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The Evolution and Evolvability of Photosystem II

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Abstract

Photosystem II is the water-oxidizing and O_2-evolving enzyme of photosynthesis. How and when this remarkable enzyme arose are fundamental questions in the history of life that have remained difficult to answer. Here, recent advances in our understanding of the origin and evolution of photosystem II are reviewed and discussed in detail. The evolution of photosystem II indicates that water oxidation originated early in the history of life, long before the diversification of cyanobacteria and other major groups of prokaryotes, challenging and transforming current paradigms on the evolution of photosynthesis. We show that photosystem II has remained virtually unchanged for billions of years, and yet the nonstop duplication process of the D1 subunit of photosystem II, which controls photochemistry and catalysis, has enabled the enzyme to become adaptable to variable environmental conditions and even to innovate enzymatic functions beyond water oxidation. We suggest that this evolvability can be harnessed to develop novel light-powered enzymes with the capacity to carry out complex multistep oxidative transformations for sustainable biocatalysis.

Contents

1. INTRODUCTION

One of the most remarkable enablers of life is the capacity to harness light as an energy source. Photosynthesis has sustained a thriving biodiversity for billions of years, it has sustained human society through agriculture and the availability of fossil fuels, and many of us are hopeful that photosynthesis holds lessons on, if not solutions for, how to tackle some of the sustainability challenges we must urgently address (83, 138, 154). Despite the omnipresence of photosynthesis in our day-to-day life, its origin remains a mystery. There is very little we know with certainty about its early evolution, and the subject has remained largely speculative for nearly a century (27). Yet today we have achieved a highly detailed understanding of the structure and function of many of the components of the photosynthetic process. This advanced understanding enables us to look at their origin with fresh eyes and to begin piecing together the puzzle of the deep and long history of photosynthesis with ever greater confidence.

In photosynthesis, the photochemical conversion of energy is carried out by photosystems (see the sidebar titled Photosystem Versus Reaction Center). Photosystems are large molecular complexes made up of several protein subunits, pigments, and other cofactors found within cytoplasmic or specialized membrane systems of bacteria or in organelles of bacterial origin in eukaryotes. Broadly speaking, photosystems absorb light, which is then used to trigger a stepwise electron transfer process known as charge separation (31). Reducing equivalents flow through an electron transfer chain that, coupled with proton translocation across the membrane, is used to generate nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) to power the metabolic process of the cell. These metabolic processes can include CO_2 and N_2 fixation.

Charge separation: a process of electron transfer in the photosystem through the chain of cofactors; as the negative charge is transferred to the next cofactor, it becomes physically distanced from the positive one

Two forms of photosynthesis can be distinguished: (*a*) oxygenic photosynthesis, in which a photosystem couples charge separation to the extraction of protons and electrons from water, liberating O_2, and (*b*) anoxygenic photosynthesis, where water oxidation cannot occur, and therefore the ultimate source of electrons and protons is derived from inorganic compounds such as H_2, H_2S, or ferrous iron, none of which are directly oxidized by a photosystem. Several groups

PHOTOSYSTEM VERSUS REACTION CENTER

The terms photosystem and reaction center (RC) can be considered somewhat equivalent but may imply different meanings depending on context or specialism. Often, the expression reaction center has been associated with bacterial reaction centers, or, in other words, with the photochemical complexes used in anoxygenic photosynthesis. Therefore, a researcher might use the expression purple bacterial reaction center to refer to the type II reaction center of the Proteobacteria, or use RC1 or GsbRC to refer to the type I reaction center of the green sulfur bacteria (Chlorobia). The term photosystem has been historically associated with the complexes of cyanobacteria and photosynthetic eukaryotes, although this usage is also not strict. Reaction center can also refer specifically to the part of the complex where the photochemical reactions occur, which is also referred to as the core domain or the reaction center core. Here, we use the term photosystem to mean the full complex, with all its subunits and components, as it is found in a functional state, regardless of whether it is used in anoxygenic or oxygenic photosynthesis. In addition, we use the term photochemical core to refer to the part of the photosystem where charge separation occurs.

of bacteria have photosystems without being coupled to CO_2 fixation, thus requiring organic carbon for growth: These are known as photoheterotrophs. It is worth noting that no other metabolic process is as productive as oxygenic photosynthesis, which is solely responsible for well over 99% of all fixed carbon in the biosphere (109). The photosystem-mediated oxidation of water also made O_2 available to life, which, despite being a by-product, stores much of the energy of photosynthesis (120, 121). It also led to the formation of the ozone layer and the success of aerobic respiration and enabled the rise of eukaryotes. Therefore, in the story of life and the planet itself, the evolution of the photosystems takes center stage.

This review focuses on the evolution of photosystem II (PSII), the water-splitting and O_2-evolving enzyme. Through the analysis of the evolution of PSII, the evolution of other photosystems can also be unraveled. The review focuses on the recontextualization of the origin and evolution of PSII in light of recent advances in the elucidation of photosystem structure and function and in our expanded view of the planet's biodiversity. We emphasize how the evolution of PSII is a continuous process that has never stopped and suggest ways in which the evolvability of the photosystem could be used. For an excellent introduction and primer on the evolution of photosynthesis, the review by Hohmann-Marriott & Blankenship (68) in this journal is recommended.

2. THE EARLY EVOLUTION OF THE PHOTOSYSTEMS

2.1. The Origin of the Photosystems

All known photosystems have a single origin (92). This is unambiguously understood because all photosystems share a common architectural theme that consists of 2 protein units, each totaling 11 symmetrically arranged transmembrane (TM) helices (an important exception is described below). The membrane-embedded protein units, also known as reaction center proteins, bind a set of chlorophyll and/or bacteriochlorophyll pigments used in light harvesting and redox reactions, as shown in **Figure 1**, many of which are placed at conserved positions across all photosystems. The first 8 TM helices from the N terminus bind the pigments used in light harvesting, and the last 3 TM helices bind the pigments directly involved in charge separation, sandwiched between the 2 units.

The photosystems can be divided into the type I photosystems (i.e., type I reaction centers) and the type II photosystems (i.e., type II reaction centers). The two types are distinguished by a large

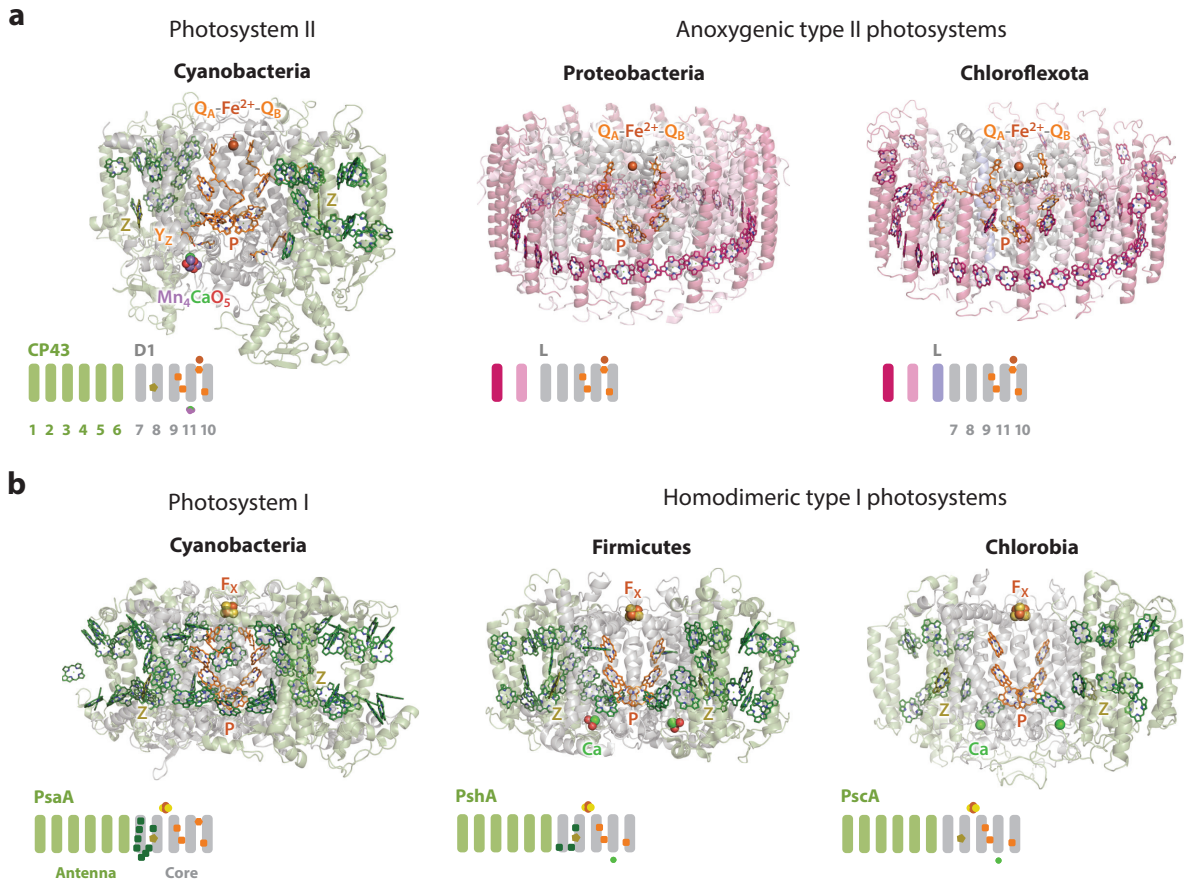


Figure 1

Structural overview of the photosystems: (*a*) type II and (*b*) type I. For clarity, only the conserved photosystem core subunits are shown. The protein scaffold is shown in transparent ribbons. The first 6 TM helices of the core subunit are shown in light green, and the remaining 5 helices are shown in light gray. Chlorophyll and bacteriochlorophyll pigments involved in light harvesting are shown as green sticks, while those involved in photochemical charge separation are shown as orange sticks. A particular antenna chlorophyll/bacteriochlorophyll bound by the eighth TM helix conserved in PSII and the type I photosystems is marked with a Z and shown in olive green. Below each structure, a schematic representation of the TM helices of a monomer are shown (in colors matching the structure above). The positions of chlorophylls and bacteriochlorophylls bound by the last 5 TM helices, as well as some key cofactors, are shown. The cryoEM structure of the anoxygenic type II photosystem of the green nonsulfur bacterium (Chloroflexota) *Roseiflexus castenholzii* featured a unique additional TM helix in the L subunit, marked in light purple, and does not show homology to other TM helices of the photosystems. The light-harvesting complex of the anoxygenic type II photosystems, shown in dark and light pink, have a separate origin to that of the antenna of the other photosystems. For clarity, only the tetrapyrrole ring of the chlorophyll and bacteriochlorophyll molecules is shown. Structures used are PSII from *Thermosynechococcus vulcanus* (Cyanobacteria), PDB ID 3wu2; anoxygenic type II photosystems from *Thermochromatium tepidum* (Proteobacteria), PDB ID 5y5s, and *Roseiflexus castenholzii* (Chloroflexota), PDB ID 5yq7; PSI from *Thermosynechococcus elongatus* (Cyanobacteria), PDB ID 1jb0; and homodimeric type I photosystems from *Heliobacterium modesticaldum* (Firmicutes), PDB ID 5v8k, and *Chlorobaculum tepidum* (Chlorobia), PDB ID 6M32. Abbreviations: cryoEM, cryogenic electron microscopy; PDB ID, Protein Data Bank identification; PSI, photosystem I; PSII, photosystem II; TM, transmembrane.

set of features, the most prominent of which are the nature and arrangement of cofactors involved in accepting electrons. For example, type II photosystems use a Q_A-Fe^{2+}-Q_B structural motif, where Q stands for quinone and Q_B is an exchangeable terminal electron acceptor (**Figure 1**). The exact type of quinone varies from lineage to lineage. By contrast, type I photosystems use a Fe_4S_4 cluster, also known as F_X, as a terminal electron acceptor. There are also structural differences in the folding of the 3 C-terminal TM helices and how some of the redox pigments are oriented, which distinguish both types (29).

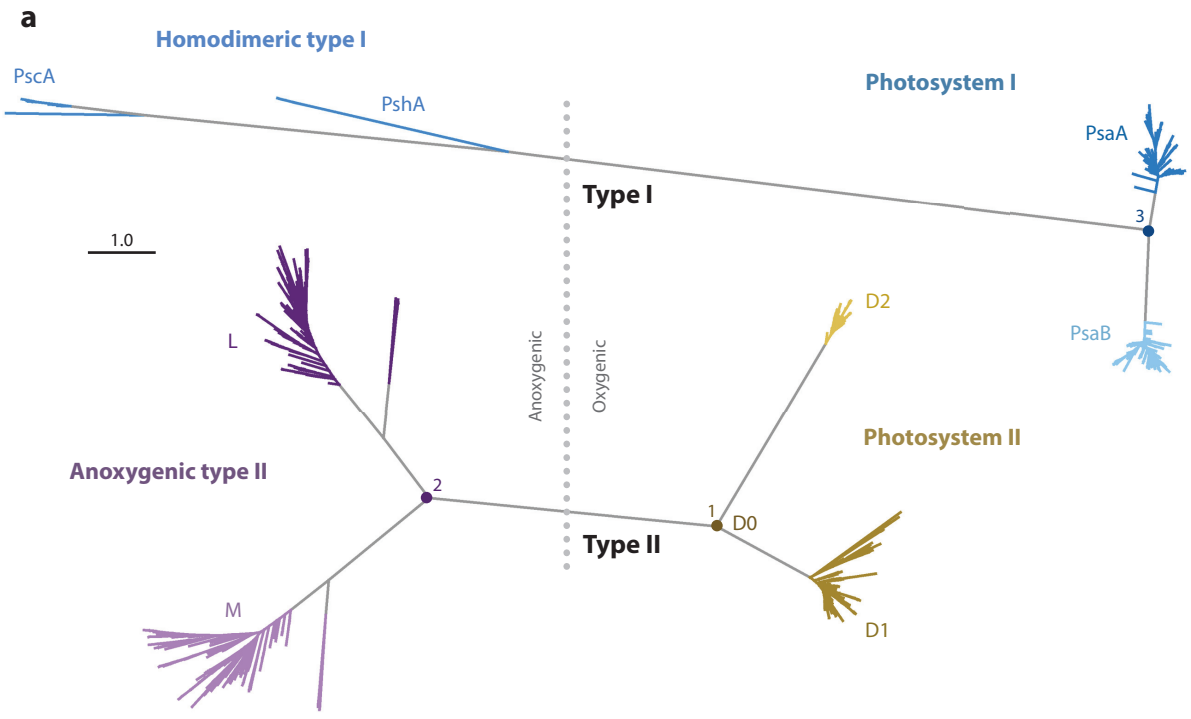
Each photosystem type can also be further divided into two subtypes: those that are used in anoxygenic photosynthesis and those used in oxygenic photosynthesis. All type I photosystems used in anoxygenic photosynthesis are homodimers, meaning that the two protein units, each one a monomer, are identical. Homodimeric type I photosystems retain near-perfect symmetry, and the core subunit is encoded in a single gene. The type I photosystems used in oxygenic photosynthesis are instead heterodimers, meaning that the two monomers are no longer identical and are thus encoded in two separate genes.

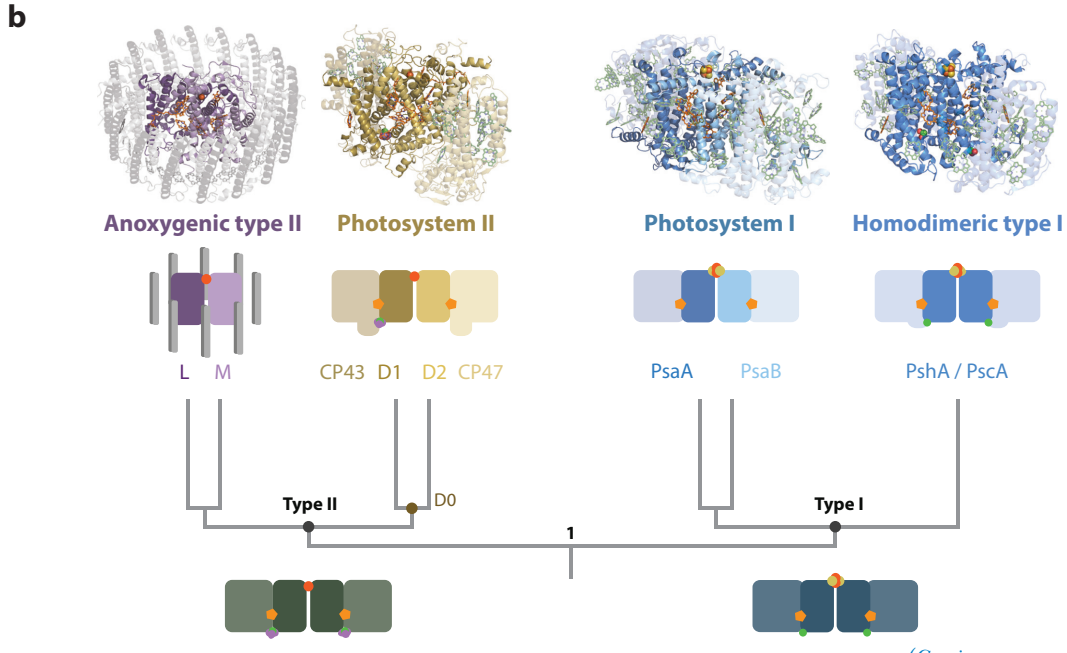
All type II photosystems are heterodimers. The type II photosystem used in oxygenic photosynthesis, PSII, is characterized by the 11 TM helices of a monomer structured as two separate protein subunits. One subunit has the first 6 TM helices binding most, but not all, of the pigments involved in light harvesting, and the other has the remaining 5 TM helices. This arrangement means that the conserved core is encoded in four separate genes. In addition, PSII harbors a catalytic site made up of 4 Mn atoms and a Ca atom bridged by 5 O atoms, usually referred to as the Mn_4CaO_5 cluster. The type II photosystem used in anoxygenic photosynthesis lacks a water-oxidizing complex, and unlike all other photosystems, it also lacks the first 6 TM helices of each monomeric unit. Instead, it uses a unique light-harvesting complex not shared with any other photosystem.

One major difference between anoxygenic and oxygenic photosynthesis is that the former employs either a type I or type II photosystem, but never both, while the latter requires both photosystems working in cooperation (68). The two photosystems of oxygenic photosynthesis are known as photosystem I (PSI) and photosystem II (PSII).

This categorization of the photosystems as either type I or type II is our first approximation of their evolution, as these structural and functional differences reflect phylogenetic relationships (**Figure 2**). In this way, it is possible to confidently infer that one of the earliest stages in the evolution of the photosystems was the acquisition of the traits that led to the specialization of type I and type II photosystems. What cannot be resolved with the same level of confidence are the evolutionary pressures that led to this specialization at an ecological or physiological level. At the same time, these evolutionary relationships, which are structural and functional by their nature, do not firmly indicate that one type is older than the other or that one gave rise to the other. In other words, this stage in the evolution of the photosystems is better described as the rise and parallel differentiation of the two types of photosystems from a single origin.

The second stage, which is well preserved in the phylogenetic record of the photosystems, is the subsequent specialization of each type into the forms now found in anoxygenic and oxygenic photosynthesis. This stage is constrained by three independent and unambiguous gene duplication events, which resulted in the evolution of the heterodimeric photosystem cores (marked as points 1–3 in **Figure 2*a***). In this way, one may deduce that after the emergence of the two types, a deep evolutionary dichotomy occurred, leading to the parallel evolution of photosystem lineages that are specific to anoxygenic and oxygenic photosynthesis. A first-level implication of this deduction is that no direct evidence can be found indicating that one lineage is older than the other or that one gave rise to the other. Therefore, the evolutionary relationship of the photosystems disagrees with the well-accepted paradigm that oxygenic photosynthesis emerged from anoxygenic





(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Evolution of the photosystem core subunits. (*a*) Maximum likelihood phylogenies of type I (*top*) and II (*bottom*) photosystems. The deep dichotomy between anoxygenic and oxygenic lineages is highlighted by the vertical dotted line. Points 1, 2, and 3 mark unambiguous duplication events. No evidence can be found that PSI and PSII, which are required in oxygenic photosynthesis, originated from photosystems used in anoxygenic photosynthesis, but the duplications suggest a history of the oxygenic lineage before the rise and diversification of cyanobacteria. The scale bar denotes amino acid substitutions per site. (*b*) A schematic representation of the phylogenies in panel *a* with added structural detail. Point 1 marks the structural and functional specialization of type I and type II photosystems, occurring before the diversification of phylum-specific subtypes. Structural relationships suggest that the ancestral type II photosystem was more like PSII than the anoxygenic type II photosystem, as discussed in the main text. Abbreviations: PSI, photosystem I; PSII, photosystem II.

photosynthesis (27, 100). This conclusion becomes more evident when the photosystems are compared against each other, considering the new, better resolved, and more accessible structures, as discussed in Section 3. While the occurrence of these duplications has been understood for quite some time without much debate, what they imply for the evolution of photosynthesis has been overlooked, to an extent because of historical reasons (some of which are discussed in Section 2.2).

But how did photosystems originate in the first place? The exact origins of the photosystems remain unknown. The photosystem core subunits have no homology to any other type of protein. In fact, while there is clear sequence identity within each type, only traces of identity remain between the two types. These traces are found at key locations around certain pigments (23, 122, 144). A plausible distant relationship is the proposed evolutionary link with the core subunit *b* of the cytochrome bc/b_6f complex, involved in both photosynthetic and respiratory electron transport chains. This was pointed out by Xiong & Bauer (156), based on partial sequence alignments and structural comparisons with type II photosystems. While the scenarios they provide (156) should be considered with care given the resources available at the time, the structural similarities they identify are intriguing and have not been scrutinized or investigated since then.

2.2. A Brief History of the Study of the Evolution of the Photosystems

It is often assumed that anoxygenic photosynthesis gave rise to oxygenic photosynthesis. This paradigm has been used to make interpretations of the evolution of photosynthesis since well before the concept of a photosystem was firmly established (14, 142). From this idea, one can derive the following corollary: The photosystems in anoxygenic photosynthesis originated before and gave rise to those in oxygenic photosynthesis. This in turn can be understood as PSII emerging from an anoxygenic type II photosystem. The latter is considered to be, or to resemble, the photosystem found in purple bacteria (Proteobacteria, **Figure 1**).

The first published scenario for the evolution of the photosystems was presented by Olson (98) in 1970, who first suggested that "system I" gave rise to "system II." This proposal was explicitly based on the rationale that as organic matter from the primordial soup was depleted, the evolutionary pressure to use ever-weaker electron donors pushed one photosystem toward the higher electron potentials that eventually allowed the oxidation of water (98). This rationale is an explicit reconceptualization of van Niel's (142, 143) earlier thoughts on the evolution of photosynthesis, which was in turn an interpretation of Oparin et al.'s (102) origin of life scenario. Van Niel (142, 143) proposed that purple and green sulfur bacteria, which use H_2S or H_2 as electron donors, represented intermediate stages between chemoautotrophs, emerging from the primordial soup and green plants. In Olson's (98) sense, system II refers to water-splitting PSII because it was not clear then that there was an anoxygenic type II photosystem, leaving system I as the only photosystem known in bacteria capable of anoxygenic photosynthesis. In the early 1980s and thereafter,

Chemoautotrophs: organisms that obtain energy from the oxidation of electron donors, either organic or inorganic, without the use of light

once researchers understood that the purple bacteria had a photosystem that was related to PSII (12, 99, 117, 151), it was eventually deemed the primitive one from which PSII arose by acquiring those traits that enabled oxygen evolution (see, for example, 2, 13, 99, 112).

Great Oxidation Event (GOE): refers to the first time in the history of life, about 2.3 billion years ago, when the concentrations of O_2 rose permanently by several orders of magnitude

Giga-annum (Ga): a measuring unit of time denoting billions of years; it can also be read as billions of years ago

Within the context of the paradigm that anoxygenic gave rise to oxygenic photosynthesis, two competing models for the evolution of the photosystems became widely popularized toward the end of the 1980s and the start of the 1990s. They were the result of early attempts at explaining the distribution of photosynthetic bacteria in the tree of life, as 16S ribosomal RNA sequences and phylogenies became available. The first one was an elaboration of Olson's (98) 1970 scenario, but it considered that both types of photosystems originated before bacteria started to diversify, as amply discussed by Olson & Pierson (101, 106). This model implied widespread loss of photosynthesis across bacteria and retained the idea that type I photosystems led to type II photosystems. The second model did not question the order in which the photosystems evolved but contrasted one of the first parsimony-based phylogenies of type II photosystem subunits against a 16S ribosomal RNA phylogeny. It argued that photosystems evolved in a lineage of bacteria and were later scattered across the tree of life via horizontal gene transfer. It was formally proposed by Blankenship in 1992 (11, but see also 8). Variations of these models have been proposed over the years, discussing the exact nature of the primordial photosystem, the nature of the earliest-evolving photosynthetic bacteria, or the relative dominance of loss of photosynthesis versus horizontal gene transfer (see 23 for a compilation of these, and for more recent examples, see 65, 84, 86, 127).

Based on the validity of this paradigm, it is postulated that at some point an anoxygenic type II and a type I photosystem came together in an anoxygenic ancestor of cyanobacteria prior to the evolution of oxygenic photosynthesis. This remains an unproven assumption (27). One classic model argues that having two photosystems represents an ancestral state retained in oxygenic photosynthesis, and the other argues that they were acquired via horizontal gene transfer. Whatever the case, both models demonstrate how the paradigm of the evolution of photosynthesis, often taken unquestionably as fact, is still built upon unproven assumptions and speculative ideas.

The validity of the paradigm that anoxygenic gave rise to oxygenic photosynthesis appears consistent with the well-supported observation that prior to the Great Oxidation Event (GOE), occurring at about 2.3 Ga, the concentrations of O_2 on the planet were orders of magnitude below present atmospheric levels (34, 76). However, it cannot be overstated that detecting biogenic O_2 signatures in the oldest geochemical record remains challenging. The notion that oxygenic photosynthesis could have appeared by at least 3.0 Ga is now considered likely (see 34, 110 for recent reviews; for a contrasting view, see 74, 131). Nonetheless, geological evidence for oxygenic photosynthesis before 3.0 Ga and up to the oldest available rocks has been considered in the literature (51, 69, 111, 116, 148), but the evidence is often thought to be inconclusive or problematic. Remarkably, geochemical evidence alone has not proven that anoxygenic photosynthesis originated before oxygenic photosynthesis, and the interpretation of the geochemical record has relied on biological evidence or assumptions to affirm the validity of the paradigm (see, for example, 77, p. 56, or 107, p. 126, for a more recent example, and 102, 147 for more historical accounts).

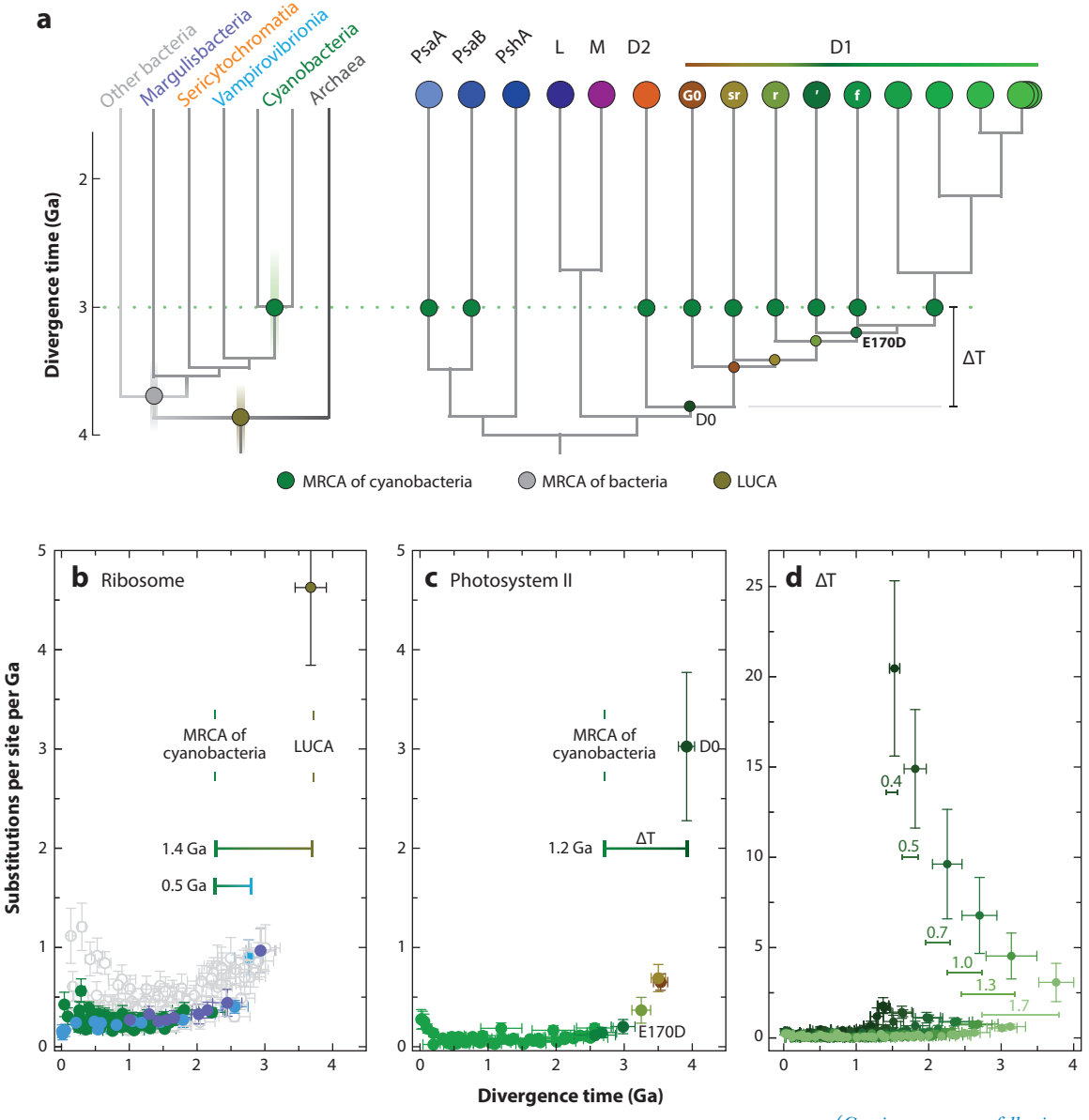
In the past few years, the validity of the paradigm has come into question as an outcome of detailed molecular evolution studies of the photosystems (25, 27, 96). These studies have highlighted two key aspects of the evolution of photosynthesis that have been largely obscured by historical assumptions: firstly, that the relationships of the photosystem core subunits do not agree with predictions and rationales derived from the paradigm and, secondly, that PSII has retained a greater number of ancestral traits than the anoxygenic type II photosystem, traits that are integral to supporting water oxidation catalysis. This is the subject of the following section.

3. THE ORIGIN OF PHOTOSYSTEM II AND WATER OXIDATION

3.1. The Emergence of Cyanobacteria

Most recent common ancestor (MRCA): synonym of last common ancestor; the most recent individual from which all members of a given group are descended

To scrutinize the evolution of photosynthesis, it is essential to define the properties of the most recent common ancestor (MRCA) of cyanobacteria capable of oxygenic photosynthesis (**Figure 3**). In the tree of life, this is technically defined as the node marking the speciation of the group that contains the genus *Gloeobacter* and their recently discovered relatives *Aurora vandensis* (61) and *Anthocerotibacter panamensis* (108) and the group that contains all other strains. This ancestor would not appear in any way atypical if found today, as it had a heterodimeric PSII capable of



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Photosystem II evolution versus the species tree of bacteria. (*a*) A representation of a species tree of life around cyanobacteria and as a function of time, following approximate timing as determined in Reference 50, and compared with that of photosystem core subunits as compiled from previous work (26, 30, 96). The ages are not meant to be absolute but should contrast the amount of time that the different diversification processes are implied to have taken. The green circles denote the MRCA of cyanobacteria. This MRCA had a set of core photosystem subunits similar to those inherited by all described strains, including several D1 subunits. D0 denotes the duplication leading to D1 and D2. ΔT denotes the span of time between the duplication that leads to D1 and D2 and the MRCA of cyanobacteria. The fading stripes around the divergence time for the LUCA and the MRCA of bacteria and cyanobacteria represent the lack of certainty on the estimation of these dates. (*b*) Plot of the change in the rates of protein evolution as a function of time, calculated for a concatenated data set of ribosomal proteins. Each point in the graph represents a node in a timed phylogeny (molecular clock) similar to that shown in panel *a* (*left*). The two bars denote the span of time from the MRCA of cyanobacteria to the point of divergence of their closest relatives (0.5 Ga), or to the LUCA (1.4 Ga). Even when the former is 1.4 Ga, the large distance between archaeal and bacterial ribosomes results in a very large rate of evolution at the LUCA, which decreases exponentially. (*c*) A comparable plot for a molecular clock of D1 and D2 subunits, following a diversification pattern as shown in panel *a* (*right*). The trace shows the rates of evolution that are necessary to have a scenario where ΔT is about 1.2 Ga. The traces shown in panel *d* represent scenarios for the evolution of D1 and D2 in which D0 has been made to be consecutively younger and younger, yielding a range of subsequently smaller ΔT. Smaller ΔT could also result from an older MRCA of cyanobacteria relative to D0. Whatever the case, a ΔT of 0.5 Ga would imply rates of PSII early evolution that would surpass the fastest-evolving proteins in biology, such as antibodies or peptide toxins, which is inconsistent with the structure and function of photosystems and other enzymes of comparable complexity. Data for panels *b–d* are from References 30 and 96. Abbreviations: Ga, billion years; LUCA; last universal common ancestor; MRCA, most recent common ancestor; PSI, photosystem I; PSII, photosystem II; ΔT, span of time between duplication.

water oxidation and a heterodimeric PSI, both with nearly all their components and subunits. Therefore, the MRCA of cyanobacteria can be used as a confident proxy to determine a minimum age for the evolution of oxygenic photosynthesis. It means, however, that the origin of oxygenic photosynthesis antedates the MRCA of cyanobacteria by some undefined amount of time. One can wonder: If there was oxygenic photosynthesis by 3.0 Ga, did the O_2 producers live before or after the MRCA of cyanobacteria? Efforts to time this ancestor have remained difficult, with molecular clocks providing a range of dates spanning 2 billion years, between 3.5 and 1.5 Ga (9, 10, 53, 127). In addition, the fossil record of putative cyanobacteria before 2.0 Ga has remained difficult to interpret unambiguously (44, 119). Nonetheless, a notable recent study by Fournier et al. (50) implementing an expanded diversity of taxa, novel calibrations, and a large number of constraints from horizontal gene transfer events produced an estimate for this ancestor between 3.3 and 2.7 Ga under the authors' preferred models, with a mean age centered at about 3.0 Ga.

Timing the MRCA of cyanobacteria, while setting a minimum age for the origin of oxygenic photosynthesis, does not answer the question of when exactly a photosystem capable of water oxidation appeared for the first time. As a conceptual exercise, and to model different scenarios for the evolution of oxygenic photosynthesis, it is useful to define more precisely the span of time between the MRCA of cyanobacteria and a point in time that is linked to the origin of photosynthetic water oxidation. The evolution of PSII provides us with such a point: namely, the duplication events that enabled its transition from a homodimer into the heterodimeric photosystem that was inherited by the MRCA of cyanobacteria. As we discuss in the next section, the homodimeric PSII was well on its way toward the evolution of water oxidation chemistry, if it was not already O_2 evolving. Consequently, we can now study the evolutionary history of oxygenic photosynthesis before the MRCA of cyanobacteria by focusing on the span of time between these core gene duplications and the MRCA of cyanobacteria, a period that we refer to as ΔT (**Figure 3**). Thus, if ΔT is short, it could be concluded that the origin of oxygenic photosynthesis occurred not long before the MRCA of cyanobacteria, and it would imply that the process evolved rapidly. However, if ΔT is long, there is a chance that the start of the process can no longer be placed in an ancestor that can technically be defined as a cyanobacterium.

Molecular clock: a computational method used to time evolutionary events using sequence data, phylogenetic trees, and some time points known as calibrations

Cyanobacteria's closest relatives are the Vampirovibrionia and Sericytochromatia (132), which are now considered to be part of the same phylum (**Figures 3** and **4*a***). These three in turn are related to the recently described phylum Margulisbacteria. While Vampirovibrionia, Sericytochromatia, and Margulisbacteria remain poorly characterized, no genomic evidence has yet emerged for a photosynthetic representative within these clades. The best-described representatives all appear to be part of diverse symbiotic associations within eukaryotes (45, 62, 133, 141). Cyanobacteria, their close nonphotosynthetic relatives, and Margulisbacteria are part of the Terrabacteria (7) (**Figure 4*a***), a multiphylum-level grouping, which includes Chloroflexota,

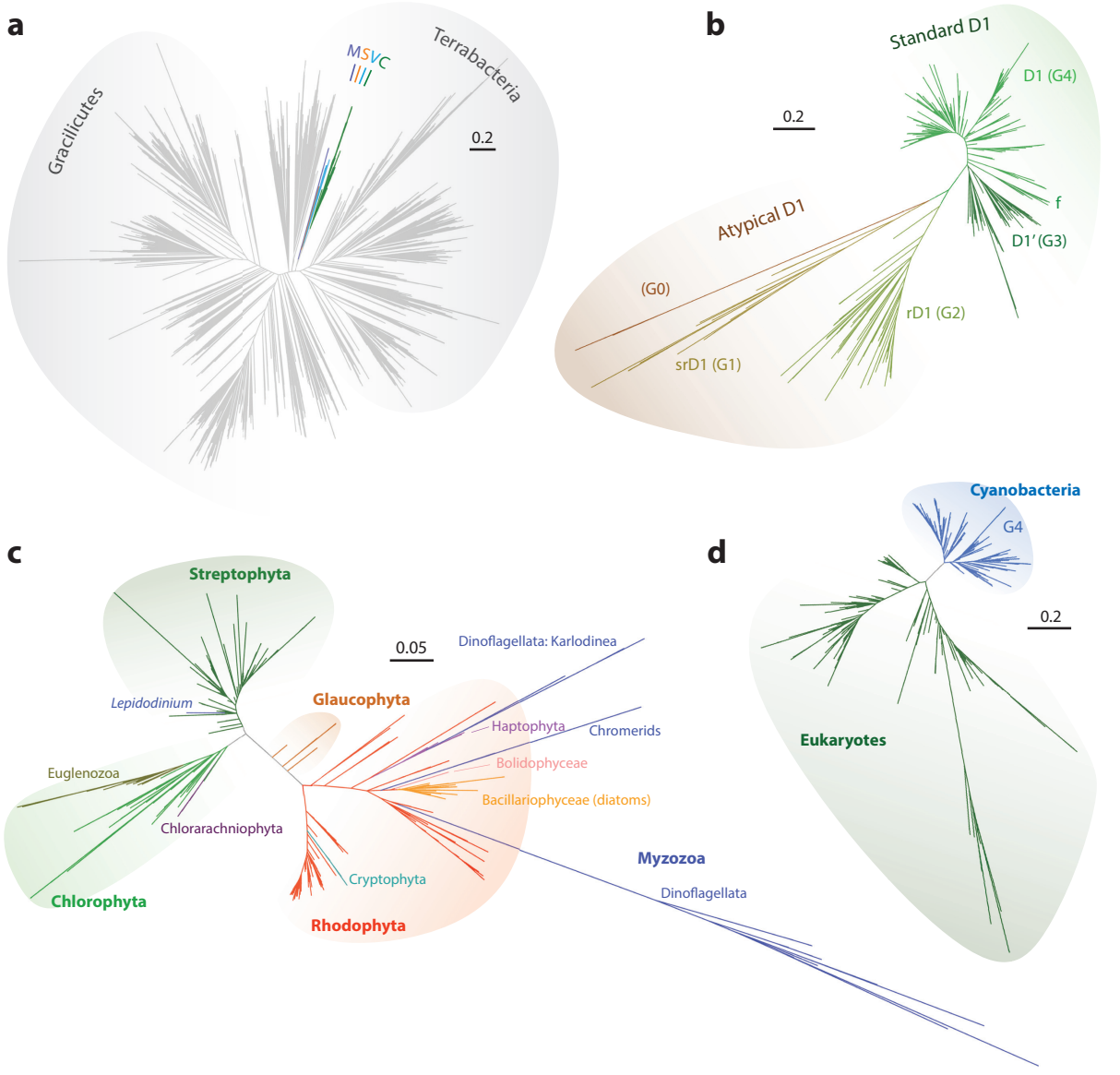


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Figure 4 (*Figure appears on preceding page*)

Phylogenetic trees of bacteria and D1. (*a*) An unrooted ML phylogeny of bacteria taken from the latest release of the Genome Taxonomy Database (07-RS207) inferred from a concatenated data set of highly conserved proteins across the domain Bacteria (105). It illustrates that the phylum Cyanobacteria and its closest relatives exist within the larger diversity of bacteria in a crowded tree of life. (*b*) An unrooted ML phylogeny of cyanobacterial D1 proteins highlighting the distinct groups. (*c*) The evolution of D1 in photosynthetic eukaryotes. The phylogeny is consistent with the complex evolution of plastids and shows the distinct primary clades of photosynthetic eukaryotes, including the green algae (Chlorophyta) and land plants (Streptophyta), the red algae (Rhodophyta), and the glaucophytes. The latter did not make a monophyletic clade, but the different sequences within the group clustered close to each other. The phylogeny of D1 recapitulates reasonably well secondary and tertiary endosymbiosis in the origin of important clades such as Euglenozoa and the dinoflagellates. There is a high degree of variation in the rates of amino acid substitutions of different D1, suggesting diverse evolutionary pressures and adaptations. (*d*) A tree of D1 protein sequences of eukaryotes together with a selection of standard (G4) cyanobacterial sequences. The tree shows a diversification process of D1 in eukaryotes that can surpass that of cyanobacteria in accumulated change over relatively shorter spans of times. Scale bars denote the number of amino acid substitutions per site. Branch support values have been omitted for clarity. Abbreviations: ML, maximum likelihood; MSVC, Margulisbacteria, Sericytochromatia, Vampirovibrionia, and Cyanobacteria; rD1, rogue D1; srD1, super-rogue D1.

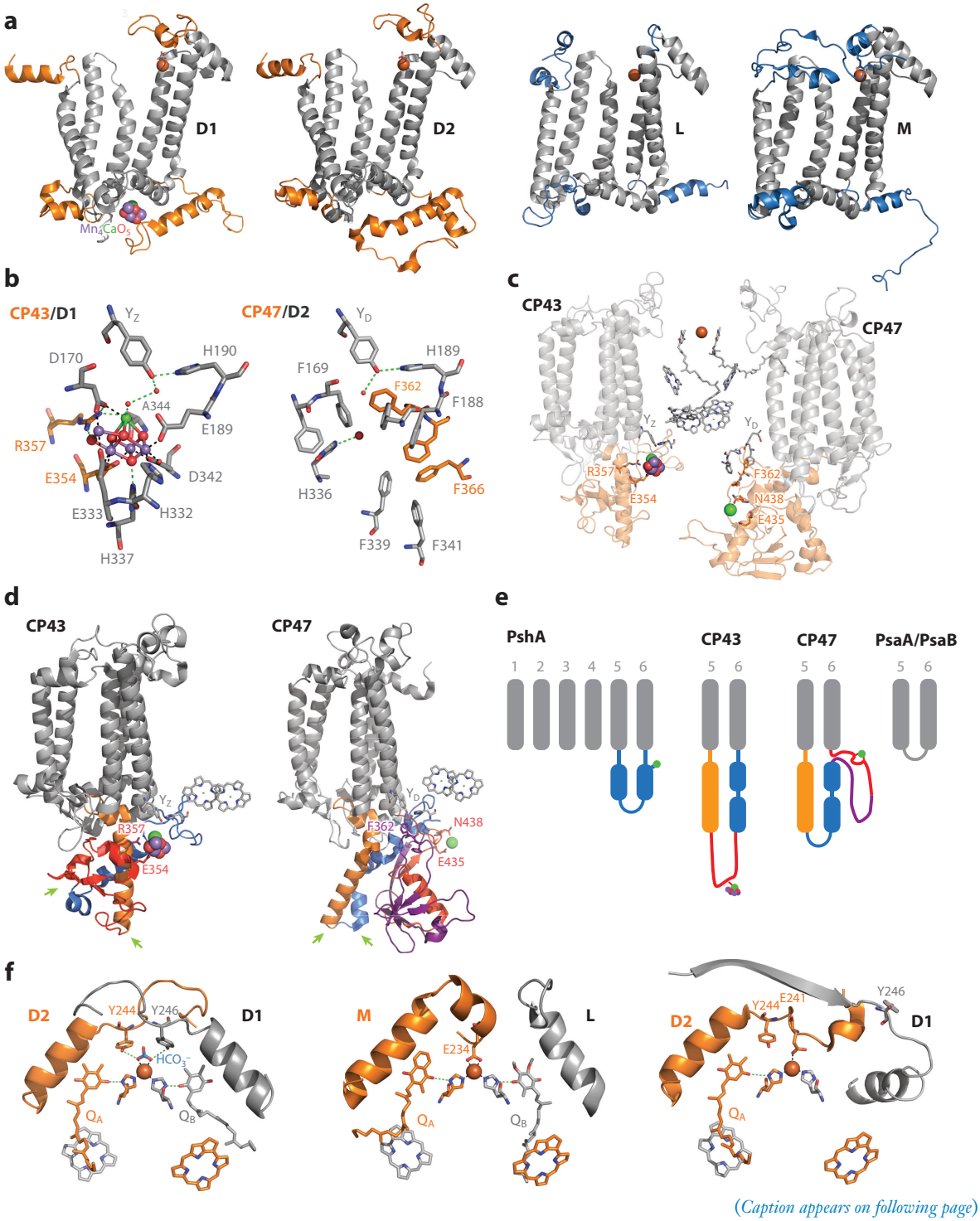
Firmicutes, and others. Terrabacteria in turn make a sister group to a collection of phyla referred to as Gracilicutes (38) or Hydrobacteria (7), which includes Proteobacteria, Acidobacteriota, the class Chlorobia, and others. The importance of this conceptualization of the evolution of oxygenic photosynthesis is that the larger the ΔT, the greater the chance that the emergence of water oxidation occurred in an ancestor that includes other lineages beyond the cyanobacteria, because the branches of a tree of life converge toward the root (**Figure 3**).

3.2. The Water-Splitting Homodimeric Photosystem II

The PSII complex from the model cyanobacterium *Synechocystis* sp. PCC 6803 contains at least 21 protein subunits, of which only 4 represent the conserved structural core with homology to other photosystems (59). In addition, the set includes 13 small membrane–embedded peripheral subunits that surround the complex and 4 extrinsic subunits that shelter the electron donor side where the Mn_4CaO_5 cluster is found. These accessory subunits are not shared with any other photosystem, and there are lineage-specific variations in subunit composition. The conserved core is made up of D1, D2, CP43, and CP47. CP43 and CP47 are the antenna subunits, and they bind the bulk of the chlorophylls and carotenoids involved in light harvesting and photoprotection. They each make up the first 6 TM helices of the photosystem monomeric unit. D1 and D2 bind the pigments and cofactors involved in photochemistry and water oxidation, in addition to a set of peripheral chlorophylls and carotenoids that functionally link D1 and D2, the photochemical core, to the antenna subunits (**Figure 1**). They each make up the last 5 TM helices of the conserved monomeric unit. While the four subunits are encoded in four separate genes, at a structural and evolutionary level, the photosystem is better understood as a dimer consisting of CP43/D1 and CP47/D2. CP43/D1 and CP47/D2 diverged after gene duplications and are thus homologous. It can be concluded, therefore, that at some point before the MRCA of cyanobacteria, PSII was homodimeric.

All cofactors involved in electron transfer reactions are bound symmetrically by D1 and D2, yet the Mn_4CaO_5 cluster is bound asymmetrically by CP43 and D1, and no cluster is found on the CP47/D2 side of the photosystem, as shown in **Figures 1** and **5**. The following question arises: Did water oxidation and oxygen evolution originate when the photosystem was a homodimer, or was this capability added to the photosystem only after it had already become a heterodimer? Answering this question is crucial because it would have implications not only for how water oxidation emerged, but also for the timing of the origin of oxygenic photosynthesis (**Figure 3**).

The high degree of structural symmetry between the two monomers, combined with our highly detailed understanding of the function of PSII, allows us to reconstruct many traits of the photosystem at the homodimeric stage. For example, the photosystem would have looked



(*Caption appears on following page*)

Figure 5 (*Figure appears on preceding page*)

Structural comparisons of type II photosystem core subunits. (*a*) Comparison of D1 and D2 of PSII and L and M of the anoxygenic type II photosystem. The transmembrane helices are shown in gray ribbons and interconnecting loops in orange (D1/D2) and blue (L/M). Notice the high degree of structural conservation between D1 and D2, showing nearly identical folds, while L and M show greater asymmetry. (*b*) Coordination sphere of the Mn_4CaO_5 cluster in CP43/D1 and the equivalent region in CP47/D2. (*c*) Comparison of the extrinsic domain of CP43 and CP47 relative to the positions of the redox active Y_Z and Y_D, respectively. (*d*) Comparison of the extrinsic domain of CP43 and CP47 located between the fifth and sixth helices, highlighting a domain swap: The swapped region (*red ribbon*) includes the two residues that bind the cluster, E354 and R357 in CP43, which occupy homologous positions to E435 and N438 in CP47, respectively. Two green arrows mark the location of the swap. (*e*) A schematic representation and comparison with the situation in the heliobacterial photosystem (PshA) and PSI (PsaA/PsaB). (*f*) Comparison of the acceptor side of type II photosystems: (*left*) structure of PSII showing the highly conserved symmetry, including the binding of bicarbonate (HCO_3^-) at the nonheme Fe^{2+} site; (*middle*) the acceptor side from the structure of the proteobacterial anoxygenic type II photosystem, showing asymmetric binding of the nonheme Fe^{2+} via M-E234; and (*right*) the structure of an assembly intermediate PSII complex, showing the asymmetric binding of the nonheme Fe^{2+} via D2-E241. Abbreviations: PSI, photosystem I; PSII, photosystem II. Panels *a*, *b*, and *c* adapted from Cardona et al. (30) (CC BY 4.0). Panels *d* and *e* adapted from Oliver et al. (96) (CC BY 4.0).

very similar to the heterodimeric PSII inherited by cyanobacteria, given that the structural folds of each monomeric unit are highly conserved, overlapping almost perfectly (**Figure 5**). This is impressive because they share less than 30% sequence identity, indicating that this structural arrangement has been under strong evolutionary pressure since early in its history. The homodimer also had nearly all the cofactors and pigments found in the heterodimer, since most of them are found at conserved positions in each monomer. It would have had some small peripheral subunits surrounding the photosystem, but it is unclear whether it had the extrinsic polypeptides, as these bind asymmetrically in the heterodimer (24, 30).

The midpoint redox potential for the oxidation of water to O_2 is +0.82 V under ambient conditions (4) and about +0.73 V under the effectively anaerobic conditions of the early Earth prior to widespread oxygenation (48). The midpoint redox potential of chlorophyll in an organic solvent is about +0.8 V (71), which is similar to that for water oxidation. To split water, PSII generates over +1.2 V of oxidizing potential via a chlorophyll cation radical, $P^{\bullet+}$. This radical in turn oxidizes a tyrosine, Y_Z, found in the D1 subunit, which is hydrogen bonded to a nearby histidine residue (H190) within the same subunit. Y_Z oxidation by $P^{\bullet+}$ leads to the formation of a neutral tyrosine radical. The tyrosine radical in turn oxidizes the Mn_4CaO_5 cluster. In a cycle of four consecutive light-driven charge separation events, two water molecules are fully oxidized, four reducing equivalents enter the electron transport chain, four protons are released into the positive side of the membrane (thylakoid lumen), and O_2 is released.

A first clue on the nature of the ancestral homodimeric PSII is revealed by the Y_Z-H190 redox active pair in D1. This is strictly conserved in D2 as the redox active Y_D-H189 (**Figure 5*b***), indicating that the homodimer had the capacity to oxidize tyrosine residues and therefore had sufficient power to oxidize water. Inspired by this observation, Rutherford & Nitschke (114) first discussed the possibility of a homodimeric photosystem capable of splitting water on each side of the complex, losing one catalytic site as the system evolved to be heterodimeric. The PSII crystal structures later revealed a vestigial catalytic site in D2 found at the equivalent position of the Mn_4CaO_5 cluster, near the Y_D-H189. Instead of appearing as ligands to a cluster, a hydrophobic patch of phenylalanine residues was found filling in a cavity (**Figure 5*b***), bolstering the case for a homodimeric photosystem that was unlike any of the anoxygenic ones known.

The Mn_4CaO_5 cluster is located in D1, which provides six of the seven ligands. The seventh ligand, E354, is provided by the CP43 antenna through an extrinsic domain that reaches into the catalytic site (**Figure 5*c–e***). A second residue, R357, provides important hydrogen bonds to the cluster and gets within 4 Å of the Ca atom. Sequence and structural comparisons indicate that a structural rearrangement occurred in the homologous site in the CP47 subunit during

heterodimerization, contributing to the loss of ligands (96). The rearrangement resulted in the extrinsic domain inserting two additional phenylalanine residues into the catalytic site on the CP47/D2 ancestor, contributing to the formation of the hydrophobic patch. At the same time, the residues at homologous sites to E354 and R357 in CP43 now appear to bind a Ca atom of unknown function in some cyanobacterial PSII complexes.

Additional evidence for an ancestral homodimeric photosystem with two catalytic sites on each side of the dimer was provided using ancestral sequence reconstruction (ASR), a computational approach that enables ancestral sequences at each point in a phylogeny to be inferred from the sequence alignment data (63). ASR of D1 and D2 showed that there is a high probability that the ancestor of D1 and D2, which we have dubbed D0 (**Figures 2** and **3**), would have been very similar to D1, sharing nearly 70% sequence identity. In other words, since duplication, D1 has retained the larger fraction of the predicted ancestral states relative to D2. While this may seem unexpected or counterintuitive, it can be seen in the phylogeny shown in **Figure 2*a***, where the branch leading from the duplication point to D1 is half the length of that leading to D2. It means that fewer amino acid substitutions have occurred in D1 compared with D2, relative to the duplication point D0. The ASR data suggest that most ligands to the Mn_4CaO_5 cluster were retained from D0 (**Figure 6*a***), except for aspartate at position 170 (D170), which was predicted to have been ancestrally a glutamate (96).

The structure of PSII shows that at the homodimeric stage the system had many, if not all, of the traits needed to split water, including enough oxidizing power, the redox-active tyrosine residues, and a coordination sphere suitable to house a catalytic cluster. The structure of the homodimeric type I photosystem from a member of the Firmicutes, *Heliobacterium modesticaldum* (57), revealed that the photosystem had symmetrically positioned Ca-binding sites exactly where Ca is bound in the Mn_4CaO_5 cluster (**Figure 7**), in addition to a remarkable set of structural similarities to PSII. These similarities include a linkage to the extrinsic domain of the antenna and coordination from the C-terminal carboxylic group from the core subunit (29). The Ca-binding site was later found to be conserved in the distantly related homodimeric type I photosystems of the green sulfur bacterium *Chlorobaculum tepidum* (36) and *Chloracidobacterium thermophilum*, a representative of the phylum Acidobacteriota (47). (See Reference 55 and **Figure 7** for more detailed descriptions of these Ca-binding sites.) This previously unknown feature of the homodimeric type I photosystems suggests that the water-oxidizing cluster was built upon a structural blueprint that can be traced to the first photosystems before the specialization that led to types I and II (**Figure 2**). It also highlights the importance of the antenna for the construction of the Mn_4CaO_5 cluster. The well-conserved nature of the interaction between the antenna and the photochemical core in type I photosystems and PSII (**Figure 7**), regardless of whether they are encoded in the same or separate genes, indicates that PSII never acquired its antenna subunits but that they have been retained since before the initial divergence of types I and II. It can be now confirmed without ambiguity that the architecture of the anoxygenic type II photosystem is atypical, not primordial, and that at some point early in its evolutionary history, the ancestral antenna domain was lost. An outcome of this was the evolution of a new antenna system, the light-harvesting complex 1 (LH1), which characteristically surrounds the streamlined photochemical core.

3.3. The Mechanism of Water Oxidation in the Homodimeric Photosystem II

PSII is not just a water-oxidizing enzyme; it is also a Mn-oxidizing one. Assembly of the catalytic cluster, a process known as photoactivation, starts with the light-driven oxidation of soluble Mn^{2+} (6). Photoactivation does not require any chaperone or protein assembly machinery and occurs both in vivo and in vitro. This means that the evolution of water oxidation did not require the innovation of specialized assembly machinery for the cluster to emerge. When considering the

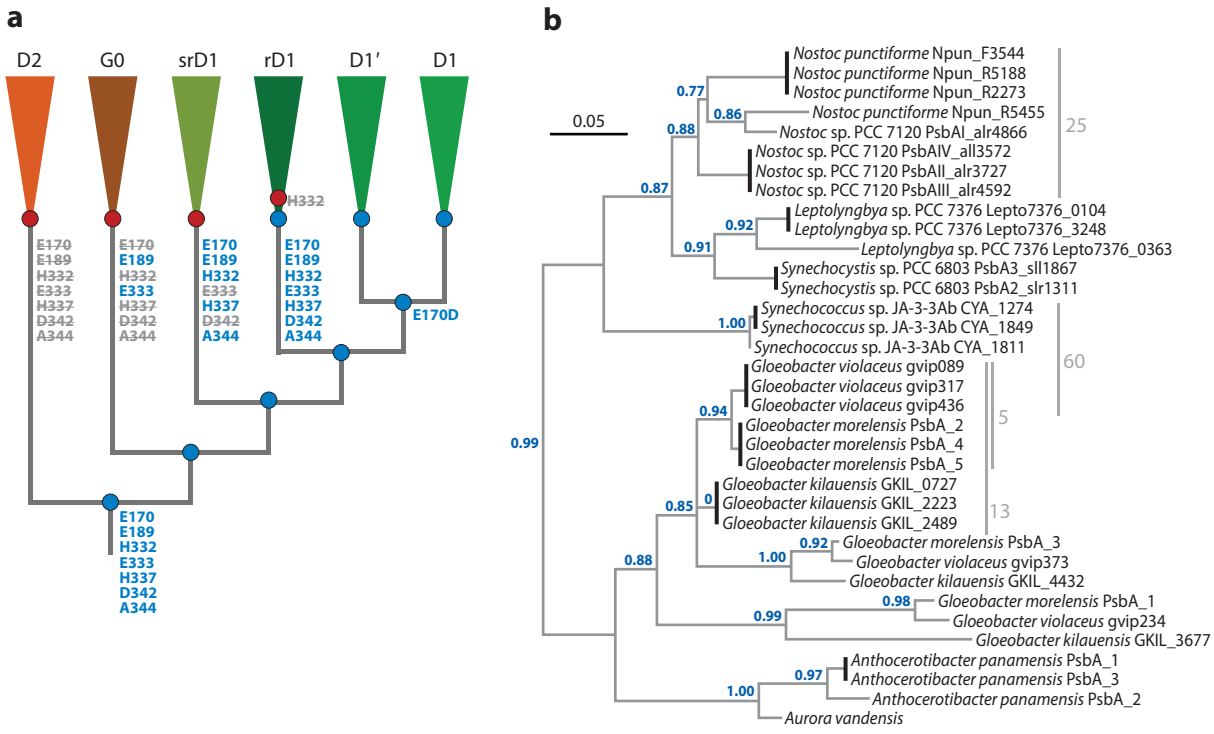


Figure 6

D1 evolution at two scales. (*a*) Ancestral sequence reconstruction of D1 ligands of the Mn_4CaO_5 cluster across a D1 and D2 phylogeny. Residues in blue denote the presence of that ligand in the ancestral sequence, and strikethrough residues in gray denote absence. Blue circles indicate a predicted capacity to support water oxidation, and red circles indicate the loss of water oxidation function. (*b*) ML phylogeny of standard D1 in *Gloeobacter*, their closest relatives, and a selection of distant relatives. Vertical lines in black show sequences that are identical. The three identical D1 sequences from *Gloeobacter violaceus* differ at 5 and 13 positions when compared with those of *Gloeobacter morelensis* and *Gloeobacter kilauensis*, respectively, and by 60 residues when compared with those of *Synechococcus* sp. JA-3-3Ab (*gray vertical lines to the right* of the two sets of sequences). The three sets of identical sequences in *Nostoc punctiforme* differ at 25 positions when compared with the set of identical sequences in *Nostoc* sp. PCC 7120. In the long term, the emergence of identical sequences occurs via duplication, which is compensated by losses in a continuous evolutionary process. In the short term, sequences maintain perfect identity via a process of concerted evolution, in which a substitution can be erased or copied and pasted onto other paralogs via intrachromosomal homologous recombination. The horizontal scale bar denotes amino acid substitutions per site. Abbreviations: ML, maximum likelihood; rD1, rogue D1; srD1, super-rogue D1.

molecular mechanism of water oxidation in a homodimeric system, it is useful to consider the process starting from the oxidation of Mn^{2+} and leading to cluster assembly. Next, we consider the function of the predicted homodimeric PSII.

One of the reasons that type II photosystems are heterodimeric is thought to relate to the oxidation of quinones (113). While cofactors are symmetrically arranged, electron transfer initiates on the D1-bound cofactors. Electrons are transferred from the photochemical chlorophylls to Q_A, which is immobile and bound by D2, and from Q_A to Q_B, which is exchangeable and bound by D1. Upon double reduction and protonation, Q_B leaves the site to be replaced by a new quinone (43). The photochemical core of the ancestral homodimeric PSII can be conceptually visualized as a double-D1 photosystem. Charge separation would occur on both sides, as still occurs in type I photosystems, while the quinone-binding sites would be like that of Q_B, in that they would allow their exchange on both sides upon complete reduction.

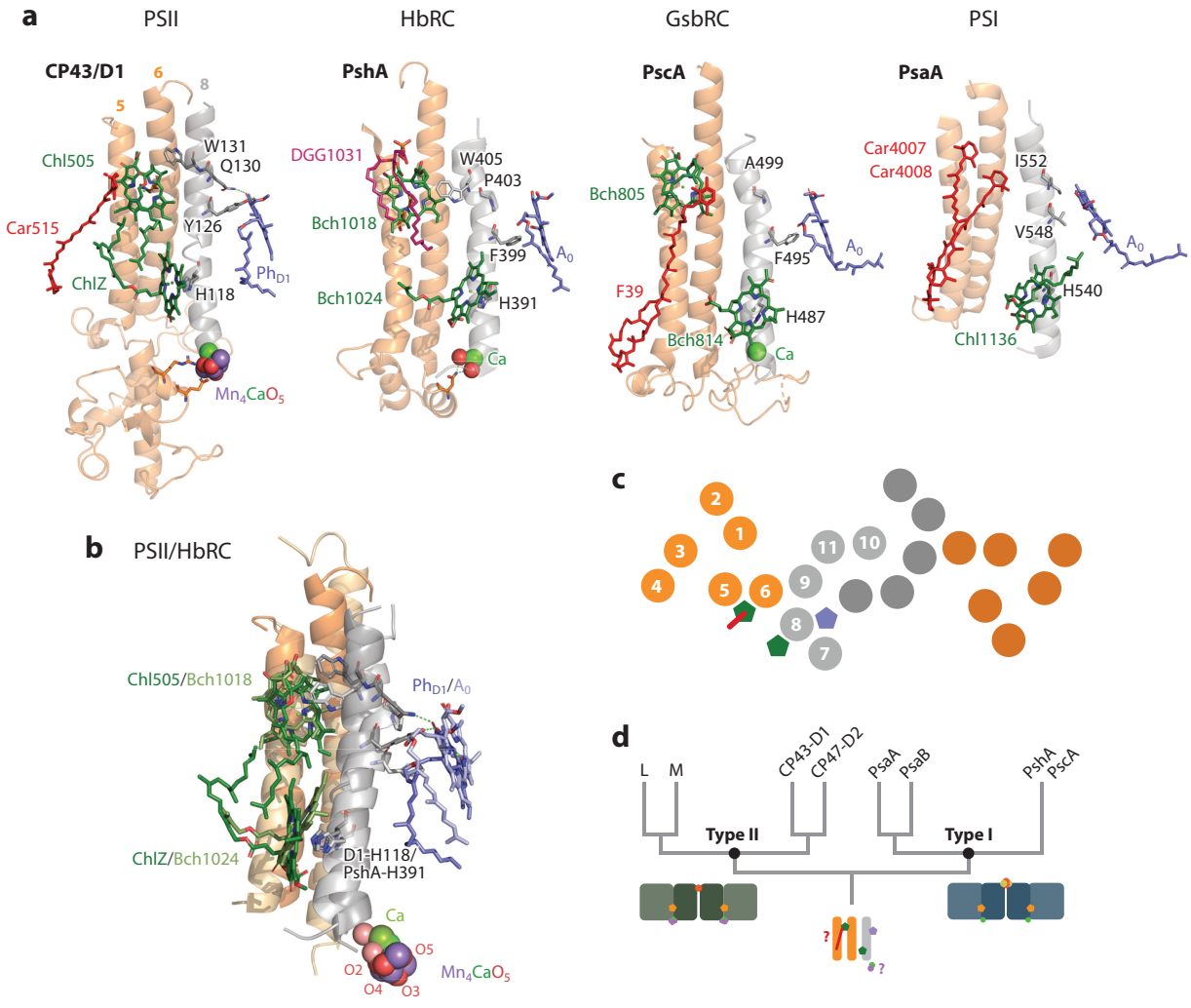


Figure 7

Structural comparisons of PSII and type I photosystems. (*a*) Structural visualization of the region around the fifth, sixth, and eighth TM helices. HbRC denotes the heliobacterial photosystem (Firmicutes) and GsbRC, the green sulfur bacterial photosystem (Chlorobia). (*b*) Structural overlap of the same region in PSII and the HbRC, showing a high degree of structural conservation, including the position of the respective Ca atoms. (*c*) A schematic top-view representation of the position of the TM helices and pigments shown in panels *a* and *b*. Pigments found at homologous positions are shown in similar colors. Antenna Chl and Bch are shown in green. Note that ChlZ-H118 (PSII), Bch1024-H391 (HbRC), Bch814-H487 (GsbRC), and Chl1136-H540 (PSI) occupy homologous positions. A0 denotes the primary acceptor of type I photosystems (*light purple*). The overlap of the Ca-binding site and the water-oxidizing cluster shows that the oxygen atom of a water molecule bound to the Ca in the HbRC overlaps with an oxygen atom in the water-oxidizing cluster known as O2. Carotenoids (*red pigments*) appear at a homologous position in PSII and the GsbRC near the conserved pigments Chl505 and Bch805, respectively. In PSI, the equivalent chlorophyll to Car505 has been displaced in PsaA by a second carotenoid. This feature of PsaA is also conserved in PsaB (*not shown*). In the HbRC, the equivalent carotenoid is replaced by a lipid. These comparisons indicate that the earliest photosystems before the type I and type II divergence had a similar pigment-carotenoid/lipid arrangement at the interface between the antenna and photochemical core domains, which is retained in PSII and as schematized in panel *d*. Abbreviations: Bch, bacteriochlorophylls; Chl, chlorophylls; GsbRC, green sulfur bacterial photosystem; HbRC, heliobacterial photosystem; Ph_{D1}, pheophytin molecule bound by D1; PSI, photosystem I; PSII, photosystem II; TM, transmembrane.

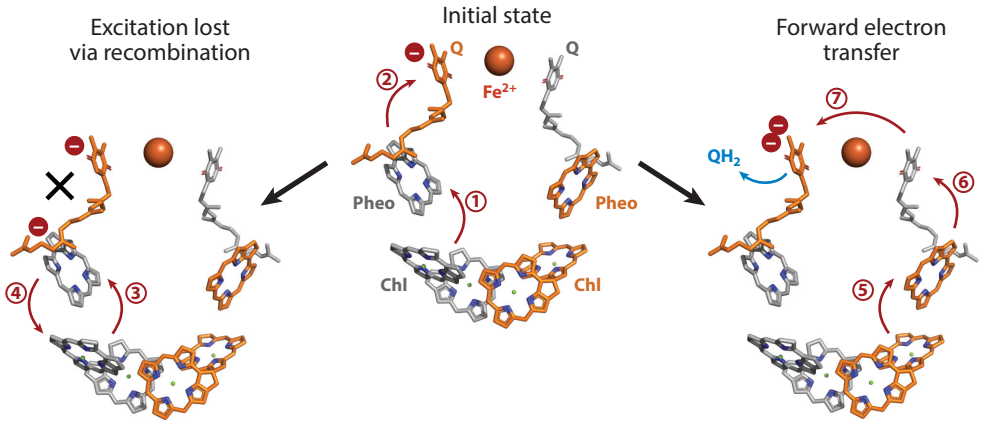


Figure 8

Schematic diagram of charge separation in the ancestral homodimeric PSII. For simplicity, the donor side is not considered in this diagram. Upon charge separation, electron transfer can occur on either side of the reaction center with equal probability. (*Center*) Pheophytin (Pheo) acts as the primary electron acceptor (①), which then reduces the quinone (②) marked as Q. (*Left*) If the second charge separation event occurs on the same side as the first (③), the pheophytin anion radical recombines with the chlorophyll (Chl) cation radical faster than semiquinone reduction can occur (④), and the excitation is lost. (*Right*) Conversely, if the second charge separation event occurs on the opposite side of the reaction center from the first (⑤ and ⑥), the quinone on that side is reduced. Disproportionation of the two semiquinones (⑦) leads to the formation of the fully reduced quinone (QH_2), which leaves the site (*curved blue arrow*) and can exchange with a new quinone from the pool.

At the acceptor side, a quinone-reducing photosystem would have several inefficiencies, which have been discussed before and are still relevant (113). Firstly, if charge separation occurs on a side without a bound quinone, the electron will bounce back to reduce the photochemical pigments, a process known as recombination, and therefore the energy in the photon is effectively lost. Recombination results in triplet chlorophyll formation, which would lead to photodamage if O_2 were present (115). Secondly, if charge separation occurs on a side with a quinone that is already reduced by a single electron (semiquinone), the second reduction cannot occur (**Figure 8**). This is the case in heterodimeric PSII, and it is thought to be because the protonation and conformational changes required for the semiquinone to be reduced are too slow in comparison with the lifetime of the immediate electron donor (31, 88), the pheophytin anion, thus favoring recombination (**Figure 8**). Successful forward electron transfer in the homodimer is expected to occur from semiquinone to semiquinone via disproportionation.

Disproportionation: a redox reaction in which a compound of intermediate oxidation state converts to two compounds, one of a higher and one of a lower oxidation state

At the electron donor side, although there is evidence for two catalytic sites available in the homodimer, it is less clear whether the homodimeric PSII would have been able to photoactivate two Mn_4CaO_5 clusters at the same time. In the homodimer, the cation radical of $P^{\bullet+}$ could oxidize either one of the redox tyrosine residues with equal probability. However, binding of Ca^{2+} or oxidation of Mn^{2+} to Mn^{3+} on one side of the photosystem could create electrostatic effects that shift the equilibrium constants so that further oxidation events are more likely to occur on that same side. This would result in the preferential photoactivation of a cluster on one side over the other, but it does not mean that activation of the second cluster would necessarily be impeded. In heterodimeric PSII, the presence or absence of the cluster triggers strong asymmetric effects on function. For example, when the Mn_4CaO_5 cluster is removed, the rate of Y_Z oxidation is slowed by two orders of magnitude (66) and the midpoint potential of Q_A is shifted by ~150 mV (17, 73). In fact, the shift in the potential of Q_A is attributed to the binding or release of the Ca^{2+} in the

cluster (17, 81). One can wonder if such asymmetric effects can be traced back to the homodimer. However, such asymmetric effects could be neutralized by deprotonation events or the binding of Cl^-. In this way, if there is plenty of light and a good sink of electrons away from the enzyme, photoactivation of both sites could occur with similar probability.

Reactive oxygen species (ROS): includes species such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and superoxide ($^\bullet O_2^-$)

Of all the ligands that bind the cluster in D1, ASR predicts that only D170 is different in the ancestral homodimer, where it is present as a glutamate. D170 not only binds the Mn_4CaO_5 cluster but it is also essential in the photoactivation process as it is the site where the first Mn^{2+} atom binds with high affinity. As a result, several studies have used the D170E mutant to understand its function but never within an evolutionary context (16, 21, 70, 93). The D170E mutant retains 60–100% of the wild type's maximal rate of oxygen evolution but has a water oxidation cycle with steps that appear more stable. This comes at a cost to photoactivation efficiency, which is reduced to about a fifth versus the intact system. Interestingly, Nixon & Diner (93) reported that the equilibrium constant for one of the state transitions (S_2 to S_3) in the catalytic cycle was 1.5 to 3 times larger in the D170E mutant than in the native enzyme. These longer-lived and more stable states in a primordial water oxidation cycle might have been advantageous if recombination were more frequent in the homodimer, but this would have been at the expense of photoactivation efficiency. We can speculate, therefore, that the transition from E170 to D170 may have been favored once more robust photoprotection and repair processes were in place.

While the prediction of cluster ligands is a good indication that the homodimeric PSII could or was nearly able to split water, there are enough uncertainties within ASR and the sequence alignments to warrant caution (96), and ligands alone are not sufficient evidence to demonstrate oxygen evolution activity. For example, when investigating photoactivation, Chen et al. (35) observed the light-induced binding of 18 Mn ions per PSII center when Ca was absent from their buffers. Building upon this work, Chernev et al. (37) were able to characterize the formation of Mn oxide nanoparticles in PSII samples devoid of the cluster and the extrinsic subunits. The authors suggested that nanoparticle formation led to water oxidation catalysis and subsequently to the Mn_4CaO_5 cluster. However, it was not explicitly specified whether the Mn^{2+}-oxidizing chemistry would be occurring before or after heterodimerization. Nonetheless, similar chemistry is compatible with a homodimeric PSII. Whether Mn^{2+} oxidation without water oxidation occurred as a transitional stage in the evolution of PSII, and if it occurred, whether it existed for short or long expanses of time, remain unclear. Using geochemical evidence from the 2.41 Ga Koegas Subgroup of the Kaapvaal Craton of South Africa, Johnson et al. (74) speculated that Mn deposits in the location have characteristics consistent with such a Mn^{2+}-oxidizing photosystem incapable of water oxidation. (For a recent review detailing historical scenarios for the origin of the water-splitting cluster, see 95.)

Additional evidence for the evolution of water oxidation before heterodimerization can be found in some structural features of PSII that evolved before the ancestral duplications. These features have functions that are absent in all other photosystems but retain ancestral symmetry and that help protect the system against the formation of reactive oxygen species (ROS) or optimize function in the face of continuous ROS-mediated damage. Three of these features stand out:

1. The structural conservation of the peripheral chlorophylls and binding sites, known as $ChlZ_{D1}$, $ChlZ_{D2}$, and associated carotenoids, have a role in protective electron transfer pathways and quenching dangerous excited states (30, 31).
2. The separation of the antenna and photochemical core not only optimizes the economics of gene expression and protein translation but also allows efficient repair by enabling FtsH quality control proteases to access D1 and D2 (82). It is well known that the turnover of D1 degradation is under 1 h (158), while D2 is ~3 h, and CP43/CP47 is ~10 h. In comparison,

the PSI subunits (PsaA/PsaB) have a lifetime of ~30–75 h (158). The antenna and core split is therefore well understood in the context of supporting water oxidation, and it happened before the ancestral duplications that enabled heterodimerization.

3. A unique bicarbonate-binding site is built around a highly conserved symmetrical fold provided by D1 and D2 and involving the nonheme Fe^{2+} (**Figure 5*f***). Researchers demonstrated in 2016 (17) that when the intracellular concentration of CO_2 is limiting, the overreduction of the electron transfer chain leads to the formation of a long-lived Q_A semiquinone state, which subsequently triggers the release of bicarbonate from the nonheme Fe^{2+}. The release of bicarbonate modifies the midpoint redox potential of Q_A, decreasing the probability of recombination that can trigger the formation of ROS (115). More recently, it was shown that under stressful conditions and in the absence of bicarbonate, O_2 can bind at the nonheme Fe^{2+}, leading to the formation of superoxide (49). Conditions can be analogous to those created under assembly, photoactivation, photoinhibition, and repair of PSII (49). Surprisingly, new cryoEM structures of PSII assembly intermediates have shown that bicarbonate is displaced by a glutamate residue from the D2 subunit, which creates a highly asymmetric state (155, 159). Such a state was also seen in another PSII structure that was devoid of the Mn_4CaO_5 cluster (58). Glutamate coordination of the nonheme Fe^{2+} is proposed to trigger a protective state. This symmetric bicarbonate-binding site, which serves a protective role, therefore evolved before heterodimerization and does not exist in the anoxygenic type II photosystems. It is rather peculiar, from an evolutionary standpoint, that this asymmetric state is similar to native anoxygenic type II photosystems, where the nonheme Fe^{2+} is permanently bound by a glutamate provided by the M subunit (**Figure 5*f***).

Last universal common ancestor (LUCA): the most recent individual prokaryote from which all forms of life are descended; the point of divergence of the domains Archaea and Bacteria

3.4. Timing the Origin of Photosystem II and Its Implications

As we have seen, the homodimeric PSII was unlike any anoxygenic type II photosystem. Therefore, we can use ΔT to gain a sense of the span of time between an early well-defined stage in the evolution of oxygenic photosynthesis and the MRCA of cyanobacteria (**Figure 3**). Analyses exploiting molecular clocks to calculate and simulate the rates of evolution of the core subunits with varying ΔT concluded that this span of time can comfortably be over 1 Ga (30, 96). It was found that even when ΔT is very large, an exponential decrease in the rates of evolution is required (**Figure 3*b***), with the fastest rates placed at the point of duplication (e.g., D0) and decaying rapidly, stabilizing at current slow rates before the MRCA of cyanobacteria.

It is useful to think about protein evolution in rates of change measured in amino acid substitutions per site per Ga (δ Ga^{-1}). Take two identical sequences evolving independently from each other at a rate of 1 amino acid substitution per site per Ga. Then, assuming all sites have an equal chance of changing, it would take 1 Ga for each sequence to have accumulated on average 1 mutation per site. This means that it would take less than 0.5 Ga for both sequences to lose all sequence identity (78). Sequences that have remained highly conserved over periods of billions of years, or sequences predicted to be in the last universal common ancestor (LUCA), evolve slowly, at rates well below 1 δ Ga^{-1}. Molecular clocks use a protein sequence alignment and time constraints (e.g., fossil calibrations, geochemistry, general assumptions, and/or other molecular clocks) to calculate the rates of protein evolution in amino acid changes per site per unit of time across a phylogenetic tree. Today, molecular clocks use statistics to infer the uncertainty generated by sparse calibration points and the fact that different clades, sequences, and amino acid sites can evolve at different rates.

In the molecular clock analyses of Fournier et al. (50), they calculated divergence times on a phylogeny of concatenated ribosomal proteins that include a great diversity of bacteria spanning

the distance between Gracilicutes and Terrabacteria (**Figures 3** and **4*a***). The span of time between the mean age of the MRCA of cyanobacteria and that of the divergence of their closest nonphotosynthetic relatives, Vampirovibrionia, was about 0.5 Ga. The span of time between the MRCA of cyanobacteria and the root of the tree used was less than 0.75 Ga. The average rate of evolution for the ribosomal proteins was found to be 0.38 δ Ga^{-1}. Using a similar data set of concatenated ribosomal proteins that included a different collection of bacterial and archaeal sequences, and a different set of calibration points, Oliver et al. (96) estimated nearly identical spans of time between the respective groups, with an average rate for bacterial ribosomal protein evolution of about 0.41 δ Ga^{-1}, albeit with all ages shifted to be younger. These comparisons indicate that the origin of oxygenic photosynthesis, as recapitulated by the evolution of PSII laid over a tree of life (**Figure 3**), not only antedates cyanobacteria by a long time but could be approaching the origin of life.

PSII is among the slowest-evolving enzymes known, with rates of protein evolution of about 0.1 δ Ga^{-1} (30). In other words, PSII core subunits evolve slower than the ribosomes (**Figure 3*b***). To put this into context, if the core subunits of PSII were evolving three times faster (which is still slower than ribosomal proteins), their entire evolution within cyanobacteria and plastids would be compressed to just about 250 million years. Moreover, PSII also evolves more slowly than other enzymes known to be older than the LUCA, such as the core subunits of ATP synthase or RNA polymerase (96). PSII evolves at about a fifth of the rate of anoxygenic type II photosystems (30) and about a third of the rate of type I photosystems (26) under conservative scenarios. These observations indicate that among all photosystems, PSII is the most likely to have retained ancestral characteristics, which explains the numerous unexpected similarities with the homodimeric type I photosystems that the new structural studies have revealed (29, 36, 55). It also warns against the assumption that all traits that make PSII special from the point of view of water oxidation are novel rather than ancestral.

The rate of evolution of PSII is so slow, and the distance between CP43/D1 and CP47/D2 is so large, despite the high degree of structural conservation, that for ΔT to be 1 Ga, the rate at the point of duplication had to be over 50 times greater than any rates measured for these subunits since cyanobacteria and plastids started to diversify (**Figure 3*b***). This is a strong evolutionary constraint because if the span of time (ΔT) is assumed to be shorter, the rate of evolution would increase with a power law function, quickly reaching implausible levels (**Figure 3*b***), even when the span of time is relatively large, that is, several hundred million years. The exponential decay in the rates since the point of duplication also implies that the homodimeric stage was short lived.

While at first glance an early origin stands in stark contrast with all that is known about the early evolution of life, it may all be a matter of interpretation. An early origin of aerobic respiration, apparently, and controversially, tracing back to the LUCA, has been repeatedly inferred for the last two decades (18, 33, 42, 72, 103, 150). The same can be said of superoxide dismutases and other ROS-handling enzymes that have repeatedly been inferred to be found in the LUCA, if not emerging very early prior to the diversification of bacteria and before the diversification of cyanobacteria (15, 64, 80, 103, 130, 160). Jabłońska & Tawfik (72), in their recent study, inferred that there were two O_2-using enzymes in the LUCA, including the core subunit of the O_2 reductases and superoxide dismutase. Moreover, 22 protein families of O_2-using enzymes were assigned to the MRCA of Terrabacteria and Gracilicutes, including enzymes required for the degradation of fatty acids, sterols, and aromatic amino acids. At the same time, another study suggested that the establishment of the genetic code required O_2 (60). Other studies have suggested that even if photosynthetic oxygen evolution started before 4.0 Ga, the levels of O_2 would have remained low due to internal feedback in the long-term natural cycles of nutrients (1). It was also suggested recently that the oxygenation of the planet could have been delayed due to the increased rate of

collisions of large impactors from space during the Archean Eon (85). The origin of water oxidation as suggested by the evolution of PSII can explain an early emergence of aerobic respiration and ROS-handling systems and could challenge perspectives that assume the origin and early diversification of life occurring in the absence of light.

Archean Eon: a geologic eon spanning the time between 4.0 and 2.5 billion years ago

4. PHOTOSYSTEM II: AN EVOLVABLE ENZYME

4.1. Photosystem II Has Evolved Tunability

Cyanobacteria are known to carry in their genomes multiple copies of the *psbA* genes encoding paralogs of the D1 subunit. The number varies widely from 1 gene up to 11 (125), although most strains have between 3 and 6, of which a few are often identical or nearly identical (28). Today, at least 7 different distinct groups of D1 have been recognized on the basis of evolutionary relationships (125), displaying a large degree of sequence variation (28, 91). Of these groups, 3 are considered atypical due to the accumulation of amino acid substitutions that would impede water oxidation function in PSII (28). The remaining D1 groups are represented by sequences that are known to support water oxidation or are predicted to do so based on the amino acid sequence, and we will refer to these as standard D1 (**Figures 3** and **4*b***). The exact array of D1 types found in cyanobacteria is highly species specific. Indeed, the phylogeny of D1 suggests that the *psbA* gene is under strong selection pressure to duplicate often (**Figure 6*b***), which is compensated by loss (118). This is seen in most clades of cyanobacteria, including basal ones. For example, several strains of the distantly related *Gloeobacter* and *Nostoc* species contain at least 5 copies of standard D1, of which 3 are identical (**Figure 6*b***). When the set of identical sequences are compared against each other, within the genus *Gloeobacter* and between lineages, they turn out to be different, suggesting a degree of concerted evolution beyond recurrent duplication and loss (118). Nevertheless, duplicated genes eventually drift and accumulate change. Most mutations would have been deleterious, leading to gene loss, but a few would have been beneficial under suitable conditions, leading to the emergence of new properties. These could range from the fine-tuning of preexisting function to the acquisition of a new enzymatic activity.

The multiplicity of D1 subunits enables a strain to adapt to changing conditions. Before in-depth studies of the phylogeny of D1 subunits, two PSII acclimation mechanisms were recognized. One is the stress-induced increase in the expression of *psbA* copies encoding identical D1 proteins, which is thought to boost the repair process under increased ROS-induced photodamage (89, 90). A second mechanism can be activated when conditions change, resulting in the expression of a *psbA* gene encoding a distinct D1 subunit with amino acid substitutions that tune the system for better performance under the new condition. The best characterized examples are the variant forms of D1 that confer enhanced PSII function under high light intensity and the variants that enhance function under low-O_2 conditions. The high-light forms are characterized by a small number of substitutions that fine-tune the redox potential of some of the cofactors. In particular, a change of glutamine to glutamate at position 130 increases the redox potential of the pheophytin molecule bound by D1 (134, 135). The change enhances performance by diminishing the chance of the formation of chlorophyll triplet states that can react with O_2 and lead to ROS and, often, damage (41, 115, 134). Within the standard forms of D1, the Q130E transition, as well as the E130Q transition, has occurred repeatedly (28), suggesting that the evolution of fine-tuning amid changing conditions is a continuous evolutionary process that optimizes trade-offs between productive and photoprotective states (145).

Another type of D1 is a variant expressed under microoxic conditions usually referred to as D1′ (129, 137). PSII with a D1′ has been reported to show alterations in some of the electron and proton transfer steps during the catalytic cycle (136). Crawford et al. (39) showed that under

ambient concentrations of O_2, a D1′-PSII was more sensitive to high light than the normally expressed D1-PSII, but under low-O_2 conditions, the D1′-PSII was more robust in high light than the D1-PSII. At this stage, it is still unclear how the different amino acid substitutions of the D1′ alters the properties of PSII to achieve fine-tuning. Phylogenetically, the D1′ shows a great diversity of sequences with a broad distribution across species, with a few showing the accumulation of mutations that would impair function (28, 125).

4.2. The Atypical Photosystem II Complexes

Murray (91) first characterized sequences for two types of D1, which were missing some of the residues that bind the Mn_4CaO_5 cluster and featured many differences when compared with the well-studied standard D1. These were dubbed rogue D1 (rD1) and super-rogue D1 (srD1). They make distinct and consistent phylogenetic clades (28, 91, 125) (**Figures 4*b*** and ***6a***). The exact role of rD1 is not clear, but srD1 has been shown to confer the capacity to synthesize chlorophyll *f* as part of an acclimation response that enables oxygenic photosynthesis to occur in far-red light (700 to 800 nm) (52, 67, 94). Another atypical type of D1 of unknown function has been found exclusively in the genome of two *Gloeobacter* strains (28, 118). These show substantial erosion of the sequence, accelerated rates of evolution, and mutations that are likely to disrupt assembly into a PSII complex—signs that the sequences may be in a process of extinction from the phylogenetic record.

A good fraction of cyanobacteria with rD1 are diazotrophic, which has led to the hypothesis that rD1 is inserted into PSII to inactivate the complex and allow nitrogenase to function in the absence of O_2 (139, 149). This is backed up by transcriptional analyses of various cyanobacteria subjected to light-dark cycles, which show that transcription of rD1 is upregulated in the dark when N_2 fixation occurs (87, 97, 139). However, not all N_2-fixing cyanobacteria contain an rD1, and some rD1 sequences have been found in cyanobacteria that do not fix N_2 (91, 125). Separate studies of different unicellular N_2-fixing cyanobacteria grown under light-dark cycles have found that active PSII content drops at the onset of darkness with rD1 being inserted into PSII at a later point in the dark cycle (87, 128). However, rD1-PSII was only detected in a small fraction of the centers. Expression of the rD1-encoding *psbA* gene has also been detected under artificial photoinhibitory conditions (79) and in heterotrophically grown cyanobacteria (104). In another study, a strain producing only rD1-PSII was shown to be incapable of photoautotrophic growth or O_2 evolution, but no alternative function for the complex was discovered (149). The exact role and associated molecular mechanisms of this puzzling variant of PSII still remain to be determined.

A small but highly diverse fraction of cyanobacteria has the capacity to remodel the photosystems and the phycobilisome light-harvesting complex to drive oxygenic photosynthesis with light beyond the standard visible range (52, 94). This is known as far-red light photoacclimation (FaRLiP). The response involves the inducible expression of 1 or 2 gene clusters that contain in total about 20 genes, encoding alternative versions of both photosystem and phycobilisome subunits. The response also involves the capacity to produce two pigments with red-shifted absorption, chlorophyll *d* and *f*. FaRLiP-capable cyanobacteria are globally distributed but restricted to environments that can become enriched in far-red light, when the visible light has been filtered out. Examples of these include microbial mats, soil, rock, and stromatolites (3). Within this set of genes, a highly divergent form of D1 was found, the srD1, which was later identified as the key factor that enabled chlorophyll *f* synthesis (67). Initial characterization of the srD1 suggested that it binds with itself to form what could be the first homodimeric type II photosystem ever discovered (124). Later on, additional studies demonstrated that srD1 assembled into a standard PSII complex in strains with and without its endogenous D1 subunits; however, no homodimers were detected (140).

Proterozoic Eon: a geologic eon spanning the time between 2.5 billion and 541 million years ago

Chlorophyll *f* synthesis involves the selective oxidation of a methyl group to a formyl group in the chlorophyll ring. The exact mechanism is not yet clearly understood, but it is thought that a free-radical mechanism involving the activation of O_2 is required (54). It was demonstrated using site-directed mutagenesis that only two amino acid substitutions of a standard D1 sequence allowed PSII to produce chlorophyll *f* (140): M127Q and G128D. They are located at the interface with the CP43 subunit and within binding distance of an antenna chlorophyll, which was highlighted by Trinugroho et al. (140) as the potential active site. Currently, the enzyme involved in the synthesis of chlorophyll *d*, which is also required for FaRLiP, has not been identified, and there is no indication that it is a modified photosystem (19, 20).

For FaRLiP to work, the newly made chlorophyll *f* and *d* pigments are bound to modified photosystems that optimize light absorption and photochemistry to far-red light (94). The remodeling of PSII requires 5 of the 35 chlorophyll *a* molecules to be replaced by 4 chlorophyll *f* and 1 chlorophyll *d* molecules. The latter is positioned as a key cofactor within the redox pigments of the photochemical core and initializes charge separation upon light absorption. The position was first determined using spectroscopy measurements and sequence modeling (94) and was later confirmed with the release of a cryogenic electron microscopy (cryoEM) FaRLiP PSII structure (58). A second chlorophyll *f* was modeled in the CP43 subunit and the remaining three in the CP47 subunit (58). FaRLiP was suggested to be inherited vertically, with no evidence found yet for the horizontal transfer of a full gene cluster (3). If this is the case, the MRCA of FaRLiP cyanobacteria likely occurred after the GOE during the early Proterozoic Eon (3, 15, 50). Furthermore, a recent study on the evolution of FaRLiP PSII showed evidence of transitional stages that could indicate earlier and simpler adaptations to far-red light tracing back to before the MRCA of cyanobacteria (56).

A puzzling feature of the overall diversity of D1 proteins is that the atypical ones appear to branch earlier than the standard forms (28, 125). Cardona et al. (28) suggested that the MRCA of cyanobacteria, like most of its descendants, had a multiplicity of D1 proteins, implying that the history of D1 duplications is long. Based on the observation that the atypical sequences had a basal position, they suggested that the pattern of diversification recapitulated the emergence of the Mn_4CaO_5 cluster starting from a Mn^{2+}-oxidizing homodimeric PSII (28). However, after a closer inspection of the sequence alignments, and with the aid of ASR, it was possible to show that the atypical sequences emerged from forms of D1 that were able to support water oxidation (96) yet still evolved from ancient duplication events. These ancestral D1 proteins were also found to have likely had glutamate at position 170, instead of the standard D170, as inherited from the homodimer. The pattern of loss of ligands was found to be somewhat random, such that all ligands can be recovered within the diversity of rD1, and all but two for srD1 (**Figure 6*a***). One reasonable conclusion is that what makes the atypical sequences different is not so much their ancestral traits but their novel ones.

4.3. Evolution of Photosystem II in Photosynthetic Eukaryotes

Given the high degree of sequence conservation of the standard core photosystem subunits in cyanobacteria and photosynthetic eukaryotes, researchers have argued that these exist as a frozen metabolic accident impervious to change over billions of years (126). While this is true when just the standard form of the enzyme is considered, it is also true that PSII is evolvable and has never stopped evolving since the early history of life. This evolvability is clearly demonstrated by the variety of D1 and other core subunit paralogs that abound within cyanobacteria. By contrast, the lack of D1 and other photosystem subunit paralogs in the plastid genomes could be seen as PSII fundamentally being frozen in eukaryotes, with all innovation revolving around the emergence of distinct supercomplexes of PSII and peripheral light-harvesting antennae (22, 40). However, a survey of all eukaryote D1 revealed a remarkable diversity of sequences across clades

(**Figures 4*c*,*d***), some of which have accumulated substantial change, in particular, but not exclusively, within the dinoflagellates, which is a group of secondary algae with fascinating evolutionary traits (152) and rapidly evolving plastid genes (163). The survey also revealed D1 paralogs within the nuclear genome of some plants. For example, the genome of the hot pepper (*Capsicum annuum*) has 14 D1 paralogs, not including the original plastid sequence; the genome of *Amborella trichopoda*, a basal angiosperm, has at least 8 paralogs of D1. Some of these paralogs show substantial change, from amino acid substitutions to domain deletions and fragmentations. It is well known that plastid DNA can be copied and integrated into the nuclear genome (5, 162), and while most of the genes quickly become nonfunctional and are eventually deleted, these are the types of genetic features that generate diversity and lead to innovations (162). Whether these isoforms have any functions or are targeted to the chloroplast need to be demonstrated.

5. PERSPECTIVES

5.1. A View to the Past: Recontextualizing the Evolution of Photosynthesis

The evolution of PSII challenges the way we have traditionally understood the emergence of photosynthesis and early life. The availability of new structural data of the homodimeric type I photosystems has put this into stark relief. PSII can be seen now as a slow-evolving enzymatic complex retaining numerous primordial and ancestral traits, which enabled the emergence of water oxidation at a time that defies paradigms. By contrast, the anoxygenic type II photosystem appears now as atypical and streamlined, as if it had emerged from a PSII-like complex caught in a partial state of assembly or disassembly. From this perspective, taking structural and phylogenetic data together, one may postulate that the anoxygenic type II photosystems could have originated from a gene duplication event of a core photosystem subunit that would have assembled into a photosystem, structurally and functionally, like a homodimeric PSII. The duplicated gene drifted and acquired a new function by a mechanism similar to that which led to the evolution of D2 or the atypical D1 forms. These events likely happened early and rapidly, before or during the early diversification of bacteria. Given that the primordial type II photosystem was similar to PSII, it raises the following question: Was the ancestral specialization of type I and II photosystems actually driven by the necessity to optimize linear electron flow out of water and into CO_2 fixation? In conclusion, there is a real possibility that there was never a substantial period in the history of life that could be considered to have occurred before the evolution of oxygenic photosynthesis.

5.2. A View to the Future: Photosystem II for Sustainable Biocatalysis

PSII is an evolvable enzyme. This evolvability projects the exciting new opportunity that this complex enzyme could be engineered to tackle some of the pressing sustainability challenges that we face, well beyond the enhancement of photosynthetic function (32). For example, the tremendous oxidative power of PSII could be harnessed for biocatalysis beyond water oxidation, to drive complex, energy-demanding, multistep oxidative chemical transformations powered by light. There is an urgent need to transition to a greener and more sustainable chemical production (153, 164) because many reactions involve energy-intensive processes with toxic by-products (146). By contrast, enzymatic biocatalysis can operate under mild conditions with greater regio-, stereo- and enantioselectivity and therefore has drawn much attention in recent decades as a more sustainable alternative to conventional synthesis processes (46, 123). The unique architecture of PSII, optimized for efficient electron and proton extraction, can be tuned for new substrates, exploiting protein engineering techniques such as directed evolution (157, 161). The catalytic site could be altered to bind different transition metals to suit a specific need, and in the absence of water oxidation, photodamage may not be a major concern. One imagines that such evolved photosystems

could be useful for both in vivo and in vitro applications. Our detailed atomic understanding of the structure and function of the photosystem makes the integration of rational design strategies with evolution-based methods ideal. The evolution of the chlorophyll *f* synthase demonstrates that this is possible, that there is potential for catalytic mechanisms to emerge beyond what can be currently conceived by intuitive means, and, to top it all, that it might be achieved with the replacement of a single subunit: D1. A similar concept could be applied to PSI to power reductive transformations when chimerized with a reductive enzyme, as suggested before (75). While this vision may represent a major research endeavor, the development of photosystems with novel properties could usher in a new era of photosynthesis research.

SUMMARY POINTS

1. Photosystem II originated deeper in the evolutionary history of life than previously believed.
2. It is likely that photosynthetic water oxidation originated substantially before the diversification of cyanobacteria and possibly before other major groups of bacteria.
3. The anoxygenic type II photosystems were ancestrally similar to water-splitting photosystem II.
4. Photosystem II has never stopped evolving.
5. The catalytic D1 subunit of photosystem II is under a continuous process of gene duplication and drift, enabling adaptability and the emergence of novel functions.
6. The catalytic D1 subunit, used as a proxy for the evolvability of photosystem II, shows large and unrecognized diversity in photosynthetic eukaryotes.
7. The evolvability of photosystem II is a trait that could be harnessed to develop light-driven biocatalysis to power energy-demanding and complex oxidative transformations.

FUTURE ISSUES

1. Combining synthetic biology with ancestral sequence reconstruction will enable the biochemical and biophysical characterization of ancestral photosystem core subunits spanning billions of years.
2. A greater understanding of the changes in the rates of genome evolution of cyanobacteria and their relatives will enable a more robust estimation of their time of origin.
3. New insights into the origin of the photochemical processes that led to the emergence of photosynthesis and biological water oxidation may be found within the context of the origin and early diversification of life, and vice versa.
4. Further genetic, physiological, biochemical, and biophysical studies are required to understand how the great diversity of D1 and other core subunits optimize or alter photosystem function.
5. Synthetic biology and directed evolution approaches will facilitate the engineering of photosystems with novel catalysis for greener biotechnological applications.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Alcott LJ, Mills BJW, Poulton SW. 2019. Stepwise Earth oxygenation is an inherent property of global biogeochemical cycling. *Science* 366:1333–37
2. Allen JP, Williams JC. 2011. The evolutionary pathway from anoxygenic to oxygenic photosynthesis examined by comparison of the properties of photosystem II and bacterial reaction centers. *Photosynth. Res.* 107:59–69
3. Antonaru LA, Cardona T, Larkum AWD, Nurnberg DJ. 2020. Global distribution of a chlorophyll *f* cyanobacterial marker. *ISME J.* 14:2275–87
4. Armstrong FA. 2008. Why did nature choose manganese to make oxygen? *Philos. Trans. R. Soc. B* 363:1263–70
5. Ayliffe MA, Scott NS, Timmis JN. 1998. Analysis of plastid DNA-like sequences within the nuclear genomes of higher plants. *Mol. Biol. Evol.* 15:738–45
6. Bao H, Burnap RL. 2016. Photoactivation: the light-driven assembly of the water oxidation complex of photosystem II. *Front. Plant Sci.* 7:578
7. Battistuzzi FU, Hedges SB. 2009. A major clade of prokaryotes with ancient adaptations to life on land. *Mol. Biol. Evol.* 26:335–43
8. Beanland TJ. 1990. Evolutionary relationships between "Q-type" photosynthetic reaction centers: hypothesis-testing using parsimony. *J. Theor. Biol.* 145:535–45
9. Betts HC, Puttick MN, Clark JW, Williams TA, Donoghue PCJ, Pisani D. 2018. Integrated genomic and fossil evidence illuminates life's early evolution and eukaryote origin. *Nat. Ecol. Evol.* 2:1556–62
10. Blank CE, Sánchez-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
11. Blankenship RE. 1992. Origin and early evolution of photosynthesis. *Photosynth. Res.* 33:91–111
12. Blankenship RE, Parson WW. 1978. The photochemical electron transfer reactions of photosynthetic bacteria and plants. *Annu. Rev. Biochem.* 47:635–53
13. Blankenship RE, Sadekar S, Raymond J. 2007. The evolutionary transition from anoxygenic to oxygenic photosynthesis. In *Evolution of Primary Producers in the Sea*, ed. PG Falkowski, AH Knoll, pp. 21–35. Burlington, MA: Academic
14. Blum HF. 1937. On the evolution of photosynthesis. *Am. Nat.* 71:350–62
15. Boden JS, Konhauser KO, Robbins LJ, Sanchez-Baracaldo P. 2021. Timing the evolution of antioxidant enzymes in cyanobacteria. *Nat. Commun.* 12:4742
16. Boerner RJ, Nguyen AP, Barry BA, Debus RJ. 1992. Evidence from directed mutagenesis that aspartate 170 of the D1 polypeptide influences the assembly and/or stability of the manganese cluster in the photosynthetic water-splitting complex. *Biochemistry* 31:6660–72
17. **Brinkert K, De Causmaecker S, Krieger-Liszkay A, Fantuzzi A, Rutherford AW. 2016. Bicarbonate-induced redox tuning in Photosystem II for regulation and protection. *PNAS* 113:12144–49**
18. Brochier-Armanet C, Talla E, Gribaldo S. 2009. The multiple evolutionary histories of dioxygen reductases: implications for the origin and evolution of aerobic respiration. *Mol. Biol. Evol.* 26:285–97

17. Discovery of the function of bicarbonate at the acceptor side of photosystem II.

19. Bryant DA, Hunter CN, Warren MJ. 2020. Biosynthesis of the modified tetrapyrroles—the pigments of life. *J. Biol. Chem.* 295:6888–925
20. Bryant DA, Shen G, Turner GM, Soulier N, Laremore TN, Ho MY. 2020. Far-red light allophycocyanin subunits play a role in chlorophyll *d* accumulation in far-red light. *Photosynth. Res.* 143:81–95
21. Campbell KA, Force DA, Nixon PJ, Dole F, Diner BA, Britt RD. 2000. Dual-mode EPR detects the initial intermediate in photoassembly of the photosystem II Mn cluster: the influence of amino acid residue 170 of the D1 polypeptide on Mn coordination. *J. Am. Chem. Soc.* 122:3754–61
22. Cao P, Pan X, Su X, Liu Z, Li M. 2020. Assembly of eukaryotic photosystem II with diverse light-harvesting antennas. *Curr. Opin. Struct. Biol.* 63:49–57
23. Cardona T. 2015. A fresh look at the evolution and diversification of photochemical reaction centers. *Photosynth. Res.* 126:111–34
24. Cardona T. 2016. Reconstructing the origin of oxygenic photosynthesis: Do assembly and photoactivation recapitulate evolution? *Front. Plant Sci.* 7:257
25. Cardona T. 2017. Photosystem II is a chimera of reaction centers. *J. Mol. Evol.* 84:149–51
26. Cardona T. 2018. Early Archean origin of heterodimeric photosystem I. *Heliyon* 4:e00548
27. Cardona T. 2019. Thinking twice about the evolution of photosynthesis. *Open Biol.* 9:180246
28. Cardona T, Murray JW, Rutherford AW. 2015. Origin and evolution of water oxidation before the last common ancestor of the Cyanobacteria. *Mol. Biol. Evol.* 32:1310–28
29. Cardona T, Rutherford AW. 2019. Evolution of photochemical reaction centres: more twists? *Trends Plant Sci.* 24:1008–21
30. Cardona T, Sánchez-Baracaldo P, Rutherford AW, Larkum AWD. 2019. Early Archean origin of photosystem II. *Geobiology* 17:127–50
31. Cardona T, Sedoud A, Cox N, Rutherford AW. 2012. Charge separation in photosystem II: a comparative and evolutionary overview. *Biochim. Biophys. Acta Bioenerg.* 1817:26–43
32. Cardona T, Shao S, Nixon PJ. 2018. Enhancing photosynthesis in plants: the light reactions. *Essays Biochem.* 62:85–94
33. Castresana J, Saraste M. 1995. Evolution of energetic metabolism: the respiration-early hypothesis. *Trends Biochem. Sci.* 20:443–48
34. Catling DC, Zahnle KJ. 2020. The Archean atmosphere. *Sci. Adv.* 6:eaax1420
35. Chen C, Kazimir J, Cheniae GM. 1995. Calcium modulates the photoassembly of photosystem II $(Mn)_4$-clusters by preventing ligation of nonfunctional high-valency states of manganese. *Biochemistry* 34:13511–26
36. Chen J-H, Wu H, Xu C, Liu X-C, Huang Z, et al. 2020. Architecture of the photosynthetic complex from a green sulfur bacterium. *Science* 370:eabb6350
37. Chernev P, Fischer S, Hoffmann J, Oliver N, Assunção R, et al. 2020. Light-driven formation of manganese oxide by today's photosystem II supports evolutionarily ancient manganese-oxidizing photosynthesis. *Nat. Commun.* 11:6110
38. Coleman GA, Davín AA, Mahendrarajah TA, Szánthó LL, Spang A, et al. 2021. A rooted phylogeny resolves early bacterial evolution. *Science* 372:eabe0511
39. Crawford TS, Hanning KR, Chua JP, Eaton-Rye JJ, Summerfield TC. 2016. Comparison of D1′- and D1-containing PS II reaction centre complexes under different environmental conditions in *Synechocystis* sp. PCC 6803. *Plant Cell Environ.* 39:1715–26
40. Croce R, van Amerongen H. 2020. Light harvesting in oxygenic photosynthesis: Structural biology meets spectroscopy. *Science* 369:eaay2058
41. Cser K, Vass I. 2007. Radiative and non-radiative charge recombination pathways in Photosystem II studied by thermoluminescence and chlorophyll fluorescence in the cyanobacterium *Synechocystis* 6803. *Biochim. Biophys. Acta Bioenerg.* 1767:233–43
42. David LA, Alm EJ. 2011. Rapid evolutionary innovation during an Archaean genetic expansion. *Nature* 469:93–96
43. De Causmaecker S, Douglass JS, Fantuzzi A, Nitschke W, Rutherford AW. 2019. Energetics of the exchangeable quinone, Q_B, in Photosystem II. *PNAS* 116:19458–63
44. Demoulin CF, Lara YJ, Cornet L, Francois C, Baurain D, et al. 2019. Cyanobacteria evolution: insight from the fossil record. *Free Radic. Biol. Med.* 140:206–23

28. The first highly detailed evolutionary study of the D1 proteins of photosystem II.

30. The first attempt at resolving the evolution of photosystem II and water oxidation as a function of time.

45. Di Rienzi SC, Sharon I, Wrighton KC, Koren O, Hug LA, et al. 2013. The human gut and groundwater harbor non-photosynthetic bacteria belonging to a new candidate phylum sibling to Cyanobacteria. *eLife* 2:e01102

46. Dong J, Fernandez-Fueyo E, Hollmann F, Paul CE, Pesic M, et al. 2018. Biocatalytic oxidation reactions: a chemist's perspective. *Angew. Chem. Int. Ed. Engl.* 57:9238–61

47. Dong S, Huang G, Wang C, Wang J, Sui S-F, Qin X. 2022. Structure of the Acidobacteria homodimeric reaction center bound with cytochrome *c*. *Nat. Commun.* 13:7745

48. Ducluzeau AL, Schoepp-Cothenet B, van Lis R, Baymann F, Russell MJ, Nitschke W. 2014. The evolution of respiratory O_2/NO reductases: an out-of-the-phylogenetic-box perspective. *J. R. Soc. Interface* 11:20140196

49. Fantuzzi A, Allgöwer F, Baker H, McGuire G, Teh WK, et al. 2022. Bicarbonate-controlled reduction of oxygen by the Q_A semiquinone in Photosystem II in membranes. *PNAS* 119:e2116063119

50. Fournier GP, Moore KR, Rangel LT, Payette JG, Momper L, Bosak T. 2021. The Archean origin of oxygenic photosynthesis and extant cyanobacterial lineages. *Proc. Biol. Sci.* 288:20210675

51. Frei R, Crowe SA, Bau M, Polat A, Fowle DA, Døssing LN. 2016. Oxidative elemental cycling under the low O_2 Eoarchean atmosphere. *Sci. Rep.* 6:21058

52. Gan F, Zhang S, Rockwell NC, Martin SS, Lagarias JC, Bryant DA. 2014. Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light. *Science* 345:1312–17

53. Garcia-Pichel F, Lombard J, Soule T, Dunaj S, Wu SH, Wojciechowski MF. 2019. Timing the evolutionary advent of cyanobacteria and the later Great Oxidation Event using gene phylogenies of a sunscreen. *mBio* 10:e00561-19

54. Garg H, Loughlin PC, Willows RD, Chen M. 2017. The $C2^1$-formyl group in chlorophyll *f* originates from molecular oxygen. *J. Biol. Chem.* 292:19279–89

55. Gisriel CJ, Azai C, Cardona T. 2021. Recent advances in the structural diversity of reaction centers. *Photosynth. Res.* 149:329–43

56. Gisriel CJ, Cardona T, Bryant DA, Brudvig GW. 2022. Molecular evolution of far-red light-acclimated photosystem II. *Microorganisms* 10:1270

57. Gisriel CJ, Sarrou I, Ferlez B, Golbeck JH, Redding KE, Fromme R. 2017. Structure of a symmetric photosynthetic reaction center–photosystem. *Science* 357:1021–25

58. Gisriel CJ, Shen G, Ho MY, Kurashov V, Flesher DA, et al. 2022. Structure of a monomeric photosystem II core complex from a cyanobacterium acclimated to far-red light reveals the functions of chlorophylls *d* and *f*. *J. Biol. Chem.* 298:101424

59. Gisriel CJ, Wang J, Liu J, Flesher DA, Reiss KM, et al. 2022. High-resolution cryo-electron microscopy structure of photosystem II from the mesophilic cyanobacterium, *Synechocystis* sp. PCC 6803. *PNAS* 119:e2116765118

60. Granold M, Hajieva P, Tosa MI, Irimie FD, Moosmann B. 2018. Modern diversification of the amino acid repertoire driven by oxygen. *PNAS* 115:41–46

61. Grettenberger CL, Sumner DY, Wall K, Brown CT, Eisen JA, et al. 2020. A phylogenetically novel cyanobacterium most closely related to *Gloeobacter*. *ISME J.* 14:2142–52

62. Gruber-Vodicka HR, Leisch N, Kleiner M, Hinzke T, Liebeke M, et al. 2019. Two intracellular and cell type-specific bacterial symbionts in the placozoan *Trichoplax* H2. *Nat. Microbiol.* 4:1465–74

63. Hanson-Smith V, Kolaczkowski B, Thornton JW. 2010. Robustness of ancestral sequence reconstruction to phylogenetic uncertainty. *Mol. Biol. Evol.* 27:1988–99

64. Harada M, Akiyama A, Furukawa R, Yokobori SI, Tajika E, Yamagishi A. 2021. Evolution of superoxide dismutases and catalases in cyanobacteria: occurrence of the antioxidant enzyme genes before the rise of atmospheric oxygen. *J. Mol. Evol.* 89:527–43

65. Harel A, Karkar S, Cheng S, Falkowski PG, Bhattacharya D. 2015. Deciphering primordial cyanobacterial genome functions from protein network analysis. *Curr. Biol.* 25:628–34

66. Hays AM, Vassiliev IR, Golbeck JH, Debus RJ. 1998. Role of D1-His190 in proton-coupled electron transfer reactions in photosystem II: a chemical complementation study. *Biochemistry* 37:11352–65

67. Ho MY, Shen G, Canniffe DP, Zhao C, Bryant DA. 2016. Light-dependent chlorophyll *f* synthase is a highly divergent paralog of PsbA of photosystem II. *Science* 353:aaf9178

45. Discovery of a nonphotosynthetic sister lineage of cyanobacteria.

50. A state-of-the-art molecular clock analysis timing the evolution of cyanobacteria and using constraints from many horizontal gene transfer events.

52. Discovery of the far-red light photoacclimation response involving extensive remodeling of the photosystems.

57. The first crystal structure of a homodimeric type I photosystem.

68. Hohmann-Marriott MF, Blankenship RE. 2011. Evolution of photosynthesis. *Annu. Rev. Plant Biol.* 62:515–48
69. Homann M, Heubeck C, Airo A, Tice MM. 2015. Morphological adaptations of 3.22 Ga-old tufted microbial mats to Archean coastal habitats (Moodies Group, Barberton Greenstone Belt, South Africa). *Precambrian Res.* 266:47–64
70. Hwang HJ, McLain A, Debus RJ, Bumap RL. 2007. Photoassembly of the manganese cluster in mutants perturbed in the high affinity Mn-binding site of the H_2O-oxidation complex of photosystem II. *Biochemistry* 46:13648–57
71. Ishikita H, Saenger W, Biesiadka J, Loll B, Knapp EW. 2006. How photosynthetic reaction centers control oxidation power in chlorophyll pairs P680, P700, and P870. *PNAS* 103:9855–60
72. Jabłońska J, Tawfik DS. 2021. The evolution of oxygen-utilizing enzymes suggests early biosphere oxygenation. *Nat. Ecol. Evol.* 5:442–48
73. Johnson GN, Rutherford AW, Krieger A. 1995. A change in the midpoint potential of the quinone Q_A in Photosystem II associated with photoactivation of oxygen evolution. *Biochim. Biophys. Acta Bioenerg.* 1229:202–7
74. Johnson JE, Webb SM, Thomas K, Ono S, Kirschvink JL, Fischer WW. 2013. Manganese-oxidizing photosynthesis before the rise of cyanobacteria. *PNAS* 110:11238–43
75. Kanygin A, Milrad Y, Thummala C, Reifschneider K, Baker P, et al. 2020. Rewiring photosynthesis: a photosystem I-hydrogenase chimera that makes H_2 *in vivo*. *Energy Environ. Sci.* 13:2903–14
76. Kasting JF, Walker JCG. 1981. Limits on oxygen concentration in the prebiological atmosphere and the rate of abiotic fixation of nitrogen. *J. Geophys. Res.* 86:1147–58
77. Kharecha P, Kasting J, Siefert J. 2005. A coupled atmosphere-ecosystem model of the early Archean Earth. *Geobiology* 3:53–76
78. Kimura M, Ota T. 1971. On the rate of molecular evolution. *J. Mol. Evol.* 1:1–17
79. Kiss E, Kós PB, Chen M, Vass I. 2012. A unique regulation of the expression of the *psbA*, *psbD*, and *psbE* genes, encoding the D1, D2 and cytochrome b559 subunits of the Photosystem II complex in the chlorophyll *d* containing cyanobacterium *Acaryochloris marina*. *Biochim. Biophys. Acta Bioenerg.* 1817:1083–94
80. Knoops B, Loumaye E, Van Der Eecken V. 2007. Evolution of the peroxiredoxins. *Subcell. Biochem.* 44:27–40
81. Krieger A, Weis E, Demeter S. 1993. Low-pH-induced Ca^{2+} ion release in the water-splitting system is accompanied by a shift in the midpoint redox potential of the primary quinone acceptor Q_A. *Biochim. Biophys. Acta Bioenerg.* 1144:411–18
82. Krynická V, Shao S, Nixon PJ, Komenda J. 2015. Accessibility controls selective degradation of photosystem II subunits by FtsH protease. *Nat. Plants* 1:15168
83. Kselíková V, Singh A, Bialevich V, Čížková M, Bišová K. 2022. Improving microalgae for biotechnology—from genetics to synthetic biology—moving forward but not there yet. *Biotechnol. Adv.* 58:107885
84. Magnabosco C, Moore KR, Wolfe JM, Fournier GP. 2018. Dating phototrophic microbial lineages with reticulate gene histories. *Geobiology* 16:179–89
85. Marchi S, Drabon N, Schulz T, Schaefer L, Nesvorny D, et al. 2021. Delayed and variable late Archaean atmospheric oxidation due to high collision rates on Earth. *Nat. Geosci.* 14:827–31
86. Martin WF, Bryant DA, Beatty JT. 2018. A physiological perspective on the origin and evolution of photosynthesis. *FEMS Microbiol. Rev.* 42:205–31
87. Masuda T, Bernát G, Bečková M, Kotabová E, Lawrenz E, et al. 2018. Diel regulation of photosynthetic activity in the oceanic unicellular diazotrophic cyanobacterium *Crocosphaera watsonii* WH8501. *Environ. Microbiol.* 20:546–60
88. Müh F, Glöckner C, Hellmich J, Zouni A. 2012. Light-induced quinone reduction in photosystem II. *Biochim. Biophys. Acta Bioenerg.* 1817:44–65
89. Mulo P, Sakurai I, Aro E-M. 2012. Strategies for *psbA* gene expression in cyanobacteria, green algae and higher plants: from transcription to PSII repair. *Biochim. Biophys. Acta Bioenerg.* 1817:247–57
90. Mulo P, Sicora C, Aro EM. 2009. Cyanobacterial *psbA* gene family: optimization of oxygenic photosynthesis. *Cell. Mol. Life Sci.* 66:3697–710

72. A phylogenetic mapping of the origin of O_2-using enzymes along the diversification of bacteria.

91. Murray JW. 2012. Sequence variation at the oxygen-evolving centre of Photosystem II: a new class of 'rogue' cyanobacterial D1 proteins. *Photosynth. Res.* 110:177–84
92. Nitschke W, Rutherford AW. 1991. Photosynthetic reaction centers: variations on a common structural theme? *Trends Biochem. Sci.* 16:241–45
93. Nixon PJ, Diner BA. 1992. Aspartate 170 of the photosystem II reaction center polypeptide D1 is involved in the assembly of the oxygen-evolving manganese cluster. *Biochemistry* 31:942–48
94. Nurnberg DJ, Morton J, Santabarbara S, Telfer A, Joliot P, et al. 2018. Photochemistry beyond the red limit in chlorophyll f-containing photosystems. *Science* 360:1210–13
95. Oliver N, Avramov AP, Nürnberg DJ, Dau H, Burnap RL. 2022. From manganese oxidation to water oxidation: assembly and evolution of the water-splitting complex in photosystem II. *Photosynth. Res.* 152:107–33
96. Oliver T, Sánchez-Baracaldo P, Larkum AW, Rutherford AW, Cardona T. 2021. Time-resolved comparative molecular evolution of oxygenic photosynthesis. *Biochim. Biophys. Acta Bioenerg.* 1862:148400
97. Olsen MT, Nowack S, Wood JM, Becraft ED, LaButti K, et al. 2015. The molecular dimension of microbial species: 3. Comparative genomics of *Synechococcus* strains with different light responses and *in situ* diel transcription patterns of associated putative ecotypes in the Mushroom Spring microbial mat. *Front. Microbiol.* 6:604
98. Olson JM. 1970. The evolution of photosynthesis. *Science* 168:438–46
99. Olson JM. 1981. Evolution of photosynthetic reaction centers. *Biosystems* 14:89–94
100. Olson JM, Blankenship RE. 2004. Thinking about the evolution of photosynthesis. *Photosynth. Res.* 80:373–86
101. Olson JM, Pierson BK. 1987. Evolution of reaction centers in photosynthetic prokaryotes. *Int. Rev. Cytol.* 108:209–48
102. Oparin AI, Braunshtein AE, Pasynski AG, Pavlovskaya TE. 1959. *The Origin of Life on the Earth*. London: Pergamon
103. Ouzounis CA, Kunin V, Darzentas N, Goldovsky L. 2006. A minimal estimate for the gene content of the last universal common ancestor: exobiology from a terrestrial perspective. *Res. Microbiol.* 157:57–68
104. Park JJ, Lechno-Yossef S, Wolk CP, Vieille C. 2013. Cell-specific gene expression in *Anabaena variabilis* grown phototrophically, mixotrophically, and heterotrophically. *BMC Genom.* 14:759
105. Parks DH, Chuvochina M, Rinke C, Mussig AJ, Chaumeil P-A, Hugenholtz P. 2022. GTDB: an ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. *Nucleic Acids Res.* 50:D785–94
106. Pierson BK, Olson JM. 1989. Evolution of photosynthesis in anoxygenic photosynthetic procaryotes. In *Microbial Mats: Physiological Ecology of Benthic Microbial Communities*, ed. Y Cohen, E Rosenberg, pp. 402–27. Washington, DC: Am. Soc. Microbiol.
107. Planavsky NJ, Crowe SA, Fakhraee M, Beaty B, Reinhard CT, et al. 2021. Evolution of the structure and impact of Earth's biosphere. *Nat. Rev. Earth Environ.* 2:123–39
108. Rahmatpour N, Hauser DA, Nelson JM, Chen PY, Villarreal A JC, et al. 2021. A novel thylakoid-less isolate fills a billion-year gap in the evolution of Cyanobacteria. *Curr. Biol.* 31:2857–67.e4
109. Raven JA. 2009. Contributions of anoxygenic and oxygenic phototrophy and chemolithotrophy to carbon and oxygen fluxes in aquatic environments. *Aquat. Microb. Ecol.* 56:177–92
110. Reinhard CT, Planavsky NJ. 2022. The history of ocean oxygenation. *Annu. Rev. Mar. Sci.* 14:331–53
111. Rosing MT, Frei R. 2004. U-rich Archaean sea-floor sediments from Greenland–indications of >3700 Ma oxygenic photosynthesis. *Earth Planet. Sci. Lett.* 217:237–44
112. Rutherford AW. 1989. Photosystem II, the water-splitting enzyme. *Trends Biochem. Sci.* 14:227–32
113. Rutherford AW, Faller P. 2003. Photosystem II: evolutionary perspectives. *Philos. Trans. R. Soc. B* 358:245–53
114. Rutherford AW, Nitschke W. 1996. Photosystem 2 and the quinone–iron-containing reaction centers: comparisons and evolutionary perspectives. In *Origin and Evolution of Biological Energy Conversion*, ed. H Baltscheffsky, pp. 143–75. New York: VCH
115. Rutherford AW, Osyczka A, Rappaport F. 2012. Back-reactions, short-circuits, leaks and other energy wasteful reactions in biological electron transfer: redox tuning to survive life in O_2. *FEBS Lett.* 586:603–16

98. The first published scenario for the evolution of the photosystems.

116. Satkoski AM, Beukes NJ, Li W, Beard BL, Johnson CM. 2015. A redox-stratified ocean 3.2 billion years ago. *Earth Planet. Sci. Lett.* 430:43–53
117. Sauer K. 1979. Photosynthesis—the light reactions. *Annu. Rev. Phys. Chem.* 30:155–78
118. Saw JH, Cardona T, Montejano G. 2021. Complete genome sequencing of a novel *Gloeobacter* species from a waterfall cave in Mexico. *Genome Biol. Evol.* 13:evab264
119. Schirrmeister BE, Sánchez-Baracaldo P, Wacey D. 2016. Cyanobacterial evolution during the Precambrian. *Int. J. Astrobiol.* 15:187–204
120. Schmidt-Rohr K. 2015. Why combustions are always exothermic, yielding about 418 kJ per mole of O_2. *J. Chem. Educ.* 92:2094–99
121. Schmidt-Rohr K. 2021. O_2 and other high-energy molecules in photosynthesis: why plants need two photosystems. *Life* 11:1191
122. Schubert WD, Klukas O, Saenger W, Witt HT, Fromme P, Krauss N. 1998. A common ancestor for oxygenic and anoxygenic photosynthetic systems: a comparison based on the structural model of photosystem I. *J. Mol. Biol.* 280:297–314
123. Sheldon RA. 2020. Catalytic oxidations in a bio-based economy. *Front. Chem.* 8:132
124. Shen G, Canniffe DP, Ho MY, Kurashov V, van der Est A, et al. 2019. Characterization of chlorophyll *f* synthase heterologously produced in *Synechococcus* sp. PCC 7002. *Photosynth. Res.* 140:77–92
125. Sheridan KJ, Duncan EJ, Eaton-Rye JJ, Summerfield TC. 2020. The diversity and distribution of D1 proteins in cyanobacteria. *Photosynth. Res.* 145:111–28
126. Shi T, Bibby TS, Jiang L, Irwin AJ, Falkowski PG. 2005. Protein interactions limit the rate of evolution of photosynthetic genes in cyanobacteria. *Mol. Biol. Evol.* 22:2179–89
127. Shih PM, Hemp J, Ward LM, Matzke NJ, Fischer WW. 2017. Crown group Oxyphotobacteria postdate the rise of oxygen. *Geobiology* 15:19–29
128. Sicora CI, Chis I, Chis C, Sicora O. 2019. Regulation of PSII function in *Cyanothece* sp. ATCC 51142 during a light-dark cycle. *Photosynth. Res.* 139:461–73
129. Sicora CI, Ho FM, Salminen T, Styring S, Aro EM. 2009. Transcription of a "silent" cyanobacterial *psbA* gene is induced by microaerobic conditions. *Biochim. Biophys. Acta Bioenerg.* 1787:105–12
130. Ślesak I, Ślesak H, Kruk J. 2012. Oxygen and hydrogen peroxide in the early evolution of life on Earth: *in silico* comparative analysis of biochemical pathways. *Astrobiology* 12:775–84
131. Slotznick SP, Johnson JE, Rasmussen B, Raub TD, Webb SM, et al. 2022. Reexamination of 2.5-Ga "whiff" of oxygen interval points to anoxic ocean before GOE. *Sci. Adv.* 8:eabj7190
132. Soo RM, Hemp J, Hugenholtz P. 2019. Evolution of photosynthesis and aerobic respiration in the cyanobacteria. *Free Radical Biol. Med.* 140:200–5
133. Soo RM, Woodcroft BJ, Parks DH, Tyson GW, Hugenholtz P. 2015. Back from the dead; the curious tale of the predatory cyanobacterium *Vampirovibrio chlorellavorus*. *PeerJ* 3:e968
134. Sugiura M, Azami C, Koyama K, Rutherford AW, Rappaport F, Boussac A. 2013. Modification of the pheophytin redox potential in *Thermosynechococcus elongatus* Photosystem II with PsbA3 as D1. *Biochim. Biophys. Acta Bioenerg.* 1837:139–48
135. Sugiura M, Boussac A. 2014. Some Photosystem II properties depending on the D1 protein variants in *Thermosynechococcus elongatus*. *Biochim. Biophys. Acta Bioenerg.* 1837:1427–34
136. Sugiura M, Ogami S, Kusumi M, Un S, Rappaport F, Boussac A. 2012. Environment of Tyr_Z in photosystem II from *Thermosynechococcus elongatus* in which PsbA2 is the D1 protein. *J. Biol. Chem.* 287:13336–47
137. Summerfield TC, Toepel J, Sherman LA. 2008. Low-oxygen induction of normally cryptic *psbA* genes in cyanobacteria. *Biochemistry* 47:12939–41
138. Tan C, Xu P, Tao F. 2022. Carbon-negative synthetic biology: challenges and emerging trends of cyanobacterial technology. *Trends Biotechnol.* 40:1488–502
139. Toepel J, Welsh E, Summerfield TC, Pakrasi HB, Sherman LA. 2008. Differential transcriptional analysis of the cyanobacterium *Cyanothece* sp. strain ATCC 51142 during light-dark and continuous-light growth. *J. Bacteriol.* 190:3904–13
140. Trinugroho JP, Beckova M, Shao SX, Yu JF, Zhao ZY, et al. 2020. Chlorophyll *f* synthesis by a super-rogue photosystem II complex. *Nat. Plants* 6:238–44

140. Characterization of a photosystem II complex with catalysis beyond water oxidation.

141. Utami YD, Kuwahara H, Igai K, Murakami T, Sugaya K, et al. 2019. Genome analyses of uncultured TG2/ZB3 bacteria in 'Margulisbacteria' specifically attached to ectosymbiotic spirochetes of protists in the termite gut. *ISME J.* 13:455–67
142. van Niel CB. 1949. The comparative biochemistry of photosynthesis. *Am. Sci.* 37:371–83
143. van Niel CB. 1956. Evolution as viewed by the microbiologist. In *The Microbe's Contribution to Biology*, ed. AJ Kluyver, CB van Niel, pp. 155–76. Cambridge, MA: Harvard Univ. Press
144. Vermaas WF. 1994. Evolution of heliobacteria: implications for photosynthetic reaction center complexes. *Photosynth. Res.* 41:285–94
145. Vinyard DJ, Gimpel J, Ananyev GM, Mayfield SP, Dismukes GC. 2014. Engineered Photosystem II reaction centers optimize photochemistry versus photoprotection at different solar intensities. *J. Am. Chem. Soc.* 136:4048–55
146. Wahart AJC, Staniland J, Miller GJ, Cosgrove SC. 2022. Oxidase enzymes as sustainable oxidation catalysts. *R. Soc. Open Sci.* 9:211572
147. Walker JCG. 1980. Atmospheric constraints on the evolution of metabolism. *Orig. Life* 10:93–104
148. Wang XL, Planavsky NJ, Hofmann A, Saupe EE, De Corte BP, et al. 2018. A Mesoarchean shift in uranium isotope systematics. *Geochim. Cosmochim. Acta* 238:438–52
149. Wegener KM, Nagarajan A, Pakrasi HB. 2015. An atypical *psbA* gene encodes a sentinel D1 protein to form a physiologically relevant inactive photosystem II complex in cyanobacteria. *J. Biol. Chem.* 290:3764–74
150. Weiss MC, Sousa FL, Mrnjavac N, Neukirchen S, Roettger M, et al. 2016. The physiology and habitat of the last universal common ancestor. *Nat. Microbiol.* 1:16116
151. Williams JC, Steiner LA, Ogden RC, Simon MI, Feher G. 1983. Primary structure of the M subunit of the reaction center from *Rhodopseudomonas sphaeroides*. *PNAS* 80:6505–9
152. Wisecaver JH, Hackett JD. 2011. Dinoflagellate genome evolution. *Annu. Rev. Microbiol.* 65:369–87
153. Wohlgemuth R, Twardowski T, Aguilar A. 2021. Bioeconomy moving forward step by step—a global journey. *N. Biotechnol.* 61:22–28
154. Wu A, Hammer GL, Doherty A, von Caemmerer S, Farquhar GD. 2019. Quantifying impacts of enhancing photosynthesis on crop yield. *Nat. Plants* 5:380–88
155. Xiao Y, Huang G, You X, Zhu Q, Wang W, et al. 2021. Structural insights into cyanobacterial photosystem II intermediates associated with Psb28 and Tsl0063. *Nat. Plants* 7:1132–42
156. Xiong J, Bauer CE. 2002. A cytochrome *b* origin of photosynthetic reaction centers: an evolutionary link between respiration and photosynthesis. *J. Mol. Biol.* 322:1025–37
157. Yang Y, Arnold FH. 2021. Navigating the unnatural reaction space: directed evolution of heme proteins for selective carbene and nitrene transfer. *Acc. Chem. Res.* 54:1209–25
158. Yao DCI, Brune DC, Vermaas WFJ. 2012. Lifetimes of photosystem I and II proteins in the cyanobacterium *Synechocystis* sp. PCC 6803. *FEBS Lett.* 586:169–73
159. Zabret J, Bohn S, Schuller SK, Arnolds O, Möller M, et al. 2021. Structural insights into photosystem II assembly. *Nat. Plants* 7:524–38
160. Zamocky M, Janecek S, Koller F. 2000. Common phylogeny of catalase-peroxidases and ascorbate peroxidases. *Gene* 256:169–82
161. Zeymer C, Hilvert D. 2018. Directed evolution of protein catalysts. *Annu. Rev. Biochem.* 87:131–57
162. Zhang G-J, Dong R, Lan L-N, Li S-F, Gao W-J, Niu H-X. 2020. Nuclear integrants of organellar DNA contribute to genome structure and evolution in plants. *Int. J. Mol. Sci.* 21:707
163. Zhang Z, Green BR, Cavalier-Smith T. 2000. Phylogeny of ultra-rapidly evolving dinoflagellate chloroplast genes: a possible common origin for sporozoan and dinoflagellate plastids. *J. Mol. Evol.* 51:26–40
164. Zimmerman JB, Anastas PT, Erythropel HC, Leitner W. 2020. Designing for a green chemistry future. *Science* 367:397–400