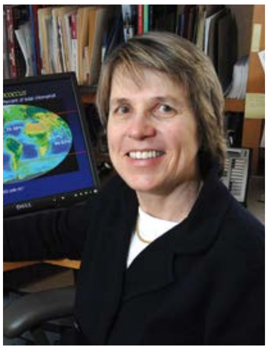

MARINE MICROORGANISMS, THE BIOSPHERE, AND *PROCHLOROCOCCUS*

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Introduction

The significance of Microbial Life in the Oceans

The oceans cover two thirds of our planet and sustain its climate. This function depends in no small part on life in the oceans – primarily microbial life. Microorganisms are the primary engines that drive dynamic ocean processes; some of them carry out half of the photosynthesis on our planet, and others are responsible for recycling essential nutrients so the cycle of life can continue. Averaging concentrations of 1,000,000,000 cells per liter of seawater, there are an estimated 100 billion billion billion microbial cells in the oceans, each containing 1,000 to 50,000 genes. These genes contain the 'information' that sustains the ocean's metabolism; they represent the largest reservoir of genetic information and biochemical diversity on Earth, and play a key role in buffering the Earth's climate system. Much as our understanding of the human genome is improving modern medicine, understanding the oceans microbial meta-genome will help us manage the critical biospheric processes in the coming years.

Over the past decade or two there has been a revolution in the genomic sciences that has allowed us to make tremendous advances in 'decoding' the ocean's genome. The tools of genomics that were developed to study the human genome, have been applied with great success in studying marine microbial ecosystems, and over the past decade we have been able to capitalize on this success. These tools have revealed not only astounding diversity, but they have illuminated patterns and processes relevant to the microbial players, and their interactions with one another, and the environment.

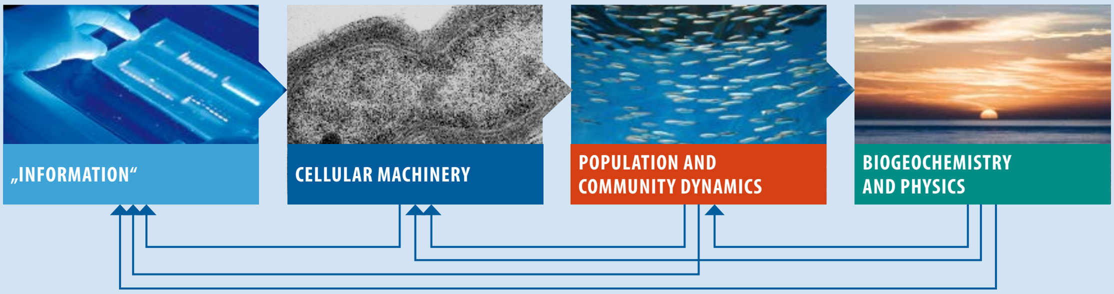
At the same time, there has also been a revolution in the Earth Sciences giving rise to an entirely new field called Geobiology. Studies of past geochemical states of our planet, along with global analyses of its present day composition, have led to the inescapable conclusion that the biosphere, hydrosphere, and geosphere of the Earth have co-evolved over evolutionary time. Living organisms not only adapt to the world around them, but their collective metabolisms shape the chemical and physical environment. Our planet is shaped by life — by the information encoded in the collective DNA of the biosphere. Thus the challenge now before us is to understand how the information in the genomes of organisms influences the properties of the biosphere, geosphere and hydrosphere — and vice versas (Box 1). We need to bring these disciplines together into a cross-cutting enterprise that is informed by advances in genomics, complexity theory, data assimilation and information management, and catalyzed by a new perspective on the properties of life.

Features of Marine Microbial Systems

Life first evolved in the sea. It is in this 'primordial soup' that microbial systems began to self-organize into complex communities where the metabolic products of one group were exploited as substrates by another - continuously developing variations on the theme of carbon and energy acquisition. As a result, the majority of genes in life's inventory – including our own - are probably represented in marine microbes and they are the central actors in shaping the biogeochemical balance of our planet. Without marine microbes there would be no oxygen in the atmosphere, for example, the global nitrogen cycle would be sluggish at best.

Box 1: Life, Emergent Properties, and *Prochlorococcus* as a model system

One cannot understand life in the oceans without studying it at all scales of biological organization. Each scale has properties that emerge from the interactions of the component parts, and those properties feed back on the lower levels of organization and influence the emergent behavior of all components over time. The information in the DNA of living things contains the information that shapes most of their cellular machinery. This machinery is organized into living organisms, which in turn form populations of species, which interact to form the communities that self-organize into ecosystems. These living systems shape the geochemistry of our planet, and have a profound influence on Earth System Processes.



Cross Scale Systems Biology

Prochlorococcus **has a number of features that make it ideal as a model system for studying cross-scale systems biology – an approach designed to not only understand microbial life in the oceans, but also its role in shaping the biogeochemistry of ocean ecosystems.**

- It is the simplest form of life: It creates biomass from sunlight, CO_2, inorganic nutrients, and the information in roughly 2000 genes
- It is the most abundant photosynthetic cell in the oceans. There are an estimated 1027 *Prochlorococcus* in the global oceans, and often 100,000,000 cells per liter. They are responsible for 50 % of the photosynthesis over vast regions.
- It is easily isolated into culture for studies of its growth patterns and dependencies and its small genome simplified genomic approaches.
- It has a simple 'life style' — passively floating in a relatively well mixed fluid medium — allows one to easily recreate its natural environment in the laboratory
- Its natural habitat is well known and circumscribed, and the availability of cellular requirements in the wild can be measured making it possible to link genomic properties with agents of natural selection in the environment.

The ocean ecosystem is an eminently tractable system for studying microbial meta-genomics with how it interfaces with geobiology. Microbes are distributed in the oceans on spatial and temporal scales that are readily and reproducibly sampled and measured. Likewise, their liquid environment makes it relatively easy to measure the physical and chemical variables that influence their distribution and abundance. Thus it is possible to study their properties and dynamics while at the same time studying the properties of the environment with which they have co-evolved. This is the fundamental data necessary to understand what shapes living systems.

The most compelling reason for studying marine microbial systems is their intrinsic value for planetary maintenance. We must understand how they work, in order to develop models that can help us understand past and future states of the planet. Without this predictive capability, it is impossible to design rational conservation practices. One of the most promising approaches to understanding this system is through the study of model organisms. That is, picking one species and studying everything there is to know about it – across all scales of organization from the genome to the ocean ecosystem (Box 1). For the past 30 years I have been studying the marine cyanobacterium (blue green alga), *Prochlorococcus*, for this purpose.

Prochlorococcus – A model system

Prochlorococcus is one of the 'stars' of microbial life in the sea (Figs. 1,2). Discovered only 30 years ago (Fig 3), it is the smallest and most abundant photosynthetic cell in the global oceans (Box 1). It thrives in the mid-latitude oceans, often single-handedly carrying out over 50% of the photosynthesis over vast stretches of the upper 200 meters of sunlit ocean waters. As such, it is responsible for a significant fraction of the fixation of atmospheric carbon dioxide into living matter in the oceans, and for providing sustenance to the rest of the food web.

Over the past 3 decades my laboratory has been studying the ecology and evolution of *Prochlorococcus*, trying to unravel its role in marine ecosystems. We view it as a model organism for understanding what we call "cross-scale systems biology" (Box 1). In the course of our studies we have discovered that within this single microbial species exists an enormous amount of physiological and genetic diversity - with each lineage of cells adapted to slightly different environmental conditions. In this way the *Prochlorococcus* 'Federation', as we call it (Fig. 4), thrives as a global collective – maintaining its populations from ocean to ocean, season to season, and along longitudinal gradients. We have observed, for example, that the *Prochlorococcus* cells in the Atlantic Ocean contain many more genes involved in phosphorus acquisition than those in the Pacific. This serves them well, as phosphorus concentrations in the Atlantic are an order of magnitude lower than those in the Pacific, and limit the productivity in the central Gyres of this ocean basin. Similarly, *Prochlorococcus* cells in areas of the oceans that are limited by iron availability carry many more genes for iron acquisition than those in regions that have a surfeit of iron delivered to them via atmospheric dust. It is through this kind of finely tuned genetic diversity that *Prochlorococcus* is able to dominate the seas.

In recent years we have begun to study how *Prochlorococcus* interacts with other microorganisms in its environment and the results have changed the way we think about microbial communities. The unusually streamlined genome of *Prochlorococcus* cells is possible because other microbes in is environment carry out certain critical cellular functions that *Prochlorococcus* cells can no longer carry out

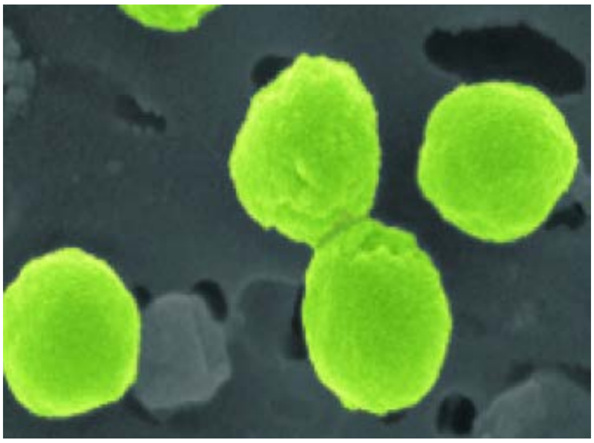


Figure 1: Microscopic image of *Prochlorococcus*, the most abundant photosynthetic cell in the oceans. Each cell is about 1 micron in diameter, which is about 1/100 the width of a human hair. The paired cells in the center have just undergone cell division - the process through which the cells multiply in the oceans. They divide in half about once each day and are eaten by other members of the food web at about the same rate; thus their populations in the oceans remain relatively stable. Research over the past decade has shown that this remarkable stability emerges from the extraordinary genetic and physiological diversity embodied in the billion billion billion *Prochlorococcus* cells in the global oceans. (Photo by Anne Thompson – green color added to black and white photograph)

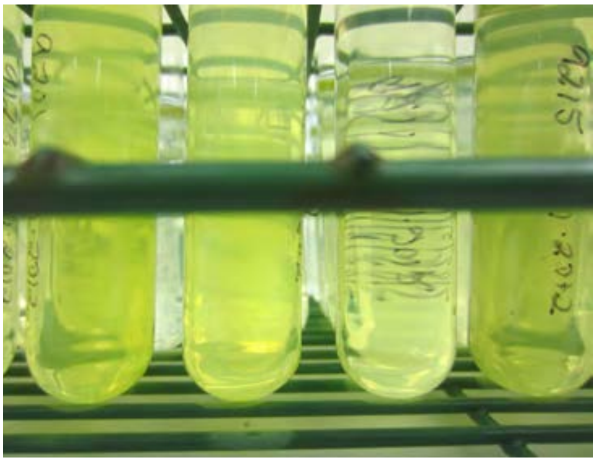


Figure 2: *Prochlorococcus* cells that have been isolated into culture. Each test tube contains about 100,000,000 cells. The green color is the photosynthetic pigment chlorophyll, with which the cells harvest light energy and convert it to organic carbon – the material that forms the base of the food web in ocean ecosystems. My laboratory maintains the largest collection of of *Prochlorococcus* cultures in the world. They have been isolated from diverse regions around the world's oceans and carry genetic information that reflects the particular ocean habitat from which they were captured. Through decoding the genomes of these cultures, we have been able to learn a lot about which environmental variables are most important in shaping the 'metabolism' of different ocean ecosystems. (Photo by Jessie Berta-Thompson)

because they have 'shed' critical genes over evolutionary time. In return, *Prochlorococcus* excretes organic compounds that these co-evolved microorganisms need for carbon and energy. The cooperative metabolic web reflected in this, and many other, 'exchanges' in the microbial marketplace, changes our view of the ecosystem; we we now see the microbial community as a co-evolved super-organism rather than an assemblage of discrete species interacting haphazardly via a fluid milieu.

Prochlorococcus cells multiply themselves by photosynthesizing, growing, and dividing in half (Fig. 1). In the wild, they increase in size during the day, and divide in half by night – such that the entire population doubles roughly once every one or two days. Yet the total population size remains fairly constant over vast stretches of the ocean. This is because the cells die, or are eaten, at a rate that matches their growth rate – testimony to the complexity that endows the system with its remarkable stability. What are the sources of mortality for *Prochlorococcus* in the sea? We know they can be eaten by a diversity of micro-zooplankton – small primitive cells that can engulf bacteria – but we know little about them. What we understand much better is the role of viruses in killing *Prochlorococcus*. Viruses attach to the cells, inject their DNA through the cell membrane, and take over *Prochlorococcus*' cellular machinery. They then redirect it so they can multiply themselves, burst and kill the *Prochlorococcus* 'host' cell, and drift off into the sea to find another cell to infect.

One of the most fascinating aspects of the infection of *Prochlorococcus* by viruses was the discovery that the viruses carry genes that they have 'stolen' from their host cells over the course of evolution. They have incorporated these genes into their own genomes, and employ them when infect the host cell to help them redirect host metabolism toward their own ends. Over vast stretches of evolutionary time these genes drift back and forth between host and virus genomes, all the while evolving so that the molecules they encode have slightly different functions. Thus their temporary 'residency' in the virus actually serves to create diversity in the gene pool upon which natural selection can operate. As a result, we have come to view the viruses as representing an extended gene pool for the *Prochlorococcus* Federation. Yes, viruses cause the death of

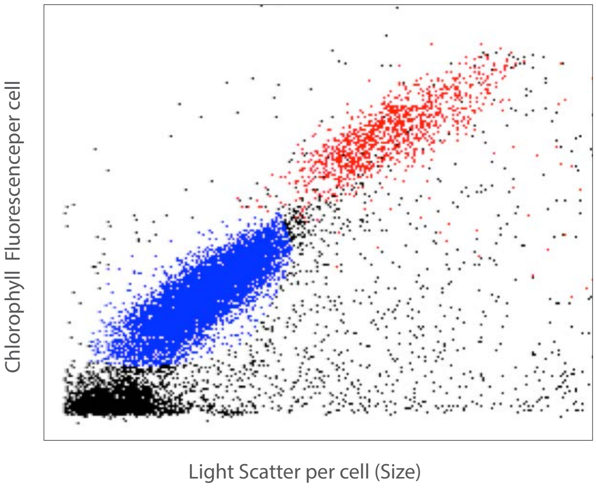


Figure 3: How was Prochlorococcus discovered and how do we study it in the wild? This tiny cell- the most abundant phytoplankter in the sea – eluded the detection of oceanographers before 1985 because it is so small it is not easily visualized using standard microscopic techniques. It was ultimately discovered using a flow cytometer, which is an instrument that focuses a laser on a tiny capillary tube through which a sample is injected. As cells flow past the laser, they scatter light in proportion to the size of the cell, and excite chlorophyll molecules, which give off a characteristic signal. In this image each dot is an individual microorganism in the sample positioned in the frame according to its characteristic fluorescence and light scatter. Prochlorococcus –here colored blue – is the smallest cell in the oceans that has chlorophyll fluorescence.

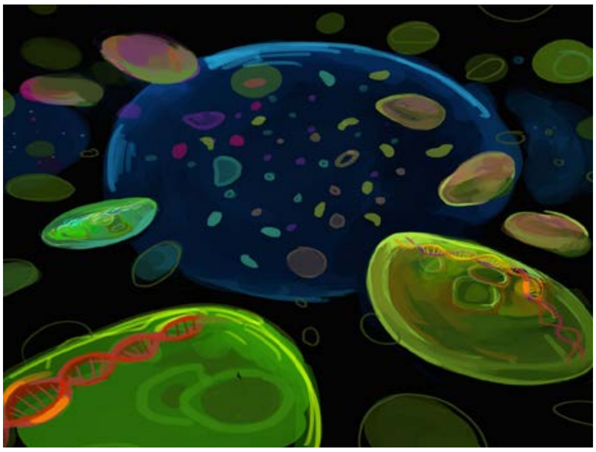


Figure 4: The Prochlorococcus "Federation". Artist's rendering of a drop of seawater containing thousands of Prochlorococcus cells. The individual cells carry slightly different genomic information. This diversity contributes to the stability of Prochlorococcus in the world oceans, as well as the ecosystems it supports.
(Artist: Carly Sanker, MIT)

individual cells, but those cells have millions of clones of themselves floating in the oceans. So the cost of the death of some may be well worth the benefit of the expanded gene pool contributing to the finely tuned diversity in the Federation.

These are but a few examples of the things we have learned by studying *Prochlorococcus*. This tiny cell has been my muse for 30 years, and just keeps pointing the way to new and interesting features of ocean ecosystems. In the past few years for example, we discovered that *Prochlorococcus* releases tiny membrane-bound vesicles into seawater that are packed with genes. This inspired us to look carefully at seawater to see if other species of bacteria also release vesicles. Indeed they do! It appears that this is a very common feature of ocean ecosystems, one that escaped the attention of oceanographers until *Prochlorococcus* pointed the way. What function do these vesicles serve in the microbes that produce them? Why would they shed such precious resources into the seawater? The fact that so many of the microbial species in the oceans are doing it tells us that they must serve some general function. Could this be a mechanism for all the bacteria to share genetic information? Might this play a role in establishing and maintaining the co-evolved meta-metabolism of the microbial community? We look forward to unraveling some of these mysteries - with the help of *Prochlorococcus*, of course.

Petersen Prize

Events during my visit to GEOMAR

I was honored to receive the Petersen Prize in November 2010, which made it possible for me to visit GEOMAR for the first time. My visit was quite short because of time constraints on my end, but while there I gave three lectures. One was a public lecture in conjunction with the Prize – "Tiny Cells, Global Impact" in which I describe the important role of marine micro-organisms in maintaining the biosphere. The second lecture was given in the context of a symposium on marine cyanobacteria that was held during my stay at the Institute. I gave a lecture on my research on *Prochlorococcus*: "From single cells to global metagenomics: What *Prochlorococcus* and its phage can tell us about life in the oceans." Finally, I was asked by my host to give a lecture describing my experience as a member of the MIT committee on Women in the School of Science that uncovered unintentional discrimination against women faculty at MIT. This committee led to a widely-publicized effort on the part of the MIT Administration to: (1) increase the number of women faculty, 2) identify barriers to equality, and (3) develop mechanisms for removing them . The title of the lecture I gave was: How MIT began to 'move the needle' vis a vis women faculty representation and equity". It was followed by a very thoughtful discussion among the participants of the challenges we face in building a scientific community with diversity and equity built into its design.

Sustained Impact

I enjoyed my visit to GEOMAR very much, and found it enormously intellectually stimulating. My host, Julie LaRoche shares my interest in marine cyanobacteria, and we are both involved in the International GEOTRACES program thus we had much to discuss. GEOTRACES is an international ocean research program designed to increase our understanding of the role of trace metals –such as iron, zinc, and manganese – in regulating life in the sea and the global processes that depend on it. Scientists from approximately 35 countries have been involved in the program, which is designed to study all major ocean basins over the next decade. The mission of this enormous project is to: "identify processes and quantify fluxes that control the distributions of key trace elements at isotopes in the ocean, and to establish the sensitivity of these distributions to changing environmental conditions". As described above, the genes *Prochlorococcus* caries have much to tell us about the selective pressures in the environment, and trace elements that are essential for life are among some of the strongest selective agents in ocean ecosystems. My laboratory just received funding to sequence the DNA of samples from many GEOTRACES cruises so we can see what it can tell us about the role of these metals in various ocean ecosystems throughout the globe.