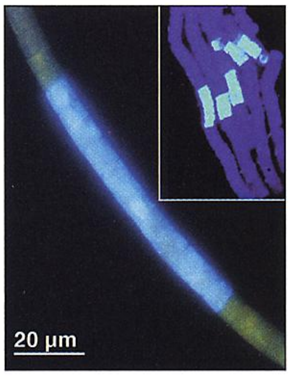
Evolution of Nitrogen Fixation

THE PROCESSES OF OXYGENIC PHOTOSYNTHESIS and nitrogen fixation in the cyanobacterium *Trichodesmium* are elegantly documented by Ilana Berman-Frank and colleagues as being separated temporally and spatially (Reports, "Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium *Trichodesmium*," 16 Nov., p. 1534). The evolution of dual separation of the processes in this genus contrasts with the spatial separation evolved in heterocyst-containing cyanobacteria in which nitrogen fixation is confined to these specialized cells. It also contrasts with the strategy devised by other nonheterocystous cyanobacteria of fixing nitrogen only in low light.

In the Early Archean oceans, such protective mechanisms were necessarily adaptive survival responses to what must have been both an early appearance of free oxygen and a loss of ammonia, life's ultimate source of nitrogen (*1*). Although the effect of free oxygen on nitrogen fixation is usually emphasized in evolutionary scenarios, the process is more sensitive to ammonia. Production of the heterocyst is repressed by the presence of ammonia, and both the synthesis of the nitrogenase enzyme and the activation of dinitrogen fixation itself are inhibited by even micromolar amounts of it.



Fluorescent antibodies reveal the localization of nitrogen fixation activity in the cyanobacterium *Trichodesmium*.

CREDIT: FROM I. BERMAN-FRANK *ET AL.*, *SCIENCE* **294**, 1534 (2001).

In the globally anaerobic Archean worlds envisioned by many, no oxygen is available for oxidative destruction of ammonia, and the water-soluble ammonium ion should have been present after atmospheric rainout to the oceans. With such a world, it is awkward to argue that such an adenosine triphosphate–expensive enzyme would have evolved to convert nitrogen to its most reduced form under conditions where even small amounts of reduced nitrogen should have been available. This is especially true for nonoxygen-producing prokaryotic anaerobes that are phylogenetically more primitive than the cyanobacteria (for example, methanogens). Therefore, the early environmental destruction of ammonia in the Early Archean (*2*) seems likely to be more critical to the evolution and use of nitrogen fixation than is the absence of, or repression by, free oxygen. Indeed, some environmental free oxygen should have been available in the Archean such that primitive anaerobic organisms living in locally anaerobic environments (as many do today) could have temporary encounters with this compound. Such encounters with oxygen would provide pressure to evolve, with increasing sophistication, the various mechanisms and protective devices against it. This would be especially true for those early phototrophs coupling Photosystems I and II to produce free oxygen both internally to themselves and externally to their ecosystem neighbors.

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References and Notes

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Evolution and the Fossil Record, B. Runnegar, J. W. Schopf, Eds. (Paleontological Society Short Courses in Paleontology, Paleontological Society, Pittsburgh, PA, 1988), pp. 114–129; in *Early Life on Earth*, S. Bengtson, Ed. (Columbia Univ. Press, New York, 1994), pp. 36–47.

2. The loss of ammonia in the Early Archean should have taken place by photochemical destruction as well as by oxidative loss, both processes placing evolutionary pressure on all forms of life to evolve some way of obtaining this important material.

Response

TOWE ARGUES THAT AN ENERGETICALLY expensive enzyme such as nitrogenase would not have evolved in the early environment of the Archean oceans where reduced forms of nitrogen were available, and that the impetus for nitrogenase evolution coincided with the oxygenation of the atmosphere (by cyanobacteria) and loss of ammonia via oxidation.

The availability of fixed forms of nitrogen would certainly have influenced the evolution of biological fixation of nitrogen and of the enzyme responsible, nitrogenase. Evidence demonstrates that nitrogenase is a highly conserved enzyme in eubacteria and cyanobacteria, with phylogenetic analyses clearly suggesting a single ancestral origin for the catalytic subunits of the enzyme complex (*1*) that preceded the oxygenation of the atmosphere and the oxidative destruction of ammonia (NH_3). The presence of nitrogen-fixing organisms as early as 3.3 billion years ago implies that reduced nitrogen would have already been scarce. Indeed, current models propose that CH_4 and not CO_2 warmed the planet, thereby limiting NO formation from N_2 and CO_2 (*2*). Additionally, ultraviolet radiation would cause rapid dissociation of NH_3 in the atmosphere with little fallout to the oceans (*2, 3*).

Furthermore, the nitrogenase complex is nonspecific and reduces triple-bond molecules such as hydrogen azide, nitrous oxide, acetylene, and hydrogen cyanide. Primitive forms of nitrogenase might have evolved as a N_2 respiratory enzyme (N_2 being an accessible electron sink for anaerobic heterotrophs under the reducing conditions) or as a detoxyase that would have detoxyified cyanides and other prevalent molecules in the ancient oceans (*4, 5*). With the loss of free ammonia and cyanides, nitrogenase would have evolved to become the prevalent biological mechanism for nitrogen acquisition before the oxygenation of the atmosphere and the advent of nitrification (*3, 5*).

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CORRECTIONS AND CLARIFICATIONS

LETTERS: "Minimizing effects of CO_2 storage in oceans" by G. H. Rau, K. Caldeira (11 Jan., p. 275). The amount of carbon ingassed and stored by the oceans each year was mistakenly edited to read ~2 picograms per year instead of ~2 petagrams.

LETTERS: "Etymology of epigenetics," letter by H. Rubin, response by C.-t. Wu (21 Dec., p. 2477). Misinterpretation of the response during the editing process led to an erroneous statement in note 4, which implies that C. H. Waddington discussed "epigentics" in his 1939 book *An Introduction to Modern Genetics*; Waddington only alluded to the concept, leaving his formal definition of "epigenetics" to later.