Were Precambrian oceans devoid of planktonic cyanobacteria? Insights from metabolism

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Abstract

Studies on biospheric evolution often assume planktonic cyanobacteria existed in Precambrian oceans, but that their productivity was limited due to various factors. However, available evidence suggests that planktonic cyanobacteria only colonized the open ocean near the Neoproterozoic-Phanerozoic boundary, close to the when a period of atmospheric oxygenation triggered the rise of animals. It is an open question if earlier planktonic marine cyanobacteria went extinct, possibly due to snowball Earth glaciations, or if cyanobacteria colonized the open ocean for the first time during this period. If the latter is true, consensus explanations are lacking for why cyanobacteria did not colonize the oceans sooner. Here I review the reconstructed metabolic evolution of marine picocyanobacteria, which perform ~25% of oceanic CO_2-fixation and which emerged in the early Phanerozoic. These reconstructions provide insights into how the marine biosphere overcame earlier constraints on productivity, and how the rise of arthropods may have been key to allowing cyanobacteria to make the evolutionary leap from the benthos to the open ocean, helping transform the biosphere and Earth as a whole. The idea that photosynthesis was largely limited to shallow continental shelf waters during the Precambrian has implications for interpreting large-scale patterns of biospheric productivity and Earth history.

Introduction

The oxygenation of Earth's atmosphere resulted from an interplay of multiple biological and geological processes but was ultimately driven by the photosynthetic production of oxygen and the long-term sequestration of associated organic carbon in Earth's mantle, crust and oceans (1,2). Understanding large-scale patterns in biospheric productivity over Earth history (3) is key to reconstructing the rise of oxygen in Earth's atmosphere. Biospheric productivity is determined by the size and spatial extent of different ecosystems in which oxygenic photosynthesis occurs, and the rate of photosynthesis in those ecosystems.

Both aspects are challenging to precisely constrain in reconstructions of early Earth environments as they depend on inferences from geochemical proxies and fossil/biomarker records that become sparser and more ambiguous in deep time, especially for the deep open ocean (4-6). This in tun impacts molecular clock calculations of the evolution of major groups of photosynthetic organisms, which depend on external constraints from the geologic record (7-9). Together this leads to generally greater uncertainty regarding the scale of primary production when extrapolating further back in Earth history.

Studies of metabolism can help address these uncertainties by suggesting linkages between the geologic and genomic records. Developing systematic frameworks of metabolic evolution identifies large-scale tradeoffs and forces that shape ecosystems and the biogeochemical cycles they mediate, informing interpretations of ecosystem change in deep time. I will illustrate these ideas using marine picocyanobacteria, consisting of the sister lineages *Prochlorococcus* (10) and marine *Synechococcus* (11). Marine picocyanobacteria perform ~20-25% of CO_2-fixation in extant oceans (12,13) and their rise may have been linked to general biospheric revolutions in the early Phanerozoic (541 Mya – present) that ultimately led to the rise of macroscopic eukaryotic life (14-16). As background I will first review geologically-informed frameworks of the productivity of the marine biosphere during the Proterozoic (2500 Mya – 541 Mya), as well as evidence regarding the timelines of evolution of planktonic marine cyanobacteria in the open ocean. I will then review emerging frameworks for the metabolic evolution of marine picocyanobacteria and end by discussing their implications for large-scale patterns of biospheric productivity over Earth history.

Biospheric productivity in Proterozoic oceans

Atmospheric oxygen underwent a dramatic rise in the early Proterozoic (17-19) update from here onward, but then remained low relative to modern levels until a second dramatic rise occurred between the late Proterozoic and early Phanerozoic (20-22). Various constraints on productivity of the Proterozoic marine biosphere have been proposed (Fig. 1), several of which involve the interplay between metals and the macronutrients nitrogen and phosphorus. For example, it has been suggested that molybdenum and other metals essential to the biological nitrogen cycle would have been scavenged by sulfide ions in sulfide-rich (i.e. euxinic) waters, leading to nitrogen-limitation of photosynthesis (23,24). Euxinic conditions were likely limited to relatively productive coastal waters, but models suggest that such environments could still have acted as an effective global sink of molybdenum due to large-scale ocean circulation processes (24). Similarly, it has been suggested that the high levels of ferrous iron (Fe^{2+}) in the mostly anoxic

Proterozoic oceans (25,26) would have scavenged free phosphate, leading to phosphorus limitation of photosynthesis (27,28).

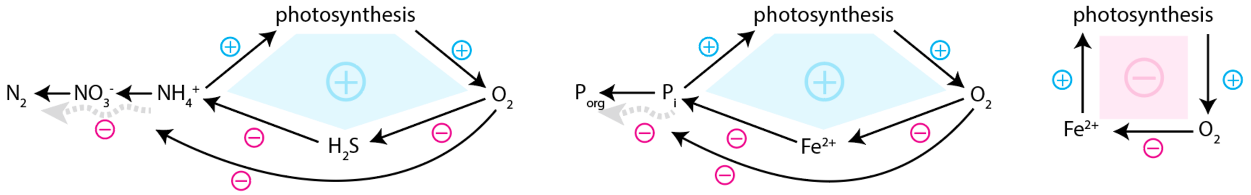

Fig. 1. Proposed constraints on productivity of the ocean biosphere during the Proterozoic. Blue positive signs are positive, stimulatory effects, while pink negative signs are negative, inhibitory effects. Availability of nutrients (nitrogen, phosphorus, iron) stimulates photosynthesis and thereby O_2 production. Nitrogen and phosphorus availability are proposed to have been limited due to chemical feedbacks (23,24,27,28), as well as due to differential rates within the biological cycles of both elements, the latter represented as dashed grey arrows (29,30). Increased O_2 levels would have helped eliminate these negative feedbacks, leading to overall self-amplifying cycles (blue shading) that may have been stuck in local minima. In contrast, iron availability is proposed to have *become* limiting under rising O_2 levels due to its decreasing solubility, leading to an overall self-damping cycle (pink shading). See text for further details. Note: phytoplankton can use multiple forms of nitrogen, but the universal entry point of nitrogen into metabolism for all life is ammonia. Abbreviations: P_i = inorganic phosphorus (i.e. phosphate), P_{org} = organic phosphorus

Other proposals focus on limitations arising due to differences in the rates of processes within the biological cycles of nitrogen and phosphorus under conditions of low oxygen (Fig. 1). That is, when oxygen is low, denitrification (i.e. conversion of NO_3^- to N_2 gas) rates are predicted to outpace nitrification (i.e. conversion of NH_4^+ to NO_3^-), preventing NO_3^- from accumulating, thereby driving nitrogen-loss (i.e. outgassing of N_2 from the ocean) and nitrogen limitation (29). Similarly, when oxygen is low, remineralization of organic phosphate to inorganic phosphate is predicted to have been suppressed, driving or exacerbating phosphorus-limitation (30).

These proposals share two common properties. One is that a rise in oxygen toward Phanerozoic levels ultimately acts to remove preceding nutrient limitations (Fig. 1). That is, oxygenating the ocean removes both sulfides and ferrous iron, thereby eliminating the scavenging of molybdenum (31) and phosphorus (32), respectively. Simultaneously, a rise in oxygen levels increases the rate of phosphorus remineralization (30) and the rate of nitrification relative to denitrification, thereby counteracting nitrogen loss (29) (Fig. 1). Relative to Proterozoic sediments, Phanerozoic sediments show an increase in the levels of molybdenum (31) and phosphorus (28,32), as well as the onset of a stable oceanic nitrate pool (33,34), consistent with these proposals. However, it should be noted that most of the geochemical records used to make inferences of ocean nutrient inventories are largely derived from shallow continental shelf environments (28,31-34), leading to inevitable uncertainties regarding the nutrient landscape of the open ocean. Still, molecular clocks suggest that nitrogen-fixing marine planktonic cyanobacteria did not expand into the open ocean until the late-Neoproterozoic (35), consistent with nutrient-poor (or at least nitrogen-poor) oceans before this time.

The second common feature of these proposals is they generally assume that planktonic cyanobacteria were present in the open ocean, but with their productivity suppressed due to nutrient limitations. Together these features create the potential for non-linear dynamics in the oxygenation of the ocean and atmosphere. That is, increases in oxygen will be transient so long as the marine biosphere remains in the grip of outlined nutrient limitations, but can be become self-sustaining if they are significant enough to begin loosening constraints on productivity of pre-existing planktonic cyanobacteria (Fig. 1) (36). This has led the argument that a large-scale redox perturbation due to snowball Earth played a key role in triggering the transition to an oxygenated ocean, effectively by freeing cyanobacteria from constraints on their productivity (36). Proposals that seek to explain the time-course of atmospheric oxygenation through long-term changes in geologic processes rather than through changes in constraints on biospheric productivity often similarly assume – either implicitly or explicitly – that cyanobacteria were present in the open ocean (37-40). However, the validity of the assumption that planktonic cyanobacteria existed in the open ocean in the Proterozoic is not clear.

A different dynamic emerges in a proposal that colleagues and I put forward (41,42), in which productivity of Proterozoic oceans was limited at least in part due to a negative feedback built into the molecular machinery of photosynthesis itself (Fig. 1). That is, photosynthesis depends on iron, but iron is insoluble in the presence of oxygen. Thus, as oxygenation proceeds, iron becomes less available, lowering the production of oxygen, thus creating a self-damping dynamic that contrasts with the ultimately self-amplifying dynamics as in the proposals outlined above (Fig. 1). Indeed, this proposed dynamic is independent of the ocean inventories of nitrogen and phosphorus, suggesting that external perturbations (36) or long-term changes in geologic processes (37-40) may have been insufficient to drive oxygenation of the open ocean, and that biological innovations could have been necessary to unlock the full productivity potential of the ocean (42). If the latter view is correct, it in turn raises questions on the nature of those innovations, and why they did not, or could not, happen sooner.

Reconstructed timelines for the evolution of planktonic marine cyanobacteria

Evaluating proposals for primary production in the open ocean in deep time requires considering the evidence for the existence of planktonic marine cyanobacteria throughout Earth history. Indirect evidence comes from the geologic carbon isotope record, which shows nearshore-to-offshore gradients in the ^{13}C-content of sedimentary carbonates that could be consistent with export of organic carbon from surface waters into sediments in the Proterozoic (43) and Archaean (44). However, observed gradients are weak, and conclusions regarding export processes thereby depend on assumptions of high levels of atmospheric CO_2 (43,44) needed to maintain liquid oceans on the early Earth due to lower solar luminosity (45). Precise levels of CO_2 relative to other potential greenhouse gases (particularly CH_4) remain debated (46,47), leaving uncertainty about the magnitude of potential export processes. Moreover, offshore isotopic records are limited in number and typically come from depositional environment that extend only a few tens of kilometer beyond the shelf break (43,44). In modern oceans continental shelves and near-shore open ocean waters are connected through physical transport processes driven by tides, wind stress and general ocean circulation (48,49). This leaves open the possibility that observed carbon isotopic gradients could have been generated by primary production within continental shelves followed by lateral transport of organic carbon to deeper waters.

Direct evidence specific to the evolution of cyanobacteria ultimately comes from two sources: fossils and biomarkers available in ancient rocks (50,51), and the record of molecular evolution available in genomes of extant cyanobacteria (8,9,14). The fossil record is often less definitive for cyanobacteria than for eukaryotes due to their smaller size and simpler structural morphologies (52,53). Targeted genome sequencing efforts (e.g. 54) and biomarker analyses (e.g. 55) have increased the number of structural morphologies in rocks tied to cyanobacterial taxa, but the numbers remain limited and essentially all are derived from shallow depositional environments and assigned to taxa not exclusively associated with a planktonic lifestyle (for detailed reviews, see Refs 8,50,56). Biomarkers in turn suggest that Proterozoic marine environments were dominated by cyanobacterial photosynthesis (51), but the assignment of these molecules to specific cyanobacterial taxa remains uncertain and samples containing them were obtained from shallow depositional settings (51). In summary, Proterozoic fossil/biomarker records offer no direct evidence of planktonic cyanobacteria in the deep open ocean (50,56).

The genomic record, in turn, is clearer. Most molecular clock calculations suggest that ancestors of marine picocyanobacteria colonized the ocean in the late Neoproterozoic (1000 – 541 Mya) or early Phanerozoic (9,14,57-59), although origins in the early Neoproterozoic have also been found (60). In general, molecular clock calculations of divergence times and associated uncertainty ranges are strongly dependent on the external constraints that are used, and as outlined above, the number of constraints available from the geologic record are very limited for cyanobacteria (8,50,56). To help address this gap, a recently emerging approach is to use horizontal gene transfer (HGT) events as an additional source of constraint on molecular clock calculations (61). That is, if the topologies of both the donor and recipient trees within the phylogenies of genes undergoing HGT are sufficiently similar to those of the species trees of both groups, and either group has an absolute date constraint, this constraint can be propagated across both groups (61). Using this approach leads to a calculated mean age of 424 Mya (range 491 – 340 Mya) for crown group marine picocyanobacteria, and a calculated mean age of 576 Mya (range 666 – 492 Mya) for 'total group' picocyanobacteria, the latter of which includes groups from freshwater and brackish environments (9). These ages are consistent with other recent results (57,62) but reduce calculated uncertainties by several hundred million years. In other words, molecular clock calculations suggest that the cyanobacterial branch leading to crown group marine picocyanobacteria evolved over a ~150-million-year period that came after the last Neoproterozoic snowball Earth (i.e. the Marinoan glaciation), which ended ~632 Mya (63).

In summary, the genomic record suggests that marine picocyanobacteria most likely colonized the ocean in the early Phanerozoic, while the fossil and biomarker records offer no evidence of planktonic marine cyanobacteria before then. This leaves open two basic possibilities: 1) planktonic marine cyanobacteria *did* exist in the open ocean during the Precambrian, but went extinct, possibly due to snowball Earth glaciations, with evidence of their existence erased or still to be discovered, or 2) cyanobacteria colonized the open ocean for the first time in the early Phanerozoic. If the latter is true, why did cyanobacteria not colonize the ocean sooner? For insights into these questions, I will next look at evidence from metabolic reconstructions.

Metabolic evolution of marine picocyanobacteria

The metabolic makeup of a cell reflects how it transforms environmental inputs to make additional copies of itself. Metabolic pathways are governed by various ecological tradeoffs, for example due to differences in their energetic or protein biomass requirements (64), the metals needed in enzyme cofactors (65), the nature and redox state of the inorganic nutrients that cells assimilate (66), or the relative amounts of carbon, nitrogen, or other elements, that are generated in the case of catabolic pathways. Metabolic innovations that occur over microbial evolution thus reflect changes in the ecosystems they are part of, whether due to changes or perturbations in the Earth system or due to the colonization of new environments. Relating metabolic diversity to Earth history requires developing systematic frameworks of metabolic evolution. Colleagues and I have been pursuing this through the integration of phylogenetic and metabolic network reconstructions (41,67). This allows us to pinpoint specific innovations that occur along major divergences within microbial phylogenies and infer the underlying tradeoffs and driving forces acting on them. Identifying these tradeoffs and evolutionary driving forces at major branches in microbial phylogenies offers opportunities for linking the timeline of these branches as calculated using molecular clocks with records of ecosystem change observed in the rock record.

We have taken this approach to reconstruct the metabolic evolution of marine picocyanobacteria, starting with the metabolic core, i.e. the pathways involved in photosynthesis, electron transfer and carbon-fixation (Fig. 2A). The metabolic core is where light energy and CO_2 are combined to generate the carbon backbones of all biomolecules making up the cell. Because everything else depends on it, the evolution of the metabolic core is highly constrained and any changes that do occur reflect strong selective pressures (68). In our reconstructions, we found that the metabolic core of marine picocyanobacteria underwent a large-scale remodeling as they diverged from other ancestral cyanobacteria (Fig. 2) (41). That is, many genes involved in core carbohydrate metabolism or photosynthesis were lost, gained or replaced through horizontal gene transfer, while the pigments and stoichiometry of the photosynthetic machinery were also modified (Fig. 2). Metabolic changes include the addition of several pathways for the exudation of intermediates of core carbohydrate metabolism, suggesting that selection has favored an increase in organic carbon exudation of cells (41). This whole-sale reorganization of core pathways indicates that the metabolism of marine picocyanobacteria came under intense selection as they colonized the open ocean, reflecting the unique challenges this environment presents.

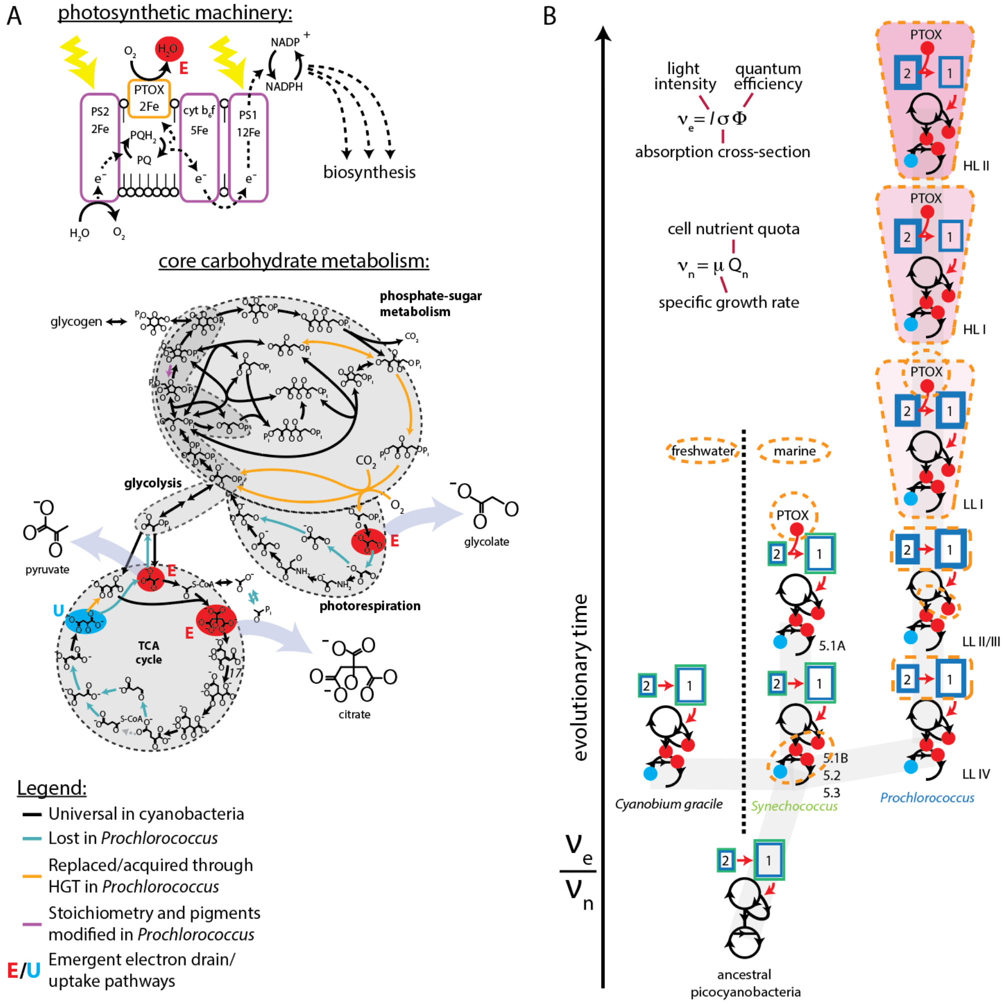

Fig. 2. Reconstructed metabolic evolution of marine picocyanobacteria. Left panel: Remodeling of the metabolic core, with genes lost highlighted in green, genes gained in orange, changes to photosystems/pigments in purple, and universal genes in black. The addition of pathways for exuding organic carbon (or water, in the case of the electron transport chain) are highlighted in red and a putative pathway for taking up organic carbon highlighted in blue. Right panel: metabolic innovations in lineages of *Synechococcus* and *Prochlorococcus* are highlighted with dashed circles and occur along an axis defined by the relative supplies of electrons and nutrients (i.e. ν_e/ν_n). Little black diagrams represent core carbohydrate metabolism with direction of carbon flow indicated by arrows, green/blue boxed 1's and 2's represent photosystems I and II, and red arrows represent electron flux. Increasing pink shading high-light adapted *Prochlorococcus* ecotypes represents acquisition of UV/light stress genes that allow cells to withstand higher levels of light, thereby generating an increased photosynthetic electron flux. Figure is adapted from Refs (40,41).

A deeper understanding of the selective forces driving the metabolic innovations of marine picocyanobacteria comes from examining them in their physiological and ecological context. That is, the metabolic remodeling is most extensive in *Prochlorococcus*, populations of which undergo a process of depth-based niche partitioning in warm oligotrophic waters, with more recently diverging "high-light (HL) adapted" clades dominating at the surface and deeper-branching "low-light (LL) adapted" clades dominating at the bottom of the euphotic zone (69,70). The resulting higher photon fluxes received by HL adapted cells are accompanied by modifications in the stoichiometries and pigments of photosystems (71-73) that act to increase the cellular absorption cross-section (41), together driving an increase in the achievable photosynthetic electron flux (ν_e).

This evolutionary increase in the photosynthetic electron flux (ν_e) in more recently diverging *Prochlorococcus* cells coincides with changes in their usage of inorganic nutrients. That is, *Prochlorococcus* cells are smaller and grow slower than *Synechococcus* cells (10,74), lowering the uptake flux of all resources required for growth. This is accompanied by a process of evolutionary streamlining, in which the membrane (75), genome (76), proteome (77), transcriptome (78) and metabolism (41) are modified in ways that lower the cellular contents of phosphorus, nitrogen and iron, the three main inorganic nutrients limiting phytoplankton growth in the ocean (79). Collectively these changes are inferred to drive a decrease in the assimilation flux (ν_n) of limiting nutrients, and thus an increase in the electron-to-nutrient flux ratio of cells (ν_e/ν_n) over the course of evolution in marine picocyanobacteria (Fig. 2B). The addition of pathways for exudation of organic carbon in the metabolic core thus reflects a growing excess flux of electrons and fixed carbon that is released from picocyanobacterial cells into the environment.

Selection for increases in ν_e/ν_n and associated removal of excess electrons through exudation of organic carbon can in turn be understood as follows. First, the increase in ν_e/ν_n has progressed the furthest in *Prochlorococcus*, which dominates in the most nutrient-poor regions of the open ocean (12,69), suggesting a link between metabolic evolution and nutrient assimilation. Second, photosynthetic electron flux (ν_e) carries solar energy into metabolism, suggesting metabolic evolution has increased the energy flux density, or metabolic power, of cells. Third, the free energy cost of nutrient assimilation scales with the nutrient gradient across cellular membranes (i.e. $\Delta G_r \propto \ln([n]_{in}/[n]_{out})$ for nutrient n). Taken together this led us to conclude that the reconstructed evolutionary increase in ν_e/ν_n in marine picocyanobacteria reflects a necessary increase in metabolic power of cells to allow growth at decreasing nutrient concentrations, and that added pathways for exuding organic carbon reflects the inevitable "carbon exhaust" of nutrient assimilation (41,42).

The above view of metabolic evolution in marine picocyanobacteria led us to propose a "depth-displacement" model for the evolutionary niche partitioning of *Prochlorococcus* clades. In this model, new innovations that increase ν_e/ν_n and allow cells to grow at lower nutrient concentrations draw nutrients down near the surface, restricting cells from deeper branching clades to regions deeper in the water column (41). This view of *Prochlorococcus* macroevolution is consistent with the distribution of nitrate assimilation genes in *Prochlorococcus* (80). That is, nitrate assimilation genes are absent in most deeply branching clades of *Prochlorococcus* but are retained in recently diverging HL-adapted clades, where genomic analyses suggest they have been inherited through a process of vertical descent that reaches back to crown group *Prochlorococcus*. Further, the frequency of nitrate assimilation genes in HL-adapted clades is enhanced under conditions of nitrogen-limitation (81). Together this suggests that conditions of nitrogen limitation in surface waters provided the selective pressure to retain nitrate assimilation genes in surface-dwelling clades, but that these genes were lost in deeper-branching clades as they were displaced further down the water column where general availability of nitrogen from other sources increases (80). This consistent with the depth-displacement model of *Prochlorococcus* evolution as emerged from metabolic reconstructions (41).

The emerging framework for the metabolic evolution of marine picocyanobacteria has implications for understanding how the marine biosphere overcame proposed constraints on productivity during the Proterozoic (Fig. 1). First, by lowering the nutrient concentration at which cells can grow, the metabolic innovations of marine picocyanobacteria increase their potential for expanding into the ocean in the face of proposed pre-existing nutrient limitation resulting from abiotic chemical processes in ancient oceans (23,24,27,28), as well as from ecological process (29,30) in scenarios where other planktonic cyanobacteria did exist in the open ocean before the rise of marine picocyanobacteria. By increasing ocean productivity, the global ecological expansion of marine picocyanobacteria would have helped drive oxygenation of the ocean, thereby loosening nutrient controls on productivity, helping to consolidate the transition toward an oxygenated state (Fig. 1). Although molecular clocks independently suggest that the flux of nitrogen fixation into the ocean increased in the late Neoproterozoic (35), it remains possible that ocean nutrient inventories of the deep open ocean were higher than is estimated from geochemical proxies largely derived from shallow-water sediments (27,28,31-34). In that scenario, the innovations of marine picocyanobacteria could in part reflect an adaption to the most nutrient-poor parts of the open ocean.

Second, the metabolic evolution of marine picocyanobacteria may have also helped counteract the negative feedback on productivity involving iron (Fig. 1C). That is, in today's oxygenated ocean iron is insoluble but is made bioavailable through complexation with organic carbon (82,83). The products of the exudation pathways added to the metabolic core of marine picocyanobacteria (Fig. 2) belong to the broad class of "weak" ligands that are a major source of iron in the ocean (82,83). Thus, as marine picocyanobacteria expanded into the open ocean, they would have helped gradually increase the inventory of iron ligands, thereby helping counteract the decreased availability of iron for photosynthesis due to the diminishing solubility of iron in oxygenated waters. Indeed, modeling results suggest that ocean microbes collectively tune the total inventory of accessible iron through similar feedbacks, such that it maximizes ocean productivity (84). Importantly, this interplay of positive and negative feedbacks in the availability of iron for photosynthesis is independent of the ocean inventories of nitrogen and phosphorus and is thus consistent with the late emergence of planktonic marine cyanobacteria even if there was sufficient nitrogen and phosphorus available in earlier times.

Emergence of a planktonic lifestyle in marine picocyanobacteria: the "chitin raft hypothesis"

While the reconstructed evolution of core metabolism in marine picocyanobacteria provides insight into how the marine biosphere overcame earlier constraints on productivity (Fig. 1), it does not address the questions of why these innovations happened when they did, or why cyanobacteria may not have colonized the open ocean sooner. Here another metabolic trait that we recently discovered in marine picocyanobacteria provides relevant context: chitin degradation (85). Chitin is a carbon polymer that is highly abundant in the ocean and is largely derived from the exoskeletons of arthropods (86). The existence of genes for chitin degradation in marine picocyanobacteria is unexpected, as chitin exists mostly as particles, and marine picocyanobacteria are generally considered to live a planktonic lifestyle (14,87). However, we showed that marine *Synechococcus* and deep-branching *Prochlorococcus* attach to chitin particles and display enzymatic activity of chitinase, the main extracellular enzyme involved in cleaving chitin polymers into smaller units that cells can take up (85). We also showed that expression of chitin degradation genes and other attachment-associated genes increases when cells are exposed to chitosan, a solubilized form of chitin, and that *Prochlorococcus* obtains a growth boost from exposure to chitin when grown at low light levels. These results demonstrate that chitin degradation is an active functional trait in marine picocyanobacteria, changing our view of their ecology.

The existence of chitin attachment and degradation capabilities in marine picocyanobacteria informs macroevolutionary frameworks. First, combined phylogenetic and phylogenomic analyses indicate genes necessary for chitin degradation were acquired through horizontal gene transfer (likely from unspecified planctomycetes) along the branch leading to crown group marine picocyanobacteria, and that the trait was lost during the evolution of *Prochlorococcus*, but remains universally preserved in its deepest branches and in marine *Synechococcus* (85). As mentioned above, the branch leading from other picocyanobacteria to crown group marine picocyanobacteria evolved over the period of ~576 – 424 Mya (9), which overlaps with the period during with both fossils and molecular clocks indicate that marine arthropods underwent a major ecological expansion (88-91). This suggests that the early evolution of marine arthropods and marine picocyanobacteria was intertwined and may have played a role in the inferred oxygenation of shallow continental shelf waters in the late Neoproterozoic that is inferred to have preceded eventual oxygenation of the deep ocean in the early Phanerozoic (21,22).

To further understand the relevance of the acquisition of the chitin attachment and degradation trait to the ecological rise of marine picocyanobacteria, it is instructive to consider microbial lifestyle in general. Global surveys suggest that outside of lakes and oceans, microbial biomass exists predominantly within aggregates attached to surfaces due to the collective stress buffering and nutrient recycling conditions such communities create (92). Most microbes, including cyanobacteria, can facultatively switch between planktonic and aggregated states, and while there are no single universal stressors driving aggregation across all microbial systems, common triggers include salinity, UV radiation and nutrient stress (92-96), all of which are elevated in surface waters of the open ocean. These stresses were moreover likely exacerbated in the Precambrian, when UV levels were higher due to a less-developed ozone layer (97), and oceanic nutrient inventories may have been lower (23,24,27-35). How then, did planktonic cyanobacteria colonize the open ocean in the face of stresses that favor aggregation? In response to this question, we proposed the “chitin raft hypothesis” (Fig. 4). In this hypothesis (85), the exoskeleton molts that arthropods produce as a byproduct of their growth provided surfaces where picocyanobacteria could aggregate and find shelter from environmental stresses, while picocyanobacteria in turn provided a food source for arthropods. Over millions of years some *Prochlorococcus* lineages evolved the necessary adaptations that eventually allowed them to make a secondary transition of fully planktonic lifestyle.

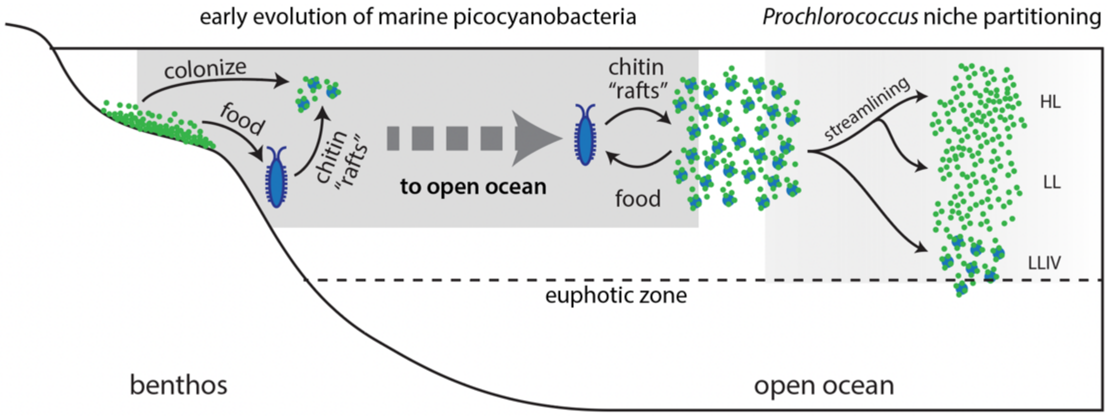

Fig. 3. Chitin raft hypothesis. Colonization of the open ocean by marine picocyanobacteria is proposed to have a collective, ecosystem-level process involving two stages. In the first stage, picocyanobacteria colonized chitin particles resulting from the waste of co-evolving arthropods, allowing them to maintain their facultatively surface-aggregated lifestyle and find protection from environmental stresses, while in turn providing arthropods a source of food. This association allowed both groups to expand out into the deep open ocean. Later, after acquiring adaptations to better withstand the stresses of life in the open ocean, some lineages of *Prochlorococcus* made a secondary evolutionary transition to a committed planktonic lifestyle. Figure is adapted from Ref (85).

The chitin raft hypothesis for is consistent with several additional lines of evidence in marine picocyanobacteria. First, a growing number of associations of marine picocyanobacteria to particles and/or protist hosts have been identified in recent years (98), including a dinoflagellate symbiont that branches between *Prochlorococcus* and *Synechococcus* (99). This suggests surface attachments play a more significant role in the ecology and evolution of marine picocyanobacteria than historically recognized. A second line of evidence comes from the adaptations in extant lineages, including acquisition of genes for compatible solutes that protect against osmotic stress from elevated salinity (100,101) and for protection from UV and light stress (102,103), as well as cellular and macromolecular changes that lower nutrient requirements while raising nutrient affinity (41,75-78), as outlined above. By helping to adapt to the unique challenges of microbial life in the open ocean, these adaptations also mitigate against the stresses that are generally linked to cellular aggregation and surface attachment. Finally, a recent study found that the region in the Prochlorococcus tree that separates clades with chitin metabolism from those without it contains unique photophysiology and is linked to a massive expansion of photosystem genes (104). This is consistent with the photosynthetic machinery of Prochlorococcus coming under intense selection during the evolutionary transition to a fully planktonic lifestyle.

Given the general lack of evidence of planktonic cyanobacteria before the rise of marine picocyanobacteria, we may posit that the associations with arthropods was a necessary precondition for the emergence of planktonic photosynthesis in the open ocean. Indeed, this proposal highlights how lineages do not evolve in isolation, but rather in the context of their ecological interdependencies: marine picocyanobacteria depend on the wasteful byproducts (i.e. exoskeleton molts) of arthropod growth, whole arthropods in turn depend on a steady supply of food from picocyanobacteria and other particle-attached microbes. In this view, oxygenic photosynthesis could not emerge in the deep open ocean until ecosystems were complex enough such that could sustain internal

Implications for biospheric productivity and Earth history

While the chitin raft hypothesis helps resolve questions regarding both the absence of evidence for planktonic marine cyanobacteria before the rise of marine picocyanobacteria, as well as the diversity and distribution of traits within the latter, others remain unresolved. For example, planktonic nitrogen-fixing cyanobacteria also colonized the ocean sometime in the late Neoproterozoic or early Phanerozoic, and the evolution of their lifestyle has not been fully unraveled. Many marine nitrogen-fixing cyanobacteria exist as part of buoyant colonies (e.g. *Trichodesmium*) (105) or in associations with eukaryotic phytoplankton (e.g. *Richellia* or UCYN-A) (106,107), which helps address the question of how they colonized the ocean in the face of aggregation-inducing stresses. Other groups, including *Crocosphaera watsonii* (108,109), have a unicellular planktonic lifestyle, but it is not yet clear if they have unrecognized abilities to switch between planktonic and attached lifestyles as we recently discovered in marine picocyanobacteria. Further studies on the diversity of lifestyles and lifestyle-associated traits among nitrogen-fixing marine cyanobacteria, and when in time different groups diverged from each other as calculated by molecular clocks will test, or at least provide nuance, to the chitin raft hypothesis.

In terms of the geologic record, additional targeted searches for evidence of planktonic cells in Proterozoic sediments, either from fossils, particularly of small cells (50), or from biomarkers (51), and from any deep-water depositional environments, would provide important tests that could invalidate our proposal. Further examination of nearshore-to-offshore gradients in carbon isotopes can in turn provide indirect evidence of the presence and/or productivity of potential planktonic cyanobacteria in open ocean waters. If high CO_2 levels muted such signals in the Archaean and early Proterozoic (43,44), then one might expect such gradients to become stronger in the later Proterozoic when CO_2 levels are expected to have been somewhat lower (45-47), as is potentially seen in some late-Mesoproterozoic sediments (110). Increasing the number of available records to improve statistics and extending included samples to include further-offshore environments less likely to be coupled to continental shelf environments through physical transport processes will help understand whether there was significant organic carbon production in deep open oceans, potentially indicative of planktonic cyanobacteria.

If the chitin raft hypothesis holds up in the face of such added scrutiny, it can potentially help inform interpretations of larger patterns of biospheric productivity and atmospheric oxygenation in deep time. That is, if oxygenic photosynthesis was largely restricted to shallow continental shelf environments during the Precambrian, then the total productivity of the marine biosphere was limited by the total global expanse of shelf environments and the rate of nutrient supply to those environments (Fig. 4). Hence, super continental cycles (111) could have had a role in modulating productivity during the Proterozoic (112), by impacting both the total size of shelf environments and the weathering supply of nutrients through assembly and break-up processes (113-116). Furthermore, it has become increasingly clear from both geochemical studies and geophysical modeling results that there was a major increase in sub-aerial continental surface area between the late Archaean and early Proterozoic (38,117-119). In a world of continental shelf-bound oxygenic photosynthesis, the transition from a ‘water world’ to a world with large continents would have led to a major expansion of shelf environments and thus of primary production, possibly helping fuel the great oxidation event. These examples highlight how metabolic reconstructions of microbial evolution can provide mechanistic hypotheses for interpreting large-scale patterns of Earth history.

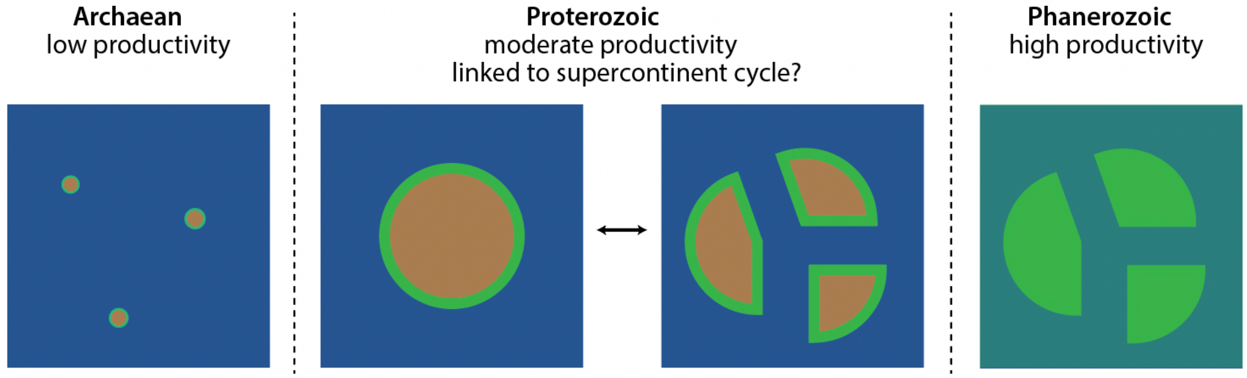

Fig. 4. Proposed model for the two-stage evolution of biospheric productivity over Earth history. Shelf-bound primary production during the Precambrian drove a first increase in productivity with the emergence of continents near the Archaean-Proterozoic boundary. Colonization of the oceans by marine picocyanobacteria and eukaryotic algae and colonization of the continents by land plants drove a second increase in productivity. Notes: green circumferences of brown landmasses represent continental shelf environments where productivity was localized during the Precambrian. Green oceans and continents in the Phanerozoic represent the global spread of chlorophyl and thus oxygenic photosynthesis.

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