Vitamin Biosynthesis and Regulation in Marine Algae

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Project Goals: To develop a systems biology approach to the study of the widespread marine alga *Micromonas* and use it to investigate gene function, pathways and consequences of environmental perturbations on primary production.

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Abstract:
Phytoplankton are important primary producers in marine environments. While the regulatory role of macronutrients on algal growth has been clearly demonstrated, the potential influence of vitamins and small metabolites remains largely unknown. Several groups of organisms have developed independence from vitamin B12, however vitamin B1 (thiamine) is required by all known members of the three domains of life. Still, it is unclear to what extent ecologically relevant marine algae require exogenous sources of B1 and which taxa can synthesize this vitamin. Furthermore, there is little information about the algal genes involved in thiamine biosynthesis, which limits hypothesis development and experimentation.

Here, we present an analysis of the thiamine biosynthetic pathway in eukaryotic algae, with targeted experimental work in the marine green alga *Micromonas*. To explore the evolutionary history of thiamine biosynthesis genes and elucidate which marine algae may have the genetic potential to synthesize B1 we performed a comparative genomics analysis of thiamine biosynthetic genes encoded by algae from different eukaryotic supergroups and by cyanobacteria. These genes were compared to those in bacteria, yeast and land plants. The analysis revealed that green algae are quite different from other eukaryotic algae (e.g. diatoms and pelagophytes), and that even within the green algae there is significant variation. For example, within the *Micromonas* genus, one strain (RCC299) appears to lack key thiamine biosynthetic enzymes, and therefore presumably requires an exogenous source, while another strain (CCMP1545) appears to encode a more complete pathway. In addition, at least one thiamine biosynthetic gene, found in *Micromonas*, and other green algae, is more akin to homologs in fungi than to the gene that performs the same biosynthetic step in land plants.

Because *Micromonas* has a widespread distribution in the ocean, we examined several of the identified putative thiamine biosynthesis genes experimentally. In addition to sampling for complete transcriptome sequencing, qPCR primers were designed to a suite of genes in each strain. Experiments were conducted to explore growth responses to thiamine deprivation in the two *Micromonas* strains. When cultured without thiamine, *Micromonas* CCMP1545 up-regulated key thiamine biosynthetic genes however neither strain seemed able to grow successfully in the absence of thiamine. qPCR primers were also designed to genes that have no known role in thiamine biosynthesis, but, based on genomic analysis, appear to be under the control of a thiamine sensing molecular switch. *Micromonas* RCC299 up-regulated these genes in response to thiamine deprivation and we hypothesize they represent previously unrecognized thiamine transporters. The disparity in expression responses between the two *Micromonas* as well as the unusual pattern of gene homology indicate that thiamine plays a role in algal physiology and ecology. However, experimental validation is critical for developing true understanding of ecological controls and niche differentiation.

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