Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 8135–8139, September 1992
Biochemistry

Photosynthetic reaction center genes in green sulfur bacteria and in photosystem 1 are related

(photosynthesis/*Chlorobium*)

MICHAEL BÜTTNER*, DIAN-LIN XIE†, HANNAH NELSON†, WILFRIED PINTHER*, GÜNTER HAUSKA*, AND NATHAN NELSON†

*Lehrstuhl für Zellbiologie und Pflanzenphysiologie, Universität Regensburg, 8400 Regensburg, Federal Republic of Germany; and †Roche Institute of Molecular Biology, Nutley, NJ 07110

Communicated by George Feher, May 6, 1992 (received for review January 20, 1992)

ABSTRACT **Oxygenic photosynthesis of chloroplasts and cyanobacteria involves two photosystems, which originate from different prokaryotic ancestors. The reaction center of photosystem 2 (PS2) is related to the well-characterized reaction center of purple bacteria, while the reaction center of photosystem 1 (PS1) is related to the green sulfur bacteria, as is convincingly documented here. An operon encoding the P840 reaction center of *Chlorobium limicola* f.sp. *thiosulfatophilum* has been cloned and sequenced. It contains two structural genes, coding for proteins of 730 and 232 amino acids. The first protein resembles the large subunits of the PS1 reaction center. Putative binding elements for the primary donor, P840 in *Chlorobium* and P700 in PS1, and for the acceptors A_o, A_1, and FeS center X are conserved. The second protein is related to the PS1 subunit carrying the FeS centers A and B. An adjacent third gene, not belonging to the reaction center, encodes a protein related to dolichyl-phosphate-D-mannose synthase from yeast. The different origins of PS1 and PS2 are discussed.**

Conversion of light into chemical free energy is fundamental for life, and photosynthetic reaction centers play a major role in this process. These are special protein–chlorophyll complexes in the core of light-harvesting photosystems, which use excitation energy to drive charge separation across the photosynthetic membranes. Two prototypes of photosynthetic reaction centers are known. One functions in the oxygen-evolving photosystem 2 (PS2) of chloroplasts and cyanobacteria, as well as in the nonoxygenic photosystem of purple bacteria. The other functions in ferredoxin reduction by photosystem 1 (PS1) of chloroplasts and cyanobacteria. A current view of the structure, common to both types, is a transmembrane heterodimeric arrangement of two proteins that hold the primary electron donor and the early electron acceptors. Two major differences between PS1-type and PS2-type reaction centers are that the former involves strongly reductive FeS centers as electron acceptors and contains many more chlorophyll molecules.

Research on PS2 was highly stimulated by the identities discovered in the genes for its heterodimer subunits, D1 and D2, and for the subunits L and M of a corresponding heterodimer in purple bacteria (1–4). Detailed modeling of the PS2 reaction center was possible because the reaction center of purple bacteria had been crystallized and its three-dimensional structure had been resolved by x-ray crystallography (4–6). Our picture of the reaction center of PS1 is lagging behind. Its structure has not been resolved yet, although crystallization has been achieved (7, 8). Interestingly, however, a similarity to the reaction center of green sulfur bacteria has been recognized (9–15). All the redox components known for PS1 (7, 8), the "special pair" of chlorophylls as electron donor, A_o and A_1 as early electron acceptors, and the subsequent FeS centers X, A, and B, have also been detected in the green sulfur bacterium *Chlorobium limicola* (11, 13), and they seem to exist as well in the distantly related *Heliobacteria* (14, 16). Moreover, the isolated reaction center from *Chlorobium* contains large subunits (10), very similar to PS1 preparations (17). The primary donor and the acceptors A_o, A_1, and X are thought to be located on these large subunits of PS1, while the two 4Fe4S centers A and B are contained in an extra subunit (7, 8).

Numerous genes from chloroplasts and cyanobacteria for the two large subunits, called *psaA* and *psaB* (8, 18), and for the polypeptide carrying the FeS centers A and B, called *psaC* (8, 19), have been sequenced. Here we report on the genes from the green sulfur bacterium *Chlorobium limicola* corresponding to *psaA/B* and *psaC*.‡

METHODS

Chlorobium limicola f.sp. *thiosulfatophilum* (Deutsche Sammlung von Mikroorganismen, Göttingen, F.R.G.) was grown, and its reaction center with the primary electron donor P840 was prepared as described (10), with minor modifications (11). From the major polypeptide at 65 kDa (see Fig. 1) proteolytic fragments were obtained either by soaking lyophilized gel pieces with staphylococcal protease V8 overnight, electroeluting the digest from the gel pieces on a second SDS gel according to Schägger and von Jagow (20), and electroblotting on Immobilon (Millipore) or by electroblotting first, digesting the 65-kDa band cut from the Immobilon filter with trypsin, and fractionating the digest by HPLC on a Serva RP8 column with a gradient from 0 to 100% acetonitrile in 0.1% trifluoroacetic acid. The peptides cut from the blot or in the HPLC fractions were sequenced in a gas-phase sequenator (Applied Biosystems). Four peptide sequences were obtained (see Fig. 3), and corresponding oligonucleotides were prepared to screen a gene bank of *Chlorobium* DNA, which we got by partial digestion with *Sau*3A1, isolation of 1- to 5-kilobase (kb) fragments, ligation into pBluescript SK (Stratagene), and transformation of *Escherichia coli* DH5α cells. Positive clones yielded overlapping sequences of 1629 base pairs (bp) (position 190–1819 in Fig. 3) and included the sequence coding for FPCLG-PVYGGTC (position 1570–1618 in Fig. 3). This dodecapeptide is related to the conserved peptides in PS1 that bind the

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Abbreviations: PS1 and PS2, photosystem 1 and 2; P700 and P840, primary electron donors of the reaction centers in PS1 and *Chlorobium*; A_o, A_1, and X, electron acceptors in the reaction center of PS1; L and M and D1 and D2, subunits of the reaction center of purple bacteria and of PS2, respectively.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M94675).

FeS center X, suggesting that we had found the desired gene. The sequenced region also contained a *Hin*dIII site (position 424 in Fig. 3). Digestion of *Chlorobium* DNA with *Eag* I yielded a fragment of about 5 kb in Southern blots that hybridized with the oligonucleotides above. To obtain the complete sequence of the region shown in Fig. 2, *Chlorobium* DNA was digested with *Eag* I and *Hin*dIII, and the two fragments (about 1 and 4 kb) reacting in the Southern blot with the 0.7-kb insert of a positive clone from the *Sau*3AI library were ligated into pBluescript SK and cloned in *E. coli* DH5α. DNA sequencing with oligonucleotide primers and dideoxynucleotides was carried out by the exonuclease III deletion technique (21). The complementary nature of the two *Eag* I–*Hin*dIII clones was proven by the sequences of several overlapping *Sau*3AI clones. All molecular biology procedures used can be found in Sambrook *et al.* (22).

RESULTS AND DISCUSSION

Cloning and Sequencing the Operon Encoding *Chlorobium* Reaction Center Proteins. The reaction center from the green sulfur bacterium *Chlorobium limicola* f.sp. *thiosulfatophilum* was purified some years ago (10, 11), and more recently it was also purified from *Heliobacteria* (12, 23). Fig. 1 shows the polypeptide pattern of the P840 reaction center from *Chlorobium*. Most prominent is a diffuse band of about 65 kDa, from which four partial peptide sequences were obtained. It corresponds to a similar band seen in PS1 preparations (17), which represents the two large reaction center subunits of PS1 of about 83 and 82 kDa (7, 8, 18). Fig. 2 shows the general arrangement of the DNA fragment containing the reaction center genes. It is 4.8 kb long and reveals three open reading frames (GenBank, accession no. M94675, gene description CHLORC). All four peptide sequences of the 65-kDa band (Fig. 1) are found in the first reading frame (see italic letters in Fig. 3), which encodes a protein of 730 amino acids, with a calculated mass of 82.24 kDa, very close to the mass of the heterodimer proteins of PS1. The second open reading frame codes for a protein of 23.87 kDa with 232 amino acids which is related to the subunit of PS1 holding the two 4Fe–4S centers A and B (see below). The third open reading frame codes for a protein of 185 amino acids, which probably does not belong to the reaction center. It is partially identical to dolichyl-phosphate-D-mannose synthase from yeast (24), but the C-terminal hydrophobic sequence is missing. Stem–loop elements are found at nucleotide positions 3659–3711 and 4392–4230, after the second and the third structural gene. Stem–loops function as transcriptional terminators in cyanobacteria (25) and may have a similar function in green bacteria. They suggest that the first two genes are transcribed as bicistronic mRNA and the third is transcribed separately. A ribosome binding site, AAGGAG, is found at position −9 from the initiator methionine. This Shine–Dalgarno sequence is very similar to the sequences in *E. coli* and cyanobacteria (25, 26). A potential Pribnow box (27) consisting of the sequence TATCAT, 309 nucleotides upstream from the initiator methionine, and a second one with a sequence of TTGATA, 17 nucleotides further on, were identified. These

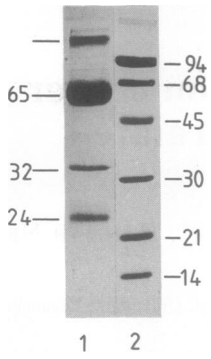

FIG. 1. Polypeptides of the reaction center preparation from *Chlorobium*. Lane 1, P840 reaction center. The top band is an incompletely unfolded, still green form of the 65-kDa band, as is observed also in preparations of the PS1 reaction center. Lane 2, protein standard. Numbers indicate molecular mass values in kDa. The reaction center was prepared, electrophoresed on SDS/15% polyacrylamide gels, and stained with Coomassie blue as described (10, 11).

sequences may constitute part of the promoter system for the operon.

Comparison of the P840 Protein with Other Reaction Center Proteins. From the hydropathy plot of the P840 protein up to 11 hydrophobic segments with a potential for forming transmembrane helices can be identified. Fig. 3 shows a fit of the P840 sequence into a consensus alignment for the two large PS1 subunits (8, 18). Of the 11 putative transmembrane segments, I–III and VI–XI of the P840 protein fit the corresponding segments in the PS1 subunits, but the region of the putative transmembrane helix V in *psaA* and *-B* products carries one negative and two positive charges in the P840 protein. On the other hand, shifting the transmembrane spans in this part of the P840 protein toward the N terminus would make the corresponding regions IV in the P840 protein and in the *psaA* and *-B* products run in opposite directions. Possibly segments IV and V are not embedded in the membrane. However, the distribution of charges in the interhelical loops, which follows the "positive inside rule" (28) when the N terminus faces the cytoplasm, favors a model with 11 transmembrane spans, with a net positive charge of 14 on the cytoplasmic surface and a net negative charge of 7 on the outer surface of the membrane. Such a folding is in accordance with the favored model for the PS1 reaction center (29).

The overall identity of amino acid residues is 15% for P840/psaA and 14% for P840/psaB (Fig. 4). It is especially pronounced in the stretch comprising helices VIII and IX, where P840, A_1, and the FeS center X may be bound. For the psaA and psaB proteins the overall identity is 45% (8).

The P840 protein contains only 20 histidines, compared to 42 and 37 in the *psaA* and *-B* products, respectively. Of these 20 histidines only 7 are found at identical positions in psaA and -B. The one in the putative helix VIII might bind P840, presumably a "special pair" of bacteriochlorophylls a, like the chlorophylls a for P700 (see ref. 8). Of the two histidines conserved in transmembrane span VI the one closer to the cytoplasmic surface may hold the primary acceptor A_o, which in PS1 is probably a chlorophyll a molecule (8), in place of the pheophytin in the reaction centers of PS2 and purple bacteria (3, 4). No histidines are conserved in the cytoplasmic halves of helices VIII and IX, which would resemble the ones in the fourth and fifth helix in the reaction center polypeptides of purple bacteria and PS2 and which ligate an Fe^{2+} ion (6). Such an iron can therefore be excluded for PS1-type reaction

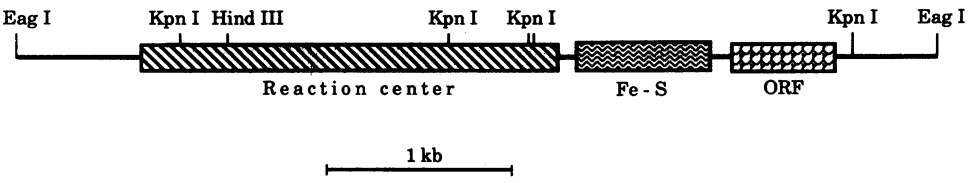

FIG. 2. Arrangement of the 4.8-kb DNA fragment. The scheme shows the two complementary clones from the *Eag* I–*Hin*dIII library with the three open reading frames and useful *Kpn* I sites. Reaction center, gene of the large reaction center subunit; Fe-S, gene of the subunit carrying FeS centers A and B; and ORF, open reading frame.

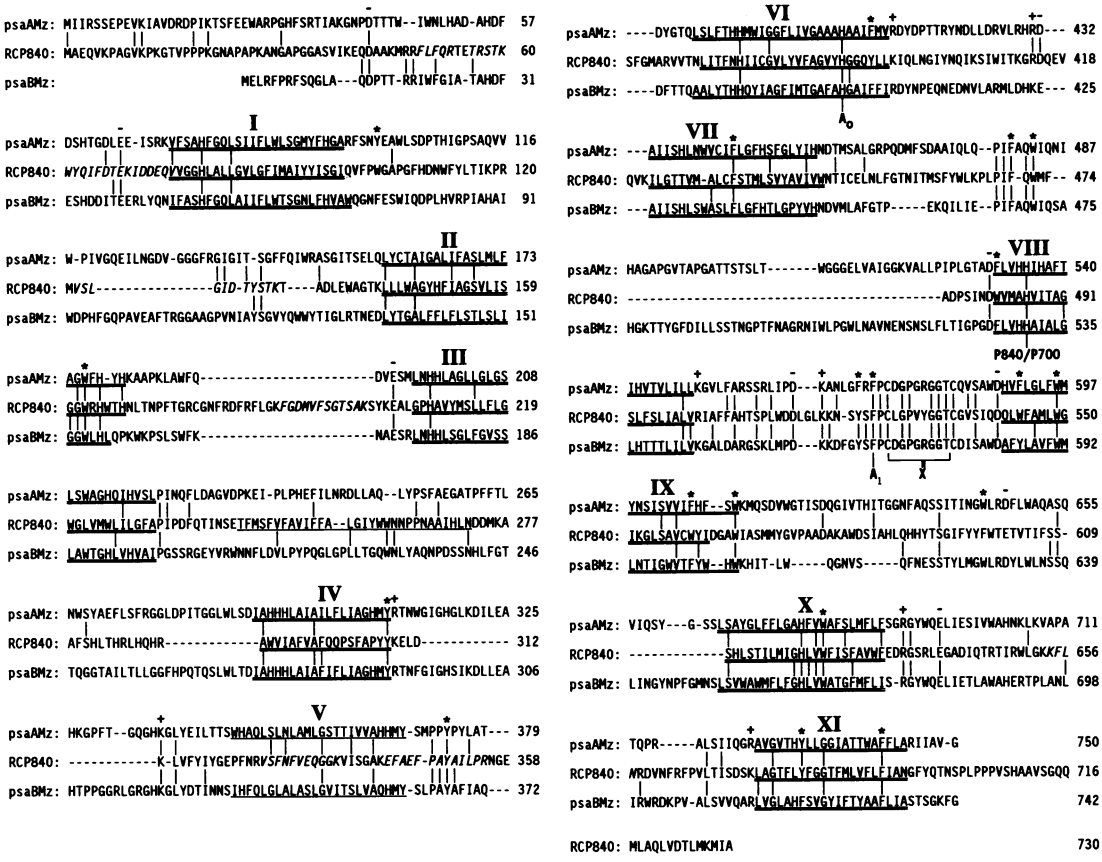

FIG. 3. Alignment of the amino acid sequence for the P840 protein with the large PS1 subunits from maize. RCP840, psaAMz, and psaBMz stand for P840 reaction center protein and the gene products of *psaA* and *psaB* from maize (18). Amino acid residues are shown in the single-letter code. The peptides obtained from the 65-kDa band in Fig. 1 are shown in italic letters. Identical residues are indicated by vertical bars. The 11 putative transmembrane spans of the *psaA* and *-B* products are marked by roman numbers. The 10 of them also found in RCP840 are double underlined, and the one not falling into place is single underlined. Conserved charges (+ and −) and aromatic residues (*) are indicated on top, and possible binding of the "special pair" P840 or P700 and of the electron acceptors A_o and A_1 and the FeS center X are indicated below the alignment. The P840-sequence was fit into an alignment of psaA and B (8, 18) by eye, with the attempt to conserve a maximum of identical residues and putative transmembrane helices.

centers, as is already suggested by the absence of any magnetic interaction of the spin-polarized EPR signal of A_1^- (32).

Most striking is the conservation of 9 out of 12 residues of psaA and -B, in the loop between the spans VIII and IX. This dodecapeptide contains two cysteines and presumably holds the 4Fe–4S center X. The *Chlorobium* peptide lacks the aspartates next to the cysteines. The homologous dodecapeptide from *Heliobacillus mobilis* also lacks these charged residues (33). As expected, the favored folding with 11

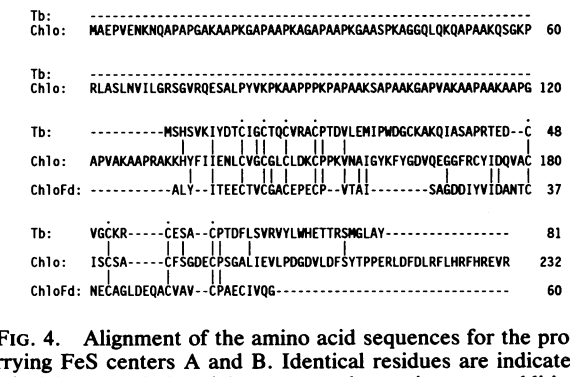

FIG. 4. Alignment of the amino acid sequences for the proteins carrying FeS centers A and B. Identical residues are indicated by vertical bars, and the eight conserved cysteines are additionally marked by dots. The *Chlorobium* sequence (Chlo) is compared to the product of *psaC* from tobacco (Tb; ref. 19). For comparison the sequence of *Chlorobium* ferredoxin (ChloFd) is included (30, 31).

transmembrane spans places the peptide with the FeS center X on the cytoplasmic surface.

Several tryptophans and other aromatic residues are conserved, especially in helix IX. These might facilitate charge transfer across the membrane. Furthermore, the first residue of the dodecapeptide binding the FeS center X is a phenylalanine. This could mediate transfer of electrons to center X from the acceptor A_1, which is thought to be a phylloquinone in PS1 (8, 34–36) and may be menaquinone in the P840 reaction center (37). In the L subunit of purple bacteria and in the D1 subunit of PS2 a phenylalanine is found in the loop between the fourth and fifth transmembrane helix (2, 4). The M and the D2 subunits contain a tryptophan in this position (2, 4). This, however, facilitates electron transfer to, not from, the quinone. An overall structural similarity of the reaction center subunits L and M in purple bacteria, and D1 and D2 in PS2 on one hand, with the C-terminal part of the large subunits in PS1 on the other, has been considered recently (refs. 15, 30, and 38; see below).

The Protein Containing FeS Centers A and B. The second gene of the transcription unit may be related to the *psaC* gene for the FeS protein in PS1 carrying the two 4Fe–4S centers A and B. The 24-kDa band in Fig. 1 could represent this protein, but this remains to be established. Fig. 4 shows its derived amino acid sequence aligned to the product of tobacco *psaC* (19). For comparison we included the 60 amino acids of *Chlorobium* ferredoxin 1, which also carries two 4Fe–4S centers (31, 39). The 13 *psaC* genes sequenced so far,

from cyanobacteria to higher plants, all correspond to small proteins of 81 amino acids and 8.8 kDa (8). However, the *Chlorobium* protein has a large N-terminal extension and a smaller C-terminal extension. These do not show any significant relation to the sequences of other subunits of PS1 (8) or any other protein in the data banks. The N-terminal extension is highly positive, containing repetitions of tetrapeptides with two alanines flanked by proline or lysine. A similar positive N-terminal stretch is found between amino acid positions 11 and 45 of the P840 gene (Fig. 3), which can be aligned with amino acid positions 84–118 of Fig. 4 with over 50% identity. These positively charged peptides on the cytoplasmic surface could function in docking the highly negative ferredoxin of *Chlorobium*, with 11 negative charges and not a single positive one (Fig. 4). It is not clear yet, however, that this ferredoxin is the redox partner of the psaC-related protein in *Chlorobium*. Of the 81 amino acids in the *psaC* products only 16 (20%) are conserved in *Chlorobium*, most prominently two clusters of four cysteines, probably binding the two 4Fe–4S centers A and B. In addition, prolines following the fourth cysteine of both clusters are conserved. A glycine is found before the second cysteines of both clusters in the *psaC* products, but only of the first cluster in *Chlorobium*. The prolines are also found in ferredoxins from *Chlorobium* and other bacteria, but not the glycines (39). While the cysteines in the first cluster are spaced twice by two and once by three amino acids in all cases, this spacing is kept in the second cluster only for the *psaC* products (Fig. 3) and some of the bacterial ferredoxins (39). In the *Chlorobium* subunit the last two cysteines of the second cluster are spaced by five amino acids instead. Since the two 4Fe–4S centers are bound by the first three cysteines of one cluster and by the fourth of the other (39), this insertion need not cause a large effect. More importantly, another observation allows the assignment of the two cysteine clusters to FeS centers A and B. Two positive charges between the second and the third cysteines of the second cluster, which could stabilize the reduced form of an FeS center, are conserved in all psaC proteins but are missing in *Chlorobium*. The redox potentials of centers A and B are turned around in *Chlorobium* in comparison to PS1, center A being more negative than center B (13). Center A thus is probably bound to the first three cysteines of the second cluster and to the fourth cysteine of the first cluster (39).

Homo- or Heterodimer? The reaction center operon contains only one gene for a large subunit in the P840 reaction center (Fig. 2). For all PS1 reaction centers two genes are known, called *psaA* and *psaB*, usually organized in one transcription unit (8). In *Chlorobium*, however, we found no signs for a second related gene in Southern blots, even under very low stringency. An example is shown in Fig. 5. Also, blots of digests with 11 other restriction enzymes revealed only one reactive fragment each (not shown). Cross-hybridization occurs between *psaA* and *-B* of PS1 and therefore is also expected for the two *Chlorobium* genes that would encode a heterodimer of the reaction center. Does *Chlorobium* live with a homodimeric reaction center? This would explain why all four peptides obtained from the 65-kDa subunit (Fig. 1) are found in the one gene presented here (Fig. 3). The probability for this is only 1 in 16 for two proteins equally accessible to proteases.

Purple bacteria, PS2 (3, 6), and probably also PS1 (7, 8) contain heterodimeric reaction centers of pseudo-*C2* symmetry. The redox centers for charge separation are organized in two branches across the membrane, one of them being favored by symmetry-breaking elements. The advantage of such an arrangement is a matter of intense discussion. At low light intensity the quantum yield may be increased in an asymmetric system, which allows the two quinone acceptors to act in series, the one in the preferred branch being reduced

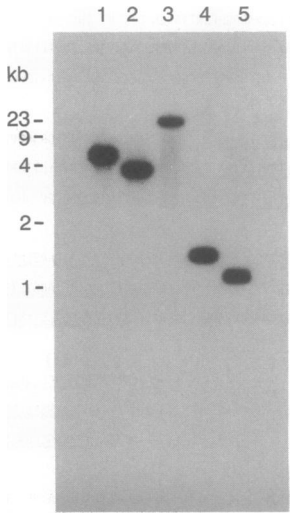

FIG. 5. Southern blot of a piece in the 4.8-kb *Eag* I fragment with *Chlorobium* DNA cut by several restriction enzymes. The DNA piece used was 750 bp long, downstream from the *Hin*dIII site marked in Fig. 2, and corresponds to 250 amino acids from the P840 protein. The DNA was cut with *Eag* I (lane 1), *Eag* I + *Hin*dIII (lane 2), *Hin*dIII (lane 3), *Kpn* I (lane 4), or *Hin*dIII + *Kpn* I (lane 5). The blot was washed with 0.15 M NaCl/0.015 M sodium citrate at 37°C.

first and staying permanently bound (6). At high light intensity photodamage may be avoided, because a permanently bound quinone in the preferred branch can accept two electrons, before triplet states are built by back-reactions, causing the formation of singlet oxygen. Perhaps triplet states were less damaging for a strictly anaerobic ancestor to *Chlorobium*, and it could live with parallel branches of electron transfer to mobile quinones, and this might have been kept after an FeS center X had been acquired. Actually, it is not known whether the quinone in *Chlorobium* reflecting A_1 is tightly bound as in PS1 (35, 36) and whether its only function is to reduce FeS center X.

In the context of a homo- versus a heterodimeric structure, the cluster of the charged residues REXEXXXK/R at the C terminus of transmembrane helix B of the L subunits in the reaction center of purple bacteria and of helix II in the D1 subunit of PS2, close to the pheophytin of the preferred branch (38), is worth mentioning. In the psaA subunit of PS1 a similar charge cluster reading RDXDXXXR is found after putative helix VI, but the P840 protein lacks it. Only the first positive charge is conserved, as in the M and D2 subunits, the positions reading KIXLXXXY. Finally, also in accordance with a homodimeric structure, the leucine zipper motif in front of the FeS X binding peptide, which has been implicated for heterodimer formation in PS1 (40), is not found in the P840 protein.

Evolutionary Implications. The conservation of significant amino acid residues, as well as the placement of hydrophobic spans in the large subunit, confirms the conclusion that *Chlorobium* has a PS1-like reaction center. Moreover, the following from putative transmembrane helix VI to IX of the large PS1-type subunits is similar to the folding from the second to the fifth helix of the smaller reaction center subunits in purple bacteria and PS2 (15, 29, 38), as indicated by the shaded regions in Fig. 6. The N-terminal part of the large PS1 subunits probably function as chlorophyll antennae. What might the ancestral gene for all types of reaction centers have looked like? The question of a small or large ancestor cannot be answered, but it is reasonable to assume that the heterodimers in PS1 and PS2 evolved by duplication of a gene for a homodimer (Fig. 6). This might have happened twice. Even three duplications could have occurred, because the PS2 subunits D1 and D2 are more closely related than subunits L and M from purple bacteria (1, 6, 41). However, mutation rates need not have been the same. They could have been slower in PS1 and PS2 reaction centers, which have many more interacting subunits. Higher stringency of mutability in plants and cyanobacteria is obvious from the high conservation of the *psaA* and *-B* products, which are over 80% identical (8). Between the two purple bacteria *Rhodo-*

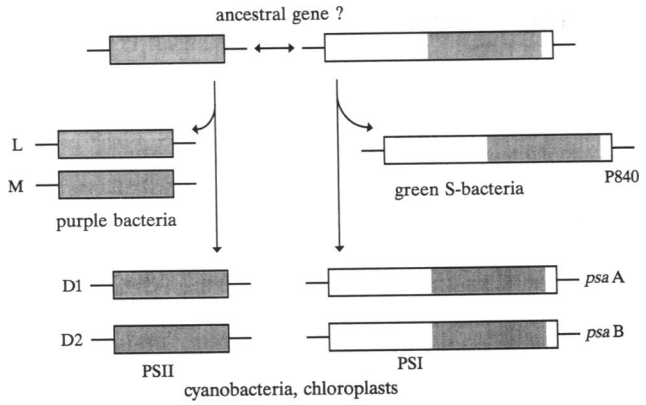

FIG. 6. Evolutionary concept of the reaction center heterodimers in oxygenic photosynthesis. L and M and D1 and D2 stand for the subunits constituting the heterodimer of the reaction center in purple bacteria and PS2, respectively, and *psaA* and *psaB* are the denotations of the genes coding for the two large subunits of PS1 (8). Shaded parts of the bars indicate related regions in all reaction centers.

pseudomonas viridis and *Rhodobacter capsulatus* only 50–60% of the residues in subunits L and M are conserved (1, 6).

The identity between PS2-type reaction centers of oxygenic photosynthesis in cyanobacteria and of nonoxygenic photosynthesis in purple bacteria is remarkably small. In spite of their relationship only 10% of the residues in the M subunit of purple bacteria and the D2 subunit of PS2 are identical, and only 13% in L and D1 (1, 6). Likewise, the identity of PS1-type reaction centers in cyanobacteria and *Chlorobium* is small as well. The P840 protein is only 15% and 14% identical to the products of *psaA* and *-B*, respectively (Fig. 3). In the FeS A and B proteins of *Chlorobium* and PS1 again only about 20% of the corresponding 81 amino acids are identical (Fig. 4), while within PS1 from cyanobacteria to higher plants more than 90% are conserved (8). The high percentage of mutational replacements pinpoints the functionally required amino acids in both prototypes of reaction centers, and thus provides valuable suggestions for directed replacements.

Although the N-terminal parts in the large subunits of PS1 and *Chlorobium* might serve as core antennas (29, 38), the proposed chlorophyll binding elements are largely not found in the P840 protein. From the hexapeptide DPTTRR at position 15 of the psaB protein, which is also found in an antenna protein of PS2 (29), PTT has been lost in the P840 protein (Fig. 3). Also, other DP dipeptides and histidines are found less frequently, likely reflecting the difference between chlorophyll and bacteriochlorophyll binding.

In conclusion, as suggested in Fig. 6, the reaction centers of PS1 and PS2 may have joined during the evolution of cyanobacteria by horizontal recombination from two different roots, one being also ancestral to the green sulfur bacteria and the other to the purple photosynthetic bacteria. Other membrane protein complexes in cyanobacteria, such as cytochrome oxidase (42), may have been acquired by similar events. Indeed, the mosaic structure of bacterial genomes, and even of individual genes, is increasingly appreciated (43).

The preparation of the reaction center by Mrs. E. Herold and the peptide sequencing by Dr. R. Deutzmann are gratefully acknowledged. G.H. is grateful for intensive discussions with W. Nitschke.

1. Michel, H., Weyer, K. A., Gruenberg, H., Dunger, I., Oesterhelt, D. & Lottspeich, F. (1986) *EMBO J.* **5,** 1149–1158.
2. Trebst, A. (1986) *Z. Naturforsch. C.* **41,** 240–245.
3. Michel, H. & Deisenhofer, J. (1988) *Biochemistry* **27,** 1–7.
4. Komiya, H., Yeates, T. O., Rees, D. C., Allen, J. P. & Feher, G. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9012–9016.
5. Deisenhofer, J., Epp, O., Miki, K., Huber, R. & Michel, H. (1985) *Nature (London)* **318,** 618–624.
6. Deisenhofer, J. & Michel, H. (1989) *EMBO J.* **8,** 2149–2170.
7. Chitnis, P. R. & Nelson, N. (1991) in *Cell Culture and Somatic Cell Genetics of Plants*, eds. Bogorad, L. & Vasil, I. K. (Academic, New York), pp. 177–224.
8. Golbeck, J. H. & Bryant, D. A. (1991) *Curr. Top. Bioenerg.* **16,** 83–177.
9. Blankenship, R. E. (1985) *Photosynth. Res.* **6,** 317–333.
10. Hurt, E. & Hauska, G. (1984) *FEBS Lett.* **168,** 149–154.
11. Nitschke, W., Feiler, U., Lockau, W. & Hauska, G. (1987) *FEBS Lett.* **218,** 283–286.
12. Trost, J. T. & Blankenship, R. E. (1989) *Biochemistry* **28,** 9898–9904.
13. Nitschke, W., Feiler, U. & Rutherford, A. W. (1990) *Biochemistry* **29,** 3834–3842.
14. Nitschke, W., Setif, P., Liebl, U., Feiler, U. & Rutherford, A. W. (1990) *Biochemistry* **29,** 11079–11088.
15. Nitschke, W. & Rutherford, A. W. (1991) *Trends Biochem. Sci.* **16,** 241–245.
16. Woese, C. R. (1987) *Microbiol. Rev.* **51,** 221–271.
17. Bengis, C. & Nelson, N. J. (1975) *J. Biol. Chem.* **250,** 2783–2788.
18. Fish, L. E., Kück, U. & Bogorad, L. (1985) *J. Biol. Chem.* **260,** 1413–1421.
19. Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongsbe, J., Obokata, J., Yamaguchi-Shinozaki, K., Kato, A., Tohdo, N., Shimada, H. & Sugiura, M. (1986) *EMBO J.* **5,** 2043–2049.
20. Schägger, H. & von Jagow, G. (1987) *Anal. Biochem.* **166,** 369–379.
21. Henikoff, S. (1984) *Gene* **28,** 351–359.
22. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
23. van de Meent, E. J., Kleinheerenbrink, F. A. M. & Amesz, J. (1990) *Biochim. Biophys. Acta* **1015,** 223–230.
24. Orlean, P., Albright, C. & Robbins, P. W. (1988) *J. Biol. Chem.* **263,** 17499–17505.
25. Cozens, A. L. & Walker, J. E. (1987) *J. Mol. Biol.* **194,** 359–383.
26. Lill, H. & Nelson, N. (1990) in *Current Research in Photosynthesis*, ed. Baltscheffsky, M. (Kluwer, Dordrecht, The Netherlands), pp. 128–136.
27. Resnikoff, W. S., Siegele, D. A., Coming, D. W. & Cross, C. A. (1985) *Annu. Rev. Genet.* **9,** 355–388.
28. von Heijne, G. (1986) *EMBO J.* **5,** 3021–3027.
29. Fish, L. E., Kück, U. & Bogorad, L. (1985) in *Molecular Biology of the Photosynthetic Apparatus*, eds. Steinback, K. E., Bonitz, S., Arntzen, C. J. & Bogorad, L. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 111–120.
30. Margulies, M. M. (1991) *Photosynth. Res.* **29,** 133–147.
31. Tanaka, M., Haniu, M., Yasunobu, K. T., Evans, M. C. W. & Rao, K. K. (1974) *Biochemistry* **13,** 2953–2959.
32. Gast, P., Thurnauer, M. C., Petersen, J. & Stehlik, D. (1987) *Photosynth. Res.* **14,** 15–30.
33. Blankenship, R. E., Trost, J., Causgrove, T., Alden, R., Brune, D., Liebl, U. & Vermaas, W. (1991) *Photochem. Photobiol.* **53,** 6S (abstr.).
34. Takahashi, Y., Hirota, K. & Katoh, S. (1985) *Photosynth. Res.* **6,** 183–192.
35. Schöder, H.-U. & Lockau, W. (1986) *FEBS Lett.* **199,** 23–27.
36. Biggins, J. (1990) *Biochemistry* **29,** 7259–7264.
37. Hauska, G. (1988) *Trends Biochem. Sci.* **13,** 415–416.
38. Robert, B. & Moenne-Loccoz, P. (1990) in *Current Research in Photosynthesis*, ed. Baltscheffsky, M. (Kluwer, Dordrecht, The Netherlands), pp. 65–88.
39. Oh-Oka, H., Takahashi, Y., Kuriyama, K., Saeki, K. & Matsubara, H. (1988) *J. Biochem. (Tokyo)* **103,** 962–968.
40. Webber, A. N. & Malkin, R. (1990) *FEBS Lett.* **264,** 1–4.
41. Beanland, T. J. (1990) *J. Theor. Biol.* **145,** 535–545.
42. Wastyn, M., Achatz, A., Molitor, V. & Peschek, G. A. (1988) *Biochim. Biophys. Acta* **935,** 217–224.
43. Smith, J. M., Dowson, C. G. & Spratt, B. G. (1991) *Nature (London)* **349,** 29–31.