Advancing the *Chromochloris zofingiensis* molecular toolkit

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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 partitioning based on the carbon source. Enabled by cutting-edge synthetic biology and genome-editing tools, we will integrate the systems data in a predictive model that will guide us in the redesigning and engineering of the metabolism in *C. zofingiensis*. This poster details recent advances our group has made in developing genetic engineering and lipid analysis tools for this emerging model organism.

Abstract:

The green alga *Chromochloris zofingiensis* has a natural ability to accumulate high amounts of energy-dense lipids and pigments in a concentrated culture. Recent and ongoing investigation of this organism has revealed key signaling and biochemical hubs in the lipid biosynthesis pathway (1,2,3). The understanding and manipulation of pathway elements require the use of modern molecular genetics tools, none of which have been optimized for this organism. Here we report on the delivery of recombinant DNA by electroporation and *Agrobacterium*-mediated transformation and on our efforts to deliver proteins by electroporation. In addition, we have adapted a relatively simple lipid extraction protocol that, coupled with TLC, provides a quick and detailed analysis of the lipid profile of *C. zofingiensis*. A pipeline combining our established UV mutagenesis protocol and TLC provides a powerful forward genetics screen for strains with aberrant lipid profiles. Of particular interest will be mutants with increased TAG accumulation or those with more efficient lipid extraction. Concurrently, we are adapting genome editing protocols for use in *C. zofingiensis* with the intention of disrupting specific genetic loci to increase lipid accumulation or facilitate extraction. Potential targets identified by RNA-Seq analysis will be discussed.

References

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2. Roth MS, Gallaher SD, Westcott DJ, Iwai M, Louie KB, Mueller M, Walter A, Foflonker F, Bowen BP, Ataii NN, Song J, Chen J-H, Blaby-Haas CE, Larabell C, Auer M, Northen TR, Merchant SS, Niyogi KK (2019) Regulation of oxygenic photosynthesis during trophic transitions in the green alga *Chromochloris zofingiensis*. *Plant Cell*, in press.
3. Roth MS, Westcott DJ, Iwai M, Niyogi KK Hexokinase functions as a molecular switch for oxygenic photosynthesis and metabolism in a green alga. Manuscript in revision.

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