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Minireview

Thinking about the evolution of photosynthesis*

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Abstract

Photosynthesis is an ancient process on Earth. Chemical evidence and recent fossil finds indicate that cyanobacteria existed 2.5–2.6 billion years (Ga) ago, and these were certainly preceded by a variety of forms of anoxygenic photosynthetic bacteria. Carbon isotope data suggest autotrophic carbon fixation was taking place at least a billion years earlier. However, the nature of the earliest photosynthetic organisms is not well understood. The major elements of the photosynthetic apparatus are the reaction centers, antenna complexes, electron transfer complexes and carbon fixation machinery. These parts almost certainly have not had the same evolutionary history in all organisms, so that the photosynthetic apparatus is best viewed as a mosaic made up of a number of substructures each with its own unique evolutionary history. There are two schools of thought concerning the origin of reaction centers and photosynthesis. One school pictures the evolution of reaction centers beginning in the prebiotic phase while the other school sees reaction centers evolving later from cytochrome *b* in bacteria. Two models have been put forth for the subsequent evolution of reaction centers in proteobacteria, green filamentous (non-sulfur) bacteria, cyanobacteria, heliobacteria and green sulfur bacteria. In the selective loss model the most recent common ancestor of all subsequent photosynthetic systems is postulated to have contained both RC1 and RC2. The evolution of reaction centers in proteobacteria and green filamentous bacteria resulted from the loss of RC1, while the evolution of reaction centers in heliobacteria and green sulfur bacteria resulted from the loss of RC2. Both RC1 and RC2 were retained in the cyanobacteria. In the fusion model the most recent common ancestor is postulated to have given rise to two lines, one containing RC1 and the other containing RC2. The RC1 line gave rise to the reaction centers of heliobacteria and green sulfur bacteria, and the RC2 line led to the reaction centers of proteobacteria and green filamentous bacteria. The two reaction centers of cyanobacteria were the result of a genetic fusion of an organism containing RC1 and an organism containing RC2. The evolutionary histories of the various classes of antenna/light-harvesting complexes appear to be completely independent. The transition from anoxygenic to oxygenic photosynthesis took place when the cyanobacteria learned how to use water as an electron donor for carbon dioxide reduction. Before that time hydrogen peroxide may have served as a transitional donor, and before that, ferrous iron may have been the original source of reducing power.

Abbreviations: BChl – bacteriochlorophyll; Chl – chlorophyll; ET – electron transfer; FMO – Fenna–Matthews–Olson protein; Ga – billion years ago; OEC – oxygen-evolving complex; PS – photosystem; RC – reaction center

Origin of photosynthetic life

The evolution of photosynthesis has long been a subject that interests and challenges scientists. As discussed below, the origin of photosynthesis is such an ancient event that definitive evidence as to how this essential biological process began and developed may be lost forever. However, some evidence can

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be assembled from a variety of sources, including geology, biogeochemistry, comparative biochemistry and molecular evolution analysis that begins to give some insights into the complex evolutionary history of photosynthesis. In this paper, we will discuss how thinking on this subject has developed over the years, and will give an account of some of the current ideas.

About 30 years ago one of us (Olson 1970) suggested that the ancestral 'photosynthesizer' may have utilized light to drive the assimilation of organic compounds left over from the prebiotic phase of chemical evolution. Since 1970 the organic soup hypothesis has thinned considerably, as the estimated levels of H_2, CH_4 and NH_3 on the early Earth have been revised downward (Kastings and Brown 1998). (Organic compounds are thought to have been synthesized from reduced inorganic compounds.) In the absence of an adequate concentration of organic soup, it would appear the ancestral photosynthesizer may have fixed carbon dioxide utilizing an external reductant such as hydrogen sulfide.

Early evidence for photosynthesis

Chemical markers

There are three main lines of evidence for the antiquity of photosynthesis: chemical markers, stromatolite fossils, and microfossils of ancient organisms. Among the chemical markers the ratio of $^{13}C/^{12}C$ in sedimentary organic carbon (kerogen) indicates a continuous record of biological CO_2 fixation that goes back 3.5–3.8 Ga (Schidlowski et al. 1983; Schidlowski 1988). Organic carbon is depleted in ^{13}C, while inorganic carbonate is not. The difference between these two forms of carbon is expressed as the relative depletion of ^{13}C in a sample;

$$\delta^{13}C = \frac{[(^{13}C/^{12}C)\text{sample} - 1]}{[(^{13}C/^{12}C)\text{standard}]} \times 10^3.$$

The geological record shows a continuous value of $-27 \pm 7‰$ for organic carbon and a continuous value of $+0.4 \pm 2.6‰$ for carbonate carbon. This record is consistent with biological CO_2 fixation dominated by RuBP carboxylase (Schidlowski et al. 1983), but is also consistent with other fixation mechanisms and does not reveal whether the CO_2 fixation was photosynthetic. Mojzsis et al. (1996) suggested that 3.8-billion-year-old carbon inclusions in rocks from Greenland were consistent with autotrophic CO_2 fixation. However, recently, Fedo and Whitehouse (2002) have questioned whether these structures are biogenic in origin.

Stromatolite fossils

Stromatolites are layered structures consisting of alternate layers of mat-forming organisms and sediment. Extant stromatolites almost always contain filamentous photosynthetic bacteria and/or cyanobacteria. There is a continuous fossil record of stromatolites from 2.8 Ga to the present, with evidence of earlier structures dated at 3.1 and 3.5 Ga (Walter 1983). A few of these fossil stromatolites contain fossils of filamentous microorganisms, and by inference these microorganisms are thought to have been photosynthetic. However, the biogenicity of the most ancient stromatolites has been questioned, and so the most ancient evidence relative to possible early forms of photosynthetic life is ambiguous. As discussed below, the same is true of putative microfossils that have been proposed to be of cyanobacterial origin. An excellent, balanced discussion of the criteria that can be used to distinguish biogenic from abiogenic stromatolites is given by Buick (2001).

Microfossils

Some of the oldest microfossils (0.9 Ga) that clearly look like modern cyanobacteria come from the Bitter Springs Formation in Australia (Schopf 1968; Schopf and Blacic 1971). However, microfossils generally accepted as coming from really ancient cyanobacteria have been dated at 2.0 Ga (Schopf 1974; Hofmann and Schopf 1983). Strong evidence that cyanobacteria-like organisms existed even before 2.5 Ga comes from chemical biomarkers (2-methylhopanoids) found in ancient rocks (Summons et al. 1999). However, it is not possible to determine from the biomarkers alone whether these cyanobacterial ancestors actually carried out oxygenic photosynthesis.

Recently, Kazmierczak and Altermann (2002) presented fossil evidence for the existence of chroococcalean cyanobacteria 2.5–2.6 Ga in what is now South Africa. More ancient fossils (2.8 and 3.5 Ga) from western Australia (Fortescue Group and Warrawoona Group) were discovered by Schopf and Walter (1983) and Awramick et al. (1983). Four types of filamentous fossil bacteria were identified, and it was suggested that some of them may have been photoautotrophic. In later publications Schopf (1993) and Schopf and Packer (1987) have proposed on the basis of morphology alone that the photoautotrophs may have been ancient oxygen-evolving cyanobacteria.

However, it is not possible to determine the physiology of a microorganism from its shape. Nevertheless, the discovery of these microfossils was extremely exciting, because they seemed to show the existence of life at a very early time in the history of the earth.

Non-photosynthetic filamentous microfossils dated at 3.2 Ga have been found in a massive sulfide deposit from the Pilbarra Craton in Australia (Rasmussen 2000) and show that filamentous fossils should not automatically be assumed to be photosynthetic.

Almost 10 years after the discoveries of Schopf and coworkers, Brasier et al. (2002) delivered a stunning blow to the earlier interpretation of the 3.5 Ga microfossil-like structures. These authors interpreted the structures as secondary artifacts formed from amorphous graphite within metalliferous hydrothermal vein chert and volcanic glass. This view is based on new studies utilizing mapping, optical and electron microscopy, digital image analysis and micro-Raman spectroscopy. Schopf et al. (2002a) replied that Raman spectroscopy did in fact show that 4 microfossil-like specimens (0.88, 2.1, 3.4 and 3.5 Ga) are composed of carbonaceous kerogen in concentrations much higher than those in the wispy clouds of finely divided particulate kerogen in which the specimens are preserved. However, as Pasteris and Wopetka (2002) pointed out, the Raman spectra alone indicate only the presence of disordered carbonaceous materials of indeterminate origin. Schopf et al. (2002b) replied that the case for biogenicity rests on three lines of evidence: cellular morphology, carbonaceous molecular-structural make-up, and carbon isotope composition. Kazmierczak and Kremer (2002) further showed that 0.4 Ga-microfossils, clearly related to modern cyanobacteria, and heated by Caledonian and/or Variscic thermal events, are almost identical in appearance to the filamentous structures in the 3.5 Ga Apex Chert. In addition, these authors suggested that the 11 filamentous microbial 'taxa' described by Schopf (1993) may be remnants of a homogeneous microbial community.

What should we believe about the status of cyanobacteria as ancient organisms? The chemical evidence (2-methylhopanoids) and recent fossil evidence seem to indicate that cyanobacteria really did exist 2.5–2.6 Ga ago. The fossil evidence for the existence of cyanobacteria 3.5 Ga ago has been seriously questioned, but there is evidence that some of the 'microfossils' described by Schopf and others may be genuine. However, it is not certain that they should be classified as cyanobacteria.

Granick hypothesis for chlorophyll biosynthesis

The evolution of complex organic photosensitizers such as chlorophylls can be rationalized by the Granick hypothesis that biosynthetic pathways recapitulate their evolution (Granick 1957; Mauzerall 1992). This hypothesis answers the problem of how biosynthetic pathways to complex products could have evolved by random mutation. The pathway is built forward with each step fulfilling a useful function, eventually to be replaced by the next step selected for improved utility.

Olson and Pierson (1987a, b) used the Granick hypothesis to construct a hypothetical evolutionary history of RCs from the biosynthetic pathways of Chl *a* and BChl *a*. They proposed that protochlorophyll *a* might have functioned in a primitive RC at some time before Chl *a* existed, and that protoporphyrin IX and Mg protoporphyrin IX might have served as RC pigments before protochlorophyll *a* existed. In agreement with Mauzerall (1978), Olson (1970, 1981) had previously interpreted the biosynthetic pathway of BChl *a* to indicate that Chl *a* had appeared before BChl *a* in evolutionary history.

Burke et al. (1993) made a sequence comparison of protochlorophyllide reductases (required for synthesis of both Chl *a* and BChl *a*) and chlorin reductases (required only for BChl *a* synthesis) in modern photosynthetic organisms and concluded that in ancient organisms only BChl *a* was synthesized. However Lockhardt et al. (1996) reanalyzed the data and concluded that ancient organisms might have synthesized both Chl *a* and BChl *a*, or just Chl *a* alone. [For more details see Olson (2001) and Blankenship (2002).]

More recently Xiong et al. (1998, 2000a, b) showed from the molecular phylogeny of several chlorophyll synthesis genes that the proteobacterial lineage (BChl *a*) is more deeply rooted than either the heliobacterial lineage (BChl *g*) or the cyanobacterial lineage (Chl *a*). Additional analysis of the genes that code for the protochlorophyllide and chlorin reductases have clearly established that these have resulted from an ancient gene duplication (Raymond et al. 2003a), in agreement with the original proposal of Burke et al. (1993). The nature of the ancient reductase that predated the gene duplication is not known, but typically ancient enzymes are both less specific and less efficient than modern enzymes (Benner 2002). This suggests that the ancient non-specific reductase may have reduced protochlorophyllide to bacteriochlorophyllide in two

successive turnovers. After the gene duplication, each reductase became specialized for reduction of a single ring. The key event in the emergence of Chl *a*-containing organisms is then predicted to be the loss of the chlorin reductase genes, leaving only the protochlorophyllide reductase enzyme.

So, the Granick hypothesis has been shown probably to be wrong with respect to the order in which Chl *a* and BChl *a* appeared in evolutionary history. But that does not disprove the hypothesis in all respects. It would appear that the Granick hypothesis can still be useful for suggesting intermediate pigments like Mg protoporphyrin IX and protochlorophyll in the evolution of photosynthesis, but the pathway for the synthesis of protoporphyrin IX probably evolved in response to evolutionary pressure for efficient electron transport cofactors such as heme in cytochrome *b*.

Reaction centers

The evolution of photosynthesis begins with the evolution of photochemical reaction centers (RCs). Two schools of thought exist concerning the origin of RCs and photosynthesis. The first school pictures the evolution of RCs/photosynthesis beginning in the prebiotic phase (e.g., Mauzerall 1992; Hartman 1998) whereas the second school sees RCs and photosynthesis evolving much later from anaerobic respiration in Bacteria (eubacteria) containing DNA, electron-transport proteins, and ATP synthase (e.g., Meyer et al. 1996; Nitschke et al. 1998). The first school envisions the evolution of photosynthesis and the origin of life to be tightly intertwined; the second school sees the origin of photosynthesis coming much later than the appearance of the common ancestor of all life. According to this view photosynthesis arose in the Bacteria after they had separated from the Archaea (archaebacteria).

A well-known proponent of the first school is David Mauzerall, author of several papers on porphyrin photochemistry. Following the Granick hypothesis that biosynthetic pathways recapitulate their evolution, Mauzerall moved down the pathway for chlorophyll synthesis to uroporphyrinogen and uroporphyrin. The highly water soluble uroporphyrin is the first porphyrin to be synthesized along the pathway to chlorophyll, and Mauzerall (1992) proposed a primitive photochemistry in which uroporphyrin is photoreduced to its radical by various electron donors. The radicals are thought to have disproportionated to uroporphyrin and dihydro-uroporphyrin. In the presence of a catalyst, the dihydro-uroporphyrin is reoxidized, forming hydrogen (Mercer-Smith and Mauzerall 1984). The uroporphyrin cycle produces hydrogen from electron donors such as ferrous ion, sulfhydryl groups, or organic molecules.

Mauzerall's primitive photosystem is far removed from the contemporary RCs found in bacteria and chloroplasts. In porphyrin photochemistry the primary charge separation leaves the originally excited porphyrin in the reduced state, whereas in contemporary RCs the primary charge separation leaves the originally excited chlorophyll in the oxidized state (Mauzerall 1977). In such a primitive photosystem all the reactions take place in aqueous solution, whereas in contemporary RCs the reactions take place between donor and acceptor molecules anchored to proteins that are embedded in membranes. The primitive photosystem might have functioned in the prebiotic phase of evolution, but something more complicated would have been necessary for the common ancestor of the Bacteria and the Archaea – something like protoporphyrin IX, which is two steps further along in the biosynthetic pathway to chlorophyll. (Protoporphyrin IX is not appreciably water soluble, but is rather lipophilic.) A more sophisticated RC (Olson and Pierson 1987b) based on a lipophilic porphyrin, an Fe–S center, a quinone, and a few simple proteins might have been able to transfer an electron across the cytoplasmic membrane from an external electron donor to an internal low potential electron acceptor.

According to the second school, RCs evolved from the membrane-spanning cytochrome *b* of the cytochrome bc_1 complex (Meyer 1994). The cytochrome *b* protein contains eight hydrophobic segments and two heme binding sites each with two histidine ligands. Each subunit could therefore have provided binding sites (histidines) for four chlorophylls. Cytochrome *b* from any extant photosynthetic organism contains seven or eight transmembrane helices, and the cytochrome b_6f complexes in *Chlamydomonas reinhardtii* and in *Synechocystsis* PCC6803 contain about one molecule of Chl *a* per molecule of cytochrome *f* (Pierre et al. 1997; Poggese et al. 1997). However, the related cytochrome bc_1 complexes from anoxygenic organisms do not contain any chlorophyllous pigments, and so this pigment may have been added at a later time for photoprotection instead of being a remnant of an ancient trait.

Nitschke et al. (1998) pointed out that cytochrome *b* and the Rieske 2Fe–2S protein, essential components of cytochrome *bc* complexes, are found in both Bacteria and Archaea. They proposed that the evolution of a primitive cytochrome *b*/Fe–S complex occurred in the common ancestor of Bacteria and Archaea before the evolution of photosynthesis in bacteria. According to this scenario the first photochemical RC would have been integrated into an existing respiratory electron transport chain.

Xiong and Bauer (2002a) have further identified sequence and structural homology between the cytochrome *b* subunit of the cytochrome bc_1 complex and RC2 core polypeptides (L, M, D1 and D2). The sequence similarity appears in a membrane-spanning region (helices B, C and D) in cytochrome *b* that contains ligands for binding hemes and quinone with a similar membrane-spanning region (helices B, C and D) in the RC2 core polypeptides that contain ligands for (bacterio)chlorophylls, (bacterio)pheophytin and a non-heme iron. Three of the four heme ligands in cytochrome *b* are conserved in the RC2 polypeptides that form ligands to (bacterio)chlorophylls and the non-heme iron. The overall helical structure within helices B, C or D of the two groups of proteins is roughly similar. Although there is less sequence similarity between cytochrome *b* and the RC domain of RC1-type reaction centers, the similarities between cytochrome *b* and RC polypeptides are consistent with a possible common origin, although the evidence for this is weak. Yanyushin (2002) has shown that *Chloroflexus aurantiacus* almost certainly does not contain a cytochrome bc_1 complex. How this observation relates to the possibility that cytochrome *b* was the precursor of the RC is not yet clear.

A variety of evidence, primarily comparative biochemical and biophysical analysis, suggested that purple bacterial reaction centers (RC2) and Photosystem (PS) II RCs from oxygenic organisms are similar in overall structure and mechanism, excepting of course the oxygen evolution complex (Nitschke and Rutherford 1991; Blankenship 1992). Similar arguments were made for the reaction center (RC1) of green sulfur bacteria (later also including heliobacteria) and PS I (Olson et al. 1976). The evidence in favor of these relationships has continued to mount, including sequence comparisons (Michel and Deisenhofer 1988), culminating in detailed structural comparisons of PS II and the purple bacterial reaction center. Further structural comparisons have suggested that even the RC1 and RC2 reaction centers share a common ancestor (see below). A recent review by Heathcote et al. (2002) summarizes this evidence.

An evolutionary relationship between RC1 and RC2 was first suggested by Robert and Moenne-Loccoz (1989, 1990) and Margulies (1991). While studying RC1 in heliobacteria, Vermaas (1994) discovered a strong similarity (33% identity and 72% similarity) between helix VI of the large subunit and helix VI of CP47, a core antenna protein of PS II in cyanobacteria and chloroplasts. Actually a moderate similarity (19–22% identity) extends over the entire N-terminal half (helices I–VI) of PshA and the corresponding parts of CP47 or CP43, a related core antenna protein of PS II. These similarities suggest an evolutionary linkage between PshA and PS II of cyanobacteria and chloroplasts. Mulkidjian and Junge (1997) extended the approach of sequence analysis to align RCs, core CPs of PS II, and light-harvesting (LH) complexes of green filamentous bacteria and proteobacteria. These alignments are shown in Figure 1. Useful conclusions are that: (1) both α- and β-polypeptides of LH1 and LH2 are similar to helix D of subunit L of bacterial RC2; (2) all five membrane-spanning helices of L, M, D1 and D2 subunits of RC2 have counterparts in RC1 with pairwise

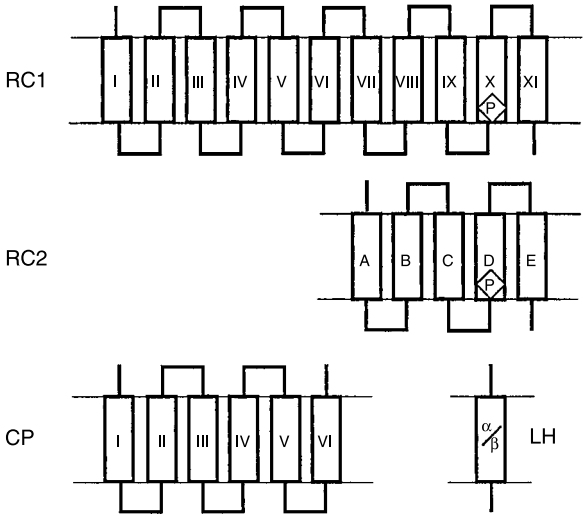

Figure 1. Homology of transmembrane helices in reaction center subunits and chlorophyll proteins. RC1 represents the RCs of heliobacteria, green sulfur bacteria and PS I. RC2 represents the RCs of proteobacteria, green filamentous bacteria and PS II. CP represents CP47 and CP43 of cyanobacteria and chloroplasts. LH represents the antenna complexes of proteobacteria and green filamentous bacteria. Homologous transmembrane helices are aligned vertically. (Reprinted with permission of Data Trace Publishing Company. Chemtracts Biochem Mol Biol 12: 468–482, © 1999.)

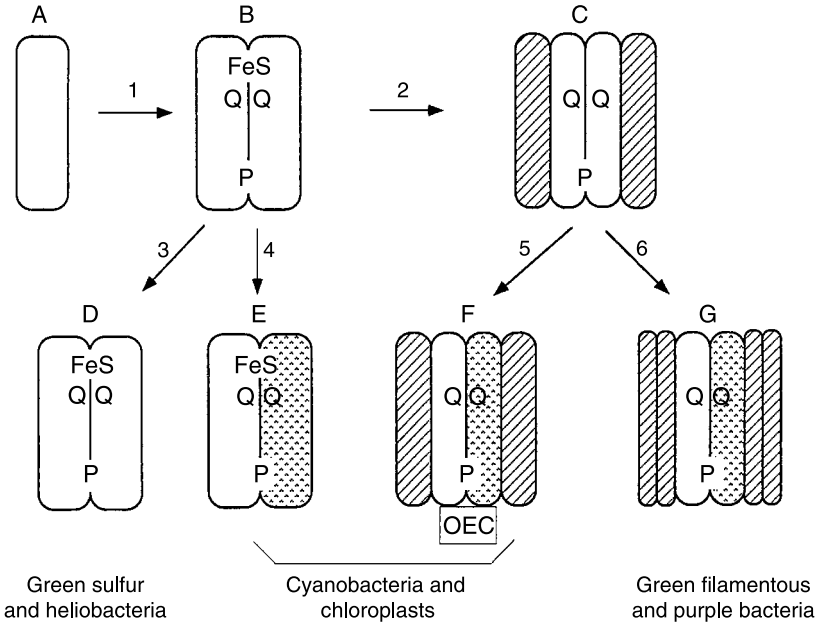

Figure 2. Hypothetical history of reaction center evolution based on protein homology. See text for details. (Reprinted with permission of Data Trace Publishing Company. Chemtracts Biochem Mol Biol 12: 468–482, © 1999.)

identity values in the range of 25–40%; and (3) RC1, RC2, CP and LH probably share a common ancestor. [Vermaas (2002) has proposed that a small number of one-helix ancestral proteins may have given rise to the various reaction center and antenna proteins shown in Figure 1.]

Schubert et al. (1998) developed a 4 Å structural model of PS I showing that PsaA and PsaB each consist of an N-terminal antenna binding domain and a C-terminal RC domain. A structural comparison of the RC domain of PS I and bacterial RC2 support the hypothesis that the two RC types (1 and 2) share a common evolutionary origin.

Selective loss model

Vermaas' (1994) interpretation of the sequence data combined with the Olson–Pierson view of the evolution of RCs (Olson 1970; Olson and Pierson 1987a, b) is shown in Figure 2. The first major evolutionary accomplishment was the creation of a homodimeric RC1 (shown as B in Figure 2), in which two chlorophylls could join to form a special pair and four cysteine residues could bind a single Fe–S center (F_X). This RC1 (B) is assumed to be the ancestral RC for all subsequent forms. The next major evolutionary changes (step 2) were a gene duplication, the loss of the Fe–S center, and the splitting of one large subunit gene into two smaller genes, one coding for CP and the other for homodimeric RC2 (shown as C in Figure 2). In step 4 there was a gene duplication and divergence that resulted in a heterodimeric RC1 (shown as E in Figure 2) containing PsaA and PsaB. In step 5 there were two gene duplications and divergences that resulted in CP43 and CP47 bound to heterodimeric RC2 (shown as F in Figure 2) containing D1 and D2. According to the Olson–Pierson view, steps 4 and 5 took place only in the cyanobacterial line of evolution and led to the evolution of two photosystems (PS I and PS II) that functioned in series for the reduction of NADP and the simultaneous oxidation of weak electron donors such as $Fe(OH)^+$.

The homodimeric RC1 (B) of heliobacteria and green sulfur bacteria (shown as D in Figure 2) may have evolved (step 3) from the ancestral RC1 (B). The heterodimeric RC2 of proteobacteria and green filamentous bacteria (shown as G in Figure 2) may have evolved (step 6) from the ancestral RC2 (C) by a gene duplication resulting in homologous subunits L and M. The light-harvesting (LH) complexes (α and β subunits) of proteobacteria and green filamentous bacteria are not related to CP but are thought to have evolved from helix D of RC2 (G) together with the loss of photochemical activity.

This interpretation is consistent with evolutionary pressures. The ancestral RC1 (B) was adapted to photoautotrophic photosynthesis in low redox envir-

onments and might well have functioned in a phototrophic bacterium that used reduced sulfur compounds as electron donors for CO_2 fixation (Olson and Pierson 1986). The evolution of RC2 in Figure 2 (C → F) was a response to the gradual increase in the redox level of the environment as the best electron donors for CO_2 fixation were used up. Since many proteobacteria and green filamentous bacteria can function as photoheterotrophs or as respiring organisms in the presence of oxygen, the evolution of these organisms containing RC2 (G) may have been a response to the accumulation of organic material and/or oxygen in the environment. The *modus operandi* for most bacteria containing RC2 is mainly cyclic electron flow that drives ATP production, whereas for most bacteria containing RC1 (D) it is mainly linear electron flow that reduces ferredoxin.

Figure 3 combines the proposed evolution of RCs with the evolution of chlorophyll synthesis genes (Xiong et al. 2000) and the evolution of cytochrome *b* (Schütz et al. 2000). The branching order for the evolution of chlorophyll synthesis genes (Figure 3) is proteobacteria → green bacteria → heliobacteria + cyanobacteria. On the other hand Gupta (2003) has proposed a different branching order for photosynthetic organisms based on non-photosynthetic proteins: heliobacteria → green filamentous bacteria → cyanobacteria → green sulfur bacteria → proteobacteria. It remains to be seen whether these two branching orders can be reconciled.

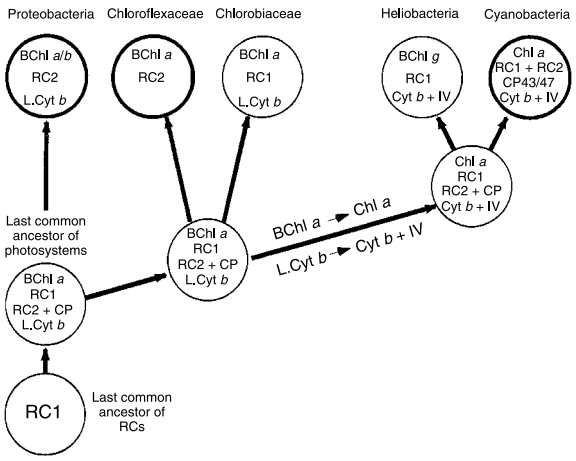

Figure 3. Proposed phylogenetic tree (selective loss model) for the evolution of BChl/Chl biosynthesis enzymes, chlorophylls, RC polypeptides and cytochrome *b* according to Olson (2001). Chlorobiaceae = green sulfur bacteria; Chloroflexaceae = green filamentous bacteria. (Reprinted from Photosynth Res 68: 91–111, © 2001.)

Fusion model

Mathis (1990), Blankenship (1992), Meyer et al. (1994, 1996), Xiong et al. (1998) and Xiong and Bauer (2002a, b) all disagree with Olson and Pierson (1987a, b) in one fundamental aspect. They propose that RC1 and RC2 evolved independently in different organisms, and then were brought together in one organism by genetic fusion to produce the cyanobacterial line of photosynthesis with two photosystems in series. Mathis (1990) proposed an evolutionary tree in which genes for RC1 were transferred from a 'heliobacterium' to the genome of a 'purple bacterium' already possessing genes for RC2. The resulting fusion became the forerunner of the cyanobacteria. Blankenship (1992) proposed that the ancestral RC evolved in two directions simultaneously as shown in Figure 4. One direction led to the evolution of RC2, and the other direction led to RC1. The L/M line to RC2 probably diverged from the D1/D2 line to RC2 before L and M (and D1 and D2) diverged from each other (Blankenship 1992). This implies that RC2 was a homodimer at the time of RC1 and RC2 divergence, after which RC1 and RC2 co-evolved in the same organism as the result of a genetic fusion between two organisms, each carrying RC1 or RC2. The cyanobacteria are the descendants of the fusion organism, and the proteobacteria are the descendants of the line

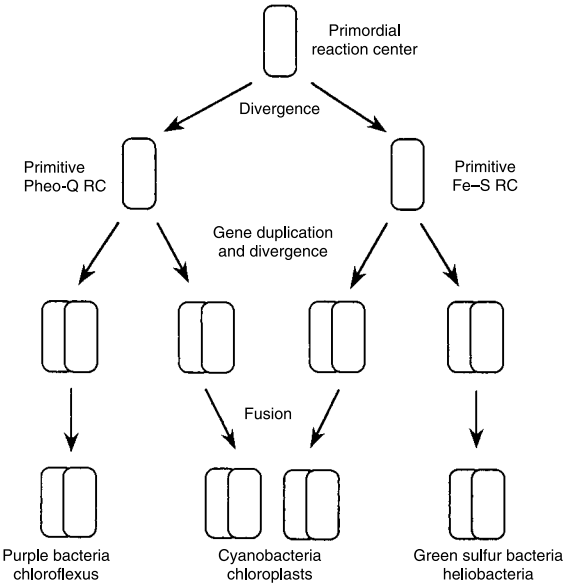

Figure 4. Evolution of photosynthetic RCs according to Blankenship (1992). (Reprinted from Photosynth Res 51: 91–111, © 1992.)

containing RC2. The green sulfur bacteria and heliobacteria are descended from the line containing RC1. [An evolutionary tree based mainly on cytochrome c_2 and high-potential iron protein (Meyer et al. 1996) more or less agrees with Blankenship's view.] The main problem with this fusion model is that the evolutionary pressures driving the various steps were not specified in the original formulation. However, Blankenship (2002) has since suggested that PS II and the OEC may have developed in an organism that lacked PS I but could still reduce CO_2 by reverse electron driven by PS II. [Greenbaum et al. (1995, 1997) thought that they had isolated CO_2-fixing mutants of *Chlamydomonas* that lacked PS I, but it turned out that their mutants contained small amounts of PS I.] The genetic fusion of such an organism and one containing PS I relatively early in the evolution of photosynthesis would have substantially increased the efficiency of CO_2 fixation. This fusion hypothesis predicts different evolutionary histories for RC1 and RC2 and is consistent with the evolution of BChl *a* before Chl *a*.

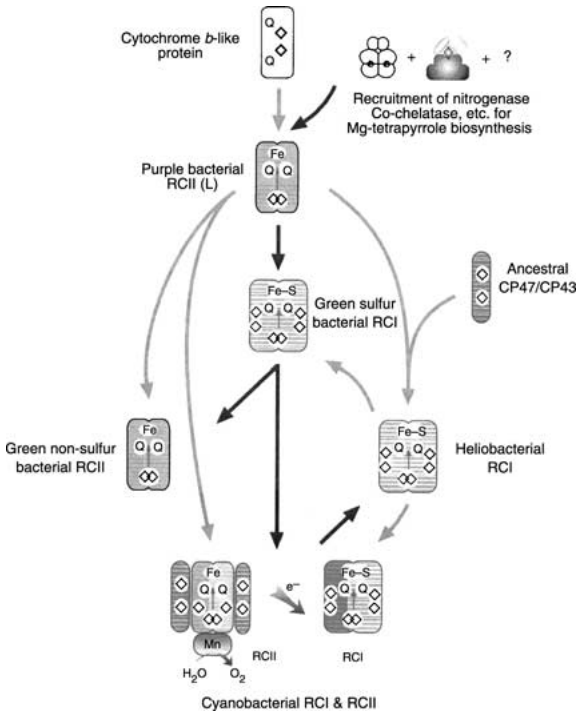

Figure 5. Evolution of photosynthetic RCs based on combined phylogenetic information for both RC apoproteins and Mg-tetrapyrrole biosynthesis enzymes according to Xiong and Bauer (2002b). Dark arrows indicate pigment biosynthesis; light arrows indicate RC apoproteins. See text for details. (Reprinted with permission of Annual Reviews. Ann Rev Plant Physiol 53: 503–521, © 2002.)

The most recent version (Figure 5) of the fusion hypothesis has been developed by Xiong and Bauer (2002a, b). In their scheme RC2 (purple bacterial/proteobacterial) evolved from a cytochrome *b*-like protein. This RC2 then evolved along three different pathways. In the first pathway RC2 (purple bacterial) evolved into RC2 (green filamentous bacterial). In the second pathway RC2 (purple bacterial) developed into RC2 (cyanobacterial), and in the third pathway to RC1 (heliobacterial) through a fusion of the gene for RC2 (purple bacterial) and the gene for an ancestral CP47/CP43 protein. From RC1 (heliobacterial) there is one evolutionary pathway to RC1 (green sulfur bacterial) and another to RC1 (cyanobacterial). The net result is that the cyanobacteria got RC1 from a 'heliobacterium' and RC2 from a 'proteobacterium'. The evolutionary pressures driving the various pathways were not specified. However, an important conclusion was drawn, that the evolutionary history of RC proteins is different from the history of pigment-synthesizing enzymes. This conclusion suggests that many of the components of the photosynthetic apparatus may have different origins. This has been independently shown to be the case for the overall genomes of photosynthetic prokaryotes, in which large scale horizontal gene transfer events have shaped the evolutionary histories of these organisms (Raymond et al. 2002, 2003b).

Antennas

LH1 and LH2 antenna complexes

LH1 complexes are found in proteobacteria and green filamentous bacteria, whereas LH2 complexes are found only in proteobacteria (Blankenship 2002). The LH1 complex consists of ca. 16 α–β subunits in the shape of a large ring which may surround the RC complex like a donut. An *in vitro* subunit of LH1 consists of a heterodimer of α and β peptides and two BChl *a* or *b* molecules (Loach and Parkes-Loach 1995). LH2 is built up from subunits consisting of a heterodimer of α and β peptides along with three molecules of BChl *a* or *b* and one molecule of carotenoid. These subunits aggregate into larger complexes in which eight or nine subunits assemble into ring-shaped units of ca. 65 Å diameter (Blankenship 2002). The α and β peptides have some sequence similarity and are likely to be related to an ancient single-helix or homodimeric antenna complex (Zuber and Brunisholz 1991).

Since both peptides are similar to helix D of subunit L (bacterial RC2), the presumed ancient single helix or homodimeric antenna complex may have been derived from helix D of an ancient subunit L (Mulkidjian and Junge 1997).

Chlorosomes

Chlorosomes are the characteristic light-harvesting complexes of green filamentous bacteria and green sulfur bacteria (Olson 1998; Blankenship and Matsuura 2003). Chlorosomes are filled with BChl *c, d* or *e* molecules in a highly aggregated state and are appressed to the cytoplasmic side of the cytoplasmic membrane. BChl *c, d* and *e* form a family of chlorophylls (chlorosome chlorophyll) that contain an hydroxyethyl group at position 3. The main esterifying alcohol is farnesol in green sulfur bacteria and stearol in green filamentous bacteria, which contain only BChl *c*. In addition to chlorosome chlorophyll all chlorosomes contain a small amount of BChl *a*. BChl *a* is associated with CsmA, a small protein in the envelope of the chlorosome (Bryant et al. 2002; Montaño et al. 2003), whereas chlorosome chlorophyll is located in the interior and is organized in rod-like structures containing little or no protein (Olson 1998).

The chlorosomes from green *filamentous* bacteria (designated F-chlorosomes) are approximately 100 nm long, 20–40 nm wide and 10–20 nm high. Chlorosomes from green *sulfur* bacteria (designated S-chlorosomes) are considerably larger with lengths from 70 to 260 nm and widths from 30 to 100 nm. All chlorosomes consist of a core and a 2–3 nm envelope. The core consists of rod elements made up of aggregated chlorosome chlorophyll, while the envelope is a monolayer of mostly galactolipid and some protein. The rod elements of S-chlorosomes are 10 nm in diameter, whereas those of F-chlorosomes are only 5.2–6 nm. The major protein component in both kinds of chlorosomes is CsmA, with a molecular mass of 5.7 kDa in F-chlorosomes and 6.3 kDa in S-chlorosomes. Although the two types of chlorosomes (F and S) show differences in size and rod element morphology, the organization of the chlorosome chlorophylls appears to be basically similar, and the *csmA*/CsmA genes and proteins appear to be homologous (Olson 1998). This suggests the F- and S- chlorosomes are descended from a common ancestor.

Blankenship (1992) first suggested that green filamentous bacteria may have been the recipient (or donor) of photosynthetic capabilities via lateral gene transfer. Some years later Olson (1998) proposed that the chlorosome was invented by green filamentous bacteria and subsequently transferred laterally to an ancestor of the green sulfur bacteria. Later it was suggested that the genes for the synthesis of light harvesting pigments in both kinds of green bacteria were laterally transferred as a package from an ancestral organism into two ancient non-photosynthetic lineages that evolved into filamentous bacteria and sulfur bacteria respectively (see Figure 3) (Olson 2001). Raymond et al. (2002), on the basis of a whole-genome analysis of *Synechocystis* sp. PCC6803 (cyanobacterium), *C. aurantiacus* (green filamentous bacterium), *Chlorobium tepidum* (green sulfur bacterium), *Rhodobacter capsulatus* (proteobacterium) and *Heliobacillus mobilis* (heliobacterium), concluded that *Chloroflexus* may have acquired phototrophy by multiple lateral gene transfers. One transfer may have imported the chlorophyll biosynthesis and chlorosome genes, and another the reaction center genes.

FMO protein

The Fenna–Matthews–Olson (FMO) protein is an unusual, water soluble chlorophyll protein found only in green sulfur bacteria (Blankenship and Matsuura 2003). It functions as an excitation transfer link between the chlorosome and the reaction center. Each subunit (365/366 amino acids) contains 7 BChl *a* molecules wrapped in a string bag of protein consisting of 15 strands of β sheet, 6 short lengths of α helix and a few regions of irregular conformation (Matthews et al. 1979; Li et al. 1997). It has been a real mystery where the FMO protein came from, but Olson and Raymond (2003) recently suggested a relationship with PscA, based on apparent sequence homology over a 220-residue segment. The inference is that the FMO protein and PscA share a common ancestor that was an RC protein.

CP43 and CP47

The core antennas CP43 and CP47 (Vermaas et al. 1987; Chisholm and Williams 1988) are Chl *a*-binding proteins closely associated with RC2 in cyanobacteria and chloroplasts (there is always one CP43 and one CP47 per RC2). These proteins consist of ca. 130 and 190 amino acids respectively, and each contains

10–12 conserved histidines that bind the Chl *a* molecules. Each protein possesses six transmembrane helices with a large hydrophilic domain between the last two helices (see Figure 1). There is a strong similarity between helix VI of CP47 and helix VI of PshA, the RC protein of heliobacteria (Vermaas 1994), and a moderate similarity between the entire N-terminal half (helices I–VI) of PshA and helices I–IV of CP47. As mentioned previously, these similarities strongly suggest an evolutionary connection between PshA and PS II. (See also the discussion under 'Reaction centers'.) CP43 and CP47 clearly have resulted from an ancient gene duplication event. In addition, CP43 has significant sequence similarity to the pcb antenna complexes from prochlorophytes and the isiA antenna complexes (also called CP43′) from iron-stressed cyanobacteria (LaRoche et al. 1996).

Phycobilisomes

Cyanobacteria and red algae contain supramolecular light-harvesting complexes called phycobilisomes, that are attached to the stromal side of the photosynthetic membrane (Blankenship 2002). These complexes can transfer excitation energy to the core complex (CP43, CP47 and RC2) with more than 95% efficiency. The phycobilisomes consist of related pairs of α and β phycobiliproteins (allophycocyanin, phycocyanin and sometimes phycoerythrocyanin and phycoerythrin). The phycobiliproteins are pigment proteins that have evolved by multiple gene duplications and divergences from an ancestral form that could form short rods (Grossman et al. 1995). The phycobiliproteins also appear to be distantly related to the globin family of proteins (Schirmer et al. 1985; Kikuchi et al. 2000).

A comprehensive review on the evolution of antennas has recently been published (Green 2003).

Transition from anoxygenic to oxygenic photosynthesis

The most ancient form of anoxygenic photosynthesis may well have used reduced sulfur compounds as electron donors for CO_2 fixation (Olson and Pierson 1986). The evolutionary pressure for the utilization of ever weaker electron donors as the environment become more oxidizing selected for a second photosystem (PS II), able to utilize weaker electron donors than could be utilized by PS I alone (Olson 1970, 1978).

One of us (Olson 1970, 1978) thought that significant quantities of hydrogen, hydrazine, hydrogen peroxide and hydroxylamine might have been produced by UV radiation from the sun, and that hydrazine and hydroxylamine might have served as early electron donors to PS II. It was further postulated that NO and NO_2^- might have served as intermediate electron donors. However there is no evidence to support these ideas, and there is no longer any serious consideration of nitrogen compounds as important to the evolution of the oxygen-evolving complex (OEC) of PS II (Olson 2001).

The most widespread source of reducing power in late Archean and early Proterozoic (2.9–1.6 Ga) seawater was ferrous iron (0.1–1.0 mM) (Walker 1983), and several authors (Olson 1978; Cohen 1984; Olson and Pierson 1987a; Pierson and Olson 1989) proposed that ferrous iron may have been an early electron donor to PS II. The common ancestor of proteobacteria and cyanobacteria might well have used $Fe(OH)^+$ as the principal electron donor for CO_2 fixation (Widdle et al. 1993; Ehrenreich and Widdle 1994; Heising and Schink 1998). Because contemporary green sulfur bacteria utilize $Fe(OH)^+$ rather poorly (Heising et al. 1999), one of us (Olson 2001) proposed that the driving force for the evolution of RC2 in addition to RC1 was the necessity to utilize $Fe(OH)^+$ effectively for CO_2 fixation in the absence of reduced sulfur compounds. However, some contemporary proteobacteria can utilize $Fe(OH)^+$ for CO_2 fixation with RC2 alone (Widdle et al. 1993; Ehrenreich and Widdle 1994).

Because the reduction of NADP in green sulfur bacteria is driven directly by RC1, the reduction of P^+ ($E_m = +0.24$ V) by $Fe(OH)^+$ ($E_m = +0.1$–0.2 V) (Heising et al. 1999) should be very slow because of the small difference in redox potential between the two. This might explain why $Fe(OH)^+$ is a poor electron donor for organisms containing only RC1. On the other hand $Fe(OH)^+$ would have been a good electron donor for P^+ ($E_m = +0.4$–0.5 V) in RC2. Linear ET through RC2 and RC1 in series (as in the Z scheme) combined with cyclic ET around either RC2 or RC1 could have been a possible mechanism for the reduction of NADP and the generation of ATP, required for CO_2 fixation by the Calvin cycle enzymes (Olson 2001). The absence of RC1 in proteobacteria does not significantly affect the quantum efficiency of the overall process (Olson 2001). However, the need to utilize $Fe(OH)^+$ may have led to the evolution of RC2 in organisms containing RC1. The further evolution of

RC1, RC2 and the OEC in cyanobacteria, according to this scheme, appears to have been driven by the necessity to replace $Fe(OH)^+$ with water as electron donor.

Several authors (McKay and Hartman 1991; Bader 1994; Rutherford and Nitschke 1996; Samuilov 1997) have suggested hydrogen peroxide (H_2O_2) as an early electron donor to PS II. Blankenship and Hartman (1998) recently proposed that H_2O_2 may have been a transitional donor and that the current OEC may be structurally related to Mn-containing catalase enzymes. The oxidation of H_2O_2 to O_2 has a redox potential of +0.27 V, slightly higher than that for the oxidation of Fe^{2+} to Fe^{3+} (Heising et al. 1999). The proposed photosynthetic oxidation of H_2O_2 might be expected to be similar to the oxidation reaction catalyzed by catalase in the breakdown of H_2O_2 to water and oxygen. Blankenship and Hartman (1998) further observed that Mn catalases have a binuclear Mn center that is structurally similar to half of the proposed tetranuclear center of the OEC (Dismukes 1996) and that under certain conditions the OEC can act as a catalase. These authors suggested that an RC2-like complex associated with an Mn catalase might have been able to evolve oxygen using H_2O_2 as electron donor. The dimanganese center is thought to have evolved to the four-manganese center of the modern OEC for the extraction of electrons from water. [Abiotically formed Mn bicarbonate complexes may have been the source of the Mn cluster of the OEC (Baranov et al. 2000; Dismukes et al. 2001).] When Blankenship and Hartman (1998) made their proposal, there was no evidence for a significant concentration of H_2O_2 ever existing on the Earth, but recently Borda et al. (2001) showed that pyrite-induced H_2O_2 formation from H_2O might well have taken place in the absence of oxygen on the early Earth.

A mechanism for oxidizing ferrous iron may have evolved in the common ancestor of proteobacteria and cyanobacteria sometime before 3 billion years ago. Ferrous iron may have been the electron donor for photosynthetic CO_2 fixation for about 1 billion years until Chl *a* replaced BChl *a* in the RCs and the 4-Mn OEC was invented. The 3.5 Ga 'cyanobacteria' discovered by Schopf and others (Schopf and Barghoorn 1967; Schopf and Walter 1983) may have carried out a ferrous iron-dependent photosynthesis that produced ferric iron. We suppose that this photosynthetically produced ferric iron, together with ferrous iron, may have been responsible for the magnetite ($Fe^{2+}Fe_2^{3+}O_4$), hematite ($Fe_2^{3+}O_3$) and siderite ($Fe^{2+}CO_3$) found in the banded iron formations formed between 3 and 1.7 billion years ago (Olson 2001). When the deposition of banded iron formations ended about 2 billion years ago (Walker 1983), the supply of ferrous iron in the sea may have been depleted to such an extent that H_2O_2 and then water replaced ferrous iron as the electron donor for photosynthesis in the cyanobacterial line of evolution. Although it is still a controversial subject, the majority view of geologists is that the atmosphere started to build up significant quantities of molecular oxygen by about 2.2.–2.4 Ga (Catling and Zahnle 2002). The ability of organisms to produce O_2 through oxygenic photosynthesis had unquestionably developed by that time, although as discussed above, a variety of processes may have prevented the accumulation of free oxygen until long after it was invented.

Endosymbiosis

This theory for the origin of chloroplasts was proposed by Schimper and Meyer in the 1880s, and further developed by Mereschkowsky early in the last century (Blankenship 2002). However, Margulis (1970) was largely responsible for its general acceptance in the 1970s. The strongest evidence for the endosymbiotic origin of chloroplasts comes from comparative genomics of chloroplasts and cyanobacteria. In almost all cases where sequence comparisons have been made, chloroplast genes cluster with homologous genes from cyanobacteria in a phylogenetic tree. The available evidence places the appearance of eukaryotic photosynthetic microorganisms (and chloroplasts) at about 2 Ga (Han and Runnegar 1992; Knoll 1992). Whether all classes of chloroplasts have derived from a single primary endosymbiotic event or instead multiple events has long been discussed. The majority of evidence presently available suggest that a single primary endosymbiotic event is sufficient to explain the distribution of chloroplasts, but the evidence is still very fragmentary on this point and it is nearly impossible to distinguish between a single endosymbiotic event versus several closely spaced independent events. The primary endosymbiotic event is distinct from secondary endosymbioses, in which a eukaryotic alga was incorporated into another cell. This has clearly happened on multiple occasions giving rise to many of the different groups of eukaryotic algae (Palmer and Delwiche 1996). The evolution of chloroplasts from cyanobacteria led to many important

structural changes, but the basic chemistry of oxygenic photosynthesis remained essentially unchanged.

Concluding remarks

This paper traces the history of thinking on how photosynthesis originated and developed, from primitive cells through anoxygenic photosynthetic bacteria, through cyanobacteria and eventually to chloroplasts. It is now clear that earlier attempts to reconstruct 'the path' of evolutionary development were doomed to failure. There have been many such paths that different parts of the photosynthetic apparatus have followed, which have not been the same in either of two classes of organisms or even in different parts of the same organism. Thus there is not one linear path that was followed. The much more complex and non-linear history that has taken place is challenging and interesting to unravel, and will undoubtedly keep scientists occupied for at least another 30 years.

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