Mechanisms of Carbon Partitioning into Chrysolaminarin, the Storage Polysaccharide of Diatoms

Michael A. Caballero[1]* (mike.caballero@colostate.edu), Graham Peers,[1] and **Andrew E. Allen[2]**

[1]Colorado State University, Fort Collins; [2]J. Craig Venter Institute, La Jolla, CA

Project Goals: Our overall goal is to reprogram metabolic networks using i*n vivo* synthetic modules to increase the flux of energy and carbon into biofuel precursors in the marine diatom *Phaeodactylum tricornutum*. This is broken down into 3 sub-goals: 1) Profile the transcriptome, proteome and metabolome to investigate cell responses to physiologically relevant conditions. 2) Identify and manipulate key factors involved in the control of inorganic C assimilation, photosynthetic efficiency and carbon partitioning. 3) Create screen and genotype a forward genetic library generation. These approaches complement our development of *Phaeodactylum* genome reconstruction modeling and our development of novel synthetic genomic tools to achieve our overall goal of increasing photosynthetic productivity.

Diatoms contribute to global carbon cycles, accounting for about one-fifth of global primary productivity. Partitioning photosynthate into storage metabolites enables flexible cellular metabolism by providing a reservoir of carbon and energy. Diatoms store carbohydrate as chrysolaminarin, a β-1,3 glucan, instead of starch or glycogen. Disrupting chrysolaminarin metabolism may direct diatom carbon partitioning from storage carbohydrates to triacylglycerol, an important metabolite for biodiesel production. However, the genes responsible for producing proteins important for chrysolaminarin metabolism remain broadly unknown. We are interested in identifying these genes, disrupting their expression, and investigating their impact on carbon partitioning.

The lack of a robust method for chrysolaminarin quantification was an initial challenge for this study. We developed a method to selectively quantify chrysolaminarin from *Phaeodactylum tricornutum* cell extracts with selective hydrolysis. Chrysolaminarin reserves were depleted overnight, decreasing from 1.69±0.27 to 0.06±0.03 pg glucose equivalents per cell, while structural carbohydrates did not significantly decrease. H-NMR structural analysis of *Phaeodactylum's* chrysolaminarin indicates a smaller polymer with less branching than commercially available laminarin. This method enables phenotyping *Phaeodactylum* mutants by chrysolaminarin accumulation, rather than established total-carbohydrate methods.

We have identified chrysolaminarin-related targets through several genomics-enabled approaches. Comparative genomics has identified several conserved UGPases in the *Phaeodactylum* genome, and are thought to be the initiating step of chrysolaminarin synthesis. We are investigating the contribution of a putatively chloroplast-localized UGPase to chrysolaminarin biology. In a complementary approach, we identified a putative, chrysolaminarin-related, phosphatase-like protein (PCP) from the *Phaeodactylum* proteome using 2D-affinity electrophoresis. PCP RNAi lines accumulated two-fold more chrysolaminarin

per cell volume than wild type.

This research seeks to identify and study new chrysolaminarin-related proteins and expands the available biochemical toolkit to characterize carbon partitioning in algae. Investigating chrysolaminarin biology will improve our understanding of diatom central carbon metabolism and informs our future bioengineering efforts to produce metabolites of interest, such as triacylglycerol for biodiesel.

Project supported by the DOE Office of Science-BER (DE-SC0008595).