Evolution and Metabolic Configuration of Nitrogen Flux in a Model Marine Diatom

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Project Goals:

We propose integration of genome-scale modeling with genome engineering to optimize energy and metabolte flux through subcellular compartments to promote efficient production of high value and fuel-related metabolites. Through the proposed research activities, we aim to construct streamlined artificial chromosomes encoding reprogrammed biological modules designed for *in vivo* optimization of electron flow efficiency, photosynthesis, and overall cellular growth while directing key metabolic precursors away from storage carbohydrates and into lipids or branched chain amino acids (BCAA). The underlying goal of the proposed research is to produce strains of diatoms encoding cellularly compartmentalized biosynthesis pathways on an artificial chromosome, with the natural genetic background altered to include knockouts of respective native genes as well as the installation of *in vivo* metabolite bioreporters.

Abstract:

Diatoms dominate phytoplankton communities by outcompeting other groups for nitrate, yet little is known about the mechanisms underpinning this ability. Genome and genome-enabled studies have shown that diatoms possess unique metabolic features compared to other phototrophs, such as mitochondrial glycolysis and the presence of a urea cycle. In diatoms, the cycle is known to be important for recovery from nitrogen limitation, however there are open questions about how the cycle is integrated within the cell-wide metabolic network. To develop a whole-cell level understanding of the impact of nitrogen source and status on *Phaeodactylum tricornutum*, we investigated gene expression and metabolic flux in in experiments aimed at eliciting shifts in nitrogen status over the short term. Using a combination of transcriptomics, proteomics, metabolomics, fluxomics, and flux balance analysis, we have arrived at a systems-level understanding of how nitrogen is assimilated and distributed within *P. tricornutum*. We found a high degree of metabolic network connectivity between the chloroplast and mitochondria of pathways at the critical intersection of carbon and nitrogen metabolism. We characterize the differentiated function of organellar GS-GOGAT cycles and describe aspartate and alanine systems used to exchange amino moieties between organelles. We also describe an arginine biosynthesis pathway that is split across organelles in diatoms, clarifying the role of the urea cycle. We propose that the unique configuration and high degree of metabolic integration between the major energy organelles allows diatoms to efficiently respond to changing nitrogen status, conferring an ecological advantage over other phytoplankton taxa.

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