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Now that the GTL grant “An integrative approach to energy, carbon, and redox metabolism in the cyanobacterium *Synechocystis* sp. PCC 6803” has come to an end, below is a summary of the main findings at Arizona State University that have been enabled by this grant. Note that this grant also had components at UCLA (proteomics; K. Faull, PI) and at the Fellowship for the Integration of Genomes (bioinformatics; R. Overbeek, PI; see <http://theseed.uchicago.edu/FIG/organisms.cgi?show=cyano>); as funding was provided directly to these institutions, I trust that they will provide you with final reports on their findings.

The broader goal of this project was to merge knowledge from genomic, metabolic, ultrastructural and other perspectives to understand how cyanobacteria live, adapt and are regulated. This understanding aids in metabolic engineering and synthetic biology efforts using this group of organisms that contribute greatly to global photosynthetic CO₂ fixation and that are closely related to the ancestors of chloroplasts. This project focused on photosynthesis and respiration in the cyanobacterium *Synechocystis* sp. PCC 6803, which is spontaneously transformable and has a known genome sequence. Modification of these fundamental processes in this organism can lead to improved carbon sequestration and hydrogen production, as well as to generation of high-quality biomass.

In our GTL-supported studies at Arizona State University we focus on cell structure and cell physiology in *Synechocystis*, with particular emphasis on thylakoid membrane formation and on metabolism related to photosynthesis and respiration. Results on (a) thylakoid membrane biogenesis, (b) fluxes through central carbon utilization pathways, and (c) distribution mechanisms between carbon storage compounds are presented in subsequent paragraphs. Together, these results help pave the way for metabolic engineering efforts that are likely to result in improved bioenergy production and carbon sequestration.

The internal thylakoid membrane system of *Synechocystis* comprises about 80% of the total membrane content of the cell, and contains membrane protein complexes involved in both photosynthesis and respiration. Thylakoid organization is rather sophisticated, with membranes occurring in multiple layers along the periphery of the cell while often connected to a rod-like structure, the thylakoid center (van de Meene et al., 2006). Thylakoid formation seems to be critically correlated with the presence of chlorophyll and not with the presence of photosynthetic complexes, as in a mutant where chlorophyll synthesis is under strict light control thylakoids are essentially absent after prolonged growth in virtual darkness, whereas thylakoid membranes form rapidly upon exposure to light (Hamad et al., 2006). In contrast, mutants lacking both photosystems retain a significant amount of thylakoids. The molecular mechanism of thylakoid formation remains largely unknown, but some proteins that may be involved with thylakoid generation have been identified. Upon overexpression of one of these proteins we found more and closer spaced thylakoids protein in *Synechocystis*, along with novel membrane structures that may be instructive in understanding membrane biogenesis. These results indicate that we now are able to generate cyanobacterial strains with increased levels of the photosynthetic apparatus and thylakoids (useful biomass) per cell (Hamad et al., 2006).

Metabolic engineering is aided by detailed insight into fluxes through main metabolic pathways. Thus far, flux data regarding central carbon utilization pathways are derived largely

from a rather indirect approach of isotope labeling and monitoring the isotopic composition of end products such as amino acids. However, cyanobacterial carbon utilization is very complex, with sugar utilization by the pentose phosphate pathway and glycolysis immediately connected with carbon fixation pathways through the Calvin-Benson-Bassham cycle. Most steps in the pathways are reversible, and several steps involve the reorganization of C-C bonds, causing rapid isotope scrambling when providing uniformly labeled ^{13}C -glucose. With improved LC/MS (liquid chromatography/mass spectrometry) techniques we have now been successful in monitoring the mass redistribution of several sugar phosphates as a function of time after adding labeled glucose. Comparing these results under different growth conditions and in specific mutants lacking particular steps in the pathways yields a detailed insight regarding *in vivo* rates of key metabolic reactions in carbon utilization (Wang et al., 2006). Moreover, we have started to develop software to model kinetic rates to fit experimental data (N. Tatonetti and W. Vermaas, unpublished).

Synechocystis sp. PCC 6803 has two main carbon storage compounds: glycogen and polyhydroxybutyrate (PHB). The compound to be accumulated has been found to primarily depend on the environmental conditions. Glycogen is found under many conditions, but under – for example- nitrate limitation PHB can make up 5-10% of the dry weight of the cell. We have explored the metabolic reasons for this apparent dichotomy in the preferred carbon storage compound in *Synechocystis*. Based on results of comparative PHB accumulation analysis as a function of environmental conditions in different mutant strains, the level of reduction of specific redox carriers in the cell is found to be a key determinant for PHB accumulation (Cai et al., 2006). With this knowledge PHB production in *Synechocystis* can now be optimized.

Cai Z, van de Meene AML, Vermaas WFJ (2006) Poly-3-hydroxybutyrate in the cyanobacterium *Synechocystis* sp. PCC 6803: Regulation of biosynthesis and physiological role in metabolism. submitted

Hamad S, Roberson RW, Vermaas WFJ (2006) Mechanisms of thylakoid membrane biogenesis in the cyanobacterium *Synechocystis* sp. PCC 6803: the role of chlorophyll. In preparation.

van de Meene AML, Hohmann-Marriott MF, Vermaas WFJ, Roberson RW (2006) The three-dimensional structure of the cyanobacterium *Synechocystis* sp. PCC 6803. *Arch Microbiol* 184: 259-270.

Wang B, Brune DC, Vermaas WFJ (2006) Dynamic analysis of metabolic fluxes through the central carbohydrate metabolism pathways of *Synechocystis* sp. PCC 6803. In preparation.

These aspects of the project build on an excellent foundation of genomic and functional data regarding *Synechocystis* sp. PCC 6803, and together provide a solid basis for metabolic engineering of this cyanobacterium to enhance solar-powered carbon sequestration and bioenergy conversion. Fueled by the very encouraging results obtained in this project, we already have attracted interest from major companies in the use of cyanobacteria for biofuel production.