Chapter 18

The Evolution of Chlorophylls and Photosynthesis

Anthony W. D. Larkum*
School of Biological Sciences, Building AO8, University of Sydney, NSW 2006, Australia

Summary

Photosynthesis evolved very early on the Earth, but after respiration, and probably after the metabolic processes for methanogenesis and sulfur oxidation. This occurred in ancestors of anoxygenic photosynthetic bacteria. An ancestral reaction center of Photosystem I and II (RCI/II) type of photosynthesis arose in which a five membrane-spanning helix (MSH) protein bound two molecules of chlorophyll (Chl)/bacteriochlorophyll (BChl) in a special pair and had a Chl/quinone primary acceptor, and this protein fused, early on, with a six MSH antenna protein. Logic suggests that the earliest photopigments were protoporphyrin IX, followed by Mg protochlorophyllide *a*, followed by Chl/BChl. It is not clear whether Chl or BChl came first. The evolution of the modern RCI type occurred later but it is not clear under what selection pressure it arose, possibly when ferric salts and sulfur compounds became more available in the Proterozoic Eon.

*Email: Alark@mail.usyd.edu.au

Bernhard Grimm, Robert J. Porra, Wolfhart Rüdiger and Hugo Scheer (eds): Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications, pp. 261–282.

The revolutionary water-splitting mechanism that liberated molecular oxygen into the environment evolved later than earlier supposed, around 2.8–3.0 gigayears ago (Ga). This occurred in chloroxybacteria, precursors of Cyanobacteria, and defined as organisms possessing Chl *a* and a water splitting apparatus. It is probable that chloroxybacteria arose with the fusion of an RCI and RCII type RCs: RC Fusion Hypothesis. During the Proterozoic Eon, chloroxybacteria diversified with the development of light-harvesting based on Chl *a, b, c* and *d* and on phycobiliproteins. The Cyanobacteria with phycobiliproteins and phycobilisomes are seen as a late development, as is the evolution of Chl *d.* Because of many sinks, molecular oxygen remained negligible in the oceans and the atmosphere until about 2.3 Ga. During the period from 2.3 Ga to 1.0–0.5 Ga, oxygen levels remained low (1% present atmospheric level). Oxygen was at first toxic to most organisms but detoxification mechanisms that evolved must have been quickly gained by the first eukaryotic cells and this opened the way for the evolution of the first mitochondriate protists. Eukaryotic algae arose by about 1.5 Ga, by endosymbiosis of chloroxybacteria in mitochondriate protists.

I. Introduction

Not withstanding the theory of punctuated equilibria (Eldredge and Gould, 1972) and the recent evidence for widespread lateral gene transfer in Bacteria and Archaebacteria (Gogarten et al., 2002; Woese, 2002; Rivera and Lake, 2004) the modern synthesis on evolutionary theory still suggests that evolution occurs by small steps. This is even more true for the evolution of enzymatic pathways. In a previous review of Chl evolution (Larkum, 1991) it was noted: 'It is perhaps surprising to note how little Darwinian evolutionary theory has entered into the thinking on Chl evolution. Thus in considering the evolution of Chl it is reasonable to suggest that in broad terms its evolution is recapitulated in the biosynthetic pathway of Chl *a* and BChl *a.*' However it was also noted that a contrary view has been taken (Horowitz, 1945) — that evolutionary pathways could be built from the top down—and this alternative has been augmented recently by a patchwork theory (Copley, 2000). The recapitulation hypothesis for Chl evolution was proposed by Granick (1957), but has gained little acceptance, with the notable exceptions of Olson (1970, 2000) and Mauzerall (1973,1978); perhaps, because of the difficulty of gaining evidence on events that occurred more than 3 gigayears ago (Ga), but also because of the emphasis, hitherto, on photosynthetic bacteria, possessing BChl, as the first photosynthetic organisms. However, if one considers the implications of the recapitulation theory, it becomes apparent that the earliest photosynthetic pigments were probably closely related to porphyrins and that Chl *a* may have preceded BChl. Even more surprisingly, it is an easy step to suggest, as Granick (1957) did (in a throwaway line), that Chl *c* or a close relative may have preceded Chl *a*. This is a hypothesis supported below.

Abbreviations: Acc. –Acaryochloris; BChl – bacteriochlorophyll; BPhe – bacteriopheophytin; CAB – chlorophyll *a/b* binding protein; CAC – chlorophyll *a/c* binding protein; CAO – chlorophyll *a* oxygenase; *Cfx. – Chloroflexus*; Chl – chlorophyll; *Chl. – Chlorobium*; D1 – D1 polypeptide of RCII coded by *psbA*; D2 – D2 polypeptide of RCII coded by *psbB*; Ga – giga years (10^9 yr) ago; *Hbt. – Heliobacterium*; IR – infrared; LHC – light-harvesting complex; MgDVP – Mg-divinyl-3,8-divinyl pheoporphyrin A_5 monomethyl ester; MSH – membrane-spanning helix; PAL – present atmospheric level; PCB– prochlorophyte Chl *a/b* binding protein; Phe – pheophytin; POR – protochlorophyllide oxido-reductase; RC – reaction center; PS I – Photosystem II; PS II – Photosystem II; RCI – reaction center of PS I; RCII – reaction center of PS II; SSU rRNA – single subunit ribosomal ribonucleic acid; UV-B – ultraviolet B radiation; UVR – ultraviolet radiation

II. The Early Earth and the Origins of Photosynthesis

The Earth formed ~4.5 Ga and very soon after this the moon formed, possibly by a collision of a smaller early planet (Kuhn, 1998). For the next 0.5 giga years the Earth was bombarded by meteors (Owen and Bar-Nun, 2001), which meant not only that the surface was too hot for life to take hold, but which also changed the planet by, i) doubling the water content so that the oceans eventually covered 70% of the Earth's surface, ii) introducing significant amounts of organic material into the Earth's mantle, which were very significant for the start of life on the early Earth, and iii) probably introducing much of the diatomic (gaseous) nitrogen into the Earth's atmosphere. By about 4.0 Ga conditions were becoming more favorable to the evolution of life and by 3.8 Ga, most experts now agree, life had started.

Conditions on this early Earth were as follows: a highly reducing atmosphere, with oxygen levels

at ~0.01% of present atmospheric levels (PAL), a relatively high level of CO_2 of 9 kPa (vs. 0.03 today), relatively high residual hydrogen remaining from Earth formation plus additional amounts from chemical processes in the mantle. Previous predictions of high methane levels seem to have been in error, as it is now thought that early organisms formed methane at a slightly later date. The Sun was, at this stage, in the early phase of its development with a solar radiation of about 80% of today's radiant flux.

It is likely, though difficult to prove, that photosynthetic organisms evolved between the period 3.7–3.5 Ga (Blankenship, 2002). However, the proposal that oxygenic photosynthetic organisms (i.e., organisms that produced oxygen) were present from ~3.5 Ga (Schopf, 1993) is now widely discounted (e.g., Brassier et al., 2002). The oldest substantial evidence for such organisms (here called 'chloroxybacteria', but elsewhere in the literature called 'Cyanobacteria') now comes from chemical 'fossils' in rocks, which are considerably younger, viz. 2.8 Ga, than those of the earlier microfossils (Brocks et al., 1999; Summons et al., 1999). In these circumstances, it seems reasonable to assert that photosynthetic bacteria began to evolve ~ 3.6 Ga, but that chloroxybacteria did not begin to evolve, with a mechanism for splitting water and releasing molecular oxygen, until ~3.0 Ga.

Thus, significant input of molecular oxygen on the Earth would not have occurred until after 3.0 Ga and the rise of oxygen in the atmosphere would have been slow because of: i) the large reservoirs of reducing sediments and gases on the early Earth; and, ii) the slow evolution of an efficient photosynthetic water splitting mechanism, and the spread of the chloroxybacterial organisms in which it evolved (see Section VI). Current evidence suggests that atmospheric and upper ocean levels of oxygen would have risen to only 1% PAL at ~ 2.3 Ga (Kasting, 2001; Bjerrum and Canfield, 2002; Shen et al., 2003) and would have remained at this level until ~1.0–0.6 Ga (Canfield and Raiswell, 1999; Anbar and Knoll, 2002; Kasting and Siefert, 2002).

This establishes a timescale for the further development of organisms on the Earth: the period from 3.0–2.0 Ga would have seen the development of aerobic bacteria and the first primitive eukaryotes, leading to the first mitochondrial eukaryotes by ~1.8 Ga (Knoll, 1999), and then to the first photosynthetic eukaryotes (algae), a short time thereafter.

III Evolution of the Pathway to the Earliest Photosynthetic Pigments

A. The Pathway to Mg-Protoporphyrin IX

An early role of porphyrinogens and porphyrins in the evolution of organisms on the primitive Earth, between 3.8–3.0 Ga, is implicated by the central role of these compounds in the formation of cytochromes, hemes, biliproteins, Chls, phytochrome, F430 and vitamin B_{12} (Hodgson and Ponnamperuma, 1968; Georgopapadakou. and Scott, 1977)(Fig. 1). Porphyrins have been produced by Urey-Miller experiments, using both electrical discharges (Hodgson and Pannamperuma, 1968) and ultraviolet radiation (Szutka, 1965; Hodgson and Baker, 1967; Simionescu et al., 1978) and they have been found in early rocks and in meteorites (Hodgson and Baker, 1964). A surface-catalysed synthesis of porphyrins with high yield, starting from pyrrole and aldehydes, on an aqueous clay suspension, has also been reported (Cady and Pinnavaia, 1978). Such abiotic syntheses are stimulated by cations and by a range of metals that coordinate with the porphyrins. Thus, it is likely that porphyrinogens accumulated on the early Earth by abiotic reactions, which were later augmented by enzyme-catalysed reactions, which could have followed the Shemin or glutamate pathway (Larkum, 1991).

Porphyrinogens are colorless, since they are hexahydroporphyrins, i.e., the closed system of conjugated double bonds that exists around the macrocycle of porphyrins, chlorins and bacteriochlorins is not present. In the modern biosynthetic pathway, the route from the porphyrinogens to the porphyrins is from uroporphyrinogen III via coproporphyrinogen III to protoporphyrinogen IX and then to protoporphyrin IX: the first natural porphyrin pigment (Fig. 1; Table 1; Chapter 1, Scheer; Chapter 13, Yaronskaja and Grimm). Alternatively uroporphyrinogen could be converted to uroporphyrin III, which is also colored (Table 1) and then on to protoporphyrin IX. In contrast to the porphyrinogens, all the relevant porphyrins, from uroporphyrin to protoporphyrin, are colored (Table 1), with intense absorption maxima between 400 and 410 nm and four minor, but characteristic, bands in the green region (Mauzerall, 1973,1978; Treibs, 1973). Since porphyrins are produced in Urey-Miller experiments, it seems very plausible that the first-formed macrocycle, uroporphyrinogen III was oxidized abiotically to a dihydro- or fully oxidized

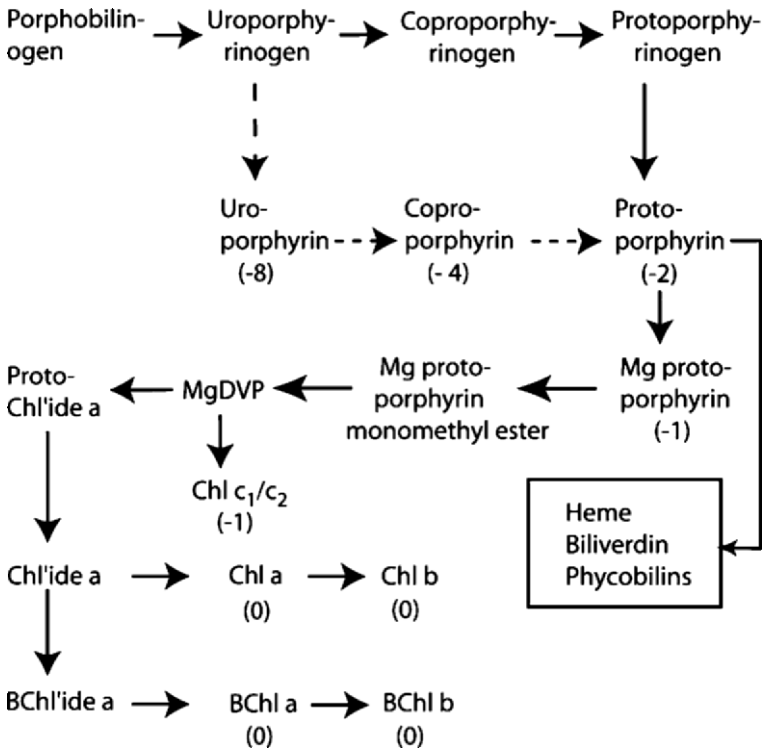

Fig. 1. Outline of the major steps in the biosynthetic pathway of chlorophyll and bacteriochlorophyll synthesis (solid lines) and another possible route for chlorophyll evolution (hatched followed by solid). Numbers in parentheses indicate molecular charge.

uroporphyrin III (Mauzerall, 1960; Mercer-Smith and Mauzerall, 1984). It is possible, therefore, that uroporphyrin took part in an early light-driven reaction (Treibs, 1973; Mercer-Smith and Mauzerall, 1984), as set out below: a reaction that presumably was abandoned long ago by these early organisms. From experimental results of several groups (Krasnovsky, 1971; Runquist and Loach, 1981; Ilani et al., 1989), it can be suggested that an early type of light-driven redox pump could have used porphyrins or porphyrinogens (Fig. 2). Such a mechanism could have been employed, for example, to oxidize organic compounds, to reduce ferric ions and to create a proton gradient (Fig. 2A). This could have been a forerunner of the reaction center (RC).

These ideas have been extended with the suggestion that the photoreaction itself could also have been involved in the oxidation of uroporphyrinogen to uroporphyrin, driven by UV radiation with a wavelength shorter than 230 nm (Mercer-Smith et al., 1985). The self-catalysed photooxidation, it is proposed, was accompanied by the formation of molecular hydrogen, with organic compounds acting as electron donors. With the absence of oxygen in the atmosphere (see above), the amount of UV radiation (UVR, 200–240 nm), penetrating to the Earth's surface would have been relatively high. However, if the recent proposal for high methane levels in the late Archean and early Proterozoic Eons is correct, then a methane haze would have reduced the UVR considerably about the time 3.5–2.7 Ga (Pavlov et al., 2003). Nonetheless, this is well after the period of evolution of porphyrins, and the proposal for UV-driven formation of uroporphyrin in the early Archean is plausible. Other mechanisms such as catalytic action of clays or serendipitous enzymic sites on other proteins or nucleic acids remain a possibility. Furthermore, the early pigments may well have evolved as a mechanism for UV protection (Larkum, 1991; Mulkidjanian and Junge, 1997) and there would have been a strong selection pressure to use this radiation for useful purposes, that is, in an early photoreaction (Fig. 2).

The role of metals in the early light absorption processes is of great interest. A number of metals, abundant in the primitive earth (Fe, Zn), may have

Table 1. Absorption characteristics of chlorophyll, bacteriochlorophyll and their colored biosynthetic precursors (adapted from Larkum, 1991)

Pigment	λ [nm] (ε [mM^{-1} cm^{-1}]) *							Reference
Uroporphyrin III	406 (215)	502 (16)	536 (9)	572 (7)		627 (4)		KM Smith, 1975
Protoporphyrin IX	404 (158)	503 (15)	536 (12)	576 (7)	605 (2)	633 (7)		KM Smith, 1975
Mg-protoporphyrin monomethyl ester	419 (100)	510 (1)	553 (6)	591 (6)				Jones, 1963
Mg-3,8-divinyl pheoporphyrin a_5 monomethyl ester	437 (10)		574 (0.5)		624 (1)			Jones, 1963
Protochlorophyll *a*	432 (102)	438 (137)	533 (4)	570 (8)	602 (7)	622 (22)		Houssier and Sauer, 1970
Chlorophyll *a*	410 (85)	430 (118)	530 (3)	578 (8)	615 (13)	662 (90)		Houssier and Sauer, 1970
Chlorophyll *b*	430 (63)	455 (175)	549 (7)		595 (13)	644 (62)		JHC Smith and Benitez, 1966
Chlorophyll *c* (combined)		447 (227)		580 (21)		628 (51)		JHC Smith and Benitez, 1966
Chlorophyll *d*	399 (81)	455 (80)	550 (7)	595 (9)		698 (88)		JHC Smith and Benitez, 1966
Bacteriochlorophyll *g**	408 (100)	418 (95)	470 (27)	575 (21)			763 (51)	Brockman and Lipinski, 1883
Bacteriochlorophyll *a*	357 (73)	392 (47)		573 (22)			770 (96)	Houssier and Sauer, 1970
Bacteriochlorophyll *b*	368 (86)	408 (77)		578 (26)			794 (106)	JHCSmith and Benitez, 1966

Absorptivity (ε) values are listed beneath the appropriate wavelengths (λ) for the pigments. Solvents, in general, diethylether; dioxane for BChl *g* and chloroform for uroporphyrin.
*For BChl g the values in parenthesis are $\varepsilon/\varepsilon_{max}$ (%)

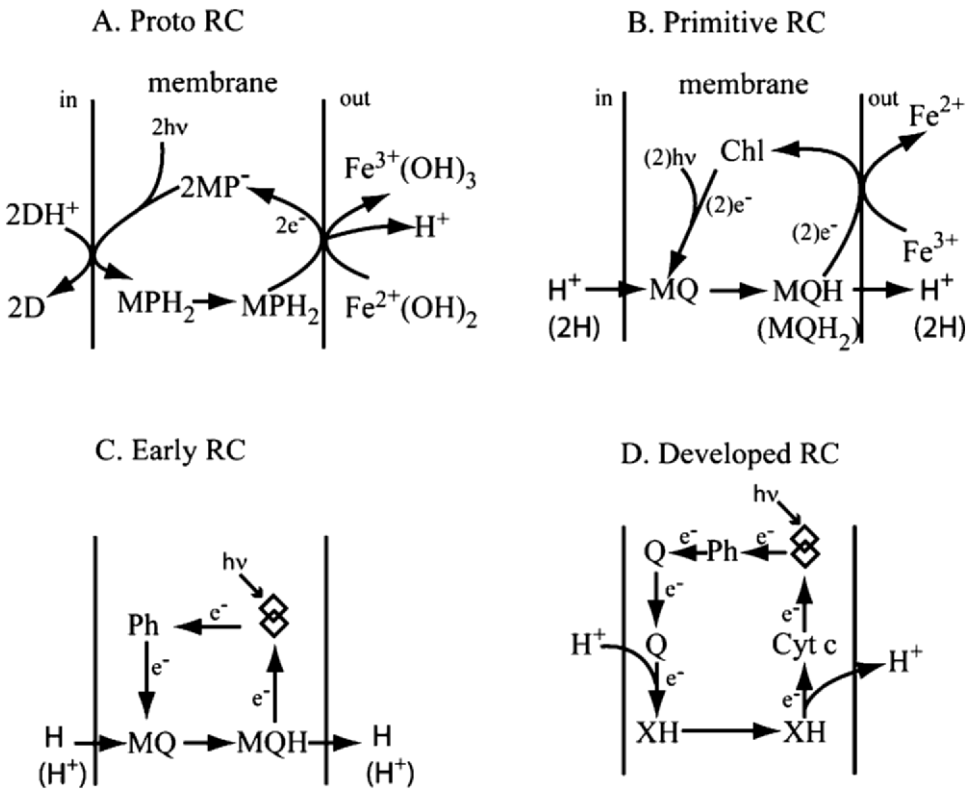

Fig. 2. Possible developments in the evolution of primitive RCs of photosynthesis linked to electron transport across a membrane (adapted from Larkum, 1991; see also Olson, 1999). Cyt *c*, cytochrome *c*: D, hydrogen donor; MP, metalloporphyrin; MQ, menaquinone; Ph, pheophytin *a*; Q, bound quinone; overlapping diamonds, special pair of Chls; XH, membrane-bound hydrogen carrier.

interacted with porphyrins and related compounds to form pigments and complexes that no longer exist. Zinc porphyrins were less successful photoxidizing agents for organic compounds than free-base porphyrins (Mauzerall, 1978; but see Zn BChl, below). However studies with porphyrin-platinum complexes, where the porphyrin and the metal catalyst are held in close association by the molecular complex, but the metal is not complexed within the tetrapyrrole ring, indicate that, with certain porphyrins, rates were higher than with the free-base (Mercer-Smith and Mauzerall, 1984). The nature of the redox reaction changes, however, with the free base: in general, the porphyrin becomes reduced in photoreactions, but this is strongly dependent on the reaction conditions. In the metal complex, as with RC Chl, the porphyrin undergoes photooxidation (Mercer-Smith and Mauzerall, 1984). Thus, evolutionary experimentation with metal complexes having long excited-state lifetimes may have led to the modern type of photooxidation of the photoactive pigment.

The next steps must have led to the formation of copro- and protoporphyrin (Fig. 1), yielding pigments with useful and similar absorption properties in the near-ultraviolet and violet (Table 1), but with greater stability, better redox properties (Mercer-Smith and Mauzerall, 1984) and much less charge [–8 for uroporphyrinogen to –2 for protoporphyrin (Mauzerall, 1978)]. The trend to lower charge is an important consideration for membrane-located systems. For the formation of coproporphyrin a non-enzymatic step could have occurred (Mauzerall, 1978) but light and near UV may also have played a part (Jacobs and Jacobs, 1984). Next, the formation of protoporphyrin IX is problematic since the reaction is thermodynamically unfavorable under non-oxidizing conditions. It has been suggested that an enzyme was needed to carry out specific oxidation of two of the four propionic residues on rings A and B (Mauzerall, 1978) but a non-enzymatic step is not impossible (Jacobs and Jacobs, 1984). Today, all organisms have a single pathway from ALA to protoporphyrin IX, the branch point to protoheme or Chl synthesis (Chapter 13, Yaronskaya and Grimm).

The final step, which differentiates heme synthesis from Chl synthesis, and produces arguably the first

'modern' pigment in photosynthesis (Table 1), is the formation of Mg-protoporphyrin IX, with a spectrum similar to Mg-protoporphyrin monomethyl ester. This step is catalyzed by Mg-protoporphyrin IX chelatase, which is made up of 3 subunits in cyanobacteria, algae and plants, encoded by *chlD*, *chlI* and *chlH*: one product (ChlD) mediates an ATP hydrolysis. This reaction could have occurred in the absence of enzymic catalysts, especially with locally high magnesium concentrations, for example, in clays, but it would have been hard to control the formation of heme vs. that of (B)Chl. Nevertheless, it was a pivotal reaction, which, however slow, would have been selected for because of the favorable spectral properties of the product. Once this was formed the next five steps on the route to (B)Chl formation would have evolved inexorably in the selection of pigments better for absorbing visible light. In this evolution the addition of the fifth isocyclic ring played a key role as discussed by many previous workers (Mauzerall, 1973,1978; Chapter 1, Scheer)

B. Strong Conclusions on the Early Evolution of Photopigments

1. The earliest light-driven reactions must be distinguished from the evolution of modern photopigments and photosynthetic systems (discussed in Section V). This evolutionary phase took place as far back as 3.7 Ga and little hard evidence remains about the evolutionary events involved.

2. Respiration preceded photosynthesis (Castresana and Sarastre, 1995; Castresana, 2001). This provided a firm base for the evolution of heme compounds and ferroprotoporphyrins. Thus some form of early cytochromes would have been in existence before the evolution of the first light-driven reactions

3. The present view is that ultraviolet radiation (UVR) was the driving force in the development of the first organisms to use light-driven reactions. This is contrary to the previous view that UVR was harmful, largely due to damage to DNA, to the development of early organisms (Olson and Pierson, 1987). According to the new view, a primitive type of photoreaction preceded, not only photosynthesis, but might have preceded heme-related respiratory systems, as well.

4. A fourth conclusion, implicit in 2., is that evolution of primitive photoactive pigments (i.e., primitive photosynthesis) occurred after the evolution of the redox-active hemes taking advantage of pigments which evolved initially to protect from UVR.

IV. Evolution of Extant Photosynthetic Pigments and Early Photosynthetic Organisms

A. Evolution of Chlorophylls and Bacteriochlorophylls

1. Evolution of the First Modern Photosynthetic Pigments

There is little doubt that the first effective photosynthetic pigments were Mg-porphyrins, such as Mg-protoporphyrin monomethyl ester. Indeed, it is the chelation of Mg^{2+} at this stage in the biosynthetic pathway that distinguishes this line of development from that of heme formation where $Fe^{2+/3+}$ is involved, the hemes have only very short excited state lifetimes. Alternatively, these Mg-compounds may have been intermediates, naturally formed without the intervention of specific enzymes, on the pathway to what may be viewed as the first true Chl, Mg-3,8-pheoporphyrin a_5 monomethyl ester (Mg-DVP, or Mg protochlorophyllide *a* and is now confirmed as a *c* type chlorophyll). The structure of this pigment has been elucidated (Helfrich et al., 1999) and its presence shown in anoxygenic photosynthetic bacteria, in two prochlorophytes and in primitive green algae such as *Micromonas* spp (Helfrich et al., 1999). It is an easy extension from there to suggest that other Chls *c*, such as Chl c_1 and Chl c_2 arose by small chemical modifications and were used in photosynthesis (Larkum, 1991, 1999). However, the early involvement of any other Chls *c* is made less likely by the absence, so far, of any pigments, other than Mg-DVP, in any prokaryotic photosynthetic organisms. It may be that replacement of Chl *c*, by (B)Chls, i.e., through reduction of a porphyrin to a chlorin or bacteriochlorin, was likely as soon as these evolved, since they are much better pigments (Larkum, 1991, 1992, 1999).

2. Evolution of the Pigments After Mg-Divinyl Pheoporphyrin

The evolutionary pathway from Mg-DVP to Chl/BChl is unclear. The evidence based on Chl/BChl biosynthesis and other photosynthesis genes is discussed in the Section IV.B. It is certain, however, that oxygenic photosynthetic bacteria, viz. chloroxybacteria, evolved from an ancient group of anoxygenic photosynthetic bacteria. However it is not proven that these earliest anoxygenic forms possessed BChl. Lockhart et al. (1996a) and Larkum (1999) suggested that the earliest enzymes were multifunctional to the extent that they produced both Chl- and BChl-type pigments from protochlorophyllide *a* and only later evolved into their present forms, which make exclusively Chl- or BChl-type pigments. There is enough experimental work to show that either of these pigments would bind to several of today's Chl- or BChl-binding proteins (Larkum, 2003; Chapter 1, Scheer; Chapter 26, Paulsen) and therefore one would expect even greater adaptability in the distant past.

The alternative hypothesis to Chl first is BChl first. While many scientists have adopted this view (Blankenship, 2002), it involves invoking arguments for i) why Chl *a* was not among the first pigments formed (above), and ii) how a BChl system was later converted into a Chl system (Blankenship, 2002).

3. Evidence from Light-Harvesting Characteristics

Larkum (1992) argued as follows: i) that (B)Chl would have been a very prevalent pigment in early photosynthetic bacteria of whatever type; ii) as these (B)Chl-based forms multiplied and were able to compete for most of the light in the violet and red regions of the spectrum, a situation would arise whereby selection pressure would induce the formation of other (B)Chls.)

With hindsight we can say that chloroxybacteria, with Chl, prevailed because: a) with the extended redox spans of Chl vs. BChl, they could use water as a source of electrons and protons; and b) because, of the Chl and BChl pigments, they were most suited to harvest that part of the Sun's spectrum with the highest flux. According to this argument, anoxygenic photosynthetic bacteria were relegated to refugia where: a) oxygen was often restricted; b) where hydrogen sources (H_2, H_2S, organic compounds) were available; and, 3) where the light climate was likely to be stripped of visible light by chloroxybacteria and/or water-quality effects. These were the sites where single-photosystem-photosynthesis, based on BChl, would be selected for and these are still the sites where anoxygenic photosynthetic bacteria are most often found, although some can now survive in aerobic environments, such as the open ocean (Kolber et al., 2001; Rappé and Giovannoni, 2003). Furthermore, we should remember that chloroxybacteria, and their descendants, Cyanobacteria, have competed for the last 1.5 Ga with algal protists and, later, with higher plants, as these came to dominate the terrestrial photosynthetic systems on Earth. As a result it is likely that many former types of chloroxybacteria/Cyanobacteria were pushed to extinction, just as many early anoxygenic photosynthetic bacteria were, and we are now left with a relict population.

4. Evolution of Chlorophylls

The diversity of Chls is discussed by Kobayashi et al. (Chapter 4), Rüdiger and Grimm (Chapter 10) and by Frigaard et al. (Chapter 15). The path of evolution of Chls proposed here is that the original MgDVP (see above) is followed and replaced by Chl *a*. Later Chl *b* and Chls *c* evolved in the light-harvesting antennae (Larkum, 1991) in deeper water or more shaded sites where light capture, especially of blue and green light, was at a premium.

Until recently, there would have been no argument over the primary role of Chl *a* among the Chls, but the discovery of *Acaryochloris (Acc.) marina* (Miyashita et al., 1996) has challenged that view. Chl *d* in *Acc. marina* is present in proportions of ~95 % of the total Chl, with Chl *a* as the only other Chl (MgDVP has not been detected). Chl *d* is the pigment of the special pair of PS I (Hu et al., 1998) and it is possible that it plays an important role in PS II and may form the special pair (Chen et al., 2005b), notwithstanding previous evidence for Chl *a* (Mimuro et al., 1999). Chl *a* and Chl *d* also act in a light-harvesting capacity (Larkum, 2003). Until the biosynthetic pathway of Chl *d* and Chl *b* in Cyanobacteria is clarified (see discussion below) no firm evolutionary conclusions can be made. However it seems more likely that Chl *d* evolved 'late,' in chloroxybacteria, under selection pressure for a particular niche that developed, as algae, Cyanobacteria and anoxygenic photosynthetic bacteria harvested most of the light penetrating algal mats or symbiotic associations such as didemnid ascidians (Kühl and Larkum, 2001), leaving only a window of

light in the 700–740 nm region (Kühl et al., 2005). This is consistent with the view that prochlorophyte Chl *a/b*-binding proteins (PCB), which bind Chl *a* and *b* or Chl *d* are a fairly late development in chloroxybacteria (Zhang et al., 2004). Another possibility, put forward by Blankenship and Hartmann (1998), is that it was one of the intermediates on their proposed evolutionary pathway from BChl-based organisms to Chl-based organisms.

Chl *b* occurs in prochlorophytes (Cyanobacteria), green algae, euglenoids, chlorarachniophytes, charophytes and land plants (Larkum, 2003) and is bound to either a PCB protein, in prochlorophytes, or a Chl *a/b*-binding (CAB) light-harvesting protein in all the others. Chl *c* (c_1, c_2 c_3, etc) is found in a variety of chromophytic algae (Larkum, 2003) and is bound to a Chl *a/c*-binding (CAC) light-harvesting protein. MgDVP is bound either to PCB in prochlorophytes or to CAB proteins in prasinophytes [primitive green algae (Larkum, 2003)]. Divinyl Chl *a* and divinyl Chl *b* are found in deep water strains of the prochlorophyte *Prochlorococcoccus marinus* (Partensky and Garczarek, 2003) and this seems to be an adaptation to match the spectra of these forms to the prevailing blue light at depth in the ocean (Ting et al., 2002). It is accomplished by deletion of the gene for reduction of the divinyl group at position 5.

Chl *b* almost certainly arose in chloroxybacteria and was passed on to Cyanobacteria. Larkum (1992) proposed that Chl *b* arose as one of the earliest forms of accessory Chls, well before phycobiliproteins evolved, in response to selection pressure to increase light harvesting in the visible light spectrum.

Since Raven (1996) showed that the addition of MgDVP would make a significant difference to the light harvesting capacity of a unicellular organism with Chl *a* plus Chl *b*, it is clear that the addition of Chl *b* would have made a much greater contribution, since the red peak is so much larger in Chl *b* vs. MgDVP (Table 1). Furthermore, a special role of Chl *b* in binding and stabilizing antenna Chl proteins has been suggested by Hoober and Eggink (2001) and a similar argument was also made for Chls *c* (Hoober and Eggink, 2001). However, Larkum (2003) has pointed out that the contribution of Chls *c* is less obvious than that of Chl *b*; Chl *c* may play some other role, such as facilitating the transfer of resonant energy from carotenoids to Chl *a*. It is important to note that Chl *c* (MgDVP, Chl c_1, Chl c_2, Chl c_3, etc.) is a violet/blue absorbing pigment. Against this is the evidence that in the peridinin Chl complex, no Chl *c* is present, yet the transfer efficiency is very high (Larkum and Barrett, 1983) and the binding is very tight (two Chl *a* and eight peridinin; Hofmann et al., 1996), but this may have more to do with the special chemistry of peridinin, which has a very long excited life time of 150 ps in solution.

Both Chl *b* and Chl *d* have (oxygenated) formyl groups not present in Chl *a*. This oxygenation step might be taken to imply the need for molecular oxygen in the environment (Larkum and Barrett, 1983; but see Raymond and Blankenship, 2004). In the case of Chl *b* it is now known that the methyl group of Chl *a* is oxygenated to a formyl group by a Chl *a* oxygenase (CAO)(Tomitani et al., 1999), which is found in prochlorophytes, green algae and higher plants. However, a gene for CAO is absent in *Prochlorococcus* (Rocap et al., 2003). The biosynthetic pathway for Chl *d* is not known.

From the phylogenetic point of view the big question is why chromophytes adopted the same light-harvesting chlorophyll-binding complex (LHC) as those algae that utilize Chl *b*, but chose a different light harvesting Chl? In the prochlorophytes Chl *a*, Chl *b* and MgDVP are all bound onto a single PCB antenna protein and in eukaryotic micromonads the same pigments are bound onto a CAB antenna protein (see above). Perhaps, the answer is explained most easily by a stochastic event that produced an organism at the prokaryotic level viz. a chloroxybacterium, that possessed only Chl *c* and which gained a gene for CAC by lateral transfer. Larkum (1991,1992) suggested that Chl *c* (viz. MgDVP) was the first modern Chl to evolve in a photosynthetic system, to be rapidly replaced by Chl *a*, but that Chls *c* then took on an early light-harvesting role. However no Chls *c,* apart from MgDVP, have yet been found in Cyanobacteria.

5. Evolution of Bacteriochlorophylls

The diversity of BChls is discussed by Frigaard et al. (Chapter 15). All 'true' BChls have a bacteriochlorin ring structure (i.e., the porphyrin ring is reduced in rings B and D; Chapter 1, Scheer). Based on its widespread occurrence and its proximity to protochlorophyllide *a* on the biosynthetic pathway, it is logical to see BChl *a* as the earliest BChl. The bacteriochlorin macrocycle of BChls is brought about today by the reduction of ring B by three enzymes, coded by the genes, *bchX*, *bchY* and *bchZ*. These genes are clearly homologous to the genes that code for the

light-independent reductase that reduces the D ring to form the chlorin macrocycle from the porphyrin macrocycle; inferences based on these gene sequence similarities are discussed in the next section (Section IV B). However, BChl *a* is not produced simply by the reduction of the B ring: the vinyl group on ring A (of Chl) is replaced by an acetyl group in BChl *a* (Fig. 3). Consequently, it is possible to imagine a number of other schemes (Fig. 2, Larkum, 1991). It has been suggested that BChl *g* and Chl *a* are isomers, which could be more than a coincidence (Margulis and Obar, 1985; Olson and Pierson, 1987; Xiong et al., 2000; Dismukes et al., 2001). Blankenship and Hartman (1998) proposed that Chl *d* (Section IV.A.4) was an evolutionary intermediate between BChl *a* and Chl *a*. While the spectral properties of BChl *b, c, d,* and *e* might make them attractive in terms of evolutionary pathways, their detailed structural properties are not so easy to incorporate into schemes of BChl/Chl evolution (Larkum, 1991). BChl *c, d* and *e* (chlorins) have their Q_y absorption band in the red or near infrared (IR) (Table 1, Chapter 1, Scheer): BChl *a, b* and *g* (bacteriochlorins) absorb in the IR (~773, 794 and 764 nm, respectively), and also have a relatively intense Q_X-band near 580 nm. For BChls *c, d* and *e,* which are Mg-chlorins (Chapter 1, Scheer; Chapter 15, Friegaard et al.) this band is minor (λ_{max} ~ 600 nm). In all cases the Soret band is in the UV-A to violet region of the visible spectrum, so anoxygenic photosynthetic bacteria can use visible light if necessary. However, in the niches in which these bacteria exist (algal mats, muds, sediments of hot springs, etc.), UV and violet light is generally filtered out, so that near IR radiation forms the major energy source, as it does also for *Acc. marina* (see Section IV.A.4). BChls are generally esterified by either farnesol or phytol alcohols, but in the case of BChl *c*, *d* and *e*, many other alcohols can be used (Chapter 1, Scheer).

Further progress in discussing the evolutionary pathways involved should be possible when we have more detail about biosynthetic pathways. This will come when we have more whole genome sequences for the anoxygenic photosynthetic bacteria and for the Chl *d*-containing organism, *Acc. marina*.

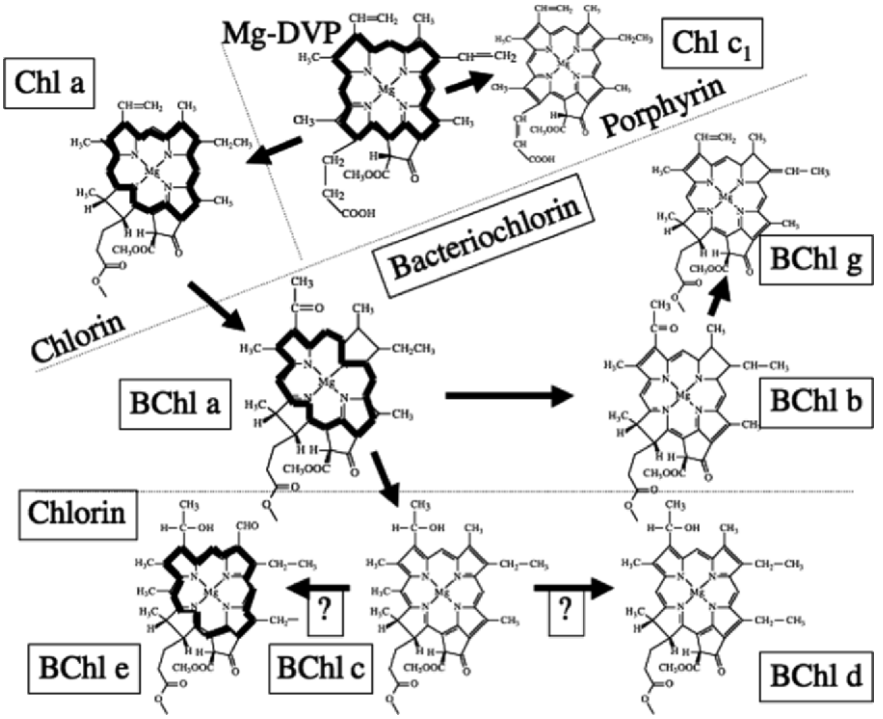

Fig. 3. Chemical structures of chlorophylls and bacteriochlorophylls with indications of possible routes on the evolution of their biosynthesis. Heavy lines indicate the π-bond resonance pathway (these are not shown, for convenience, for all the bacteriochlorophylls, see Chapter 23, Steiner and Fowler). The esterified hydrocarbon tail of various chlorophylls and bacteriochlorophylls is not shown.

B. Molecular Phylogenetic Analyses

The use of gene sequences to reconstruct evolutionary history is a potentially powerful tool, with perhaps its greatest success so far being the theoretical division of life into three domains, Archaebacteria, Bacteria and Eukarya (Woese, 1987). This was done on the basis of the aligned sequence of small subunit ribosomal RNA (SSU rRNA). Despite this success, there is still some doubt about the general applicability of this approach, especially in terms of prokaryote evolution (Rivera and Lake, 2004). Photosynthesis is a particularly good example of the difficulties encountered at the prokaryotic level since, when applied to photosynthetic bacteria, the results are not easily understood (Woese, 1987; Blankenship, 1992): there is no clear evolutionary development, in terms of photosynthesis, of RCI (PS I) or RCII (PS II) anoxygenic photosynthetic bacteria, and the origin of Cyanobacteria is apparently not a development of any of these earlier lines. The conclusion is that there has either been massive lateral transfer of genes and suites of genes across widely separated taxa or the SSU rRNA trees are not reliable, or both. Certainly there is now far-reaching evidence for lateral transfer both from whole genome studies, in a variety of organisms (Gogarten et al., 2002; Woese, 2002; Xiong and Bauer, 2002) and particularly in photosynthetic organisms (Raymond et al., 2002).

Molecular phylogenetic analysis was used to test the question of whether Chl or BChl evolved first. Burke et al. (1993) made tree reconstructions based on the genes coding for the enzymes that reduce porphyrin ring to the chlorin ring and the chlorin ring to the bacteriochlorin ring: this is the light-independent protochlorophyllide oxido-reductase (POR) dark mechanism, not the light-activated POR mechanism that replaces it in higher plants. Photosynthetic bacteria have two sets of genes: *bchL/bchN/bchB* reduce the porphyrin ring D to a chlorin and *bchlX/bchY/bchlZ* reduce the chlorin ring B to a bacteriochlorin. Cyanobacteria, and all descendants of this oxygenic photosynthetic line, algae and higher plants, have just one set of enzymes, differing in detail but homologous to those found in anoxygenic photosynthetic bacteria: *chlL/chlN/chlB* enzymes. All the subunits of these reductases are homologous and show distant homology with *nif* (nitrogen fixation) genes, which Burke et al. (1993) used as the out-group. The latter, using well-founded phylogenetic reconstruction based on maximum likelihood, found that the *bchX* gene was the earliest branch within the Chl/BChl reductases. The authors argued that this meant that the earliest reductases were BChl reductases, suggesting that BChl preceded Chl in evolution.

This view was contested by Lockhard et al. (1996a), who showed that the results of Burke et al. (1993) were not supported when only sites-free-to-vary were analyzed. Lockhart et al. (1996a) argued that there is not enough information in the sequence data to reach a significant conclusion. They also argued that even if the result of Burke et al. (1993) was the correct one, this would not be a definitive indication that BChl preceded Chl: Burke et al. (1993) put forward the hypothesis that an early version of the reductase could carry out both steps (reduction of ring B and ring D). Lockhart et al. (1996a) further suggested that this would mean that both Chl *a* and BChl *a* would have been formed. The final Chl/BChl molecules are formed by esterifying with a phytyl tail, and in the case of BChl after modification of the vinyl group on ring A to a acetyl group (Fig. 3). Only by further evolution, Lockhart el al. (1996a) proposed, were the specific enzymes (*chlL*, *bchX*, *bchN*) evolved. Therefore, it was impossible to decide whether Chl or BChl came first; probably the answer was that both arose together. Thus, it can be argued that Chl may have operated in the earliest photosynthetic mechanism(s) alongside BChl. Perhaps this condition persisted until the rise of chloroxybacteria (Section II). The rise of chloroxybacteria would have placed such strong selective pressure on anoxygenic photosynthetic bacteria that in the latter organisms only BChl was selected (see Section IV.A.3).

More recently it has been possible to assemble all the genes for Chl/BChl reductases (Xiong et al., 2000) and to produce phylogenetic trees of all the major anoxygenic/oxygenic taxa (Xiong et al., 2000; Xiong and Bauer, 2002). The first analyses, based on *bchL*/*chlL* indicated that the purple sulfur bacteria were the oldest photosynthetic group, while the heliobacteria (often proposed as an early evolutionary group) were placed closest to the Cyanobacteria (often placed as a late group). This general arrangement was supported by other genes (*bchH*/*chlH*, *petB*, *bchI*/*chlI*/*bchD*/*chlD* and RCII-type genes). However it should be remembered that the actual taxon and the photosynthetic mechanism that each taxon possesses, cannot be easily connoted, since, as shown by Raymond et al. (2002), it is highly probable that there has been a great deal of lateral gene transfer within the Eubacteria involving large parts

of the photosynthetic apparatus. Therefore, the possession of a particular photosynthetic apparatus does not indicate the taxonomic position of a particular anoxygenic bacterial group. To further complicate the issue, Jermiin et al. (2001) have shown that the analyses of Xiong et al. (2000) are not powerful enough to make a full discrimination of the true tree. Based on an analysis of informative sites, Jermiin et al. (2001) showed that the position of heliobacteria, close to Cyanobacteria, was not firm and, overall, the information in the data available is not sufficient to accurately predict the true tree (also see Gupta et al., 1999; Gupta, 2003).

The recent proliferation of whole genomes has ushered in a new era of phylogenetic analysis, which gives a new impetus to evolutionary studies. Methods for assessing the information in phylogenetic terms are still at an early stage. While, at present, it is possible to see in qualitative terms how genes or suites of genes have been inherited, assessment in reliable quantitative terms, afforded by gene order and gene sequence analysis, is in its infancy (House et al., 2003). For Eubacteria there are many whole genomes at present and the list is increasing rapidly (see http://www.genomesonline.com) although some outstanding omissions from anoxygenic bacteria also exist. To date 14 whole genomes have been sequenced from cyanobacteria, including the recent deep-water *Synechococcus* WH8102 (Palenik et al., 2003), and at least another two for *Prochlorococcus* (Dufresne et al., 2003; Rocap et al., 2003). There are three whole nuclear genomes available for algae (a red (*Cyanidioschyzon*), a diatom (*Thalassiosira*) and a green (*Chlamydomonas*) and others are being done, and as well as a number of plastid genomes (Douglas et al., 2003). In higher plants, the whole genomes of *Arabidopsis thaliana* and rice were sequenced and several others will be completed shortly. This patchy record gives a tantalizing glimpse of the evolution of photosynthetic systems, which is most clear for the anoxygenic photosynthetic bacteria, but as more whole genomes are added the situation should yield many new insights into evolutionary pathways (Rivera and Lake, 2004).

In summary the emerging picture indicates that there has been much lateral gene transfer involving the photosynthetic apparatus (Raymond et al., 2002; Xiong and Bauer, 2002) and, in future, the combined use of sets of genes will also be required. However, if there has been lateral transfer of photosynthesis genes there may well have been lateral transfer of whole suites of genes, thus making a 'natural' classification and phylogeny of bacteria an impossible task, as discussed by Woese (2002) and Gogarten et al. (2002).

C. Some Strong Conclusions on Anoxygenic Photosynthetic Bacterial Evolution

a) The evolution of bacterial photosynthesis was a very early event but was preceded by the evolution of respiratory enzymes.

b) Anoxygenic photosynthetic bacteria preceded oxygenic photosynthetic organisms, but these may not resemble modern photosynthetic bacteria.

c) A homodimeric structure of the RC preceded a heterodimeric structure, but this does not necessarily mean that *Chloroflexus* (*Cfx.* and allies) and *Heliobacter* (*Hbt.* and allies) have the oldest photosynthetic apparatus (even if lateral gene transfer has not occurred).

d) Oxygenic photosynthesis emerged at a later time, that is not accurately known, either by a fusion or fission hypothesis of a reaction center of PS I-type (RCI) and a reaction center of PS II-type (RCII).

e) Chloroxybacteria, an ancient lineage, gave rise to the extant Cyanobacteria (including prochlorophytes and *Acaryochloris* and allies) relatively recently and after much evolution and lateral transfer of genes and suites of genes.

V. Reaction Centers

A. Earliest Reaction Centers

In the strict sense, photosynthesis is a vectorial charge transfer across a membrane causing oxidation on one side and reduction on the other, powered by a Chl-, or, more loosely, a porphyrin-based light reaction. Lake and coworkers (Lake et al., 1985) proposed a group within the bacteria called the photocytes (including the Eubacteria and Halobacteria) on the basis of carotenoid content and light-driven systems. This proposal would mean that a common ancestor gave rise to both light-driven systems. However, this

has not received wide support (Gouy and Li, 1989). Bacteriorhodopsin is a light-driven proton pump (Oesterhelt and Tittor, 1989) that does not involve Chl, and occurs in archaebacteria and an increasing number of eubacteria (Rappé and Giovannnoni, 2003). It has been suggested, for example by Lake et al. (1985), that bacteriorhodopsin may represent a precursor of early photosynthetic mechanisms, but one with less evolutionary potential than the light-driven redox mechanism.

Speculations on the functioning of primitive mechanisms are summarized in Fig. 2. Mauzerall and colleagues (Mauzerall, 1978; Ilani and Mauzerall, 1981; Mercer-Smith and Mauzerall, 1984; Ilani et al., 1989) have explored, over many years, the possible involvement of Chl precursors in such charge transfer reactions. They have also shown that a number of porphyrinogens and porphyrins can carry out rapid vectorial reductions across artificial membranes (Woodle et al., 1987; Ilani et al., 1989). Iron salts might well have been involved as electron acceptors and donors in such systems (Borowska and Mauzerall, 1987) (see Fig. 2A). It has even been suggested that the geologically ancient and abundant Banded Iron Formations may have resulted from abiotic reactions (Cairns-Smith, 1978) as occurs, for instance, when UV radiation acts on ferrous salts to cause reduction of water and the release of hydrogen gas (Borowska and Mauzerall, 1987, 1988). It is also possible that early RCs were harnessed to oxidize ferrous salts and cause the reduction of organic compounds (Borowska and Mauzerall, 1988)(see Fig. 2B) or as a proton pump. Iron may have been the coordinated metal in some of these early RCs, but the poor photoactivity and the strong redox properties of Fe-porphyrins make it much more likely that Mg- or Zn-porphyrins were favored for light (and UV) reactions, and that Fe-porphyrins acted as the intermediary electron carriers from earliest times.

There is now strong evidence for respiration preceding photosynthesis (Castresana and Sarastre, 1995; Castresana, 2001); it is likely that this involved use of nitrates, ferrous salts and a number of other electron sinks, rather than the early use of oxygen on the Earth: since oxygen would have been scarce until photosynthetic oxygen evolution evolved. Nevertheless an important outcome, if this assumption is correct, is that the first photosynthetic organisms could form ATP by a proton motive force mechanism and thus could fix CO_2 by a Rubisco-based or another mechanism (linked to a reductive NAD mechanism as in extant anoxygenic photosynthetic bacteria).

B. Evolution of Modern Reaction Centers

Evolution of the RCs must have led eventually to modern photosynthetic RCs. The crystal structures of the RCI and RCII (Jordan et al., 2001; Zouni et al., 2001; Ferreira et al., 2004) now give abundant evidence for homology between these two RCs. In anoxygenic photosynthetic bacteria no organism has been found with both a RCI and a RCII type of RC, and these organisms are divided into those with a RCI-type and those with a RCII-type mechanism (Baymann et al., 2001; Fyfe et al., 2002). Heterodimers (pairs of similar, but not identical, polypeptides) are a feature of many RCs, including the filamentous photosynthetic bacterium *Cfx. aurantiacus* (Shiozawa et al., 1989). Of the RCI-types, however, two RCIs are homodimeric: *Hbt. chlorum* and *Chlorobium* (*Chl.*) (see Fig. 4). There is strong homology, on the one hand, between the L and M polypeptides of purple bacteria and, on the other, the D1 and D2 polypeptides of PS II (Michel and Deisenhofer, 1988; Baymann et al., 2002). There is also strong homology between the L and M polypeptides of green filamentous photosynthetic bacteria and those of purple bacteria (Ovchinnikof et al., 1988a,b). This suggests that from an early stage, perhaps even before the evolution of BChl *a*/Chl *a*, all RCs are phylogenetically related. It seems reasonable to suggest that the original RC was homodimeric like the RCI-types of *Hbt.* and *Chl.* However, they were probably not at all similar and possibly resembled the RCII-type.

The existence of the special pair may have been an inevitable consequence of the structure of Chl and indeed may have been the reason why the Chls developed their present chemical structure. However, the recent X-ray crystal structures mentioned above illustrate that this may be an over-simplification, since in RCI the special pair turns out to be formed from one molecule of Chl *a* and one molecule of Chl *a´*, whereas in RCII the two Chl molecules involved may not overlap sufficiently to form a special pair (Zouni et al., 2001; Ferreira et al., 2004).

It would be useful if gene sequence analysis could be applied effectively to RCs to ascertain their relationship. However, the long periods of time involved and the degree of divergence (<30% similarity of peptide residues) means that any such inferences would be very weak (Baymann et al., 2001; Xiong et al., 2000). However, the structural information

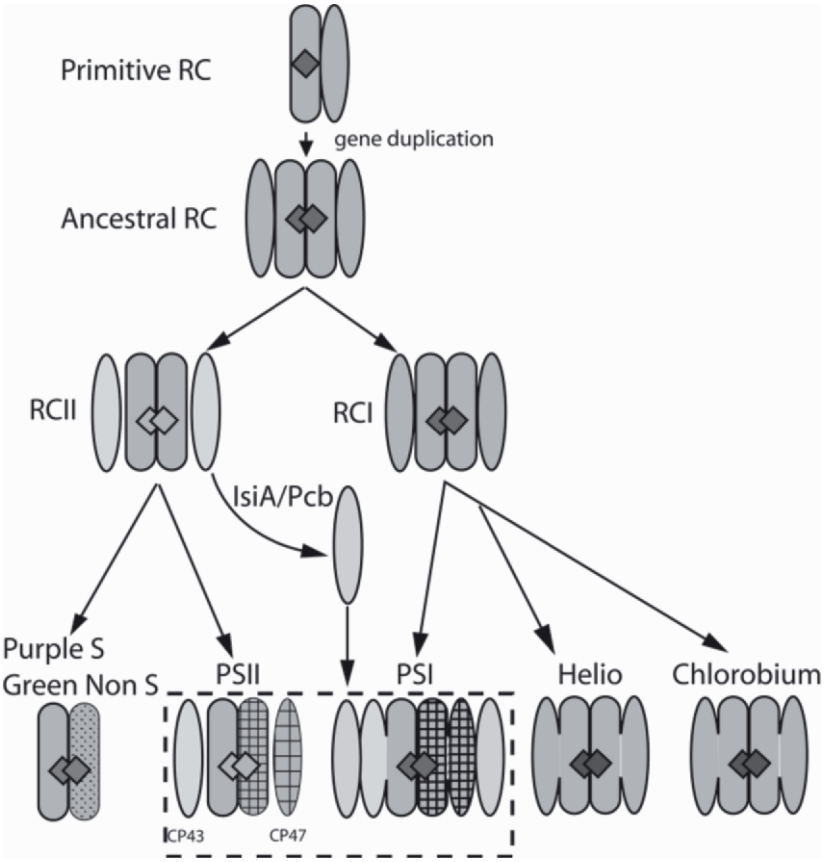

Fig. 4. Outline of the evolution of the ancestral RC and its transition into modern RCs, assuming an early, independent evolution of the six membrane spanning helix antenna protein. Helio, Heliobacteria; isiA, iron stress-induced chlorophyll protein A (CP43´); PCB, prochlorophyte chlorophyll *a/b* binding protein.

concerning homologous polypeptide sequences is very strong (Fyfe et al., 2002). Zhang et al. (2004) have presented evidence that the scheme of Fig. 4 is not correct. In the future it should be possible to incorporate sequence data with specific 3D information of active- or coenzyme-sites and so construct a much more reliable phylogeny. Another untested method is to use the presence or absence of accessory polypeptides; however, none of these avenues of evidence currently add to the debate.

Clearly, gene duplication and differentiation is an important aspect of photosynthetic systems. Most RCs are the result of a gene duplication (see above). It is probable that, initially, each polypeptide acted independently and that the two Chls/BChls of each polypeptide acted as a special pair (van Gorkom, 1987). Gene duplication of the original polypeptide may have been favored because it facilitated the formation of the special pair.

The similar arrangement of the two core RCII polypeptides in both purple photosynthetic bacteria (L and M) and oxygenic phototrophs (D1 and D2) suggests that gene duplication occurred at an early stage. However, the order of these events in the two organisms is undecided (Beanland, 1990; Lockhart et al., 1996b). It might be logical to think that the differentiation of the RC occurred before the separation of these two lines of evolution, but it is possible that the opposite occurred. A more important selection pressure, however, in later stages, but not in the early stage, may have been the need, in the case of RCII, to transfer two electrons, in quick succession, to the tightly-bound primary quinones on each polypeptide, to prevent the decay of the semiquinones (Dutton, 1986). It is tempting to see the two-electron gating mechanism of PS II and the RC of purple bacteria as occurring at this stage but it may have been a later development for protecting the semiquinones from reacting with molecular oxygen generated by the water-splitting system (Ort, 1986). In this case the

situation may be one of convergent evolution, but the gating mechanisms are very similar suggesting that these two gating systems are homologous (Zouni et al., 2001).

It is also interesting to note that there is a quinone (phylloquinone), acting at the electron acceptor site, A_1 of RCI (Jordan et al., 2001). This may suggest that the RCII-type is closer to the ancestral form, in this respect, and gave rise to the RCI-type by the evolution of the secondary FeS acceptors (F_X, F_A and F_B). Such acceptors occur today to varying degrees in extant anoxygenic photosynthetic bacteria with an RCI-type RC (Golbeck, 2003) (Fig. 4). It is possible that only when iron became available in estuarine and oceanic waters in the Proterozoic Eon, due to molecular oxygen liberated by chloroxybacteria (Section III.C), that the FeS acceptor systems became possible. Earlier the RC of *Hbt. chlorum,* called an RC-1q type (Olson and Pierson, 1987) was considered to be a likely early ancestor to RCI and RCII (Vermaas, 1994), and there are proponents of this view today (Gupta et al., 1999; Gupta, 2003). However, the phylogenetic position of *Hbt.* is equivocal (see Section IV.B).

The existence of pheophytin (Phe) and bacteriopheophytin (BPhe) in type II RCs (Michel and Deisenhofer, 1988; Zouni et al., 2001; Chapter 4, Kobayashi et al.) has been a puzzle and could be taken as an indication of an earlier biosynthetic route to Chl via Phe. However, the role of Phe/BPhe as the primary acceptor of the 'special pair' suggests that this role emerged later than that of Chl (in the 'special pair'). It also suggests that a specific mechanism evolved quite early to remove the Mg^{2+} from only the Chl in that specific position and that this mechanism has been conserved. It has to be noted that in PS II, unlike purple sulfur bacteria RC, the Chls of the 'special pair' are relatively far apart and excitonic coupling is weak (Zouni et al., 2001; Fereira et al., 2004). This may be a special adaptation to the need for a more oxidizing redox potential in the RCII (see Section VI.B). It should also be noted that in RCI, the special pair has one Chl *a* and one Chl *a′* (Jordan et al., 2001; Chapter 4, Kobayashi et al.), which requires a special mechanism for the formation of Chl *a′* and its insertion into the special pair.

A summary of the likely events in the evolution of modern RCs is given in the scheme of Fig. 4. It proposes that the ancestral RC of five membrane spanning helices (5 MSHs) was joined by an ancestral light harvesting protein (6 MSHs) which belongs to the family which gave rise to CP43, CP47, IsiA and PCB proteins in Cyanobacteria (La Roche et al., 1996). However, this RC-antenna fusion hypothesis is by no means the universal favorite. Xiong et al. (2000) have proposed that the ancestral protein was an 11 MSH unit (Mulkijanian and Junge, 1997; Baymann et al., 2001), which split later in some organisms to a 5 MSH RC and a 6 MSH antenna (RC antenna fission hypothesis). The latter hypothesis is supported by fewer facts, in that it has to account for the emergence of the 11 MSH protein and the evolutionary developments that led from there to the RCs of extant anoxygenic photosynthetic bacteria and PS I and PS II. Proof of which alternative is most likely should be possible by careful analysis of conserved sequences and conserved geometry among the various RCs.

VI. Evolution of Oxygenic Photosynthesis

A. Evolution of the First Organisms to Evolve Oxygen

As discussed in Section II, the existence of organisms, which split water in photosynthesis is not known from fossilized remains, but can be inferred from chemical 'fossils.' These organisms certainly came from anoxygenic photosynthetic bacteria. However, the relationship of these organisms, which are here called chloroxybacteria (Section II.B), to extant anoxygenic photosynthetic bacteria is not clear (Section V). A clearer picture may emerge from whole genome studies of anoxygenic photosynthetic bacteria, but, as already apparent, much lateral transfer has occurred (Raymond et al., 2002; Rivera and Lake, 2004) and a definitive scheme may be elusive.

The evolution of the ability to split water and evolve oxygen in photosynthesis, driven by the need to provide reducing equivalents for the reduction of CO_2, was the second great innovation in photosynthesis after the invention of the RC. As discussed in Section II, this occurred in chloroxybacteria as early as 3.0 Ga. There are two important considerations: i) how the water splitting apparatus evolved, and ii) how the arrangement of two different photosystems in series came about.

B. Evolution of the Water-Oxidizing Complex

The best working hypothesis for the evolutionary developments that gave rise to water-splitting is still that of Olson (1970): that selective pressure, for the evolution of two populations of photoreactions with

overlapping, but non-identical redox spans, came into play as suitable electron donors (including H_2, H_2S and simple organic compounds) were used up on the early Earth. A crucial point is that there developed a pool of quinone in the photosynthetic membrane of an evolving chloroxybacterium, which could either be reduced or oxidized by adjacent photosystems: the use of quinones, which is quite different in Cyanobacteria from anoxygenic photosynthetic bacteria, still needs explanation (Baymann et al., 2001). It is proposed that consumption of available oxidants in the environment forced the oxidizing end of the proto-PS II (Olson, 1970, 2000; Dismukes et al., 2001) to change to more oxidizing E_h values, oxidizing thereby a range of intermediate compounds and finally able to oxidize water.

Only on the very early Earth, where H_2 and other reducing sources were more readily available, would the opportunity for a great abundance of anoxygenic photosynthetic organisms have occurred, which is in contrast to the relative scarcity of these organisms on the Earth today. This might have been in the period up to 3.5 Ga, after which out-gassing and microbial activity would have considerably reduced earlier levels of H_2, CH_4, H_2S, etc., (Kasting and Siefert, 2002). With the exhaustion of these sources and the oxygenation of environments following the 'invention' of oxygen evolution, these organisms would have been driven to restricted niches in the environment, with later accommodation in a small number of instances to aerobic metabolism. This had implications for a number of associated reactions because of the increased exposure to singlet oxygen and the damage due to reactive oxygen species (Larkum, 2003).

Three proposals have been put forward for compounds that were precursors to water in supplying electrons to RCII: 1) Formate and similar weak oxidants (Olson, 1970); 2) hydrogen peroxide (Blankenship and Hartman, 1998); and 3) bicarbonate (HCO_3^-) (Dismukes et al., 2001).

Almost certainly a number of compounds preceded bicarbonate, although there is no evidence that hydrogen peroxide was ever very abundant. Bicarbonate has merit because it is abundant in seawater (currently ~2 mM) and would have been more concentrated in the past when CO_2 levels were higher (Walker et al., 1983; Rye et al., 1995)]. There is also a well-known bicarbonate effect on PS II (Dismukes et al., 2001) and a putative site for bicarbonate has recently been indicated at the site of oxygen evolution (Ferreira et al., 2004). Furthermore, carbonic anhydrase activity is found associated with PS II (Dismukes et al., 2001; Stemler, 2002) which may well enhance the rate of photosynthesis by speeding up the movement of CO_2 to the stroma (or cytoplasm of chloroxybacteria/Cyanobacteria) (Raven and Beardall, 2003).

The evolution of the Mn-complex, the S-state cycle and the extrinsic polypeptides which modulate the Mn site are all still speculative and will advance rapidly with knowledge of the exact mechanism by which water is split. The current proposal for the water-splitting tetra-manganese complex, which has been the 'holy grail' for photosynthesis workers, is an asymmetric manganese-oxo cubane structure ($CaMn_4O_4$) with three Mn and one Ca in the cubane assemblage, and one Mn displaced to one side (Ferreira et al., 2004). Ideas on how this structure catalyses the splitting of water are still speculative (Dismukes and van Willigen, 2005) and therefore it is difficult to speculate on how this complex evolved. Suggestions in the past have involved a Mn-dependent catalase enzyme (Dismukes, 1996). However, the Ferreira et al. (2004) structure indicates the Mn-Ca complex to be ligated to D1, CP43 and CP47 polypeptides, so a further evolutionary link to another polypeptide is difficult to imagine.

Dismukes et al. (2001) give a scheme for the evolution of water-splitting starting from a conventional green non-sulfur photosynthetic bacterium; Fig. 5 is based on that scheme, with the exception that Chl *a* is considered a possibility in the RC from the beginning, a possible replacement for Chl *d* in the RC.

C. Fusion or Fission Hypotheses for Water Splitting Photosynthesis

As pointed out above, it is attractive to propose that RCI and RCII arose from an ancestral RC, within a single chloroxybacterium (RC Fission Hypothesis)(Olson, 1970, 2000). This hypothesis has the disadvantage that it implies the secondary evolution, from this primitive chloroxybacterium, of anoxygenic photosynthetic bacteria with either RCI or RCII. This hypothesis, although logically compelling, conflicts with our preconceived idea that anoxygenic bacteria preceded chloroxybacteria. For those who hold to the precedence of anoxygenic bacteria, the alternative hypothesis is that two lines evolved, giving rise to the RCI- and RCII-types of modern anoxygenic photosynthetic bacteria; later, fusion of these two into one organism gave rise to chloroxybacteria/Cyanobacteria, which over time

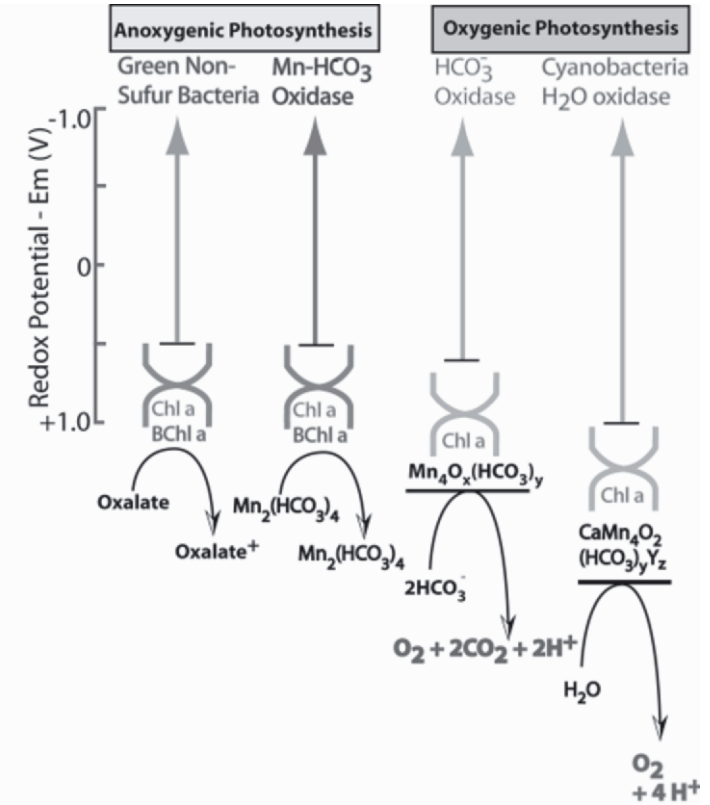

Fig. 5. Possible stages in the evolution of photosynthetic water splitting and oxygen formation. Adapted from Dismukes et al., 2001.

evolved into the modern PS I and PS II (RC Fusion Hypothesis). Olson (1970, 2000) preferred the RC Fission Hypothesis while others have preferred the RC Fusion Hypothesis (Blankenship, 1992; Baymann et al., 2001). Vermaas (1994) suggested that the ancestral RC was close to the *Hbt* RC. It will be seen from Fig. 4 that basically both hypotheses incorporate an ancestral RC, although not necessarily in the form of a *Hbt.*-like RC. With a fission hypothesis at least one fairly difficult lateral transfer is involved (Baymann et al., 2001).

VII. Light-Harvesting Chlorophyll Proteins

In all photosynthetic organisms there is a clear requirement to have a light harvesting mechanism to supply absorbed light energy to the RCs so that the latter can operate at reasonable efficiency. The antenna proteins, which bind the Chl, BChl and other light-harvesting proteins, clearly evolved almost as soon as photosynthesis itself and may be seen as one of the major advances of Chl-based photosynthesis compared to retinal (bacteriorhodopsin)-based photosynthesis. It is perhaps for this reason that the origin of the various light-harvesting Chl/BChl proteins is so obscure.

Chls/BChls are bound to specific proteins in their functional light-harvesting states, but surprisingly these proteins are quite different between the anoxygenic photosynthetic bacteria and the oxygenic photosynthetic organisms (Green, 2003; Green and Parson, 2003). Thus the anoxygenic photosynthetic bacteria have as their principle light harvesting proteins, LH1 and LH2, which bear little similarity to each other (Green and Parson, 2003). The crystal structure of LH2 has been determined at the 2.3 Å resolution (Cogdell et al., 1999). In chloroxybacteria and modern Cyanobacteria an ancient light harvesting antenna has given rise recently to a group of antenna proteins that, under certain circumstances, form a ring around PS I (Bibby et al., 2001, 2003; Boekema et al. 2001; Chen et al., 2005b). In PS II similar proteins form a supramolecular complex attached to dimeric

or quadrimeric PS II units. These antenna proteins bind Chl *a*, and additionally, in prochlorophytes, Chl *b,* and sometimes MgDVP (Larkum et al., 1994), and in *Acc. marina*, Chl *d*. They are recently derived from CP43 (Zhang et al., 2004) but are related to the ancient antenna 6 MSH protein that fused with the 5 MSH RC protein early in the evolution of all extent photosynthetic organisms (Zhang et al., 2004; Section V.B).

In algae and higher plants, another light-harvesting antenna protein occurs: a light-harvesting Chl-binding protein. In green algae, euglenoids and *Chlorarachnion* there is a family of LHC proteins (CAB proteins) that bind Chl *a* and Chl *b* (Durnford, 2003). In Chl *c*-containing algae, a homologous family of proteins bind Chl *a* and Chl *c* (CAC proteins)(Durnford, 2003). In red algae, a small group of homologous LHC proteins bind only Chl *a*, and are only found attached to PS I (Grabowski et al., 2001).

The evolutionary origin of these LHC antenna proteins, which are 3 MSH proteins, appears to be from a single high light-induced protein (HLIP) MSH protein found in cyanobacteria. This HLIP protein, with a homologous single MSH, functions as a Chl carrier protein (Dolganov et al., 1995), but does not function in light-harvesting. In higher plants an early light-inducible protein (ELIP), with two homologous MSHs has been found (Heddad and Adamska, 2000). Thus the 3 MSH LHC antenna may have arisen by two serial gene duplications, via possibly a PsbS-type protein (with 4 MSH)(Larkum, 2003).

Nothing is known of how the IsiA, PCB antenna proteins of some chloroxybacteria/cyanobacteria were replaced by the entirely distinct family of light-harvesting antenna proteins (Green, 2003; Larkum, 2003). It appears to have taken place during the early stages of the symbioses that gave rise to plastids. This is one of the great challenges for understanding evolution of the photosynthetic mechanisms in eukaryotic algae.

VIII. Outlook

The basis on which the evolution of photosynthesis can be assessed will change dramatically in the near future as whole genome studies reveal the genes and gene structure of many of the photosynthetic organisms that lie at the heart of the questions now being asked. Such studies will require new techniques for comparing complex data sets. However, they promise answers to many of the questions posed here. Side by side with these studies will come detailed crystallographic and functional studies of RCs and their ancillary apparatus. And on the broader scale, there will be a growing consensus of the physical and chemical environment of the early Earth, its atmosphere and its oceans: this should prove a crucial tool in deciding the course of past photosynthetic events.

References

Anbar AD and Knoll AH (2002) Proterozoic ocean chemistry and evolution: A bioinorganic bridge? Science 297: 1137–1142

Baymann F, Brugna M, Mühlenhoff U and Nitschke W (2001) Daddy, where did (PS)I come from? Biochim Biophys Acta 1507: 291–310

Beanland TJ (1990) Evolutionary relationship between 'Q–type' photosynthetic reaction centers: Hypothesis testing using parsimony. J Theoret Biol 145: 535–545

Bibby T, Mary I, Nield J and Barber J (2003) Lowlight-adapted *Prochlorococcus* species possess specific antennae for each photosystem. Nature 424: 1051–1054

Bibby TS, Nield J, Partensky F and Barber J (2001) Oxyphotobacteria. Antenna ring around photosystem I. Nature 413: 590

Bjerrum CJ and Canfield DE (2002) Ocean productivity before about 1.9 Gyr ago limited by phosphorus adsorption onto iron oxides. Nature 417: 159–162

Blankenship RE (1992) Origin and early evolution of photosynthesis. Photosynthesis Res 33, 91–111

Blankenship RE (2002) Molecular Mechanisms of Photosynthesis. Blackwell Science Ltd, Oxford

Blankenship RE and Hartman H (1998) The origin and evolution of oxygenic photosynthesis. Trends Biochem Sci 23: 94–97

Borowska Z and Mauzerall D (1987) Efficient near ultraviolet light-induced formation of hydrogen by ferrous hydroxide. Origin Life Evol Biosphere 17: 251–259

Borowska Z and Mauzerall D (1988) Photoreduction of carbon dioxide by aqueous ferrous ions: An alternative to the strongly reducing atmosphere for the chemical origin of life. Proc Natl Acad Sci USA 85: 6577–6580

Brassier MD, Green OR, Jephcoat AP, Kleppe AK, van Kranendonk MJ, Lindsay JF, Steele A and Grassineau NV (2002) Questioning the evidence for Earth's oldest fossils. Nature 416: 76–81

Brockman H Jr and Lipinski A (1983) Bacteriochlorophyll *g*. A new bacteriochlorophyll from *Heliobacterium chlorum*. Arch Mikrobiol 136: 17–25

Brocks JJ, Logan GA, Buick R and Summons RE (1999) Archean molecular fossils and the early rise of eukaryotes. Science 285: 1033–1036

Burke DH, Hearst JE and Sidow A (1993) Early evolution of photosynthesis: Clues from nitrogenase and chlorophyll iron proteins. Proc Natl Acad Sci USA 90: 7134–7138

Cady SS and Pinnavaia TJ (1978) Porphyrin intercalation in mica-type silicates. Inorg Chem 17: 1501–1507

Cairns-Smith AG (1978) Precambrian solution photochemistry,

inverse segregation, and banded iron formations. Nature 276: 807,

Canfield DE and Raiswell, R (1999) The evolution of the sulfur cycle. Amer J Sci 299: 697–723

Castresana J (2001) Comparative genomics and bioenergetics. Biochim. Biophys Acta 1505: 147–162

Castresana J and Sarastre N (1995) Evolution of energetic metabolism: The respiration-early hypothesis. Trends Biochem Sci 20: 443–448

Chen M, Bibby TS, Nield J, Larkum AWD and Barber J (2005a) Iron effect on formation and localization of antenna system binding with chlorophyll *d*. Biochim Biophys Acta 1708: 367–374

Chen M, Telfer A, Lin S, Pascal A, Larkum AWD and Blankenship RE (2005b) The nature of the Photosystem II reaction centre in the chlorophyll *d* containing prokaryote, *Acaryochloris marina*. Photochem Photobiol Sci 4: 1060–1064

Cogdell RJ, Isaacs NW, Howard TD, Mcluskey K, Fraser NJ and Prince SM (1999) How photosynthetic bacteria harvest solar energy. J Bacteriol 181: 3869–3879

Copley SD (2000) Evolution of a metabolic pathway for degradation of a toxic xenobiotic: The patchwork approach. Trends Biochem Sci 25: 261–266

Dismukes GC (1996) Manganese enzymes with binuclear active sites. Chem Rev 96: 2909–2926

Dismukes GC and van Willigen RT (2005) Manganese: The oxygen-evolving complex and models. In: King R (ed) Encyclopedia of Inorganic Chemistry II. Wiley Interscience, in press

Dismukes GC, Klimov VV, Varanov, SV, DasGupta J and Tyryshkin A (2001) The origin of atmospheric oxygen on Earth: The innovation of oxygenic photosynthesis. Proc Natl Acad Sci USA 98: 2170–2175

Dolganov NA, Bhaya D and Grossman AR (1995) Cyanobacterial protein with similarity to the chlorophyll *a/b* binding-protein of higher plants — evolution and regulation. Proc Natl Acad Sci USA 92: 636–640

Douglas SE, Raven JA and Larkum AWD (2003) The algae and their general characteristics. In: Larkum AWD, Douglas SE and Raven JA (eds) Photosynthesis in Algae, pp 1–10. Kluwer Academic Publishers, Dordrecht

Dufresne A, Salanoubat M, Partensky F, Artiguenave F, Axmann I M, Barbe V, Duprat S, Galperin MY, Koonin EV, Le Gall F, Makarova KS, Ostrowski M, Oztas S, Robert C, Rogozin IB, Scanlan DJ, Tandeaau de Marsac N. Weissenbach J, Wincker P, Wolf Y and Hess WR (2003) Genome sequence of cyanobacterium *Prochlorococcus marinus* SS120, a nearly minimal oxyphototrophic genome. Proc Natl Acad Sci USA 100: 10020–10025

Durnford, D (2003) Genes for Chl *a/b* and Chl *a/c* light harvesting proteins. In: Larkum AWD, Douglas Sand Raven JA (eds) Photosynthesis in Algae, pp 63–82, Kluwer Academic Publishers, Dordrecht

Dutton PL (1986) Energy transduction in anoxygenic photosynthesis. In: Staehelin LA and Arntzen CJ (eds) Encyclopedia of Plant Physiology, Photosynthesis III, pp 197–232. Springer-Verlag, Berlin

Eldredge N and Gould SJ (1972) Punctuated equilibria: An alternative to phyletic gradualism. In: Schopf TJM (ed) Models in Paleobiology, pp 82–115. Freeman, Cooper and Co., San Francisco

Ferreira KN, Iverson TM, Maghlaoui K, Barber J and Iwata S (2004) Architecture of the photosynthetic oxygen-evolving center. Science 303: 1831–1838

Fyfe PK, Jones MR and Heathcote P (2002) Insights into the evolution of the antenna domains of Type-I and Type-II photosynthetic reaction centers through homology modeling. FEBS Lett 530: 117–123

Georgopapadakou NH and Scott AI (1977) On B_{12} biosynthesis and evolution. J Theor Biol 69: 381–384

Golbeck JH (2003) The binding of cofactors to Photosystem I analyzed by spectroscopic and mutagenesis methods. Ann Rev Biophys Biomolec Struct 32: 237–256

Gogarten JP, Doolittle WF and Lawrence JC (2002) Prokaryotic evolution in the light of gene transfer. Mol Biol Evol 19: 2226–2238

Gouy M and Li W-P (1989) Phylogenetic analysis based on rRNA sequence supports the archaebacterial rather than eocyte tree. Nature 339: 145–147

Grabowski B, Cunningham FX, and Gantt E (2001) Chlorophyll and carotenoid binding in a simple red algal light-harvesting complex crosses phylogenetic lines. Proc Natl Acad Sci USA 98: 2911–2916

Granick S (1957) Speculations on the origins and evolution of photosynthesis. Ann NY Acad Sci 69: 292– 301

Green BR (2003) The evolution of light-harvesting antennas. In: Green BR and Parson WF (eds) Light Harvesting Antennas in Photosynthesis, pp 129–168. Kluwer Academic Publishers, Dordrecht

Green BR and Parson WF (eds) (2003) Light Harvesting Antennas in Photosynthesis. Kluwer Academic Publishers, Dordrecht

Gupta RS (2003) Evolutionary relationships among photosynthetic bacteria. Photosynth Res 76: 173–183

Gupta RS, Mukhtar T and Singh B (1999) Evolutionary relationships among photosynthetic prokaryote (*Heliobacterium chlorum, Chloroflexus aurantiacus*, cyanobacteria, *Chlorobium tepidum* and proteobacteria): Implication regarding the origin of photosynthesis. Mol Microbiol 32: 893–906

Heddad M and Adamska I (2000) Light stress-regulated two-helix proteins in *Arabidopsis thaliana* related to the chlorophyll *a/b*-binding gene family. Proc Natl Acad Sci USA 97: 3741–3746

Helfrich M, Ross A, King GC, Turner AG and Larkum AWD (1999) Identification of [8-vinyl]-protochlorophyllide *a* in phototrophic prokaryotes and algae: Chemical and spectroscopic properties. Biochim Biophys Acta 1410: 262–272

Hodgson GW and Baker GL (1964) Evidence for porphyrin in the Orgueil meteorite. Nature 202: 125–127

Hodgson GW and Baker GL (1967) Porphyrin abiogenesis from pyrrole and formaldehyde under simulated geochemical conditions. Nature 216: 29–32

Hodgson GW and Ponnamperuma C (1968) Prebiotic porphyrin genesis: Porphyrins from electric discharge in methane, ammonia and water vapour. Proc Natl Acad Sci USA 31: 153–158

Hofmann E, Wrench PM, Sharples FP, Hiller RG, Welte W and Diederichs K (1996) Structural basis of light-harvesting by Carotenoids: Peridinin-Chlorophyll-Protein from *Amphidinium carterae*. Science 272: 1788–1791

Hoober JK and Eggink LL (2001) A potential role of chlorophylls *b* and *c* in assembly of light-harvesting complexes. FEBS Lett 489: 1–3

House CH, Runnegar B and Fitz-Gibbon ST (2003) Geobiological analysis using whole genome-based tree building applied to the

bacteria, Archaea, and Eukarya. Geobiology 1: 15–26
Houssier C and Sauer K (1970) Circular dichroism and magnetic circular dichroism of chlorophyll and protochlorophyllide pigments. J Am Chem Soc 92: 779–790
Hu Q, Miyashita H, Iwasaki I Kurano N, Miyachi S, Iwaki M and Itoh S (1998) A Photosystem I reaction center driven by chlorophyll *d* in oxygenic photosynthesis. Proc Natl Acad Sci USA 95: 13319–13323
Ilani A and Mauzerall D (1981) The potential span of photoredox reactions of porphyrins and chlorophyll at the lipid bilayer-water interface. Biophys J 35: 79–92
Ilani A, Woodle M and Mauzerall D (1989) Photoinduced electron transfer across lipid bilayers containing magnesium octaethylporphyrin. Photochem Photobiol 29: 673–679
Jacobs JM and Jacobs NJ (1984) Protoporphyrinogen oxidation, an enzymatic step in heme and chlorophyll synthesis: Partial characterization of the reaction in plant organelles, and comparison with mammalian and bacterial systems. Arch Biochem Biophys: 229: 312–319
Jermiin LJ, Blankenship RE, Lockhart PJ and Larkum AWD (2001) Phylogenetic reconstruction of ancient photosynthetic lineages using chlorophyll and bacteriochlorophyll biosynthetic genes. In: PS2001: Proceedings 12th International Congress on Photosynthesis, S09–12. CSIRO Publishing, Melbourne (CD-ROM)
Jones OTG (1963) The production of magnesium protoporphyrin monomethyl ester by *Rhodopseudomonas spheroides*. Biochem J 86: 429–435
Jordan P, Fromme P, Witt HT, Klukas O Saenger W and Krauss N. (2001) Three-dimensional structure of cyanobacterial Photosystem I at 2.5 Ångstrom resolution. Nature 411: 909–917
Kasting JF (2001) The rise of atmospheric oxygen. Science 293: 819–820
Kasting JF and Siefert JL (2002) Life and the evolution of Earth's atmosphere. Science 296: 1066–1068
Knoll AH (1999) Paleontology — A new molecular window on early life. Science 285: 1025–1026
Kolber Z.S, Plumley FG, Lang AS, Beatty JT, Blankenship RE, Vandover CL, Vetriani C, Koblizek, M, Cathgeber C and Falkowski P (2001) Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. Science 292: 2492–2495
Krasnovsky AA (1971) The evolution of photochemical electron transfer systems. In: Krimball AP and Oro J (eds) Prebiotic and Biochemical Evolution, pp 207–216. North Holland, Amsterdam
Kühl M and Larkum AWD (2001) The microenvironment and photosynthetic performance of *Prochloron* sp. in symbiosis with didemnid ascidians. In: Seckbach J (ed) Symbiosis, pp 273–290. Kluwer Academic Publishers, Dordrecht
Kühl M, Chen M, Ralph P, Schreiber U and Larkum AWD (2005) Niche and photosynthesis of chlorophyll *d*-containing cyanobacteria. Nature 433: 820
Kuhn KF (1998) In Quest of the Universe. Jones and Barlett, Boston
La Roche J, van der Staay GWM, Ducret A, Aebersold R, Li R, Golden SS, Hiller RG, Wrench PM, Larkum AWD and Green BR. (1996) Independent evolution of the prochlorophyte and green plant chlorophyll *a/b* light-harvesting proteins. Proc Natl Acad Sci USA 93: 15244–15248
Lake JA., Clarke MW, Henderson E, Fay SP, Oaks M, Scheinman A, Thornber JP and Mah RA (1985) Eubacteria, Halobacteria, and the origin of photosynthesis: The photocytes. Proc Natl Acad Sci USA 82: 3716–3720
Larkum AWD (1991) The evolution of chlorophylls. In: Scheer H (ed) Chlorophylls, pp 367–383. CRC Press, Boca Raton
Larkum AWD (1992). Evolution of photosynthetic systems. In: Murata (ed) Research in Photosynthesis, Vol III, pp 475–482, Kluwer Academic Publishers, Dordrecht
Larkum AWD (1999) The evolution of algae. In: Seckbach J (ed) Enigmatic Microorganisms and Life in Extreme Environments, pp 31–48. Kluwer Academic Publishers, Dordrecht
Larkum AWD (2003) Light-harvesting systems in algae In: Larkum AWD, Douglas S and Raven JA (eds) Photosynthesis in Algae, pp 277–304. Kluwer Academic Publishers, Dordrecht
Larkum AWD and Barrett J (1983) Light-harvesting systems in algae. Adv Bot Res 10: 1–221
Larkum AWD and Vesk M (2003) Algal plastids: Their fine structure and properties. In: Larkum AWD, Douglas S and Raven JA (eds) Photosynthesis in Algae, pp 11–28. Kluwer Academic Publishers, Dordrecht
Larkum AWD, Scaramuzzi C, Cox GC, Hiller RG and Turner AG (1994) Light-harvesting chlorophyll *c*-like pigment in *Prochloron*. Proc Natl Acad Sci USA 91: 679–683
Lockhart P, Howe CJ, Bryant DA, Beanland TJ and Larkum AWD (1992) Substitutional bias may preclude phylogenetic inference of chloroplast origins. J Mol Evol 34: 153–162
Lockhart PJ, Larkum AWD, Steel MA, Wardell P and Penny D (1996a) Evolution of chlorophyll and bacteriochlorophyll: The problem of invariant sites in sequence analysis. Proc Natl Acad Sci USA 93: 1930–1934
Lockhart PJ, Steel MA and Larkum AWD (1996b) Gene Duplication and the evolution of photosynthetic reaction centers. FEBS Lett 385: 193–196
Margulis LM and Obar R (1985) Heliobacterium and the origin of chrysoplasts. BioSystems, 17: 317–325
Mauzerall D (1960) The condensation of porphobilinogen to uroporphyrinogen. J Am Chem Soc 82: 2605–2609
Mauzerall D (1973) Why chlorophyll? Ann NY Acad Sci 206: 483–494
Mauzerall D (1978) Porphyrins, chlorophyll and photosynthesis. In: Trebst AA and Avron M (eds) Encyclopedia of Plant Physiology, Vol V, pp 117–124. Springer, New York
Mercer-Smith JA and Mauzerall DC (1984) Photochemistry of porphyrins: A model for the origin of photosynthesis. Photochem Photobiol 39: 397–405
Mercer-Smith, JA, Raudino A and Mauzerall DC (1985) A model for the origin of photosynthesis. III The ultraviolet photochemistry of uroporphyrinogen. Photochem Photobiol 42: 239–244
Michel H and Deisenhofer J (1988) Relevance of the photosynthetic reaction center from purple bacteria to the structure of Photosystem II. Biochemistry 27: 1–7
Mimuro M., Akimoto S, Yamazaki I, Miyashita H and Miyachi S (1999) Fluorescence properties of chlorophyll *d*-dominating alga, *Acaryochloris marina*: Studies using time-resolved fluorescence microscopy on whole cells. Biochim Biophys Acta 1412: 37–46
Miyashita H, Ikemoto H, Kurano N, Adachi K, Chilara M and Miyachi S (1996) Chlorophyll *d* as a major pigment. Nature 383: 402
Mulkidjanian AY and Junge W (1997) On the origin of photo-

synthesis as inferred from sequence analysis — a primordial UV-protector as common ancestor of reaction centers and antenna proteins. Photosynth Res 51: 27–42
Olson JM (1970) The evolution of photosynthesis. Science 168: 438–446
Olson JM (1999) Early evolution of chlorophyll-based photosynthesis. Chemtracts 12: 468–482
Olson JM (2000) 'Evolution of photosynthesis' (1970). Re-examined thirty years later. Photosynth Res 68: 95–117
Olson JM and Pierson BK (1987) Evolution of reaction centers in photosynthetic prokaryotes. Int Rev Cytol 108: 209–248
Ort DR (1986) Energy transduction in oxygenic photosynthesis: An overview of structure and mechanism. In: Staehelin LA and Arntzen CJ (eds) Encyclopedia of Plant Physiology, Photosynthesis III, pp 143–196. Springer, Berlin
Oesterhelt D, Tittor J (1989) Two pumps, one principle: Light-driven ion transport in halobacteria. Trends Biochem Sci 14: 57–61
Ovchinnikof YA, Abdulaev NG, Zolotarev AS, Shmukler BE, Zargarov AA, Kutuzov MA, Telezhinskaya TN and Levina NB (1988a) Photosynthetic reaction centre of *Chloroflexus aurantiacus*. 1. Primary structure of the L-subunit. FEBS Lett 231: 237–242
Ovchinnikof YA, Abdulaev NG, Shmukler BE, Zargarov AA, Kutuzov MA, Telezhinskaya TN, Levina NB and Zolotarev AS (1988b) Photosynthetic reaction centre of *Chloroflexus aurantiacus*. Primary structure of the M-subunit. FEBS Lett 232: 364–368
Owen TC and Bar-Nun A (2001) Contributions of icy planetisimals to the Earth's early atmosphere. Origins Life Evol Biosphere 31: 435–458
Palenik B, Brahamsha B, Larimer FW, Land M, Hauser L, Chain P, Lamerdin J, Regala W, Allen EE, McCarren J, Paulsen I, Dufresne A, Partensky F, Webb EA and Waterbury J (2003) The genome of a motile marine *Synechococcus*. Nature 424: 1037–1042
Partensky F and Garczarek L (2003) The photosynthetic apparatus of chlorophyll *b*- and *d*-containing oxyphotobacteria. In: Larkum AWD, Douglas S and Raven JA (eds) Photosynthesis in Algae, pp 29–62. Kluwer Academic Publishers, Dordrecht
Pavlov AA, Hurtgen MT, Kasting JF and Arthur MA (2003) Methane-rich Proterozoic atmosphere? Geology 31: 87–90
Rappé MS and Giovannoni SJ (2003) The unknown microbial majority. Ann Rev Microbiol 57: 369–394
Raven JA (1996) The bigger the fewer: Size, taxonomic diversity and the range of chlorophyll(ide) pigments in oxygen-evolving marine photolithotrophs. J Mar Biol Ass UK 76: 211–217
Raven JA and Beardall J (2003) Carbon acquisition mechanisms of algae: Carbon dioxide diffusion and carbon dioxide concentrating mechanisms. In: Larkum AWD, Douglas S and Raven JA (eds) Photosynthesis in Algae, pp 225–244. Kluwer Academic Publishers, Dordrecht
Raymond J and Blankenship RE (2004) Biosynthetic pathways, gene replacement and the antiquity of life. Geobiology 2: 199–203
Raymond J, Zhaxybayeva O, Gogarten JP, Gerdes SV and Blankenship RE (2002) Whole-genome analysis of photosynthetic prokaryotes. Science 298: 1616–1620
Rivera MC and Lake J (2004) The ring of life provides evidence for a genome fusion origin of eukaryotes. Nature 431: 152–155
Rocap G, Larimer FW, Lamerdin J, Mafattl S, Chain P, Ahigren NA, Areliano A, Coleman M, Hauser L, Hess WR, Johnson ZI, Land M, Lindell D, Post AF, Regala W, Shah M, Shaw SL, Steglich C, Sullivan MB, Ting CS, Tolonen A, Webb EA, Zinser ER and Chisholm SW (2003) Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. Nature 424: 1042–1047
Runquist JA and Loach PA (1981) Catalysis of electron transfer across phospholipid bilayers by iron-porphyrin complexes. Biochim Biophys Acta 637: 231–244
Rye R, Kuo PH and Holland HD (1995) Atmospheric carbon dioxide concentration before 2.2 billion years ago. Nature 378: 603–605
Schopf JW (1993) Microfossils of the early archean apex chert: New evidence on the antiquity of life. Science 260: 640–646
Shen Y, Knoll AH and Walter MR 2003 Evidence for low sulphate and anoxia in a mid-Proterozoic marine basins. Nature 423: 632–635
Shiozawa JA, Lottspeich F, Osterhelt D and Feick R (1989) The primary structure of *Chloroflexus aurantiacus* reaction center polypeptides. Eur J Biochem 180: 75–84
Simionescu CI, Simionescu BC, Mora R and Leanca M (1978) Porphyrin-like compounds genesis under simulated geochemical conditions. Origins Life Evol Biosphere 9: 103–114
Smith KM (1975) Appendix: Atomic absorption spectra. In: Smith KM (ed) Porphyrins and Metalloporphyrins, p 871. Elsevier, Amsterdam
Smith JHC and Benitez A (1955) Chlorophyll analysis in plant materials. In: Paech K and Travey MV (eds) Modern Methods of Plant Analysis, Vol 4, pp 142–165. Springer, Berlin
Stemler AJ (2002) The bicarbonate effect, oxygen evolution, and the shadow of Otto Warburg. Photosynth Res 73: 177–183
Summons RE, Jahnke LL, Hope JM and Logan GA (1999) 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. Nature 400: 554–557
Szutka A (1965) Probable synthesis of porphine-like substances during chemical evolution. In: Fox SW (ed) Origin of Prebiological Systems and Their Molecular Matrices, p 245. Academic Press, New York
Ting CS, Rocap G, King J and Chisholm SW (2002) Cyanobacterial photosynthesis in the oceans: The origin and significance of divergent light-harvesting strategies. Trends Microbiol 10: 134–142
Tomitani A, Okada K, Miyashita H, Matthijs HCP, Ohno T and Tanaka A (1999) Chlorophyll *b* and phycobilins in the common ancestor of cyanobacteria and chloroplasts. Nature 400: 159–162
Treibs A (1973) On the chromophores of porphyrin systems. Ann NY Acad Sci 206: 97–115
Van Gorkom HJ (1987) Evolution of photosynthesis. In: Amesz J (ed) Photosynthesis, pp 343–350. Elsevier, Amsterdam
Vermaas WFJ (1994) Evolution of heliobacteria: Implications for photosynthetic reaction center complexes. Photosynth Res 41: 285–294
Walker JGC, Klein C., Schidlowski M, Schopf JW, Stevenson DJ and Walker MR (1983) Environmental evolution of the Archean-early Proterozoic Earth. In: Schopf JW (ed) Earth's Earliest Biosphere, pp 260–290. Princeton University Press, Princeton
Woese CR (1987) Bacterial Evolution. Microbiol Rev 51: 221–271
Woese CR (2002) On the evolution of cells. Proc Natl Acad Sci

USA 99: 8742–8747

Wolfe GR, Cunningham FX, Durnford D, Green BR and Gantt E (1994) Evidence for a common origin of chloroplasts with light-harvesting complexes of different pigmentation. Nature 367: 566–568

Woodle M, Zhang JW and Mauzerall D (1987) Kinetics of charge transfer at the lipid bilayer-water interface on the nanosecond time scale. Biophys J 52: 577– 586

Xiong J and Bauer CE (2002) Complex evolution of photosynthesis. Annu Rev Plant Biol 53: 503–521

Xiong J, Fischer M, Inoue K, Nakahara M and Bauer CE (2000) Molecular evidence for the early evolution of photosynthesis. Science 289: 1724–1729

Zhang Y, Jermiin L and Larkum AWD (2004) Phylogenetic analysis of light-harvesting antenna peptides from plants and bacteria. In: van der Est A and Bruce D (eds) Photosynthesis: Fundamental Aspects and Global Perspectives. Proc. 13th International Congress on Photosynthesis, Montréal, p. 745. International Society of Photosynthesis Research

Zouni A, Witt HT, Kern J, Fromme P, Krauss N, Saenger W and Orth P (2001) Crystal structure of Photosystem II from *Synechococcus elongatus* at 3.8 Ångstrom resolution. Nature 409: 739–743