82. Integrating energy transduction from light absorption to biofuel precursors in the alga *Phaeodactylum tricornutum*.

Graham Peers[1]* (graham.peers@colostate.edu), Nathan Sindt[1], Michael Caballero[1], Seijin Park[1], Corey Broeckling[1], **Bernhard Ø. Palsson[2]**, **Chris L. Dupont[3]**, **Andrew E. Allen[3]**

[1]Colorado State University, Fort Collins; [2]University of California, San Diego; [3]J. Craig Venter Institute, San Diego, California

Goals: Overall goal -- Reprogram metabolic networks using i*n vivo* synthetic modules to increase the flux of energy and carbon into biofuel precursors. Goal 1) Profiling the transcriptome, proteome and metabolome to investigate cell responses to physiologically relevant conditions. Goal 2) Identify and manipulate key factors involved in the control of inorganic C assimilation, photosynthetic efficiency and regulation of lipid synthesis. Goal 3) Forward genetic library generation, screening and genotyping. 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 productivity.

We are using a systems biology approach to understand and increase the efficiency of photon and carbon utilization in the model marine diatom *Phaeodactylum tricornutum*. Our approach spans photo---physiology observations to metabolic modeling and pathway manipulation to identify important pinch---points of metabolism and increase the overall yield of fuel precursors. The work reported in this abstract is one avenue of this project.

We observed changes associated with cell metabolism during a shift from excess light energy to light fluxes that limit growth. This represents a switch in cellular energy states from one where energy is being shunted to storage products such as carbohydrate (chrysolaminarin) or lipid (triacylglycerol) to a condition where these storage products are being consumed to maintain cell division. This switch also captures the process of photoacclimation, whereby light-limited cells begin to increase cellular pigments concentrations – this process is a well recognized target for improving overall photosynthetic efficiency in dense cultures of algae like those found in an industrial scale algal photobioreactor.

We have identified a series of differentially regulated, novel targets for increasing yields of lipids. These include 4 putative components of chrysolaminarin biosynthesis and a putative acyl-CoA-dehydrogenase that may participate in the oxidation of fatty acid. We are using a novel knock-down vector to investigate the role of these proteins in carbon partitioning. We have also identified several proteins that play likely play role in photoacclimation including three light harvesting antenna proteins (LHCF5, -16 and -14) that are highly upregulated in response to low light.

We are working to increase data analyses throughput for the rapid, automated analyses of shotgun metabolomics data. We are developing software code that will query spectral databases to ID experimentally derived shotgun metabolite data sets. This will greatly increase the speed that metabolites can be identified. We are also developing tools to detect and quantify highly polar metabolites associated with the primary steps of photosynthesis (e.g. NADPH, fructose-6-phosphate).

The physiology of photosynthesis is dynamic over the course of a natural day/night cycle. *Phaeodactylum* induces a pathway to maintain an oxidized plastoquinone pool during the highest light fluxes of the afternoon and this is independent of non-photochemical quenching. We are currently investigating the role

of alternate electron transport pathways in this photoprotective response and are quantifying the energy flow through this pathway using membrane-inlet mass spectrometry techniques.

This research is supported by the Office of Biological and Environmental Research in the DOE Office of Science grant # DE-SC0008595. MC is supported by a NSF graduate student fellowship.