

Photosynthesis: what color was its origin?

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Abstract

Recent studies using geological and molecular phylogenetic evidence suggest several alternative evolutionary scenarios for the origin of photosynthesis. The earliest photosynthetic group is variously thought to be heliobacteria, proteobacteria or a precursor of cyanobacteria, organisms whose photosynthetic pigments make them different colors.

The origin of photosynthesis using tetrapyrrole compounds (such as chlorophylls) has long been one of the most complex and challenging issues in biology. Many schools of thought have emerged, each with its own assumptions and with evidence supporting a particular origin of photosynthesis. A number of recently published landmark papers [1-3] have contributed further to the debate.

To get a better grasp of the important issue, one needs to first understand the distribution of extant photosynthetic groups and the types of photosynthetic apparatus within each group. Within the prokaryotic domain, there are five main groups of bacteria that perform tetrapyrrole-based photosynthesis. They are proteobacteria (also known as purple bacteria), heliobacteria, Chloroflexi (filamentous bacteria also known as green non-sulfur bacteria), Chlorobi (green sulfur bacteria) and cyanobacteria. Cyanobacteria are known evolutionary progenitors of chloroplasts in algae and plants which evolved at a later stage [4]. Therefore, to understand the early evolution of photosynthesis, one needs to focus on the photosynthetic prokaryotes. The types of photosynthesis and photosynthetic apparatus in these groups of organisms also vary. Cyanobacteria contain two types of photosystems (type I, also known as Fe-S type, and type II, also known as quinone type) and carry out oxygen-evolving photosynthesis. The other four groups of photosynthetic bacteria contain only one type of photosystem and perform non-oxygen-evolving photosynthesis. Proteobacteria and Chloroflexi are known to contain a simplified type II photosystem whereas Chlorobi and heliobacteria contain a simplified type I photosystem only.

In addition, the chlorophyll pigments contained in the photosystems of these bacterial groups also differ structurally with cyanobacteria having chlorophyll *a*, heliobacteria having bacteriochlorophyll *g*, and the other three groups synthesizing various bacteriochlorophylls from *a* to *e*. These pigments absorb light at different frequencies and thus have slightly different colors. The question of the original nature of the most ancestral photosynthetic apparatus can thus be metaphorically encapsulated by asking the color of the first photosynthetic apparatus. (It needs to be pointed out that the actual colors of the photosynthetic organisms are often masked by non-chlorophyll pigments such as carotenoids and phycobilins).

Geological evidence on the origin of photosynthesis

The advent of photosynthesis is known from geological studies to be a very ancient event. The earliest evidence for biological carbon fixation was dated to 3.8 billion years ago (or Giga-annum, Ga) [5,6] from the isotopic composition of sedimentary rocks. The best known fossil evidence of the earliest photosynthetic forms of life has been dated to 3.5 Ga and was found to contain filamentous cellular structures [7,8]. From their morphology alone, Schopf and co-workers [7,8] proposed that these structures were oxygen-evolving cyanobacteria. This finding is significant in that it suggests that photosynthetic organisms were the earliest forms of life on Earth and that oxygen-evolving photosynthesis started in the early Archean age [9]. The finding remains controversial, however, because it is difficult to determine cell physiology on the basis of the shape of the structures in ancient

microfossils. More recently, Brasier *et al.* [10] challenged the early interpretation of the microfossils by suggesting (using data from electron microscopy, digital imaging and Raman spectroscopy) that the structures were in fact artifacts of amorphous graphite. However, emerging geochemical studies [11,12] seem to have reconfirmed the biogenic nature of the microfossils and thus reversed the conclusion of Brasier *et al.* [10].

Despite the controversies surrounding the 3.5 Ga fossils, there are some other microfossils thought to be cyanobacteria that are more likely to be genuine, the oldest of which were dated to 2.6-2.7 Ga by more reliable biomarkers [13,14]. This timing is significant because it predates slightly the early rise of oxygen on Earth, which was about 2.3 Ga [15-17]. Recently, Tice and Lowe [1] showed additional evidence of photosynthetic carbon fixation by filamentous microbial mats found in 3.4 Ga cherts (flint-like sedimentary rocks) in completely anoxic environments. Their geochemical analysis further ruled out the possibility that the primary electron donor for the carbon fixation could be H₂O (as used by plants), Fe²⁺ or H₂S. Instead, the primary electron source for this type of photosynthesis is most likely to have been hydrogen, which was abundant in the atmosphere in the early Archean age. The result is consistent with the view that the early photosynthesis was most likely to have been carried out by anoxygenic photosynthetic bacteria rather than cyanobacteria.

Molecular phylogenetic evidence on the evolution of photosynthesis

Although the geological records provide the timing information for the evolutionary events, finding the sequence with which the five main groups of photosynthetic microorganisms evolved from a common ancestor requires molecular phylogenetic analysis of the genetic components of extant photosynthetic organisms. The use of molecular sequences to discover this ordering has, however, so far generated even greater controversies than the study of microfossils. Various hypotheses have been proposed and various methodologies used in the course of reconstructing the early history of photosynthesis.

Studies of whole organisms and genomes

In the early days of molecular phylogenetics, bacterial relationships were usually resolved using 16S ribosomal RNA (rRNA) [18,19], which allowed classification and identification of the major bacterial groups. From the 16S rRNA phylogenetic trees, the evolutionary pathway of the five photosynthetic bacterial groups can be compiled, giving Chloroflexi as the earliest photosynthetic lineage, with heliobacteria as the second, followed by Chlorobi, cyanobacteria and proteobacteria, in that order [4] (Figure 1a).

Gupta *et al.* [20] used heat shock proteins (Hsp60 and Hsp70) as the molecular markers and relied heavily on conserved insertions and deletions (indels) in the sequence alignment to derive phylogenetic trees for the photosynthetic bacterial groups. The results led to the conclusion that the heliobacterial group was the most ancestral out of the photosynthetic groups and that the evolutionary pathway followed a linear order, with Chloroflexi branching second, then cyanobacteria, Chlorobi and proteobacteria in that order (Figure 1b).

With the rapid accumulation of bacterial whole-genome sequence data, phylogenetic relationships are now more often studied at the whole-genome level to obtain a clearer picture of bacterial evolution. Raymond *et al.* [21] analyzed one representative genome from each of the five photosynthetic taxa and discovered highly incongruent evolutionary patterns among the five genomes. They observed 15 possible tree topologies from the commonly shared proteins encoded by all five genomes. To resolve the evolutionary pattern for photosynthesis further, the authors [21] then compiled a set of 'photosynthesis-specific' and 'photosynthesis-related' genes and performed phylogenetic analysis on each gene product, but they failed to reach a phylogenetic consensus. This confirms that bacterial genome evolution involves extensive lateral gene transfer, which also had a role in the development of the photosynthetic apparatus.

Recently, Mulikjanian *et al.* [2] analyzed 15 cyanobacterial genomes and derived a set of genes commonly shared by all the genomes, in the form of a minimal cyanobacterial genome. The photosynthesis-related portion of the minimal gene set was found to be much larger than the gene set previously derived by the Blankenship group [21] because many genes are specific to cyanobacteria. The more comprehensive nature of the cyanobacterial gene set prompted the conclusion that cyanobacteria were the most ancestral phototroph. As the conclusion was not drawn from accepted rooted phylogenies using *bona fide* photosynthesis genes found in all photosynthetic lineages, however, the logic behind this proposal seems weak.

Studies using chlorophyll biosynthesis markers

Because (bacterio)chlorophylls are integral components of the photosynthetic apparatus, enzymes involved in the biosynthesis of this pigment (encoded by the *bch* genes) could be used as specific indicators for the evolution of photosynthesis. The main advantage of this set of markers is their ubiquitous presence among all the photosynthetic bacterial groups. Most of the *bch* trees can be unambiguously rooted, because a composite tree can be constructed with a reliable outgroup from a different but homologous enzyme family.

The analysis of the Bch enzymes has been instrumental in testing the long-standing Granick hypothesis [22], which states that biosynthetic pathways recapitulate their

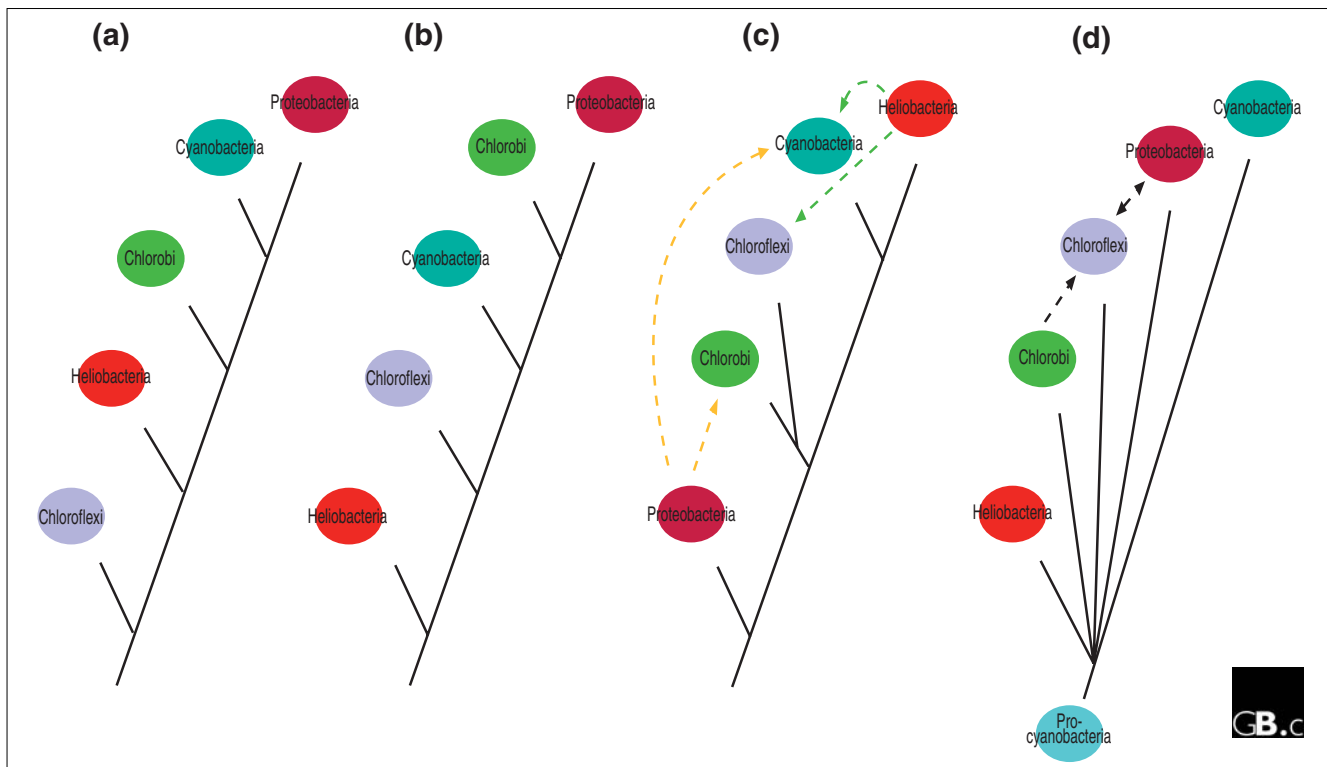


Figure 1
 Four representative scenarios for the early evolution of the photosynthetic process among photosynthetic bacteria. **(a)** The branching order of photosynthetic organisms based on 16S rRNA [4,18,19]. **(b)** The branching order for photosynthetic organisms proposed by Gupta and coworkers [20] using heat shock proteins; **(c)** the branching order that we [29] derived from analysis of (bacterio)chlorophyll biosynthesis genes (yellow arrows represent the direction of lateral gene transfer for the type II reaction center; green arrows represent the direction of lateral gene transfer for the type I reaction center); and **(d)** the branching order evolutionary pathway derived from analysis of photosynthesis genes by Galperin and coworkers [2] (black arrows represent the direction of lateral gene transfer for some photosynthesis genes).

evolution: in a multi-step biosynthetic pathway, products produced in early steps would evolutionarily predate products produced in later steps. As a general guide to biochemical evolution, this hypothesis makes sense, but when it is used to reconstruct the evolutionary history of photosynthesis, it may generate erroneous conclusions.

In the chlorophyll biosynthesis pathway, chlorophyll *a* biosynthesis requires shorter steps and appears before bacteriochlorophyll *a* [23]. According to the Granick hypothesis, this would indicate that cyanobacteria (which contain chlorophyll *a*) predate anoxygenic photosynthetic bacteria (containing bacteriochlorophyll *a*) [24,25]. Therefore, by applying the Granick hypothesis, one would conclude that photosynthesis originated with cyanobacteria. This view agrees with that of Mulikidjanian *et al.* [2]. But the molecular phylogenetic analysis of a number of enzymes involved in (bacterio)chlorophyll biosynthesis, performed by my group and others [26-28] using carefully selected outgroups for rooting the trees, indicates that the anoxygenic photosynthetic lineages are almost certain to be more deeply rooted than the oxygenic cyanobacterial and chloroplast lineages. Proteobacteria

seem to be the earliest evolving among the anoxygenic lineages, suggesting that bacteriochlorophyll *a* predates chlorophyll *a* during evolution. A Bayesian analysis that we subsequently performed on the dataset delineated the sequence of evolution for (bacterio)chlorophyll biosynthesis [29] (Figure 1b). In this scenario, the pigment biosynthesis genes were laterally transferred from proteobacteria to Chlorobi, from which the lineage bifurcated to Chloroflexi and cyanobacteria, which gave rise to heliobacteria (Figure 1c). This result seems to contradict the Granick hypothesis. A simple explanation for this paradox could be that gene loss of some of the *bch* genes occurred during the evolution of the genes in the cyanobacterial lineage, leading to a shortened biosynthesis pathway.

Studies using reaction centers

The reaction center is the core of the (bacterio)chlorophyll-containing protein complex where the primary electron transfer event takes place during photosynthesis. Because of their central importance, reaction center proteins have naturally become the focus of study for the evolutionary pathway of photosynthesis. However, the main difficulty of

using the reaction center proteins as molecular markers is the extremely high divergence of the sequences between the two types of reaction center, making it difficult to derive an evolutionary scenario for all five photosynthetic bacterial groups. Currently, several hypotheses have been put forward to postulate the origin and developmental pathway of the photosynthetic apparatus. Generally, they fall within two schools of thought, the selective loss model and the fusion model.

The selective loss model

The selective loss model [24,25,30] postulates an ancestral photosynthetic organism, similar to oxygenic cyanobacteria, containing both types of reaction center. A subsequent loss of one of the reaction center types gave rise to a single reaction center found in extant anoxygenic photosynthetic bacteria. The model suggests that organisms like cyanobacteria were present in the prebiotic phase, when life first originated. This view can find initial support from the 3.5 Ga microfossil study by Schopf and coworkers [7,8].

The most recent support for the selective loss theory came from Mulkidjanian *et al.* [2], who believe that the enlarged photosynthesis core gene set suggests a cyanobacterial origin of photosynthesis. Given the compelling geological evidence that anoxygenic photosynthesis evolved before oxygenic photosynthesis, the authors [2] offered a revised selective loss model in which a group termed 'procyanobacteria', which was largely similar to extant cyanobacteria but did not evolve oxygen, was the most ancient phototroph and that it subsequently spread photosynthesis genes to other anoxygenic photosynthetic bacterial groups by lateral gene transfer and large-scale gene loss (Figure 1d).

The authors [2] further postulated that procyanobacteria, as the photosynthetic progenitors, contained the type I reaction center only. This idea was based primarily on the geological evidence of Tice and Lowe [1] that the 3.4 Ga phototroph performed hydrogen-based photosynthesis. According to Mulkidjanian *et al.* [2], only procyanobacteria were suitable for this type of photosynthesis. Chlorobi and heliobacteria were excluded from consideration because they do not contain the Calvin cycle (in which a six carbon sugar molecule is synthesized by fixing CO₂ and combining it with a five carbon molecule, 1,5-ribulose biphosphate). Chloroflexi and proteobacteria were excluded because hydrogen is too reducing for the quinone-type reaction centers that they contain. Both arguments seem weak because green sulfur bacteria (Chlorobi) are known to fix CO₂ not through the Calvin cycle but through the reductive tricarboxylic acid (TCA) cycle [31] (the traditional citric acid cycle running in reverse), which uses hydrogen or reduced sulfur compounds as electron donors. In addition, it is well established that Chlorobi, Chloroflexi and proteobacteria can use hydrogen as the sole electron donor and CO₂ the sole electron acceptor for photoautotrophic growth [32] (that is, growth that solely

depends on light and inorganic nutrients). In contrast, normal cyanobacterial cells are not capable of using hydrogen as the sole electron donor in photosynthesis. Though some specialized cyanobacterial cells such as heterocysts (nitrogen fixing cells with multi-layered cell walls) are capable of anoxygenic photosynthetic electron transfer using the type I photosystem only with hydrogen or sulfur compounds serving as electron donors, this special type of differentiated cells are considered a relatively recent evolutionary invention [33].

The fusion model

The fusion model [4,34] postulates that the type I and type II reaction centers could have been established independently in two different ancestral lineages (one in proteobacteria or Chloroflexi and the other in heliobacteria or Chlorobi) before being brought together into one lineage to produce the cyanobacterial dual photosystem. The model envisages the photosynthetic apparatus as having evolved from simple to complex, which seems more reasonable than the opposite scenario.

A colleague and I proposed one version of the fusion model [29], in which the direction of reaction-center evolution was inferred from a Bayesian analysis. The most ancestral form of the reaction center was proposed to be a type II reaction center of proteobacterial origin. The subsequent divergence of the proteobacterial lineage into Chloroflexi and cyanobacteria gave rise to the extant type II reaction center in these two lineages. The type I reaction center, which was thought to be relatively late evolving, may have been formed through a fusion by the primordial type II reaction center and a light-harvesting antenna protein (which contains chlorophyll pigments that harvest light energy and transfer it to the reaction center). We proposed that the gene fusion event occurred in a heliobacterial lineage, resulting in an enlarged reaction center. This type I-like reaction center later diverged into those found in Chlorobi and cyanobacteria. The arrival of both types of reaction centers in cyanobacteria enabled the later establishment of a linear electron transfer between the two. Our proposed evolutionary scenario for the reaction centers is distinct from the evolutionary pathway for (bacterio)chlorophyll biosynthesis (Figure 1c), which adds an additional layer of lateral gene transfer relative to the 16S rRNA evolutionary pathway.

In view of the lack of obvious sequence similarity between the two types of reaction centers, which makes it difficult to derive a common evolutionary tree for them, a new approach was adopted by the Blankenship group [3], based on both structure and sequence. Because of the known structural similarity of the two types of reaction centers, Blankenship and coworkers [3] first aligned the conserved core structures of two reaction centers, which exposed the structurally corresponding residues. The structurally aligned residues were then used to construct a sequence alignment that was

then used to build a unified phylogeny of the reaction centers. The reaction center trees were unrooted and thus did not allow direct inference of the most ancestral reaction center. If a midpoint rooting technique were used, however, the trees would suggest that the earliest reaction center was anoxygenic and probably a homodimeric complex.

In conclusion, although no consensus for the evolutionary history of photosynthetic apparatus has yet emerged, it is widely accepted that it is a very complex process involving multi-layered lateral gene transfer [35]. The lateral gene transfer events can seem so complex that the origin of photosynthesis could become an intractable issue. As a solution to the problem, instead of assuming that all genes are equally important in their ability to reveal the early evolutionary history of photosynthesis, we [29] suggested focusing on a sub-process, (bacterio)chlorophyll biosynthesis, as the factor most likely to have determined the advent of photosynthesis. Along with the development of the most important elements of the photosynthetic apparatus, a functional apparatus could have been assembled through a multi-staged recruitment of reaction center proteins and antenna proteins, which could conceivably have had separate evolutionary histories and performed different functions before the recruitment. The recruitment process may have undergone several intermediate stages, producing products with various degrees of complexity. In essence, the precise picture of early evolution of photosynthesis still remains to be understood. To reveal the true color of the origin of photosynthesis will require years of painstaking biogeochemistry and molecular phylogenetic studies.

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