Evolution: A Fixed-Nitrogen Fix in the Early Ocean?

A new study asserts that a late evolutionary leap in cyanobacterial nitrogen fixation terminated a long history of nitrogen-limited primary production in the ocean — and contributed to a dramatic increase in biospheric oxygen coincident with the rise of animals.

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Conventional wisdom holds that phosphorus (P) is the ultimate limiting nutrient in the ocean — when viewed through the geologic lens of millions to billions of years — because the recycling processes that resupply P are tied to equally long-term tectonic cycles of mountain building and erosion. Nitrogen (N_2), after all, dominates our atmosphere. Some organisms, including certain cyanobacteria, can convert N_2 to ammonia (NH_3), which is then readily available to a far wider range of life. This process of N_2 fixation (diazotrophy) should in theory be able to keep pace with the delivery of P to the ocean via rivers and the slow breakdown of mountains. Now, in a recent issue of *Current Biology*, Sanchez-Baracaldo *et al.* [1] ask us to consider a decidedly less uniformitarian view: that the N_2-fixing capacity of the early ocean, for more than 80% of Earth's 4.5 billion-year history, was inhibited until the emergence of planktonic, unicellular diazotrophic cyanobacteria less than a billion years ago during the Neoproterozoic (Figure 1). They assert that this apparent evolutionary delay was a consequence of marine deficiencies in molybdenum (Mo) and other trace metals that are essential co-factors in nitrogenase enzymes that convert N_2 to bioavailable NH_3.

The history of nutrients in the ocean is intimately tied to the history of oxygen (O_2) in the biosphere [2]. O_2 conditions at Earth's surface largely regulate nutrient delivery and availability in the ocean, while nutrients fuel primary production and burial of organic matter, which in turn drive oxygen accumulation. The first permanent accumulation of O_2 in the atmosphere occurred roughly 2.3 billion years ago (Ga), during what is widely known as the Great Oxidation Event (GOE). This first step was followed soon after by a dramatic increase and then mysterious drop to relatively low levels at ca. 2 Ga, and for a billion 'boring' years, oxygen persisted at low levels of perhaps only 0.1% or less of present-day O_2 levels.

The so-called 'boring' billion was clearly named with tongue placed firmly in cheek [3,4]. 'Remarkable' more appropriately describes the combination of factors that came together to yield the low biogeochemical and evolutionary baseline that endured from roughly 2.0 Ga to 1.0 Ga, including the generally low abundance and diversity of eukaryotic organisms that is the hallmark of this period. To start with, dominantly low oxygen levels in the atmosphere would have precluded pervasive oxygenation of the deep ocean; indeed, the ocean may have remained mostly oxygen-free for the duration of this interval [5] — long after the GOE. From studies of the modern Black Sea, we know that anoxia in deep marine waters favors at least local accumulation of hydrogen sulfide (H_2S) and that these sulfide-rich waters are characteristically poor in key trace metals that support nitrogen fixation, particularly Mo and vanadium (V). (Anoxic, sulfidic conditions are rare today beneath our oxygen-rich atmosphere, but they are widespread in the Black Sea, due in part to its weak connection to the open ocean.) This relationship between anoxia and nutrient limitation suggests that the 'boring' billion lacked sufficient Mo and V to support copious, efficient N_2 fixation and that the ocean may have been starved of this life-sustaining element [6]. One solution to dealing with low levels of Mo and V in the ocean might lie with alternative nitrogenase pathways reliant on iron (Fe) rather than Mo and V, but the available data generally point to lower efficiencies for these processes [7].

Recent numerical models have explored the extent of sulfidic conditions required to draw the metals down to levels that may have limited N_2 fixation [8]. These estimates jibe well with Mo-limited cyanobacterial activity identified in the lab [9] and overarching views of an iron-rich anoxic deep ocean with sulfide limited to the biologically productive ocean margins [10,11]. From this vantage point, we can imagine a series of negative feedbacks among ocean redox, extents of N_2 fixation, primary production in the ocean, and oxygen accumulation in the atmosphere that together favored a redox stasis within a relatively narrow range that limited eukaryotic diversity and overall biospheric oxygenation. For example, organic compounds from primary production that settle to the deeper ocean can foster O_2 loss through aerobic respiration, as in the Black Sea, and these deep reducing conditions then favor the reactions that remove Mo from seawater, thus limiting further primary production. This feedback is compounded by the observation that microbiology can drive NH_3 (or NH_4^+ more precisely) back to N_2 under anoxic conditions — via the so-called 'anammox' reaction.

The logical follow-up question is what factors finally shattered the low evolutionary, ecological and oxygenation ceiling that covered the 'boring' billion. In fact, several circumstances probably played a role in the dramatic rise in oxygen that occurred during the Neoproterozoic Era, roughly 1.0 Ga to 0.5 Ga. The formation of the supercontinent Rodinia and associated mountain building may have increased the mass of nutrients, including the bioessential metals, delivered to the ocean; associated sediment delivery would have enhanced the burial of photosynthetically produced organic matter, which in turn released oxygen to the atmosphere. Likewise, global glaciations during a 'snowball Earth' episode within the period known as the Cryogenian (~850–635 million years ago) may have accelerated the delivery of nutrients — specifically, P and the metals required to help N_2 fixation keep pace with the enhanced P inputs [12,13]. Finally, evolutionary events such as diversification among algae further facilitated oxygen contributions to the atmosphere.

Explaining the relative roles of these various controls is a grand challenge that lies with resolving a chicken-and-egg argument. For example, did the

'snowball' glaciations trigger the rise of oxygen, or were they a product of a rise? Did increasing oxygen catalyze an algal diversification that led to greater burial of organic matter and thus further oxygenation? Through feedbacks, might the answer lie with a complex combination of interrelated processes? And now Sanchez-Baracaldo et al. [1] give us something else to think about, the possibility of delayed evolutionary development of the marine cyanobacterial lineage responsible for abundant N_2-fixation today — unicellular, planktonic varieties — stemming from limitations in trace metal availability.

The fundamentally new view of global nitrogen cycle evolution proposed by Sanchez-Baracaldo et al. [1] is presented with supporting arguments. Specifically, the authors first use genomic data to construct a suite of three phylogenetic histories describing the deep evolutionary relationships among cyanobacterial groups. With these evolutionary histories as a framework, the authors then perform molecular clock analyses with the aim of establishing the absolute timing of nodes within the phylogeny — and thus the timing of key evolutionary events within the cyanobacterial clade. This analysis yields a Cryogenian age for the evolutionary emergence of marine planktonic nitrogen fixers, as well as a series of cyanobacterial lineages that make a substantial contribution to marine biomass and net primary productivity on the modern Earth [14].

Such an analysis is not without its potential pitfalls, however. Notable uncertainties are associated with variable nucleotide substitution rates and with the problems inherent in any implementation of fossil calibration constraints [15] — in other words, calibration of molecular clocks, particularly in very deep time, is a tricky business. Moreover, the model implicitly relies on the notion that freshwater habitats were initially much more favorable than the ocean for N_2-fixing cyanobacteria in terms of bioessential metal availability and further suggests that cyanobacteria moved from freshwaters such as lakes to the ocean about 2.4 Ga. Freshwater is usually deficient in Mo, even compared to the Mo-poor early ocean [16], so it is not a logical cradle for the earliest diazotrophy. Also, the evolutionary delays the authors
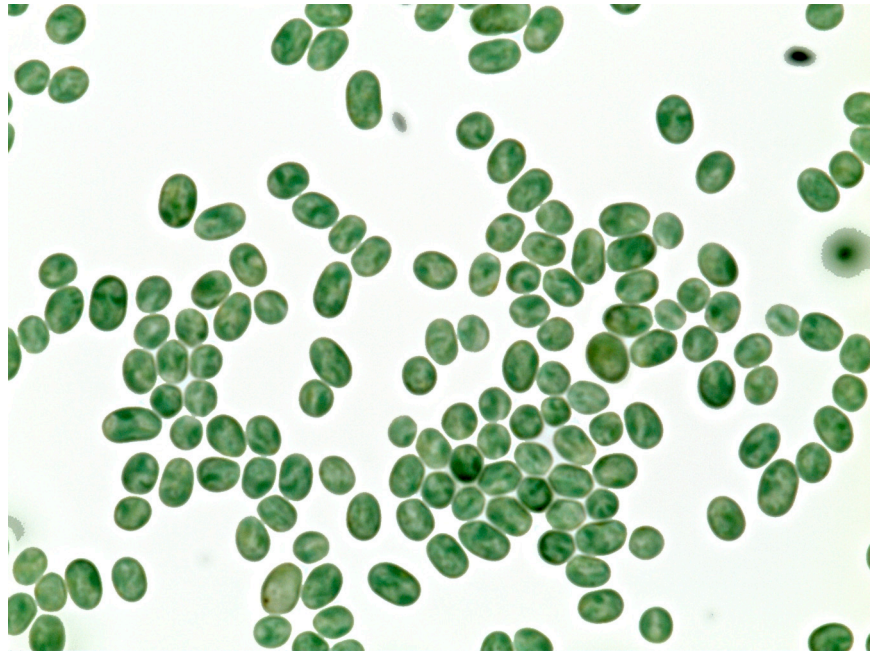

Figure 1. Planktonic cyanobacteria.
Photomicrograph of modern *Cyanothece*, an example of the important marine planktonic nitrogen-fixing cyanobacterial lineage that Sanchez-Baracaldo et al. [1] assert arrived relatively late in Earth history. Photo courtesy of M. Liberton and H. Pakrasi, Washington University.

describe may come as a surprise given that the geochemical record of O_2 production by cyanobacteria may extend back to at least $\sim$3.0 to 2.5 Ga (reviewed in [2]), including the possibility of localized 'oases' of oxygen production in the shallow photic ocean. Further, the marine fossil record of forms capable of cell differentiation dates back to at least 2.1 Ga [17]. Finally, the authors' notion of a protracted phylogenetic progression among cyanobacteria from freshwater to marine forms is complicated by the observation of modern cyanobacterial genera that thrive equally well in both settings.

Sanchez-Baracaldo et al. [1] tell us that rising trace metal content of seawater in the wake of the 'boring' billion [13,18] favored the diversification of N_2-fixing cyanobacteria that stimulated primary production in the ocean and thus ocean–atmosphere oxygenation. Something else, then, must have triggered the increasing metal contents, which spawned the evolutionary event among cyanobacteria. Was it a different driver of ocean oxygenation — a precursor? Again, we are faced with the chicken-and-egg dilemma. In all

likelihood, big jumps in biospheric oxygenation are best explained by first-order (tectonic or evolutionary) triggers and the cascade of positive feedbacks they put into motion that led to further oxygenation (including possible cyanobacterial diversification).

The assumed late timing of cyanobacterial diversification begs other questions. For example, we are left wondering how the persistent availability of fixed nitrogen, at times, at likely high levels, was maintained in the earlier ocean long before the end of the 'boring' billion and their hypothesized evolutionary diversification event. Evidence for substantial early nitrogen availability in the ocean includes numerous examples of old rocks rich in organic compounds and, in particular, suggestions of an interval between the GOE and the 'boring' billion of intense and prolonged organic burial (>100 million years) likely well beyond what we see today, along with high oxygen concentrations [19,20] — all more than a billion years before the authors' proposed cyanobacterial innovation.

End-member arguments are a risk when it comes to interpreting oxygenation of the early Earth. Rather,

we imagine a billion years of low oxygen during Earth's middle chapters maintained by a mix of tectonic and biogeochemical controls. Foremost among those throttles were trace metal (Mo and V) deficiencies in the low-oxygen ocean and less efficient Fe nitrogenase — with the net effect of sustained limitations in fixed nitrogen that constrained primary production and corresponding oxygen release at low levels. What did it take to break the low-oxygen sustaining negative feedbacks? Our money is on a combination of first-order tectonic, evolutionary, and climatic controls woven together. Once tipped in the right direction, the result may have been a network of positive feedbacks that ultimately raised biospheric oxygen concentrations to a new, higher state. And now, thanks to the thought-provoking results of Sanchez-Baracaldo *et al.* [1], temporal patterns of evolution within the cyanobacterial lineage, particularly in the ocean, must be considered more carefully in that mix.

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Nutrition: Rejection Is the Fly's Protection

Animals need to ingest a full set of essential amino acids through their diet. A new study in *Drosophila* larvae describes how activation of the kinase GCN2 in three dopaminergic neurons mediates the rejection of amino-acid-imbalanced food.

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The word homeostasis was coined in the early 20th century by Walter Cannon and popularized in his book *The Wisdom of the Body* [1]. The notion of nutrient homeostasis assumes that animals have an optimal nutrient intake, critical for both health and wellbeing, and that they should be able to select or reject food accordingly. The behavioral and physiological mechanisms underlying nutrient homeostasis are starting to be revealed and are an intense field of research within neuroscience. Whilst the senses, primarily smell and taste, are known to play an important role in food selection, it is becoming increasingly apparent that the 'quality control' of food continues after ingestion [2].

Studies on energy homeostasis found that animals can select metabolizable carbohydrates over non-metabolizable carbohydrates, independent of their sensory properties [3]. Furthermore, the formation of stable associative memories requires exposure to metabolizable carbohydrates, while the sensory properties of that sugar are secondary to the reinforcement [4–6].

Some of the earliest research on post-ingestive mechanisms focused on amino acid homeostasis. It was observed that amino-acid-deprived rodents rejected diets deficient in essential amino acids, and that post-ingestive amino-acid assessment involved a region of their olfactory cortex, known anatomically as the anterior piriform cortex (APC) [7]. A