On the Non-Oxygenic Origins of Thylakoids

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Abstract

Recent studies have shed light on the emergence and evolution of oxygenic photosynthesis. It is likely that the ancestor of Terrabacteria already possessed two distinct reaction centers, and that the ability to split water via photosystem II arose early in their evolution. Consequently, it is equally probable that the emergence of thylakoid membranes played a crucial role in the accumulation of oxygen in the atmosphere. However, the study of thylakoid emergence remains less explored than the origin of photosystems. With a highly synchronized process involving numerous assembly factors, the biogenesis of thylakoid membranes suggests the existence of intermediate evolutionary states in the emergence of these compartments. Constrained by the sulfide-rich environment of the Archean, I propose a non-oxygenic origin of thylakoid membranes, which would have been initially devoted to alternative electron flows.

Main text

Recent findings have significantly advanced our understanding of the origins of oxygenic photosynthesis (OxyP) and oxygenation of Earth during the Great Oxidation Event (GOE), 2.4 Gya (Bekker et al. 2004). Nishihara et al. (2024) phylogenomic analysis based on DNA-RNA binding proteins, along with phylogenetic trees of bacteriochlorophyll and chlorophyll synthetases, revealed that the common ancestor of Terrabacteria—a group of bacteria considered to be associated with terrestrial colonization, which includes cyanobacteria (Battistuzzi and Hedges 2009)—already possessed two homodimeric photosystems (PS), was capable of CO_2 fixation, and performed anoxygenic photosynthesis (Nishihara et al. 2024). In such a scenario, photosynthesis evolved among Terrabacteria through a series of gene losses, so that today, cyanobacteria are the only prokaryotes that retain both photosynthetic reaction centers (RC) (Nishihara et al. 2024). One key characteristic of cyanobacteria is that the PSII D1 subunit, containing a Mn_4CaO_5 cluster, is responsible for water splitting and oxygen release as a by-product (Oliver et al. 2023). This capability of D1 must have evolved between the emergence of the common ancestor of Terrabacteria and cyanobacteria, though the exact timeframe remains unclear (Nishihara et al. 2024). However, recent evidence suggests that a homodimeric form of PSII, as opposed to the heterodimeric form of modern cyanobacteria, may have been capable of water oxidation at least one billion years before cyanobacteria emerged (Cardona et al. 2019; Oliver et al. 2021; Oliver et al. 2023) which is estimated to have occurred between 2.5 Ga (Boden et al. 2021) and 3.1 – 3.3 Ga (Shih et al. 2017). Altogether, these findings imply that sister group to cyanobacteria, such as Vampirovibrionales (Soo et al. 2017; Soo et al. 2019) and Sericytochromatia (Parks et al. 2017), were once photosynthetic and may have even been capable of water splitting (Oliver et al. 2021). The existence of such an organism before the current root of cyanobacteria raises an important question about the GOE: could its later onset have been delayed by the absence of some crucial structure?

In most cyanobacteria, OxyP occurs in the membrane of dedicated cellular compartments, the thylakoids. However, one group of cyanobacteria, the Gloeobacterales, lack thylakoid membranes (TM) and performs OxyP in specialized regions of the cytoplasmic membrane (CM) (Rippka et al. 1974; Guglielmi et al. 1981; Rexroth et al. 2011; Rahmatpour et al. 2021). Interestingly, Gloeobacterales represent the earliest-diverging cyanobacterial lineage, as confirmed by multiple phylogenomic analyses (Criscuolo and Gribaldo 2011; Shih et al. 2013; Soo et al. 2014; Uyeda et al. 2016).

The structural advantage offered by TM, in terms of the number of PS per cell, compared to the ancestral state still observed in Gloeobacterales, has recently been hypothesized as a plausible explanation for the GOE (Guéguen and Maréchal 2022). Nevertheless, despite extensive studies on the origin of OxyP and PS, the evolution of TM has been relatively understudied so far. Tan et al. (2024) recently investigated TM emergence by analyzing metagenome-assembled genomes (MAGs) from Thermostichales, the lineage immediately following Gloeobacterales in the cyanobacterial phylogeny

(Tan et al. 2024). A duplication of the *PspA* gene, further equipped with a C-terminal extension, occurring in the last common ancestor of cyanobacteria with TM led to the emergence of the VIPP1 protein, which is thought to be involved in the biogenesis of TM (Tan et al. 2024). The TM consist of three key glycolipids, also found in CM: monogalactosyldiacylglycerol (MGDG, >50% of lipid content), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol (SQDG), the latter absent in Gloeobacterales (Rast et al. 2015). MGDG facilitates TM curvature (Bottier et al. 2007), DGDG stabilizes thylakoid stacking through hydrogen bonding (Demé et al. 2014) whereas the role of SQDG is unclear. The process by which TM are formed, and how their lipid ratios differ compared to those in the CM, has been the subject of three main hypotheses: membrane fusion, vesicular transport, or direct lipid transport via soluble carriers (Jouhet et al. 2007; Rast et al. 2015). The involvement of VIPP1, a vesicle-inducing protein, seems to favor the second hypothesis. Nevertheless, the role of VIPP1 in cyanobacteria remains to be clarified: it is indeed essential for TM biogenesis in *Synechocystis* sp. PCC 6803 (Gao and Xu 2009), but not in *Synechococcus* sp. PCC 7002 (Zhang et al. 2014). In addition to VIPP1, other proteins present in cyanobacteria (CPSAR1 (Garcia et al. 2010), THF1 (Wu et al. 2011), CPRabA5e (Karim et al. 2014)) have been identified as crucial for TM lipid biogenesis in Arabidopsis, although their role in cyanobacteria has never been investigated.

The transition from the ancestral Gloeobacter-like state, with a linear electron transfer (LET) chain functioning in the CM, to the modern state, where all components are localized in TM, goes beyond the formation of lipidic membranes. Indeed, the integration of LET complexes into TM during biogenesis is a highly regulated stepwise process involving numerous assembly factors (AF) that act as chaperones, notably for transporting PS subunits from CM to TM (**Figure 1**) (Rast et al. 2015). The most studied element is PSII, whose RC biogenesis starts in CM with the precursor of D1, pD1. D1 transitions through the *pratA*-defined membrane (PDM), where D1 assembles with D2, leading to RC photoactivation (Klinkert et al. 2004; van de Meene et al. 2006; Rast et al. 2019). PDM, named after the *pratA* gene, also depends on *curT* (Rast et al. 2019) and *ancM* (Ostermeier et al. 2022), and serves as a contact point between TM and CM, playing a functional role in TM biogenesis (Rast et al. 2019). Once the PSII RC is assembled, it is first incorporated into TM with the CP43 and CP47 antenna complexes, the PSII subunits (Q, U, O, V), then dimerizes and associates with the phycobilisome on the outer TM face (Heinz et al. 2016). Thirty-four AF, among which twelve also known in cyanobacteria, coordinate this process (**Table 1**). Elements of PSI have been identified in CM, particularly PsbA (Rast et al. 2015; H. Yang et al. 2015), but no evidence suggests that the PSI RC is assembled and activated outside the TM. While the stepwise assembly of PSI is less understood, at least thirteen AF are also associated with it, with only one detected outside the TM (**Table 1)**, rather supporting early PSI integration within TM during evolution. Some subunits of Cytb6f and ATPase complexes have also been detected in the CM, hinting to stepwise assembly, but few corresponding AF have been reported so far (**Table 1)**.

The complexity of the biogenesis process suggests intermediate states, which have never been studied or even theorized but likely existed in the past and ultimately led to TM as we know them today. The emergence of cyanobacteria with TM occurred around the GOE (Sánchez-Baracaldo et al. 2017; Fournier et al. 2021; Sánchez-Baracaldo et al. 2022) or in early Proterozoic (Shih et al. 2017), in a sulfide-rich environment (Scott et al. 2011), although sulfide is highly toxic to OxyP as a known inhibitor of PSII (Cohen et al. 1986; Garcia-Pichel and Castenholz 1990; Miller and Bebout 2004). To address this contradiction, I propose an evolutionary model for the origin of the TM (**Figure 1**), where these intermediate states were linked to the alternative electron flow (AEF). Indeed, complete LET integration requires the highest number of complexes to be transferred from CM to TM, with six complexes, except for NDH1-related AEF. By contrast, AEF involves between two and six complexes (**Figure 2**). It is likely that there was once an ancestral organism possessing a primordial TM system dedicated to AEF, such as anoxygenic photosynthesis (AnoxyP), while maintaining a complete LET in its CM. Such an intermediary state would have provided a key evolutionary advantage for the survival of early cyanobacteria. Interestingly, the very recent discovery of the sulfide quinone oxidoreductase (SQR) gene, involved in anoxygenic H_2S-related photosynthesis, in the early-diverging Thermostichales (Tan et al. 2024), further lends supports to this hypothesis

Figures & Table

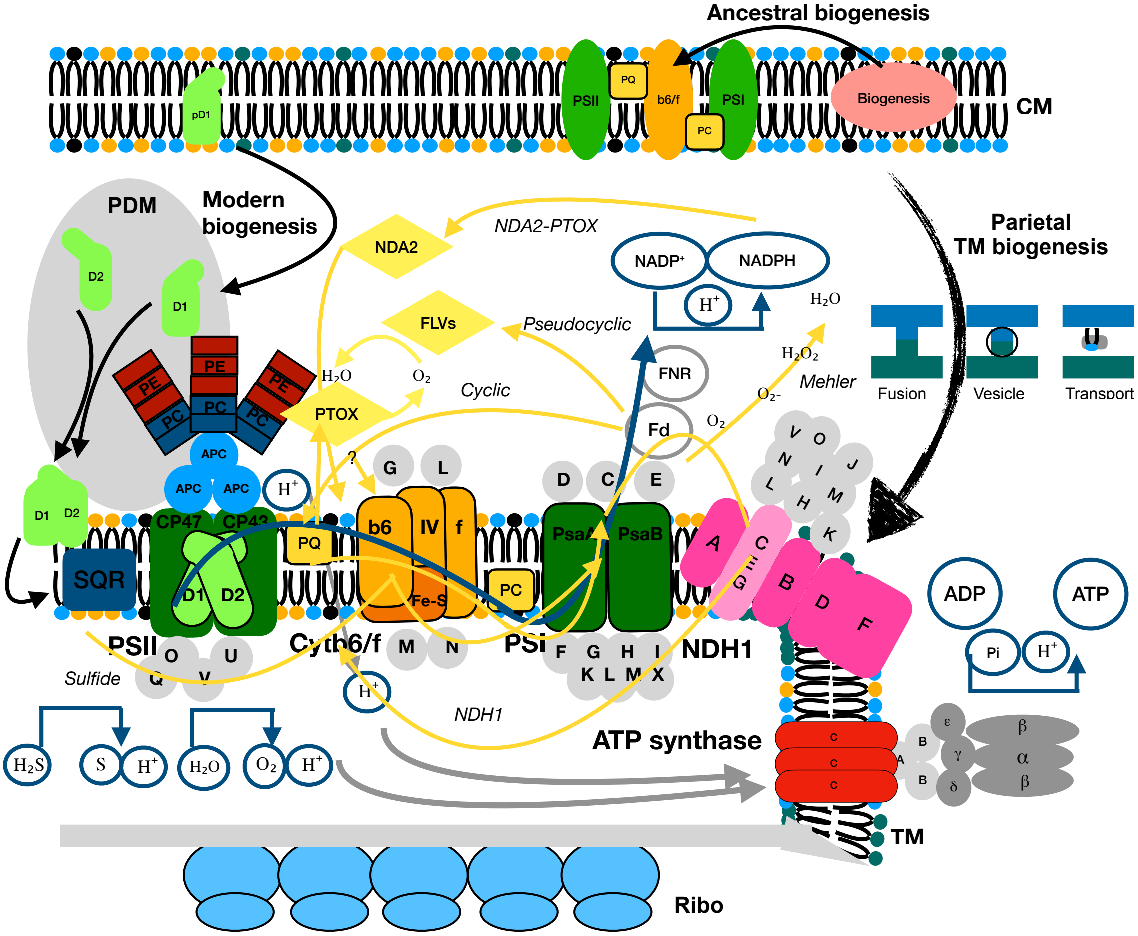
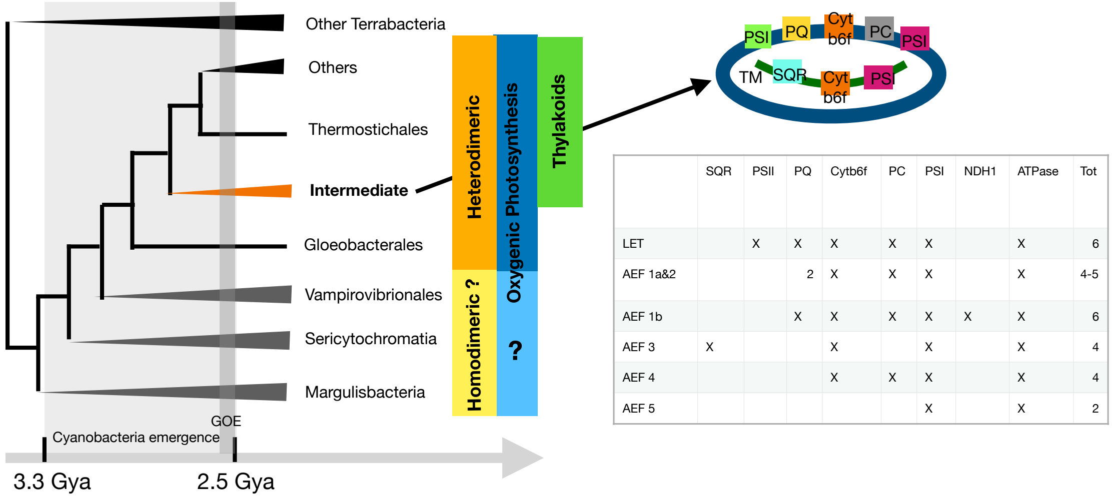


Figure 1: Schematic view of thylakoid membrane biogenesis and composition, along with LET and AEF.

CM and the **TM** are composed of the same lipidic components: **PG**, **MGDG, DGDG**, with proportions varying between the two membranes. **SQDG** are not represented due to their unclear role and absence in Gloeobacterales. **MGDG** is involved in the curvature of **TM**. Three scenarios have been proposed for the biogenesis of **TM** from **CM**. In the primitive 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**. The outer face of TM, on the cytosolic side, is rich in ribosomes (**Ribo**). **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**. **AEF** are represented in yellow and include: **1a**. **the cyclic electron flow**, which reduces PSI components while bypassing PSII, giving electrons directly to PQ and creating ΔpH; **1b**. the cyclic pathway which also utilizes the **NDH1** complex to generate ΔpH; **2. the pseudo-cyclic flow**, where electrons are transferred to **FLV** to reduce O_2 to H_2O, with electrons being cyclically transferred back to PSI via **Cytb6f**, generating ΔpH; **3. the sulfide pathway (AnoxyP)**, where H_2S is oxidized by **SQR**, and electrons are transferred to **Cytb6f** before reaching PSI, generating ΔpH; **4.** the pathway utilizing **PTOX**, which reduces O_2 to H_2O using electrons directly from PQ after excitation of PSII or **4b** from NADPH via NADPH reductase; **5. the Mehler reaction** which 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 thylakoid membranes, although the mechanism remains unclear. **TM** Thylakoid membrane, **CM** Cytoplasmic membrane, **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, **APC Allophycocyanin**; **FNR** ferredoxin-NADP$^+$ reductase, **FLV** flavodiiron proteins**, SQR** sulfide quinone oxidoreductase, **PTOX** Plastid Terminal Oxidase**, Lipids: PG** phosphatidylglycerol (blue), **MGDG** monogalactosyldiacylglycerol (green**), DGDG** digalactosyldiacylglycerol (yellow), **SQDG** sulfoquinovosyldiacylglycerol, **Ribo** Ribosome, **LET** Linear Electron Transfer, **AEF** Alternative Electron flow. Modified from (Cohen et al. 1986; Blankenship 2010; Rast et al. 2015; Heinz et al. 2016; Rast et al. 2019; Huokko et al. 2021; Eckardt et al. 2024).



	SQR	PSII	PQ	Cytb6f	PC	PSI	NDH1	ATPase	Tot
LET		X	X	X	X	X		X	6
AEF 1a&2			2	X	X	X		X	4-5
AEF 1b			X	X	X	X	X	X	6
AEF 3	X			X		X		X	4
AEF 4				X	X	X		X	4
AEF 5						X		X	2

Figure 2: Evolutionary Hypothesis of Thylakoid Membranes.

The evolutionary tree of cyanobacteria and their allies is depicted to highlight 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. The complexity of thylakoid biogenesis suggests the existence of intermediate evolutionary states. The hypothesis involving the fewest transfers of complexes between CM and

TM, and the most likely given the sulfide-rich environmental conditions of the Archaean era, points to alternative electron flows (AEF). The table summarizes the number of complexes involved in the different AEF.

Complexes	Assembly factors
PSI (13)	**Alb3 (PDM, TM) ; VIPP1 (TM) ; Ycf3 (TM) ; Ycf4 (TM) ; Ycf37 (TM)** ; Y3IP1 (TM) ; PPD1 (TM) ; Psa2 (L) ; **RubA (TM) ; Hcf101 (S) ; CnfU (S)** ; APO1 (S); **Ycf51 (TM)**
PSII (34)	**ChlG (CM, PDM) ; CtpA (PDM, CM); CyanoP (CM) ;** HliA, HliB (PDM) **; HliC, HliD (PDM) ;** Pittc (-) **;** Pam68 (PDM, TM) **; PratA (PDM) ; Psb27 (TM) ; Psb28 (TM) ;** Psb29 (-) **;** Psb32 (TM) **; Psb34 (-) ;** Psb35 (PDM) **; SecY (PDM) ;** RubA (CM) **;** Sll0408 (L) **;** Sll0606 (CM) **; Sll0933 (TM) ;** Slr0151 (TM) **;** Slr0144 (-) **;** Slr0286 (-) **;** Slr0565 (CM) **;** Slr1761 (L) **;** Slr2013 (TM, PDM) **; Ycf39 (PDM) ; Ycf48 (PDM, TM) ; YidC (PDM, TM) ;** LP2/3 (TM) **; LPA19 (L) ;** PsbP (PDM) **;** PsbN (PDM,CM)
Cytb6f (4)	HCF164 (-); trxm134 (-) ; NTA1 (-) ; DEIP1 (-)

Table 1: List of 51 known assembly factors.

AF in bold are found in cyanobacteria, all AF at the exception of Ycf51 are also found in eukaryotic chloroplasts. Localization of the AF are indicated in parentheses. Modified from (Chi et al. 2012; Rast et al. 2015; C.-C. Yang et al. 2015; Heinz et al. 2016; Johnson and Pakrasi 2022; Sandoval-Ibáñez et al. 2022; Li et al. 2023; Chen et al. 2024; Dai et al. 2024)

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Conflict of Interest

The author declare no competing interest.

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