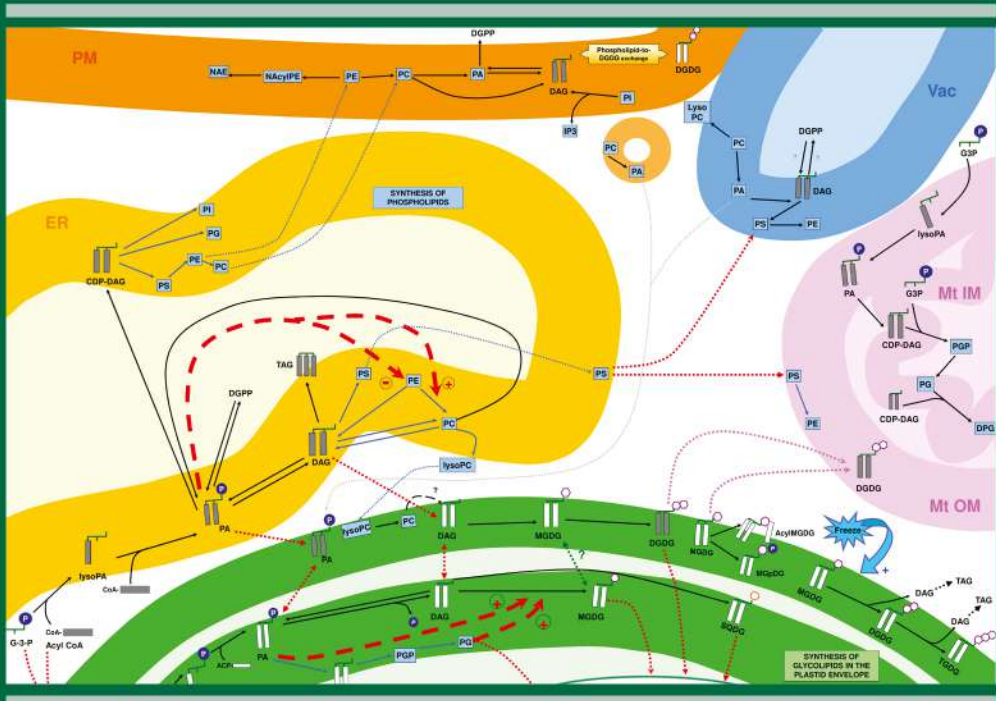


Advances in BOTANICAL RESEARCH

LIPIDS IN PLANTS AND ALGAE:
FROM FUNDAMENTAL SCIENCE TO
INDUSTRIAL APPLICATIONS



Volume 101

Edited by

FABRICE RÉBEILLÉ AND ERIC MARÉCHAL

Series Editor

JEAN-PIERRE JACQUOT





VOLUME ONE HUNDRED AND ONE

ADVANCES IN **BOTANICAL RESEARCH**

Lipids in Plants and Algae: From
Fundamental Science to Industrial
Applications

ADVANCES IN BOTANICAL RESEARCH

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Preface

The term “lipid” is a general one that encompasses a large group of molecules. The common thing about these molecules is that they are organic compounds with hydrophobic or amphiphilic properties. They derive from two different types of chemical structures that serve as building units and thus form two distinct groups: (i) ketoacyl and (ii) isoprene groups. Lipids deriving from ketoacyl groups comprise fatty acids, glycerolipids, sphingolipids, and polyketides. They are involved in a wide array of metabolic and structural functions; in particular, they are the main constituents of cell membranes and oil bodies. Lipids deriving from isoprene groups comprise sterol and prenol families. They too are involved in a large number of cellular processes, acting in membrane fluidity, protection, electron transport, or in integrated functions such as plant defense.

There is no life without lipids. The most obvious reason is the emergence of membranes as one of the critical steps in the origin of life. The chemistry of the cell is contingent on the laws of thermodynamics. As predicted by the second law of thermodynamics (entropy), spontaneous processes occur in directions that increase the overall disorder. Diffusion is a spontaneous process, and it is obvious that without boundaries the macromolecular components of a cell would spread and disperse into the environment. Life requires highly compacted and ordered processes, which imply diffusion barriers. In a world of water, amphiphilic molecules have emerged as the best candidates to create those membranes that delimit vesicles and compartments in which the reactions of life can occur efficiently. In eukaryotic and most prokaryotic cells, these molecules are essentially diacylglycerolipids (which are composed of a glycerol molecule esterified with two fatty acids), exemplified by phospholipids and glycoglycerolipids. Cell membranes are not just barriers. They also control exchanges with the external environment and participate in the transformation/production of the energy required to maintain cell activity (formation of chemical or electrical gradients; electron transport chains). Indeed, living systems are not closed systems, as defined by thermodynamics. According to the law of entropy, closed systems are in equilibrium. Living systems are open systems that must permanently maintain a state of nonequilibrium by recovering free energy from their environment. In such a nonequilibrium state, high-enthalpy substrates from the external environment are transformed into low-enthalpy products inside

the cell, providing energy and metabolic fluxes needed to synthesize the components of life and to maintain cellular activity. To fulfill these functions of exchange and import/conversion of energy, membranes interact with many specific proteins and integrate within their structure a number of other hydrophobic molecules such as sterols, carotenoids, or quinones. Membranes are therefore very complex systems that require many metabolic processes to adjust their composition and plasticity continuously.

Lipids are not only involved in the formation and composition of membranes, but they are also major actors in many other metabolic functions. Fatty acids store carbons and energy, allowing the production of more ATP during their oxidation than sugars with the same number of carbons. This is because carbon atoms in fatty acids are in a more reduced state than those of carbohydrates. From this point of view, oxidation of triacylglycerol (a glycerol molecule esterified with three fatty acids) produces twice more energy than carbohydrates and proteins on a dry weight basis. For this reason, triacylglycerols stored in oil bodies represent the main form of energy storage in animals and in many plants and algae.

Lipids are also the precursors of many compounds that play essential roles in mediating and controlling a large number of cellular processes. A few examples of compounds are as follows. Fatty acids are precursors of oxylipins, such as jasmonic acid in plants, which is involved in the control of ontogenesis and reproductive processes and in the resistance to various pathogens. In the major phylum of photosynthetic stramenopiles, prostaglandins, prostacyclins, thromboxanes, and nonenzymatically produced phytoprostanes are also major oxylipins; their study in terms of diversity of structures, evolution, and role is still in its infancy. Sterols are involved in the synthesis of steroid hormones. For example, brassinosteroids are plant steroids essential for normal growth and development. Cross talks exist between metabolic and signaling roles. Lipid compounds such as phosphatidic acid and diacylglycerol are metabolic hubs controlling various metabolic pathways. In particular, phosphatidic acid interacts with several signaling pathways, including those of the TOR signaling cascade involved in cell stress response, adipogenesis, autophagy, or insulin signaling in animals. Other examples include sphingosine-1-phosphate and phosphatidylinositol phosphates that are, respectively, involved in calcium mobilization and calcium-mediated activation of protein kinase C. These few examples illustrate how lipids encompass an immense molecular diversity; they have structural, metabolic, bioenergetic, and signaling roles, pointing to a sophisticated network of reactions and control mechanisms

at the very heart of cellular functions. Today, there is still much to discover and understand.

Why is it important to study lipid metabolism in plants and algae from a societal perspective? There are several reasons for that: health, sustainable production of valuable compounds, and green chemistry. From a nutritional point of view, several fatty acids are considered essential because they are either not or poorly synthesized by human beings. This is the case for linoleic acid (LA, 18:2^{Δ^{9,12}}) of the ω-6 series, α-linolenic acid (ALA, 18:3^{Δ^{9,12,15}}) of the ω-3 series, and the ω-3 very-long-chain polyunsaturated fatty acids (VLC-PUFAs) such as eicosapentaenoic acid (EPA, 20:5^{Δ^{5,8,11,14,17}}) or docosahexaenoic acid (DHA, 22:6^{Δ^{4,7,10,13,16,19}}). A deficiency of the first two is rare in humans due to their easy availability in diet (plant oils), but this is not the case for VLC-PUFAs that are not produced at a high level in land plants. The actual diet source for these fatty acids is fish oil, which is not a sustainable source considering the overexploitation of fish stocks and their contamination by toxic substances such as heavy metals. Likewise today, squalene is obtained from shark liver oil. Squalene, the precursor of sterols, is extensively used in the cosmetic industry for its anti-oxidant properties. Alternative and more sustainable sources have to be found in order to fulfill the high societal demand for these compounds. Marine algae and other marine protists are likely candidates. Today, they are emerging as interesting alternatives for the production of a number of valuable compounds, including VLC-PUFAs (human health), squalene (cosmetic industry), carotenoids, and pigments (food industry). Plants and algae are also promising models for the development of nonpetroleum chemistry. In this context, chemistry based on renewable lipids from plants or algae could replace the controversial use of fossil carbon and indirectly contribute to the reduction of CO₂ emissions. For example, unsaturated oils could serve for manufacturing biosourced polyurethane foams, useful for thermal insulation. Likewise, squalene from algal cultures could serve to produce isoprene units as raw material to synthesize a diverse range of industrial products, including synthetic rubber and a wide variety of elastomers. Fatty acids have chemical structures and fuel properties similar to those of hydrocarbons. Fatty acids with medium chain length and low unsaturation can be converted to biofuel. Third-generation biofuels are those obtained from algae, thus preserving agricultural land for human consumption.

To meet the strong societal demand for these various products, lipid production in plants and algae must be “optimized.” The most sought-after phenotypic traits include oleaginous cells and tissues enriched in

triacylglycerol (oil) or cells accumulating pigments (carotenoids). It is clear that the “optimization” of these systems requires a complete understanding of (i) their endogenous regulation and (ii) their integration within the metabolism as a whole. This will then allow the rational development of the most appropriate engineering strategies to modify and adapt these organisms for commercial and societal purposes.

This book includes comprehensive and authoritative reviews by leading experts on lipid metabolism in plants and algae. We thank them for their time and effort. The objective of the book is to bring together the recent and rapidly growing experimental information on this challenging and current topic. Some very recent fields in algal and plant lipid science are addressed here, providing key reviews that are missing in the current literature. In the book, the reader will find chapters on the physical structure of membranes, their composition, formation, and renewal. The book also includes chapters that deal with storage lipids, lipid droplets, and plastoglobules; their formations; and their roles in cell physiology and lipid metabolism. The dynamic mechanisms of acyl flux, fatty acid desaturation, and sterol biogenesis are also reviewed in detail. Finally, new information is presented on lipids as signaling molecules in plants and microalgae.

We believe that the book will be useful as a starting point for undergraduate and graduate students, as well as for researchers who wish to pursue specialized studies in this area.

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Biophysical properties of glycerolipids and their impact on membrane architecture and biology

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Abstract

Cellular membranes have a remarkable variety of lipids, and different organelles have different lipid compositions. Changes in lipid composition can alter the surface charge, thickness, and fluidity of a membrane—characteristics that affect, for example, photosynthesis efficiency or vesicular trafficking. Therefore, for proper organelle function, these parameters must be kept within an appropriate range and must be regulated. Due to their low mobility, plant and algae are dependent on their environment and face sudden changes such as light, temperature or osmotic variations, that will affect

membrane features. This review focus on the physical and structural properties of glycerolipids and their impact on membrane specificities in response to environmental cues. Here, we present an overview of the methods that are currently used to establish biophysical membrane properties. We then describe the common glycerolipids present in plants and algae, with their characteristic and their distribution within cell membranes. In the light of the organelle lipid composition, we illustrate how membranes are able to sense and adapt their architecture to maintain their homeostasis and their properties in response to environmental stresses. Thanks to the improved techniques recently available to study membranes in their native context, we are now discovering that the regulation of membrane properties by lipids is far more complex and entangled than it was originally thought.



1. Introduction

The capacity of glycerolipids to self-organize in water is the physical basis for spontaneous membrane formation, and renders membranes virtually impermeable to polar solutes. This fundamental principle enabled the first cells to segregate their internal constituents from the external environment and also occurs within eukaryotic cells to form organelles. Whereas in principle a single lipid species is sufficient to generate a barrier to solute transfer, membrane lipids in eukaryotic cells are characterized by a remarkable structural diversity. In addition, the lipid composition and transbilayer arrangement among organelles show striking variations, and there is compelling evidence that the collective properties of the bulk lipids play a profound part in defining organelle identity and function (Holthuis & Menon, 2014).

Of particular interest are the lipid-induced changes in the membrane physical properties such as bilayer thickness, lipid packing density and surface charge that will shape organelle architecture and sort membrane protein (Bigay & Antonny, 2012; Singh & Mittal, 2016). In this review, we focus on glycerolipids that constitute the bulk of cell membrane. We present the biophysical approaches that can help us to determine the physicochemical properties of glycerolipids, the glycerolipid composition of membranes in plant and alga cells and the influence of environmental stresses on physical properties of membranes *in vivo* with three examples: thermic stress, osmotic stress and the evolution of plastid architecture.



2. Physicochemical properties and analysis methods

Lipid membrane domains are characterized by a distinctive protein and/or lipid composition. These domains confer specific properties to the

membrane leading to original structure and function. Membranes are qualified as a mosaic fluid referring to the state of the lipidic phase. Lipid membranes are able to adjust to environmental parameters, thus maintaining the homeostasis of the cell (Martonosi, Kracke, Taylor, Dux, & Peracchia, 1985; Shinitzky, 1984). Moreover, lipids are able to form a bilayer structure, but also non-bilayer structures. These lipid conformations lead to specific physicochemical properties, essential for protein functions. This section describes some methods to explore lipid and fatty acid structures.

2.1 Membrane visualization techniques

The first microscopic observations described the plasma membrane only as a physical barrier between the cytoplasm and the outside of the cell. Scientists improved their knowledge about membranes and proposed some hypothesis on their structure and the presence of proteins around. The history of membrane discoveries is well described in the Lombard review (Lombard, 2014). A first complete model of bilayer was described in 1957 by Fernandez-Moran and Finean after their study of myelin sheath using electronic microscopy and X-rays diffraction (Fernandez-Moran & Finean, 1957). It was finally in 1972 that Singer and Nicolson described the fluid mosaic model as a lipid bilayer containing proteins more or less embedded in the membrane (Singer & Nicolson, 1972). This membrane model becomes a reference model for the next studies. Compared to the past, the resolution of membrane visualization techniques has improved significantly. In this section, we present a non-exhaustive list of biophysical techniques that are widely used to establish biophysical membrane properties.

2.1.1 Classical microscopy technique

As mentioned above, the first discoveries and studies about membranes have been made by electron microscopy. The resolution of microscopes varies a lot, depending on the technique used. For example, the resolution of the optical microscopy is $0.2\mu\text{m}$, while that for transmission electron microscopy is around 0.04nm . In optical microscopy, the light is directed to a flatten object with a resolution that could not be achieved by eyes. By addition of a laser that emitted a specific wavelength, fluorescence microscopy permits to visualize a fluorescent dye in the cell. This technique can resolve the location of proteins or lipids inside a cell (Calvez, Jouhet, Vie, Durmort, & Zapun, 2019). Other techniques commonly used in biology are the confocal microscopy and the transmission electron microscopy (TEM). The first one can analyze cross sections without any impact of the

light emitted outside the focal plan. The object appears in three dimensions. Confocal microscopy is usually coupled with fluorescence microscopy. The TEM technique consists of a very thin object placed under an electron beam. Then, the electronic picture is converted into an image. A 3D reconstruction of an object is possible after analysis by a focused ion beam-scanning electron microscopy (FIB-SEM) where the ionic beam cuts the object and the electron beam visualizes the cross section. Thanks to all these microscopy techniques, structures, contact sites of organelles, location of proteins can be visualized.

An example of application using microscopy techniques are the studies on chloroplast. The structure of the chloroplast was first discovered by Hugo von Mohl with optical microscopy (Staelin, 2003). The electron microscopy techniques have been the principal tool for understanding thylakoid architecture and its functional organization. It is clearly possible to measure the size of thylakoids, count the number of grana, and measure the thickness of stroma thylakoids. Also, the two membranes of the chloroplast can be distinguished. Using 3D reconstructions, the structures inside the organelle and contacts between organelles can be understood. More recently, thanks to the laser confocal microscopy, Johnson and his collaborators have investigated the changes in the structural reorganization of photosynthetic membranes during the setup of photoprotective functions (Johnson, Brain, & Ruban, 2011).

2.1.2 Cryo-electron microscopy technique

The development of new sample preparation techniques such as cryo-electron microscopy (Cryo-EM) allows the possibility to establish structures at similar resolution than X-ray crystallography. Unlike preparation method for electron microscopy analysis requiring sample fixation in resin bed, samples analyzed by cryo-EM need to be cooled down into vitreous state before visualization (Lepault, 1985). The resolution of cryo-EM is better than 4 Å, higher than other electron microscopy techniques. Cryo-EM is widely used to study protein structure at atomic level into a bilayer membrane. It is possible to determine protein structure, liposome lamellarity, size, shape and ultrastructure (Mio & Sato, 2018). Demurtas and his collaborators used cryo-EM to visualize and understand the structure of the cubic lipid phase in the context of drug delivery system applications (Demurtas et al., 2015).

2.1.3 Freeze-fracture electron microscopy

Freeze-fracture electron microscopy is a method for studying the structure of biological samples at low and medium resolution (20 Å) (Gulik-Krzywicki, 1997). This technique is useful to investigate lipid structures, or protein insertion in the membrane. For example, it is possible to distinguish lamellar phase from the ripple phase, but only with ordered fatty acids. Indeed, with disordered acyl chains, the lipid structures are not well preserved after freezing. The Meyer and Richter's review describes some structures with or without proteins (Meyer & Richter, 2001). Cubic membrane architecture was also studied using this method (Delacroix, Gulik-Krzywicki, Mariani, & Luzzati, 1993).

Briefly, the preparation consists in freezing quickly the sample at very low temperature with liquid nitrogen (-170°C) or liquid propane (-200°C). Then, the ice block is fractured by a shock, always at low temperature and under pressure. The fracture happens along the line with the minimum resistance. For example, for a cell or a vesicle, two types of fractures exist: (1) the fracture can happen in the middle of the cell or vesicle, showing the inside of the sample, or (2) the fracture separates the bilayer leaflets in two monolayers, showing the center of the membrane. Proteins cannot be cut and are always intact (Shechter, 1984).

2.1.4 Atomic force microscopy (AFM)

The atomic force microscopy technique (AFM) is a very-high-resolution type of scanning probe microscopy with a resolution of a nanometer. An AFM generates images by scanning a small cantilever over the surface of a sample. The sharp tip on the end of the cantilever contacts the surface, bends the cantilever and transfers the signal by changing the amount of a laser light reflected by the cantilever into a photodiode. The AFM has three major abilities: force measurement, topographic imaging, and manipulation. It is capable of displaying the surface topology of protein complexes of hydrated membranes (Johnson et al., 2011; Kirchhoff, Lenhart, Buchel, Chi, & Nield, 2008; Sarkis et al., 2014; Wood et al., 2018). The organization of photosystem II (PSII) and cytochrome *b6f* complexes were also done by AFM (Johnson, Vasilev, Olsen, & Hunter, 2014). Nowadays, AFM is mainly used for protein structure into a membrane, but in the future this technique could progress to study the lipid organization.

2.2 Fatty acid phase transition measurement

The fluidity of the lipid bilayer influences many basic membrane functions. The lipid phase transition can affect the protein activity. Indeed, Ray et al. show that the activity of the phospholipase A₂ is mainly impacted by the lipid phase and less by the depth of insertion in the membrane (Ray, Scott, & Tatulian, 2007).

Membrane fluidity strongly depends on the lipid composition of the bilayer, on the length and the degree of unsaturation of fatty acids, on the temperature and on the state of the lipidic phase. Indeed, inside a membrane bilayer, hydrocarbon chains can adopt two main conformations: (1) an ordered conformation where fatty acids are rigid and stretched, it is called the gel phase (L_{β}), or (2) a disordered conformation where fatty acids are liquid-crystalline, less stretched, the fluid phase (L_{α}). The rippled gel phase, between gel and fluid phases, is sometimes present and named P_{β} . The phase structures are well described in the following review (Koynova & Tenchov, 2013). The lipid phase transition between gel and fluid phases is characterized by a specific temperature T_m , depending on the length and unsaturations of the carbon chains. This phase transition temperature can be measured using various physical techniques. On this part, a representative list of techniques is presented.

2.2.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is a relatively rapid, straightforward, and non-perturbing technique for studying the thermotropic phase behavior of hydrated lipid dispersions, or reconstituted lipid model of biological membranes (Demetzos, 2008; Lewis, Mannock, & McElhaney, 2007). It is a thermoanalytical technique that measures and compares the differences of energy (heat) between the sample and known reference. In other terms, the transition between gel to fluid conformation corresponds to a fusion of fatty acid chains, leading to the absorption of heat. Conversely, the transition between fluid to gel is characterized by a crystallization of fatty acid chains and heat release. The DSC measures the heat absorbed or released.

Sample and reference are sealed in an aluminum pan, and the temperature increases or decreases at a constant rate. In a typical thermogram, two main peaks are visible: the exothermic peak (fusion, heat released) and the endothermic peak (crystallization, heat absorbed). This result means that before the melting temperature, lipids are in gel phase, and beyond the crystallization peak, lipids are in fluid phase. In a lipid-protein system, the modification of the DSC peak area indicates that protein conformational

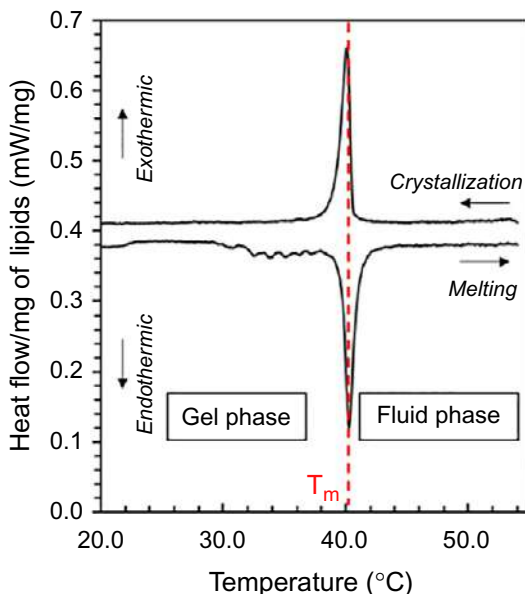


Fig. 1 DSC thermogram of DPPC in water. The thermogram shows the crystallization and melting peaks at the phase transition temperature. The phase transition between gel and fluid phase of DPPC is at 40°C.

changes alter the nature of lipid–protein interactions (McElhaney, 1986). The following figure shows the DSC thermogram of dipalmitoylphosphatidylcholine (DPPC, PC 16:0/16:0) (Fig. 1).

2.2.2 Solid-state nuclear magnetic resonance (SS-NMR)

SS-NMR spectroscopy is used to study lipid dynamics in membrane models. Two types of NMR are used for lipid analysis: (1) ^{31}P NMR gives information on the polar head of phospholipid (Dufourc, Mayer, Stohrer, Althoff, & Kothe, 1992) and (2) ^2H NMR on deuterated lipids gives dynamics of the acyl chains (Davis, 1983). The shape of the ^2H spectrum is different between fatty acids in gel or fluid phase. Indeed, in gel phase, fatty acids are fully elongated and highly ordered leading to a wide spectrum, while disordered fluid fatty acids lead to a narrower spectrum (Furlan et al., 2020). The difference of value of the first moment M1 between the two phases determinates the phase transition temperature (Fig. 2). As an example, the influence of polyunsaturated fatty acids on membrane organization was widely studied with this technique (Shaikh, Kinnun, Leng, Williams, & Wassall, 2015).

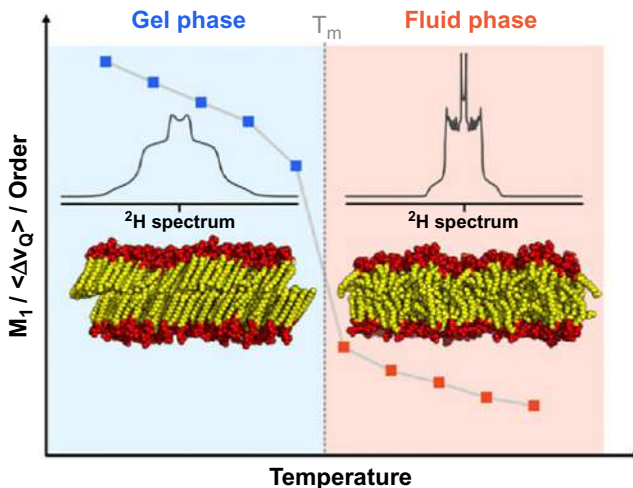


Fig. 2 The shape of the ^2H NMR spectrum of lipid membrane depending on the fatty acid crystalline state. The shape of the spectrum and the first moment M_1 value determine the lipid chain phase transition temperature T_m between gel and fluid. In gel phase, fatty acids are fully elongated and highly ordered leading to a wide spectrum, while disordered fluid fatty acid chains lead to a narrower spectrum (Furlan et al., 2020).

2.2.3 Electron spin resonance (ESR) spectroscopy

ESR spectroscopy is a non-destructive tool for studying membrane dynamics. It can measure both rotational and translational diffusion of membrane proteins and lipids. Briefly, ESR measures the transition between the electron spin energy levels. The sample is placed in the microwave resonator located between two magnet poles. The magnetic field of the studied molecule is scanned and the first derivative of the microwave radiation absorption is recorded. In order to detect phase transitions, the order parameter, $2A_{\text{per}}$, is plotted as a function of temperature. For example, Fig. 3 shows the pretransition between the gel and rippled phases at 28°C , and the main phase transition at 40°C for DPPC lipid (Pali & Pesti, 1996).

2.2.4 Fluorescence anisotropy

The measurement of fluorescence anisotropy is commonly used for the description of phase transitions (Kawski, 1993; van der Meer, van Hoeven, & van Blitterswijk, 1986; van Hoek, Vos, & Visser, 1987). As an example, by using this technique, Smutzer and Yeagle measured the phase transition temperature of a 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) membrane with addition of various amounts of dehydroergosterol, a

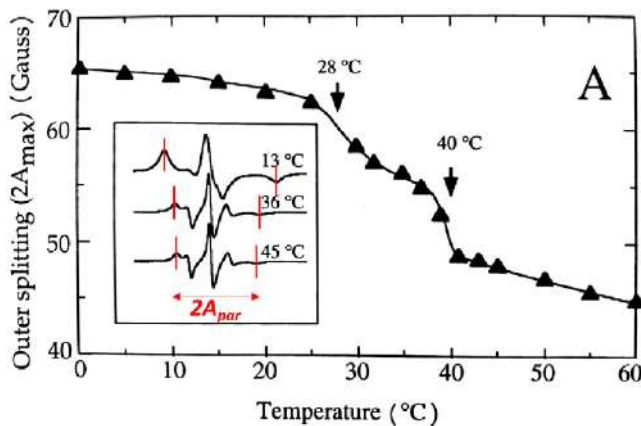


Fig. 3 Lipid phase transition using ESR. The plot of the spectral parameter $2A_{par}$ as a function of the temperature leads to the phase transition temperature of DPPC. Arrows indicate the pre-transition temperature at 28 °C, and the main transition at 40 °C. Adapted from Pali T, Pesti M (1996) Phase transition of membrane lipids. In *Manual on membrane lipids*, R. Prasad (ed) pp. 80–93. Berlin: Springer.

fluorescent molecule similar to cholesterol. They describe the sterol-rich domains thanks to this molecule (Smutzer & Yeagle, 1985). Biological membranes contain autofluorescent molecules, such as quinones, benzoic acid derivatives, alkaloids, etc. that can be used for biochemical, physiological or imaging studies (Donaldson, 2020). Different techniques exist, such as steady-state and time-resolved fluorescence anisotropy (SSFA and TRFA, respectively), fluorescence quenching (FQ) and intramolecular fluorescence energy transfer (IFET) (Esquembre, Ferrer, Gutierrez, Mallavia, & Mateo, 2007).

All lipids have their own phase diagram showing phase transition temperatures between each phase. This phase transition temperature is measured by the techniques described above. As described by Koynova and Tenchov, it is important to notice that the fatty acid chain length increases the fusion temperature, while the presence of unsaturation decreases the phase transition temperature. The polar head of lipids also has an impact on the phase transition of the lipid. In addition, the fusion temperature of fatty acid chains is dependent on the number of methyl groups, the position of the unsaturation on the carbon chain, and the environment such as pH (Koynova, Wang, & Macdonald, 2008). The following figure shows some examples of lipid phase transition temperatures depending on their fatty acid and polar head composition (Fig. 4).

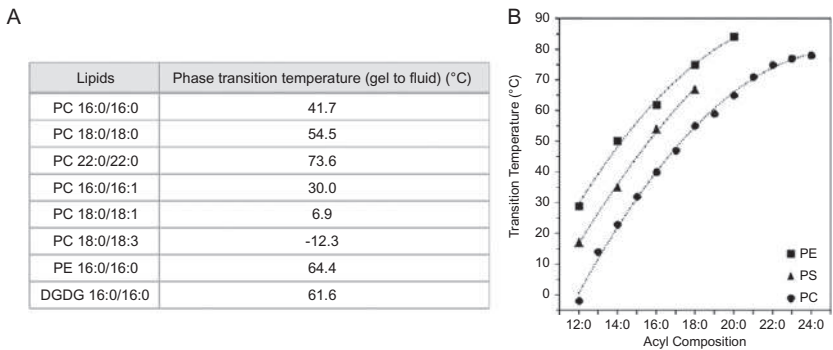


Fig. 4 Phase transition temperature depending on the fatty acid chain and lipid polar head. The length of fatty acid chains increases the fusion temperature while the unsaturation decreases it. The polar head also affects the temperature. (A) Phase transition temperature of lipids with different fatty acid chain lengths (Tenchov & Koynova, 2017). (B) Influence of fatty acid chains and polar heads on the phase transition temperature. Panel (B) adapted from Avanti Polar Lipids data.

2.3 Organized structure of membranes

As explained above, fatty acid chains can be frozen (gel phase) or liquid (fluid phase) inside membranes, leading to specific biophysical properties. Moreover, because lipids self-assemble to limit contacts between hydrophobic parts and water molecules, organized structures emerge (Fig. 5). First, lipids can adopt a structure composed of stacked bilayers (L_{α}) separated by water layers, whose thickness depends on the water content in the system. Second, the hexagonal conformation depends on the relative size of the polar heads compared to that of the fatty acid chains. If the volume occupied by the polar head is larger than the volume occupied by the fatty acid chains, which is mainly the case for lysolipids, a tubular hexagonal I (HI) conformation is formed with polar heads outside the tube and fatty acids inside. Conversely, if the volume occupied by the polar head is smaller than the volume occupied by fatty acid chains, an inverted hexagonal phase (HII) is formed, with the polar heads inside the tube and the fatty acids chains pointing outside. HII forming lipids are sometimes able to switch from a HII phase to a lamellar phase through an intermediate cubic phase (Fig. 5) by lowering the temperature (Tenchov & Koynova, 2012). All these phase transitions are spontaneous and reversible (Siegel & Tenchov, 2008). Most biological membranes are close to phase boundaries or in phase equilibrium between HII and L_{α} , allowing the formation of HII phase

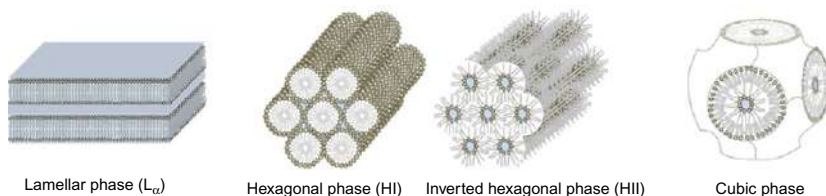


Fig. 5 Organized structures of lipids. Lipids self-organized in different structures, depending on the lipid composition and the water content in the system. Lamellar phase (L_{α}) is composed by stacked bilayers separated by a water layer. The hexagonal (HII) conformation is formed by lipids whose polar heads occupy more space than the fatty acid tail, i.e., lysolipids. This lipid can also form micelles. The inverted hexagonal (HIII) phases are adopted by conic lipids whose polar heads occupy less space than the fatty acid tails, i.e., PE, PA, MGDG. In this structure, the polar heads are inside the tube containing water and fatty acid chains outside. The cubic structure is derived from HIII forming lipids depending on the temperature and water amount. *Adapted from Jouhet J (2013) Importance of the hexagonal lipid phase in biological membrane organization. Frontiers in Plant Science 4: 494.*

nanodomains in the membrane (Jouhet, 2013). Biophysical techniques allow the study of these lipid structures. In this section, only a few of these techniques are presented.

2.3.1 X-ray and neutron diffraction analysis

X-ray diffraction and neutron diffraction are similar techniques, one using X-rays beams whereas the other is using neutron beams. They both provide information on the membrane organization, the bilayer and water layer thicknesses, and the bending rigidity. Samples are placed in a beam of thermal or cold neutrons to obtain a diffraction pattern that provides information on the structure of the material. Due to their different scattering properties, neutrons and X-rays provide complementary information: X-rays are suited for superficial analysis, strong X-rays from synchrotron radiation are suited for shallow depths or thin specimens, while neutrons having a higher penetration depth are suited for bulk samples.

As DSC technique, X-ray and neutron diffractions can also determine the phase transition of lipids. A large diffraction peak around 0.46 nm characterizes disordered fatty acids, while ordered fatty acids are characterized by a smaller and thin diffraction peak, at 0.42 nm (Finean & Hutchinson, 1988; Lis & Quinn, 1991). A plot of the lipid peak position against the temperature shows the phase transition temperature. Many studies report lecithin bilayer characteristics, such as the conformation of the fatty acid

chains and the phase transition temperature, using X-ray diffraction (Janiak, Small, & Shipley, 1979; Tardieu, Luzzati, & Reman, 1973). These bilayer parameters for synthetic lecithin permit to anticipate the structural behavior of natural lipids and other lipid classes.

Structure of membranes can be solved, and hypothesis on the role of lipids in the membrane can be formulated. Indeed, samples are single component or mixes of lipids, and so, depending on the concentration of each lipid class, membranes are not similarly structured. For example, the thylakoid-like lipid mixture, composed with MGDG/DGDG/PG/SQDG (respectively monogalactosyldiacylglycerol, digalactosyldiacylglycerol, phosphatidylglycerol, sulfoquinovosyldiacylglycerol), self-organizes in inverted hexagonal phase at low humidity and in lamellar phase at high humidity, as shown in the following figure (Fig. 6) (Demé, Cataye, Block, Marechal, & Jouhet, 2014).

One major advantage of neutron diffraction over X-ray diffraction is that the latter is rather insensitive to the presence of hydrogen (H) in a structure, whereas the nuclei ^1H and ^2H (i.e., Deuterium, D) are strong scatterers for neutrons. The greater scattering power of protons and deuterons means that the position of hydrogen in a crystal and its thermal motions can be

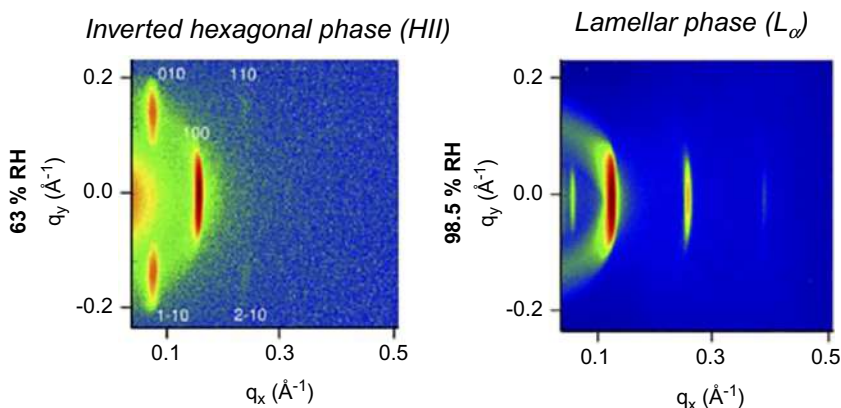


Fig. 6 Diffraction patterns of a thylakoid-like lipid mixture, obtained at the D16 diffractometer (ILL, Grenoble, France). The reconstituted membranes are composed of MGDG/DGDG/PG/SQDG (100/48/17/17 mol%) and measured in a dedicated chamber under controlled humidity. At low hydration (63% humidity, left) lipids self-organize in HII phase, while at high hydration (98.5% humidity right) lipids are in lamellar phase. Adapted from Demé B, Cataye C, Block MA, Marechal E, Jouhet J (2014) Contribution of galactoglycerolipids to the 3-dimensional architecture of thylakoids. *The FASEB Journal* 28: 3373–83.

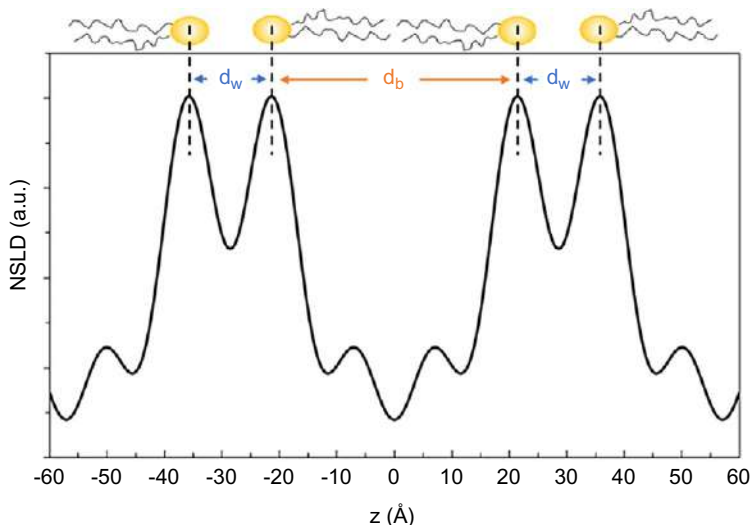


Fig. 7 Neutron scattering length density (NSLD) profile. Using neutron diffraction, the NSLD profile can be established by discrete Fourier analysis. The NSLD profile gives access to the bilayer thickness (d_b) and to the water layer thickness (d_w). At 8% D_2O solvent contrast, due to the high SLD of lipid headgroups, the maxima in the profile correspond to the polar head layer while the H-rich fatty acid chain ends correspond to the minima.

determined with greater precision. Using a 8% D_2O solvent contrast, the bilayer and water layer thickness can be calculated from the neutron scattering length density (NSLD) profile (Kucerka, Nieh, Pencer, Sachs, & Katsaras, 2009). As shown in the Fig. 7, at this specific solvent contrast and due to the H content of lipid molecules, the maxima correspond to the lipid polar heads, while the minima correspond to the center of the bilayer. Because the hydration can be increased in the humidity chamber, its impact on the membrane organization can be followed by calculating the NSLD profile for each humidity.

Other methods providing information on the lipid phase structure are X-ray diffraction (Luzzati & Husson, 1962) or ^{31}P NMR (Epend, D'Souza, Berno, & Schlame, 2015). They enable bilayer thickness calculation using similar method as neutron diffraction.

2.3.2 Membrane compressibility

The membrane compressibility can be estimated by looking at the Bragg peak profiles (or Bragg sheet) in neutron diffraction patterns, from which the bending rigidity and the compression modulus of the membrane are

extracted. These two parameters give information on the physical properties of the membrane, especially the capacity of the bilayer stack to be compressed and bent.

Studying monolayer of lipids in Langmuir trough also gives information on the compressibility of the membrane. Many studies use this technique to see the impact of an addition of a protein or another lipid inside the monolayer. For example, Phan and Shin studied the effect of cardiolipin on membrane morphology. They conclude that the addition of cardiolipin increases membrane elasticity (Phan & Shin, 2015). Nintenberg et al. give insight into the reaction mechanism and binding properties of MGD1, the enzyme responsible for the bulk synthesis of monogalactosyldiacylglycerol (MGDG), the major lipid of photosynthetic membranes. In this paper, the authors aim to understand the role of phosphatidylglycerol (PG) in MGD1 binding. Indeed, a membrane of PG has a high affinity for MGD1 like MGDG membrane. Conversely, digalactosyldiacylglycerol (DGDG) membrane has a negative effect and tends to exclude the protein (Nintenberg et al., 2020). The membrane composition is crucial for the protein binding and activity.

2.3.3 Molecular dynamics simulations

Membranes can be modeled and their organization simulated using molecular dynamics simulation methods. Simulation of the repulsive and attractive charges, physical movements of atoms and molecules are generated *in silico*. A snapshot at the equilibrium of the system gives information on the lipid polar head and the water molecules orientation between two bilayers. This method is often used in parallel with other physical techniques. For example, molecular dynamics simulations on phosphatidylcholine (PC) and DGDG membranes confirmed the lamellar organization of these two lipids and allowed to calculate the attractive forces between two adjacent bilayers. It showed that DGDG bilayers are six times more attractive than PC bilayers (Kanduč et al., 2017).

Other physics techniques are available to study lipid membranes, such as small angle neutron and X-ray scattering (SANS and SAXS), reflectometry or circular dichroism. All these techniques can also investigate the protein mobility and insertion in the membrane.

By using all these techniques, biophysical properties of lipid membranes can be established. However, lipids are often classified depending on their chemical structures. In higher plants and algae, three main lipid classes coexist: glycerolipids, sphingolipids and isoprenoid lipids such as sterols and

carotenoids. This chapter focuses on glycerolipids, the main lipid component in membranes, and their influence on membrane architecture. Because organelle membranes have a different lipid composition, they will display different biophysical properties and therefore different architectures.

3. Structure and distribution of glycerolipids

In plants and algae, glycerolipids consist of a glycerol backbone originating from glycerol-3-phosphate to which one to three fatty acids are linked by an ester bond. Glycerolipids containing two fatty acids may have a polar head, and they form the matrix of biological membranes. Glycerolipids containing three fatty acids are called triacylglycerols, or oils, and accumulate in the form of droplets within the cells (Fig. 8).

In plants and algae, less than a dozen classes of glycerolipids are sufficient to compose most biological membranes. Among them, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), the major constituents of extra-plastidial membranes, are in the phospholipids class. The bulk of photosynthetic membranes is mainly composed by non-phosphorus galactolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). In algae, there is a third type of membrane glycerolipids, called betaine lipids, which are non-phosphorous and localized in extra-plastidial membranes (Li-Beisson, Thelen, Fedosejevs, & Harwood, 2019). This section describes the structure and the cellular localisation of these glycerolipids.

The localization of each lipid is closely related to their synthesis place, such as the chloroplast, the endomembrane system and the mitochondria. In the environment, organisms are exposed to nutrients starvations, biotic and abiotic stresses, which often require modifications of their membrane

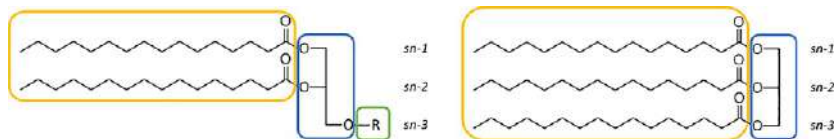


Fig. 8 Glycerolipids structures. Membrane glycerolipids (on the left) consist of a glycerol backbone (in blue) with two fatty acids (in yellow) esterified on the *sn*-1 and *sn*-2 positions and a variable polar head (in green) on the *sn*-3 position. The triacylglycerol, present in lipid droplet or oil (on the right), contains three fatty acids esterified on the glycerol backbone.

lipid composition for surviving. Lipid trafficking exist between the three compartments thanks to vesicles or contact sites, but they are still not well understood.

In this section, the structure and localization of glycerolipids are mainly based on three biological models: *Arabidopsis thaliana* (Angiosperm), *Chlamydomonas reinhardtii* (Chlorophyta) and *Phaeodactylum tricornutum* (Heterokonta), respectively, the higher plant model, the green microalgae model and the diatom (brown microalgae) model. Indeed, these three models gather most of the literature on plant and alga glycerolipid knowledge.

3.1 Structure and biosynthesis of fatty acids

In plants and algae, fatty acids are carboxylic acids with even hydrocarbon chains of 14 up to 24 carbons and a terminal carboxyl group. In photosynthetic organisms, the synthesis of fatty acids takes place inside the stroma of chloroplasts. The characterization of the fatty acid synthesis pathways is well established in *Arabidopsis thaliana*. The extrapolation in algae, derived from primary (such as *Chlamydomonas reinhardtii*) or secondary endosymbiosis (such as *Phaeodactylum tricornutum*), helps to understand the fatty acid synthesis pathways in these organisms. However, many steps concerning the trafficking of fatty acids are still unknown. The fatty acid synthesis pathways and enzymes involved are not detailed in this chapter, but are well detailed in various reviews (Boudiere et al., 2014; Li-Beisson et al., 2019).

Fatty acids are diversified by their chain length, but also by the number of double bond in the carbon chain, also called unsaturation. In plants, membrane lipids contain mainly fatty acids with 16 or 18 carbons, while in microalgae, fatty acids up to 24 carbons are present. Table 1 lists some of the commonly occurring fatty acids in *Arabidopsis thaliana* and different microalgae.

On saturated fatty acids, desaturases can add unsaturations on specific positions in the carbon chain. Fatty acids can have one unsaturation (mono-unsaturated fatty acids), and up to six (polyunsaturated fatty acids). Two types of unsaturations exist, modifying the shape of the carbon chain and membrane fluidity (Fig. 9). As for saturated fatty acids, *trans*-unsaturation leads to a straight carbon chain. In vivo, the *trans*-configuration is present in one fatty acid, the 16:1 Δ 3, only in *sn*-2 position of phosphatidylglycerol (PG) of thylakoid membrane (Dubacq & Tremolieres, 1983). All other double bonds in fatty acids are in *cis*-conformation, providing a rigid

Table 1 List of the most common fatty acids in higher plants and different microalgae.

Fatty acids (mol%)	Name	Angiosperm (plant)	Chlorophyta (green microalgae)		Heterokonta (brown microalgae)		Cyanobacteria
		<i>A. thaliana</i> ^a	<i>C. reinhardtii</i> ^b	<i>C. vulgaris</i> ^c	<i>P. tricornutum</i> ^a	<i>M. gaditana</i> ^a	<i>S. maxima</i> ^c
14:0	Myristic	0.07	–	0.7	4.3	2.5	–
16:0	Palmitic	17.8	22	14.4	15.2	34.0	35.8
16:1	Palmitoleic	3.7	7	4.0	22.6	32.0	0.9
16:2	Hexadecadienoic	–	2	5.3	3.5	0.7	4.6
16:3	Hexadecatrienoic	1.5	3	4.9	8.6	0.2	–
18:0	Stearic	0.5	2	1.6	2.8	1.8	1.5
18:1	Oleic (<i>cis</i>) Elaidic (<i>trans</i>)	26.2	16	17.6	0.9	3.2	5.0
18:2	Linoleic	12.2	8	12.0	2.7	1.7	16.3
18:3	Linolenic	38.2	25	15.8	1.4	0.4	18.2
18:4	Stearidonic	–	2	–	–	0.1	–
20:1	Eicosenoic	–	–	–	0.3	0.1	–
20:2	Eicosadienoic	–	–	–	0.4	1.1	0.6
20:4	Arachidonic	–	–	–	1.8	1.5	–
20:5	EPA	–	–	–	30.3	20.1	–
22:1	Erucic	–	–	–	–	0.4	–
22:5	Eicosapentaenoic	–	–	–	0.4	–	–
22:6	DHA	–	–	0.3	4.2	–	–
24:0	Lignoceric	–	–	0.2	0.7	–	0.6

^a(Jouhet et al., 2017).^b(Giroud, Gerber, & Eichenberger, 1988).^c(Odes & Pire, 2001).

Fatty acid composition in one plant, *Arabidopsis thaliana*, two green microalgae, *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, two brown microalgae *Phaeodactylum tricornutum* and *Microchloropsis gaditana* and one cyanobacteria *Spirulina maxima*. The values are in mol%. DHA: decosahexaenoic acid, EPA: eicosapentaenoic acid.

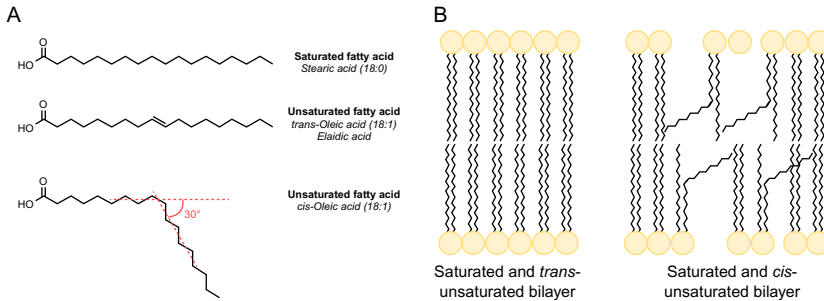


Fig. 9 Fatty acid and membrane structures. Fatty acids can be saturated or unsaturated (A). Two types of unsaturation exist: the *trans*-unsaturation extends the chain structure and the *cis*-unsaturation provides a rigid kink of 30° in the carbon chain. (B) Membranes containing lipids with saturated and *trans*-unsaturated fatty acids are more rigid than a membrane with *cis*-unsaturated fatty acids. The kink in the fatty acid chains maintains space between adjacent lipid molecules, influencing the membrane permeability.

kink of 30° in the hydrocarbon chain, solved by molecular dynamics on 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine lipid (PLPC 16:0–18:2) (Hyvonen, Rantala, & Ala-Korpela, 1997). The length and degree of unsaturation of fatty acids determines their impact on the membrane biophysical properties: saturated fatty acids with short chains form viscous membranes, while unsaturated fatty acids form membranes that are more fluid (Ibarguren, Lopez, & Escriba, 2014; Maulucci et al., 2016).

Inoue and collaborators studied the impact of adding saturated or unsaturated fatty acids in a saturated bilayer of dipalmitoylphosphatidylcholine (DPPC). They concluded that the long chains of saturated and *trans*-monounsaturated fatty acids raise the main phase transition temperature of DPPC bilayer and rigidify the lipid acyl chain in gel phase, while *cis*-monounsaturated fatty acid exhibits a minimal perturbation on the physical properties of the bilayer (Inoue, Yanagihara, Misono, & Suzuki, 2001). Mouritsen writes that the enzymatic activity of desaturases and elongases can directly affect membrane fluidity by increasing the abundance of mono- and polyunsaturated fatty acids in membrane phospholipids (Mouritsen, 2005). In agreement, Maulucci et al. show that if the polyunsaturated fatty acid content increases, the membrane is more fluid, and vice versa (Maulucci et al., 2016). Moreover, if saturated fatty acids, with their straight tails, are compressed, they press on each other, making a dense and fairly rigid membrane. However, if unsaturated fatty acids are compressed, the kinks in their tails maintain some space between adjacent lipid molecules, thus influencing membrane permeability (de Mendoza & Pilon, 2019). Molecular dynamics simulations and biochemical measurements

indicate that polyunsaturated fatty acids induce membrane curvature (Pinot et al., 2014; Risselada & Marrink, 2009). They also influence the membrane thickness because 22:6 chains have an average length of 8.2 Å at 41 °C compared to 14.2 Å for 18:1 chains (Fernandes, Castanho, & Garcia de la Torre, 2002).

Therefore, membranes adapt their fatty acid composition depending on their function and architecture. For example, organelles and vesicles, that generally display highly curved membranes, have an enrichment of polyunsaturated fatty acids that decreases the membrane rigidity (Takamori et al., 2006; Yang, Sugiura, Ikegami, Konishi, & Setou, 2012). Indeed, the lack of polyunsaturated fatty acids compromises vesicle formation (Tixier-Vidal, Picart, Loudes, & Bauman, 1986). Organisms are also capable to change the proportion of unsaturated fatty acids in their membranes in response to environmental stress, such as cold temperature (see next section).

Plants display fatty acids with two major chain lengths, C16 and C18, synthesized within the chloroplast. The desaturation of fatty acids on the glycerolipids lead to 16:3 from 16:0, and 18:3 from 18:1. Outside the chloroplast, only desaturation of fatty acids containing 18 carbons or more occurs (Heemskerk, Schmidt, Hammer, & Heinz, 1991). Glycerolipids esterified by two fatty acids on the position *sn*-1 and *sn*-2 of the glycerol-3-phosphate backbone form the phosphatidic acid (PA), first precursor of lipid biosynthesis. In plants, fatty acid chain length at the *sn*-2 position indicates the place of synthesis for the diacylglycerol backbone. The starting point of the plastidial or “prokaryotic” pathway is the synthesis of PA in the chloroplast envelope by the sequential action of acyl-ACP/glycerol-3-phosphate acyltransferase and acyl-ACP/lysophosphatidic acid acyltransferase. These enzymes produce almost exclusively 18:1/16:0 PA (Frentzen, Heinz, McKeon, & Stumpf, 1983). This is in agreement with the finding that isolated chloroplasts (in which only the prokaryotic pathway can operate) synthesize PA, diacylglycerol, glycolipid and PG, which contain only C16 fatty acids at the *sn*-2 position (Heinz & Roughan, 1983; Roughan, Holland, & Slack, 1980). However, the microsomal acyltransferase isoenzymes, which are responsible for PA synthesis in the endoplasmic reticulum, or “eukaryotic” pathway, give rise to lipids that contain a C18 fatty acid at the *sn*-2 position and either C16 or C18 fatty acids at the *sn*-1 position (Frentzen et al., 1983). Because both signatures are found in Arabidopsis galactolipids (C16 and C18 at the *sn*-2 position), a traffic of diacylglycerol backbone occurs from the endoplasmic reticulum to the chloroplast. The trigalactosyldiacylglycerol transporter (TGD) is

essential for this transport (Xu, Moellering, Muthan, Fan, & Benning, 2010). However, the nature of the transferred molecule, PA, DAG or PC, is still under debate (Block & Jouhet, 2015).

In microalgae, the fatty acid synthesis also takes place in the chloroplast but afterwards differences with land plants occur. While the fatty acid chain length in *Chlamydomonas reinhardtii* is similar to *A. thaliana*, plastidial lipids have a C16 fatty acid at the *sn*-2 position and lack C18 fatty acid at the *sn*-2 position, suggesting at first that there is no traffic from ER to chloroplast. However, Kim et al. identified a lysophosphatidic acid acyl transferase in the ER able to put a C16 fatty acid in *sn*-2 position (Kim, Terng, Riekhof, Cahoon, & Cerutti, 2018). Moreover, TGD machinery is present in green algae (Warakanont et al., 2015), confirming the existence of a diacylglycerol backbone traffic from the ER to the plastid in these organisms. In addition, a plastidial $\Delta 4$ desaturase is present producing high amount of glycolipid 16:4 (Giroud et al., 1988; Zauner, Jochum, Bigorowski, & Benning, 2012), a typical feature from green algae.

Marine algae are rich in very-long chain polyunsaturated fatty acid (Li-Beisson et al., 2019). For example, 20:5 is found at the *sn*-1 position in glycolipids in the diatom *Phaeodactylum tricornutum*, whereas the *sn*-2 position hosts a C16 fatty acid, suggesting also a plastidial origin for the diacylglycerol backbone. However, the fatty acid 20:5 is not synthesized in the chloroplast but is obtained by the elongation and desaturation in the ER, probably from the 16:0 by the ω -pathway before its return to the chloroplast (Petroutsos et al., 2014; Smith et al., 2021). Nowadays, this traffic is still not known. At the opposite of *A. thaliana*, there is no evidence of 18:0 fatty acid synthesis in the plastid (Zulu, Zienkiewicz, Vollheyde, & Feussner, 2018) and 16:1 is produced by the desaturation of 16:0 inside the plastid that will be exported outside the chloroplast in other membrane lipids (Smith et al., 2021).

Glycerolipids are classified from the polar heads present at the *sn*-3 position of the diacylglycerol backbone. The biosynthesis of glycerolipids occurs in two main organelles: the endoplasmic reticulum for phospholipid and betaine lipid biosynthesis, and the chloroplast for glycoglycerolipid and phosphatidylglycerol (PG) biosynthesis. Phosphatidylglycerol can also be synthesized in mitochondria, as well as diphosphatidylglycerol (DPG, also called cardiolipin). The biosynthesis of lipids is not described in details in this chapter, only the main steps are presented here and summarized in Fig. 10. More details about the enzymes involved in each step and their regulation are well described in the Li-Beisson's review (Li-Beisson et al., 2019).

3.2 Phospholipids

Phospholipids are the most abundant class of glycerolipids in non-plastidial membranes in plants. The polar head of phospholipids contains a phosphate group, and may have in addition, various alcohols linked to the phosphorous group leading to different phospholipids, as shown in Fig. 11.

In plants, the phospholipid synthesis takes place in the endoplasmic reticulum. As previously described, phosphatidic acid (PA) is the precursor of phospholipid synthesis. In plants, PA serves as substrate to synthesize CDP-diacylglycerol (CDG-DAG) by CDP-diacylglycerol synthase (CDS) or DAG by phosphatidic acid phosphatase (PAP) or phosphatidic hydrolase (PAH). CDP-DAG is used by phosphatidylinositol synthase (PIS) and

Fig. 10 Glycerolipids biosynthesis in higher plant *A. thaliana*, green microalgae *C. reinhardtii*, and brown microalgae *P. tricornutum*. The fatty acid synthesis occurs in chloroplast and fatty acid are exported in the endoplasmic reticulum (ER) for further desaturation and elongation. The glycolipid (galactolipids MGDG and DGDG, sulfolipid SQDG) synthesis takes place in the chloroplast. Acyl-SQDG (ASQD) synthesis is unknown, but probably also occurs in the chloroplast compartment. The phospholipid and betaine lipid synthesis occurs in the ER. Only the phosphatidylglycerol (PG) can be synthesized in the ER as well as in the chloroplast. The polyunsaturated fatty acids can be transported from the ER to the chloroplast through the DAG lipid pool using the TGD transporter. In *A. thaliana*, lipids containing a C16 signature at the *sn*-2 position of the glycerol backbone are synthesized in the chloroplast and followed the “prokaryotic” pathway, while lipids containing a C18 signature at the *sn*-2 position are synthesized in the ER and followed the “eukaryotic” pathway. In *P. tricornutum*, the return of elongated fatty acids from the ER to the chloroplast is called the ω -pathway. Lipids present in *A. thaliana* are in dark green, *C. reinhardtii* light green and *P. tricornutum* brown. The chloroplast of *A. thaliana* and *C. reinhardtii* is surrounded by two membranes (green ones), while the chloroplast of *P. tricornutum* is surrounded by four membranes (two green and two brown). The lipid structure and the fatty acid composition of each compartments are written on the bottom of each compartment representation. For an aesthetic reason, all enzymes involved in lipids synthesis are not placed inside the membranes. ACC: acetyl-CoA carboxylase, BTA: betaine lipid synthase, CPT: CDP-choline:DAG choline phosphotransferase, CL: cardiolipin, CLS: cardiolipin synthase, DGAT: acyl-CoA:DAG acyltransferase, DGD: DGDG synthase, EPT: CDP-ethanolamine:DAG ethanolamine phosphotransferase, FATA: fatty acid thioesterase A/B, GPAT: glycerol-3-phosphate acyl transferase, KAS: ketoacyl-ACP synthase, LC-FACS: long chain acyl-CoA synthetase, LPAAT: 1-acyl-glycerol-3-phosphoacyltransferase, MCMT: malonyl-CoA:ACP acyltransferase, MGD: MGDG synthase, PAP: phosphatidic acid phosphatase, PCT: phosphatidic acid cytidyl transferase, PDH: pyruvate dehydrogenase, PEMT: phosphatidylethanolamine N-transferase, PGS: phosphatidylglycerol synthase, PIS: phosphatidylinositol synthase, PPC: phosphocholine, PPE: phosphoethanolamine, PS: phosphatidylserine, PSS1: PS synthase SQD1/2: SQDG synthases.

Structure	Name	Global charge	Organization
	Phosphatidic acid (PA)	-	HII
	Phosphatidylethanolamine (PE)	0	HII
	Phosphatidylcholine (PC)	0	L _α
	Phosphatidylglycerol (PG)	-	L _α
	Phosphatidylserine (PS)	-	HII
	Phosphatidylinositol (PI)	-	L _α
	Cardiolipin (DPG)	-	HII

Fig. 11 Structure of the phosphoglycerolipids class. R_1 , R_2 are the two fatty acids esterified on the *sn*-1 and *sn*-2 position of the glycerol backbone. The global charge of the lipid is shown: negative charge (−) or neutral charge (0). The organization into the membrane is also mentioned: inverted hexagonal (HII) or lamellar (L_α).

phosphatidylglycerol synthase (PGS) to form respectively phosphatidylinositol (PI) and phosphatidylglycerol (PG). DAG is the precursor of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) through the Kennedy pathway (Dubots et al., 2012) with the respective enzyme activities CDP-ethanolamine:DAG ethanolamine phosphotransferase (EPT) and CDP-choline:DAG choline phosphotransferase (CPT). Because plants cannot find choline in their environment, the major pool of choline is synthesized by the trimethylation of phosphoethanolamine (PPE) by the phosphoethanolamine-*N*-methyltransferases (PEMTs) to form phosphocholine (PPC). Then, phosphocholine is esterified in *sn*-3 position of the DAG backbone to form phosphatidylcholine (Nakamura, 2021). PG is the only

phospholipid synthesized both in ER and plastid membranes. Quantitatively, PC is the most abundant phospholipid in photosynthetic organisms (Table 2). So far, in *A. thaliana*, almost all the enzymes involved in phospholipid synthesis have been identified and localized (Nakamura, 2021).

In plants, there is also a small amount of phosphatidylserine (PS). This lipid is synthesized from PE by the PS synthase (PSS) in the ER compartment. PS is mentioned as a signal lipid because it can interact with proteins present at low level, such as in the auxin signaling pathway (Colin & Jaillais, 2020). Finally, cardiolipin is a diphosphatidylglycerol lipid (DPG) containing four fatty acids bound to two glycerol moieties, which are linked by two phosphate groups to a third glycerol. In eukaryotes, cardiolipin is exclusively located in the inner mitochondrial membrane. It is synthesized by condensation of PG on CDP-DAG molecule, a reaction catalyzed by the cardiolipin synthase (CLS) (Katayama, Sakurai, & Wada, 2004; Zhou, Peisker, & Dormann, 2016).

In algae, genes responsible for phospholipid synthesis could be identified by sequence homology. Except for PG, that has also probably a dual location in ER and plastids, the phospholipid synthesis is predicted to be located in the ER. Therefore, by analogy, phospholipid synthesis pathways are supposed to be similar in plants and algae. However, *C. reinhardtii* lacks PC in its lipids composition, due to the absence of PEMT and PEAMT genes (Hirashima, Toyoshima, Moriyama, & Sato, 2018).

The physicochemical properties of phospholipids are described in Fig. 11. While phospholipids are mainly anionic due to the negative charge of the phosphate group, only PE and PC are zwitterionic because of the ammonium positive charge that compensates the phosphate negative charge. Due to differential ion concentrations between the inside and the outside of the cell compartments, membranes are polarized. In plants, there is an “electrostatic membrane territory” due to an electrostatic gradient from the plasma membrane to the tonoplast. For example, the plasma membrane is the most electronegative while the ER-derived compartments are neutral (Platre et al., 2018; Simon et al., 2016). The surface charges of membrane compartments are a key determinant to control the location of many proteins. Anionic lipids are major regulators of fundamental cellular processes, such as cell signaling, cell division, membrane trafficking, gene expression as described in the review by Noack and Jaillais (2020).

Thanks to neutron diffraction studies, the membrane organization of phospholipids were determined. The polar heads of PA and PE are small compared to their fatty acid volumes, leading to inverted hexagonal (HII)

Table 2 Lipid composition in higher plants and microalgae.

	Angiosperm (plant)	Chlorophyta (green microalgae)	Heterokonta (brown microalgae)	Ochrophyta (brown microalgae)	Cyanobacteria
Lipids (%)	<i>A. thaliana</i> ^a	<i>C. reinhardtii</i> ^b	<i>P. tricornutum</i> ^c	<i>M. gaditana</i> ^c	<i>S. platensis</i> ^d
MGDG	48.9	38.2	42.3	13.2	10.3
DGDG	14.5	14.8	8.5	5.8	6.44
SQDG	1.6	5	19.6	11.1	11.4
ASQD	—	— ^e	tr.	—	—
PG	8.6	7.7	6.8	5.3	25.9
PC	19.7	—	12.2	7.5	—
PE	6.7	6.1	1.1	2.5	—
PI	—	2.3	2.2	2.9	—
PS	—	—	—	—	—
DGTS	—	25.8	—	14.0	—
DGTA	—	—	4.5	—	—

^a(Kelly, Froehlich, & Dormann, 2003).^b(Riekhof, Ruckle, Lydic, Sears, & Benning, 2003).^c(Jouhet et al., 2017).^d(Xue et al., 2002).^eASQD was not analyzed in Xue et al. (2002) work but it is present in *C. Reinhardtii* (Riekhof et al., 2003).

Arabidopsis thaliana has six major lipids, three glycerolipids and three phospholipids. The composition in the green microalgae *Chlamydomonas reinhardtii* is similar to plant but DGTS replaces PC. The brown microalgae *Phaeodactylum tricornutum* and *Microchloropsis gaditana* have the same composition, with DGTA or DGTS, respectively. *Spinalina platensis* a cyanobacteria is only composed by four lipids: MGDG, DGDG, SQDG and PG. ASQD: acyl-sulfoquinovosyldiacylglycerol, DGDG: digalactosyldiacylglycerol, DGTA: 1,2-diacylglycerol-3-O-2'-(hydroxymethyl)-(N,N,N-trimethyl)-β-alanine, DGTS: 1,2-diacylglycerol-3-O-4'-(N,N,N-trimethyl)-homoserine, MGDG: monogalactosyldiacylglycerol, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PG: phosphatidylglycerol, PI: phosphatidylinositol, PS: phosphatidylserine, SQDG: sulfoquinovosyldiacylglycerol.

organization. DPG and PS organization is also hexagonal. PC, PG and PI are bilayer forming lipids. Bradshaw looked at the headgroup orientation of PI lipid in the membrane. Indeed, because PI-specific enzymes recognize this lipid, they investigated the conformation of PI headgroup. They concluded that the inositol ring in the headgroup extends perpendicular to the membrane surface (Bradshaw, Bushby, Giles, Saunders, & Reid, 1996).

3.3 Glycoglycerolipids

In the plant kingdom, glycoglycerolipids, including galactolipids, mono-galactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and the sulfolipid sulfoquinovosyldiacylglycerol (SQDG), are prevalent components of photosynthetic membranes, representing around 80% of membrane lipids in chloroplasts (Block, Dorne, Joyard, & Douce, 1983). Their syntheses occur in the plastid envelope. DAG is the precursor of MGDG and SQDG. They are synthesized by MGDG synthases and SQDG synthases, respectively. The DAG precursor is synthesized within the chloroplast from PA by a PAP, and displays the plastid signature with a C16 fatty acid on the *sn*-2 position. Galactolipids synthesized from this DAG share this signature. However, as indicated previously, a DAG backbone trafficking exists between the ER and the chloroplast, leading to a pool of MGDG and SQDG with C18 fatty acid on position *sn*-2, characteristic of the non-plastid pathway. MGDG synthases add a galactose from UDP-galactose on the *sn*-3 position of the glycerol backbone in β -anomeric position (Kelly et al., 2003), while SQDG synthase adds a sulfoquinovose group on the *sn*-3 position of DAG (Yu, Xu, & Benning, 2002). By glycosylation of MGDG by DGDG synthases, a second galactose is added in α -anomeric position. The position of DGDG polar head was also solved by neutron diffraction, it is oriented parallel to the plane of the bilayer such that the galactose moieties are tightly packed at the bilayer surface into a 0.8 nm thick polar layer (McDaniel, 1988).

Acyl-SQDG (ASQD) has been identified in some algae, such as *Chlorella vulgaris* (Morimoto, Murakami, Nagatsu, & Sakakibara, 1993), *C. reinhardtii* (Riekhof et al., 2003), *P. tricornutum* (Naumann et al., 2011) and dinoflagellate (personal observation). The ASQD structure is a SQDG backbone with an addition of a third fatty acid on the carbon 2 of the sulfoquinovose. ASQD location in the cell is not known but is probably chloroplastic because it derives from SQDG localized in this compartment. The third fatty acid on the sugar is usually the longest polyunsaturated fatty acid within the

cell: 18:3 for *C. reinhardtii*, 20:5 for *P. tricornutum* and 22:6 for dinoflagellate (Riekhof et al., 2003). The location and the insertion of the third fatty acid on the membrane is not defined. We propose two hypotheses: (1) the third fatty acid is placed in the same membrane as the other two, resulting in a flattening of the polar head on the membrane, or (2) the third fatty acid binds an adjacent membrane, maintaining the two bilayers in contact. Preliminary neutron diffraction data are in favor of the first hypothesis (personal communication).

While galactolipids are uncharged, sulfolipids are negatively charged due to the sulfate group (Fig. 12). Because the polar head of MGDG is small, this lipid self-organizes in HII phase. DGDG and SQDG, with a larger polar head, are in lamellar organization. Many neutron diffraction studies have been done to understand galactolipid membrane organization. For example, it was shown that the architecture of thylakoid membrane depends on the MGDG/DGDG ratio. DGDG is important to keep the lamellar organization (Demé et al., 2014). Another study shows the influence of DGDG on membrane stacking in comparison with PC (Kanduč et al., 2017). However, the properties of SQDG and ASQD are not yet well understood and these lipids need further studies.

In plants, the envelope of the chloroplast is delimited by two membranes, the outer and the inner membranes, whose lipid composition has been

Structure	Name	Global charge	Organization
	Monogalactosyldiacylglycerol (MGDG)	0	HII
	Digalactosyldiacylglycerol (DGDG)	0	L _α
	Sulfoquinovosyldiacylglycerol (SQDG)	-	L _α
	Acyl-sulfoquinovosyldiacylglycerol (ASQD)	-	Unknown

Fig. 12 Structure of the glycolipids class. R₁, R₂ are the two fatty acids esterified on the *sn*-1 and *sn*-2 position of the glycerol backbone. The global charge of the lipid is shown: negative charge (−) or neutral charge (0). The organization into the membrane is also mentioned: inverted hexagonal (HII) or lamellar (L_α). The organization of ASQD into the membrane is still unknown.

characterized. The inner envelope of chloroplasts and thylakoids contains MGDG, DGDG, SQDG and PG, while the outer envelope contains PC and PI in addition to MDGD, DGDG and PG (Block, Dorne, Joyard, & Douce, 1984; Douce, Holtz, & Benson, 1973; Douce & Joyard, 1990). In *C. reinhardtii*, the lipid composition of the chloroplast envelope and thylakoid membranes are similar to the plant chloroplast composition, but without PC and with a small amount of PE in the envelope. Betaine lipids (see below) are also present in the chloroplast envelope, but probably not in thylakoid membranes (Mendiola-Morgenthaler, Eichenberger, & Boschetti, 1985). In algae deriving from the secondary endosymbiosis event, such as *Phaeodactylum tricornutum*, the chloroplast is surrounded by four membranes whose lipid composition is unknown.

These two classes of lipids, phospholipids and glycolipids, are found in plant and alga membranes. In lower plants and algae, a third class of membrane glycerolipids is present, the betaine lipids that are absent in seed plants (angiosperms and gymnosperms) (Sato, 1992).

3.4 Betaine lipids

Betaine lipids are phosphate-free glycerolipids structurally related to phosphatidylcholine (PC) (Fig. 13). The presence of betaine lipids was first noted in a chrysophyte *Ochromonas danica* by Nichols and Appleby (1969).

Regarding evolution, betaine lipids are present exclusively in non-flowering plants, as well as lichen and fungi (Kunzler & Eichenberger, 1997). 1,2-diacylglyceryl-3-O-4'-(N,N,N-trimethyl)-homoserine (DGTS)

Structure	Name	Global charge	Organization
	1,2-diacylglyceryl-3-O-4'-(N,N,N-trimethyl)-homoserine (DGTS)	+/-	L _α ?
	1,2-diacylglyceryl-3-O-2'-(hydroxymethyl)-(N,N,N-trimethyl)-β-alanine (DGTA)	+/-	L _α ?
	1,2-diacylglyceryl-3-O-carboxy-(hydroxymethyl)-choline (DGCC)	+/-	L _α ?

Fig. 13 Betaine lipids structures. R₁, R₂ are the two fatty acids esterified on the *sn*-1 and *sn*-2 position of the glycerol backbone. The three lipids are neutrally charged (+/-). The organization of the betaine lipids are still unknown but probably lamellar (L_α).

is found in many green algae, ferns, mosses, and in some algal species derived from secondary endosymbiosis. 1,2-diacylglycerol-3-O-2'-(hydroxymethyl)-(N,N,N-trimethyl)- β -alanine (DGTA) and 1,2-diacylglycerol-3-O-carboxy-(hydroxymethyl)-choline (DGCC) are mostly found in brown algae such as *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*, respectively (Canavate, Armada, Rios, & Hachero-Cruzado, 2016; Guschina & Harwood, 2006; Sato, 1992). DGCC is a common constituent of Haptophyceae (Kato, Sakai, Adachi, Ikemoto, & Sano, 1996). In green algae, a high amount of betaine lipids is often linked to a low amount or lack of PC (Kalisch, Dormann, & Holz, 2016; Sato, 1992). For example, the green alga *Chlamydomonas reinhardtii* does not have PC but a high level of DGTS (Sakurai, Mori, & Sato, 2014).

The betaine lipids location is still elusive. However, Eichenberger studied the lipid composition of chloroplasts in many groups such as Chlorophyta, Bryophyta and Pteridophyta, and found that the amount of DGTS is insignificant, suggesting DGTS is an extraplastidial constituent (Eichenberger, Araki, & Müller, 1993). Also, the plasma membrane of *Dunaliella salina* contains a high concentration of DGTS (Sheffer, Fried, Gottlieb, Tietz, & Avron, 1986). The location of DGTS and DGTA by immunoelectron microscopy showed that these two betaine lipids are found in non-plastidial membranes (Kunzler, Eichenberger, & Radunz, 1997). First experiments made on *Pavlova lutheri* showed that DGCC is also found in non-plastid cell membranes, but further investigations are needed (Eichenberger & Gribo, 1997). All these works suggest that betaine lipids are located in non-plastidial membranes.

The polar parts of DGTS and DGTA are structural isomers (Fig. 13). However, the betaine lipid biosynthesis pathway is elucidated only for DGTS that occurs in the ER with the BTA1 enzyme (Riekhof, Sears, & Benning, 2005). Some researches were conducted to elucidate the biosynthesis pathway of DGTA that derives from DGTS (Vogel & Eichenberger, 1992) and DGCC (Kato et al., 1996), but the enzymes responsible for its synthesis are not yet known. The organization of betaine lipids in the membrane is also not known, but we can hypothesize that betaine lipids are bilayer forming lipids, alike PC. Indeed, PC and betaine lipids share similar chemical features: they are zwitterionic with a positive charge bared by a trimethylamine and a negative charge by a phosphate group (PC) or a carboxylic group (betaine lipids).

To summarize, by analogy with plants and by predicting the location of lipid synthesizing enzymes, some rules can be proposed: plastid membranes

are mainly composed of glycoglycerolipids while phosphoglycerolipids and betaine lipids constitute non-plastidial membranes. The lipid composition of some organisms is shown in Table 2. The biosynthesis pathways of lipids in *A. thaliana*, *C. reinhardtii* and *P. tricornutum* are summarized in Fig. 10. These particular lipid compositions confer original properties to the compartment to which they belong.



4. Adaptation to a changing environment by rearranging membrane properties

4.1 Influence of fatty acid tail in membrane fluidity: Adaptation to thermal stress

As seen above, changing the fatty acid chain length and unsaturation has a huge impact on membrane properties. The acyl tails of plant and alga membrane lipids are enriched in long and unsaturated fatty acid chains and their saturation state heavily influences the viscosity of the hydrophobic membrane core (Harwood & Jones, 1989; Sarcina, Murata, Tobin, & Mullineaux, 2003). Temperature changes the physical properties of all molecules in the cell, thereby altering enzyme kinetics, protein binding, membrane fluidity, and protein folding. High temperatures lead to increased fluidity of the membrane. During chilling temperatures the opposite occurs, the rigidity of the membrane increases, leading to the loss of the bilayer structure and membrane leakiness during freezing (Thomashow, 1999). Regulation of the fluidity gets through the modulation of fatty acid desaturases (FAD) that are unstable at elevated temperature and over-expressed during cold stress (Niu & Xiang, 2018).

The study of FAD regulation from different organisms have revealed a conceptual convergence: (i) the physical properties of the membrane can activate a signal transduction pathway that controls the expression of FAD genes and (ii) unsaturated fatty acids, the product of desaturases, act as negative signaling molecules that turn the pathway off (Aguilar & de Mendoza, 2006). However, the perception of temperature stress is different depending on the temperature: heat perception is mediated by denaturated protein with Heat Shock Protein and light receptors such as phytochrom B (Lamers, van der Meer, & Testerink, 2020). Although studies have indicated that there are several ion channels that function as heat sensors in animal cells (Ramot, MacInnis, & Goodman, 2008; Yao, Liu, & Qin, 2011), the plasma membrane thermosensor in plants long remained unidentified. The existence of primary sensors for heat stress was revealed in the plasma membrane

of *Physcomitrium patens*, which study supports the conclusion that heat-mediated calcium channels are probably regulated by membrane fluidity (Saidi, Finka, & Goloubinoff, 2011; Saidi et al., 2009). Further evidence of the role of these calcium channels as “thermosensors” in plants is needed.

Perception of cold stress is better understood. In cyanobacteria, the cold sensor Hik33 is involved in the activation of FADs. The structural characteristics of Hik33 suggest that cold stress might promote a conformational change of one of its transmembrane domain, which would trigger the dimerisation of Hik33 and its activation. However, the way in which Hik33 perceives cold-induced changes in membrane fluidity at the sub-molecular level remains to be clarified. Hik33 can be considered as a “multistress sensor” in *Synechocystis* because it can perceive also light, nutrient and hyperosmotic stresses (Mikami & Murata, 2003). Another histidine kinase, DesK, was identified as a cold sensor that regulates the cold inducible expression of the $\Delta 5$ desaturase in *Bacillus subtilis* (Aguilar, Hernandez-Arriaga, Cybulski, Erazo, & de Mendoza, 2001). Temperature sensing of DesK involves a built-in instability caused by a group of hydrophilic residues located near the N-terminus of the first transmembrane segment (Fig. 14). At lower temperature, the membrane is thicker due to a more ordered packing of lipids, and these residues are buried in the lipid phase, triggering the kinase conformation of DesK. At higher temperatures, the membrane becomes thinner with more disordered lipids, and hydrophilic residues are pushed toward the cytoplasm, promoting the required conformational changes to switch to the phosphatase conformation (Inda et al., 2014).

In plants, there is no evidence of membrane fluidity change triggering cold signaling. Two sensors were identified, COLD1 in *Oriza sativa* and the protein kinase OST1 in *Arabidopsis thaliana*, but the perception mechanisms are not known. COLD1 interacts with the protein RGA1 during cold temperatures, resulting in increased GTPase activity, which activates, in turn, an unknown calcium influx channel. OST1 is activated upon myristoylation of its interactor EGR2 in response to cold temperatures (Lamers et al., 2020).

Therefore, plants and algae adapt their membrane composition to face changing temperatures. In plants, during both heat and cold stress, TAG content is increased serving as a storage of polyunsaturated lipids. Indeed, heat stress reduces the quantities of chloroplast glycerolipids containing 18:3 acyl chains but increased 18:3-containing PC, DAG, and TAG. TAG is proposed to serve as acyl carrier for lipid turnover and β -oxidation

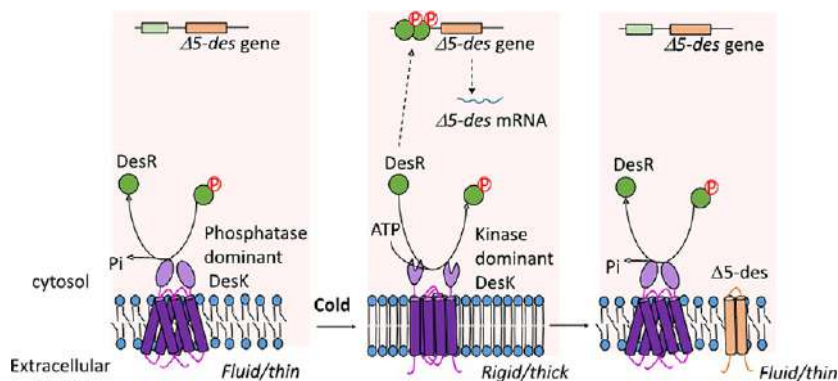


Fig. 14 Influence of membrane thickness and rigidity on the thermosensor DesK. The thermosensor DesK is composed of transmembrane domains and a cytosolic histidine kinase/phosphatase domain acting at the top of a regulatory cascade controlling the synthesis of unsaturated fatty acids in *Bacillus subtilis*. Following a drop in temperature, the membrane spans sense an increase in bilayer thickness due to an increased ordering of the lipid acyl chains by the burial of hydrophilic residues. This promotes a kinase dominant state of DesK, which autophosphorylates and then transfers the phosphate group to DesR. P-DesR activates transcription of the *des* gene coding for a $\Delta 5$ -desaturase. $\Delta 5$ -Des desaturates the acyl chains of membrane phospholipids, increasing membrane fluidity. This change promotes the phosphatase-dominant state of DesK, leading to DesR dephosphorylation and turning off transcription of the *des* gene. Adapted from Holthuis JC, Menon AK (2014) Lipid landscapes and pipelines in membrane homeostasis. *Nature* 510: 48–57.

of chloroplast-derived fatty acids (Higashi, Okazaki, Myouga, Shinozaki, & Saito, 2015). HIL1 (Heat Induce Lipase 1) encodes a chloroplastic lipase that releases 18:3 in the *sn*-1 position from MGDG. HIL1 is activated by heat stress, suggesting it has an important role in the lipid remodeling process induced by heat stress in plants (Higashi et al., 2018). On the opposite, desaturase activity is stimulated during cold conditions to increase desaturation of lipids in the lipid bilayer of chloroplast and other membranes to maintain membrane fluidity (Lamers et al., 2020). The hydrophobic fatty acyl chains might also be shortened to maintain the membrane fluidity (Barrero-Sicilia, Silvestre, Haslam, & Michaelson, 2017; Pittera et al., 2018).

In eukaryotes, lipid species that increase membrane viscosity—sterols and sphingolipids—are depleted from energy-transducing membranes, i.e., chloroplast and mitochondria (van Meer, Voelker, & Feigenson, 2008). Therefore, the level of fatty acid unsaturation drives most of the membrane fluidity. Cold and heat stress modulating fatty acid unsaturation have therefore an influence on the activity of these organelles. For instance, an increase

in thylakoid fatty acid saturation inhibits the rate of linear photosynthetic electron transport (Oquist, 1982; Vigh, Horvath, van Hasselt, & Kuiper, 1985), whereas a decrease in fatty acid saturation in mitochondria membranes is connected to an increase of quinone diffusion during respiration (Budin et al., 2018).

To summarize, membrane fluidity is affected by temperature. A homeostasis of the membrane viscosity and fluidity is necessary for the survival of plants and algae and to preserve metabolic reactions occurring in the membrane, such as photosynthesis or respiration. Fatty acid desaturases are therefore regulated to preserve these properties. However, as indicated earlier, membranes contain an equilibrium of HII and bilayer forming lipids and this equilibrium must be preserved for membrane integrity.

4.2 Homeostasis of the membrane tension and curvature: Effect of mechanical stress

The various organelles (ER, Golgi, endosome, plasma membrane) associated with the secretory pathway have differences in lipid composition that help shaping their specialized tasks. For example, they do not have the same membrane thickness throughout the secretory pathway, ER membrane being thinner than plasma membrane. This implies that transmembrane domains of ER proteins contain two amino acids less than transmembrane domains of plasma membrane proteins (Singh & Mittal, 2016). Therefore, the collective action of bulk lipids determines organelle function by influencing generic physical membrane parameters such as fluidity, thickness, lipid packing density and surface charges, despite the fact that specific lipids are also able to modulate locally the physical properties of membranes (Noack & Jaillais, 2020). For proper organelle function, these physical parameters must be kept within an appropriate range (Holthuis & Menon, 2014).

We already saw that freezing causes the formation of non-bilayer structures, such as the HII phase in the plasma and chloroplast membranes, inducing in turn membrane lesions and damaging the cell membrane integrity (Barrero-Sicilia et al., 2017). In *Arabidopsis thaliana*, during freezing, the chloroplast enzyme SFR2 is activated in addition to the diacylglycerol acyltransferase DGAT1 to remove MGDG and to produce TAG, thus preventing membrane disruption by removing HII forming lipids (Arisz et al., 2018; Moellering, Muthan, & Benning, 2010). SFR2 is activated by acidification of the cytosol, a process that occurs during freezing

(Barnes, Benning, & Roston, 2016; Barnes, Elowsky, & Roston, 2019), but the mechanism of perception of this acidification is not known.

Perception mechanism of hypo-osmotic or hyper-osmotic stresses is starting to emerge. These stresses increase membrane surface tension produced either by osmotic swelling of the cell or by shrinking of the plasma membrane prevented by the attachment of the membrane to the cell wall. How plant and alga cells are able to distinguish between these two stresses is not fully understood but they perceive membrane property changes and trigger cell signals to recover their initial properties. The primary sensors that mediate such rapid responses to mechanical changes are ion channels and they are called mechanosensors (Kefauver, Ward, & Patapoutian, 2020).

In plants, four kinds of mechanosensors have been identified so far: MscS, Piezo, OSCA and MCA. No study was done on algae so far. MscS, for mechanosensitive channel small conductance, is a family widely distributed from bacteria to animals, yeast and plants. Land plants encode 10 MscS-like (MSL) genes that are grouped into three categories on the basis of their subcellular location (Hamilton, Schlegel, & Haswell, 2015). Group I and group II are expressed in mitochondria and plastids, where they have an osmoregulatory role. *For example*, MSL2 and MSL3 control plastid size, shape, and perhaps division during normal plant development by altering ion flux in response to changes in membrane tension (Haswell & Meyerowitz, 2006). During hypo-osmotic stress, such as flooding or rehydration, they prevent epidermis plastid lysis allowing the efflux of osmolytes from the plastid into the cytosol (Veley, Marshburn, Clure, & Haswell, 2012). Group III reside at the plasma membrane, where their roles remain an active area of research. In *Arabidopsis thaliana*, MSL8 and MSL10 have been shown to be mechanosensitive ion channels, with roles in pollen survival, stress-induced cell death and cell swelling in seeds (Basu & Haswell, 2020; Basu, Shoots, & Haswell, 2020; Hamilton & Haswell, 2017). Recent structures of MSL1 reveal a multimeric architecture shared with bacterial MscS. An amphipathic helix on the cytosol leaflet is bound to several lipids that may be important for channel opening in response to mechanical stimuli (Rasmussen, Flegler, Rasmussen, & Bottcher, 2019; Reddy, Bavi, Lu, Park, & Perozo, 2019) (Fig. 15A). It is proposed that when cells are exposed to hypo-osmotic shock, the resultant cell swelling induces an increase in membrane tension that will move the amphipathic helices and increase the probability of opening the channel. When open, these channels allow ions and other small osmolytes to flow out of the cell, reducing internal osmotic pressure and protecting the membrane from lysis (Hamilton & Haswell, 2017).

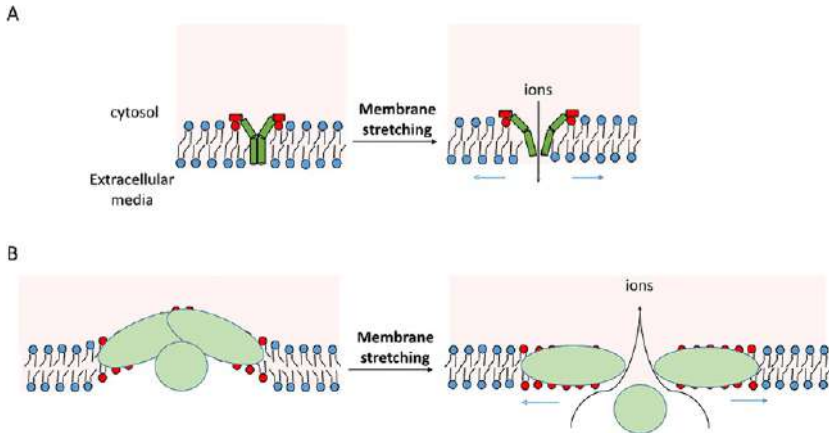


Fig. 15 “Force from lipids” models of mechanosensing (A) The dragging model. Lipids interact with an amphipathic helix and drag it outwards upon membrane expansion. MscS, for example, has an amphipathic helix on the internal leaflet (red) that drives a tilt to the pore-lining helix (green) as it is “dragged” outward under tension opening the channel to allow the efflux of ion. (B) Membrane dome model. Channel curvature within the membrane induced by the mechanosensor stores energy. During mechanic stress inducing stretching of the membrane, PIEZOs, for example, expand and flatten, gating the pore. Adapted from Kefauver JM, Ward AB, Patapoutian A (2020) *Discoveries in structure and physiology of mechanically activated ion channels. Nature* 587: 567–576.

PIEZOs are large trimeric proteins with a triskelion or three-blade propeller architecture. The three blade domains extend outward within the lipid bilayer and an extracellular cap domain resides below the central pore (Fig. 15B). PIEZOs have an unusually large number of transmembrane domain per protomer; 38 transmembrane helices per subunit are resolved in the mammalian PIEZO2 structure (Wang et al., 2019), matching previous membrane topology predictions for PIEZO1 (Coste et al., 2015). Amphipathic helices are known to sense and/or induce membrane curvature (Saotome et al., 2018). The curved form of PIEZOs could cause a local distortion to the native cell membrane or result in its preferred location to a membrane domain of similar curvature. Membrane tension would flatten the piezo trimer and open the cation channel. Most plant species harbor a single Piezo protein but it is absent in Stramenopiles (brown algae) (Cox, Bavi, & Martinac, 2017). It is involved in limiting the systemic movement of plant viruses (Zhang et al., 2019) and is required for proper root penetration in compacted environments creating mechanical stresses (Mousavi et al., 2021). AtPIEZO protein might directly alleviate mechanical

pressure in columella cells by protecting cell wall integrity and/or by transducing Ca^{2+} signals to other parts of the root such as the elongation zone (Mousavi et al., 2021).

Genes coding for *MCA* (*mid1*-complementing activity) orthologs are found exclusively in the plant kingdom. *Arabidopsis thaliana* contain two genes that have redundant functions. *MCA* mediates Ca^{2+} influx upon mechanical stimulation, such as hypo-osmotic shock and membrane stretch, and facilitates, alike *PIEZO*, penetration of the root in a hard medium (Nakagawa et al., 2007). In contrast to *PIEZO* proteins, the polypeptides of *MCA* have a single transmembrane segment and assemble as a homotetramer to form a Ca^{2+} -permeable channel (Kamano et al., 2015; Shigematsu et al., 2014). The mechanism of mechanoperception is not known.

The last known family, *OSCA* (osmosensing calcium antiporter)/*TMEM63* (transmembrane protein 63) proteins, constitutes the largest family of mechanosensitive channels. Initially described as hyperosmolarity sensors in *A. thaliana*, *OSCA* proteins have recently been shown to be pore-forming, inherently mechanosensitive channels that are conserved across plants and animals (Kefauver et al., 2020). *OSCA*s are stretch-activated at a high threshold compared to *PIEZO* channels (Zhang et al., 2018). In plants, mutations in *OSCA1.1* reduce the influx of cytosolic Ca^{2+} in response to hyperosmolarity stress. Mutations prevent Ca^{2+} signal to be generated and funneled to downstream events, such as the activation of *ASK1* protein kinase, *ABA* accumulation and stomatal closure, leading to reduction of water loss (Yuan et al., 2014). *OSCA* may act as a stretch sensor that coordinates the movements of the transmembrane helices, similar to the model for *PIEZO* mechanosensing (Liu, Wang, & Sun, 2018; Maity et al., 2019).

At present, two basic models are used to describe the gating of mechanosensitive channels by mechanical force. The “force-from-lipids” model favors the view that force transmitted through the lipid bilayer directly gates the channels, whereas the “force-from-filament” model favors the notion of channel gating via cytoskeleton or cell wall tethers that are directly connected to the mechanosensitive channels. The force-from-filament model is not developed here because it does not involve lipids. Force-from-lipids was validated for *MscS*, *Piezo* and *OSCA* from liposome or artificial bilayer experiments where no cytoskeleton or cell wall is present (Kefauver et al., 2020). Force-from-lipids manifests itself in many ways that

are not mutually exclusive. Two main examples are hydrophobic mismatch and bilayer curvature. Given that the lipid bilayer is practically incompressible, membrane stretching thins the lipid bilayer, which results in hydrophobic mismatch between the hydrophobic length of the transmembrane domain of a channel and the bilayer. To minimize the mechanical strain on the bilayer, two hypotheses are proposed: (1) lipids will move laterally in the bilayer to surround the channel with lipids of matching size and shape, which may influence helix tilt, and thus mechanosensitive channel gating, by shifting the equilibrium between open and closed conformations; (2) lipids bound to an amphipathic helix connected to the pore will move in the membrane away from the pore, dragging the amphipathic helix and straightening the channel as the membrane equilibrates under tension. This will favor the opening of the channel (Fig. 15A) (Hamill & Martinac, 2001). A different type of membrane distortion is a change in curvature (Fig. 15B). Channel activity can be modulated by the addition of conical lipids or amphipathic molecules that tend to bend membranes (Martinac et al., 2018). For example, the addition of lysoPC that will tend to flatten the curved membrane is able to activate OSCA (Zhang et al., 2018). Furthermore, the intrinsic curvature of PIEZO proteins will favor membrane bending and when tension is applied to the membrane by an atomic force microscopy cantilever, PIEZO1 protein reconstituted in planar membranes undergoes substantial area expansion implying the opening of the channel (Lin et al., 2019). Of course, these structural and mechanistic similarities exist in other channels, where instead of force-from-lipids, the gating stimuli can be membrane potential, pH, or ligand binding. Therefore, not only mechanosensors are sensitive to force-from-lipids. For example, [water permeability](#) of individual aquaporin channels depends on composition, thickness, and elasticity of the lipid bilayer (Tong, Briggs, & McIntosh, 2012).

Hydrophobic mismatch can also be used to regulate lipid synthesis and to maintain membrane homeostasis. The most studied regulation by hydrophobic mismatch is the balance between PE and PC that is controlled by the cytidine triphosphate (CTP): phosphocholine cytidyltransferase (CCT). This enzyme is the limiting step for PC synthesis; it transfers CTP to phosphocholine to form CDP-choline which is thereafter transferred to a DAG to form PC. CCT contains an amphipathic helix—called domain M—that silences the activity of the enzyme in its soluble form (Lee, Taneva, Holland, Tieleman, & Cornell, 2014). In the presence of PC

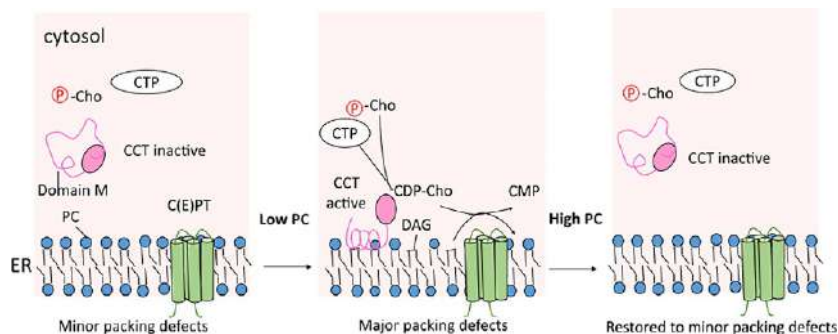


Fig. 16 Control of the CTP:phosphocholine cytidyltransferase (CCT) activity by membrane defect. CCT, the rate-limiting enzyme in PC biosynthesis, interconverts between an inactive soluble and active membrane bound form in response to changes in membrane lipid packing density. An amphipathic helix, called domain M, silences the activity of the enzyme in its soluble form. Lipid packing defects arising from increased levels of conical lipids (for example, DAG or PE) are sensed by domain M, creating a hydrophobic face for membrane binding. Association of domain M with membranes relieves self-inhibition, increasing the affinity of CCT for its substrate CTP. This then enhances biosynthesis of PtdCho to prevent transition of the membrane into a porous state. C(E)PT, choline/ethanolaminephosphotransferase. *Adapted from Holthuis JC, Menon AK (2014) Lipid landscapes and pipelines in membrane homeostasis. Nature 510: 48–57.*

bilayers with a high content of HII forming lipids such as DAG or PE, domain M undergoes a conformational switch that creates a hydrophobic surface for membrane binding (Attard, Templer, Smith, Hunt, & Jackowski, 2000) (Fig. 16). Association with membranes relieves self-inhibition and drastically increases the affinity of CCT for its substrate CTP. By contrast, membrane association and activity of CCT is reduced when DAG/PC or PE/PC ratios are low. Thus, CCT seems to act as a general sensor of lipid packing defects that signals a demand for PC biosynthesis to allow membrane expansion or to prevent its transition into a porous state (Holthuis & Menon, 2014). Consistent with this model, in mammals, CCT is recruited to lipid droplets in the growing phase to allow production of more PC molecules to surround the expanding oily core. It will be interesting to investigate the BTA1 enzyme involved in betaine lipid synthesis to see if they share the same kind of regulation.

Finally, to illustrate the influence of glycerolipid properties on membrane architecture, we describe in the next paragraph the changes occurring in one organelle, the chloroplast, through the life cycle of the land plant, and architectural differences existing between different photosynthetic organisms.

4.3 Plastid lipid composition has an influence on plastid architecture and therefore on plastid compartmentalization and function

Plastids are typical organelles present in photosynthetic eukaryotes. They derived from the endosymbiosis of a cyanobacteria and are conserved through the evolution up to higher plants and through the alga kingdoms. They have a unique lipid composition that is conserved throughout all types of plastids whereas they can adopt different membrane architecture (Fig. 17). One hypothesis for the conservation of the plastid lipid composition is their specific biophysical properties.

As indicated earlier, plastid membranes are mainly composed of glycolipids MGDG, DGDG, SQDG and one phospholipid PG with a particular fatty acid in the *sn*-2 position, a 16:1 Δ 3 in *trans*-conformation. The fact that 16:1 t-PG is only associated with the photosynthetic machinery and conserved throughout the evolution (Nichols, Harris, & James, 1965; Roughan & Boardman, 1972), suggests a crucial structural or functional role in eukaryotic photosynthesis (Boudiere et al., 2014; Wada & Murata, 2007). However, plants devoid of 16:1 t-PG are growing normally with minor defects in photosynthesis (Browse, McCourt, & Somerville, 1985; Horn, Smith, Clark, Froehlich, & Benning, 2020; McCourt, Browse, Watson, Arntzen, & Somerville, 1985), leaving the conservation of 16:1 t-PG as an unsolved mystery. As described above, MGDG and DGDG are neutral lipids, the first one forming HII phases whereas the second one bilayer structures. As a functional consequence, DGDG forms and stabilizes membrane bilayers, whereas MGDG is likely to favor curvature of thylakoids (Gounaris, Sen, Brain, Quinn, & Williams, 1983). On the opposite, SQDG and PG are anionic lipid and are both bilayer forming lipid. SQDG is able to replace PG during phosphate starvation indicating that both lipids share common properties. All these lipids associate specifically with protein complexes including photosystems, explaining why they might be conserved through the evolution (Boudiere et al., 2014).

In angiosperms, plastids can adopt different architectures (Fig. 17A). They start in meristem tissues as proplastids. Proplastids are small plastids, about 1 μ m in diameter, and less complex at the structural level than other plant plastids. They are colorless, can change their morphology and may content a variable amount of tubular-like internal membranous compartments, as well as starch depots. By division and differentiation, they give rise to the rest of the plant plastids. In hypocotyls and roots of growing seedlings, proplastids usually develop into a number of colorless plastids commonly

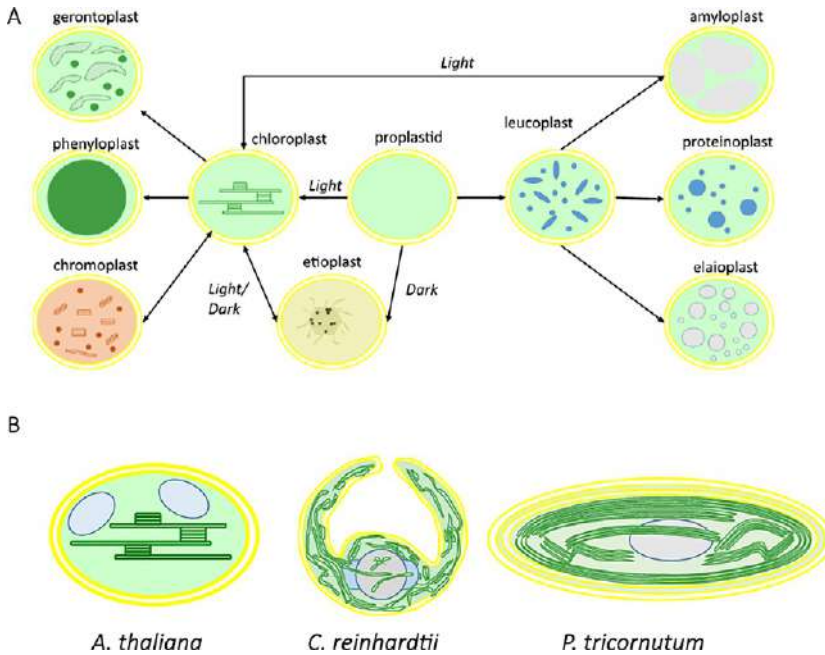


Fig. 17 Diversity and differentiation of plastids depending on tissue type, developmental stages and organisms. (A) Diversity of plastids in land plants. Arrows indicate transitions between different plastid types. The proplastid is the progenitor plastid, point of differentiation, developing into either etioplast or chloroplast depending on exposure to darkness or light, respectively. Proplastids are also able to develop into the intermediate plastid, the leucoplast, that may further differentiate into either amylo-, protein- or elaioplasts, accumulating starch, proteins or oils, respectively. Chloroplasts can also further differentiate depending on environmental stimuli and plant cell types into chromoplasts, phenyloplast and the dying gerontoplast, in which resources are recycled and redistributed. Chromoplast and phenyloplast accumulate phytochemicals such as carotenoids and phenyl-propanoids, respectively. (B) Architectures of thylakoid membranes. Models of the diverse structures of thylakoid membranes (green) in *Arabidopsis thaliana*, *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*. Pyrenoids are shown in gray, starch in blue, envelope membranes are indicated in yellow, thylakoid in green. Panel (A) adapted from Knudsen C, Gallage NJ, Hansen CC, Moller BL, Laursen T (2018) Dynamic metabolic solutions to the sessile life style of plants. *Natural Product Reports* 35: 1140–1155. Panel (B) adapted from Rast A, Heinz S, Nickelsen J (2015) Biogenesis of thylakoid membranes. *Biochimica et Biophysica Acta* 1847: 821–30.

summarized under the term leucoplasts. This group does not develop a network of internal membranes and therefore are delimited only by the plastid envelope. It comprises amyloplasts, proteinoplast, and elaioplasts. In later stages, they may also develop in other parts of the plant. These colorless plastids develop even if tissues are exposed to light (Lieber, 2015).

Chevalier, Blanvillain, & Pfannschmidt, 2018). Amyloplasts can reversibly differentiate into photosynthetic chloroplasts when exposed to light, as exemplified by the greening of potato tubers. In contrast, differentiation of proplastids into elaioplasts and proteinoplasts is non-reversible (Knudsen, Gallage, Hansen, Moller, & Laursen, 2018). Elaioplasts play a key role in storage and biosynthesis of lipids and oils, including terpenoids, which in citrus fruits are then exported into secretory pockets. Proteinoplasts (or proteoplasts) are defined as the site of protein and lipoprotein storage in the plant cell, and these plastids do not apparently encompass biosynthesis or storage roles for phytochemicals (Knudsen et al., 2018).

In the dark, seedlings follow a developmental program called skotomorphogenesis (Solymosi & Schoefs, 2010). In cotyledons of such seedlings, proplastids develop into etioplasts. Etioplasts are characteristic for this developmental program and represent an intermediate stand-by state of chloroplast formation. They do not develop a thylakoid membrane system, but paracrystalline structures, i.e., three dimensional lattice membrane structures called prolamellar bodies (PLBs) from which flattened lamellar prothylakoids (PTs) are radiated. PLBs are composed of regular arrangements of NADPH, the enzyme protochlorophyllide-oxidoreductase (POR), the chlorophyll precursor protochlorophyllide (Pchl_{id}) and galactolipids (Bastien et al., 2016).

The paracrystalline PLB structure is identified by its specific composition, in which polar lipids supposedly play a part in the formation and maintenance of such unique cubic arrangements. The lipid composition of PLBs is similar to that of the thylakoid membrane: in PLBs of wheat etioplasts, MGDG and DGDG account for approximately 50% and 30%, respectively, of total glycerolipids but with a slight increase of the MGDG/DGDG ratio compared with thylakoids (Selstam & Sandelius, 1984). This increase of the ratio MGDG/DGDG in PLB was supposed to be the main driver in favor of cubic arrangement of the membrane instead of bilayer organization. Such hypothesis was mainly based on the known roles of the conically shaped MGDG and the bilayer-forming DGDG in the bending of etioplast membranes (Demé et al., 2014). Expression of a dexamethasone inducible artificial microRNA targeting MGD1 in dark-grown *Arabidopsis* seedlings reduced MGD1 mRNA level by 70% as compared with the control, which resulted in 36% loss of MGDG without affecting DGDG content (Fujii, Kobayashi, Nagata, Masuda, & Wada, 2017). The decrease of MGDG in etioplasts only slightly changed the lattice structure of PLBs, whereas it strongly impaired the elongation of

PTs and the formation and oligomerisation of Pchl_{ide}–POR complexes in membranes. On the opposite, in the dark-grown *dgd1* mutant, DGDG content was reduced by 80% as compared with the wild type and the lattice structure of PLBs was strongly disordered while PTs were severely underdeveloped (Fujii, Kobayashi, Nagata, Masuda, & Wada, 2018). These data demonstrate the essential roles of galactolipids in the formation of internal membranes and membrane-associated processes in etioplasts but the ratio MGDG/DGDG is not the only actor of the cubic architecture of PLB.

More generally, PLB, similar to other cubic membranes in nature such as alveolar surface of mammalian lungs, retina cells, cuticle of butterfly, etc., separates aqueous phases into a two-channel system corresponding, respectively, to the future stroma and lumen compartment. This enables different molecular composition and, therefore, function (Mezzenga et al., 2019). The size of the water channels, controlled by the lipid and pigment composition that will drive the scale of the structure, can exclude or authorize the location of certain molecules on one particular side of the membrane (Bykowski et al., 2020). In such way, we can directly link the ultrastructural features of PLBs and PTs, length-scale in particular, with new possible biological functions, such as the segregation of proteins in different membrane domains. Tomographic reconstructions show that ATP synthase monomers are enriched in the PTs, whereas plastid ribosomes and POR are in the tubular lattice (Floris & Kuhlbrandt, 2021).

With light exposure, etioplasts rapidly differentiate into chloroplasts to allow photo-autotrophic growth. The differentiation from etioplasts to chloroplasts after light exposure involves the dynamic transformation of PLBs to thylakoids, which is accompanied by changes in pigment compositions from Pchl_{ide} to chlorophyll and accumulation of other photosynthetic components including photosystem (PS) I, PSII, and the light-harvesting complex (Fujii, Wada, & Kobayashi, 2019). The dynamic conversion of etioplasts into chloroplasts could operate, at least partly, via a cubic-to-lamellar lipid-phase transition. This phase transition is a spontaneous process and the released energy could be used as a driving force to change the plastid inner architecture (Demé et al., 2014; Pipitone et al., 2021).

Chloroplasts are the most studied plastid because they contain the photosynthetic membranes, the thylakoids. However, thylakoid architecture is highly different depending on light intensity and quality, and also on the organism it belongs to (Fig. 17B). In angiosperms, thylakoids comprise a sum of stroma lamellae, which turns around the grana stacks as a

right-handed helix, connecting individual grana disks by narrow membrane protrusions (Daum et al., 2014). According to the helical model, the granum has a roughly cylindrical shape comprising a variable number of stacked granal thylakoids with a flat discoid shape and typical diameters in the range of 400–600 nm (Lambrev & Akhtar, 2019). In *Chlamydomonas reinhardtii*, thylakoids were most commonly found in stacks of variable length but uniform spacing, with a number of thylakoids per stack ranging from 2 to 15, with a median of 3 thylakoids (Engel et al., 2015). In the stroma, in contrast to the disordered arrangement of Rubisco in higher plants, this enzyme is concentrated in membrane-less organelles called pyrenoids, a highly dynamic liquid-like body packed with Rubisco and traversed by thylakoid membranes (Freeman Rosenzweig et al., 2017). In *Phaeodactylum tricornutum*, the 3D structure of the photosynthetic membranes show the presence of three parallel layers of stacked thylakoids, connected between them, as well as the presence of a pyrenoid (Flori et al., 2017; Pysznik & Gibbs, 1992). Why these organisms display different membrane organizations whereas their constituting lipids and proteins are conserved? One explanation could be the different proportion of lipid classes, or differences in the fatty acid composition or pigment content such as chlorophyll or carotenoid. For example, diatoms are richer in SQDG than green algae and land plants, contain very long polyunsaturated fatty acid chains and their photosystems comprise chlorophyll *c* and fucoxanthin instead of chlorophyll *a* and xanthophyll (Tables 1 and 2).

As a common feature, in most photosynthetic eukaryotes, the thylakoid membranes are subdivided into appressed regions that face adjacent membranes within thylakoid stacks and non-appressed regions that freely face the stroma. In both plants and algae, as in etioplast, membrane architecture might be used to isolate proteins. Indeed, PSII and PSI appear to be segregated to the appressed and non-appressed membranes, respectively (Flori et al., 2017; Goodenough & Staehelin, 1971; Pribil, Labs, & Leister, 2014; Wietrzynski & Engel, 2021; Wollman, Olive, Bennoun, & Recouvreur, 1980). This lateral heterogeneity is believed to coordinate photosynthesis by concentrating different reactions within specialized membrane domains, while the redistribution of light-harvesting complex II (LHCII) antennas between these domains may enable adaptation to changing environmental conditions (Minagawa & Tokutsu, 2015; Nawrocki, Santabarbara, Mosebach, Wollman, & Rappaport, 2016).

The stacking of thylakoids to form grana or appressed membranes depends on several parameters that are still not completely understood.

Forces that sustain thylakoid stacking were listed by (Chow, Kim, Horton, & Anderson, 2005). The LHCII of PSII is supposed to drive the structural dynamics of grana stacking in higher plants; phosphorylations of LHCII and PSII are involved in state transition implying stacking and destacking of thylakoids (Tikkanen & Aro, 2012). However, LHCII is apparently not the only one factor driving thylakoid stacking. Galactolipids, mainly DGDG, favor also membrane stacking by forming hydrogen bonds between the galactose polar heads screening repulsive electrostatic contribution of the charged lipids PG and SQDG (Demé et al., 2014; Kanduč et al., 2017).

Formation of thylakoids needs an intense traffic of material from the inner membrane to the thylakoid network. Vesicles accumulating in the stroma near the chloroplast envelope could organize such a traffic. Indeed, a study uncovered the presence of such vesicles in mature chloroplasts, proplastids, etioplasts and under a variety of conditions, supporting the hypothesis of vesicle-mediated transport (Lindquist, Solymosi, & Aronsson, 2016). However, other mechanisms using the non-bilayer properties of MGDG could also participate, such as flattened invaginations from the inner membrane and HII intermediate structures (Bastien et al., 2016; Garab, Ughy, & Goss, 2016).

Maintaining a constant MGDG/DGDG ratio in thylakoid membranes (at least under standard growth conditions) seems crucial for the stability and functional integrity of these membranes (Dormann & Benning, 2002). Signatures of non-bilayer phases in thylakoid were found by ^{31}P NMR and by the fluorescence of the lipid-phase-sensitive dye merocyanine-540 (Krumova, Dijkema, et al., 2008; Krumova, Koehorst, et al., 2008). More recently, higher-resolution NMR data have identified on the stromal and luminal side of thylakoid membranes the coexistence of lamellar and non-lamellar phases, tentatively assigned to inverted hexagonal HII and isotropic phases, respectively (Garab et al., 2017). This HII/L α coexistence would for instance explain the influence of MGDG on non-photochemical quenching (NPQ) via the violaxanthin de-epoxidase activity (Schaller et al., 2010), because this enzyme requires inverted hexagonal lipid structures for its activity (Jahns, Latowski, & Strzalka, 2009).

In angiosperms, during the course of plant development, chloroplasts are matured in gerontoplast, chromoplast or phenyloplast (Fig. 17A). In senescing leaves, gerontoplasts are generated as the last ontogeny stage of chloroplasts. These plastids no longer harbor functional DNA. How exactly the degradation of the thylakoid membrane systems occurs is not fully understood, but during degradation more and bigger lipid-rich

micro-compartment, called plastoglobuli, are formed. In fleshy fruits such as tomatoes, ripening is associated with differentiation of green fruit chloroplasts into ripe fruit chromoplasts. Chromoplasts are recognized by massive accumulation of carotenoid pigments that give the red, orange and yellow colors to the plant structures. Upon maturity, the concentration of carotenoids, stored in the plastoglobuli, increases and may result in the formation of carotenoid crystals. Recent studies on re-differentiated chloroplasts demonstrated that plastoglobuli may serve as biosynthetic units as well as storage sites of phenylpropanoids. Based on studies in the vanilla pod that focused on their ability to accumulate vanillin glucoside, a phenylpropanoid derived defense compound, these plastids were named phenyloplasts. The transition from chloroplasts to phenyloplasts proceeds by a decline of photosynthetic capacity, thylakoid lamellae and chlorophyll content, associated with accumulation of high concentrations of phenylpropanoid-derived glucosides (Knudsen et al., 2018). Plastoglobuli are also present in chloroplasts but they are much smaller than in phenyloplasts or chromoplasts. In contrast to the thylakoid membrane, plastoglobuli are composed of neutral lipids (triacylglycerol, phytol esters, and free fatty acids), prenylquinones (α -tocopherol, plastoquinone, plastochromanol, and phylloquinone), as well as carotenoids—the last category being especially prevalent in the plastoglobuli of chromoplasts (van Wijk & Kessler, 2017).

A possible function of plastoglobuli that has not been considered so far is that they serve as dynamic lipid reservoirs for thylakoid membranes. Indeed, plastoglobuli sizes vary and they contain thylakoid lipids at their surface (van Wijk & Kessler, 2017). A challenge on this hypothesis relies on the observation that plastoglobuli monolayers are connected only with the outer thylakoid membrane leaflet (Kirchhoff, 2019). Because thylakoid membranes most likely do not contain flippases (Jouhet, Marechal, & Block, 2007), the question arises of how the inner leaflet can profit from a dynamic lipid exchange mechanism between plastoglobuli and the outer leaflet. A possibility is the formation of non-bilayer hexagonal II phases that can act as lipid carousels between two lipid monolayers in thylakoid membranes (Garab et al., 2016). In addition to the “lipid-exchange” mechanism to maintain the thylakoid lipid/protein ratio, plastoglobuli also might adjust the thylakoid lipid content by remobilization of lipids (van Wijk & Kessler, 2017).

To summarize, by adapting lipid class distribution, plastids are able to modulate their membrane architecture, allowing the formation of membrane domains and favoring protein or pigment segregation. All these modulation needs to be highly dynamic to face sudden change in the

environment such as light intensity, temperature variation or osmotic stress. How plants and algae transduce the perception of these changes to modify their lipid metabolism and membrane composition remains a huge and challenging question.

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References

- Aguilar, P. S., & de Mendoza, D. (2006). Control of fatty acid desaturation: A mechanism conserved from bacteria to humans. *Molecular Microbiology*, 62, 1507–1514.
- Aguilar, P. S., Hernandez-Arriaga, A. M., Cybulski, L. E., Erazo, A. C., & de Mendoza, D. (2001). Molecular basis of thermosensing: A two-component signal transduction thermometer in *Bacillus subtilis*. *The EMBO Journal*, 20, 1681–1691.
- Arisz, S. A., Heo, J. Y., Koevoets, I. T., Zhao, T., van Egmond, P., Meyer, A. J., et al. (2018). Diacylglycerol acyltransferase1 contributes to freezing tolerance. *Plant Physiology*, 177, 1410–1424.
- Attard, G. S., Templer, R. H., Smith, W. S., Hunt, A. N., & Jackowski, S. (2000). Modulation of CTP:Phosphocholine cytidyltransferase by membrane curvature elastic stress. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 9032–9036.
- Barnes, A. C., Benning, C., & Roston, R. L. (2016). Chloroplast membrane remodeling during freezing stress is accompanied by cytoplasmic acidification activating SENSITIVE TO FREEZING2. *Plant Physiology*, 171, 2140–2149.
- Barnes, A. C., Elowsky, C. G., & Roston, R. L. (2019). An Arabidopsis protoplast isolation method reduces cytosolic acidification and activation of the chloroplast stress sensor SENSITIVE TO FREEZING 2. *Plant Signaling & Behavior*, 14, 1629270.
- Barrero-Sicilia, C., Silvestre, S., Haslam, R. P., & Michaelson, L. V. (2017). Lipid remodelling: Unravelling the response to cold stress in Arabidopsis and its extremophile relative *Eutrema salsugineum*. *Plant Science*, 263, 194–200.
- Bastien, O., Botella, C., Chevalier, F., Block, M. A., Jouhet, J., Breton, C., et al. (2016). New insights on thylakoid biogenesis in plant cells. *International Review of Cell and Molecular Biology*, 323, 1–30.
- Basu, D., & Haswell, E. S. (2020). The mechanosensitive Ion Channel MSL10 potentiates responses to cell swelling in Arabidopsis seedlings. *Current Biology*, 30, 2716–2728 (e6).
- Basu, D., Shoots, J. M., & Haswell, E. S. (2020). Interactions between the N- and C-termini of the mechanosensitive ion channel AtMSL10 are consistent with a three-step mechanism for activation. *Journal of Experimental Botany*, 71, 4020–4032.
- Bigay, J., & Antonny, B. (2012). Curvature, lipid packing, and electrostatics of membrane organelles: Defining cellular territories in determining specificity. *Developmental Cell*, 23, 886–895.
- Block, M. A., Dorne, A. J., Joyard, J., & Douce, R. (1983). Preparation and characterization of membrane fractions enriched in outer and inner envelope membranes from spinach chloroplasts. I. Electrophoretic and immunochemical analyses. *The Journal of Biological Chemistry*, 258, 13273–13280.

- Block, M. A., Dorne, A., Joyard, J., & Douce, R. (1984). Preparation and characterization of membrane fractions enriched in outer and inner envelope membranes from spinach chloroplasts. In C. Sybesma (Ed.), *Advances in photosynthesis research* (pp. 27–30). Dordrecht: Springer.
- Block, M. A., & Jouhet, J. (2015). Lipid trafficking at endoplasmic reticulum-chloroplast membrane contact sites. *Current Opinion in Cell Biology*, 35, 21–29.
- Boudiere, L., Michaud, M., Petroustos, D., Rebeille, F., Falconet, D., Bastien, O., et al. (2014). Glycerolipids in photosynthesis: Composition, synthesis and trafficking. *Biochimica et Biophysica Acta*, 1837, 470–480.
- Bradshaw, J. P., Bushby, R. J., Giles, C. C., Saunders, M. R., & Reid, D. G. (1996). Neutron diffraction reveals the orientation of the headgroup of inositol lipids in model membranes. *Nature Structural Biology*, 3, 125–127.
- Browse, J., McCourt, P., & Somerville, C. R. (1985). A mutant of Arabidopsis lacking a chloroplast-specific lipid. *Science*, 227, 763–765.
- Budin, I., de Rond, T., Chen, Y., Chan, L. J. G., Petzold, C. J., & Keasling, J. D. (2018). Viscous control of cellular respiration by membrane lipid composition. *Science*, 362, 1186–1189.
- Bykowski, M., Mazur, R., Buszewicz, D., Szach, J., Mostowska, A., & Kowalewska, L. (2020). Spatial Nano-morphology of the Prolamellar body in etiolated Arabidopsis thaliana plants with disturbed pigment and Polyprenol composition. *Frontiers in Cell and Development Biology*, 8, 586628.
- Calvez, P., Jouhet, J., Vie, V., Durmort, C., & Zapun, A. (2019). Lipid phases and cell geometry during the cell cycle of Streptococcus pneumoniae. *Frontiers in Microbiology*, 10, 351.
- Canavate, J. P., Armada, I., Rios, J. L., & Hachero-Cruzado, I. (2016). Exploring occurrence and molecular diversity of betaine lipids across taxonomy of marine microalgae. *Phytochemistry*, 124, 68–78.
- Chow, W. S., Kim, E. H., Horton, P., & Anderson, J. M. (2005). Granal stacking of thylakoid membranes in higher plant chloroplasts: The physicochemical forces at work and the functional consequences that ensue. *Photochemical & Photobiological Sciences*, 4, 1081–1090.
- Colin, L. A., & Jaillais, Y. (2020). Phospholipids across scales: Lipid patterns and plant development. *Current Opinion in Plant Biology*, 53, 1–9.
- Coste, B., Murthy, S. E., Mathur, J., Schmidt, M., Mechoukhi, Y., Delmas, P., et al. (2015). Piezo1 ion channel pore properties are dictated by C-terminal region. *Nature Communications*, 6, 7223.
- Cox, C. D., Bavi, N., & Martinac, B. (2017). Origin of the force: The force-from-lipids principle applied to piezo channels. *Current Topics in Membranes*, 79, 59–96.
- Daum, B., Quax, T. E., Sachse, M., Mills, D. J., Reimann, J., Yildiz, O., et al. (2014). Self-assembly of the general membrane-remodeling protein PVAP into seven-fold virus-associated pyramids. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 3829–3834.
- Davis, J. H. (1983). The description of membrane lipid conformation, order and dynamics by ²H-NMR. *Biochimica et Biophysica Acta*, 737, 117–171.
- de Mendoza, D., & Pilon, M. (2019). Control of membrane lipid homeostasis by lipid-bilayer associated sensors: A mechanism conserved from bacteria to humans. *Progress in Lipid Research*, 76, 100996. <https://doi.org/10.1016/j.plipres.2019.100996>.
- Delacroix, H., Gulik-Krzywicki, T., Mariani, P., & Luzzati, V. (1993). Freeze-fracture electron microscope study of lipid systems. The cubic phase of space group Pm3n. *Journal of Molecular Biology*, 229, 526–539.
- Demé, B., Cataye, C., Block, M. A., Marechal, E., & Jouhet, J. (2014). Contribution of galactoglycerolipids to the 3-dimensional architecture of thylakoids. *The FASEB Journal*, 28, 3373–3383.

- Demetzos, C. (2008). Differential scanning calorimetry (DSC): A tool to study the thermal behavior of lipid bilayers and liposomal stability. *Journal of Liposome Research*, 18, 159–173.
- Demurtas, D., Guichard, P., Martiel, I., Mezzenga, R., Hebert, C., & Sagalowicz, L. (2015). Direct visualization of dispersed lipid bicontinuous cubic phases by cryo-electron tomography. *Nature Communications*, 6, 8915.
- Donaldson, L. (2020). Autofluorescence in plants. *Molecules*, 25, 2393.
- Dormann, P., & Benning, C. (2002). Galactolipids rule in seed plants. *Trends in Plant Science*, 7, 112–118.
- Douce, R., Holtz, R. B., & Benson, A. A. (1973). Isolation and properties of the envelope of spinach chloroplasts. *The Journal of Biological Chemistry*, 248, 7215–7222.
- Douce, R., & Joyard, J. (1990). Biochemistry and function of the plastid envelope. *Annual Review of Cell Biology*, 6, 173–216.
- Dubacq, J. P., & Tremolieres, A. (1983). Occurrence and function of Phosphatidylglycerol Containing Delta-3-trans-Hexadecenoic acid in photosynthetic lamellae. *Physiol Veg*, 21, 293–312.
- Dubots, E., Botte, C., Boudiere, L., Yamaryo-Botte, Y., Jouhet, J., Marechal, E., et al. (2012). Role of phosphatidic acid in plant galactolipid synthesis. *Biochimie*, 94, 86–93.
- Dufourc, E. J., Mayer, C., Stohrer, J., Althoff, G., & Kothe, G. (1992). Dynamics of phosphate head groups in biomembranes. Comprehensive analysis using phosphorus-31 nuclear magnetic resonance lineshape and relaxation time measurements. *Biophysical Journal*, 61, 42–57.
- Eichenberger, W., Araki, S., & Müller, D. G. (1993). Betaine lipids and phospholipids in brown algae. *Phytochemistry*, 34, 1323–1333.
- Eichenberger, W., & Gribo, C. (1997). Lipids of Pavlova lutheri: Cellular site and metabolic role of DGCC. *Phytochemistry*, 45, 1561–1567.
- Engel, B. D., Schaffer, M., Kuhn Cuellar, L., Villa, E., Plitzko, J. M., & Baumeister, W. (2015). Native architecture of the Chlamydomonas chloroplast revealed by in situ cryo-electron tomography. *eLife*, 4, e04889.
- Epand, R. M., D'Souza, K., Berno, B., & Schlame, M. (2015). Membrane curvature modulation of protein activity determined by NMR. *Biochimica et Biophysica Acta*, 1848, 220–228.
- Esquembre, R., Ferrer, M. L., Gutierrez, M. C., Mallavia, R., & Mateo, C. R. (2007). Fluorescence study of the fluidity and cooperativity of the phase transitions of zwitterionic and anionic liposomes confined in sol-gel glasses. *The Journal of Physical Chemistry. B*, 111, 3665–3673.
- Fernandes, M. X., Castanho, M. A., & Garcia de la Torre, J. (2002). Brownian dynamics simulation of the unsaturated lipidic molecules oleic and docosahexaenoic acid confined in a cellular membrane. *Biochimica et Biophysica Acta*, 1565, 29–35.
- Fernandez-Moran, H., & Finean, J. B. (1957). Electron microscope and low-angle x-ray diffraction studies of the nerve myelin sheath. *The Journal of Biophysical and Biochemical Cytology*, 3, 725–748.
- Finean, J. B., & Hutchinson, A. L. (1988). X-ray diffraction studies of lipid phase transitions in cholesterol-rich membranes at sub-zero temperatures. *Chemistry and Physics of Lipids*, 46, 63–71.
- Flori, S., Jouneau, P. H., Bailleul, B., Gallet, B., Estrozi, L. F., Moriscot, C., et al. (2017). Plastid thylakoid architecture optimizes photosynthesis in diatoms. *Nature Communications*, 8, 15885.
- Floris, D., & Kuhlbrandt, W. (2021). Molecular landscape of etioplast inner membranes in higher plants. *Nature Plants*, 7, 514–523.
- Freeman Rosenzweig, E. S., Xu, B., Kuhn Cuellar, L., Martinez-Sanchez, A., Schaffer, M., Strauss, M., et al. (2017). The eukaryotic CO₂-concentrating organelle is liquid-like and exhibits dynamic reorganization. *Cell*, 171, 148–162 (e19).

- Frentzen, M., Heinz, E., McKeon, T. A., & Stumpf, P. K. (1983). Specificities and selectivities of glycerol-3-phosphate acyltransferase and monoacylglycerol-3-phosphate acyltransferase from pea and spinach chloroplasts. *European Journal of Biochemistry*, 129, 629–636.
- Fujii, S., Kobayashi, K., Nagata, N., Masuda, T., & Wada, H. (2017). Monogalactosyldiacylglycerol facilitates synthesis of photoactive Protochlorophyllide in Etioplasts. *Plant Physiology*, 174, 2183–2198.
- Fujii, S., Kobayashi, K., Nagata, N., Masuda, T., & Wada, H. (2018). Digalactosyldiacylglycerol is essential for Organization of the Membrane Structure in Etioplasts. *Plant Physiology*, 177, 1487–1497.
- Fujii, S., Wada, H., & Kobayashi, K. (2019). Role of Galactolipids in plastid differentiation before and after light exposure. *Plants (Basel)*, 8, 357.
- Furlan, A. L., Laurin, Y., Botcazon, C., Rodriguez-Moraga, N., Rippa, S., Deleu, M., et al. (2020). Contributions and limitations of biophysical approaches to study of the interactions between amphiphilic molecules and the plant plasma membrane. *Plants (Basel)*, 9, 648.
- Garab, G., Ughy, B., & Goss, R. (2016). Role of MGDG and non-bilayer lipid phases in the structure and dynamics of chloroplast thylakoid membranes. *Sub-Cellular Biochemistry*, 86, 127–157.
- Garab, G., Ughy, B., Waard, P., Akhtar, P., Javornik, U., Kotakis, C., et al. (2017). Lipid polymorphism in chloroplast thylakoid membranes – as revealed by ³¹P-NMR and time-resolved merocyanine fluorescence spectroscopy. *Scientific Reports*, 7, 13343.
- Giroud, C., Gerber, A., & Eichenberger, W. (1988). Lipids of Chlamydomonas-Reinhardtii – analysis of molecular-species and intracellular site(S) of biosynthesis. *Plant and Cell Physiology*, 29, 587–595.
- Goodenough, U. W., & Staehelin, L. A. (1971). Structural differentiation of stacked and unstacked chloroplast membranes. Freeze-etch electron microscopy of wild-type and mutant strains of Chlamydomonas. *The Journal of Cell Biology*, 48, 594–619.
- Gounaris, K., Sen, A., Brain, A. P. R., Quinn, P. J., & Williams, W. P. (1983). Phase-separation of non-bilayer lipids in thylakoid membranes of chloroplasts. *Biophysical Journal*, 41, A315.
- Gulik-Krzywicki, T. (1997). Freeze-fracture transmission electron microscopy. *Current Opinion in Colloid & Interface Science*, 2, 137–144.
- Guschina, I. A., & Harwood, J. L. (2006). Lipids and lipid metabolism in eukaryotic algae. *Progress in Lipid Research*, 45, 160–186.
- Hamill, O. P., & Martinac, B. (2001). Molecular basis of mechanotransduction in living cells. *Physiological Reviews*, 81, 685–740.
- Hamilton, E. S., & Haswell, E. S. (2017). The tension-sensitive ion transport activity of MSL8 is critical for its function in pollen hydration and germination. *Plant & Cell Physiology*, 58, 1222–1237.
- Hamilton, E. S., Schlegel, A. M., & Haswell, E. S. (2015). United in diversity: Mechanosensitive ion channels in plants. *Annual Review of Plant Biology*, 66, 113–137.
- Harwood, J. L., & Jones, A. L. (1989). Lipid Metabolism in Algae. *Advances in Botanical Research*, 16, 1–53.
- Haswell, E. S., & Meyerowitz, E. M. (2006). MscS-like proteins control plastid size and shape in Arabidopsis thaliana. *Current Biology*, 16, 1–11.
- Heemskerk, J. W., Schmidt, H., Hammer, U., & Heinz, E. (1991). Biosynthesis and desaturation of prokaryotic galactolipids in leaves and isolated chloroplasts from spinach. *Plant Physiology*, 96, 144–152.
- Heinz, E., & Roughan, P. G. (1983). Similarities and differences in lipid metabolism of chloroplasts isolated from 18:3 and 16:3 plants. *Plant Physiology*, 72, 273–279.

- Higashi, Y., Okazaki, Y., Myouga, F., Shinozaki, K., & Saito, K. (2015). Landscape of the lipidome and transcriptome under heat stress in *Arabidopsis thaliana*. *Scientific Reports*, 5, 10533.
- Higashi, Y., Okazaki, Y., Takano, K., Myouga, F., Shinozaki, K., Knoch, E., et al. (2018). HEAT INDUCIBLE LIPASE1 remodels Chloroplastic Monogalactosyldiacylglycerol by liberating alpha-linolenic acid in *Arabidopsis* leaves under heat stress. *Plant Cell*, 30, 1887–1905.
- Hirashima, T., Toyoshima, M., Moriyama, T., & Sato, N. (2018). Evolution of the phosphatidylcholine biosynthesis pathways in green algae: Combinatorial diversity of methyltransferases. *Journal of Molecular Evolution*, 86, 68–76.
- Holthuis, J. C., & Menon, A. K. (2014). Lipid landscapes and pipelines in membrane homeostasis. *Nature*, 510, 48–57.
- Horn, P. J., Smith, M. D., Clark, T. R., Froehlich, J. E., & Benning, C. (2020). PEROXIREDOXIN Q stimulates the activity of the chloroplast 16:1(Delta3trans) FATTY ACID DESATURASE4. *The Plant Journal*, 102, 718–729.
- Hyvonen, M. T., Rantala, T. T., & Ala-Korpela, M. (1997). Structure and dynamic properties of diunsaturated 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine lipid bilayer from molecular dynamics simulation. *Biophysical Journal*, 73, 2907–2923.
- Ibarguren, M., Lopez, D. J., & Escriba, P. V. (2014). The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health. *Biochimica et Biophysica Acta*, 1838, 1518–1528.
- Inda, M. E., Vandenbranden, M., Fernandez, A., de Mendoza, D., Ruyschaert, J. M., & Cybulski, L. E. (2014). A lipid-mediated conformational switch modulates the thermosensing activity of DesK. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 3579–3584.
- Inoue, T., Yanagihara, S., Misono, Y., & Suzuki, M. (2001). Effect of fatty acids on phase behavior of hydrated dipalmitoylphosphatidylcholine bilayer: Saturated versus unsaturated fatty acids. *Chemistry and Physics of Lipids*, 109, 117–133.
- Jahns, P., Latowski, D., & Strzalka, K. (2009). Mechanism and regulation of the violaxanthin cycle: The role of antenna proteins and membrane lipids. *Biochimica et Biophysica Acta*, 1787, 3–14.
- Janiak, M. J., Small, D. M., & Shipley, G. G. (1979). Temperature and compositional dependence of the structure of hydrated dimyristoyl lecithin. *The Journal of Biological Chemistry*, 254, 6068–6078.
- Johnson, M. P., Brain, A. P., & Ruban, A. V. (2011). Changes in thylakoid membrane thickness associated with the reorganization of photosystem II light harvesting complexes during photoprotective energy dissipation. *Plant Signaling & Behavior*, 6, 1386–1390.
- Johnson, M. P., Vasilev, C., Olsen, J. D., & Hunter, C. N. (2014). Nanodomains of cytochrome b6f and photosystem II complexes in spinach grana thylakoid membranes. *Plant Cell*, 26, 3051–3061.
- Jouhet, J. (2013). Importance of the hexagonal lipid phase in biological membrane organization. *Frontiers in Plant Science*, 4, 494.
- Jouhet, J., Lupette, J., Clerc, O., Magneschi, L., Bedhomme, M., Collin, S., et al. (2017). LC-MS/MS versus TLC plus GC methods: Consistency of glycerolipid and fatty acid profiles in microalgae and higher plant cells and effect of a nitrogen starvation. *PLoS One*, 12, e0182423.
- Jouhet, J., Marechal, E., & Block, M. A. (2007). Glycerolipid transfer for the building of membranes in plant cells. *Progress in Lipid Research*, 46, 37–55.
- Kalisch, B., Dormann, P., & Holz, G. (2016). DGDG and glycolipids in plants and algae. *Sub-Cellular Biochemistry*, 86, 51–83.
- Kamano, S., Kume, S., Iida, K., Lei, K. J., Nakano, M., Nakayama, Y., et al. (2015). Transmembrane topologies of Ca²⁺-permeable mechanosensitive channels MCA1 and MCA2 in *Arabidopsis thaliana*. *The Journal of Biological Chemistry*, 290, 30901–30909.

- Kanduč, M., Schlaich, A., de Vries, A. H., Jouhet, J., Marechal, E., Deme, B., et al. (2017). Tight cohesion between glycolipid membranes results from balanced water-headgroup interactions. *Nature Communications*, 8, 14899.
- Katayama, K., Sakurai, I., & Wada, H. (2004). Identification of an *Arabidopsis thaliana* gene for cardiolipin synthase located in mitochondria. *FEBS Letters*, 577, 193–198.
- Kato, M., Sakai, M., Adachi, K., Ikemoto, H., & Sano, H. (1996). Distribution of betaine lipids in marine algae. *Phytochemistry*, 42, 1341–1345.
- Kawski, A. (1993). Fluorescence anisotropy – theory and applications of rotational depolarization. *Critical Reviews in Analytical Chemistry*, 23, 459–529.
- Kefauver, J. M., Ward, A. B., & Patapoutian, A. (2020). Discoveries in structure and physiology of mechanically activated ion channels. *Nature*, 587, 567–576.
- Kelly, A. A., Froehlich, J. E., & Dormann, P. (2003). Disruption of the two digalactosyldiacylglycerol synthase genes DGD1 and DGD2 in *Arabidopsis* reveals the existence of an additional enzyme of galactolipid synthesis. *Plant Cell*, 15, 2694–2706.
- Kim, Y., Terng, E. L., Riekhof, W. R., Cahoon, E. B., & Cerutti, H. (2018). Endoplasmic reticulum acyltransferase with prokaryotic substrate preference contributes to triacylglycerol assembly in *Chlamydomonas*. *Proceedings of the National Academy of Sciences of the United States of America*, 115, 1652–1657.
- Kirchhoff, H. (2019). Chloroplast ultrastructure in plants. *The New Phytologist*, 223, 565–574.
- Kirchhoff, H., Lenhart, S., Buchel, C., Chi, L., & Nield, J. (2008). Probing the organization of photosystem II in photosynthetic membranes by atomic force microscopy. *Biochemistry*, 47, 431–440.
- Knudsen, C., Gallage, N. J., Hansen, C. C., Moller, B. L., & Laursen, T. (2018). Dynamic metabolic solutions to the sessile life style of plants. *Natural Product Reports*, 35, 1140–1155.
- Koynova, R., & Tenchov, B. (2013). Recent patents on nonlamellar liquid crystalline lipid phases in drug delivery. *Recent Patents on Drug Delivery & Formulation*, 7, 165–173.
- Koynova, R., Wang, L., & Macdonald, R. C. (2008). Cationic phospholipids forming cubic phases: Lipoplex structure and transfection efficiency. *Molecular Pharmaceutics*, 5, 739–744.
- Krumova, S. B., Dijkema, C., de Waard, P., Van As, H., Garab, G., & van Amerongen, H. (2008). Phase behavior of phosphatidylglycerol in spinach thylakoid membranes as revealed by ³¹P-NMR. *Biochimica et Biophysica Acta*, 1778, 997–1003.
- Krumova, S. B., Koehorst, R. B., Bota, A., Pali, T., van Hoek, A., Garab, G., et al. (2008). Temperature dependence of the lipid packing in thylakoid membranes studied by time- and spectrally resolved fluorescence of Merocyanine 540. *Biochimica et Biophysica Acta*, 1778, 2823–2833.
- Kucerka, N., Nieh, M. P., Pencer, J., Sachs, J. N., & Katsaras, J. (2009). What determines the thickness of a biological membrane. *General Physiology and Biophysics*, 28, 117–125.
- Kunzler, K., & Eichenberger, W. (1997). Betaine lipids and zwitterionic phospholipids in plants and fungi. *Phytochemistry*, 46, 883–892.
- Kunzler, K., Eichenberger, W., & Radunz, A. (1997). Intracellular localization of two betaine lipids by cell fractionation and immunomicroscopy. *Zeitschrift fuer Naturforschung, C: Journal of Biosciences*, 52, 487–495.
- Lambrev, P. H., & Akhtar, P. (2019). Macroorganisation and flexibility of thylakoid membranes. *The Biochemical Journal*, 476, 2981–3018.
- Lamers, J., van der Meer, T., & Testerink, C. (2020). How plants sense and respond to stressful environments. *Plant Physiology*, 182, 1624–1635.
- Lee, J., Taneva, S. G., Holland, B. W., Tieleman, D. P., & Cornell, R. B. (2014). Structural basis for autoinhibition of CTP:Phosphocholine cytidyltransferase (CCT), the regulatory enzyme in phosphatidylcholine synthesis, by its membrane-binding amphipathic helix. *The Journal of Biological Chemistry*, 289, 1742–1755.

- Lepault, J. (1985). Cryo-electron microscopy of helical particles TMV and T4 polyheads. *Journal of Microscopy*, 140, 73–80.
- Lewis, R. N., Mannock, D. A., & McElhaney, R. N. (2007). Differential scanning calorimetry in the study of lipid phase transitions in model and biological membranes: Practical considerations. *Methods in Molecular Biology*, 400, 171–195.
- Li-Beisson, Y., Thelen, J. J., Fedosejevs, E., & Harwood, J. L. (2019). The lipid biochemistry of eukaryotic algae. *Progress in Lipid Research*, 74, 31–68.
- Liebers, M., Chevalier, F., Blanvillain, R., & Pfannschmidt, T. (2018). PAP genes are tissue- and cell-specific markers of chloroplast development. *Planta*, 248, 629–646.
- Lin, Y. C., Guo, Y. R., Miyagi, A., Levring, J., MacKinnon, R., & Scheuring, S. (2019). Force-induced conformational changes in PIEZO1. *Nature*, 573, 230–234.
- Lindquist, E., Solymski, K., & Aronsson, H. (2016). Vesicles are persistent features of different plastids. *Traffic*, 17, 1125–1138.
- Lis, L. J., & Quinn, P. J. (1991). The application of synchrotron X-radiation for the study of phase transitions in lipid model membrane systems. *Journal of Applied Crystallography*, 24, 48–60.
- Liu, X., Wang, J., & Sun, L. (2018). Structure of the hyperosmolality-gated calcium-permeable channel OSCA1.2. *Nature Communications*, 9, 5060.
- Lombard, J. (2014). Once upon a time the cell membranes: 175 years of cell boundary research. *Biology Direct*, 9, 32.
- Luzzati, V., & Husson, F. (1962). The structure of the liquid-crystalline phasis of lipid-water systems. *The Journal of Cell Biology*, 12, 207–219.
- Maity, K., Heumann, J. M., McGrath, A. P., Kopcho, N. J., Hsu, P. K., Lee, C. W., et al. (2019). Cryo-EM structure of OSCA1.2 from *Oryza sativa* elucidates the mechanical basis of potential membrane hyperosmolality gating. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 14309–14318.
- Martinac, B., Bavi, N., Ridone, P., Nikolaev, Y. A., Martinac, A. D., Nakayama, Y., et al. (2018). Tuning ion channel mechanosensitivity by asymmetry of the transbilayer pressure profile. *Biophysical Reviews*, 10, 1377–1384.
- Martonosi, A., Kracke, G., Taylor, K. A., Dux, L., & Peracchia, C. (1985). The regulation of the Ca^{2+} transport activity of sarcoplasmic reticulum. *Society of General Physiologists Series*, 39, 57–85.
- Maulucci, G., Cohen, O., Daniel, B., Sansone, A., Petropoulou, P. I., Filou, S., et al. (2016). Fatty acid-related modulations of membrane fluidity in cells: Detection and implications. *Free Radical Research*, 50, S40–S50.
- McCourt, P., Browse, J., Watson, J., Amtzen, C. J., & Somerville, C. R. (1985). Analysis of photosynthetic antenna function in a mutant of *Arabidopsis thaliana* (L.) lacking trans-Hexadecenoic acid. *Plant Physiology*, 78, 853–858.
- McDaniel, R. V. (1988). Neutron diffraction studies of digalactosyldiacylglycerol. *Biochimica et Biophysica Acta*, 940, 158–164.
- McElhaney, R. N. (1986). Differential scanning calorimetric studies of lipid-protein interactions in model membrane systems. *Biochimica et Biophysica Acta*, 864, 361–421.
- Mendiola-Morgenthaler, L., Eichenberger, W., & Boschetti, A. (1985). Isolation of chloroplast envelopes from *Chlamydomonas*. Lipid and polypeptide composition. *Plant Science*, 41, 97–104.
- Meyer, H. W., & Richter, W. (2001). Freeze-fracture studies on lipids and membranes. *Micron*, 32, 615–644.
- Mezzenga, R., Seddon, J. M., Drummond, C. J., Boyd, B. J., Schroder-Turk, G. E., & Sagalowicz, L. (2019). Nature-inspired design and application of Lipidic Lyotropic liquid crystals. *Advanced Materials*, 31, e1900818.
- Mikami, K., & Murata, N. (2003). Membrane fluidity and the perception of environmental signals in cyanobacteria and plants. *Progress in Lipid Research*, 42, 527–543.

- Minagawa, J., & Tokutsu, R. (2015). Dynamic regulation of photosynthesis in *Chlamydomonas reinhardtii*. *The Plant Journal*, 82, 413–428.
- Mio, K., & Sato, C. (2018). Lipid environment of membrane proteins in cryo-EM based structural analysis. *Biophysical Reviews*, 10, 307–316.
- Moellering, E. R., Muthan, B., & Benning, C. (2010). Freezing tolerance in plants requires lipid remodeling at the outer chloroplast membrane. *Science*, 330, 226–228.
- Morimoto, T., Murakami, N., Nagatsu, A., & Sakakibara, J. (1993). Studies on Glycolipids .7. Isolation of 2 New Sulfoquinovosyl Diacylglycerols from the Green-Alga *Chlorella Vulgaris*. *Chemical & Pharmaceutical Bulletin*, 41, 1545–1548.
- Mouritsen, O. G. (2005). Lively lipids provide for function. In T. F. Collection (Ed.), *Life — As a matter of fat: The emerging science of Lipidomics* (pp. 129–136). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Mousavi, S. A. R., Dubin, A. E., Zeng, W. Z., Coombs, A. M., Do, K., Ghadiri, D. A., et al. (2021). PIEZO ion channel is required for root mechanotransduction in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, 118, e2102188118.
- Nakagawa, Y., Katagiri, T., Shinozaki, K., Qi, Z., Tatsumi, H., Furuichi, T., et al. (2007). *Arabidopsis* plasma membrane protein crucial for Ca^{2+} influx and touch sensing in roots. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 3639–3644.
- Nakamura, Y. (2021). Headgroup biosynthesis of phosphatidylcholine and phosphatidylethanolamine in seed plants. *Progress in Lipid Research*, 82, 101091.
- Naumann, I., Klein, B. C., Bartel, S. J., Darsow, K. H., Buchholz, R., & Lange, H. A. (2011). Identification of sulfoquinovosyldiacylglycerides from *Phaeodactylum tricornutum* by matrix-assisted laser desorption/ionization QTrap time-of-flight hybrid mass spectrometry. *Rapid Communications in Mass Spectrometry*, 25, 2517–2523.
- Nawrocki, W. J., Santabarbara, S., Mosebach, L., Wollman, F. A., & Rappaport, F. (2016). State transitions redistribute rather than dissipate energy between the two photosystems in *Chlamydomonas*. *Nature Plants*, 2, 16031.
- Nichols, B. W., & Appleby, R. S. (1969). The distribution and biosynthesis of arachidonic acid in algae. *Phytochemistry*, 8, 1907–1915.
- Nichols, B. W., Harris, P., & James, A. T. (1965). The biosynthesis of trans- δ^3 -hexadecenoic acid by *Chlorella vulgaris*. *Biochemical and Biophysical Research Communications*, 21, 473–479.
- Nitenberg, M., Makshakova, O., Rocha, J., Perez, S., Marechal, E., Block, M. A., et al. (2020). Mechanism of activation of plant monogalactosyldiacylglycerol synthase 1 (MGD1) by phosphatidylglycerol. *Glycobiology*, 30, 396–406.
- Niu, Y., & Xiang, Y. (2018). An overview of biomembrane functions in plant responses to high-temperature stress. *Frontiers in Plant Science*, 9, 915.
- Noack, L. C., & Jaillais, Y. (2020). Functions of anionic lipids in plants. *Annual Review of Plant Biology*, 71, 71–102.
- Oquist, G. (1982). Seasonally induced changes in acyl lipids and fatty acids of chloroplast thylakoids of *Pinus silvestris*: A correlation between the level of unsaturation of monogalactosyldiglyceride and the rate of electron transport. *Plant Physiology*, 69, 869–875.
- Otles, S., & Pire, R. (2001). Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. *Journal of AOAC International*, 84, 1708–1714.
- Pali, T., & Pesti, M. (1996). Phase transition of membrane lipids. In R. Prasad (Ed.), *Manual on membrane lipids* (pp. 80–93). Berlin: Springer.
- Petroutsos, D., Amiar, S., Abida, H., Dolch, L. J., Bastien, O., Rebeille, F., et al. (2014). Evolution of galactoglycerolipid biosynthetic pathways— from cyanobacteria to primary plastids and from primary to secondary plastids. *Progress in Lipid Research*, 54, 68–85.

- Phan, M. D., & Shin, K. (2015). Effects of cardiolipin on membrane morphology: A Langmuir monolayer study. *Biophysical Journal*, 108, 1977–1986.
- Pinot, M., Vanni, S., Pagnotta, S., Lacas-Gervais, S., Payet, L. A., Ferreira, T., et al. (2014). Lipid cell biology. Polyunsaturated phospholipids facilitate membrane deformation and fission by endocytic proteins. *Science*, 345, 693–697.
- Pipitone, R., Eicke, S., Pfister, B., Glauser, G., Falconet, D., Uwizeye, C., et al. (2021). A multifaceted analysis reveals two distinct phases of chloroplast biogenesis during de-etiolation in *Arabidopsis*. *eLife*, 10, e62709.
- Pittera, J., Jouhet, J., Breton, S., Garczarek, L., Partensky, F., Marechal, E., et al. (2018). Thermoacclimation and genome adaptation of the membrane lipidome in marine *Synechococcus*. *Environmental Microbiology*, 20, 612–631.
- Platre, M. P., Noack, L. C., Doumane, M., Bayle, V., Simon, M. L. A., Maneta-Peyret, L., et al. (2018). A combinatorial lipid code shapes the electrostatic landscape of plant Endomembranes. *Developmental Cell*, 45, 465–480 (e11).
- Pribil, M., Labs, M., & Leister, D. (2014). Structure and dynamics of thylakoids in land plants. *Journal of Experimental Botany*, 65, 1955–1972.
- Pysznik, A. M., & Gibbs, S. P. (1992). Immunocytochemical localization of the photosystem I and the fucoxanthin-chlorophylla/c light-harvesting complex in the diatom *Phaeodactylum tricornutum*. *Protoplasma*, 208–217.
- Ramot, D., MacInnis, B. L., & Goodman, M. B. (2008). Bidirectional temperature-sensing by a single thermosensory neuron in *C. elegans*. *Nature Neuroscience*, 11, 908–915.
- Rasmussen, T., Flegler, V. J., Rasmussen, A., & Bottcher, B. (2019). Structure of the Mechanosensitive Channel MscS embedded in the membrane bilayer. *Journal of Molecular Biology*, 431, 3081–3090.
- Ray, S., Scott, J. L., & Tatulian, S. A. (2007). Effects of lipid phase transition and membrane surface charge on the interfacial activation of phospholipase A2. *Biochemistry*, 46, 13089–13100.
- Reddy, B., Bavi, N., Lu, A., Park, Y., & Perozo, E. (2019). Molecular basis of force-from-lipids gating in the mechanosensitive channel MscS. *eLife*, 8, e50486.
- Riekhof, W. R., Ruckle, M. E., Lydic, T. A., Sears, B. B., & Benning, C. (2003). The sulfolipids 2'-O-acyl-sulfoquinovosyldiacylglycerol and sulfoquinovosyldiacylglycerol are absent from a *Chlamydomonas reinhardtii* mutant deleted in SQD1. *Plant Physiology*, 133, 864–874.
- Riekhof, W. R., Sears, B. B., & Benning, C. (2005). Annotation of genes involved in glycerolipid biosynthesis in *Chlamydomonas reinhardtii*: Discovery of the betaine lipid synthase BTA1Cr. *Eukaryotic Cell*, 4, 242–252.
- Risselada, H. J., & Marrink, S. J. (2009). Curvature effects on lipid packing and dynamics in liposomes revealed by coarse grained molecular dynamics simulations. *Physical Chemistry Chemical Physics*, 11, 2056–2067.
- Roughan, P. G., & Boardman, N. K. (1972). Lipid composition of pea and bean leaves during chloroplast development. *Plant Physiology*, 50, 31–34.
- Roughan, P. G., Holland, R., & Slack, C. R. (1980). The role of chloroplasts and microsomal fractions in polar-lipid synthesis from [1-¹⁴C]acetate by cell-free preparations from spinach (*Spinacia oleracea*) leaves. *The Biochemical Journal*, 188, 17–24.
- Saidi, Y., Finka, A., & Goloubinoff, P. (2011). Heat perception and signalling in plants: A tortuous path to thermotolerance. *The New Phytologist*, 190, 556–565.
- Saidi, Y., Finka, A., Muriel, M., Bromberg, Z., Weiss, Y. G., Maathuis, F. J., et al. (2009). The heat shock response in moss plants is regulated by specific calcium-permeable channels in the plasma membrane. *Plant Cell*, 21, 2829–2843.
- Sakurai, K., Mori, N., & Sato, N. (2014). Detection and characterization of phosphatidylcholine in various strains of the genus *Chlamydomonas* (Volvocales, Chlorophyceae). *Journal of Plant Research*, 127, 641–650.

- Saotome, K., Murthy, S. E., Kefauver, J. M., Whitwam, T., Patapoutian, A., & Ward, A. B. (2018). Structure of the mechanically activated ion channel Piezo1. *Nature*, 554, 481–486.
- Sarcina, M., Murata, N., Tobin, M. J., & Mullineaux, C. W. (2003). Lipid diffusion in the thylakoid membranes of the cyanobacterium *Synechococcus* sp.: Effect of fatty acid desaturation. *FEBS Letters*, 553, 295–298.
- Sarkis, J., Rocha, J., Maniti, O., Jouhet, J., Vie, V., Block, M. A., et al. (2014). The influence of lipids on MGD1 membrane binding highlights novel mechanisms for galactolipid biosynthesis regulation in chloroplasts. *The FASEB Journal*, 28, 3114–3123.
- Sato, N. (1992). Betaine lipids. *Botanical Magazine Tokyo*, 105, 185–197.
- Schaller, S., Latowski, D., Jemiola-Rzeminska, M., Wilhelm, C., Strzalka, K., & Goss, R. (2010). The main thylakoid membrane lipid monogalactosyldiacylglycerol (MGDG) promotes the de-epoxidation of violaxanthin associated with the light-harvesting complex of photosystem II (LHCII). *Biochimica et Biophysica Acta*, 1797, 414–424.
- Selstam, E., & Sandelius, A. S. (1984). A comparison between Prolamellar bodies and Prothylakoid membranes of Etioplasts of dark-grown wheat concerning lipid and polypeptide composition. *Plant Physiology*, 76, 1036–1040.
- Shaikh, S. R., Kinnun, J. J., Leng, X., Williams, J. A., & Wassall, S. R. (2015). How polyunsaturated fatty acids modify molecular organization in membranes: Insight from NMR studies of model systems. *Biochimica et Biophysica Acta*, 1848, 211–219.
- Shechter, E. (1984). *Membranes biologiques: structure, transports, bioénergétique*. (Masson (ed) Paris).
- Sheffer, M., Fried, A., Gottlieb, H. E., Tietz, A., & Avron, M. (1986). Lipid composition of the plasma-membrane of the halotolerant alga, *Dunaliella salina*. *Biochimica et Biophysica Acta - Biomembranes*, 857, 165–172.
- Shigematsu, H., Iida, K., Nakano, M., Chaudhuri, P., Iida, H., & Nagayama, K. (2014). Structural characterization of the mechanosensitive channel candidate MCA2 from *Arabidopsis thaliana*. *PLoS One*, 9, e87724.
- Shinitzky, M. (1984). Membrane fluidity in malignancy. Adversative and recuperative. *Biochimica et Biophysica Acta*, 738, 251–261.
- Siegel, D. P., & Tenchov, B. G. (2008). Influence of the lamellar phase unbinding energy on the relative stability of lamellar and inverted cubic phases. *Biophysical Journal*, 94, 3987–3995.
- Simon, M. L., Platre, M. P., Marques-Bueno, M. M., Armengot, L., Stanislas, T., Bayle, V., et al. (2016). A PtdIns(4)P-driven electrostatic field controls cell membrane identity and signalling in plants. *Nature Plants*, 2, 16089.
- Singer, S. J., & Nicolson, G. L. (1972). The fluid mosaic model of the structure of cell membranes. *Science*, 175, 720–731.
- Singh, S., & Mittal, A. (2016). Transmembrane domain lengths serve as signatures of organismal complexity and viral transport mechanisms. *Scientific Reports*, 6, 22352.
- Smith, R., Jouhet, J., Gandini, C., Nekrasov, V., Marechal, E., Napier, J. A., et al. (2021). Plastidial acyl carrier protein Delta9-desaturase modulates eicosapentaenoic acid biosynthesis and triacylglycerol accumulation in *Phaeodactylum tricornutum*. *The Plant Journal*.
- Smutzer, G., & Yeagle, P. L. (1985). A fluorescence anisotropy study on the phase behavior of dimyristoylphosphatidylcholine/cholesterol mixtures. *Biochimica et Biophysica Acta*, 814, 274–280.
- Solymosi, K., & Schoefs, B. (2010). Etioplast and etio-chloroplast formation under natural conditions: The dark side of chlorophyll biosynthesis in angiosperms. *Photosynthesis Research*, 105, 143–166.
- Staehelin, L. A. (2003). Chloroplast structure: From chlorophyll granules to supra-molecular architecture of thylakoid membranes. *Photosynthesis Research*, 76, 185–196.

- Takamori, S., Holt, M., Stenius, K., Lemke, E. A., Grønborg, M., Riedel, D., et al. (2006). Molecular anatomy of a trafficking organelle. *Cell*, 127, 831–846.
- Tardieu, A., Luzzati, V., & Reman, F. C. (1973). Structure and polymorphism of the hydrocarbon chains of lipids: A study of lecithin–water phases. *Journal of Molecular Biology*, 75, 711–733.
- Tenchov, B., & Koynova, R. (2012). Cubic phases in membrane lipids. *European Biophysics Journal*, 41, 841–850.
- Tenchov, B., & Koynova, R. (2017). Cubic phases in phosphatidylethanolamine dispersions: Formation, stability and phase transitions. *Chemistry and Physics of Lipids*, 208, 65–74.
- Thomashow, M. F. (1999). PLANT COLD ACCLIMATION: Freezing tolerance genes and regulatory mechanisms. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50, 571–599.
- Tikkanen, M., & Aro, E. M. (2012). Thylakoid protein phosphorylation in dynamic regulation of photosystem II in higher plants. *Biochimica et Biophysica Acta*, 1817, 232–238.
- Tixier-Vidal, A., Picart, R., Loudes, C., & Bauman, A. F. (1986). Effects of polyunsaturated fatty acids and hormones on synaptogenesis in serum-free medium cultures of mouse fetal hypothalamic cells. *Neuroscience*, 17, 115–132.
- Tong, J., Briggs, M. M., & McIntosh, T. J. (2012). Water permeability of aquaporin-4 channel depends on bilayer composition, thickness, and elasticity. *Biophysical Journal*, 103, 1899–1908.
- van der Meer, B. W., van Hoeven, R. P., & van Blitterswijk, W. J. (1986). Steady-state fluorescence polarization data in membranes. Resolution into physical parameters by an extended Perrin equation for restricted rotation of fluorophores. *Biochimica et Biophysica Acta*, 854, 38–44.
- van Hoek, A., Vos, K., & Visser, A. J. W. G. (1987). Ultrasensitive time-resolved polarized fluorescence spectroscopy as a tool in biology and medicine. *IEEE Journal of Quantum Electronics*, 23, 1812–1820.
- van Meer, G., Voelker, D. R., & Feigenson, G. W. (2008). Membrane lipids: Where they are and how they behave. *Nature Reviews. Molecular Cell Biology*, 9, 112–124.
- van Wijk, K. J., & Kessler, F. (2017). Plastoglobuli: Plastid microcompartments with integrated functions in metabolism, plastid developmental transitions, and environmental adaptation. *Annual Review of Plant Biology*, 68, 253–289.
- Veley, K. M., Marshburn, S., Clure, C. E., & Haswell, E. S. (2012). Mechanosensitive channels protect plastids from hypoosmotic stress during normal plant growth. *Current Biology*, 22, 408–413.
- Vigh, L., Horvath, I., van Hasselt, P. R., & Kuiper, P. J. (1985). Effect of frost hardening on lipid and fatty acid composition of chloroplast thylakoid membranes in two wheat varieties of contrasting hardness. *Plant Physiology*, 79, 756–759.
- Vogel, G., & Eichenberger, W. (1992). Betaine lipids in lower plants. Biosynthesis of DGTS and DGTA in *Ochromonas danica* (Chrysophyceae) and the possible role of DGTS in lipid metabolism. *Plant and Cell Physiology*, 33, 427–436.
- Wada, H., & Murata, N. (2007). The essential role of phosphatidylglycerol in photosynthesis. *Photosynthesis Research*, 92, 205–215.
- Wang, L., Zhou, H., Zhang, M., Liu, W., Deng, T., Zhao, Q., et al. (2019). Structure and mechanogating of the mammalian tactile channel PIEZO2. *Nature*, 573, 225–229.
- Warakanont, J., Tsai, C. H., Michel, E. J., Murphy, G. R., 3rd, Hsueh, P. Y., Roston, R. L., et al. (2015). Chloroplast lipid transfer processes in *Chlamydomonas reinhardtii* involving a TRIGALACTOSYLDIACYLGLYCEROL 2 (TGD2) orthologue. *The Plant Journal*, 84, 1005–1020.
- Wietrzynski, W., & Engel, B. D. (2021). Chlorophyll biogenesis sees the light. *Nature Plants*, 7, 380–381.

- Wollman, F. A., Olive, J., Bennoun, P., & Recouvreur, M. (1980). Organization of the photosystem II centers and their associated antennae in the thylakoid membranes: A comparative ultrastructural, biochemical, and biophysical study of *Chlamydomonas* wild type and mutants lacking in photosystem II reaction centers. *The Journal of Cell Biology*, 87, 728–735.
- Wood, W. H. J., MacGregor-Chatwin, C., Barnett, S. F. H., Mayneord, G. E., Huang, X., Hobbs, J. K., et al. (2018). Dynamic thylakoid stacking regulates the balance between linear and cyclic photosynthetic electron transfer. *Nature Plants*, 4, 116–127.
- Xu, C., Moellering, E. R., Muthan, B., Fan, J., & Benning, C. (2010). Lipid transport mediated by Arabidopsis TGD proteins is unidirectional from the endoplasmic reticulum to the plastid. *Plant & Cell Physiology*, 51, 1019–1028.
- Xue, C. H., Hu, Y. Q., Saito, H., Zhang, Z. H., Li, Z. J., Cai, Y. P., et al. (2002). Molecular species composition of glycolipids from *Spirulina platensis*. *Food Chemistry*, 77, 9–13.
- Yang, H. J., Sugiura, Y., Ikegami, K., Konishi, Y., & Setou, M. (2012). Axonal gradient of arachidonic acid-containing phosphatidylcholine and its dependence on actin dynamics. *The Journal of Biological Chemistry*, 287, 5290–5300.
- Yao, J., Liu, B., & Qin, F. (2011). Modular thermal sensors in temperature-gated transient receptor potential (TRP) channels. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 11109–11114.
- Yu, B., Xu, C., & Benning, C. (2002). Arabidopsis disrupted in SQD2 encoding sulfolipid synthase is impaired in phosphate-limited growth. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 5732–5737.
- Yuan, F., Yang, H., Xue, Y., Kong, D., Ye, R., Li, C., et al. (2014). OSCA1 mediates osmotic-stress-evoked Ca^{2+} increases vital for osmosensing in Arabidopsis. *Nature*, 514, 367–371.
- Zauner, S., Jochum, W., Bigorowski, T., & Benning, C. (2012). A cytochrome b5-containing plastid-located fatty acid desaturase from *Chlamydomonas reinhardtii*. *Eukaryotic Cell*, 11, 856–863.
- Zhang, Z., Tong, X., Liu, S. Y., Chai, L. X., Zhu, F. F., Zhang, X. P., et al. (2019). Genetic analysis of a piezo-like protein suppressing systemic movement of plant viruses in Arabidopsis thaliana. *Scientific Reports*, 9, 3187.
- Zhang, M., Wang, D., Kang, Y., Wu, J. X., Yao, F., Pan, C., et al. (2018). Structure of the mechanosensitive OSCA channels. *Nature Structural & Molecular Biology*, 25, 850–858.
- Zhou, Y., Peisker, H., & Dormann, P. (2016). Molecular species composition of plant cardiolipin determined by liquid chromatography mass spectrometry. *Journal of Lipid Research*, 57, 1308–1321.
- Zulu, N. N., Zienkiewicz, K., Vollheyde, K., & Feussner, I. (2018). Current trends to comprehend lipid metabolism in diatoms. *Progress in Lipid Research*, 70, 1–16.