Molecular Cloning and Sequence of the B880 Holochrome Gene from
Rhodospirillum rubrum*

Jacques Bérard, Gilles Bélanger, Pierre Corriveau, and Gabriel Gingras†

From the Département de Biochimie, Université de Montréal, C. P. 6128, Montréal, Québec H3C 3J7, Canada

Restriction fragments of genomic Rhodospirillum rubrum DNA were selected according to size by electrophoresis followed by hybridization with [32P]mRNA encoding the two B880 holochrome polypeptides. The fragments were cloned into Escherichia coli C600 with plasmid pBR327 as a vector. The clones were selected by colony hybridization with [32P]-holochrome-mRNA and counterselected by hybridization with Rs. rubrum ribosomal RNA, a minor contaminant of the mRNA preparation. Chimeric plasmid pRR22 was shown to contain the B880 genes by hybrid selection of B880 holochrome-mRNA. We report a restriction map of its 2.2-kilobase insert and the sequence of a 430 base pair fragment thereof. Genes α and β are nearly contiguous, indicating that they are transcribed as a single operon. The predicted amino acid sequences coincide with the sequences of the α and β polypeptides established in other laboratories, except for additional C-terminal tails of 10 and 13 amino acid residues, respectively. We suggest that these tail sequences may serve, during membrane assembly, to give these intrinsic membrane proteins their peculiar orientation with their C-terminus facing the periplasm and their N terminus facing the cytoplasm. Intraspecific sequence homology between the α and β genes of Rs. rubrum is low, showing no evolutionary relatedness. This is in contrast to the high interspecific homology between the corresponding sequences of Rs. rubrum and Rhodopseudomonas capsulata B880 genes.

The two main protein complexes involved in the trapping and primary conversion of light energy by photosynthetic organisms are the holochrome or antenna proteins and the photoreaction center proteins. These proteins are both intrinsic membrane proteins and they both carry chlorophyll. In the Rhodospirillales, as a group, a long-standing problem has been the organization of the genes coding for the photosynthetic machinery (1). Of particular interest are the genes of the photoreaction center and of the antenna holochromes. The main concern of this article is the genes encoding the holochrome protein complex of Rhodospirillum rubrum. Unlike most other photosynthetic bacteria, Rs. rubrum is thought to contain a single holochrome designated as B880 because of its maximum absorption wavelength (880 nm) in the near-infrared. The protein has recently been shown to contain two low molecular weight polypeptides (2, 3), α and β, the sequences of which have been determined (4–6). On the basis of membrane digestions with proteinase K, it has been concluded that the N-terminal sequences of both polypeptides protrude from the cytoplasmic side of the intracytoplasmic membranes and that their C-termini are oriented towards the periplasmic side (7).

Our approach to the characterization of the genes encoding the photosynthetic machinery of Rs. rubrum has relied on the purification (8) and use as a molecular probe of B880 holochrome mRNA. With this probe, we have selected a 5.1-kb HindIII fragment of Rs. rubrum genomic DNA and have cloned it into Escherichia coli C600 with plasmid pBR327 (9) as a vector. This insert contains the two B880 structural genes, the photoreaction center L subunit gene and part of the M gene. A 2.2-kb fragment was subcloned and its sequence was determined in part. The sequences of the B880 α and β genes are only poorly homologous with one another. However, there is high interspecific sequence homology between the B880 α genes and between the β genes of Rs. rubrum and Rps. capsulata. Their organization, similar to that of Rps. capsulata (10, 11), confirms our suggestion, based on in vitro translational patterns of holochrome-mRNA, that gene β precedes gene α in transcriptional order. An interesting finding is that, in Rs. rubrum, the predicted sequence of gene products α and β have tailing sequences at their C-terminal ends. Since these carboxyl-terminal sequences are not found in Rs. rubrum B880 holchrome polypeptides, their processing probably plays a role in membrane assembly.

EXPERIMENTAL PROCEDURES

Materials—Tris and PIPES buffers, T4 DNA ligase, and all restriction endonucleases were from Boehringer Mannheim. Bacterial alkaline phosphatase, T4 polynucleotide kinase, phenol, urea, and agarose (ultrapure) were from Bethesda Research Laboratories. [%32P]ATP (1.11 107Bq/mo1) and L-[3,4,5-3H]leucine were from American Hydrazine and dimethyl sulfate were from Aldrich Chemical Co. Antibiotics and all other reagents were from Sigma. Nitrocellulose and Elutip-D columns were from Schleicher & Schuell.

Bacterial Strains and Plasmids—Rs. rubrum strain S1 (ATCC 11170) was grown photosynthetically in the medium of Cohen-Bazire et al. (12) as described before (13). E. coli C600 (F–, thi-1, thr-1, leuB6, lacY1, ton A21, SupE44, lambda+) was used as a host in the transformation experiments. This strain was grown in the Luria-Bertani medium. When needed, ampicillin or tetracycline were added to the medium at final concentrations of 50 and 15 µg/ml, respectively. Plasmid pBR327 (amp’, tet’) (9) was used as cloning vector.

Preparation of DNA—Plasmid DNA was amplified by addition of 100 µg/ml of chloroamphenicol to logarithmically growing cells of E. coli C600. Extraction of plasmid DNA was followed by centrifugation to equilibrium in ethidium bromide/CsCl gradients (14, 15). Chromosomal DNA was extracted from Rs. rubrum cells (5 g fresh weight) by a modification of the method of Blin and Stafford (16). In this

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† To whom correspondence should be addressed.

1 The abbreviations used are: kb, kilobase; PIPES, 1,4-piperazine-diethanesulfonic acid.
2 G. Bélanger, J. Bérard, and G. Gingras (1985) Eur. J. Biochem., in press.
modification, 150 μg/ml of proteinase K was used and the solution was replenished in enzyme after 1.5 h of incubation. After extraction with phenol/chloroform, nucleic acid was recovered by ethanol precipitation. The pellet (14 mg of nucleic acid) was treated with pancreatin DNase-free RNase. The yield of DNA was 6.6 mg.

Selection of Restriction Fragments by DNA-RNA Hybridization—Samples of purified DNA were subjected to a 24-h digestion with HindIII restriction endonuclease and electrophoresed on horizontal 0.7% agarose gels in 89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA (pH 8.0). A mixture of EcoRI and HindIII fragments of phage A were used as molecular weight markers. The DNA was transferred to nitrocellulose and hybridized (17) to 32P-labeled probe. The probe was a Rs. rubrum holochrome-mRNA fraction purified by molecular sieve filtration followed by sucrose gradient-density centrifugation. The RNA probe (2 μg) was 5’-dephosphorylated by treatment with bacterial alkaline phosphatase and labeled with [γ-32P]ATP using a phage T4 polynucleotide kinase. Autoradiography was at ~70°C on Kodak XAR 5 film with Dupont Cronex intensifying screens.

Cloning Procedures—Plasmid pBR327 was digested with endonuclease HindIII and treated with bacterial alkaline phosphatase. Genomic DNA fragments of size corresponding to those selected by RNA-DNA hybridization were isolated by electrophoresion from agarose gels (18, 19). The eluted DNA was dialyzed against 20 mM Tris-HCl (pH 7.3), 200 mM NaCl, 1 mM EDTA, concentrated and freed of agarose contaminates by filtration on 0.45-μm cellulose acetate filters and by subsequent chromatography on Elutip-D columns. The pooled DNA fragments were ligated to pBR327 with T4 DNA ligase.

Transformation of E. coli C600 was by the CaCl2 procedure of Mandel and Higa (20). Recombinants were selected among the antibiotic-resistant transformants by looking for tetracycline-sensitive colonies on LB plates. Recombinant colonies were transferred onto Colony/Plaque Screen (New England Nuclear) disks and their DNA was denatured (21, 22). Colony hybridization was either with purified mRNA or with ribosomal 16S and 23S RNA fractions that did not stimulate incorporation of [3H]leucine in a control in E. coli S-30 translation systems. Only those colonies that hybridized exclusively with holochrome-mRNA were selected.

Identification of Recombinants by Hybrid Selection of mRNA—Purified plasmid DNA from selected recombinants was restricted with endonuclease HindIII and used in the hybrid selection procedure of Miller et al. (23). Hybridizations were carried out in 100 μl of 65% formamide, 10 mM PIPES (pH 6.4), 0.4 M NaCl with 30 μg of 32P-holochrome-mRNA purified according to Belanger et al. The hybridization temperature was varied from 48 to 37°C with 20 min of incubation at each 1°C step. After translation of the selected mRNA, the translation products were immunoprecipitated and analyzed by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels (24).

DNA Sequencing—This was according to the Maxam and Gilbert chemical method (25).

RESULTS AND DISCUSSION

The probe that we used, an enriched holochrome-mRNA fraction, probably also contains other RNA species such as fragments of 23 S and 16 S ribosomal RNA (8). Since the bacterial genome contains several copies of the genes coding for ribosomal RNA (26), the use of this probe heavily skewed the procedure towards selection of ribosomal RNA clones. Taking this difficulty into account, we adopted the following cloning strategy: 1) assess the size of those HindIII fragments that hybridize with the holochrome-mRNA fraction, 2) elute the corresponding bands from the agarose gel and transform E. coli C600 with these fragments using plasmid pBR327 as a vector, 3) select the transformants, first by their resistance to ampicillin and their sensitivity to tetracycline and then by colony hybridization with holochrome-mRNA, 4) counterselect these transformants by colony hybridization with ribosomal RNA, 5) authenticate the selected recombinants by hybrid selection of mRNA, and 6) sequence the gene.

Molecular Cloning of the B880 Holochrome Gene—Rs. rubrum genomic DNA, digested to completion with endonuclease HindIII and electrophoresed on agarose gel, was transferred to nitrocellulose and hybridized with the 32P-labeled holochrome-mRNA fraction. The bands with a positive response were of about 2.2, 3.4, 5.1, and 23 kb. Corresponding but slightly wider slices were cut out of preparative gels, electroleuted, and inserted into the HindIII site of plasmid pBR327. Only the 5.1-kb band gave rise to transformants that hybridized with the holochrome-mRNA fraction but not with ribosomal RNA. A restriction map showed the inserts of two corresponding plasmids, pRR51 and pRR51', to have opposite orientations in the plasmid.

Subcloning of a Region Containing the Holochrome Genes—
To facilitate the subsequent experiments, we next attempted to localize the holochrome genes on the 5.1-kb fragment by hybridization with purified holochrome-mRNA. Hybridization was carried out on nitrocellulose blots of BamHI and PstI restriction fragments. Positive hybridization was obtained with a 2.2-kb HindIII-PstI subfragment and more precisely within BamHI and PstI sites on this subfragment. Excision from pRR51 with endonucleases EcoRI and PstI yielded the 2.2-kb fragment with a 29-base pair appendix taken from between the HindIII and EcoRI sites of pBR327. This fragment of mixed origin was inserted between the EcoRI and PstI sites of plasmid pBR327 to form the chimeric plasmid pRR22 which was subcloned in E. coli. Fig. 1 shows the restriction map of the 2.2-kb insert.

Hybrid Selection of mRNA—As a verification that the 2.2-kb insert carried the gene encoding the B880 holochrome, we performed a selection of mRNA (23) using plasmid pRR22 for affinity binding. In this experiment, the plasmid was bound to nitrocellulose and incubated with holochrome-mRNA purified as described elsewhere. The mRNA was eluted from the solid support and used to program an E. coli S-30 cell-free translation system into incorporating [3H]leucine. The translation products were precipitated either with acetone or with antibodies directed against the holochrome polypeptides. The precipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by fluorography as described elsewhere. As expected, plasmid pBR327 alone does not retain any mRNA: the translation products are the same whether translation is directed by S-30 endogenous mRNA (Fig. 2, lane A) or by purified holochrome-mRNA "selected" by plasmid pBR327 (Fig. 2, lane B). Neither lanes A nor B contain the α and β polypeptides that can be made under the direction of purified holochrome-mRNA as illustrated in Fig. 2, lane E. However, plasmid pRR22 clearly does retain holochrome-mRNA activity as shown in lanes C and D where the translation products were of about 2.2, 3.4, 5.1, and 23 kb.
were precipitated, respectively, with specific antibodies or with acetone (more details in legend to Fig. 2).

Search for Modified Copies of the Holochrome Gene—Since the sequence analysis (see below) gave unexpected results, it was deemed important to check whether other forms of the gene might be present in the *Rs. rubrum* genome. To this aim, we digested genomic DNA with restriction endonucleases, separated the fragments by agarose gel electrophoresis, and transferred them to nitrocellulose. A 268-nucleotide segment of the 2.2-kb insert was obtained by digestion with restriction endonucleases BamHI and XhoI, isolated by polyacrylamide gel electrophoresis and by fluorography. *Lanes A* and *B*, total translation products programmed, respectively, with no exogenous RNA and with purified holochrome-mRNA after selection and release from plasmid pRR22, *Lanes C* and *D*, respectively, immunoprecipitated and acetone-precipitated translation products directed by purified holochrome-mRNA selected by affinity binding with plasmid pRR22. *Lane E*, acetone-precipitated translation products directed by purified holochrome-mRNA. Total incorporation was 34,000 cpm for experiments shown in *lanes A* and *B*, 710,000 cpm with purified holochrome-mRNA (lane E), 334,900 cpm with selected holochrome mRNA (lanes C and D). Equal amounts of radioactivity were applied to each lane to emphasize the absence of polypeptides α and β in lane *B*.

Sequence Analysis of the B880 Holochrome Gene—The sequence was determined by the chemical method of Maxam and Gilbert (25). The *arrows* at the bottom of Fig. 1 indicate the sequencing strategy. The sequence of Fig. 4 was deter-

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**Fig. 2.** Hybrid selection of B880 holochrome mRNA. Plasmid pRR22 was bound to nitrocellulose and incubated with holochrome-mRNA for affinity binding. The mRNA was eluted from the solid support and used to program an *E. coli* S-30 cell-free translation system into incorporating [3H]leucine. The translation products were precipitated either with acetone (total translation products) or with antibodies directed against the holochrome polypeptides. The precipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by fluorography. *Lanes A* and *B*, total translation products programmed, respectively, with no exogenous RNA and with purified holochrome-mRNA after selection and release from plasmid pRR22. *Lanes C* and *D*, respectively, immunoprecipitated and acetone-precipitated translation products directed by purified holochrome-mRNA selected by affinity binding with plasmid pRR22. *Lane E*, acetone-precipitated translation products directed by purified holochrome-mRNA. Total incorporation was 34,000 cpm for experiments shown in *lanes A* and *B*, 710,000 cpm with purified holochrome-mRNA (lane E), 334,900 cpm with selected holochrome mRNA (lanes C and D). Equal amounts of radioactivity were applied to each lane to emphasize the absence of polypeptides α and β in lane *B*.

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**Fig. 3.** Search for different forms of the B880 holochrome gene in *Rs. rubrum*. Genomic DNA was limit-digested with restriction endonucleases HindIII, BamHI, SalI, SphI, and PvuI. The digests were electrophoresed on 0.7% agarose gel and transferred to nitrocellulose. Hybridization was with a 268-nucleotide fragment cut out of plasmid pRR22 by means of restriction endonuclease BamHI and XhoI and 32P-labeled at their 5' terminus.

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**Fig. 4.** Sequence of the *Rs. rubrum* B880 holochrome genes. The deduced amino acid sequence is also presented. The underlined regions were not found experimentally (4-8) in the amino acid sequences of polypeptides α and β.
mined on both strands over its entire length. It contains two open reading frames, each beginning by an AUG codon and terminating by a UAA stop codon. Putative structural genes β and α are respectively preceded by a space of 11 and 10 nucleotides center-to-center between the predicted Shine-Dalgarno (27) ribosome-binding site of the mRNA sequence AGGAG(G) and the AUG start codon. The termination signal of gene β overlaps with the ribosome-binding site of gene α. As shown in Fig. 5, this sequence is complementary to the 3’ terminal sequence from 16 S rRNA in both Rs. rubrum and E. coli (27, 28). The precedence of gene β, and perhaps also a weaker homology of the gene α ribosome-binding site, may explain the higher translation rates observed in E. coli S-30 cell-free translation systems programmed with Rs. rubrum holochrome-mRNA. The short distance between genes α and β and our observation (not shown) that a BamHI to PstI DNA fragment forms a stable hybrid with the 0.62-kb holochrome-mRNA coding for both polypeptides α and β indicate that the two genes are transcribed as an operon. Preliminary sequence work shows that the AUG start codon of the gene encoding the L subunit of the photoreaction center is at some 112 nucleotides downstream from the UAA termination codon of gene α. This resembles the situation found in Rp. capsulata (10, 11).

Fig. 4 also gives the predicted amino acid sequence of the gene product, using the known sequence of the holochrome polypeptides (4–6) to determine the reading phase. The two sequences coincide for such a long stretch as to exclude an erroneous reading frame assignment. However, there are differences (underlined in Fig. 4): the sequence of putative gene β predicts alanine for the N-terminal residue instead of glutamic acid, as reported (6). This difference may perhaps be related to these authors’ 50% recovery of the N-terminal residue and to their finding phenylthiohydantoin derivatives eluting close to alanine and leucine along with the partially liberated N-terminal amino acid. However, the other major difference, consisting of 13 and 10 residue stretches, respectively, at the C-terminal ends of the predicted β and α polypeptides must receive a completely different sort of explanation (see below).

Codon Usage—Table I shows the codon usage in genes α and β. Comparison with the codon usage in the ribulose-diphosphate carboxylase gene (1401 base pair) (29) shows some interesting similarities. Some codons are not used in both ribulose-diphosphate carboxylase and B880: GCA for alanine, ATA for isoleucine, CCT and CCA for proline, ACA for threonine, and GTA for valine. Leu is coded for primarily by CGT (12/19 in B880 and 22/33 in ribulose-diphosphate carboxylase), neither gene making use of codons CTA and GTA. The G + C content of the coding sequence of B880 is 57.8% compared to 63.7% in the whole genomic DNA (30) and 65.1% in ribulose-diphosphate carboxylase.

Sequence Homology—To detect possible sequence homology between the genes encoding the α and β subunits of the B880 holochrome of Rs. rubrum, the corresponding coding

| Rs. rubrum | Rs. rubrum | Rs. rubrum | Rs. rubrum |
|-----------|-----------|-----------|-----------|
| 16S rRNA  | 16S rRNA  | 16S rRNA  | 16S rRNA  |
| 5’ ATT GAGG UAGG .. 3’ | 5’ ATT GAGG UAGG .. 3’ | 5’ ATT GAGG UAGG .. 3’ | 5’ ATT GAGG UAGG .. 3’ |
| 3’ UUU UCCUG ACCA .. 5’ | 3’ UUU UCCUG ACCA .. 5’ | 3’ UUU UCCUG ACCA .. 5’ | 3’ UUU UCCUG ACCA .. 5’ |
| Fig. 5. Shine-Dalgarno B880 holochrome-mRNA sequences predicted from the DNA sequence. These sequences are complementary to the 3’ terminal end of the Rs. rubrum and E. coli 16 S ribosomal RNA. |

Table I

| Codon usage in the B880 holochrome genes of Rs. rubrum |
|-------------|-------------|-------------|-------------|
| Ala | GCT | 5 | GCC | 6 | GCA | 0 | GCG | 0 |
| Arg | CGT | 2 | CGC | 2 | CGA | 0 | CGG | 0 |
| Asn | AAT | 0 | AAC | 2 |
| Asp | GAT | 1 | GAC | 1 |
| Cys | TGT | 0 | TGC | 0 |
| Gln | CAA | 1 | CAG | 4 |
| Glu | GAA | 6 | GAG | 2 |
| Gly | GGT | 1 | GGC | 4 | GGA | 1 | GGG | 1 |
| His | CAT | 0 | CAC | 3 |
| Ile | ATT | 4 | ATC | 3 | ATA | 0 |
| Leu | TTA | 0 | TGG | 1 | CTT 2 | CTC | 4 | CTA | 0 | CTG | 12 |
| Lys | AAA | 0 | AAG | 4 |
| Met | ATG | 4 |
| Phe | TTT | 3 | TTC | 7 |
| Pro | CCT | 0 | CCC | 2 | CCA | 0 | CGG | 4 |
| Ser | TCT | 1 | TCC | 2 | TCA | 0 | TCG | 5 | AGT | 0 | AGC | 3 |
| Thr | ACT | 2 | ACC | 6 | ACA | 0 | ACG | 2 |
| Trp | TGG | 6 |
| Tyr | TAT | 0 | TAC | 2 |
| Val | GTT | 4 | GTC | 4 | GTA | 0 | GTG | 2 | END* TAA 2 | TAG | 0 | TGA | 0 |

* END, termination codon.

![Fig. 6. Dot matrix analysis of sequence homology between the B880 holochrome genes of Rs. rubrum and Rp. capsulata.](image-url)

The dot matrix program is from Zweig (31) and the Rp. capsulata sequence is from Youvan et al. (10, 11). Some interspecific homology was found between the coding regions of genes on the one hand and β on the other (Fig. 6). For this plot, a window of 15 nucleotides was used with a stringency of 12/15, stringency being defined as the number of matches within the window for printing a dot in the homology search. The homology between genes β is high over most of their length and is strictly in phase, indicating evolutionary relatedness. There is a break in homology between genes β and α, corresponding to a 33-base pair insertion in the β gene of Rs. rubrum with respect to its Rp. capsulata counterpart. This also causes a displacement of the diagonal line. Fig. 6 also shows a high homology between the α genes of the two organisms. Such high homology between the B880 genes of these two species is in line with the general sequence homology found by inter-
specific cross-hybridization experiments carried out among different photosynthetic bacteria (32).

Fig. 7 shows the alignment of the B880 gene sequences of *Rs. rubrum* and *Rp. capsulata* according to maximum homology. The predicted amino acid sequences also show high homology, but where the substituted residues are expected to fulfill similar functions (Leu/Ile, Asp/Glu, Thr/Ser, Lys/Arg) are indicated by dashed lines.

**Fig. 7. Alignment of the *Rs. rubrum* and *Rp. capsulata* B880 holochrome genes according to maximum homology of their nucleotide sequences (see Fig. 6).** The deduced amino acid sequences are also presented. The sequence of *Rp. capsulata*(10,11) are in italics. Regions of perfect homology, according to the predicted amino acid sequences, are boxed. Some regions of probable functional homology are indicated by dashed lines.

As pointed out by Brunisholz et *al.* (6,7), polypeptides α and β contain three regions: 1) a polar/charged N-terminal region of 12 (for α) and 20 (for β) residues, 2) a hydrophobic region of 21 (for α) and 23 (for β) residues with the proper length to span once the 32-Å hydrophobic layer of the membrane and 3) a C-terminal polar region. This last region would be even more polar and longer in the gene products predicted here and may thus be involved in membrane assembly. This suggestion is all the more tempting in the absence of a signal peptide (44,45) whose function of solubilization and anchorage in the hydrophobic stretch of 21 to 23 residues.

Two main scenarios of membrane assembly may be envisaged according to whether it is assumed to be post- or cotranslational. In the first case, the trigger signal mechanism (37,38) might be invoked. Accordingly, the C-terminal sequence, together with the polar N-terminal region, might insure the solubility of the proteins in the cytoplasm. Processing of the predicted extension by a cytoplasmic peptidase might cause a solubility and/or conformational change that would trigger membrane integration of the proteins. In this respect, there is evidence for a conformational change in the B880 holochrome proteins of *Rp. capsulata* after contact with the membrane (46).

In a cotranslational assembly mechanism, the polar/charged N terminus would stay at the membrane-to-cytoplasm interface while the hydrophobic stretch would penetrate the hydrophobic interior. The C-terminal polar tail would be pulled through the membrane by the free energy decrease afforded by the interaction of the hydrophobic domains of the proteins and of the membrane. A further thermodynamic pull might be afforded by the proteolysis of the C-terminal extension, perhaps in the periplasmic compartment. A cotranslational mechanism might facilitate the concerted assembly of the α and β polypeptides with each other and with the L and M subunits of the photosynthetic reaction center whose genes are in sequential order with the B880 genes (10,11). The H subunit may also play a pivotal role in this assembly (47,48).

The only other known instance of carboxyl-terminal processing of a membrane polypeptide is that of the 32-kDa *Qb*-protein of chloroplast. In that case, membrane assembly appears to be posttranslational and is thought to occur via the membrane trigger mechanism (49).

**REFERENCES**

1. Saunders, V. A. (1978) *Microbiol. Rev.* 42, 357-384
2. Cogdell, R. J., Lindsay, G., Valentine, J. & Durant, I. (1982) *FEBS Lett.* 150, 151-154
3. Picorel, R., Belanger, G. & Gingras, G. (1983) *Biochemistry* 22, 2491-2497
4. Brunisholz, R. A., Cundet, P. A., Theiler, R. & Zuber, H. (1981) *FEBS Lett.* 129, 150-154
5. Gogel, G. E., Parkes, P. S., Louch, P. A., Brunisholz, R. A. & Zuber, H. (1983) *Biochim. Biophys. Acta* TA 746, 32-39
6. Brunisholz, R. A., Suter, F. & Zuber, H. (1984) *Hoppe-Seyler's Z. Physiol. Chem.* 365, 675-688
7. Brunisholz, R. A., Wieken, V., Suter, F., Bachofen, R. & Zuber, H. (1984) *Hoppe-Seyler's Z. Physiol. Chem.* 365, 689-701
8. Belanger, G., Berard, J. & Gingras, G. (1984) in *Advances in Photosynthesis Research* (Sybesma, C., ed) Vol. IV, pp. 591-594, Martinus Nijhoff/Dr W. Junk, The Hague, The Netherlands
9. Soberon X, Covarrubias, L. & Bolivar, F. (1980) *Gene (Amst.)* 9, 287-305
10. Youvan, D. C., Alberti, M., Begush, H., Bylina, E. J. & Hearst, J. (1984) *Proc. Natl. Acad. Sci. U.S.A.* 81, 189-192
11. Youvan, D. C., Bylina, E. J., Alberti, M., Begush, H. & Hearst, J. E. (1984) *Cell* 37, 949-957
22. Benton, W. & Davis, R. (1977) Science 196, 180–182
23. Miller, J. S., Patterson, B. M., Ricciardi, R. P., Cohen, L. & Roberts, B. E. (1983) Methods Enzymol. 111, 650–674
24. Lasemmler, U. K. (1970) Nature (Lond.) 227, 689–688
25. Maxam, A. M. & Gilbert, W. (1980) Methods Enzymol. 65, 449–560
26. Nomura, M., Gourse, R. & Baughman, G. (1984) Annu. Rev. Biochem. 53, 75–117
27. Shine, J. & Dalgarno, L. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 1342–1346
28. Gibson, J., Stackebrandt, E., Zablen, L. B., Gupta, R. & Woese, C. R. (1979) Curr. Microbiol. 3, 59–64
29. Nargang, F., McIntosh, L. & Somerville, C. (1984) Mol. Gen. Genet. 193, 220–224
30. Vanyushin, B. F., Belozersky, A. N., Kokurina, N. A. & Kadirova, D. X. (1968) Nature (Lond.) 218, 1066–1067
31. Zweig, S. E. (1984) Nucleic Acids Res. 12, 767–776
32. Britsy, J. T. & Cohen, S. N. (1983) J. Bacteriol. 154, 1440–1445
33. Gerber, G. E., Gray, C. P., Wildenauer, D. & Khorana, H. G. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5286–5290
34. Abdulaev, N. G., Feigina, M. Yu., Kiselev, A. V., Ovchinikov, Yu. A., Drachev, L. A., Kaslen, A. D., Khitrina, L. V. and Skulachev, V. P. (1978) FEBS Lett. 90, 190–194
35. Ovchinikov, Yu. A., Abdulaev, N. G., Feigina, M. Y., Kiselev, A. V. & Lohanov, N. (1979) FEBS Lett. 100, 219–223
36. Walter, J. E., Carne, A. F. & Schmitt, H. W. (1979) Nature (Lond.) 278, 653–654
37. Wickner, W. (1979) Annu. Rev. Biochem. 48, 23–45
38. Wickner, W. (1980) Science 210, 861–868
39. Benson, S. A. & Silhavy, T. J. (1983) in Gene Function in Prokaryotes (Beckwith, J., Davies, J. & Gallant, J. A., eds) pp. 253–290, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
40. Stoeck, T. L., Koziersz, J. J., Singh, M. K., Reddy, G. & Köhler, H. (1978) Biochemistry 17, 1216–1222
41. Katz, F. N., Rothman, J. E., Lingappa, V. R., Blobel, G. & Lodish, H. F. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3278–3282
42. Rothman, J. E. & Lennard, J. (1977) Science 195, 743–753
43. Williams, D. G., Jenkins, R. & Tanner, M. J. A. (1979) Biochem. J. 181, 477–493
44. Blobel, G. & Dobberstein, B. (1975) J. Cell Biol. 67, 935–951
45. Blobel, G. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 1496–1500
46. Dierstein, R. (1984) Eur. J. Biochem. 138, 509–518
47. Chory, J., Donohue, T. J., Varga, A. R., Stashefin, I. A. & Kaplan, S. (1984) J. Bacteriol. 159, 540–554
48. Peters, J., Takemoto, J. & Drews, G. (1983) Biochemistry 22, 5660–5667
49. Marder, J. B., Goloubinoff, P. & Edelman, M. (1984) J. Biol. Chem. 259, 3900–3908