20. Systems biology of nitrogen assimilation in the diatom Phaeodactylum tricornutum

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Project Goals: Genome-scale metabolic models are fundamental for the analysis of cellular processes at a systems level and represent an ideal organizational framework for analyses of functional genomics, experimental work and computational studies. In recent years, there has been an increasing interest in high-quality metabolic reconstructions of phototrophic organisms and robust computational tools to integrate ‘omic’ data from these organisms within genome-scale models. The approach of the project is to combine cutting-edge genome manipulation and physiological characterization with metabolic modeling. The ultimate goal is the exploration of next-generation biofuels through a comprehensive understanding of light-driven lipid metabolism in the model marine diatom Phaeodactylum tricornutum.

The eukaryotic microalgae diatoms hold great promise for bioproduction of fuels and higher value chemicals and recent advances by our group have greatly improved the ability to genetically engineer diatoms. Given their evolutionary history of serial endosymbiotic events with increasingly evolved exosymbionts, diatoms contain novel combinations of the biochemical pathways, making them alluring alternative bioproduction systems. However, this also means our understanding of central metabolism, including nitrogen assimilation, is lacking. To address this knowledge gap, we used physiological characterizations, genetic manipulations, transcriptomics, metabolomics, and proteomics to examine the distribution and expression of proteins involved in nitrogen assimilation and metabolism in the pennate diatom Phaeodactylum tricornutum. Cellular measurements of carbon and nitrogen and fourier transform infrared spectrometry profiles revealed dramatic and rapid shifts in cellular nitrogen contents, specifically proteins, with changes in nitrogen availability. They also highlighted a remarkable rapidity in protein synthesis and growth recovery upon the addition of nitrogen to nitrogen-limited cultures, a phenotype of diatoms with ecological implications. Protein localizations revealed both mitochondrial and chloroplast localizations for complete Glutamine-synthase-Glutamate synthase (GS-GOGAT) pathways for ammonia assimilation. The localizations of urease and nitrite reductase suggest that urea and nitrite are assimilated in the mitochondria and chloroplast respectively. Fluxomics with ¹⁵urea and ¹⁵nitrate confirmed this hypothesis but also highlighted variations in the metabolic profiles of diatoms grown on different nitrogen sources. Short term (<1 hours) multi-time point transcriptomic and proteomic experiments show that the addition of nitrate, nitrite, urea, and ammonia result in divergent expression profiles of proteins centrally involved in nitrogen assimilation. In summary, diatoms have specialized systems for the assimilation of different nitrogen compounds that are very rapidly regulated (<15 minutes) by the availability of the various compounds. All of these results were incorporated into a constraint-based metabolic model of nitrogen assimilation in P. tricornutum, which reveals key metabolite exchanges between subcellular compartments and carbon metabolism while guiding future experiments.
Figure: Modeled metabolic flux and protein expression in nitrogen starved diatoms

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