Final Report

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"The Evolution of Photosynthesis and the Transition from an Anaerobic to an Aerobic World"

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Introduction

This project was focused on elucidating the evolution of photosynthesis, in particular the evolutionary developments that preceded and accompanied the transition from anoxygenic to oxygenic photosynthesis. Development of this process has clearly been of central importance to evolution of life on Earth. Photosynthesis is the mechanism that ultimately provides for the energy needs of most surface-dwelling organisms. Eukaryotic organisms are absolutely dependent on the molecular oxygen that has been produced by oxygenic photosynthesis. In this project we have employed a multidisciplinary approach to understand some of the processes that took place during the evolution of photosynthesis.

In this project, we made excellent progress in the overall area of understanding the origin and evolution of photosynthesis. Particular progress has been made on several more specific research questions, including the molecular evolutionary analysis of photosynthetic components and biosynthetic pathways (2, 3, 5, 7, 10), as well as biochemical characterization of electron transfer proteins related to photosynthesis and active oxygen protection (4, 6, 9). Finally, several review and commentary papers have been published (1, 8, 11).

A total of twelve publications arose out of this grant, references to which are given below. Some specific areas of progress are highlighted and discussed in more detail.

The Role of Horizontal Gene Transfer in the Evolution of Photosynthesis

Understanding the evolutionary development of photosynthesis has long been a subject of great interest (11). Previous phylogenetic analyses of photosynthetic bacteria have necessarily used a limited subset of genes to infer relationships among these organisms, often resulting in incongruent results. It is now clear that horizontal gene transfer (HGT) has been a major force in the evolution of photosynthesis. The evolutionary trajectory of different parts of the photosynthetic apparatus has been different, so that no single evolutionary scheme can hope to describe what has been a very complex and nonlinear process.

Whole-genome data have allowed us to make an extensive comparison of representatives of each of the five known groups of photosynthetic bacteria and may help to reconcile multiple lines of disparate phylogenetic evidence centered on them (2, 5, 7). In agreement with other recent whole-genome analyses, HGT appears to be an integral aspect of prokaryote evolution, and genetic components of the photosynthetic apparatus have crossed species lines nonvertically. The robustness of the trees that we interpreted as evidence for HGT has recently been confirmed using additional sequences from other taxa. This argues strongly against reconstruction artifacts due to long-branch attraction of sparsely sampled taxa.

Origin of the Oxygen Evolving Complex and the Transition to Oxygenic Photosynthesis

Perhaps the most striking difference between the more primitive anoxygenic bacterial photosynthetic reaction centers and photosystem II of oxygenic organisms is the dramatically increased complexity of the photosystem II reaction center, exemplified by the much larger number of protein subunits in photosystem II. There are more than 25 distinct subunits in photosystem II compared to three or four in the bacterial reaction center. These include several extrinsic subunits that comprise or closely interact with the oxygen evolving complex (OEC), as well as a suite of low molecular weight single transmembrane peptides peripheral to the core reaction center. In many cases, the functions of these subunits have not been established, and for the most part their evolutionary origin is a complete mystery. None of these proteins have unambiguous homologs outside of the cyanobacterial domain, though interestingly a small number are distantly similar to one another (10).

The intriguing conclusion from this work is that the oxygenic photosystem evolved within a remarkably narrow window of geologic time and, moreover, has remained largely unchanged with respect to protein subunit composition for perhaps 2 billion years. As yet to be clarified and central to our understanding of the early evolution of oxygenic photosynthesis is whether the modern complexity of the photosystem II reaction center – especially the extrinsic and peripheral subunits – preceded and thereby possibly facilitated the water oxidation reaction or, conversely, developed in conjunction with or subsequent to water oxidation, where the function might have been within the context of protection, regulation, or repair.

Evolution of Biosynthetic Pathways

There is a deep relationship between the two central metabolic pathways of photosynthesis and nitrogen fixation. This connection is clearly manifest at the level of the genes that code for the structural proteins for nitrogenase as well as some of the enzymes that are important in chlorophyll biosynthesis (3, 12). We have found that there is another major class of enzymes that are related to both these classes, but have not previously been reported and appear to be basal in the tree and therefore may represent a more primitive biochemistry that predates the appearance both photosynthesis and nitrogen fixation (12).

Publications from this Project

1. Blankenship R. E. (2001) Molecular evidence for the evolution of photosynthesis, *Trends in Plant Science*, **6**: 4-6.

2. Blankenship, R. E., Raymond, J., Lince, M., Larkum, A. W. D., Jermiin, L. S., Lockhart, P. J., Zhaxybayeva, O. and Gogarten J. P. (2001) Evolution of photosynthetic antennas and reaction centers. *PS 2001 Proceedings: 12th International Congress of Photosynthesis*, CSIRO Publishing, Collingwood, Victoria, Australia. (published only electronically).

3. Jermiin, L. S., Blankenship, R. E., Lockhart, P. J. and Larkum A. W. D. (2001) Phylogenetic reconstruction of ancient photosynthetic lineages using chlorophyll and bacteriochlorophyll biosynthetic genes. *PS 2001 Proceedings: 12th International Congress of Photosynthesis*, CSIRO Publishing, Collingwood, Victoria, Australia. (published only electronically).

4. Bond, C., Blankenship R. E., Freeman, H., Guss, J. M., Maher, M., Selvaraj, F., Wilce, M. and Willingham, K. (2001) Crystal structure of auracyanin, a 'blue' copper protein from the green thermophilic photosynthetic bacterium *Chloroflexus aurantiacus*, *J. Molec. Biol.*, **306**: 47-67.

5. Raymond, J. R., Zhaxybayeva, O., Gerdes, S., Gogarten, J. P. and Blankenship R.E. (2002) Whole genome analysis of photosynthetic prokaryotes, *Science*, **298**: 1616-1620.

6. Rooney, M. D., Honeychurch, M. J., Selvaraj, F. M., Blankenship, R. E., Bond, A. M. and Freeman, H. C. (2003) A thin-film electrochemical study of 'blue' copper proteins, auracyanin A and auracyanin B, from the photosynthetic bacterium *Chloroflexus aurantiacus*: The reduction potential as a function of pH, *J. Biolog. Inorg. Chem*, **8**: 306-317.

7. Raymond, J., Zhaxybayeva, O., Gogarten, J. P. and Blankenship R.E. (2003) Evolution of photosynthetic prokaryotes: a maximum liklihood mapping approach. *Phil. Trans of the Royal Soc. B,* **358**: 223-230.

8. Raymond, J. and Blankenship, R. E. (2003) Horizontal gene transfer in eukaryotic algal evolution. *Proc. Nat'l. Acad. Sci. USA* **100**: 7419-7420.

9. Lancaster V. R., LoBrutto, R., Selvaraj, F. M. and Blankenship, R. E. (2004) Cambialistic superoxide dismutase in the thermophilic photosynthetic bacterium *Chloroflexus aurantiacus*. *J. Bacteriol.* **186**: 3408-3414.

10. Raymond, J. R. and Blankenship, R.E. (2004) The evolutionary development of the protein complement of photosystem 2, *Biochim. Biophys. Acta*, **1655:** 133-139.

11. Olson, J. M. and Blankenship, R. E. (2004) Thinking about the evolution of photosynthesis. *Photosynth. Res.* **80**: 373-386.

12. Raymond, J., Siefert, J., Staples, C. and Blankenship, R. E. (2004) The natural history of nitrogen fixation, *Molecular Biology and Evolution*, **21**: 541-554.