ANNUAL REVIEWS

Annual Review of Plant Biology

Chloroplast Lipids and Their Biosynthesis

Georg Hölzl and Peter Dörmann

Institute of Molecular Physiology and Biotechnology of Plants (IMBIO), University of Bonn, 53115 Bonn, Germany; email: doermann@uni-bonn.de

Annu. Rev. Plant Biol. 2019. 70:51–81

First published as a Review in Advance on February 20, 2019

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-050718-100202

Keywords

phospholipid, galactolipid, sulfolipid, fatty acid, phosphate deprivation, photosynthesis

Abstract

Chloroplasts contain high amounts of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) and low levels of the anionic lipids sulfoquinovosyldiacylglycerol (SQDG), phosphatidylglycerol (PG), and glucuronosyldiacylglycerol (GlcADG). The mostly extraplastidial lipid phosphatidylcholine is found only in the outer envelope. Chloroplasts are the major site for fatty acid synthesis. In *Arabidopsis*, a certain proportion of glycerolipids is entirely synthesized in the chloroplast (prokaryotic lipids). Fatty acids are also exported to the endoplasmic reticulum and incorporated into lipids that are redistributed to the chloroplast (eukaryotic lipids). MGDG, DGDG, SQDG, and PG establish the thylakoid membranes and are integral constituents of the photosynthetic complexes. Phosphate deprivation induces phospholipid degradation accompanied by the increase in DGDG, SQDG, and GlcADG. During freezing and drought stress, envelope membranes are stabilized by the conversion of MGDG into oligogalactolipids. Senescence and chlorotic stress lead to lipid and chlorophyll degradation and the deposition of acyl and phytyl moieties as fatty acid phytyl esters.

Contents

1. LIPID COMPOSITION IN PLANT CHLOROPLASTS

Chloroplasts are the hallmark organelles of the plant cell. Thylakoid membranes harbor the photosynthetic complexes, and numerous essential biochemical pathways, including the synthesis of chlorophyll, aromatic amino acids, and fatty acids, are chloroplast localized. The thylakoids represent the largest membrane system in the leaf mesophyll cell. Therefore, it is not surprising that approximately 80% of all leaf glycerolipids are found in chloroplasts (91). Thylakoids harbor a highly characteristic set of glycolipids and phospholipids. Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) make up approximately 52% and 26%, respectively, of chloroplast lipids (15, 35). They carry one or two galactoses, respectively, bound to diacylglycerol (DAG). The first galactose is bound in β-anomeric configuration to DAG, while the second galactose in DGDG is α-anomeric (**Figure 1**). Oligogalactolipids carrying two, three, or more galactoses (all in β-anomeric configuration) are not observed under normal conditions but accumulate during stress (138). Similarly, acylated MGDG (acyl-MGDG) with a third fatty acid at the C6 position of the galactose occurs with very low abundance (approximately 1%) in healthy leaves but accumulates during freezing (57). Sulfoquinovosyldiacylglycerol (SQDG) is an anionic glycolipid carrying a C6-sulfonated deoxyglucose and accounts for approximately 6.5% of thylakoid lipids. The anionic glucuronosyldiacylglycerol (GlcADG), carrying a glucuronosyl head group, is below the detection limit but increases during phosphate deprivation. The thylakoids contain one anionic phospholipid, phosphatidylglycerol (PG), which amounts to approximately 9.5%. While the lipid composition of the inner chloroplast envelope (including 49% MGDG, 30% DGDG, 5% SQDG, 8% PG) resembles that of thylakoids, the outer envelope reveals a very distinct composition of 17% MGDG, 29% DGDG, 6% SQDG, 10% PG, and, in addition, 32% phosphatidylcholine (PC) (15). PC is restricted to the outer leaflet of the outer envelope of chloroplasts (34). The high PC content in the outer chloroplast envelope and in the endoplasmic reticulum (ER) membrane suggests that these two membrane systems are biochemically related and possibly connected via

MGDG: monogalactosyldiacylglycerol

DGDG: digalactosyldiacylglycerol

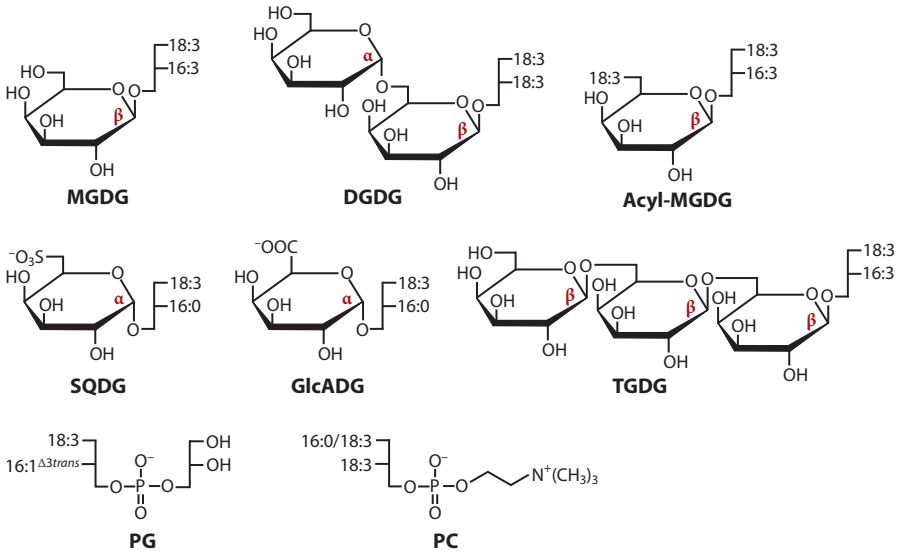
SQDG: sulfoquinovosyldiacylglycerol

GlcADG: glucuronosyldiacylglycerol

PG: phosphatidylglycerol

PC: phosphatidylcholine

a



b

Fatty acid	Carbon atoms : double bonds (abbreviation)	Structure
Oleic acid	18:1	
Linoleic acid	18:2	
α-Linolenic acid	18:3	
12-Oxophytodienoic acid	OPDA	
Palmitic acid	16:0	
3*trans*-Hexadecenoic acid	16:1$^{\Delta 3trans}$	
Hexadecatrienoic acid	16:3	

Figure 1

Structures of chloroplast lipids. (*a*) MGDG and DGDG are the most abundant galactolipids in chloroplasts. Oligogalactolipids (e.g., TGDG) have lower abundance but accumulate during freezing. SQDG and another anionic glycolipid, GlcADG, accumulate during phosphate deprivation. PC and PG are the only phospholipids in chloroplasts. Anomeric configurations of the sugars are depicted in red. PC is restricted to the outer envelope. Molecular species shown are characteristic for *Arabidopsis*. (*b*) Fatty acids typically found in chloroplast lipids. *X*:*Y* indicates the number of carbon atoms and the number of double bonds in the acyl chains. Abbreviations: DGDG, digalactosyldiacylglycerol; GlcADG, glucuronosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; OPDA, 12-oxophytodienoic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; SQDG, sulfoquinovosyldiacylglycerol; TGDG, trigalactosyldiacylglycerol.

contact sites (see Section 2.3). Additional lipids of the chloroplast were detected in low amounts by phosphorylation experiments, i.e., phosphatidylinositol, phosphatidylinositol-phosphate, and phosphorylated derivatives of MGDG and DGDG (109), but it is unclear whether they represent authentic chloroplast components.

18:3: abbreviation for a fatty acid (the ratio of carbons to double bonds)

Plastoglobules: small, spherical lipid droplets in the stroma of chloroplasts, surrounded by a lipid monolayer, containing proteins and filled with neutral lipids

Chloroplasts/plastids are the major sites for fatty acid synthesis in the plant cell (115). Palmitic acid (16:0) and unsaturated C18 fatty acids (oleic acid, $18:1^{\Delta 9 cis}$; linoleic acid, $18:2^{\Delta 9 cis,12 cis}$; and α-linolenic acid, $18:3^{\Delta 9 cis,12 cis,15 cis}$) are highly abundant in chloroplasts (**Figure 1**). Unsaturated C16 fatty acids are found in PG (3*trans*-hexadecenoic acid, $16:1^{\Delta 3 trans}$) and MGDG (7*cis*,10*cis*,13*cis*-hexadecatrienoic acid, $16:3^{\Delta 7 cis,10 cis,13 cis}$) in some plant species like *Arabidopsis* (104). 12-Oxophytodienoic acid (OPDA) is derived from 18:3 by action of 13-lipoxygenase, 13-allene oxide synthase, and allene oxide cyclase. After export from the chloroplast, OPDA serves as a precursor for jasmonic acid synthesis in the peroxisome. OPDA exists in free and lipid-bound forms. In *Arabidopsis*, it is found in the DAG moieties of MGDG and DGDG and in the C6 position of galactose of acyl-MGDG (the so-called arabidopsides) (114). Furthermore, triacylglycerol and fatty acid phytyl esters are found in chloroplasts. These neutral lipids accumulate during stress and are mostly found in plastoglobules, which are small lipid droplets in the stroma enclosed by a lipid monolayer membrane (140). In addition, plastoglobules contain further neutral lipids (e.g., tocopherol, phylloquinol), structural proteins (e.g., fibrillins), and enzymes and are connected with the thylakoid membranes (140).

Excellent review articles on fatty acid synthesis (115), glycerolipids in general (22, 100, 106), SQDG (13), and storage lipids (153) were previously published in this journal. A collection of lipid biosynthesis pathways and genes is available at the *Arabidopsis* Acyl-Lipid Metabolism database *ARALIP* (**http://aralip.plantbiology.msu.edu**) (91). Furthermore, comprehensive reviews on chloroplast isoprenoid lipids were published (30, 140, 142). Important isoprenoid lipids of chloroplasts include chlorophyll, carotenoids, and prenylquinols (plastoquinol-9, phylloquinol, tocopherol).

The different lipid classes from chloroplasts can be separated by thin-layer chromatography followed by measuring fatty acids as methyl esters via gas chromatography (145). The development of liquid chromatography–mass spectrometry (LC-MS) provided the means to directly measure intact membrane glycerolipids without derivatization. Two strategies for LC-MS analyses of glycerolipids were established: direct-infusion tandem MS measurements (shotgun lipidomics or electrospray ionization–tandem MS) and separation of lipids by high-performance LC prior to MS (117, 147). These powerful LC-MS methods provide the means to acquire quantitative results for hundreds of glycerolipid molecular species in just a few minutes with unprecedented accuracy, and they thus allow the characterization of even minor lipid changes during stress or in plant mutants.

2. ORIGIN OF FATTY ACIDS FOR CHLOROPLAST LIPIDS

2.1. Fatty Acid De Novo Synthesis

Some enzymatic functions of fatty acid or glycerolipid synthesis in *Arabidopsis* are based on small gene families, while others are derived from single locus genes (91) (**Table 1**). The significance of these differences is unclear. Null mutations in many single locus genes of fatty acid synthesis cause lethality during gamete or embryo development, in agreement with the essential function of fatty acids in the plant cell, whereas mutations in later steps of glycerolipid synthesis oftentimes affect the establishment of photosynthetically active chloroplasts (**Table 1**).

Approximately 95% of fatty acids in the plant cell are produced by the plastidial fatty acid synthase (FAS) (116). Mitochondria produce only minor amounts of fatty acids, mostly octanoic acid (8:0) as a precursor for lipoic acid, the cofactor for oxidative decarboxylation reactions. Chloroplast FAS enzymes belong to the type I FAS represented by individual polypeptides, as found in prokaryotes. Acetyl–coenzyme A (CoA) is first converted into malonyl-CoA by

Table 1 Genes involved in chloroplast lipid biosynthesis in *Arabidopsis*

Gene	Enzymatic function	Protein	Localization	Mutant[a]	Mutant growth phenotype[a]	Reference(s)
Heteromeric plastidial ACCase						
At2g38040	α-Carboxyltransferase	α-CT, CAC3	Stroma	NA		132
AtCg00500 (plastome)	β-Carboxyltransferase	β-CT, accD	Stroma	NA		126
At5g35360	Biotin carboxylase	BC, CAC2	Stroma	NA		134
At5g15530	Biotin carboxyl carrier protein	BCCP2	Stroma	*cac1b*		26, 90
At5g16390	Biotin carboxyl carrier protein	BCCP1	Stroma	*cac1a*	Embryolethal	26, 90
Plastidial fatty acid synthase						
At3g05020	ACP	ACP1	Stroma	NA		60
At1g54580	ACP	ACP2	Stroma	NA		60
At1g54630	ACP	ACP3	Stroma	NA		60
At4g25050	ACP	ACP4	Stroma	NA		60
At5g27200	ACP	ACP5	Stroma	NA		60
At2g30200	Malonyl-CoA:ACP malonyltransferase	MCMT	Stroma	*emb3147*	Embryolethal	23
At1g62640	3-Ketoacyl-ACP synthase III	KASIII	Stroma	*kas3*	Low temperature–sensitive	135
At5g46290	3-Ketoacyl-ACP synthase I	KASI	Stroma	*kas1*	Semidwarf, chlorotic	148
At1g74960	3-Ketoacyl-ACP synthase II	KASII	Stroma	*fab1*, *kas2*	Low temperature–sensitive	24, 149
At1g24360	3-Ketoacyl-ACP reductase	KAR	Plastid	NA		43
At1g62610	3-Ketoacyl-ACP reductase	KAR	Plastid	NA		43
Ag3g46170	3-Ketoacyl-ACP reductase	KAR	Plastid	NA		43
At3g55290	3-Ketoacyl-ACP reductase	KAR	Plastid	NA		43
At3g55310	3-Ketoacyl-ACP reductase	KAR	Plastid	NA		43
At2g22230	Hydroxyacyl-ACP dehydratase	HAD	Plastid	NA		17
At5g10160	3R-hydroxyacyl-ACP dehydratase	HAD	Plastid	NA		17
At2g05990	Enoyl-ACP reductase	ENR1, MOD1	Plastid	*mod1*, *enr1*	Premature cell death	107
At2g43710	Stearoyl-ACP Δ9-desaturase	FAB2	Plastid	*fab2*, *ssi2*, *fad1*	Dwarf, defense response	69, 70, 92, 129, 136
At5g16240	Stearoyl-ACP Δ9-desaturase	DES1	Plastid	NA		70
At3g02610	Stearoyl-ACP Δ9-desaturase	DES2	Plastid	NA		70
At5g16230	Stearoyl-ACP Δ9-desaturase	DES3	Plastid	NA		70
At3g02620	Stearoyl-ACP Δ9-desaturase	DES4	Plastid	NA		70
At3g02630	Stearoyl-ACP Δ9-desaturase	DES5	Plastid	NA		70
At1g43800	Stearoyl-ACP Δ9-desaturase	DES6	Plastid	*sad6*	Drought signaling	80
Prokaryotic lipid synthesis						
At1g32200	Glycerol-3-phosphate acyltransferase	GPAT, ATS1	Stroma	*act1*, *ats1*	Dwarf	85, 154
At4g30580	LPA acyltransferase	LPAT1, ATS2	Plastid	*ats2*	Embryolethal	77, 157
At1g15080	PA phosphatase	LPP2	Plastid	*lpp2*		72
At2g01180	PA phosphatase	LPP1	Plastid	*lpp1*		121
At3g50920	PA phosphatase	LPPε1	Plastid	*lppε1*		112

(*Continued*)

Table 1 (*Continued*)

Gene	Enzymatic function	Protein	Localization	Mutant[a]	Mutant growth phenotype[a]	Reference(s)
At5g66450	PA phosphatase	LPPε2	Plastid	*lppε2*		112
At5g03080	PA phosphatase	LPPγ	Plastid	*Lppγ*		112
Chloroplast membrane–bound desaturases						
At4g27030	16:0-PG Δ3*trans*-desaturase	FAD4	Plastid	*fad4*		20, 46
At3g15850	16:0-MGDG Δ7-desaturase	FAD5, ADS3	Plastid	*fad5*, *ads3*	Low temperature–sensitive	56, 86
At4g30950	16:1/18:1 galactolipid ω6 desaturase	FAD6	NA	*fat6*, *fadC*	Low temperature–sensitive	18, 41
At3g11170	16:2/18:2 galactolipid ω3 desaturase	FAD7	NA	*fad7*, *fadD*		21, 63
At5g05580	16:2/18:2 galactolipid ω3 desaturase	FAD8	NA	*fad8*		49, 101
Galactolipid synthesis						
At4g31780	MGDG synthase	MGD1	IE	*mgd1*, *emb2797*	Albino	65, 83, 130
At5g20410	MGDG synthase	MGD2	OE	*mgd2*		7, 81
At2g11810	MGDG synthase	MGD3	OE	*mgd3*		7, 81
At3g11670	DGDG synthase	DGD1	OE	*dgd1*	Dwarf, chlorotic	31, 32
At4g00550	DGDG synthase	DGD2	OE	*dgd2*		74, 75
At3g06510	Galactolipid:galactolipid galactosyltransferase	GGGT, SFR2	OE	*sfr2*	Sensitive to freezing	44, 103, 137
At2g42690	Acylated galactolipid associated phospholipase 1	AGAP1	OE/cytosol	*agap1*		114
Sulfolipid synthesis						
At3g56040	UDP-glucose pyrophosphorylase	UGP3	Stroma	*ugp3*		119
At4g33030	UDP-sulfoquinovose synthase	SQD1	Stroma	*sqd1*		40, 108
At5g01220	UDP-sulfoquinovose:DAG sulfoquinovosyltransferase	SQD2	IE	*sqd2*		158
Plastidial PG synthesis						
At2g45150	CDP-DAG synthase	CDS4	Plastid	*cds4*		54
At4g60620	CDP-DAG synthase	CDS5	Plastid	*cds5*		54
At2g39290	PGP synthase	PGP1	Mitochondrion, plastid	*pgp1*, *jov*	Dwarf, chlorotic	11, 51, 152
At3g58830	PGPP phosphatase	PGPP1	Plastid	*pgpp1*		93, 161
Eukaryotic lipid synthesis						
At3g24110	Acyl-ACP thioesterase A1	FATA1	Plastid	NA		125
At4g13050	Acyl-ACP thioesterase A2	FATA2	Plastid	NA		NA
At1g08510	Acyl-ACP thioesterase B	FATB	Plastid	*fatB*	Dwarf	16, 33
At3g57280	Fatty acid exporter	FAX1	IE	*fax1*		89
At1g35290	Acyl lipid thioesterase	ALT1	Plastid	NA		123
At1g35250	Acyl lipid thioesterase	ALT2	Plastid	NA		123
At1g68260	Acyl lipid thioesterase	ALT3	Plastid	NA		123
At1g68280	Acyl lipid thioesterase	ALT4	Plastid	NA		123
At4g23850	Long chain acyl-CoA synthetase	LACS4	ER	*lacs4*		66
At1g77590	Long chain acyl-CoA synthetase	LACS9	Envelope	*lacs9*		66
At5g60620	Acyl-CoA:glycerol-3-phosphate acyltransferase	GPAT9	ER	NA		50

(*Continued*)

Table 1 (*Continued*)

Gene	Enzymatic function	Protein	Localization	Mutant[a]	Mutant growth phenotype[a]	Reference(s)
At3g57650	Acyl-CoA:LPA acyltransferase	LPAT2	ER	*lpat2*	Lethal for ♀ gametes	78
At3g09560	PA phosphatase	PAH1	ER, cytosol	*pah1*		38, 111
At5g42870	PA phosphatase	PAH2	Cytosol	*pah2*		38, 111
PC synthesis						
At2g26830	Choline ethanolamine kinase	CEK4	Plasma membrane	*cek4*	Embryolethal	94
At1g48600	Phosphomethylethanolamine methyltransferase	PMEAMT, NMT2, CPUORF31	Probably cytosol	*pmeatmt*		12
At1g73600	Phosphoethanolamine methyltransferase	NMT3	NA	NA		28
At3g18000	Phosphoethanolamine methyltransferase	PEAMT, NMT1, XIPOTL1	NA	*xpl1*	Altered roots	28
At2g32260	CTP:phosphocholine cytidylyltransferase	CCT1	Microsomal	*cct1*		64
At4g15130	CTP:phosphocholine cytidylyltransferase	CCT2	Microsomal	*cct2*		64
At1g13560	CDP-choline:DAG phosphocholine transferase	AAPT1	NA	*aapt1*	*aapt1 aapt2* embryolethal	97
At3g25585	CDP-choline:DAG phosphocholine transferase	AAPT2	NA	*aapt2*		97
At3g12120	18:1-PC ω6 desaturase	FAD2	ER	*fad2*		120
At2g29980	18:1-PC ω3 desaturase	FAD3	ER	*fad3*		5, 19
PC, PG, and MGDG hydrolysis						
At3g05630	Phospholipase D	PLDζ2	NA	*pldζ2*		29, 88
At3g16785	Phospholipase D	PLDζ1	NA	*pldζ1*		88
At1g07230	Nonspecific phospholipase C	NPC1	NA	*npc1*		110
At2g26870	Nonspecific phospholipase C	NPC2	Plastid	*npc2*	*npc2 npc6* embryolethal	110, 113
At3g03520	Nonspecific phospholipase C	NPC3	NA	*npc3*		110
At3g03530	Nonspecific phospholipase C	NPC4	Plasma membrane	*npc4*		110
At3g03540	Nonspecific phospholipase C	NPC5	Cytosol	*npc5*		48, 110
At3g48610	Nonspecific phospholipase C	NPC6	Plastid	*npc6*		110, 113
At3g61680	PG-specific plastid lipase	PLIP1	Plastid	*plip1*		143
At1g02660	MGDG-specific plastid lipase	PLIP2	Plastid	*plip2*		144
At3g62590	PG-specific plastid lipase	PLIP3	Plastid	*plip3*		144
At2g44810	Phospholipase A1	DAD1	Plastid	*dad1*	Anther dehiscence	39
At4g13550	Heat-inducible lipase	HIL1	Plastid	*hil1*	Less thermo-tolerant	59
Isoprenoid ester synthesis						
At1g54570	Phytyl ester synthase 1	PES1	Plastid	*pes1*		96
At3g26840	Phytyl ester synthase 2	PES2	Plastid	*pes2*		96
Eukaryotic lipid import into the plastid						
At3g20320	PA-binding protein of ABC transporter	TGD2	IE	*tgd2*		10
At1g19800	Permease-like protein of ABC transporter	TGD1	IE	*tgd1*		151

(*Continued*)

Table 1 (*Continued*)

Gene	Enzymatic function	Protein	Localization	Mutant[a]	Mutant growth phenotype[a]	Reference(s)
At1g65410	ATPase of ABC transporter	TGD3, NAP11, ABCI13	IE	*tgd3*		99
At3g06960	Plastid lipid import protein	TGD4	OE	*tgd4*		150
At1g27695	Plastid lipid import protein	TGD5	IE	*tgd5*		42
Chloroplast membrane structure						
At4g01490	Curvature thylakoid 1A	CURT1A	Thylakoid	*curt1A*		4
At2g46820	Curvature thylakoid 1B	CURT1B	Thylakoid	*curt1B*		4
At1g46480	Curvature thylakoid 1C	CURT1C	Thylakoid	*curt1C*		4
At4g43440	Curvature thylakoid 1D	CURT1D	Thylakoid	*curt1D*		4
At1g58860	Vesicle inducing protein in plastids 1, inner membrane protein of 30 kilodaltons	VIPP1, IM30	IE	*vipp1*, *im30*		58

[a]Only null mutants were included. Blank cells indicate mutants that grow normally or show conditional phenotypes.
Abbreviations: ACCase, acetyl–coenzyme A carboxylase; ACP, acyl carrier protein; CoA, coenzyme A; DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; ER, endoplasmic reticulum; IE, inner envelope; MGDG, monogalactosyldiacylglycerol; NA, not available; OE, outer envelope; PC, phosphatidylcholine; PG, phosphatidylglycerol.

acetyl-CoA carboxylase (ACCase). *Arabidopsis* contains two ACCases, a homomeric polypeptide in the cytosol, and a heteromeric multisubunit form in the chloroplast. The homomeric form provides malonyl-CoA for fatty acid elongation and secondary metabolite production, while the heteromeric form produces malonyl-CoA for fatty acid de novo synthesis. In dicotyledonous plants like *Arabidopsis*, the chloroplast ACCase consists of four subunits: α-carboxyltransferase (α-CT/CAC3), β-carboxyltransferase (β-CT/accD), biotin carboxyl carrier protein (which has two isoforms: BCCP1/CAC1A and BCCP2/CAC1B), and biotin carboxylase (BC/CAC2) (26, 90, 126, 132, 134). All genes are nuclear encoded except β-CT/accD which is plastome encoded (134). The plastid ACCase is essential for *Arabidopsis*, because the *cac1a* mutant deficient in *BCCP1* expression is embryolethal (90). Mutations in *cac1b* have only minor consequences presumably because the expression of *BCCP2* is lower than that of *BCCP1/CAC1A* (90). Many monocotyledonous plants contain homomeric ACCase forms in the cytosol and chloroplast but do not contain heteromeric ACCase. Aryloxyphenoxypropionate and cyclohexanedione herbicides specifically target the carboxyltransferase domain of the plastid homomeric ACCase in grasses, thus interfering with growth of monocotyledonous weeds (159).

The next step in fatty acid synthesis is the transfer of malonate from malonyl-CoA to acyl carrier protein (ACP) by malonyl-CoA:ACP malonyltransferase (MCMT). A mutation in *MCMT* (*emb3147* mutant) is embryolethal (23). Fatty acids are produced by two carbon elongation reactions using malonyl-ACP as a donor (**Figure 2**). *Arabidopsis* contains eight ACP isoforms to which the acyl chains are bound, and five ACP isoforms are localized to the chloroplast (60) (**Table 1**). During the first elongation reaction, acetyl-CoA is condensed with malonyl-ACP producing acetoacetyl-ACP by 3-ketoacyl-ACP synthase 3 (KASIII), one of the three chloroplast KASs. Partial loss of KASIII activity in the *Arabidopsis kas3* mutant results in a decrease in fatty acid production and an increased sensitivity to low temperature (135). Acetoacetyl-ACP is reduced to 3R-hydroxybutyryl-ACP by 3-ketoacyl-ACP reductase (KAR) (43), and hydroxyacyl-ACP dehydratase (HAD) converts 3R-hydroxybutyryl-ACP into 2*trans*-butenoyl-ACP (17), which again is reduced to butyryl-ACP by enoyl-ACP reductase (ENR) (107). *Arabidopsis mod1* mutant plants with strongly reduced ENR activity show premature cell death (107). The condensation

ACP: acyl carrier protein

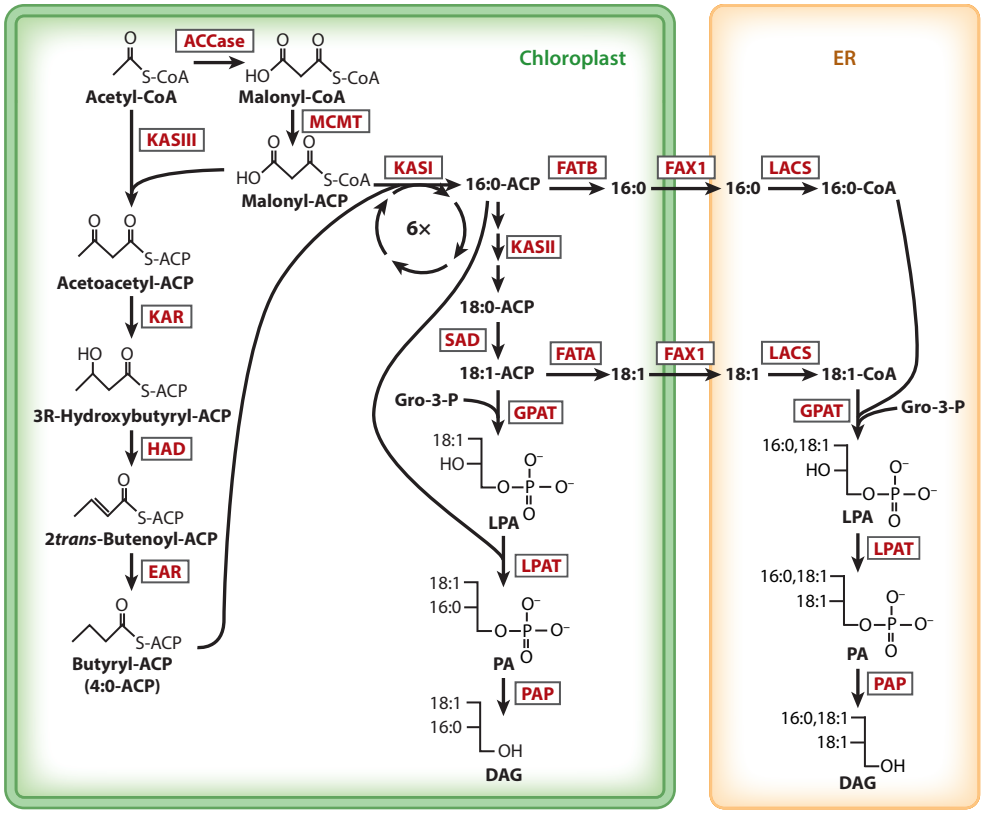


Figure 2

Fatty acid de novo synthesis and assembly of DAG backbones. The predominant proportion of fatty acids in plant cells is synthesized in chloroplasts. Acetyl-CoA is converted into malonyl-CoA, and the malonyl group is transferred to ACP. Acetyl-CoA is condensed with malonyl-ACP producing acetoacetyl-ACP. The 3-ketoacyl-ACPs are converted into acyl-ACP, and additional condensation reactions finally result in the production of 16:0-ACP and 18:0-ACP. 18:0-ACP is readily desaturated. 16:0 and 18:1 are used for prokaryotic lipid synthesis in the chloroplast or, after export, for eukaryotic lipid synthesis at the ER. Enzyme names are indicated in red. Abbreviations: ACCase, acetyl–coenzyme A carboxylase; ACP, acyl carrier protein; CoA, coenzyme A; DAG, diacylglycerol; EAR, enoyl-ACP reductase; ER, endoplasmic reticulum; FATB, fatty acyl-ACP thioesterase B; FAX, fatty acid exporter; GPAT, glycerol-3-phosphate acyltransferase; Gro, glycerol; HAD, hydroxyacyl-ACP dehydratase; KAR, 3-ketoacyl–acyl carrier protein reductase; KAS, 3-ketoacyl–acyl carrier protein synthase; LACS, long-chain acyl–coenzyme A synthetase; LPA, lysophosphatidic acid; LPAT, lysophosphatidic acid acyltransferase; MCMT, malonyl–coenzyme A:acyl carrier protein malonyltransferase; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; SAD, stearoyl–acyl carrier protein Δ9 desaturase.

of butyryl-ACP with malonyl-ACP is catalyzed by KASI, which is specific for 4:0-ACP up to 14:0-ACP. The *kas1* mutant shows reduced fatty acid contents, semidwarfism, and disrupted embryo development (148). During each elongation cycle, 3-ketoacyl-ACP is converted into the corresponding acyl-ACP by KAR, HAD, and ENR activities. The last condensation of 16:0-ACP with malonyl-ACP yielding 18:0-ACP is catalyzed by KASII. The *fab1* mutant carrying a mutation in *KASII* contains increased amounts of 16:0 and dies at low temperature (24, 149). A large fraction of 18:0-ACP is readily converted into oleoyl-(18:1$^{\Delta 9 cis}$)ACP by stearoyl-ACP Δ9-desaturase (SAD) (129, 136). *Arabidopsis* contains seven *SAD* genes, but the FAB2 protein contributes most of the leaf SAD activity. The *fab2* (*ssi2*) mutant of *Arabidopsis* contains increased amounts of 18:0, is dwarfed when grown at 22°C, and shows an increased defense response (69, 92). A mutation of

another SAD desaturase, DES6, affects drought and hypoxia signaling in *Arabidopsis* crown galls (80). In conclusion, 16:0-ACP and 18:1$^{\Delta 9 cis}$-ACP are the main products of chloroplast fatty acid de novo synthesis.

sn: stereospecific numbering of glycerol; in natural glycerolipids, acyl groups are bound at positions *sn*1 and *sn*2, and the head group is bound at *sn*3

Prokaryotic lipid: a lipid derived from diacylglycerol produced in the chloroplast

Eukaryotic lipid: a lipid derived from diacylglycerol produced at the endoplasmic reticulum

PE: phosphatidylethanolamine

2.2. Two Sites for the Assembly of Lipid Precursors

The building blocks for chloroplast lipids are phosphatidic acid (PA) and DAG (**Figure 2**). While fatty acids are exclusively derived from chloroplast FAS, PA is produced both in chloroplasts and at the ER, depending on the plant species. PA is produced in chloroplasts via acylation of glycerol-3-phosphate by acyl-ACP:glycerol-3-phosphate acyltransferase (GPAT, ATS1) and subsequent acyl transfer onto lysophosphatidic acid (LPA) by acyl-ACP:LPA acyltransferase (LPAT, ATS2) (**Table 1**). The acyltransferases ATS1 and ATS2 are specific for 16:0-ACP and 18:1-ACP, respectively. Therefore, the main product of the chloroplast pathway is (*sn*1,*sn*2) 18:1,16:0-PA (45). Because the *sn* distribution in 18:1,16:0-PA reflects that of cyanobacteria, the chloroplast pathway of lipid synthesis was designated the prokaryotic pathway (124, 160). Leaky mutations in *ATS1* cause a loss of prokaryotic-type galactolipids. Further downregulation of ATS1 expression in the *ats1–1* mutant by RNAi resulted in reduced PG content and decreased growth (85, 154). Mutations in the second acyltransferase ATS2 are embryolethal (77, 157).

After dephosphorylation of PA by one of several plastid-localized lipid phosphate phosphatases (LPP) (72, 112, 121), the resulting DAG is employed for glycolipid synthesis (see Sections 3.1 and 3.2). Introduction of double bonds into the acyl groups of galactolipids by different desaturases (FAD5, FAD6, FAD7, and FAD8) results in the conversion of a large proportion of 16:0 and 18:1 into 16:3 and 18:2/18:3, respectively, in *Arabidopsis* (18, 20, 21, 41, 46, 63, 86, 101, 128). The *fad5* and *fad6* mutants, which contain decreased amounts of 16:3 and 16:3/18:3, respectively, show strong growth reduction and leaf chlorosis at 6°C but not at 22°C. Galactolipids with a glycerol backbone of 18:3,16:0, 18:3,16:1, or 18:3,16:3 (*sn*1,*sn*2) are the characteristic products of the prokaryotic pathway. A large number of plant species containing the prokaryotic pathway—including *Arabidopsis*—were designated 16:3 plants (104).

A certain proportion of acyl-ACPs is not used for prokaryotic lipid synthesis but is hydrolyzed by the acyl-ACP thioesterases FATA (specific for 18:1-ACP) or FATB (specific for saturated acyl-ACPs, particularly 16:0-ACP), which release free fatty acids (33). A mutation in *fatB* causes reduced growth and deficiency in 16:0, particularly in extraplastidial lipids (16). *Arabidopsis* contains four additional plastidial acyl lipid thioesterases (ALTs) specific for medium chain, saturated and 3-keto acyl-ACPs. The ALT sequences are different from FATA and FATB, and the corresponding enzymes were suggested to be involved in secondary metabolite production (123). The FAX1 protein, which localizes to the inner envelope, was suggested to be involved in fatty acid export from the chloroplast (89). This hypothesis was based on findings that in the *Arabidopsis fax1* mutant, some prokaryotic lipid species are increased, whereas eukaryotic lipids are decreased, and that α-linolenic import into the yeast fatty acid transport mutant *fat1* was restored by introducing *Arabidopsis FAX1* cDNA (89). Next, fatty acids are converted into acyl-CoAs by one of the nine acyl-CoA synthetases (131). At the ER, LPA is produced by the ER-localized GPAT (50). PA synthesis at the ER is catalyzed by LPAT2. Mutations in LPAT2 are lethal for female but not for male gametogenesis (78). Because of different substrate specificities of GPAT and LPAT2, 16:0,18:1-PA and 18:1,18:1-PA are the major products of the ER-localized lipid synthesis pathway. DAG is released from ER-localized PA by PA phosphatases (PAH1, PAH2) (38, 111). This pathway was termed the eukaryotic pathway because of its association with the ER, which originates from the eukaryotic host cell. DAG is the precursor for PC and phosphatidylethanolamine (PE) synthesis at the ER (see Section 4.1). 18:1 bound to PC is further desaturated to 18:2 and

18:3 by the desaturases FAD2 and FAD3 (5, 19, 120). Thus, 16:0,18:3-PC and 18:3,18:3-PC, i.e., lipids carrying 18:3 at *sn*2, are major products of the eukaryotic pathway. Plant species (e.g., pea) that lost the prokaryotic pathway during evolution are devoid of 16:0 and 16:3 in the *sn*2 position of galactolipids (124). These plants entirely depend on the synthesis of their chloroplast lipids via the eukaryotic pathway and are characterized by the accumulation of galactolipids carrying 18:3 at the *sn*2 position. Numerous species termed 18:3 plants depend exclusively on the eukaryotic pathway (104).

Trigalactosyldiacylglycerol (TGDG): an oligogalactolipid with all galactoses in β-anomeric configuration

TGD complex: a protein complex of up to five subunits mediating transport of lipid precursors from the endoplasmic reticulum to chloroplasts

UDP-Gal: uridine diphosphate–galactose

2.3. Import of Lipid Precursors into the Chloroplast

DAG employed for galactolipid and sulfolipid synthesis is derived from the eukaryotic pathway or from the eukaryotic and prokaryotic pathways, depending on the plant species. The nature of the eukaryotic precursor imported into the chloroplast is still unclear. The outer leaflet of the outer envelope contains PC, but chloroplasts lack cytidine-diphosphate (CDP)-choline:DAG phosphocholine transferase activity. Therefore, import of PC or a PC metabolite into the chloroplast is required. It was suggested that lyso-PC is imported into chloroplasts (105), and a plastidial lyso-PC acyltransferase could be involved in the reacylation of lyso-PC (14). Acyl-CoA required for the reacylation could be derived from the long chain acyl-CoA synthetases LACS9 (outer envelope) or LACS4 (ER) (66). In agreement with this scenario, the *lacs9 lacs4* double mutant contains reduced proportions of eukaryotic chloroplast lipids.

Chloroplast envelope membranes harbor a multisubunit lipid import complex termed TGD for trigalactosyldiacylglycerol (TGDG), because corresponding *tgd* mutants accumulate the oligogalactolipid TGDG (see Section 5.4). In addition, the *tgd* mutants accumulate triacylglycerol (TAG) and PA. Five proteins (TGD1, TGD2, TGD3, TGD4, and TGD5) are involved in ER-to-chloroplast lipid transfer (10, 42, 99, 150, 151). TGD1, TGD2, and TGD3 form a bacterial-type ABC (adenosine triphosphate (ATP) binding cassette) lipid transport complex in the inner envelope. TGD1 is a permease-like protein, TGD3 represents a small ATPase, and TGD2 is a PA-binding protein. TGD2 establishes a lipid transport conduit between the two envelopes. TGD4 localizes to the outer envelope and is associated with ER membranes where it presumably mediates PA import to the outer envelope (150). The glycine-rich TGD5 protein in the envelope membranes physically interacts with TGD1, TGD2, TGD3, and TGD4. TGD5 was suggested to facilitate lipid transfer across the aqueous intermembrane space by bridging TGD4 with TGD1, TGD2, and TGD3, to form a lipid transport super complex (42). The nature of the lipid transferred by the TGD complex still remains unclear, but PA was suggested to be the most likely candidate.

Physical contact sites between the ER and the outer chloroplast membrane were previously observed (1). Such contact sites were suggested to be involved in lipid import into the chloroplast (presumably via the TGD complex) and in the export of acyl groups from the chloroplast to the ER (probably by FAX1). Corroborating evidence for the existence of ER-to-outer-envelope contact sites was obtained by demonstrating that ER-localized enzymes have access to lipid substrates in the chloroplast (102). It remains to be shown whether these sites include membrane-to-membrane associations or even membrane hemifusions.

3. GLYCOLIPID SYNTHESIS IN CHLOROPLASTS

3.1. Galactolipid Synthesis

MGDG synthases catalyze the first step of galactolipid synthesis by transferring one galactose from uridine diphosphate (UDP)-galactose (UDP-Gal) onto DAG (**Figure 3**) (37). MGDG

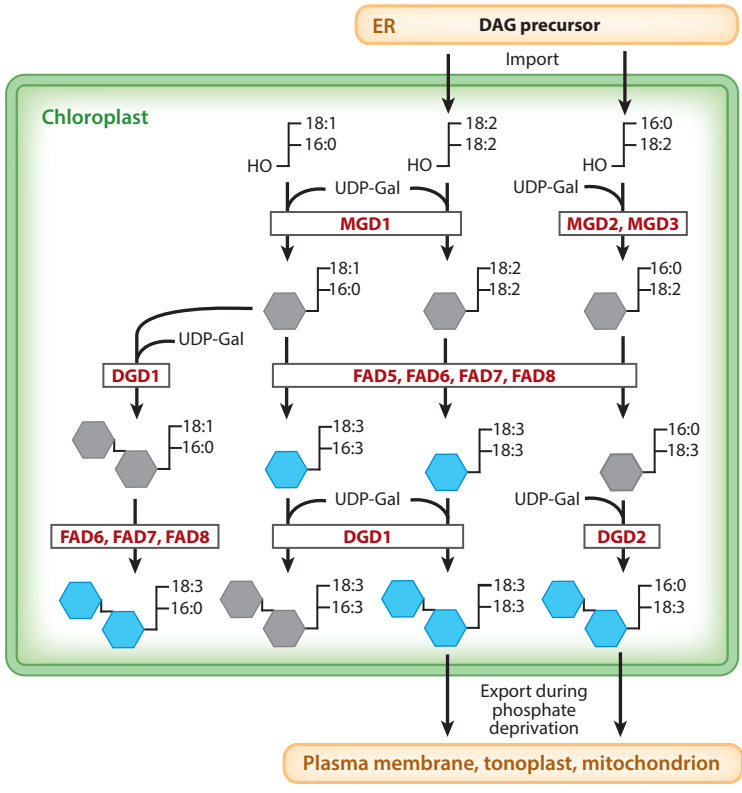


Figure 3

Galactolipid synthesis. MGDG is produced in *Arabidopsis* by MGD1 via transfer of a galactose from UDP-galactose onto DAG derived from the prokaryotic/plastidial (DAG-18:1,16:0) or eukaryotic/ER (DAG-18:2,18:2) pools. DGD1 converts MGDG and UDP-galactose into DGDG. Different plastidial desaturases (FAD5, FAD6, FAD7, and FAD8) introduce double bonds in MGDG and DGDG (22). The predominant MGDG and DGDG species are shown with blue hexagons. Minor galactolipids are shown with gray hexagons (75). A second pathway involving MGD2/MGD3 and DGD2 is mainly activated under phosphate deprivation. DGDG produced by DGD2 and to some extent by DGD1 is exported under phosphate deprivation to extraplastidial membranes. Enzyme names are indicated in red. Abbreviations: DAG, diacylglycerol; DGD, digalactosyldiacylglycerol synthase; DGDG, digalactosyldiacylglycerol; ER, endoplasmic reticulum; FAD, fatty acid desaturase; Gal, galactose; MGD, monogalactosyldiacylglycerol synthase; MGDG, monogalactosyldiacylglycerol; UDP, uridine diphosphate.

synthases are members of the glycosyltransferase family 28 (GT28) as outlined in the CAZy database (**http://www.cazy.org**) (98). GT28 glycosyltransferases produce β-anomeric linkages. Two MGDG synthase classes are found in plants, types A and B (7, 130). MGD1 from *Arabidopsis*, a type A enzyme, localizes to the inner envelope, whereas MGD2 and MGD3, type B enzymes, localize to the outer envelope. MGD1 is highly expressed in green tissues, whereas expression of MGD2 and MGD3 is observed only in specific organs; the latter two proteins are highly expressed in leaves under phosphate deprivation (7, 81) (see Section 5.3). Expression of MGD1 (but neither MGD2 nor MGD3) is upregulated by light and cytokinin resulting in rapid accumulation of galactolipids (155). In addition, MGD1 is activated in a redox-dependent manner probably by reduced thioredoxin (155). Redox modification by thioredoxin transmits information about the redox-state of the photosynthetic apparatus and thus regulates photosynthesis-related enzymes.

Furthermore, MGD1 activity is stimulated by the acidic phospholipids PA and PG, which act synergistically. PA, which is presumably bound in an allosteric manner, shows the stronger effect (37).

UDP-Glc: uridine diphosphate–glucose

The second step of galactolipid synthesis is catalyzed by DGDG synthases, which transfer a galactose from UDP-Gal onto MGDG (**Figure 3**) (75). DGDG synthases belong to the family GT4 of retaining glycosyltransferases as indicated by the CAZy database (98). These enzymes form α-linkages of the terminal galactose leading to the unique head group structure of DGDG. *Arabidopsis* contains two DGDG synthases, DGD1 and DGD2, differing in the presence of a long N-terminal extension in DGD1 (N-DGD1). This extension contains the information for targeting to the outer envelope, and it is involved in lipid transfer between the two envelope membranes (see Section 5.1) (76). The glycosyltransferase activity is found in the C-terminal part of DGD1 (74). DGD1 is responsible for the bulk synthesis of DGDG in *Arabidopsis* because its disruption in the *dgd1* mutant leads to a 90% reduction in the amount of DGDG (32). The *dgd1* mutant shows dwarfed growth, reduced photosynthetic activity with altered chloroplast ultrastructure, and grana-free stroma areas. DGD2 also localizes to the outer envelope, and its expression is highly upregulated under phosphate deprivation (53) (see Section 5.3). Its disruption in the *dgd2* mutant has no further consequences for the DGDG content, photosynthesis, or growth under normal conditions (75). The *dgd1 dgd2* double mutant is completely devoid of DGDG, which leads to infertility, further impairment in growth, and decreased photosynthetic activity.

According the endosymbiont theory, chloroplasts are derived from a cyanobacterial ancestor, which was engulfed by a eukaryotic progenitor cell. Chloroplasts and cyanobacteria differ in their pathways of galactolipid biosynthesis. Recent cyanobacteria, including *Synechocystis*, first synthesize monoglucosyldiacylglycerol (MGlcDG) by MgdA, which is converted into MGDG by the epimerase MgdE (6, 8). DGDG in *Synechocystis* is synthesized by DgdA via galactosylation of MGDG (9). MgdA and DgdA are only distantly related to the plant MGDG synthases and DGD1. Homologs of plant MGD1 were identified in the filamentous *Chloroflexus* and *Roseiflexus* bacteria (62). It was proposed that eukaryotic MGDG synthases were obtained by horizontal gene transfer from an ancient member of the Chloroflexi in parallel with the integration of the cyanobacterial endosymbiont. The divergence of types A and B MGDG synthases occurred approximately 323 million years ago in the common ancestor of Spermatophyta. DGD1-like and DGD2-like sequences are present in all plants and most eukaryotic algae, but their evolutionary origin remains enigmatic (76).

3.2. Sulfolipid and Glucuronosyllipid Synthesis

SQDG biosynthesis is localized to the chloroplast and comprises three enzymatic steps (**Figure 4**). UDP-glucose (UDP-Glc) is produced from uridine triphosphate (UTP) and glucose-1-phosphate by the unique stroma-localized UDP-Glc pyrophosphorylase UGP3 (119). UDP-Glc derived from the cytosol cannot be used for SQDG synthesis because *Arabidopsis ugp3* mutants are SQDG deficient. This result appears in contrast to the galactolipid pathway, which depends on UDP-Gal derived from the cytosol (14).

UDP-sulfoquinovose synthase (SQD1/SqdB) also localizes to the stroma and converts UDP-Glc and sulfite into UDP-sulfoquinovose (40, 108). SQD1 (plants, algae) and SqdB (cyanobacteria, bacteria, archaea) sequences are highly conserved and show sequence similarity with and are structurally related to sugar nucleotide epimerases and hydratases (13). The addition of the sulfite to the C6 carbon of the glucose presumably includes an unsaturated UDP-4-ketoglucose-5-ene intermediate (108). The transfer of sulfoquinovose to DAG (SQDG synthesis) is catalyzed by SQD2 in plants and eukaryotic algae. SQD2 is a glycosyltransferase of the GT4 family specific

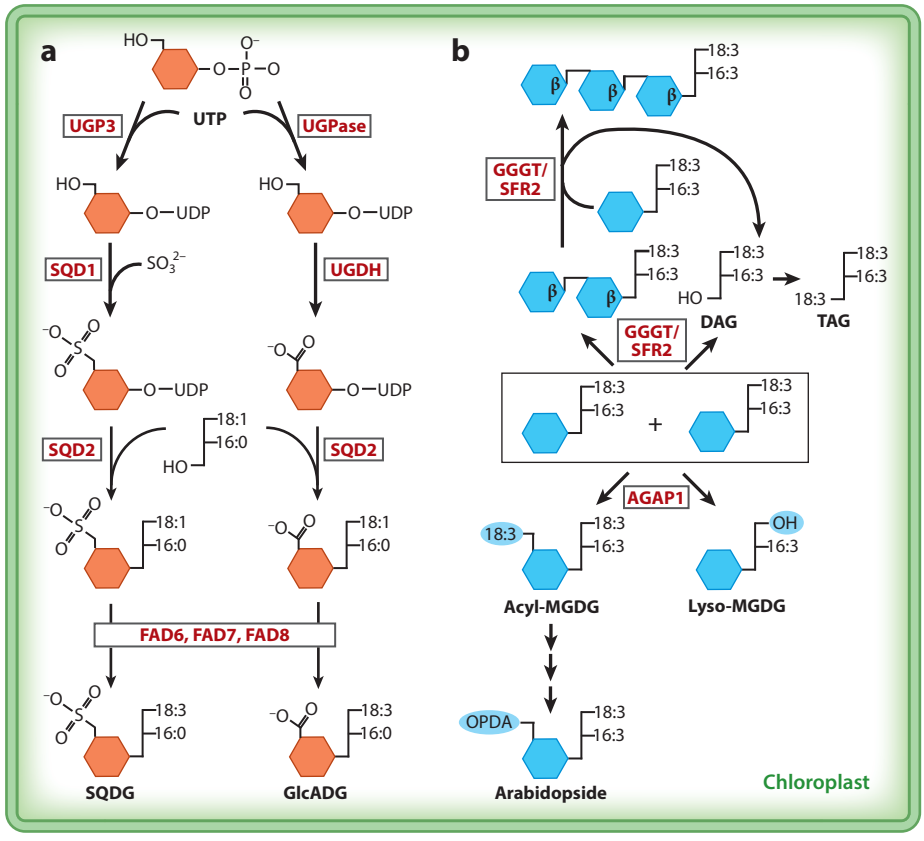


Figure 4

Sulfolipid, glucuronosyllipid, oligogalactolipid, and acyl-galactolipid synthesis. (*a*) Glucose-1-phosphate is converted into UDP-glucose by a chloroplast UGPase (UGP3). Sulfite is attached to the C6 carbon of glucose by SQD1, and sulfoquinovose is transferred to DAG by SQD2. In the other pathway, the C6 of UDP-glucose (produced by an unknown UGPase different from UGP3) is oxidized to UDP-glucuronic acid by an unknown UGDH. Glucuronic acid is transferred to DAG by SQD2, resulting in the production of GlcADG. 18:1 groups in SQDG and GlcADG are desaturated by FAD6, FAD7, and FAD8. DAG for SQDG synthesis can be derived from the prokaryotic pathway (as shown) or the eukaryotic pathway (16:0/18:2,18:2). Orange hexagons represent glucose-derived residues. (*b*) A galactose is transferred from one MGDG to another in a head group–disproportioning reaction catalyzed by GGGT/SRF2 giving rise to β,β-DGDG and DAG. Subsequent galactose transfer from another MGDG leads to the formation of β,β,β-TGDG. DAG is converted into TAG by an unknown acyltransferase. Acyl transfer via a disproportioning reaction between two MGDG molecules is catalyzed by AGAP1 to form acyl-MGDG. In *Arabidopsis*, the acyl group of acyl-MGDG can be oxidized to OPDA, giving rise to an arabidopside. Blue hexagons indicate galactose. Enzyme names are indicated in red. Abbreviations: AGAP, acylated galactolipid associated phospholipase; DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; FAD, fatty acid desaturase; GGGT, galactolipid:galactolipid galactosyltransferase; GlcADG, glucuronosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; OPDA, 12-oxophytodienoic acid; SFR, sensitive to freezing; SQD1, UDP-sulfoquinovose synthase; SQD2, sulfoquinovosyldiacylglycerol synthase; SQDG, sulfoquinovosyldiacylglycerol; TAG, triacylglycerol; TGDG, trigalactosyldiacylglycerol; UDGH, uridine diphosphate–glucose dehydrogenase; UDP, uridine diphosphate; UGP, uridine diphosphate–glucose pyrophosphorylase; UTP, uridine triphosphate.

for α-glycosidic linkages and is inserted in the inner envelope (158). SQDG synthesis is mediated by SqdX in cyanobacteria and by SqdD in bacteria. SQD2 and SqdX show high sequence similarity and can be traced back to a common ancestor, whereas the bacterial SqdD shows only low sequence similarity to SQD2/SqdX and therefore might have evolved independently (13, 158). UGP3, which produces UDP-Glc, belongs to the type B UDP-Glc pyrophosphorylases, conserved in higher plants, with homologs in unicellular green algae (119).

Recently, GlcADG, another anionic glycolipid containing glucuronic acid, was detected in low amounts in different plants (118). GlcADG is also synthesized by SQD2 in line with its α-anomeric head group, but it accumulates only under phosphate deprivation (**Figure 4**). The sugar donor for GlcADG synthesis is probably UDP-glucuronic acid derived from an unknown UDP-Glc dehydrogenase (UGDH). *Arabidopsis ugp3* mutants still accumulate GlcADG. Therefore, UDP-Glc for UDP-glucuronic acid production cannot be derived from plastidial UGP3.

4. PHOSPHOLIPID SYNTHESIS

4.1. Phosphatidylcholine Synthesis

PC is synthesized at the ER following the head group activation pathway (**Figure 5**). Choline is converted into phosphocholine by choline ethanolamine kinase (CEK). A mutation in *cek4* causes embryolethality, indicating that CEK4 is the most important isoform (94). Alternatively, phosphocholine can be produced by three methylation reactions starting with phosphoethanolamine. *Arabidopsis* harbors three genes involved in the conversion of phosphoethanolamine to phosphocholine. Deletion of the phosphoethanolamine methyltransferase XIPOTL1 in the *xpl1* mutant causes alterations in root morphology, while deletion of phosphomethylethanolamine methyltransferase (*PMEAMT*) has only minor effects on plant growth (12, 28). Phosphocholine is converted into CDP-choline, catalyzed by cytidine triphosphate (CTP):phosphocholine cytidylyltransferase (CCT). *Arabidopsis* contains two *CCT* genes whose expression is differentially regulated at low temperature. Lipid contents in leaves of *cct1* and *cct2* single mutants are similar to lipid amounts in the wild type (64). CCT1 activity is stimulated by PA derived from the PA phosphatases PAH1 and PAH2, thereby causing an increased net PC synthesis (27). The activated phosphocholine head group is transferred to DAG by CDP-aminoalcohol:DAG phosphoaminoalcohol transferases (AAPTs). *Arabidopsis* contains two AAPT enzymes employing both CDP-ethanolamine and CDP-choline as substrates. Single mutants of *aapt1* and *aapt2* are viable, but the *aapt1 aapt2* double mutant is embryolethal, emphasizing the importance of PC and PE synthesis for cellular metabolism (97).

4.2. Phosphatidylglycerol Synthesis

PG is synthesized by the CDP-DAG (activated lipid backbone) pathway in the mitochondrion, ER, and chloroplast (**Figure 5**). Activation is achieved via the transfer of cytidine monophosphate (CMP) from CTP onto PA, resulting in the formation of CDP-DAG [CDP-DAG synthase (CDS)/CTP:PA cytidylyltransferase]. *Arabidopsis* contains two CDS proteins (CDS4, CDS5) targeted to the chloroplast. While single mutants grow like the wild type, *cds4 cds5* double mutant plants contain reduced amounts of plastidial PG (54). Next, PA is transferred from CDP-DAG onto glycerol-3-phosphate by PG-phosphate synthase (PGP). *Arabidopsis* harbors two PGPs. PGP1 is dually targeted to the mitochondrion and chloroplast, and it gives rise to the lone PGP activity in the chloroplasts. Thus, mutations in the *PGP1* gene (*JOVTENKY*) affect plastidial PG synthesis and thylakoid biogenesis, resulting in pale leaves (11, 51, 152). Finally, phosphate is hydrolyzed from PG-phosphate by PG-phosphate phosphatase (PGPP). The *Arabidopsis* PGPP1

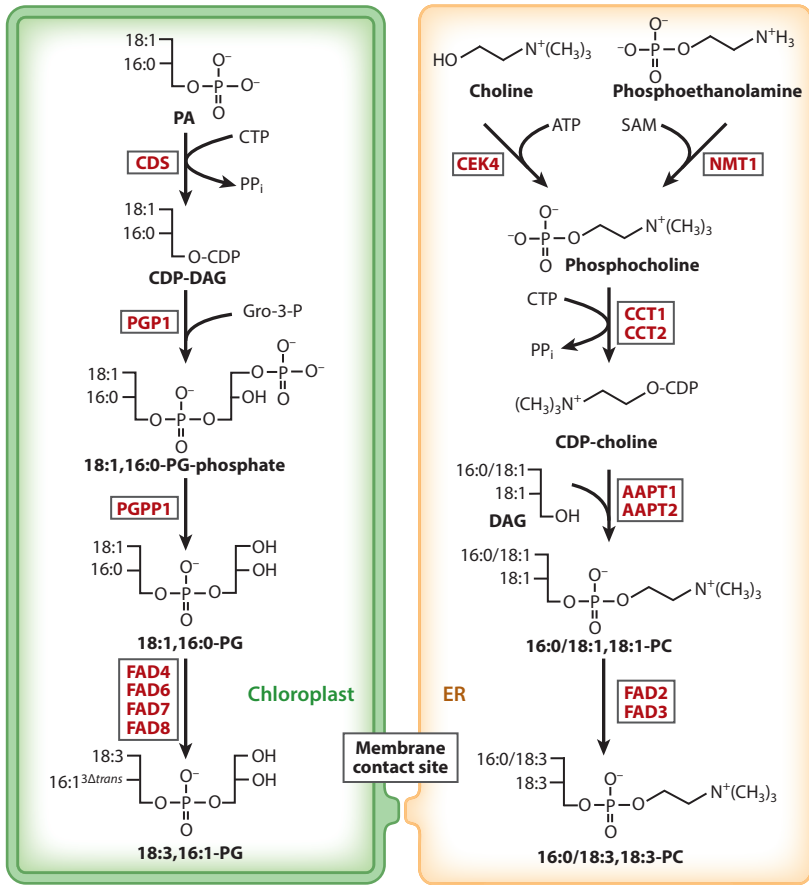


Figure 5

PC and PG synthesis. PG is synthesized in chloroplasts following the CDP-DAG pathway. CDP-DAG is produced from PA and CTP by CDS. DAG is transferred onto glycerol-3-phosphate giving rise to PG after dephosphorylation. The Δ3*trans* double bond is introduced into *sn*2–16:0-PG by FAD4. FAD6, FAD7, and FAD8 convert 18:1 in PG into 18:2 and 18:3. PC is synthesized at the ER from DAG and the activated head group, CDP-choline, by AAPT. CDP-choline is derived from phosphocholine and CTP via the CCT reaction. Phosphocholine can be produced directly by phosphorylation of choline or by successive methylation of phosphoethanolamine. Double bonds are introduced into 18:1 of PC by FAD2 and FAD3. PC, lyso-PC, or PA might be transferred from the ER to the outer chloroplast envelope presumably via contact sites. Abbreviations: AAPT, cytidine diphosphate–aminoalcohol:diacylglycerol phosphoaminoalcohol transferase; CCT, cytidine triphosphate:phosphocholine cytidylyltransferase; CDP, cytidine diphosphate; CDS, cytidine diphosphate–diacylglycerol synthase; CEK, choline ethanolamine kinase; CTP, cytidine triphosphate; DAG, diacylglycerol; ER, endoplasmic reticulum; FAD, fatty acid desaturase; NMT, phosphoethanolamine *N*-methyltransferase; PA, phosphatidic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; PGP, phosphatidylglycerol-phosphate synthase; PGPP, phosphatidylglycerol–phosphate phosphatase; PP_i, diphosphate; SAM, *S*-adenosyl-methionine.

protein localizes to the chloroplast and is crucial for plastidial PG synthesis, as shown by the analysis of the corresponding *pgpp1* mutant (93, 161). In *Arabidopsis*, PG is mostly derived from the prokaryotic pathway. While 18:1 at *sn1* is converted into 18:2 and 18:3 by the plastidial desaturases FAD6, FAD7, and FAD8, a Δ3*trans* double bond is specifically introduced into *sn*2–16:0 of PG by FAD4 (20, 46).

5. FUNCTIONS OF CHLOROPLAST LIPIDS

5.1. Establishment of Chloroplast Subcompartments

Bilayer-forming lipids: membrane lipids with large head groups that form bilayers due to their cylindrical shape, e.g., digalactosyldiacylglycerol, phosphatidylcholine, and phosphatidylglycerol

Nonbilayer-forming lipids: membrane lipids with small head groups that form inverted micelles due to their cone-like shape, e.g., monogalactosyldiacylglycerol and phosphatidylethanolamine

Lipids establish the membranes for compartmentalization of the chloroplast, and they provide the matrix for embedding the photosynthetic protein complexes. The characteristics of the membranes are determined by the distribution of the proteins and lipids and their physicochemical characteristics. The regulation and the maintenance of a critical ratio of bilayer-forming lipids to nonbilayer-forming lipids is a prerequisite for functional membranes. The nonbilayer-forming lipid MGDG with its small head group and its highly desaturated fatty acids exhibits a cone-like shape and forms an inverted hexagonal (H_{II}) phase (inverted micelles or tubular structures) when dispersed in aqueous solutions, while the other thylakoid lipids DGDG, SQDG, and PG exhibit a cylindrical shape and form lamellar L_α phases (bilayers) (146). Lipid mixtures containing MGDG and DGDG in a ratio of 1:1 form H_{II} phases in vitro. A lamellar (bilayer) organization can only be observed with lower MGDG contents or after addition of proteins. This explains why thylakoid membranes, which contain approximately 50% MGDG but are highly packed with proteins (approximately 70%), can establish stable bilayers (79).

Thylakoid grana are characterized by their high curvature at the margins. It was suggested that the nonbilayer-forming properties of MGDG stabilize these curved structures. However, later the CURVATURE THYLAKOID 1 proteins (CURT1A, CURT1B, CURT1C, and CURT1D) were identified as the major factors establishing grana curvature (4) (**Figure 6**). The CURT1 proteins are highly enriched in the marginal regions of grana and stabilize membrane curvature, as demonstrated by analyzing *curt1* mutants, which contain lobe-like thylakoids and fewer margins; CURT1 overexpressing lines show slimmer but taller grana with more layers and margins. A further role for the establishment of the 3D thylakoid structure was shown for DGDG because it contributes to membrane stacking via hydrogen bonds between the polar head groups of adjacent bilayers (71).

The biogenesis of thylakoid membranes starts with small vesicles and lamellae in the proplastids and results in the establishment of the complex 3D structures in the mature chloroplasts. MGDG and DGDG, which are synthesized in the inner and outer envelopes, respectively, are distributed to the thylakoids by involving transport processes across the two envelope membranes and to the growing thylakoids. The N-terminal domain of DGD1 (N-DGD1) mediates the access of DGD1 (outer envelope) to MGDG produced in the inner envelope for galactosylation and the translocation of DGDG from the outer envelope to the inner envelope probably via membrane associations in the presence of PA (76) (**Figure 6**). Different scenarios have been proposed for the translocation of lipids from the inner envelope to the thylakoids. During early thylakoid development, stromal invaginations are formed and subsequently fused leading to the establishment of thylakoid lamellae, which are finally separated from the inner envelope (83). Alternatively, lipids might be transported by vesicles mediated by the inner membrane protein of 30 kilodaltons/vesicle-inducing protein in plastids 1 (IM30/VIPP1) (84). But a lack of evidence has limited further support of the vesicle-inducing function of VIPP1 or the existence of a vesicle-trafficking system between the inner envelope and thylakoid membranes (55). IM30/VIPP1 forms highly ordered oligomeric ring structures. Loss of IM30/VIPP1 in *Arabidopsis* mutants affects thylakoid architecture. A recent study suggests that the establishment of contact zones between thylakoids and the inner envelope is mediated by the oligomeric IM30/VIPP1 ring structure in the presence of Mg^{2+} (58) (**Figure 6**). The IM30/VIPP1 protein association leads to the enrichment of MGDG at the contact zones followed by destabilization and fusion of the two membranes and release of the protein (55).

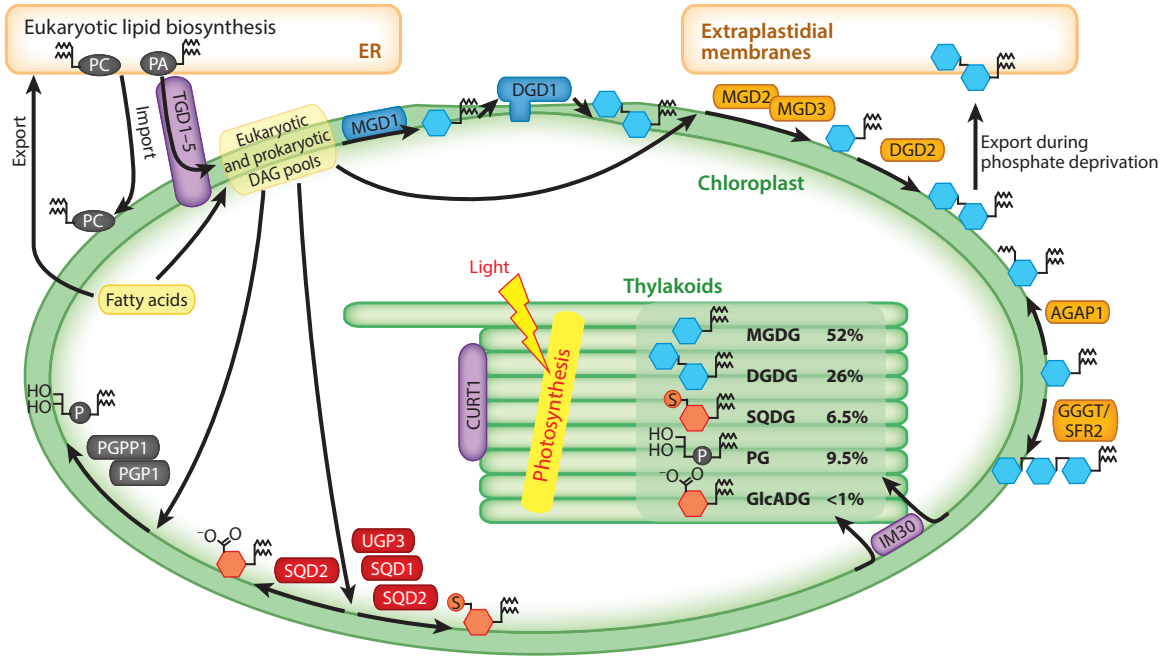


Figure 6

Glycerolipid synthesis and transport in chloroplasts. Fatty acids are employed for glycerolipid synthesis in the chloroplast (prokaryotic pathway) or, after export, are used for eukaryotic lipid synthesis at the ER. Eukaryotic lipid precursors (presumably PA) are reimported into the chloroplast through the TGD transport complex. PC or lyso-PC might serve as alternative lipids transported from the ER to the chloroplast. MGDG and DGDG are produced through the MGD1/DGD1 pathway, with galactolipid shuffling between the inner and outer membranes mediated by the N-terminal domain of DGD1. DGDG derived from the MGD2/MGD3/DGD2 pathway is exported to extraplastidial membranes. PG, SQDG, and GlcADG are produced in the envelope membranes. Thylakoids are derived from the inner envelope by lipid transport mediated by IM30/VIPP1. The characteristic lipid composition of thylakoids is essential for optimal efficiency of photosynthesis. CURT1 proteins at the thylakoid margins stabilize highly curved membrane regions. Under stress, MGDG in the outer envelope is converted into acyl-MGDG and oligogalactolipids. Blue hexagons indicate galactose and orange hexagons depict glucose-derived residues. Also shown are galactolipid synthesis (*blue text boxes*), stress-induced galactolipid synthesis (*orange text boxes*), lipid transport or membrane curvature (*purple text boxes*), sulfolipid and GlcADG synthesis (*red text boxes*), and phospholipid synthesis (*gray text boxes*). Abbreviations: AGAP, acylated galactolipid associated phospholipase; CURT, curvature thylakoid; DAG, diacylglycerol; DGD, digalactosyldiacylglycerol synthase; DGDG, digalactosyldiacylglycerol; ER, endoplasmic reticulum; GGGT, galactolipid:galactolipid galactosyltransferase; GlcADG, glucuronosyldiacylglycerol; IM30, inner membrane protein of 30 kilodaltons; MGD, monogalactosyldiacylglycerol synthase; MGDG, monogalactosyldiacylglycerol; P, phosphate group; PA, phosphatidic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; PGP, phosphatidylglycerol-phosphate synthase; PGPP, phosphatidylglycerol–phosphate phosphatase; SFR, sensitive to freezing; SQD1, UDP-sulfoquinose synthase; SQD2, sulfoquinovosyldiacylglycerol synthase; SQDG, sulfoquinovosyldiacylglycerol; TGD, trigalactosyldiacylglycerol related; UGP, uridine diphosphate–glucose pyrophosphorylase; VIPP, vesicle inducing protein in plastids.

Further lipid structures of the chloroplasts are the plastoglobules and stromules. Plastoglobules are lipid droplets established at thylakoid regions with high curvature by blistering out from the outer leaflet of the thylakoid membrane (140). Plastoglobules are surrounded by a lipid monolayer, which is physically connected to the stroma leaflet of the thylakoid membrane. They can be found in high number in etioplasts and in plastids of senescent leaves, and they accumulate during stress. Plastoglobules serve as chloroplast subcompartments for lipid biosynthesis and storage (see Section 5.3). Stromules are tubular extensions of the inner and outer chloroplast envelopes into the cytosol. They are filled with stroma but devoid of thylakoids, and they increase in number during stress (52). Stromules associate with extraplastidial membranes, including the plasma

membrane, mitochondria, and ER, and they therefore could be involved in lipid exchange between organelles. Furthermore, stromule numbers are increased during phosphate deprivation in a strigolactone-dependent manner, suggesting that they could also be required for DGDG export from the plastids (141).

5.2. Role of Lipids in Photosynthesis

The central function of the thylakoid membrane is to enable the generation of an electrochemical potential difference and a proton gradient for ATP synthesis. The characteristic lipid composition of thylakoids is highly conserved in photosynthetic organisms. The four lipids MGDG, DGDG, SQDG, and PG establish the matrix for embedding the protein complexes of the photosynthetic electron transport chain and the mobile electron carriers plastoquinone and plastocyanine. The proteins account for approximately 70% of the membrane area; the remaining 30% consists of lipids, which form shells around and contribute to the flexibility of the protein complexes (79). In addition to their roles as membrane building blocks, thylakoid lipids provide specific functions as integral components of the photosynthetic complexes (82). DGDG and PG are indispensable for the trimerization and stability of light-harvesting complex II (LHCII) complexes. DGDG also mediates the contact between adjacent trimers presumably for dissipation of excess excitation energy. All four chloroplast lipids were detected as integral components in PSII from cyanobacteria and plants. It was suggested that the lipids contribute to the local mobility of the D1 protein during repair after photodamage. MGDG and PG were found close to the cyanobacterial PSI core polypeptides. The different electron transfer rates through branches A and B were explained by associations with PG or MGDG, respectively. Chloroplast lipids are also important for cytochrome $b_{6/f}$ and ATP synthase activities. Analysis of the different mutants (see Sections 3.1 and 3.2) demonstrated the importance of chloroplast lipids for photosynthesis. However, the complete loss of a membrane lipid such as MGDG or DGDG does not always allow for inference of its specific functions for photosynthesis. To address this problem, a bacterial glucosyltransferase involved in glucosylgalactosyldiacylglycerol synthesis was introduced into the *Arabidopsis dgd1* mutant. Growth of transformed plants was complemented, whereas photosynthetic activity remained compromised, indicating that the specific head group of DGDG is crucial for optimal photosynthesis (61).

The xanthophyll cycle describes the conversion of violaxanthin via the intermediate antheraxanthin into zeaxanthin by violaxanthin de-epoxidase (VDE) under high light. Under low light, the reverse reaction is catalyzed by zeaxanthin epoxidase. The xanthophyll cycle contributes to the dissipation of excess excitation energy (nonphotochemical quenching) and thereby protects the photosynthetic complexes against photodamage. VDE localizes to the thylakoid lumen and is regulated by lumen pH and by binding to MGDG. The acidification of the lumen by photosynthetic activity under high light activates the enzyme and stimulates its binding to MGDG-enriched domains. VDE activation requires binding to nonbilayer lipid-rich domains, which form H_{II} phases. In chloroplasts, H_{II} phases can be established by MGDG, but in vitro VDE can also be stimulated by binding to PE (87). In line with this scenario, MGDG deficiency in the *Arabidopsis mgd1–1* mutant leads to impaired operation of the xanthophyll cycle (65).

5.3. Lipid Changes During Phosphate Deprivation

Nitrogen, sulfur, and phosphate are macronutrients with a strong impact on the growth of plants and algae. The proportion of nitrogen in leaves bound to the N-containing phospholipids (PE and PC) is rather low compared to protein-bound nitrogen. Thus, nitrogen deprivation does not result in a decrease in PE or PC, but, instead, the plants undergo chlorosis and senescence (47).

A large amount of sulfur in the leaves is bound to cysteine and methionine in proteins, whereas SQDG contains only minor amounts of sulfur. In line with this finding, there is no evidence for the degradation of sulfolipid for sulfur mobilization in plants. In contrast, SQDG does serve as a sulfur source during the early adaptation to sulfur starvation in *Chlamydomonas* (133).

Phosphate is an essential mineral indispensable for plants and microorganisms. Hence, plants and microorganisms developed adaptation mechanisms to cope with phosphate limitation by increasing the uptake of or reducing the demand for phosphate. While the inorganic phosphate in a leaf mesophyll cell strongly varies, about one third of the organic phosphate is bound respectively to nucleic acids, esters (e.g., sugar phosphates), and phospholipids (122). Therefore, the regulation of phospholipid accumulation represents a target for reducing phosphate demand. The most significant lipid change during phosphate deprivation is the replacement of phospholipids (PC, PE, and PG) with phosphorus-free glycolipids (DGDG, SQDG, and GlcADG). Membrane lipid remodeling during phosphate starvation involves the induction of genes encoding enzymes for galactolipid and sulfolipid synthesis and degradation of phospholipids.

When plants are grown on low-phosphate medium, DGDG accumulates and is exported from the plastid to replace extraplastidial phospholipids (53). During phosphate deprivation, expression of the genes of MGD2 and MGD3 (81) and DGD1 and DGD2 is induced (74, 75). MGD2, MGD3, and DGD2 form a DGDG synthesis module in the outer envelope, which is upregulated under phosphate deprivation and produces DGDG exported to extraplastidial membranes. MGDG is not directly involved in the phospholipid exchange during phosphate deprivation, and its proportion is not increased, but it has an important role as substrate for DGDG synthesis. Auxin is required for the induction of MGD2 and MGD3 expression, and it is also responsible for increased root growth during phosphate starvation that requires the synthesis of additional amounts of DGDG for membrane proliferation. The expanded root surface area is a prerequisite for increased phosphate uptake. MGD3 is the main isoform involved in the phosphate-deprivation response. DGD2-derived DGDG shows a very peculiar molecular species composition (16:0,18:3-DGDG), different from DGD1-dependent DGDG (mostly 18:3,18:3-DGDG). DGD1 is also upregulated, and it contributes to DGDG accumulation under phosphate deprivation. Large amounts of DGDG (up to 25% of membrane glycerolipids) accumulate in the plasma membrane and tonoplast in root cells of phosphate-deprived oat, where DGDG replaces phospholipids, primarily PC. In addition, the proportions of glucosylceramide and sterol glucoside, two alternative phosphate-free glycolipids, are increased (2). DGDG is transported to the plasma membrane, the vacuole (2), and mitochondria, presumably via direct contact sites with chloroplasts (67).

Phospholipids are degraded by different phospholipases, and DAG is released as a precursor for glycolipid synthesis during phosphate limitation. During the early response to phosphate deprivation, a transient increase in PC was observed, accompanied by an increasing DAG pool and reduction of PE and PG (68). Expression of different phospholipases is induced under phosphate deprivation, e.g., nonspecific phospholipases C4 (NPC4) and NPC5. NPC4 is localized to the plasma membrane and is presumably involved in the hydrolysis of plasma membrane phospholipids. NPC5, a cytosolic isoform, was suggested to contribute to phospholipid degradation during phosphate deprivation (48). Alternatively, phospholipids can be hydrolyzed by phospholipase D (PLD) and the product, PA, is then further degraded by PA hydrolases to yield DAG. Expression of the two PLDs PLDζ1 and PLDζ2 is induced upon phosphate deprivation (29, 88). In the *pldζ1* and *pldζ2* single and double mutants, DGDG accumulation under phosphate deprivation is only partially affected. Therefore, the proportions of DAG and PA that are produced by the different phospholipases (NPC4, NPC5, PLDζ1, PLDζ2) under phosphate deprivation remain unclear. PA released from phospholipids by PLDζ1 or PLDζ2 can be further hydrolyzed by PAH1 and PAH2, yielding DAG (38, 111). PAH1 and PAH2 are required for normal growth and are expressed in

all tissues and developmental stages. The two enzymes are soluble with access to different membranes. Double mutants of *pah1 pah2* show increased levels of PA and PC in leaves, accompanied by a reduction of DGDG and MGDG. The PG and SQDG contents of the chloroplasts are not affected in the double mutant, suggesting that DAG produced by PAH1 and PAH2 is exclusively of eukaryotic origin.

SQDG represents a surrogate lipid for PG in the chloroplast under phosphate deprivation. The mutual exchange of PG/SQDG provides the means to adjust the ratio of these two anionic glycerolipids and to establish a certain ionic charge of the membrane (40, 127, 158). The accumulation of SQDG under phosphate deprivation is correlated with an induced expression of the genes UGP3, SQD1, and SQD2. The loss of SQDG has minor effects on growth and photosynthesis, but *sqd2* mutant plants suffer more severely from phosphate starvation than the *pgp1–1* mutant, whose PG content is decreased (156, 158). Double mutant plants of *sqd2 pgp1–1* show a strong growth retardation and defects in photosynthesis. Their response to phosphate deprivation is compromised (156).

The mechanism of the substitution of phospholipids with SQDG or DGDG is highly relevant for marine ecosystems. Cyanobacteria (e.g., *Prochlorococcus*) in phosphate-limited aquatic environments contain considerable amounts of SQDG (139). Some *Prochlorococcus* strains accumulate SQDG up to 66% of total membrane lipids, the only phospholipid is PG with 2%, and the remaining lipids are MGDG and DGDG. These cyanobacteria are often the dominating species among phytoplankton. The replacement of phospholipids with SQDG and DGDG or other glycolipids decreases the phosphorus demand and enables these organisms to compete with phospholipid-rich heterotrophic bacteria in oligotrophic environments.

Another plant lipid that accumulates under phosphate deprivation is GlcADG, which, like SQDG, is synthesized by SQD2 (118). GlcADG only accumulates during phosphate deprivation, but it is not found in plants grown under full nutrition conditions. GlcADG might serve as a surrogate for acidic phospholipids, like SQDG. It is still present in the SQDG-free *ugp3* and *sqd1* mutants but absent from *sqd2*. Therefore, *sqd2* is the only one of the three mutants that lacks both SQDG and GlcADG, which might explain why the *sqd2* mutant is more strongly affected (e.g., leaf bleaching) under phosphate deprivation than *ugd3* or *sqd1*.

5.4. Chloroplast Lipid Turnover and Adaptation to Abiotic Stress

Chloroplast lipids are subject to constant turnover and are degraded during stress or senescence. Different plastidial lipases acting on phospholipids or galactolipids were characterized in recent years. The phospholipases PLDζ1, PLDζ2, NPC4, and NPC5 of *Arabidopsis* are presumably involved in phospholipid hydrolysis during phosphate deprivation (see Section 5.3). *Arabidopsis* contains four additional NPC proteins, two of which (NPC2, NPC6) localize to the chloroplast (110, 113). NPC2 and NPC6 hydrolyze PE and PC and might be involved in DAG production for the eukaryotic pathway in the plastids. NPC activity in the chloroplast is essential for gamete development of *npc2 npc6*, and only double homozygous mutant seeds are embryolethal (113).

A plastid galactolipid degradation lipase 1 (PGD1) from *Chlamydomonas* hydrolyzes acyl groups from MGDG but not from DGDG (36). The *Chlamydomonas pgd1* mutant shows a reduced flux of acyl groups from MGDG to TAG during nitrogen deprivation, demonstrating that a large proportion of fatty acids from galactolipids are deposited in TAG during stress. The increase in TAG accompanied by the degradation of galactolipids during stress has also been described for *Arabidopsis* (47, 73).

Arabidopsis contains several lipases including the phospholipase A1 (PLA1) enzymes deficient in anther dehiscence 1 (DAD1) and dongle 1 (DGL1), which were suggested to hydrolyze chloroplast lipids accompanied by the release of 18:3 for subsequent OPDA and jasmonate production.

However, DGL1 localizes to lipid droplets but not to chloroplasts, and DAD1 and DGL1 were shown to be not essential for jasmonate production (39).

Plastid lipase 1 (PLIP1), a PG-specific plastidial PLA1 with preference for PG containing *sn2*–16:1$^{\Delta 3\,trans}$ releases 18:2 and 18:3, which are then exported to the cytosol and incorporated into PC and TAG (143). Therefore, PLIP1 contributes to fatty acid export from chloroplasts to the ER for eukaryotic lipid biosynthesis. Two additional genes of the PLIP family, *PLIP2* and *PLIP3*, are involved in releasing fatty acids—in particular 18:3—from MGDG or PG, respectively, in the chloroplast. Free 18:3 released by PLIP2 or PLIP3 can be converted into OPDA. Overexpression of PLIP2 or PLIP3 in *Arabidopsis* results in jasmonate accumulation accompanied by reduced growth. This growth retardation can be rescued by introduction of the *coi1* mutation affecting jasmonate signaling (144). PLIP2 and PLIP3 show preferences for prokaryotic MGDG and PG carrying 16:3 or 16:1$^{\Delta 3\,trans}$, respectively, at *sn2*. The scenario that galactolipids are the precursors for jasmonate production is also corroborated by the finding that jasmonate homeostasis is disturbed in the *Arabidopsis dgd1* mutant. In fact, the growth reduction of *dgd1* is based on jasmonate overproduction rather than on the lack of the lipid building block DGDG or on decreased photosynthetic activity (95). Therefore, the content of MGDG or the ratio of MGDG to DGDG might be critical for inducing release of 18:3 for OPDA and jasmonate production.

During heat stress, chloroplast membranes are stabilized by decreasing the ratio of MGDG to DGDG. This is mediated in part by stimulating DGDG synthesis as shown by the finding that the DGDG-deficient *dgd1* mutant reveals decreased thermotolerance (25). In addition, a chloroplast heat-inducible lipase (HIL1), which specifically hydrolyzes 18:3 from MGDG, is induced and contributes to MGDG degradation under high temperature (59).

Free fatty acids released from chloroplast lipids can be deposited as fatty acid phytyl esters after esterification with phytol released from chlorophyll degradation. Fatty acid phytyl esters accumulate in the plastoglobules during stress to prevent the accumulation of free fatty acids and free phytol, which are toxic due to their detergent-like characteristics (47). Two genes, phytyl ester synthase 1 (*PES1*) and *PES2*, which belong to the esterase/lipase/thioesterase (ELT) family, are responsible for fatty acid phytyl ester synthesis in *Arabidopsis* (96). In tomato, PYP1, another acyltransferase of the ELT family, is responsible for xanthophyll ester synthesis. The tomato pale yellow petals 1 (*pyp1*) mutant is deficient in the synthesis of xanthophyll esters in the petals (3). Thus, ELT acyltransferases are involved in the synthesis of different prenyl esters in chloroplasts.

In addition to chloroplast lipid degradation by lipases, MGDG in the outer envelope can be acylated or converted into oligogalactolipids, thereby adjusting the ratio of bilayer-forming lipids to nonbilayer-forming lipids. MGDG can be acylated with a third acyl group at the galactose C6 position under stress (57). The acyl-MGDG content in plants is very low, but under wounding or freezing stress, a large proportion of MGDG is converted into acyl-MGDG (114). The MGDG acyltransferase (acylated galactolipid associated phospholipase 1, AGAP1) produces acyl-MGDG by a disproportioning reaction of MGDG accompanied by the release of lyso-MGDG (57) (**Figure 4**). AGAP1 also belongs to the PLA1 family and presumably localizes to the outer envelope and cytosol (114). As the membrane packing characteristics of acyl-MGDG and lyso-MGDG differ from MGDG, induction of AGAP1 activity contributes to membrane stabilization during stress (114). In some plant families including the Brassicaceae, OPDA is bound at the C6 position of acyl-MGDG, linking acyl-MGDG synthesis with jasmonate production. Oxygenated fatty acids, e.g., OPDA, have been identified not only at the C6 position of galactose but also bound to the glycerol backbone, particularly in MGDG in *Arabidopsis* (so-called arabidopsides).

Another stress-dependent modification of MGDG includes the synthesis of oligogalactolipids with two, three (TGDG), or four galactoses in a processive manner by the galactolipid:galactolipid galactosyltransferase (GGGT), which is independent from DGD1 or DGD2 (75, 138). GGGT

employs MGDG but not UDP-Gal as a sugar donor and a second MGDG as the primary acceptor (**Figure 4**). All galactoses are bound in the β configuration. GGGT, which is a glycosylhydrolase located to the outer envelope, is also known as SENSITIVE TO FREEZING 2 (SFR2) and confers tolerance to freezing (44, 103, 137). Its activity is stimulated during stress to reduce the proportion of the nonbilayer-forming lipid MGDG in favor of bilayer-forming oligogalactolipids. At the same time, DAG released from MGDG is converted into TAG.

SUMMARY POINTS

1. Chloroplasts synthesize the predominant proportion of the fatty acids in a plant cell.
2. Thylakoid membranes contain two galactolipids [monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG)], a phospholipid (phosphatidylglycerol) and a sulfolipid.
3. Precursors for chloroplast lipids are produced at the endoplasmic reticulum (ER) and inside the chloroplast.
4. Lipids are transported between chloroplast and ER and between the two envelope membranes and the thylakoid membranes.
5. Under phosphate deprivation, the levels of phospholipids decrease, and the amounts of DGDG, sulfolipid, glucuronosyllipid, and acyl-MGDG increase.
6. The outer envelope membrane is subject to lipid remodeling under stress to decrease the proportion of the nonbilayer-forming lipid MGDG and to increase bilayer-forming oligogalactolipids.
7. Chloroplast lipids are indispensable for oxygenic photosynthesis; they are integral components of photosynthetic protein complexes and are constituents of mechanisms to protect the photosynthetic machinery against high light.
8. The genes involved in chloroplast galactolipid synthesis are not derived from their evolutionary progenitors, cyanobacteria, but were instead obtained by lateral gene transfer from other bacteria.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We would like to thank Ernst Heinz (University of Hamburg) for critically reading this manuscript.

LITERATURE CITED

1. Andersson MX, Goksör M, Sandelius AS. 2007. Optical manipulation reveals strong attracting forces at membrane contact sites between endoplasmic reticulum and chloroplasts. *J. Biol. Chem.* 282(2):1170–74
2. Andersson MX, Larsson KE, Tjellström H, Liljenberg C, Sandelius AS. 2005. Phosphate-limited oat: the plasma membrane and the tonoplast as major targets for phospholipid-to-glycolipid replacement and stimulation of phospholipases in the plasma membrane. *J. Biol. Chem.* 280(30):27578–86

3. Ariizumi T, Kishimoto S, Kakami R, Maoka T, Hirakawa H, et al. 2014. Identification of the carotenoid modifying gene *PALE YELLOW PETAL 1* as an essential factor in xanthophyll esterification and yellow flower pigmentation in tomato (*Solanum lycopersicum*). *Plant J.* 79(3):453–65
4. Armbruster U, Labs M, Pribil M, Viola S, Xu W, et al. 2013. *Arabidopsis* CURVATURE THYLAKOID1 proteins modify thylakoid architecture by inducing membrane curvature. *Plant Cell* 25(7):2661–78
5. Arondel V, Lemieux B, Hwang I, Gibson S, Goodman H, Somerville C. 1992. Map-based cloning of a gene controlling omega-3 fatty acid desaturation in *Arabidopsis*. *Science* 258(5086):1353–55
6. Awai K, Kakimoto T, Awai C, Kaneko T, Nakamura Y, et al. 2006. Comparative genomic analysis revealed a gene for monoglucosyldiacylglycerol synthase, an enzyme for photosynthetic membrane lipid synthesis in cyanobacteria. *Plant Physiol*. 141(3):1120–27
7. Awai K, Maréchal E, Block MA, Brun D, Masuda T, et al. 2001. 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*. *PNAS* 98(19):10960–65
8. Awai K, Ohta H, Sato N. 2014. Oxygenic photosynthesis without galactolipids. *PNAS* 111(37):13571–675
9. Awai K, Watanabe H, Benning C, Nishida I. 2007. Digalactosyldiacylglycerol is required for better photosynthetic growth of *Synechocystis* sp. PCC6803 under phosphate limitation. *Plant Cell Physiol*. 48(11):1517–23
10. Awai K, Xu C, Tamot B, Benning C. 2006. A phosphatidic acid-binding protein of the chloroplast inner envelope membrane involved in lipid trafficking. *PNAS* 103(28):10817–22
11. Babiychuk E, Müller F, Eubel H, Braun H-P, Frentzen M, Kushnir S. 2003. *Arabidopsis* phosphatidylglycerophosphate synthase 1 is essential for chloroplast differentiation, but is dispensable for mitochondrial function. *Plant J.* 33:899–909
12. BeGora MD, Macleod MJR, McCarry BE, Summers PS, Weretilnyk EA. 2010. Identification of phosphomethylethanolamine N-methyltransferase from *Arabidopsis* and its role in choline and phospholipid metabolism. *J. Biol. Chem.* 285(38):29147–55
13. Benning C. 1998. Biosynthesis and function of the sulfolipid sulfoquinovosyl diacylglycerol. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49:53–75
14. Bertrams M, Wrage K, Heinz E. 1981. Lipid labelling in intact chloroplasts from exogenous nucleotide precursors. *Z. Naturforsch. C* 36:62–70
15. Block MA, Dorne AJ, Joyard J, Douce R. 1983. Preparation and characterization of membrane fractions enriched in outer and inner envelope membranes from spinach chloroplasts. II. Biochemical characterization. *J. Biol. Chem.* 258(21):13281–86
16. Bonaventure G, Salas JJ, Pollard MR, Ohlrogge JB. 2003. Disruption of the *FATB* gene in *Arabidopsis* demonstrates an essential role of saturated fatty acids in plant growth. *Plant Cell* 15(4):1020–33
17. Brown A, Affleck V, Kroon J, Slabas A. 2009. Proof of function of a putative 3-hydroxyacyl-acyl carrier protein dehydratase from higher plants by mass spectrometry of product formation. *FEBS Lett.* 583(2):363–68
18. Browse J, Kunst L, Anderson S, Hugly S, Somerville C. 1989. A mutant of *Arabidopsis* deficient in the chloroplast 16:1/18:1 desaturase. *Plant Physiol*. 90(2):522–29
19. Browse J, McConn M, James D Jr., Miquel M. 1993. Mutants of *Arabidopsis* deficient in the synthesis of α-linolenate. Biochemical and genetic characterization of the endoplasmic reticulum linoleoyl desaturase. *J. Biol. Chem.* 268(22):16345–51

20. Isolation of *fad4*, the first desaturase mutant from *Arabidopsis*, by screening of a mutant population for alterations in leaf fatty acid composition.

20. Browse J, McCourt P, Somerville CR. 1985. A mutant of *Arabidopsis* lacking a chloroplast-specific lipid. *Science* 227:763–65

21. Browse J, McCourt P, Somerville C. 1986. A mutant of *Arabidopsis* deficient in C18:3 and 16:3 leaf lipids. *Plant Physiol*. 81(3):859–64
22. Browse J, Somerville C. 1991. Glycerolipid synthesis: biochemistry and regulation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42:467–506
23. Bryant N, Lloyd J, Sweeney C, Myouga F, Meinke D. 2011. Identification of nuclear genes encoding chloroplast-localized proteins required for embryo development in *Arabidopsis*. *Plant Physiol*. 155(4):1678–89

24. Carlsson AS, LaBrie ST, Kinney AJ, von Wettstein-Knowles P, Browse J. 2002. A *KAS2* cDNA complements the phenotypes of the *Arabidopsis fab1* mutant that differs in a single residue bordering the substrate binding pocket. *Plant J.* 29(6):761–70
25. Chen J, Burke JJ, Xin Z, Xu C, Velten J. 2006. Characterization of the *Arabidopsis* thermosensitive mutant *atts02* reveals an important role for galactolipids in thermotolerance. *Plant Cell Environm.* 29(7):1437–48
26. Choi JK, Yu F, Wurtele ES, Nikolau BJ. 1995. Molecular cloning and characterization of the cDNA coding for the biotin-containing subunit of the chloroplastic acetyl-coenzyme A carboxylase. *Plant Physiol.* 109(2):619–25
27. Craddock CP, Adams N, Bryant FM, Kurup S, Eastmond PJ. 2015. PHOSPHATIDIC ACID PHOSPHOHYDROLASE regulates phosphatidylcholine biosynthesis in *Arabidopsis* by phosphatidic acid-mediated activation of CTP:PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE activity. *Plant Cell* 27(4):1251–64
28. Cruz-Ramírez A, López-Bucio J, Ramírez-Pimentel G, Zurita-Silva A, Sánchez-Calderon L, et al. 2004. The *xipotl* mutant of *Arabidopsis* reveals a critical role for phospholipid metabolism in root system development and epidermal cell integrity. *Plant Cell* 16(8):2020–34
29. Cruz-Ramírez A, Oropeza-Aburto A, Razo-Hernández F, Ramírez-Chávez E, Herrera-Estrella L. 2006. Phospholipase DZ2 plays an important role in extraplastidic galactolipid biosynthesis and phosphate recycling in *Arabidopsis* roots. *PNAS* 103(17):6765–70
30. DellaPenna D, Pogson BJ. 2006. Vitamin synthesis in plants: tocopherols and carotenoids. *Annu. Rev. Plant Biol.* 57:711–38
31. Dörmann P, Balbo I, Benning C. 1999. *Arabidopsis* galactolipid biosynthesis and lipid trafficking mediated by DGD1. *Science* 284(5423):2181–84
32. Dörmann P, Hoffmann-Benning S, Balbo I, Benning C. 1995. Isolation and characterization of an *Arabidopsis* mutant deficient in the thylakoid lipid digalactosyl diacylglycerol. *Plant Cell* 7(11):1801–10
33. Dörmann P, Voelker TA, Ohlrogge JB. 1995. Cloning and expression in *Escherichia coli* of a novel thioesterase from *Arabidopsis thaliana* specific for long-chain acyl-acyl carrier proteins. *Arch. Biochem. Biophys.* 316(1):612–18
34. Dorne AJ, Joyard J, Block MA, Douce R. 1985. Localization of phosphatidylcholine in outer envelope membrane of spinach chloroplasts. *J. Cell Biol.* 100(5):1690–97
35. Douce R. 1974. Site of biosynthesis of galactolipids in spinach chloroplasts. *Science* 183(4127):852–53
36. Du Z-Y, Lucker BF, Zienkiewicz K, Miller TE, Zienkiewicz A, et al. 2018. Galactoglycerolipid lipase PGD1 is involved in thylakoid membrane remodeling in response to adverse environmental conditions in *Chlamydomonas*. *Plant Cell* 30(2):447–65
37. Dubots E, Audry M, Yamaryo Y, Bastien O, Ohta H, et al. 2010. Activation of the chloroplast monogalactosyldiacylglycerol synthase MGD1 by phosphatidic acid and phosphatidylglycerol. *J. Biol. Chem.* 285(9):6003–11
38. Eastmond PJ, Quettier A-L, Kroon JTM, Craddock C, Adams N, Slabas AR. 2010. PHOSPHATIDIC ACID PHOSPHOHYDROLASE1 and 2 regulate phospholipid synthesis at the endoplasmic reticulum in *Arabidopsis*. *Plant Cell* 22:2796–811
39. Ellinger D, Stingl N, Kubigsteltig II, Bals T, Juenger M, et al. 2010. DONGLE and DEFECTIVE IN ANTHER DEHISCENCE1 lipases are not essential for wound- and pathogen-induced jasmonate biosynthesis: Redundant lipases contribute to jasmonate formation. *Plant Physiol.* 153(1):114–27
40. Essigmann B, Güler S, Narang RA, Linke D, Benning C. 1998. Phosphate availability affects the thylakoid lipid composition and the expression of *SQD1*, a gene required for sulfolipid biosynthesis in *Arabidopsis thaliana*. *PNAS* 95(4):1950–55
41. Falcone DL, Gibson S, Lemieux B, Somerville C. 1994. Identification of a gene that complements an *Arabidopsis* mutant deficient in chloroplast ω6 desaturase activity. *Plant Physiol.* 106(4):1453–59
42. Fan J, Zhai Z, Yan C, Xu C. 2015. *Arabidopsis* TRIGALACTOSYLDIACYLGLYCEROL5 interacts with TGD1, TGD2, and TGD4 to facilitate lipid transfer from the endoplasmic reticulum to plastids. *Plant Cell* 27(10):2941–55

31. Isolation of the digalactosyldiacylglycerol synthase DGD1 and establishment of the entire galactolipid synthesis pathway in *Escherichia coli*.

35. Localization of monogalactosyldiacylglycerol, digalactosyldiacylglycerol, oligogalactolipids, phosphatidylcholine, and galactolipid synthesis activity to the chloroplast envelope.

43. Fisher M, Sedelnikova SE, Martindale W, Thomas NC, Simon JW, et al. 2000. Crystallization of the NADP-dependent β-keto acyl-carrier protein reductase from *Brassica napus*. *Acta Crystallogr*. 56(1):86–88
44. Fourrier N, Bédard J, Lopez-Juez E, Barbrook A, Bowyer J, et al. 2008. A role for *SENSITIVE TO FREEZING2* in protecting chloroplasts against freeze-induced damage in *Arabidopsis*. *Plant J*. 55:734–45
45. Frentzen M, Heinz E, McKeon TA, Stumpf PK. 1983. Specificities and selectivities of glycerol-3-phosphate acyltransferase and monoacylglycerol-3-phosphate acyltransferase from pea and spinach chloroplasts. *Eur. J. Biochem*. 129(3):629–36
46. Gao J, Ajjawi I, Manoli A, Sawin A, Xu C, et al. 2009. FATTY ACID DESATURASE4 of *Arabidopsis* encodes a protein distinct from characterized fatty acid desaturases. *Plant J*. 60(5):832–39
47. Gaude N, Bréhélin C, Tischendorf G, Kessler F, Dörmann P. 2007. Nitrogen deficiency in *Arabidopsis* affects galactolipid composition and gene expression and results in accumulation of fatty acid phytyl esters. *Plant J*. 49(4):729–39
48. Gaude N, Nakamura Y, Scheible W-R, Ohta H, Dörmann P. 2008. Phospholipase C5 (NPC5) is involved in galactolipid accumulation during phosphate limitation in leaves of *Arabidopsis*. *Plant J*. 56:28–39
49. Gibson S, Arondel V, Iba K, Somerville C. 1994. Cloning of a temperature-regulated gene encoding a chloroplast ω-3 desaturase from *Arabidopsis thaliana*. *Plant Physiol*. 106(4):1615–21
50. Gidda SK, Shockey JM, Rothstein SJ, Dyer JM, Mullen RT. 2009. *Arabidopsis thaliana* GPAT8 and GPAT9 are localized to the ER and possess distinct ER retrieval signals: functional divergence of the dilysine ER retrieval motif in plant cells. *Plant Physiol. Biochem*. 47(10):867–79
51. Hagio M, Sakurai I, Sato S, Kato T, Tabata S, Wada H. 2002. Phosphatidylglycerol is essential for the development of thylakoid membranes in *Arabidopsis thaliana*. *Plant Cell Physiol*. 43(12):1456–64
52. Hanson MR, Hines KM. 2018. Stromules: probing formation and function. *Plant Physiol*. 176(1):128–37
53. Härtel H, Dörmann P, Benning C. 2000. DGD1-independent biosynthesis of extraplastidic galactolipids after phosphate deprivation in *Arabidopsis*. *PNAS* 97(19):10649–54
54. Haselier A, Akbari H, Weth A, Baumgartner W, Frentzen M. 2010. Two closely related genes of *Arabidopsis* encode plastidial cytidinediphosphate diacylglycerol synthases essential for photoautotrophic growth. *Plant Physiol*. 153(3):1372–84
55. Heidrich J, Thurotte A, Schneider D. 2017. Specific interaction of IM30/Vipp1 with cyanobacterial and chloroplast membranes results in membrane remodeling and eventually in membrane fusion. *Biochim. Biophys. Acta* 1859(4):537–49
56. Heilmann I, Mekhedov S, King B, Browse J, Shanklin J. 2004. 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*. 136(4):4237–45
57. Heinz E. 1967. Acylgalaktosyldiglycerid aus Blatthomogenaten. *Biochim. Biophys. Acta* 144(2):321–32
58. Hennig R, Heidrich J, Saur M, Schmüser L, Roeters SJ, et al. 2015. IM30 triggers membrane fusion in cyanobacteria and chloroplasts. *Nat. Commun*. 6:7018
59. Higashi Y, Okazaki Y, Takano K, Myouga F, Shinozaki K, et al. 2018. A lipase gene, *HEAT INDUCIBLE LIPASE1*, is involved in remodeling chloroplastic monogalactosyldiacylglycerol by liberating α-linolenic acid in *Arabidopsis* leaves under heat stress. *Plant Cell* 30:1887–905
60. Hloušek-Radojčić A, Post-Beittenmiller D, Ohlrogge JB. 1992. Expression of constitutive and tissue-specific acyl carrier protein isoforms in *Arabidopsis*. *Plant Physiol*. 98(1):206–14
61. Hölzl G, Witt S, Kelly AA, Zähringer U, Warnecke D, et al. 2006. Functional differences between galactolipids and glucolipids revealed in photosynthesis of higher plants. *PNAS* 103(19):7512–17
62. Hölzl G, Zähringer U, Warnecke D, Heinz E. 2005. Glycoengineering of cyanobacterial thylakoid membranes for future studies on the role of glycolipids in photosynthesis. *Plant Cell Physiol*. 46(11):1766–78
63. Iba K, Gibson S, Nishiuchi T, Fuse T, Nishimura M, et al. 1993. A gene encoding a chloroplast ω-3 fatty acid desaturase complements alterations in fatty acid desaturation and chloroplast copy number of the *fad7* mutant of *Arabidopsis thaliana*. *J. Biol. Chem*. 268(32):24099–105

64. Inatsugi R, Kawai H, Yamaoka Y, Yu Y, Sekiguchi A, et al. 2009. Isozyme-specific modes of activation of CTP:phosphorylcholine cytidylyltransferase in *Arabidopsis thaliana* at low temperature. *Plant Cell Physiol.* 50(10):1727–35
65. Jarvis P, Dörmann P, Peto CA, Lutes J, Benning C, Chory J. 2000. Galactolipid deficiency and abnormal chloroplast development in the *Arabidopsis MGD synthase 1* mutant. *PNAS* 97(14):8175–79
66. Jessen D, Roth C, Wiermer M, Fulda M. 2015. 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.* 167(2):351–66
67. Jouhet J, Maréchal E, Baldan B, Bligny R, Joyard J, Block MA. 2004. Phosphate deprivation induces transfer of DGDG galactolipid from chloroplast to mitochondria. *J. Cell Biol.* 167(5):863–74
68. Jouhet J, Maréchal E, Bligny R, Joyard J, Block MA. 2003. Transient increase of phosphatidylcholine in plant cells in response to phosphate deprivation. *FEBS Lett.* 544(1–3):63–68
69. Kachroo A, Lapchyk L, Fukushige H, Hildebrand D, Klessig D, Kachroo P. 2003. Plastidial fatty acid signaling modulates salicylic acid- and jasmonic acid-mediated defense pathways in the *Arabidopsis ssi2* mutant. *Plant Cell* 15(12):2952–65
70. Kachroo A, Shanklin J, Whittle E, Lapchyk L, Hildebrand D, Kachroo P. 2007. The *Arabidopsis* stearoyl-acyl carrier protein-desaturase family and the contribution of leaf isoforms to oleic acid synthesis. *Plant Mol. Biol.* 63(2):257–71
71. Kanduč M, Schlaich A, de Vries AH, Jouhet J, Maréchal E, et al. 2017. Tight cohesion between glycolipid membranes results from balanced water–headgroup interactions. *Nat. Commun.* 8:14899
72. Katagiri T, Ishiyama K, Kato T, Tabata S, Kobayashi M, Shinozaki K. 2005. An important role of phosphatidic acid in ABA signaling during germination in *Arabidopsis thaliana*. *Plant J.* 43(1):107–17
73. Kaup MT, Froese CD, Thompson JE. 2002. A role for diacylglycerol acyltransferase during leaf senescence. *Plant Physiol.* 129(4):1616–26
74. Kelly AA, Dörmann P. 2002. *DGD2*, an *Arabidopsis* gene encoding a UDP-galactose-dependent digalactosyldiacylglycerol synthase is expressed during growth under phosphate-limiting conditions. *J. Biol. Chem.* 277(2):1166–73
75. Kelly AA, Froehlich JE, Dörmann P. 2003. Disruption of the two digalactosyldiacylglycerol synthase genes *DGD1* and *DGD2* in *Arabidopsis* reveals the existence of an additional enzyme of galactolipid synthesis. *Plant Cell* 15(11):2694–706
76. Kelly AA, Kalisch B, Holzl G, Schulze S, Thiele J, et al. 2016. Synthesis and transfer of galactolipids in the chloroplast envelope membranes of *Arabidopsis thaliana*. *PNAS* 113(38):10714–19
77. Kim HU, Huang AHC. 2004. Plastid lysophosphatidyl acyltransferase is essential for embryo development in *Arabidopsis*. *Plant Physiol.* 134(3):1206–16
78. Kim HU, Li Y, Huang AHC. 2005. Ubiquitous and endoplasmic reticulum-located lysophosphatidyl acyltransferase, LPAT2, is essential for female but not male gametophyte development in *Arabidopsis*. *Plant Cell* 17(4):1073–89
79. Kirchhoff H, Mukherjee U, Galla H-J. 2002. Molecular architecture of the thylakoid membrane: lipid diffusion space for plastoquinone. *Biochemistry* 41(15):4872–82
80. Klinkenberg J, Faist H, Saupe S, Lambertz S, Krischke M, et al. 2014. Two fatty acid desaturases, STEAROYL-ACYL CARRIER PROTEIN Δ^9-DESATURASE6 and FATTY ACID DESATURASE3, are involved in drought and hypoxia stress signaling in *Arabidopsis* crown galls. *Plant Physiol.* 164(2):570–83
81. Kobayashi K, Awai K, Nakamura M, Nagatani A, Masuda T, Ohta H. 2009. Type-B monogalactosyldiacylglycerol synthases are involved in phosphate starvation-induced lipid remodeling, and are crucial for low-phosphate adaptation. *Plant J.* 57:322–31
82. Kobayashi K, Endo K, Wada H. 2016. Roles of lipids in photosynthesis. *Subcell. Biochem.* 86:21–49
83. Kobayashi K, Kondo M, Fukuda H, Nishimura M, Ohta H. 2007. Galactolipid synthesis in chloroplast inner envelope is essential for proper thylakoid biogenesis, photosynthesis, and embryogenesis. *PNAS* 104(43):17216–21
84. Kroll D, Meierhoff K, Bechtold N, Kinoshita M, Westphal S, et al. 2001. *VIPP1*, a nuclear gene of *Arabidopsis thaliana* essential for thylakoid membrane formation. *PNAS* 98(7):4238–42

85. Kunst L, Browse J, Somerville C. 1988. Altered regulation of lipid biosynthesis in a mutant of *Arabidopsis* deficient in chloroplast glycerol-3-phosphate acyltransferase activity. *PNAS* 85:4143–47
86. Kunst L, Browse J, Somerville C. 1989. A mutant of *Arabidopsis* deficient in desaturation of palmitic acid in leaf lipids. *Plant Physiol*. 90(3):943–47
87. Latowski D, Åkerlund HE, Strzałka K. 2004. Violaxanthin de-epoxidase, the xanthophyll cycle enzyme, requires lipid inverted hexagonal structures for its activity. *Biochemistry* 43(15):4417–20
88. Li M, Welti R, Wang X. 2006. Quantitative profiling of *Arabidopsis* polar glycerolipids in response to phosphorus starvation. Roles of phospholipases Dζ1 and Dζ2 in phosphatidylcholine hydrolysis and digalactosyldiacylglycerol accumulation in phosphorus-starved plants. *Plant Physiol*. 142(2):750–61
89. Li N, Gügel IL, Giavalisco P, Zeisler V, Schreiber L, et al. 2015. FAX1, a novel membrane protein mediating plastid fatty acid export. *PLOS Biol*. 13(2):e1002053
90. Li X, Ilarslan H, Brachova L, Qian H-R, Li L, et al. 2011. Reverse-genetic analysis of the two biotin-containing subunit genes of the heteromeric acetyl-coenzyme A carboxylase in *Arabidopsis* indicates a unidirectional functional redundancy. *Plant Physiol*. 155(1):293–314
91. Li-Beisson Y, Shorrosh B, Beisson F, Andersson MX, Arondel V, et al. 2013. Acyl-lipid metabolism. *The Arabidopsis Book* 11:e0161. **https://doi.org/10.1199/tab.0161**
92. Lightner J, Wu J, Browse J. 1994. A mutant of *Arabidopsis* with increased levels of S stearic acid. *Plant Physiol*. 106(4):1443–51
93. Lin Y-C, Kobayashi K, Hung C-H, Wada H, Nakamura Y. 2016. *Arabidopsis* phosphatidylglycerophosphate phosphatase 1 involved in phosphatidylglycerol biosynthesis and photosynthetic function. *Plant J*. 88(6):1022–37
94. Lin Y-C, Liu Y-C, Nakamura Y. 2015. The choline/ethanolamine kinase family in *Arabidopsis*: essential role of CEK4 in phospholipid biosynthesis and embryo development. *Plant Cell* 27(5):1497–511
95. Lin Y-T, Chen L-J, Herrfurth C, Feussner I, Li H-M. 2016. Reduced biosynthesis of digalactosyldiacylglycerol, a major chloroplast membrane lipid, leads to oxylipin overproduction and phloem cap lignification in *Arabidopsis*. *Plant Cell* 28(1):219–32
96. Lippold F, vom Dorp K, Abraham M, Hölzl G, Wewer V, et al. 2012. Fatty acid phytyl ester synthesis in chloroplasts of *Arabidopsis*. *Plant Cell* 24(5):2001–14
97. Liu Y, Wang G, Wang X. 2015. Role of aminoalcoholphosphotransferases 1 and 2 in phospholipid homeostasis in *Arabidopsis*. *Plant Cell* 27(5):1512–28
98. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucl. Acids Res.* 42:D490–95
99. Lu B, Xu C, Awai K, Jones AD, Benning C. 2007. A small ATPase protein of *Arabidopsis*, TGD3, involved in chloroplast lipid import. *J. Biol. Chem.* 282:35945–53
100. Mazliak P. 1973. Lipid metabolism in plants. *Annu. Rev. Plant Physiol.* 24:287–310
101. McConn M, Hugly S, Browse J, Somerville C. 1994. A mutation at the *fad8* locus of *Arabidopsis* identifies a second chloroplast ω-3 desaturase. *Plant Physiol*. 106(4):1609–14
102. Mehrshahi P, Stefano G, Andaloro JM, Brandizzi F, Froehlich JE, DellaPenna D. 2013. Transorganellar complementation redefines the biochemical continuity of endoplasmic reticulum and chloroplasts. *PNAS* 110(29):12126–31

103. The long-sought galactolipid:galactolipid galactosyltransferase was identified as a stress-dependent enzyme not involved in bulk galactolipid synthesis.

103. Moellering ER, Muthan B, Benning C. 2010. Freezing tolerance in plants requires lipid remodeling at the outer chloroplast membrane. *Science* 330(6001):226–28

104. Mongrand S, Bessoule J-J, Cabantous F, Cassagne C. 1998. The $C_{16:3}/C_{18:3}$ fatty acid balance in photosynthetic tissues from 468 plant species. *Phytochemistry* 49:1049–64
105. Mongrand S, Cassagne C, Bessoule JJ. 2000. Import of lyso-phosphatidylcholine into chloroplasts likely at the origin of eukaryotic plastidial lipids. *Plant Physiol*. 122:845–52
106. Moore TS Jr. 1982. Phospholipid biosynthesis. *Annu. Rev. Plant Physiol.* 33:235–59
107. Mou Z, He Y, Dai Y, Liu X, Li J. 2000. Deficiency in fatty acid synthase leads to premature cell death and dramatic alterations in plant morphology. *Plant Cell* 12(3):405
108. Mulichak AM, Theisen MJ, Essigmann B, Benning C, Garavito RM. 1999. Crystal structure of SQD1, an enzyme involved in the biosynthesis of the plant sulfolipid headgroup donor UDP-sulfoquinovose. *PNAS* 96(23):13097–102

109. Müller MO, Meylan-Bettex M, Eckstein F, Martinoia E, Siegenthaler PA, Bovet L. 2000. Lipid phosphorylation in chloroplast envelopes: evidence for galactolipid CTP-dependent kinase activities. *J. Biol. Chem.* 275(26):19475–81
110. Nakamura Y, Awai K, Masuda T, Yoshioka Y, Takamiya K, Ohta H. 2005. A novel phosphatidylcholine-hydrolyzing phospholipase C induced by phosphate starvation in *Arabidopsis*. *J. Biol. Chem*. 280(9):7469–76
111. Nakamura Y, Koizumi R, Shui G, Shimojima M, Wenk MR, et al. 2009. *Arabidopsis* lipins mediate eukaryotic pathway of lipid metabolism and cope critically with phosphate starvation. *PNAS* 106(49):20978–83
112. Nakamura Y, Tsuchiya M, Ohta H. 2007. Plastidic phosphatidic acid phosphatases identified in a distinct subfamily of lipid phosphate phosphatases with prokaryotic origin. *J. Biol. Chem.* 282(39):29013–21
113. Ngo AH, Lin Y-C, Liu Y-C, Gutbrod K, Peisker H, et al. 2018. A pair of nonspecific phospholipases C, NPC2 and NPC6, are involved in gametophyte development and glycerolipid metabolism in *Arabidopsis*. *New Phytol*. 219:163–75
114. Nilsson AK, Johansson ON, Fahlberg P, Kommuri M, Töpel M, et al. 2015. Acylated monogalactosyl diacylglycerol: prevalence in the plant kingdom and identification of an enzyme catalyzing galactolipid head group acylation in *Arabidopsis thaliana*. *Plant J*. 84(6):1152–66
115. Ohlrogge JB, Jaworski JG. 1997. Regulation of fatty acid synthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:109–36
116. Ohlrogge JB, Kuhn DN, Stumpf PK. 1979. Subcellular localization of acyl carrier protein in leaf protoplasts of *Spinacia oleracea*. *PNAS* 76(3):1194–98
117. Okazaki Y, Kamide Y, Hirai M, Saito K. 2013. Plant lipidomics based on hydrophilic interaction chromatography coupled to ion trap time-of-flight mass spectrometry. *Metabolomics* 9(1):121–31
118. Okazaki Y, Otsuki H, Narisawa T, Kobayashi M, Sawai S, et al. 2013. A new class of plant lipid is essential for protection against phosphorus depletion. *Nat. Commun.* 4:1510
119. Okazaki Y, Shimojima M, Sawada Y, Toyooka K, Narisawa T, et al. 2009. A chloroplastic UDP-glucose pyrophosphorylase from *Arabidopsis* is the committed enzyme for the first step of sulfolipid biosynthesis. *Plant Cell* 21(3):892–909
120. Okuley J, Lightner J, Feldmann K, Yadav N, Lark E, Browse J. 1994. Arabidopsis *FAD2* gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. *Plant Cell* 6(1):147–58
121. Pierrugues O, Brutesco C, Oshiro J, Gouy M, Deveaux Y, et al. 2001. Lipid phosphate phosphatases in *Arabidopsis*. Regulation of the *AtLPP1* gene in response to stress. *J. Biol. Chem.* 276(23):20300–8
122. Poirier Y, Thoma S, Somerville C, Schiefelbein J. 1991. Mutant of *Arabidopsis* deficient in xylem loading of phosphate. *Plant Physiol*. 97(3):1087–93
123. Pulsifer IP, Lowe C, Narayaran SA, Busuttil AS, Vishwanath SJ, et al. 2014. Acyl-lipid thioesterase1–4 from *Arabidopsis thaliana* form a novel family of fatty acyl–acyl carrier protein thioesterases with divergent expression patterns and substrate specificities. *Plant Mol. Biol.* 84(4–5):549–63
124. Roughan G, Slack R. 1984. Glycerolipid synthesis in leaves. *Trends Biochem. Sci.* 9(9):383–86
125. Salas JJ, Ohlrogge JB. 2002. Characterization of substrate specificity of plant FatA and FatB acyl-ACP thioesterases. *Arch. Biochem. Biophys.* 403(1):25–34
126. Sasaki Y, Hakamada K, Suama Y, Nagano Y, Furusawa I, Matsuno R. 1993. Chloroplast-encoded protein as a subunit of acetyl-CoA carboxylase in pea plant. *J. Biol. Chem.* 268(33):25118–23
127. Sato N, Hagio M, Wada H, Tsuzuki M. 2000. Requirement of phosphatidylglycerol for photosynthetic function in thylakoid membranes. *PNAS* 97(19):10655–60
128. Schmidt H, Heinz E. 1993. Direct desaturation of intact galactolipids by a desaturase solubilized from spinach (*Spinacia oleracea*) chloroplast envelopes. *Biochem. J.* 289(3):777–82
129. Shanklin J, Somerville C. 1991. Stearoyl-acyl-carrier-protein desaturase from higher plants is structurally unrelated to the animal and fungal homologs. *PNAS* 88(6):2510–14
130. Shimojima M, Ohta H, Iwamatsu A, Masuda T, Shioi Y, Takamiya K. 1997. Cloning of the gene for monogalactosyldiacylglycerol synthase and its evolutionary origin. *PNAS* 94(1):333–37
131. Shockey JM, Fulda MS, Browse JA. 2002. *Arabidopsis* contains nine long-chain acyl-coenzyme A synthetase genes that participate in fatty acid and glycerolipid metabolism. *Plant Physiol.* 129(4):1710–22

116. Localization of acyl carrier protein and fatty acid synthesis to chloroplasts of plants.

118. Discovery of the new chloroplast lipid glucuronosyldiacylglycerol previously only known from bacteria.

124. Characterization of the prokaryotic and eukaryotic pathways of glycerolipid synthesis.

128. Direct evidence for galactolipid-linked desaturation in chloroplasts.

130. Isolation of the monogalactosyldiacylglycerol (MGDG) synthase gene *MGD1* involved in the production of MGDG, the most abundant lipid in the biosphere.

132. Shorrosh BS, Savage LJ, Soll J, Ohlrogge JB. 1996. The pea chloroplast membrane-associated protein, IEP96, is a subunit of acetyl-CoA carboxylase. *Plant J.* 10(2):261–68
133. Sugimoto K, Sato N, Tsuzuki M. 2007. Utilization of a chloroplast membrane sulfolipid as a major internal sulfur source for protein synthesis in the early phase of sulfur starvation in *Chlamydomonas reinhardtii*. *FEBS Lett.* 581(23):4519–22
134. Sun J, Ke J, Johnson JL, Nikolau BJ, Wurtele ES. 1997. Biochemical and molecular biological characterization of *CAC2*, the *Arabidopsis thaliana* gene coding for the biotin carboxylase subunit of the plastidic acetyl-coenzyme A carboxylase. *Plant Physiol.* 115(4):1371–83
135. Takami T, Shibata M, Kobayashi Y, Shikanai T. 2010. De novo biosynthesis of fatty acids plays critical roles in the response of the photosynthetic machinery to low temperature in *Arabidopsis*. *Plant Cell Physiol.* 51(8):1265–75
136. Thompson GA, Scherer DE, Foxall-Van Aken S, Kenny JW, Young HL, et al. 1991. Primary structures of the precursor and mature forms of stearoyl-acyl carrier protein desaturase from safflower embryos and requirement of ferredoxin for enzyme activity. *PNAS* 88(6):2578–82
137. Thorlby G, Fourrier N, Warren C. 2004. The *SENSITIVE TO FREEZING2* gene, required for freezing tolerance in *Arabidopsis thaliana*, encodes a β-glucosidase. *Plant Cell* 16:2192–203
138. van Besouw A, Wintermans JFGM. 1978. Galactolipid formation in chloroplast envelopes. I. Evidence for two mechanisms in galactosylation. *Biochim. Biophys. Acta* 529(1):44–53
139. Van Mooy BAS, Rocap G, Fredricks HF, Evans CT, Devol AH. 2006. Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments. *PNAS* 103(23):8607–12
140. van Wijk KJ, Kessler F. 2017. Plastoglobuli: plastid microcompartments with integrated functions in metabolism, plastid developmental transitions, and environmental adaptation. *Annu. Rev. Plant Biol.* 68:253–89
141. Vismans G, van der Meer T, Langevoort O, Schreuder M, Bouwmeester H, et al. 2016. Low-phosphate induction of plastidal stromules is dependent on strigolactones but not on the canonical strigolactone signaling component MAX2. *Plant Physiol.* 172(4):2235–44
142. Vranová E, Coman D, Gruissem W. 2013. Network analysis of the MVA and MEP pathways for isoprenoid synthesis. *Annu. Rev. Plant Biol.* 64:665–700
143. Wang K, Froehlich JE, Zienkiewicz A, Hersh HL, Benning C. 2017. A plastid phosphatidylglycerol lipase contributes to the export of acyl groups from plastids for seed oil biosynthesis. *Plant Cell* 29(7):1678–96
144. Wang K, Guo Q, Froehlich JE, Hersh HL, Zienkiewicz A, et al. 2018. Two abscisic acid-responsive plastid lipase genes involved in jasmonic acid biosynthesis in *Arabidopsis thaliana*. *Plant Cell* 30(5):1006–22
145. Wang Z, Benning C. 2011. *Arabidopsis thaliana* polar glycerolipid profiling by thin layer chromatography (TLC) coupled with gas-liquid chromatography (GLC). *J. Vis. Exp.* 49:e2518
146. Webb MS, Green BR. 1991. Biochemical and biophysical properties of thylakoid acyl lipids. *Biochim. Biophys. Acta* 1060:133–58
147. Welti R, Li W, Li M, Sang Y, Biesiada H, et al. 2002. Profiling membrane lipids in plant stress responses: role of phospholipase Dα in freezing-induced lipid changes in *Arabidopsis*. *J. Biol. Chem.* 277(35):31994–2002
148. Wu G-Z, Xue H-W. 2010. *Arabidopsis* β-ketoacyl-[acyl carrier protein] synthase I is crucial for fatty acid synthesis and plays a role in chloroplast division and embryo development. *Plant Cell* 22(11):3726–44
149. Wu J, James DW Jr., Dooner HK, Browse J. 1994. A mutant of *Arabidopsis* deficient in the elongation of palmitic acid. *Plant Physiol.* 106(1):143–50
150. Xu C, Fan J, Cornish AJ, Benning C. 2008. Lipid trafficking between the endoplasmic reticulum and the plastid in *Arabidopsis* requires the extraplastidic TGD4 protein. *Plant Cell* 20:2190–204
151. Xu C, Fan J, Riekhof W, Froehlich JE, Benning C. 2003. A permease-like protein involved in ER to thylakoid lipid transfer in *Arabidopsis*. *EMBO J.* 22(10):2370–79
152. Xu C, Härtel H, Wada H, Hagio M, Yu B, et al. 2002. The *pgp1* mutant locus of *Arabidopsis* encodes a phosphatidylglycerolphosphate synthase with impaired activity. *Plant Physiol.* 129(2):594–604
153. Xu C, Shanklin J. 2016. Triacylglycerol metabolism, function, and accumulation in plant vegetative tissues. *Annu. Rev. Plant Biol.* 67:179–206

147. The first application of mass spectrometry to the quantitative measurement of membrane glycerolipids in plants.

154. Xu C, Yu B, Cornish AJ, Froehlich JE, Benning C. 2006. Phosphatidylglycerol biosynthesis in chloroplasts of *Arabidopsis* mutants deficient in acyl-ACP glycerol-3-phosphate acyltransferase. *Plant J.* 47(2):296–309
155. Yamaryo Y, Kanai D, Awai K, Shimojima M, Masuda T, et al. 2003. Light and cytokinin play a cooperative role in MGDG synthesis in greening cucumber cotyledons. *Plant Cell Physiol.* 44(8):844–55
156. Yu B, Benning C. 2003. Anionic lipids are required for chloroplast structure and function in *Arabidopsis. Plant J.* 36(6):762–70
157. Yu B, Wakao S, Fan J, Benning C. 2004. Loss of plastidic lysophosphatidic acid acyltransferase causes embryo-lethality in *Arabidopsis. Plant Cell Physiol.* 45(5):503–10
158. Yu B, Xu C, Benning C. 2002. *Arabidopsis* disrupted in *SQD2* encoding sulfolipid synthase is impaired in phosphate-limited growth. *PNAS* 99(8):5732–37
159. Zagnitko O, Jelenska J, Tevzadze G, Haselkorn R, Gornicki P. 2001. An isoleucine/leucine residue in the carboxyltransferase domain of acetyl-CoA carboxylase is critical for interaction with aryloxyphenoxypropionate and cyclohexanedione inhibitors. *PNAS* 98(12):6617–22
160. Zepke HD, Heinz E, Radunz A, Linscheid M, Pesch R. 1978. Combination and positional distribution of fatty acids in lipids from blue-green algae. *Arch. Microbiol.* 119:157–62
161. Zhou Y, Hölzl G, vom Dorp K, Peisker H, Melzer M, et al. 2017. Identification and characterization of a plastidial phosphatidylglycerophosphate phosphatase in *Arabidopsis thaliana*. *Plant J.* 89(2):221–34