1 at position *sn*-2 of MGDG, and that (*ii*) 16:1- and subsequently formed 16:2- and 16:3-MGDG species *are not* utilized by DGD1 and DGD2.

The sulfolipid is simply produced by transfer of a sulfoquinovosyl from UDP-Sq on a DAG, by the action of an SQDG synthase, or SQD2, in *Arabidopsis* [35] (Fig. 3, enzyme 6). This enzyme was localized in the chloroplast [35], catalyzing the production of SQDG in the IEM, consistently with earlier biochemical evidence [129,130]. Recently, it was shown that in angiosperms, SQD2 could also catalyze the synthesis of glucoronosyldiacylglycerol (GlcADG), a quantitatively minor lipid that accumulate upon phosphate starvation and might play a role as surrogate for PI and/or PE [131].

4.2.2. Evidence of channeling and enzymatic regulations of the biosynthetic fluxes producing MGDG, DGDG, SQDG and PG

The establishment of a refine balance between each lipid class in thylakoid membrane requires controlling and regulatory mechanisms. Based on genetic and biochemical studies, some general guidelines can be deduced.

- First, some reactions catalyzed by apparently dissociated enzymes operate in series, with substrates captured as soon as they are produced to feed the downstream enzyme, following a process known as 'channeling'. The synthesis of PG thus appears as a channeling from prokaryotic PA to PG (prokaryotic PA →→ PG) [44,119]. Likewise, the analyses of mutants, grown in various supplies of phosphate support the occurrence of a cooperation of MGD and DGD proteins with a possible DAG → MGDG → DGDG channeling. DGD1 (in the OEM) seems to operate downstream MGD1 (in the IEM), whereas DGD2 (in the OEM) seems to operate downstream MGD2 and MGD3 (in the OEM) following Pi deprivation [24,132].
- Second, some lipid intermediates can act as effectors of enzymes acting in the same or different lipid biosynthetic pathways. Recombinant and native MGD1 from *Arabidopsis* is thus strongly activated by very low proportions of PA, an upstream precursor in the same pathway, or by higher proportions of PG [133,134].

The different mechanisms that govern the establishment of the thylakoid lipid profile integrate transcriptional, post-translational and enzymological regulatory mechanisms, which are still poorly known [55]. Future challenges include their characterizations.

5. Lipid trafficking

The chloroplast is not isolated in the cytosol, and the cooperation of other compartments is particularly important in the overall lipid homeostasis at the cellular level. Import of eukaryotic precursors is reflected by the high proportion of eukaryotic signatures in plastid galactoglycerolipids. These imported precursors derive from non-plastidic phosphoglycerolipids. In the current understanding of this process, PA produced by phospholipases D (PLDs) might be transferred across the plastid envelope by an ABC transporter (TGD complex) [24]. In the IEM, imported PA could then be dephosphorylated, thus generating DAG used for galactoglycerolipid syntheses. DAG produced by phospholipases C (PLCs) or endomembrane phosphatidic acid phosphatases [135], might also be transported to the plastid envelope by an unknown mechanism, but this route is currently debated. The import of PC, at least in the cytosolic leaflet of the OEM [136] might involve a lyso-PC intermediate [137,138]. The search for components of the eukaryotic pathway has benefited of the analyses of the effect of phosphate starvation on the remodeling of lipids, triggering a transient accumulation of PC, its hydrolysis and net conversion into galactoglycerolipids [139]. Study of the transcriptome of *Arabidopsis* in response to low phosphate combined with genetics have pointed genes coding for PLDs (PLDζ1, PLDζ2) and PLCs (NPC4, NPC5) and involved in the supply of precursors for MGDG (reviewed by [1,24,108]).

In addition to the TGD machinery, ER-chloroplast lipid trafficking could alternatively occur at the level of PLAM (plastid associated membranes) systems [140] or even via direct biochemical continuity between ER and plastids [141]. Other puzzling questions regarding lipid trafficking required for the biogenesis of thylakoids include lipid transfers between the IEM and the OEM, lipid lateral movements and from one leaflet to another within envelope membranes, lipid trafficking from the chloroplast to the endomembranes and mitochondrion and, eventually, lipid trafficking from the IEM to the thylakoids (for review [1,24,55,106,108]). Transport of lipids via vesicle trafficking inside plastids has been suggested in various studies [48,142–145], but has not been unambiguously demonstrated so far.

6. Conclusions and future challenges

In this review, we have shown that MGDG, DGDG, PG and SQDG have been conserved from cyanobacteria to primary plastids. The relative proportions of each lipid result from highly regulated and compartmentalized metabolic pathways. The MGDG/DGDG ratio appears critical for the appropriate structure of thylakoid membranes. The SQDG/PG and, at least in eukaryotes, the galactoglycerolipid/phosphoglycerolipid ratios are finely tuned in response to the availability of phosphate, and in relation with the cellular phosphate homeostasis. Concerning the photosynthetic function, MGDG, DGDG and PG are important stabilizing components for PSII. SQDG seems to act as a 'stand-in-actor', playing part of PG roles upon phosphate starvation. These lipids also play other roles, in particular in eukaryotes. Future challenges include the understanding of lipid metabolic pathways as a highly regulated system, the molecular characterization of lipid trafficking machineries, and the complex evolution history that has preserved these lipid classes from cyanobacteria to chloroplasts.

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