Chapter 7

Molecular Genetics of Lipid Metabolism in the Model Green Alga *Chlamydomonas reinhardtii*

Eric R. Moellering
Department of Biochemistry and Molecular Biology and Department of Energy-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA

Rachel Miller
Cell and Developmental Biology Program and Department of Energy-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA

Christoph Benning*
Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA

Summary

Research focusing on microalgae is currently experiencing a renaissance due to the potential of microalgae for providing biofuels without competing with food crops. Despite this potential, our knowledge of neutral and membrane lipid metabolism in microalgae is very limited, and opportunities to explore lipid metabolism in microalgae and contrast it to plant lipid metabolism abound. The unicellular green alga *Chlamydomonas reinhardtii* is currently the best genetic and genomic model for microalgal lipid research. This chapter summarizes the current knowledge of lipid metabolism in this alga. *Chlamydomonas* lipid metabolism differs in some aspects from that of seed plants. For example, *Chlamydomonas* lacks phosphatidylcholine and has in its place the betaine lipid diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine. This has important implications for lipid trafficking and lipid modification.

*Author for Correspondence, e-mail: benning@msu.edu

H. Wada and N. Murata (eds.), Lipids in Photosynthesis: Essential and Regulatory Functions, pp. 139–155.

These distinct aspects of algal lipid metabolism combined with the lower number of genes involved in lipid metabolism in *Chlamydomonas* provide several opportunities for basic research aimed at a more in-depth understanding of lipid metabolism in eukaryotic photosynthetic organisms in general.

I Introduction

Lipid biosynthesis in plants has been studied for decades and our current molecular understanding of lipid metabolism in plants is substantial. Genes encoding enzymes of glycerolipid biosynthesis and fatty acid desaturation have been identified by genetic and biochemical means (Ohlrogge and Browse, 1995; Joyard et al., 1998; Frentzen, 2004; Benning and Ohta, 2005; Holzl and Dörmann, 2007), and the first examples of components involved in lipid trafficking between the plastid and the endoplasmic reticulum (ER) are being discovered (Jouhet et al., 2007; Benning, 2008). Annotation of the *Arabidopsis* genome sequence (The Arabidopsis Genome Initiative, 2000) has led to the identification of novel genes, which likely encode proteins involved in lipid biosynthesis, trafficking, and catabolism (Beisson et al., 2003).

Like *Arabidopsis*, the eukaryotic green alga *Chlamydomonas reinhardtii* is a well established model for the study of different processes of general relevance, such as photosynthesis (Niyogi, 1999) and post-transcriptional gene silencing (Wu-Scharf et al., 2000). Beyond these, *Chlamydomonas* research has provided substantial insights into processes more specific to unicellular algae, e.g., phototaxis and flagellar function (Silflow and Lefebvre, 2001), nutrient acquisition (Davies et al., 1994, 1996, 1999), and microalgal metabolism (Grossman et al., 2007). The recent completion of the *Chlamydomonas* genome sequence (Merchant et al., 2007), as well as the development of insertional mutagenesis (Tam and Lefebvre, 1993), RNA interference (RNAi) methods (Fuhrmann et al., 2001; Sineshchekov et al., 2002), and a molecular map (Kathir et al., 2003) make *Chlamydomonas* an attractive model to study gene function by genetic or direct molecular analysis. Preliminary annotations of lipid genes present in the genome of *Chlamydomonas* were recently published (Riekhof et al., 2005b; Riekhof and Benning, 2008). Based on these attributes, *Chlamydomonas* has great promise for the analysis of the biosynthesis and physiological functions of different lipids.

Availability of a suitable microalgal model system is timely, as microalgae are increasingly discussed as a biomass resource for the production of biofuels that does not have to compete with the agricultural production of food crops (Hu et al., 2008). While *Chlamydomonas reinhardtii* itself is not a candidate species for the commercial production of biofuels, it still is the best studied microalga at the genetic and genomic level. Moreover, *Chlamydomonas* is related to other unicellular green algae that are commercially used, e.g., *Dunalliella salina*, and *Chlamydomonas* has been reported to accumulate triacylglycerols (TAGs) under conditions of nutrient deprivation (Weers and Gulati, 1997) or high light (Picaud et al., 1991). *Chlamydomonas* also synthesizes TAGs from lipids supplied in the medium (Grenier et al., 1991). To fill in the gaps in knowledge, efforts are currently underway in our lab to genetically dissect the biosynthesis of TAGs and its regulation in *Chlamydomonas*, and to identify genes that might be useful for the engineering microalgal production strains.

Abbreviations: ACP – Acyl carrier protein; CDP-DAG – CDP-diacylglycerol; DAG – Diacylglycerol; DGTS – Diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine; DGDG – Digalactosyl-diacyl-glycerol; ER – Endoplasmic reticulum; FAS – Fatty acid synthase; MGDG – Monogalactosyldiacylglycerol; PA – Phosphatidic acid; PC – Phosphatidylcholine; PE – Phosphatidylethanolamine; PG – Phosphatidylglycerol; PI – Phosphatidylinositol; PS – Phosphatidylserine; PUFA – Polyunsaturated fatty acid; RNAi – RNA interference; SQDG – Sulfoquinovosyldiacylglycerol; TAG – Triacylglycerol.

II General Differences in Lipid Metabolism between *Chlamydomonas* and Seed Plants

As elaborated below, many aspects of lipid metabolism follow common pathways that were presumably established during the evolution of chloroplasts of green algal and plant ancestors (Reyes-Prieto et al., 2007). However, at least

two possibly related aspects of lipid metabolism in *Chlamydomonas* differ from lipid metabolism in seed plants (Fig. 1). Most prominently, *Chlamydomonas* is unable to synthesize the otherwise common phosphoglycerolipid phosphatidylcholine (PC). Instead, it produces the non-phosphorus betaine lipid diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine (DGTS) (Eichenberger and Boschetti, 1977). This lipid is similar in structure and function to PC (Fig. 2) and is thought to substitute for PC in *Chlamydomonas* (Sato and Murata, 1991; Sato, 1992; Moore et al., 2001). Interestingly, PC is central to lipid metabolism in developing seeds or leaves where it serves as substrate for fatty acid modifying enzymes, such as desaturases (Browse and Somerville, 1991; Ohlrogge and Browse, 1995; Wallis and Browse, 2002), or possibly as the lipid transferred between the ER and the plastid (Jouhet et al., 2007; Benning, 2008).

Precursors of thylakoid lipid biosynthesis in many plants are derived from two pathways (Mongrand et al., 1998), the plastid and the ER

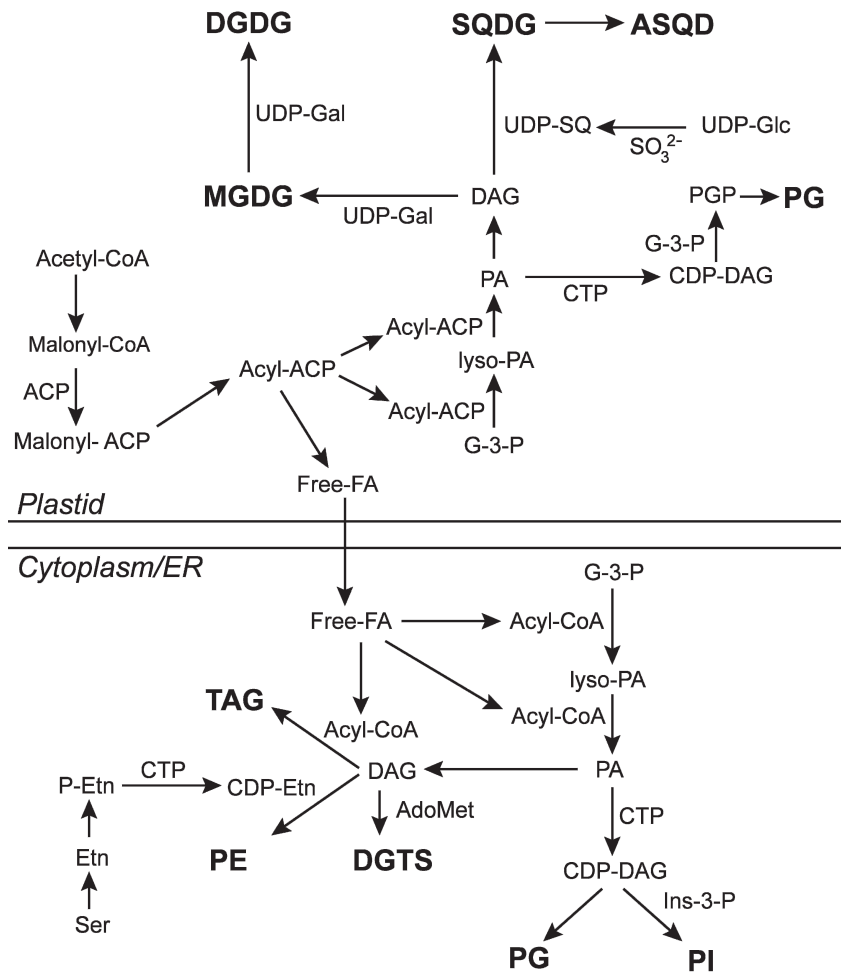

Fig. 1. Overview of glycerolipid biosynthesis in Chlamydomonas. Endproducts are shown in bold. Abbreviations: ACP, acyl carrier protein; AdoMet, S-adenosylmethionine; ASQD, 2′-O-acyl-sulfoquinovosyldiacylglycerol; CDP, cytidine 5′-diphosphate; CoA, coenzyme A; CTP, cytidine 5′-triphosphate; DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; DGTS, diacylglyceryl-N,N,N-trimethylhomoserine; Etn, ethanolamine; FA, fatty acid; G3-P, glycerol 3-phosphate; Glc, glucose; Ins-3-P, inositol 3-phosphate; MGDG, monogalactosyldiacylglycerol; P-Etn, phosphoethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGP, phosphatidylglycerolphosphate; PI, phosphatidylinositol; PA, phosphatidic acid; Ser, serine; SQ, sulfoquinovose; SQDG, sulfoquinovosyldiacylglycerol; TAG, triacylglycerol; UDP, uridine 5′-diphosphate (modified with permission from Fig. 1 in Riekhof et al., 2005b).

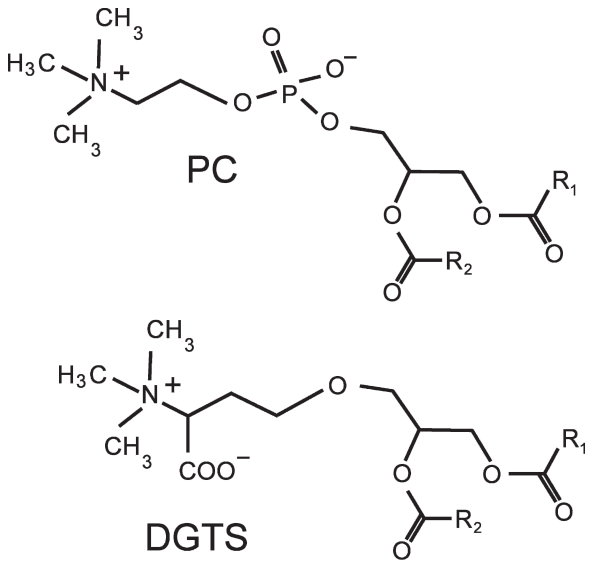

Fig. 2. Structural similarity between phosphatidylcholine (PC) and betaine lipid (DGTS).

pathways. This two pathway hypothesis was formulated by Roughan and coworkers based on labeling experiments (Roughan et al., 1980; Roughan and Slack, 1982) and later confirmed by mutant analysis in *Arabidopsis* (Browse and Somerville, 1991; Wallis and Browse, 2002). Thylakoid lipid molecular species derived from either of the two pathways can be distinguished based on their fatty acid composition (Heinz and Roughan, 1983), and fluxes through the two pathways have been determined (Browse et al., 1986). While a large number of plant species have lost the ability to de novo assemble thylakoid lipids, such as the dominant galactoglycerolipids in the plastid, nearly all reported plant species derive at least a fraction of their thylakoid lipids from precursors assembled at the ER (Mongrand et al., 1998) requiring import of lipid precursor into the plastid. However, detailed compositional analysis of lipids and labeling studies suggest that in *Chlamydomonas* all thylakoid lipids are assembled de novo in the plastid (Giroud et al., 1988). Thus, it is possible that the lack of PC and the lack of trafficking of lipid precursors from the ER to the plastid in *Chlamydomonas* are related if PC is a critical intermediate in ER-to-plastid lipid trafficking. Because in the betaine lipid, DGTS, the head group moiety is ether-linked to the diacylglyceryl moiety (Fig. 2), *Chlamydomonas* might lack an enzyme to break this ether linkage. This ether linkage is more stable than the phosphate ester linkage in phosphoglycerolipids. Therefore, the conversion of DGTS into the galactoglycerolipid precursor diacylglycerol might not be possible in *Chlamydomonas*.

Aside from the betaine lipid, *Chlamydomonas* and many other microalgae contain a rich set of polyunsaturated fatty acids (Fig. 3) not present in most seed plants, which will be discussed in detail below.

III Membrane Glycerolipid Biosynthesis

A Fatty Acid Synthesis and Incorporation into Glycerolipids

De novo synthesis of fatty acids is localized to the chloroplast of *Chlamydomonas* cells (Sirevag and Levine, 1972). The common ancestral origin of green algal and seed plant plastids is particularly apparent in many homologous components of the fatty acid biosynthetic machinery. For example, bioinformatic analysis of the *Chlamydomonas* genome has identified genes for the full suite of enzymes required for the conversion of acetyl-CoA to acylated-acyl carrier protein (ACP), including the multimeric bacterial-type acetyl-CoA carboxylase and fatty acid synthase complexes (Riekhof et al., 2005b; Riekhof and Benning, 2008). These enzymes are essential for fatty acid biosynthesis in plants (and presumably algae), which predominantly produce 16:0-ACP and 18:1-ACP as the result of desaturation of 18:0-ACP by a soluble stearoyl-ACP Δ9 desaturase (Browse and Somerville, 1991; Shanklin and Somerville, 1991). In plants, fatty acids are incorporated directly into chloroplast membrane glycerolipids by stepwise acylation of glycerol 3-phosphate to form phosphatidic acid (*sn1*–18:1, *sn2*–16:0-PA) by glycerol 3-phosphate:acyl-ACP acyltransferase (GPAT), which shows substrate specificity for 18:1-ACP, and then by lysophosphatidate:acyl-ACP acyltransferase (LPAT, 16:0-ACP specific) (Kunst et al., 1988; Browse and Somerville, 1991; Murata and Tasaka, 1997; Kim and Huang, 2004; Xu et al., 2006).

Fatty acids are also assembled into glycerolipids at the ER where isoforms of the plastid

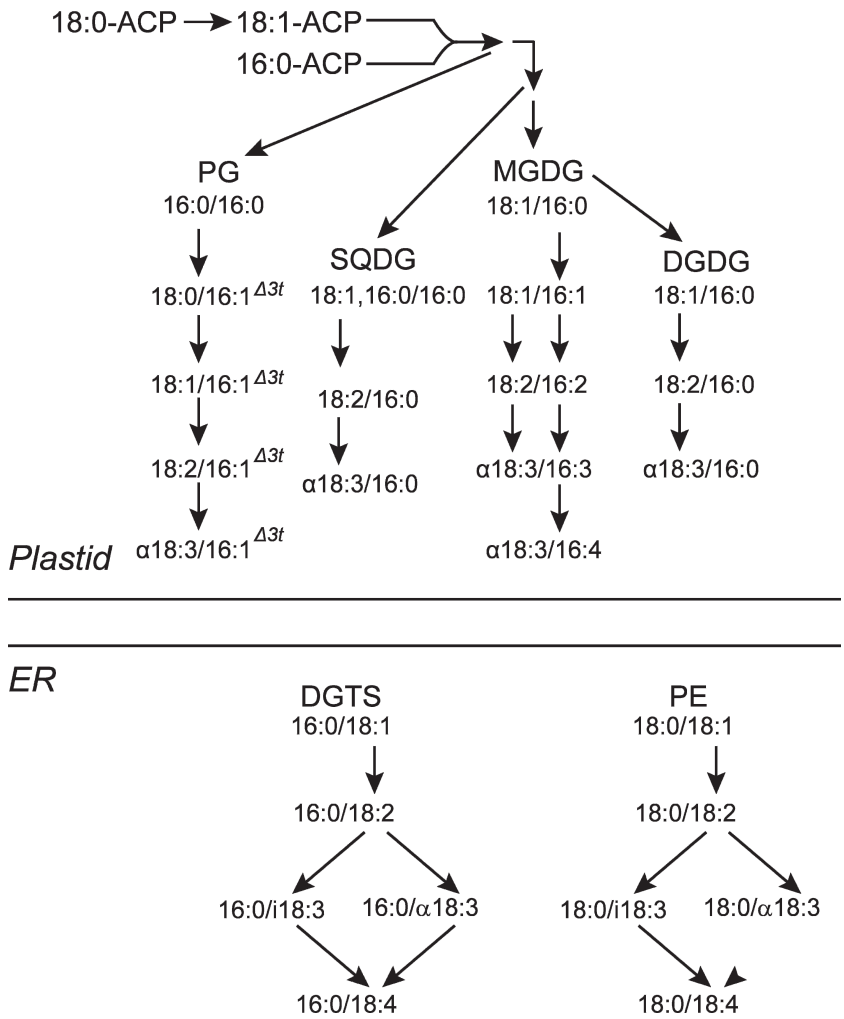

Fig. 3. Overview of acyl-chain desaturation in Chlamydomonas. Glycerolipid abbreviations are the same as those in Fig. 1. Fatty acids are referred to by the standard abbreviation "carbon atoms:double bonds." Fatty acids at the sn-1 and sn-2 positions of the glyceryl moiety are indicated. Double bond positions within the fatty acid chain and/or common names of the fatty acids are as follows: 16:0, palmitic acid; $16:1^{\Delta 7}$, plamitoleic acid or $16:1^{\Delta 3t}$ in plastidic PG; $16:2^{\Delta 7,10}$; $16:3^{\Delta 7,10,13}$; for 16:4 the double bond position is not known; 18:0, stearic acid; $18:1^{\Delta 9}$, oleic acid or in some lipids $18:1^{\Delta 11}$, vaccenic acid; $\alpha 18:2^{\Delta 9,12}$, αlinoleic acid; $\alpha 18:3^{\Delta 9,12,15}$, αlinoleic acid; $i18:3^{\Delta 5,9,12}$, pinolenic acid; $18:4^{\Delta 5,9,12,15}$, coniferonic acid. The predominant molecular species of SQDG is 16:0/16:0 (modified with permission from Fig. 2 in Riekhof et al., 2005b).

acyltransferases are present and have been characterized in *Arabidopsis* (Zheng et al., 2003; Kim et al., 2005). Putative orthologs of the plant GPAT and LPAT genes are annotated in the final *Chlamydomonas* genome draft (Riekhof et al., 2005b; Riekhof and Benning, 2008). Candidates for the plastid PA phosphatase, which produces the diacylglycerol precursors for the biosynthesis of non-phosphorus lipids in the plastid, have been recently identified in *Arabidopsis* (Nakamura et al., 2007). However, there is currently no good candidate in the *Chlamydomonas* genome predicted to encode this enzyme (Riekhof et al., 2005b; Riekhof and Benning, 2008).

B Chloroplast Membrane Lipids

The overall structural organization of membranes in the chloroplast of *Chlamydomonas* and seed plant chloroplasts is essentially identical, where the inner and outer envelope membranes enclose an extensive thylakoid membrane system in which the

photosynthetic apparatus is embedded. Genetic studies of *Arabidopsis* have identified many of the genes responsible for the biosynthesis of chloroplast membrane lipids, and have revealed the essential role that lipid composition plays in optimal photosynthetic function (Vijayan et al., 1998; Dörmann and Benning, 2002; Wallis and Browse, 2002; Benning and Ohta, 2005). Though the genetic study of glycerolipid metabolism in *Chlamydomonas* has far fewer documented examples, detailed biochemical analysis of this alga's lipid composition has long confirmed the presence of the major chloroplast membrane lipids found in land plants – including the galactoglycerolipids mono- and digalactosyldiacylglycerol (MGDG and DGDG), sulfoquinovosyldiacylglycerol (SQDG), and the phosphoglycerolipid phosphatidylglycerol (PG) (Giroud et al., 1988).

As in plants, galactoglycerolipids are the predominant membrane glycerolipid class in *Chlamydomonas*, where they make up a majority of the chloroplast membrane lipids (Janero and Barrnett, 1981a; Giroud et al., 1988). In *Arabidopsis*, the bulk of galactolipid biosynthesis involves two enzymatic steps, whereby MGDG is formed from diacylglycerol (DAG) and UDP-galactose (UDP-Gal) substrates by MGDG synthase (MGD1), and DGDG is formed from MGDG and UDP-Gal by DGDG synthase (DGD1) (Benning and Ohta, 2005). Genes encoding MGDG and DGDG synthases have been identified in the *Chlamydomonas* genome as orthologs of the *Arabidopsis* genes *MGD1* and *DGD1*, respectively (Riekhof et al., 2005b; Riekhof and Benning, 2008). *MGD1* and *DGD1* are single-copy genes in *Chlamydomonas*, which differs from that of the *MGD1*, *2*, *3* and *DGD1*, *2* paralogs found in the *Arabidopsis* genome. Molecular analysis of the *Arabidopsis MGD2*, *3* and *DGD2* genes has revealed their role in a galactolipid biosynthetic pathway that is transcriptionally induced during phosphate deprivation, and is proposed to provide galactolipids for extraplastidic membranes (Härtel et al., 2000; Kelly and Dörmann, 2002; Jouhet et al., 2004). The apparent lack of this induced galactolipid pathway in *Chlamydomonas* suggests a distinct lipid metabolic response to phosphate limitation, or a lack of need for one; however, to date the galactolipid biosynthetic genes of *Chlamydomonas* have not been studied in detail at the molecular level to test these hypotheses.

The sulfolipid sulfoquinovosyldiacylglycerol (SQDG) has long been studied in the context of its role in photosynthetic membranes, not only due to its prevalence in photosynthetic eukaryotes and prokaryotes, but also because of its association with photosynthetic pigment–protein complexes (Menke et al., 1976; Gounaris and Barber, 1985; Pick et al., 1985; Stroebel et al., 2003). However, the more recent discovery of SQDG and/or the genes and enzymes involved in SQDG biosynthesis in non-photosynthetic bacteria as summarized in (Cedergren and Hollingsworth, 1994; Benning et al., 2008), has clearly indicated that the role of sulfolipids is not limited to the function of photosynthetic membranes. The biosynthesis of SQDG is carried out in two enzymatic steps in *Arabidopsis* by SQD1, which catalyzes the formation of UDP-sulfoquinovose from UDP-Glc and sulfite, and SQD2, which transfers the sulfoquinovose moiety from UDP-sulfoquinovose to DAG, forming SQDG (Essigmann et al., 1998; Sanda et al., 2001; Yu et al., 2002). A single copy ortholog of *SQD1* is present in *Chlamydomonas*, and two possible orthologs of *Arabidopsis SQD2* are found in the genome (Yu et al., 2002; Riekhof et al., 2003). Recently, a *Chlamydomonas* mutant deleted in *SQD1* (Δ*sqd1*) and completely lacking sulfolipid has been studied (Riekhof et al., 2003). Phenotypic analysis of Δ*sqd1* revealed a reduced growth rate during phosphate-limiting conditions, under which the SQDG level was found to double in wild-type cells. This is similar to what has been observed in sulfolipid-deficient mutants in other organisms, such as *Arabidopsis*, which showed impaired growth after severe phosphate limitation (Yu et al., 2002), and in the photosynthetic purple bacterium *Rhodobacter sphaeroides* (Benning et al., 1993). In both *Chlamydomonas* and *Arabidopsis*, the increase in SQDG levels under phosphate-limiting conditions is accompanied by a decrease in PG, resulting in little net change in the amount of anionic glycerolipids. These results suggest a role for SQDG in partially replacing PG during phosphate limitation in order to maintain thylakoid membrane function (Riekhof et al., 2003). However, during sulfur (S) limitation a large decrease in SQDG and concomitant increase in PG has been observed in *Chlamydomonas* (Sugimoto et al., 2008), and SQDG was shown to be a major

internal S-source for protein synthesis in the early phases of the S-starvation response (Sugimoto et al., 2007).

In addition, Δ*sqd1* showed sensitivity to a photosystem II inhibitor under normal growth conditions (Riekhof et al., 2003). This is consistent with another *Chlamydomonas* SQDG-deficient mutant, *hf-2*, which was first discovered as a high chlorophyll fluorescence mutant, and was later found to be impaired in photosystem II stability and showed increased sensitivity to a PSII inhibitor, which could be partially restored by SQDG addition (Sato et al., 1995a, b; Minoda et al., 2002, 2003). However, whether the *hf-2* mutant is impaired in growth during phosphate limitation has not been reported, nor has the exact molecular defect in this mutant been determined. Interestingly, detailed biochemical analysis of the *Δsqd1* mutant also led to the discovery of the novel sulfolipid derivative, 2′-*O*-acylsulfoquinovosyldiacylglycerol (ASQD), which was also not produced in *Δsqd1* (Riekhof et al., 2003). Due to the loss of both sulfolipids in *Δsqd1*, the specific roles played by SQDG and ASQD in *Chlamydomonas* and phenotypes associated with *Δsqd1* can only be fully interpreted after the identification and characterization of the acyltransferase catalyzing ASQD production has been undertaken.

Phosphatidylglycerol (PG) is presumably the only major phospholipid component in thylakoid membranes of seed plants, and biochemical analysis of thylakoid lipid composition has confirmed this to be the case in *Chlamydomonas* (Janero and Barrnett, 1981b; Mendiola-Morgenthaler et al., 1985). While the gene encoding the final enzyme in PG biosynthesis, phosphatidylglycerolphosphate (PGP) phosphatase, remains unknown in plants and algae (Beisson et al., 2003), the putative genes encoding the enzymes that catalyze the formation of the two intermediates, CDP-DAG synthetase and phosphatidylglycerolphosphate synthase, have been identified (Riekhof et al., 2005b; Riekhof and Benning, 2008), but not yet confirmed. While neither the single gene encoding the CDP-DAG synthetase or the two putative plastid paralogs encoding phosphatidylglycerolphosphate synthase have been studied at the molecular/genetic level, PG deficient mutants, *mf 1* and *mf 2*, have been isolated and studied in great biochemical detail (Garnier et al., 1987; Maroc et al., 1987; Garnier et al., 1990; Maanni et al., 1998; Dubertret et al., 2002; Pineau et al., 2004). The *mf 1*, *2* mutants were first isolated as low fluorescent strains lacking functional Photosystem II (PS II), as well as an oligomeric form of the light-harvesting chlorophyll antenna (CPII) (Maroc et al., 1987; Dubertret et al., 1994). It was also shown that both *mf 1* and *mf 2* contained approximately 30% of wild-type PG levels and lacked Δ3-*trans*-hexadecenoic acid ($16{:}1^{\Delta 3trans}$ [carbons:double bonds$^{\Delta positions}$]) (Maroc et al., 1987; Dubertret et al., 1994), a fatty acid that is specifically esterified to chloroplastic PG in both *Arabidopsis* and *Chlamydomonas* (Browse et al., 1985; Garnier et al., 1987; Giroud et al., 1988). Addition of a preparation of spinach leaf PG containing $16{:}1^{\Delta 3trans}$ to *mf-2* cells restored the ability to form oligomeric CP II, while 18:0 PG additions did not, and 16:0 PG did so only weakly so (Garnier et al., 1990; Dubertret et al., 1994). A PG-deficient mutant in *Arabidopsis*, *pgp1*, which is defective in the chloroplastid isoform of PGP synthase has been found to be photosynthetically impaired with decreased quantum yield through PSII, but did not lack $16{:}1^{\Delta 3trans}$ PG (Xu et al., 2002; Hagio et al., 2002; Babiychuk et al., 2003). Similarly, two *Synechocystis* PG deficient mutants showed altered PSII activity and required exogenous PG for phototropic growth (Hagio et al., 2000; Sato et al., 2000).

Taken together with the contrasting findings from analyses of the *Arabidopsis fad4* mutant, which lacks $16{:}1^{\Delta 3trans}$, but is otherwise not affected in chloroplast PG content and also shows no apparent photosynthetic defects (Browse et al., 1985), it can currently only be concluded that in general PG plays an important role in photosynthetic membrane biogenesis and function, and it seems possible that the $16{:}1^{\Delta 3trans}$ PG form could be essential in some organisms (e.g., *Chlamydomonas*), but is of conditional importance or dispensable in others. It is certain however, that the elucidation of the exact molecular defects in the *Chlamydomonas mf 1*, *2* mutants, and the identification of the genes encoding FAD4 activity as well as the elusive plant/algal PGP phosphatase that catalyzes the final step in PG biosynthesis, will be prerequisite to gaining a better understanding of the roles PG plays in the photosynthetic membranes in various species.

C Extrachloroplastic Membrane Lipid Metabolism

In eukaryotic photoautotrophs the bulk of extraplastidic membrane glycerolipids is assembled in the ER from acyl-CoA thioesters, which are formed from free fatty acids after their liberation from acyl-ACPs in the plastid (see Fig. 1). While other extraplastidic sites for lipid synthesis are known (e.g., mitochondria), the ER localized pathway is predominant, and in most plants the ER lipid assembly pathway significantly contributes to thylakoid membrane biogenesis. As such, a discussion of the analogous pathways in *Chlamydomonas* is merited. As mentioned above, *Chlamydomonas* lacks the capability for PC biosynthesis (Giroud et al., 1988) and genes predicted to encode enzymes involved in PC biosynthesis are not present in its genome (Riekhof et al., 2005b; Riekhof and Benning, 2008). Instead, it contains the non-phosphorous zwitterionic betaine lipid DGTS (Fig. 2) in its membranes (Eichenberger and Boschetti, 1977; Janero and Barrnett, 1982), which has similar biophysical properties to PC (Sato and Murata, 1991). DGTS has also been found in other algal species, e.g. (Eichenberger, 1982), prokaryotes like the purple bacterium *Rhodobacter sphaeroides*, e.g. (Benning et al., 1995; Hofmann and Eichenberger, 1996), and in non-seed plants, such as ferns, e.g. (Sato and Furuya, 1983; Eichenberger, 1993), but appears to be absent in seed plants. Labeling studies suggest that the biosynthesis of DGTS is similar in all organisms studied (Sato, 1988, 1991; Sato and Kato, 1988; Vogel and Eichenberger, 1992; Hofmann and Eichenberger, 1996). It begins with the transfer of the 3-amino 3-carboxypropyl residue from *S*-adenosylmethionine (AdoMet) to DAG catalyzed by AdoMet:DAG 3-amino-3-carboxypropyltransferase activity followed by successive methylation of the amino group by an AdoMet-dependent *N*-methyltransferase (Fig. 1). The two genes encoding these catalytic activities, *btaA* and *btaB*, were first identified in *R. sphaeroides* (Klug and Benning, 2001; Riekhof et al., 2005a).

More recently, a single gene sufficient for DGTS biosynthesis in *Chlamydomonas*, *Bta1*, was identified in the genome (Riekhof et al., 2005b). The encoded protein Bta1 is similar in its N-terminal domain to bacterial BtaB and in its C-terminal domain to BtaA and the predicted catalytic function of each Bta1 domain was confirmed by mutagenesis (Riekhof et al., 2005b). The bifunctionality observed in Bta1 as a fusion of two prokaryotic enzymes active in the same pathway into a single polypeptide is a common theme in plants (Moore, 2004), and could represent an improvement in DGTS biosynthesis by eliminating the need for coordinated regulation of two independent gene products or permitting substrate channeling. In addition, the presence of DGTS and concomitant lack of PC are perhaps related to the absence of additional galactolipid biosynthetic pathways seen in seed plants, e.g., MGD2, MGD3 and DGD2 in *Arabidopsis* (Benning and Ohta, 2005). As this alternative galactolipid pathway is believed to be involved in replacing phospholipids in extraplastidic membranes with DGDG during phosphate deprivation, the constitutive replacement of PC with the non-phosphorous DGTS has perhaps obviated the need to replace PC by extraplastidic DGDG. Interestingly, DGTS was tentatively identified as a component of purified *Chlamydomonas* thylakoids (Janero and Barrnett, 1981b, 1982) and chloroplast envelope membranes (Mendiola-Morgenthaler et al., 1985). However, whether DGTS is indeed present in the chloroplast membranes of *Chlamydomonas* or plays a specific function in thylakoids remains to be confirmed.

Phosphatidylserine (PS) is a minor component of extraplastidic membranes of plants and can be decarboxylated to phosphatidylethanolamine (PE) in plants and other organisms (Vance and Steenbergen, 2005; Nerlich et al., 2007). However, PS is absent in *Chlamydomonas* membranes (Giroud et al., 1988) and genes encoding the phosphatidylserine synthase or relevant phospholipid base exchange enzymes were not detected in the genome (Riekhof et al., 2005b; Riekhof and Benning, 2008). As such, the biosynthesis of PE, which is a known component of extraplastidic membranes in *Chlamydomonas*, is likely only carried out by a single pathway (Fig. 1). Genes encoding a serine decarboxylase which produces ethanolamine, an ethanolamine kinase and CTP:phosphoethanolamine cytidylyltransferase the combined activities of which produce CDP-ethanolamine, and a CDP-ethanolamine:DAG ethanolamine phosphotransferase, which produces PE, have been identified in the genome (Riekhof et al., 2005b; Riekhof and Benning, 2008).

Recently, the gene encoding the CTP: phosphoethanolamine cytidylyltransferase has been characterized by heterologous expression in *Escherichia coli*, and the expression of the respective gene was found to be up-regulated during the reflagellation of *Chlamydomonas* cells (Yang et al., 2004). Phosphatidylinositol (PI) is a minor component of *Chlamydomonas* membranes, and genes required for its biosynthesis, including inositol-3-phosphate synthase and CDP-DAG:inositol phosphotransferase, are present in the genome (Riekhof et al., 2005b; Riekhof and Benning, 2008). The PI biosynthetic enzymes of *Chlamydomonas* have been studied at the biochemical level, and PI synthesis was found to be highest in the microsomal fraction, suggesting its association with the ER (Blouin et al., 2003).

Extraplastidic PG biosynthesis is known to be associated with both the ER and mitochondria, and three isoforms of phosphatidylglycerolphosphate synthase are encoded in the genome, each with differential targeting prediction probabilities for subcellular localization to the mitochondria, chloroplast or cytosol (Riekhof et al., 2005b; Riekhof and Benning, 2008). However, a detailed analysis of these proteins and their respective genes is not yet available in *Chlamydomonas*. An extraplastidic candidate for CDP-DAG synthase, which provides one of the substrates for phosphatidylglycerolphosphate synthase, has been identified in the genome (Riekhof et al., 2005b). However, as in the case of chloroplast PG biosynthesis, no gene encoding an extraplastidic phosphatidylglycerolphosphate phosphatase is currently known.

IV Fatty Acid Desaturation

Biochemical studies in *Chlamydomonas* have indicated that further desaturation of 16:0 and 18:1 acyl groups occurs after the production of the major glycerolipids, in a manner similar to plants (Giroud et al., 1988). Furthermore, the *Chlamydomonas* fatty acid profile is known to change markedly in response to various environmental conditions, including CO_2 concentration, as well as nitrogen and phosphorous limitation (Tsuzuki et al., 1990; Weers and Gulati, 1997). The elucidation of fatty acid desaturase (*FAD*) genes in *Arabidopsis* is a classic example of the power of genetic and molecular biological approaches in solving biological problems, which prove to be largely intractable through a strictly biochemical approach (Browse and Somerville, 1991; Wallis and Browse, 2002). The identification of many of the *Chlamydomonas* desaturase gene candidates by their similarity to *Arabidopsis* orthologs, combined with a handful of studies of *Chlamydomonas* desaturase mutants and characterization of cloned *FAD* genes, has provided a reasonable picture of the fatty acid desaturation pathways in this alga (Fig. 3).

Putative orthologs for the plastidic desaturases encoded by *Arabidopsis FAD5* (MGDG palmitate-Δ7-desaturase), *FAD6* (ω6-desaturase), and *FAD7* or *FAD8* (encoding ω3-desaturase isozymes) are present in the *Chlamydomonas* genome (Riekhof et al., 2005b; Riekhof and Benning, 2008). Of these, only the *Chlamydomonas FAD6* gene has been studied to date at the molecular/genetic level (Sato et al., 1995b, 1997). The *hf-9* mutant was first isolated by its high chlorophyll fluorescence phenotype, and detailed lipid analysis revealed an apparent defect in ω6-desaturase activity as it showed marked decreases in both 16- and 18-carbon polyunsaturated fatty acyl groups, with concomitant increases in $16{:}1^{\Delta 7}$ and $18{:}1^{\Delta 9}$ (Sato et al., 1995b). The *hf-9* mutant had an increased doubling time and showed reduced photosynthetic O_2 evolution as well as an altered chloroplast ultrastructure (Sato et al., 1995b). The *Chlamydomonas FAD6* gene (first described as *DES6*) was subsequently cloned and found to be highly similar to cyanobacterial Δ12- and seed plant ω6-desaturases, and was also shown to complement the *hf-9* mutant desaturation defects (Sato et al., 1997). However, it did not restore the photosynthetic defects, suggesting that these phenotypes arose from a mutation in another gene (or possibly from multiple loci). As such, the roles of polyunsaturated fatty acids (PUFAs) in the assembly or maintenance of optimally functioning photosynthetic membranes in *Chlamydomonas* cannot be easily deduced from analysis of *hf*-9. The function of PUFAs in this regard have been studied and found to differ in mutants of both plants and cyanobacteria. The *Arabidopsis fad6 fad2* double mutant, which lacks both plastidic and ER ω6-desaturases exhibited severe growth and photosynthetic defects (McConn and Browse, 1998). In contrast,

a mutant of *Synechocystis* sp. PC 6,803 lacking PUFAs had no observable photosynthetic defects under normal growth conditions (Gombos et al., 1992). Thus, it still remains to be determined whether the importance of PUFAs in photosynthetic membrane function in *Chlamydomonas* is more similar to that of seed plants or cyanobacteria. Other plastidic desaturases still have no gene candidates in *Chlamydomonas*. As noted above, the desaturase producing $16{:}1^{\Delta 3trans}$ specifically on plastidic PG is still not identified at the molecular level in plants or algae, although mutants lacking this fatty acid have been obtained in *Arabidopsis* and *Chlamydomonas* (Browse et al., 1985; Maroc et al., 1987). Likewise, a gene encoding Δ4 desaturase activity, which is specific for MGDG-esterified 16-carbon acyl groups based on biochemical studies (Giroud et al., 1988), and presumably produces both the $16{:}3^{\Delta 4,7,10}$ and $16{:}4^{\Delta 4,7,10,13}$ found in *Chlamydomonas* is currently unidentified.

The extraplastidic ω6- and ω3-desaturases, which produce $18{:}2^{\Delta 9,12}$ and $18{:}3^{\Delta 9,12,15}$, are encoded by *FAD2* and *FAD3*, respectively, in *Arabidopsis*, and putative orthologs of these genes have been identified in the *Chlamydomonas* genome (Riekhof et al., 2005b; Riekhof and Benning, 2008). The extraplastidic *Chlamydomonas* lipids DGTS and PE have been shown to contain significant amounts of $18{:}3^{\Delta 5,9,12}$ and $18{:}4^{\Delta 5,9,12,15}$ esterified to the respective *sn*-2 positions of the glycerol back bone (Giroud et al., 1988); these Δ5-unsaturated fatty acids are also found in gymnosperms (Mongrand et al., 2001; Wolff and Christie, 2002). Recently a *Chlamydomonas* gene, *CrDES*, encoding a "front-end" type Δ5-desaturase was identified by a bioinformatics approach through its similarity to a known Δ5-desaturase from the liverwort *Marchantia polymorpha* (Kajikawa et al., 2006). Heterologous expression of *CrDES* in *Pichia pastoris* and analysis of desaturase activity indicated that while the primary substrates were $18{:}2^{\Delta 9,12}$ and $18{:}3^{\Delta 9,12,15}$, low but detectable levels of endogenous $18{:}1^{\Delta 9}$ desaturation were also observed (Kajikawa et al., 2006). Transgenic tobacco plants constitutively expressing the *CrDES* gene exhibited strikingly high levels of $18{:}3^{\Delta 5,9,12}$ and $18{:}4^{\Delta 5,9,12,15}$ (which are normally absent), with the highest combined yield reaching ~45% of leaf total fatty acids, and no apparent morphological phenotypes (Kajikawa et al., 2006). The biological roles of these Δ5-unsaturated fatty acids in the organisms which produce them are largely unknown, and the identification of the *CrDES* gene responsible for $18{:}3^{\Delta 5,9,12}$ and $18{:}4^{\Delta 5,9,12,15}$ production in *Chlamydomonas* will allow for this gene to be targeted for suppression through RNAi technology.

V Neutral Lipid Metabolism

To date, little research has been done on neutral glycerolipid synthesis in *Chlamydomonas*. However, there is an increasing focus on oil production in microalgae due to its potential role as a feedstock for biodiesel or jet fuels (Hu et al., 2008), and as a source of commercial oils and fatty acids (Spolaore et al., 2006). Beginning in the late 1970s, the Department of Energy initiated a two decade-spanning research effort, the Aquatic Species Program, to investigate the possibility of obtaining biodiesel from microalgae (Sheehan et al., 1998). Researchers screened numerous algal strains for oil production, and found many that accumulated oil up to 75% dry weight (Benamotz and Tornabene, 1985; Bigogno et al., 2002; Chisti, 2007). *Chlamydomonas* also accumulates oil in the form of triacylglycerol under certain conditions, as already mentioned above (Weers and Gulati, 1997).

In most algae species, oil production is triggered by environmental stress, suggesting that triacylglycerol plays a role in microalgae beyond energy storage. One of the main stresses investigated is nutrient deprivation, with nitrogen deprivation being the most common condition used. Growing the chlorophyte *Neochloris oleoabundans* in growth media limited for nitrogen resulted in lipids being accumulated up to 56% of the total dry weight, with 80% of that being triacylglycerol (TAG) (Tornabene et al., 1983). The eustigmatophyte *Nannochloropsis* gave similar results (Suen et al., 1987) as did the chlorophyte *Parietochloris incise* (Merzlyak et al., 2007). Growing the chlorophyte *Haematococcus pluvialis* in nitrogen-free medium led not only to an increase in total lipid content, but also to a change in fatty acid composition, with an increase in oleic acid (Zhekisheva et al., 2002). Other nutrient deficiencies can trigger lipid accumulation. For example, silicon deficiency leads to increased lipid content (mainly in the form of TAG) in the diatom

Cyclotella cryptica (Roessler, 1988). Phosphate limitation leads to an increase in TAG and overall lipid levels in some green algae, and to a decrease in lipid content in others (Khozin-Goldberg and Cohen, 2006).

Other factors, such as light, temperature and growth phase also affect oil accumulation in microalgae. The effect of temperature on lipid accumulation varies between strains, with some reporting increases in lipid levels, and others decreases (Richardson et al., 1983; Dempster and Sommerfeld, 1998; de Swaaf et al., 1999). Inhibition of cell cycle in the chlorophyte *Chlorella* by high pH leads to an accumulation of TAG similar to that due to nutrient deprivation, suggesting that environmental stress may indirectly trigger TAG synthesis by inhibiting growth, rather than directly (Guckert and Cooksey, 1990). High light intensity has been shown to increase the ratio of TAG to total lipids, although the total lipid level can remain the same or decrease (Zhekisheva et al., 2002; Khotimchenko and Yakovleva, 2004, 2005). In the chlorophyte *Dunaliella bardawil*, the accumulation of TAG under high-light stress is linked to an accumulation of β-carotene, which suggests that TAG accumulation may help to protect the chloroplasts from photooxidative damage (Benamotz et al., 1989; Rabbani et al., 1998).

The biochemistry of TAGs in microalgae has not been studied, including that in the genetic model *Chlamydomona*s. Previous research has indicated that lipid synthesis in *Chlamydomonas* is homologous to that in plants (Fig. 1), and possibly simpler (Riekhof et al., 2005b; Riekhof and Benning, 2008). Therefore, it is likely that many general aspects of TAG synthesis in *Chlamydomonas* follow that of plants. One common path for TAG synthesis in seed plants is the Kennedy pathway, which involves the stepwise addition of fatty-acyl groups to a glycerol-3-phosphate to form PA, which is converted to DAG by phosphatidic acid phosphatase; DAG is further acylated by diacylglycerol acyltransferase to form TAG, as discussed further below (Kennedy, 1961). Two genes encoding putative extraplastidic phosphatidic acid phosphatases have been identified in the *Chlamydomonas* genome, but have not yet been studied in molecular detail (Riekhof et al., 2005b). Several studies in many different plant species have also indicated that DAG derived from the PC pool also contributes substantially to TAG biosynthesis (Ohlrogge and Browse, 1995). Clearly, PC plays no role in TAG biosynthesis in *Chlamydomonas*, and its role in plants is not universal, as studies of mesocarp microsomes in avocado indicated that only the Kennedy pathway was active (Stobart and Stymne, 1985). It may be possible that the assumed functional analog of PC in *Chlamydomonas*, DGTS, is an intermediate in the biosynthesis of TAG. However, no biochemical studies to determine this have been undertaken to date, and utilization of the DGTS pool to provide DAG precursors would require an as yet unidentified enzyme to remove the ether-linked trimethylhomoserine head group. Regardless of the precursors used in forming DAG, the final step is catalyzed by diacylglycerol acyltransferases, or DGATs, which transfer a fatty acid from acyl-CoA to diacylglycerol. DGATs have been isolated and characterized from several plant species, including *Arabidopsis* (Routaboul et al., 1999; Zou et al., 1999; Hobbs et al., 1999), maize (Zheng et al., 2008) and castor beans (Kroon et al., 2006). The *Chlamydomonas* genome contains a number of putative DGAT isoforms yet to be studied in molecular detail (Riekhof and Benning, 2008; R. Miller and C. Benning, unpublished, 2009).

*Chlamydomona*s may not only utilize the Kennedy pathway for TAG synthesis. Indeed, an alternate pathway for TAG synthesis involves phospholipid: diacylglycerol acyltransferases, or PDATs, to generate TAG using a phospholipid as a fatty acid donor, rather than acyl-CoA. PDATs have also been found in plants (Dahlqvist et al., 2000) and represent a possible second type of enzyme that is also present in *Chlamydomonas* (Riekhof and Benning, 2008). Given the induction of TAG biosynthesis by different stresses, it is likely that the mechanism for the regulation of TAG synthesis differs in *Chlamydomonas* from that in seed plants, which often produce oil during a specific phase of their life cycle and in specialized tissues.

VI Perspectives

The study of lipid metabolism in microalgae is experiencing a renaissance due to their potential for the production of large quantities of biomass

in general, and specifically due to their ability to accumulate neutral lipids following nutrient deprivation. However, during the past decade much of basic research on lipid metabolism in photosynthetic organisms was focused on cyanobacteria and seed plants, in particular, the genetic and genomic model *Arabidopsis*. *Chlamydomonas* has been developed over the years as an excellent genetic model as well, however, not necessarily for the study of lipid metabolism. The availability of the *Chlamydomonas* genomic sequence (Merchant et al., 2007) has made the application of knowledge on well studied lipid metabolism in seed plants to this model alga relatively facile using comparative genomics. The result is a testable hypothesis of lipid metabolism in *Chlamydomonas* (Fig. 1) based on genome annotation (Riekhof et al., 2005b; Riekhof and Benning, 2008), which provides a wealth of opportunities to students of lipid metabolism. Those researchers interested in studying basic lipid metabolism in photosynthetic organisms might wonder what novel concepts research on *Chlamydomonas* could contribute beyond research on *Arabidopsis*. The answer lies in the fact that lipid metabolism in *Chlamydomonas* appears simpler and in some aspects drastically different from that in seed plants as discussed in detail above. The reduced redundancy in *Chlamydomonas* versus *Arabidopsis* permits testing of hypotheses on the function of parallel pathways, e.g., galactoglycerolipid biosynthesis (Härtel et al., 2000), present in *Arabidopsis*. Moreover, the unicellular organization of *Chlamydomonas* and its resulting lifestyle requires completely different input for the regulation of TAG biosynthesis (Hu et al., 2008) than the developmental regulation of storage lipid metabolism in developing seeds of *Arabidopsis* (Santos-Mendoza et al., 2008). The absence of PC in *Chlamydomonas* challenges concepts about the role of this lipid as a central metabolite in lipid trafficking and lipid modification in plants. How widespread the replacement of PC by the betaine lipid DGTS in microalgae is not known at this time, but it would be important to explore, if *Chlamydomonas* is to become the model for the engineering of microalgal biofuel-producing strains.

Acknowledgements

Work on lipid biosynthesis in plants and algae in the Benning lab is currently supported, in part, by grants from the US Air Force Office of Scientific Research, the US National Science Foundation, the US Department of Energy, the Great Lakes Bioenergy Research Center, BASF-Plant Sciences, the MSU Center of Excellence for the Structural Biology Membrane Proteins, and the Michigan Agricultural Experiment Station.

References

Babiychuk E, Müller F, Eubel H, Braun HP, Frentzen M and Kushnir S (2003) *Arabidopsis* phosphatidylglycerophosphate synthase 1 is essential for chloroplast differentiation, but is dispensable for mitochondrial function. Plant J 33: 899–909

Beisson F, Koo AJ, Ruuska S, Schwender J, Pollard M, Thelen JJ, Paddock T, Salas JJ, Savage L, Milcamps A, Mhaske VB, Cho Y and Ohlrogge JB (2003) *Arabidopsis* genes involved in acyl lipid metabolism. A 2003 census of the candidates, a study of the distribution of expressed sequence tags in organs, and a web-based database. Plant Physiol 132: 681–697

Benamotz A and Tornabene TG (1985) Chemical profile of selected species of microalgae with emphasis on lipids. J Phycol 21: 72–81

Benamotz A, Shaish A and Avron M (1989) Mode of action of the massively accumulated β-carotene of *Dunaliella bardawil* in protecting the alga against damage by excess irradiation. Plant Physiol 91: 1040–1043

Benning C (2008) A role for lipid trafficking in chloroplast biogenesis. Prog Lipid Res 47: 381–389

Benning C and Ohta H (2005) Three enzyme systems for galactoglycerolipid biosynthesis are coordinately regulated in plants. J Biol Chem 280: 2397–2400

Benning C, Beatty JT, Prince RC and Somerville CR (1993) The sulfolipid sulfoquinovosyldiacylglycerol is not required for photosynthetic electron transport in *Rhodobacter sphaeroides* but enhances growth under phosphate limitation. Proc Natl Acad Sci USA 90: 1561–1565

Benning C, Huang ZH and Gage DA (1995) Accumulation of a novel glycolipid and a betaine lipid in cells of *Rhodobacter sphaeroides* grown under phosphate limitation. Arch Biochem Biophys 317: 103–111

Benning C, Garavito RM, Shimojima M (2008) Sulfolipid biosynthesis and function in plants. In: Hell R, Dahl C, Knaff D and Leusteck T (eds) Sulfur Metabolism in Phototrophic Organisms. Springer, Dordrecht, pp. 185–200

Bigogno C, Khozin-Goldberg I, Boussiba S, Vonshak A and Cohen Z (2002) Lipid and fatty acid composition of the green oleaginous alga *Parietochloris incisa*, the richest plant source of arachidonic acid. Phytochemistry 60: 497–503

Blouin A, Lavezzi T and Moore TS (2003) Membrane lipid biosynthesis in *Chlamydomonas reinhardtii*. Partial characterization of CDP-diacylglycerol:*myo*-inositol 3-phosphatidyltransferase. Plant Physiol Biochem 41: 11–16

Browse J and Somerville C (1991) Glycerolipid biosynthesis: Biochemistry and regulation. Annu Rev Plant Physiol Plant Mol Biol 42: 467–506

Browse J, McCourt P and Somerville C (1985) A mutant of *Arabidopsis* lacking a chloroplast-specific lipid. Science 227: 763–765

Browse J, Warwick N, Somerville CR and Slack CR (1986) Fluxes through the prokaryotic and eukaryotic pathways of lipid synthesis in the "16:3" plant *Arabidopsis thaliana*. Biochem J 235: 25–31

Cedergren RA and Hollingsworth RI (1994) Occurrence of sulfoquinovosyl diacylglycerol in some members of the family *Rhizobiaceae*. J Lipid Res 35: 1452–1461

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25: 294–306

Dahlqvist A, Stahl U, Lenman M, Banas A, Lee M, Sandager L, Ronne H and Stymne H (2000) Phospholipid: diacylglycerol acyltransferase: An enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. Proc Natl Acad Sci USA 97: 6487–6492

Davies JP, Yildiz F and Grossman AR (1994) Mutants of *Chlamydomonas* with aberrant responses to sulfur deprivation. Plant Cell 6: 53–63

Davies JP, Yildiz FH and Grossman A (1996) Sac1, a putative regulator that is critical for survival of *Chlamydomonas reinhardtii* during sulfur deprivation. EMBO J 15: 2150–2159

Davies JP, Yildiz FH and Grossman AR (1999) Sac3, an Snf1-like serine/threonine kinase that positively and negatively regulates the responses of *Chlamydomonas* to sulfur limitation. Plant Cell 11: 1179–1190

Dempster TA and Sommerfeld MR (1998) Effects of environmental conditions on growth and lipid accumulation in *Nitzschia communis* (Bacillariophyceae). J Phycol 34: 712–721

De Swaaf ME, de Rijk TC, Eggink G and Sijtsma L (1999) Optimisation of docosahexaenoic acid production in batch cultivations by *Crypthecodinium cohnii*. J Biotech 70: 185–192

Dörmann P and Benning C (2002) Galactolipids rule in seed plants. Trends Plant Sci 7: 112–118

Dubertret G, Mirshahi A, Mirshahi M, Gerard-Hirne C and Tremolieres A (1994) Evidence from in vivo manipulations of lipid composition in mutants that the Δ^3-*trans*-hexadecenoic acid-containing phosphatidylglycerol is involved in the biogenesis of the light-harvesting chlorophyll a/b-protein complex of *Chlamydomonas reinhardtii*. Eur J Biochem 226: 473–482

Dubertret G, Gerard-Hirne C and Tremolieres A (2002) Importance of *trans*-Δ^3-hexadecenoic acid containing phosphatidylglycerol in the formation of the trimeric light-harvesting complex in *Chlamydomonas*. Plant Physiol Biochem 40: 829–836

Eichenberger W (1982) Distribution of diacylglyceryl-*O*-4′-(*N*,*N*,*N*-trimethyl)homoserine in different algae. Plant Sci Lett 24: 91–95

Eichenberger W (1993) Betaine lipids in lower plants. Distribution of DGTS, DGTA and phospholipids, and the intracellular localization and site of biosynthesis of DGTS. Plant Physiol Biochem 31: 213–221

Eichenberger W and Boschetti A (1977) Occurrence of 1(3),2-diacylglyceryl-(3)-*O*-4′-(*N*,*N*,*N*-trimethyl)homoserine in *Chlamydomonas reinhardtii*. FEBS Lett 88: 201–204

Essigmann B, Güler S, Narang RA, Linke D and Benning C (1998) Phosphate availability affects the thylakoid lipid composition and the expression of *SQD1*, a gene required for sulfolipid biosynthesis in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 95: 1950–1955

Frentzen M (2004) Phosphatidylglycerol and sulfoquinovosyldiacylglycerol: anionic membrane lipids and phosphate regulation. Curr Opin Plant Biol 7: 270–276

Fuhrmann M, Stahlberg A, Govorunova E, Rank S and Hegemann P (2001) The abundant retinal protein of the *Chlamydomonas* eye is not the photoreceptor for phototaxis and photophobic responses. J Cell Sci 114: 3857–3863

Garnier J, Maroc J and Guyon D (1987) Characterization of new strains of photosynthetic mutants of *Chlamydomonas reinhardtii*. IV. Impaired excitation energy transfer in three low fluorescent mutants. Plant Cell Physiol 28: 1117–1131

Garnier J, Wu B, Maroc J, Guyon D and Tremolieres A (1990) Restoration of both an oligomeric form of the light-harvesting antenna CP II and a fluorescence state II-state I transition by Δ^3-*trans*-hexadecenoic acid-containing phosphatidylglycerol in cells of a mutant of *Chlamydomonas reinhardtii*. Biochim Biophys Acta 1020: 153–162

Giroud C, Gerber A and Eichenberger W (1988) Lipids of *Chlamydomonas reinhardtii*. Analysis of molecular species and intracellular site(s) of biosynthesis. Plant Cell Physiol 29: 587–595

Gombos Z, Wada H and Murata N (1992) Unsaturation of fatty acids in membrane lipids enhances tolerance of the cyanobacterium *Synechocystis* PCC 6803 to low-temperature photoinhibition. Proc Natl Acad Sci USA 89: 9959–9963

Gounaris K and Barber J (1985) Isolation and characterisation of a photosytem II reaction center lipoprotein complex. FEBS Lett 188: 68–72

Grenier G, Guyon D, Roche O, Dubertret G and Tremolieres A (1991) Modification of the membrane fatty-acid composition of *Chlamydomonas reinhardtii* cultured in the presence of liposomes. Plant Physiol Biochem 29: 429–440

Grossman AR, Croft M, Gladyshev VN, Merchant SS, Posewitz MC, Prochnik S and Spalding MH (2007) Novel metabolism in *Chlamydomonas* through the lens of genomics. Curr Opin Plant Biol 10: 190–198

Guckert JB and Cooksey KE (1990) Triglyceride accumulation and fatty acid profile changes in *Chlorella* (Chlorophyta) during high pH-induced cell cycle inhibition. J Phycol 26: 72–79

Hagio M, Gombos Z, Varkonyi Z, Masamoto K, Sato N, Tsuzuki M and Wada H (2000) Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis. Plant Physiol 124: 795–804

Hagio M, Sakurai I, Sato S, Kato T, Tabata S and Wada H (2002) Phosphatidylglycerol is essential for the development of thylakoid membranes in *Arabidopsis thaliana*. Plant Cell Physiol 43: 1456–1464

Härtel H, Dörmann P and Benning C (2000) DGD1-independent biosynthesis of extraplastidic galactolipids following phosphate deprivation in *Arabidopsis*. Proc Natl Acad Sci USA 97: 10649–10654

Heinz E and Roughan G (1983) Similarities and differences in lipid metabolism of chloroplasts isolated from 18:3 and 16:3 plants. Plant Physiol 72: 273–279

Hobbs DH, Lu C and Hills MJ (1999) Cloning of a cDNA encoding diacylglycerol acyltransferase from *Arabidopsis thaliana* and its functional expression. FEBS Lett 452: 145–149

Hofmann M and Eichenberger W (1996) Biosynthesis of diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine in *Rhodobacter sphaeroides* and evidence for lipid-linked N methylation. J Bacteriol 178: 6140–6144

Holzl G and Dörmann P (2007) Structure and function of glycoglycerolipids in plants and bacteria. Prog Lipid Res 46: 225–243

Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M and Darzins A (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54: 621–639

Janero DR and Barrnett R (1981a) Cellular and thylakoid-membrane glycolipids of *Chlamydomonas reinhardtii* 137 $^{+}$. J Lipid Res 22: 1119–1125

Janero DR and Barrnett R (1981b) Cellular and thylakoid-membrane phospholipids of *Chlamydomonas reinhardtii* 137 $^{+}$. J Lipid Res 22: 1126–1130

Janero DR and Barrnett R (1982) Isolation and characterization of an ether-linked homoserine lipid from the thylakoid membrane of C*hlamydomonas reinhardtii* 137 $^{+}$. J Lipid Res 23: 307–316

Jouhet J, Marechal E, Baldan B, Bligny R, Joyard J and Block MA (2004) Phosphate deprivation induces transfer of DGDG galactolipid from chloroplast to mitochondria. J Cell Biol 167: 863–874

Jouhet J, Marechal E and Block MA (2007) Glycerolipid transfer for the building of membranes in plant cells. Prog Lipid Res 46: 37–55

Joyard J, Teyssier E, Miege C, Berny-Seigneurin D, Marechal E, Block MA, Dorne AJ, Rolland N, Ajlani G and Douce R (1998) The biochemical machinery of plastid envelope membranes. Plant Physiol 118: 715–723

Kajikawa M, Yamato KT, Kohzu Y, Shoji S, Matsui K, Tanaka Y, Sakai Y and Fukuzawa H (2006) A front-end desaturase from *Chlamydomonas reinhardtii* produces pinolenic and coniferonic acids by ω 13 desaturation in methylotrophic yeast and tobacco. Plant Cell Physiol 47: 64–73

Kathir P, LaVoie M, Brazelton WJ, Haas NA, Lefebvre PA and Silflow CD (2003) Molecular map of the *Chlamydomonas reinhardtii* nuclear genome. Euk Cell 2: 362–379

Kelly AA and 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: 1166–1173

Kennedy EP (1961) Biosynthesis of complex lipids. Fed Proc 20: 934–940

Khotimchenko SV and Yakovleva IM (2004) Effect of solar irradiance on lipids of the green alga *Ulva fenestrata* Postels *et* Ruprecht. Bot Mar 47: 395–401

Khotimchenko SV and Yakovleva IM (2005) Lipid composition of the red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance. Phytochemistry 66: 73–79

Khozin-Goldberg I and Cohen Z (2006) The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte *Monodus subterraneus*. Phytochemistry 67: 696–701

Kim HU and Huang AH (2004) Plastid lysophosphatidyl acyltransferase is essential for embryo development in *Arabidopsis*. Plant Physiol 134: 1206–1216

Kim HU, Li Y and Huang AH (2005) Ubiquitous and endoplasmic reticulum-located lysophosphatidyl acyltransferase, LPAT2, is essential for female but not male gametophyte development in *Arabidopsis*. Plant Cell 17: 1073–1089

Klug RM and Benning C (2001) Two enzymes of diacylglyceryl-*O*-4′-(*N*,*N*,*N*-trimethyl)homoserine biosynthesis are encoded by *btaA* and *btaB* in the purple bacterium *Rhodobacter sphaeroides*. Proc Natl Acad Sci USA 98: 5910–5915

Kroon JTM, Wei WX, Simon WJ and Slabas AR (2006) Identification and functional expression of a type 2 acyl-CoA: diacylglycerol acyltransferase (DGAT2) in developing castor bean seeds which has high homology to the

major triglyceride biosynthetic enzyme of fungi and animals. Phytochemistry 67: 2541–2549

Kunst L, Browse J and Somerville C (1988) Altered regulation of lipid biosynthesis in a mutant of *Arabidopsis* deficient in chloroplast glycerol-3-phosphate acyltransferase activity. Proc Natl Acad Sci USA 85: 4143–4147

Maanni AE, Dubertret G, Delrieu M, Rochon A and Tremolieres A (1998) Mutants of *Chlamydomonas reinhardtii* affected in phosphatidylglycerol metabolism and thylakoid biogenesis. Plant Physiol Biochem 36: 609–619

Maroc J, Tremolieres A, Garnier J and Guyon D (1987) Oligomeric form of the light-harvesting chlorophyll a + b-protein complex CP-II, phosphatidyldiacylglycerol, Δ3-*trans*-hexadecenoic acid and energy transfer in *Chlamydomonas reinhardtii*, wild type and mutants. Biochim Biophys Acta 893: 91–99

McConn M and Browse J (1998) Polyunsaturated membranes are required for photosynthetic competence in a mutant of *Arabidopsis*. Plant J 15: 521–530

Mendiola-Morgenthaler L, Eichenberger W and Boschetti A (1985) Isolation of chloroplast envelopes from *Chlamydomonas*. Lipid and polypeptide composition. Plant Sci Lett 41: 97–104

Menke W, Radunz A, Schmid GH, Koenig F and Hirtz RD (1976) Intermolecular interactions of polypeptides and lipids in the thylakoid membrane. Z Naturforsch [C] 31: 436–444

Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Marechal-Drouard L, Marshall WF, Qu LH, Nelson DR, Sanderfoot AA, Spalding MH, Kapitonov VV, Ren Q, Ferris P, Lindquist E, Shapiro H, Lucas SM, Grimwood J, Schmutz J, Cardol P, Cerutti H, Chanfreau G, Chen CL, Cognat V, Croft MT, Dent R, Dutcher S, Fernandez E, Fukuzawa H, Gonzalez-Ballester D, Gonzalez-Halphen D, Hallmann A, Hanikenne M, Hippler M, Inwood W, Jabbari K, Kalanon M, Kuras R, Lefebvre PA, Lemaire SD, Lobanov AV, Lohr M, Manuell A, Meier I, Mets L, Mittag M, Mittelmeier T, Moroney JV, Moseley J, Napoli C, Nedelcu AM, Niyogi K, Novoselov SV, Paulsen IT, Pazour G, Purton S, Ral JP, Riano-Pachon DM, Riekhof W, Rymarquis L, Schroda M, Stern D, Umen J, Willows R, Wilson N, Zimmer SL, Allmer J, Balk J, Bisova K, Chen CJ, Elias M, Gendler K, Hauser C, Lamb MR, Ledford H, Long JC, Minagawa J, Page MD, Pan J, Pootakham W, Roje S, Rose A, Stahlberg E, Terauchi AM, Yang P, Ball S, Bowler C, Dieckmann CL, Gladyshev VN, Green P, Jorgensen R, Mayfield S, Mueller-Roeber B, Rajamani S, Sayre RT, Brokstein P, Dubchak I, Goodstein D, Hornick L, Huang YW, Jhaveri J, Luo Y, Martinez D, Ngau WC, Otillar B, Poliakov A, Porter A, Szajkowski L, Werner G, Zhou K, Grigoriev IV, Rokhsar DS and Grossman AR (2007) The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. Science 318: 245–250

Merzlyak MN, Chivkunova OB, Gorelova OA, Reshetnikova IV, Solovchenko AE, Khozin-Goldberg I and Cohen Z (2007) Effect of nitrogen starvation on optical properties, pigments, and arachidonic acid content of the unicellular green alga *Parietochloris incisa* (Trebouxiophyceae, Chlorophyta). J Phycol 43: 833–843

Minoda A, Sato N, Nozaki H, Okada K, Takahashi H, Sonoike K and Tsuzuki M (2002) Role of sulfoquinovosyl diacylglycerol for the maintenance of photosystem II in *Chlamydomonas reinhardtii*. Eur J Biochem 269: 2353–2358

Minoda A, Sonoike K, Okada K, Sato N and Tsuzuki M (2003) Decrease in the efficiency of the electron donation to tyrosine Z of photosystem II in an SQDG-deficient mutant of *Chlamydomonas*. FEBS Lett 553: 109–112

Mongrand S, Besoule J-J, Cabantous F and Cassagne C (1998) The C16:3/C18:3 fatty acid balance in photosynthetic tissues from 468 plant species. Phytochemistry 49: 1049–1064

Mongrand S, Badoc A, Patouille B, Lacomblez C, Chavent M, Cassagne C and Bessoule JJ (2001) Taxonomy of gymnospermae: multivariate analyses of leaf fatty acid composition. Phytochemistry 58: 101–115

Moore B (2004) Bifunctional and moonlighting enzymes: lighting the way to regulatory control. Trends Plant Sci 9: 221–228

Moore TS, Du Z and Chen Z (2001) Membrane lipid biosynthesis in *Chlamydomonas reinhardtii*. In vitro biosynthesis of diacylglyceryltrimethylhomoserine. Plant Physiol 125: 423–429

Murata N and Tasaka Y (1997) Glycerol-3-phosphate acyltransferase in plants. Biochim Biophys Acta 1348: 10–16

Nakamura Y, Tsuchiya M and Ohta H (2007) Plastidic phosphatidic acid phosphatases identified in a distinct subfamily of lipid phosphate phosphatases with prokaryotic origin. J Biol Chem 282: 29013–29021

Nerlich A, von Orlow M, Rontein D, Hanson AD and Dörmann P (2007) Deficiency in phosphatidylserine decarboxylase activity in the *psd1 psd2 psd3* triple mutant of *Arabidopsis* affects phosphatidylethanolamine accumulation in mitochondria. Plant Physiol 144: 904–914

Niyogi KK (1999) Photoprotection revisited: Genetic and molecular approaches. Annu Rev Plant Physiol Plant Mol Biol 50: 333–359

Ohlrogge J and Browse J (1995) Lipid biosynthesis. Plant Cell 7: 957–970

Picaud A, Creach A and Tremolieres A (1991) Studies on the stimulation by light of fatty-acid synthesis in *Chlamydomonas reinhardtii* whole cells. Plant Physiol Biochem 29: 441–448

Pick U, Gounaris K, Weiss M and Barber J (1985) Tightly bound sulfolipids in chloroplast CF_0-CF_1. Biochim Biophys Acta 808: 415–420

Pineau B, Girard-Bascou J, Eberhard S, Choquet Y, Tremolieres A, Gerard-Hirne C, Bennardo-Connan A, Decottignies P, Gillet S and Wollman FA (2004) A single mutation that causes phosphatidylglycerol deficiency impairs synthesis of photosystem II cores in *Chlamydomonas reinhardtii*. Eur J Biochem 271: 329–338

Rabbani S, Beyer P, Von Lintig J, Hugueney P and Kleinig H (1998) Induced β-carotene synthesis driven by triacylglycerol deposition in the unicellular alga *Dunaliella bardawil*. Plant Physiol 116: 1239–1248

Reyes-Prieto A, Weber AP and Bhattacharya D (2007) The origin and establishment of the plastid in algae and plants. Annu Rev Genet 41: 147–168

Richardson K, Beardall J and Raven JA (1983) Adaptation of unicellular algae to irradiance - an analysis of strategies. New Phytol 93: 157–191

Riekhof WR and Benning C (2008) Glycerolipid biosynthesis. In: Stern D and Harris EH (eds) The Chlamydomonas Sourcebook: Organellar and Metabolic Processes, 2. Elsevier, Dordrecht, pp. 41–68

Riekhof WR, Ruckle ME, Lydic TA, Sears BB and Benning C (2003) The sulfolipids 2′-*O*-acyl-sulfoquinovosyldiacylglycerol and sulfoquinovosyldiacylglycerol are absent from a *Chlamydomonas reinhardtii* mutant deleted in SQD1. Plant Physiol 133: 864–874

Riekhof WR, Andre C and Benning C (2005a) Two enzymes, BtaA and BtaB, are sufficient for betaine lipid biosynthesis in bacteria. Arch Biochem Biophys 441: 96–105

Riekhof WR, Sears BB and Benning C (2005b) Annotation of genes involved in glycerolipid biosynthesis in *Chlamydomonas reinhardtii*: discovery of the betaine lipid synthase BTA1Cr. Euk Cell 4: 242–252

Roessler PG (1988) Effects of silicon deficiency on lipid composition and metabolism in the diatom *Cyclotella cryptica*. J Phycol 24: 394–400

Roughan PG and Slack CR (1982) Cellular organization of glycerolipid metabolism. Ann Rev Plant Physiol 33: 97–132

Roughan PG, Holland R and Slack CR (1980) The role of chloroplasts and microsomal fractions in polar-lipid synthesis from [1–^{14}C]acetate by cell-free preparations from spinach (*Spinacia oleracea*) leaves. Biochem J 188: 17–24

Routaboul JM, Benning C, Bechtold N, Caboche M and Lepiniec L (1999) The TAG1 locus of *Arabidopsis* encodes for a diacylglycerol acyltransferase. Plant Physiol Biochem 37: 831–840

Sanda S, Leustek T, Theisen M, Garavito M and Benning C (2001) Recombinant *Arabidopsis* SQD1 converts UDP-glucose and sulfite to the sulfolipid head precursor UDP-sulfoquinovose in vitro. J Biol Chem 276: 3941–3946

Santos-Mendoza M, Dubreucq B, Baud S, Parcy F, Caboche M and Lepiniec L (2008) Deciphering gene regulatory networks that control seed development and maturation in *Arabidopsis*. Plant J 54: 608–620

Sato N (1988) Dual role of methionine in the biosynthesis of diacylglyceryltrimethylhomoserine in *Chlamydomonas reinhardtii*. Plant Physiol 86: 931–934

Sato N (1991) Lipids in *Cryptomonas* CR-1. II. Biosynthesis of betaine lipids and galactolipids. Plant Cell Physiol 32: 845–851

Sato N (1992) Betaine lipids. Bot Mag Tokyo 105: 185–197

Sato N and Furuya M (1983) Isolation and identification of diacylglyceryl-*O*-4′-(*N*,*N*,*N*-trimethyl)-homoserine from the fern *Adiantum capillus-veneris* L. Plant Cell Physiol 24: 1113–1120

Sato N and Kato K (1988) Analysis and biosynthesis of diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine in cells of *Marchantia* in suspension culture. Plant Sci 55: 21–25

Sato N and Murata N (1991) Transition of lipid phase in aqueous dispersions of diacylglyceryltrimethylhomoserine. Biochim Biophys Acta 1082: 108–111

Sato N, Sonoike K, Tsuzuki M and Kawaguchi A (1995a) Impaired photosystem II in a mutant of *Chlamydomonas reinhardtii* defective in sulfoquinovosyl diacylglycerol. Eur J Biochem 234: 16–23

Sato N, Tsuzuki M, Matsuda Y, Ehara T, Osafune T and Kawaguchi A (1995b) Isolation and characterization of mutants affected in lipid metabolism of *Chlamydomonas reinhardtii*. Eur J Biochem 230: 987–993

Sato N, Fujiwara S, Kawaguchi A and Tsuzuki M (1997) Cloning of a gene for chloroplast omega6 desaturase of a green alga, *Chlamydomonas reinhardtii*. J Biochem 122: 1224–1232

Sato N, Hagio M, Wada H and Tsuzuki M (2000) Requirement of phosphatidylglycerol for photosynthetic function in thylakoid membranes. Proc Natl Acad Sci USA 97: 10655–10660

Shanklin J and Somerville C (1991) Stearoyl-acyl-carrier-protein desaturase from higher plants is structurally unrelated to the animal and fungal homologs. Proc Natl Acad Sci USA 88: 2510–2514

Sheehan J, Dunahay T, Benemann J, and Roessler PA (1998) Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae. US Department of Energy National Renewable Energy Laboratory, http://www1.eere.energy.gov/biomass/pdfs/biodiesel_from_algae.pdf

Silflow CD and Lefebvre PA (2001) Assembly and motility of eukaryotic cilia and flagella. Lessons from *Chlamydomonas reinhardtii*. Plant Physiol 127: 1500–1507

Sineshchekov OA, Jung KH and Spudich JL (2002) Two rhodopsins mediate phototaxis to low- and high-intensity light in *Chlamydomonas reinhardtii*. Proc Natl Acad Sci USA 99: 8689–8694

Sirevag R and Levine RP (1972) Fatty acid synthetase from *Chlamydomonas reinhardtii*. J Biol Chem 247: 2586–2591

Spolaore P, Joannis-Cassan C, Duran E and Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101: 87–96

Stobart AK and Stymne S (1985) The regulation of the fatty acid composition of the triacyglycerols in microsomal preparations from avocado mesocarp and the developing cotyledons of safflower. Planta 163: 119–125

Stroebel D, Choquet Y, Popot JL and Picot D (2003) An atypical haem in the cytochrome b_6f complex. Nature 426: 413–418

Suen Y, Hubbard JS, Holzer G and Tornabene TG (1987) Total lipid production of the green alga *Nannochloropsis* sp. QII under different nitrogen regimes. J Phycol 23: 289–296

Sugimoto K, Sato N and 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. FEBS Lett 581: 4519–4522

Sugimoto K, Midorikawa T, Tsuzuki M and Sato N (2008) Upregulation of PG synthesis on sulfur-starvation for PSI in *Chlamydomonas*. Biochem Biophys Res Commun 369: 660–665

Tam LW and Lefebvre PA (1993) Cloning of flagellar genes in *Chlamydomonas reinhardtii* by DNA insertional mutagenesis. Genetics 135: 375–384

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408: 796–815

Tornabene TG, Holzer G, Lien S and Burris N (1983) Lipid composition of the nitrogen starved green alga *Neochloris oleoabundans*. Enz Microb Tech 5: 435–440

Tsuzuki M, Ohnuma E, Sato N, Takaku T and Kawaguchi A (1990) Effects of CO_2 concentration during growth on fatty acid composition in microalgae. Plant Physiol 93: 851–856

Vance JE and Steenbergen R (2005) Metabolism and functions of phosphatidylserine. Prog Lipid Res 44: 207–234

Vijayan P, Routaboul JM and Browse J (1998) A genetic approach to investigating membrane lipid structure and photosynthetic function. In: Siegenthaler P-A and Murata N (eds) Lipids in Photosynthesis: Structure, Function and Genetics. Kluwer, Dordrecht, pp. 263–285

Vogel G and Eichenberger W (1992) Betaine lipids in lower plants. Biosynthesis of DGTS and DGTA in *Ochromonas danica* (Chrysophyceae) and the possible role of DGTS in lipid metabolism. Plant Cell Physiol 33: 427–436

Wallis JG and Browse J (2002) Mutants of *Arabidopsis* reveal many roles for membrane lipids. Prog Lipid Res 41: 254–278

Weers P and Gulati R (1997) Growth and reproduction of *Daphnia galeata* in response to changes in fatty acids, phosphorus, and nitrogen in *Chlamydomonas reinhardtii*. Limnol Oceanogr 42: 1586–1589

Wolff RL and Christie WW (2002) Structures, practical sources (gymnosperm seeds), gas-liquid chromatographic data (equivalent chain lengths), and mass spectrometric characteristics of all-*cis* 5-olefinic acids. Eur J Lipid Sci Technol 104: 234–244

Wu-Scharf D, Jeong BR, Zhang CM and Cerutti H (2000) Transgene and transposon silencing in *Chlamydomonas reinhardtii* by a DEAH-Box RNA helicase. Science 290: 1159–1162

Xu C, Härtel H, Wada H, Hagio M, Yu B, Eakin C and Benning C (2002) The *pgp1* locus of *Arabidopsis* encodes a phosphatidylglycerol synthase with impaired activity. Plant Physiol 129: 594–604

Xu C, Cornish AJ, Froehlich JE and Benning C (2006) Phosphatidylglycerol biosynthesis in chloroplasts of *Arabidopsis* mutants deficient in acyl-ACP glycerol-3-phosphate acyltransferase. Plant J 47: 296–309

Yang W, Mason CB, Pollock SV, Lavezzi T, Moroney JV and Moore TS (2004) Membrane lipid biosynthesis in *Chlamydomonas reinhardtii*: expression and characterization of CTP:phosphoethanolamine cytidylyltransferase. Biochem J 382: 51–57

Yu B, Xu C and Benning C (2002) *Arabidopsis* disrupted in SQD2 encoding sulfolipid synthase is impaired in phosphate-limited growth. Proc Natl Acad Sci USA 99: 5732–5737

Zhekisheva M, Boussiba S, Khozin-Goldberg I, Zarka A and Cohen Z (2002) Accumulation of oleic acid in *Haematococcus pluvialis* (Chlorophyceae) under nitrogen starvation or high light is correlated with that of astaxanthin esters. J Phycol 38: 325–331

Zheng P, Allen WB, Roesler K, Williams ME, Zhang S, Li J, Glassman K, Ranch J, Nubel D, Solawetz W, Bhattramakki D, Llaca V, Deschamps S, Zhong GY, Tarczynski MC and Shen B (2008) A phenylalanine in DGAT is a key determinant of oil content and composition in maize. Nat Genet 40: 367–372

Zheng Z, Xia Q, Dauk M, Shen W, Selvaraj G and Zou J (2003) *Arabidopsis* AtGPAT1, a member of the membrane-bound glycerol-3-phosphate acyltransferase gene family, is essential for tapetum differentiation and male fertility. Plant Cell 15: 1872–1887

Zou J, Wei Y, Jako C, Kumar A, Selvaraj G and Taylor DC (1999) The *Arabidopsis thaliana* TAG1 mutant has a mutation in a diacylglycerol acyltransferase gene. Plant J 19: 645–653