

Unveiling *Prochlorococcus* The Life and Times of the Ocean's Smallest Photosynthetic Cell

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Our task now is to resynthesize biology; put the organism back into its environment; connect it again to its evolutionary past; and let us feel that complex flow that is organism, evolution, and environment united.

Carl Woese, 2004

A few years ago, the woman who cuts my hair—who has become a part of the fabric of my life over the years—finally worked up the courage to ask me about my research. I explained that we study a very small microorganism that lives in the surface oceans—a tiny single-celled plant, discovered only 25 years ago. “You’ve been studying the same organism for 25 years? That must get *really* tedious!” she said as she snipped away at my hair for the 100th time.

It is difficult to describe the thrill of studying *Prochlorococcus*. The name alone is enough to stop a conversation. Far from being tedious, studying this extraordinary little cell is like opening a present every day. It is a gift, and a responsibility. When people ask

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me about it I usually launch into my “photosynthesis appreciation” lecture, trying to convince them that nearly all life on Earth comes from photosynthesis: making life from sunlight, air, and water. If I succeed at that, which is not easy, I go on to tell them that half of global photosynthesis is done by microscopic phytoplankton in the oceans and that *Prochlorococcus* is the smallest and most abundant member of this “invisible forest.” There are over a trillion trillion *Prochlorococcus* organisms in the global oceans, and we did not know that they existed until a few decades ago. People find this extremely difficult to believe. I would too, had I not been lucky enough to live through its story.

Like most scientific advances, the unveiling of *Prochlorococcus* involved new technologies, diverse approaches, teamwork, and luck. Until about 40 years ago, we thought that all phytoplankton were between 5 to 100 μm in diameter, because this was all we could easily see under a microscope. (I use the editorial “we” throughout this essay for simplicity, but it refers to many people involved in this story; see last page for elaboration.) In the 1970s, advances in microscopy revealed that the oceans were filled with even smaller photosynthetic cells, about 1 μm in diameter, that were 10 times more abundant than the larger phytoplankton. Because of their unusual pigments, these cells, ultimately named *Synechococcus*, appeared as tiny orange beacons. Higher-resolution images of their populations revealed subtly different variants, and one of the smaller variants was more abundant in deep water and appeared more green than orange on collection filters. This was *Prochlorococcus*. But as is often the case with major discoveries, its photo sat unnoticed on the journal page for a decade. Around that same time, Dutch oceanographers discovered an “unidentified chlorophyll derivative” in samples from the North Sea that was particularly abundant in particles smaller than 1 μm . This was, in fact, a pigment that we now know is uniquely characteristic of *Prochlorococcus*, but they had no way of knowing that at the time.

Over a decade later, the pieces of the *Prochlorococcus* puzzle began to fall into place. An instrument became available to oceanographers that employs lasers to study the pigment and light scatter properties of microbes in seawater. It was ideal for studying *Synechococcus* because of its distinct orange pigmentation, so study

it we did. After several years, we began to notice some signals emerging from the electronic noise of the instrument that suggested the presence of cells that were not orange but green. We ignored them for a while, thinking they were just an extension of the noise signal. But eventually we noticed that the signals were stronger in samples from deeper water, where a cell might need more pigment to harvest the dwindling sunlight. This could not be ignored. It soon became clear that there were photosynthetic microbes smaller than *Synechococcus* in the oceans, with a different pigment, that were 10 times more abundant. There were 100 million of these cells in a liter of seawater.

Over the next few years, we determined that these were the very cells that had been photographed a decade earlier and dubbed *Synechococcus* variants. And we learned that they contained divinyl chlorophyll, which has properties identical to the “chlorophyll derivative” that Dutch oceanographers had described passing through their 1- μm -pore-size filters years before. We discovered further that the cells also contained chlorophyll *b*, a pigment that is typically found in “green plants” found on land. With growing affection, we began to call our newly discovered cells “little greens.” Their detailed structure bore an uncanny resemblance to chloroplasts, the small oval bodies in plant cells where photosynthesis takes place, which are known to be evolutionarily derived from microbial cells through an ancient symbiotic union. No microbe had been identified that both resembled a chloroplast and contained the telltale chlorophyll *b*. Had we found the missing link? Were our little greens living fossils? Before we knew the answer to these questions, we had to give the cells a proper name. We chose *Prochlorococcus*—“little round progenitors of chloroplasts.” We would soon regret this.

Around that time, a revolution was occurring in evolutionary biology. Scientists had learned that the relatedness among organisms could be measured by comparing the DNA sequences of genes that are shared universally across all living things. By sequencing these genes in *Prochlorococcus*, we could ask directly, does *Prochlorococcus* share a recent common ancestor with the chloroplasts of higher plants? The answer was no. *Prochlorococcus*'s closest relative is, perhaps not surprisingly, *Synechococcus*, and neither of them

shares a recent common ancestor with chloroplasts. We had come full circle. *Prochlorococcus* is just a smaller version of *Synechococcus*, with an unusual set of pigments. But once a microbe has a name, it takes a lot of effort to change it. *Prochlorococcus* stuck.

The first *Prochlorococcus* cells isolated into culture came from a 120-m depth in the Sargasso Sea. Soon thereafter, another was isolated from the surface waters of the Mediterranean Sea, and the strains were named SS120 and MED4, respectively. It became clear immediately that although they shared the “signature” characteristics of *Prochlorococcus*, MED4 and SS120 were not the same: MED4 could grow at high light intensities that killed SS120, while SS120 could grow under extremely low-light conditions that could not sustain MED4; i.e., the cells were adapted to the light intensities found where they were captured. We called them high- and low-light-adapted “ecotypes.” It was slowly dawning on us that it was through the layering of these ecotypes that *Prochlorococcus*—the collective—was able to fill the sunlit 200 m of the oceans. We had captured two of them. How many more were there? Did they differ in other ways?

Over the years that followed, we isolated more strains of *Prochlorococcus* from many different oceans and depths. We sequenced their diagnostic genes and used them to develop a family tree of sorts. The strains could be grouped into two broad clusters—either high- or low-light adapted. Within the high-light-adapted group there were two additional clusters. What differentiated them? We went off to sea and mapped their distributions along a north to south transect of the Atlantic Ocean, letting the cells tell us, through their relative abundances, what environments most suited them. The answer was clear. One ecotype dominated the warm tropical and subtropical waters, and the other the colder waters at high latitudes. Further experiments showed that strains differed not only in their temperature preferences but also in their abilities to exploit different nitrogen and phosphorus sources. Thus, *Prochlorococcus* is a federation of sorts, divvying up the oceans according to light, temperature, and nutrients. What else? How many different evolutionary paths have these cells taken?

While we were growing cultures and mucking about at sea, the DNA sequence of the entire human genome, containing roughly

20,000 genes, was completed and released with much fanfare. This left a lot of DNA sequencing machines available for other projects, and because of its small size, photosynthesizing ability, and global reach, *Prochlorococcus* MED4 was among the first microbes to have its genome completely sequenced. While I was very excited about this opportunity, I could not shake the absurd feeling that we were invading the inner life of this tiny cell that had drifted unnoticed in the oceans for millions and millions of years. My feeling grew into a sense of responsibility—a need to bring it the respect it deserves. One does get attached.

The first thing we learned from the genome sequencing project was that MED4 is very streamlined, even for a microorganism, containing only about 1,700 genes. So far, this represents the minimum amount of information (DNA is simply information in chemical form) necessary to create life out of elemental components: sunlight, carbon dioxide, water, and other essential nutrients drawn from seawater. This cell is truly the “essence” of life. As a photosynthesizer, it can do what humans cannot, even with all of our technology: it can split water using sunlight and make hydrogen and oxygen—all with only 1,700 genes.

In studying evolution, it is the difference between things that holds the answers, so we sequenced the genomes of 12 *Prochlorococcus* strains spanning the family tree. There are 1,200 genes shared by all of them. This is its essential core—the bare bones of being *Prochlorococcus*. Each strain is endowed with 500 to 1,200 additional genes, some shared with some (but not all) of their cousins. These non-core genes give each strain its unique character. Some determine, for example, what nutrients the cells can scavenge, how well they can protect themselves from high light, and what their outer surface looks like to predators. But the functions of most of these extra genes are a total mystery to us. They hold important keys to understanding the sheer abundance and persistence of *Prochlorococcus* in the oceans and have much to tell us about the selective forces that have shaped both the ancient and recent oceans. We think of the cells as little “reporters” who use a language we only partially understand.

The sheer number of these reporters and the genes they carry is astounding. So far, every new strain that has been sequenced has

revealed an average of 200 completely new genes. Thus, the size of the global *Prochlorococcus* federation gene pool—the so-called pan genome—must be enormous. Is each cell unique, like a snowflake? We know this cannot be true, because the cells reproduce by making identical daughter cells. Each cell grows by day, basking in the sun's energy, and each divides into two by night. They all do this in unison, coordinated by the daily pulse of energy. For every cell that is produced, there is another that is eaten by small predatory cells that must rely on others for their food. This keeps the *Prochlorococcus* population in check and begins the flow of energy through the marine food web.

Because they reproduce by making identical copies of themselves, we know that at any moment in time there must be lineages of identical *Prochlorococcus* cells in the oceans. But how many lineages are there? What is the rate of genetic change and how does it occur? While we don't have answers to these questions, we are finding clues where we might least expect them: viruses. There are millions of viruses in a milliliter of ocean water, some of which use *Prochlorococcus* cells as their hosts. A virus is simply a rudimentary set of genes packaged in a protein coat. In order to reproduce, it has to inject its DNA into a host cell, take over its machinery, and use it to make more virus particles. Sometimes the viral DNA loops into the host genome and assumes a holding pattern until the time is right to take over the host and make more virus particles. Viral and host genes get exchanged on occasion, and if not detrimental to host or virus, the shuffled gene persists through generations in the recipient. It may even become an asset over time. What is an asset to a virus, of course, has to be a liability for the host cell it successfully infects. But that cell has, we assume, many identical clones in the ocean—sister and daughter cells—that will carry on its heritage and likely never see that particular virus again. So one can think of a host cell as simply playing a role in maintaining the diversity of the federation by keeping the gene shufflers in business. And gene shuffling, over time and space, might just be a key contributor to the global diversity, structure, and stability of the *Prochlorococcus* federation.

When I look at our emerald green laboratory cultures of captive *Prochlorococcus*, my mind quickly turns to their wild

cousins, drifting freely in the world oceans. I am reminded that while they carry out a respectable fraction of the photosynthesis on our planet, they escaped our attention until a few decades ago. What else of this magnitude are we not seeing? Will we find it before human activities have completely dominated the oceans, as they have the land? As I write this, commercial ventures are gearing up to fertilize the oceans to trigger phytoplankton blooms, designed to draw carbon dioxide out of the atmosphere to abate global warming. If carried out on grand scales this approach would, by design, dramatically alter the marine food web. I have repeatedly voiced the position that commercialized ocean fertilization is an ill-advised climate mitigation strategy. Some have argued, only partially in jest, that my protests are simply a disguised concern for *Prochlorococcus*. On the contrary, these tiny cells occupied our planet long before humans, and they will surely outlast us. They can photosynthesize. They thrive through diversity. Their federation can adapt. And, as one of my graduate students once put it, they have a time-tested strategy: “Grow slowly, and endure.”

*Because this essay was written for the nonscientist, I omitted specific attributions for ease of narrative. In using the editorial “we” I am assuming the role of spokesperson for the many scientists who were involved in this story. There are a few people who played pivotal roles in the initial chapter of “the unveiling,” however, who should be recognized: Robert Olson, using a flow cytometer, was the first to notice the “little greens”; Ralph Goericke and John Waterbury played central roles in putting the puzzle together; and Brian Palenik isolated them into culture. These contributions set the stage for all that followed. Since those early days, there have been many—too many to mention by name—who played key roles in giving *Prochlorococcus* a voice. May they continue to do so!*

