Mineral Nutrition and Energy Pathways Regulate Lipid Accumulation and Photosynthesis

Tim L. Jeffers*[1] (tim_jeffers@berkeley.edu), Shivani Upadhyaya*[1] (shivani.u@berkeley.edu), Kim Hixson,[2] Sam Purvine,[2] Carrie Nicora,[2] Ryan McCombs,[1] Mary Lipton,[2] **Krishna K. Niyogi**,[1,3,4] Melissa S. Roth[1]

[1]Department of Plant and Microbial Biology, University of California, Berkeley; [2]Pacific Northwest National Laboratory; [3]Lawrence Berkeley National Laboratory; [4]Howard Hughes Medical Institute, University of California, Berkeley

Project Website: https://sites.google.com/view/czofingiensis/home

Project Goals: Our overarching research goal is to design and engineer high-level production of biofuel precursors in photoautotrophic cells of the unicellular green alga *Chromochloris zofingiensis*. Our strategy involves using large-scale multi-'omics systems analysis to understand and model the genomic basis for how the energy metabolism of the cell is redirected based on the carbon source. Here, we focus on elucidating the effects of nutrients and the master regulator target of rapamycin (TOR) kinase on regulation of photosynthesis and metabolism to ultimately improve production of biofuels and bioproducts.

Microalgae have the potential to become a major source of biofuels and bioproducts without exacerbating environmental problems. Photosynthetic microbes can utilize solar energy, grow quickly, consume CO_2, and be cultivated on non-arable land. However, there are presently considerable practical limitations in the photosynthetic production of biofuels from microalgae, resulting in low productivity and high costs. Insight into regulation of photosynthesis and metabolism will accelerate bioengineering of microalgae to maximize production of biofuels and bioproducts.

Photosynthetic organisms respond to multiple signals to produce, use, and store energy in diverse environments. We have exploited proteomics in the oleaginous green alga *Chromochloris zofingiensis* to understand the influence of essential nutrients and master regulators of energy pathways on photosynthesis and the accumulation of biofuel precursors. Previously, we showed that glucose (Glc) sensing and signaling via hexokinase (HXK1) increases growth, enhances triacylglycerol accumulation (TAGs), and completely switches off photosynthesis (1, 2). However, here, we found that the switching off of photosynthesis only occurs under iron deficiency. Multifactorial proteomics revealed that *C. zofingiensis* switches off photosynthesis to prioritize iron resources for respiration and *de novo* fatty acid synthesis. Our combinatorial iron and Glc experimental design effectively identified novel candidates involved in photosynthetic regulation, lipid accumulation, and iron partitioning across algae and plants.

Furthermore, we investigated the role of TOR kinase in Glc perception in *C. zofingiensis*. In the plant *Arabidopsis thaliana*, Glc sensing and signaling pathways are regulated by TOR kinase, and together they are important for the regulation of growth and metabolism. In algae, in contrast, the role of TOR kinase in glucose signaling and regulation of photosynthesis is unknown. Using the TOR kinase domain inhibitor AZD8055, we found that TOR inhibition causes inhibition of photosynthesis and accumulation of TAG and ketocarotenoids in *C. zofingiensis*, a phenotype similar to the Glc-mediated switch. However, TOR inhibition in the presence of glucose prevents biomass accumulation, whereas glucose on its own promotes accumulation of biomass. Using a phosphoproteomic approach, we identified novel targets of TOR kinase, whose phosphorylation changes in response to TOR kinase inhibition by AZD. Intriguingly, many proteins involved in TAG degradation appear to be targets of phosphorylation by TOR kinase. We also found that inhibition of TOR kinase resulted in mobilization of major membrane lipids into TAGs and not *de novo* TAG biosynthesis as was observed upon Glc addition. Altogether, these studies show the power of using *C. zofingiensis* for gene discovery and pathway elucidation related to nutrient physiology, photosynthesis, and carbon flow regulation, which will enable metabolic redesign and bioengineering for sustainable biofuels.

References

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