Final Technical Report

This information was submitted electronically to the DOE RIMS system on June 8, 2004

Agency project number: **ER63232-1018220-0007203**
Principal Investigator name: **Timothy J. Donohue**
Project title: **The Molecular Basis for Metabolic and Energetic Diversity**
UW fund-account number: **144-KL89**
Award period: **9/15/01-9/14/05**

Research Progress:

1. **We have used experimental and computational analysis of *R. sphaeroides* photosynthesis and other gene expression networks (Kaplan, Gomelsky, Donohue) (3-9, 12, 13, 15-18, 20).** We have identified many new candidate photosynthesis genes with expression patterns that varied as a function of light intensity. Results from these experiments suggest there are many more light-regulated aspects of the photosynthetic lifestyle of this bacterium than previously appreciated. Ongoing genetic analysis confirms that mutations in some of these newly-identified photosynthesis block the ability of cells to use solar energy in the laboratory.

We also carried out transcriptome and computational analysis of individual *R. sphaeroides* regulons. This identified additional genes that are directly regulated by individual transcription factors and refined the consensus sequence for master regulators of photosystem development. We also showed that PpsR indirectly regulates genes that do not contain the PpsR-binding sites, e.g. *puf* and *puhA* operons. This suggests that PpsR plays a more global role as a regulator of photosystem development than what was assumed before. A similar computational and microarray analysis of PrrA target genes has identified many new candidate promoters that are controlled by this master regulator of photosynthesis. We have begun bioinformatic, genetic and biochemical experiments aimed at elucidating the interactions of transcriptional pathways controlling photosystem development (PrrBA and AppA-PpsR).

We carried out computational analysis designed to cluster oxygen-dependent genes in *R. sphaeroides* based on the transcriptome data for cells grown between 30% and 0% oxygen. As a result, new statistical tools for clustering expression profiles from DNA microarrays have been developed.

2. **We have analyzed the assembly, bioenergetic role and regulatory functions of the aerobic respiratory chain (Donohue, Edwards, Hosler, Kaplan)(10, 11, 14){Hiser, 2000 #4612}.** We have used computational, genetic and biochemical approaches to map the flow of electrons through the major bioenergetic pathways of this bacterium. From this, we have formulated and tested predictions for the major and minor routes of electron transport to the 5 predicted terminal oxidases; these are now being tested by genetic and physiological experiments.

We have demonstrated a direct role for the *R. sphaeroides* cyt cbb_3 terminal oxidase of the aerobic respiratory chain in controlling the activity of a master energy homeostasis pathway, PrrBA. This presents an opportunity for analyzing the ability of a cytochrome oxidase to act as a direct modulator of a second bioenergetic machine like the photosynthetic apparatus.

Given the important bioenergetic and regulatory role of the aerobic respiratory chain, we have analyzed assembly of this machine. One of these proteins, Cox11p is a copper protein that plays a critical, yet poorly-understood, role cytochrome oxidase assembly in many bacteria and organelles. We have (for the first time) succeeded in expressing the soluble domain of Cox11p as a fusion protein with thioredoxin in *E. coli* and shown that the protein binds copper. This and a series of mutant Cox11p proteins are being used to analyze the role of copper in this protein and determine the role of Cox11p homologs in assembly of Cu-dependent bioenergetic machines.

3. **In collaboration with GTL researchers at Pacific Northwest National Laboratory, we analyzed and localized the *R. sphaeroides* proteome (Kaplan, Donohue, Smith)(1, 2, 19).** We have determined the entire proteome of cells grown under different energy states and localizes what fraction of the proteome is contained in the major subcellular fractions of the cell, including the photosynthetic apparatus. This has identified many new and unknown proteins within this specialized solar energy harvesting machine that we would like to analyze with future DOE support.

GTL Papers

1. **Callister, S. J., M. A. Dominguez, C. D. Nicora, X. Zeng, C. L. Tavano, S. Kaplan, T. J. Donohue, R. D. Smith, and M. S. Lipton.** 2006. Application of the accurate mass and time tag approach to the proteome analysis of sub-cellular fractions obtained from *Rhodobacter sphaeroides* 2.4.1. aerobic and photosynthetic cell cultures. J. Proteome Res. **5:**1940-1947.
2. **Callister, S. J., C. D. Nicora, X. Zeng, J. H. Roh, M. A. Dominguez, C. L. Tavano, M. E. Monroe, S. Kaplan, T. J. Donohue, R. D. Smith, and M. S. Lipton.** 2006. Comparison of aerobic and photosynthetic *Rhodobacter sphaeroides* 2.4.1 proteomes. Journal of Microbiological Methods **67:**424-436.
3. **Choudhary, M., Y. X. Fu, C. Mackenzie, and S. Kaplan.** 2004. DNA sequence duplication in *Rhodobacter sphaeroides* 2.4.1: Evidence of the ancient and essential partnership between chromosomes I and II. J. Bacteriol. **186:**2019-2027.
4. **Gomelsky, M., and G. Klug.** 2002. BLUF: a novel FAD-binding domain involved in sensory transduction in microorganisms. Trends Biochem Sci **27:**497-500.
5. **Kapil G. Gadkar, F. J. D. I. I. I. J. S. E. R. M.** 2005. Estimating optimal profiles of genetic alterations using constraint-based models. Biotechnology and Bioengineering **89:**243-251.
6. **Kauffman, K. J., B. A. Ogunnaike, and J. S. Edwards.** 2006. Designing experiments that aid in the identification of regulatory networks. Brief Funct Genomic Proteomic **4:**331-342.
7. **MacKenzie, C., M. Choudhary, F. W. Larimer, P. F. Predki, S. Stilwagen, J. P. Armitage, R. D. Barber, T. J. Donohue, J. P. Hosler, J. Newman, J. P. Shapliegh, R. E. Sockett, J. Zeilstra-Ryalls, and S. Kaplan.** 2001. The home stretch, a preliminary analysis of the nearly completed genome of *Rhodobacter sphaeroides* 2.4.1. Photosynthesis Research **70:**19-41.
8. **Mahadevan, R., J. S. Edwards, and F. J. Doyle, III.** 2002. Dynamic Flux Balance Analysis of Diauxic Growth in Escherichia coli. Biophys. J. **83:**1331-1340.
9. **Moskvin, O. V., L. Gomelsky, and M. Gomelsky.** 2005. Transcriptome Analysis of the Rhodobacter sphaeroides PpsR Regulon: PpsR as a Master Regulator of Photosystem Development. J. Bacteriol. **187:**2148-2156.
10. **Oh, J., II, I.-J. Ko, and S. Kaplan.** 2003. Digging deeper: uncovering genetic loci which modulate photosynthesis gene expression in Rhodobacter sphaeroides 2.4.1. Microbiology **149:**949-960.
11. **Oh, J. I., I. J. Ko, and S. Kaplan.** 2004. Reconstitution of the *Rhodobacter sphaeroides* cbb_3-PrrBA Signal Transduction Pathway *in vitro*. Biochemistry **43:**7915-7923.
12. **Pappas, C. T., J. Sram, O. V. Moskvin, P. S. Ivanov, R. C. Mackenzie, M. Choudhary, M. L. Land, F. W. Larimer, S. Kaplan, and M. Gomelsky.** 2004. Construction and validation of the *Rhodobacter sphaeroides* 2.4.1 DNA microarray: Transcriptome flexibility at diverse growth modes. J. Bacteriol. **186:**4748-4758.
13. **Roh, J. H., W. E. Smith, and S. Kaplan.** 2004. Effects of oxygen and light intensity on transcriptome expression in *Rhodobacter sphaeroides* 2.4.1: redox active gene expression profile. J. Biol. Chem. **279:**9146-9155.

14. **Smith, D., J. Gray, L. Mitchell, W. E. Antholine, and J. P. Hosler.** 2005. Assembly of Cytochrome-c Oxidase in the Absence of Assembly Protein Surf1p Leads to Loss of the Active Site Heme. J. Biol. Chem. **280:**17652-17656.
15. **Tavano, C. L., J. C. Comolli, and T. J. Donohue.** 2004. The role of *dor* gene products in controlling the P2 promoter of the *Rhodobacter sphaeroides* cytochrome c_2 gene, *cycA*. Microbiology **150:**1893-1899.
16. **Tavano, C. L., A. Podevels, and T. J. Donohue.** 2005. Gene products required to recycle reducing power produced under photosynthetic conditions. J. Bacteriol. **187:**5249-5258.
17. **Venugopal Mikkilineni, R. D. M. J. M. J. R. D. G. M. C. B. O. J. S. E.** 2004. Digital quantitative measurements of gene expression. Biotechnology and Bioengineering **86:**117-124.
18. **Zeng, X., M. Choudhary, and S. Kaplan.** 2003. A second and unusual *pucBA* operon of *Rhodobacter sphaeroides* 2.4.1: genetics and function of the encoded polypeptides. J. Bacteriol. **185:**6171-6184.
19. **Zeng, X., J. H. Roh, S. J. Callister, C. L. Tavano, T. J. Donohue, M. L. Lipton, and S. Kaplan.** Proteomic characterization of the *Rhodobacter sphaeroides* photosynthetic apparatus. Submitted.
20. **Zhou, S., E. Kvikstad, A. Kile, J. Severin, D. Forrest, R. Runnheim, C. Churas, T. S. Anantharaman, J. W. Hickman, C. Mackenzie, T. J. Donohue, S. Kaplan, and D. C. Schwartz.** 2003. Whole-genome shotgun optical mapping of *Rhodobacter sphaeroides* strain 2.4.1. Genome Research **13:**2142-2151.