86. Regulation of Cellular Nitrogen Metabolism in the Model Marine Diatom *Phaeodactylum tricornutum*

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Project Goals:
While the complete genome sequence of a centric and pennate diatom, forward and reverse genetic techniques and *in silico* modeling have enabled our laboratory and others to begin characterizing unknown genes, pathways and interactions; nevertheless, key information sets necessary to a systems biology approach to diatom biology remain undeveloped. Our proposed goals focus on two critical gaps in the diatom knowledge base: *i)* although *in silico* models of carbon and nitrogen metabolism depend on information about subcellular locations of metabolic pathway constituents, very few have yet to be experimentally verified; *ii)* overall pathways and mechanisms controlling cellular carbon and nitrogen sensing, assimilation, and flux, in diatoms remain largely undescribed and have not been formally linked to lipid metabolism. Using a combination of transcriptomics, proteomics, phosphoproteome, metabolomic and stable isotope metabolite flux profiling in steady state cultures, along with directed enzyme localization and biochemistry experiments, we will evaluate lipid metabolism within the overall context of cellular nitrogen and carbon metabolism.

Abstract:
The unique evolutionary footprint of diatoms may have fostered the evolution of peculiar and unique biochemical pathways contributing to the ecological success of diatoms in the modern ocean. Most notably, a complete metazoan-like urea cycle appears to have been acquired from the host of the secondary endosymbiotic event that gave rise to the Chl c algae. In metazoans, the urea cycle is involved in the catabolism of amino acids and the generation of urea for export. The presence of the urea degrading enzyme urease, acquired from the endosymbiont, strongly suggests an alternative function in diatoms. In marine diatoms, which are frequently subjected to nitrogen limitation, we hypothesize that the urea cycle functions in an anabolic capacity to repackage and recycle inorganic C and N from both endogenous and exogenous sources (Allen et al., 2011). Like green algae and vascular plants, diatom genomes also appear to encode plastid targeted Glutamine Synthetase-Glutamine oxoglutarate aminotransferase (GS-GOGAT) components; unlike green lineage eukaryotes, however, diatoms also express distinct mitochondrially targeted GS-GOGAT genes. This mitochondrial GS-GOGAT cycle, in tandem with a mitochondrial urease, might allow for a rapid redistribution of urea cycle-derived nitrogen metabolites to amino acids following the cessation of nutrient limitation. We propose that a two-part uptake system, involving a plant-like outer membrane transporter and a metazoan-like mitochondrial transporter, delivers urea from the extracellular mileau to the mitochondria. Genomic analyses and metabolite flux studies show that the ammonium produced by urease is assimilated using a complete GS- GOGAT cycle found in the mitochondria, with ancillary fixation through CPS III and the urea cycle. In contrast, nitrate-derived ammonium is clearly assimilated through a plastid-localized GS-GOGAT cycle, with a transfer to the urea cycle metabolite pool via arginosuccinate synthase. Comparative genomic analyses suggests this bifurcated nitrogen assimilation system may be present in other phytoplankton of the chromaveolate lineage. RNAi and TALEN mediated knockdown of mitochondrial urease and mitochondrial and

chloroplast localized GS levels are providing additional insights into overall cellular regulation of nitrogen metabolism.

Nitrate reductase (NR) is also enzyme central to overall cellular nitrogen assimilation and metabolism. NR was predominantly believed to be involved in reduction of nitrate as part of nitrogen assimilation. However, mounting evidence suggests a multifunctional role in marine diatoms. First, NR is highly upregulated under cold temperature-high light conditions; this been hypothesized to suggest that NR provides an alternative electron sink for photosynthetically derived electons and reductants that are in excess due to an imbalance between carbon assimilation and growth (Lomas and Gilbert 1999; Parker and Armbrust 2005).. Additionally, in NR-YFP transgenic overexpressors, nitric oxide production is greatly increased. This signaling molecule has been implicated in apotosis and cell-cell signaling in diatoms; although the source of NO in plant cells remains controversial, the peroxisome and NR have each independently, but never together, been implicated in NO production. It is tempting to speculate that NR could be fueling NO production in diatom peroxisomes. In any case, it appears clear that NR is at the center of nitrogen assimilation, signaling, and energy balance. In order to investigate this in more detail we have performed a series of immunolocalization experiments intended to examine NR localization *in vivo* in response to cellular nitrogen status and nitrogen source. NR localization appears to oscillate between the cytosol, peroxisome, and association with the vacuole. RNAseq, proteomic, phosphoproteomic, and metabolomic experiments aimed at preliminary characterization of the diatom response to cellular nitrogen status and nitrogen source have also been performed. In conjunction with the various data types collected to date, an initial genome-scale model of nitrogen metabolism has been constructed.

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