

A Systems Level Study of the Marine Diatom *Phaeodactylum* Reveals an Unexpected Mitochondrial Fatty Acid Beta Oxidation Pathway.

Graham Peers^{1*} (graham.peers@colostate.edu), Denis Jallet,¹ Denghui Xing,¹ Mark Moosburner,² and Andrew Allen²

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

Project Goals: Overall goal - Reprogram metabolic networks using *in 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 accumulation. 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.

Photosynthetic organisms balance heterotrophic and autotrophic metabolisms across the day to supply their requirements for growth and survival. We investigated the system level response of the diatom *Phaeodactylum tricornerutum* during a shift from excess light, wherein energy is stored in compounds such as triacylglycerol (1), to light fluxes that limit the reaction rates of the photosynthetic system. Despite this shift, cells maintained maximal growth rates for 24 hours. Transcriptomic, proteomic and metabolomic data were fit to our previously established genome scale model of metabolism (2). This suggested that lipid catabolism fueled the rapid growth rates and photosynthetic remodeling required to capture a reduced light flux. *Phaeodactylum* was predicted to use both the plant-type peroxisomal fatty acid beta oxidation cycle as well as the cycle found in the mitochondria (animal-type). We found that the mitochondrial pathway was upregulated in response to low light, and is also dynamically regulated on a day/night cycle. The first step of this beta oxidation pathway is catalyzed by an Acyl-CoA Dehydrogenase (ACAD) and, in *Phaeodactylum*, this enzyme appears to be encoded by a gene resulting from a recent horizontal gene transfer from bacteria. We knocked out this gene using a CRISPR-Cas9 approach and found that mutant cells had slightly impaired growth rates compared to WT cells during growth in day/night conditions. Lipidomics studies revealed that the primary Triacylglycerol species, containing primarily 16:0 and 16:1 fatty acids, were not catabolized at night in the mutant. We also verified that this ACAD was targeted to the mitochondria and that a recombinant enzyme was able to catalyze the oxidation of palmitoyl-CoA (16:0). Mutant strains hyper-accumulated lipid following the amelioration of nitrogen starvation, suggesting that disrupting mitochondrial beta oxidation may be an effective mechanism to channel carbon to biofuel precursors in some conditions. We believe that this is the first characterization of triacylglycerol catabolism occurring by the animal-type pathway in a photosynthetic organism.

Publications

1. Jallet, D., Caballero, M.A., Gallina, A.A., Youngblood, M. and **Peers, G.** (2016) Photosynthetic physiology and biomass partitioning in the model diatom *Phaeodactylum tricornutum* grown in a sinusoidal light regime. *Algal Research* 18: 51-60.
2. Levering, J., Broddrick, J., Dupont, C.L., **Peers, G.**, Beeri, K., Mayers, J., Gallina, A.A., Allen, A.A., Palsson, B.O., Zengler, K. (2016) Genome scale model reveals metabolic basis of biomass partitioning in a model diatom. *PLOS ONE* DOI:10.1371/journal.pone.0155038

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