ABSTRACT

Author: Yu King Hing, Nathaphon
Institution: Purdue University
Degree Received: May 2021
Title: A Multi-Omic Characterization of the Calvin-Benson-Bassham Cycle in Cyanobacteria
Major Professor: John A. Morgan

Cyanobacteria are photosynthetic organisms with the potential to sustainably produce carbon-based end products by fixing carbon dioxide from the atmosphere. Optimizing the growth or biochemical production in cyanobacteria is an ongoing challenge in metabolic engineering. Rational design of metabolic pathways requires a deep understanding of regulatory mechanisms. Hence, a deeper understanding of photosynthetic regulation of the influence of the environment on metabolic fluxes provides exciting possibilities for enhancing the photosynthetic Calvin-Benson-Bassham cycle. One approach to study metabolic processes is to use omic-level techniques, such as proteomics and fluxomics, to characterize varying phenotypes that result from different environmental conditions or different genetic perturbations.

This dissertation examines the influence of light intensity on enzymatic abundances and the resulting Calvin-Benson-Bassham cycle fluxes using a combined proteomic and fluxomic approach in the model cyanobacteria *Synechocystis* sp. PCC 6803. The correlation between light intensity and enzymatic abundances is evaluated to determine which reactions are more regulated by enzymatic abundance. Additionally, carbon enrichment data from isotopic labelling experiments strongly suggest metabolite channeling as a flexible and light-dependent regulatory mechanism present in cyanobacteria. We propose and substantiate biological mechanisms that explains the formation of metabolite channels under specific redox conditions.

The same multi-omic approach was used to examine genetically modified cyanobacteria. Specifically, genetically engineered and conditionally growth-enhanced *Synechocystis* strains

overexpressing the central Calvin-Benson-Bassham cycle enzymes FBP/SBPase or transketolase were evaluated. We examined the effect of the heterologous expression of each of these enzymes on the Calvin-Benson-Bassham cycle, as well as on adjacent central metabolic pathways. Using both proteomics and fluxomics, we demonstrate distinct increases in Calvin-Benson-Bassham cycle efficiency as a result of lowered oxidative pentose phosphate pathway activity. This work demonstrates the utility of a multi-omic approach in characterizing the differing phenotypes arising from environmental and genetic changes.