Algal Lipids and Their Metabolism

2

Irina A. Guschina and John L. Harwood

1 Introduction

Algal lipids can be divided into two main groups: the non-polar lipids (acylglycerols, sterols, free (non-esterified) fatty acids, hydrocarbons, wax and steryl esters) and polar lipids (phosphoglycerides, glycosylglycerides) (Gunstone et al. 2007). They are essential constituents of all living cells where they perform important functions.

Phosphoglycerides, glycosylglycerides and sterols are essential structural components of biological membranes. These lipids maintain specific membrane functions and provide the permeability barrier surrounding cells and between organelles within cells, as well as providing a matrix for various metabolic processes. Some polar lipids may act as key intermediates (or precursors of intermediates) in cell signalling pathways (e.g. inositol lipids, sphingolipids, oxidative products of polyunsaturated fatty acids). The non-polar lipids, mainly triacylglycerols (TAG), are abundant storage products which can be easily catabolised to provide metabolic energy (Gurr et al. 2002). Waxes commonly contribute to the extracellular surface layers covering different parts of higher plants. Moreover, they may act (in the form of wax esters) as energy stores especially in some organisms from cold water habitats (Guschina and Harwood 2007, 2008).

Algae comprise a large group of photosynthetic, heterotrophic organisms from different phylogenetic groups, representing many taxonomic divisions. They are distributed worldwide, inhabiting predominantly fresh- and seawater ecosystems. The ability of algae to adapt to environmental conditions is reflected in an exceptional variety of lipids as well as a number of unusual compounds. Many algae accumulate substantial amounts of non-polar lipids, mostly in the form of TAG or hydrocarbons, and these levels may reach up to 20–50% of dry cell weight. These oleaginous species have been considered as promising sources of oil for biofuels, such as surrogates of gasoline, kerosene and diesel, being both renewable and carbon neutral. The potential advantages of algae as a source of oil for biofuels include their ability to grow at high rates exhibiting a rapid biomass doubling time (usually 1–6 days) and producing 10–20 times more oil (ha^{-1} $year^{-1}$) than any oil crop plant. Algae can grow in saline, brackish and coastal seawater with little competition. They may utilize growth nutrients from wastewater sources and sequester carbon dioxide from emitted flue gases, thereby providing additional environmental benefits. Moreover, algae can produce valuable co- and by-products including carotenoids (β-carotene, astaxanthin, canthaxanthin and lutein), other pigments (phycocyanin and phycoerythrin), ω-3 fatty acids (eicosapentaenoic and docosahexaenoic acids), vitamins (tocopherols, vitamin B12 and provitamin A), polysaccarides and proteins. Thus, algae exhibit superior attributes to terrestrial crop plants as bioenergy sources. Moreover, in most cases algae will not compete for habitats used to produce food crops.

In spite of several technical limitations associated with existing technologies in the production of economically-viable algal oil, further research in this area is needed and such studies will clearly benefit from a better understanding of lipid metabolism and accumulation in algal cells. At present, relatively little information is available on lipid biosynthesis and its regulation in algae. Moreover, the lack of information about control mechanisms for lipid synthesis in different algal species limits our attempts to manipulate lipid metabolism in algae. However, some promising achievements in genetic and metabolic manipulations in higher plants are useful examples/directions to follow.

In the present chapter we will give an overview of lipid composition and lipid metabolism in algae with a special emphasis on the production of algal oils and/or their metabolism for biofuel applications. Previous useful reviews of algal lipids are Harwood and Jones (1989), Thompson (1996), Harwood (1998a) and Guschina and Harwood (2006a).

I.A. Guschina (✉) • J.L. Harwood
School of Biosciences, Cardiff University,
Museum Avenue, CF10 3AX Cardiff, Wales, UK
e-mail: guschinaia@cardiff.ac.uk; harwood@cardiff.ac.uk

M.A. Borowitzka and N.R. Moheimani (eds.), *Algae for Biofuels and Energy*, Developments in Applied Phycology 5,
DOI 10.1007/978-94-007-5479-9_2, © Springer Science+Business Media Dordrecht 2013

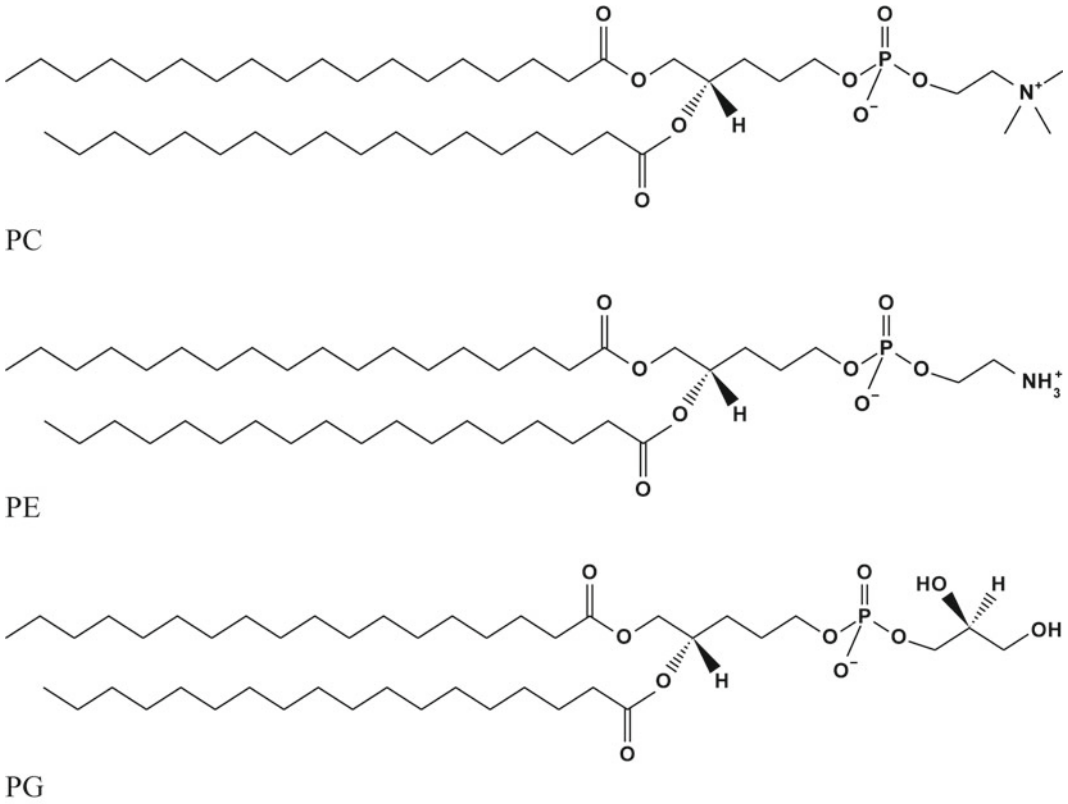

Fig. 2.1 Examples of the major phosphoglycerides of algae. *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol

2 Algal Lipids

2.1 Polar Glycerolipids

2.1.1 Phosphoglycerides

The basic structure of phosphoglycerides (phospholipids) is a glycerol backbone metabolically derived from glycerol 3-phosphate to which are esterified hydrophobic acyl groups at the 1- and 2-positions, and phosphate is esterified to the *sn*-3 position with a further link to a hydrophilic base group. Three phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), are the major phosphoglycerides identified in most algae species (Fig. 2.1). In addition, phosphatidylserine (PS), phosphatidylinositol (PI) and diphosphatidylglycerol (DPG) (or cardiolipin) may also present in different algal cells in appreciable amounts. Phosphatidic acid is usually a minor component but is an important metabolic intermediate and may be a signalling compound.

The phospholipids are located in the extrachloroplast membranes with the exception of PG. This phospholipid is present in substantial quantities in thylakoid membranes. PG accounts for around 10 and 20% of the total polar glycerolipids in eukaryotic green algae. An unusual fatty acid, Δ^3-*trans*-hexadecenoic acid (16:1(3 t)), is present in all eukaryotic photosynthetic organisms, being highly enriched at the *sn*-2 position of PG (for review see Tremolieres and Siegenthaler 1998). The *trans*-configuration of the double bond and its Δ^3 position are both very unusual for naturally-occurring fatty acids (El Maanni et al. 1998). A possible role of PG-16:1(3 t) in photosynthetic membranes will be discussed below.

Several unusual phospholipids have been isolated from algae. A sulfonium analog of phosphatidylcholine has been identified in diatoms (Anderson et al. 1978a, b; Bisseret et al. 1984). In this lipid, a sulphur atom replaces the nitrogen atom of choline. This phosphatidylsulfocholine (PSC) completely replaces PC in a non-photosynthetic diatom, *Nitzschia alba*, whereas in four other diatom species both lipids were found with PSC at levels corresponding to 6–24% of the total PC + PSC fraction. Low levels of PSC (less than 2%) were also reported for the diatoms *Cyclotella nana* and *Navicula incerta* as well as for a *Euglena* spp. (Bisseret et al. 1984).

A novel lipid constituent was isolated from brown algae. It was identified as phosphatidyl-O-[N-(2-hydroxyethyl)glycine] with the glycine derivative as headgroup (PHEG) (Eichenberger et al. 1995). This lipid was present in all 30 brown algal species analysed in the range 8–25 mol% of total phospholipids. This common lipid of brown algae has been shown to be accumulated in the plasma membrane of gametes of the brown alga *Ectocarpus*. Arachidonic acid

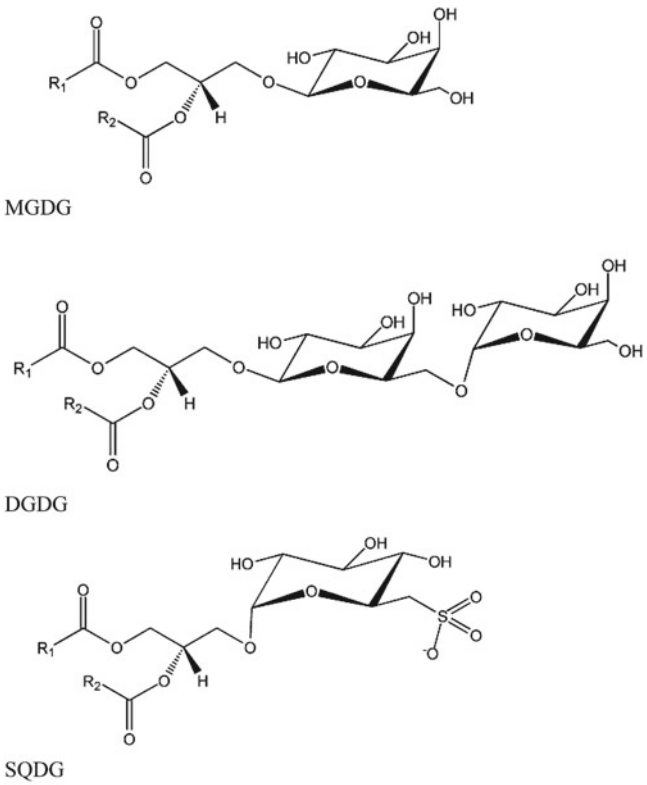

Fig. 2.2 The structures of the main glycosylglycerides of algae. *R1* and *R2* are the two fatty acyl chains. *MGDG* monogalactosyldiacylglycerol; *DGDG* digalactosyldiacylglycerol; *SQDG* sulfoquinovosyldiacylglycerol

(20:4n-3) and 20:5n-3 (eicosapentaenoic acid; EPA) were dominant in PHEG and represent 80 and 10%, respectively. Based on this finding, a special role of PHEG as an acyl donor for pheromone production and its possible participation in the fertilization of brown algae has been proposed (Eichenberger et al. 1995).

2.1.2 Glycosylglycerides

Glycosylglycerides (glycolipids) are characterized by a 1,2-diacyl-*sn*-glycerol moiety with a mono- or oligosaccharide attached at the *sn*-3 position of the glycerol backbone. The major plastid lipids, galactosylglycerides, are uncharged, polar lipids. They contain one or two galactose molecules linked to the *sn*-3 position of the glycerol corresponding to 1,2-diacyl-3-O-(β-D-galactopyranosyl)-*sn*-glycerol (or monogalactosyldiacylglycerol, MGDG) and 1,2-diacyl-3-O-(α-D-galactopyranosyl)-(1 → 6)-O-β-D-galactopyranosyl-*sn*-glycerol (or digalactosyldiacylglycerol, DGDG) (Fig. 2.2). In plants, MGDG and DGDG account for 40–55% and 15–35% of the total lipids in thylakoid membranes, respectively Harwood (1998a). Another class of glycosylglyceride is a sulfolipid, sulfoquinovosyldiacylglycerol, or 1,2-diacyl-3-O-(6-deoxy-6-sulfo-α-D-glucopyranosyl)-*sn*-glycerol (SQDG) (Fig. 2.2). It is present in both photosynthetic and in non-photosynthetic membranes of algae and may reach up to 30% of total lipids as found in the raphidophycean alga *Chattonella antiqua* (Harwood and Jones 1989). SQDG is unusual because of its sulfonic acid linkage. The sulfonoglucosidic moiety (6-deoxy-6-sulfono-glucoside) is described as sulfoquinovosyl and its sulfonic residue carries a full negative charge at physiological pH (see review by Harwood and Okanenko 2003).

Plastid galactolipids are characterised by a very high content of polyunsaturated fatty acids (Harwood 1998a). Thus, MGDG in fresh water algae contains α-linolenic (C18:3n-3) as the major fatty acid, and C18:3n-3 and palmitic acid (C16:0) are dominant in DGDG and SQDG. The glycolipids from some algal species, e.g. green algae *Trebouxia* spp., *Coccomyxa* spp., *Chlamydomonas* spp., *Scenedesmus* spp., may also be esterified with unsaturated C16 acids, such as hexadecatrienoic (C16:3n-3/C16:3n-2) and hexadecatetraenoic (C16:4) (Guschina et al. 2003; Arisz et al. 2000). In contrast, the plastidial glycosylglycerolipids of marine algae contain, in addition to C18:3n-3 and C16:0, some very-long-chain polyunsaturated fatty acids, e.g. arachidonic (C20:4n-6), eicosapentaenoic acid (C20:5n-3), docosahexaenoic (C22:6n-3) as well as octadecatetraenoic acid (18:4n-3) (Harwood and Jones 1989).

In an extract of the marine chloromonad *Heterosigma carterae* (Raphidophyceae), a complex mixture of SQDGs with C16:0, C16:1n-7, C16:1n-5, C16:1n-3 and C20:5n-3 as the main fatty acids has been identified (Keusgen et al. 1997). MGDG from the marine diatom *Skeletonema costatum* contains another unusual fatty acid, C18:3n-1, at a relatively high amount (about 25%) (D'Ippolito et al. 2004).

In some species of algae, a few unusual glycolipids have been identified in addition to MGDG, DGDG and SQDG. Trigalactosylglycerol has been found in *Chlorella* spp. (Harwood and Jones 1989). It has also been shown that glycolipids may contain sugars other than galactose (e.g., mannose and rhamnose) as reported for some red algae (Harwood and Jones 1989). An unusual glycolipid, sulfoquinovosylmonogalactosylglycerol (SQMG) was isolated from the marine red alga, *Gracilaria verrucosa* (Son 1990).

A carboxylated glycoglycerolipid, diacylglyceryl glucuronide (DGGA) has been described in *Ochromonas danica* (Chrysophyceae) and in *Pavlova lutheri* (Haptophyceae) (Eichenberger and Gribi 1994, 1997). This glycolipid accounts for about 3% of the glycerolipids in *O. danica*. Its predominant molecular species contained a C20:4/C22:5-combination of fatty acids. C22:5n-6 (44.4% of total FA) and C22:6n-3 acids (18.9%) were present in DGGA from the haptophyte *Pavlova lutheri* (Pavlovophyceae) (Eichenberger and Gribi 1997).

A new glycoglycerolipid with a rare 6-deoxy-6-aminoglucose moiety, avrainvilloside, has been reported for the marine green alga *Avrainvillea nigricans* (Andersen and Taglialatela-Scafati 2005). Three minor new glycolipids were also found

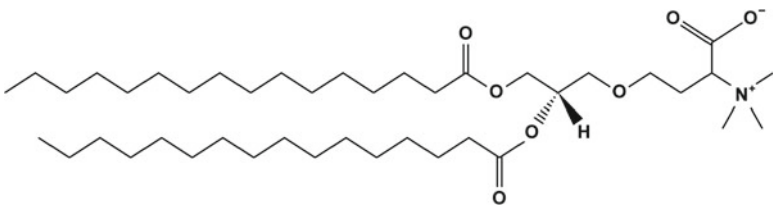

Fig. 2.3 The main betaine lipid of algae, 1,2-diacylglyceryl-3-*O*-4′-(*N,N,N*-trimethyl)-homoserine (DGTS)

in crude methanolic extracts of the red alga, *Chondria armata* (Al-Fadhli et al. 2006). They were identified as 1,2-di-O-acyl-3-O-(acyl-6′-galactosyl)-glycerol (GL_{1a}), the sulfonoglycolipid2-O-palmitoyl-3-O-(6′-sulfoquinovopyranosyl)-glycerol and its ethyl ether derivative. GL_{1a} has been mentioned as the first example of a glycolipid acylated at the 6' position of galactose which occurred naturally (Al-Fadhli et al. 2006).

In algae (as in higher plants and cyanobacteria), glycolipids are located predominantly in photosynthetic membranes and their role in photosynthesis is discussed below.

2.1.3 Betaine Lipids

Betaine lipids have a betaine moiety as a polar group which is linked to the *sn*-3 position of glycerol by an ether bond. Betaine lipids contain neither phosphorus nor carbohydrate groups. 1,2-diacylglyceryl-3-*O*-4′-(*N,N,N*-trimethyl)-homoserine (DGTS), 1,2-diacylglyceryl-3-*O*-2′-(hydroxymethyl)-(*N,N,N*-trimethyl)-β-alanine (DGTA) and 1,2-diacylglyceryl-3-*O*-carboxy-(hydroxymethyl)-choline (DGCC) are three types of betaine lipids identified in algae (Dembitsky 1996) (Fig. 2.3). They are all zwitterionic at neutral pH since their molecules have a positively charged trimethylammonium group and a negatively charged carboxyl group (Fig. 2.3).

Betaine lipids are common components of algae (as well as ferns, bryophytes, lichens, some fungi and protozoans), but they are not found in higher plants, either gymnosperms or angiosperms. The taxonomic distribution of betaine lipids in various groups of algae has been reviewed in detail by Dembitsky (1996) and Kato et al. (1996).

The fatty acid composition of DGTS varies significantly between freshwater and marine species. So, in freshwater algae mainly saturated fatty acids (C14:0 and C16:0) were found at the *sn*-1 position of the glycerol backbone and C18 acids (predominantly C18:2n-6 and C18:3n-3) at the *sn*-2 position. DGTS in marine algae can contain very long chain polyunsaturated fatty acids at both the *sn*-1 and *sn*-2 positions. For example, in the marine eustigmatophyte UTEX 2341 (previously identified as *Chlorella minutissima*) which produced DGTS at unusually high levels (up to 44% of total lipids), DGTS was exceptionally rich in EPA. The latter's level constituted over 90% of total fatty acids of DGTS in this alga (Gladu et al. 1995; Haigh et al. 1996).

A structural similarity between betaine lipids and phosphatidylcholine (as well as their taxonomical distribution with a reciprocal relationship between PC and betaine lipids in many algal species) has led to the suggestion that betaine lipids, especially DGTS, are more evolutionarily primitive lipids which, in lower plants, play the same functions in membranes that PC does in higher plants and animals (Dembitsky 1996).

2.1.3.1 Role of Polar Glycerolipids and Their Fatty Acids in Photosynthesis

Photosynthesis is a key process of converting atmospheric carbon dioxide into numerous metabolites, and it is pivotal for many metabolic pathways involved in the production of new biomass. To harness the potential of algae to grow rapidly and to accumulate lipids in large amounts, a deeper understanding of photosynthetic metabolism and especially its regulation in algae may be useful. In this part of our chapter, we would like to give a brief review of the role of lipids as important structural and regulatory compounds of chloroplast membranes. For more detailed information on the role of lipids in photosynthesis refer to Jones (2007) and Wada and Murata (2010).

The unique lipid composition in chloroplast membranes (e.g. high level of fatty acid unsaturation, the presence of PG-16:1(3 t) as well as the galactosylglycerides which are mainly located in these cell organelles) has been suggested to be important for normal photosynthetic function (Murata and Siegenthaler 1998). Investigation of a series of *Chlamydomonas* mutants with specific alterations in lipid composition has been shown to be a powerful tool to study structure-function relationships. To examine the role of SQDG in thylakoid membranes, Sato and co-workers (Sato et al. 2003a) compared the structural and functional properties of photosystem II (PSII) between a mutant of *Chlamydomonas reinhardtii* defective in SQDG (*hf-2*) and the wild type. Through characterization of the photosynthetic apparatus of an SQDG-defective mutant, it has been suggested that SQDG is involved in maintenance of the normal properties of PSII (Sato et al. 2003a). Selected mutants of *C. reinhardtii* lacking Δ^3-*trans*-hexadecenoic acid-containing phosphatidylglycerol (PG-16:1(3 t)) have been used to study a possible role of this lipid in the biogenesis and trimerization of the main light-harvesting chlorophyll-protein complex, the LHCII (El Maanni et al. 1998; Dubertret et al. 2002; Pineau et al. 2004). From a number of experiments where PG-16:1(3 t) was reincorporated into the photosynthetic membranes of the living mutants, it has been concluded that PG plays a crucial role in the LHCII

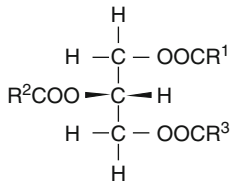

Fig. 2.4 Triacylglycerol structure. R^1, R^2 and R^3 are (usually different) fatty acyl chains

trimerization process. Moreover, 16:1(3 t) confers special properties to the PG molecule allowing high affinity interactions with some specific sites in the chlorophyll-protein complex (Dubertret et al. 2002; Pineau et al. 2004). An excellent review providing information on possible functions for PG in photosynthesis is that by Domonkos et al. (2008).

The role and contribution of lowered unsaturation of chloroplast lipids to adaptation and tolerance of photosynthesis to high temperature has been shown when studying a mutant of *C. reinhardtii* (*hf-9*) with impaired fatty acid desaturation of its chloroplast lipids (Sato et al. 1996).

2.2 Non-polar Storage Lipids

2.2.1 Triacylglycerols

Triacylglycerols (Fig. 2.4) are accumulated in many algae species as storage products. The level of TAG accumulation is very variable (Fig. 2.5) and may be stimulated by a number of environmental factors (see below). When algal growth slows down and there is no requirement for the synthesis of new membrane compounds, the cells divert fatty acids into TAG synthesis before conditions improve and there is a need for further growth.

It has been shown that, in general, TAG synthesis is favoured in the light period when TAG is stored in cytosolic lipid bodies and then reutilized for polar lipid synthesis in the dark (Thompson 1996). Nitrogen deprivation seems to be a major factor which is important for the stimulation of TAG synthesis. Many algae sustain a two- to three-fold increase in lipid content, predominantly TAG, under nitrogen limitation (Thompson 1996). Algal TAG are generally characterized by saturated and monounsaturated fatty acids. However, some oleaginous species may contain high levels of long chain polyunsaturated fatty acids in TAG (Table 2.1). The dynamics of arachidonic acid accumulation in TAG has been studied in the green alga *Parietochloris incisa* (Bigogno et al. 2002a). They found that arachidonyl moieties were mobilised from storage TAG into chloroplast lipids when recovering from nitrogen starvation (Bigogno et al. 2002a; Khozin-Goldberg et al. 2000, 2005). In this alga, PUFA-rich TAG have been hypothesised to be metabolically active in serving as a reservoir for specific fatty acids. During adaptation to sudden changes in environmental conditions, when the de novo synthesis of PUFA would be slow, PUFA-rich TAG may provide specific acyl groups for polar lipids thus enabling a rapid adaptive reorganisation of the membranes (Khozin-Goldberg et al. 2005; Makewicz et al. 1997).

The biosynthesis of TAG in algae is discussed in a later section.

2.2.2 Hydrocarbons

Some algae are known and characterised by their capacity to synthesise and accumulate a significant amount of hydrocarbons and have, therefore, excellent capability for biodiesel production. One of the most promising species in this algal group is *Botryococcus braunii*. This green colonial fresh water microalga has been recognised for some time as having good potential as a renewable resource for the production of liquid hydrocarbons (Metzger and Casadevall 1991; Metzger and Largeau 2005). It is of interest, that geochemical analysis of petroleum has shown that botryococcene- and methylated squalene-type hydrocarbons, presumably generated by microalgae ancestral to *B. braunii*, may be the source of today's petroleum deposits (Eroglu and Melis 2010).

The structure of hydrocarbons from *B. braunii* varies depending on the race, and *B. braunii* has been classified into A, B, and L races depending on the type of hydrocarbons synthesised. Thus, the A race produces up to 61% (on a dry biomass basis) of non-isoprenoid dienic and trienic hydrocarbons, odd numbered n-alkadienes, mono-, tri, tetra-, and pentaenes, from C25 to C31, which are derived from fatty acids. Race B yields C30–C37 highly unsaturated isoprenoid hydrocarbons, termed botryococcenes and small amounts of methyl branched squalenes. Race L produces a single tetraterpenoid hydrocarbon known as lycopadiene (Rao et al. 2007a, b). Botryococcenes are extracted from total lipids in the hexane-soluble fraction and can be converted into useful fuels by catalytic cracking (Raja et al. 2008). It has been reported that on hydrocracking, the distillate yields 67% gasoline, 15% aviation turbine fuel, 15% diesel fuel, and 3% residual oil. The unit area yield of oil is estimated to be from 5000 to 20,000 gal acre^{-1} year^{-1} (7,700–30,600 L ha^{-1} year^{-1}). This is 7–30 times greater that the best oil crop, palm oil (63 5 gal acre^{-1} year^{-1} = 973 L ha^{-1} year^{-1}) (Raja et al. 2008).

In general, the hydrocarbon content in *B. braunii* varies between 20 and 50% of dry weight depending upon the environmental conditions. In natural populations, the content of botryococcenes varies from 27–86% of dry cell mass and may be affected by various growth conditions. Nitrogen limitation has been shown to lead to a 1.6-fold increase in lipid content in this species (Singh and Kumar 1992). Anaerobiosis under nitrogen-deficient conditions also led to a greater lipid production in comparison to anaerobiosis in nitrogen-sufficient medium. Growth of *B. braunii* (race A) and production of hydrocarbons has been shown to be influenced by

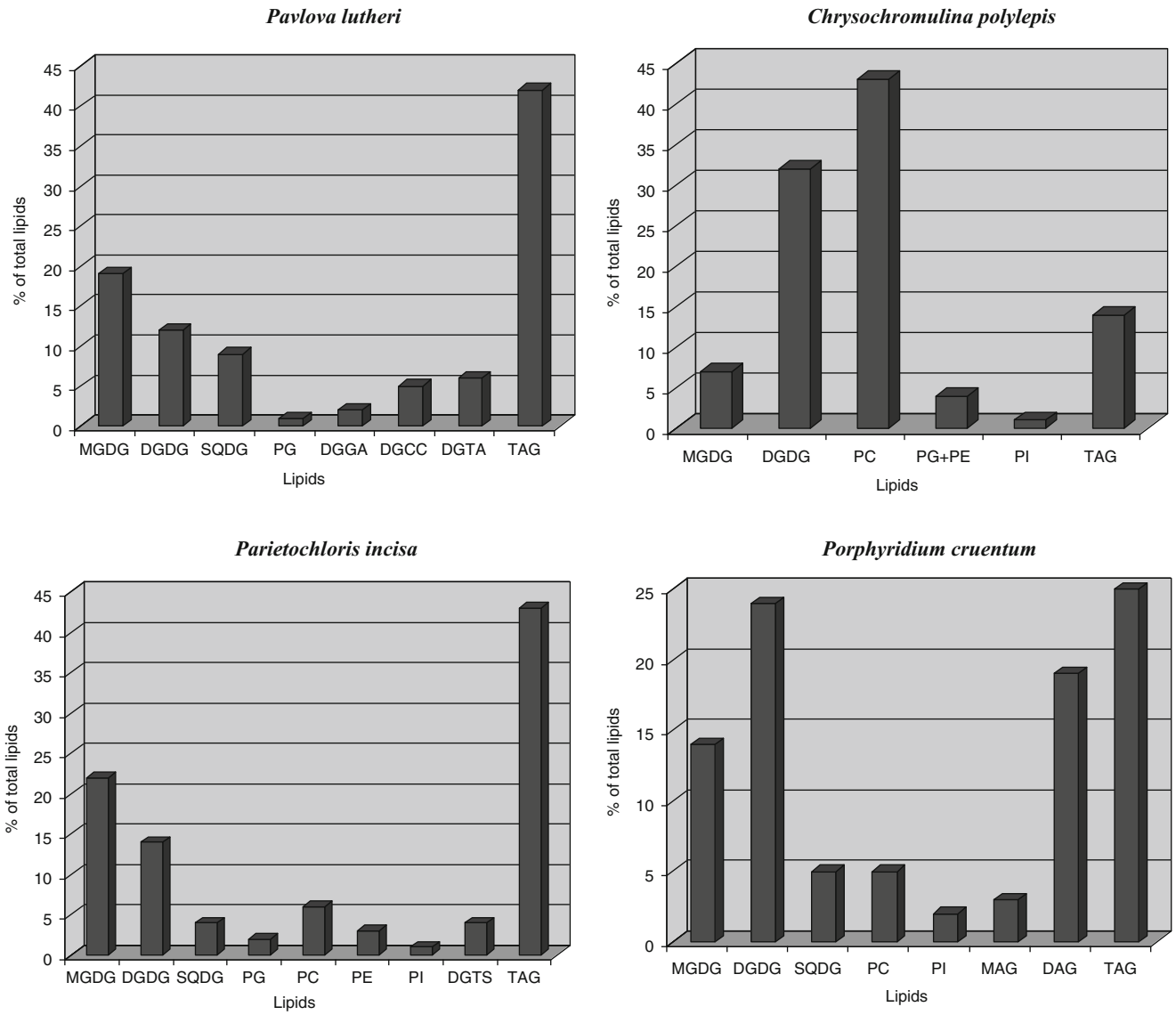

Fig. 2.5 Glycerolipid composition of selected species of algae. *Pavlova lutheri* (Eichenberger and Gribi 1997); *Chrysochromulina polylepis* (John et al. 2002); *Parietochloris incise* (Bigogno et al. 2002a); *Porphyridium cruentum,* (Alonso et al. 1998). Abbreviations: *MGDG* monogalactosyldiacylglycerol, *DGDG* digalactosyldiacylglycerol, *SQDG* sulfoquinovosyldiacylglycerol, *PG* phosphatidylglycerol, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PI* phosphatidylinositol, *DGTS* diacylglyceryltrimethylhomoserine,*DGTA* diacylglycerylhydroxymethyltrimethylalanine, *DGGA* diacylglycerylglucuronide, *DGCC* diacylglycerylcarboxyhydroxymethylcholine, *MAG* monoacylglycerol, *DAG* diacylglycerol, *TAG* triacylglycerol. The lipids were quantified on the basis of their fatty acid contents

different levels of salinity and CO_2 (Vazquez-Duhalt and Arredondo-Vega 1991; Rao et al. 2007a, b).

The biomass was found to increase with increasing concentrations (from 17 to 85 mM) of NaCl and the maximum biomass yield was achieved in 17 and 34 mM salinity (Rao et al. 2007a). Maximum hydrocarbon contents (28%, wt/wt) were observed in 68 mM salinity. The total lipid content of this alga was also affected by salinity varying from 24 to 28% (wt/wt) whereas in control it was 20% (Rao et al. 2007a). Stearic and linoleic acids were dominant in control cultures while palmitoleic and oleic acids were in higher proportions in algae grown at two different salinities (34 and 85 mM NaCl) (Rao et al. 2007a). The biomass production and hydrocarbon yield have been shown to be also increased with increasing concentrations of CO_2 in cultures (from 0.5 to 2%) (Rao et al. 2007b). Maximum hydrocarbon content was found at 2% CO_2 (Rao et al. 2007b).

The growth of *B. braunii* B70 and the size of oil granules in cells can be significantly increased by an addition of low concentrations of glucose (2–10 mM) to the culture medium (Tanoi et al. 2011). The possibility of using wastewater from a soybean curd (SCW) manufacturing plant as a growth

Table 2.1 Fatty acid distribution reported in TAG from selected algae species

	14:0	16:0	16:1	16:2	16:3	16:4	18:0	18:1	18:1	18:2	18:3	18:4	20:2	20:3	20:4	20:5	22:5	22:6
Algae				n-4	n-4	n-1		n-9	n-7	n-6	n-3	n-3		n-6	n-6	n-3		n-3
Eustigmatophyceae																		
Nannochloropsis sp.	18.8	41.6	33.7	–	–	–	1.0	3.8	–	1.1	–	–	–	–	–	–	–	–
Chlorophyceae																		
Parietochloris Incisa	–	8.4	0.4[a]	–	–	–	3.1	18.0	4.0	14.1	0.4[b]	–	–	1.1	47.1	0.7	–	–
Rhodophyceae																		
Porphyridium cruentum	1.6	21.1	1.5	–	–	–	3.7	4.0	0.9	12.2	–	–	1.0	1.1	24.2	15.9	–	–
Bacillariophyceae																		
Phaedactylum tricornutum	4.3	13.3	17.4	4.8	2.1	0.5	2.5	1.4	0.1	1.0	–	–	–	–	3.7	35.5	–	1.2
Prymnesiophyceae																		
Isochrysis galbana	4.8	12.3	21.7	–	–	–	1.2	4.9	1.2	2.2	1.5	7.2	–	–	–	25.6	1.2	8.1
Haptophyceae																		
Pavlova lutheri	8.6	39.2	32.9	–	–	–	tr.	2.1[c]	–	3.7	–	–	4.3	–	–	7.0	–	1.1

The positions of double bonds were assigned following capillary gas-liquid chromatography but were not confirmed by other methods. Dashes mean none detected, tr. = trace. Only the major fatty acids present are shown

Nannochloropsis sp. (grown under low light conditions) (Sukenik et al. 1993); *Parietochloris incisa* (stationary phase culture analysed) (Bigogno et al. 2002a); (Eichenberger and Gribi 1997); *Isochrysis galbana, Porphyridium cruentum, Phaeodactylum tricornutum* (Alonso et al. 1998)

[a]0.4 – 16:1 represents C16:1n-11 isomer

[b]0.4 – 0.7% of C18:3n-6 also present

[c]2.1 – sum of two isomers present. 16:1 is a mixture if isomers

promoter of *B. braunii* strain BOT-22 has been evaluated (Yonezawa et al. 2012). The growth and hydrocarbon accumulation were significantly higher in the cultures with 1 and 2% SCW. An addition of SCW also caused a shift in the hydrocarbon profile from $C_{34}H_{58}$ to $C_{32}H_{54}$ (Yonezawa et al. 2012). In addition, higher production of hydrocarbons in *B. braunii* Bot-144 (race B) has been achieved when it is grown under red light (Baba et al. 2012).

Although *B. braunii* can be found in all climatic zones, its habitats are restricted to freshwater or brackish water. Recently, a marine microalga, *Scenedesmus* sp. (strain JPCC GA0024, tentatively identified as *S. rubescens*), has been characterised for biofuel production (Matsunaga et al. 2009). It has been shown that the maximum biomass of 0.79 $g.L^{-1}$ could be obtained in 100% artificial seawater without additional nutrients for 11 days. The lipid content reached 73% of dry biomass under starvation conditions (no nutrient addition), which is equivalent to that of *B. braunii* (Matsunaga et al. 2009). Among non-polar lipids, aliphatic hydrocarbons were estimated as 0.6% of dry biomass in nutrient-rich medium. This value was higher than other hydrocarbon-producing cyanobacterial species (0.025–0.12%) but significantly lower than that of *B. braunii* (Matsunaga et al. 2009).

An understanding of hydrocarbon biosynthetic pathways and their regulation may provide an important tool for metabolic manipulation and increasing the yield of hydrocarbons in potential algal species. In this direction, some achievements have been demonstrated when studying hydrocarbon biosynthesis in *B. braunii*. From a number of radiolabelling experiments, it has been shown that oleic acid (but not palmitic or stearic acids) was a precursor (through chain elongation-decarboxylation reactions) for non-isoprenoid hydrocarbon production in the A race of *B. braunii* (Templier et al. 1984; Laureillard et al. 1988). The suggested mechanism of biosynthesis was also confirmed by experiments where thiols were used as known inhibitors of hydrocarbon formation in various higher plants (Templier et al. 1984).

The production of triterpenoid hydrocarbons isolated from race B of *B. braunii*, botryococcene and squalene, both of which are putative condensation products of farnesyl diphosphate, has also been studied (Okada et al. 2000). In order to understand better the regulation involved in the formation of these hydrocarbons, a squalene synthase (SS) gene was isolated and characterised from *B. braunii* (Okada et al. 2000). Comparison of the *Botryococcus* SS (BSS) with SS from different organisms showed 52% identity with *Nicotiana tabacum*, 51% with *Arabidopsis thaliana*, 48% with *Zea mays*, 40% with rat, 39% with yeast and 26% with *Zymomonas mobilis*. Expression of full-length and carboxy-terminus truncated BSS cDNA in *Escherichia coli* resulted in significant levels of bacterial SS enzyme activity but no botryococcene synthase activity (Okada et al. 2000). Later, botryococcene synthase (BS) enzyme activity was reported for *B. braunii* (Okada et al. 2004). It was shown that BS enzyme activity was correlated with the accumulation of botryococcenes during a *B. braunii* culture growth cycle, which was different from the profile of SS enzyme activity (Okada et al. 2004). Recently, high yields of squalene production have been achieved and measured in plants engineered for trichome specific expression of a soluble form of squalene synthase targeted to the chloroplast (Chappell 2009). Thus, it has been demonstrated that the unique biochemistry of *Botryococcus* can be engineered into other organisms thereby providing new tools for the manipulation of algal oil production. Recently, some additional studies to define the botryococcene biosynthetic pathway and to identify the genes coding for these unique enzymological transformations have been conducted (Niehaus et al. 2011). Three squalene synthase-like (SSL) genes have been identified, and it has been shown that the successive action of two distinct SSL enzymes was required for botryococcene biosynthesis (Niehaus et al. 2011).

3 Biosynthesis of Glycerolipids

3.1 Fatty Acid and Polar Glycerolipid Biosynthesis

Detailed discussions of plant/algal glycerolipid biosynthesis are available from a number of detailed reviews to which the reader is referred (Roughan and Slack 1982; Harwood et al. 1988; Harwood and Jones 1989; Browse and Somerville 1991; Dörmann 2005; Hu et al. 2008). In plants, biosynthesis of fatty acids and glycerolipids involves cooperation of two subcellular organelles, plastids and the endoplasmic reticulum (ER) (Fig. 2.6) and for eukaryotic algae this is probably also the case.

Higher plants synthesise palmitate, stearate and oleate through a pathway located in the plastid. This is one of the primary pathways of lipid metabolism and the main *de novo* source of the acyl chains of complex lipids. It begins with acetyl-CoA and then uses malonyl-acyl carrier protein (ACP) as the two-carbon donor (Fig. 2.7).

The acetyl-CoA needed for this synthesis comes ultimately from photosynthesis. The actual process of de novo synthesis to produce long-chain saturated fatty acids involves the participation of two enzymes, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). In most plants, the chloroplastic ACC is a multiprotein complex containing several functional proteins (a biotin carboxyl carrier protein, biotin carboxylase and two different subunits of the carboxyltransferase).

FAS is the second major enzyme complex involved in de novo fatty acid formation. The plant FAS is a Type II dissociable multiprotein complex (Harwood 1996) (like the *E. coli* system and unlike that of animals). Thus, the individual proteins that make up FAS can be isolated and their function

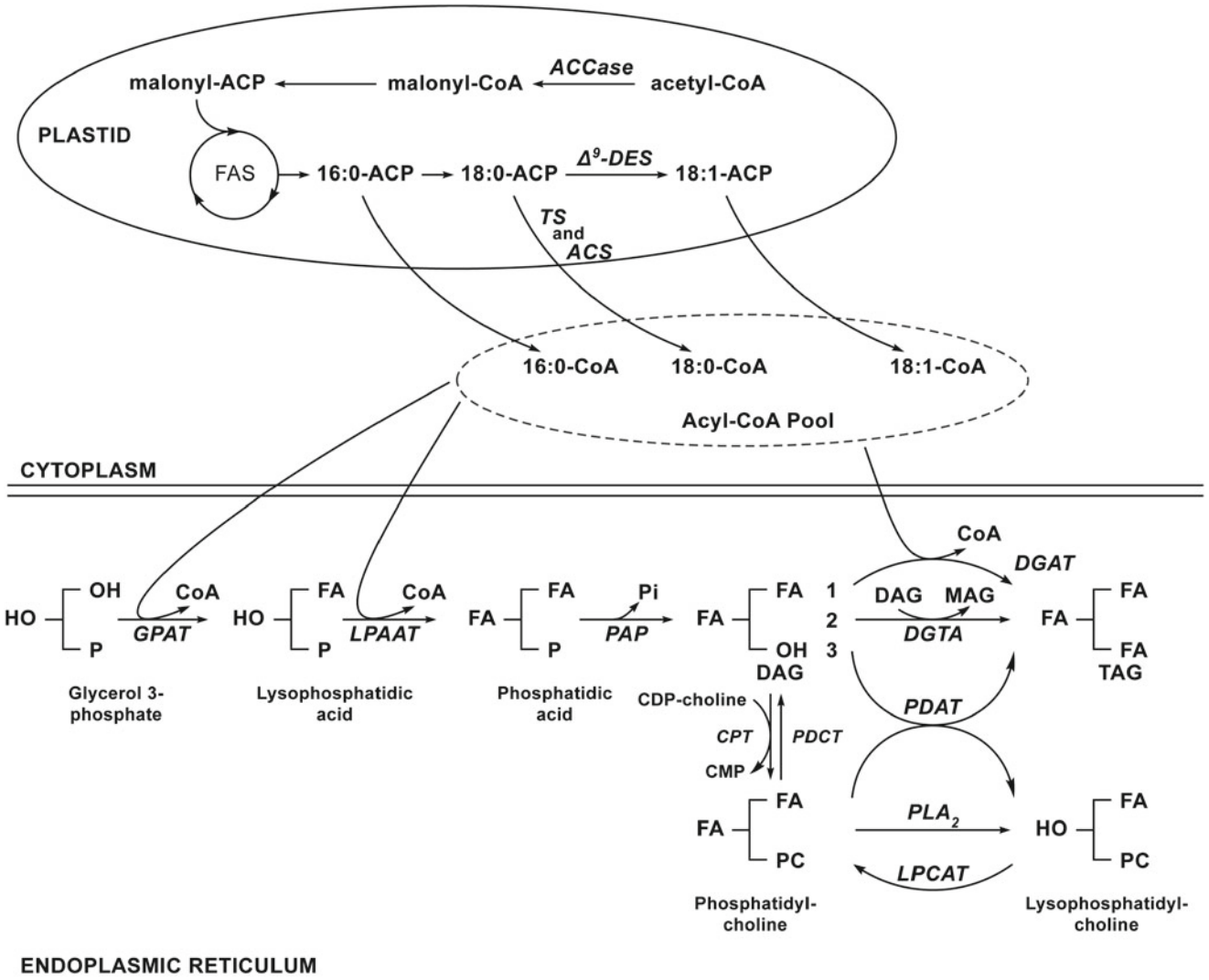

Fig. 2.6 Simplified scheme of TAG biosynthesis in plants. *ACCase* acetyl-CoA carboxylase, *ACP* acyl carrier protein, *ACS* acyl-CoA synthase, *CPT* CDP-choline:1,2-diacylglycerol cholinephosphotransferase, *Δ9-DES* Δ^9-desaturase, *DGAT* DAG acyltransferase, *DGTA* diacylglycerol:diacylglycerol transacylase, *FAS* fatty acid synthase, *GPAT* glycerol 3-phosphate acyltransferase, *LPAAT* lysophosphatidate acyltransferase, *LPCAP* lysophosphatidylcholine acyltransferase, *PAP* phosphatidate phosphohydrolase, *PDAT* phospholipid:diacylglycerol acyltransferase, *PLA_2* phospholipase A_2, *TE* acyl-ACP thioesterase, *PDCT* phosphatidylcholine:diacylglycerol cholinephosphotransferase

demonstrated separately. The first condensation reaction in fatty acid synthesis is catalysed by β-ketoacyl-ACP synthase III (KAS III) that uses acetyl-CoA and malonyl-ACP substrates to give a 4C-keto-intermediate. Successive reduction, dehydration, and a second reduction then produce a 4C fatty acid, butyrate, with all reactions taking place while esterified to acyl carrier protein (ACP). The next six condensations are catalysed by KAS I to produce 6-16C fatty acids. The final reaction between palmitoyl-ACP and malonyl-ACP uses KAS II and results in synthesis of stearate. The remaining enzymes of FAS are β-ketoacyl-ACP reductase, β-hydroxylacyl-ACP dehydrase and enoyl-ACP reductase (Fig. 2.7).

Many enzymes involved in fatty acid synthesis (β-ketoacyl-ACP reductase, β-ketoacyl-ACP synthase, acyl-ACP thioesterase, β-ketoacyl-CoA synthase and β-ketoacyl-CoA reductase) have been either up- or down-regulated in higher plants (Guschina and Harwood 2008). From these studies, it has been concluded that malonyl-CoA is a potential limiting factor affecting the final oil content and, thus, ACCase is a key enzyme in the complex reactions of fatty acid synthesis. Indeed, the enzyme shows high flux control for lipid synthesis in the light (Page et al. 1994). ACC is a soluble Class 1 biotin-containing enzyme that catalyses the ATP-dependent formation of malonyl-CoA from bicarbonate and acetyl-CoA. The product, malonyl-CoA, is used for de novo synthesis of fatty acids inside plastids. In addition, malonyl-CoA is needed for elongation of fatty acids on the endoplasmic reticulum as well as for synthesis of various secondary

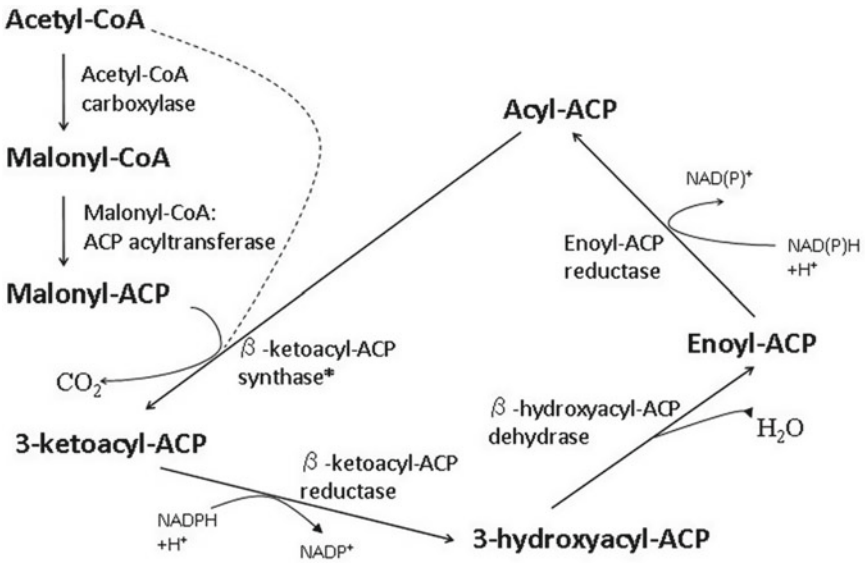

Fig. 2.7 Simplified scheme of de novo fatty acid synthesis in plants. *β-Ketoacyl-ACP synthase (KAS III) catalyses the first reaction of condensation using acetyl-CoA and malonyl-ACP as substrates. The next six condensation reactions are catalysed by KAS I. The final condensation between palmitoyl-ACP and malonyl-ACP is catalysed by KAS II

metabolites in the cytosol. As expected from such requirements, two isoforms of ACC are found in plants, the second of which is extra-chloroplastic (presumed to be cytosolic) and is a multifunctional protein. These isoforms have distinct properties which give rise to their different susceptibility to herbicides (Alban et al. 1994; Harwood 1996). Some success has been achieved in increasing ACCase activity and an associated increase of oil yield by 5% as a result of targeting of a cytosolic version of the enzyme to rapeseed plastids (Roesler et al. 1997).

In algae, ACCase has been purified and characterised from the diatom *Cyclotella cryptica* and it showed a high similarity to higher plant ACCase (Roessler 1990). ACCase from this alga was not inhibited by cyclohexanedione or aryloxyphenoxypropionic acid herbicides as strongly as monocotyledon ACCase but was strongly inhibited by palmitoyl-CoA. In this respect, the diatom enzyme more closely resembled ACCase from dicotyledonous plants than the enzyme from monocotyledonous plants (Roessler 1990). In *Isochrysis galbana,* grown under various environmental conditions, lipid synthesis and accumulation were related to the *in vitro* activity and cellular abundance of ACCase (Sukenik and Livne 1991). Later, the gene encoding ACCase in *C. cryptica* was cloned and characterized (Roessler and Ohlrogge 1993), and some attempts to over-express the ACCase gene have been reported (Hu et al. 2008). Although the experiments did not lead to increased oil production, this still remains one of the possible engineering approaches towards increasing algal oil production.

The fatty acids produced in plastids can be incorporated into the plastid pool of phosphatidate which can be subsequently converted into chloroplast lipids, MGDG, DGDG, SQDG and PG. Similar to cyanobacteria, algal glycerolipids synthesised through this pathway in plastids, have C16 fatty acids esterified at the *sn*-2 position of glycerol and either C16 or C18 fatty acids at the *sn*-1 position of their glycerol skeleton. Such lipids and the pathway responsible for their biosynthesis are called "prokaryotic". Within the ER, glycerolipids are synthesized by the core glycerol 3-phosphate ("Kennedy") pathway with TAG (see below) and phosphoglycerides as products (Gurr et al. 2002) (Fig. 2.6). Diacylglycerol (DAG) originating from a pool of endoplasmic reticulum PC, may be transferred from ER to plastids and be used there as a substrate for synthesis of chloroplast lipids. The *sn*-2 position of glycerolipids from this pathway is esterified with C18 fatty acids. These lipids and the pathway are designated as "eukaryotic". The distinct character of the esterification of the *sn*-2 position of glycerolipids in plastids and the ER, respectively, can be explained by the substrate specificities of lysophosphatidate acyltransferases (Gurr et al. 2002).

According to the above, the fatty acid composition of MGDG allows higher plants to be divided into two groups: 16:3 and 18:3 plants. MGDG from 16:3-plants is esterified with both C16 and C18 acids, and produced through both prokaryotic and eukaryotic pathways, whereas MGDG from 18:3 plants is esterified mainly with C18 acids and synthesised almost exclusively using the eukaryotic pathway (Roughan and Slack 1982).

It is believed that green algae and algae which contain PUFA of no more than 18 carbon atoms are similar to higher plants in so far as their metabolism is generally concerned (Khozin et al. 1997). So, green algae such as *C. vulgaris* and *Chlorella kessleri* have been shown to contain both prokaryotic and eukaryotic types of MGDG (with C16 and C18 acids at the sn-2 position) (see Sato et al. 2003b). Moreover, the existence of a eukaryotic pathway in *C. kessleri* has been proven by a number of radiolabelling experiments (Sato et al.

2003b). The authors suggested that the physiological function of the eukaryotic pathway in this alga is to supply chloroplast membranes with 18:3/18:3-MGDG which may improve their functioning and, hence, be favoured during evolution into land plants (Sato et al. 2003b).

However, algae species with C20 PUFA as well as algae where PC is substituted with betaine lipids have been show to possess differences from higher plants and more complex pathways (Giroud et al. 1988; Cho and Thompson 1987; Khozin et al. 1997; Eichenberger and Gribi 1997). Based on results from the betaine lipid-containing *Pavlova lutheri*, it has been concluded that extraplastid DGCC was involved in the transfer of fatty acids from the cytoplasm and, thus, in the biosynthesis of MGDG (Eichenberger and Gribi 1997). Moreover, these authors suggested that individual fatty acids rather than DAGs were transferred from the cytoplasm to the chloroplast and were incorporated into MGDG by an exchange mechanism (Eichenberger and Gribi 1997).

In the red microalga *Porphyridium cruentum,* EPA-containing galactolipids have been shown to be both eukaryotic and prokaryotic types (Khozin et al. 1997). The analysis revealed the presence of EPA and AA at the *sn*-1 position and C16 fatty acids, mainly C16:0, at the *sn*-2 position in prokaryotic molecular species. In the eukaryotic molecular species both positions were esterified by EPA or arachidonic acid. However, based on studies using radiolabelled precursors, the authors suggested that both prokaryotic and eukaryotic molecular species were formed in two pathways, ω6 and ω3, which involved cytoplasmic and chloroplastic lipids (Khozin et al. 1997). In the ω6 pathway, cytoplasmic C18:2-PC was converted to 20:4ω6-PC whereas in the minor ω3 pathway, C18:2-PC was first desaturated to 18:3ω3 and then converted into 20:5ω3-PC using the same desaturases and elongases as the ω6 pathway. The diacylglycerol moieties of the products were exported to the chloroplast to be galactosylated into their respective MGDG molecular species (Khozin et al. 1997).

Biosynthesis of the betaine lipid, DGTS, has been studied in *C. reinhardtii* using [^{14}C-carboxyl]-*S*-adenosyl-*L*-methionine (Moore et al. 2001). It has been shown that S-adenosylmethionine was the precursor used for both the homoserine moiety and the methyl groups. The activity was associated with the microsomal fraction and did not occur in the plastid (Moore et al. 2001). The discovery of the betaine synthase gene ($BTA1_{Cr}$) has been also recently reported for this alga (Riekhof et al. 2005).

The synthesis of phosphatidylinositol was also studied in *C. reinhardtii* (Blouin et al. 2003). Their data provided evidence for the operation of both of the biosynthetic pathways which had been described in plant and animal tissues previously. One reaction involved CDP-diacylglycerol and was catalyzed by PI synthase (CDP-diacylglycerol: *myo*-inositol 3-phosphatidyltransferase). In the second reaction (which did not in fact result in net PI formation), a free inositol was exchanged for an existing inositol headgroup. The major site of PI biosynthesis in *C. reinhardtii* was the microsomal (containing endoplasmic reticulum (ER)) fraction (Blouin et al. 2003).

3.2 Biosynthesis of TAG

As mentioned above, glycerolipids are synthesized within the ER by the core glycerol 3-phosphate pathway with TAG, phosphoglycerides and glycosylglycerides as major products (Gurr et al. 2002). The first two reactions in this Kornberg-Price pathway to TAG are the formation of phosphatidic acid by the stepwise acylation of glycerol 3-phosphate (Fig. 2.6). These reactions are catalysed by two distinct acyltransferases which are specific for positions sn-1 and sn-2. Membrane-bound glycerol 3-phosphate acyltransferase (GPAT) initiates the process by transferring the acyl chain from acyl-CoA to the sn-1 position of glycerol 3-phosphate with the formation of lysophosphatidic acid (monoacylglycerol 3-phosphate) (Eccleston and Harwood 1995; Manaf and Harwood 2000). One report has been published on the gene for the membrane-bound form of GPAT (Weselake et al. 2009), which is believed to have a low selectivity for different acyl chains. (The soluble chloroplast form of GPAT, which uses acyl-ACP substrates, has, however, been well studied). The transfer of acyl chains from acyl-CoAs to the sn-2 position to form phosphatidic acid, is catalyzed by lysophosphatidic acid acyltransferase (LPAAT) which, in plants, prefers unsaturated acyl chains (Voelker and Kinney 2001). The phosphatidic acid is then dephosphorylated to produce diacylglycerol (DAG). The final step in the pathway is the addition of a final fatty-acyl group to the sn-3 position of DAG to produce TAG. It is catalyzed by diacylglycerol acyltransferase (DGAT), an enzyme unique to TAG biosynthesis. In plants, two unrelated genes have been shown to encode DGAT enzymes. One form (DGAT 1) is related to acyl-CoA:cholesterol acyltransferase, whereas a second form (DGAT 2) does not resemble any other known genes.

Recent studies in plants provide evidence for alternative reactions for TAG synthesis in plants. In one of these reactions, a fatty acid residue is directly transferred from the sn-2 position of PC to DAG forming lyso-PC and TAG. This is referred to a phospholipid:diacylglycerol acyltransferase (PDAT). There is also a reaction involving acyl transfer between two molecules of DAG (i.e. DAG:DAG transacylase) (Stobart et al. 1997). Another enzyme which probably plays a key role in exchanging the diacylglycerol from phosphatidylcholine for the bulk pool and, hence, allowing entry of polyunsaturated fatty acids into TAG synthesis is phosphatidylch oline:diacylglycerol cholinephosphotransferase (PDCT) (Lu

et al. 2009). It remains to be seen whether all these enzymes exist in algae and, in addition, how important they are for TAG formation.

Since DGAT is a key mediator of plant TAG biosynthesis, over-expression of DGAT genes has been suggested as a promising strategy to boost TAG yield. It is known that DGAT is an integral endoplasmic reticulum protein presented in oil bodies and plastids. As mentioned above, two classes of DGAT have been isolated: DGAT1 protein consists of nine to ten putative transmembrane domains whereas DGAT2 only contains two such domains (Lung and Weselake 2006). DGAT2 has been shown to be important for the production of TAG in several cases where unusual fatty acids are involved. In contrast, DGAT1 seems to be quantitatively most important for TAG synthesis when common fatty acids are esterified (see Weselake et al. 2009; Li et al. 2010a).

The potential benefit of acyl-CoA:diacylglycerol acyltransferase-transformed plants has been demonstrated. Thus, over-expression of *AtDGAT1* in tobacco leaves produced up to seven-fold increase in TAG content of the tissue. Seed-specific expression of *AtDGAT1* in *Arabidopsis* led to an increase of 11–28% in seed oil content and similar studies have been made with oilseed rape (Weselake et al. 2008, 2009). These studies have confirmed the important role of DGAT in regulating the quantity of seed TAGs.

As to TAG biosynthesis in algae, one GPAT and two DGATs have been identified and characterized (Xu et al. 2009; Wagner et al. 2010; Guihéneuf et al. 2011). A membrane-bound GPAT was isolated from the marine diatom *Thalassiosira pseudonana*. This enzyme has been shown to prefer saturated C16 fatty acid as a substrate and to play a significant role in determining the fatty acid profile in glycerolipids (Xu et al. 2009). Three putative DGAT two genes were identified from the green alga *Osteococcus tauri* by a database search in its genome (Wagner et al. 2010). For two of the cDNA sequences (OtDGAT2A and B), enzyme activity has been determined by heterologous expression in *Saccharomyces cerevisiae* mutant strains which had impaired TAG metabolism (Wagner et al. 2010). DGAT1 isolated from the diatom microalga *Phaeodactylum tricornutum* (*PtDGAT1*) showed a high homology to several functionally characterised higher plant DGAT1 proteins, and functional expression of *PtDGAT1* was achieved in *S. cerevisiae* (Guihéneuf et al. 2011).

The recent advances in the identification of genes involved in algal lipid metabolism have been thoroughly reviewed by Khozin-Goldberg and Cohen (2011).

It has been recently demonstrated that *C. reinhardtii* may employ a distinct pathway that uses DAG derived almost exclusively from the chloroplast to produce TAG (Fan et al. 2011).

4 Factors Affecting Lipid Composition and Lipid Productivity of Algae

Knowledge of how various cultivation conditions affect the lipid composition and productivity of algae is important for choosing optimal growth conditions for better growth rates, biomass production and the level of TAG accumulation as well as for the modulation of oil fatty acid composition. The latter should not be ignored for at least two reasons. Firstly, the fatty acid profile determines the optimal biofuel characteristics. Thus, it has been concluded from various studies that biodiesel with high levels of methyl oleate or palmitoleate will have excellent characteristics with regard to ignition quality and fuel stability. In many oleaginous algae, TAG and other lipids contain very high levels of polyunsaturated fatty acids which are excellent for the cold stability of the fuel but not desirable for its other characteristics. The second reason is that valuable n-3 fatty acids are co-products of the biofuel technological process and their increased yield, which may be manipulated by growth conditions, can be highly desirable for high value nutraceuticals.

4.1 General Growth Conditions

4.1.1 Temperature

Light and temperature are probably most important and well-studied factors influencing the lipid and fatty acid composition of algae. Changes to the lipids of photosynthetic tissues (and other organisms) as a response to different temperatures and/or light conditions have been recently reviewed (Harwood 1998b; Guschina and Harwood 2006a, b; Morgan-Kiss et al. 2006; Guschina and Harwood 2009a, b). It is believed that many of the lipid changes alter the physical properties of membranes which allows their unimpaired functioning in important physiological processes including photosynthesis, respiration and membrane transport.

For alterations in environmental temperature, changes in fatty acid unsaturation are the most common modification in membrane lipids observed (Harwood 1998b). Low temperature modification of lipid composition has been extensively analysed in the green alga, *Dunaliella salina* (Thompson 1996). A temperature shift from 30 to 12°C increased the level of lipid unsaturation in this alga significantly (Thompson 1996). Retailoring the molecular species of pre-existing PE and PG (especially an increase in molecular species with two unsaturated fatty acids) was noted as a quick response to the temperature shift (Thompson 1996). In addition, a rise in C18:3/C16:1-PG from 48 to 57% and a concomitant decrease in C18:2/C16:1-PG from 34 to 26% of total chloroplast PG was correlated with a significant alteration in the threshold temperature of thermal denaturation of the photosynthetic

apparatus (Thompson 1996). No effect of temperature shift on the content of the acidic lipids, SQDG and PG, has been noticed in *C. reinhardtii* (Sato et al. 2000). However, in the marine haptophyte alga *P. lutheri,* significant changes in lipid class content and fatty acid composition have been reported for cultures grown at 15°C compared to 25°C (Tatsuzawa and Takizawa 1995). Lower temperatures resulted in increased relative amounts of the polyunsaturated fatty acids, EPA and DHA. In addition, the relative percentage of betaine lipids, PG and SQDG increased when algae were cultivated at 15°C with a concomitant decrease in the levels of TAG and MGDG.

Lowering of the growth temperature increased the proportion of the eukaryotic molecular species of MGDG, especially C20:5/C20:5 MGDG, in the red microalga *Porphyridium cruentum* (Adlerstein et al. 1997). A special role for these molecular species in adaptation of PUFA-rich algae to low growth temperatures has been suggested (Adlerstein et al. 1997). Such algae (as well as *Parietochloris incisa*) accumulate high levels of arachidonic acid (AA) in the storage TAG and the reported transfer of this acid from TAG to membrane lipids has been suggested as an adaptive mechanism to low temperature stress (Khozin-Goldberg et al. 2000; Bigogno et al. 2002b).

In two green microalgae, *C. vulgaris* and *B. braunii*, increased growth temperatures led to a decrease in the relative content of more unsaturated intracellular fatty acids, especially trienoic species, while the composition of fatty acids secreted into a medium was unchanged (Sushchik et al. 2003). A decrease in cultivation temperature from 25 to 10°C resulted in an elevation of the relative proportion of oleate in the green alga *Selenastrum capricornutum* (McLarnon-Riches et al. 1998). In contrast to the general expected increase in the proportion of fatty acid unsaturation levels, a decrease in linoleate and stearidonate (C18:4) at lower temperatures has been also shown in this alga (McLarnon-Riches et al. 1998).

In cultures of the haptophyte *I. galbana* grown at 15 and 30°C, lipids and fatty acids were analysed and compared (Zhu et al. 1997). At 30°C, total lipids accumulated at a higher rate with a slight decrease in the proportion of non-polar lipids, an increase in the proportion of glycosylglycerides but no change in the proportion of phospholipids. Higher levels of α-linolenate and DHA with a corresponding decrease in linoleate, monounsaturated and saturated fatty acids were found in the cells grown at 15°C.

Four tropical Australian microalgal species, a diatom *Chaetoceros* sp., two cryptomonads, *Rhodomonas* sp. and *Cryptomonas* sp., and an unidentified prymnesiophyte, cultured at five different temperatures showed decreased levels of EPA and DHA at higher cultivation temperatures (Renaud et al. 2002). Similarly, the content of EPA and PUFAs in marine diatom *Phaeodactylum tricornutum* have been shown to be higher at lower temperatures when comparing cultures grown at 10, 15, 20, or 25°C (Jiang and Gao 2004).

In general, lower growth temperatures lead to increased levels of unsaturated fatty acids in algae although the details of these alterations may vary from species to species. Moreover, some additional subtle alterations may be often seen in many algae rather than a simple correlation of increased unsaturation with lower temperatures. The general alterations in fatty acid unsaturation in algae mirror similar changes observed in other eukaryotes as well as cyanobacteria (Harwood 1998b).

4.1.2 Light

Light intensity also influences algal lipid metabolism and, therefore, lipid composition (Harwood 1998b). In general, high light intensities usually lead to oxidative damage of polyunsaturated fatty acids. In *Nannochloropsis* sp., the degree of unsaturation of fatty acids decreased with increasing irradiance, especially the percentage of total n-3 fatty acids (from 29 to 8% of total fatty acids) mainly due to a decrease of EPA (Fabregas et al. 2004). In other EPA-producing algae (*P. tricornutum* and *Monodus subterraneus*) this tendency had been noticed previously when increasing light intensity had caused a reduction in EPA accumulation (cited by Adlerstein et al. 1997). High light exposure (300 μmol photons.m^{-2}.s^{-1}) decreased the total phospholipid content and increased the level of non-polar lipids, namely TAG, in the filamentous green alga *Chladophora* sp. (Napolitano 1994).

Variations in lipid composition were studied in the marine red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance (Khotimchenko and Yakovleva 2005). Light intensity caused significant alterations in both storage and structural lipids. Exposure of this alga to low light intensity (8–10% of the incident photosynthetically active radiation (PAR)) resulted in increased levels of some cell membrane lipids, especially SQDG, PG and PC, whereas cultivation of algae at higher light intensities (70–80% of PAR) increased the level of storage TAG. The light conditions used did not change the total fatty acid composition in *T. crinitus*, although there were a few changes noticed in the fatty acid composition of individual lipids (Khotimchenko and Yakovleva 2005).

In the green alga *Ulva fenestrata*, the relative amounts of MGDG, SQDG and PG increased 2–3.5 times when grown at 24% of the incident photosynthetically active radiation (PAR) compared to algae cultured at 80% PAR (Khotimchenko and Yakovleva 2004). In contrast, the relative contents of DGDG and betaine lipid, as well as the relative proportions of fatty acids in TAG, MGDG and SQDG, were not affected by light intensity (Khotimchenko and Yakovleva 2004).

Light:dark cycles also have a significant effect on algal lipid composition. As an example, a detailed study of various

light regimes on lipids of the diatom, *Thalassiosira pseudonana,* may be cited (Brown et al. 1996). The light regimes used were 100 µmol photons.m^{-2}.s^{-1} on a 12:12 h light:dark (L:D) cycle; 50 µmol photons.m^{-2}.s^{-1} on a 24:0 h L:D cycle and 100 µmol photons.m^{-2}.s^{-1} on a 24:0 h L:D cycle. An increased percentage of TAG and a reduced percentage of the total polar lipids were found for the cells grown under 100 µmol photons.m^{-2}.s^{-1} continuous light. The fatty acid composition of algae in the logarithmic growth stage under the two continuous light regimes showed no differences, whereas the cell grown under 100 µmol photons.m^{-2}.s^{-1} and 12:12 h L:D conditions contained a higher proportion of PUFAs and a lower proportion of saturated and monounsaturated fatty acids (Brown et al. 1996).

Low light decreased the relative percentage of palmitate and increased in those of palmitoleate and α-linolenate in two green freshwater algae *Cladophora* sp. and *Spirogyra* sp. (Napolitano 1994). Dark treatment caused a decrease in the relative proportion of oleate and an increase in that of linoleate in the green alga *S. capricornutum* (McLarnon-Riches et al. 1998). In the dinoflagellate *Prorocentrum minimum*, dark exposure led to a reduced content of TAG and galactosylglycerides, while the total content of phospholipids changed little with decreased PC, PE and PG and increased PS, PA and PI levels. The decrease of TAG and galactosylglycerides was in parallel to an increase in the activity of β-oxidation and isocitrate lyase indicating that TAG and galactosylglycerides were utilized as alternative carbon sources by the cells under non-photosynthetic growth conditions (McLarnon-Riches et al. 1998).

Lipid production in *P. cruentum* has been studied under different culture conditions (Oh et al. 2009). A higher lipid accumulation (19.3%, w.w^{-1}) was demonstrated using a 12:12 h light:dark cycle and a 25°C growth temperature compared to 35°C (Oh et al. 2009).

In general, light will stimulate the algal biomass production. Stimulation of fatty acid and membrane lipid, mainly chloroplast, synthesis are normally expected as a result of an increase in light intensity.

4.1.3 Salt Concentrations

Some algae exhibit an excellent ability to tolerate high salt concentrations. The genus *Dunaliella,* growing in the wide range of salinities, is a good example as well as an useful model in studying mechanisms of such resistance (Azachi et al. 2002). It has been shown that the expression of β-ketoacyl-coenzyme A (CoA) synthase (KCS) (which catalyzes the first step in fatty acid elongation) was induced in the cells of *D. salina* transferred from 0.5 to 3.5 M NaCl (Azachi et al. 2002). In these cells, a considerably higher ratio of 18C (mostly unsaturated) to 16C (mostly saturated) fatty acids was also noted. The authors suggested that the salt-induced KCS, together with fatty acid desaturases, may play a role in adapting intracellular membranes to function in the high internal glycerol concentrations used to balance the external osmotic pressure created by high salt (Azachi et al. 2002). (However, it must be noted that such a proposal assumes that KCS is responsible for 18C fatty acid production rather than fatty acid synthase).

In *D. salina* cells, the lipid content was manipulated by salt stress and nitrogen limitation, and it reached a value of about 38% in cells grown at 16% NaCl combined with 2.5 mM unspecified nitrogen salts (Abd El-Baky et al. 2004). These conditions also increased the relative proportion of PUFAs, in particular the C18:3n-3 and C16:4n-3 fatty acids (Abd El-Baky et al. 2004).

An increase in the initial salt concentration from 0.5 M NaCl to 1.0 M resulted in an increase (from 60 to 67%) of intracellular lipid content in *Dunaliella tertiolecta* (Takagi et al. 2006). The further increase in lipid content up to 70% has been achieved when 0.5 or 1.0 M NaCl were added at mid-log phase or the end of log phase during cultivation with the initial NaCl concentration of 1.0 M (Takagi et al. 2006).

4.1.4 pH

Lipids are also affected during growth of algae at extreme pH. Thus, it has been shown that alkaline pH stress increased the TAG percentage accumulation and decreased the relative level of membrane lipids in *Chlorella* sp. (Guckert and Cooksey 1990). The effects of pH on the lipid and fatty acid composition of a *Chlamydomonas* sp., isolated from a volcanic acidic lake and *C. reinhardtii*, obtained from an algal collection (Institute of Applied Microbiology, Tokyo) have been studied and compared (Tatsuzawa et al. 1996). Fatty acids in the polar lipids were more saturated in the unidentified *Chlamydomonas* sp. than those in *C. reinhardtii* when grown under the same conditions. The TAG content (as % of total lipids) was also higher in *Chlamydomonas* sp. grown at pH 1 than that in the cells cultivated at higher pH. The increase in saturation of fatty acids in membrane lipids of *Chlamydomonas* was proposed to be an adaptive reaction to low pH in order to decrease membrane lipid fluidity (Tatsuzawa et al. 1996).

4.1.5 Nutrients

Nutrient availability affects significantly the lipid composition of algae, and a number of broad effects of nutrient limitation have been reported as important modulators of algal lipid biosynthesis. It is accepted, that when algal growth slows down as a result of nutrient deficiency, and there is no requirement for the synthesis of new membrane compounds, the cells can transfer fatty acids into their storage lipids before conditions improve. There are many examples showing that algal species deficient in nutrients can more than double their lipid and TAG content (see Thompson 1996; Guschina and Harwood 2006a).

Nutrient-deficiency has been shown to affect markedly the lipid composition of the freshwater diatom *Stephanodiscus minutulus* when grown under silicon, nitrogen, or phosphorus limitation (Lynn et al. 2000). An increase in TAG accumulation and a decrease of polar lipids (as % of total lipids) was noticed in all of the nutrient-limited cultures (Lynn et al. 2000). An increase in TAG percentages (from 69 to 75% of total lipids) together with phospholipids (from 6 to 8%) was reported for the microalga *P. tricornutum* as a result of reduced nitrogen concentration. In contrast, the proportion of galactolipids decreased from 21 to 12% in nitrogen-starved cells (Alonso et al. 2000).

In *Chlamydomonas moewusii*, nutrient-limitation resulted in alterations in the fatty acid composition of the chloroplast lipids, PG and MGDG (Arisz et al. 2000). The PUFAs, C16:3, C16:4 and C18:3, which were present in the plastidic galactolipids, and C16:1(Δ3-*trans*), specific for plastidic PG, decreased under nutrient-limited conditions. This may, possibly, be due to a reduction in the intracellular content of chloroplasts although that was not specifically examined. The synthesis of storage lipids has been suggested to be stimulated by depletion of nutrients and this was consistent with the rise in the levels of C16:1 and C18:1 which were prominent in storage lipids (Arisz et al. 2000).

Euglena gracilis has been cultivated under various conditions of autotrophy and photoheterotrophy in order to estimate the contribution of lactate (a carbon source) and ammonium phosphate (a nitrogen source) to its metabolism (Regnault et al. 1995). Effects of increasing ammonium phosphate concentration on lipid composition were noticed only when lactate was depleted. Such conditions increased the content of galactolipids rich in polyunsaturated 16C and 18C fatty acids as well as the ratio of MGDG/DGDG. Excess of nitrogen did not change the content of medium chain (12-14C) acids but induced a reduction of 22C acids. When ammonium phosphate was absent in the cultural medium, increasing the lactate concentration led to a decrease in all plastid lipids, whereas the accumulation of storage lipids (enriched with myristate and palmitate) increased. Biosynthesis of 18C PUFAs was reduced as indicated by the accumulation of oleate (Regnault et al. 1995).

The effects of sodium nitrate as a nitrogen source on cell growth and lipid accumulation has been studied in a green alga, *Neochloris oleoabundans*, one of the most promising oil-rich microalgal species (Li et al. 2008). The highest lipid cell content (0.40 $g.g^{-1}$ dry weight) was obtained at the lowest sodium nitrate concentration (3 mM), whereas a higher lipid productivity of 0.133 $g.L^{-1}.day^{-1}$ was achieved at 5 mM with a lipid cell content of 0.34 $g.g^{-1}$ (Li et al. 2008).

Other studies with *N. oleoabundans* showed that the best growth was obtained when the algae were cultivated at 30°C under conditions of nitrogen-sufficiency and CO_2 supplementation (Gouveia et al. 2009). However, the maximum lipid content (56% of dry weight) was shown after 6 days of nitrogen depletion without CO_2 supplementation (Gouveia et al. 2009).

Similar results for nitrogen limitation were also found during cultivation of microalgae *Chlorella* sp. with urea (Hsieh and Wu 2009). Initial urea concentrations of 0.025, 0.050, 0.100, 0.150 and 0.200 $g.L^{-1}$ were used to investigate its effect on cell growth and lipid productivity in batch cultures. The microalgae showed the highest total lipid content (0.661 $g.g^{-1}$ dry weight) when cultured with the lowest concentration of urea. However, the maximum lipid productivity of 0.124 $g.L^{-1}.day^{-1}$ was shown for cells in media containing 0.100 $g.L^{-1}$ urea (Hsieh and Wu 2009).

In experiments with *Scenedesmus obliquus*, lipid accumulation has been studied under various culture conditions including nitrate, phosphate, sodium thiosulfate and glucose supplementation (Mandal and Mallick 2009). Lipid accumulation was found to be more affected by the concentrations of nitrate, phosphate and sodium thiosulphate than glucose supplementation to the growth media (Mandal and Mallick 2009). The most significant accumulation of lipids (43% of dry cell weight) was recorded under N-deficiency (against 2.7% of lipids of dry cell weight under control conditions). Under P-deficiency and thiosulphate supplementation lipid accumulation also increased (up to 30% of dry cell weight) (Mandal and Mallick 2009).

The lipid composition of seven species of marine algae has been studied when cultured in phosphorus-limiting conditions (Reitan et al. 1994). Such conditions caused an increase in total lipid content in *P. tricornutum, Chaetoceros* sp., and in *P. lutheri*, but a decrease of that in the green flagellates, *Nannochloris atomus* and *Tetraselmis* sp. A higher relative content of palmitate and oleate and lower levels of C18:4*n*-3, EPA and DHA have been shown for the more severe nutrient-limited conditions of cultivation (Reitan et al. 1994). In contrast, for phosphorus-starved cells of the green alga *Chlorella kessleri*, an elevated level of unsaturated fatty acids has been reported in all the individual lipids identified, namely PC, PG, DGDG, MGDG and SQDG (El-Sheek and Rady 1995). These studies reveal considerable variation in the way that individual algal species react to nutrient limitation.

In *C. reinhardtii*, the acidic lipids in thylakoid membranes have been studied under sulfur- and phosphorus-starved cultivation (Sato et al. 2000). Sulfur-limited cells lost the most of their SQDG as compared with normal conditions. In this organism, PG content increased by two-fold, representing a compensatory mechanism for the reduced level of the other anionic lipid, SQDG. In agreement, *C. reinhardtii* grown in a media with limited phosphorus, showed a 40% decrease in PG and a concomitant increase in the SQDG content. In general, the replacement of membrane phospholipids by non-phosphorus glycolipids and betaine lipids under phosphate limitation has

been demonstrated in many organisms, including higher plants, photosynthetic bacteria, and algae (e.g., Benning et al. 1995; Härtel et al. 2000; Andersson et al. 2003; Jouhet et al. 2007).

The effect of phosphate starvation on the lipid and fatty acid composition has been studied in some detail in the fresh water eustigmatophyte *Monodus subterraneus* (Khozin-Goldberg and Cohen 2006). Incubation of this alga in media with decreasing phosphate concentrations (175, 52.5, 17.5 and 0 μM) resulted in a gradual decrease in the relative EPA concentration whereas the cellular total lipid content increased, mainly due to TAG accumulation. In phosphate-depleted cells, the proportion of phospholipids reduced from 8.3 to 1.4% of total lipids (Khozin-Goldberg and Cohen 2006).

A general reduction in the degree of fatty acid unsaturation as a response to elevated CO_2 concentration has been reported for several species of green algae (Thompson 1996). In *C. kessleri*, cells grown under low CO_2 (0.04% CO_2 compared to 2% CO_2) showed elevated contents of α-linolenate, especially at both *sn*-1 and *sn*-2 positions of MGDG and DGDG, and also at the *sn*-2 position of PC and PE (Sato et al. 2003c). CO_2 has also been shown to change the content and composition of fatty acids and chloroplast lipids in the unicellular halophilic green alga *Dunaliella salina* (resistant to CO_2 stress) (Muradyan et al. 2004). The response was seen after a one day-long increase in CO_2 concentration from 2 to 10% and resulted in an increase in the total amount of fatty acids on a dry weight basis by 30% (Muradyan et al. 2004).

Effects of CO_2 concentration on the biomass production and lipid accumulation of *Nannochloropsis oculata* has been investigated in a semicontinuous culture (Chiu et al. 2009). Based on the results, this microalga was thought to be best grown in a semicontinuous system aerated with 2% CO_2 and operated by 1-day replacement for long-term biomass production and the higher lipid yield (Chiu et al. 2009). A highly CO_2 tolerant alga, *Chloroccoccum littorale*, has a good potential for aquacultural fatty acid production, and photoautotrophic fatty acid accumulation was investigated in this alga in the presence of inorganic carbon and nitrate at a light intensity of 170 μmol photons.m^{-2}.s^{-1} (Ota et al. 2009). The results of this study showed that fatty acid synthesis was increased at low CO_2 concentrations after nitrate depletion with a controlled HCO_3^-/CO_2 ratio. The relative FA content was 34 wt.% on a dry weight basis under the conditions of 22°C, light intensity of 170 μmol photons.m^{-2}.s^{-1} and CO_2 concentration of 5% with O_2-free gas and this content was comparable with plant seed oils (Ota et al. 2009).

Biomass and lipid productivities of *C. vulgaris* were studied and compared under autotrophic, heterotrophic and mixotrophic growth conditions (Liang et al. 2009). While autotrophic growth did provide a higher cellular lipid content (38% of dry cell weight), the lipid productivity was much lower compared with that during heterotrophic growth with acetate, glucose, or glycerol. Optimal cell growth (2 g.L^{-1}) and lipid productivity (54 mg.L^{-1}.day^{-1}) were obtained using glucose or glycerol at 1% (wt.v^{-1}) whereas higher concentrations were inhibitory (Liang et al. 2009).

To increase the biomass, corn powder hydrolysate (CPH) was supplied as substrate for heterotrophic growth of microalgae *Chlorella photothecoides* (Xu et al. 2006). Growth of *C. photothecoides* and its lipid accumulation using glucose or cassava starch hydrolysate (CSH) as carbon sources have also been compared (Wei et al. 2009). Their data demonstrated that the highest biomass (15.8 g.L^{-1}) and the maximum total lipid yield (4.19 g.L^{-1}) were obtained when CSH was used as a carbon source. In addition, glucose, but not glycerol, has been shown to be a suitable carbon source for the heterotrophic growth of *Porphyridium cruentum* (Oh et al. 2009). The lipid class and fatty acid composition of the green microalga *Chlorella zofingiensis* have been compared under photoautotrophic and heterotrophic cultivation conditions (Liu et al. 2011). Heterotrophic cells fed with 30 g.L^{-1} of glucose were shown to increase lipid yield by 900% and the content of oleic acid (from 17.9 to 35.2% of total fatty acids) in comparison to photoautotrophic cells. Thus, it was concluded that oils from heterotrophic *C. zofingiensis* appeared to be more suitable for biodiesel production (Liu et al. 2011).

For *Scenedesmus obliquus*, lipid accumulation was boosted up to 2.16 g.L^{-1}, to give a value about 40-fold higher in comparison to control conditions, when the cells were pregrown in the optimised medium supplemented with 1.5% glucose (Mandal and Mallick 2009). It should also be noted that the presence of palmitate and oleate as its major fatty acids makes *S. obliquus* biomass a very suitable feedstock for algal-based biodiesel (Mandal and Mallick 2009). To induce the lipid accumulation in *Scenedesmus* sp LX1, algal cells were treated with an antialgal allelochemical, ethyl-2-methyl acetoacetate (EMA) (Xin et al. 2010). Under EMA concentrations of 1.0–2.0 mg.L^{-1} the relative TAG content (about 20 wt.% in control cultures) and TAG productivity (about 23 mg.L^{-1} in control cells) were increased by 79 and 40%, respectively (Xin et al. 2010).

Another efficient approach to increase the cellular TAG content is to shift carbon flux from energy-rich storage compounds, such as starch, to TAG biosynthesis. This has been supported by a number of studies including the inhibition of starch biosynthesis in *C. reinhardtii* and using stress induction of TAG accumulation in *C. reinhardtii* starchless mutants (Wang et al. 2009; Li et al. 2010a, b).

5 Conclusion and Future Directions

Algae provide many potential advantages as feedstocks for biofuel production in comparison to oil crop plants. Although many advances have been made in studying lipid metabolism

in algae, a more detailed understanding of lipid (especially TAG) formation as well as regulation of the carbon flux in general is necessary in order to optimize TAG biosynthesis in algae. For this purpose, the identification of all genes encoding the key enzymes (ACCases, DGAT etc.) controlling TAG synthesis is an important priority. Mutagenesis and enzyme modulators may be useful tools in understanding the primary regulatory mechanisms for lipid pathways in different algae species. These approaches may lead to some additional strategies for enhancing algal oil content. In addition, more attention should be paid to the fatty acid profiles in TAG in relation to optimal biofuel characteristics.

Finally, many results on oil improvement and the regulation of lipid biosynthesis in oil-crop plants (as mentioned in this chapter) can be useful for comparative purposes and to provide clues as to useful potential strategies for algal lipid accumulation.

Acknowledgement Recent research in the authors' laboratory has been supported financially by grants from the BBSRC, DuPont, NERC, the Royal Society and Syngenta for which we are grateful.

References

Abd El-Baky HH, El Baz FK, El-Baroty GS (2004) Production of lipids rich in omega-3 fatty acids from the halotolerant alga *Dunaliella salina*. Biotechnology 3:102–108

Adlerstein D, Bigogno C, Khozin I, Cohen Z (1997) The effect of growth temperature and culture density on the molecular species composition of the galactolipids in the red microalga *Porphyridium cruentum* (Rhodophyta). J Phycol 33:975–979

Alban C, Baldet P, Douce R (1994) Localisation and characterisation of two structurally different forms of acetyl-CoA carboxylase in young pea leaves, of which one is sensitive to aryloxylphenoxypropionate herbicides. Biochem J 300:557–565

Al-Fadhli A, Wahidulla S, D'Souza L (2006) Glycolipids from the red alga *Chondria armata* (Kütz.) Okamura. Glycobiology 16:902–915

Alonso DL, Belarbi E-H, Rodriguez-Ruiz J, Segura CI, Gimenez A (1998) Acyl lipids of three microalgae. Phytochemistry 47:1473–1481

Alonso DL, Belarbi E-H, Fernandez-Sevilla JM, Rodriguez-Ruiz J, Grima EM (2000) Acyl lipid composition variation related to culture age and nitrogen concentration in continuous cultures of the microalga *Phaeodactylum tricornutum*. Phytochemistry 54:461–471

Andersen RJ, Taglialatela-Scafati O (2005) Avrainvilloside, a 6-deoxy-6-aminoglucoglycerolipid from the Greek alga *Avrainvillea nigricans*. J Nat Prod 68:1428–1430

Anderson R, Livermore BP, Kates M, Volcani BE (1978a) The lipid composition of the non-photosynthetic diatom *Nitzschia alba*. Biochim Biophys Acta 528:77–88

Anderson R, Kates M, Volcani BE (1978b) Identification of the sulfolipids in the non-photosynthetic diatom *Nitzschia alba*. Biochim Biophys Acta 528:89–106

Andersson MX, Stridh MH, Larsson KE, Liljenberg C, Sandelius AS (2003) Phosphate-deficient oat replaces a major portion of the plasma membrane phospholipids with the galactolipid digalactosyldiacylglycerol. FEBS Lett 537:128–132

Arisz SA, van Himbergen JAJ, Musgrave A, van den Ende H, Munnik T (2000) Polar glycerolipids of *Chlamydomonas moewusii*. Phytochemistry 53:265–270

Azachi M, Sadka A, Fisher M, Goldshlag P, Gokhman I, Zamir A (2002) Salt induction of fatty acid elongase and membrane lipid modifications in the extreme halotolerant alga *Dunaliella salina*. Plant Physiol 129:1320–1329

Baba M, Kikuta F, Suzuki I, Watanabe MM, Shiraiwa Y (2012) Wavelength specificity of growth, photosynthesis, and hydrocarbon production in the oil-producing green alga *Botryococcus braunii*. Bioresour Technol 109:266–270

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

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

Bigogno C, Khozin-Goldberg I, Cohen Z (2002b) Accumulation of arachidonic acid-rich triacylglycerols in the microalga *Parietochloris incisa* (Trebouxiophyceae, Chlorophyta). Phytochemistry 60:135–143

Bisseret P, Ito S, Tremblay P-A, Volcani BE, Dessort D, Kates M (1984) Occurrence of phosphatidylsulfocholine, the sulfonium analog of phosphatidylcholine in some diatoms and algae. Biochim Biophys Acta 796:320–327

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

Brown MR, Dunstan GA, Norwood SJ, Miller KA (1996) Effects of harvest stage and light on the biochemical composition of the diatom *Thalassiosira pseudonata*. J Phycol 32:64–73

Browse J, Somerville CR (1991) Glycerolipid synthesis: biochemistry and regulation. Annu Rev Plant Physiol Plant Mol Biol 42:467–506

Chappell J (2009) Defining the biological origins of petroleum reserves and oil shales: the biochemical wizardry of *Botryococcus braunii*. International seminar on algal biofuel, Antofagasta, Chile, 7–8 October 2009

Chiu S-Y, Kao C-Y, Tsai M-T, Ong S-C, Chen C-H, Lin C-S (2009) Lipid accumulation and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration. Bioresour Technol 100:833–838

Cho SH, Thompson GA Jr (1987) On the metabolic relationships between monogalactosyldiacylglycerol and digalactosyldiacylglycerol molecular species in *Dunaliella salina*. J Biol Chem 262:7586–7593

D'Ippolito G, Tucci S, Cutignano A, Romano G, Cimino G, Miralto A et al (2004) The role of complex lipids in the synthesis of bioactive aldehydes of the marine diatom *Skeletonema costatum*. Biochim Biophys Acta 1686:100–107

Dembitsky VM (1996) Betaine ether-linked glycerolipids: chemistry and biology. Prog Lipid Res 35:1–51

Domonkos I, Laczkó-Dobos H, Gombos Z (2008) Lipid-assisted protein–protein interactions that support photosynthetic and other cellular activities. Prog Lipid Res 47:422–435

Dörmann P (2005) Membrane lipids. In: Murphy DJ (ed) Plant lipids. Biology, utilisation and manipulation. Blackwell, Oxford, pp 123–161

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

Eccleston VS, Harwood JL (1995) Solubilisation, partial purification and properties of acyl-CoA;glycerol-3-phosphate acyltransferase from avocado (*Persea americana*) fruit mesocarp. BBA 1257:1–10

Eichenberger W, Gribi C (1994) Diacylglyceryl-α-D-glucuronide from *Ochromonas danica* (Chrysophyceae). Plant Physiol 124:272–276

Eichenberger W, Gribi C (1997) Lipids of *Pavlova lutheri*: cellular site and metabolic role of DGCC. Phytochemistry 45:1561–1567

Eichenberger W, Bigler P, Gfeller H, Gribi C, Schmid CE (1995) Phosphatidyl-O-[N-(2-hydroxyethyl)glycine](PHEG), a new glycerophospholipid from brown algae (Phaeophyceae). Plant Physiol 146:398–404

El Maanni A, Dubertret G, Delrieu M-J, Roche O, Tremolieres A (1998) Mutants of *Chlamydomonas reinhardtii* affected in phosphatidylglycerol metabolism and thylakoid biogenesis. Plant Physiol Biochem 36:605–619

El-Sheek MM, Rady AA (1995) Effect of phosphorus starvation on growth, photosynthesis and some metabolic processes in the unicellular green alga *Chlorella kessleri*. Phyton 35:139–151

Eroglu E, Melis A (2010) Extracellular terpenoid hydrocarbon extraction and quantification from the green microalgae *Botryococcus braunii* var. Showa. Bioresour Technol 101:2359–2366

Fabregas J, Maseda A, Dominquez A, Otero A (2004) The cell composition of *Nannochloropsis* sp. changes under different irradiances in semicontinuous culture. World J Microbiol Biotechnol 20:31–35

Fan J, Andre C, Xu C (2011) A chloroplast pathway for de novo biosynthesis of triacylglycerol in *Chlamydomonas reinhardtii*. FEBS Lett 585:1985–1991

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

Gladu PK, Patterson GW, Wikfors GH, Smith BC (1995) Sterol, fatty acids, and pigment characteristics of UTEX 2341, a marine eustigmatophyte identified previously as *Chlorella minutissima* (Chlorophyceae). J Phycol 31:774–777

Gouveia L, Marques AE, da Silva TL, Reis A (2009) *Neochloris oleabundans* UTEX#1185: a suitable renewable lipid source for biofuel production. J Ind Microbiol Biotechnol 36:821–826

Guckert JB, 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

Guihéneuf F, Leu S, Zarka A, Khozin-Goldberg I, Khalilov I, Boussiba S (2011) Cloning and molecular characterization of a novel acyl-CoA:diacylglycerol acyltransferase 1-like gene (*PtDGAT1*) from the diatom *Phaeodactylum tricornutum*. FEBS J 278:3651–3666

Gunstone FD, Harwood JL, Dijkstra AJ (eds) (2007) The lipid handbook, 3rd edn. Taylor & Francis, Boca Raton, 791 pp

Gurr MI, Harwood JL, Frayn KN (2002) Lipid biochemistry. An introduction, 5th edn. Blackwell, Oxford, 320 pp

Guschina IA, Harwood JL (2006a) Lipids and lipid metabolism in eukaryotic algae. Prog Lipid Res 45:160–186

Guschina IA, Harwood JL (2006b) Mechanisms of temperature adaptation in poikilotherms. FEBS Lett 580:5477–5483

Guschina IA, Harwood JL (2007) Complex lipid biosynthesis and its manipulation in plants. In: Ranalli P (ed) Improvement of crop plants for industrial end use. Springer, Dordrecht, pp 253–279

Guschina IA, Harwood JL (2008) Chemical diversity of lipids. In: Wiley encyclopedia of chemical biology. Wiley, Hoboken, pp 1–31

Guschina IA, Harwood JL (2009a) The versatility of algae and their lipid metabolism. Biochimie 91:679–684

Guschina IA, Harwood JL (2009b) Algal lipids and effect of the environment on their biochemistry. In: Arts MT, Brett MT, Kainz MJ (eds) Lipids in aquatic ecosystems. Springer, New York, pp 1–24

Guschina IA, Dobson G, Harwood JL (2003) Lipid metabolism in cultured lichen photobionts with different phosphorus status. Phytochemistry 64:209–217

Haigh WG, Yoder TF, Ericson L, Pratum T, Winget RR (1996) The characterisation and cyclic production of highly unsaturated homoserine lipid in *Chlorella minutissima*. Biochim Biophys Acta 1299:183–190

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

Harwood JL (1996) Recent advances in the biosynthesis of plant fatty acids. Biochim Biophys Acta 1301:7–56

Harwood JL (1998a) Membrane lipids in algae. In: Siegenthaler P-A, Murata N (eds) Lipids in photosynthesis: structure, function and genetics. Kluwer Academic Publishers, Dordrecht, pp 53–64

Harwood JL (1998b) Involvement of chloroplast lipids in the reaction of plants submitted to stress. In: Siegenthaler P-A, Murata N (eds) Lipids in photosynthesis: structure, function and genetics. Kluwer Academic Publishers, Dordrecht, pp 287–302

Harwood JL, Jones AL (1989) Lipid metabolism in algae. Adv Bot Res 16:1–53

Harwood JL, Okanenko AA (2003) Sulphoquinovosyl diacylglycerol – the sulpholipid of higher plants. In: Abrol YP, Ahmad A (eds) Sulfur in plants. Kluwer, Dordrecht, pp 189–219

Harwood JL, Pettitt TP, Jones AL (1988) Lipid metabolism. In: Rogers LJ, Gallon JR (eds) Biochemistry of the Algae and Cyanobacteria. Clarendon, Oxford, pp 49–67

Hsieh C-H, Wu W-T (2009) Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. Bioresour Technol 100:3921–3926

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

Jiang H, Gao K (2004) Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). J Phycol 40:651–654

John U, Tillmann U, Medlin LK (2002) A comparative approach to study inhibition of grazing and lipid composition of a toxic and non-toxic clone of *Chrysochromulina polylepis* (Prymnesiophyceae). Harmful Algae 1:45–57

Jones MR (2007) Lipids in photosynthetic reaction centres: structural roles and functional roles. Prog Lipid Res 46:56–87

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

Kato M, Sakai M, Adachi K, Ikemoto H, Sano H (1996) Distribution of betaine lipids in marine algae. Phytochemistry 42:1341–1345

Keusgen M, Curtis JM, Thibault P, Walter JA, Windust A, Ayer SW (1997) Sulfoquinovosyl diacylglycerols from the alga *Heterosigma carterae*. Lipids 32:1101–1112

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

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

Khozin I, Adlerstein D, Bigogno C, Heimer YM, Cohen Z (1997) Elucidation of the biosynthesis of eicisapentaenoic acid in the microalga *Porphyridium cruentum*. II. Studies with radiolabeled precursors. Plant Physiol 114:223–230

Khozin-Goldberg I, 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

Khozin-Goldberg I, Cohen Z (2011) Unraveling algal lipid metabolism: recent advances in gene identification. Biochimie 93:91–100

Khozin-Goldberg I, Yu HZ, Adlerstein D, Didi-Cohen S, Heimer YM, Cohen Z (2000) Triacylglycerols of the red microalga *Porphyridium cruentum* can contribute to the biosynthesis of eukaryotic galactolipids. Lipids 35:881–889

Khozin-Goldberg I, Shrestha P, Cohen Z (2005) Mobilization of arachidonyl moieties from triacylglycerols into chloroplastic lipids following recovery from nitrogen starvation of the microalga *Parietochloris incisa*. Biochim Biophys Acta 1738:63–71

Laureillard J, Largeau C, Casadevall E (1988) Oleic acid in the biosynthesis of the resistant biopolymers of *Botryococcus braunii*. Phytochemistry 27:2095–2098

Li Y, Horsman M, Wang B, Wu N, Lan CQ (2008) Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. Appl Microbiol Biotechnol 81:629–636

Li R, Yu K, Hildebrand DF (2010a) *DGAT1, DGAT2*, and *PDAT* expression in seeds and other tissues of epoxy and hydroxy fatty acid accumulating plants. Lipids 45:145–157

Li Y, Han D, Hu G, Sommerfeld M, Hu Q (2010b) Inhibition of starch synthesis results in overproduction of lipids in *Chlamydomonas reinhardtii*. Biotechnol Bioeng 107:258–268

Liang Y, Sarkany N, Cui Y (2009) Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic conditions. Biotechnol Lett 31:1043–1049

Liu J, Huang J, Sun Z, Zhong Y, Jiang Y, Chen F (2011) Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*: assessment of algal oils for biodiesel production. Bioresour Technol 102:106–110

Lu C, Xin Z, Miquel M, Browse J (2009) An enzyme regulating triacylglycerol composition is encoded by the *ROD1* gene of *Arabidopsis*. Proc Natl Acad Sci USA 106:18837–18842

Lung S-C, Weselake RJ (2006) Diacylglycerol acyltransferase: a key mediator of plant triacylglycerol synthesis. Lipids 41:1073–1088

Lynn SG, Kilham SS, Kreeger DA, Interlandi SJ (2000) Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). J Phycol 36:510–522

Makewicz A, Gribi C, Eichenberger W (1997) Lipids of *Ectocarpus fasciculatus* (Phaeophyceae). Incorporation of [1-^{14}C]oleate and the role of TAG and MGDG in lipid metabolism. Plant Cell Physiol 38:952–960

Manaf AM, Harwood JL (2000) Purification and characterisation of acyl-CoA:glycerol 3-phosphate acyltransferase from oil palm (*Elaeis guineensis*) tissues. Planta 210:318–328

Mandal S, Mallick N (2009) Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. Appl Microbiol Biotechnol 84:281–291

Matsunaga T, Matsumoto M, Maeda Y, Sugiyama H, Sato R, Tanaka T (2009) Characterization of marine microalga, *Scenedesmus* sp. strain JPCC GA0024 towards biofuel production. Biotechnol Lett 31:1367–1372

McLarnon-Riches CJ, Rolph CE, Greenway DLA, Robinson PK (1998) Effects of environmental factors and metals on *Selenastrum capricornutum*. Phytochemistry 49:1241–1247

Metzger P, Casadevall E (1991) Botryococcoid ethers, ether lipids from *Botryococcus braunii*. Phytochemistry 30:1439–1444

Metzger P, Largeau C (2005) *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. Appl Microbiol Biotechnol 66:486–496

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

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Huner NPA (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Mol Biol Rev 70:222–252

Muradyan EA, Klyachko-Gurvich GL, Tsoglin LN, Sergeyenko TV, Pronina NA (2004) Changes in lipid metabolism during adaptation of the *Dunaliella salina* photosynthetic apparatus to high CO_2 concentration. Russ J Plant Physiol 51:53–62

Murata N, Siegenthaler P-A (1998) Lipids in photosynthesis: an overview. In: Siegenthaler P-A, Murata N (eds) Lipids in photosynthesis: structure, function and genetics. Kluwer Academic Publishers, Dordrecht, pp 1–20

Napolitano GE (1994) The relationship of lipid with light and chlorophyll measurement in freshwater algae and periphyton. J Phycol 30:943–950

Niehaus TD, Okada S, Devarenne TP, Watt DS, Sviripa V, Chappell J (2011) Identification of unique mechanisms for triterpene biosynthesis in *Botryococcus braunii*. PNAS 108:12260–12265

Oh SH, Han JG, Kim Y, Ha JH, Kim SS et al (2009) Lipid production in *Porphyridium cruentum* grown under different culture conditions. Jpn J Biosci Bioeng 108:429–434

Okada S, Chappell J, Devarenne TP (2000) Molecular characterization of squalene synthase from the green microalga *Botryococcus braunii*, race B. Arch Biochem Biophys 373:307–317

Okada S, Devarenne TP, Murakami M, Abe H, Chappell J (2004) Characterization of botryococcene synthase enzyme activity, a squalene synthase-like activity from the green microalga *Botryococcus braunii*, Race B. Arch Biochem Biophys 422:110–118

Ota M, Kato Y, Watanabe H, Watanabe M, Sato Y, Smith RL Jr, Inomata H (2009) Fatty acid production from a highly CO_2 tolerant alga, *Chlorococcum littorale*, in the presence of inorganic carbon and nitrate. Bioresour Technol 100:5237–5242

Page RA, Okada S, Harwood JL (1994) Acetyl-CoA carboxylase exerts strong flux control over lipid synthesis in plants. Biochim Biophys Acta 1210:369–372

Pineau B, Girard-Bascou J, Eberhad S, Choquet Y, Tremolieres A, Gerard-Hirne C et al (2004) A single mutation that causes phosphatidylglycerol deficiency impairs synthesis of photosystem II cores in *Chlamydomonas reinhardtii*. Eur J Biochem 271:329–338

Raja R, Hemaiswarya S, Ashok Kumar N, Sridhar S, Rengasamy R (2008) A perspective on the biotechnological potential of microalgae. Crit Rev Microbiol 34:77–88

Rao AR, Dayananda C, Sarada R, Shamala TR, Ravishankar GA (2007a) Effects of salinity on growth of green alga *Botryococcus braunii* and its constituents. Bioresour Technol 98:560–564

Rao R, Sarada R, Ravishankar GA (2007b) Influence of CO_2 on growth and hydrocarbon production in *Botryococcus braunii*. J Microbiol Biotechnol 17:414–419

Regnault A, Chevrin D, Chammai A, Piton F, Calvayrac R, Mazliak P (1995) Lipid composition of *Euglena gracilis* in relation to carbon-nitrogen balance. Phytochemistry 40:725–733

Reitan KI, Rainuzzo JR, Olsen Y (1994) Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. J Phycol 30:972–977

Renaud SM, Thinh LV, Lambrinidis G, Parry DL (2002) Effects of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 211:195–214

Riekhof WR, Sears BB, Benning C (2005) Annotation of genes involved in glycerolipid biosynthesis in *Chlamydomonas reinhardtii:* discovery of the betaine lipid synthase $BTA1_{Cr}$. Eukaryot Cell 4:242–252

Roesler K, Shintani D, Savage L, Boddupalli S, Ohlrogge J (1997) Targeting of the Arabidopsis homomeric acetyl-coenzyme A carboxylase to plastids of rapeseed. Plant Physiol 113:75–81

Roessler PG (1990) Purification and characterization of acetyl-CoA carboxylase from the diatom *Cyclotella cryptica*. Plant Physiol 92:73–78

Roessler PG, Ohlrogge JB (1993) Cloning and characterization of the gene that encodes acetyl-coenzyme A carboxylase in the alga *Cyclotella cryptica*. J Biol Chem 268:19254–19259

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

Sato N, Sonoike K, Kawaguchi A, Tsuzuki M (1996) Contribution of lowered unsaturation levels of chloroplast lipids to high temperature tolerance of photosynthesis in *Chlamydomonas reinhardtii*. J Photochem Photobiol B 36:333–337

Sato N, Hagio M, Wada H, Tsuzuki M (2000) Environmental effects on acidic lipids of thylakoid membranes. In: Harwood JL, Quinn PJ (eds) Recent advances in the biochemistry of plant lipids. Portland Press Ltd, London, pp 912–914

Sato N, Aoki M, Maru Y, Sonoike K, Minoda A, Tsuzuki M (2003a) Involvement of sulfoquinovosyl diacylglycerol in the structural integrity and heat-tolerance of photosystem II. Planta 217:245–251

Sato N, Tsuzuki M, Kawaguchi A (2003b) Glycerolipid synthesis in *Chlorella kessleri* 11h I. Existence of a eukaryotic pathway. Biochim Biophys Acta 1633:27–34

Sato N, Tsuzuki M, Kawaguchi A (2003c) Glycerolipid synthesis in *Chlorella kessleri* 11h II. Effect of CO_2 concentration during growth. Biochim Biophys Acta 1633:35–42

Singh Y, Kumar HD (1992) Lipid and hydrocarbon production by *Botryococcus* spp. under nitrogen limitation and anaerobiosis. World J Microbiol Biotechnol 8:121–124

Son BW (1990) Glycolipids from *Gracilaria verrucosa*. Phytochemistry 29:307–309

Stobart K, Mancha M, Lenman M, Dahlqvist A, Stymne S (1997) Triacylglycerols are synthesised and utilized by transacylation reactions in microsomal preparations of developing safflower (*Carthamus tinctorius*) seeds. Planta 203:58–66

Sukenik A, Livne A (1991) Variations in lipid and fatty acid content in relation to acetyl-CoA carboxylase in the marine prymnesiophyte *Isochrysis galbana*. Plant Cell Physiol 32:371–378

Sukenik A, Yamaguchi Y, Livne A (1993) Alterations in lipid molecular species of the marine eustigmatophyte *Nannochloropsis* sp. J Phycol 29:620–626

Sushchik NN, Kalacheva GS, Zhila NO, Gladyshev MI, Volova TG (2003) A temperature dependence of the intra- and extracellular fatty acid composition of green algae and cyanobacterium. Russ J Plant Physiol 50:374–380

Takagi M, Karseno, Yoshida T (2006) Effect of salt concentration on intracellular accumulation of lipids and triacylglycerols in marine microalgae *Dunaliella* cells. J Biosci Bioeng 3:223–226

Tanoi T, Kawachi M, Watanabe MM (2011) Effects of carbon source on growth and morphology of *Botryococcus braunii*. J Appl Phycol 23:25–33

Tatsuzawa H, Takizawa E (1995) Changes in lipid and fatty acid composition of *Pavlova lutheri*. Phytochemistry 40:397–400

Tatsuzawa H, Takizawa E, Wada M, Yamamoto Y (1996) Fatty acid and lipid composition of the acidophilic green alga *Chlamydomonas* sp. J Phycol 32:598–601

Templier J, Largeau C, Casadevall E (1984) Mechanism of non-isoprenoid hydrocarbon biosynthesis in *Botryococcus braunii*. Phytochemistry 23:1017–1028

Thompson GAJ (1996) Lipids and membrane function in green algae. Biochim Biophys Acta 1302:17–45

Tremolieres A, Siegenthaler P-A (1998) Role of acyl lipids in the function of photosynthetic membranes in higher plants. In: Siegenthaler P-A, Murata N (eds) In lipids in photosynthesis: structure, function and genetics. Kluwer Academic Publishers, Dordrecht, pp 145–173

Vazquez-Duhalt R, Arredondo-Vega BO (1991) Haloadaptation of the green alga *Botryococcus braunii* (Race A). Phytochemistry 30:2919–2925

Voelker T, Kinney AJ (2001) Variations in the biosynthesis of seed-storage lipids. Annu Rev Plant Physiol Plant Mol Biol 52:335–361

Wada H, Murata N (2010) Lipids in photosynthesis: essential and regulatory functions. Springer, Dordrecht, 474 pp

Wagner M, Hoppe K, Czabany T, Heilmann M, Daum G, Feussner I, Fulda M (2010) Identification and characterization of an acyl-CoA:diacylglycerol acyltransferase 2 (DGAT2) gene from the microalga *O. tauri*. Plant Physiol Biochem 48:407–416

Wang ZT, Ullrich N, Joo S, Waffenschmidt S, Goodenough U (2009) Algal lipid bodies: stress induction, purification, and biochemical characterization in wild-type and starchless *Chlamydomonas reinhardtii*. Eukaryot Cell 8:1856–1868

Wei A, Zhang X, Wei D, Chen G, Wu Q, Yang S-T (2009) Effects of cassava starch hydrolysate on cell growth and lipid accumulation of the heterotrophic microalgae *Chlorella protothecoides*. J Ind Microbiol Biotechnol 36:1383–1389

Weselake RL, Shah S, Tang M, Quant PA, Snyder CL, Furukawa-Stoffer TL, Zhu W, Taylor DC, Zou J, Kumar A, Hall L, Laroche A, Rakow G, Raney P, Moloney MM, Harwood JL (2008) Metabolic control analysis is helpful for informed genetic manipulation of oilseed rape (*Brassica napus*) to increase seed oil content. J Exp Bot 59:3543–3549

Weselake RJ, Taylor DC, Rahman H, Laroche A, McVetty PBE, Harwood JL (2009) Increasing the flow of carbon into seed oil. Biotechnol Adv 27:866–878

Xin L, Hong-ying H, Yin-hu W (2010) Enhancement effect of ethyl-2-methyl acetoacetate on triacylglycerols production by a freshwater microalga, *Scenedesmus* sp. LX1. Bioresour Technol 101:9819–9821

Xu H, Miao X, Wu Q (2006) High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. J Biotechnol 126:499–507

Xu J, Zheng Z, Zou J (2009) A membrane-bound glycerol-3-phosphate acyltransferase from *Thalassiosira pseudonana* regulates acyl composition of glycerolipids. Botany 87:544–551

Yonezawa N, Matsuura H, Shiho M, Kaya K, Watanabe MM (2012) Effects of soybean curd wastewater on the growth and hydrocarbon production of *Botryococcus braunii* strain BOT-22. Bioresour Technol 109:304–307

Zhu CJ, Lee YK, Chao TM (1997) Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* TK1. J Appl Phycol 9:451–457