A Hard Life for Cyanobacteria

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GEOCHEMISTRY

A Hard Life for Cyanobacteria

The discovery of calcification inside present-day cyanobacteria raises questions about their geologic record.

Robert Riding

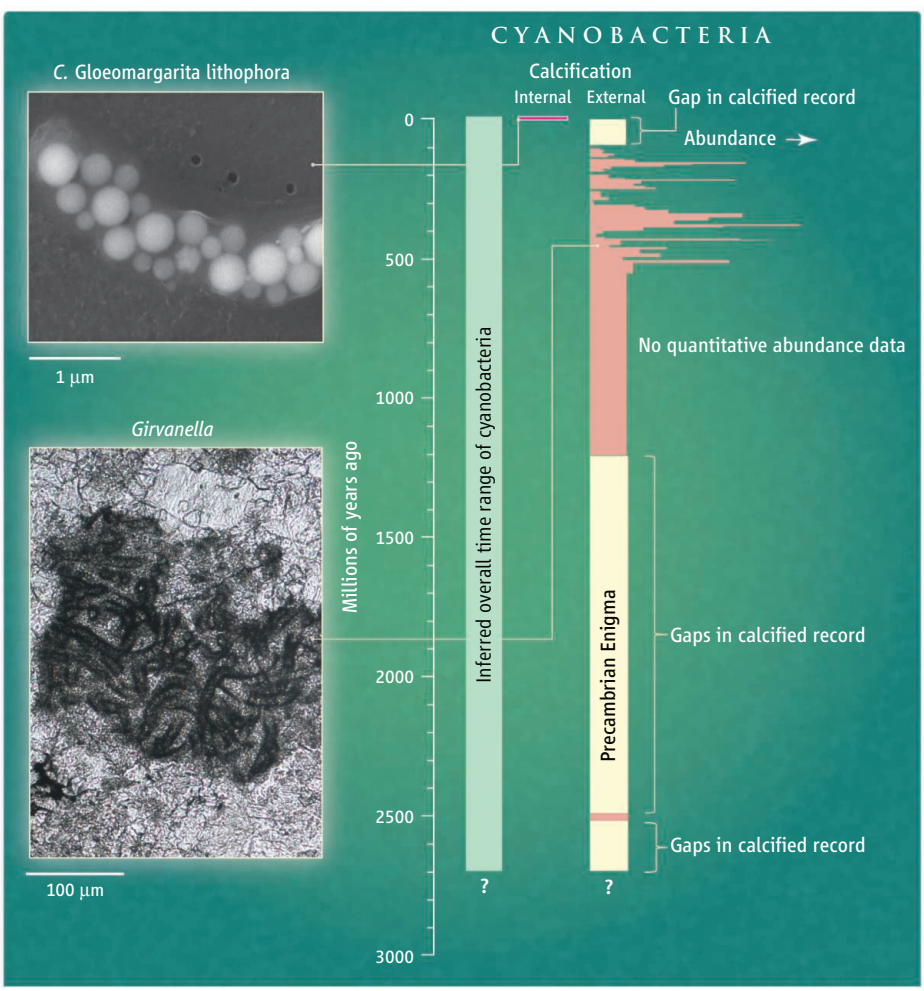
Many organisms precipitate minerals (*1*), and sometimes the reasons appear obvious. Humans, for example, rely on biominerals to provide vital skeletal support. But interpreting biomineralization can also be challenging. On page 459 in this issue, Couradeau *et al*. (*2*) show that the cyanobacterium *Candidatus* Gloeomargarita lithophora from Alchichica, an alkaline lake in Mexico, produces the amorphous mineral benstonite. This rare carbonate has a more complicated composition than common calcium carbonate minerals such as calcite. Furthermore, the particular variety of benstonite produced by the bacterium contains almost as much barium, magnesium, and strontium as calcium. But the point that focuses attention is that the benstonite occurs inside the bacterial cells.

Intracellular inclusions of various types are widespread in bacteria (*3*). Most do not contain minerals, but there are some well-known examples that do, including magnetic iron crystals that aid orientation in magnetotactic bacteria, and granules that store sulfur in sulfur bacteria (*3*). However, intracellular calcification has been unknown until now in cyanobacteria. As Couradeau *et al*. show, the benstonite inclusions in Gloeomargarita are spherical and less than 0.5 μm across. The cells themselves are only a few micrometers long; some are packed with inclusions. The authors suggest that the cells and their biomineralization may have previously been overlooked because of their small size and possibly also their scarcity.

Extracellular calcification in cyanobacteria has long been recognized (*4*). It typically involves calcite crystals that can form on the cell surface (*5*) but most commonly, and most durably, impregnate the protective mucilaginous sheath around the cells (*6*). The sheath structure itself may play a role in the calcification process (*4*), but two environmental influences are crucial: the carbonate saturation state of the adjacent water, which affects precipitation of calcium carbonate minerals (*7*, *8*), and the availability of dissolved carbon dioxide, which affects photosynthesis (*5*, *9*). When carbon dioxide levels are low, present-day cyanobacteria turn to other sources of dissolved inorganic carbon, such as bicarbonate, for photosynthesis. This requires conversion of the bicarbonate to carbon dioxide, which raises pH just outside the cells and promotes calcification if water chemistry favors precipitation (*6*).

To account for calcification in Gloeomargarita, Couradeau *et al*. also infer a key role for photosynthesis. But they suggest that the excess alkalinity produced by photosynthesis is trapped in the cell by the benstonite inclusions, rather than being exported as in the case of sheath calcification. The authors argue convincingly that both the intracellular location of the benstonite and its composition, which differs from that of other carbonate minerals deposited in Lake Alchichica, suggest a degree of cellular control over the biomineralization process. They hypothesize that the benstonite granules could act as ballast to help Gloeomargarita live at the bottom of the lake.

The discovery of a cyanobacterium with intracellular mineral inclusions may help to understand the geologic record of cyanobacteria (see the figure). Despite often being



Extracellular and intracellular calcification in cyanobacteria. Organic and silicified fossils, together with evidence for oxygen, suggest that cyanobacteria have existed for at least the past ~2700 million years (green column) (*12*). Yet sheath-calcified cyanobacterial fossils, exemplified by *Girvanella*, have a very episodic distribution in marine environments (right column); before 1200 million years ago, there is only one recorded example 2500 million years ago (*13*). Calcified sheath abundance data for the past 550 million years are from (*10*). Couradeau *et al*. now report evidence for intracellular calcification in Gloeomargarita from present-day Lake Alchichica, Mexico (*2*). Geologic evidence for such internal calcification may, however, be difficult to find.

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less than 20 μm across, calcified cyanobacterial sheaths have a good, but highly episodic, microfossil record (*10*), reflecting the response of sheath calcification to environmental effects. Well-calcified cyanobacterial sheaths first became conspicuous in the marine geologic record about 1200 million years ago (*11*), remained relatively common until about 100 million years ago, and today are scarce in seawater but locally abundant in fresh water (*6*). Their scarcity in rocks older than about 1200 million years has attracted particular attention. It seems that sheath calcification rarely occurred in marine cyanobacteria during the first half of their history. A possible explanation for this "Precambrian Enigma" (*6*) is that the high carbon dioxide levels during this period meant that cyanobacteria did not need to use bicarbonate (*6*).

It is not yet known whether the controlled intracellular calcification shown by Gloeomargarita has a geologic record. If it does, it might be much less erratic than the one for calcified sheaths. Couradeau *et al*. suggest that ancient relatives of Gloeomargarita may indeed have carried out intracellular calcification. They reason that Gloeomargarita has ancestral features and that the alkalinity export involved in sheath calcification might require cellular mechanisms that did not evolve until later.

However, it may be difficult to find geologic evidence. Tiny benstonite inclusions like those in Gloeomargarita would not be easy to identify with confidence in Precambrian rocks. Recognizing this difficulty, Couradeau *et al*. suggest that carbonate deposits containing barium and strontium, present in the benstonite, might provide a better geologic indicator for this style of biomineralization than the granules themselves.

Couradeau *et al*.'s discovery raises some intriguing possibilities, but assessing their importance for the fossil record will be challenging. In addition to searching for scarce calcified sheaths, we now need to be on the lookout for subtle traces of intracellular calcification. This is likely to cause some head scratching. At least we know why our skulls are so well calcified. Or do we?

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MOLECULAR BIOLOGY

Reprogramming the Genetic Code

Incorporating unnatural amino acids into proteins presents challenges in expanding the genetic code.

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The genetic code provides rules by which a genome is decoded to produce proteins of defined amino acid composition and sequence. These rules, which specify 61 codons (triplets of nucleotides) that code for the 20 common amino acids, and 3 codons that signal the termination of protein synthesis, are near-universally conserved in living organisms. Despite conservation of this code and the translational machinery that enforces it, a growing body of work addresses the challenges in reprogramming the genetic code. Designer amino acids, created by synthetic chemistry, can now be incorporated into specific sites in proteins of interest in vitro, in cells, and most recently in a whole animal (see the figure). These routes to unnatural polymer synthesis and evolution are already facilitating the study of cellular processes including protein interactions, protein conformational changes, posttranslational modification biology, and the kinetics of protein transport and cell signaling with a new level of molecular precision (*1*). Emerging developments may allow the synthesis and evolution of new materials and therapeutics.

The fidelity of natural protein synthesis is maintained by specific aminoacylation of a transfer RNA (tRNA) with an amino acid, and the ribosomal decoding of each tRNA in response to a cognate codon on a messenger RNA (mRNA) (*2*, *3*). Incorporating unnatural amino acids (with different chemical and physical properties that differ from those of the common amino acids) into proteins requires methods for loading them onto tRNAs and unique codons that can be specifically decoded by "orthogonal" tRNAs, which have been engineered not to be recognized as substrates for endogenous aminoacyl–tRNA synthetases. Chemical methods to aminoacylate orthogonal tRNAs designed to recognize a stop codon (the amber codon), and the use of these tRNAs in translation reactions in vitro, provided the first general route to incorporating unnatural amino acids into proteins (*4*). Recent advances have expanded the scope of in vitro protein synthesis. For example, quadruplet codons (four nucleotides) that can be read, albeit poorly, on the natural ribosome have been used to incorporate several unnatural amino acids into a single protein (*5*). In vitro translation can also be reconstituted from purified factors, allowing particular aminoacyl–tRNA synthetases and tRNAs to be omitted from the translation reaction. The resulting "blank" codons can then be reassigned to new amino acids by introducing orthogonal tRNAs bearing anticodons complementary to the blank codons (*6*, *7*). Ribozymes that catalyze the aminoacylation of tRNAs with unnatural amino acids and other monomers including alpha hydroxy acids (*8*) have provided an accessible alternative to chemical aminoacylation of tRNAs. This method has allowed the synthesis and directed evolution of unnatural peptides and cyclic peptides bearing a range of unnatural amino acids and monomers (*8*). Thus far, in vitro approaches have been used to synthesize and evolve antibiotic-like molecules and to label proteins for fluorescence resonance energy transfer experiments (*5*, *8*).

Extending unnatural protein synthesis to cells has also seen progress. The injection of a chemically aminoacylated orthogonal tRNA, engineered to recognize an amber codon, into *Xenopus laevis* oocytes has facilitated new insight into the structure and function of membrane proteins, including

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