

The beauty in small things revealed

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Oxygenic photosynthesis accounts for nearly all the primary biochemical production of organic matter on Earth. The byproduct of this process, oxygen, facilitated the evolution of complex eukaryotes and supports their/our continuing existence. Because macroscopic plants are responsible for most terrestrial photosynthesis, it is relatively easy to appreciate the importance of photosynthesis on land when one views the lush green diversity of grasslands or forests. However, Earth is the “blue planet,” and oceans cover nearly 75% of its surface. All life on Earth equally depends on the photosynthesis that occurs in Earth’s oceans.

A rich diversity of marine phytoplankton, found in the upper 100 m of oceans, accounts only for $\approx 1\%$ of the total photosynthetic biomass, but this virtually invisible forest accounts for nearly 50% of the net primary productivity of the biosphere (1). Moreover, the importance of these organisms in the biological pump, which traps CO_2 from the atmosphere and stores it in the deep sea, is increasingly recognized as a major component of the global geochemical carbon cycle (2). It seems obvious that it is as important to understand marine photosynthesis as terrestrial photosynthesis, but the contribution of marine photosynthesis to the global carbon cycle was grossly underestimated until recently. Satellite-based remote sensing (e.g., NASA sea-wide field sensor) has allowed more reliable determinations of oceanic photosynthetic productivity to be made (refs. 1 and 2; see Fig. 1).

In this issue of PNAS, Dufresne *et al.* (3) report the genomic sequence for *Prochlorococcus marinus* strain SS120 (see Fig. 2), a low-light-adapted cyanobacterial ecotype isolated from the Sargasso Sea. As every microbiologist inherently knows, little things can be the cause of much greater things that are often of the utmost importance, and this is especially true for marine picophytoplankton. The diminutive organisms of the genera *Prochlorococcus* sp. and the closely related marine *Synechococcus* sp. (see below) account for as much as two-thirds of the CO_2 fixation that occurs in the oceans, and hence these organisms could be responsible for nearly one-third of the primary biomass production on Earth (Fig. 1). To understand the global carbon cycle, it is obvious that one must understand the physiological characteristics of marine cyanobacteria. Genomic analysis can provide all-encompassing and

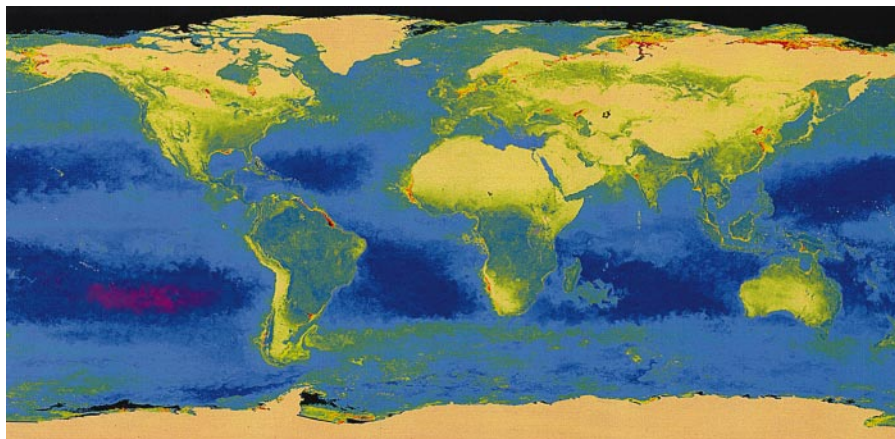


Fig. 1. Depiction of the amount of chlorophyll present in the oceans and the amount of vegetation on land. Purple and blue represent low levels of chlorophyll, and green, yellow, and red indicate progressively higher concentrations. Brown pixels show areas with little vegetation on land, and blue-green pixels represent areas of dense vegetation. [Image provided by ORBIMAGE (Copyright 2003, Orbital Imaging Corporation) and processing by NASA Goddard Space Flight Center.]

penetrating insights into the biochemical and physiological secrets of an organism.

Probably because of their small sizes and low cell densities, the most abundant photosynthetic organisms in the oceans were not discovered until very recently. The marine picophytoplankton clade includes organisms of two closely related cyanobacterial genera. Marine *Synechococcus* species, first described nearly 25 years ago (4, 5), are typically more abundant in the upper 25 m of the ocean where white or blue-green light is available for photosynthesis (6). Although all isolates of *Synechococcus* synthesize chlorophyll *a*, they are a heterogeneous assemblage that can be divided into two distinct subgroups. Members of the first group are found in coastal waters, have phycobilisomes for light harvesting but usually do not produce phycoerythrin, and are salt-tolerant but do not have elevated salt requirements for growth. Members of the second group produce phycobilisomes containing phycoerythrin as the major light-harvesting protein, have elevated salt requirements, and are abundant in the euphotic zone of both coastal and open-ocean regions of temperate and tropical oceans (7).

Organisms of the genus *Prochlorococcus*, first reported by Chisholm *et al.* (8, 9) only 15 years ago, reach much higher cell densities than marine *Synechococcus* and predominate at depths greater than ≈ 25 m, where blue-violet light penetrates (6). *Prochlorococcus* sp. dominate phytoplankton communities in

most tropical and temperate open-ocean ecosystems (6, 10) and, with diameters of only 0.5–0.7 μm , are the smallest known oxyphototrophs. They lack the phycobilisome light-harvesting complexes found in typical cyanobacteria and instead contain chlorophyll–protein complexes known as Pcb’s (11–13), which contain divinyl chlorophyll *a* and chlorophyll *b*, signature molecules unique to this genus (8, 10). *Prochlorococcus* strains are divided into two distinctly different ecotypes that are adapted for growth in different ecological niches (10). Ecotypes adapted to high light intensities occupy the well illuminated but nutrient-depleted upper 100-m layer of the water column. Ecotypes adapted to low-light environments preferentially grow at the bottom of the euphotic zone (80–200 m) where nutrients are more abundant.

What are some of the general characteristics of *P. marinus* that can be deduced from its genomic sequence? Perhaps the answer to this question is best given as “just the basics, please.” *P. marinus* SS120 has a single circular chromosome of 1.75 Mbp that is predicted to encode 1,884 ORFs. Most of these ORFs have assigned functions (67%) or are conserved hypothetical proteins (21%). Only ≈ 230 ORFs have no detectable homologs in the databases. Because *P. marinus* is an oxygen-

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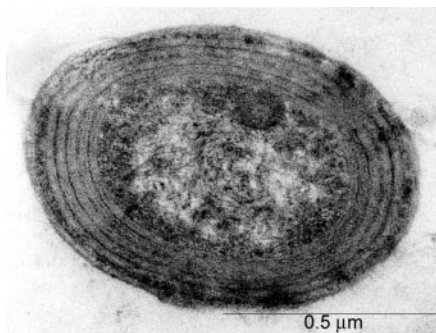


Fig. 2. Transmission electron micrograph of an ultrathin section of *P. marinus* SS120 showing the closely appressed thylakoids that distinguish this organism from marine *Synechococcus* sp. (Photograph courtesy of William Li and Frederic Partensky.)

evolving photolithoautotroph, it must encode all the proteins required for oxygenic photosynthesis, including the biosynthesis of chlorophyll, carotenoids, quinones, heme, the photosynthetic electron transport chain and light-harvesting proteins, and the Calvin cycle. Unlike most other cyanobacteria, however, it generally lacks duplicate genes for photosystem II components (e.g., *psbA* and *psbD*) and tetrapyrrole biosynthesis (e.g., *hemN*, *acsF*, and *hox*). Because *P. marinus* is an autotroph, it must have the ability to synthesize all cellular constituents, including amino acids, nucleotides, coenzymes, etc., from CO₂ and mineral salts. The only inorganic carbon uptake system detected is the sodium-dependent bicarbonate transporter, and, interestingly, no carbonic anhydrase could be identified. *P. marinus* SS120 has carboxysomes, organelles in which CO₂ fixation by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) occurs, but the carboxysome shell proteins as well as the large and small subunits of Rubisco are more similar to homologs in proteobacteria such as *Acidithiobacillus* species than to those in freshwater cyanobacteria (13, 14). *P. marinus* SS120 cannot utilize nitrate, nitrite, urea, or cyanate as N-sources and apparently utilizes only ammonia and amino acids although some other *Prochlorococcus* sp. strains possess a nitrite transporter and nitrite reductase and thus can utilize nitrite (15). Although it cannot synthesize the typical cyanobacterial N-storage material cyanophycin, it may store nitrogen in the polyamine spermidine. Other biochemical systems show greatly reduced complexity: DNA repair, chaperones, transport systems, intermediary metabolism, nitrogen metabolism, and pilus biogenesis and motility are all missing or less-diversified than in other

freshwater cyanobacteria. In most open-ocean waters on Earth, phytoplankton growth is limited by the availability of iron (2), an element that is required for building the photosynthetic machinery, electron transport proteins, and certain enzymes. The *P. marinus* genome lacks genes for siderophore production and several iron transporters found in most freshwater cyanobacteria, although an ATP-binding cassette transporter, which may also transport iron chelates (16), is present for Fe⁺³ acquisition.

A particularly striking example of mini-mization is the nearly complete absence of signal transduction and environmental stress-response (two-component) systems. Only five histidine kinases and six response regulators are encoded in the *P. marinus* genome. In comparison to other cyanobacteria, regulatory proteins such as protein kinases, phosphatases, cyclic nucleotide-binding proteins, photoreceptors, group III σ factors, and regulators of RNA polymerase σ factors are absent or rare in the genome. Finally, few (≈ 11) DNA-binding transcriptional regulators are found in the genome, which leads one to conclude that environmental sensing and transcriptional regulation have limited importance in the physiology of *P. marinus*.

The genomes of two other *P. marinus* strains (MED4 and MIT9313) (17) and marine *Synechococcus* sp. strain WH8102 (18) were also sequenced recently. *P. marinus* MED4, a high-light-adapted strain isolated from the Mediterranean Sea, has a genome of 1.66 Mbp that encodes $\approx 1,716$ ORFs. Together with *P. marinus* SS120, these organisms likely possess nearly minimal genomes for oxyphototrophs. Interestingly, these genomes are very similar in size to those of chemolithoautotrophic eubacteria and Archaea (1.55–1.75 Mbp). The genomes of low-light-adapted *P. marinus* strain MIT9313 (2.41 Mbp, 2,275 ORFs) and *Synechococcus* sp. WH8102 (2.43 Mbp, 2,527 ORFs) encode >500 –800 additional ORFs. These genomes are similar in size to those of the thermophilic cyanobacterium *Thermosynechococcus elongatus* (2.59 Mbp, 2,475 ORFs) (19) as well as that of *Chlorobium tepidum* (2.15 Mbp, 2,288 ORFs), a moderately thermophilic, green sulfur bacterium that is a strictly anaerobic photolithoautotroph (20). However, all these genomes are considerably smaller than those of most other cyanobacteria, which have genomes from ≈ 3.5 to ≈ 15 Mbp (21, 22).

In an essay on the physiological consequences of small size, Raven (23) concluded that a spherical cell with a volume of $0.10 \mu\text{m}^3$, precisely the volume of an average *P. marinus* cell, might approach the lower size limit for marine photolithoautotrophs (*Escherichia coli* has a volume of $\approx 1.0 \mu\text{m}^3$). One benefit of small size is less self-shading (the “package” effect) and hence more efficient light capture. A second advantage is the very large increase in cell surface to volume ratio. This leads to a very substantial improvement in nutrient acquisition for permease-mediated solutes, although the surface area for resource loss is also increased. An important mechanism for minimizing cell size is to reduce both the types as well as the

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numbers of catalysts (i.e., proteins or enzymes). Considering all the organisms mentioned above, the maintenance energy requirements, coding complexity, and size of extremely successful prokaryotes in relatively constant (oligotrophic oceans, hot springs, etc.), energy- or nutrient-limited environments seem to have been effectively minimized during evolution. Most of these organisms have also significantly reduced their ability to adapt to their environments and to control gene expression at the transcriptional level. However, being minimalistic clearly does not necessarily mean that *Prochlorococcus* sp. or these other prokaryotes are less competitive, because other phenomena such as microdiversity and rapid diversification (24) allow other mechanisms of adaptation to occur. It is interesting to note that one of the smallest but most successful organisms on Earth, *Pelagibacter ubique* (SAR11), which was also originally detected in the Sargasso Sea and may account for $\approx 20\%$ of the prokaryotic cells in the ocean (25), has a volume of $0.01 \mu\text{m}^3$ and an estimated genome size of ≈ 1.5 Mbp (26). Yes, small things can be simple and yet highly successful on a global scale.

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