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Sequencing, Chromosomal Inactivation, and Functional Expression in *Escherichia coli* of *ppsR*, a Gene Which Represses Carotenoid and Bacteriochlorophyll Synthesis in *Rhodobacter sphaeroides*

ROBERT J. PENFOLD AND JOHN M. PEMBERTON*

Microbiology Department, The University of Queensland, St. Lucia, Brisbane 4072, Australia

Received 22 November 1993/Accepted 10 March 1994

Sequencing of a DNA fragment that causes *trans* suppression of bacteriochlorophyll and carotenoid levels in *Rhodobacter sphaeroides* revealed two genes: *orf-192* and *ppsR*. The *ppsR* gene alone is sufficient for photopigment suppression. Inactivation of the *R. sphaeroides* chromosomal copy of *ppsR* results in overproduction of both bacteriochlorophyll and carotenoid pigments. The deduced 464-amino-acid protein product of *ppsR* is homologous to the CrtJ protein of *Rhodobacter capsulatus* and contains a helix-turn-helix domain that is found in various DNA-binding proteins. Removal of the helix-turn-helix domain renders PpsR nonfunctional. The promoter of *ppsR* is located within the coding region of the upstream *orf-192* gene. When this promoter is replaced by a *lacZ* promoter, *ppsR* is expressed in *Escherichia coli*. An *R. sphaeroides* DNA fragment carrying *crtD'*, *-E*, and *-F* and *bchC*, *-X*, *-Y*, and *-Z'* exhibited putative promoter activity in *E. coli*. This putative promoter activity could be suppressed by PpsR in both *E. coli* and *R. sphaeroides*. These results suggest that PpsR is a transcriptional repressor. It could potentially act by binding to a putative regulatory palindrome found in the 5' flanking regions of a number of *R. sphaeroides* and *R. capsulatus* photosynthesis genes.

Rhodobacter sphaeroides is a metabolically versatile gram-negative bacterium which is able to grow by aerobic and anaerobic respiration, fermentation, and anoxygenic photosynthesis. Its photosynthetic apparatus is relatively simple, consisting of two types of light-harvesting antennae (LH-I and LH-II), a reaction center (RC) complex, and various electron transport proteins. Bacteriochlorophyll (Bch) and carotenoid (Crt) photopigments are noncovalently bound to the LH-I, LH-II, and RC complexes (28). Most of the genes for photosynthesis are clustered in a 45-kb region of the chromosome in both *R. sphaeroides* and in its close relative *Rhodobacter capsulatus* (12). Numerous *bch* and *crt* genes, encoding Bch and Crt biosynthetic enzymes, are flanked by the *puh* and *puf* operons which encode RC and LH-I complex proteins. The *puc* operon, which encodes LH-II proteins, lies outside the cluster. It is located some 18 kb from *puhA* (47). Recent results from *R. capsulatus* show that a number of photosynthesis gene operons, including *crt*, *bch*, *puf*, and *puh* operons, are transcriptionally linked into superoperons (49).

Oxygen and, to a lesser extent, light regulate the photosynthetic apparatus. When oxygen tension drops below a threshold level, synthesis of the photosynthetic apparatus is induced, even in the absence of light. Under low oxygen conditions, light intensity regulates both the composition and concentration of the photosynthetic apparatus, with production being maximal at low light intensity (11, 13).

This pattern of oxygen and light regulation is, in general, repeated at the level of gene transcription (4, 28, 29). While progress has been made in uncovering the transcriptional regulatory mechanisms governing expression of the *puf*, *puh*, and *puc* operons (4, 28, 29, 31, 43, 49), little is known about the more weakly regulated *bch* and *crt* operons. Two lines of evidence from *R. capsulatus* suggest that the *bch* and *crt* operons might be regulated differently from other photosynthesis operons. *Escherichia coli* σ^{70}-like promoter sequences and a putative regulatory palindrome have been found upstream of several *bch* and *crt* genes but are not found upstream of the *puf* and *puh* operons (2). Additionally, the regulatory protein RegA activates *puf*, *puh*, and *puc* transcription under anaerobic conditions but does not affect photopigment genes (43). We have previously reported the cloning of a gene (*ppsR*) from the photosynthesis gene cluster of *R. sphaeroides* which represses Bch and Crt levels in both *R. sphaeroides* and *R. capsulatus* (39). In the present paper, we present evidence that PpsR may be a transcriptional repressor of photosynthesis genes and propose a putative model for such a role.

MATERIALS AND METHODS

Bacteria, plasmids, and cell growth. The bacterial strains and plasmids used in this study are listed in Table 1. All strains were grown on Z medium (39); *E. coli* and *Pseudomonas stutzeri* were grown at 37°C, and *R. sphaeroides* was grown at 32°C. For aerobic growth, cultures were inoculated into 250-ml flasks containing 10 ml of Z broth to a density of 10^8 cells per ml. Flasks were shaken at 350 rpm for 18 h in the dark. Photosynthetic growth conditions have been previously described (39).

DNA manipulations and sequencing. Alkaline lysis plasmid isolation, blunt ending of DNA termini, and ligations were performed by standard protocols (42). Restriction digests were performed in KGB buffer (22). Transformations were carried out as described elsewhere (10), except that a 10-min heat shock at 37°C was included. Both strands of the pJP5381 insert were commercially sequenced by U.S. Biochemical, Cleveland, Ohio. TFASTA (36) and TBLASTN (1) were used for homology searches against translated GenBank (release 80.0) and EMBL (release 37.0) data bases.

Conjugal DNA transfer. For mobilization of suicide plasmids into *R. sphaeroides*, ca. 10^8 cells of S17-1 (λ*pir*) carrying the suicide plasmid were mixed with an equal number of *R. sphaeroides* cells in 0.4 ml of 0.9% (wt/vol) saline. The matings were spotted onto Z plates, and the plates were incubated at

* Corresponding author. Phone: (61-7) 365-4640. Fax: (61-7) 365-4620.

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description[a]	Reference or source
R. sphaeroides		
RS7162	Restrictionless; Sm^r Rif^r	39
RS7166	Restrictionless; carotenoidless (blue green phenotype); Sm^r Rif^r	This laboratory
E. coli		
JM109	Host for α-complementation cloning vectors	51
JM109 (λ*pir*)	JM109 lysogenized with phage lambda carrying the *pir* gene; allows replication of R6K-based suicide vectors	40
S17-1	Mobilizing strain; carries chromosomally integrated derivative of RP4; Sm^r Tp^r	44
S17-1 (λ*pir*)	S17-1 lysogenized with phage lambda carrying the *pir* gene; allows replication of R6K-based suicide vectors	V. de Lorenzo
P. stutzeri		
JMP2536	Restrictionless; Sm^r	38
Plasmids		
pUC19	Cloning vector; Ap^r	51
pRK415	Broad-host-range mobilizable cloning vector; Tc^r	27
pKK232-8	Promoter probe vector with promoterless *cat* gene; Ap^r	8
pJP116	pHC79Δ*Bam*HI::Tn*5* with the *crt* genes of *R. sphaeroides*; Ap^r Km^r	37
pJP5350	pJP5011 with a 2.6-kb *Sma*I fragment carrying *ppsR*	39
pJP5603	R6K-based suicide vector; Km^r	40
R751::Tn813	Conjugative cointegrative plasmid	7
pJP5222	pUC19 with the 2.5-kb *Bam*HI fragment of pJP116	This work
pJP5223	pUC19 with the 5.0-kb *Bam*HI fragment of pJP116	This work
pJP5224	pUC19 with the 7.1-kb *Bam*HI fragment of pJP116	This work
pJP5226	pUC19 with the 3.4-kb *Bam*HI fragment of pJP116	This work
pJP5227	pUC19 with the 3.5-kb *Bam*HI fragment of pJP116	This work
pJP5380	pUC19 with the 2.6-kb *Sma*I fragment of pJP5350	This work
pJP5381	pUC19 with the 2.6-kb *Sma*I fragment of pJP5350; reverse orientation to that of pJP5380	This work
pJP5385	pJP5380 with a 518-bp *Sac*I deletion	This work
pJP5386	pRK415 with the 2.0-kb *Hin*dIII-*Eco*RI fragment of pJP5385	This work
pJP5404	pRK415 with the 1.9-kb *Pst*I fragment of pJP5381	This work
pJP5405	pRK415 with the 1.9-kb *Pst*I fragment of pJP5381; reverse orientation to that of pJP5404	This work
pJP5406	pRK415 with the 2.5-kb *Hin*dIII-*Eco*RI fragment of pJP5380	This work
pJP5407	pRK415 with the 2.5-kb *Hin*dIII-*Eco*RI fragment of pJP5381	This work
pJP5408	pJP5381 with a 204-bp *Nco*I deletion	This work
pJP5409	pRK415 with the 2.3-kb *Hin*dIII-*Eco*RI fragment of pJP5408	This work
pJP5450	pJP5603 with the 0.2-kb *Bgl*II-*Sac*I fragment of pJP5381	This work
pJP5472	pKK232-8 with the 7.1-kb *Bam*HI fragment of pJP5224	This work
pJP5475	R751::Tn*813*::pJP5472 cointegrate	This work

[a] Sm, streptomycin; Rif, rifampin; Tp, trimethoprim; Ap, ampicillin; Tc, tetracycline; Km, kanamycin.

32°C for 4 days. Mating growth was resuspended in saline and plated out on Z plates containing 30 μg of rifampin and 50 μg of kanamycin per ml and on Z plates containing only rifampin. Mobilization of plasmids into *P. stutzeri* JMP2536(pJP116) (38) was performed similarly, except that S17-1 was used in place of S17-1(λ*pir*), matings were of 4 h duration, and exconjugants were selected on Z plates containing 50 μg of kanamycin and 50 μg of tetracycline per ml.

Quantitation of photopigment levels. Photopigment levels were quantitated as previously described (39).

Nucleotide sequence accession number. The nucleotide sequence shown in Fig. 1 is located in the GenBank data base under accession no. L19596.

RESULTS

Sequences of the *ppsR* and *orf-192* genes. The *ppsR* gene had previously been localized to a 2.6-kb *Sma*I fragment in pJP5350 (39). This *Sma*I fragment was cloned into pUC19 in both orientations to produce pJP5380 and pJP5381. Both strands of the pJP5381 insert were sequenced and searched for open reading frames. *R. sphaeroides* genes show a strong bias against codons ending in A or T, which provides a valuable means of identifying genes. Two open reading frames with codon usage in good agreement with that of other *R. sphaeroides* genes were identified (data not shown). *orf-192* (nucleotides 38 to 553) is predicted to encode a 171-amino-acid (18-kDa) protein, while *ppsR* (nucleotides 689 to 2082) is predicted to encode a 464-amino-acid (51-kDa) protein (Fig. 1). The translational starts of *orf-192* and *ppsR* are preceded by purine-rich stretches, complementary to the 3′ end of *R. sphaeroides* 16S rRNA (15), which may function as ribosome-binding sites (Fig. 1). No potential terminator sequences were detected downstream of either gene.

A search of the protein data bases revealed that the PpsR protein is a homolog of the *R. capsulatus* CrtJ protein (PIR protein data base accession no. S17813). The PpsR and CrtJ proteins share 53% amino acid identity, which rises to 83% when conservative changes are allowed (Fig. 2A). Use of the local homology program TBLASTN (1) detected several pro-

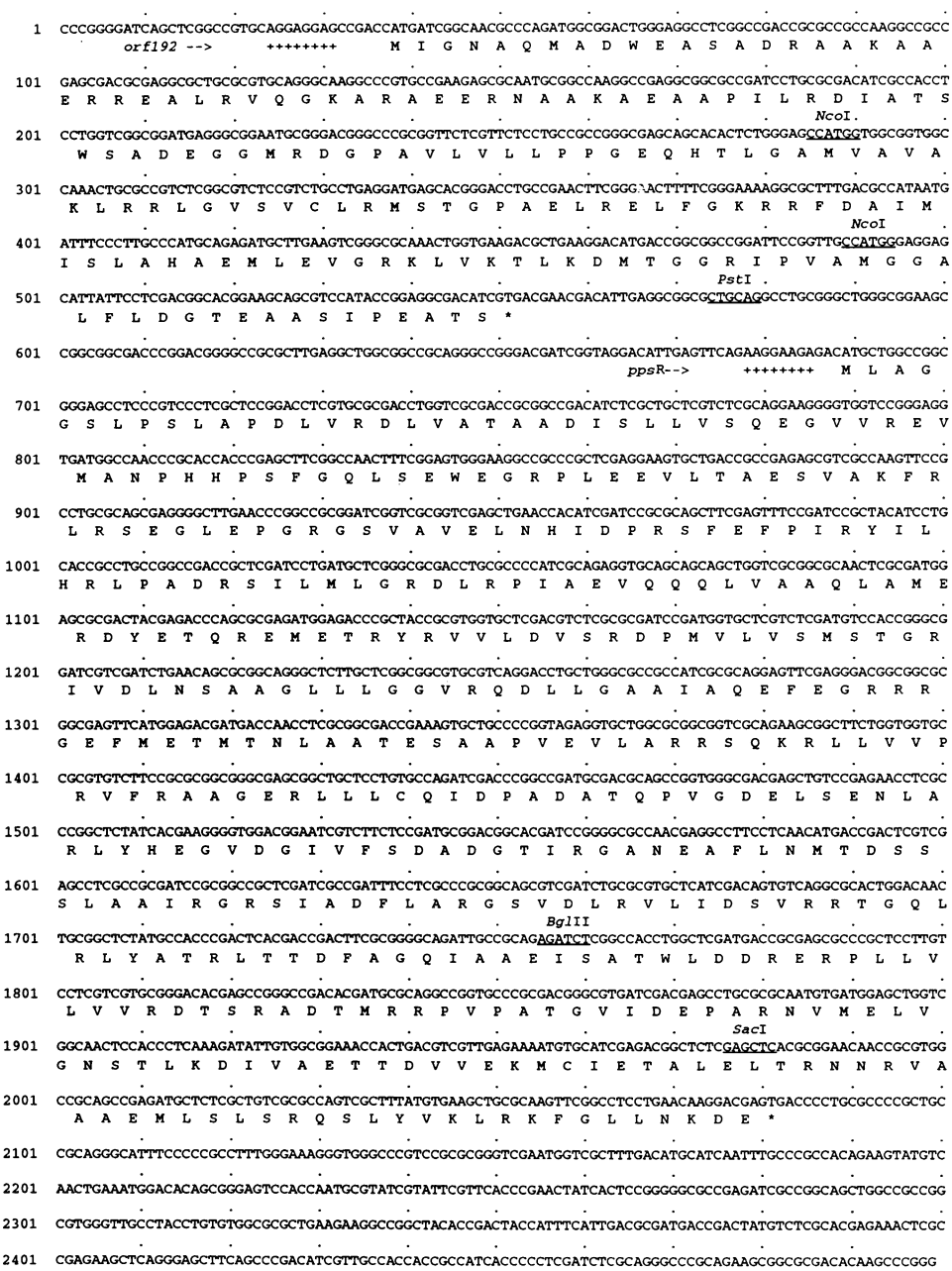

FIG. 1. Nucleotide sequence of the 2,499-bp *Sma*I insert of pJP5381. Only the sense strand is shown. Transcriptional directions of *orf-192* (nucleotides 38 to 553) and *ppsR* (nucleotides 689 to 2082) are indicated by arrows. Single-letter amino acid codes, translated regions; plus signs, potential ribosome-binding sites; asterisks, stop codons; underlining, restriction enzyme sites.

teins (RteB, PilR, NtrC, HydG, FlbD, HoxA, and Fis) with homology to the C-terminal region of PpsR (Fig. 2B). With the exception of Fis, the proteins are all response regulators (NtrC class) of two-component regulatory systems (see references given in Fig. 2B). Since the homology between PpsR and the response regulators is limited to the C-terminal region, it appears that PpsR either is an atypical response regulator or is not one at all. The region of homology spans a helix-turn-helix (HTH) domain found in many DNA-binding proteins (14, 23 [see also references in Fig. 2B]). The fact that homology between PpsR and CrtJ is particularly high in the C-terminal region (Fig. 2A) suggests that DNA binding may be important in PpsR-CrtJ function. The only protein in the data bases to share significant homology with Orf-192 was the *R. capsulatus* Orf-192 protein (PIR protein data base accession no. S17812) The proteins share 35% amino acid identity, which rises to 58% when conservative changes are allowed (data not shown). In *R. capsulatus*, *crtJ* is located just downstream from *orf-192*, which parallels the gene arrangement in *R. sphaeroides*. A biological function for the Orf-192 protein remains unelucidated. However, it is worth noting that many two-component regulatory systems involve close linkage between the sensor and regulator genes. If PpsR is an atypical response regulator, then the Orf-192 protein may well act as its sensor.

A

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PpsR        MLAGGS-------LPSLAPDLVRDLVATAADISLLVSQEGVVREVMANPHHPSPGQLSEW
CrtJ        VTSDGSFLMRREALQRVSPDLLADIVTSACDIALVVSPGRVVESVMVNPQFGSAERFAAW
            . ..**       *  ..***. *.*..* **.*.**   **  ** **.  *  ... *

PpsR        EGRPLEEVLTAESVAKFRLR-SEGLEPGRGSVAVELNHIDPRSFEFPIRYILHRLPADRS
CrtJ        QGARLSQLFSPESAQKLENRLADGPEPGR-SLQLELTH-AADAFTLPVRYTITRSGEDGT
            .*  *  .....** *.  * ..* **** *. .** *  . .* .*.** . *   * .

PpsR        ILMLGRDLRPIAEVQQQLVAAQLAMERDYETQREMETRYRVVLDVSRDPMVLVSMSTGRI
CrtJ        LLLIGRDMQPLAEVQQQLVKAQLALERDYEAQREIETRYRVLLEAHPAPLLIVSMSTGRI
            .*..***..*.******** ****.*****.***.******.*.    *...********

PpsR        VDLNSAAGLLLGGVRQDLLGAAIAQEFEGRRRGEFMETMTNLAATESAAPVEVLARRSQK
CrtJ        ADLNLAAAAMIGATRAELIDAPVGQELDGRRRGEFLENLAKIAGSDPLGAVELTIRRSRR
             *** **. ..*. * .*..*...**..*******.* ....*.... ..**.  ***..

PpsR        RLLVVPRVFRAAGERLLLCQIDPADATQPVGDELSENLARLYHEGVDGIVFSDADGTIRG
CrtJ        KVTVTATLFRAAGDRLLLCRLGEAEARRTRVDDTVELSERLFLKGIDAMVFLDADGTIRA
            .. * . .*****.*****... *.* .   *.  *   **.  *.*..** *******.

PpsR        ANEAFLNMTDSSSLAAIRGRSIADFLARGSVDLRVLIDSVRRTGQLRLYATRLTTDFAGQ
CrtJ        ANDAFLYLTDAGSAALVQGRSFADFLSRGAVDLNVLLDNVKRIGHLRHYVTRLNTDFSGQ
            **.*** .**..* * ..***.****.**.*** **.*.*.* *.** * *** ***.**

PpsR        IAAEISATWLDDRERPLLVLVVRDTSRADTMRRPVPATGVIDEPARNVMELVGNSTLKDI
CrtJ        VTVELSATLFHDRATPTIALVIRDSNLADATR--IMPGMASNEGLRNVMQMVGYATLRDI
            .. *.*** ..**  * .  **.**.. **. *  . .    .*  ****..** .**.**

PpsR        VAETTDVVEKMCIETALELTRNNRVAAAEMLSLSRQSLYVKLRKFGLLNKD-E
CrtJ        VSETTEIIEKMCIETALELTGNNRVAAAELLSLSRQSLYVKLRKFGLLSKDAE
            *.***...************ ********.******************.** *
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B

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PpsR        421 EKMCIETALELTRNNRVAAAEMLSLSRQSLYVKLRKPG
CrtJ        426 EKMCIETALELTGNNRVAAAELLSLSRQSLYVKLRKPG  36
PilR        399 ERKLIMQALEETRWNRTAAAQRLGLTFRSMRYRLKKLG  18
RteB        395 DKERILRALKQANGNRSVAAELLGIGRTTLYSKLEEYG  16
HoxA        441 EAVVLKEALLRHRWNKTHAAKELGLSRGGLRQKLLRPG  16
NtrC        436 EYPLILAALTATRGNQIKAADLLGLNRNTLRKKIRELG  16
HydG        400 EKEVILAALEKTGGNKTEAARQLGITRKTLLAKLSR.   15
FlbD        401 EQQLIIDTLEHCLGNRTHAANILGISIRTLRNKLKEYS  13
Fis          59 EQPLLDMVMQYTRGNQTRAALMMGINRGTLRKKLKKYG  12
NifA        481 ERQRLIAALEKAGWVQAKAARLLGMTPRQVAYRIQIMD   7
                        ******************
                          helix-turn-helix

Con             E---I--ALE-TR-NR--AA--LGL-R--L--KL---G
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FIG. 2. (A) Alignment of the PpsR and CrtJ (PIR protein data base accession no. S17813) proteins. Asterisks, amino acid identity; dots, conservative replacements. Gaps have been introduced to maximize the alignment. (B) Conserved HTH domain present in PpsR, CrtJ, *Bacteroides thetaiotaomicron* RteB (45), *P. aeruginosa* PilR (24, 25), *Rhizobium meliloti* NtrC (48), *Klebsiella pneumoniae* NifA (14), *E. coli* HydG (46), *C. crescentus* FlbD (41), *Alcaligenes eutrophus* HoxA (16), and *E. coli* Fis (26). The numbers of residue identities with PpsR are indicated in the righthand column. The putative HTH domain (14, 23) is indicated by asterisks. The consensus (Con) sequence shows residues that are present in at least half of the sequences.

The *ppsR* gene is sufficient for photopigment suppression. Subcloning was performed to determine if both *orf-192* and *ppsR* were required for photopigment suppression. A *Pst*I site at position 579 separates the two genes (Fig. 1). *ppsR* was subcloned, in both orientations, as a 1.9-kb pJP5381 *Pst*I fragment into pRK415 to create pJP5404 and pJP5405. As controls, the inserts of pJP5380 and pJP5381 (carrying both *orf-192* and *ppsR*) were cloned as *Hin*dIII-*Eco*RI fragments into pRK415 to create pJP5406 and pJP5407, respectively.

To monitor *ppsR* expression, *P. stutzeri* JMP2536(pJP116) was used. This strain is orange because of expression of the *R. sphaeroides crt* cluster carried by pJP116 (38). When a functional copy of *ppsR* is introduced, pigmentation changes from orange to white because of the action of PpsR (38a). Plasmids pRK415, pJP5404, pJP5405, pJP5406, and pJP5407 were mobilized into *P. stutzeri* JMP2536(pJP116) by using *E. coli* S17-1. Exconjugants carrying pRK415 and pJP5405 were orange, while those carrying pJP5404, pJP5406, and pJP5407 were white. Therefore, in the case of pJP5404, the *ppsR* gene alone was sufficient for photopigment suppression.

These results showed that without subcloning, *ppsR* expression was independent of insert orientation (pJP5406/pJP5407); however, with the *Pst*I subcloning, it became orientation dependent (pJP5404/pJP5405). This suggested that a native *ppsR* promoter was removed in the *Pst*I subcloning, rendering *ppsR* expression dependent on a pRK415 promoter. This implies that the native *ppsR* promoter is located between nucleotides 1 and 579 in the pJP5381 sequence and, furthermore, that it is functional in *P. stutzeri*. *ppsR* expression by pJP5404 was presumably due to either the upstream *lacZ* or *tet* promoter of pRK415. The functionality of the *E. coli lacZ* promoter in *P. stutzeri* would not be surprising, since we have previously shown that ColE1 replicons are maintained in this organism (38).

Localization of the *ppsR* promoter. To further localize the promoter, a deletion was generated by using *Nco*I. There are two *Nco*I sites in pJP5381, located at positions 286 and 490 in the *orf-192* coding region (Fig. 1). To remove the DNA between these sites, plasmid pJP5381 was digested with *Nco*I and self-ligated under dilute conditions to create pJP5408. The insert of pJP5408 was cloned as a *Hin*dIII-*Eco*RI fragment into pRK415 to create pJP5409. This plasmid was tested in *P. stutzeri* JMP2536(pJP116) for PpsR activity as described for the *Pst*I subcloning. Exconjugants carrying pJP5409 were orange. Thus, the native *ppsR* promoter is located between nucleotides 286 and 490 within the *orf-192* coding region. This type of situation is not unknown in *Rhodobacter* species; *bchZ* and *orf-1696* harbor promoters within their coding regions for the downstream *puf* and *puh* operons, respectively (6, 33, 34, 54). Inspection of the 204-bp *Nco*I region failed to reveal any obvious promoter sequences. However, little is known about the structure of *Rhodobacter* promoters.

The HTH domain is required for PpsR activity. A single *Sac*I site at position 1981 is found in the pJP5381 insert sequence (Fig. 1). Deletion of sequence between the insert and polylinker *Sac*I sites in pJP5380 would remove the last 33 amino acids of PpsR, including the HTH domain. It would also add 10 amino acids to the truncated PpsR protein via a limited PpsR-LacZ′ fusion (data not shown). Plasmid pJP5380 was digested with *Sac*I and was self-ligated under dilute conditions to form pJP5385. The pJP5385 insert was cloned as a *Hin*dIII-*Eco*RI fragment into pRK415 to create pJP5386. This plasmid was tested in *P. stutzeri* JMP2536(pJP116) for PpsR activity as described for the *Pst*I subcloning. No PpsR activity was detected, indicating the necessity for the HTH domain.

Inactivation of the chromosomal *ppsR* gene. An efficient method of gene inactivation involves a single crossover event between a target gene and an internal fragment of that gene (40). The *ppsR* sequence contains internal *Bgl*II (position 1756) and *Sac*I (position 1981) sites (Fig. 1). By using JM109(λ*pir*), the 226-bp *Bgl*II-*Sac*I fragment from pJP5381 was cloned into the *Bam*HI and *Sac*I sites of the suicide vector pJP5603 to produce pJP5450. Plasmid pJP5450 was mobilized into *R. sphaeroides* RS7162 and RS7166 by S17-1(λ*pir*). RS7162 produces Bch and Crt, whereas RS7166 produces only Bch. Exconjugant/recipient values of 1.4×10^{-7} (RS7162) and 3.0×10^{-7} (RS7166) were obtained.

Approximately half of the RS7162 and RS7166 exconjugant colonies exhibited a mutant phenotype; Southern blot analysis confirmed insertion into *ppsR* (data not shown). RS7162 mutants were dark red, while RS7166 mutants were dark green, phenotypes suggestive of photopigment overproduction. Indeed, quantitation of photopigment levels in cells grown

under aerobic conditions revealed that the mutants produced approximately five times as much Bch and Crt as the parents (Table 2). The periphery of wild-type *R. sphaeroides* colonies is pale because of exposure to oxygen (30). In contrast, mutant colonies were pigmented throughout, suggesting that oxygen suppression of Bch and Crt biosynthesis was reduced or absent in the mutants. The RS7162 overproducer mutant was capable of photosynthetic growth, and its spectrum was similar to that of RS7162. The spectrum of the RS7166 overproducer mutant showed a broad peak centered at 475 nm that was not present in the RS7166 spectrum (data not shown).

Both mutant phenotypes, but particularly the dark green one, were quite unstable. Subculturing after any extended period invariably resulted in a variety of secondary mutants with altered pigmentation. The intensity of color in many of these mutants suggested that pigments were still being overproduced.

Cloning a putative promoter from the *R. sphaeroides* photosynthesis gene cluster that functions in *E. coli*. Plasmids pJP5222, pJP5223, pJP5224, pJP5226, and pJP5227 contain *Bam*HI fragments cloned from the *R. sphaeroides* photosynthesis gene cluster (Table 1). Together, these fragments span 22 kb of the photosynthesis gene cluster. By aligning our genetic-physical map (39 and unpublished data) with other *Rhodobacter* maps (12), it could be estimated that this 22-kb section encompassed photosynthesis genes from *bchJ* to *bchA* (data not shown). To clone putative promoters from this region, pKK232-8 was used. When a fragment with promoter activity is cloned upstream of the pKK232-8 promoterless *cat* gene, Cat is synthesized and cells containing the construct can grow on media containing chloramphenicol. The aforementioned plasmids were digested with *Bam*HI, ligated to similarly digested pKK232-8, and transformed into JM109 with selection on Z plates containing 7.5 μg of chloramphenicol per ml. Only the pJP5224 transformation produced any colonies. Plasmid DNAs were extracted from four clones and separately digested with *Bam*HI and *Pst*I. All plasmids contained the 7.1-kb *Bam*HI fragment of pJP5224 in the same orientation. One of the plasmids, pJP5472, was retained. The aforementioned map alignments predicted that the 7.1-kb *Bam*HI fragment would carry all or part of the *crtD*, *-E*, and *-F* and *bchC* and *-A* genes.

PpsR suppression of a putative *R. sphaeroides* promoter in *E. coli*. JM109(pJP5472) was separately transformed with pRK415, pJP5404, pJP5405, pJP5406, and pJP5407 with selection on Z plates containing 100 μg of ampicillin and 20 μg of tetracycline per ml. Transformant cultures were streaked on plates containing 20 μg of tetracycline per ml and various levels of chloramphenicol and the plates were scored for growth of single colonies. Chloramphenicol resistance, mediated by the putative *R. sphaeroides* promoter(s), was diminished by pJP5404 but was unaffected by the other plasmids (Table 3). With pJP5404, this result indicated that *ppsR* could be expressed in *E. coli* when it was supplied with an upstream *lacZ* promoter. The diminution of pJP5472 chloramphenicol resistance by pJP5404 suggested that PpsR might be binding to a site(s) on pJP5472 to block transcription. The absence of any effect with pJP5406 and pJP5407 showed that although the native *ppsR* promoter was functional in *P. stutzeri*, it was not functional in *E. coli*. Furthermore, the lack of effect with pJP5406 indicated that read-through transcription from the *lacZ* promoter through *orf-192* was not occurring. This suggested termination of *lacZ*-mediated transcription in this region.

PpsR suppression of the putative *R. sphaeroides* promoter in *R. sphaeroides*. To eliminate possible artifacts involved with the use of *E. coli*, it was decided to test for repression of pJP5472 promoter activity in a wild-type background. To transfer pJP5472 to *R. sphaeroides*, transposon-mediated cointegration was used (7). JM109(pJP5472) was mated with JM109 R751::Tn*813* and exconjugants selected on Z plates containing 100 μg of ampicillin and 50 μg of trimethoprim per ml. Cointegrates between the two plasmids were isolated by mating exconjugants with JM109 (Rif^r) and then by selection on Z plates containing 100 μg of rifampin, 100 μg of ampicillin, and 50 μg of trimethoprim per ml. A R751::Tn*813*::pJP5472 cointegrate thus isolated was designated pJP5475.

JM109(pJP5475) was separately mated with *R. sphaeroides* RS7162 (PpsR$^+$) and RS7162 *ppsR*::pJP5450 (PpsR$^-$) with selection on plates containing 100 μg of streptomycin, 50 μg of trimethoprim, and 5 μg of ampicillin per ml. Exconjugants were then tested for resistance to chloramphenicol (Table 3). The diminution of pJP5472-mediated chloramphenicol resistance in the PpsR$^+$ strain of *R. sphaeroides* compared with the PpsR$^-$ strain paralleled the *E. coli* result, which suggested that the same process might be occurring in both species.

TABLE 2. Aerobic photopigment production by *R. sphaeroides* strains with and without PpsR

Strain	Photopigment (μg/mg of protein)	
	Bch	Crt
RS7162	3.43	2.68
RS7162 (*ppsR*::pJP5450)	16.43	11.91
RS7166	1.26	ND[a]
RS7166 (*ppsR*::pJP5450)	5.05	ND[a]

[a] ND, not determined.

DISCUSSION

Comparison of *R. sphaeroides* and *R. capsulatus* genetic maps suggests that the photosynthetic gene clusters of both species are very similar in terms of size (ca. 45 kb) and organization (12). A similar photosynthesis gene cluster is found in the more distantly related *Rhodospirillum centenum* (5, 53). On the basis of similar chromosomal positions, it was

TABLE 3. Repression of pJP5472 putative promoter activity by PpsR in *E. coli* and *R. sphaeroides*

Strain	Growth[a] on Cm[b]				
	1.0	2.5	5.0	7.5	10.0
E. coli JM109 (pJP5472)					
pRK415	+	+	+	+/−	−
pJP5404	+	+/−	−	−	−
pJP5405	+	+	+	+/−	−
pJP5406	+	+	+	+/−	−
pJP5407	+	+	+	+/−	−
R. sphaeroides					
	1.0	2.5	5.0	7.5	10.0
RS7162(pJP5475) (PpsR$^+$)	+	−	−	−	−
RS7162(pJP5475) (PpsR$^-$)	+	+	+	+/−	−

[a] Growth was scored as formation of single colonies (+), the absence of single colonies (−) or limited formation of single colonies (+/−).

[b] Cm, chloramphenicol. For *E. coli*, test plates contained 20 μg of tetracycline per ml and various levels of Cm (in micrograms per milliliter), while for *R. sphaeroides*, test plates contained 100 μg of streptomycin, 50 μg of trimethoprim, and 5 μg of ampicillin per ml and various levels of Cm (in micrograms per milliliter).

predicted that *ppsR* might be a homolog of the *R. capsulatus crtJ* gene (39). This has now been confirmed by sequencing. While inactivation of *ppsR* results in overproduction of Bch and Crt, inactivation of *crtJ* results in the loss of Crt (55). The reason for the apparent disparity between PpsR and CrtJ function is unknown.

Inactivation of the chromosomal copy of *ppsR* produces genetically unstable mutants that overproduce photopigments. Overproducer mutant colonies are pigmented throughout, which suggests that oxygen control of photopigment synthesis is absent or reduced. The combination of Bch and oxygen can lead to photooxidative killing (3); thus, the genetic instability may reflect selective pressure against producing high Bch levels. The absence of Crt, which provides some protection against photooxidative killing, is the probable reason for the particularly high instability of RS7166 overproducer mutants. Photopigment overproducer mutants may prove useful in the study of photopigment enzymes. Historically, difficulties have been encountered in attempts to purify photopigment biosynthetic enzymes (28). The use of overproducer mutants could potentially facilitate such purifications by providing a more concentrated source of the biosynthetic enzyme.

Genetically unstable dark red mutants of *R. sphaeroides* were first isolated almost four decades ago (20). Three dark red mutants, isolated via three separate routes, have subsequently been described (30). While these mutants showed normal anaerobic photopigment levels and light regulation, they produced 5 to 50 times more Bch and Crt under aerobic conditions. The PpsR$^-$ overproducer mutants produce ca. five times more Bch and Crt under aerobic conditions. Recently, genetically unstable dark red mutants were isolated that produced increased levels of Bch, Crt, and LH-II under aerobic conditions (32).

Excluding cyanobacteria, a number of so-called aerobic photosynthetic bacteria that produce Bch in the presence of oxygen have been isolated (17). Since many are closely related to photosynthetic species, it is possible that they are derived from phototrophs. Inactivation or deletion of *ppsR* could be a requisite step in such an evolutionary transition. Interestingly, a mutant of *R. centenum* that, unlike the wild type, shows oxygen repression of photopigment synthesis has been isolated (52).

Alignments of *R. sphaeroides* and *R. capsulatus* genetic-physical maps predicted that the 7.1-kb *Bam*HI insert of pJP5472 would carry all or part of the *crtD*, *-E*, and *-F* and *bchC* and *-A* genes. The *bchA* locus is now known to be composed of three genes (*bchX*, *-Y*, and *-Z*) in both *R. sphaeroides* (34) and *R. capsulatus* (33). Alignment of a restriction map derived from *R. sphaeroides bchCXYZ* (34) and *crtD* (18) sequence data with the pJP5472 restriction map revealed that the 7.1-kb *Bam*HI fragment carries *crtD'*, *crtEF*, and *bchCXYZ'* (Fig. 3). It does not carry the *puf* promoter(s) located in the downstream coding region of *bchZ* (33, 34, 54).

Recently, an unusual superopersonal gene organization in *R. capsulatus* has been described (for a review, see reference 49). Adjacent *crtEF*, *bchCXYZ*, and *puf* operons are transcribed in the same direction and are cotranscribable. Transcription may begin at the initiation sites of any of these three operons and continue through the downstream operons. A study of the *R. sphaeroides bchCXYZ* operon has yielded preliminary evidence for a superoperonal gene organization (34). It is possible then that the putative promoter activity of pJP5472 is due to transcription of *bchCXYZ'* and/or *crtEF-bchCXYZ'* (Fig. 3).

In *R. capsulatus*, promoters resembling *E. coli* σ^{70} promoters have been found upstream of the *crtEF* (2) and *bchCXYZ* (2, 33) operons. A *puf*-like promoter is also found upstream of the *bchCXYZ* operon (50). However, recent experiments suggest that it is the σ^{70}-like promoter rather than the *puf*-like promoter that is utilized for transcription of the *bchCXYZ* operon (33). Although it is not commented upon, a σ^{70}-like promoter (TTGACA-17 bp-AATGTT) is also evident upstream of the *R. sphaeroides bchCXYZ* operon (34). The existence of *E. coli* σ^{70}-type promoters upstream of the *crtEF* and *bchCXYZ* operons would provide a rationale for pJP5472 promoter activity in *E. coli*. This is supported by the finding that the σ^{70}-like promoter 5' to *bchCXYZ* in *R. capsulatus* functions as a promoter in *E. coli* (3). While it is possible that the promoter activity of pJP5472 is artifactual, the fact that it is specifically reduced by PpsR in both *E. coli* and *R. sphaeroides* argues against this.

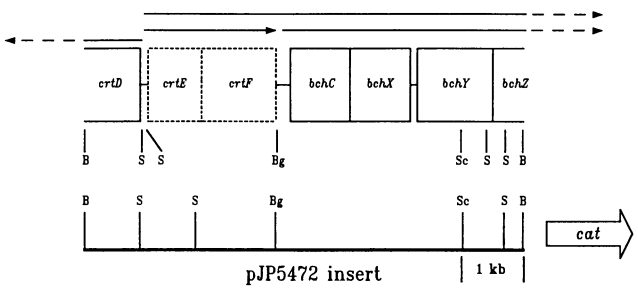

FIG. 3. Restriction map of the 7.1-kb *Bam*HI insert in pJP5472. The map is aligned with a restriction map generated from sequence data of *crtD* (18) and the *bchCXYZ* operon (34). Location of *crtEF* is based on *R. capsulatus* sequence data (2). Arrowed lines, potential RNA transcripts; dashed sections, transcription beyond the bounds of the 7.1-kb *Bam*HI insert (see text for details). The transcriptional direction of the promoterless *cat* gene in pJP5472 is indicated. B, *Bam*HI; S, *Sma*I; Bg, *Bgl*II; Sc, *Sac*I. Scale is in kilobases.

Thus, several lines of evidence raise the possibility that PpsR is a transcriptional repressor. As well as reducing the putative promoter activity of pJP5472 in both *E. coli* and *R. sphaeroides*, PpsR causes photopigment repression in *R. sphaeroides*, in *R. capsulatus*, and in *P. stutzeri* and *Paracoccus denitrificans* strains carrying the cloned *crt* genes of *R. sphaeroides* (38, 39). Conversely, inactivation of *ppsR* results in overproduction of Bch and Crt. An especially highly conserved region between PpsR and CrtJ carries an HTH domain that is found in various DNA-binding proteins. This putative DNA-binding region of PpsR is essential for PpsR activity.

Negative regulation mediated by repressors often occurs through overlap of the repressor binding site with the regulated promoter. Interestingly, a putative regulatory palindrome overlapping the σ^{70}-like promoters has been found upstream of the *R. capsulatus crtEF* (2) and *bchCXYZ* (33) operons. Although it has not been commented upon, a similar arrangement is evident upstream of the *R. sphaeroides bchCXYZ* operon (34). In *R. capsulatus*, the putative regulatory palindrome has also been found upstream of *crtA*, *-D*, and *-I*, the *puc* operon (2), and a possible *bchFNBHLM-orf-1696-puhA* superoperon (6, 9). In *R. sphaeroides*, it has also been found upstream of *crtD* (18) and the *puc* operon (31). The presence of the palindrome upstream of the *bch*, *crt*, and *puc* genes in both *R. sphaeroides* and *R. capsulatus* suggests possible coregulation of these three photosynthetic components (2, 4, 33).

A plausible scenario for PpsR repression of pJP5472 promoter activity is that PpsR binds to the putative regulatory palindrome located upstream of *crtEF* and *bchCXYZ*, preventing transcription by blocking RNA polymerase access to the overlapping σ^{70}-like promoters.

TGTAA	8 bp	TTACA	*R. capsulatus* consensus	(2)
TGTCA	8 bp	TGACA	*R. sphaeroides puc*	(31)
TGTCA	8 bp	TTACA	*R. sphaeroides crtD*	(18)
TGTCC	8 bp	TGACA	*R. sphaeroides bchCXYZ*	(34)
TGTCA	7 bp	TCACA	*P. aeruginosa sipR*	(35)
CGGCA	7 bp	CCGCA	*C. crescentus ftr*	(35, 41)
TGTCG	6 bp	CRACA	NifA consensus	(21)
TGCACY	5 bp	GGTGCA	NtrC consensus	(21)
TGTGT	6-10 bp	ACACA	prokaryotic consensus	(19)

FIG. 4. Comparison of palindromes. R, purine; Y, pyrimidine. Residues appearing in at least 80% of cases are underlined. References are listed in the right column.

Some support for this possibility comes from the PpsR HTH domain. This region shows high homology with RteB, PilR, NtrC, HydG, FlbD, and HoxA, all members of the NtrC class of response regulators. To a lesser degree, it also shows homology with NifA, which is structurally related to NtrC (21) (Fig. 2B). Furthermore, the NtrC and NifA HTH domains are homologous to the HTH domains of a variety of regulatory proteins, including λ cII, λ Cro, Crp, LacI, GalR, TrpR, AraC, and LexA (14). These HTH domain homologies suggest that the proteins might bind to homologous DNA-binding sites. Indeed, NtrC and NifA consensus sequences (21), a possible binding site for the *Pseudomonas aeruginosa* PilR protein (24, 35), a possible binding site for the *Caulobacter crescentus* FlbD protein (41), and a prokaryotic consensus sequence (19) (derived from the recognition sites of NifA, AraC, CAP, LacI, GalR, LexA, TnpR, LysR, and λ cII) all show some similarity to the putative regulatory palindromes of *R. sphaeroides* and *R. capsulatus* (Fig. 4). It will be interesting to see if similar putative DNA-binding sites are found upstream of genes regulated by RteB, HydG, and HoxA.

The following is one possible explanation for the results presented. Under aerobic conditions, PpsR represses transcription of *bch*, *crt*, and *puc* operons by binding to upstream palindromes. Removal of oxygen derepresses transcription because of either less PpsR being synthesized or PpsR being modified such that it binds with less affinity. Since the LH-II complex is the major photopigment binding site, the coregulation of *bch*, *crt*, and *puc* operons may help to protect the cell from photooxidative killing by ensuring a minimal free Bch pool. Consistent with this conjecture, the palindrome upstream of *bchCXYZ* in *R. capsulatus* has been shown to be involved in the formation of a DNA-protein complex (33).

The proposed model suggests that mutation of *ppsR* would result in overproduction of Bch, Crt, and LH-II under aerobic conditions. In this study, we have shown this to be the case with Bch and Crt. Another study has reported high levels of Bch, Crt, and LH-II under aerobic conditions due to mutation of an uncharacterized gene designated *oxyB* (32). While the location of *oxyB* does not quite correspond to that of *ppsR*, there is some discrepancy between different *R. sphaeroides* maps (12, 47); thus, the genes may still be found to be identical. It should be noted that the *puf* and *puh* operons are transcriptionally linked to upstream photopigment operons (6, 9, 49, 54). Therefore, inactivation of *ppsR* could also lead to increased levels of RC and LH-I under aerobic conditions.

Another prediction stemming from the model is that mutation of an upstream palindrome should produce increased transcription of the downstream gene under aerobic conditions. Mutations in either half of a palindrome found upstream from the *R. sphaeroides puc* operon resulted in partial derepression of *puc* operon expression under aerobic conditions (31). For reasons unknown, this effect was seen only when upstream sequence was also present. A similar study of the palindrome upstream from the *R. capsulatus bchCXYZ* operon provided somewhat different results. While a mutation in the left half of the palindrome led to increased aerobic transcription of *bchCXYZ*, a mutation in the right half produced the opposite; these results are consistent with either aerobic binding of a repressor or anaerobic binding of an activator (33). The results presented indicate that PpsR is involved in the regulation of photopigment levels, possibly as a transcriptional repressor. However, it is still possible that control is at some level other than that of transcription. Further experiments are in progress to test the proposed model.

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