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DARWIN REVIEW

Origin of cyanobacterial thylakoids via a non-vesicular glycolipid phase transition and their impact on the Great Oxygenation Event

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Abstract

The appearance of oxygenic photosynthesis in cyanobacteria is a major event in evolution. It had an irreversible impact on the Earth, promoting the Great Oxygenation Event (GOE) ~2.4 billion years ago. Ancient cyanobacteria predating the GOE were *Gloeobacter*-type cells lacking thylakoids, which hosted photosystems in their cytoplasmic membrane. The driver of the GOE was proposed to be the transition from unicellular to filamentous cyanobacteria. However, the appearance of thylakoids expanded the photosynthetic surface to such an extent that it introduced a multiplier effect, which would be more coherent with an impact on the atmosphere. Primitive thylakoids self-organize as concentric parietal uninterrupted multilayers. There is no robust evidence for an origin of thylakoids via a vesicular-based scenario. This review reports studies supporting that hexagonal II-forming glucolipids and galactolipids at the periphery of the cytosolic membrane could be turned, within nanoseconds and without any external source of energy, into membrane multilayers. Comparison of lipid biosynthetic pathways shows that ancient cyanobacteria contained only one anionic lamellar-forming lipid, phosphatidylglycerol. The acquisition of sulfoquinovosyldiacylglycerol biosynthesis correlates with thylakoid emergence, possibly enabling sufficient provision of anionic lipids to trigger a hexagonal II-to-lamellar phase transition. With this non-vesicular lipid-phase transition, a framework is also available to re-examine the role of companion proteins in thylakoid biogenesis.

Keywords: Cyanobacteria, *Gloeobacter*, Great Oxygenation Event, hexagonal II lipid, monogalactosyldiacylglycerol synthase, monoglucosyldiacylglycerol, monogalactosyldiacylglycerol, phosphatidylglycerol, sulfoquinovosyldiacylglycerol, thylakoid,

Introduction

The appearance of oxygenic photosynthesis is a major event in the early evolution of life and had an irreversible impact on the Earth. This bioenergetic and trophic innovation, coupling water splitting upon exposure to sunlight with the reduction of atmospheric carbon dioxide, occurred in the cyanobacterial ancestral lineage (Cardona, 2019, 2018; Cardona *et al.*, 2019;

Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; MGlcDG, monoglucosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdGro, phosphatidylglycerol.

Sanchez-Baracaldo and Cardona, 2020). It deeply modified ecosystems, positioning cyanobacteria as prominent primary producers at the base of trophic networks, and allowed the development of a tremendous biodiversity of secondary and tertiary consumers. At geological scale, the production of oxygen as a by-product of water splitting enabled the oxygenation of the atmosphere during the Great Oxygenation Event (GOE), 2.4–2.32 billion years ago (bya) (Bekker *et al.*, 2004; Lyons *et al.*, 2014).

Later, oxygenic photosynthesis was acquired by Eukaryota of the Archaeplastida phylum, by the integration of an intracellular organelle, the chloroplast, deriving from the engulfment of a close relative of the deep-branching cyanobacterium *Gloeomargarita*, combined with components acquired by gene transfers from a variety of other sources (Sato and Awai, 2017; Sato and Takano, 2017; Marechal, 2018; Ponce-Toledo *et al.*, 2019). Following this unique endosymbiotic event, the expansion of marine eukaryotic phytoplankton correlated with the oxygenation of the oceans during the Neoproterozoic Oxygenation Event (NOE), 800–600 million years ago. This so-called 'rise of algae' is thought to have created food webs with even more efficient nutrient and energy transfers, driving ecosystems toward larger and more complex organisms, including animals (Brocks *et al.*, 2017).

Access to increasing genomic data and refined reconstructions of molecular phylogenies have improved our understanding of the early evolution of photosynthetic reaction center proteins (Cardona, 2019, 2018; Cardona *et al.*, 2019; Sanchez-Baracaldo and Cardona, 2020). Schematically, oxygenic photosynthesis requires two sets of dimeric reaction centers, one of type II (in photosystem II, PSII) and one of type I (in PSI), all deriving from a unique origin in an ancestral anoxygenic photosynthetic prokaryote. Whereas single PSII-like or PSI-like reaction centers have evolved independently in various clades of anoxygenic phototrophic bacteria (Hohmann-Marriott and Blankenship, 2011), crown group cyanobacteria inherited both a heterodimeric version of PSII, where water splitting occurs, and a heterodimeric PSI (Sanchez-Baracaldo and Cardona, 2020). Recent estimates of the age of PSII and PSI gene families coincide with geological evidence of oxygen traces throughout a division of Precambrian time called the Archean eon (4–2.5 bya). This implies that oxygenic photosynthesis was established by 3.0 bya, long before the GOE (Cardona, 2018; Cardona *et al.*, 2019). It is still unresolved whether a 'protocyanobacterium' acquired the two sets by gene duplication of anoxygenic photosystems (Martin *et al.*, 2018) or via gene transfers (Raymond *et al.*, 2002). Among plausible scenarios, a versatile protocyanobacterium containing two reaction centers, namely, a hydrogen sulfide (H_2S)-splitting PSI (photolithotrophy) with an expression controlled by fluctuating H_2S concentrations, and a PSII (photoorganotrophy), might have developed in shallow water in the vicinity of temporary hydrothermal springs. This protocyanobacterium, having a flexible metabolism adapted to alternating redox conditions, might have acquired oxygenic photosynthesis by mutations in the direction of the constitutive expression of both Type I and Type II reaction centers, and an extraction of electrons, first from manganese and secondarily from water. This hypothesis was termed the 'redox switch hypothesis for the first cyanobacteria' (Allen, 2014, 2016).

Given that oxygenic photosynthesis predated the GOE by about 0.5–1 billion years, what was the actual driver of this major atmospheric upheaval, if not the appearance of photosynthesis per se? The extant diversity of cyanobacteria evolved after the GOE (Sanchez-Baracaldo and Cardona, 2020). Most ancient cyanobacteria are likely to have populated low-salinity habitats and dry environments (Blank and Sanchez-Baracaldo, 2010) as small unicellular forms close to present-day *Gloeobacter* (Mares *et al.*, 2019; Sanchez-Baracaldo and Cardona, 2020; Raven and Sanchez-Baracaldo, 2021). The GOE was proposed to be the consequence of the ability of cyanobacteria to form multicellular filaments, resembling extant *Pseudanabaena*, facilitating the formation of microbial mats, a feature supposed to increase their ecological dominance to such an extent that atmospheric oxygen underwent this dramatic increase (Sanchez-Baracaldo and Cardona, 2020).

An alternative hypothesis, based on studies reviewed here, is that an atmospheric change as important as the GOE requires an extraordinary multiplier effect that multicellularity, which introduces no additive effect, is not sufficient to explain. The transition from *Gloeobacter*-type to other cyanobacteria is marked by the emergence of internal membrane sacs, known as thylakoids, which allowed significant gain in the area of the 'solar panels' accommodating the photosystems (Mares *et al.*, 2019). This review highlights the role of lipid components in the membranes of cyanobacteria, which have been disregarded until now among the determinant processes involved in the origin of thylakoids.

The emergence of thylakoids in early cyanobacteria had a multiplier effect on the photosynthetic surface per cell

Thylakoids are evidently not required for oxygenic photosynthesis, since *Gloeobacter* hosts its photosystems in its cytoplasmic (or inner) membrane (Raven and Sanchez-Baracaldo, 2021). The evolution of thylakoids starts with the appearance of continuous linear sacs with several concentric, uninterrupted layers (Mares *et al.*, 2019). Based on a spherical cell with a diameter of 1 μm (volume=0.53 μm^3), a *Gloeobacter* membrane surface would be ~3.14 μm^2. Taking into account that in *Gloeobacter*, photosynthetic proteins are concentrated in domains representing less than one-third of the cytosolic membrane (Rexroth *et al.*, 2011), the actual photosynthetic surface is in the 1 μm^2 range. By contrast, in a *Cyanobium*-like coccal cell of the same size (Mares *et al.*, 2019), with four concentric thylakoid membranes lining the plasma membrane

(so-called parietal thylakoids), the photosynthetic surface would reach ~10 μm^2. Parietal thylakoid fascicules encountered in *Synechocystis*-like coccal or *Leptolyngbya*-like filamentous cyanobacteria (Mares *et al.*, 2019) with four layers would reach about the same photosynthetic surface per cell. In cyanobacteria containing a parallel or radial arrangement of thylakoids (Mares *et al.*, 2019) the photosynthetic surface can increase even further. An *Arthrospira*-like cell with 50 radial thylakoids or a *Cyanothece*-like cell with 50 parallel thylakoids can reach a photosynthetic surface in the range of 50–70 μm^2, based on the summed area of flattened cisternae, within the same volume. In the course of evolution, following chloroplast integration in Archaeplastida, thylakoids evolved in the direction of an increased surface and organization in functional domains with the development of stacks and grana, which do not form in cyanobacteria. In the cells of eukaryotic microalgae, the volume of the plastid is commonly greater than 10 μm^3 (Uwizeye *et al.*, 2021), whereas in vascular plants, such as maize, the volume of mesophyll cell chloroplasts can reach volumes greater than 100 μm^3 (Kutik *et al.*, 2004). Based on a 10–100 μm^3 chloroplast containing 50 thylakoids, a gross calculation gives a photosynthetic surface of 10^{-3}–10^{-2} mm^2 per chloroplast. Overall, a population of 1 million *Gloeobacter*-type cells harbors a global photosynthetic surface of ~1 mm^2, whereas in 1 million *Arthrospira*- or *Cyanothece*-like cells this area can easily exceed 50–70 mm^2. Because of this obvious multiplier effect, the appearance of thylakoids therefore seems to be the most plausible determinant in the GOE.

One would naturally examine extant protein machinery(ies) involved in thylakoid biogenesis to trace back its origin and look for evidence supporting this hypothesis. The difficulty lies in the still unresolved process(es) governing the biogenesis of this membrane system, most notably because this question is mainly posed considering protein components only. We advocate here that the spontaneous organization of lipids, following an appropriate biosynthetic sequence, would be sufficient to produce very large amounts of membrane surfaces in nano- to microseconds. This process does not require the budding of vesicles from the cyanobacterial plasma membrane or the chloroplast inner envelope membrane. In fact, the traditional hypothesis of a 'vesicular-based' biogenesis of thylakoids finds little support in ultrastructural analyses of cyanobacteria and chloroplasts, where the observation of inner membrane invaginations is extremely rare (Botté *et al.*, 2011a; Boudiere *et al.*, 2012; Bastien *et al.*, 2016; Mares *et al.*, 2019; Gupta *et al.*, 2021). Some reports highlight the presence of vesicles in eukaryotic proplastids before their conversion into chloroplasts (Mechela *et al.*, 2019), but the lack of such a vesicular compartment in cyanobacteria disqualifies this mechanism as the origin of thylakoids in ancient clades. Likewise, the focus on *Synechocystis* 'thylakoid convergence zones', where thylakoids merge into high-curvature membranes in close contact with the plasma membrane, also observed in some chloroplasts (Gupta *et al.*, 2021), does not account for the biogenesis of more primitive parietal thylakoids organized in concentric and uninterrupted layers (Mares *et al.*, 2019). Here, we do not disregard the fact that protein components evolved in the direction of helping, organizing, guiding, or orchestrating the process of thylakoid biogenesis, leading to the very sophisticated and efficient processes observed today, but we assume that no evidence supports their determinant role in the early stages.

Thylakoids emerged from a mixture of lipids allowing a non-vesicular biogenesis of membranes

Membrane bilayers are made of polar lipids, which harbor a 'polar head group' exposed to the surface, and a hydrophobic 'tail' inside the core of the bilayer. How can a lipid bilayer form without any source of vesicles protruding or budding from a parent membrane and merging to produce a large surface? An answer, developed here, comes from the biophysical properties of polar lipids. Depending on the equilibrium between polar head interactions and repulsive forces between the hydrophobic tails, each of the polar lipids can self-organize and form so-called 'phases', with either a positive curvature (e.g. micellar tubules, also known as 'hexagonal I', or *HexI*), no curvature ('lamellar', or *Lm*), or a negative curvature ('inverted hexagonal', hexagonal II, or *HexII*) (Jouhet, 2013) (Fig. 1). These properties have attracted attention for a long time, with a focus on lipids promoting a *Lm* → *HexII* transition, since the disturbance introduced by the *HexII* phase was considered essential for the promotion of highly curved and fused domains (Gawrisch *et al.*, 1992; Kuzmin *et al.*, 2001; Toombes *et al.*, 2002; Jouhet, 2013) (Fig. 2A). Few thermodynamic studies are available to evaluate all possible phase transitions in the different lipid mixtures found in nature. Our knowledge relies on model lipids such as phosphatidylethanolamine (PtdEtn), whose organization in *Lm* or *HexII* phases depends on its chemical structure, that is, the length and level of desaturation of acyl chains; physical properties, such as hydration or temperature; and the presence of other lipids (Gawrisch *et al.*, 1992; Harper *et al.*, 2001; Mannock *et al.*, 2001; Toombes *et al.*, 2002) (Fig. 2A).

It is essential to keep in mind that so-called '*HexII* lipids' are also found in membrane bilayers, either because in their pure state they can form both *HexII* or *Lm* phases depending on temperature, hydration, etc., or because when they are mixed with *Lm* lipids, the lamellar phase is the more stable organization. In past studies, *Lm* → *HexII* was the direction examined in the search for plausible membrane fusion mechanisms. The enthalpy of dioleoyl-PtdEtn phase transition (ΔH) measured by differential scanning calorimetry had a positive value (+0.3 kcal mol^{-1} = 1.255 kJ mol^{-1}), meaning that energy external to the system had to be provided (Gawrisch *et al.*, 1992) (Fig. 2A). It was further shown that neither *Lm* → *HexII* nor *HexII* → *Lm* kinetics had any significant deviation from a power-law

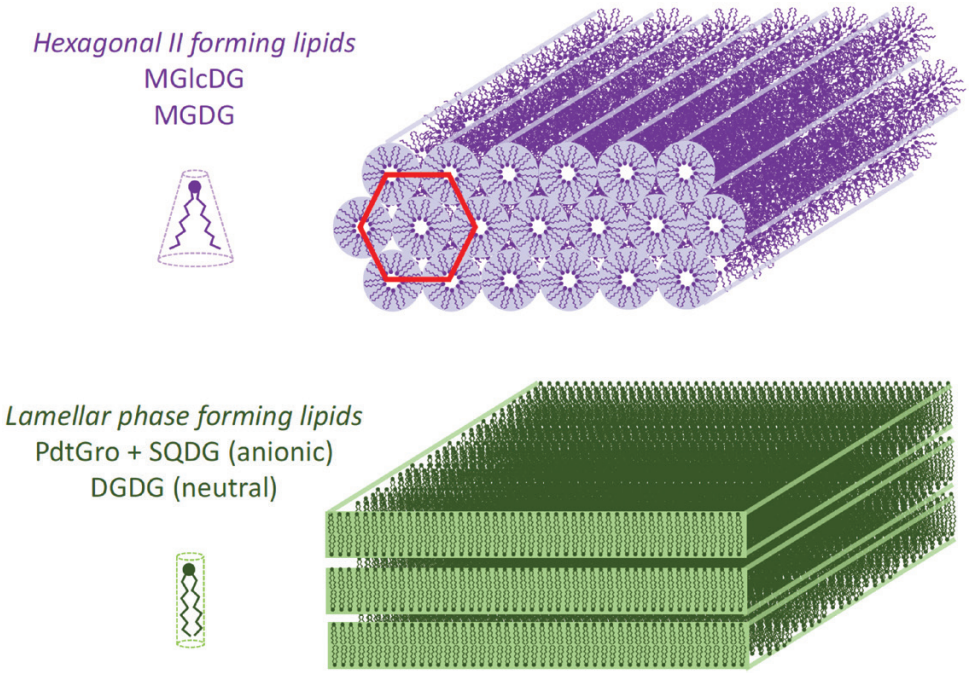

Fig. 1. Hexagonal II and lamellar lipid phases. Lipids with a polar head (shown as a solid circle) that is relatively smaller than the volume occupied by their hydrophobic tail (acyl chains) have a molecular shape that fits within a truncated cone. They induce a negative curvature and favor self-assembly into inverted tubular micelles (hexagonal II or *HexII* phase, shown in purple). Arrays of tubular micelles are regular, with seven tubules having a hexagonal section, as shown in red. Lipids with a similar cross-sectional area for their polar head and their hydrophobic tail have a molecular shape that fits within a cylinder. They form bilayers (lamellar or *Lm* phase, shown in green). Some lipids can form both structures, based on some discrete structural differences, such as the number of double bonds in their acyl moiety, or on different physicochemical conditions (temperature, hydration level). Lipid mixtures can adopt one conformation or the other, based on the relative proportions of *HexII*-forming and *Lm*-forming lipids in the mixture. Cyanobacterial lipids comprise *HexII*-forming (monoglucosyldiacylglycerol, MGlcDG; monogalactosyldiacylglycerol, MGDG) and *Lm*-forming (phosphatidylglycerol, PtdGro; sulfoquinovosyldiacylglycerol, SQDG; digalactosyldiacylgycerol, DGDG) lipids.

relationship: a relative change in the *Lm* phase results in a proportional relative change in *HexII*, and vice versa (Toombes *et al.*, 2002; Garab, 2016). The *Lm* ↔ *HexII* transition is therefore remarkable for the symmetry between the forward and reverse dynamics (Toombes *et al.*, 2002). A corollary is that the *HexII* → *Lm* transition for this pure lipid species may release some energy and therefore occur spontaneously. Similarly, spontaneous *HexII* → *Lm* transition of dioleoyl-PtdEtn could occur by changing the hydration level (Gawrisch *et al.*, 1992). The *HexII* → *Lm* phase transition provides a solution to the unresolved question of the emergence of early membrane systems, especially with such extended surfaces as thylakoids (Fig. 2B).

Let us first consider the lipid composition of cyanobacteria in a simplified evolutionary context. A variety of chemical structures can produce impermeable membranes delineating cells (Jia *et al.*, 2019). We have evidence of two distinct options for the structure of polar lipids in primordial membranes, based on their conservation in the three domains of life. These primordial lipids are all built on a three-carbon glycerol scaffold (numbered *sn*-1, *sn*-2, and *sn*-3). On the one hand, Archaea are bounded by membranes made of isoprenoid hydrocarbon chains linked via ether bonds to an *sn*-2,3-glycerol phosphate backbone, and multiple derivatives with two polar heads, which can form lipid monolayers (Imachi *et al.*, 2020; Salvador-Castell *et al.*, 2021). On the other hand, Bacteria and Eukaryota are primarily bounded by membranes made of acyl-glycerolipids, deriving from a precursor containing fatty acids linked via ester bonds to an *sn*-1,2- glycerol phosphate backbone (Imachi *et al.*, 2020). It is established that in Archaea, the presence of phytanyl chains, ether bonds, and bipolar lipids confers a high stability on their membranes (Guler *et al.*, 2009; Balleza *et al.*, 2014; Garcia-Arribas *et al.*, 2015). By contrast, in Bacteria and Eukaryota, acyl-glycerolipids may confer a higher flexibility, especially via the controlled addition of double bonds to the acyl chains, tuning the lateral fluidity of membranes, but also via the balance between *HexII* and *Lm* lipids (de Kruijff, 1997; Garab, 2016). Increasing evidence supports that eukaryogenesis originated from the merger between an archaeal host and a bacterial endosymbiont related to modern α-proteobacteria (Imachi *et al.*, 2020). This raises the puzzling question of the loss of Archaea-type lipids in Eukaryota. One hypothesis is that the integrated α-proteobacterium gave rise to a proto-mitochondrion, producing ATP by specific protein machineries preferring Bacteria-type membranes, which were subsequently conserved (Sojo, 2019). In these initial membrane

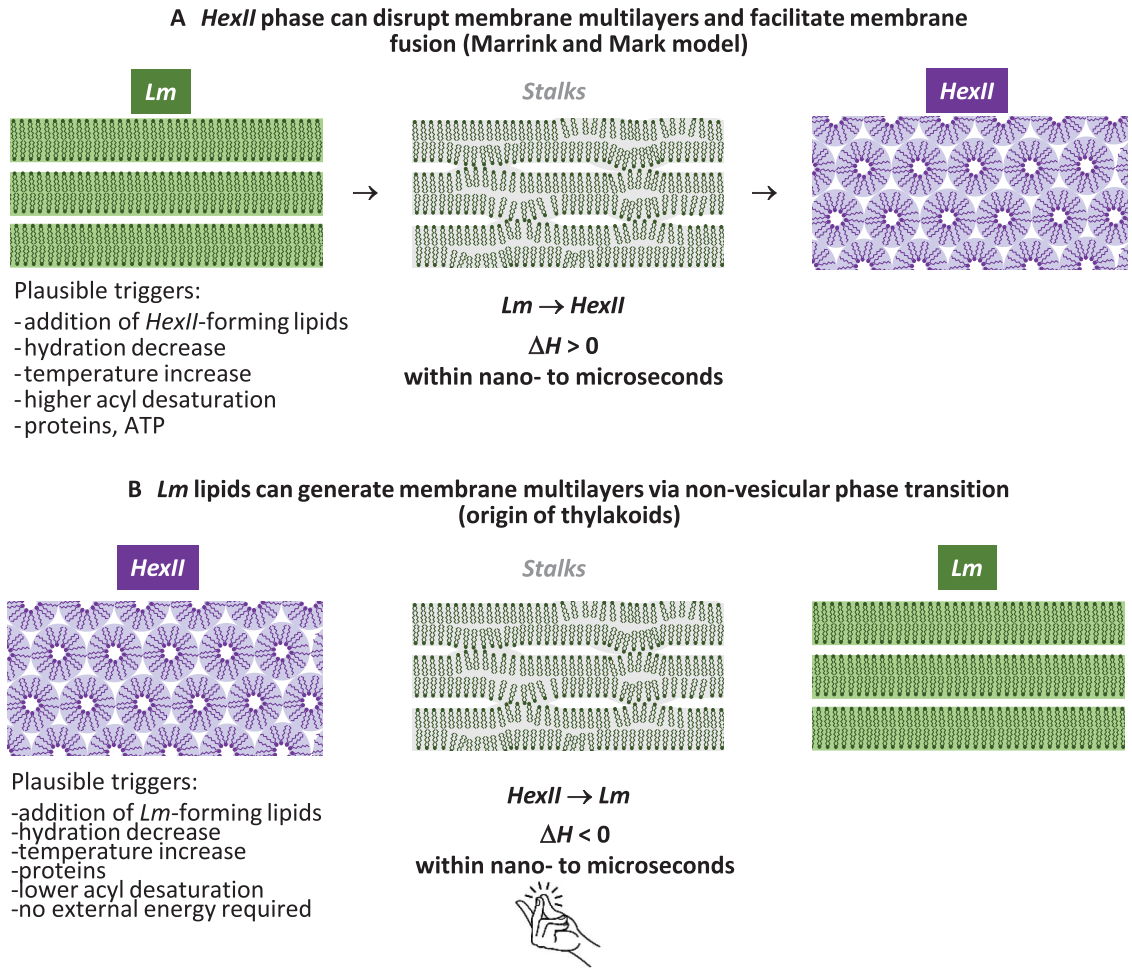

Fig. 2. Non-vesicular lipid phase transition at the origin of thylakoids. (A) *Lm* → *HexII* phase transition as a driving process for membrane fusion. Based on work with the model lipid PtdEtn, the enthalpy of the *Lm* → *HexII* phase transition is positive, indicating the requirement for an external source of energy. The intermediary phase, forming so-called 'stalks', is shown based on the model of Marrink and Mark (2004). (B) *HexII* → *Lm* phase transition as a driving process for thylakoid emergence. Based on the reversibility principle, the complete phase transition is spontaneous, occurs within nanoseconds, and is facilitated by a variety of possible triggers, including the addition of *Lm*-forming lipids.

matrices, other lipidic structures and hydrophobic polypeptides were integrated, contributing to the multitude of functions harbored by cell membranes.

Acyl-glycerolipids with a polar head containing a phosphate group, such as the *HexII* lipid PtdEtn or the *Lm* lipid phosphatidylcholine (PtdCho), are conserved in the majority of Bacteria and Eukaryota clades, with the notable exception of crown cyanobacteria, which contain only one phospholipid, phosphatidylglycerol (PtdGro) (Yuzawa *et al.*, 2012; Petroutsos *et al.*, 2014; Sato and Awai, 2016) (Fig. 3A). PtdGro is anionic and its proportion is regulated in response to phosphate availability (Frentzen, 2004; Boudiere *et al.*, 2014). The conservation of PtdGro is consistent with its role in the assembly and functioning of photosynthesis components (Boudiere *et al.*, 2014), supported by the lethality of PtdGro-less mutants of cyanobacteria (Hagio *et al.*, 2000; Sakurai *et al.*, 2003a, b; Kopecna *et al.*, 2015; Kobori *et al.*, 2018) and plants (Xu *et al.*, 2002; Babiychuk *et al.*, 2003; Xu *et al.*, 2006). Such a membrane composition parsimonious in phospholipids supports the idea that early cyanobacteria populated an environment where phosphorus was limiting, at least to meet their basal metabolic demand.

The lipid composition of *Gloeobacter* membranes is simple, containing three lipid classes in addition to PtdGro: monoglucosyldiacylglycerol (MGlcDG), monogalactosyldiacylglycerol (MGDG), and digalactosyldiacylglycerol (DGDG) (Rexroth *et al.*, 2011; Yuzawa *et al.*, 2012; Sato, 2015; Sato and Awai, 2016) (Fig. 3A). MGlcDG and MGDG are *HexII* lipids, whereas DGDG and PtdGro are *Lm* lipids (Jouhet, 2013). It is interesting to note that the addition of *HexII*-forming MGlcDG could rescue a bacterial *Escherichia coli* mutant lacking the *HexII*-forming lipid PtdEtn (Wikstrom *et al.*, 2004), indicating that a *HexII* lipid could replace another *HexII* lipid, thus filling a vital role governed by its biophysical properties. The plasma membrane of *Gloeobacter* has only traces of MGlcDG (Sato, 2015; Sato and Awai, 2016). It has been shown to contain

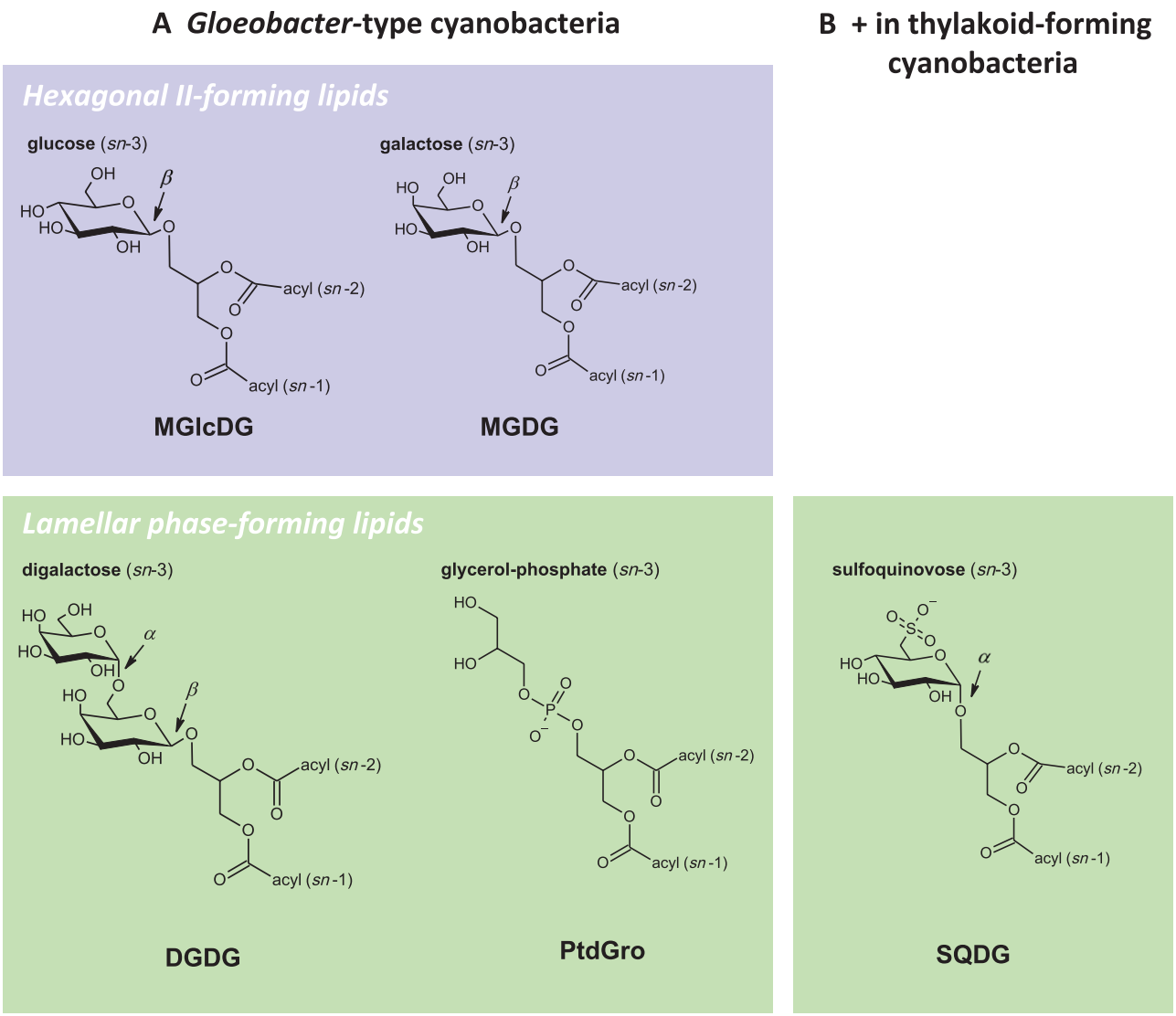

Fig. 3. Cyanobacterial polar lipids. (A) *Gloeobacter*-type polar lipids consist of two *HexII* lipid classes, monoglucosyldiacylgycerol (MGlcDG) and monogalactosyldiacylglycerol (MGDG), and two *Lm*-forming lipids, digalactosyldiacylglycerol (DGDG) and phosphatidylglycerol (PtdGro). (B) All other cyanobacteria contain a third *Lm*-forming lipid, sulfoquinovosyldiacylglycerol (SQDG). The α or β anomery of glycosylation linkages is indicated. PtdGro is the sole cyanobacterial phospholipid, and its proportion is controlled by the availability of phosphorus in the environment. PtdGro and SQDG are anionic lipids.

segregated bioenergetic domains, some with low chlorophyll content being MGDG-rich (~70% MGDG, ~22% DGDG, and ~8% PtdGro), whereas high-chlorophyll domains were more balanced in their lipid composition (~55% MGDG, ~35% DGDG, and ~10% PtdGro) (Rexroth *et al.*, 2011) (Fig. 4A). These latter domains also contain photosynthetic components, namely, PSII, PSI, and ATP synthase. It is very important to note that the relative MGDG/DGDG ratio differs in the two membrane domains: this indicates that although DGDG is produced from MGDG, some regulatory mechanisms uncouple its final proportion from the available amount of substrate. This is consistent with the multitude of roles played by DGDG, which need to be finely controlled, including controlling the stability of the photosynthetic machinery (Awai *et al.*, 2007; Sakurai *et al.*, 2007; Boudiere *et al.*, 2014; Apdila and Awai, 2018). The MGDG-rich domains at the plasma membrane appear therefore to be ideal platforms to promote *HexII* → *Lm* phase transition. Nevertheless, the $HexII_{(MGlcDG,\ MGDG)}/Lm_{(DGDG,\ PtdGro)}$ balance is still insufficient in *Gloeobacter* to promote the formation of thylakoids. In addition, because PtdGro is essential for the stability of photosystems (Hagio *et al.*, 2000; Xu *et al.*, 2002; Sakurai *et al.*, 2003a, b; Babiychuk *et al.*, 2003; Xu *et al.*, 2006; Kopecna *et al.*, 2015; Kobori *et al.*, 2018), a minimal quantity of this anionic lipid should be included in thylakoids, and the production of excessive amounts of PtdGro in the bulk of membrane lipids appears difficult to maintain in a phosphate-limiting environment.

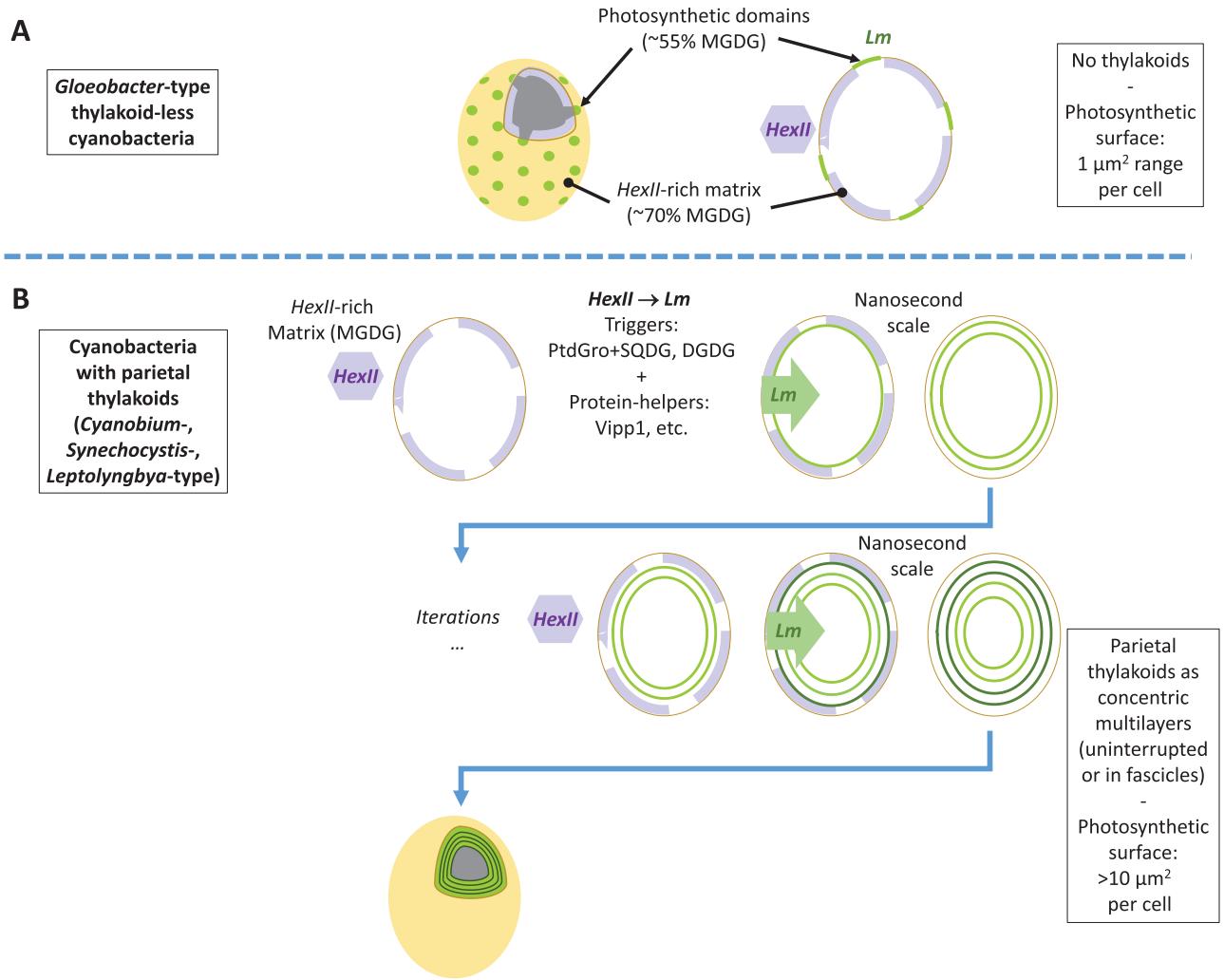

Fig. 4. Origin of thylakoids in early cyanobacteria. (A) *Gloeobacter*-type cyanobacteria have membrane domains where photosystems are concentrated, with a balanced composition of *HexII*-forming and *Lm*-forming lipids, in a MGDG-rich matrix. This matrix could form an internal *HexII* phase, shown in purple, used for *Gloeobacter* function (some enzymes need a *HexII* phase to operate). The level of *Lm*-forming lipids, and most importantly of PtdGro, is not sufficient to trigger any *HexII* → *Lm* phase transition. For a 1 µm diameter cell, the photosynthetic surface is in the range of 1 µm^2. (B) In early cyanobacteria containing SQDG, following the transfer of genes required for its synthesis, *Lm*-forming lipids can trigger serial *HexII* → *Lm* phase transitions at the origin of concentric parietal thylakoids. Parietal thylakoids can be made of uninterrupted layers in *Cyanobium*-type cells (as shown) or as fascicles in *Synechocystis*- or *Leptolyngbya*-type cells. For a 1 µm diameter cell with four concentric membranes, the photosynthetic surface is >10 µm^2. In *Arthrospira*-like cells with 50 radial thylakoids or *Cyanothece*-like cells with 50 parallel thylakoids, the photosynthetic surface is in the range of 50–70 µm^2 for the same volume. The multiplier effect introduced by thylakoids is consistent with a determinant role in the GOE, over or in addition to alternative features, such as the development of multicellular filaments.

How could thylakoids emerge from a *HexII* → *Lm* transition process? An absolute prerequisite is a topological segregation of domains producing lipids in *HexII* phase from those promoting *Lm* bilayers. MGDG-rich domains in the *Gloeobacter* plasma membrane make this condition plausible (Fig. 4A). The enzymes synthesizing MGlcDG and MGDG should be distant enough from those consuming them to produce the *Lm*-forming DGDG. The galactolipid biosynthesis pathway in *Gloeobacter* was shown to be similar to that in all other cyanobacteria. First, MGlcDG is produced by a monoglucosyldiacylglycerol synthase (MgdA) (Awai *et al.*, 2006; Apdila and Awai, 2018); then, the glucosyl polar head is epimerized by an unknown mechanism into a galactosyl residue, thus forming MGDG; eventually, DGDG is formed by the action of a digalactosyldiacylglycerol synthase (DgdA) similar to that identified in other cyanobacteria (Sakurai *et al.*, 2007; Awai *et al.*, 2007; Apdila and Awai, 2018). The activity converting MGlcDG into MGDG in *Gloeobacter* was measured (Sato, 2015) but the corresponding enzyme has yet to be characterized molecularly. It has been tentatively called MgdX (Apdila and Awai, 2018). MGlcDG does not accumulate, and this lipid is barely detected in *Gloeobacter*, in support of a physical proximity between MgdA and MgdX *in vivo*. With a few exceptions, major cyanobacterial clades which have radiated

from a *Gloeobacter*-like ancestor contain another epimerase, called MgdE, shown to convert MGlcDG into MGDG (Awai *et al.*, 2014). Since MGlcDG can accumulate, even in small but detectable amounts in MgdE-containing cyanobacteria (Sato and Awai, 2016; Apdila and Awai, 2018), one can suppose that MgdE is topologically separated from MgdA. The galactolipid pathway thus evolved in the direction of a topological separation of each step synthesizing each of its components, MGlcDG, MGDG, and DGDG. Based on membrane fractionation and enzymatic measurements, MgdA activity was detected in both thylakoid and plasma membranes of *Anacystis* (Omata and Murata, 1986) and *Synechocystis* (Selao *et al.*, 2014), whereas DgdA appears to be restricted to the plasma membrane (Selao *et al.*, 2014). This topological segregation of MGlcDG- and MGDG-synthesizing enzymes from DGDG-synthesizing enzymes may have enabled a transient accumulation of *HexII* lipids (Fig. 4A).

One could consider that the most important *HexII* lipid in this process would be the most abundant one, that is, MGDG, but genetic studies in cyanobacteria do not support this strict requirement. The knockout of *mgdA* was lethal in *Synechocystis* (Apdila and Awai, 2018; Awai *et al.*, 2006), probably because this enzyme is the committing step for the formation of three lipid classes, namely, MGlcDG, MGDG, and DGDG. The knockout of the second enzyme, *mgdE*, led to the hyperaccumulation of MGlcDG in *Synechocystis*, reaching nearly 70% of the total proportion of lipids and replacing all MGDG and DGDG, which could not be produced. The *Synechocystis mgdE* mutant was viable and contained thylakoids (Awai *et al.*, 2006; Apdila and Awai, 2018). This indicated that MGDG was not strictly required for thylakoid biogenesis, but that MGlcDG or MGDG, as *HexII* lipids, may be indistinctly usable. The *Synechocystis mgdE* mutation also indicated that thylakoid bilayers, if generated from MGlcDG instead of MGDG, did not strictly require DGDG as a necessary *Lm*-forming lipid. Taking the evidence together, thylakoids might have emerged from a *Gloeobacter*-type cyanobacterium, in which a specific biosynthetic platform allowed the accumulation of a *HexII* lipid, most likely MGDG, although MGlcDG might have been appropriate as well, reminiscent of the complementation of viable *E. coli* PtdEtn mutants by the external supply of MGlcDG (Wikstrom *et al.*, 2004).

A 'trigger' should then be added to this system, to promote the transition of a *HexII* phase into a *Lm* one. We discussed above that the model lipid PtdEtn could operate such a transition by discrete changes of its chemical structure, in particular the level of desaturation of its acyl chains, or by modulations in temperature or hydration, and that the enthalpy of the *Lm* → *HexII* transition was positive (Gawrisch *et al.*, 1992). Bearing in mind the mechanism of membrane fusions, the modeling of the *Lm* → *HexII* transition showed that, given an initial multilamellar configuration and with appropriate provision of external energy (increasing the temperature), a formation of so-called 'stalks' (elongated structures) between bilayers could be observed on a nanosecond timescale (Fig. 2A). The stalks were not stable and they subsequently elongated in a cooperative manner, thus leading to the formation of an inverted *HexII* phase (Marrink and Mark, 2004). Thylakoids may have simply emerged from the reverse process (Fig. 2B), from a MGlcDG/MGDG *HexII* matrix converted within nano- to microseconds into piled multilamellar membranes. It was reported that a decrease in the number of double bonds per molecule of MGDG was sufficient to promote the conversion from *HexII* structure to *Lm* phase (Gounaris and Barber, 1983). The study of MGDG mixed with other thylakoid lipids has also shown that the presence of various *Lm* lipids added to MGDG triggered a spontaneous *HexII* → *Lm* transition upon increasing hydration (Deme *et al.*, 2014). The trigger could therefore simply be a *Lm* lipid, such as any lipid of this type present in cyanobacterial membranes. One should now identify which of the *Lm*-forming lipid would play this role at the beginning.

Another difference between *Gloeobacter*-type and subsequent cyanobacteria is the absence of a fifth lipid class, the anionic sulfoquinovosyldiacylglycerol (SQDG) (Fig. 3B). The absence of the equipment to synthesize SQDG in *Gloeobacter*, that is, the *sqdB* and *sqdX* genes, was interpreted as an ancestral trait (Sato *et al.*, 2016), although a secondary loss has also been suggested (Sato and Awai, 2016). Here, it may be interesting to remind one of the scenarios reported above for the origin of oxygenic photosynthesis, starting from a protocyanobacterium containing two reaction centers, one being an H_2S-splitting PSI controlled by fluctuating H_2S concentrations (the redox switch hypothesis for the first cyanobacteria) (Allen, 2014, 2016). It may be that, in addition to living in a phosphorus-limiting environment, the protocyanobacteria were exposed to reduced forms of sulfur, such as H_2S, rather than oxidized forms, such as sulfate (H_2SO_4). The acquisition of the sulfolipid synthesis pathway by horizontal gene transfer would then be a key event in the radiation of early cyanobacteria. In thylakoid-containing cyanobacteria, mutants lacking SQDG are either affected, such as in *Synechocystis*, or show no apparent phenotype under optimal growth condition, such as in *Synechococcus* (Apdila and Awai, 2018), leading to the common idea that this lipid could be to some extent dispensable (Sato *et al.*, 2016). It is now established in cyanobacteria, but also in chloroplasts of vascular plants, that the function of SQDG is linked to that of PtdGro, temporarily replacing this anionic phospholipid in the instance of phosphate shortage in the environment (Boudiere *et al.*, 2014; Frentzen, 2004; Shimojima, 2011). The gain is clear: cyanobacteria with SQDG have acquired an anionic surrogate to replace PtdGro, and can overcome more severe depletions of phosphorus in the environment. Thanks to SQDG, anionic membrane lipids are no longer limiting. This gain is an advantage when competing with SQDG-lacking cyanobacteria, and in any case, when competing with bacteria with phospholipid-rich membranes in a phosphorus-deprived context. This also allows the availability of sufficient amounts of an anionic *Lm*-forming lipid for the expensive formation of

an additional membrane system. Together, SQDG and PtdGro, and to some extent DGDG, can be used as lipid triggers, some kind of catalysts, for the nanosecond-scale $HexII_{(MGlcDG,\ MGDG)} \rightarrow Lm_{(MGlcDG,\ MGDG,\ DGDG,\ PdtGro,\ SQDG)}$ transition (Fig. 4B).

Was DGDG necessary at the origin of thylakoids? This could make sense, as SQDG and PtdGro are anionic, and their use, although essential for photosystems, can thus introduce repulsive electrostatic forces between membrane bilayers, which may prevent the formation of tightly stacked membranes. Some arguments contradict this idea, however, most importantly that the *mgdE* mutant of *Synechocystis* can still make thylakoids even though it has lost all of its DGDG (Awai *et al.*, 2006; Apdila and Awai, 2018). One should note that *Synechocystis* thylakoids can organize as stacks in spite of the strong anionic charges harbored by the polar head groups of its *Lm*-forming lipids. Protein components may contribute to this stable architecture in *Synechocystis*, but these proteins may have been missing in the original steps. Indeed, DGDG has been shown to stabilize membrane stacks, including in lipid mixtures mimicking those of photosynthetic membranes (Deme *et al.*, 2014; Kanduc *et al.*, 2017). Thus, we cannot assess with certainty the potential role of DGDG at the origin of thylakoids, but this lipid would have been advantageous for the formation of stacked thylakoid primordia.

The hypothesis defended here relies on the fact that all the lipid actors but one, SQDG, were present in *Gloeobacter*-type early cyanobacteria. SQDG introduces a more flexible and robust membrane lipid metabolism by the tuning of the SQDG/PtdGro balance in response to phosphorus in the environment. *HexII* lipids, mostly MGDG, could be produced concentrically toward the inside of the cell, followed by the transfer of *Lm* lipids, namely, the anionic PtdGro and SQDG, and the neutral DGDG (Fig. 4B). These *Lm* lipids would then act as triggers of a *HexII* → *Lm* phase transition. This provides an explanation for the non-vesicular biogenesis of thylakoids, and for the initial formation of concentric, uninterrupted layers (Mares *et al.*, 2019).

Role of proteins

A *HexII* ↔ *Lm* phase transition is of the 'first order', that is, it involves a latent heat or phase transition enthalpy, releasing or absorbing a fixed and typically large amount of energy, thus differing from a 'second order' or 'continuous phase' transition. The dynamics of the dramatic lipid rearrangements occurring during this transition, although very rapid, has limiting rate steps in topological transitions (Toombes *et al.*, 2002). Protein actors could have played a role at the origin of thylakoid formation by acting in the dynamics of *Lm* lipid transfers to a *HexII* matrix and the 'nucleation' of *HexII* → *Lm* transition.

Gloeobacter contains a homolog of Vipp1, which is found in all other oxygenic photosynthetic organisms (Raven and Sanchez-Baracaldo, 2021). The Vipp1 protein has been functionally associated with thylakoid biogenesis in a multitude of biological models, by various genetic, structural, or biochemical studies (Mechela *et al.*, 2019), but the precise understanding of its role, at the molecular level, remains puzzling, in spite of recent advances in the resolution of its structural organization (Gupta *et al.*, 2021). *Gloeobacter* Vipp1 lacks a ~30 amino acid extension found in thylakoid-containing organisms (Raven and Sanchez-Baracaldo, 2021), and this protein plays a primitive function which is still elusive. In thylakoid-containing *Synechocystis* or in the chloroplast of the green alga *Chlamydomonas reinhardtti*, *vipp1* mutations lead to the formation of aberrant thylakoid convergence zones (Gupta *et al.*, 2021; Nordhues *et al.*, 2012). This has led to the idea that Vipp1 gained a new role in the course of evolution, acting at the level of highly bent regions of thylakoids (which are absent in cyanobacteria containing concentric thylakoids with uninterrupted layers). Of interest here, Vipp1 has a strong affinity for lipids, particularly anionic lipids including but not restricted to PtdGro, *in vitro* and *in vivo* (Li *et al.*, 1994; Kroll *et al.*, 2001; Otters *et al.*, 2013; Heidrich *et al.*, 2016; McDonald *et al.*, 2017). Structural studies suggest a possible role of Vipp1 in guiding some lipids from a donor membrane to thylakoid primordia (Gupta *et al.*, 2021). If Vipp1 played this function for all thylakoid lipids, one should expect to detect innumerable complexes connecting the inner membrane, Vipp1, and thylakoids, but instead these are very rarely observed (Gupta *et al.*, 2021). Little evidence is therefore available to support the suggestion that Vipp1 would act in the transfer of the bulk of thylakoid lipids, and in any case, a role in a massive vesicular-based process is not supported. Vipp1 may have acquired a new function in early cyanobacteria, guiding some anionic lipids toward the inside of the cell, thus 'helping' the nucleation of thylakoid bilayer formation. The Vipp1 ancestral protein in *Gloeobacter* might therefore have evolved in the direction of the guidance of thylakoid biogenesis based on its initial biophysical properties, interacting with other lipids or proteins.

In this review, we have not elaborated any scenario for the evolution of Vipp1 or other proteins (Raven and Sanchez-Baracaldo, 2021). We simply suggest that proteins present in early cyanobacteria, completed with others acquired later on as a result of gene transfers, might have been integrated based on their intrinsic biophysical properties. This is supported by studies showing that the cyanobacterial Vipp1 C-terminal domain associates with lipid bilayers and modulates membrane fusion and fluxes of lipids and proteins between membranes (Hennig *et al.*, 2015, 2017; Saur *et al.*, 2017). Here, we have not addressed the question of the loading of thylakoids with all kinds of other components, proteins, and cofactors, including the photosystems and ATP synthase, which might also require guiding machineries. This could maybe rely on *HexII* glycolipids, which are found in association with reaction centers from anoxygenic photosynthetic bacteria to cyanobacteria, all algae, and plants (Mizoguchi *et al.*, 2013). A photosynthetic reaction center from *Blastochlorus viridis*, a species containing *HexII* glycolipid, was shown to form a so-called 'lipidic sponge

phase' crystal structure (Wohri *et al.*, 2009). It has long been known that in energy-transducing membranes, *HexII* lipids are preferentially associated with the part of the bilayers in which the coupling factors are embedded, that is, MGDG in the outer layer of plant thylakoids, PtdEtn on the cytosolic face of *Rhodobacter sphaeroides* chromatophores, and both PtdEtn and cardiolipin in the inner monolayer of the internal mitochondrial membrane (Rawyler *et al.*, 1987). In this context, a parsimonious hypothesis is that at the origin of thylakoids, photosynthetic components were loaded during the *HexII* → *Lm* phase transition.

The endosymbiotic origin of chloroplasts supports the conserved role of *HexII* lipids in the formation of their thylakoids

A vast array of evidence supports an origin of the chloroplast in Archaeplastida cells (Glaucophyta, Rhodophyta, and Chlorophyta) following a unique primary endosymbiosis event, in which a cyanobacteria-like ancestor related to extant *Gloeomargarita* was engulfed by an unknown eukaryotic host cell (Petroutsos *et al.*, 2014; Sato and Awai, 2017; Sato and Takano, 2017; Marechal, 2018; Ponce-Toledo *et al.*, 2019). This view is, however, simplistic, since numerous genes coding for chloroplast proteins have no ancestor in crown cyanobacteria, but rather in other clades not discussed here (Ball *et al.*, 2013; Cenci *et al.*, 2017; Sato *et al.*, 2020). It is extremely important to note that all cyanobacterial lipids have been conserved, except one, MGlcDG (Boudiere *et al.*, 2014; Petroutsos *et al.*, 2014; Sato and Awai, 2016; Sato and Awai, 2017). The four lipids MGDG, DGDG, PtdGro, and SQDG are thus present in all prokaryotic and eukaryotic thylakoids. Only when photosynthesis was lost secondarily could plastids 'lose' their glycolipids (Botté *et al.*, 2011b, 2013, 2018; Botté and Marechal, 2014).

How could galactolipids be synthesized in chloroplasts, if the committing step of MGlcDG synthesis is not present? Chloroplast MGDG is not synthesized by a cyanobacterial pathway, but in one step by the action of a monogalactosyldiacylglycerol synthase (MGD) acquired secondarily by horizontal gene transfer from a bacterium (Yuzawa *et al.*, 2012; Petroutsos *et al.*, 2014; Sato and Awai, 2017). The production of DGDG is catalyzed by a cyanobacterial-type enzyme in Glaucophyta and one branch of Rhodophyta but, again, by another type of digalactosyldiacylglycerol synthase (DGD), also acquired secondarily (Petroutsos *et al.*, 2014; Sato and Awai, 2017). Some well-studied Archaeplastida models, such as Arabidopsis, show clear topological separation between the MGD enzyme involved in thylakoid biogenesis, called MGD1, in the inner envelope membrane, and the DGD acting at the outer envelope membrane (Awai *et al.*, 2001; Froehlich *et al.*, 2001; Boudiere *et al.*, 2012; Petroutsos *et al.*, 2014), supporting that *HexII* lipids can transiently accumulate. In models where such topological separation is not fully demonstrated, such as *Chlamydomonas* chloroplasts, most representations show MGD in the inner envelope membrane separated from DGD in the outer envelope membrane (Hurlock *et al.*, 2014; Li-Beisson *et al.*, 2015). In *Chlamydomonas,* other strategies were also shown to ensure an accumulation of an MGDG pool, by the rapid desaturation of MGDG fatty acids, generating molecular species that cannot be used by DGD enzymes (Yang *et al.*, 2015). Such 'locking' of specifically unsaturated MGDG species that are more prone to form *HexII* structures (Gounaris *et al.*, 1983) is also observed in Arabidopsis and other photosynthetic eukaryotes (Dolch and Marechal, 2015). Thus, all chloroplasts present in cells of the immense biodiversity of photosynthetic Eukaryota conserved cyanobacterial lipids, without the corresponding cyanobacterial genes, and the new biosynthetic pathway retained initial important features, or even improved on them, such as mechanisms ensuring that *HexII* MGDG can accumulate.

The molecular characterization of plant chloroplast MGD1 is remarkably coherent with the hypothesis elaborated here. In biomimetic membranes, it was shown that Arabidopsis MGD1 had unique biophysical properties. MGD1 is soluble and its membrane binding is promoted by the presence of its product, MGDG (Sarkis *et al.*, 2014), supporting the existence of a positive feedback loop leading to a local overaccumulation of *HexII* lipids. MGD1 binding is inhibited by DGDG, reinforcing the initial segregation of MGDG from DGDG detailed above (Sarkis *et al.*, 2014). The activity of MGD1 requires the presence of anionic lipids, including PtdGro and phosphatidic acid (Makshakova *et al.*, 2020; Dubots *et al.*, 2010, 2012), reminiscent of the activation of the cyanobacterial MgdA enzyme by another anionic lipid, SQDG (Selao *et al.*, 2014). This emphasizes the likely role of anionic lipids as triggers for thylakoid biogenesis. When MGD1 activity is lowered with the application of a specific inhibitor, the proportion of *HexII* MGDG decreases and some membrane invaginations appear at the inner envelope membrane (Botté *et al.*, 2011a), indicating not a vesicular-based formation of thylakoids, but rather an excess of *Lm*-forming lipids. Similar results were obtained in a null *MGD1* mutant of Arabidopsis, which germinated as small albinos only in the presence of sucrose, with seedlings containing traces of galactolipids (Kobayashi *et al.*, 2007). Invaginations of the inner envelope, which were not seen in mature wild-type chloroplasts, were also observed in this mutant, simply due to an excess of *Lm* lipids over *HexII* ones (Kobayashi *et al.*, 2007). Although this phenomenon was initially suggested as evidence for the formation of thylakoids from the budding of the inner envelope membrane, it fits with the non-vesicular model developed here, reflecting an uncontrolled excess of *Lm* lipids in this membrane. Consistently, the growth of the *MGD1* mutant in low-phosphate conditions, activating the expression of the MGDG synthase isoforms MGD2 and MGD3, allowed the synthesis of *HexII* lipids in chloroplasts and the formation of thylakoid structures (Kobayashi *et al.*, 2013). Nevertheless, the level of MGDG remained low under these conditions, and photosynthetic activity was still abolished (Kobayashi *et al.*,

2013), indicating that the production of the right amount of MGDG at the right place, by MGD1 located in the inner envelope membrane, could not be achieved by the action of MGD2 and MGD3 located at the outer envelope membrane.

A very recent study has shown that MGD enzymes could also play roles outside the chloroplast, in the cytosol. It was shown that during the development of the Arabidopsis pollen tube, MGD2 accumulated in the cytosol, following the expression of mRNAs controlled by a specific RNA-binding protein (Billey *et al.*, 2021). This raises questions, since pollen tubes do not contain chloroplasts. Nevertheless, pollen tubes are known to accumulate galactolipids (Botté *et al.*, 2011a; Nakamura *et al.*, 2009). In green tissues, Arabidopsis MGD2 had been localized at the outer envelope membrane of the chloroplast, but some cytosolic localization had also been observed based on the imaging of proteins fused with a fluorescent reporter (Awai *et al.*, 2001). It is therefore plausible that in addition to their role in chloroplast architecture, the soluble eukaryotic MGD enzymes may have introduced a powerful system for integrating lipid metabolism from different compartments. One possibility is that MGD2 might be able to bind its diacylglycerol substrate from one cytosolic membrane and cargo it to another one, including the chloroplast, where MGDG synthesis should occur, or possibly other cell compartments.

Altogether, the chloroplast MGD biosynthesis system performs much better than the primitive MgdA/MgdE system of the *Gloeomargarita*-type cyanobacterium integrated following primary endosymbiosis. This may be one of the reasons for the substitution of the MgdA/MgdE/DgdA pathway by an MGD/DGD pathway early in the radiation of Archaeplastida. A *HexII* → *Lm* phase transition is therefore the most likely scenario for the non-vesicular biogenesis of thylakoids in chloroplasts as well (Bastien *et al.*, 2016). Protein actors, such as Vipp1, have become more and more important in the orchestration of thylakoid biogenetic processes, possibly guiding anionic lipids, including chloroplastic lipids such as PtdGro, and also extraplastidial lipids such as phosphoinositides (Gupta *et al.*, 2021). The increase in size of chloroplasts compared with cyanobacteria, the development of larger photosynthetic surfaces per cell, some structural innovations such as the appearance of pyrenoids or grana, and the bioenergetic coupling of chloroplasts within the eukaryotic metabolism led to a supplementary multiplier effect, which was likely determinant for the 'rise of algae' during the Neoproterozoic period, and the oxygenation of oceans known as the NOE.

Concluding remarks

The origin of internal membrane compartments is one of the most fascinating and difficult questions in the evolution of cells. Textbooks highlight that one of the differences between the three domains of life, the Archaea and Bacteria on the one hand, and the Eukaryota on the other hand, lies in the level of subcellular compartmentalization, which is absent or poor in the former, and prolific, sophisticated, and dynamic in the latter. Such a statement suggests that cell compartmentalization should be accomplished the way it is described in a model Eukaryota cell, that is, either by vesicles budding from one parent membrane merging to form a novel compartment, and/or by various membrane protrusions, fusion mechanisms, and so on. The biophysical rearrangements of membrane lipids during a fusion process were shown to have a positive enthalpy and require an external source of energy, usually provided by ATP or GTP. Such reorganizations of membrane architecture are not spontaneous and involve dedicated protein machineries.

There is no robust evidence for an origin of thylakoids via a vesicular-based scenario, first because of the lack of any ultrastructural observation of vesicles supposed to protrude from the plasma membrane toward the inside of the cell, but also because primitive thylakoids are concentric, and we do not see where thylakoid biogenesis could be initiated. Here, we reviewed studies converging into a simple hypothesis based on the stepwise production of *HexII* lipids at the inner periphery of the cytosolic membrane of an early cyanobacterium, which turned within nanoseconds and without any external source of energy into multilayered pro-thylakoids. Comparison of lipid biosynthetic pathways suggests that most ancient cyanobacteria could not achieve thylakoid biogenesis due to the lack of anionic *Lm* lipids, represented only by a minor phospholipid whose proportion is controlled by the level of phosphorus in the environment. The acquisition of the biosynthetic pathway producing a sulfolipid seemed critical for a regular provision of sufficient amounts of anionic lipids, allowing the *HexII* → *Lm* phase transition to occur, thus forming parietal thylakoids. We designed this model before the very recent description of the spatiotemporal biogenesis process of thylakoid membranes in the rod-shaped cyanobacterium *Synechococcus elongatus* PCC 7942 (Huokko *et al.*, 2021). In this cyanobacterium, the plasma membrane and concentric thylakoid layers have no physical connections, and newly synthesized thylakoid membrane fragments emerge between the plasma membrane and pre-existing thylakoids (Huokko *et al.*, 2021). This is strikingly reminiscent of the model described in Figs 2A and 4, and illustrates the power of the *HexII* → *Lm* phase transition not only at the origin of early thylakoids, but also in biogenetic processes observed today. With this non-vesicular lipid-phase transition, a framework is therefore also available to re-examine the role of companion proteins, such as Vipp1, whose characterization in a hypothetical vesicular-based biogenetic process remains unsatisfying in both cyanobacteria and chloroplasts. It is time to address these questions by considering lipids and proteins together, as well as additional challenging issues, such as the integration of membrane proteins and cofactors in the course of thylakoid biogenesis. Eventually, first-order phase transitions are not restricted to changes in lipid phases. They are also involved in RNA and protein structural changes between liquid phases (Hardenberg *et al.*, 2020; Rhine *et al.*, 2020; Guo *et al.*,

2021), which might provide clues on extremely rapid, nearly instantaneous processes, which cannot yet be comprehended with more classical biochemical, structural, or cell biology approaches. Taking all these phase transitions into consideration might improve our understanding of other steps in cell evolution.

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Author contributions

NG and EM developed the hypothesis discussed in this paper, and contributed to the writing of the manuscript.

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