New advances in diatom functional genomics: Gene knockout methodology and genome-wide mapping of transcription factor binding sites

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Diatoms, which comprise 20-40% of all phytoplankton, thrive throughout the world's oceans. They are single-celled eukaryotes, drawing down atmospheric CO_2 and producing oxygen by photosynthesis. Phytoplankton biomass anchors the base of the ocean food chain, and their storage of excess energy as lipids drives the algal biofuel and food industries. To build biomass and lipids diatoms take up bicarbonate and nitrogen, in the form of nitrate. The uptake and assimilation of nitrate by diatoms is a tightly-coordinated, energy-demanding, multi-protein, multi-organelle effort. To describe the passage and transformation of nitrate through the cell, transgenic lines for twelve primary, nitrate-related genes have been produced in the marine model-diatom, *Phaeodactylum tricornutum* by CRISPR-Cas9 knockout methods. The genes chosen were identified by annotation and prior transcriptomic, time-course expression studies. CRISPR knockouts of two genes encoding outer membrane nitrate transporters (Phatr3_J26029 and J54560), tentatively described by comparative analysis as low and high sensitivity nitrate transporters, show distinctly different growth profiles. The CRISPR knockout line for nitrate reductase (Phatr3_J54983), which catalyzes the first step nitrate assimilation, does not grow on nitrate, whereas its growth ammonium is unchanged. Changes in growth and/or fluorescence (RFUs) have been tracked for the individual CRISPR knockouts for the coupled enzymes: GSII and GOGAT in the chloroplast (Phatr3_J51092 and J24739) and the GSIII and GOGAT(m) in the mitochondria (Phatr3_J22357 and J20342); here, phenotypic changes, while apparent, are more subtle. Within the chloroplast, growth phenotypes for CRISPR-knockout lines for two nitrite reductases (Phatr2_J12902 and Phatr3_EG02286) indicate potential diel roles in nitrite reduction. Previous research suggests that diatoms may store nitrate in excess of their immediate needs; CRISPR-knockout lines for two putative vacuolar nitrate transporters (Phatr3_EG01952 and J28245) have been shown to grow more slowly than WT cells. Experiments in progress may to determine if sluggish growth is directly related to nitrate uptake into the vacuole. In bacteria and plants, external and intra-cellular sensing of nitrate abundance enable cells to regulate transcriptomic responses; a CRISPR-knockout line for a nitrate sensing kinase (Phatr3_J21961), identified by homology in *P. tricornutum,* points to possible interaction with one of the outer membrane nitrate transporters.

Additionally, diatoms change patterns of gene expression as they adjust to environmental conditions, such as shifting nutrient availability or light levels. Transcription factors (TF) bind specific DNA sequences to regulate the expression of nearby genes, making them important components of this cellular response. In the model diatom *Phaeodactylum tricornutum,* little is known about which genes are regulated by specific TFs, as only a few of the 212 annotated TFs have been functionally investigated. Improved knowledge of TFs and their binding sites (TFBS) is essential to construct a systems level model of gene expression and is also valuable for enhancing the molecular toolbox for genetic engineering of diatoms. Using a new, low-cost,

high-throughput technique called DAP-seq (DNA affinity purification sequencing) we are mapping transcription factors binding sites genome-wide. Initial rounds of DAP-seq have been successfully completed, on 30 TFs selected on the basis of their transcriptional sensitivity to nutrient perturbations (Fe, N). We will expand this effort to include all annotated TFs in the *P. tricornutum* genome, greatly expanding functional knowledge of DNA regulatory elements and transcriptional regulation.

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