Prochlorococcus
An adventure in Microbial Oceanography
(or the power of the model system approach)

Penny Chisholm
C-MORE Summer Course
June 4, 2014
Prochlorococcus
An adventure in Microbial Oceanography
(or the power of the model system approach)

C-MORE Summer Course
June 4, 2014
Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles
- Integrative Systems Biology
The BIG discovery - began the paradigm shift

Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium

In marked contrast to their freshwater counterparts, marine planktonic cyanobacteria are restricted to a few nostocalean genera, of which only *Trichodesmium* is capable of forming extensive water blooms\(^1\). We report here the widespread occurrence of a small, marine, chroococcalean cyanobacterium belonging to the genus *Synechococcus*.

Natural water samples were filtered through 0.2 \(\mu\)m Nuclepore filters, counterstained with Irgalan black\(^4\). The filters were examined with a Zeiss Standard microscope equipped with Neofluar objectives and an epifluorescent illumination system containing a 100-W halogen lamp, a BP 450–500 excitation filter, a LP 528 barrier filter and a FT 510 chromatic beam splitter. Using this system, phycoerythrin-containing cyanobacteria fluoresce orange and can be distinguished from phytoplankters that fluoresce red.

Phycoerythrin-rich unicellular cyanobacteria were observed at seven stations in the Arabian Sea in January 1977, at three stations off the coast of Peru in March 1978, in Slope Water north of the Gulf Stream in April 1978, and periodically in Woods Hole Harbor. In the relatively rich waters of the Arabian Sea and off the coast of Peru, the population varied from \(10^4\) to \(10^5\) cells ml\(^{-1}\) within the euphotic zone (Table 1). The greatest number of cells was found within the top 20 m of the water column, with occasional cells being observed as deep as 400 m. In contrast, the surface sample collected from Slope Water north

---

*John B. Waterbury
Stanley W. Watson
Robert R. L. Guillard
Larry E. Brand*

Department of Biology,
Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts 02543

© Macmillan Journals Ltd 1979
Johnson and Seiburth 1979
Chroococcoid cyanobacteria in the sea:
A ubiquitous and diverse phototrophic biomass.
Limnology and Oceanography 24(5):928-935.

That same year...

“Type II Synechococcus”
Meanwhile, a few years later, a new pigment was discovered...

Gieskes and Kraay, 1983
And that pigment is enriched in the < 1 µm fraction

Gieskes and Kraay, 1983

Chl a “derivative” > Chl a in small fraction

unfractionated

< 1µm fraction

Gieskes and Kraay, 1983
Enter Flow Cytometry.....

...for the study of single cells
And someone with the courage to take it to sea…

What was I thinking?

Rob Olson
We set out to study *Synechococcus*, and noticed something else…

Based on their fluorescence excitation/emission spectrum - suspected chl b, typical of green algae. Called them “**Little Greens**”
Finally, and electron micrograph – It’s a prokaryote!

Synechococcus

“Little Greens”

John Waterbury
CLOSURE I: Johnson and Sieburth’s “Type II Synechococcus” was the same as our cells
What is their pigment composition?

HPLC pigment analysis of unidentified picoplankton

<table>
<thead>
<tr>
<th>Pigment</th>
<th>0.8 μm filtrate (95% pure)</th>
<th>FCM sort</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorophyll a₁</td>
<td>2.0 fg cell⁻¹</td>
<td>present</td>
</tr>
<tr>
<td>chlorophyll b₁</td>
<td>2.7 fg cell⁻¹</td>
<td>present</td>
</tr>
<tr>
<td>zeaxanthin</td>
<td>0.6 fg cell⁻¹</td>
<td></td>
</tr>
<tr>
<td>α carotene</td>
<td>0.3 fg cell⁻¹</td>
<td></td>
</tr>
<tr>
<td>lutein</td>
<td>not detected</td>
<td></td>
</tr>
<tr>
<td>chlorophyll a₂</td>
<td>not detected</td>
<td></td>
</tr>
<tr>
<td>β carotene</td>
<td>not detected</td>
<td></td>
</tr>
</tbody>
</table>
CLOSURE II:

Gieskes and Kraay’s “chlorophyll α derivative” was the divinyl chlorophyll α of the “Little Greens”
But what do we call them?

What else is prokaryotic and has Chlorophyll b?

Prochloron

Prochlorothrix

So we called them “Prochlorophytes”
A novel free-living prochlorophyte abundant in the oceanic euphotic zone

Sallie W. Chisholm, Robert J. Olson*, Erik R. Zettler*, Ralf Goericke†, John B. Waterbury* & Nicholas A. Welschmeyer†

48–425 Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
* Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA
† Harvard University, Cambridge, Massachusetts 02138, USA
1992 Cultures

Some time later.....

Lead to the name: *Prochlorococcus*

(“coccoid prokaryote with chl b”)
It *IS* closely related to *Synechococcus*, and not to *Prochloron* or *Prochlorothrix*.
What exactly is *Prochlorococcus*?

- Smallest cell in the oceans that fluoresces red
- Contains Divinyl Chl a and Chl b
- Oceanic cyanobacterium, 0.6 - 0.8 µm diameter
- Smallest (size and genome), and most abundant photosynthetic cell on Earth
Prochlorococcus is the ‘minimal life’ form:
Smallest amount of information that can make life from scratch

Light + CO₂ + N,P,S,Fe, Co...etc. (no organic compounds) + 1600 genes

LIFE

Geosphere

Biosphere
Can be significant fraction of ocean chlorophyll

Record concentration:
700,000 cells ml$^{-1}$

Global photosynthesis $\approx 5$ Gt C yr$^{-1}$
Prochlorococcus is basically a slightly smaller Synechococcus, with a different light harvesting system

Ting et al 2006
They share (and partition) the “small size bin”

Prochlorococcus

Synechococcus

DuRand et al 2001
The World of *Prochlorococcus*…

**Dynamic**

**Turnover Time**

1 – 3 days

```
Dilute

5 pM Co
0.2 nM Fe
1 nM P
10 nM N
```
Our Goal:

Study *Prochlorococcus* at all scales of organization
Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles
- Integrative Systems Biology
Tight Cell Cycle Synchrony

Zinser, Lindell et al. 2009
Can see it growing in the wild

1 April 1992

<table>
<thead>
<tr>
<th>Time</th>
<th>540</th>
<th>800</th>
<th>1100</th>
<th>1410</th>
<th>1700</th>
<th>2000</th>
<th>2300</th>
<th>220</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (m)</td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
<td>60</td>
<td>75</td>
<td>90</td>
<td>100</td>
<td>125</td>
</tr>
</tbody>
</table>

Equatorial Pacific

Daniel Vaulot
Prochlorococcus growth rate as a function of depth (measured by cell cycle progression)
Gene expression highly choreographed

Transcripts of 80% genes oscillate
... in the wild too!

Wild Pacific Prochlorococcus

Ottesen et al & DeLong, SCIENCE, in press
Multispecies diel transcriptional oscillations in open ocean heterotrophic bacterial assemblages

Zinser et al & Chisholm 2009
Choreography of the transcriptome, photophysiology and cell cycle of a minimal photoautotroph
Reminder: Structure of the ocean habitat

...physical and chemical gradients =

Niche dimensions!
Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles
- Integrative Systems Biology
Light adaptation defines ecotypes…

Whole Genome Phylogeny
(matches rRNA ITS phylogeny)

Low Light Adapted

Hi Light Adapted

Moore, Rocap, Kettler and more, 1990 - 2006
...leading to niche differentiation

First demonstrated by West et al 2001

Malmstrom et al 2010
...leading to niche differentiation

Cell Concentration (Cells L$^{-1}$)

Depth Integrated Abundance

First demonstrated by West et al 2001

Malmstrom et al 2010
Temperature differentiates the two High Light ecotypes
So there are meaningful layers, within layers, of diversity (leads to stability)

Prochlorococcus ecotypes
“The NATLs” are very interesting
Not HL-Adapted, but intermediate taxonomic position

Prochlorococcus ecotypes
NATLs appear to:
handle high light better than other LL ecotypes…

…and deep mixing better than some HL ecotypes.

Abundance (cells mL$^{-1}$)

Zinser et al 2007
HYPOTHESIS: NATLs better adapted to fluctuating light

BATS time series

Malmstrom et al 2010
Back to the lab…

“The NATLs” are more resistant to light shock than other LL strains

Malmstrom et al 2010
Flashing forward: What do the genomes tell us?

Coleman and Chisholm 2007
Overview

- History
- The Cell
- Niche Dimensions of Prochlorococcus
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles
- Integrative Systems Biology
Beginning to understand *Prochlorococcus* niche differentiation in *two dimensions*

**BUT Remember…**

**Ecological Niche:**

*n*-dimensional hypervolume

n-2 dimensions to go!
Enter Genomics:
Genomes of 13 strains (now 45)

- What genes are “core’ i.e. shared by all?
- How many unique genes in the gene pool of ALL Prochlorococcus?
- Their functions?
The core and flexible genomes – 12 strains

What is the global pan-genome?

- **5736** ‘Flexible Genes’ (w/ 45 genomes -12,000)
- **1250** Shared Core Genes (now about 1000)

Kettler, Martiny et al. 2007

<table>
<thead>
<tr>
<th>Number of Genomes</th>
<th>Number of Genomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2000</td>
<td>4000</td>
</tr>
</tbody>
</table>

Number of genomes vs. Pair-wise comparison
Global Pan-genome is huge

Total of 57,792 genes

FLEXIBLE genes give us clues as to environmental pressures

- Viral defense
- Nutrient acquisition
- Stress responses

- 80% from distant phyla
- A lot of gene swapping going on out there!
Genome size in *Prochlorococcus* is variable

Genome size in Prochlorococcus is variable

Genome size (mb)

Systematic Genome Reduction? NO

small genomes

Genes are gained and lost – with most of the action in the “leaves of the tree”

large genomes

Kettler, Martiny et al. 2007
Variability focused in genomic islands

Coleman et al. 2006
Variability focused in genomic islands

of 236 genes unique to MIT9312
80% are in islands

of 139 genes unique to MED4
74% are in islands
Exploring the full diversity through single cell genomics of wild Prochlorococcus

- Flow Sort individual cells
- Select cells for sequencing
- DNA Amplification
- Sequence Genomes

Rodrigue, Malmstrom, et al & Chisholm 2009
Remember this?

Prochlorococcus ecotypes

Prochlorococcus

Synechococcus

Cells mm\(^{-2}\)
Thousands of single cell rRNA ITS sequence, and hundreds of (partial) genomes
Astounding Diversity!
(layer upon layer…leading to stability)

- Roughly 5000 new genes added to pan-genome
- ‘Genomic backbones’ (core allele variation + associated flexible genes)

Reports

Single-Cell Genomics Reveals Hundreds of Coexisting Subpopulations in Wild Prochlorococcus

Kashtan et al 2014
Astounding Diversity!
(layer upon layer…leading to stability)

- Populations well-mixed 10 km² x 3 m (1 week)
- Each backbone sub-population > 10^{13} cells
- ≈ Effective Population size
The assembly of diversity

A PRIMARY BUILDING BLOCKS OF PROCHLOROCOCCUS DIVERSITY

Distinct variants of core gene alleles

Flexible gene pool

B Each backbone consists of a distinct variant of core gene alleles and a small distinct set of flexible genes

C Cells within the same backbone may differ in some flexible genes in island regions

D Population selected by Environment 1

Population selected by Environment 2

Kashtan et al. Science 2014
Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles
- Integrative Systems Biology
Prochlorococcus is P–challenged

- 13 amole P per cell
- N:P Ratio higher than Redfieldian (21-62) (Bertilsson et al 2003)
- Sulfo- instead of Phospho- lipids (Van Mooy et al 2006)
- 96% P is in nucleic acids (Waldbauer 2010)
**phoBR** genomic region (phosphorus acquisition) in different strains is dictated by ocean of origin and not ribotype.
Back to the oceans…
Comparing the *Prochlorococcus* populations in the Atlantic and Pacific

~10-100nM PO$_4$  
~1-10 nM PO$_4$
For *Prochlorococcus*, the only major difference between the two ecosystems is …

![Graph showing the frequency of genes for the Atlantic and Pacific sites with emphasis on P-acquisition genes.](image-url)

- P-acquisition genes
  - Arsenate reductase and arsenite efflux genes

*Coleman and Chisholm 2010*
Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles!
- Integrative Systems Biology
Prochlorococcus and N sources:

Synechococcus (like “the ancestor”)

NO\(_{-3}\) → NO\(_{-2}\) → NH\(_{4}\) → Biosynthesis

Nitrate reductase

Nitrite reductase

nirA

narB

The Prochlorococcus dogma (based on cultured isolates):

- All Prochlorococcus can use NH\(_{4}\)
- Some can use NO\(_{2}\)
- None can use NO\(_{3}\) (odd...)

Moore et al 2002
Prochlorococcus and N sources:

Synechococcus (like “the ancestor”)

\[ \text{NO}^-_3 \rightarrow \text{NO}^-_2 \rightarrow \text{NH}^+_4 \rightarrow \text{Biosynthesis} \]

Nitrate reductase

Nitrite reductase

nirA

narB

13 Genomes confirmed loss of reductase genes in Prochlorococcus

Moore et al 2002
**Prochlorococcus and N sources:**

*Synechococcus* (like “the ancestor”)

\[
\begin{align*}
\text{NO}_3^- & \rightarrow \text{NO}_2^- & \text{NH}_4^+ & \rightarrow \text{Biosynthesis} \\
\text{Nitrate reductase} & \times & \text{Nitrite reductase} & \times
\end{align*}
\]

13 Genomes confirmed loss of reductase genes in *Prochlorococcus*

**BUT...** There was some evidence for $\text{NO}_3^-$ utilization

**CULTURES**
Williams EZ, Campbell L, DiTullio G. (1999). ASLO Aquatic Sciences Meeting – reported on $\text{NO}_3^-$ utilizing *Prochlorococcus*

**WILD**
Turning to GOS again…

Adam Martiny and Paul Berube
- Recruit all fragments with *narB* gene
- Find those containing known *Prochlorococcus* gene

Cloned DNA fragments from the wild (GOS)

Similar to sequence from *Prochlorococcus* reference genome

Nitrate reductase gene on fragment with known *Prochlorococcus* gene

*Martiny et al 2009*
Abundance distribution of *Prochlorococcus narB* genes in GOS (they are not everywhere)

Martiny et al 2009
Abundance distribution of *Prochlorococcus narB* genes in GOS (they are not everywhere)

Now have many strains in culture – both HL and LL adapted

Nitrate assimilation genes gained, lost, duplicated…

Note: GOS samples only from surface waters

*Martiny et al 2009*
**Prochlorococcus isolates** (bold) that can utilize nitrate – i.e. contain narB (a number are axenic)

- We suspect that all clades will ultimately have representatives
- This trait, like P-acquisition, does not follow taxonomy
Dynamics of \textit{narB} - containing \textit{Prochlorococcus} in the wild (they are a fraction of the population)

Abundance High Light-Adapted \textit{Prochlorococcus} Clade HLII (10^5 cells/ml)

% HLII cells that contain \textit{narB} (can utilize nitrate)

Nitrate + Nitrite
Overview

- History
- The Cell
- Niche Dimensions of Prochlorococcus
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles
- Integrative Systems Biology
Genomes of 5 Wild Prochlorococcus Cells

- Expanded recruited GOS reads by 15%
- Added to hundreds of new genes to Prochlorococcus pan-genome
- New functions

Malmstrom, Rodrigue, 2012
Genomes of 5 Wild Prochlorococcus Cells

- Expanded recruited GOS reads by 15%
- Added to hundreds of new genes to Prochlorococcus pan-genome
- New functions

Malmstrom, Rodrigue, 2012

Rusch et al 2010, West et al 2011
New HL clades restricted to equatorial waters

- Warm
- Low Iron
- High P

Malmstrom, Rodrigue et al, 2012
Siderophore transport genes found in wild cells, and in one of our cultures

Genes are expressed under conditions of iron starvation in cultures

Malmstrom, Rodrigue et al, 2012
Global distributions through GOS metagenomics

Siderophore transporter

“HNLC” Clade Abundance
Siderophore transporter

Iron Dust deposition

Makes sense, so far…
Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles
- Integrative Systems Biology
Prochlorococcus loves its heterotrophs
…*but not ALL* heterotrophs

No effect of co-culture
- enhanced
- inhibited

*Prochlorococcus* MIT 9313  
*Prochlorococcus* MED4

Relative culture fluorescence

…and different strains respond differently to the same suite of heterotrophs  
(a lifetime of PhD theses!)

Evolution generates “beneficiaries” of reduced genomic content [*Prochlorococcus*] dependent on leaky “helpers,” [heterotrophs that leak catalase-peroxidase] perhaps explaining the observed non-universality of phototrophy, stress resistance, and other cellular functions...

It’s a metabolic marketplace...
...a meta-metabolic web

---

Prochlorococcus reveals another new ocean feature


- 2-5 vesicles per cell per generation
- Stable for weeks
- Global production $\sim 10^{27}-10^{28}$ vesicles/day (0.1 – 1 megatonnes carbon)

- Vesicles contain interesting cargo
Many ocean microbes produce vesicles
- A new dimension of ocean biogeochemistry

Vesicles contain DNA from...
34 phyla across all prokaryotes

Vesicles abound $10^5 - 10^6$ mL$^{-1}$

Prochlorococcus points the way!

Biller et al. 2014
What is the function of vesicle release?
Why should *Prochlorococcus* release valuable resources?

- Decoys for phage?
- Cross-feeding?
- Horizontal gene transfer?
- Synechococcus
- Growth of heterotroph

Image from Dave Scanlan
Overview

- History
- The Cell
- Niche Dimensions of *Prochlorococcus*
  - Light and Temperature
  - Genomics and Niche Dimensions:
    - Phosphorus
    - Nitrogen
    - Iron
- The Community
- Marine Vesicles!
- Integrative Systems Biology
The micro-scale complexity is humbling...

Have come to view the system as a loose network of ‘dissolved information’ temporarily housed in microbes and viruses.
...the stability of the emergent patterns is awe inspiring
It’s a co-evolved, self-organizing, COMPLEX SYSTEM...

What are the assembly rules?
Toward a “New Biology”
Integrative Systems Biology

“information” in Genes
Cellular Machinery and Physiology
Population and Community dynamics
Biogeochemistry & Physics

How life works
What designs it
“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
Many thanks to…

Rob Olson
Lisa Moore
Gabrielle Rocap
Allison Coe
Debbie Lindell
Erik Zinser
Zackary Johnson
Rex Malmstrom
Sebastien Rodrigue
Jason Bragg
Maureen Coleman
Katherine Huang
Matt Sullivan
Greg Kettler
Anne Thompson
Jessie Thompson
Katya Fros-Moniz
Andres Cubillos-Ruiz
Nadav Kashtan
Steve Biller

Adam Martiny
Kun Zhang
Paul Berube
Jake Waldbauer
Luke Thompson
Qinglu Zeng
Libusha Kelly
Peter Weiglele

Mick Follows
Mak Saito
Eric Webb
Eric Alm
Ed DeLong
George Church
Bruce Birren
Matt Henn

And many more!