Chapter 1
Lipids: From Chemical Structures, Biosynthesis, and Analyses to Industrial Applications

Yonghua Li-Beisson, Yuki Nakamura, and John Harwood

Abstract Lipids are one of the major subcellular components, and play numerous essential functions. As well as their physiological roles, oils stored in biomass are useful commodities for a variety of biotechnological applications including food, chemical feedstocks, and fuel. Due to their agronomic as well as economic and societal importance, lipids have historically been subjected to intensive studies. Major current efforts are to increase the energy density of cell biomass, and/or create designer oils suitable for specific applications. This chapter covers some basic aspects of what one needs to know about lipids: definition, structure, function, metabolism and focus is also given on the development of modern lipid analytical tools and major current engineering approaches for biotechnological applications. This introductory chapter is intended to serve as a primer for all subsequent chapters in this book outlining current development in specific areas of lipids and their metabolism.

Keywords Fatty acids • Lipid biotechnology • Lipid metabolism • Lipid analysis • Algae • Plants

Y. Li-Beisson (✉)
Institut de Biologie Environnementale et Biotechnologie, UMR 7265 CEA - CNRS - Université Aix Marseille, CEA Cadarache, Saint-Paul-lez-Durance 13108, France
e-mail: yonghua.li@cea.fr

Y. Nakamura
Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan
e-mail: nakamura@gate.sinica.edu.tw

J. Harwood
School of Biosciences, Cardiff University, Cardiff CF10 3AX, UK
e-mail: harwood@cardiff.ac.uk

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Introduction

This article is the first chapter opening our current book on 'Lipids in plant and algae development' including chapters on specific topics contributed by leading experts in their respective fields. We consider it necessary to use the first chapter to introduce what are lipids, their chemical structures and biosynthesis. We also cover topics that are less explored in the other chapters, i.e. definition, classical and new development in lipid analysis technologies, and also summarize the applications of lipids and current biotechnological goals in a variety of domains. Due to limitations of space and scope, focus is given here on knowledge gained specifically from the study of higher plants and algae.

Definition and Importance

Whereas most lipid biochemists have a working understanding of what is meant by the term lipid (derived from the Greek *lipos*- or fat) there is no generally-accepted definition. Often lipids are described as hydrophobic or amphipathic small molecules that are readily soluble in organic solvents such as chloroform, ethers or alcohols. In fact, the solubility definition can be misleading because many lipids are nearly as soluble in water as in organic solvents (Christie 2013).

Although there are a huge number of different classes of lipids, the most abundant in most organisms, including algae and higher plants, are glycerolipids (which are based on the trihydric alcohol glycerol). Other types of lipids which have often been used for the classification of algae are the pigments, principal of which are the carotenoids and the chlorophylls (or their derivatives).

Lipids play four major roles in different organisms (Gurr et al. 2002). First, they naturally form membrane structures where they represent 30–70 % of the total dry weight. Phosphoglycerides and glycosylglycerides are the main such lipids in plants and algae. Sometimes, ether lipids are important for this purpose in algae. Second, where storage lipids are present they are usually triacylglycerols (TAGs) although some algae can accumulate hydrocarbons, such as in *Botryococcus braunii* (Banerjee et al. 2002). A few plants (e.g. jojoba seeds) accumulate wax esters for storage. Third, lipids or their metabolites can act as signaling molecules. For algae this area is still in its infancy but, by comparison with higher plants, is likely to expand rapidly in the next few years. Fourth, in many organisms lipids contribute to the surface coverings. This function has not really been examined in depth for algae but is likely to be more important for macro-species. In plants, the surface coverings (including surface waxes, cutin and suberin) have been well examined (Kunst and Samuels 2009; Pollard et al. 2008; Kolattukudy 2001).

There are a number of rather specialized functions for individual classes of lipids of which photosynthesis is clearly of vital importance in algae and in many higher plant tissues (Mizusawa and Wada 2012).

Structures of Important Lipids in Algae and Higher Plants

Depending on the alga concerned, the quantitative importance of different lipids will vary. However, as mentioned above phosphoglycerides, glycosylglycerides and some ether lipids are important (Guschina and Harwood 2013). In extrachloroplastic membranes, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Fig. 1.1) are prominent while the chloroplast thylakoids typically contain three glycosylglycerides (monogalactosyldiacylglycerol, MGDG; digalactosyldiacylglycerol, DGDG; sulfoquinovosyldiacylglycerol, sulfolipid, SQDG) and phosphatidylglycerol (PG) (Fig. 1.1) (Harwood and Guschina 2009; Harwood and Jones 1989). For higher plants, the same general remarks apply (Table 1.1) although ether lipids are not usually significant.

In non-photosynthetic tissues such as roots the relative contribution of the glycosylglycerides is reduced because plastids are less abundant organelles compared with leaves (Gunstone et al. 2007).

The structure of some important algal ether lipids is shown in Fig. 1.2. Of these, DGTS (diacylglyceryltrimethylhomoserine ether lipid) is found in many green algae while DGTA (diacylglycerylhydroxymethyl trimethyl-β-alanine) and DGCC (diacylglycerylcarboxy(hydroxylmethyl) choline) are significant in some brown algae (Guschina and Harwood 2006).

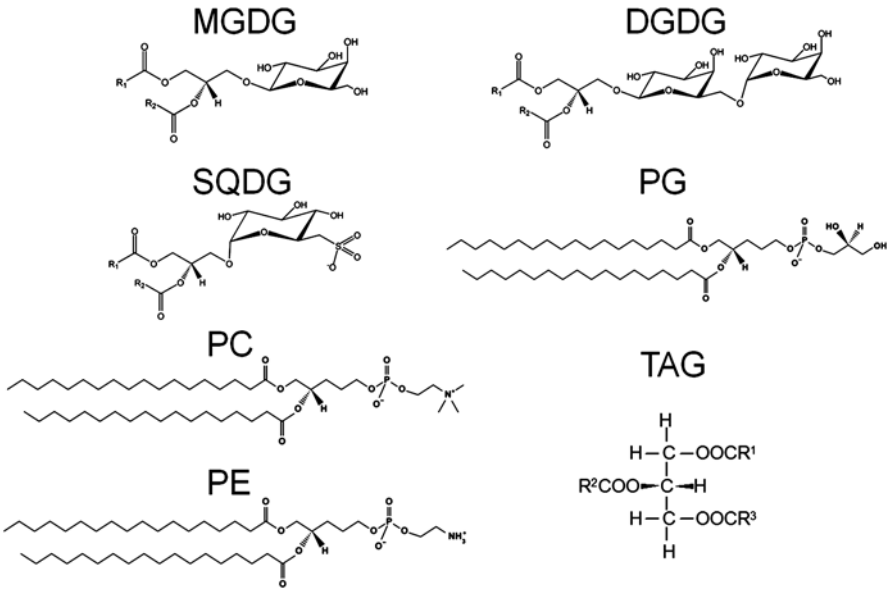

Fig. 1.1 Some examples of major membrane and storage lipid structures. *R* represents any acyl group. Abbreviations: *MGDG* monogalactosyldiacylglycerol, *DGDG* digalactosyldiacylglycerol, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *SQDG* sulfoquinovosyldiacylglycerol, *TAG* triacylglycerol

Table 1.1 Lipid composition of some plant tissues

	% total lipids									
	MGDG	DGDG	SQDG	PC	PE	PG	PI	DPG	TAG	Other
Potato tuber	6	16	1	26	13	1	6	1	15	15[a]
Apple fruit	1	5	1	23	11	1	6	1	5	48[b]
Soybean	tr.	tr.	tr.	4	2	tr.	2	tr.	88	4
Maize leaf	42	31	5	6	3	7	1	tr.	tr.	5
Clover leaf	46	28	4	7	5	6	1	tr.	tr.	3

	Fatty acid composition (% total)						
	16:0	16:1[c]	18:0	18:1	18:2	18:3	Other
Rapeseed (LEAR) oil	4	–	2	62	20	10	2
Palm oil	44	tr.	4	39	11	1	1
Palm kernel oil	9	tr.	2	15	2	tr.	72[d]
Castor bean oil	1	–	tr.	3	5	–	91[e]
Maize leaf	8	4	2	7	8	66	5
Pea leaf	12	3	1	2	22	56	4
Spinach leaf	13	3	tr.	7	16	56	5[f]

For more information see Gunstone et al. (2007), Gurr et al. (2002), and Harwood (1980)
Fatty acids are abbreviated with the numbers before and after the colon showing the number of carbon atoms and the number of double bonds, respectively. 18:1 is oleate, 18:2 is linoleate and 18:3 is α-linolenate
Abbreviations: *PI* phosphatidylinositol, *DPG* diphosphatidylglycerol (cardiolipin), *tr.* trace (<0.5), the others as in Fig. 1.1
[a]Cerebroside, sterol glycoside and acyl sterol glycosides are significant
[b]Cerebrosides, sterol glycosides and sterols are significant
[c]In leaf tissue, *trans*-3-hexadecenoate is a significant component of the PG fraction
[d]Contains 48 % laurate and 16 % myristate
[e]Contains 90 % ricinoleate
[f]a '16:3-plant' with a prokaryotic-like fatty acid metabolism and 5 % *cis*-7, 10, 13-hexadecatrienoic acid, almost entirely located in MGDG

The storage compound, i.e. TAG, is also shown in Fig. 1.1. Accumulation of this lipid is usually dependent on algal growth conditions and can be enhanced by (nutrient) stress (Hu et al. 2008). This is important for the potential commercial exploitation of algae such as for the provision of long-chain polyunsaturated fatty acids. For higher plants, TAG is almost the exclusive lipid accumulated and typical oil seeds may have up to 70 % dry weight as lipid. Plants contain a spectacular variety of fatty acids within their accumulated oils although most commercially-important crops have palmitate, oleate and linoleate as major components. However, many plant TAGs have niche markets including as renewable materials for the chemical industry. Two examples are shown in Table 1.1 where palm kernel oil is used in the detergent industry and castor oil as a lubricant (Murphy 2005; Carlsson et al. 2011; Vanhercke et al. 2013). On the other hand, rapeseed and palm oils contain the usual fatty acids (above) as their main components.

All the above compounds are acyl lipids. Therefore, within each lipid class there will be a host of molecular species, each containing different combinations and

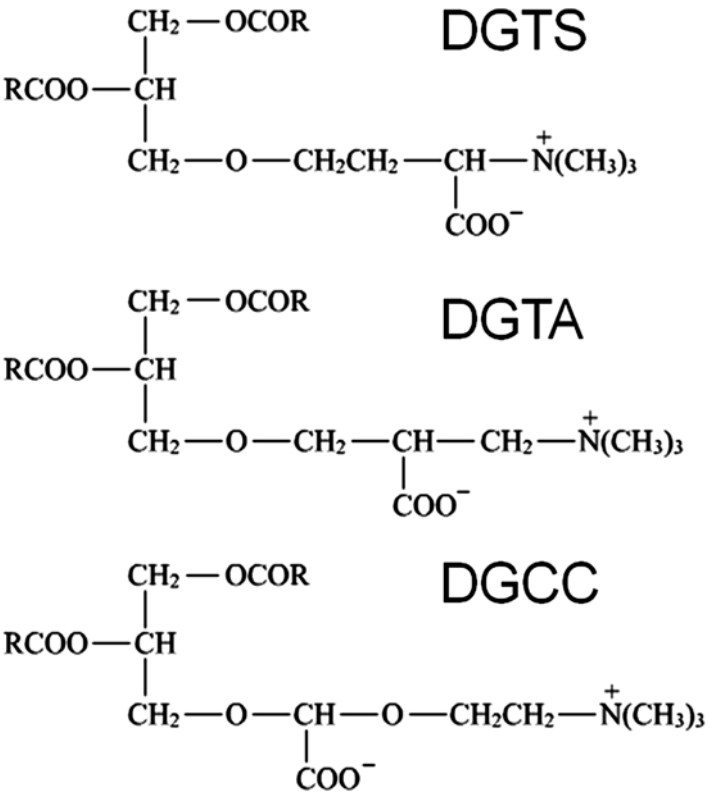

Fig. 1.2 Examples of structures of ether lipids found in algae. *R* represents any acyl group. Abbreviations: *DGTS* 1,2-diacylglyceryl-3-*O*-4′-(*N,N,N*-trimethyl)-homoserine, *DGTA* 1,2-diacylglyceryl-3-*O*-2′-(hydroxymethyl)-(*N,N,N*-trimethyl)-β-alanine,*DGCC*1,2-diacylglyceryl-3-*O*-carboxy-(hydroxymethyl)-choline

positional distributions of fatty acids. Freshwater algae contain similar fatty acids to terrestrial plants although their proportions vary considerably. In general, freshwater algae contain higher proportions of 16C (carbon) and less 18C fatty acids than plants. Unlike marine algae, 20C (or 22C) polyunsaturated fatty acids (PUFA) are usually absent (or only present in trace amounts) in freshwater species. However, in those algae growing in high-salt environments, such as the Dead Sea, arachidonate (ARA) and n-3 eicosapentaenoate (EPA) are often major components (Pohl and Zurheide 1979; Harwood and Jones 1989).

The leaves, shoots and roots of higher plants are dominated by palmitate as the main saturated fatty acid, oleate as the principal monounsaturated and linoleate and α-linolenate as the prominent polyunsaturated acids. The latter is particularly enriched in chloroplast thylakoids and, hence, in leaf tissue (Table 1.1).

Different Algae and Plants Often Contain Contrasting Lipid Compositions

Algae are a very diverse group of organisms so, unsurprisingly, their lipid compositions can vary markedly. The fatty acid compositions of a range of different algae are shown in Table 1.2. As mentioned above, 16C unsaturated fatty acids are often

Table 1.2 Fatty acid composition of different algae

	Fatty acid composition (% total)												
	14:0	16:0	16:1	16:2	16:3	16:4	18:1	18:2	18:3	18:4	20:4	20:5	22:6
Freshwater spp.													
Scenedesmus obliquus	1	35	2	tr.	tr.	15	9	6	30	20	–	–	–
Chorella vulgaris	2	26	8	7	2	–	2	33	20	–	–	–	–
Chlamydomonas reinhardtii	1	21	4	1	4	23	7	6	31	2	–	–	–
Salt-tolerant spp.													
Ankistrodesmus sp.	tr.	13	3	1	1	14	25	2	29	2	–	1	–
Isochrysis sp.	16	15	4	1	1	–	21	5	4	17	tr.	tr.	9
Nannochlorsis atomus	1	20	10	4	15	–	5	10	22	3	2	3	tr.
Marine phytoplankton													
Monochrysis lutheri (Chrysophyceae)	10	13	22	5	7	1	3	1	tr.	2	1	18	7
Olisthodiscus spp. *(Xanthophyceae)*	8	14	10	2	2	1	4	4	6	18	2	19	2
Lauderia borealis (Bacillariophyceae)	7	12	21	3	12	1	2	1	tr.	–	1	3	–
Amphidinium carterale (Dinophyceae)	3	24	1	1	tr.	–	5	1	2	15	–	14	25
Dunaliella salina (Chlorophyceae)	tr.	41	15	tr.	–	–	11	8	19	–	–	–	–
Hemiselmis brunescens (Cryptophyceae)	1	13	3	3	tr.	tr.	2	tr.	9	30	tr.	14	–
Marine macroalgae													
Fucus vesiculosus (Phaeophyceae)	tr.	21	2	tr.	–	tr.	26	10	7	4	15	8	–
Chondrus crispus (Rhodophyceae)	tr.	34	6	tr.	–	–	9	1	1	4	18	22	–
Ulva lactuca (Chlorophyceae)	1	18	2	tr.	1	18	9	2	17	24	1	2	tr.

Fatty acids are abbreviated with the figure before the colon indicating the number of carbon atoms and the figure after the colon showing the number of double bonds. See Harwood and Jones (1989) for sources of information. Although the precise structure of individual fatty acids reported was not always determined the unsaturated components are likely to be mainly 16:1n-7, 16:2n-4, 16:3n-4 or 16:3n-3, 16:4n-3, 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3, 20:4n-6, 20:5n-3, 22:6n-3

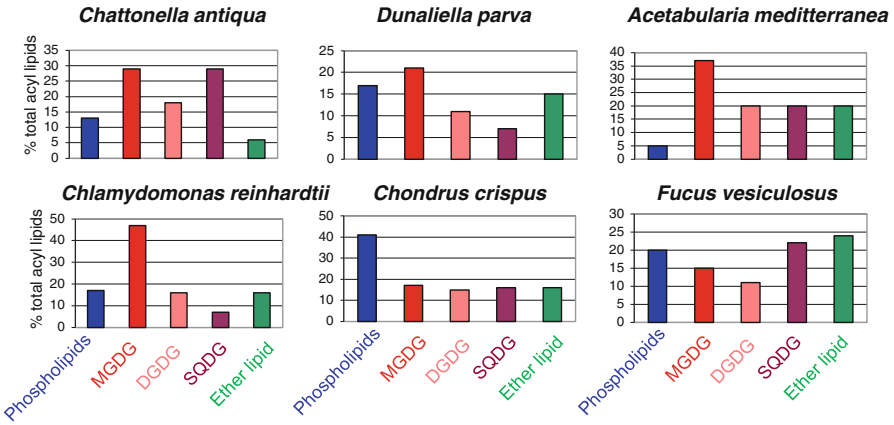

Fig. 1.3 The acyl lipid composition of some algae. Abbreviations: Phospholipids include PC and PE; ether lipids include DGTS and DGTA. Other abbreviations as detailed in Fig. 1.1 legend

significant while 20 or 22C PUFA are prominent in marine or salt-tolerant species. Since algae are often at the bottom of food chains then nutritionally-important PUFA (EPA or DHA i.e. docosahexaenoic acid), often referred to as 'fish oil components', are extremely important and may be more exploited commercially in the future.

Just as the fatty acid composition of algae varies considerably so does their relative acyl lipid content. Some examples are shown in Fig. 1.3. For more information, the reader is referred to Ratledge and Cohen (2010), Guschina and Harwood (2006), Harwood and Scrimgeour (2007), and Harwood and Jones (1989). By comparison, the acyl lipid compositions of higher plant tissues are much more consistent. Thus, in Table 1.1, monocotyledon leaves (e.g. maize) are rather similar to those of dicotyledons (e.g. clover). Moreover, the overall fatty acid compositions of leaves are also consistent even when a particular species (e.g. spinach) may have some subtle differences in metabolism (see Table 1.1). More details of higher plant lipids and their distribution will be found in Harwood and Gunstone (2007) and Murphy (2005). Nonetheless, little is known about the evolutionary advantage or physiological needs whereby particular cell types preferentially synthesize one type of fatty acid rather than another.

Biosynthesis of Major Glycerolipids and Their Cellular Functions

Lipid biosynthesis is a complex hub, so the detailed lipid metabolic map may be somewhat overwhelming. Here, we present a simplified view of lipid biosynthesis (Fig. 1.4). We emphasize the aspects that will be covered by the subsequent chapters

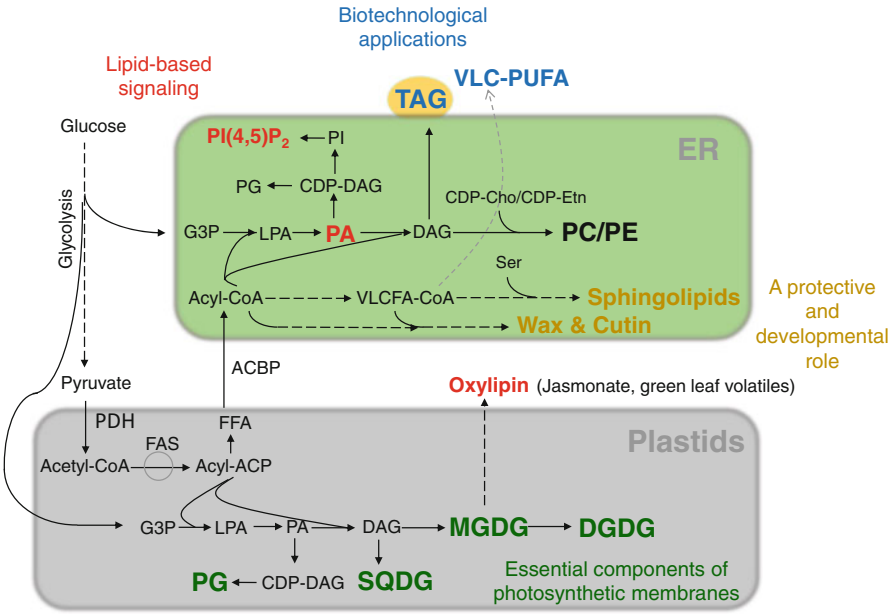

Fig. 1.4 A simplified view of lipid biosynthesis in green photosynthetic cells. Abbreviations: *ACBP* acyl-CoA binding protein, *CDP-DAG* cytidinediphosphate-diacylglycerol, *CoA* coenzyme A, *FAS* fatty acid synthase, *FFA* free (non-esterified) fatty acid, *PA* phosphatidic acid, *PI* phosphatidylinositol, *VLC-PUFA* very long chain (>20C) polyunsaturated fatty acids, other abbreviations as detailed in Fig. 1.1 legend

in this book. For more complete information on current knowledge of acyl-lipid metabolism, readers are referred to Li-Beisson et al. (2013) for the higher plant model *Arabidopsis thaliana*; and to Li-Beisson et al. (2015) and Liu and Benning (2013) for the model green microalga *Chlamydomonas reinhardtii*.

Carbon Source and Two-Carbon by Two-Carbon Fatty Acid Elongation in the Plastid

Most of lipid classes discussed in this book contain both glycerol and fatty acids, thus their biosyntheses share some common pathways. After carbon fixation through photosynthetic reactions, glycolysis provides two major substrates for biosynthesis of glycerolipids, i.e. glycerol 3-phosphate (G3P) and acetyl-Coenzyme A (CoA). Acetyl-CoA is the major source for plastidial fatty acid biosynthesis. Although two reactions have been proposed to supply acetyl-CoA for de novo fatty acid synthesis, now several lines of evidence indicate that pyruvate is the major source (Schwender et al. 2003; Bao et al. 1998; Lin et al. 2003). This reaction is catalyzed by pyruvate dehydrogenase (PDH). The contribution of plastidial acetyl-CoA synthetase (ACS)

to supply acetyl-CoA for fatty acid synthesis is considered marginal in several plant tissue types (Bao et al. 1998; Lin et al. 2003). In algae, genes encoding orthologous enzymes to PDH and ACS occur, but their detailed contribution to fatty acid synthesis awaits experimental evidence.

The first committed step is catalyzed by acetyl-CoA carboxylase (ACCase) producing malonyl-CoA. The chain elongates 2C at a time, and these reactions are catalyzed by four well characterized enzymes together called fatty acid synthase (FAS), and the reactions terminate, in most species, when the chain length reaches 16C or 18C. The acyl chain length beyond 16C or 18C can be further elongated by ER-resident fatty acid elongases to produce very-long-chain fatty acid (VLCFA)-CoA (>20C), which are substrates for sphingolipids or surface lipids such as waxes and cutin (Kunst and Samuels 2009).

Glycerolipid Assembly

Acyl-chains produced in the chloroplasts serve either as substrates for chloroplastic glycerolipid biosynthesis or are exported to the ER where they act as an acyl donor for ER-located glycerolipid synthesis pathways. Glycerolipids are assembled by two sequential transfers of acyl chains to G3P resulting in production of phosphatidic acid (PA), which occurs in both plastids and the ER (Kennedy pathway) (Kennedy 1956) (Fig. 1.4). PA is a substrate for both diacylglycerol (DAG) and cytidine diphosphate-DAG (CDP-DAG), which are further converted to different classes of glycerolipids. In addition, PA itself functions as a signaling molecule. In chloroplasts, four major glycerolipid classes, PG, MGDG, DGDG, SQDG, are synthesized. MGDG, DGDG and SQDG are synthesized from DAG, whereas PG is produced from CDP-DAG. These lipid classes are synthesized exclusively in chloroplasts except PG, which can be also produced in the ER and mitochondria. In the ER, major phospholipid classes are synthesized, including PA, PC, PE, PG, PI and PS. PC and PE are produced from DAG using CDP-Cho or CDP-Etn, respectively, while PS is synthesized from PE by a base-exchange reaction (Yamaoka et al. 2011). PI is synthesized from CDP-DAG, which is also a substrate of PG biosynthesis. The major storage lipid TAG is synthesized from DAG by two complementary reactions, acyl-CoA dependent and acyl-CoA independent reactions catalyzed by a diacylglycerol acyltransferase (DGAT) and a phospholipid: diacylglycerol acyltransferase (PDAT), respectively (Zhang et al. 2009).

Major Cellular Functions of Lipids

The galactolipids (MGDG and DGDG) are the most predominant glycerolipid class in photosynthetic tissues of plants as well as algal cells under photoautotrophic growth. MGDG and DGDG are essential in the development and function of

thylakoids and entire plastids. PG is the only phospholipid produced in the chloroplasts and it is an essential component in the center of photosystem II.

As well as their role in plastid function, galactolipids are also substrates for production of oxylipins, because polyunsaturated fatty acids released from the galactolipids are preferred substrates for oxidation reactions catalyzed by lipoxygenases (Feussner and Wasternack 2002). Initial biosynthetic steps to jasmonic acid (JA), a representative oxylipin signal, occur in chloroplasts. Moreover, galactolipids are the substrates of various volatile oxylipin derivatives, named green leaf volatiles, in plant signaling, response and communications. PI can be further phosphorylated to produce phosphatidylinositol phosphates (or phosphoinositides). A representative phosphoinositide, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), has functions in lipid signaling (Boss and Im 2012).

Surface lipids represent an essential role of lipids to coat the outermost surfaces of plant tissues to protect from environmental stresses. Therefore, their metabolism is tightly linked to the developmental regulation of plant body shape. Sphingolipids are a lipid class containing a backbone of aliphatic amino alcohols. They are major components in plasma membranes, tonoplast and endomembranes, playing structural and bioactive roles.

Major Tools in Lipid Analyses: From Chemical Composition to Lipid Imaging

As a first step toward attributing functions to lipids, chemical composition analyses of the tissue studied are obviously important. Due to their hydrophobic nature, lipid analyses usually start with their extraction into an organic solvent, which remove many hydrophilic cellular components such as carbohydrate, sugars and proteins. The extracted lipids contained in the solvent are usually dried down with a stream of nitrogen gas. An estimation of total lipid content can be determined by dry weight at this stage; but this measurement takes into account the weight of all solvent-soluble components and, thus, represents a total of lipophilic compounds including fatty acids, glycerolipids, sphingolipids, triterpenoids and chlorophyll or other pigments as well as non-lipid contaminants. To further evaluate the quantity and composition of each class of lipids, chromatographic methods have been developed since the 1950s. Two most popular and affordable ones are those of Thin Layer Chromatography (TLC) and Gas Chromatography (GC).

A recent method that has been developed for plant tissues in 2003 is that of direct infusion electron spray ionization-mass spectrometry (ESI-MS/MS), as well as liquid chromatography-mass spectrometry (ESI-MS/MS). Both methods are referred to as lipidomics, allowing a snapshot of all lipid molecular species present in a sample. Mass spectrometry imaging has also been developed and used in analyzing plant cells, which gives additional information in relation to lipid chemical composition. The major characteristics of each technique are summarized in Fig. 1.5.

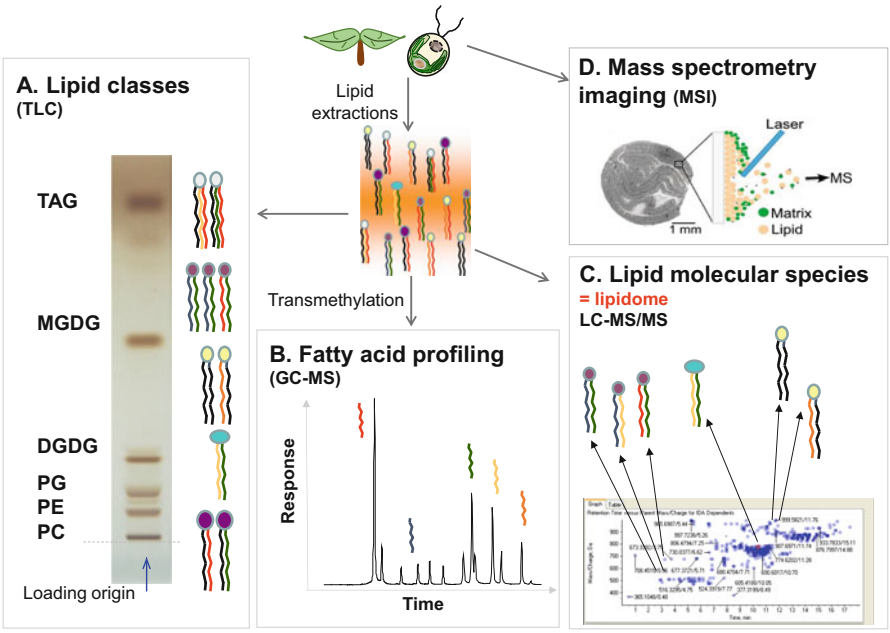

Fig. 1.5 A summary of major lipid analytical tools. Abbreviations: *GC-MS* gas chromatography – mass spectrometry, *LC-MS/MS* liquid chromatography-tandem mass spectrometry. Other abbreviations as detailed in Fig. 1.1. We acknowledge Dr. Fred Beisson in conception of this figure

These methods can be used alone or in combination. Below we describe in more detail some of the above mentioned analytical tools.

Thin Layer Chromatography (TLC)

TLC is probably one of the most highly used lipid analytical tools. It is highly reproducible and low cost, which makes it accessible to all laboratories interested in lipids and was developed over 60 years ago. It is simple in setup requiring a glass tank, a glass plate pre-coated with a thin layer of silica gel (stationary phase), and organic solvents (mobile phase). After the sample (i.e. the lipid extracts) has been applied to one end of the plate, a solvent or solvent mixture is drawn up the plate via capillary action. Because different lipid classes possess different polarity, each lipid class ascends the TLC plate at different rates. Thus separation is achieved. An example of separation of polar lipids is shown in Fig. 1.5a. For separation of some complex lipids, two-dimensional TLC plate can also be applied.

Most lipids are colorless. Therefore once they are separated, several methods can be used to reveal them. These methods can be classified as destructive or non-destructive. A common technique is to spray the TLC plate with concentrated sulfuric acid in methanol, followed by charring in an oven, when lipids turn dark. This method renders all carbon-rich compounds a black color and, thus, is suitable for universal detection. Iodine vapor is widely used but may cause problems with poly-unsaturated fatty acids which cannot always be recovered intact. Indeed, because iodine adds to double bonds it will stain unsaturated lipids more intensely than saturated compounds. Otherwise, specific or preferential staining of particular lipid classes are also available; for example, molybolic acid staining is often used for phospholipids staining because molybolic acid renders compounds containing phosphate ester show up as blue spots on a TLC plate; and betaine lipid can be detected by spraying the TLC plate with Dragendorff's reagent spray solution. For non-destructive and more general detection, spraying the TLC plates with a solution of primulin (0.01 % w/v in acetone-water 60:40 v/v) is popular. After spraying and allowing it to dry under the hood, lipids can be visualized under UV as distinct light spots, which can be recovered from the silica gel with organic solvent, prior to analyses by other means.

During the visualization, if quantification is also desirable, then authentic standards can be applied alongside. Quantification can be obtained based on a densitometry method. More accurate quantification can be further obtained if the lipids are to be recovered, and then analyzed by GC-FID. Other information related to TLC methods can be found in Christie et al. (2007) and the AOCS Lipid Library website (http://lipidlibrary.aocs.org/index.html).

Gas Chromatography (GC)

In plants and algal cells, the majority of lipids contain fatty acids, such as glycerolipids, sphingolipids, wax esters and monomers of cutin and suberins. These fatty acid-containing lipids can be converted to fatty acid methyl esters (FAMEs), which can be analyzed by GC. GC is often coupled to a Flame Ionization Detector (GC-FID) or Mass Spectrometer (GC-MS). Fatty acids are provisionally identified based on their retention time and then the mass split patterns used to compare with authentic standards. Quantification can be achieved with addition of an internal standard, often heptadecanoic acid (17:0 fatty acid) because this odd-chain fatty acid is usually absent in eukaryotic cells.

Conversion of acyl-lipids to their respective FAMEs can be catalyzed either by an acid- or a base- mediated transmethylation reaction. In most cases, both methods are inter-changeable. Having said these, it is worth noting that base catalysts do not esterify free (non-esterified) fatty acids (FFAs) and neither trans-esterify amide-bond fatty acids in sphingolipids; whereas acid catalysts are usually too strong and are not suited for certain fatty acids with sensitive functional groups, such as epoxy, cyclopropane or cylopropene rings (Bao et al. 2002). Although free fatty acids are

only very minor cellular components, largely due to their detergent properties, derivation of FFAs themselves can be achieved by using a mixture of 1-ethyl-3-3-dimethylaminopropylcarbodiimide containing methanolic solution (Kaczmarzyk and Fulda 2010). This ability to measure the content of FFA alone (without the bulk of glycerolipids) is desirable in some genetic engineering studies.

FAMEs are then subjected to separation, identification and quantification by GC-FID or GC-MS. An example of elution of FAMEs from cell extracts of *Chlamydomonas reinhardtii* is shown in Fig. 1.5b. For detailed methods on preparation of fatty acid derivatives and GC conditions, please consult the AOCS Lipid Library site (http://lipidlibrary.aocs.org/topics/ester_93/index.htm).

Lipidomics

With the development in modern analytical tools, especially in mass spectrometry, we can now not only determine the total lipid content, lipid classes, or fatty acid profile for a given sample, but also we can determine the type, number and quantity of each lipid molecular species present in a tissue (Fig. 1.5c). The determination of the total number and type of lipid molecular species present in a sample is called 'lipidomics'. In general well over 200 lipid molecular species have been identified in plants and algal cells (Welti et al. 2002, 2007; Nguyen et al. 2013; Liu et al. 2013). Application of modern lipidomic tools to the green lineage was first established in the Kansas Lipidomics Centre (http://www.k-state.edu/lipid/lipidomics/) funded and directed by Professor Ruth Welti since 2003 and has since also been setup and routinely operated in a few other laboratories including the author's laboratory at CEA Cadarache.

Lipidomic analysis is one sub-branch of metabolomics, in that the former deal principally with hydrophobic molecules. Currently, two major types of lipidomics are in use, direct-infusion electron spray-MS/MS (ESI-MS/MS) or separation by liquid chromatography followed by mass spectrometry (LC-MS/MS). Most lipid types can be analyzed by either method. Organelle or sub-cellular lipidomics can be achieved by extracting lipids from isolated subcellular organelles or fractions. Due to the requirement for authentic standards, lipidomics are often used in a comparative context, for example, comparing wild-type to a mutant, or comparing one developmental stage to another, comparing one condition to a stress condition etc. The power of lipidomics in linking genes to function can be seen in numerous publications (Welti et al. 2002; Yoon et al. 2012). As compared to other analytical tools, lipidomics profiling gives an instaneous snapshot of the lipidomic state of a cell, interpretation of which can be combined together with transcriptomic and proteomic dataset thus allowing a systematic understanding of cell physiology. Nonetheless, it has also the disadvantages that the apparatus is still quite expensive, and the handling and processing of data needs specialist knowledge. This means that it is not accessible to most laboratories.

Lipid Imaging: From Lipophilic Stains to Mass Spectrometry Imaging

The above mentioned methods have largely focused on the chemical analyses of lipid compositions. As with other subcellular components, lipids can also be visualized. Classical techniques of lipid imaging include first staining with a lipophilic dye such as Nile red followed by observation under a microscope; or can also be visualized by electron microscopic techniques. Examples of these analytical tools in advancing our understanding of gene functions are abundant, and some examples can be found in Li-Beisson et al. (2013), Cagnon et al. (2013), and Xie et al. (2014).

Recently a coordinated analysis of the lipid composition with their sub-cellular localizations at a high spatial resolution have been developed, and one such approach is called mass spectrometry imaging (MSI) (Fig. 1.5d). MSI integrates in situ visualization with chemical based lipidomics. Three major types of MSI are now available, which differ in their ionization sources, i.e. secondary ion MS (SIMS), desorption electrospray ionization (DESI), laser desorption/ionization (MALDI)-MS. For detailed description of these platforms, readers are referred to Horn et al. (2012), Horn and Chapman (2014) and other references there-in. These technologies have been applied to a number of plant tissues.

Biotechnological Applications of Lipids Derived from Algae and Plants

Over several hundred fatty acid structures have been identified in nature, many of which are present only in particular species. For example, ricinoleic acid is found almost exclusively in castor bean. Due to their large structural diversity, lipid applications are also diverse including sectors like food, chemicals, bioenergy, and nutraceuticals and the cosmetics industry. Among all lipid structures presented, oils are the most exploitable ones for two major reasons: oils are one of the most abundant lipids stored in cells; and industrial processes of oil extraction and processing are affordable and represent a mature technology as compared to extraction and recovery of membrane lipids (i.e. galactolipids and phospholipids).

The most abundant fatty acid species present in most plant and algae cells are less than ten. Fatty acids with secondary modifications (for example oxygenation) or unusual chain length were often called unusual fatty acids. These ‘unusual’ fatty acids can be of particular industrial value. However, they are often synthesized naturally only in some non-agronomic plant species, or are present in a less-easily accessible form such as polymers coating plant surfaces (Li and Beisson 2009).

The current major biotechnological objectives in the area of plant/algal lipid research include: (i), increasing the productivity of current oilseed crops to meet the increasing demand of the world population; (ii), introducing novel genes thus allowing synthesis and production of unusual fatty acids in the oil fraction of existing

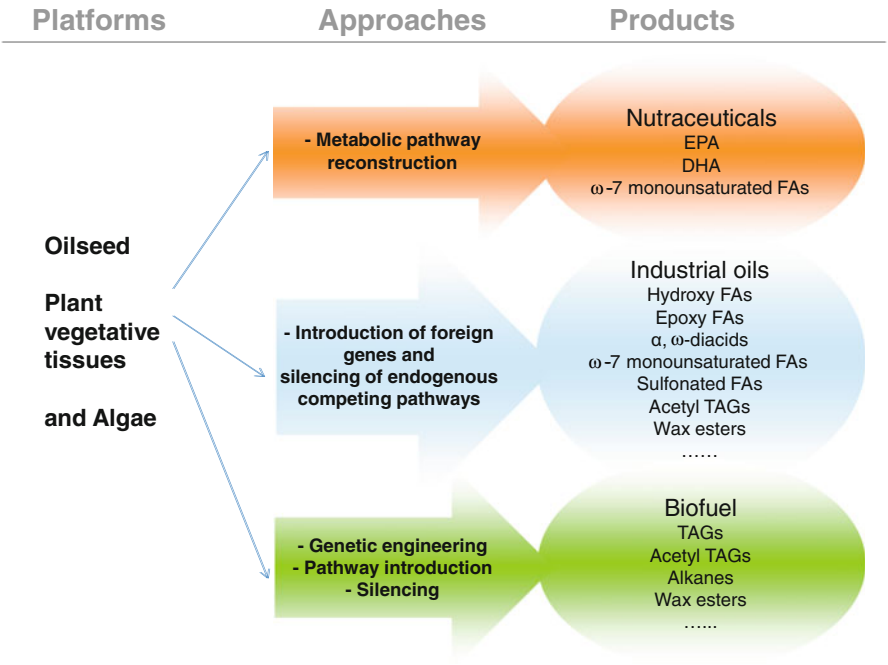

Fig. 1.6 Some examples of biotechnological applications of lipids and the approaches used toward these purposes

crop species; (iii), increasing oil/energy content of plant vegetative tissues or algal cells, thus establishing new platforms for oil production and having the advantage of not competing with oil crops for food usage; and (iv), pathway reconstruction for production of very long chain polyunsaturated fatty acids for the nutraceutical industry (Fig. 1.6). Abundant successful proof-of-the-concept examples are available in the literature (Napier and Graham 2010; Jaworski and Cahoon 2003; Lu et al. 2011). Now one major challenge for the next step is the commercialization of these findings, thus requiring field trials, techno-economic and life cycle analyses.

Conclusion

In this chapter, we have highlighted a few key points related to lipids: structures, biosynthesis, analysis and applications, which we consider important to appreciate the following chapters in this book. Part of this book describes the latest developments in the functions of lipids in photosynthesis, and part of this book deals with the roles of lipids in plant and algal development and signaling; emerging platforms for industrial applications of lipids are also covered.

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