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

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1 **Title**

2 Origin of Cyanobacteria thylakoids via a non-vesicular glycolipid phase transition and impact  
3 on the Great Oxygenation Event

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31 **Highlight and Abstract**

32 The quest for thylakoid origin resists vesicular-based scenarios. An unexplored hypothesis is  
33 based on Hexagonal II-forming glycolipids, turned within nanoseconds and without any  
34 external energy source into membrane multilayers.

35 **Abstract**

36 Appearance of oxygenic photosynthesis in Cyanobacteria is a major event in the evolution of  
37 Life. It had an irreversible impact on our planet, promoting the Great Oxygenation Event  
38 (GOE), ~2.4 b.y.a. Ancient Cyanobacteria predating the GOE were *Gloeobacter*-type cells,  
39 having no thylakoids. They hosted photosystems in their cytoplasmic membrane. The driver  
40 of the GOE was proposed to be the transition from unicellular to filamentous Cyanobacteria.  
41 However, the appearance of thylakoids expanded the photosynthetic surface by multiple logs:  
42 this multiplier effect would be more coherent with an impact on the atmosphere. Primitive  
43 thylakoids self-organize as concentric parietal uninterrupted multilayers. The quest for their  
44 origin resists vesicular-based scenarios. This review reports studies supporting that *Hexagonal*  
45 *II*-forming gluco- and galactolipids at the periphery of the cytosolic membrane could be  
46 turned within nanoseconds and without any external source of energy into membrane  
47 multilayers. Comparison of lipid biosynthetic pathways further shows that ancient  
48 Cyanobacteria contained only one anionic *Lamellar*-forming lipid, phosphatidylglycerol.  
49 Acquisition of sulfoquinovosyldiacylglycerol biosynthesis correlates with thylakoid  
50 emergence, possibly enabling a sufficient provision of anionic lipids to trigger an *Hexagonal*  
51 *II*-to-*Lamellar* phase transition. With this non-vesicular lipid-phase transition, a framework is  
52 also available to reexamine the role of companion proteins in thylakoid biogenesis processes.

53 **Keywords**

54 Cyanobacteria; thylakoid; *Gloeobacter*; monoglucosyldiacylglycerol;  
55 monogalactosyldiacylglycerol; Hexagonal II lipid; phosphatidylglycerol;  
56 sulfoquinovosyldiacylglycerol; MGD; Great Oxygenation Event

57 **Abbreviations**

58 DGDG, digalactosyldiacylglycerol

59 MGDG, monogalactosyldiacylglycerol

60 MGlcDG, monoglucosyldiacylglycerol

61 SQDG, sulfoquinovosyldiacylglycerol

62 PtdCho, phosphatidylcholine

63 PtdEtn, phosphatidylethanolamine

64 PtdGro, phosphatidylglycerol

65

## 66 Main Text

### 67 1 Introduction

68 The appearance of oxygenic photosynthesis is a major event in the early evolution of Life and  
69 had an irreversible impact on our planet. This bioenergetic and trophic innovation, coupling  
70 H<sub>2</sub>O splitting upon exposure to sun light with the reduction of atmospheric CO<sub>2</sub>, occurred in  
71 the Cyanobacteria ancestral lineage (Sanchez-Baracaldo and Cardona, 2020; Cardona, 2019,  
72 2018; Cardona *et al.*, 2019). It deeply modified ecosystems, positioning Cyanobacteria as  
73 prominent primary producers at the base of trophic networks, and allowed the development of  
74 a tremendous biodiversity of secondary and tertiary consumers. At geological scale, the  
75 production of O<sub>2</sub> as a byproduct of water splitting enabled the oxygenation of the atmosphere  
76 during the Great Oxygenation Event (GOE), 2.4-2.32 billion years ago (b.y.a.) (Bekker *et al.*,  
77 2004; Lyons *et al.*, 2014).

78 Later, oxygenic photosynthesis was acquired by Eukaryota of the Archaeplastida phylum, by  
79 integration of an intracellular organelle called the ‘chloroplast’, deriving from the engulfment  
80 of a close relative of the deep-branching *Gloeomargarita* cyanobacterium, combined with  
81 components acquired by gene transfers from a variety of other sources (Ponce-Toledo *et al.*,  
82 2019; Sato and Awai, 2017; Sato and Takano, 2017; Marechal, 2018). Following this unique  
83 endosymbiotic event, the expansion of marine eukaryotic phytoplankton correlated with the  
84 oxygenation of the oceans during the Neoproterozoic Oxygenation Event (NOE), 800-600  
85 million years ago. This so-called ‘rise of Algae’ is thought to have created food webs with  
86 even more efficient nutrient and energy transfers, driving ecosystems towards larger and more  
87 complex organisms, including animals (Brocks *et al.*, 2017).

88 Access to increasing genomic data and refine reconstructions of molecular phylogenies have  
89 improved our understanding of the early evolution of photosynthetic reaction center proteins  
90 (Sanchez-Baracaldo and Cardona, 2020; Cardona, 2019, 2018; Cardona *et al.*, 2019).  
91 Schematically, oxygenic photosynthesis requires two sets of dimeric reaction centers, one of  
92 type II (in photosystem II, PSII) and one of type I (in PSI), all deriving from a unique origin  
93 in an ancestral anoxygenic photosynthetic prokaryote. Whereas single PSII-like or PSI-like  
94 reaction centers have evolved independently in various clades of anoxygenic phototrophic  
95 Bacteria (Hohmann-Marriott and Blankenship, 2011), crown group Cyanobacteria inherited  
96 both an heterodimeric versions of PSII, where water-splitting occurs, and an heterodimeric  
97 PSI (Sanchez-Baracaldo and Cardona, 2020). Recent estimates of the age of PSII and PSI  
98 gene families coincide with geological evidence of oxygen traces throughout a division of  
99 Precambrian time called the Archean eon (4-2.5 b.y.a.). This implies that oxygenic  
100 photosynthesis was established by 3.0 b.y.a., long before the GOE (Cardona, 2018; Cardona  
101 *et al.*, 2019). It is still unresolved whether a ‘protocyanobacteria’ acquired the two sets by  
102 gene duplication of anoxygenic photosystems (Martin *et al.*, 2018) or *via* gene transfers  
103 (Raymond *et al.*, 2002). Amongst plausible scenarios, a versatile protocyanobacteria  
104 containing two reaction centers, i.e. an H<sub>2</sub>S splitting PSI (photolithotrophy) with an  
105 expression controlled by fluctuating hydrogen sulfide concentrations, and a PSII  
106 (photoorganotrophy), might have developed in shallow water at the vicinity of temporary  
107 hydrothermal springs. This protocyanobacteria having a flexible metabolism adapted to  
108 alternating redox conditions, might have acquired oxygenic photosynthesis by mutations in  
109 the direction of a constitutive expression of both Types I and II reaction centers, and an  
110 extraction of electrons, firstly from manganese and secondarily from water. This hypothesis  
111 was termed the ‘Redox switch hypothesis for the first Cyanobacteria’ (Allen, 2016; Allen,  
112 2014).

113 Since oxygenic photosynthesis predated the GOE by about 0.5 to 1 b.y., what was the actual  
114 driver of this major atmospheric upheaval if not photosynthesis appearance *per se*? The extant  
115 diversity of Cyanobacteria evolved after the GOE (Sanchez-Baracaldo and Cardona, 2020).  
116 Most ancient Cyanobacteria are likely to have populated low salinity habitats and dry  
117 environments (Blank and Sanchez-Baracaldo, 2010) as small unicellular forms close to  
118 present-day *Gloeobacter* (Sanchez-Baracaldo and Cardona, 2020; Mares *et al.*, 2019; Raven  
119 and Sanchez-Baracaldo, 2021). The GOE was proposed to be the consequence of the ability  
120 of Cyanobacteria to form multicellular filaments, resembling extant *Pseudanabaena*,  
121 facilitating the formation of microbial mats, a feature supposed to increase their ecological  
122 dominance to such an extent that atmospheric O<sub>2</sub> underwent this dramatic increase (Sanchez-  
123 Baracaldo and Cardona, 2020).

124 An alternative hypothesis based on studies reviewed here is that such an important  
125 atmospheric change as the GOE requires an extraordinary multiplier effect that  
126 multicellularity, introducing no additive effect, is not sufficient to explain. The transition from  
127 *Gloeobacter*-type to other Cyanobacteria, is marked by the emergence of internal membranes  
128 sacks, known as ‘thylakoids’, allowing a significant gain in the area of the ‘solar panels’  
129 accommodating photosystems (Mares *et al.*, 2019). This review highlights the role of lipid  
130 components in Cyanobacteria membranes, disregarded until now in the determinant processes  
131 at the origin of thylakoids.

## 132 **2 Emergence of thylakoids in early Cyanobacteria had a multiplier effect on the** 133 **photosynthetic surface per cell.**

134 Thylakoids are evidently not required for oxygenic photosynthesis since *Gloeobacter* hosts its  
135 photosystems in its cytoplasmic (or inner) membrane (Raven and Sanchez-Baracaldo, 2021).  
136 The evolution of thylakoids starts with the appearance of continuous linear sacks with several  
137 concentric, uninterrupted layers (Mares *et al.*, 2019). Based on a spherical cell with a 1 μm  
138 diameter (volume = 0.53 μm<sup>3</sup>), a *Gloeobacter* membrane surface would be ~3.14 μm<sup>2</sup>. Taking  
139 into account that in *Gloeobacter*, photosynthetic proteins are concentrated in domains  
140 representing less than a third of the cytosolic membrane (Rexroth *et al.*, 2011), the actual  
141 photosynthetic surface is in the 1 μm<sup>2</sup> range. By contrast, in a *Cyanobium*-like coccid cell of  
142 same size (Mares *et al.*, 2019), with four concentric thylakoids lining the plasma membrane  
143 (so-called parietal thylakoids), the photosynthetic surface would reach ~20 μm<sup>2</sup>. Parietal  
144 thylakoid fascicules encountered in *Synechocystis*-like coccid or *Leptolyngbya*-like  
145 filamentous cyanobacteria (Mares *et al.*, 2019) with four layers would reach about the same  
146 photosynthetic surface per cell. Cyanobacteria containing parallel or radial arrangement of  
147 thylakoids (Mares *et al.*, 2019) can increase the photosynthetic surface even further. An  
148 *Arthrospira*-like cell with 50 radial thylakoids or a *Cyanothece*-like cell with 50 parallel  
149 thylakoids can reach a photosynthetic surface in the range of 50-70 μm<sup>2</sup>, based on the  
150 summed area of flattened cisternae, within the same volume. In the course of evolution,  
151 following chloroplast integration in Archaeplastida, thylakoids evolved in the direction of an  
152 increased surface and organization in functional domains with the development of stacks and  
153 grana, which do not form in Cyanobacteria. In the cells of eukaryotic microalgae, the volume  
154 of the plastid is commonly higher than 10 μm<sup>3</sup> (Uwizeye *et al.*, 2021) whereas in vascular  
155 plants, such as maize, the volume of mesophyll cell chloroplasts can reach volumes higher  
156 than 100 μm<sup>3</sup> (Kutik *et al.*, 2004). Based on a 10-100 μm<sup>3</sup> chloroplast containing 50  
157 thylakoids, a gross calculation gives a photosynthetic surface of 10<sup>-3</sup>-10<sup>-2</sup> mm<sup>2</sup> per  
158 chloroplast. Overall, a population of 1 million *Gloeobacter*-type cells harbors a global  
159 photosynthetic surface of about 1 mm<sup>2</sup>, whereas 1 million of *Arthrospira*- or *Cyanothece*-like

160 cells can easily exceed 50-70 mm<sup>2</sup>. Because of this obvious multiplier effect, the appearance  
161 of thylakoids seems therefore the most plausible determinant in the GOE.

162 One would naturally examine extant protein machinery(ies) involved in thylakoid biogenesis  
163 to trace back its origin and look for evidence supporting this hypothesis. The difficulty lies in  
164 the still unresolved process(es) governing the biogenesis of this membrane system, most  
165 notably because this question is mainly posed considering protein components only. We  
166 advocate here that spontaneous organization of lipids, following an appropriate biosynthetic  
167 sequence, would be sufficient to produce very large amounts of membrane surfaces in nano-  
168 to microseconds. This process does not require vesicles budding from the Cyanobacteria  
169 plasma membrane or the chloroplast inner envelope membrane. Actually, the traditional  
170 hypothesis for a ‘vesicular-based’ biogenesis of thylakoids finds little support in  
171 ultrastructural analyses of Cyanobacteria and chloroplasts, where the observation of inner  
172 membrane invaginations is extremely rare (Mares *et al.*, 2019; Botte *et al.*, 2011a; Boudiere *et*  
173 *al.*, 2012; Bastien *et al.*, 2016; Gupta *et al.*, 2021). Some reports highlight the presence of  
174 vesicles in eukaryotic proplastids prior their conversion into chloroplasts (Mechela *et al.*,  
175 2019), but the lack of such vesicular compartment in Cyanobacteria disqualifies this  
176 mechanism at the origin of thylakoids in ancient clades. Likewise, the focus on *Synechocystis*  
177 ‘thylakoid convergence zones’ where thylakoids merge into high-curvature membranes in  
178 close contact with the plasma membrane, also observed in some chloroplasts (Gupta *et al.*,  
179 2021), do not account for the biogenesis of more primitive parietal thylakoids organized in  
180 concentric and uninterrupted layers (Mares *et al.*, 2019). Here, we do not disregard the fact  
181 that protein components evolved in the direction of helping, organizing, guiding, orchestrating  
182 the process of thylakoid biogenesis, leading to the very sophisticated and efficient processes  
183 observed today, but we assume that no evidence support their determinant role in the early  
184 stages.

### 185 **3 Thylakoids emerged from a mixture of lipids allowing a non-vesicular biogenesis of** 186 **membranes**

187 Membrane bilayers are made of polar lipids, harboring a ‘polar head group’ exposed to the  
188 surface, and hydrophobic ‘tails’ inside its core. How can a lipid bilayer form without any  
189 source of vesicles, protruding or budding from a parent membrane, merging to produce large  
190 surfaces? An answer developed here comes from the biophysical properties of polar lipids.  
191 Depending on the equilibrium between polar head interactions and repulsive forces between  
192 hydrophobic tails, each of the polar lipid can self-organize and form so called ‘phases’, either  
193 with a positive curvature (e.g. micellar tubules, also known as ‘hexagonal I’, or *HexI*), or no  
194 curvature (‘lamellar’, or *Lm*) or with a negative curvature (‘inverted hexagonal’, or *HexII*)  
195 (Jouhet, 2013). These properties have attracted the attention for long, with a focus on lipids  
196 promoting an *Lm* → *HexII* transition, since the disturbance introduced by inverted hexagonal  
197 phase was considered essential for the promotion of highly bent and fused domains (Jouhet,  
198 2013; Gawrisch *et al.*, 1992; Toombes *et al.*, 2002; Kuzmin *et al.*, 2001). Little  
199 thermodynamic studies are available to evaluate all possible phase transitions in the different  
200 lipid mixtures found in nature. Our knowledge relies on model lipids such as  
201 phosphatidylethanolamine (PtdEtn), which organization in *Lm* or *HexII* phases depends on its  
202 chemical structure, *i.e.* the length and desaturation level of acyl chains; physical properties,  
203 such as hydration or temperature; and the presence of other lipids (Gawrisch *et al.*, 1992;  
204 Toombes *et al.*, 2002; Mannock *et al.*, 2001; Harper *et al.*, 2001).

205 It is essential to keep in mind that so-called “*HexII* lipids” are also found in membrane  
206 bilayers, either because in their pure state they can form both *HexII* or *Lm* phases depending

207 on temperature, hydration, etc., or because when mixed with *Lm* lipids, the lamellar phase is  
208 the more stable organization. In past studies, *Lm* → *HexII* was the direction examined in the  
209 search for plausible membrane fusion mechanisms. The enthalpy of dioleoyl-PtdEtn phase  
210 transition ( $\Delta H$ ) measured by differential scanning calorimetry had a positive value (+0.3  
211 kcal.mol<sup>-1</sup> = 1.255 kJ.mol<sup>-1</sup>), meaning that energy external to the system had to be provided  
212 (Gawrisch *et al.*, 1992). It was further showed that neither the *Lm* → *HexII* nor the *HexII* →  
213 *Lm* kinetics had any significant deviation from a power-law relationship: a relative change in  
214 the *Lm* phase results in a proportional relative change in *HexII* and *vice versa* (Toombes *et al.*,  
215 2002; Garab, 2016). The *Lm* ↔ *HexII* transition is therefore remarkable for the symmetry  
216 between forward and reverse dynamics (Toombes *et al.*, 2002). A corollary is that the *HexII*  
217 → *Lm* transition for this pure lipid species may release some energy, and therefore occur  
218 spontaneously. Same spontaneous *HexII* → *Lm* transition of dioleoyl-PtdEtn could occur by  
219 changing the hydration level (Gawrisch *et al.*, 1992). The *HexII* → *Lm* phase transition  
220 provides a solution to the unresolved question of the emergence of early membrane systems,  
221 especially with such extended surface as thylakoids.

222 Let us first consider the lipid composition of Cyanobacteria in a simplified evolutionary  
223 context. A variety of chemical structures can produce impermeable membranes delineating  
224 cells (Jia *et al.*, 2019). We have evidence of two distinct options for the structure of polar  
225 lipids in primordial membranes, based on their conservation in the three domains of life.  
226 These primordial lipids are all built on a three-carbon glycerol scaffold (numbered *sn*-1, *sn*-2,  
227 *sn*-3). On the one hand, Archaea are bounded by membranes made of isoprenoid hydrocarbon  
228 chains linked *via* ether bonds to an *sn*-2,3-glycerol phosphate backbone, and multiple  
229 derivatives with two polar heads, which can form lipid monolayers (Salvador-Castell *et al.*,  
230 2021; Imachi *et al.*, 2020). On the other hand, Bacteria and Eukaryota are primarily bounded  
231 by membranes made of acyl-glycerolipids, deriving from a precursor containing fatty acids  
232 linked *via* ester bonds to an *sn*-1,2- glycerol phosphate backbone (Imachi *et al.*, 2020). It is  
233 established that in Archaea, the presence of phytanyl chains, ether bonds and bipolar lipids  
234 confer a high stability to their membranes (Guler *et al.*, 2009; Garcia-Arribas *et al.*, 2015;  
235 Balleza *et al.*, 2014). By contrast, in Bacteria and Eukarya, acyl-glycerolipids may confer a  
236 higher flexibility, especially *via* the controlled addition of double bonds to the acyl chains,  
237 tuning the lateral fluidity of membranes, but also with the balance between *HexII* and *Lm*  
238 lipids (Garab, 2016; de Kruijff, 1997). Increasing evidence support that Eukaryogenesis  
239 originated from the merger between an archaeal host and a bacterial endosymbiont related to  
240 modern  $\alpha$ -proteobacteria (Imachi *et al.*, 2020). This raises the puzzling question of the loss of  
241 Archaea-type lipids in Eukarya. An hypothesis is that the integrated  $\alpha$ -proteobacteria gave  
242 rise to a proto-mitochondrion, producing ATP by specific protein machineries preferring  
243 Bacteria-type membranes, which were subsequently conserved (Sojo, 2019). In these initial  
244 membrane matrices, other lipidic structures and hydrophobic polypeptides were integrated,  
245 contributing to the multitude of functions harbored by cell membranes.

246 Acyl-glycerolipids with a polar head containing a phosphate group, such as the *HexII* lipid  
247 PtdEtn, or phosphatidylcholine (PtdCho), an *Lm* lipid, are conserved in the majority of  
248 Bacteria and Eukarya clades, with the notable exception of crown Cyanobacteria who only  
249 contain one phospholipid, phosphatidylglycerol (PtdGro) (Petroustos *et al.*, 2014; Yuzawa *et al.*,  
250 2012; Sato and Awai, 2016). PtdGro is anionic and its proportion is regulated in response  
251 to phosphate availability (Boudiere *et al.*, 2014; Frentzen, 2004). The conservation of PtdGro  
252 is consistent with its role in the assembly and functioning of photosynthesis components  
253 (Boudiere *et al.*, 2014), supported by the lethality of PtdGro-less mutants of Cyanobacteria  
254 (Kopečna *et al.*, 2015; Hagio *et al.*, 2000; Sakurai *et al.*, 2003a, b; Kobori *et al.*, 2018) and

255 plants (Babiychuk *et al.*, 2003; Xu *et al.*, 2002; Xu *et al.*, 2006). Such membrane composition  
256 parsimonious in phospholipids supports the idea that early Cyanobacteria populated an  
257 environment where phosphorous was limiting, at least to meet their basal metabolic demand.

258 The lipid composition of *Gloeobacter* membranes is simple, containing three lipid classes in  
259 addition to PtdGro: monoglucosyldiacylglycerol (MGlcDG), monogalactosyldiacylglycerol  
260 (MGDG) and digalactosyldiacylglycerol (DGDG) (Rexroth *et al.*, 2011; Yuzawa *et al.*, 2012;  
261 Sato and Awai, 2016; Sato, 2015). MGlcDG and MGDG are *HexII* lipids, whereas DGDG  
262 and PtdGro are *Lm* lipids (Jouhet, 2013). It is interesting to note that the addition of *HexII*-  
263 forming MGlcDG could rescue a bacterial *Escherichia coli* mutant lacking *HexII*-forming  
264 PtdEtn (Wikstrom *et al.*, 2004), indicating that an *HexII* lipid could replace another *HexII*  
265 lipid, thus filling a vital role governed by its biophysical properties. The plasma membrane of  
266 *Gloeobacter* has only traces of MGlcDG (Sato and Awai, 2016; Sato, 2015). It was shown to  
267 contain segregated bioenergetic domains, some with low-chlorophyll content being MGDG-  
268 rich (~70% MGDG, ~22% DGDG and ~8% PtdGro), whereas high-chlorophyll domains were  
269 more balanced in their lipid composition (~55% MGDG, ~35% DGDG, ~10% PtdGro)  
270 (Rexroth *et al.*, 2011). These latter domains also contain photosynthetic components, *i.e.* PSII,  
271 PSI and ATP-synthetase. It is very important to note that the relative MGDG/DGDG ratio  
272 differs in the two membrane domains: this indicates that although DGDG is produced from  
273 MGDG, some regulatory mechanisms uncouple its final proportion from the available amount  
274 of substrate. This is consistent with the multitude of roles played by DGDG which need to be  
275 finely controlled, including the stability of the photosynthetic machinery (Boudiere *et al.*,  
276 2014; Apdila and Awai, 2018; Sakurai *et al.*, 2007; Awai *et al.*, 2007). The MGDG-rich  
277 domains at the plasma membrane appear therefore as ideal platforms to promote *HexII* → *Lm*  
278 phase transition. Nevertheless, the  $HexII_{(MGlcDG, MGDG)} / Lm_{(DGDG, PtdGro)}$  balance is still  
279 insufficient in *Gloeobacter* to promote the formation of thylakoids. In addition, because  
280 PtdGro is essential for the stability of photosystems (Kopečna *et al.*, 2015; Haggio *et al.*, 2000;  
281 Sakurai *et al.*, 2003a, b; Koberi *et al.*, 2018; Babiychuk *et al.*, 2003; Xu *et al.*, 2002; Xu *et al.*,  
282 2006), a minimal quantity of this anionic lipid should be included in thylakoids, and the  
283 production of exceeding amounts of PtdGro in the bulk of membrane lipids appears difficult  
284 to maintain in a phosphate-limiting environment.

285 How could thylakoids emerge from an *HexII* → *Lm* transition process? An absolute  
286 prerequisite is a topological segregation of domains producing lipids in *HexII* phase from  
287 those promoting *Lm* bilayers. MGDG-rich domains in *Gloeobacter* plasma membrane makes  
288 this condition plausible. The enzymes synthesizing MGlcDG and MGDG should be distant  
289 enough from those consuming them to produce the *Lm* forming DGDG. The galactolipid  
290 biosynthesis pathway in *Gloeobacter* was shown to be similar to that in all other  
291 Cyanobacteria. Firstly MGlcDG is produced by a monoglucosyldiacylglycerol synthase  
292 (MgdA) (Apdila and Awai, 2018; Awai *et al.*, 2006), then the glucosyl polar head is  
293 epimerized by an unknown mechanism into a galactosyl residue, thus forming MGDG;  
294 eventually DGDG is formed by the action of a digalactosyldiacylglycerol synthase (DgdA)  
295 similar to that identified in other Cyanobacteria (Apdila and Awai, 2018; Sakurai *et al.*, 2007;  
296 Awai *et al.*, 2007). The activity converting MGlcDG into MGDG in *Gloeobacter* was  
297 measured (Sato, 2015), but the corresponding enzyme is still uncharacterized molecularly. It  
298 was tentatively called MgdX (Apdila and Awai, 2018). MGlcDG does not accumulate and  
299 this lipid is barely detected in *Gloeobacter*, in support of a physical proximity between MgdA  
300 and MgdX *in vivo*. With a few exceptions, major Cyanobacteria clades who have radiated  
301 from a *Gloeobacter*-like ancestor contain another epimerase, called MgdE shown to convert  
302 MGlcDG into MGDG (Awai *et al.*, 2014). Since MGlcDG can accumulate, even in small but



303 detectable amounts in MgdE-containing Cyanobacteria (Sato and Awai, 2016; Apdila and  
304 Awai, 2018), one can suppose that MgdE is topologically separated from MgdA. The  
305 galactolipid pathway thus evolved in the direction of a topological separation of each step  
306 synthesizing each of its components, MGlCDG, MGDG and DGDG. Based on membrane  
307 fractionation and enzymatic measurements, MgdA activity was detected in both thylakoid and  
308 plasma membranes of *Anacystis* (Omata and Murata, 1986) and *Synechocystis* (Selao *et al.*,  
309 2014), whereas DgdA appears restricted to the plasma membrane (Selao *et al.*, 2014). This  
310 topological segregation of MGlCDG and MGDG synthesizing enzymes from DGDG  
311 synthesizing ones may have enabled a transient accumulation of *HexII* lipids.

312 One could consider that the most important *HexII* lipid in this process would be the most  
313 abundant one, *i.e.* MGDG, but Cyanobacteria genetic studies do not support this strict  
314 requirement. The knock-out of *mgdA* was lethal in *Synechocystis* (Apdila and Awai, 2018;  
315 Awai *et al.*, 2006), probably because this enzyme is the committing step for the formation of  
316 three lipid classes, *i.e.* MGlCDG, MGDG and DGDG. The knock-out of the second enzyme,  
317 *mgdE*, led to the hyper-accumulation of MGlCDG in *Synechocystis*, reaching nearly 70% of  
318 the total proportion of lipids, replacing all MGDG and DGDG which could not be produced.  
319 The *Synechocystis mgdE* mutant was viable and contained thylakoids (Awai *et al.*,  
320 2006; Apdila and Awai, 2018). This indicated that MGDG was not strictly required for  
321 thylakoid biogenesis, but that MGlCDG or MGDG, as *HexII* lipids, may be indistinctly usable.  
322 *Synechocystis mgdE* mutation also indicated that thylakoid bilayers, if generated from  
323 MGlCDG instead of MGDG, did not strictly require DGDG as a necessary *Lm* forming lipid.  
324 Altogether, thylakoids might have emerged from a *Gloeobacter*-type Cyanobacteria, in which  
325 a specific biosynthetic platform allowed the accumulation of an *HexII* lipid, most likely  
326 MGDG, although MGlCDG might have been appropriate as well, reminiscent of the  
327 complementation of viable *E. coli* PtdEtn mutants by external supply of MGlCDG (Wikstrom  
328 *et al.*, 2004).

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

348 Another difference between *Gloeobacter*-type and subsequent Cyanobacteria is the absence of  
349 a fifth lipid class, *i.e.* the anionic sulfoquinovosyldiacylglycerol (SQDG). The absence of the  
350 equipment to synthesize SQDG in *Gloeobacter*, *i.e.* the *sqdB* and *sqdX* genes, was interpreted

351 as an ancestral trait (Sato *et al.*, 2016), although a secondary loss was also suggested (Sato  
352 and Awai, 2016). Here, it may be interesting to remind one of the scenarios reported above for  
353 the origin of oxygenic photosynthesis, starting from a protocyanobacteria containing two  
354 reaction centers, one being an H<sub>2</sub>S splitting PSI controlled by fluctuating hydrogen sulfide  
355 concentrations (redox switch hypothesis for the first Cyanobacteria) (Allen, 2016; Allen,  
356 2014). It may be that, in addition to living in a phosphorus-limiting environment, the proto-  
357 cyanobacteria were exposed to reduced forms of sulfur such as hydrogen sulfide (H<sub>2</sub>S), rather  
358 than oxidized ones such as sulfate (H<sub>2</sub>SO<sub>4</sub>). The acquisition of the sulfolipid synthesis  
359 pathway by horizontal gene transfer would then be a key event in the radiation of early  
360 Cyanobacteria. In thylakoid-containing Cyanobacteria, mutants lacking SQDG are either  
361 affected, such as in *Synechocystis*, or with no apparent phenotype under optimal growth  
362 condition, such as in *Synechococcus* (Apdila and Awai, 2018), leading to the common idea  
363 that this lipid could be to some extent dispensable (Sato *et al.*, 2016). It is now established in  
364 Cyanobacteria, but also in chloroplasts of vascular plants that SQDG function is linked to that  
365 of PtdGro, replacing temporarily this anionic phospholipid in case of phosphate shortage in  
366 the environment (Boudiere *et al.*, 2014; Frentzen, 2004; Shimojima, 2011). The gain is clear:  
367 Cyanobacteria with SQDG have acquired an anionic surrogate to replace PtdGro, and can  
368 overcome stronger depletions of phosphorus in the environment. Thanks to SQDG, anionic  
369 membrane lipids are no more limiting. This gain is an advantage when competing with  
370 SQDG-missing Cyanobacteria, and in any case, when competing with Bacteria with  
371 phospholipid-rich membranes in a phosphorus-deprived context. This also allows the  
372 availability of sufficient amounts of an anionic *Lm*-forming lipid for the expensive formation  
373 of an additional membrane system. Together, SQDG and PtdGro, and to some extent DGDG,  
374 can be used as lipid triggers, some kind of catalysts, for the nanosecond-scale *HexII*<sub>(MGlCDG,</sub>  
375 <sub>MGDG)</sub> → *Lm*<sub>(MGlCDG, MGDG, DGDG, PtdGro, SQDG)</sub> transition.

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

389 The hypothesis defended here relies on the fact that all the lipid actors but one, SQDG, were  
390 present in *Gloeobacter*-type early Cyanobacteria. SQDG introduces a more flexible and  
391 robust membrane lipid metabolism by the tuning of the SQDG/PtdGro balance in response to  
392 phosphorus in the environment. *HexII* lipids, mostly MGDG, could be produced  
393 concentrically toward the inside of the cell, followed by the transfer of *Lm* lipids, *i.e.* anionic  
394 PtdGro and SQDG and neutral DGDG. These *Lm* lipids would then act as triggers of an *HexII*  
395 → *Lm* phase transition. This gives an explanation to the non-vesicular biogenesis of  
396 thylakoids, and to the initial formation of concentric, uninterrupted layers (Mares *et al.*,  
397 2019).

#### 398 4 Role of proteins

399 An *HexII* ↔ *Lm* phase transition is of ‘first-order’, *i.e.* involving a latent heat or phase  
400 transition enthalpy, releasing or absorbing a fixed and typically large amount of energy, thus  
401 differing from a ‘second order’, or ‘continuous phase transition’. The dynamics of the  
402 dramatic lipid rearrangements occurring during this transition, although very rapid, has  
403 limiting rate steps in topological transitions (Toombes *et al.*, 2002). Protein actors could have  
404 played a role at the origin of thylakoid formation, by acting in the dynamics of *Lm* lipids’  
405 transfers to an *HexII* matrix and the ‘nucleation’ of *HexII* → *Lm* transition.

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

432 In this paper, we did not elaborate any scenario for the evolution of Vipp1 or others proteins  
433 (Raven and Sanchez-Baracaldo, 2021). We simply suggest that proteins present in early  
434 Cyanobacteria, completed with others acquired later on, following gene transfers, might have  
435 been integrated based on their intrinsic biophysical properties. This is supported by studies  
436 showing that Cyanobacteria Vipp1 C-terminal domain associates with lipid bilayers and  
437 modulates membrane fusion and fluxes of lipids and proteins between membranes (Saur *et al.*,  
438 2017; Hennig *et al.*, 2017; Hennig *et al.*, 2015). Here, we did not address the question of the  
439 loading of thylakoids with all kinds of other components, proteins and cofactors, including the  
440 photosystems and ATP-synthetase, also requiring guiding machineries. This could maybe rely  
441 on *HexII* glycolipids, which are found in association with reaction centers from anoxygenic  
442 photosynthetic Bacteria to Cyanobacteria, all algae and plants (Mizoguchi *et al.*, 2013). A  
443 photosynthetic reaction center from *Blastochloris viridis*, a species containing *HexII*  
444 glycolipid, was thus shown to form a so-called ‘lipidic sponge phase’ crystal structure (Wohri  
445 *et al.*, 2009). It is known for long that in energy-transducing membranes, *HexII* lipids are  
446 preferentially associated with that part of the bilayers in which the coupling factors are

447 embedded, *i.e.* MGDG in the outer layer of plant thylakoids, PtdEtn on the cytosolic face of  
448 *Rhodobacter sphaeroides* chromatophores, and both PtdEtn and cardiolipin in the inner  
449 monolayer of internal mitochondrial membrane (Rawlyer *et al.*, 1987). In this context, a  
450 parsimonious hypothesis is that at the origin, photosynthetic components were loaded during  
451 the *HexII* → *Lm* phase transition.

## 452 **5 The endosymbiotic origin of chloroplasts supports the conserved role of *HexII*** 453 **lipids in the formation of their thylakoids**

454 A vast array of evidence support an origin of the chloroplast in Archaeplastida cells  
455 (Glaucomphyta, Rhodophyta and Chlorophyta) following a unique primary endosymbiosis  
456 event, by engulfment of a Cyanobacteria-like ancestor related to extant *Gloeomargarita* inside  
457 an unknown eukaryotic host cell (Ponce-Toledo *et al.*, 2019; Sato and Awai, 2017; Sato and  
458 Takano, 2017; Marechal, 2018; Petroutsos *et al.*, 2014). This view is however simplistic,  
459 since numerous genes coding for chloroplast proteins find no ancestor in crown  
460 Cyanobacteria, but rather in other clades not discussed here (Ball *et al.*, 2013; Cenci *et al.*,  
461 2017; Sato *et al.*, 2020). It is extremely important to note that all Cyanobacteria lipids have  
462 been conserved, except one, MGLcDG (Sato and Awai, 2017; Petroutsos *et al.*, 2014; Sato and  
463 Awai, 2016; Boudiere *et al.*, 2014). The four lipids MGDG, DGDG, PtdGro and SQDG are  
464 thus present in all prokaryotic and eukaryotic thylakoids. Only when photosynthesis was lost  
465 secondarily, could plastids ‘lose’ their glycolipids (Botte and Marechal, 2014; Botte *et al.*,  
466 2018; Botte *et al.*, 2011b; Botte *et al.*, 2013).

467 How could galactolipids be synthesized in chloroplasts, if the committing step of MGLcDG  
468 synthesis is not present? Chloroplast MGDG is not synthesized by a Cyanobacteria pathway,  
469 but in one-step by the action of a monogalactosyldiacylglycerol synthase, or *MGD* acquired  
470 secondarily by horizontal gene transfer from a bacterium (Sato and Awai, 2017; Petroutsos *et al.*  
471 *et al.*, 2014; Yuzawa *et al.*, 2012). The production of DGDG is catalyzed by a Cyanobacteria-  
472 type enzyme in Glaucomphyta and one branch of Rhodophyta, but, again, by another type of  
473 digalactosyldiacylglycerol synthase, called *DGD*, also acquired secondarily (Sato and Awai,  
474 2017; Petroutsos *et al.*, 2014). Some well-studied Archaeplastida models, like *Arabidopsis*  
475 show clear topological separation between the *MGD* enzyme involved in thylakoid  
476 biogenesis, called *MGD1* in the inner envelope membrane, and the *DGD* acting at the outer  
477 envelope membrane (Froelich *et al.*, 2001; Boudiere *et al.*, 2012; Petroutsos *et al.*, 2014;  
478 Awai *et al.*, 2001), supporting that *HexII* lipids can transiently accumulate. In models where  
479 such topological separation is not fully demonstrated, like *Chlamydomonas* chloroplasts, most  
480 representations show *MGD* in the inner envelope separated from *DGD* in the outer envelope  
481 membrane (Hurlock *et al.*, 2014; Li-Beisson *et al.*, 2015). In *Chlamydomonas*, other strategies  
482 were also shown to ensure an accumulation of a MGDG pool, by the rapid desaturation of  
483 MGDG fatty acids, generating molecular species, which cannot be used by *DGD* enzymes  
484 (Yang *et al.*, 2015). Such ‘locking’ of specifically unsaturated MGDG species, more prone to  
485 form *HexII* structures (Gounaris *et al.*, 1983) is also observed in *Arabidopsis* and other  
486 photosynthetic eukaryotes (Dolch and Marechal, 2015). Thus, all chloroplasts present in cells  
487 of the immense biodiversity of photosynthetic Eukaryota conserved Cyanobacteria lipids,  
488 without the corresponding Cyanobacteria genes, and the new biosynthesis pathway retained  
489 initial important features, even improved, like mechanisms ensuring that *HexII* MGDG can  
490 accumulate.

491 The molecular characterization of plant chloroplast *MGD1* is remarkably coherent with the  
492 hypothesis elaborated here. In biomimetic membranes, it was shown that *Arabidopsis* *MGD1*  
493 had unique biophysical properties. *MGD1* is soluble and its membrane binding is promoted

494 by the presence of its product, MGDG (Sarkis *et al.*, 2014) supporting the existence of a  
495 positive-feedback loop leading to a local over-accumulation of *HexII* lipids. MGD1 binding is  
496 inhibited by DGDG, reinforcing this initial segregation of MGDG from DGDG detailed above  
497 (Sarkis *et al.*, 2014). The activity of MGD1 requires the presence of anionic lipids, including  
498 PtdGro and phosphatidic acid (Makshakova *et al.*, 2020; Dubots *et al.*, 2010; Dubots *et al.*,  
499 2012), reminiscent of the activation Cyanobacteria MgdA enzyme by another anionic lipid,  
500 SQDG (Selao *et al.*, 2014). This stresses the likely role of anionic lipids as triggers for  
501 thylakoid biogenesis. When lowering MGD1 activity with a specific inhibitor, the proportion  
502 of *HexII* MGDG decreases and some membrane invagination appear at the inner envelope  
503 membrane (Botte *et al.*, 2011a), not indicating a vesicular-based formation of thylakoids, but  
504 rather an excess of *Lm* forming lipids. Similar results were obtained in a null *MGD1* mutant of  
505 *Arabidopsis*, germinating as small albinos only in the presence of sucrose, with seedlings  
506 containing traces of galactolipids (Kobayashi *et al.*, 2007). Invaginations of the inner  
507 envelope, which were not seen in mature WT chloroplasts, were also observed in this mutant,  
508 simply due to an excess of *Lm* lipids over *HexII* ones (Kobayashi *et al.*, 2007). Although this  
509 phenomenon was initially suggested as an evidence for formation of thylakoids from the  
510 budding of inner envelope membrane, it fits with the non-vesicular model developed here,  
511 reflecting an uncontrolled excess of *Lm* lipids in this membrane. Consistently, the growth of  
512 the *MGD1* mutant in low phosphate conditions, activating the expression of the MGDG  
513 synthase isoforms *MGD2* and *MGD3*, allowed a synthesis of *HexII* lipids in chloroplasts and  
514 the formation of thylakoid structures (Kobayashi *et al.*, 2013). Nevertheless, the level of  
515 MGDG remained low under these conditions, and photosynthetic activity was still abolished,  
516 (Kobayashi *et al.*, 2013), indicating that the production of the right amount of MGDG at the  
517 right place, by MGD1 located in the inner envelope membrane, could not be reached by the  
518 action of MGD2 and MGD3 located at the outer envelope membrane.

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

533 Altogether, the chloroplast MGD biosynthesis system performs much better than the primitive  
534 MgdA/MgdE system of the *Gloeomargarita*-type cyanobacterium integrated following  
535 primary endosymbiosis. It may be one of the reasons for the substitution of the  
536 MgdA/MgdE/DgdA pathway by a MGD/DGD pathway early in the radiation of  
537 Archaeplastida. An *HexII* → *Lm* phase transition is therefore the most likely scenario for the  
538 non-vesicular biogenesis of thylakoids in chloroplasts as well (Bastien *et al.*, 2016). Protein  
539 actors, such as VIPP1, have become more and more important in the orchestration of  
540 thylakoid biogenetic processes, possibly guiding anionic lipids, including chloroplastic lipids  
541 such as PtdGro, but also extraplastidial lipids such as phosphoinositides (Gupta *et al.*, 2021).

542 The increase in size of chloroplasts compared to Cyanobacteria, the development of larger  
543 photosynthetic surfaces per cell, some structural innovations like the appearance of pyrenoids  
544 or grana, the bioenergetic coupling of chloroplast within eukaryotic metabolism, etc, led to a  
545 supplementary multiplier effect, likely determinant for the ‘rise of Algae’ during the  
546 Neoproterozoic period, and the oxygenation of oceans known as the NOE.

## 547 **6 Concluding remarks**

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

560 The formation of thylakoids in Cyanobacteria resists vesicular-based scenarios, firstly because  
561 of the lack of any ultrastructural observation of vesicles supposed to protrude from the plasma  
562 membrane toward the inside of the cell, but also because primitive thylakoids are concentric,  
563 and we do not see where thylakoid biogenesis could be initiated. Here, we reviewed studies  
564 converging into a simple hypothesis based on the stepwise production of *HexII* lipids, at the  
565 inner periphery of the cytosolic membrane of an early cyanobacterium, turned within  
566 nanoseconds and without any external source of energy into multilayered pro-thylakoids.  
567 Comparison of lipid biosynthetic pathways suggests that most ancient Cyanobacteria could  
568 not achieve thylakoid biogenesis due to the lack of anionic *Lm* lipids, only represented by a  
569 minor phospholipid with a proportion controlled by phosphorus level in the environment. The  
570 acquisition of the biosynthesis pathway producing a sulfolipid seemed critical for a regular  
571 provision of sufficient amounts of anionic lipids, allowing the *HexII* → *Lm* phase transition to  
572 occur thus forming parietal thylakoids. We designed this model before the very recent  
573 description of the spatio-temporal biogenesis process of thylakoid membranes in the rod-  
574 shaped cyanobacterium *Synechococcus elongatus* PCC 7942 (Huokko *et al.*, 2021). In this  
575 cyanobacterium, the plasma membrane and concentric thylakoid layers have no physical  
576 connections and newly synthesized thylakoid membrane fragments emerge between the  
577 plasma membrane and pre-existing thylakoids (Huokko *et al.*, 2021). This is strikingly  
578 reminiscent of the model described in Figs. 2A and 4 and illustrates the power of the *HexII-to-*  
579 *Lm* phase transition not only at the origin of early thylakoids, but also in biogenetic processes  
580 observed today. With this non-vesicular lipid-phase transition, a framework is therefore also  
581 available to reexamine the role of companion proteins, such as Vipp1, which characterization  
582 in a hypothetical vesicular-based biogenetic process remains unsatisfying in both Cyanobacteria  
583 and chloroplasts. It is time to address these questions by considering lipids and proteins  
584 together, with additional challenging issues, such as the integration of membrane proteins and  
585 cofactors in the course of thylakoid biogenesis. Eventually, first-order phase transitions are  
586 not restricted to changes in lipid phases. They are also involved in RNA and protein structural  
587 changes between liquid phases (Hardenberg *et al.*, 2020; Guo *et al.*, 2021; Rhine *et al.*, 2020),  
588 which might provide clues on extremely rapid, nearly instant processes, which cannot be  
589 comprehended yet with more classical biochemical, structural or cell biology approaches.

590 Taking into consideration all these phase transitions might improve our understanding of  
591 other steps in cell evolution.

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599 NG and EM contributed to the development of the hypothesis developed in this paper. NG  
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## **9 References**

**Allen JF.** 2014. Origin of Oxygenic Photosynthesis from Anoxygenic Type I and Type II Reaction Centers. In: Golbeck J, van der Est A, eds. *Biophysics for the Life Sciences.*, Vol. 11. Springer, New York, NY., 433-450.

**Allen JF.** 2016. A Proposal for Formation of Archaean Stromatolites before the Advent of Oxygenic Photosynthesis. *Frontiers in Microbiology* **7**, 1784

**Apdila ET, Awai K.** 2018. Configuration of the sugar head of glycolipids in thylakoid membranes. *Genes Genetic Systems* **92**, 235-242.

**Awai K, Kakimoto T, Awai C, Kaneko T, Nakamura Y, Takamiya K, Wada H, Ohta H.** 2006. Comparative genomic analysis revealed a gene for monoglucosyldiacylglycerol synthase, an enzyme for photosynthetic membrane lipid synthesis in cyanobacteria. *Plant Physiology* **141**, 1120-1127.

**Awai K, Marechal E, Block MA, Brun D, Masuda T, Shimada H, Takamiya K, Ohta H, Joyard J.** 2001. Two types of MGDG synthase genes, found widely in both 16:3 and 18:3 plants, differentially mediate galactolipid syntheses in photosynthetic and nonphotosynthetic tissues in *Arabidopsis thaliana*. *Proceedings of the National Academy of Science U S A* **98**, 10960-10965.

**Awai K, Ohta H, Sato N.** 2014. Oxygenic photosynthesis without galactolipids. *Proceedings of the National Academy of Science U S A* **111**, 13571-13575.

**Awai K, Watanabe H, Benning C, Nishida I.** 2007. Digalactosyldiacylglycerol is required for better photosynthetic growth of *Synechocystis* sp PCC6803 under phosphate limitation. *Plant and Cell Physiology* **48**, 1517-1523.

**Babiychuk E, Muller F, Eubel H, Braun HP, Frentzen M, Kushnir S.** 2003. *Arabidopsis* phosphatidylglycerophosphate synthase 1 is essential for chloroplast differentiation, but is dispensable for mitochondrial function. *Plant Journal* **33**, 899-909.

**Ball SG, Subtil A, Bhattacharya D, Moustafa A, Weber AP, Gehre L, Colleoni C, Arias MC, Cenci U, Dauvillee D.** 2013. Metabolic effectors secreted by bacterial pathogens: essential facilitators of plastid endosymbiosis? *Plant Cell* **25**, 7-21.

- Balleza D, Garcia-Arribas AB, Sot J, Ruiz-Mirazo K, Goni FM.** 2014. Ether- versus Ester-Linked Phospholipid Bilayers Containing either Linear or Branched Apolar Chains. *Biophysical Journal* **107**, 1364-1374.
- Bastien O, Botella C, Chevalier F, Block MA, Jouhet J, Breton C, Girard-Egrot A, Maréchal E.** 2016. New Insights on Thylakoid Biogenesis in Plant Cells. *International Review of Cell and Molecular Biology* **323**, 1-30.
- Bekker A, Holland HD, Wang PL, Rumble D, 3rd, Stein HJ, Hannah JL, Coetzee LL, Beukes NJ.** 2004. Dating the rise of atmospheric oxygen. *Nature* **427**, 117-120.
- Billey E, Hafidh S, Cruz-Gallardo I, Litholdo CG, Jean V, Carpentier MC, Picart C, Kumar V, Kulichova K, Marechal E, Honys D, Conte MR, Deragon JM, Bousquet-Antonelli C.** 2021. LARP6C orchestrates posttranscriptional reprogramming of gene expression during hydration to promote pollen tube guidance. *Plant Cell. In press*
- Blank CE, Sanchez-Baracaldo P.** 2010. Timing of morphological and ecological innovations in the cyanobacteria--a key to understanding the rise in atmospheric oxygen. *Geobiology* **8**, 1-23.
- Botte CY, Deligny M, Rocchia A, Bonneau AL, Saidani N, Hardre H, Aci S, Yamaro-Botte Y, Jouhet J, Dubots E, Loizeau K, Bastien O, Brehelin L, Joyard J, Cintrat JC, Falconet D, Block MA, Rousseau B, Lopez R, Marechal E.** 2011a. Chemical inhibitors of monogalactosyldiacylglycerol synthases in Arabidopsis thaliana. *Nature Chemical Biology* **7**, 834-842.
- Botte CY, Marechal E.** 2014. Plastids with or without galactoglycerolipids. *Trends in Plant Science* **19**, 71-78.
- Botte CY, McFadden GI, Yamaro-Botte Y.** 2018. Isolating the Plasmodium falciparum Apicoplast Using Magnetic Beads. *Methods in Molecular Biology* **1829**, 205-212.
- Botte CY, Yamaro-Botte Y, Janouskovec J, Rupasinghe T, Keeling PJ, Crellin P, Coppel RL, Marechal E, McConville MJ, McFadden GI.** 2011b. Identification of plant-like galactolipids in Chromera velia, a photosynthetic relative of malaria parasites. *Journal of Biological Chemistry* **286**, 29893-29903.
- Botte CY, Yamaro-Botte Y, Rupasinghe TW, Mullin KA, MacRae JI, Spurck TP, Kalanon M, Shears MJ, Coppel RL, Crellin PK, Marechal E, McConville MJ, McFadden GI.** 2013. Atypical lipid composition in the purified relict plastid (apicoplast) of malaria parasites. *Proceedings of the National Academy of Science U S A* **110**, 7506-7511.
- Boudiere L, Botte CY, Saidani N, Lajoie M, Marion J, Brehelin L, Yamaro-Botte Y, Satiat-Jeunemaitre B, Breton C, Girard-Egrot A, Bastien O, Jouhet J, Falconet D, Block MA, Marechal E.** 2012. Galvestine-1, a novel chemical probe for the study of the glycerolipid homeostasis system in plant cells. *Molecular Biosystems* **8**, 2023-2035, 2014.
- Boudiere L, Michaud M, Petroustos D, Rebeille F, Falconet D, Bastien O, Roy S, Finazzi G, Rolland N, Jouhet J, Block MA, Marechal E.** 2014. Glycerolipids in photosynthesis: composition, synthesis and trafficking. *Biochimica Et Biophysica Acta* **1837**, 470-480.
- Brocks JJ, Jarrett AJM, Sirantoine E, Hallmann C, Hoshino Y, Liyanage T.** 2017. The rise of algae in Cryogenian oceans and the emergence of animals. *Nature* **548**, 578-581.
- Cardona T, Sanchez-Baracaldo P, Rutherford AW, Larkum AW.** 2019. Early Archean origin of Photosystem II. *Geobiology* **17**, 127-150.
- Cardona T.** 2018. Early Archean origin of heterodimeric Photosystem I. *Heliyon* **4**, e00548.



- Cardona T.** 2019. Thinking twice about the evolution of photosynthesis. *Open Biology* **9**, 180246.
- Cenci U, Bhattacharya D, Weber AP, Colleoni C, Subtil A, Ball SG.** 2017. Biotic Host-Pathogen Interactions As Major Drivers of Plastid Endosymbiosis. *Trends in Plant Science* **22**, 316-328.
- de Kruijff B.** 1997. Biomembranes. Lipids beyond the bilayer. *Nature* **386**, 129-130.
- Deme B, Cataye C, Block MA, Marechal E, Jouhet J.** 2014. Contribution of galactoglycerolipids to the 3-dimensional architecture of thylakoids. *FASEB Journal* **28**, 3373-3383.
- Dolch LJ, Marechal E.** 2015. Inventory of fatty acid desaturases in the pennate diatom *Phaeodactylum tricornutum*. *Marine Drugs* **13**, 1317-1339.
- Dubots E, Audry M, Yamaro Y, Bastien O, Ohta H, Breton C, Marechal E, Block MA.** 2010. Activation of the chloroplast monogalactosyldiacylglycerol synthase MGD1 by phosphatidic acid and phosphatidylglycerol. *Journal of Biological Chemistry* **285**, 6003-6011.
- Dubots E, Botte C, Boudiere L, Yamaro-Botte Y, Jouhet J, Marechal E, Block MA.** 2012. Role of phosphatidic acid in plant galactolipid synthesis. *Biochimie* **94**, 86-93.
- Frentzen M.** 2004. Phosphatidylglycerol and sulfoquinovosyldiacylglycerol: anionic membrane lipids and phosphate regulation. *Current Opinion in Plant Biology* **7**, 270-276.
- Froehlich JE, Benning C, Dörmann P.** 2001. The digalactosyldiacylglycerol (DGDG) synthase DGD1 is inserted into the outer envelope membrane of chloroplasts in a manner independent of the general import pathway and does not depend on direct interaction with monogalactosyldiacylglycerol synthase for DGDG biosynthesis. *Journal of Biological Chemistry* **276**, 31806-31812.
- Garab G.** 2016. Self-assembly and structural-functional flexibility of oxygenic photosynthetic machineries: personal perspectives. *Photosynthetic Research* **127**, 131-150.
- Garcia-Arribas AB, Sot J, Balleza D, Ruiz-Mirazo K, Alonso A, Goni FM.** 2015. Effects of ether groups and acyl-chain branching on lipids tested by Atomic Force Microscopy. *European Biophysics Journal with Biophysics Letters* **44**, S111-S111.
- Gawrisch K, Parsegian VA, Hajduk DA, Tate MW, Gruner SM, Fuller NL, Rand RP.** 1992. Energetics of a Hexagonal Lamellar Hexagonal-Phase Transition Sequence in Dioleoylphosphatidylethanolamine Membranes. *Biochemistry* **31**, 2856-2864.
- Gounaris K, Barber J.** 1983. Monogalactosyldiacylglycerol - the Most Abundant Polar Lipid in Nature. *Trends in Biochemical Sciences* **8**, 378-381.
- Gounaris K, Mannock DA, Sen A, Brain APR, Williams WP, Quinn PJ.** 1983. Poly-Unsaturated Fatty Acyl Residues of Galactolipids Are Involved in the Control of Bilayer Non-Bilayer Lipid Transitions in Higher-Plant Chloroplasts. *Biochimica Et Biophysica Acta* **732**, 229-242.
- Guler SD, Ghosh DD, Pan JJ, Mathai JC, Zeidel ML, Nagle JF, Tristram-Nagle S.** 2009. Effects of ether vs. ester linkage on lipid bilayer structure and water permeability. *Chemistry and Physics of Lipids* **160**, 33-44.
- Guo Q, Shi X, Wang X.** 2021. RNA and liquid-liquid phase separation. *Noncoding RNA Research* **6**, 92-99.

- Gupta TK, Klumpe S, Gries K, Heinz S, Wietrzynski W, Ohnishi N, Niemeyer J, Spaniol B, Schaffer M, Rast A, Ostermeier M, Strauss M, Plitzko JM, Baumeister W, Rudack T, Sakamoto W, Nickelsen J, Schuller JM, Schroda M, Engel BD.** 2021. Structural basis for VIPP1 oligomerization and maintenance of thylakoid membrane integrity. *Cell* **184**, 3643-3659 e3623.
- Hagio M, Gombos Z, Varkonyi Z, Masamoto K, Sato N, Tsuzuki M, Wada H.** 2000. Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis. *Plant Physiology* **124**, 795-804.
- Hardenberg M, Horvath A, Ambrus V, Fuxreiter M, Vendruscolo M.** 2020. Widespread occurrence of the droplet state of proteins in the human proteome. *Proceedings of the National Academy of Science U S A* **117**, 33254-33262.
- Harper PE, Mannock DA, Lewis RN, McElhaney RN, Gruner SM.** 2001. X-ray diffraction structures of some phosphatidylethanolamine lamellar and inverted hexagonal phases. *Biophysical Journal* **81**, 2693-2706.
- Heidrich J, Wulf V, Hennig R, Saur M, Markl J, Sonnichsen C, Schneider D.** 2016. Organization into Higher Ordered Ring Structures Counteracts Membrane Binding of IM30, a Protein Associated with Inner Membranes in Chloroplasts and Cyanobacteria. *Journal of Biological Chemistry* **291**, 14954-14962.
- Hennig R, Heidrich J, Saur M, Schmuser L, Roeters SJ, Hellmann N, Woutersen S, Bonn M, Weidner T, Markl J, Schneider D.** 2015. IM30 triggers membrane fusion in cyanobacteria and chloroplasts. *Nature Communication* **6**, 7018.
- Hennig R, West A, Debus M, Saur M, Markl J, Sachs JN, Schneider D.** 2017. The IM30/Vipp1 C-terminus associates with the lipid bilayer and modulates membrane fusion. *Biochimica Et Biophysica Acta Bioenergy* **1858**, 126-136.
- Hohmann-Marriott MF, Blankenship RE.** 2011. Evolution of photosynthesis. *Annual Review of Plant Biology* **62**, 515-548.
- Huokko T, Ni T, Dykes GF, Simpson DM, Brownridge P, Conradi FD, Beynon RJ, Nixon PJ, Mullineaux CW, Zhang P, Liu LN.** 2021. Probing the biogenesis pathway and dynamics of thylakoid membranes. *Nature Communication* **12**, 3475.
- Hurlock AK, Roston RL, Wang K, Benning C.** 2014. Lipid trafficking in plant cells. *Traffic* **15**, 915-932.
- Imachi H, Nobu MK, Nakahara N, Morono Y, Ogawara M, Takaki Y, Takano Y, Uematsu K, Ikuta T, Ito M, Matsui Y, Miyazaki M, Murata K, Saito Y, Sakai S, Song C, Tasumi E, Yamanaka Y, Yamaguchi T, Kamagata Y, Tamaki H, Takai K.** 2020. Isolation of an archaeon at the prokaryote-eukaryote interface. *Nature* **577**, 519-525.
- Jia TZ, Chandru K, Hongo Y, Afrin R, Usui T, Myojo K, Cleaves HJ, 2nd.** 2019. Membraneless polyester microdroplets as primordial compartments at the origins of life. *Proceedings of the National Academy of Science U S A* **116**, 15830-15835.
- Jouhet J.** 2013. Importance of the hexagonal lipid phase in biological membrane organization. *Frontiers in Plant Science* **4**, 494.
- Kanduc M, Schlaich A, de Vries AH, Jouhet J, Marechal E, Deme B, Netz RR, Schneck E.** 2017. Tight cohesion between glycolipid membranes results from balanced water-headgroup interactions. *Nature Communication* **8**, 14899.

- Kobayashi K, Kondo M, Fukuda H, Nishimura M, Ohta H.** 2007. Galactolipid synthesis in chloroplast inner envelope is essential for proper thylakoid biogenesis, photosynthesis, and embryogenesis. *Proceedings of the National Academy of Science U S A* **104**, 17216-17221.
- Kobayashi K, Narise T, Sonoike K, Hashimoto H, Sato N, Kondo M, Nishimura M, Sato M, Toyooka K, Sugimoto K, Wada H, Masuda T, Ohta H.** 2013. Role of galactolipid biosynthesis in coordinated development of photosynthetic complexes and thylakoid membranes during chloroplast biogenesis in Arabidopsis. *Plant Journal* **73**, 250-261.
- Kobori TO, Uzumaki T, Kis M, Kovacs L, Domonkos I, Itoh S, Krynicka V, Kuppusamy SG, Zakar T, Dean J, Szilak L, Komenda J, Gombos Z, Ughy B.** 2018. Phosphatidylglycerol is implicated in divisome formation and metabolic processes of cyanobacteria. *Journal of Plant Physiology* **223**, 96-104.
- Kopečna J, Pilný J, Krynicka V, Tomcala A, Kis M, Gombos Z, Komenda J, Sobotka R.** 2015. Lack of Phosphatidylglycerol Inhibits Chlorophyll Biosynthesis at Multiple Sites and Limits Chlorophyllide Reutilization in Synechocystis sp. Strain PCC 6803. *Plant Physiology* **169**, 1307-1317.
- Kroll D, Meierhoff K, Bechtold N, Kinoshita M, Westphal S, Vothknecht UC, Soll J, Westhoff P.** 2001. VIPP1, a nuclear gene of Arabidopsis thaliana essential for thylakoid membrane formation. *Proceedings of the National Academy of Science U S A* **98**, 4238-4242.
- Kutik J, Hola D, Kocova M, Rothova O, Haisel D, Wilhelmova N, Ticha I.** 2004. Ultrastructure and dimensions of chloroplasts in leaves of three maize (*Zea mays* L.) inbred lines and their F-1 hybrids grown under moderate chilling stress. *Photosynthetica* **42**, 447-455.
- Kuzmin PI, Zimmerberg J, Chizmadzhev YA, Cohen FS.** 2001. A quantitative model for membrane fusion based on low-energy intermediates. *Proceedings of the National Academy of Science U S A* **98**, 7235-7240.
- Li HM, Kaneko Y, Keegstra K.** 1994. Molecular cloning of a chloroplastic protein associated with both the envelope and thylakoid membranes. *Plant Molecular Biology* **25**, 619-632.
- Li-Beisson Y, Beisson F, Riekhof W.** 2015. Metabolism of acyl-lipids in *Chlamydomonas reinhardtii*. *Plant Journal* **82**, 504-522.
- Lyons TW, Reinhard CT, Planavsky NJ.** 2014. The rise of oxygen in Earth's early ocean and atmosphere. *Nature* **506**, 307-315.
- Makshakova O, Breton C, Perez S.** 2020. Unraveling the complex enzymatic machinery making a key galactolipid in chloroplast membrane: a multiscale computer simulation. *Scientific Reports* **10**, 13514.
- Mannock DA, Lewis RN, McElhaney RN, Harper PE, Turner DC, Gruner SM.** 2001. An analysis of the relationship between fatty acid composition and the lamellar gel to liquid-crystalline and the lamellar to inverted nonlamellar phase transition temperatures of phosphatidylethanolamines and diacyl- $\alpha$ -D-glucosyl glycerols. *European Biophysical Journal* **30**, 537-554.
- Marechal E.** 2018. Primary Endosymbiosis: Emergence of the Primary Chloroplast and the Chromatophore, Two Independent Events. *Methods in Molecular Biology* **1829**, 3-16.
- Mares J, Strunecky O, Bucinska L, Wiedermannova J.** 2019. Evolutionary Patterns of Thylakoid Architecture in Cyanobacteria. *Frontiers in Microbiology* **10**, 277.

- Marrink SJ, Mark AE.** 2004. Molecular view of hexagonal phase formation in phospholipid membranes. *Biophysical Journal* **87**, 3894-3900.
- Martin WF, Bryant DA, Beatty JT.** 2018. A physiological perspective on the origin and evolution of photosynthesis. *FEMS Microbiological Reviews* **42**, 205-231.
- McDonald C, Jovanovic G, Wallace BA, Ces O, Buck M.** 2017. Structure and function of PspA and Vipp1 N-terminal peptides: Insights into the membrane stress sensing and mitigation. *Biochimica Et Biophysica Acta Biomembranes* **1859**, 28-39.
- Mechela A, Schwenkert S, Soll J.** 2019. A brief history of thylakoid biogenesis. *Open Biology* **9**, 180237.
- Mizoguchi T, Harada J, Yoshitomi T, Tamiaki H.** 2013. A variety of glycolipids in green photosynthetic bacteria. *Photosynthetic Research* **114**, 179-188.
- Nakamura Y, Kobayashi K, Ohta H.** 2009. Activation of galactolipid biosynthesis in development of pistils and pollen tubes. *Plant Physiology Biochemistry* **47**, 535-539.
- Nordhues A, Schottler MA, Unger AK, Geimer S, Schonfelder S, Schmollinger S, Rutgers M, Finazzi G, Soppa B, Sommer F, Muhlhaus T, Roach T, Krieger-Liszkay A, Lokstein H, Crespo JL, Schroda M.** 2012. Evidence for a role of VIPP1 in the structural organization of the photosynthetic apparatus in *Chlamydomonas*. *Plant Cell* **24**, 637-659.
- Omata T, Murata N.** 1986. Glucolipid synthesis activities in cytoplasmic and thylakoid membranes from the cyanobacterium *Anacystis nidulans*. *Plant and Cell Physiology* **27**, 485-490.
- Otters S, Braun P, Hubner J, Wanner G, Vothknecht UC, Chigri F.** 2013. The first alpha-helical domain of the vesicle-inducing protein in plastids 1 promotes oligomerization and lipid binding. *Planta* **237**, 529-540.
- Petroutsos D, Amiar S, Abida H, Dolch LJ, Bastien O, Rebeille F, Jouhet J, Falconet D, Block MA, McFadden GI, Bowler C, Botte C, Marechal E.** 2014. Evolution of galactoglycerolipid biosynthetic pathways--from cyanobacteria to primary plastids and from primary to secondary plastids. *Progress in Lipid Research* **54**, 68-85.
- Ponce-Toledo RI, Lopez-Garcia P, Moreira D.** 2019. Horizontal and endosymbiotic gene transfer in early plastid evolution. *New Phytology* **224**, 618-624.
- Raven JA, Sanchez-Baracaldo P.** 2021. Gloeobacter and the implications of a freshwater origin of Cyanobacteria. *Phycologia*. *In press*
- Rawlyer A, Unitt MD, Giroud C, Davies HF, Mayor JP, Harwood JL, Siegenthaler PA.** 1987. The transmembrane distribution of galactolipids in chloroplast thylakoids is universal in a wide variety of temperate climate plants. *Photosynthetic Research* **11**, 3-13.
- Raymond J, Zhaxybayeva O, Gogarten JP, Gerdes SY, Blankenship RE.** 2002. Whole-genome analysis of photosynthetic prokaryotes. *Science* **298**, 1616-1620.
- Rexroth S, Mullineaux CW, Ellinger D, Sendtko E, Rogner M, Koenig F.** 2011. The plasma membrane of the cyanobacterium *Gloeobacter violaceus* contains segregated bioenergetic domains. *Plant Cell* **23**, 2379-2390.
- Rhine K, Vidaurre V, Myong S.** 2020. RNA Droplets. *Annual Review of Biophysics* **49**, 247-265.

- Sakurai I, Hagio M, Gombos Z, Tyystjarvi T, Paakkanen V, Aro EM, Wada H.** 2003a. Requirement of phosphatidylglycerol for maintenance of photosynthetic machinery. *Plant Physiology* **133**, 1376-1384.
- Sakurai I, Hagio M, Gombos Z, Tyystjarvi T, Paakkanen V, Aro EM, Wada H.** 2003b. Role of phosphatidylglycerol in photosystem II. *Plant and Cell Physiology* **44**, S192-S192.
- Sakurai I, Mizusawa N, Wada H, Sato N.** 2007. Digalactosyldiacylglycerol is required for stabilization of the oxygen-evolving complex in photosystem II. *Plant Physiology* **145**, 1361-1370.
- Salvador-Castell M, Golub M, Erwin N, Deme B, Brooks NJ, Winter R, Peters J, Oger PM.** 2021. Characterisation of a synthetic Archeal membrane reveals a possible new adaptation route to extreme conditions. *Communications Biology* **4**, 653.
- Sanchez-Baracaldo P, Cardona T.** 2020. On the origin of oxygenic photosynthesis and Cyanobacteria. *New Phytologist* **225**, 1440-1446.
- Sarkis J, Rocha J, Maniti O, Jouhet J, Vie V, Block MA, Breton C, Marechal E, Girard-Egrot A.** 2014. The influence of lipids on MGD1 membrane binding highlights novel mechanisms for galactolipid biosynthesis regulation in chloroplasts. *FASEB Journal* **28**, 3114-3123.
- Sato N, Awai K.** 2016. Diversity in Biosynthetic Pathways of Galactolipids in the Light of Endosymbiotic Origin of Chloroplasts. *Frontiers in Plant Science* **7**, 117
- Sato N, Awai K.** 2017. "Prokaryotic Pathway" Is Not Prokaryotic: Noncyanobacterial Origin of the Chloroplast Lipid Biosynthetic Pathway Revealed by Comprehensive Phylogenomic Analysis. *Genome Biology and Evolution* **9**, 3162-3178.
- Sato N, Kamimura R, Tsuzuki M.** 2016. Dispensability of a sulfolipid for photoautotrophic cell growth and photosynthesis in a marine cyanobacterium, *Synechococcus* sp. PCC 7002. *Biochemical and Biophysical Research Communications* **477**, 854-860.
- Sato N, Takano H.** 2017. Diverse origins of enzymes involved in the biosynthesis of chloroplast peptidoglycan. *Journal of Plant Research* **130**, 635-645.
- Sato N, Yoshitomi T, Mori-Moriyama N.** 2020. Characterization and biosynthesis of lipids in *Paulinella micropora* MYN1. Evidence for efficient integration of chromatophores into cellular lipid metabolism. *Plant and Cell Physiology* **61**, 869-881
- Sato N.** 2015. Is Monoglucosyldiacylglycerol a Precursor to Monogalactosyldiacylglycerol in All Cyanobacteria? *Plant and Cell Physiology* **56**, 1890-1899.
- Saur M, Hennig R, Young P, Rusitzka K, Hellmann N, Heidrich J, Morgner N, Markl J, Schneider D.** 2017. A Janus-Faced IM30 Ring Involved in Thylakoid Membrane Fusion Is Assembled from IM30 Tetramers. *Structure* **25**, 1380-1390 e1385.
- Selao TT, Zhang L, Arioiz C, Wieslander A, Norling B.** 2014. Subcellular localization of monoglucosyldiacylglycerol synthase in *Synechocystis* sp. PCC6803 and its unique regulation by lipid environment. *PLoS One* **9**, e88153.
- Shimojima M.** 2011. Biosynthesis and functions of the plant sulfolipid. *Progress in Lipid Research* **50**, 234-239.
- Sojo V.** 2019. Why the Lipid Divide? Membrane Proteins as Drivers of the Split between the Lipids of the Three Domains of Life. *Bioessays* **41**, e1800251.

**Toombes GES, Finnefrock AC, Tate MW, Gruner SM.** 2002. Determination of L-alpha-H-II phase transition temperature for 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine. *Biophysical Journal* **82**, 2504-2510.

**Uwizeye C, Decelle J, Jouneau PH, Flori S, Gallet B, Keck JB, Bo DD, Moriscot C, Seydoux C, Chevalier F, Schieber NL, Templin R, Allorent G, Courtois F, Curien G, Schwab Y, Schoehn G, Zeeman SC, Falconet D, Finazzi G.** 2021. Morphological bases of phytoplankton energy management and physiological responses unveiled by 3D subcellular imaging. *Nature Communication* **12**, 1049.

**Wikstrom M, Xie J, Bogdanov M, Mileykovskaya E, Heacock P, Wieslander A, Dowhan W.** 2004. Monoglucosyldiacylglycerol, a foreign lipid, can substitute for phosphatidylethanolamine in essential membrane-associated functions in *Escherichia coli*. *Journal of Biological Chemistry* **279**, 10484-10493.

**Wohri AB, Wahlgren WY, Malmerberg E, Johansson LC, Neutze R, Katona G.** 2009. Lipidic sponge phase crystal structure of a photosynthetic reaction center reveals lipids on the protein surface. *Biochemistry* **48**, 9831-9838.

**Xu CC, Hartel H, Wada H, Hagio M, Yu B, Eakin C, Benning C.** 2002. The *pgp1* mutant locus of *Arabidopsis* encodes a phosphatidylglycerolphosphate synthase with impaired activity. *Plant Physiology* **129**, 594-604.

**Xu CC, Yu B, Cornish AJ, Froehlich JE, Benning C.** 2006. Phosphatidylglycerol biosynthesis in chloroplasts of *Arabidopsis* mutants deficient in acyl-ACP glycerol-3-phosphate acyltransferase. *Plant Journal* **47**, 296-309.

**Yang W, Wittkopp TM, Li X, Warakanont J, Dubini A, Catalanotti C, Kim RG, Nowack EC, Mackinder LC, Aksoy M, Page MD, D'Adamo S, Saroussi S, Heinnickel M, Johnson X, Richaud P, Alric J, Boehm M, Jonikas MC, Benning C, Merchant SS, Posewitz MC, Grossman AR.** 2015. Critical role of *Chlamydomonas reinhardtii* ferredoxin-5 in maintaining membrane structure and dark metabolism. *Proceedings of the National Academy of Science U S A* **112**, 14978-14983.

**Yuzawa Y, Nishihara H, Haraguchi T, Masuda S, Shimojima M, Shimoyama A, Yuasa H, Okada N, Ohta H.** 2012. Phylogeny of galactolipid synthase homologs together with their enzymatic analyses revealed a possible origin and divergence time for photosynthetic membrane biogenesis. *DNA Research* **19**, 91-102.