87. Inactivation of *Phaeodactylum tricornutum* urease gene using TALEN-based targeted mutagenesis

Philip D. Weyman [1], Karen Beeri [2], Stephane C. Lefebvre [2], Josefa Rivera [2], Adam L. Heuberger[3], Graham Peers[4], Christopher L. Dupont(Co-PI) [2], **Andrew E. Allen (PI)**[2,5*]

[1]J. Craig Venter Institute, Department of Synthetic Biology and Bioenergy, 4120 Torrey Pines Rd., La Jolla, California, 92037 USA, [2]J. Craig Venter Institute, Department of Microbial and Environmental Genomics, 4120 Torrey Pines Rd., La Jolla, California, 92037 USA, [3]Colorado State University, Proteomics and Metabolomics Facility, Fort Collins, Colorado, 80523 USA, [4]Colorado State University, Department of Biology, Fort Collins, Colorado, 80523 USA, [5]Integrative Oceanography Division, Scripps Institution of Oceanography, University of California, San Diego, CA 92093, United States

Project Goals: ***Targeted Genetic Manipulation and Systems Biology*:** Our initial genome scale modeling and targeted genetic manipulation activities will focus on cellular modules fundamental for cellular regulation of primary metabolism and overall photosynthetic efficiency. These include pyrenoid formation and function in regulation of the diatom carbon concentrating mechanisms (CCM), a quantitative understanding of linear and alternative electron flow through photosystems (Nogales et al. 2012), a functional characterization of metabolites that are exchanged between the chloroplast and mitochondria and their carrier proteins and manipulation of metabolite partitioning to storage lipids. We will employ an iterative approach of computational modeling followed by genetic manipulation and physiological characterization to characterize these systems.

Abstract:
We describe a method for genome editing in the model diatom *Phaeodactylum tricornutum* using transcription activator-like effector nucleases (TALENs). For a proof of concept, the gene encoding for the enzyme urease was targeted for interruption. A mix of both heterozygous and homozygous mutants was obtained using TALEN-assisted homologous recombination. Southern blot and PCR analyses of genomic DNA confirmed efficient and specific insertion of the knockout cassettes within the urease gene. Western blot analyses confirmed lack of urease in homozygous lines, which were unable to grow on urea as sole nitrogen source. The homozygous urease knockout lines are able to grow on other nitrogen sources and metabolomics analysis revealed a buildup of metabolites indicative of disruption of the urea cycle such as urea, arginine, and ornithine. Numerous high carbon metabolites were enriched in the homozygous mutant, suggesting a disruption of cellular C and N repartitioning. The presented method improves the molecular toolkit for diatoms and clarifies the role of urease in the urea cycle.

DOE-DE-SC0008593, Optimization of Energy Flow Through Synthetic Metabolic Modules