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Evolution of photosynthetic antennas and reaction centers

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Introduction

The origin and early evolution of photosynthesis took place at least 3500 million years ago and perhaps substantially earlier. Stromatolites and microfossils from Western Australia that derive from photosynthetic organisms are the oldest direct evidence for life on Earth. Modern photosynthetic organisms are found only in the bacteria and their descendents such as chloroplasts. This suggests that photosynthesis, while unquestionably an ancient process, arose after the separation of the three domains of life and that the last common ancestor of all extant life was not photosynthetic.

All reaction centers appear to have a common evolutionary origin and architecture. In contrast, antennas have arisen multiple times and employ a remarkable range of pigments and organizational principles.

Current evidence from a variety of sources suggests that the metabolic process of photosynthesis is a mosaic made up of numerous parts assembled from a variety of sources. Therefore, there is no single linear branching pathway that describes the evolutionary development of photosynthesis. Instead, numerous parts that mostly originated for other functions were subsequently incorporated as elements of the photosynthetic apparatus.

The chlorophyll biosynthetic pathway includes some steps borrowed from heme biosynthesis and other steps in which genes have been recruited from other pathways.

The earliest photosynthetic organisms were anoxygenic. Later forms developed the ability to oxidize water and produce molecular oxygen. This transition from anoxygenic to oxygenic photosynthesis is of great importance, as it permitted the development of advanced life forms, yet it is still very poorly understood.

Results and Discussion

Geological and Biological Context

The Earth is 4.55 billion years old. During the first 500 million years after formation, it was subjected to very heavy bombardment from asteroids and comets, which probably killed any early life. Despite these inhospitable conditions, life did begin and persist on Earth. By 3.8 billion years ago there is evidence for autotrophic carbon fixation (Mojzsis et al., 1996). The early atmosphere was probably almost completely devoid of O_2 (Kasting, 1993), although small amounts of O_2 could be formed by photolysis of water or CO_2 and may have provided the selection pressure for development of enzymes that detoxify reactive oxygen species and primitive oxidase systems.

The earliest life forms were probably not photosynthetic. The overall structure of the tree of life as determined by 16S rRNA shown in Fig. 1 argues against the idea that the earliest life forms were photosynthetic. All cells that are capable of chlorophyll-based photosynthesis are found in the bacterial domain, with the exception of eukaryotic phototrophs, which were unquestionably formed by the lateral transfer event of endosymbiosis. Based on this fact, it is reasonable to propose that photosynthesis arose somewhere in the radiation of bacterial species, and that the last common ancestor was not a photosynthetic cell.

The oldest evidence for photosynthetic life consists of 3.5 billion year old microfossils and stromatolites from Western Australia (Schopf, 1993). These are almost certainly the remains of photosynthetic organisms, and have been widely interpreted to be similar to modern day cyanobacteria. However, no definitive evidence is available to indicate whether these organisms evolved oxygen. The oxygen content of the atmosphere gradually increased beginning at about 2 billion years ago (Fig. 2). This increase results from production of O_2 by oxygenic photosynthetic organisms. This represents the latest time for the invention of oxygen evolution. It may have been invented significantly earlier, but the oxygen did not initially build up in the atmosphere until pools of reduced material were oxidized and the reduced carbon produced was buried and therefore sequestered on a geologic timescale (des Marais, 2000). Biomarker evidence indicates that cyanobacteria were present by 2.5 billion years ago (Summons et al. 1999).

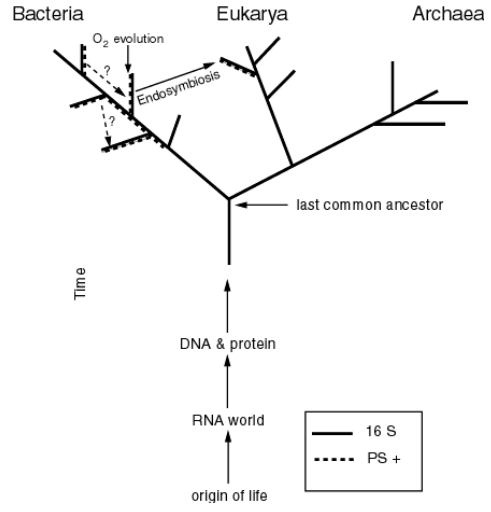


Fig. 1. Tree of life by 16S rRNA showing that photosynthesis is limited to the bacterial domain.

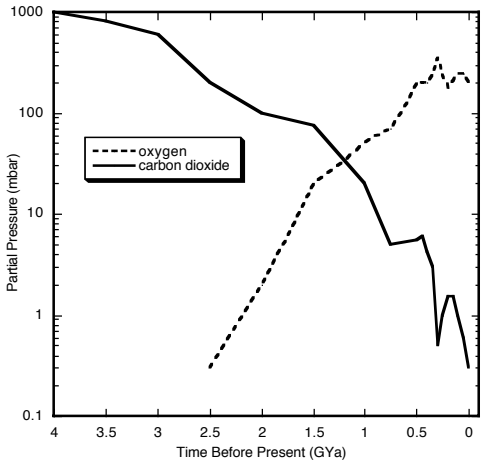


Fig. 2 History of oxygen and carbon dioxide in the atmosphere. Adapted from Blankenship (2002).

Origin and evolution of reaction centers

Photosynthetic reaction centers can easily be grouped into two distinct classes, those that have pheophytin and quinones as early acceptors (type 2) and those that have Fe-S centers as early acceptors (type 1). These two classes of reaction centers include the purple bacteria, green nonsulfur bacteria and photosystem 2 in the type 2, and the heliobacteria, green sulfur bacteria and photosystem 1 in the type 1 (Fig. 3). There is minimal sequence identity between the two main classes of reaction centers, so that from sequence analysis alone it is difficult to tell if they have a common ancestor or represent two independent inventions of reaction centers. Recent structural studies of various reaction centers have strongly suggested that all reaction centers have a common structural core and that the two classes almost certainly have descended from a distant common ancestor (Schubert et al. 1998).

All reaction centers have a dimeric protein core. In most systems, the dimer is a heterodimer, made up of two similar but distinct proteins. This is true for the purple and green nonsulfur bacteria, (L and M proteins), photosystem 2 (D1 and D2 proteins) and photosystem 1 (PsaA and PsaB proteins). These heterodimers are almost certainly derived from a duplication of a single ancestral gene that coded for a homodimeric complex, followed by divergence. However, two classes of organisms, the heliobacteria and the green sulfur bacteria, have retained the homodimeric complex. A scenario for the gradual conversion of a primitive monomeric reaction center to a homodimer and finally to a heterodimer is shown in Fig. 4 (Blankenship 1992; 2002). Whether or not the monomeric reaction center existed or was functional as a photosynthetic complex and what might be the evolutionary precursor of the reaction center is not known.

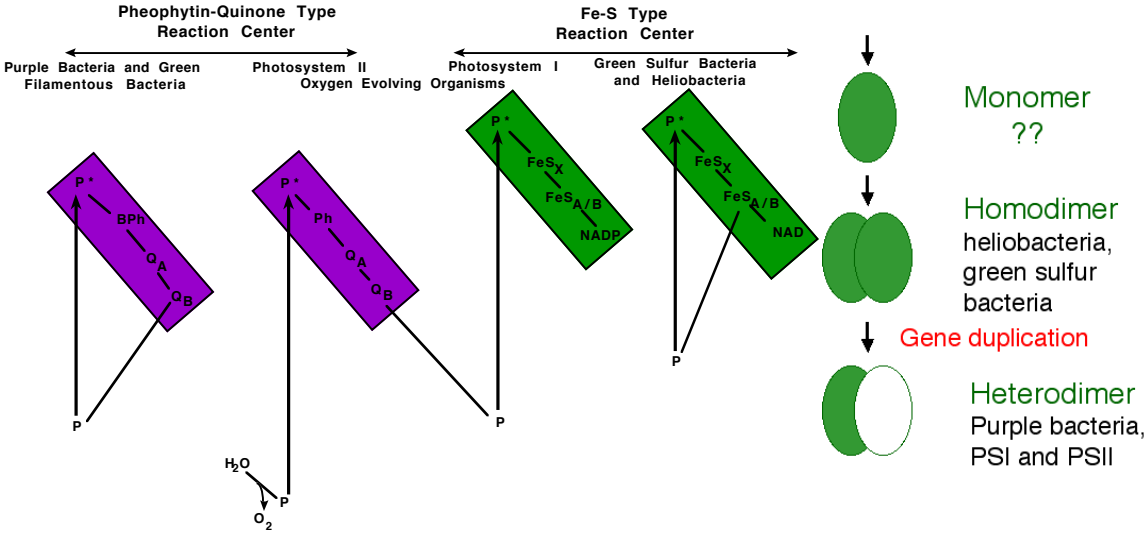


Fig. 3. Electron transport diagrams for photosynthetic reaction centers, showing the pheophytin-quinone and Fe-S types or reaction centers. Adapted from Blankenship, (1992)

Fig. 4 Evolutionary scenario for the development of heterodimer reaction centers, from a homodimer ancestor and possibly a monomeric ancestor.

Origin and evolution of antennas

Antenna complexes are remarkably varied in both pigment composition and overall structural arrangement. These different designs include the bacterial LH1 and LH2 type of integral membrane antenna complex, found in the purple bacteria and the green nonsulfur bacteria, the chlorosome peripheral antenna complex, found in the green sulfur and green nonsulfur bacteria, the phycobilisome complex, found in cyanobacteria and red algae, and the LHCI

and LHCII type of integral membrane antennas found in eukaryotic oxygenic photosynthetic organisms. Each of these complexes (as well as some other types not discussed here) has unique structural features and in many cases unique pigments. Within each major class, the evolutionary development can often be traced. However, the major classes of antennas appear to be independent evolutionary innovations. This suggests that antennas were invented several times during the evolution of photosynthesis, and this in turn suggests that the earliest forms perhaps did not have antennas and that they have developed in response to specific environmental selection pressures. For example, the chlorosome antenna system is optimized to collect light in very dim environments. It therefore incorporates a large amount of pigment with relatively little protein, making it biosynthetically cheap and therefore well adapted to an environment with low energy flux.

Pigment biosynthesis enzymes

One of the challenges to obtaining an overall picture of the evolution of photosynthesis that spans the entire range of photosynthetic life is that many if not most of the various subsystems that make up the photosynthetic apparatus are not homologous in all classes of organisms. This is especially clear for the antenna systems discussed above, but is also true for reaction centers, electron transport components and even carbon fixation enzymes. Within each of these groups, evolutionary relatedness can be traced, but a wider scale picture is difficult to obtain. Even among systems that may be homologous, such as various types of reaction centers, the very different functional roles of different complexes may seriously confuse evolutionary analysis. However, there is one group of genes that promises to provide a set of clearly homologous sequences that code for proteins that are carrying out the same chemistry in all cases. These sequences are the genes for the (bacterio)chlorophyll (and carotenoid) biosynthesis enzymes, at least for the majority of the pathway where all related pigments have essentially the same biosynthetic steps (Beale, 1999).

We have carried out analysis of the Light Independent Protochlorophyllide OxidoReductase (LI-POR) enzyme that carries out the reduction of the C-17-C-18 double bond (Fig. 5). This is the step that converts the porphyrin precursor to a chlorin. This change makes the pigment more asymmetric, thereby increasing the oscillator strength and shifting the main absorption band into the red region of the spectrum. The result is that the pigments are much more suited to light absorption for photosynthetic energy storage. Previous work has clearly established the homology of this reductase, which is coded for by the *bchL, bchN* and *bchB* genes in anoxygenic organisms and the *chlL, chlN* and *chlB* genes in oxygenic organisms (Burke et al. 1993; Xiong et al. 2000). In addition, these genes are related to genes that code for the reduction of the C-7-C-8 double bond in bacteriochlorophyll *a* containing organisms, the *bchX, bchY* and *bchZ* genes, and exhibit a distant but unambiguous similarity to the nitrogenase enzyme that reduces dinitrogen, coded for by the *nifH, nifD* and *nifK* genes.

The results of the analysis of the *bchL* gene and its homologs *chlL* and *nifH* are shown in Fig. 6. This analysis was carried out using an exhaustive maximum likelihood analysis using PROTML (Adachi and Hasegawa, 1996) followed by model averaging across “good trees” (Jermiin et al. 1997). The results support the relationships described above and strongly suggest that the ancestral gene for a reductase duplicated and diverged at least twice, to create the nif-type dinitrogen reductases and the porphyrin reductases. The porphyrin reductases then duplicated a second time to produce the LNB and XYZ complexes, each of which is more specialized to reduce one of the two double bonds in the porphyrin or chlorin macrocycle. This strongly suggests that the intermediate form of the reductase was nonspecific in its ability to reduce both double bonds and probably was able to produce bacteriochlorophyll-type pigments with two successive turnovers.

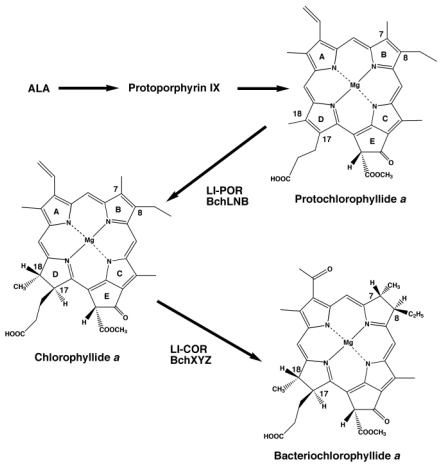


Fig. 5. Later steps in the chlorophyll and bacteriochlorophyll biosynthetic pathway, showing the reductions of the C-17-C-18 and C-7-C-8 double bonds by the LI-POR and LI-COR enzymes.

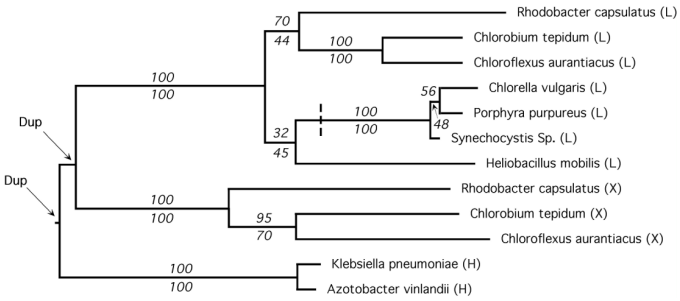


Fig. 6. Maximum likelihood analysis of the BchL, BchX, ChlL and NifH protein sequences. Two gene duplications are indicated. The dashed line indicates the advent of oxygenic photosynthesis.

Whole genome analyses

We have carried out preliminary whole genome analyses of genomes from four representative photosynthetic prokaryotes. The organisms chosen were a cyanobacterium, *Synechocystis* PCC 6803, a purple bacterium, *Rhodobacter capsulatus*, a green sulfur bacterium, *Chlorobium tepidum* and a green nonsulfur bacterium, *Chloroflexus aurantiacus*. According to the traditional analysis using 16S rRNA, these are very widely dispersed groups among the bacteria. The analyses were carried out by using identified open reading frames (ORFs) or running an ORF finding routine on each genome (Walker and Koonin, 1997) and then blasting the ORFs into each of the other three genomes. Only those genes that found each other as the most similar in all four genomes were considered further. Maximum likelihood analysis was carried out on all three unrooted four taxon trees for each gene. These genes were then mapped onto a triangular barycentric coordinate system so that genes that strongly support a particular topology map to one corner, while genes that do not distinguish the topologies map to the center of the triangle. Genes that are not present in all four genomes are eliminated from the analysis. The results are shown in Fig. 7. The largest number of genes, 131, 114 and 96 at the 90, 95 and 99% posterior probabilities, support the topology that clusters *Synechocystis* with *Chloroflexus* and *Chlorobium* with *Rhodobacter*. While some properties of sequence data may result in evolutionary trees that do not reflect the true gene phylogeny (Lockhart and Cameron, 2001) the observations of different topologies is also likely to be characteristic of a mosaic makeup of the bacterial genomes due to significant amounts of lateral gene transfer among organisms (Doolittle, 1999).

The genes that map to the three corners of the triangle are representative of all classes of proteins in the organisms, so that it is not apparent that certain metabolic pathways or functions have been transferred as a unit. This is shown in Fig. 8, in which the Clusters of Orthologous Groups (COG) (Tatusov et al., 2001) classes of the genes that support the three topologies are indicated. Work is underway to further analyze the genes in each of the corners of the triangle. The results of these analyses clearly indicate that large amounts of lateral transfer have taken place during the evolutionary development of these organisms. Exactly how much of that lateral transfer involved the photosynthetic apparatus and how it has influenced the evolution of photosynthesis remains to be established.

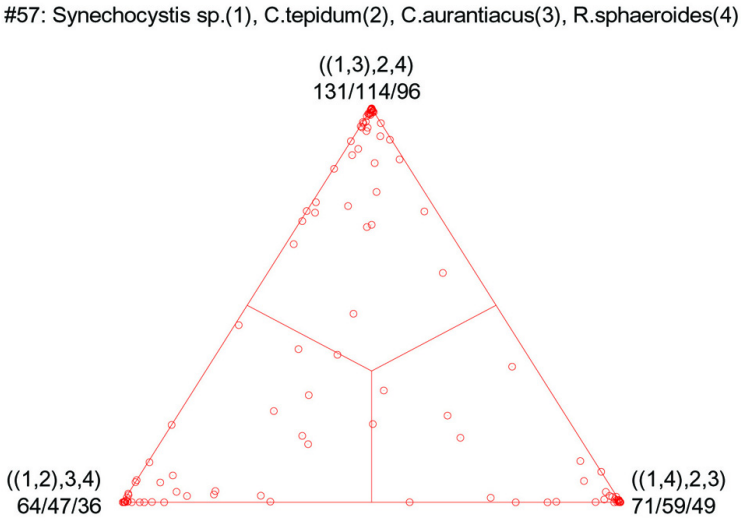
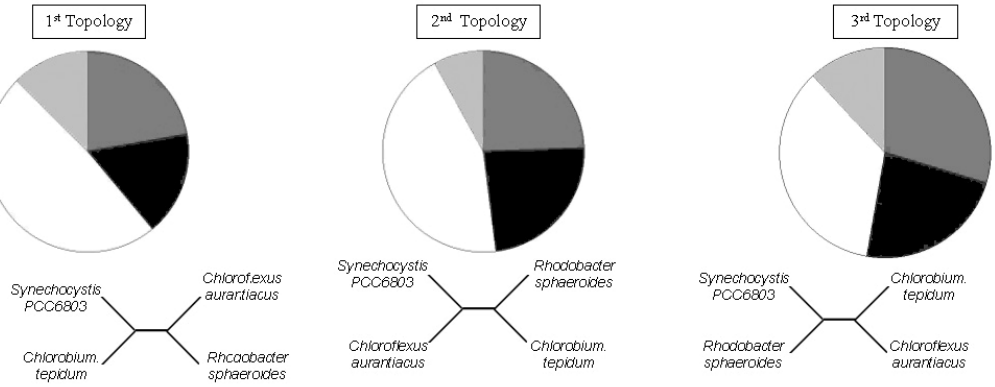


Fig. 7. Whole genome analysis of four photosynthetic organisms.



COG Categorization and Functional Assignment		Topology #1	Topology #2	Topology #3
J+K+L	**Information Storage and Processing**	**32**	**85**	**57**
J	Translation, ribosomal structure, and biogenesis	16	45	37
K	Transcription	8	8	0
L	DNA replication, recombination, and repair	8	32	20
D+...+T	**Cellular Processes**	**24**	**82**	**44**
D	Cell division and chromosome partitioning	0	4	0
M	Cell envelope/outer membrane biogenesis	4	24	17
N	Cell motility and secretion	4	18	4
O	Posttranslational modification, protein turnover, chaperones	12	24	10
P	Inorganic ion transport and metabolism	4	12	13
T	Signal transduction	0	0	0
C+...+I	**Metabolism**	**70**	**152**	**67**
C	Energy production and conversion	5	16	8
E	Amino acid transport and metabolism	21	36	17
F	Nucleotide transport and metabolism	0	28	21
G	Carbohydrate transport and metabolism	8	12	8
H	Coenzyme metabolism	24	40	8
I	Lipid metabolism	12	20	5
R+S	**Poorly characterized**	**18**	**28**	**23**
R	General function prediction only	13	24	19
S	Function unknown	5	4	4

Fig. 8. COG analysis of genes of the whole genome analysis.

The Advent of Oxygenic Photosynthesis

One of the most important yet poorly understood aspects of the evolutionary development of photosynthesis concerns the development of oxygenic photosynthesis, in which H_2O is oxidized to O_2 by photosystem II. The near infrared photons that drive the photosytems of anoxygenic bacteria contain insufficient energy to oxidize water. Therefore, an essential development is the change from long wavelength absorbing bacteriochlorophylls to the

shorter wavelength absorbing chlorophylls as the principal photopigments (Blankenship and Hartman, 1998). The key event that led to this transition is the loss of the BchXYZ enzyme system that reduces ring B (see Fig. 6). In addition, the Mn cluster that is directly involved in the oxidation of H_2O must be incorporated into the complex. The evolutionary origin of this cluster is not known, but has been suggested to have originated from either Mn catalase enzymes (Blankenship and Hartman, 1998) or Mn bicarbonate inorganic complexes (Dismukes et al. 2001).

Conclusions

The picture that is slowly emerging from many studies is that the evolutionary development of photosynthesis is a very complex process that cannot be described by a simple linear branching evolutionary diagram. Rather, photosynthesis emerged by recruiting and modifying genes encoding components of a number of other preexisting metabolic pathways, along with a few key innovations and probably a number of lateral gene transfer events. The resulting view is that, like many metabolic pathways, photosynthesis is a mosaic process that has no single well-defined evolutionary origin. Photosynthesis in different classes of organisms or even different portions of the photosynthetic apparatus in a single organism may have significantly different evolutionary histories. This concept is illustrated schematically in Fig. 9. Information from a wide range of sources and disciplines, including molecular evolution studies of complete genome sequences, biochemistry and geology, needs to be assembled and integrated in order to provide a deep understanding of the evolution of photosynthesis.

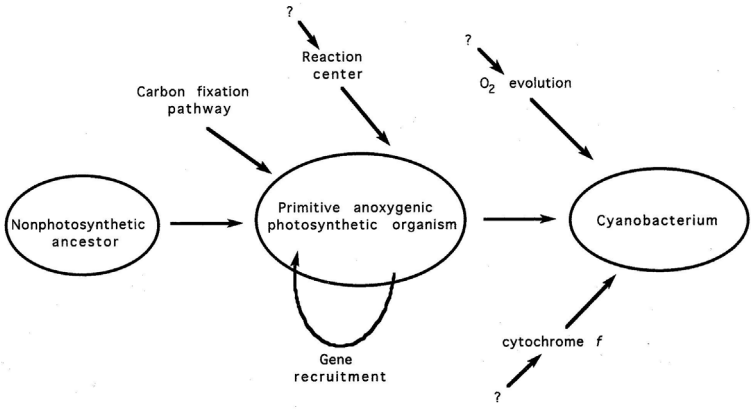


Fig. 9. Schematic diagram of the evolution of photosynthesis, including recruitment of genes from other metabolic pathways and the acquisition of other genes by lateral transfer. Figure adapted from Blankenship (2001).

Acknowledgements

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