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On the origin of photosynthesis as inferred from sequence analysis

A primordial UV-protector as common ancestor of reaction centers and antenna proteins

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Abstract

Sequence alignments between membrane-spanning segments of pheophytin-quinone-type photosynthetic reaction centers, FeS-type photosynthetic reaction centers, core chlorophyll-proteins of PS II, chlorophyll *a*/*b*-containing antenna proteins of plants and light-harvesting complexes of purple bacteria led us to postulate a large common ancestral pigment-carrying protein with more than 10 membrane spans. Its original function as a UV-protector of the primordial cell is discussed. It is conceivable that a purely dissipative photochemistry started still in the context of the UV-protection. We suggest that mutations causing the *loss* of certain porphyrin-type pigments led to the acquisition of redox cofactors and paved the way for a gradual transition from dissipative to productive photochemistry.

Abbreviations: BR – bacteriorhodopsin; BRC2 – bacterial photosynthetic reaction centers of type 2; CAB – chlorophyll *a*/*b* containing proteins; CP – chlorophyll-proteins; FeS_x – iron-sulfur cluster, the primary electron acceptor of RC1; LH – light-harvesting complexes of purple bacteria; P – primary electron donor of RC1; P′ – primary electron donor of RC2; P′* – electronically excited state of P′; P680 – primary electron donor of PS II; PS I – Photosystem I; PS II – Photosystem II; Q, quinone; Q_A, Q_B – primary and secondary quinone electron acceptors of RC2; RC – photosynthetic reaction center; RC1 – photosynthetic reaction centers of type 1, RC2 – photosynthetic reaction centers of type 2, Y_Z – redox active tyrosine-161 on D1 subunit, the fast electron donor of PS II; Y_D – redox active tyrosine-161 on D2 subunit

Introduction

Photosynthesis is driven by photosynthetic reaction centers (RC) that are associated with pigmented antenna complexes which collect sunlight. Reaction centers, formed by intrinsic membrane polypeptides, catalyse the charge separation between a special dimeric chlorophyll moiety, the primary electron donor, and a series of electron acceptors, thereby creating oxidative and reductive power at opposite sides of the protein (and of the membrane). The most abundant reaction centers are photosystem I (PS I) and photosystem II (PS II) of green plants and cyanobacteria. Other RC resemble either PS I or PS II and have been classified accordingly (Olson and Pierson 1987). PS I as well as the reaction centers of green sulfur bacteria and of Heliobacteriaceae are distinguished by iron-sulfur clusters at the acceptor side (*FeS-type RC or RC1*, see (Golbeck 1994; Blankenship 1994; Feiler and Hauska 1995; Amesz 1995; Nitschke et al. 1996). In PS II as well as in the reaction centers of purple bacteria and of Chloroflexaceae two bound quinones centered by a

non-heme iron and preceded by a (bacterio)pheophytin serve as electron acceptors (*pheophytin-quinone-type RC or RC2* (Michel and Deisenhofer 1988; Blankenship 1992; Rutherford and Nitschke 1996). Only in PS II the oxidative power of the primary donor is high enough to extract electrons from water; the reaction is catalyzed by a Mn-cluster and a redox-active tyrosine residue Y_Z. (Henceforth we denote the bacterial RC2 as BRC2 to distinguish them from PS II.) Other intrinsic membrane pigment-protein complexes that are found in green plants belong either to the *chlorophyll- proteins* (*CP*) (the best studied representatives, CP43 and CP47, form the core antenna of PS II (Bricker 1990; Sayre and Wrobel-Boerner 1994) or to the *chlorophyll a/b* containing proteins (*CAB*) that serve as external antenna both for PS I and PS II (Pichersky and Green 1990; Green et al. 1992; Green and Kühlbrandt 1995; van der Stay et al. 1995). The *light-harvesting complexes* of purple bacteria (*LH*) comprise a separate class (Zuber and Brunisholz 1991; Zuber and Cogdell 1995).

These chlorophyll-proteins are quite different. The crystal structure of the BRC2 of purple bacteria shows two innermost membrane subunits, L and M (of about 30 kD), each forming five transmembrane α-helices (Deisenhofer et al. 1984, 1995; Allen et al. 1987). Based on sequence similarities, the D1 and D2 subunits in the very center of PS II were modeled according to this template (Trebst 1986; Michel and Deisenhofer 1988). The center of PS I is also a dimer of PsaA and PsaB subunits, each of about 70–80 kD. The low-resolution crystal structure of PS I suggests 11 transmembrane α-helices for each center subunit of PS I (Fromme et al. 1996). The chlorophyll-proteins of PS II, CP43 and CP47, contain six predicted transmembrane spans each (Bricker 1990; Sayre and Wrobel-Boerner 1994). The crystal structure of LHCII-I, a CAB protein, shows long intersecting membrane-spanning α-helices A and B (Kühlbrandt et al. 1994). From their sequence similarity it has been proposed that CAB genes originated from a duplication of a short gene coding for a single α-helix which was followed by gene fusion (Green et al. 1991; Green and Kühlbrandt 1995). LH complexes of bacteria are arranged as rings of many identical units (Zuber and Cogdell 1995). The crystal structure of LH2 of *Rhodopseudomonas acidophilla* shows that each unit is a heterodimer of single-helical α- and β-polypeptides (McDermott et al. 1995).

In spite of drastic differences between these pigment–protein complexes it has been speculated about their cognate origin (Olson and Pierson 1987; Mathis 1990; Nitschke and Rutherford 1991; Zuber and Brunisholz 1991; Blankenship 1992; Büttner et al. 1992; Golbeck 1993; Vermaas 1994; Meyer 1994; Kuhn et al. 1994; Blankenship 1994; Fromme et al. 1996). Sequence analysis, however, has not yet led to a comprehensive evolutionary scheme. This has prompted our search for sequence alignments between the named five classes of chlorophyll-protein complexes. The alignments were restricted to the established and assumed transmembrane helices and emphasized those conserved amino acids as flags that may serve as ligands to pigments and cofactors. We arrived at a detailed concept for the origin of reaction centers and antenna proteins from a large common ancestor protein. We suggest that this common ancestor served as an UV-protector of the ancient cell, and discuss how the productive photochemistry originated from a dissipative, i.e. a UV-protecting one.

Sequence alignments between reaction centers and antenna complexes

The percentage of sequence identity *inside* each class of pigment-proteins is poor (about 20% in the average; see Liebl et al. (1993); Komiya et al. (1988); Bricker (1990); Pichersky and Green (1990) and Zuber and Brunisholz (1991) for representative alignments of RC1, RC2, CP, CAB proteins and bacterial LH, respectively). Hence even less similarity may be expected between different classes. Still, Vermaas (1994) has noted significant similarity between the predicted membrane spans I–VI from the N-terminus of the RC1 polypeptide of *Heliobacterium mobilis* and the six predicted membrane spans of CP47 and suggested a common ancestry of these two proteins. Kuhn and co-workers (1994) have noted a common motif [xS/THL/IDWx_8F/T] in the L and M subunits of BRC2 and in the PsaA and PsaB subunits of PS I.

Crystal structure analysis of bacterial RC2 (Deisenhofer et al. 1984, 1995), of the CAB protein LHCII-I (Kühlbrandt et al. 1994) and of the bacterial LH (McDermott et al. 1995) showed that the pigments and cofactors are clustered in α-helical domains, either transmembrane ones or oriented in parallel to the membrane surface. Gaps and deletions in these domains would change the relative position and orientation of chromophore(s) and do not occur frequently. Therefore we aligned in this work only the transmembrane helices, established ones or assumed ones based on hydropathy plots. The usual way to establish evolu-

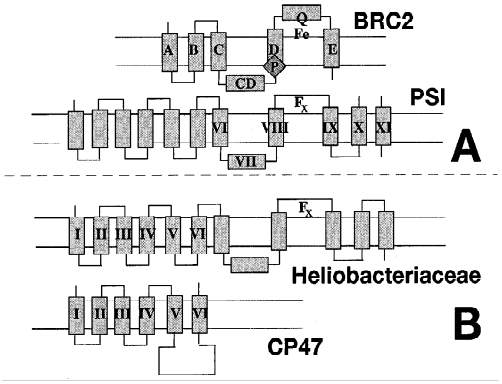

Figure 1. Preliminary alignment of the transmembrane spans of RC1, BRC2 and CP47 as inferred from work by Vermaas (1994) and Kuhn et al. (1994). We suggest that the hydrophobic segment VII of RC1 subunit lies outside of the membrane. This implied the existence of an additional membrane span to retain eleven membrane helices which are seen in the crystal structure of PS I (Fromme et al. 1996). The traditional numeration of membrane spans VI–XI was retained throughout the work, although the span VI is, in fact, the 7th one in Figure 1A. See text for further details.

tionary relations between distantly related sequences is to trace the conservation of the functionally important residues (this approach was used previously in suggesting the relatedness between the bacterial RC2 and PS II (Michel and Deisenhofer 1988), between various RC1 (Büttner et al. 1992; Liebl et al. 1993)) and in the work of Vermaas (1994) cited above). For the pigment-proteins these are, at the first hand, the ligands of pigments. We considered histidine residues as the key markers, as they are the most frequent ligands of cofactors (chlorophylls, hemes, quinones, etc.) in the energy-transferring membrane proteins (Gennis 1989; Pakrasi and Vermaas 1992). (Still the Mg-atom in (bacterio)chlorophylls may be also coordinated by arginine, glutamine, glutamate, asparagine, aspartate, methionine and even backbone carbonyls (Zuber and Brunisholz 1991; Kühlbrandt et al. 1994; McDermott et al. 1995)).

The high resolution structure of BRC2 (Deisenhofer et al. 1984, 1995) served as an anchor for the alignment of the predicted α-helical stretches of the other pigment protein complexes. In the crystal structure of *Rps. viridis* BRC2 a conserved [xS/THL/IDWx_8F/T] motif is contained in a short α-helix, which connects helices C and D from the periplasmic side (helix CD), whereas in PS I it is found in the predicted VIIth hydrophobic segment (Kuhn et al. 1994). We took this motif as a reference and, as shown in Figure 1A, considered the helices A-E of BRC2 as counterparts of the predicted hydrophobic segments IV–VI and VIII–IX of PS I center subunits under the assumption that helix VII, similarly to helix CD in BRC2, is the one that is seen to be located laterally from the lumenal side in the low-resolution crystal structure (Fromme et al. 1996). (The similarity of this attribution with the one proposed by Meyer (1994) is accidental. The latter was based on the view that the primary donor in RC1 is bound by the conserved histidine in the membrane span VIII. Recently this was disproved by Cui et al. (1995)). Assuming that the RC1 subunit of Heliobacteriaceae is structurally similar to those of PS I (Liebl et al. 1993), we compiled the attribution between RC subunits of PS I and BRC2 in Figure 1A with the one between RC subunit of Heliobacteriaceae and CP47 (Figure 1B), thereby following the work of Vermaas (1994).

Figure 1 attributes each α-helix of the BRC2 subunit to a certain membrane span in RC1 subunits and in CP47. This attribution was provisional; note, for example, that transmembrane spans VI in the RC subunits of Heliobacteriaceae and PS I do not overlap in Figure 1. However, using the scheme in Figure 1 as a guideline, we aligned sets of primary sequences of the RC2 subunits with those of RC1, CP, CAB proteins and bacterial LH as shown in Figure 2. Only the sequences of the membrane segments are depicted; the established membrane spans are indicated by solid lines; those predicted from hydropathy plots are depicted by dots. The positions of cofactor ligands are underlined. Colour scheme: established ligands (from crystal structures) are depicted in red. The residues that are suggested to occupy the positions of cofactor ligands due to their conservation, on the basis of structural data and/or by analogy are shown in violet. The meaning of the blue coloured residues is explained in the next section.

To reveal the extent of similarity between the five classes of proteins, we ‘extracted’ the best pairwise alignments of membrane segments belonging to proteins of different classes from Figure 2 and depicted them in Figure 3. The pairwaise alignments are sorted in five rows respective to the corresponding RC2 helices. For convenience we started from alignments with the C-terminal helix E of BRC2 sequence (Figure 3-I) and moved towards the N-terminal helix A (Figure 3-V). In the pairwise alignments the identical residues are shown in green, the residues with conserved aromaticity, electrical charge and/or capacity to serve as ligands are depicted in brown. Other cases of conservative exchange were neglected for simplicity.

Alignments in Figure 2 and Figure 3 specified the working scheme in Figure 1 and revealed the following new features on the evolutionary relations between different membrane chlorophyll-proteins (see the figure legends for the further details):

1. In contrast to previous suggestions (see e.g. Komiya et al. (1988)), the histidine residue in helix E that coordinates the none-heme iron in BRC2 is likely not homologous to the one in helix E of PS II which is proposed to perform a similar function (Figures 2 and 3-IB).
2. The conserved histidine residue in helix D of BRC2 which binds the bacteriochlorophyll of the primary donor (His-L173 and His-M200 in the BRC2 of *Rps. viridis*) found a counterpart only in the RC1 subunit of *Hb. mobilis* (Figures 2 and 3-IIA) but not in the other RC1 sequences.
3. Both α- and β-polypeptides of LH1 and LH2 showed similarity with helix D of the L-subunit of *Rps. viridis* RC2 (Figure 3-IIB, 3-IIE) and with the corresponding (see Figure 1) predicted helix VIII of *Hb. mobilis* RC1 (Figure 3-IIC, 3-IID).
4. In the alignment, which started from helix C of BRC2 (see Figures 2 and 3-IIIB, 3-IIIC), a histidine residue conserved in the VIth hydrophobic segments of RC1 and CP found a corollary by the conservation of aromaticity in this position in all RC2. In the crystal structure of *Rps. viridis* BRC2 the relevant Phe-121 on the L-subunit and Phe-148 on the M-subunit are in a very close proximity to the respective bacteriopheophytins ($\sim$ 4 Å, (Deisenhofer et al. 1995)). Moreover, the substitution of Phe-L121 for a histidine was shown to lead to the binding of a bacteriochlorophyll instead of bacteriopheophytin (when Phe-97 in the adjacent helix B was substituted for a less bulky valine or cysteine (Heller et al. 1995)). The other histidine residue in this alignment row, also conserved both in the predicted VIth hydrophobic segments of RC1 and CP, aligned with Tyr-161 on D1 and D2 subunits of PS II, the redox-cofactors of water oxidation Y_Z and Y_D.
5. Searching for the counterpart of helix B in the CP sequences, we failed to find the expected (from Figure 1) resemblance with helices V of CP43 and CP47. However the sequence stretches similar to those of helix B of RC2 were found in the adjacent long loop which connects the predicted membrane spans V and VI both in CP43 and CP47 (Figure 3-IVB).
6. In accordance with Figure 1 the predicted Vth hydrophobic segment in the PsaA subunit of PS I showed resemblance with helix B of RC2 (Figure 3-IVA). However, contrary to our expectations (Figure 1) the hydrophobic segment V of *Hb. mobilis* RC subunit predicted by Liebl et al. (1993) neither resembled the Vth hydrophobic segments of the other three primary structures of RC1 nor helix B of RC2 (Figure 2). Instead, it was similar to helix A of RC2 (Figure 3-VA). More thorough inspection of the RC1 sequences revealed some similarity, on the one hand, between hydrophobic segment V of *Hb. mobilis* and a sequence stretch between predicted hydrophobic segments IV and V of PS I and *Cl. limicola* and, on the other hand, between predicted hydrophobic segment V of PS I and *Cl. limicola* and a sequence stretch between hydrophobic segments V and VI of *Hb. mobilis* (Figure 2). Hence, it is plausible that the region around hydrophobic segment V of RC1 contains not one, but two transmembrane spans. To retain the overall numeration of hydrophobic segments of RC1 from Figure 1 (which was based on the one from the work of Liebl et al. (1993)) we denote

Figure 2. Alignment of the five membrane α-helices of RC2 with the corresponding membrane spans of RC1, CP43, CAB proteins and bacterial LH. Six sequences of RC2 (L and M subunits of *Rps. viridis* and *Chloroflexus aurantiacus* and D1 and D2 subunits of *Synechocystis* sp. PCC 6803) were compared with four sequences of RC1 (PsaA, PsaB subunits of maize PS I and the single subunits of homodimeric RC1 from *Heliobacterium mobilis* and *Chlorobium limicola*). The sequence of CP43 from *Synechocystis* sp. PCC 6803 was taken as representative of CP; the similarity between the CP47 sequence and those of *Heliobacterium mobilis* RC1 subunit has been shown before (Vermaas 1994). The most complete set of CAB genes obtained for tomato, *Lycopersicon esculentum*, was used as their sequence source. The sequences of the α-polypeptides of *Rps. viridis* LH1 and *Rhodocyclus gelatinosus* (Rc. gel.) LH2, along with the sequences of the β-polypeptides of *Rhodopseudomonas acidophila* and *Rhodopseudomonas palustris* LH2 and *Rc. gelatinosus* LH1 are shown as representatives of bacterial LH. See text for the colour scheme. Sequence sources for Figures 2 and 3: [1] – Michel et al. (1986); [2] – Ovchinnikov et al. (1988a); [3] – Ovchinnikov et al. (1988b); [4] – Osievacz and McIntosh (1987); [5] – Williams and Chisholm (1987); [6] – Chisholm and Williams (1988); [7] – Liebl et al. (1993); [8] – Fish et al. (1985); [9] – Büttner et al. (1992); [10] – Pichersky and Green (1990); [11] – Zuber and Brunisholz (1991); [12] – Efimov et al. (1988a); [13] – Smart and McIntosh (1991); [14] – Vermaas et al. (1987); [15] – Efimov et al. (1988b); [16] – Smart and McIntosh (1991); [17] – Mühlenhoff et al. (1993); [18] – Shinozaki et al. (1986).


RC2
Helix A
Helix B
BRC2
L_R.viridis[1]
M_R.viridis[1]
M_C.aurantiacus[2]
L_C.aurantiacus[3]
PSII
D1_S_6803[4]
D2_S_6803[5]
Chl?
CP
Helix V
Connecting loop
CP43_S_6803[6]
RC1
Helix V'
Helix V''
Hb.mobilis[7]
PsaB_maize[8]
PsaA_maize[8]
Cl.limicola[9]
Chl?
CAB proteins
Helix A
LHCII-I[10]
Helix B
LHCI-IV[10]
RC2
Helix C
Helix D
Helix E
BRC2
BPheo
P
Je
Fe
PSII
Pheo?
Yz,Yd
P680?
Fe?
Fe?
CP
Helix VI
CP43_S_6803[6]
RC1
Helix VI
Helix VIII
Helix IX
Chl?
Chl?
Chl?
Chl?
Bacterial LH
α-polypeptide
[11]
[11]
Chl
Chl
β-polypeptide
[11]
[11]
[11]
Chl
Chl

these two segments as V′ and V″ in our alignments.

7. Both transmembrane helices A and B of CAB proteins showed sequence similarity with the predicted membrane span V″ of the PsaA subunit of PS I (Figure 3-IVC, 3-IVD).

Hence, all the five membrane spanning α-helices of the center subunits of RC2 have found counterparts in RC1, CP, CAB proteins and bacterial LH. The percentage of identity in the pairwise alignments in Figure 3 was, as a rule, in the range of 25–40%, i.e. similar to those observed, in average, for the transmembrane segments of proteins belonging to the same class (see Figure 2 and Liebl et al. (1993); Komiya et al. (1988); (Bricker 1990); Pichersky and Green (1990); Zuber and Brunisholz (1991)). In most cases the similarity between transmembrane segments belonging to different classes was revealed after aligning the conserved established or assumed chlorophyll/cofactor-binding residues. Moreover, one can easy check in Figure 3 that shifting of these residues relative to each other leads to the sharp decrease of the degree of identity. In cases when more than one such ligand was present in a helix, the distance in terms of the number of residues between them varied between different transmembrane helices of *the same protein*, but it was conserved between the corresponding helices of proteins which belong to *different* classes. It was 10 residues for helix B, 13 residues for helix C and 16 residues for helix D of RC2 and for their counterparts in RC1, CP and bacterial LH. This is hardly coincidental. Taken together with previously revealed identity stretches (Vermaas 1994; Kuhn et al. 1994) it rather implied the origin of RC1, RC2, CP, CAB proteins and bacterial LH from a common ancestor.

Hypothesis: On the origin of the photosynthetic function

In all known reaction centers the primary donor, a (bacterio)chlorophyll dimer, is sequestered between

Figure 3. Pairwise alignments of membrane segments belonging to proteins of different classes. *Panel I*: Alignments starting from helix E in RC2. We used the conservation of the [FF/WM/SL] motif (containing a cluster of aromatic amino acid residues) to align helix E of RC2 and helix IX of RC1 (A). Note that an asparagine which is conserved in the middle of both PS I sequences and is likely to be a ligand (see Figure 2) found a counterpart in the sequence of the *Rps. viridis* L subunit. Another pairwise alignment, between the M subunit of *Rps. viridis* and the D2 subunit of PS II yielded 8 identical out of a total of 15 residues (B). *Panel II*: Alignments starting from helix D in RC2. We attributed helix D of BRC2 to the hydrophobic segment VIII of RC1 (Figure 1). In BRC2, helix D contains two conserved histidines, one that coordinates the (bacterio)chlorophylls of the primary donor and another one, 16 residues away, that contributes to the binding of the non-heme iron and quinone electron acceptors. The alignment based on two histidines as markers is corroborated by 7 identical amino acids out of 18 in the pairwise alignment between the L-subunit of *Rps. viridis* and the RC1 subunit of *Hb. mobilis* (A). Note that the conserved histidine in the middle of helix VIII of RC1 is corroborated by another potent ligand, asparagine, in the sequence of the L subunit of *Rps. viridis*. The α-polypeptides of LH1 also contains 16 residues between liganding histidine residue and strictly conserved arginine. As one can see, both α-polypeptides of LH1 and LH2 resemble helix D of the L-subunit of *Rps. viridis* RC2 (B, note a segment with 7 residues out of 8 identical between the α-polypeptide of LH2 of *Rc. gelatinosus* and the L subunit of *Rps. viridis*) and segment VIII of *Hb. mobilis* RC1 (C). Although the β-polypeptides of LH contain two conserved histidines at a distance of 17 residues, the similarity with the segment VIII of *Hb. mobilis* was obvious (D, 8 of 20 residues were identical including a rather unique aspartate in the middle of a hydrophobic span). *Panel III*: Alignments starting from helix C in RC2. The counterpart of helix C of RC2 are the predicted VIth membrane spans of RC1 and CP (see Figure 1). The similarity between this span in *Hb. mobilis* and the predicted VIth span of CP47 has been already noted by Vermaas ((Vermaas 1994), see Figure 1B). We found the similarity between the same span of *Hb. mobilis* RC1 and the predicted span VI of CP43 (A). In RC2 there is only one histidine, on subunit M of *Rps. viridis*, which might be considered as a counterpart to the conserved histidines of RC1 and CP (Figure 2). It is corroborated by an arginine residue, another possible chlorophyll ligand, in the other BRC2 (see the text for further details). B, a pairwise alignment between the hydrophobic segment VI of *Hb. mobilis* and helix C of the L subunit of *Rps. viridis*; 8 out of 23 residues are identical. C, pairwaise alignments between helix C of RC2 and the hydrophobic segment VI of CP. *Panel IV:* Alignments starting from helix B in RC2. The alignment based on histidines present in the helices B of the D1 and D2 subunits (Figure 2) and in the predicted membrane span V″ of PS I and of the *Cl. limicola* RC subunit gave a clue to establish the similarity between PsaA of PS I and RC2 sequences (A). It was noteworthy that both putative chromophore binding sites that we tentatively identified in helix B of RC2 (Figure 2) could be aligned with possible chlorophyll ligands in the sequence stretches similar to those of helix B of RC2 which we found both in CP43 and CP47 (B). The indication of the conserved Arg residues in helices B of RC2 as (former) pigment-binding site is supported 1) by their alignment with conserved histidines in CP43 and 47, 2) by the presence of histidine in this position in the M subunit of *C. aurantiacus* and 3) by their 'ligating' function in the modern BRC2 (see text). The sequence similarity between the membrane span V″ of PS I and both large transmembrane helices A and B of CAB proteins correlate with the presence of charged residues in both sequences (C and D). It has been established for LHCII-I by crystal structure analysis that some of them (depicted in red) serve as ligands for chlorophylls (Kühlbrandt et al. 1994). It is conceivable that they perform the same function in PS I. *Panel V:* Alignments starting from helix A in RC2. That the hydrophobic segment V′ of *Hb. mobilis* resembled helix A of RC2 (A) corroborated the observation by Vermaas (1994) of a correspondence between the predicted Vth hydrophobic regions of CP47 and of the RC1 subunit of *Hb. mobilis*, respectively. The former one (in the view of the exposed similarity between helix B of RC2 and the segment located between the membrane spans V and VI of CP, see Figures 2 and 3-IV) also corresponds to helix A of RC2 (B).

I. Helix E of RC2:

A) RC2 *versus* span IX of RC1

```
D2_barley[12]    HFFML      W
PsaB_maize[8]    FWMLN      F

D2_barley[12]    N  HFF L V W
Hb.mobilis[7]    Q  FF LQV  F

L_R.viridis[1]   H LGLF  N
PSaA_maize[8]    LGLF  N

M_R.viridis[1]   H  FFSL V  A
Hb.mobilis[7]    FFSLQV  A
```

B) BRC2 *versus* PSII

```
M_R.viridis[1]   S HRW FF L V V
D2_barley[12]    S KRW HFF L V V
```

II. Helix D of RC2:

A) RC2 *versus* hydrophobic span VIII of RC1

```
L_R.viridis[1]   H     F  NA A GLH
Hb.mobilis[7]    H     F  HA A GLH
```

B) RC2 *versus* α-subunit of bacterial LH

```
L_R.viridis[1]   P H  V  F V NAL LHGGL
B800_Rc.gel.[11] P    V  V  V AL LHGGL

L_R.viridis[1]   P H  V  F F NAL H GL
B1015_R.vir.[11] P R  V  F Y  AL H GL
```

C) span VIII of RC1 *versus* α-subunit of LH

```
Hb.mobilis[7]    H  FV A H IA  HF
B1015_R.vir.[11] R  FV  V  IA  HF
```

D) span VIII of RC1 *versus* β-subunit of LH

```
Hb.mobilis[7]    H V D  F A HAIA  H
B800_R.acid.[11] H V D  A A AIA  H

Hb.mobilis[7]    H V D  FVA HAIA  HF
B800_R.pal.[11]  H V D  TRIF AIA  HF

Hb.mobilis[7]    H  D FV HA A  HF
B880_Rc.gel.[11] H  Q FV  A A  HF
```

E) RC2 *versus* β-subunit of bacterial LH

```
L_R.viridis[1]   H  V  F  F A A  H
B880_Rc.gel.[11] H  VQG F  A A  H
```

III. Helix C of RC2:

A) span VI of RC1 *versus* span VI of CP43

```
Hb.mobilis[7]    H V G  F  G  FH
CP43_6803[6]     H V G  F  G  WH

PsaA_6803[13]    L T H  W  G  F  VG  HA
CP43_6803[6]     L T H  V  G  F  VG  HA
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B) RC2 *versus* span VI of RC1

```
L_R.viridis[1]   HV L F V  MF  VF R
Hb.mobilis[7]    QV L H V  MF  VF H
```

C) RC2 *versus* span VI of CP

```
M_C.auarant.[2]  Y  YG  A  LY  I  R
CP47_6803[14]    W  FG  A  LF  I  H

M_C.auarant.[2]  YLA  F  A  YLV  R
CP43_6803[6]     WLA  H  A  FLV  H
```

IV. Helix B of RC2:

A) RC2 *versus* membrane span V'' of RC1

```
D1_barley[15]    LHF LG A  R
PsaB_S.6803[16]  LHF LG A  R

D1_S.6803[4]     G PYQ V FHF  R  E
C1.limicola[9]   G PFN V FNF  R  E

D2_S.6803[5]     H  L  ML R  I
psaA_maize[8]    H  L  ML    I

L_C.aurant.[3]   F  T  A  MMR  I  D
psaA_S.sp.[17]   Y  T  HA A  MM   I  H
```

B) "long loop" of CP *versus* RC2

```
CP43_tobacco[18] G  F  LR  W  N  D SRL
D2_S.6803[5]     G  F  LH  F  R  E SRL

CP47_S.6803[11]  GGLF  A N  G  W H  K
D2_S.6803[5]     GGLW  A H  G  F R  R

CP47_S.6803[14]  GG FR  A N  GI  W H  E
D1_S.6803[4]     GG YQ  H H  GI  Y GR  R
```

C) span V'' of RC1 (PSI) *versus* helix B of CAB proteins

```
psaA_maize[8]    H Q  N AMLG  V  H
LHCII-I[10]      E E  H R AMLG  V  E

psaA_S.sp.[17]   HAQL  N AM  L I V Q
CP24[10]         EAEL H R AM  L I V Q
```

D) span V'' of RC1 (PSI) *versus* helix A of CAB proteins

```
psaA_maize[8]    H QL  NLAML  V H
LHCI-IV[10]      E EL N RLAML  VQH
```

V. Helix A of RC2:

A) RC2 *versus* Vth membrane span of *Hb. mobilis* RC1

```
M_C.aurant.[2]   FY  WNAV  F W  V FA  YN
Hb.mobilis[7]    FF  WEAV  F F  V FA  WN
```

B) RC2 *versus* Vth membrane span of CP43

```
D2_S.6803[5]     WSG  AL  V  WY
CP43_S.6803[6]   WSG  AL  V  WF
```

two polypeptides, each binding one chlorin moiety. The likely reason for this is that the charge separation between the primary donor and acceptors is preceded by the formation of a charge transfer state inside this dimer (Zinth et al. 1995). Nitschke and co-workers (1996) speculated that the ancestral reaction center might be formed from an association of two proteins which, as monomers, had carried another, yet unknown function. An obvious function that implied the existence of ancient pigmented proteins was the protection of the cell interior and DNA from the hazards of UV-light before the ozone layer was formed from the photosynthetically produced oxygen. Larkum has suggested that the need for UV-protection contributed to the invention of ancient RC which was followed by the development of chlorophyll-carrying antenna proteins (Larkum 1991). In contrast to this view we hypothesize that the primordial RC was formed by the dimerization of *preexisting* pigment-carrying antenna proteins, belonging to the ancient UV-protectors.

Support for this hypothesis can be found in the series of alignments presented in Figure 2. In the transmembrane spans studied here, the UV-absorbing aromatic amino acid residues (shown in blue) are not randomly distributed but rather clustered with each other and with chlorophyll/cofactor ligands (sometimes replacing the latter in the alignment rows). Several other residues that might participate in the UV-protection (depicted in light blue) are also seen in these clusters. (Histidine, methionine and cysteine, along with aromatic residues, may undergo photooxidation (Bensasson et al. 1983). Arginine, glutamine and asparagine have relatively high absorbance in the far UV (Handbook of Biochemistry and Molecular Biology, 1976); besides, along with histidine, aspartate, glutamate and lysine they may also serve as ligands). We suggest that these blue clusters, most conserved in RC1, mark the ancient sinks for UV-quanta. Capturing of the latter by these clusters decreased the probability of photo-cleavage by decreasing the life time of the excited state due to the excitonic interaction between the densely packed UV-absorbing residues. (The inclusion of excitonically interacting chromophoric polymer units is frequently used to increase the photostability of technical polymers (Shlyapintokh 1984)). In the next step, excitation was transferred to nearby pigments. (The ability of the carboxyhemoglobin to transfer UV-excitation to its heme moiety with a quantum yield of almost unity has been demonstrated already by Bücher and Kaspers (1947). In the extensive studies by Schreckenbach et al. (1978), Kalisky et al. (1981) and Polland et al. (1986) the quantum yield of the UV-excitation transfer from aromatic amino acid residues to retinal in bacteriorhodopsin has been estimated as 0.7–0.8). In this case a rapid internal conversion to the lowest excited singlet state of the pigment occurred. (This reaction is accompanied by significant release of thermal energy, and its rapid dissipation over a large volume is advantageous. The elasticity of α-helices to which porphyrins are attached and their large number were, perhaps, important to prevent thermal damage.) The lower was the energy of this singlet state, the better it was for the UV-protection. Chlorophyll is particularly favorable in this respect, it has two main excitation levels: one (the Soret band) in the near UV, close to the emission maxima of aromatic amino acids and the low-energy one, (the Q_y band) in the red. The transition from the Soret into the Q_y band with partial energy dissipation is very efficient. The enhancement of the Q_y band and its shift to the red in the conceivable evolutionary row from protoporphyrin IX via protochlorophyll *c* and chlorophyll *c* to chlorophyll *a* (Olson and Pierson 1987; Larkum 1991) increased the potential of chlorophyll not only as an absorber of visible light but also as a UV-protector.

Summarizing, *each* chlorin chromophore may be considered as a small photoreactive center (trap) operating in the UV region and surrounded by an antenna formed by interacting UV-absorbing amino acid residues. The actual arrangement of the aromatic residues around chlorin pigments in the crystal structure of BRC2 (Deisenhofer et al. 1984, 1995) is in line with this view.

As shown in Figure 2 (see blue coding colour), three UV-trapping layers are seen in each α-helix: two close to the membrane-water interface and one, less pronounced, in the middle. The three layered arrangement might have originally served to improve the packing of UV-absorbing residues with pigments and to increase the absorption cross section of the membrane. As an important side effect it paved the way for vectorial electron transfer between an electron donor (e.g. chlorophyll dimer) at one side, via an intermediate porphyrin carrier in the middle of the membrane to a secondary electron acceptor at the opposite side of the membrane. It is conceivable that the ancient function of this vectorial electron transfer was to prevent the trapping of excitation energy by the long-living and therefore hazardous triplet states of pigments. (The invention of carotenoids as triplet quenchers probably occured at a later stage. In Heliobacteriaceae they are present in small amounts, and they do not par-

ticipate in photoprotection as could be judged from the absence of the triplet transfer from bacteriochlorophylls to carotenoids (Smit and Amesz 1988).) Even the most primitive and completely reversible charge separation machinery was already advantageous in dissipating energy. Only later the stability of a charge separation across the membrane may have led to the 'secondary' function which is to gain useful work.

The transformation from the ancestral UV-protection to the productive photochemistry was, in our view, driven by mutations which *prevented* the binding of (bacterio)chlorophylls. It has been demonstrated by W. Vermaas and co-workers that point mutations which destroy the ability to bind a chlorophyll molecule destabilize the polypeptide and hinder its correct assembly (Pakrasi and Vermaas 1992; Shen et al. 1993). The empty cavity at the former position of a chlorophyll is of considerable size, and often it may expose the charges of the former chlorophyll ligands. It is conceivable that such cavities attracted cofactors (e.g. quinones or FeS complexes) that restabilised the polypeptide and were further useful (because of their absorption in the visible or near UV-portion of the spectrum) for the dissipation of UV-light. This reasoning may help to explain, how the water-splitting system in PS II might gradually evolved. The redox-active tyrosines in PS II are probably remnants of ancient porphyrin-binding sites (see Figure 2 and Figure 3-III). It is conceivable that Mn-atoms were trapped in the respective cavity where they performed a primitive function, disposing of UV-quanta and, perhaps, supplying electrons, in a facultative way, to the primary donor. It is worth noting, that a Mn-cluster containing electron vacancies might dissipate the energy of a *single* UV-quantum via formation of hydrogen peroxide from water. The ability to produce dioxygen from water at the expense of *four* low-energy quanta of red light may have *gradually* developed at a later stage of evolution.

Our view of the mechanism of UV-protection in the primordial cell conforms with the data on UV-damage in modern PS II. Although the action spectrum of this damage resembles the spectrum of a set of aromatic amino acid residues (Jones and Kok 1966), the photosynthetic cofactors which, as a rule, absorb at longer wave-lengths, namely chlorophylls, quinone acceptors and the Mn-cluster, have been shown to be specifically targeted (Bornman 1989; Karukstis 1991; Barbato et al. 1995). This might be explained by the channeling of the UV-excitation energy from the nearby UV-absorbing residues to the pigments and cofactors and by their destruction (after their capacity to dump off the UV-quanta is exhausted). UV damage of PS II might be considered as a reversal of evolution: upon UV-treatment 'PS II reaction centers are transformed into dissipative sinks for excitation energy' (cited from Karukstis (1991)).

The bacteriorhopsin (BR)-based photosynthesis in archaebacteria (see Lanyi (1993) for a review) might also have evolved *gradually* from a UV-protecting precursor function. After occluding a retinal moiety, an ancient membrane protein acquired the ability to discard of UV-quanta: the excitation energy, channeled from aromatic amino acid residues to retinal, was utilized to induce an isomerization of the latter. (An UV-quantum triggers, with quantum yield of 0.8, the *all-trans* $\rightarrow$ 13-*cys* isomerization of retinal followed by a normal photocycle even in the modern BR (Kalisky et al. 1981).) For the second turnover it was essential that the retinal returned into the initial isomeric state by slow thermal relaxation (which was gradually coupled with proton transfer later) or driven by the next UV-quantum. The blue light on the 13-*cys* retinal is known to shortcut the BR photocycle by triggering a 13-*cys* $\rightarrow$ *all-trans* back photoreaction (Kalisky et al. 1977; Ormos et al. 1978; Druckman et al. 1992). This feature, known as a blue light quenching of proton transfer, which is purely dissipative and counterproductive when viewed from the function of modern BR (Grishanin et al. 1996), may be a remnant of the ancient two-photon UV-protecting photocycle.

Evolutionary relations between photosynthetic pigment–protein complexes

Based on the foregoing alignments (Figures 2 and 3) we propose a large pigment-containing protein, with at least 11 transmembrane helices, supposedly the UV-protector of the ancient cell, as the common progenitor (Figure 4). Most probably it originated from the amplification of a simpler porphyrin-binding motif (simlar, e.g. to those used by the heme-binding helices of cytochromes *b*). To all likelihood the assembly of a large pigment–protein with *tens* of pigments became possible only after invention of Mg-porphyrins (chlorophylls) which may be coordinated by a single amino acid residue (contrary to Fe-porphyrins which need two of them). We suppose that only at this stage the deletions (insertions) in the transmembrane helices were valuable for the packing of many pigment-carrying helices. After a stable structure was formed,

the evolutionary value of further deletions (insertions) decreased dramatically.

The dimerization of this large protein initiated a primitive photochemistry of the ancient RC. The complementation of the ancient reaction center by the iron-sulfur clusters yielded ancient RC1 and started the reduction of $NAD(P)^+$.

The evolutionary line leading to RC2 is characterized by the loss of four transmembrane helices at the N-terminus of the ancient RC and of two further helices (X and XI) at its C-terminus. The conserved histidine residues in helix D of BRC2 which bind the bacteriochlorophylls of the primary donor have no counterparts in RC1 of plants (PS I) and *C. limicola* (Figure 2). This corroborates the recent data that the chlorophylls which form the primary donor in PS I are, to all likelihood, attached to the conserved histidine residues in the helix X ((Krabben et al. 1995) and P. Fromme, personal communication). Hence, it is conceivable that the gene fission which led to the ancestral RC2 was accompanied by the loss of the original binding site for the primary electron donor. A new interface has formed between two 'truncated' subunts. The formation of new special pair (P′) by chlorophylls attached to the histidine residues from the lumenal side of helix VIII (still conserved in Heliobacterialceae) contributed, perhaps, to the stabilization of this new interface. Interestingly, that the former chlorophyll ligands might participate in this stabilization. For example, the crystal structure of *Rps. viridis* RC (Deisenhofer et al. 1995) shows that the conserved arginine residues in helices B which overlap with possible ligands in CP sequences (see Figures 2 and 3-IV) perform exactly this function: Arg-103 on the L-subunit 'ligates' the M subunit (via Tre-M253 of helix E) and *vice versa*, Arg-M130 'ligates' the L subunit (via Asp-L218). In a similar way the conserved 'ligating' residues in helices C which overlap with histidines in the sequences of RC1 and CP (see Figures 2 and 3-III) stabilize the RC of *Rps. viridis* from the opposite lumenal side: Arg-L135 forms a hydrogen bond with Ser-L251 of helix E, whreas His-M162 forms a similar bridge with Tre-M285, the homologue to Ser-L251.

Another ancestral line started from a gene fission separating seven α-helices from the N-terminus of the ancient RC (or of the primordial UV-protector itself) and led to CP43, CP47 and related proteins (Bricker 1990). The structural data (Bricker 1990; Sayre and Wrobel-Boerner 1994) indicate that the segment that corresponds to the span 6 of the ancient CP lies outside the membrane both in the modern CP47 and CP43. Due to this the predicted span VI both in CP43 and CP47 is oriented in the membrane with its C-side pointing to the stroma (Bricker 1990; Sayre and Wrobel-Boerner 1994), i.e. opposite to the orientation of helix C in the crystal structure of BRC2 (Deisenhofer et al. 1984, 1995).

Two long helices of CAB proteins bear similarity with the predicted transmembrane segment V (or V″ in the numeration introduced here) of PS I which corresponds to the membrane span 6 in the ancient RC1 (Figures 2 and 3-IVC, 3-IVD). It is conceivable that this segment started an independent life after being cleaved out to become the ancestor of the CAB protein family after gene duplication. Other descendants of this cleaved fragment are, perhaps, the single-helical and presumably chlorophyll-binding proteins which were recently discovered in *Synochococcus* sp. (Miroshnichenko-Dolganov et al. 1995) and in *Synechocystis* sp. PCC 6803 (W. Vermaas, personal communication). They carry sequence resemblance with A and B helices of CAB proteins. It is noteworty, the [RxAMI/LG] motif, which is common for CAB proteins and the presumable helix V″ of PsaA of PS I (see Figure 3-IVC) is conserved also in this protein (Miroshnichenko-Dolganov et al. 1995). Although the function of this small protein is still unclear, it has been demonstrated that its induction in the cyanobacterial cell in response to UV-illumination is approximately 10 times stronger than in response to red light (Miroshnichenko-Dolganov et al. 1995).

Both α- and β-polypeptides of bacterial LH proteins are likely related to helix 8 of the ancient RC2 (as one might judge from similarity patterns with helix D of RC2 and hydrophobic segment VIII of *H. mobilis* shown in Figures 2 and 3-IIB-E). This common origin implies the relatedness of α- and β-polypeptides of LH to each other.

It is conceivable that the ancient UV-protector may also have evolved into another line(s) of non-photosynthetic enzymes by loosing its porphyrins and/or substituting them by smaller cofactors of different chemical nature. In this light it is not surprising that the [xS/THL/IDWx_8F/T] motif from the CD helix of bacterial RC2 is found not only in PS I, but also in the NDhH subunit of the NADH-plastoquinone oxidoreductase (Kuhn et al. 1994).

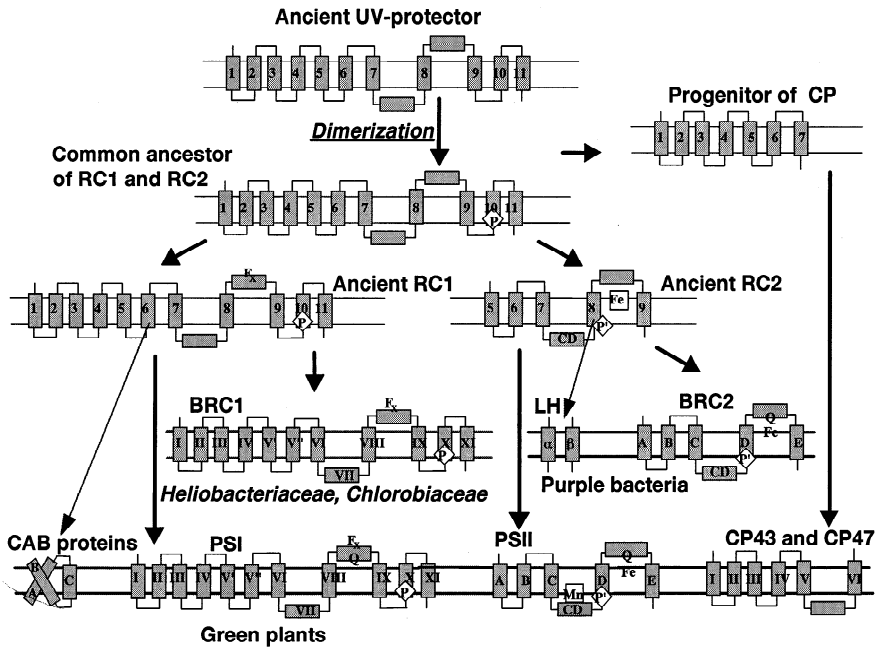

Figure 4. Evolutionary scheme for polypeptides of photosynthetic pigment-carrying proteins as derived from the sequence analysis. Although the RC2 is shown evolving from an ancient reaction center, its independent evolution directly from the UV-protector can not be excluded. The special pair pigments independently invented by RC1 and RC2 are depicted as P and P′, respectively. The scheme emphasizes that PS I, PS II, CP43, CP47 and CAB proteins are simultaneously present in thylakoid membranes of green plants whereas BRC2 and LH complexes are present in the membrane of modern purple bacteria. See text for the further explanations.

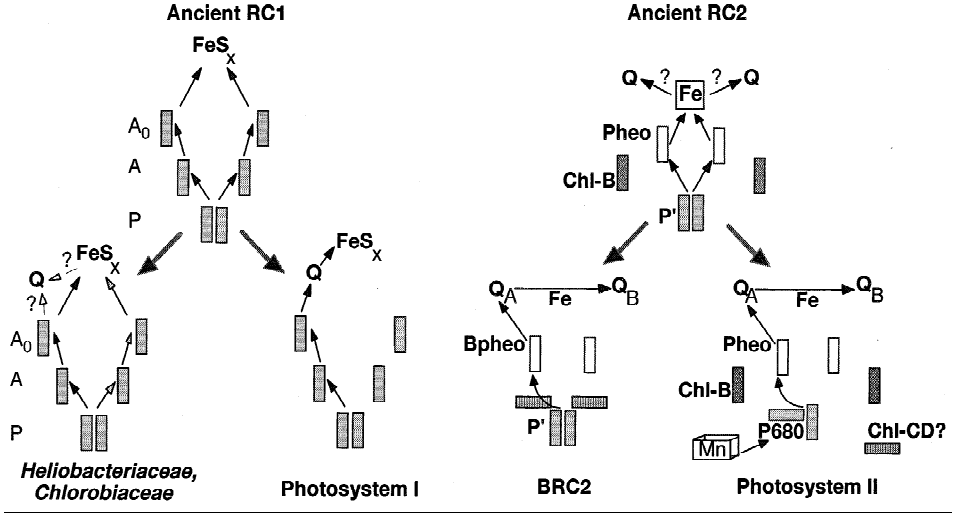

Figure 5. Evolutionary scheme for pigment arrangement in various RC1 and RC2. The question on the functional identity of both electron transfer branches in the modern RC1 of Heliobacteriaceae and Chlorobiaceae is left open. The putative rather orthogonal arrangement of two chlorophylls of P680 is based on data from (Van Mieghem et al. 1991; Noguchi et al. 1993; Mulkidjanian et al. 1996). See text for further explanations.

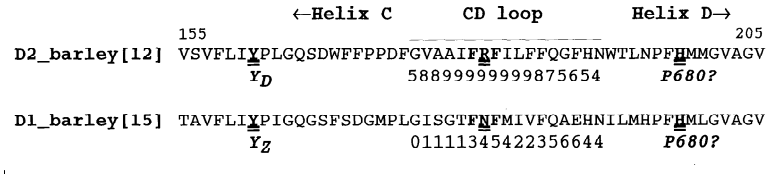

Figure 6. Primary sequences of the polypeptide loop connecting transmembrane helices C and D in the subunits D1 and D2 of PS II. The probability index for an α-helical structure (which varies from 0 to 9 and was determined with the help of PREDICTPROT mail server of EMBL as described in (Rost and Sander 1993; Rost et al. 1994; Rost and Sander 1994)) is indicated for both CD-loops. See the legend to Figure 2 for sequence sources.

On the template of a photosynthetic reaction center

As argued above, the charge separation machineries in the modern RC1 and RC2 evolved independently. Both capitalized, although in a different way, on the layered arrangement of UV-absorbing and chlorophyll-liganding amino acid residues in the membrane. The structure of PS I (Schubert et al. 1995; Fromme et al. 1996) shows a chlorophyll dimer near the lumenal side as a primary electron donor (P) and two another chlorin pairs: one in the middle of the membrane and another closer to the stromal side (denoted as A and A_0 in Figure 5). Even this low resolution structure of PS I clearly shows that all six core pigments are bound by the transmembrane α-helices. In BRC2, however, the middle pair of pigments, the accessory bacteriochlorophylls, are not bound by transmembrane helices but by the laterally lying CD-helices (loops). This means that the similarity between the arrangement of core pigments of PS I and BRC2 is only seeming. The structures of the charge separation machineries are analogous, but not homologous in RC1 and RC2.

As we suggested above, it is conceivable that the formation of RC2 took place at the interface between two truncated RC1-type subunits after two helices from the C-terminus were lost. Whereas P′ of RC2, analogous to P of RC1, is bound by histidine residues close to the lumenal surface, the primary electron acceptor, (bacterio)pheophytin, belongs to the outer UV-protecting layer, as follows from our alignments (Figure 2). The edge-to-edge distance between P′ and the presumable Mg-containing predecessor of (bacterio)pheophytin in the ancient RC2 was supposedly too large (it is still ~ 10 Å in the modern BRC2) to achieve a kinetically competent charge separation. As can be seen from the alignment in Figure 2 and as it was discussed above, a (bacterio)pheophytin molecule performs the function of the primary acceptor in all RC2 as the result of a substitution of a histidine residue (present in this position in RC1 and CP) for an aromatic amino acid. This pheophytinization might be driven by the necessity to increase the irreversibility of the charge separation. It accelerated the electron transfer from P'^* and hindered the back reaction because the midpoint potential of (bacterio)pheophytins is on the average 200 mV higher than the midpoint potential of corresponding (bacterio)chlorophylls (Watanabe and Kobayashi 1991). In the case of BRC2 the further kinetic improvement was achieved by pulling the CD helix, with a bound bacteriochlorophyll, more deeply into the membrane thus providing an additional electron carrier, accessory bacteriochlorophyll, between P′ and bacteriopheophytin (Figure 5). (The low-resolved structure of PS I shows at least 6 chlorophylls which are positioned laterally from the lumenal side (Fromme et al. 1996); this implies that some chlorophylls are still bound by the connecting loops even in the modern PS I).

PS II contains two additional conserved histidines in helices B of both subunits D1 and D2. They are currently assumed to bind two additional chlorophyll molecules (Chl-B in Figure 5) which may be involved in the transfer of excitation to the primary donor of PS II, P680 (see (Mulkidjanian et al. 1996) and references therein). The alignment series (Figures 2 and 3-IVA) shows that these two conserved histidines may be traced also in PS I and in the RC1 of *Cl. limicola*.

The CD loops of D1 and D2 subunits of PS II contain [FNF] and [FRF] amino acid triplets, respectively (Figure 6), which resemble the putative porphyrin-binding motifs that one may find in various sequences of RC1 and RC2 (see Figure 2 and also (Liebl et al. 1993)). The CD loop of D1 is assumed to be involved in the binding/assembly of the Mn-cluster (Nixon and Diner 1992); perhaps therefore its secondary structure is distorted when compared with the CD loop of D2 (Figure 6). The spectroscopic data do not support the presence of a chlorophyll molecule on D1 in the position of the accessory bacteriochlorophyll (see

Mulkidjanian et al. 1996). Moreover the presence of a chlorophyll molecule close to such strong oxidants as P680 and the water oxidase is unlikely. The CD loop of D2, on the contrary, is not known to carry any special function. It is, to all likelihood, an α-helix (see Figure 6) and may form a binding site for a chlorophyll ring. This chlorophyll may be either far enough from P′ and the water-splitting machinery not to be photooxidised (if the CD helix of D2 is not positioned so deeply inside the membrane as it happened in the case of BRC2, see Chl-CD in Figure 5) or, on the contrary, may be close to P680 as suggested by W. Vermaas (personal communication). In the latter case, to avoid photooxidation, it forms part of P680 itself.

It is conceivable that the non-heme iron is a remnant of one more porphyrin of the ancient RC2. The histidine residues in helices D that bind the none-heme iron in RC2 seem to be homologous to those that form the bacteriochlorophyll-binding site in the α- and β-polypeptides of LH (Figures 2 and 3-IIB-E). Thus, it can not be excluded that the secondary electron acceptor in the ancient RC2 was initially a porphyrin (most probably a Fe-porphyrin trapped on the interface between two subunits of the ancient RC2) which was involved in the charge separation (note that the none-heme iron in PS II can be still photoreduced). The presumable loss of the ability to accommodate the porphyrin ring (that might happen independently in the evolutionary lines leading to PS II and BRC2) implied the necessity to provide two additional ligands to the vitally important iron atom. This may explain why the iron-liganding histidines in helices E of PS II and BRC2 (Figures 2 and 3-IB) are non-homologous.

It follows from this analysis that the ancient RC1 and RC2 were probably formed by a chain of porphyrin-type pigments which crossed the membrane. The quinones, to all likelihood, were involved as electron acceptors on a later stage. (Their presence in the membrane was perhaps, originally also coupled with the UV-protection of the cell as they are good (and mobile!) fluorescence quenchers (Bensasson et al. 1983)). That the extraction of the whole quinone content from the membranes of Heliobacteriaceae do not influence the rate of charge separation between P and FeS_x (Kleinherenbrink et al. 1993), may indicate that participation of phylloquinone as an electron intermediate between A_o and FeS in PS I is a secondary acquisition specific only to PS I, but not to the RC1 of Heliobacteriaceae and Chlorobiaceae.

Conclusions and prospects

We arrived at a comprehensive evolutionary scheme with the following features: *Structurally*, two classes of reaction centers and three classes of antenna proteins were traced back to one common ancestor, a large protein with more than 10 transmembrane spans. The large size of this protein and its primary structure are most closely conserved by RC1 of Heliobacteriaceae. This is in line with previous speculations (Olson and Pierson 1987; Vermaas 1994). RC2 and three classes of antenna proteins are smaller and were traced back to gene cleavage events.

Series of alignments in Figures 2 and 3 offer a framework for structure predictions of pigment–protein complexes based on those whose crystal structures have been disclosed. For the latter the structural similarity between the homologous segments may be now traced.

Functionally, the ancestral protein may have served to the primordial cell as a UV-protector before the creation of the ozone shield. The photosynthetic function may have evolved *gradually* as follows:

a) The common ancestral pigment–protein carried UV-absorbing residues and pigments in three layers clustered at either side and in the middle of the membrane. The layering could have helped to promote energy transfer towards chlorins/porphyrins which, by rapid internal conversion, partially degraded the potentially harmful UV-quanta. The relics of this mechanism may be still operative in modern phototrophs.

b) A purely dissipative photochemistry (reversible charge separation) started still in the context of UV-protection.

c) The charge separation machinery was invented at least twice in the course of evolution, separately for RC1 and RC2.

d) The loss of certain pigments caused by mutations in their binding sites and the acquisition of redox cofactors, which filled the gaps and re-stabilized the protein, may have paved the way to proceed from dissipative to productive photochemistry.

A strange cycle of evolution: Billions of years ago the predecessors of photosynthetic organisms started to protect life from UV-light at the microscopic scale. Later, they developed the photosynthetic function as a further means to dispose of UV-quanta. The secondary product of the photosynthetic activity, oxygen, being transformed into atmospheric ozone by UV-rays, raised the protective function to the global level and provided

the conditions for the development of aerobic life and of mankind. Ironically, the destruction of this ozone shield may now evoke the need for the ancient UV-protecting machinery again.

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