Design Principles Controlling Hydrogen Metabolism in Phototrophic Organisms

Task 1

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Project goals: To obtain a systems-level understanding of the biological barriers that control hydrogen metabolism and prevent sustained H_2 photoproduction in the green alga *Chlamydomonas reinhardtii*.

Photobiological H_2 production from water is a clean, non-polluting and renewable technology. Although the potential light conversion efficiency to H_2 by biological organisms is theoretically high (about 10%), the system is currently limited by biochemical and engineering constraints. The specific objectives of this research are covered by two Tasks: (1) development, testing, validation and utilization of novel high-throughput assays to identify photosynthetic organisms with altered H_2-producing activities, thus leading to the discovery of novel strategies to circumvent known biochemical limitations (this task is now completed); and (2) deconvolution of the network of metabolic pathways centered on six ferredoxin homologs found in Chlamydomonas, aimed at understanding reductant flux in photobiological hydrogen production, and identifying targets for future metabolic pathway engineering strategies to reduce flux to non-productive pathways.

In completing Task 1, we developed a novel high-throughput assay that allows the screening of up to 500K algal colonies per minute for H_2-production characteristics. This assay is now in use by eight different labs; it is notable for its ease of use as a bench-top screen for H_2-producing organisms whose H_2 production systems are essentially O_2-intolerant. The assay uses the H_2-sensing system of *Rhodobacter capsulatus* and couples the sensor to activation of a GFP signal (Wecker et al., 2011). We validated this assay with well-characterized mutants that are either low or high H_2-producers (Wecker and Ghirardi, 2014). We then used the assay on a *C. reinhardtii* insertional mutagenesis library and isolated four strains of *C. reinhardtii* capable of high-light H_2 production. These mutants showed up to 180-fold greater levels of H_2 production at elevated light levels in comparison to the wild type strain. The greatest high-light H_2-production increase is putatively the result of a mutation in a pherophorin gene. We are complementing this mutation to determine if the mutant with a replacement gene regains its original phenotype.

We have also created a model system for developing photosynthetic H_2 production. We inserted a heterologous hydrogenase from *Clostridium acetobutylicum* into our *R. capsulatus* sensor strain. The imported enzyme produces H_2, and the H_2 produced in turn causes the organism to fluoresce. Moreover, H_2 production can be repeatedly induced by dark cycles followed by illumination. This

system therefore represents a novel means of testing, developing and selecting for *in vivo* hydrogenase activity, for photosynthetic H_2 production, and for metabolic support of H_2 production with real time output of hydrogen production reported on a cellular level. A manuscript is near completion.

Publications:

1. Wecker, M.S. and M.L. Ghirardi, *High-throughput biosensor discriminates between different algal H_2-photoproducing strains*. Biotechnology and Bioengineering, 2014. 111(7): p. 1332-1340.
2.
3. Wecker, M.S. and M.L. Ghirardi, *Expression and Photosynthetic Activity of a Clostridial [FeFe] Hydrogenase in the Bacterium Rhodobacter capsulatus* (In Preparation)

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