

RESEARCH HIGHLIGHT

Interesting things come in small packages

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Abstract

Population metagenomics reveals the reduced metabolic capacities of a marine nitrogen-fixing cyanobacterium that lacks many of the signature features of typical cyanobacteria.

In life, it's said that few things are certain except death and taxes. Similarly, in biology, while there are exceptions to many 'rules,' there do seem to be a few certainties that have stood the test of time. One of these is the general organization and structure of the photosynthetic apparatus in chlorophyll-containing green plants and cyanobacteria, in which the photosynthetic electron-transport chain consists of two photosystems, one of which generates oxygen. However, a study by Jonathan Zehr and colleagues recently published in *Nature* (Tripp *et al.* [1]) presents an unprecedented exception to the general concept of what constitutes the essential core genome of free-living chlorophyll-containing cyanobacteria.

The story begins with molecular surveys of nitrogen-fixing microorganisms in ocean surface waters. Biological nitrogen fixation is carried out only by certain species of bacteria (for example, cyanobacteria and rhizobia) and archaea (for example, some methanogens), and represents a crucial component of the global nitrogen cycle, converting atmospheric nitrogen into biologically available nutrients. Nitrogen fixation is of special interest to oceanographers and biogeochemical modelers, as it helps feed vast nutrient-poor regions of the open ocean. Microscopic surveys of ocean plankton had initially indicated that only a few species - in particular large filamentous cyanobacteria such as *Trichodesmium* - were responsible for the bulk of open-ocean nitrogen fixation [2]. Later, however, molecular surveys for nitrogenase genes (which encode the enzymes responsible for nitrogen fixation) suggested that microorganisms other than filamentous cyanobacteria might be important in

open-ocean nitrogen fixation [3]. Along with measurements of open-ocean nitrogen fixation in cell fractions of less than 10 μm [4], these data indicated that unicellular cyanobacteria [3] are also important players in marine biological nitrogen fixation.

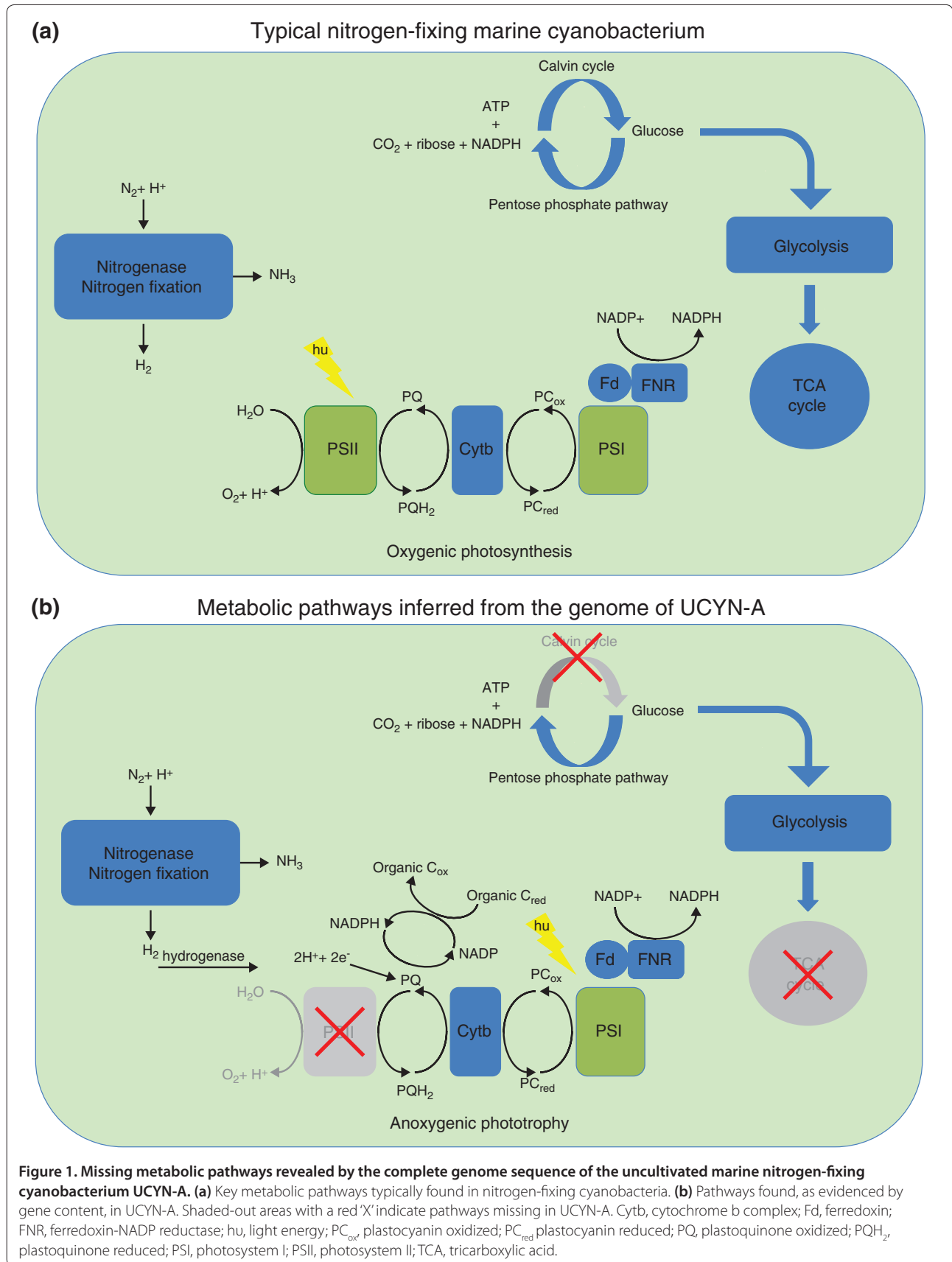
The first hurdle that Tripp *et al.* [1] had to overcome is a common one in microbial ecology: although these nitrogen-fixing cyanobacteria are abundant and widely distributed, no one has yet succeeded in culturing them so far, despite repeated attempts [5]. One way around this problem is to use novel cultivation methods employing endpoint dilution strategies, which have proved remarkably successful for many microbes that resist standard cultivation methodologies [6]. Unfortunately, end-point dilution cultivation has not proven effective for the unicellular nitrogen-fixing cyanobacteria, due in part to their lower abundance relative to other co-occurring bacteria, like *Prochlorococcus* and *Pelagibacter* species.

Instead of cultivation, Tripp *et al.* implemented a more direct approach to sequence their genomes. First, Zehr's group collected a seawater sample from the Hawaii Ocean time series station ALOHA that was fortuitously enriched in one type of nitrogen fixing cyanobacteria (called UCYN-A). This sample was analyzed via flow cytometry, and the UCYN-A cells were collected via fluorescence activated cell sorting. DNA was extracted from around 5,000 UCYN-A flow-sorted cells, further amplified by multiple displacement amplification (MDA) [7] and pyrosequenced [8].

Although it was not possible to assemble a complete genome for UCYN-A initially, the results obtained from large contigs were surprising. These initial data suggested that UCYN-A was not a typical nitrogen-fixing cyanobacterium. As predicted, the entire nitrogenase operon was present, verifying that these bacteria have the potential to fix nitrogen. Surprisingly, however, although 79% of core cyanobacterial genes were identified, a number of key functional and genetic elements found in all other cyanobacteria were absent in UCYN-A (Figure 1). For example, genes for the entire photosystem II (PSII) apparatus, including genes for PSII-associated photopigments, were absent; like green plants, the photosynthetic electron-transport pathway of cyanobacteria characteristically contains both photosystem I, which enables ATP generation, and the oxygen-generating

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photosystem II. In addition, genes for carbon fixation, including those for the key carbon-fixation enzyme ribulose-1,5-bisphosphate carboxylase, were absent. These results were puzzling, and suggested that UCYN-A are unlike any previously described cyanobacteria, given their lack of PSII and oxygen-generating photosynthesis, as well as the absence of carbon-fixation genes. And yet they appeared to have retained their capacity for nitrogen fixation. These preliminary observations set the stage for the next *tour de force*, the complete genome sequencing of the uncultivated UCYN-A [1].

Improvements in the length of reads for pyrosequencing, paired-end assembly strategies, and gap closure by contig pooling and PCR allowed Tripp *et al.* [1] to assemble the complete UCYN-A genome. A full genome assembly was also facilitated by the fact that populations of UCYN-A, like those of the cyanobacterium *Crocospaera* [9], appear genetically homogeneous and display little of the intra-population sequence diversity that typifies other planktonic microbial groups such as *Prochlorococcus* and *Pelagibacter*. At 1.44 Mbp, the UCYN-A genome is a full 4 Mbp smaller than those of closely related nitrogen-fixing marine cyanobacteria, such as *Crocospaera watsonii* and *Cyanothece* sp. ATCC 51142. Paradoxically, its coding density (81% coding DNA) was much less than the 97% coding DNA typical of other planktonic microbes with comparably small genomes, such as *Prochlorococcus* and *Pelagibacter*. As predicted from phylogenetic relationships, most open reading frames in the UCYN-A genome shared significant, if not the greatest, similarity to the genome of the unicellular coastal cyanobacterium *Cyanothece* sp. ATCC 51142. The UCYN-A genome was also unusual in that it occurs in two different arrangements, which are likely to be the consequence of recombination around two inverted-repeat rRNA operons, resulting in an inversion of about half of the whole chromosome. This situation is similar to that found in some cyanobacteria and in all chloroplasts, and (along with other data discussed below) led the authors to speculate that UCYN-A may be on a similar evolutionary path to that of the ancestor of present-day chloroplasts [1].

In addition to the unusual global genomic features described above, UCYN-A also lacks several fundamental metabolic pathways, including the tricarboxylic acid (TCA) cycle, the Calvin cycle for carbon fixation, biosynthetic pathways for several amino acids, and purine biosynthesis (Figure 1b). Given the complete lack of PSII (and therefore the inability to generate oxygen), the lack of a carbon-fixation mechanism, the lack of a TCA cycle (also never before observed in a cyanobacterium), and other missing biosynthetic pathways, the metabolic capabilities of UCYN-A are highly reduced. And yet, the retention of both nitrogen fixation and photosystem I

(PSI, which enables light-dependent cyclic photophosphorylation (ATP synthesis)) suggests a unique combination of metabolic pathways and capabilities, unlike any described before in a cyanobacterium.

On the basis of their data, Tripp *et al.* [1] propose an interesting scheme for electron flow and energy conservation involving external electron donors (organic carbon or H₂), nitrogenase (a byproduct of which is H₂), hydrogenases, oxidative phosphorylation, PSI-catalyzed light-driven photophosphorylation, and a variety of membrane-associated electron-transport chain components (see Figure 1b for a simplified version). Considering all the above, and the fact that UCYN-A cannot fix carbon, UCYN-A might best be described as an aerobic, anoxygenic photoheterotroph (AAPH). (A photoheterotroph is an organism that can use light as an energy source but cannot fix CO₂, and so relies on organic compounds for both carbon and reducing power). Examples of AAPHs exist in several other bacterial groups, such as the purple and green phototrophic bacteria. Unlike all other known AAPHs, however, UCYN-A uses chlorophyll, and not the chemically and spectrally distinct bacteriochlorophyll, as the light-harvesting pigment. UCYN-A has apparently achieved its physiology by genome reduction, as opposed to the acquisition of photosystem genes, the route that many other AAPHs (with heterotrophic ancestors) appear to have taken.

As is common in such analyses, what is lacking in the full genome sequence of UCYN-A is as revealing as what is present. It is also worth noting that this study is one of the first to successfully demonstrate full genome assembly from a flow-sorted cell population from a complex community, enabled by MDA and pyrosequencing. Yet despite all that has been learned, this stripped-down, genome-reduced marine nitrogen-fixing UCYN-A group still remains somewhat of an enigma regarding its habitat, lifestyle and ecology. On the one hand, the field data indicate that UCYN-A populations exist as free-living, single cells that compete directly with other small planktonic microbes. On the other hand, its reduced genome size, low gene density, and lack of key biosynthetic and metabolic pathways are reminiscent of the properties of a symbiont genome. In addition, very close relatives of UCYN-A have been reported to live symbiotically with diatoms and dinoflagellates. What we know is that the UCYN-A group is an important contributor of organic nitrogen to nutrient poor regions of the ocean. Indeed, another recent report by Zehr and colleagues (Moisander *et al.* [10]) indicates that the UCYN-A group has a wider geographic range and deeper depth distribution than better-known nitrogenase-containing cyanobacteria like *Trichodesmium*, thereby extending the known extent of nitrogen fixation in the world's oceans. What is less clear is where do UCYN-A

cyanobacteria make a living and how? Are they free-living competitors for organic carbon and other nutrients in the plankton, as the field data suggest? Or are they hitch-hikers, living on or in other microbial hosts that provide them with nutrients they cannot make themselves. If the latter is true, these hosts are proving even more elusive than the nitrogen-fixing UCYN-A cyanobacteria themselves!

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