

Origin and evolution of photosynthesis: clues from genome comparison

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ABSTRACT

Fifteen complete cyanobacterial genome sequences were characterized revealing 1054 protein families (Cyanobacterial clusters of Orthologous Groups of proteins, or CyOGs), encoded in at least 14 of them. While the majority of the core CyOGs were shared with other bacteria, 84 were exclusively shared by cyanobacteria and plants and/or other plastid-carrying eukaryotes that indicate their relation to the photosynthetic machinery (hereafter denoted as photosynthetic CyOGs). Only a few photosynthetic CyOGs find counterparts in the genomes of anoxygenic phototrophic bacteria *Chlorobium tepidum*, *Rhodospseudomonas palustris*, *Chloroflexus aurantiacus* or *Heliobacillus mobilis*. These observations, coupled with the recent geological data on the properties of the ancient phototrophs, suggest that photosynthesis has originated in the cyanobacterial lineage and spread to other phyla via horizontal gene transfer under the selective pressure of the UV light, availability of electron donors and, eventually, oxygen. We propose that the first phototrophs were anaerobic ancestors of cyanobacteria that conducted anoxygenic photosynthesis using photosystem I-type reaction center, similarly to the heterocysts of modern-day filamentous cyanobacteria.

INTRODUCTION

Cyanobacteria are one of the earliest branching groups of organisms and the only known prokaryotes to carry out oxygenic photosynthesis; there is little doubt that they played a key role in the formation of atmospheric oxygen (Bekker et al. 2004). Despite the evolutionary, environmental and geochemical importance of cyanobacteria, many aspects of their life remain obscure. In the last several years, complete genome sequences of

several freshwater and marine cyanobacteria became available, providing ample sequence information for a systematic analysis of cyanobacterial genomes (see e.g. Hess, 2004). Studies of the genes shared by cyanobacteria and other photosynthetic organisms allowed delineation of the 'photosynthetic gene set' and demonstrated a significant amount of horizontal gene transfer (HGT) among photoautotrophic bacteria (Raymond et al. 2002; Sato 2002; Raymond et al. 2003; Zhaxybayeva et al. 2004). A somewhat surprising result of the latter work was that most known photosynthetic genes did not belong to the 'photosynthetic gene set'.

We compared proteins encoded in 15 complete cyanobacterial genomes, including five genomes of *P. marinus*, to define the minimal set of genes common to all cyanobacteria (Mulkidjanian et al., 2006). While the majority of such core CyOGs are genes for central cellular functions that are shared with other bacteria, 84 (hereafter photosynthetic CyOGs) are exclusively shared by cyanobacteria and plants and/or other plastid-carrying eukaryotes that indicate their relation to the photosynthetic machinery. The latter group included 35 families of uncharacterized proteins, which are likely to be previously unrecognized components of the photosynthetic machinery (see Mulkidjanian et al., 2006 for details).

The revealed set of photosynthetic CyOGs might be helpful upon addressing the identity of the first phototrophs, the subject of intense discussion in recent years (Pierson and Olson 1989; Blankenship 1992; Green, Gantt 2000; Xiong et al. 2000; Dismukes et al. 2001; Raymond et al. 2002; Gupta 2003; Raymond et al. 2003; De Las Rivas et al. 2004; Mix et al. 2004; Olson and Blankenship 2004; Allen 2005, Allen, Martin, 2007).

RESULTS AND DISCUSSION

Uneven distribution of photosynthetic genes among phototrophic bacteria

As documented elsewhere, the vast majority of CyOGs had no detectable homologs in bacterial phototrophs (Mulkidjanian et al., 2006). Non-cyanobacterial photosynthetic machineries share only a small set of proteins with cyanobacterial PSI or PSII, and even these shared proteins are different in different bacteria. Out of the six groups of cyanobacterial genes that are directly related to photosynthesis, only the genes of chlorophyll biosynthesis are shared by all other prokaryotic phototrophs (Mulkidjanian et al., 2006). It is noteworthy that the phyletic distribution of CO₂ fixation mechanism through the Calvin-Benson-Bassham cycle also proved to be very uneven. Whereas all cyanobacteria encode the full set of the Calvin cycle enzymes, *Chlorobium* and *Heliobacillus* genomes lack the genes for the pathway-specific enzymes (Mulkidjanian et al., 2006). Both these organisms encode RuBisCO-like proteins, which, however, are likely to participate in methionine salvage, rather than in CO₂ fixation (Hanson and Tabita, 2001).

Evolution of photosynthesis and horizontal gene transfer

The availability of the cyanobacterial genome core allowed us to reassess the origin of the (bacterio)chlorophyll-based photosynthesis (chlorophotosynthesis). In addition to Cyanobacteria, photosynthetic bacteria are found in the Bacteroidetes/Chlorobi group (e.g. *Chlorobium tepidum*), Firmicutes (e.g. *Heliobacillus mobilis*), Proteobacteria, and Chloroflexi (green nonsulfur bacteria, e.g. *Chloroflexus aurantiacus*). The first two phyla have photosynthetic reaction centers of the RC1 type that use FeS clusters as electron

acceptors and are similar to the cyanobacterial PSI. The reaction centers of proteobacteria and Chloroflexi (RC2-type) use bound quinones as electron acceptors and are similar to the cyanobacterial PSII. Since all these phyla, except for Cyanobacteria and, perhaps, Chlorobi, also contain non-photosynthetic representatives, evolution of photosynthesis must have included frequent loss of photosynthetic genes and/or acquisition of such genes via HGT (Blankenship, 1992, Blankenship, Olson, 2004). The HGT scenario, under which photosynthesis emerged in a certain group of bacteria and was disseminated via HGT, is supported by the fact that the photosynthesis-related proteins are often encoded on a single contiguous chromosomal region (superoperon) (Alberti et al., 1995; Xiong et al. 1998) and by the observation that photosynthetic genes can be transduced by cyanophages (Lindell et al. 2004).

The propensity of photosynthetic genes to be horizontally transferred between distantly related organisms explains their patched distribution among bacterial lineages. This propensity, in the same time, makes identification of the lineage that was the first to develop chlorophyll-based photosynthesis especially challenging.

In the past few years, photosynthesis has been proposed to have emerged in *Heliobacillus* (Vermaas 1994; Gupta 2003), *Chlorobium* (Buttner et al. 1992), *Chloroflexus* (Pierson 1994), or proteobacterial (Xiong et al. 2000) lineages (see Olson and Blankenship 2004 for a review). While the arguments in favor of proteobacteria do not appear valid (Green, Gantt, 2000; Mulkidjanian et al., 2006), there seems to be some support for each of the other candidates. Thus, *Heliobacillus* and *Chlorobium* possess apparently primitive homodimeric RCs of type I (Buttner et al. 1992; Liebl et al. 1993), whereas *Chloroflexus* is believed to be an early-branching lineage of phototrophs

(Pierson 1994). Cyanobacteria are usually not considered as a lineage where photosynthesis could have emerged because of the far greater complexity of their photosynthetic machinery. This fact, however, can be interpreted both ways. Indeed, the total number of genes involved in photosynthesis in cyanobacteria is much greater than that in any of the other prokaryotic phototrophs (see Mulkidjanian et al., 2006 for details). Only cyanobacteria possess photosynthetic reaction centers of both types, RC1 and RC2, and have chlorophyll-containing proteins whose main function is to dissipate the light energy to prevent photodamage. Thus, the majority of photosynthetic genes must have first appeared in the cyanobacterial lineage anyhow. This suggests that the core RC genes might have the same origin and that cyanobacteria also should to be considered as candidate first phototrophs.

Sequence data alone do not allow us to establish the direction for an ancient lateral gene transfer; this requires additional information from independent sources. Indeed, important clues as to the nature of the first phototrophs can be gained from geological data. Tice and Lowe (2004) have provided geological evidence that the Buck Reef Chert – a 250–400-m-thick rock running along the South African coast – was produced by phototrophic microbial communities ca. 3.4 Gy ago. Tice and Lowe defined the inhabitants of the primordial microbial communities as partially filamentous phototrophs, which, according to the carbon isotopic composition, used the Calvin cycle to fix CO₂. As argued in more detail elsewhere (Mulkidjanian et al., 2006), the mechanism of CO₂ fixation, the morphology of these organisms and their location in the upper layer of the ancient microbial mat all unite them with modern cyanobacteria. Thus, the analysis of the gene content and the geologic evidence both suggest that photosynthesis has evolved

in the cyanobacterial lineage. Since there is sufficient evidence that anoxygenic photosynthesis was already happening in the period between 3.5 and 2.5 Gy ago, i.e. prior to oxygenic photosynthesis appeared, we propose that the first phototrophs were anoxygenic ancestors of the extant cyanobacteria that relied on type 1 RCs to reduce NAD(P)^+ to NAD(P)H . These anoxygenic phototrophic ancestors of cyanobacteria (APAC) would resemble heterocysts, the specialized nitrogen-fixing cells that some modern filamentous cyanobacteria produce in response to starvation for fixed nitrogen. Heterocysts have PSI but not PSII and therefore do not conduct oxygenic photosynthesis (Haselkorn, 1998). Thus, heterocyst formation can be viewed as a recapitulation of the ancestral cyanobacterial state.

Driving forces in the origin and evolution of photosynthesis

The complexity of the photosynthetic machinery leaves no doubt that its origin and subsequent evolution must have occurred in multiple steps under constant selective pressure. This selective pressure could come from at least two key factors: the damaging effects of the UV light and the necessity for the cells to conduct redox reactions to gain and store energy. As proposed by several authors, RC1 could evolve, via multiple duplication events, from simpler chlorophyll-containing membrane proteins, similar to the HLIPs of modern cyanobacteria (Mulkidjanian, Junge 1997, 1999; Montane, Kloppstech 2000). As argued previously, these proteins might serve to protect the cell DNA from the damaging effects of UV light (Mulkidjanian, Junge 1997). Participation of the modern pigment-proteins in photosynthesis masks their ability to perform the UV-protection. Still this ability is not lost: comparison of absolute action spectra for

pyrimidine dimer formation in alfalfa seedlings versus T7 bacteriophage shows over a 100 fold decrease in the rate of damage induced by UV-irradiation of 260-280 nm in the former case (Quaite et al., 1992). The emergence of RC1 from the primordial UV protectors could be driven by the need of an alternative source of reducing power as the atmospheric hydrogen content gradually decreased owing to hydrogen escape from the atmosphere and oxidation. This could be the reason for the production by RC1 of reduced NAD(P)H, which has a redox potential similar to that of hydrogen and can replace hydrogen in certain metabolic chains, as long as the membrane hydrogenase and NADH-hydrogenase differ only in the substrate-oxidizing module (Friedrich and Scheide 2000). The charge separation in the primordial RC1 led not only to the generation of reduced compounds but also to the generation of strong oxidants, which were in shortage under primordial reducing condition. It is attractive to speculate that the cytochrome *bc₁/b₆f* complexes have emerged in the same protocyanobacterial lineage - initially as menaquinol-oxidoreductases - to complete the cyclic electron transfer around first RC1 and to derive energy from the light-generated difference in redox potentials. The appearance of such (mena)quinol-oxidizing enzymes, as well as the need for further sources of redox equivalents could drive the formation of a high-potential RC2 that could use the light energy to extract electrons from Fe(II) compounds and to use this electrons for (mena)quinol generation. This step in the evolution of photosynthesis could correspond to the appearance of banded iron formations 3.2-2.6 Gy ago (Nisbet, Sleep 2001). Further depletion of electron donors upon oxidation of the available Fe(II), as discussed by several authors (Blankenship 1992; Mulkidjanian, Junge, 1997; Dismukes et al. 2001; De Las Rivas et al. 2004, Allen, 2005, Allen, Martin, 2007) could have driven

the transition of RC2 into the water-oxidizing and oxygen-generating PS2. The oxygen was extremely poisonous for the primordial microorganisms. While other bacteria could escape in the anoxic environments, the procyanobacteria had to choose between developing oxygen-protecting gadgets and being literally burned/oxidized. Only those procyanobacteria that learned to cope with oxygen passed this major evolutionary bottleneck and became cyanobacteria.

< Fig. 1 >

In this framework, modern cyanobacteria inherited their photosynthetic apparatus from the ancestral phototrophs, whereas other phototrophic bacterial lineages obtained theirs via HGT. These transfer events must have happened at different stages of evolution: the ancestors of *Chlorobium* and *Heliobacterium* acquired their RC1 soon after its emergence when it was still homodimeric, whereas Proteobacteria and *Chloroflexus* acquired RC2 before it "learned" to oxidize water. As noted above, the anoxygenic phototrophs were subject to a weaker selective pressure from light and oxygen than cyanobacteria that remained on the surface, allowing preservation of those laterally transferred genes. In this sense, photosynthetic enzymes of anaerobic bacteria can be considered snapshots of the ancient RCs: the homodimeric RC1 (PshA) of anoxic *Heliobacillus* is probably more similar to the ancient homodimeric RC1 than the highly sophisticated heterodimeric PSI (PsaA/PsaB) of modern cyanobacteria. While the photosynthetic arsenal of cyanobacteria became increasingly complex to resist light and oxygen, the photosynthetic machineries of the buried, anoxic phototrophs evolved

towards compact mobile superoperons that could be switched on/off when needed, similarly to the genes of antibiotic resistance.

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Figure Caption

Figure 1. Distribution of the photosynthetic genes content in different lineages of phototrophs and the directions of proposed lateral gene transfer.

The phototrophic phyla are depicted in accordance with the depth of their location in modern (and, perhaps, primordial) microbial mats (Nisbet, Sleep, 2001). The text boxes show the extent of photosynthetic gene transfer between the phyla; the numbers of CyOGs transferred are indicated in parentheses. The dashed text boxes show major photosynthesis-relevant “inventions” that occurred outside the (pro)cyanobacterial lineage. The coloring of the Chloroflexi reflects the presence in this group of both green chlorosome-containing organisms (Pierson, Catenholz, 1974) and the pink ones, lacking the chlorosomes (Hanada et al., 2002). The existence of the latter suggests that the chlorosomes originated in the Chlorobi (see Frigaard, Bryant, 2005, on the relation between the chlorosomes of Chlorobi and Chloroflexi). The chlorosome-less Chloroflexi carry their photosynthetic genes in an operon that is similar in gene content and even in the gene order to the photosynthetic RC2 operon of purple bacteria (Yamada et al., 2005). Because of this similarity, no conclusion can be drawn on (i) whether the RC2 was attained by purple bacteria via Chloroflexi or the other way around and (ii) whether the bacterial light-harvesting complexes (LHC, see ref. Law et al., 2004, for a review), found in purple bacteria and Chloroflexi have originated in purple bacteria or in Chloroflexi. In purple bacteria, a new protein was recruited, the “heavy” H-subunit that cups/protects RC2 from the cytoplasmic site. In other phototrophs, the RCs are cupped either by phycobilisomes (cyanobacteria) or by chlorosomes (Chlorobi and Chloroflexi), so that part of the light excitation energy pours into the RC2 through this interface. The gene for

the H-subunit, although present in the photosynthetic gene cluster, is located separately from the genes of the other two RC subunits (Suwanto, Kaplan, 1989). In Chlorobi, a unique bacteriochlorophyll-binding FMO protein (Fenna et al, 1974) is used to mediate the excitation transfer from the chlorosome to the RC1.

The picture emphasizes that while the surface phototrophs, moving along the time arrow from the anoxic into the self-made oxygenated world, underwent a major transformation from anoxygenic procyanobacteria to cyanobacteria, the inhabitants of the more light- and oxygen protected bottom layers of microbial communities could retain their ancient traits in the course of evolution.

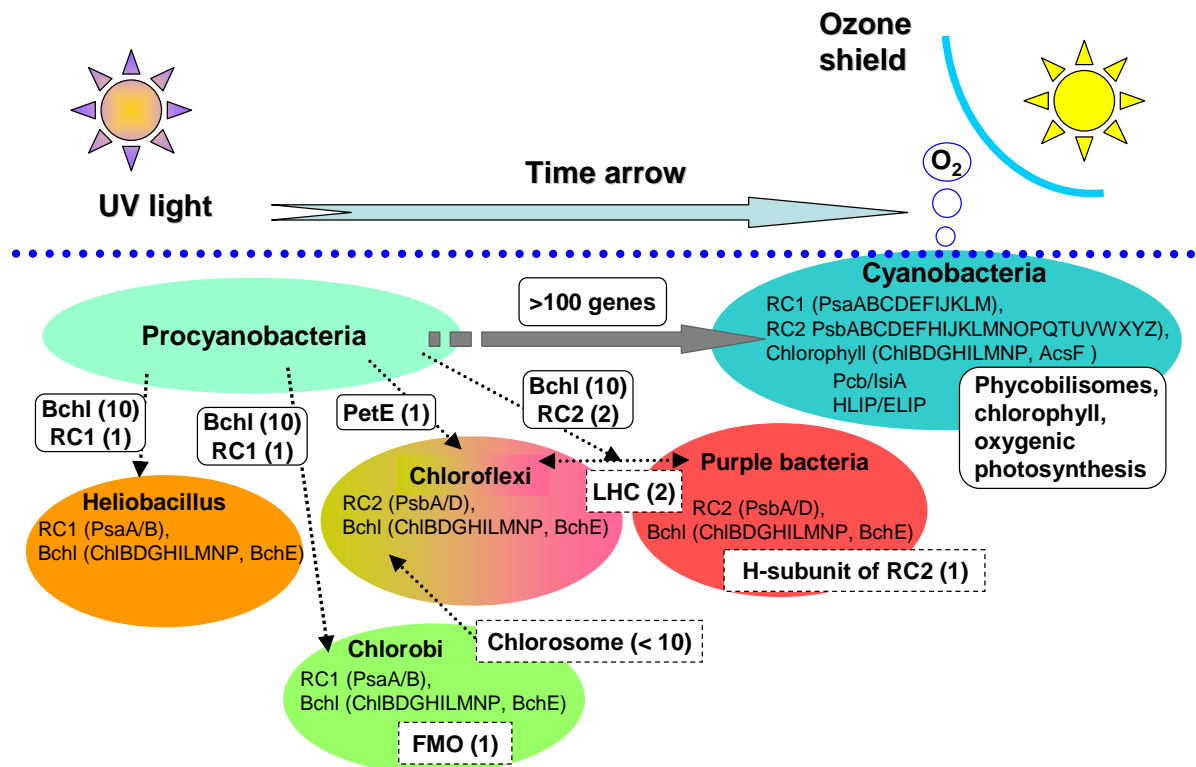


Figure 1. Distribution of the photosynthetic genes content in different lineages of phototrophs and the directions of proposed lateral gene transfer (extended version).

The phototrophic phyla are depicted in accordance with the depth of their location in modern (and, perhaps, primordial) microbial mats (1). The text boxes show the extent of photosynthetic gene transfer between the phyla; the numbers of CyOGs transferred are indicated in parentheses. The direction of the gene transfer is justified by existence of non-photosynthetic organisms in all lineages besides cyanobacteria (ref. 2 describes an apparently non-photosynthetic species of Chlorobi). The dashed text boxes show major photosynthesis-relevant "inventions" that occurred outside the (pro)cyanobacterial lineage. The coloring of the Chloroflexi reflects the presence in this group of both green chlorosome-containing organisms (3) and the pink ones, lacking the chlorosomes (4). The existence of the latter suggests that the chlorosomes originated in the Chlorobi (see ref. 5 on the relation between the chlorosomes of Chlorobi and Chloroflexi). The chlorosome-less Chloroflexi carry their photosynthetic genes in an operon that is similar in gene content and even in the gene order to the photosynthetic RC2 operon of purple bacteria (6). Because of this similarity, no conclusion can be drawn on (i) whether the RC2 was attained by purple bacteria via Chloroflexi or the other way around and (ii) whether the bacterial light-harvesting complexes (LHC, see ref. 7, 8 for reviews), found in purple bacteria and Chloroflexi have originated in purple bacteria or in Chloroflexi. In purple bacteria, a new protein was recruited, the "heavy" H-subunit that cups/protects RC2 from the cytoplasmic site. In other phototrophs, the RCs are cupped either by phycobilisomes (cyanobacteria) or by chlorosomes (Chlorobi and Chloroflexi), so that part of the light excitation energy pours into the RC2 through this interface. The gene for the H-subunit, although present in the photosynthetic gene cluster, is located separately from the genes of the other two RC subunits (9). In Chlorobi, a unique bacteriochlorophyll-binding FMO protein (10) is used to mediate the excitation transfer from the chlorosome to the RC1.

The picture emphasizes that while the surface phototrophs, moving along the time arrow from the anoxic into the self-made oxygenated world, underwent a major transformation from anoxygenic procyanobacteria to cyanobacteria, the inhabitants of the more light- and oxygen protected bottom layers could retain their traits in the course of evolution (see ref. 8, 11-17 for alternative scenarios).

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