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**IS PHOTOSYNTHESIS REALLY DERIVED FROM PURPLE BACTERIA?**

The ability to convert light energy into chemical energy must have arisen very early in the history of life. Three and a half billion years later, our problem is to reconstruct the subsequent evolution of photosynthesis by examining the five extant groups of photosynthetic prokaryotes, none of which is closely related to any of the others on the basis of the rRNA sequences or physiological/morphological characteristics. Recent progress in x-ray and electron crystallography has shown that the three-dimensional structures of both photosystem I and photosystem II of cyanobacteria and the single photosystem of purple bacteria have the same molecular design (Schubert et al. 1998), even though protein sequence similarity is fragmentary and not statistically significant. The fact that any molecular resemblances at all can be detected over this time scale is a testament to the conservative nature of cellular evolution. This structural conservation tells us that all photosynthetic reaction centers probably had a common ancestor; however, it does not mean that all photosynthetic prokaryotes had a common ancestor.

In a recent paper in *Science*, Xiong et al. (2000) have put forward the hypothesis that purple bacteria were the earliest branching photosynthetic lineage and challenged the long favored hypothesis that chlorophyll biosynthesis preceded bacteriochlorophyll biosynthesis (Granick 1965). They obtained sequences for a number of genes involved in photosynthesis from representatives of the two groups of green bacteria, the green filamentous bacterium *Chloroflexus* and the green sulfur bacterium *Chlorobium*, and compared them with previously obtained sequences from *Heliothrix* (Xiong et al. 1998), representative of a third group, and with the corresponding purple bacterial and cyanobacterial sequences. Phylogenetic trees for seven enzymes of chlorophyll/bacteriochlorophyll synthase were constructed using the three standard methods (neighboring distance, maximum parsimony, and maximum
The results were somewhat surprising. In most of the trees, *Chlorobium* and *Chloroflexus* genes were clustered, and the gram-positive obligate anaerobe *Heliobacillus* was sister group to the gram-negative oxygenic cyanobacteria. This flies in the face of all we know about the relationships of these four groups of prokaryotes (Stackebrandt et al. 1996, Madigan et al. 1996). Even more surprising, the majority of the trees showed the purple proteobacterial genes as the earliest branch, even though the proteobacteria are generally considered the most advanced of the Eubacteria (Madigan et al. 1996).

This branching pattern needs to be viewed with some caution. When a molecular sequence branches off at the base of a tree, it means that this sequence is the most divergent of the group of sequences, that is, it has accumulated more changes compared to the other sequences with which it shared a common ancestor. This can be the result of two things: it has had more time to accumulate changes since it branched off from the last common ancestor, OR it has a higher rate of evolution (i.e. of fixation of mutational changes).

The mathematical methods for minimizing the effects of different rates cannot completely remove this problem, especially over large evolutionary distances (e.g. Zhang et al. 2000). A lucid explanation of this “long-branch problem” and its effect on deep-branching trees is given by Philippe and Laurent (1998). The deep purple bacterial branch may simply mean that the pigment biosynthesis genes are evolving faster in the proteobacterial line than in the other lines.

Another problem is that only a very limited number of genes were available for comparison in all five photosynthetic groups. Although the title of the Xiong et al. paper (2000) is rather sweeping, only genes for bacteriochlorophyll/chlorophyll synthesis enzymes were analyzed. In fact, three of the genes encode subunits of the Mg chelatase (*bch*HD1/*chl*HD1), three encode subunits of the light-independent protochlorophyllide reductase (*bchBLN/ch*BLN), and one encodes the enzyme that adds the phytol tail (*bchG/ch*G), so only three independent enzymes were actually studied. The authors concluded that because purple bacteria gave rise to the earliest branch, bacteriochlorophyll synthesis must have come first. This idea was first put forward by Burke et al. (1993), but the conclusions were challenged by a reanalysis of the data (Lockhart et al. 1996). Both molecules are synthesized from chlorophyllide *a*, but bacteriochlorophyll synthesis requires three additional steps, one of which is catalyzed by the products of three genes (*bchXYZ*) in purple bacteria (Fig. 1). These three genes appear to have arisen by duplication of the three genes for protochlorophyllide reductase (Burke et al. 1993). At some point, therefore, there must have been a prokaryotic ancestor that only went as far as chlorophyllide *a*. Unfortunately, *bchXYZ* have not yet been found in the two green bacterial groups. (They would not be expected in the heliobacteria, because Bchl g is actually an isomer of chlorophyllide *a*.)

Considerable caution needs to be exercised in jumping from the phylogenetic tree of a gene family to the family tree of its owners. One of the strongest messages coming from the explosion of bacterial genome projects is that lateral gene transfer between distantly-related prokaryotes has been rampant, is ongoing, and, in some cases, involves very large blocks of DNA (Doolittle 1999, Ochman et al. 2000). It may be significant that all the genes required for synthesis of bacteriochlorophylls, carotenoids, reaction center proteins, and antenna proteins are found in single large clusters in two of these prokaryotic groups, the heliobacteria and the purple bacteria (Xiong et al. 2000). The surest way to acquire photosynthesis would be to appropriate a complete photosynthetic package from a neighbor’s genome. Whether modern gene clusters identify the owners as recipients or donors is not yet clear. Xiong et al. (2000) considered the possibility that genes were horizontally transferred from purple bacteria to the other groups but not in other directions.

We should like to propose that lateral gene transfer may have been much more multidirectional. The green filamentous *Chloroflexus* shares a unique extrinsic antenna structure (the chlorosome) with the green sulfur *Chlorobium* as well as having the greatest similarity in bacteriochlorophyll synthesis enzymes, whereas its reaction center and antenna polypeptides are closely related to those of purple bacteria and not at all like those of *Chlorobium*. *Chloroflexus* is not related to either of these groups on the basis of rRNA trees or cell wall structure, and most of its relatives are nonphotosynthetic (Stackebrandt et al. 1996). Both *Heliobacillus* and the green sulfur *Chlorobium* have what appear to be “primitive” photosystem I–like reaction centers where the chromophores are protected by a homodimer of a single protein, rather than a heterodimer of two subunits related by gene duplication (Blankenship 1994). Did one of them acquire its reaction center genes from the other?

Modern microbial mat communities provide ample opportunity for gene swapping, with *Chloroflexus*, *Chlorobium*, and purple bacteria in close proximity, often un-
derlying a layer of cyanobacteria (Pierson 1994). Microbial mats are believed to have been very common in the Precambrian era and to have given rise to sedimentary structures known as stromatolites, in which many prokaryotic microfossils have been found (Schopf and Packer 1987). The primary question of phylogenetics is to guess who came first, that is, whose ancestors were around 3.5 billion years ago when photosynthesis presumably first existed? Some authors have considered the possibility that cyanobacteria came first because many of the early microfossils resemble modern cyanobacteria in size and segmentation of wall outlines (Schopf and Packer 1987). This would imply that the other four groups acquired their photosynthesis genes piecemeal from cyanobacteria. Pierson (1994), however, has argued persuasively that Chloroflexus-like cells could equally well have given rise to similar structures.

A final point. Any hypothesis on the origin of chlorophyll biosynthesis is obligated to consider carotenoid biosynthesis for any meaningfully predictive scheme because carotenoids are required for functional photosynthetic proteins. Isoprenoid biosynthesis supplies the required precursors (isopentenyl pyrophosphate and dimethylallyl pyrophosphate) by two pathways, the 1-deoxyxylulose-5-phosphate (DOXP) and mevalonate (MVA) pathways. The apparently more “primitive” DOXP pathway occurs in cyanobacteria and is common in many bacteria. There is considerable evidence, however, for lateral gene transfer as well as selection of genes from both pathways (Boucher and Doolittle 2000).

In spite of our reservations about their conclusions, Carl Bauer and his colleagues are to be commended for undertaking the first comprehensive gene sequencing of two major prokaryotic groups (Xiong et al. 1998, 2000). We look forward to seeing more sequences from Chloroflexus and Heliobacillus and anticipate with great interest the publication of the completed Chlorobium genome (http://www.tigr.org/). Once a wider range of sequence information is available, including more information on the phylogenetic relationships of the isoprenoid pathways, it should be possible to get a clearer idea of the ancestry of photosynthetic genes.

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