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Cloning, DNA Sequence, and Expression of the *Rhodobacter sphaeroides* Light-Harvesting B800-850-α and B800-850-β Genes

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Two deoxyoligonucleotide probes were synthesized in accordance with the available amino acid sequence of the B800-850-β polypeptide from *Rhodobacter sphaeroides* and were used to isolate a 2.6-kilobase *Pst*I fragment from *R. sphaeroides* 2.4.1 chromosomal DNA. Identification of the B800-850-β and B800-850-α structural genes, *pucB* and *pucA*, was confirmed by DNA sequencing. Northern (RNA) blot analysis, using restriction endonuclease fragments from the cloned genes as probes, revealed a single *puc*-operon-specific, highly stable transcript of approximately 640 bases present in photosynthetically grown cells. In vitro transcription-translation analysis of the *puc* operon revealed that the maximum synthesis of the *puc* operon gene products was achieved when the entire 2.6-kilobase *Pst*I fragment was used as the template, although a 537-base-pair *Xma*III fragment was sufficient to direct the synthesis of *pucB* and a *pucA* fusion product.

Rhodobacter sphaeroides provides a model system in which to study both photosynthesis and membrane development. This purple non-sulfur photosynthetic bacterium can grow under both photoheterotrophic and chemoheterotrophic conditions. When growing chemoheterotrophically, *R. sphaeroides* has a typical gram-negative cell envelope structure and its growth is supported by aerobic respiration. When oxygen is removed from such a culture, a series of events is triggered which, through a process of invagination, results in the differentiation of the cytoplasmic membrane into specialized domains, resulting in the formation of the photosynthetic intracytoplasmic membrane (ICM) system. The inducible ICM is physically continuous with, but structurally and functionally distinct from, the cytoplasmic membrane. Hence, *R. sphaeroides* provides an ideal system for studying the synthesis, organization, and assembly of this highly specialized photosynthetic membrane system since this membrane can be gratuitous for cell growth under specific physiological conditions.

The ICM of *R. sphaeroides* contains three pigment-protein complexes. The light-harvesting complexes, which are the major pigment-protein complexes, have been designated B800-850 and B875 based on their near-infrared absorption maxima (5). Photons are absorbed by the B800-850 complexes, and excitation energy is transferred to the photochemical reaction center complex (the third pigment-protein complex) via the B875 complexes (5, 11, 19). The B875 and reaction center complexes are arranged as aggregates in the photosynthetic membrane in a fixed stoichiometry of approximately 15:1 (1, 19). The B800-850 complexes are arranged peripherally relative to the B875 and reaction center complexes, and the amount of the B800-850 complex is regulated inversely to the incident light intensity and noncoordinately with the B875-reaction center complexes (9, 13, 14).

The B800-850 complex, an integral membrane complex, has been purified by detergent solubilization (5). The minimal unit required for B800-850 spectral activity consists of three molecules of bacteriochlorophyll *a* (Bchl), one molecule of carotenoid, and one each of two small hydrophobic polypeptides designated B800-850-α and B800-850-β. The primary amino acid sequences of α and β have been described, and the molecular masses of these polypeptides have been determined to be 5,599 and 5,448 daltons, respectively (22).

In an effort to understand the molecular basis for the differential regulation of B800-850 complex synthesis by light and oxygen, we cloned the structural genes, designated *pucB* and *pucA*, coding for the B800-850-β and B800-850-α subunits. The available amino acid sequence (22) was used to construct synthetic deoxyoligonucleotide probes specific for the B800-850-β polypeptide, and these probes were successfully used to identify and clone the structural genes for the B800-850-β and B800-850-α polypeptides. The DNA sequence was determined, and the regulation of the transcripts encoding these polypeptides was analyzed.

(A preliminary report of this work was presented at the Fifth International Conference on Photosynthetic Procaryotes in Grindelwald, Switzerland, in 1985.)

MATERIALS AND METHODS

Bacteria, bacteriophage, and plasmids. *R. sphaeroides* 2.4.1 was grown photoheterotrophically or chemoheterotrophically as previously reported (10). *Escherichia coli* JM83 or JM103 (18) was cultured and maintained as described earlier (15). Bacteriophage M13mp19 and M13mp18 (25) were used to generate clones for dideoxy sequencing, and plasmids pUC19 and pUC18 (25) were used as vectors for cloning as previously described (15).

DNA manipulations. Large-scale preparations of plasmid DNA were purified as described by Maquat and Reznikoff (17), except the polyethylene glycol precipitation step was omitted. Restriction enzyme digestions were performed according to the manufacturer's specifications. DNA was analyzed on agarose or polyacrylamide gels, and restriction fragments were isolated from polyacrylamide gels as described previously (10). Specific plasmid constructions are described in Table 1.

RNA analysis. Total RNA was prepared as previously described (28) except that rifampin (50 μg/ml) was added at the time of cell harvesting to prevent transcription initiation during sample processing for RNA purification. Conditions used for electrophoresis of glyoxylated bulk *R. sphaeroides* RNA, transfer to GeneScreen, Northern hybridization, nick

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TABLE 1. Plasmids

Plasmid	Vector/restriction site(s)	Fragment inserted (kb)	Direction of *puc* transcription relative to the *lac* promoter[a]
pUI601	pUC19/*Pst*I	*Pst*I (2.6)	−
pUI602	pUC19/*Pst*I	*Pst*I (2.6)	+
pUI612	pUC19/*Bam*HI	*Bam*HI (1.08)	−
pUI614	pUC18/*Bam*HI, *Hin*cII	*Stu*I-*Bam*HI (0.73)	−
pUI615	pUC19/*Sma*I	*Xma*III[b] (0.54)	+
pUI616	pUC18/*Sma*I	*Xma*III (0.54)	−

[a] +, *puc* operon transcription is in the same direction as *lac* promoter; −, opposite direction.
[b] 5′ Overhangs were made blunt with DNA polymerase Klenow fragment.

translation of DNA probes, and quantitation of transcripts have been described (28).

Cloning of *puc* genes from genomic DNA. Preparation of *R. sphaeroides* 2.4.1 genomic DNA, agarose gel electrophoresis, and conditions for Southern blot analysis with deoxyoligonucleotide probe families were as previously described (10). *Pst*I-digested genomic DNA was separated by agarose gel electrophoresis, and fractions of 2 to 4 kilobases (kb) were electroeluted to DEAE paper. The DNA was purified (10), and fractions hybridizing to the deoxyoligonucleotide probes were identified by Southern hybridization analysis. The positive hybridizing fractions were ligated into the *Pst*I site of pUC19 and transformed into JM83. Recombinant plasmids containing the presumptive *puc* genes were identified by Southern blot hybridization to both mixed probes by using *Pst*I-digested plasmid DNA derived from small-scale lysates of overnight cultures (16).

DNA sequence analysis. DNA sequencing was performed by the dideoxy sequencing method of Sanger et al. (21) as described previously (10). More-recent dideoxy sequencing reactions were performed with 7-deaza-dGTP (2) using the reaction mixtures and conditions of American Bionetics, Inc. The use of 7-deaza-guanosine gave enhanced resolution of DNA sequences which exhibited regions of G · C compression during standard dideoxy sequence analysis (2). The universal 17-mer M13 primer (Collaborative Research, Inc., Lexington, Mass.), as well as an internal sequencing primer, was used for dideoxy sequencing reactions.

To generate M13 clones for dideoxy sequencing, the following DNA constructions were done. (i) The 730-base-pair (bp) *Stu*I-*Bam*HI restriction fragment was cloned into the *Hin*cII and *Bam*HI restriction sites of both M13mp18 and M13mp19. (ii) The 300-bp *Xma*III-*Ban*I and 240-bp *Ban*I-*Xma*III restriction fragments were blunt ended with DNA polymerase Klenow fragment and cloned into the *Sma*I site of M13mp19.

Preparation of [^{3}H]tyrosine-labeled chromatophores. Chromatophores were first purified from *R. sphaeroides* 2.4.1 that had been steady-state labeled with [^{3}H]tyrosine (15) and then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (6, 15).

In vitro transcription-translation analysis. Protein-synthesizing extracts of *R. sphaeroides* were prepared from chemoheterotrophically grown *R. sphaeroides* 2.4.1 (4) as modified by Kiley et al. (15) and then analyzed as previously described (15). Individual polypeptides were quantified by densitometer scanning with an LKB-Ultroscan-XL laser densitometer well within the linear range of film response.

Materials. Restriction endonucleases and DNA-modifying enzymes were purchased from Bethesda Research Laboratories (Gaithersburg, Md.) or New England BioLabs (Beverly, Mass.). DNA polymerase Klenow fragment was the product of Boehringer Manheimm, Indianapolis, Ind. [γ-^{32}P]ATP was purchased from New England Nuclear (Boston Mass.). L-[2,3,5,6-^{3}H]tyrosine (107 Ci/mmol), L-[2,5-^{3}H]histidine (57 Ci/mmol), translation-grade L-[^{35}S]methionine (1,100 Ci/mmol), and [α-^{32}P]dCTP (800 Ci/mmol) were obtained from Amersham Corp. (Arlington Heights, Ill.). GeneScreen and En^{3}Hance were obtained from New England Nuclear. The nitrocellulose used for Southern blots was from Schleicher & Schuell (Keene, N.H.). With the exception of phenol, which was redistilled before use, all other chemicals were reagent grade. The mixed oligonucleotide probes used in Southern blots were synthesized at Rocky Mountain National Laboratories with a model SAM-1 synthesizer (Biosearch, San Rafael, Calif.), whereas those used as sequencing primers were synthesized at the Biotechnology Center of the University of Illinois (Urbana-Champaign) with an Applied Biosystems model 380A DNA synthesizer.

RESULTS

Identification and cloning of the *R. sphaeroides pucB* and *pucA* genes. From the available amino acid sequence of the B800-850-β polypeptide (21), two families of deoxyoligonucleotide probes were synthesized representingthe noncoding strand for amino acids 3 to 7 [GA(T/C)GA(T/C)CT(A/T/G/C)AA(T/C)AA(A/G)GT; probe 1] and amino acids 18 to 24 [GA(A/G)GA(A/G)GT(A/T/G/C)CA(T/C)AA(A/G)CA(A/G)CT; probe 2]. The two oligonucleotide families were radioactively labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP and used as hybridization probes in Southern hybridization blots of bulk *R. sphaeroides* DNA (Fig. 1) under the conditions described by Donohue et al. (10). Specific restriction endonuclease fragments which hybridized to both families of probes were identified by a series of 10-min washes at increasing conditions of stringency, beginning with a low-stringency wash at room temperature and concluding with washes close to the theoretical melting temperature of the probe, i.e., 42 to 52°C for probe 1 and 52 to 64°C for probe 2. The identity of the second restriction endonuclease fragment present in each digest, which hybridized only to the second probe family, was not investigated further. A library of recombinant plasmids containing 2- to 4-kb *Pst*I genomic DNA restriction endonuclease fragments cloned into the vector pUC19 were screened for hybridization to both families of probes in Southern hybridization blots containing *Pst*I-digested plasmid DNA obtained from small-scale lysates of overnight cultures. From this analysis an approximately 2.6-kb *Pst*I fragment that hybridized to both mixed probes was obtained.

A restriction map of the 2.6-kb *Pst*I fragment is shown in Fig. 2. The deoxyoligonucleotide probes were further used to map the position of the *pucB* gene to the approximately 730-bp *Stu*I-*Bam*HI fragment. The *Stu*I-*Bam*HI fragment was further mapped as indicated by the dashed lines in Fig. 2, and subclones were constructed into M13mp19 and M13mp18 for DNA sequence analysis by the dideoxy-chain termination method. The DNA sequence from the *Stu*I site to the *Bam*HI site is shown in Fig. 3.

The DNA sequence was aligned with the known amino acid sequences for the *R. sphaeroides* B800-850-β and B800-850-α polypeptides (22) resulting in the identification of the structural genes for both B800-850-β (*pucB*) and B800-850-α (*pucA*). The gene order is *pucBA* as it is in

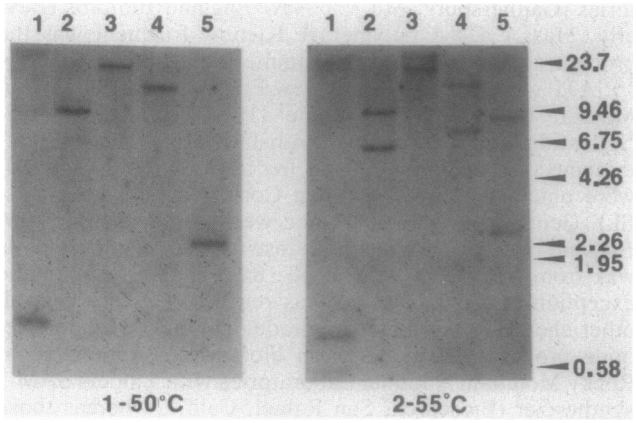

FIG. 1. Identification of the B800-850 structural genes by use of specific oligonucleotide probes in chromosomal Southern hybridization blots. Probe 1 (left) and probe 2 (right) were used as hybridization probes against Southern blots of bulk *R. sphaeroides* DNA treated with the restriction endonucleases *Bam*HI (lane 1), *Eco*RI (lane 2), *Hin*dIII (lane 3), *Pvu*II (lane 4), and *Pst*I (lane 5). Hybridizations were conducted at room temperature, and the nitrocellulose was initially washed at room temperature (two 5-min washes) in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The stringency of the washing conditions was gradually increased by a series of two 5-min washes in the same solution until temperatures close to the theoretical melting temperature, 50°C (left) or 55°C (right), of the individual oligonucleotide family were reached.

Rhodobacter capsulatus (26), and the coding region from the start of *pucB* to the end of *pucA* was 336 bp. Both genes were preceded by typical ribosomal binding sites with the initiator codon for *pucB* being GTG. As with other light-harvesting polypeptides (15, 26) and the reaction center polypeptides (8, 23, 24), there appeared to be no cleavable signal sequences preceding the mature polypeptides. The DNA sequence confirmed the existence of valine at position 24 in the

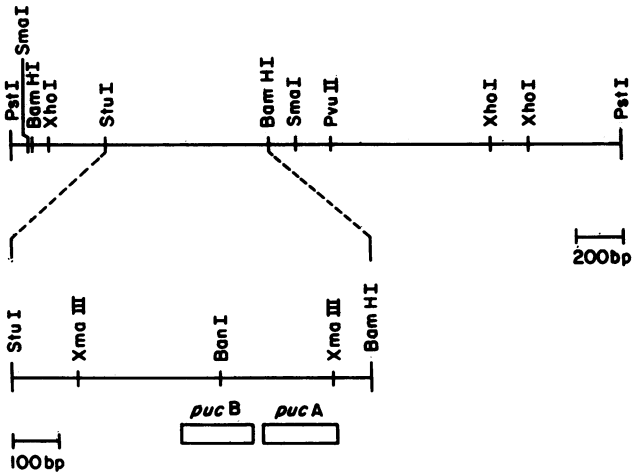

FIG. 2. Restriction map of *pucB* and *pucA* region. The 2.6-kb *Pst*I restriction fragment indicated in Fig. 1 was cloned into the plasmid vector pUC19. The oligonucleotide probes were used to map the position of the *pucB* gene to the approximately 730-bp *Stu*I-*Bam*HI restriction fragment. The *Stu*I-*Bam*HI restriction fragment was further mapped as indicated by the dashed lines, and subclones were constructed into M13mp19 for DNA sequence analysis by the dideoxy-chain termination method.

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CCTCGGACACCCTCGTTTTTGCAGCAGCGAGAGGCTGCGGGACGGCCCTGTGGGGCCGGG   60

ACAGGCAGCGTCAATTTCCCGCGCGCCTGCGGCAAAATTGTCCCTTTTCAAGCCGTTACG  120
          XmaIII
CAGGATTCCCGGCCGATCTGGCGGCCAATAAGTCGCACCCAAAACGGCCTTGTCAGCCAA  180

CACTGACATTGAATCTGTCAGCGCAATGTGACACCCATAATGCGAGCCGGGGCGGATCAG  240

AAATCGCCGACAAGGTGATCCAGGTCTCTCCGGTCTCGTCGAAGCCCGCGTGCAGGCCCT  300
                                        pucB
ACACGCAAACCGTCGATTTACCAGTTGGGAGACGACACAGTGACTGACGATCTGAACAAA  360
                          S.D.          METThrAspAspLeuAsnLys

GTCTGGCCGAGCGGCCTGACCGTTGCCGAAGCCGAAGAAGTTCATAAGCAACTCATCCTC  420
ValTrpProSerGlyLeuThrValAlaGluAlaGluGluValHisLysGlnLeuIleLeu

GGCACCCGCGTCTTCGGTGGCATGGCGCTCATCGCGCACTTCCTCGCCGCCGCTGCGACC  480
GlyThrArgValPheGlyGlyMetAlaLeuIleAlaHisPheLeuAlaAlaAlaAlaThr
                                    pucA
CCGTGGCTCGGCTGATAGGAGAAGACTGACATGACCAACGGCAAAATCTGGCTCGTGGTG  540
ProTrpLeuGly *    S.D.          METThrAsnGlyLysIleTrpLeuValVal

AAACCGACCGTCGGCGTTCCGCTGTTCCTCAGCGCTGCCGTCATCGCCTCCGTCGTTATC  600
LysProThrValGlyValProLeuPheLeuSerAlaAlaValIleAlaSerValValIle

CACGCTGCTGTGCTGACGACCACCACCTGGCTGCCCGCCTACTACCAAGGCTCGGCTGCG  660
HisAlaAlaValLeuThrThrThrThrTrpLeuProAlaTyrTyrGlnGlySerAlaAla
    XmaIII
GTCGCGGCCGGAGTAATGCTGCGCAAGGCGCGGGCCTGCGGGCCCACGCCAGCCAGTCCGT  720
ValAlaAlaGlu *                  A         A
                BamHI
GAGTTCCGAGCAGGCCGGGATCC                                        743
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FIG. 3. DNA sequence of *pucB* and *pucA* structural genes. The DNA sequence was aligned with the available amino acid sequence for B800-850-α and B800-850-β polypeptides as described by Theiler et al. (22). Both genes are preceded by ribosomal binding sites which are underlined. The DNA sequence was determined on both strands except in the region downstream of the *pucA* stop codon to the *Bam*HI site.

wild-type α polypeptide which could have been changed to a phenylalanine in the mutant R26.1 α polypeptide (22) by a single G→T transversion. Analysis of the region downstream of *pucA* revealed one region of dyad symmetry (designated A, Fig. 3) which perhaps could serve as a transcription terminator for the *puc* operon.

Genomic Southern blots. When the 2.6-kb *Pst*I fragment was used as a hybridization probe in a Southern hybridization analysis with the restriction endonuclease digests described in Fig. 1, the same pattern of hybridization was obtained as was seen with the first oligonucleotide probe (data not shown). A second band was obtained with both the *Pvu*II and the *Bam*HI restriction digests since there are restriction endonuclease sites for both of these enzymes within the 2.6-kb *Pst*I fragment.

In vitro expression from *pucB*- and *pucA*-containing plasmids. Figure 4A shows the results obtained from the analysis of the 2.6-kb *Pst*I fragment cloned into the vector pUC19 in two orientations (designated pUI602 and pUI601, Table 1) by using an *R. sphaeroides* in vitro-coupled transcription translation system with [^{3}H]histidine as a label. Two low-molecular-weight polypeptides (5,200 and 4,350) were unique to the pUI602 and pUI601 plasmid template-derived translation products (Fig. 4A, lanes 3 and 4) as compared to the vector pUC19 DNA used as a control (Fig. 4A, lane 2). These polypeptides comigrated in SDS-PAGE with authentic B800-850-β and B800-850-α, as indicated by the arrows to the right of Fig. 4A.

That the two low-molecular-weight gene products observed from pUI601 were B800-850-β and B800-850-α was determined by creation of a fusion gene product and differential amino acid labeling. A 537-bp *Xma*III fragment containing the entire *pucB* gene and the *pucA* gene lacking the last codon was cloned into the *Sma*I site of pUC19 such that the orientation of the *puc* promoter was in the same orien-

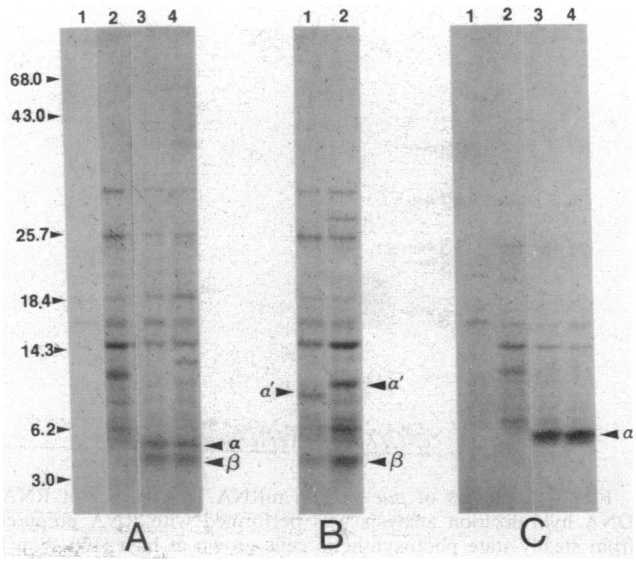

FIG. 4. In vitro transcription-translation analysis of plasmids containing *pucB* and *pucA*. The 11.5 to 18% SDS-PAGE profile of the polypeptide products synthesized after a 25-min period labeled with [^{3}H]histidine (A and B) and [^{3}H]tyrosine (C). The mobilities of the in vitro-synthesized and authentic B800-850-β and B800-850-α are indicated by the arrows at the right of each panel. The fusion products derived from pUI615 and pUI616 are indicated by arrows in panel B labeled α'. The samples in each lane are as follows: no exogenous DNA (panels A and C, lane 1), pUC19 (panels A and C, lane 2), pUI602 (panel A, lane 3; panel C, lane 4), pUI601 (panel A, lane 4; panel C, lane 3), pUI616 (panel B, lane 1), and pUI615 (panel B, lane 2).

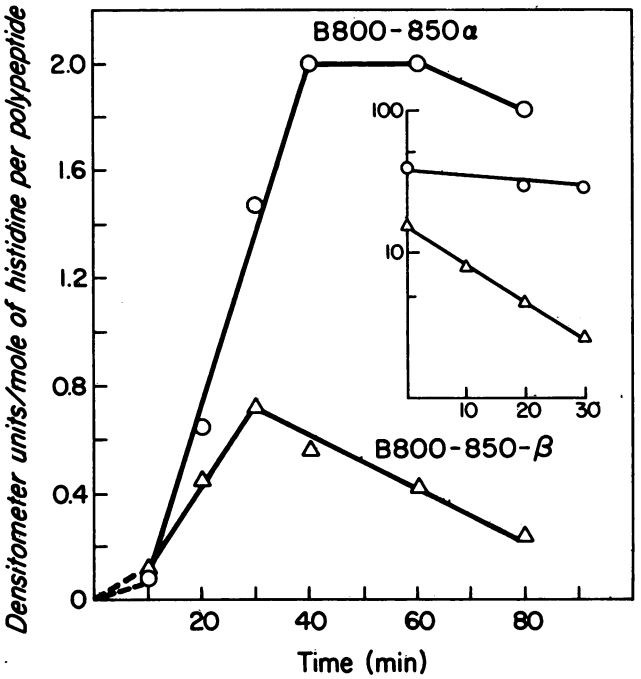

FIG. 5. In vitro synthesis of *puc*-operon-specific gene products. Kinetics of [^{3}H]histidine incorporated into B800-850-α polypeptide (○) and B800-850-β polypeptide (△). The values were calculated after integration of densitometer scans (LKB-Ultroscan-XL laser densitometer) of fluorograms well within the linear range of film response of SDS-PAGE profiles from a constant volume of in vitro-synthesized products derived from the template pUI601 (90 ng/μl) assayed at the indicated times. The integrated units were normalized for the moles of histidine per polypeptide. (Inset) Decay of B800-850-α and B800-850-β after the addition of chloramphenicol (25 μg/ml) 25 min after initiation of the synthesis reaction. Samples were removed before addition of chloramphenicol (time 0) and 10, 20, and 30 min thereafter.

tation as the *lac* promoter, and the resulting plasmid was designated pUI615 (Table 1). As expected, the size of the *pucB* gene product synthesized from pUI615 did not change (Fig. 4B, lane 2) but the size of the *pucA* gene product (designated α' in Fig. 4B, lane 2) was larger, in agreement with the size deduced from the DNA sequence of the predicted fusion polypeptide. Similarly, the *Xma*III fragment was cloned in an opposite orientation relative to the *lac* promoter to yield the plasmid pUI616 (Table 1). The size of the *pucB* gene product synthesized in vitro from this template was the same as in pUI615 (i.e., normal). The altered *pucA* gene product was observed to be a different-size α' (Fig. 4B, lane 1) than that derived from pUI615, which is consistent with the orientation of the DNA fragment and the predicted fusion polypeptide. [^{3}H]tyrosine labeling of the in vitro protein products with pUI601 and pUI602 as plasmid templates showed that only the larger α polypeptide was labeled, since B800-850-β lacks the amino acid tyrosine (Fig. 4C, lanes 3 and 4, respectively).

Examination of the kinetics of the in vitro synthesis of *pucB* and *pucA* gene products from the plasmid pUI601 revealed that there was a short but rapid coordinate increase in B800-850-β and B800-850-α which occurred during the first 30 min of the synthetic period (Fig. 5). Only during the first 10 to 15 min of synthesis was the ratio of synthesized B800-850-β to B800-850-α equimolar; thereafter the amount of B800-850-α increased relative to B800-850-β, and after 30 min there was a time-dependent disappearance of a discrete B800-850-β polypeptide. The accumulation of B800-850-α continued linearly for up to 40 min. Therefore, the apparent half-life of B800-850-β after 30 min of synthesis was measured and calculated to be 17 min. Addition of chloramphenicol to the in vitro synthesis reaction (inset, Fig. 5) to measure the decay of B800-850-β in the absence of new protein synthesis yielded a $t_{1/2}$ of 11.5 min. These results indicated that the B800-850-β polypeptide turned over during the synthetic period while the B800-850-α polypeptide was relatively stable ($t_{1/2}$, 105 min).

The results of analysis of plasmids derived from pUI601 for the production B800-850-α and B800-850-β in the *R. sphaeroides* in vitro transcription-translation system are shown in Table 2. From the data presented in Fig. 4, maximum synthesis of the gene products derived from the *puc* operon in the in vitro transcription-translation system was obtained by using either pUI601 or pUI602 as template. Since the synthesis of B800-850-β and B800-850-α from these templates was independent of the orientation of the insert DNA relative to vector sequence, we concluded that the *Pst*I fragment contained sequences required for maximal expression of the *puc* operon. Analysis of plasmid derivatives containing the *pucB* and *pucA* genes with various amounts of upstream DNA sequences showed that deletion of sequences from the *Pst*I to the *Bam*HI, *Stu*I, and *Xma*III sites, respectively, resulted in a sequential decrease in expression of the *pucB* and *pucA* gene products. Expression of the *puc* gene products from plasmids containing even the minimal sequences upstream of the start site of *pucB* should reflect promoter activity for the *puc* operon. Therefore, the pro-

TABLE 2. Expression of B800-850-β and B800-850-α from various plasmid templates in an *R. sphaeroides* transcription-translation system

DNA template[a]	% Expression[b] of:	
	B800-850-β	B800-850-α
pUI601	100	100
pUI612	66	46
pUI614	40	32
pUI616	48	22[c]

[a] Plasmid constructs are described in Table 1.

[b] The amount of B800-850-α and B800-850-β was determined by scanning densitometry (LKB-Ultroscan-XL laser densitometer) of X-ray films well within the linear range of film response of SDS-PAGE analysis of in vitro synthesis reactions from the above templates terminated at 25 min. The integrated units were normalized for the moles of incorporated [^{3}H]histidine per polypeptide (2 mol of histidine in β; 1 mol of histidine in α). Percent expression was calculated by comparing the amount of B800-850-α or -β synthesized from plasmids pUI612, pUI614, and pUI616 to pUI601.

[c] The fusion polypeptide α′ expressed from this plasmid was normalized for the 4 mol of histidine determined from the deduced amino acid sequence.

moter region(s) for the *puc* operon should be contained within the *Xma*III fragment; the *Xma*III site is 211 bp upstream of the start codon of *pucB*. We have observed that the *R. sphaeroides* and *R. capsulatus* DNA sequence can be aligned between the regions 165 and 96 bp upstream of *pucB* to yield an overall homology of 60% (Fig. 6), and this region lies downstream of the *Xma*III site.

In vivo expression of the *puc* operon. A Northern hybridization analysis was performed with RNA prepared from steady-state cells grown either chemoheterotrophically in a 30% O_2–69% N_2–1% CO_2 atmosphere or photosynthetically at high (100 W/m^2), moderate (10 W/m^2), and low (3 W/m^2) light intensites. The identification of the *puc* operon mRNA was determined by hybridization (Fig. 7) with an internal *Ban*I-*Xma*III fragment probe. A single transcript of approximately 640 nucleotides was identified from the RNA prepared from the photosynthetic cells. Since the coding region for *pucB* and *pucA* is 326 bp, a 640-nucleotide transcript would be sufficient for the expression of both genes. The 5′ end of this transcript must map downstream of the *Xma*III site since Northern hybridization with a *Stu*I-*Xma*III fragment probe did not hybridize to the *puc* transcript (data not shown). Analysis of the DNA sequence downstream of the *pucA* stop codon to the *Bam*HI site revealed one inverted repeat, which could form a stem-loop structure typical of procaryotic transcription termination sequences. If this was a transcription terminator for the *puc* operon, given the 580 bp from the *Xma*III site to the end of the inverted repeat, then the size of the transcript calculated from the Northern blots must be slightly larger than the actual transcript size. The decay of the *puc*-operon-specific mRNA was determined after the addition of rifampin (200 μg/ml) to photosynthetically growing *R. sphaeroides* (light intensity, 10 W/m^2) (Fig. 8). From these data, the half-life of the *puc*-specific mRNA was calculated to be 20.5 min, indicating that

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                  ***   **  * *** **  * *   **  ** ** *
R. capsulatus   -174 CAGTGTAAGCCCGACTTTACACTTGATCGCCGACACTT
R. sphaeroides  -165 CAGCC-AACACTGACATTGAA-TCTGTCAGCG-CAATG

                 * * ****** ****     *  ** ******* * *
                     GGGCTCCCATAGTGCGTCTCACGAGGTCGGATCACAGA  -99
                     TGACACCCATAATGCG---AGCCGGGGCGGATCAGAAA  -96
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FIG. 6. Comparison of the region upstream of the *pucB* gene from *R. capsulatus* (26) and *R. sphaeroides*. The numbers refer to the position of this homologous region relative to the *pucB* gene.

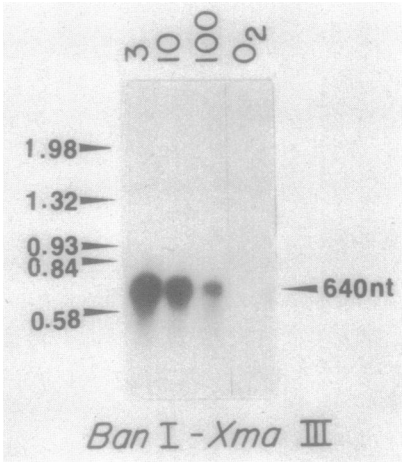

FIG. 7. Analysis of *puc* operon mRNA. Northern blot RNA-DNA hybridization analysis was performed with RNA prepared from steady-state photosynthetic cells grown at high (100 W/m^2), moderate (10 W/m^2), and low (3 W/m^2) light intensities, as well as from aerobically (30% O_2–1% CO_2–69% N_2) grown cells as described previously (10), and probed with the ^{32}P-labeled internal 240-bp *Ban*I-*Xma*III fragment.

it is a relatively stable mRNA which is similar to what has been observed for other pigment-binding protein mRNAs in both *R. sphaeroides* (29) and *R. capsulatus* (7).

Table 3 compares the levels of *puc* mRNA and B800-850-α polypeptide measured in steady-state chemoheterotrophically and photosynthetically grown cells at different light intensities. Barely detectable levels (only visible after very long film exposures) of *puc*-specific transcript were observed in aerobic cells. This is in contrast to the results obtained

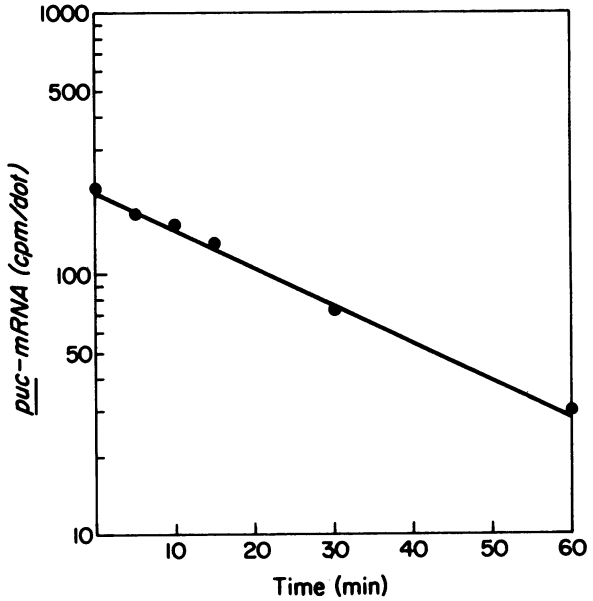

FIG. 8. Decay of *puc* mRNA. Rifampin (200 μg/ml) was added to steady-state photosynthetically growing *R. sphaeroides* (50 Klett units; 10 W/m^2 light intensity), and the amount of *puc*-specific mRNA present at the indicated times thereafter was determined by dot blot hybridization using the 240-bp *Ban*I-*Xma*III fragment as a probe and scintillation counting of the resulting filters.

TABLE 3. Levels of B800-850-α, *puc*-specific mRNA, and Bchl in cells grown under different physiological conditions

Condition	Generation time (h)	Specific Bchl[a]	*puc*-specific mRNA[b]	B800-850-α[c]
Chemoheterotrophic	3.0	ND[d]	ND[e]	
Photoheterotrophic (W/m^2)				
3[f]	10.8	9.1	4.4	2.6
10	3.0	4.6	3.2	2.1
100	3.0	2.9	1.0	1.0

[a] Determined as micrograms per milligrams of whole-cell protein.
[b] All values shown were determined from scanning densitometry of X-ray films well within the linear range of film response from the Northern blot described in Fig. 7 and which have been normalized to the amount of *puc*-specific mRNA in photoheterotrophically grown cells at 100 W/m^2.
[c] Determined by in vivo labeling with [^{3}H]tyrosine as described previously (15).
[d] ND, None detectable.
[e] ND, None detectable within this range of film response.
[f] Incident light intensity.

with the genes encoding the other Bchl-binding proteins (e.g., *puf* and *puh*), whose mRNAs are relatively abundant in chemoheterotrophically grown cells (8, 28). The amount of the *puc* transcript isolated from steady-state photosynthetic cells grown at 3 W/m^2 was 4.4-fold higher than at 100 W/m^2. Concomitant with the increase in *puc*-specific mRNA was a threefold increase in the steady-state cellular level of Bchl when the same samples were compared (Table 3). An approximately 2.6-fold increase in the level of B800-850-α was observed from chromatophores purified from cells grown at 3 W/m^2 as compared to cells grown at 100 W/m^2. Comparison of absorption spectra from crude membranes showed that the ratio of B800-850 to B875 increased by a factor of 2 from cells grown at 10 to 100 W/m^2. In contrast, the ratio of B800-850 to B875 complexes did not change significantly when the amounts of these complexes in cells grown at 10 and 3 W/m^2 were compared. However, there was an approximately twofold increase in the total amount of both spectral complexes in cells grown at light intensities of 3 W/m^2 versus those grown at 10 W/m^2 (data not shown). Therefore, a 2.6-fold increase in the amount of B800-850-α polypeptide is an under-representation of the total cellular increase in B800-850-α since equal protein loads of chromatophores were applied to the SDS-PAGE gel used in this analysis and since this value does not take into account the concurrent increase (approximately twofold) in the total amount of ICM per cell when cells grown at low and high light intensities were compared. This would predict that, when 3- versus 100-W/m^2-grown cells are compared, the total change in the amount of B800-850-α polypeptide is closer to fivefold and therefore more closely parallels the changes observed for *puc*-operon-specific mRNA levels.

DISCUSSION

The genes coding for the B800-850-β and B800-850-α polypeptides have been cloned from *R. sphaeroides* 2.4.1 genomic DNA, and the DNA sequence has been determined. The deduced amino acid sequences are in agreement with the previously published primary sequences (22). The organization of the genes (*pucB* and *pucA*) is the same as in *R. capsulatus* (26). In addition, we have shown by Northern hybridization that the two genes are encoded on the same 640-nucleotide transcript.

The *pucB* and *pucA* genes were expressed in an *R. sphaeroides*-coupled transcription-translation system using a variety of plasmid derivatives as templates. Although maximal in vitro synthesis of the *pucB* and *pucA* gene products required the entire 2.6-kb *Pst*I fragment, expression of B800-850-β and B800-850-α was observed from plasmid derivatives containing reduced amounts of DNA flanking the *puc* operon, indicating the presence of promoter sequences within 211 bp upstream of the structural genes. It is not known whether the initial reduction in expression observed from the removal of *Pst*I-*Bam*HI flanking DNA sequences was due to the removal of sequences either proximal or distal to the *puc* genes or a combination of both. Other, larger gene products were observed during in vitro transcription-translation reactions using pUI601 and pUI602 as templates, revealing the presence of other genes on this cloned DNA fragment. Similarly, additional transcripts were identified with DNA probes distal to the *puc* genes (data not shown), suggesting that there are other transcriptional units downstream of the *puc* genes although their interaction, if any, with the *puc* operon is unknown at this time. The observation by Northern hybridization that the *puc* transcript does not hybridize with restriction fragments proximal to the *Xma*III site would be consistent with the sequence distal to the first *Xma*III site, which is 211 bp proximal to the start of *pucB*, containing *puc* operon promoter sequences. It is intriguing that the *R. capsulatus* and *R. sphaeroides* DNA sequences in this region downstream of the *Xma*III site can be aligned, suggesting that these may be potential regulatory sequences which have been conserved in both species. Recently, the transcript for the *puc* operon in *R. capsulatus* has been identified (27) and the size (500 nucleotides) is consistent with that found in *R. sphaeroides* suggesting that the homologous regions could indeed be promoter regions.

We have also shown that the in vitro-synthesized B800-850-β polypeptide is labile during the synthetic period. Preliminary attempts to stabilize this gene product by addition of photosynthetic membranes or a protease inhibitor (phenylmethylsulfonylfluoride) had no effect. As previously shown in whole-cell experiments with *R. capsulatus* (7), the pigment-binding polypeptides appear to be labile in the absence of Bchl. The apparent existence of this lability in vitro may provide the basis for an assay for detecting the minimal requirements for stabilization of these polypeptides which could lead to a method for studying the assembly of Bchl protein complexes in vitro in *R. sphaeroides*. In addition, this observation may provide a means for detecting whether a specific protease is involved in the posttranslational control of these gene products. Recently, a protease has been purified from *E. coli* that acts specifically on glutamine synthetase that has been modified at a specific histidine residue by a mixed-function oxidation reaction (20). The susceptibility to oxidation is subject to metabolic regulation, thereby providing control by proteolytic turnover of the damaged enzyme. It is interesting to speculate that a similar oxidation of a histidine residue could occur on unliganded light-harvesting polypeptides, resulting in the turnover of the oxidized protein in *R. sphaeroides*. Similar instability of the *pufB* gene product in vitro has also been noted (results not shown).

Most studies on the regulation of B800-850 complex synthesis have been done by examining cells shifted from one environmental condition to another (3, 9, 12). For example,

lowering the pO_2 in an aerobically growing culture somewhat below threshold levels for ICM induction has been termed a gratuitous shift, while shifting cells from high oxygen to no oxygen in the presence of light is a nongratuitous shift. Also, photosynthetic cells can be shifted from a high to lower incident light intensity, i.e., a downshift in light intensity (9, 12). These kinds of shifts have been important in elucidating how cells respond to different environmental signals. For example, at the whole-cell level, synthesis of the B800-850 complexes, in response to increases in incident light intensity, is regulated noncoordinately relative to the synthesis of B875 complexes, although the amount of both complexes varies as a function of changes in growth conditions (12). It has been observed that one response to downshifts in light intensity is to increase the amount of the B800-850 complex which would maintain the flux of radiant energy directed to the reaction center. Lowering of the oxygen tension, which represents a gratuitous environmental shift, results in the synthesis of the B875 and reaction center first, with the B800-850 complex appearing later (12). It has been shown that the B875-α and B875-β structural genes are transcriptionally linked to the gene encoding the reaction center L and reaction center M polypeptide subunits, which are designated the *puf* operon (28). In apparent contrast to the *puc* operon, there are relatively abundant levels of *puf*-operon-specific message in highly oxygenated, chemoheterotrophically grown cells. Moreover, although *puf*-specific mRNA is present in chemoheterotrophically grown cells, there are no readily detectable B875 or reaction center spectral complexes (3, 29). Since it appears that there is a requirement for Bchl or its precursors for the translation, stabilization, or both of Bchl-binding proteins (7), the earlier appearance of the *puf* operon gene products in response to a decrease in oxygen tension may occur as a result of the relatively high levels of the *puf* transcripts already present in aerobic cells. In the presence of newly synthesized Bchl, either the RNA is translated or the gene products are stabilized and assembled into functional complexes. On the other hand, all three spectral complexes appear coordinately when cells are shifted to stringent, nongratuitous photosynthetic conditions after aerobic growth. Therefore, we consider the relationship between the expression of the *puf* and *puc* operons under these three changes in environmental parameters to be as follows. The B875 and B800-850 complexes can be expressed coordinately (downshift in light intensity or nongratuitous induction [9]), provided that some other component(s) inherent to the accumulation of the availability of complexes (e.g., Bchl) is the limiting factor and not just the transcription of *puf*- and *puc*-operon-specific mRNAs. On the other hand, noncoordinate control (gratuitous induction or a shift-up in light intensity [9]) of the B875 and B800-850 complexes reflects those regulatory parameters which represent intrinsic differences in the expression of both *puf* and *puc* operons at the transcriptional level.

Another important parameter, rarely considered when attempting to describe the cellular response to changes in environmental conditions, is the level of total cellular ICM and presumably the regulation of phospholipid movement or synthesis. Increases in the cellular levels of B875 and reaction center complexes must be accompanied by proportionate increases in the amount of ICM (8, 14), whereas increases in the amount of B800-850 complexes need not be accompanied by proportionate increases in total cellular ICM. Further experiments which examine changes in the levels of *puf*- and *puc*-operon-specific transcripts in cells undergoing the above-described shifts will be instrumental in determining the molecular basis for the differential synthesis of the two light-harvesting complexes. Comparison of the DNA sequences upstream of the *puc* operon with the *puf* operon did not yield any significant homology. However, it is clear that mechanisms must exist which signal the cell to respond to changes in both light intensity and oxygen tension.

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