Comparative metatranscriptomics identifies molecular bases for the physiological responses of phytoplankton to varying iron availability

Adrian Marchetti[a,1,2,3], David M. Schruth[a,1], Colleen A. Durkin[a], Micaela S. Parker[a], Robin B. Kodner[a], Chris T. Berthiaume[a], Rhonda Morales[a], Andrew E. Allen[b], and E. Virginia Armbrust[a,2]

[a]*School of Oceanography, University of Washington, Seattle, WA 98105; and* [b]*J. Craig Venter Institute, San Diego, CA 92121*

AUTHOR SUMMARY

Phytoplankton—microscopic algae in the uppermost, sunlit layer of the ocean—perform approximately half of Earth's photosynthesis (1). In vast regions, phytoplankton growth is limited by the availability of iron, and a resupply of iron triggers large phytoplankton blooms. In particular, a variety called diatoms responds almost immediately to iron influxes and rapidly forms the majority of phytoplankton biomass. However, the molecular bases behind diatoms' subsistence in iron-poor waters and response to iron resupply remain largely unknown. We used recently developed high-throughput sequencing methods to characterize the gene expression responses of an iron-limited plankton community to experimental iron enrichment.

We used a method known as comparative metatranscriptomics, which analyzes organisms' mRNA products in their natural environment to obtain a measure of relative gene expression. When genes are used to produce proteins, they are first "transcribed" into mRNA, forming a "transcriptome," analogous to a genome. Two high-throughput sequencing methods, 454 GS FLX and SOLiD, were used to identify genes that were being expressed (or actively transcribed) along with their relative transcript abundances in the ambient seawater (ambient library), unamended control (control library), and iron-enriched incubations (plusFe library), with the 454 sequences serving as reference for SOLiD (Fig. P1). This work was conducted at Ocean Station Papa (50° N, 145° W) in the northeastern Pacific Ocean, a well-characterized iron-limited region (2).

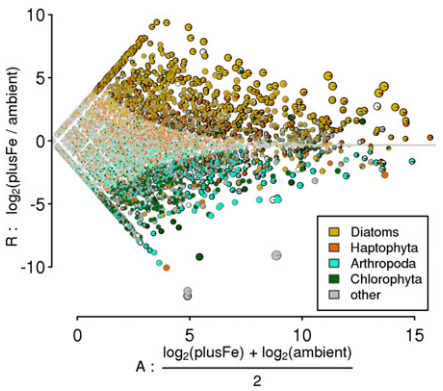

Fig. P1. Plankton community gene expression in response to iron enrichment. Each circle represents a collection of mRNA transcripts assigned to a predicted protein-encoding gene. Plotted are the fold-change ratio (*R*) and the average (*A*) of transcripts between the iron-enriched (plusFe) and ambient SOLiD libraries for a given gene. Gene circles are colored according to taxonomic affiliation (i.e., phylum). Shown are the taxa with the most assigned transcripts ranked from highest to lowest. The solid gray horizontal line indicates the cell abundance normalization ratio. Gene circles with black borders indicate transcripts that are significantly differentially expressed (adjusted *P* value <0.05) in plusFe relative to both the ambient and control libraries; gene circles with dark gray borders indicate transcripts that are significantly differentially expressed in the plusFe vs. ambient libraries only. Gene circles with light gray borders are not differentially expressed. Circles increase in size with absolute values of their coordinates to optimize visibility.

Sequences from diatom, haptophyte, and arthropod species dominated the ambient and control libraries, averaging 25%, 21%, and 18%, respectively, with a mixture of other phyla making up the remainder. After iron enrichment, the proportion of transcripts assigned to diatoms doubled, due largely to an increase from one genus of diatom, *Pseudo-nitzschia* spp. Sequences from all other phyla were found in either similar or lower proportions in the plusFe library compared with their levels in the ambient and control libraries. Over an order of magnitude, more differentially expressed genes were detected with this combined sequencing approach compared with using just the 454 sequences.

The immediate diatom response to iron enrichment is to continue expressing genes encoding non-iron–containing, less efficient proteins—rather than widespread replacement of these proteins with iron-containing, more efficient counterparts. For example, transcripts associated with the iron-free electron transfer proteins were abundant in all libraries and overrepresented after iron enrichment, suggesting that some or all diatoms continue relying on these proteins for photosynthetic electron transfer rather than switching entirely to use of the iron-containing versions present within their gene repertoires. The distinctiveness of the diatom response becomes even more apparent compared with the haptophytes, the other dominant member of the phytoplankton community after iron enrichment, which displayed a more typical response (i.e., a switch to most iron-containing proteins) to iron enrichment. Continued dependence on iron-free proteins may allow diatoms to more rapidly acclimate to returning to low-iron conditions.

We found that the addition of iron to diatoms stimulates what appears to be a system-wide pattern of gene expression in clear alleviation of chlorosis (insufficient chlorophyll production) and in preparation for rapid cell division. Specifically, we observed significant increases in transcripts encoding genes important in the biosynthesis of materials used in growth and division:

Author contributions: A.M. and E.V.A. designed research; A.M., C.A.D., and R.M. performed research; A.M. and D.M.S. contributed new reagents/analytic tools; A.M., D.M.S., C.A.D., M.S.P., R.B.K., C.T.B., A.E.A., and E.V.A. analyzed data; and A.M., D.M.S., M.S.P., and E.V.A. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The 454 and SOLiD sequences reported in this paper have been deposited in the National Center for Biotechnology Information's Sequence Read Archive (accession no. SRP006906).

[1]A.M. and D.M.S. contributed equally to this work.

[2]To whom correspondence may be addressed. E-mail: amarchetti@unc.edu or armbrust@u.washington.edu.

[3]Present address: Department of Marine Sciences, University of North Carolina, Chapel Hill, NC 27514.

See full research article on page E317 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1118408109.

nucleic and amino acids, sugars, and chlorophyll (and its precursors), as well as the prerequisites for long-chain polyamines, which may play an important role in forming diatom cell walls. Diatoms also appeared to divert most, if not all, of their newly acquired iron toward metabolic pathways involved in nitrate assimilation. Our data suggest that components of the diatom urea cycle likely play a significant role in facilitating the iron response leading to bloom formation.

We also identified putative rhodopsin-type genes in several oceanic diatoms and a haptophyte. In the marine environment, rhodopsins are generally known as prokaryotic proteins that act as light sensors for phototaxis (movement toward light sources) or as light-driven biological pumps that move protons for various purposes. Only recently has proteorhodospin been identified in eukaryotes (organisms with internal organelle membranes) (3). Diatom rhodopsins are more similar to the proton-pump variants than to sensory rhodopsins, as they contain a retinal-binding pocket and a region required for proton transport. Rhodopsin transcripts assigned to eukaryotic phytoplankton were highly abundant in the ambient and control libraries, decreasing in abundance following iron enrichment, which suggests a role for rhodopsins in dealing with low-iron conditions.

More than a dozen large-scale experiments have shown that adding iron to surface waters appreciably increases phytoplankton biomass that could potentially enhance sequestration of carbon to the deep ocean (4). Thus, iron fertilization has been proposed as a climate-change mitigation strategy. Deciphering the plankton response to iron enrichment is critical to our understanding of how such a strategy could alter ecosystem dynamics and ocean biogeochemistry. Our study identifies the molecular underpinnings of phytoplankton subsistence in iron-poor regions and their physiological response to iron enrichment, aiding understanding of a potentially important means of regulating oceanic CO_2 concentrations over geological time.

1. Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* 281:237–240.
2. Harrison PJ (2002) Station Papa time series: Insights into ecosystem dynamics. *J Oceanogr* 58:259–264.
3. Slamovits CH, Okamoto N, Burri L, James ER, Keeling PJ (2011) A bacterial proteorhodopsin proton pump in marine eukaryotes. *Nat Commun* 2:183.
4. Boyd PW, et al. (2007) Mesoscale iron enrichment experiments 1993-2005: Synthesis and future directions. *Science* 315:612–617.