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On the non-oxygenic origins of thylakoids

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Thylakoid membranes are the site of oxygenic photosynthesis, one of the most important biochemical processes on earth. The ancestral state of these membranes is represented today in *Gloeobacterales*, where they are lacking and photosynthesis instead takes place in the cytoplasmic membrane. The evolutionary transition from this ancestral state to the modern thylakoid membranes provided a major advantage, as it increased photosynthetic efficiency. However, how this significant transition occurred remains an understudied question. The biogenesis of modern thylakoid membranes relies on a highly synchronized process involving numerous assembly factors and showing important differences between the two photosystems. Together, these features suggest the existence of intermediate evolutionary states during the emergence of this compartment. Here, I propose a non-oxygenic origin of thylakoid membranes, where these intermediate states were initially dedicated to alternative electron flows. This hypothesis further addresses the paradox of cyanobacterial diversification in an euxinic environment, toxic to photosystem II.

The thylakoids question

Photosynthesis is a biochemical process of high importance on Earth, catalyzing the conversion of solar energy into carbohydrates by fixing inorganic carbon. This process originated in the Last Phototroph Common Ancestor of Terrabacteria, a group of bacteria linked to terrestrial colonization that includes cyanobacteria[1], which already possessed two types of photosynthetic reaction centers and the enzymatic machinery for CO_2 fixation, albeit in an anoxygenic form[2]. Its subsequent evolution was shaped by repeated gene losses, notably involving one of the reaction centers[2]. Today cyanobacteria are the only prokaryotes that retain both reaction centers, called photosystems (PS), and that perform oxygenic photosynthesis, in which oxygen is released as a byproduct[2]. Oxygenic photosynthesis appeared in cyanobacteria at least 3.3 billion years ago[3] and mainly takes place in the membranes of specialized cell structures called thylakoids. It involves a linear electron transfer chain from water to ferredoxin, via three transmembrane complexes, PSII, Cytochrome b6f, and PSI, and two electron carriers, plastoquinone and plastocyanin[4] (Fig. 1).

Numerous recent evolutionary studies have significantly advanced our understanding of oxygenic photosynthesis, including the origins of PS[2], their evolution[5–7], and even dating its emergence[8,9]. The selective advantage provided by thylakoid membranes (TM), in terms of number of PS per cell, has recently been proposed[10] as a plausible explanation for earth's oxygen accumulation during the Great Oxidation Event, 2.4 billion years ago[11]. Extensive studies on the origins of oxygenic photosynthesis and PS have been carried out. However, despite the important role of thylakoids on earth biogeochemistry, their origins and evolution have remained comparatively understudied. In most cyanobacteria, here referred to as *Phycobacteria*[12], oxygenic photosynthesis occurs in TM. Yet, the earliest-diverging group of cyanobacteria, the *Gloeobacterales*—as confirmed by multiple phylogenomic studies[13–16]—lacks these membranes. Instead, oxygenic photosynthesis takes place in their cytoplasmic membrane (CM)[17–20], which must thereby be the ancestral state (Fig. 1). How the transition unfolded, from this ancestral *Gloeobacterales*-like state, with a linear electron transfer chain functioning in CM, to the modern state, where all components are localized in TM, remains a biological enigma. In this evolutionary transition, two aspects must be considered: first, the emergence of the cellular compartment, and second, the transfer of complexes and the relocation of electron carriers from CM to the membrane of this new compartment.

An overview of thylakoid biogenesis

Thylakoid membrane biogenesis

TM consist of three key glycolipids, which are also present in CM: monogalactosyldiacylglycerol (MGDG, >50% of the lipid content), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol (SQDG), which is absent in *Gloeobacterales*[21]. MGDG facilitates TM curvature[22], while DGDG stabilizes thylakoid stacking via hydrogen bonds[23]. The process by which TM are formed, and how their lipid ratios differ compared to those in CM, has been the subject of four main hypotheses: membrane fusion, vesicular transport, direct lipid transport via soluble carriers[21,24] or self-assembly via a hexagonal-to-lamellar lipids phase transition[10] (Fig. 2A). Recent findings seem to support two of these hypotheses. First, the emergence of SQDG biosynthesis at the root of the *Phycobacteria* may have provided sufficient anionic lipids to enable the spontaneous formation of TM without requiring energy input[10]. Second, a recent work by Tan et al.[25] revealed that a duplication of the *PspA* gene in the ancestor of *Phycobacteria*, coupled with the acquisition of a C-terminal extension, gave rise to a vesicle-

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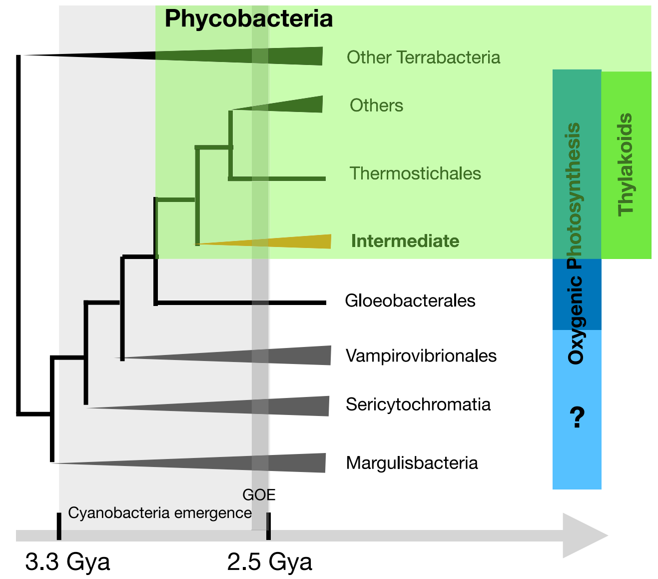

Fig. 1 | Evolutionary tree of cyanobacteria and intermediate state. The current non-photosynthetic groups (gray triangles) and the two early branching groups important in the context of TM emergence, the *Gloeobacterales* and the *Thermostichales*, are represented. Phylogeny used from ref. 85.

inducing protein, VIPP1[25]. The name of this protein, also known as IM30 and belonging to the ESCRT-III family, originates from the attribution of its hypothetical role; it is in fact a membrane fusion protein[26,27]. In *Arabidopsis*, VIPP1 shortage results in reduced thylakoid membrane content, while the complexes are correctly assembled, showing its essential role in the formation of TM but not for the transfer of complexes[26]. However, the role of VIPP1 in cyanobacteria remains to be fully understood. Indeed, in the two model organisms *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002, VIPP1 does not appear to be involved in TM biogenesis but rather in the assembly of photosynthetic complexes[28,29]. Hence, different concentrations of VIPP1 in *Synechocystis* sp. PCC 6803 affect both PSII and PSI biogenesis but not TM formation[28]. Similarly, a VIPP1 null mutant affects PSI biogenesis, leading to TM devoid of PSI in *Synechococcus* sp. PCC 7002[29]. VIPP1 has also been proposed to play an additional role in preventing proton leakage from damaged TM by forming membrane-covering structures[27]. The exact function of VIPP1, whether in TM biogenesis or in assembly of photosynthetic complexes, still needs to be clarified in the future (Tables 1, 1.1). Beyond VIPP1, proteins involved in membrane trafficking and dynamics—such as DedA proteins (putative lipid scramblases), SPFH proteins (implicated in vesicular lipid transport), and dynamin-like proteins (involved in membrane fission/fusion)[27] —as well as cyanobacterial homologs of proteins essential for TM biogenesis in *Arabidopsis thaliana*, like CPSAR1[30], THF1[31], and CPRabA5e[32], have not yet been investigated in the context of TM emergence (Tables 1, 1.2).

Photosynthetic complexes and electron carrier biogenesis

Unlike membrane structures, the transfer of photosynthetic complexes and the relocation of electron carriers has never been studied from an evolutionary perspective. To understand this transition, the biogenesis of these complexes/carriers—and specifically the proteins associated with biogenesis—provides crucial insights. The integration of transmembrane complexes into TM during biogenesis is a highly regulated stepwise process involving numerous assembly factors that act as chaperones, notably for transporting PS subunits from CM to TM (Fig. 2B)[21]. The most studied element is PSII, whose reaction center is formed by the D1 and D2 proteins. Its biogenesis starts in CM with the synthesis of the precursor of D1, pD1. D1 transitions through the *PratA*-defined membrane, where it assembles with D2, leading to RC photoactivation[33–35]. This membrane also depends on CurT[35] and AncM[36], and serves as a contact point between TM and CM, playing a structural role in TM biogenesis[35]. Once the PSII reaction center is assembled, it is first incorporated into TM with the CP43 and CP47 antenna complexes, followed by the addition of PSII subunits (Q, U, O, V)[37]. The complex then dimerizes and associates with the phycobilisome on the outer TM face[37]. Thirty-three assembly factors coordinate this process in *Arabidopsis*, among which twenty-seven orthologs are also identified in cyanobacteria (Table 2). Among these assembly factors, some are essential for PSII biogenesis, such as those involved in the translocation and maturation of pD1 (CtpA[38], YidC/SecY[37,39] translocon, Ycf48[21,40,41]), manganese delivery to D1 (PratA[33]), the synthesis of the reaction center (TerC[42]), the synthesis and delivery of chlorophyll to D1 and D2 (ChlG[39], Pitt[40], Sll0933[43], Ycf39[39]), PS heterodimerization (PsbN[42]), and antenna binding (Psb27[37,44], Psb34[43], Sll0606[45]) (Table 2). The biogenesis of PSI is significantly less understood but nonetheless involves at least eleven assembly factors (Table 2). A major difference between PSII and PSI biogenesis can nevertheless be noted, as the latter does not involve the SecY channel[46].

Huokko et al.[47] recently established a protocol to stimulate thylakoid biogenesis in *Synechococcus elongatus* PCC 7942, confirmed by cryo-electron tomography[47]. This protocol was later used by Huang et al.[48] to characterize the thylakoid proteome through membrane fractionation. Assembly factors associated with the early stage of PSII biogenesis, notably YidC/SecY, were not identified in TM; this study detected only later-stage PSII assembly factors such as Psb27, Psb28, and Psb34[48]. Interestingly, Huang et al. 2023 recovered only two assembly factors related to PSI biogenesis in TM, Ycf37 and VIPP1, the latter reported in their study as PSI-associated[48]. PSI assembly factors were recovered at a more advanced stage of TM biogenesis than those of PSII[48]. This difference in the timing of biogenesis, as well as in the assembly machinery involved (see SecY above), between PSI and PSII—which evolved through duplication from a unique ancestral PS[2,49]—suggests that their integration into TM from CM occurred at two distinct evolutionary stages. Few assembly factors for Cytb6f and ATPase, as well as NDH1, have been detected (6 for Cytb6f, of which 1 known in cyanobacteria; 6 for ATPase, of which none detected in cyanobacteria; 8 for NDH1, of which 3 known in cyanobacteria - Table 2), but their localization remains mostly unknown for the moment (Tables 1, 1.3).

The relocation of electron carriers into thylakoids also presents several unknowns. The metalloprotein plastocyanin, which was likely located in the periplasmic space in the ancestral *Gloeobacterales*-like state[50] is incorporated into the thylakoid lumen via a Sec-dependent signal[51], likely a SecA signal peptide/sequence, as observed in *Phormidium laminosum*[52]. In the latter organism, the only cyanobacterium with a characterized plastocyanin-targeting system, a 34-amino-acid N-terminal sequence is responsible for the lumen targeting[52] (Tables 1, 1.4). Copper is incorporated into plastocyanin in the thylakoid lumen after import via the transmembrane P-type ATPases CtaA and PacS[53], the latter being mainly localized in TM[54]. The biosynthesis of the lipid-soluble plastoquinone is mediated by the solanesyltransferase Slr0926[55], identified in the membranes of *Synechocystis*[56], although it remains unclear whether these membranes are thylakoid or cytoplasmic. This raises questions about how the N-terminal sequence of plastocyanin evolved in *Phycobacteria*, as well as the incorporation of SecA, PacS (Tables 1, 1.5) and Slr0926 (Tables 1, 1.6) into TM. Finally, some cyanobacteria are also able to perform anoxygenic photosynthesis using a sulfide-quinone reductase anchored in their TM[25,57,58]. The mechanism of targeting sulfide-quinone reductase to cellular membranes is unclear, although the C-terminal region is required for translocation (Tables 1, 1.7)[59].

An overview of alternative electron flows in cyanobacteria

The complexity of the stepwise biogenetic process, notably the differences between the two PS and the number of coordinated assembly factors involved, suggests the existence of evolutionary intermediate states stemming from the ancestral *Gloeobacterales*-like state. Deciphering these intermediates involves inferring what the transfer sequence of the complexes was and what the evolutionary advantages of these states could have

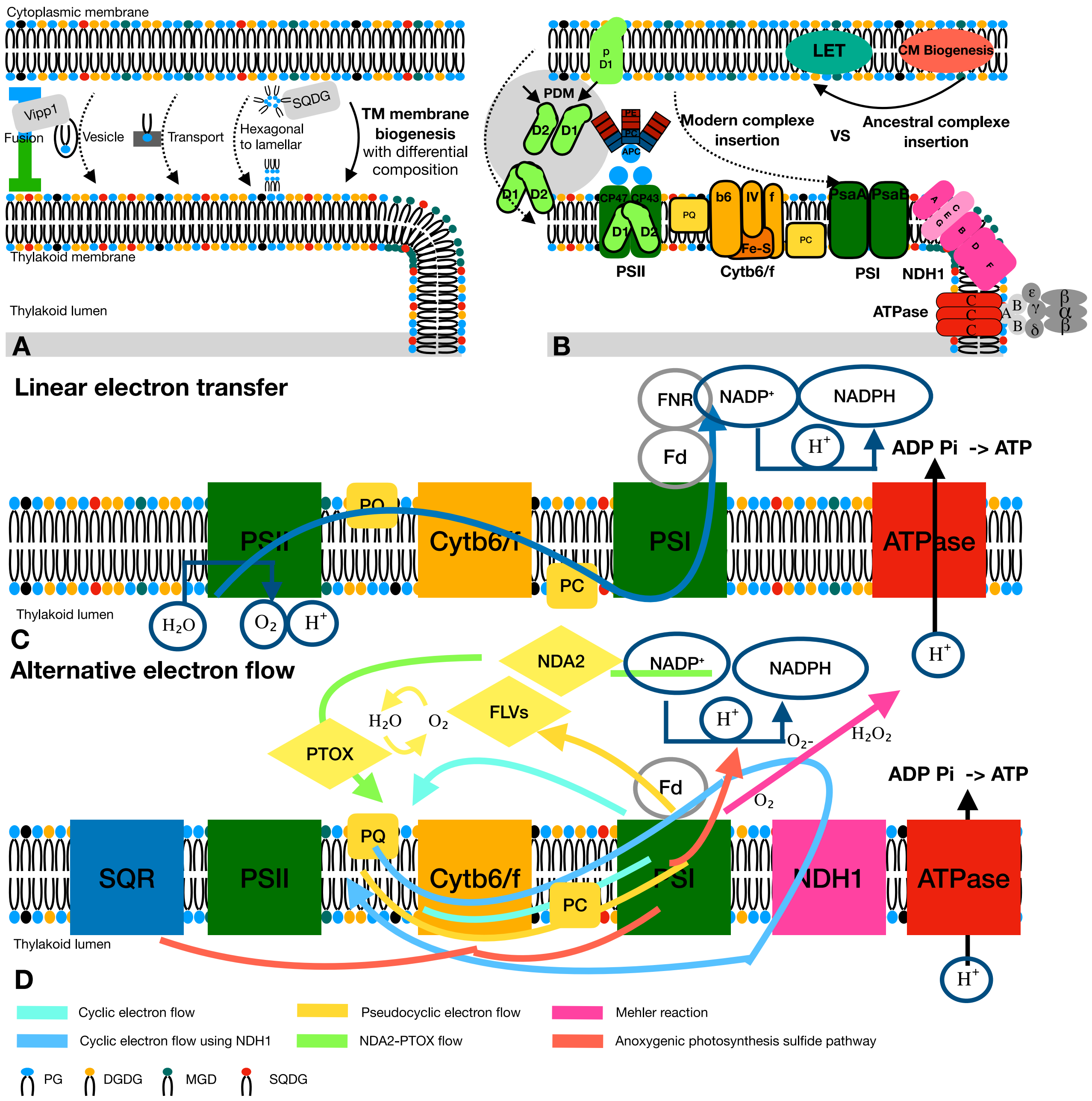

Fig. 2 | Schematic view of thylakoid membrane emergence theory, biogenesis, and the roles of linear electron transfer and alternative electron flow. A Four TM emergence theories and differential composition. CM and TM are composed of the same lipidic components: PG, MGDG, DGDG, SQDG with proportions varying between the two types of membranes. Four scenarios have been proposed for the biogenesis of TM from CM. **B** Complex biogenesis. In the ancestral state, within CM of *Gloeobacterales*, complexes of the electron transport chains are located in specialized regions of CM, which also contain the biogenic regions. In the modern state, these complexes are found in TM, with the biogenesis of certain subunits starting in CM in a highly coordinated process; specifically, the entire reaction center (RC) of PSII is assembled in PDM, outside of TM. **C** Linear Electron Transfer. LET is represented in blue. Light energy is captured by phycobilisomes, composed of PE, PC and APC, before being transmitted to the chlorophyll present in the RC of PSII. Electrons originating from the dissociation of H_2O are transferred to FNR, passing through PQ, Cytb6f, PC, PSI and Fd. **D** Alternative electron flow. The cyclic electron flow (light blue) reduces PSI components while bypassing PSII, giving electrons directly to PQ and creating ΔpH; the cyclic pathway uses the NDH1 (blue) complex to generate ΔpH; the pseudo-cyclic flow (yellow) transfers electrons to FLV to reduce O_2 to H_2O, with electrons being cyclically transferred back to PSI via Cytb6f, generating ΔpH; the PTOX pathway (green) reduces O_2 to H_2O using electrons directly from NADPH via NADPH reductase; the Mehler reaction (pink) involves reduction of O_2 to H_2O, producing different reactive oxygen species (ROS) such as O_2^- or H_2O_2. The Mehler reaction can also generate a ΔpH across TM, although the mechanism remains unclear; the sulfide pathway, AnOxyP, (orange) oxidize H_2S using SQR, and electrons are transferred to Cytb6f before reaching PSI, generating ΔpH and NADPH. PG phosphatidylglycerol (blue), MGDG monogalactosyldiacylglycerol (green), DGDG digalactosyldiacylglycerol (yellow), SQDG sulfoquinovosyldiacylglycerol (Red). APC Allophycocyanin, PC in blue: Phycocyanin, PE Phycoerythrin, PSII Photosystem II, PSI Photosystem I, Cytb6f Cytochrome b6f complex, PQ plastoquinone, PC in yellow plastocyanin, Fd ferredoxin. NDH1 NADH dehydrogenase complex 1, PDM PratA-defined membrane, FNR ferredoxin-NADP$^+$ reductase, FLV flavodiiron proteins, SQR sulfide quinone oxidoreductase, PTOX Plastid Terminal Oxidase. Data from refs. 4,10,21,35,37,47,60,81.

Table 1 | Open questions in the emergence of thylakoid membranes and the migration of photosynthetic complexes and electron carriers

1.1	**What is the role of VIPP1 in TM biogenesis: formation of intermembrane channels or assembly factor of PSI?**
1.2	What is the distribution in basal cyanobacteria of other proteins potentially involved in membrane trafficking and dynamics (DedA proteins, SPFH proteins, dynamin-like proteins, CPSAR1, THF1, and CPRabA5e)?
1.3	Is it possible to identify new assembly factors, notably using transcriptomics following Huokko's[47] protocol, particularly for ATPase?
1.4	How did the N-terminal sequence of plastocyanin, targeting it to the thylakoid lumen, evolve from the ancestral *Gloeobacterales*-like state?
1.5	How are *SecA*, *CtaA* and *PacS* integrated into TM, and what are the differences between *Gloeobacter* and *Phycobacteria*?
1.6	What is the evolutionary history of the solanesyltransferase *Slr0926*?
1.7	How is sulfide-quinone reductase integrated into TM, and what are the underlying evolutionary mechanisms?
1.8	What is the evolutionary history of flavoproteins *Flv1* and *Flv3* in basal cyanobacteria?
1.9	What is the distribution and evolutionary history of type II NAD(P)H dehydrogenase proteins, as well as cytochrome bd-quinol oxidase (*Cyd*) and plastid terminal oxidase (*PTOX*), in basal cyanobacteria?
1.10	What is the evolutionary origin of SSR2016 and SSL1217, which allow cyclic electron flow?
1.11	Which factors explain the migration of photosynthetic complexes and electron carriers—was it influenced mainly by euxinia and oxygen-independent electron flows, or did oxygen-utilizing electron flows also play a role?
1.12	What is the distribution of assembly factors of photosynthetic complexes in basal cyanobacteria, and can their structural differences explain the transition between *Gloeobacterales* and *Phycobacteria*?

been. In addition to the linear electron transfer chain of the oxygenic photosynthesis (Fig. 2C), alternative electron flows (Fig. 2D) also operate in modern TM, notably to compensate for the ATP/NADPH imbalance of oxygenic photosynthesis[60]. Among the alternative electron flows described in eukaryotic chloroplasts, three of them (Mehler, pseudocyclic, NDA2-PTOX; see Fig. 2D) use oxygen as terminal electron acceptor to generate ATP. The water-to-water reaction, also called the Mehler reaction, requires the fewest structural transfers of all electron flows and produces hydrogen peroxide, which is subsequently converted to H_2O by a peroxidase[61]. Catalase-peroxidase activity in cyanobacteria has been demonstrated in *Synechocystis*, notably via a thioredoxin peroxidase[61], suggesting that the Mehler reaction indeed occurs in cyanobacteria and can generate a proton gradient, as in eukaryotic chloroplasts, even if the ATP-generating process in itself in unknown[60]. Catalase-peroxidase activity has never been studied in basal cyanobacteria. The pseudocyclic pathway, also called Mehler-like, requires the presence of flavoproteins (FLV) and converts oxygen into water[62]. Flv1 and Flv3 are involved in this pathway in *Synechocystis*[63], where O_2 photoreduction via flavoproteins reaches about half the rate of oxygenic photosynthesis[64], without producing reactive oxygen species[63], making it an important ATP-generating pathway. Flv1 and Flv3 are present in *Gloeobacter*[65], which suggests that a Mehler-like reaction could already have operated in the *Gloeobacterales*-like ancestral state (Tables 1, 1.8). The NDA2-PTOX pathway is also present in cyanobacteria. In *Synechocystis*, three type II NAD(P)H dehydrogenases, NdbA, NdbB, and NdbC, are known[66], and PQH_2-reduced terminal oxidases, cytochrome bd-quinol oxidase (Cyd) and plastid terminal oxidase (PTOX)[66], have been identified. While NdbA has been shown to localize in TM[67], the evolution of type II NAD(P)H dehydrogenases in basal cyanobacteria has never been studied (Tables 1, 1.9). The cyclic electron flow, mediated in *Chlamydomonas* by the transmembrane proteins PGR5 and PGRL1[60], represents another source of ATP, also reaching half the maximum flux of oxygenic photosynthesis[68]. In *Synechocystis*, Ssr2016[69] and Ssl1217[70] perform analogous functions to PGR5 and PGRL1, respectively. However, the distribution and evolution of these proteins have not been investigated yet in basal cyanobacteria (Tables 1, 1.10). Another alternative cyclic electron flow involves NDH1, which is present in TM[71]. Finally, many cyanobacteria, notably basal lineages such as *Thermostichales*[25], *Pseudanabaenales*[72], *Gloeomargaritales*[25] and *Acaryochloridales*[25], are able to perform anoxygenic photosynthesis via a sulfide-quinone reductase (SQR). Two types of SQR are found in cyanobacteria: type I forms a cyanobacterial subgroup within bacteria[73], while type II has its origin in Alphaproteobacteria[74]. SQR is an evolutionarily ancient enzyme, widespread during the Proterozoic, with a complex evolutionary history including multiple horizontal gene transfers[75], notably within cyanobacteria, as observed in *Thermostichales*[25]. Interestingly, SQR I is encoded by a plasmid in *Synechocystis*[74], unlike in *Geitlerinema*[73]. The SQR enzyme would have enabled the only alternative electron flow contributing to NADPH production. It is important to note that the common denominator of these alternative electron flows is the presence in TM of at least two complexes, PSI and ATPase, which play a central role (Fig. 2D).

Evolutionary hypothesis of thylakoid emergence

To make sense of these observations, I propose an evolutionary model for the origin of TM where intermediate states were primarily associated with alternative electron flows. Structurally, such an organism would have possessed a primordial TM (PriTM) dedicated to alternative electron flows, while maintaining a complete linear electron flow for oxygenic photosynthesis within its CM. In all the scenarios considered in the remaining of this manuscript, PriTM contains at minimum PSI and ATPase. Initially, PriTM may have served simpler functions, such as storage, before complexes transfer and electron carriers relocation, after which the membranes were co-opted for photosynthetic electron transport.

The current paradigm, although not formally theorized, implies that TM are associated with oxygenic photosynthesis, and alternative electron flows—notably involving SQR—are considered secondary. Nevertheless, this modern state requires the transfer of at least four complexes and relocation of two electron carriers, without considering SQR and NDH1 (Fig. 3). Except for the Mehler reaction, which only requires the transfer of ATPase and PSI, alternative electron flows can produce ATP and/or NADPH with the transfer of just three complexes and relocation of one electron carrier. The reducing power of NADPH, generated by anoxygenic photosynthesis, would have provided a metabolic advantage for PriTM, while ATP from other alternative electron flows may have been sufficient to sustain essential cellular functions, such as heterotrophy. Most possible intermediates that rely on alternative electron flows require fewer transfers compared to the modern state; Fig. 3 provides a complete overview of the migration sequence across the different hypothetical intermediate evolutionary states. It is crucial here to make the distinction between the transfer of a complex—which requires numerous assembly factors—and the relocation of electron carriers or the SQR enzyme, which rely on a much simpler machinery. Consequently, alternative electron flows, including anoxygenic photosynthesis, may have been transferred more quickly than oxygenic photosynthesis during the evolution of TM.

This PriTM would have been situated at the periphery of the cell due to the fact that the biogenetic process is parietal[60]. From an ultrastructural perspective, this cell would have resembled modern cyanobacteria, with one exception. The nature of the phycobilisomes is uncertain, as studies,

Table 2 | List of 64 known assembly factors

Name	Complex	Localization	Function	Taxonomic distribution
Ycf3	PSI	TM[86]	Interaction with PsaA and PsaD, PSI stromal ridge assembly[87]	Chlamydomonas, Arabidopsis, slr0823 in Synechocystis[86]
Ycf4	PSI	TM[86]	Essential for PSI assembly, act as a scaffold, interact with PsaA, PsaB, PsaC, PsaD, PsaE, PsaF[86,88]	Chlamydomonas, Arabidopsis, sll0226 in Synechocystis[86]
Ycf37	PSI	?	PSI assembly or stability[89]	Chlamydomonas & Synechocystis[89]
Y3IP1	PSI	TM[86]	Interact/receptor of YCF3[90]	Arabidopsis, absent in Chlamydomonas & Cyanobacteria[86]
PPD1	PSI	L[91]	Folding of PsA and PsaB in TM; mutant unable to perform photosynthesis[91]	Arabidopsis[91]
Psa2	PSI	L[92]	Interact with PsaG, PSI assembly[92]	Arabidopsis Absent in Cyanobactreia[91,92]
RubA	PSI	TM[86]	PSI assembly[93]	Arabidopsis & Synechocystis[86]
Hcf101	PSI	S[86]	Scaffold for iron-sulfur cluster, assembly of mature PSI[94]	Arabidopsis & Chlamydomonas[86]
CnfU	PSI	?	Scaffold for iron-sulfur cluster, biogenesis of ferredoxin and& PSI[95]	Arabidopsis & Nifu-like protein in Cyanobacteria[95]
APO1	PSI	S[86]	Scaffold for iron-sulfur cluster[96]	Arabidopsis[86]
Ycf51	PSI	TM & S[97]	Interact with ycf3 and PsaC[97]	Synechocystis, absent in Arabidopsis and Chlamydomonas[97]
ChlG	PSII	CM, PDM[37]	Chlorophyll synthase, association of chlorophyll to pD1[39]	Synechocystis[39]
CtpA	PSII	PDM[37]	Cleavage of the C-terminal extension of pD1[38]	Synechocystis[38]
CyanoP, PsbP	PSII	L[37]	Stabilization of PSII-psb27 complex[37], bind to D2 pre-complex, RC assembly[43]	Arabidopsis[37] Synechocystis[43]
HliA	PSII	?	Association with CP47, chlorophyll delivery[98]	Arabidopsis Synechocystis[98]
HliB	PSII	?	Association with CP47, chlorophyll delivery[98]	Arabidopsis, Synechocystis[98]
HliC,	PSII	CM, PDM[37]	Chlorophyll delivery to D1[39]	Arabidopsis, Synechocystis[39]
HliD	PSII	CM, PDM[37]	Chlorophyll delivery to D1[39]	Arabidopsis Synechocystis[39]
Pitt	PSII	PDM, TM[37]	Chlorophyll synthesis[40]	Arabidopsis, Synechocystis[40]
Pam68, sll0933	PSII	?	Chlorophyll delivery to D1 & D2[43], binding CP47 to RC[37]	Arabidopsis, Synechocystis[21,43]
PratA	PSII	PDM[37]	Manganese transport to pD1[33]	Synechocystis[33]
Psb27	PSII	L[37]	CP43 complex stabilization and binding to RC47[37,44]	Synechocytsis[44]
Psb28	PSII	TM[37]	CP47 complex stabilization[99]	Arabidopsis, Cyanobacteria[99]
Psb29	PSII	?	Remove of PSII subunits damaged[43]	Arabidopsis, Synechocystis[43]
Psb32	PSII	TM[37]	Protection from oxidative stress[100]	Synechocystis[100]
Psb34	PSII	?	CP43 binding to RC47[43]	Arabidopsis, Cyanobacteria[43]
Psb35	PSII	?	CP47 complex stabilization[43]	Synechocystis[43]
SecY	PSII	CM[37]	Co-translational pD1 insertion, interaction with YidC[39]	Synechocystis[39]
RubA	PSII	TM[43]	Protection from Photodamage during PSII assembly[43]	Chlamydomonas[43]
Sll0408	PSII	L[37]	PSII monomer formation[101]	Arabidopsis, Cyanobacteria[101]
Sll0606	PSII	CM[37]	Attachment of CP43 and RC47[45]	Synechocystis[45]
Slr0151	PSII	TM[37]	Assembly of PSII under high light[102]	Synechocystis[102]
Slr0144	PSII	?	PSII biogenesis	Synechocystis[21]
Slr0286	PSII	?	D2 folding[103]	Synechocystis[103]
Slr0565	PSII	TM[37]	Interact with PsbO[104]	Arabidopsis[104]
Slr1761	PSII	L[37]	PSII supercomplex assembly[37]	Arabidopsis[37]
Slr2013	PSII	?	D2 folding[105]	Synechocystis[105]
Ycf39	PSII	TM[37]	Chlorophyll delivery to D1[39]	Synechocystis[39]
HCF136/Ycf48	PSII	PDM, TM, L[37]	PSII assembly, RC formation[40,41], required for heterodimerization of PSII RC[42]	Arabidopsis[42] Synechocystis[40,41]
YidC	PSII	PDM, TM[37]	Co-translational pD1 insertion, interaction with SecY[37,39,106]	Alb3 in Chlamydomonas and Arabidopsis, Synechocystis[37,39,106]
LP2	PSII	?	PSII assembly[107]	Chlamydomonas[107]
LPA19	PSII	?	pD1 maturation[108]	Arabidopsis[108]
PsbN	PSII	S[42]	Required for heterodimerization of PSII RC[42]	Arabidopsis[42]
TerC	PSII	TM[42]	Early formation of PSII RC[42]	Arabidopsis Cyanobacteria[42]
HCF164	Cytb6f	TM[86]	Disulfide reductase activity, link to cytb6f accumulation[109]	Arabidopsis, TxlA in Synechocystis [109]L
HCF222	Cytb6f	?	Chaperones activity[110]	Arabidopsis[110]

Table 2 (continued) | List of 64 known assembly factors

Name	Complex	Localization	Function	Taxonomic distribution
NTA1	Cytb6f	TM[110]	Interact with PetB, PetD, PetG, and PetN[110]	Green algae and plants[110]
DEIP1	Cytb6f	?	Interact with Pet A and PetB[111]	Arabidopsis[111]
DAC	Cytb6f	?	Interact with PetD[112]	Arabidopsis[112]
Trxm1-m4	Cytb6f	?	Cytb6f accumulation[113]	Arabidopsis[113]
Alb4	ATPase	TM[114]	Attachment of CFO and CF1[114]	Arabidopsis, Chlamydomonas[114]
Cpn60	ATPase	?	Chaperone, stromal folding, essential for CF1 assembly[115]	Arabidopsis[115]
Cpn20	ATPase	?	Chaperone, stromal folding, essential for CF1 assembly[115]	Arabidopsis[115]
PAB	ATPase	?	Interact with γ subunit[116]	Arabidopsis, Chlamydomonas[116]
CGL160	ATPase	?	CF0 assembly[117]	Arabidopsis, Chlamydomonas, absent in Cyanobacteria[117]
CGLD11	ATPase	?	CF1 assembly, interact with β subunit[115]	Arabidopsis, Chlamydomonas, absent in Cyanobacteria[115]
CCR2	NDH1	?	ndhB translocation[118]	Arabidopsis[118]
PGR3	NDH1	?	petL stabilization, Translation of ndh1 and PetL[118]	Arabidopsis[118]
SIG4	NDH1	?[118]	NDH accumulation in membranes	?
OTP84	NDH1	?	NDH stability, RNA editing activity[119]	Arabidopsis[119]
NDF5	NDH1	?	PnsB2 and PnsB3assembly and PSI-NDH1 association[120]	Angiosperms[120]
Slr1097	NDH1	S[121]	Maturation of NdhI and assembly of NDH-1L[121]	Synechocystis[121]
Ssl3829	NDH1	S[122]	Assembly of NDH1 hydrophilic arm[122]	Synechocystis[122]
Ssl3451	NDH1	S[123]	Interact with Ssl3829, accumulation of NAI130 and NAI300[123]	Synechocystis[123]

Those for which cyanobacterial and eukaryotic homologues have been identified for a long time are shown in the same row.

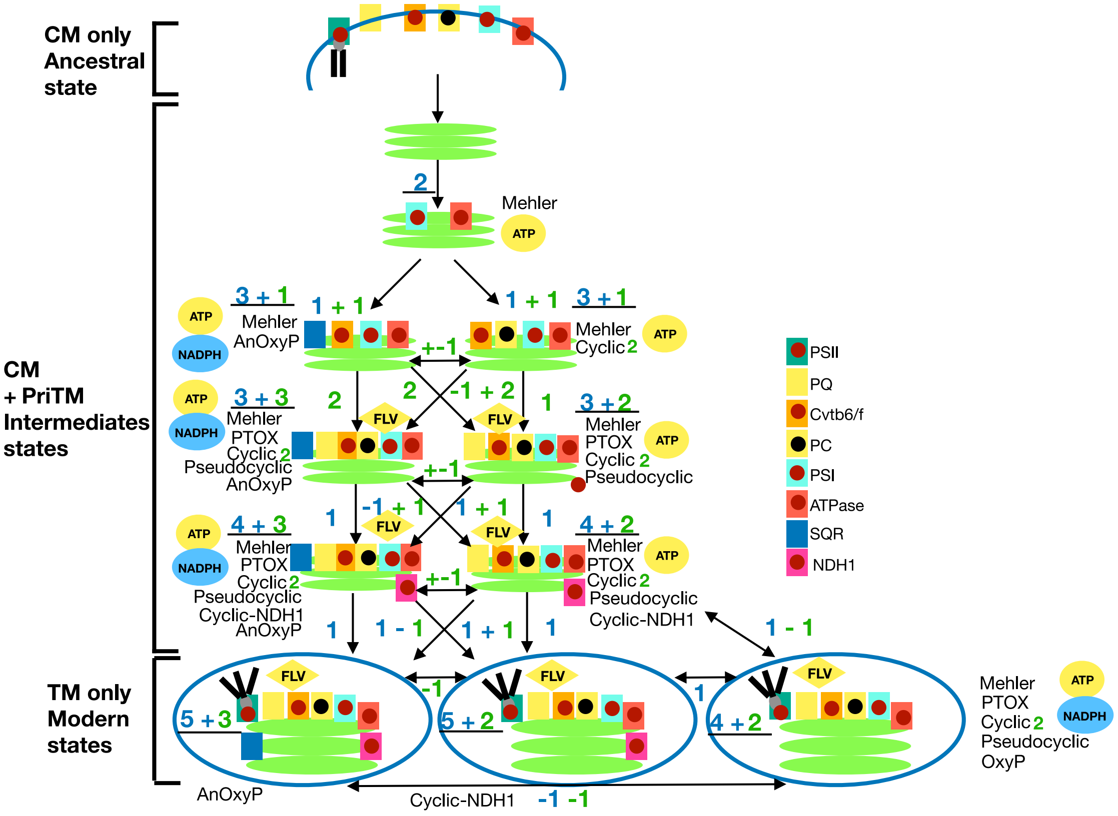

Fig. 3 | Hypothetical evolutionary states in the migration of photosynthetic complexes and electron carriers. Possible transitions from the ancestral *Gloeobacterales*-like state to the modern TM state. Complexes are represented with red dots, electron carriers in yellow, with plastocyanin distinguished by a black dot, and the SQR enzyme is shown in blue. The number of transfers, in terms of losses or gains, is shown in blue for complexes and in green for electron carriers and the SQR enzyme. Underlined numbers correspond to events required directly from the ancestral state to the intermediate state, whereas non-underlined numbers represent events between intermediate states. The advantages of evolutionary intermediate states are indicated by the presence of ATP and NADPH. Three modern states are considered, with or without NDH1 and SQR.

notably recent cryo-electron microscopy, have revealed that present-day *Gloeobacterales* possesses phycobilisomes in bundle-shaped[18] or paddel-shaped[76] forms, whereas most of *Phycobacteria* display hemidiscoidal phycobilisomes[77].

The emergence of *Phycobacteria* is estimated to have occurred slightly before the GOE[8,9,78] or during the early Proterozoic[79]. This geological timespan enclosed the transition from an anoxic ocean to a post-GOE oxic ocean, but marked by euxinic episodes—i.e., anoxic and rich in sulfide—as evidenced by sulfur isotope ratios and the presence of FeS_2 in rocks[80]. However, sulfide poses a significant challenge to oxygenic photosynthesis due to its toxicity as a potent inhibitor of PSII[81–83], responsible for water splitting, although the underlying mechanism of this toxicity is unknown. In this context, a PriTM capable of generating ATP and potentially NADPH, would have provided a key evolutionary advantage for the survival of intermediate-state cyanobacteria. Cyclic electron flows with or without NDH1 are the two pathways able of such a generation under both euxinic (i.e., anoxic condition) and oxic conditions. Alternative flows using oxygen (Mehler, pseudocyclic, NDA2-PTOX; see above) as the terminal electron acceptor could also have played a role in PriTM, particularly given the high rate of pseudocyclic flow observed in *Synechocystis*. However, this likely occurred in non-euxinic zones or in the chemocline, as oxygen is not available under euxinic condition (Tables 1, 1.11). Anoxygenic photosynthesis, owing to the presence of the SQR enzyme, would have allowed these intermediate states to operate under euxinic conditions only. The paradox of the development of oxygenic photosynthesis in sulfide-rich environments has previously been discussed in the literature[84] and is here addressed by the model of a CM dedicated to oxygenic photosynthesis and a PriTM to alternative flows. For the anoxygenic photosynthesis to have played a role in the proposed evolutionary hypothesis, the sulfide-quinone reductase — I and/or II — must have been lost in a substantial number of cyanobacteria, notably in basal lineages. Indeed, although some basal cyanobacteria carry SQR genes—apart from *Gloeobacterales*, for which no SQR has been reported—the proportion of SQR-containing taxa likely did not constitute a majority, although this has not been systematically investigated. Furthermore, the group that directly follows the evolutionary intermediates hypothesized here, the *Thermostichales*, appears to have acquired SQR types I and II through secondary lateral transfer[25]. Consequently, it is plausible, given the complex evolutionary history of this gene family, that an SQR was acquired in an intermediate evolutionary state and subsequently lost in many descendant taxa.

The alternative possibility of a transfer of PSII before PSI into PriTM does not appear to confer any evolutionary advantage without the remaining of the linear electron transfer chain and is therefore not favored here. However, a late transfer of PSII would have made oxygenic photosynthesis functional in TM and potentially triggered the GOE due to its radically increased efficiency. From an evolutionary perspective, the apparition of PriTM is proposed to have occurred after the separation of *Gloeobacterales* but before the diversification of *Phycobacteria* (Fig. 1). Future evolutionary studies on assembly factors, listed in Table 2, should help refining this hypothesis (Tables 1, 1.12). Analyses of their distribution in cyanobacteria, their evolution, especially in basal cyanobacteria, as well as the molecular dating of these assembly factors, should give insights into the transfer sequence. Table 1 lists the open evolutionary questions discussed in this manuscript that would lead to the validation or the refutation of the evolutionary hypothesis presented here.

In conclusion, the emergence of PSI-equipped PriTM dedicated to alternative electron flows facilitated ATP and potentially NADPH production, notably enabling survival in sulfide-rich environments of early earth. While these adaptations may not have directly supported long-term fitness, they represented a critical intermediate state toward the development of the complex photosynthetic systems of modern cyanobacteria.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

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Author contributions

L.C. wrote the manuscript and drew the figures.

Competing interests

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Additional information

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