Development and Validation of Novel Techniques to Understand and Address Limitations to Algal Hydrogen and Biofuels Photoproduction

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Project goals: To develop techniques that will provide a deeper understanding of algal H_2 metabolism and accelerate the development of future photobiological H_2-producing catalysts and organisms.

Photobiological H_2 production from water is a clean, non-polluting and renewable technology. The efficiency of light conversion into H_2 by biological organisms is theoretically high (about 10%). However, the system is currently limited by biochemical and engineering constraints, including the extreme O_2 sensitivity of the biological hydrogenases and the low availability of reductants to the hydrogenase due to the predominance of competing metabolic pathways. In Task 1, we developed a Rhodobacter-based H_2-sensing assay that is useful for conducting high-throughput screening for algal H_2-production mutants under fermentative conditions. The assay incorporates an agar overlay of H_2-sensing *R. capsulatus* cells that fluoresce in response to H_2 produced by an agar underlay of *C. reinhardtii* colonies. The assay is sensitive enough to distinguish one H_2-producing colony from out of thousands of non H_2-producing colonies as present on a single Petri dish. We will focus next on adapting the assay for operation under photobiological H_2-production conditions and use it to screen for microbial libraries with increased H_2 photoproduction. In parallel work, we have transferred an exogenous hydrogenase from *Clostridium acetobutylicum,* along with its attendant assembly proteins, into the H_2-reporter bacterial strain. This bacterium produces hydrogen from the exogenous hydrogenases and emits a fluorescent signal in response to its H_2 production. We will focus our work on using this system to screen for O_2-tolerant [FeFe]-hydrogenases generated through directed-evolution techniques.

In green algal chloroplasts, ferredoxins (FDXs) play a central role in the allocation of low-potential electrons from photosynthesis to the hydrogenase enzyme, as well as to numerous competing assimilatory pathways. These pathways include CO_2 fixation, nitrite reduction, glutamate synthesis, sulfite reduction, cyclic electron transport around Photosystem I, and reduction of thioredoxin for regulation of biosynthetic pathways. Under certain physiological states, these processes have the potential to compete with the hydrogenases for electrons. Moreover, there are 6 potential FDXs that could interact with the hydrogenase enzymes (HYDA1 and HYDA2) and it is not clear yet which one is the favored natural electron donor to hydrogenases under different physiological conditions. To address the issue of competitive metabolic pathways with H_2 production, Task 2 has used a two-hybrid assay to screen *C. reinhardtii* expression libraries, with HYDA2 and HYDA1 as well as the 6 FDXs as baits, followed by pull-down assays to confirm *in vitro* the results obtained *in vivo*. We have also examined pair-wise

interactions between each hydrogenase and FDX isoform to identify specific protein-protein interactions. These approaches have resulted in the identification of novel protein-protein interactions that are leading to the development of a map of the network centered on ferredoxins and involving multiple metabolic pathways in Chlamydomonas.

Publications:

Wecker MSA, Meuser JE, Posewitz MC, Ghirardi ML. Design of a new biosensor for algal H_2 production based on the H_2-sensing system of *Rhodobacter capsulatus*. *International Journal of Hydrogen Energy* 2011, **36**(17):11229-11237.

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