FINAL REPORT

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Summary

This was an extremely productive period in which we studied many aspects of gene regulation in the cyanobacterium *Synechocystis* sp. PCC 6803. The main experimental tools included the full genome microarray that we had constructed with Dr. Robert Burnap, carefully chosen physiological conditions and the ability to make selected knockout mutations in *Synechocystis*. We have concentrated on growth of cells during the diurnal cycle and especially at day-night and night-dark transitions. Our overall goal in the current proposal has been to understand the way in which the cell regulates metabolism as cells go through a typical day-night cycle. Our ultimate goal is to understand these processes and learn how to manipulate them so that we can better grow cyanobacteria under natural daylight cycles for biofuel production.

We have focused on the group 2 sigma factors and their different roles under light or dark conditions. We have shown that SigB plays a more prominent role in the dark, whereas SigD is more important in the light. However, the complete story is more complicated, and our results can best be explained using the σ cycle paradigm, as extended with the stochastic release model. In addition, SigB is at the center of a complex network that involves other group 2 sigma factors, some group 3 sigma factors and selected two-component regulatory histidine kinases. The transcript level of ribosomal proteins is a key target of gene regulation as cells transition between the light and dark. We also have found that mutants in both SigB and Hik8 have difficulty growing under short day, mixotrophic growth conditions; e.g., when the light is lowered to 8 h per day (or less), cells cannot grow in the presence of 5 mM glucose.

We have also been pursuing the importance of IsiA and its role in membrane assembly under a variety of different environmental conditions. During routine growth, we've determined that IsiA is induced as cells reach light limitation and approach stationary phase conditions. Our results, and those of other researchers, suggest that IsiA accumulation during the stationary phase may be linked to PSII protection. We have also extended our studies of MrgA, a protein first identified as induced by oxidative stress and a member of the PerR regulon. It is now clear that MrgA plays an important role in the transport of intracellular iron from storage in the bacterioferritins, to biosynthesis of metal cofactors during cell growth. We have also identified a connection between oxidative stress and the level of external exopolysaccharides produced and secreted. We have also investigated certain Photosystem II mutants that only grow under high pH conditions. Among other things, these experiments have provided new information as to the importance of Hik31 under a variety of environmental conditions.

Finally, we have shown that *Synechocystis* sp. PCC 6803 can grow under very low oxygen conditions. During the early stages of this growth, an operon that includes *psbA1* is upregulated—this is the first set of conditions in which *psbA1* is known to be transcribed. We also showed that the resulting D1' is added to PSII and is functional. There were many other genes either up or down regulated during low oxygen growth; in particular, genes for the ATP synthase and all the ribosomal proteins were down-regulated. However, in a *Δhik31* mutant, growth improved over the wild type level and these genes were no longer down regulated. These results indicated that cyanobacteria have the ability to respond to anaerobic conditions, to alter metabolism and to grow. These results will be extremely useful in further studies on biofuel production.

Project Publications

1. **Singh, A.K. and Sherman L.A.** 2005. Pleiotropic effect of a histidine kinase on carbohydrate metabolism in *Synechocystis* sp. Strain PCC 6803 and its requirement for heterotrophic growth. J. Bacteriol. **187**:2368-2376.

2. **Singh, A.K., Li, H., Bono, L. and Sherman, L.A.** 2005. Novel adaptive responses revealed by transcription profiling of a *Synechocystis* sp. PCC 6803 *ΔisiA* mutant in the presence and absence of hydrogen peroxide. Photosyn. Res. **84**:65-70.

3. **Singh, A. K., Li, H. and Sherman, L.A**. 2005. Global regulation of photosynthesis genes in response to oxidative stress in the cyanobacterium *Synechocystis* sp. PCC 6803. Photosynthesis: Fundamental Aspects to Global Perspectives (A. van der Est and D. Bruce, eds), Proceed of the 13th International Congress on Photosynthesis. pp. 691-693.

4. **Singh, A. K. and Sherman, L.A**. 2006. Iron-independent Dynamics of IsiA Production during the Transition to Stationary Phase in the Cyanobacterium *Synechocystis* sp. PCC 6803. FEMS Micro Let, 256: 159-164.

5. **Singh, A, K., Summerfield, T. C., Li, H. and Sherman, L.A.** 2006. The heat shock response in the cyanobacterium *Synechocystis* sp. Strain PCC 6803 and regulation of gene expression by HrcA and SigB. Arch. Microbiol. 186:273-286.

6. **Foster, J. S., A. K. Singh, L. J. Rothschild and Sherman L.A**. 2007. Growth-phase dependent differential gene expression in *Synechocystis* Strain sp. PCC 6803 and regulation by a group 2 sigma factor. Arch Microbiol. 187:265-279.

7. **Singh, A. K. and Sherman L. A.** 2007. Reflections on the function of IsiA, a stress-inducible cyanobacterial Chl-binding protein. Photsyn. Res. **93**:17-25.

8. **Summerfield, T. C. and L. A. Sherman**. 2007. The role of sigma factors in controlling global gene expression in light/dark transitions in the cyanobacterium *Synechocystis* sp. Strain PCC 6803. J. Bacteriol. **189**:7829-7840.

9. **Summerfield, T.C., Eaton-Rye, J.J. and Sherman, L.A**. 2007. Diffferential gene expression between a ΔPsbO:ΔPsbU mutant and a spontaneous revertant in the cyanobacterium *Synechocystis* sp. Strain PCC 6803. Photo Res. **94**:265-274.

10. **Summerfield, T.C. and Sherman, L.A**. 2008. Global transcriptional response of the alkalitolerant cyanobacterium *Synechocystis* sp. strain PCC 6803 to pH 10. Appl. Environ. Microbiol. **74**:5276-5284.

11. **Summerfield, T.C., Toepel, J. and Sherman, L.A**. 2008. Low oxygen induction of normally cryptic *psbA* genes in cyanobacteria. Biochemistry Rapid Reports. **74**:12939-12941.

12. **Foster, J.S., Havemann, S.A., Singh, A.K. and Sherman, L.A**. 2009. Role of MrgA in peroxide and light stress in the cyanobacterium *Synechocystis* sp. PCC 6803. FEMS Micro. Lett. **293**:298-304.

13. Shcolnick, S., Summerfield, T.C., Reytman, L., Sherman, L.A. and Keren, N. 2009. The mechanism of iron homeostasis in the unicellular cyanobacterium

Synechocystis sp. PCC 6803 and its relationship to oxidative stress. Plant Physiology **150**:2045-2056.

14. **Summerfield, T.C., Nagarajan, S. and Sherman, L.A**. 2011. Gene expression under low oxygen conditions and the role of the histidine kinase, Hik31 (Sll0790), in the cyanobacterium *Synechocystis* sp. PCC 6803. Microbiology, accepted for publication.