Mol Gen Genet (1989) 218:1–12

MGG

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Genetic evidence for superoperonal organization of genes for photosynthetic pigments and pigment-binding proteins in *Rhodobacter capsulatus*

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Summary. Three adjacent operons, each concerned with photosynthesis in *Rhodobacter capsulatus*, have been shown by genetic means to be cotranscribable. In the course of describing the characteristics of the *bchCA* operon, which encodes two enzymes essential for bacteriochlorophyll synthesis, we found that the expression of the *bchCA* genes is influenced by readthrough from the upstream *crtE* and *crtF* genes. The *crtE* and *crtF* genes encode enzymes required for carotenoid biosynthesis and function as an operon. Furthermore, the distal structural gene of the *bchCA* operon, *bchA*, contains within it both the major oxygen-regulated promotor (P_{puf1}) and the constitutive (P_{puf2}) promotor for the *puf* operon. Since these three operons, *crtEF*, *bchCA*, and *puf*, are all transcribed in the same direction, it appears that polymerases traversing the downstream regions may start at any of several promoters. This pattern of transcription, which is unusual among bacteria, demonstrates that the activities of individual operons in a superoperonal cluster may be affected by their positions within the cluster.

Key words: Superoperon – Overlapping operons – Photosynthetic bacteria – Bacteriochlorophyll – Puf

Introduction

Genes encoding various components of the photosynthetic apparatus were found to be clustered on the chromosome of *Rhodobacter capsulatus* (Yen and Marrs 1976; Marrs 1981), and very similar gene arrangements have subsequently been observed in *R. sphaeroides* (Sistrom et al. 1984). The significance of these and other multioperonal gene clusters might be related to lateral gene transfer or might be a remnant of the evolution of the cluster of genes. We present evidence in this work that the proximity of three operons of the photosynthetic gene cluster (*crtEF*, *bchCA*, and *puf*) in *R. capsulatus* has functional significance.

Bacteriochlorophyll (Bchl) is a photopigment essential for the photosynthetic process in the purple non-sulfur bacterium *R. capsulatus*. The *bchA* and *bchC* genes (Biel and Marrs 1983) encode proteins essential for two successive enzymatic reductions in the Bchl biosynthetic pathway, with the *bchA* gene product functioning prior to the *bchC* gene product (for a review of bacteriochlorophyll biosynthesis, see Rebeiz and Lascelles 1982). Genetic mapping has located these genes adjacent to each other, between genes encoding enzymes for carotenoid biosynthesis (*crtE* and *crtF*) and genes for the Bchl-binding reaction center and light harvesting I (LH-I) antenna proteins and the Bchl-regulating PufQ protein (the *puf* operon) (Taylor et al. 1983; Youvan et al. 1984b; Bauer et al. 1988). Two previous studies have suggested that the *bchC* and *bchA* genes comprise an operon, but contradictory conclusions were reached regarding the direction of transcription of the proposed operon (Biel and Marrs 1983; Zsebo and Hearst 1984). This conflict prompted a more detailed analysis concerning the relationship of these two genes.

In this paper, we confirm that *bchC* and *bchA* comprise an operon and demonstrate that the direction of transcription is from *bchC* toward *bchA*, thus resolving the conflict noted above. DNA sequence and interposon analyses of the extremities of the operon are described which locate the oxygen-regulated *bchCA* promoter and reveal an overlap between *bchA* and the *puf* operon. Furthermore, readthrough from a site upstream of *crtE* influences expression from the *bchCA* operon. The *crtE* and *crtF* genes also comprise an operon. These three adjacent operons (*crtEF*, *bchCA*, and *puf*) are cotranscribable.

Materials and methods

Bacterial strains, plasmids, and culture conditions. The bacterial strains and plasmids utilized in this study are listed in Table 1. Malate minimal (RCV) medium (Weaver et al. 1975) and a complex medium of 0.3% Difco Bacto-peptone and 0.3% Difco yeast extract (PY) were used to support growth of *R. capsulatus*. In addition, RCV + 2 g/l peptone and 2 g/l yeast extract (RCV 2/3 PY) was used as a buffered "rich" medium. *Escherichia coli* strains were grown in LC (Marrs 1981) media. Ampicillin, spectinomycin, and kanamycin were all used at 100 μg/ml while trimethoprim was used at 50 μg/ml for selection of resistant *E. coli*. Spectinomycin and kanamycin were used at 10 μg/ml and rifampicin at 75 μg/ml to select resistant *R. capsulatus*. Solid media employed 1.5% agar, while soft agar contained 0.7% agar.

High oxygen growth conditions consisted of 10 ml PYS (PY containing 2 mM $CaCl_2$ and 2 mM $MgSO_4$) in a

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Table 1. Strains and plasmids

Designation	Relevant markers and properties	Source/ reference
Escherichia coli		
DH5	F⁻ *endA1 hsdR17* (rK⁻ mK⁺) *supE44 thi-1* λ^- *recA1 gyrA96 relA1*	Hanahan (1983)
DH5α	F⁻ *endA1 hsdR17* (rK⁻ mK⁺) *supE44 thi-1* λ^- *recA1 gyrA96 relA1* 80dlacZm15 (*argF-laczya*) U196	Hanahan (1983)
Rhodobacter capsulatus		
BP503	*crtF129 hsd-1 str-2*	Taylor et al. (1983)
BPY109	*bchC5109 crtF129*	Derived from PY1291 as per Taylor et al. (1983)
BRP3	*bchA603 crtF129 hsd-1 str-2*	Taylor et al. (1983)
BRP17	*bchA617 crtF129 hsd-1 str-2*	Derived from BP503 as per Taylor et al. (1983)
CB1064	*rif-10 Ω::del* (*pufQ'-pufC2397*) *aadA*⁺	By GTA from CB1127 (pPH1::*Δ*sp502) donor to SB1003 recipient
CB1088	*rif-10 crtB4 puc-742*; no LH II	By GTA from SB1003 donor to MW442 recipient
CB1127	*rif-10 crtG121*; GTA overproducer	By GTA from SB1003 donor to R121 recipient
DE309	*rif-10 aadA*⁺ *bchC::Ω1 aadA*⁺ BchA⁻	This paper
DE311	*rif-10 bchA*::Kn*1*; BchA⁻	By GTA from CB1127 (pDAY1) donor to SB1003 recipient
DE319	*rif-10 aadA*⁺ *bchA::Ω1 crtB4 puc-742*; no LH II, BchA⁻	By GTA from CB1127 (pΩNaeI) donor to CB1088 recipient
DE320	*rif-10 aadA*⁺ *bchA::Ω2*; Puf_{P1}^- BchA⁻	By GTA from CB1127 (pDAY39.2) donor to SB1003 recipient
DE324	*rif-10 crtF::Ω1 aadA*⁺	This paper
DE329	*rif-10 crtF::Ω2 aadA*⁺	This paper
DE330	*rif-10 crtF::Ω3 aadA*⁺	This paper
DE331	*rif-10 bchA::Ω4 aadA*⁺; BchA⁺, puf⁻	By GTA from CB1127 (p1255exΩNcoI) donor to SB1003 recipient
DE333	*rif-10 bchA::Ω1*	By GTA from CB1127 (pΩNaeI) donor to SB1003 recipient
DE335	*rif-10 bchA::Ω3*	By GTA from CB1127 (pDAY58) donor to SB1003 recipient
MB1007	*bchC1007*	Taylor et al. (1983)
MB10110	*rif-10 bchA305*	Marrs (1981)
MW442	*crtB4 puc-742*; no LH II	Scolnik et al. (1980b)
PY1291	*crtF129*; no LH II	Scolnik et al. (1980a)
R121	*crtG121*; GTA overproducer	Scolnik et al. (1980a)
SB1003	*rif-10*	Yen and Marrs (1976)
Plasmids		
p1255ex	Amp^r, Tet^r	Bauer et al. (1988)
p1255exΩNcoI	Amp^r, Tet^r, Spec^r	Bauer et al. (1988)
pΩNaeI	Amp^r, Spec^r	This paper
pCB532	Amp^r, Spec^r	Bauer et al. (1988)
pDAY1	Amp^r, Tet^r, Kn^r	This paper
pDAY16	Amp^r, Spec^r, Kn^r	This paper
pDAY20	Amp^r	This paper
pDAY21	Amp^r	This paper
pDAY22	Amp^r	This paper
pDAY23	Amp^r, Spec^r	This paper
pDAY24	Amp^r, Spec^r	This paper
pDAY25	Amp^r, Spec^r	This paper
pDAY26	Amp^r, Spec^r, Kn^r	This paper
pDAY27	Amp^r, Spec^r, Kn^r	This paper
pDAY28	Amp^r, Spec^r, Kn^r	This paper
pDAY31	Amp^r, Spec^r	This paper
pDAY39.2	Amp^r, Spec^r	This paper
pDAY40	Amp^r, Spec^r	This paper
pDAY41	Amp^r, Kn^r	This paper
pDAY45	Amp^r, Spec^r	This paper
pDAY48	Amp^r, Spec^r	This paper
pDAY50	Amp^r, Spec^r	This paper
pDAY55	Amp^r, Spec^r	This paper
pDAY58	Amp^r, Spec^r	This paper
pDAY60	Amp^r, Spec^r, Kn^r	This paper

Table 1. (continued)

Designation	Relevant markers and properties	Source/ reference
pDPT51	Amp^r, Tm^r	Taylor et al. (1983)
pPH1	Amp^r	This paper
pPH1:*Δ*sp502	Amp^r, Spec^r	Bauer et al. (1988)
PMA117	Amp^r, Kn^r	Giuliano et al. (1988)
pNM480	Amp^r	Minton (1984)
pNM481	Amp^r	Minton (1984)
pNM482	Amp^r	Minton (1984)
pRPSE30	Amp^r, Kn^r	Taylor et al. (1983)
pU21	Amp^r, Kn^r	Youvan et al. (1985)
pUC9	Amp^r	Messing (1983)

125 ml flask shaking at 290 rpm (New Brunswick Scientific G24). Low oxygen conditions consisted of 30 ml medium in a 50 ml flask shaking at 90 rpm. Growth in the absence of oxygen was achieved by illuminating 18-ml PYS cultures in 18-ml screw capped tubes. All growth was at 35° C. For spectral scans, cultures were grown under low oxygen conditions in RCV supplemented with 0.6% glucose, 0.5% pyruvate, and 50 mM dimethyl sulfoxide (RCV$^+$; Biel and Marrs 1983).

Mating procedures. E. coli cultures containing both the plasmid to be transferred and the mobilizing plasmid pDPT51 were grown overnight in LC media without antibiotic. *R. capsulatus* cultures were grown overnight in RCV media. Equal volumes of donor and recipient were mixed in a sterile Eppendorf tube (1.4 ml), centrifuged 5 min at room temperature in an Eppendorf microfuge, then resuspended in one-half final volume of fresh RCV. Three 20 µl aliquots were spotted on "cured" or aged, partially dried RCV plates. The plates were incubated for 5 h at 35° C then overlaid without spreading with 3 ml soft PY agar containing the appropriate antibiotics.

Determination of β-galactosidase activity. Cultures were grown under conditions of high or no oxygen to a Klett (red filter) value of 50. The β-galactosidase assay was a modified version of the method described by Biel and Marrs (1983). Eight milliliters of culture were harvested by centrifugation at 27000 × *g* for 10 min at 4° C in a Sorvall SS-34 rotor. The cells were resuspended in 1.0 ml of iced Z-buffer (Miller 1972), disrupted by sonication, and centrifuged as described above. The assay was run using 50 µl of crude extract, 2.0 ml of Z-buffer, and 400 µl of a solution of 4 mg *o*-nitrophenyl-β-D-galactopyranoside/ml. Two tubes were run for each assay reaction. One tube of the set incorporated the stop solution of 1 M sodium carbonate at time 0, while the second tube was stopped only after a light to medium yellow color appeared. Both tubes were incubated at 35° C. The first tube was used as a reference sample in the Perkin Elmer lambda array 3840 spectrophotometer at 420 nm, while the second tube was used to determine absorbance. The molar extinction coefficient of nitrophenol is 4860 at pH 10.0 (Doub and Vandenbelt 1949). The protein concentration of the crude extracts was determined by the dye binding method of Bradford (1976) with crystallized bovine serum albumin (Sigma) as standard.

Gene transfer mediated crosses. Gene transfer agent (GTA) crosses were as previously described by Yen and Marrs (1976).

Transformation. Competent *E. coli* cells DH5 and DH5α were purchased from BRL and transformed following the protocol of Hanahan (1983).

Isolation and restriction analysis of plasmid DNA. Large-scale and small-scale (miniprep) isolation of plasmid DNA were performed using the alkaline lysis technique, as described by Maniatis et al. (1982). Restriction digests, ligation, bacterial alkaline phosphatase, Klenow, and T4 polymerase reactions used enzymes from IBI or New England Biolabs according to the manufacturer's specifications. Electrophoretic analyses were conducted using 0.85% agarose (Sigma) gels in 1 × TAE buffer (Maniatis et al. 1982). DNA ligations were performed according to the "in-gel ligation technique" using SeaPlaque (FMC) low melting temperature agarose (Crouse et al. 1983).

DNA sequencing. Sequencing was performed using both the Maxam-Gilbert chemical modification method (Maxam and Gilbert 1980) and the Sanger dideoxynucleotide method (Sanger et al. 1977). The *Eco*RI-Q restriction fragment was commercially cloned into pBR322, producing plasmid p1255ex, and sequenced using the Maxam-Gilbert method (Unigene Inc., Fairfield, NJ).

Plasmid construction. Ligations involving DNA fragments with noncohesive ends were preceded by conversion to blunt ends using either Klenow or T4 polymerase.

pDAY1. Plasmid PMA117 (Giuliano et al. 1988) was digested with *Sal*I enzyme and a 1.5 kp restriction fragment encoding the Tn*5* kanamycin gene (Kn cartridge, Km117) was isolated. This cartridge was ligated with a partial *Xho*I digest of p1255ex DNA (Bauer et al. 1988), a pBR322 based plasmid containing *Eco*RI-Q inserted into the unique *Eco*RI site. The mixture was transformed into *E. coli* DH5, selecting for acquired kanamycin resistance. Restriction analysis confirmed insertion of the cartridge into the first *Xho*I site of the *Eco*RI-Q fragment with retention of the second *Xho*I site intact.

pDAY16. The omega (Ω) fragment (Prentki and Krisch 1984) is a 2.0 kb DNA segment consisting of a gene coding for spectinomycin resistance flanked by transcription and translation termination signals. The *Bam*HI-digested Ω fragment was ligated into the unique *Bam*HI site of pRPSE30. Selection, following transformation, was for acquired spectinomycin resistance.

pDAY26. Plasmid p1255exΩ*Nco*I (Bauer et al. 1988) was digested with *Eco*RI and the fragment containing *Eco*RI-Q with Ω in the *Nco*I site was isolated. A partial *Eco*RI digest of pRPSE30 was ligated with the above fragment selecting for spectinomycin resistance. The final construct, pDAY26,

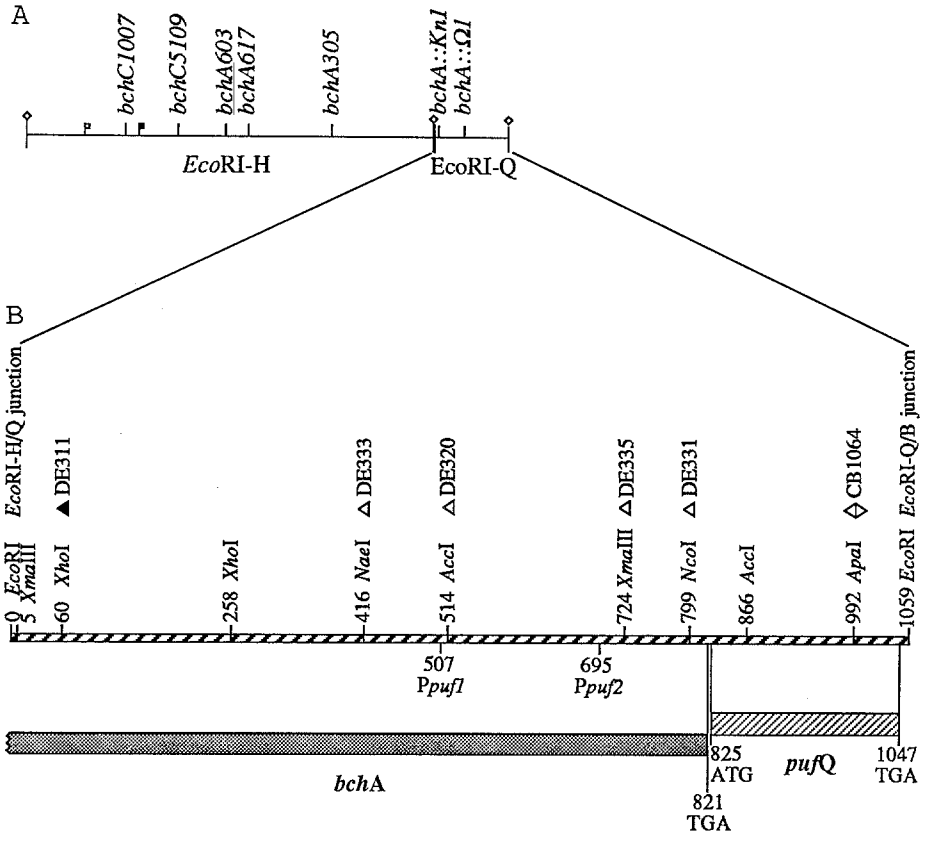

Fig. 1 A and B. Genetic-physical map of the *Rhodobacter capsulatus* *Eco*RI-H and *Eco*RI-Q fragments.
A Point mutations of the *bchC* and *bchA* genes were mapped as per Yen and Marrs (1976) and the uncertainty of their physical location is ±175 bp. Restriction enzyme sites: *Eco*RI (*diamond*); *Sph*I (*open box*); and *Bam*HI (*closed box*).
B Names of strains derived from interposon insertions (*triangles*) or insertion deletion (*double triangle*) are listed above the map. Construction of strains and BchA phenotype is as shown in Table 2. *Open* and *closed triangles* refer, respectively, to Ω and Km117 cartridges. The map positions of the *bchA* gene and the *pufQ* gene (*shaded boxes*) were determined by both genetic and nucleotide sequence analysis. *Numbers* indicate the positions of particular restriction sites (in bp) from the *Eco*RI-H/Q junction to the actual cleavage site. The *Eco*RI-Q fragment is 1059 bp in length

carries the *Eco*RI-H and *Eco*RI-Q fragments in the same orientation as the chromosome with Ω in the *Nco*I site of *Eco*RI-Q.

pΩNaeI. A *Sma*I-digested Ω fragment was ligated into the unique *Nae*I site of plasmid pCB532 (Bauer et al. 1988), selecting for spectinomycin resistance.

pDAY39.2. Plasmid p1255exΩ*Nco*I was digested with *Eco*RI and *Hin*dIII and an 804 bp piece isolated. This fragment was ligated into pUC9 (Messing 1983) that had also been digested with *Eco*RI and *Hin*dIII. The resulting plasmid contained a unique *Acc*I site (514 bp site, Fig. 1) in the *Eco*RI-Q DNA region. This vector was digested with *Acc*I and ligated with the Ω *Sma*I fragment. Selection, following transformation, was for spectinomycin resistance.

pDAY41. pRPSE30 was digested with *Bam*HI, made blunt with Klenow, and religated. Screening for loss of this unique *Bam*HI site yielded pDAY41.

pDAY58. Plasmid p1255ex was partially digested with *Xma*III and ligated with the *Sma*I fragment of Ω. The final construct, pDAY58, contains Ω in the *Xma*III site (724) of *Eco*RI-Q while retaining all other plasmid *Xma*III sites intact.

pDAY60. Plasmid pDAY58 was digested with *Eco*RI and the fragment containing *Eco*RI-Q with Ω in the *Xma*III (724) site was isolated. A partial *Eco*RI digest of pRPSE30 was ligated with the previously described fragment, selecting for spectinomycin resistance. The final construct, pDAY60, carries the *Eco*RI-H and *Eco*RI-Q fragments in the same orientation as the chromosome with Ω in the *Xma*III (724) site of *Eco*RI-Q.

pPH1:Δsp502. Plasmid pU21 (Youvan et al. 1985), carrying the *Eco*RI-Q and *Eco*RI-B/*Bam*HI-C fragments from *R. capsulatus* and a *Bam*HI kanamycin cartridge, was digested with *Bam*HI and religated to remove the kanamycin gene. The kanamycin sensitive vector produced was pPH1. Note, this vector is equivalent to pEBQ1 (Youvan et al. 1985). pPH1 was digested with *Apa*I (one site in *pufQ* and another between *puf* open reading frames C2397 and C2814, Youvan et al. 1984a). The Ω *Sma*I fragment was ligated in place of the deleted 2776 bp of *R. capsulatus* DNA (Bauer et al. 1988).

Results

Analysis of the bchA *gene size*

Extensive resequencing (this work and Adams et al. 1989) of the *Eco*RI-Q fragment, containing the *puf* operon promotor region, revealed three changes compared to our previously published sequence (Bauer et al. 1988). The corrections are as follows, with DNA sequence numbers relative to the start of the *pufB* gene(0): addition of base C between sites −575 and −576; deletion of base G at site −597; and addition of base C between sites −780 and −781. Codon preference analysis of this region (Fig. 2A) suggests the existence of an open reading frame (ORF) that terminates one base before the start of the *pufQ* gene. In order to determine the nature of this ORF, and to determine its effect on *puf* operon expression, we performed interposon insertion mutagenesis of this region (Scolnik and Haselkorn 1984).

Plasmids were constructed in which Ω or kanamycin cartridges were inserted into distinct restriction sites or deleted areas of the *Eco*RI-Q-*Eco*RI-B/*Bam*HI-C DNA frag-

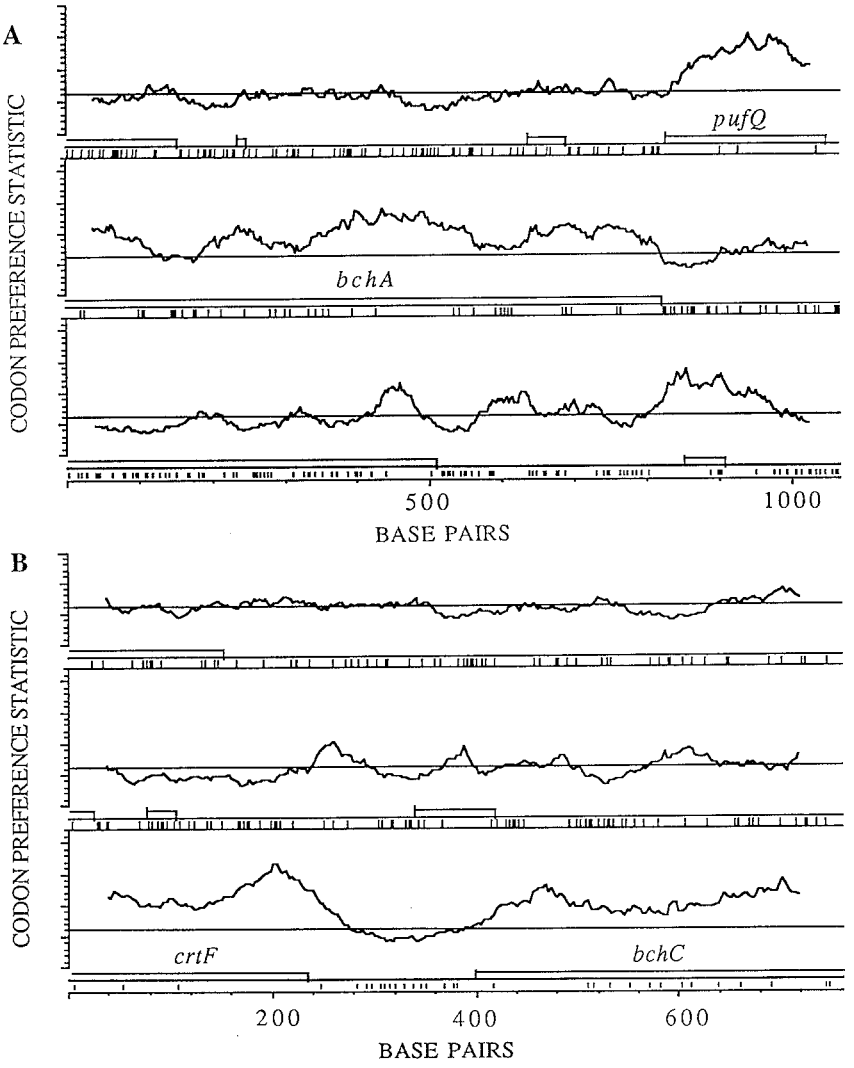

Fig. 2A and B. Codon preference plots generated using a program written by Gribskov et al. (1984). The program identifies efficiently translated genes as peaks above open reading frames (*open boxes* below peaks) that contain few rare codons (*vertical dashes* below the open reading frames). A codon frequency table used to produce the plot was obtained from previously published *pufQ, cycA, petABC, pucAB, pufBALM*, and *puhA* genes (Bauer et al. 1988; Daldal et al. 1986; Davidson and Daldal 1987; Youvan and Ismail 1985; Youvan et al. 1984a). The window size and rare codon threshold were set at 25 and 0.1, respectively.
A Codon preference plot of the *Eco*RI-Q fragment.
B Codon preference plot of the junction region between the *crtEF* and *bchCA* operons

ments (Fig. 1B). These plasmids were then each transformed into *E. coli* hosts, mobilized from the *E. coli* host into the *R. capsulatus* GTA-overproducer strain CB1127, and GTA-mediated interposon mutagenesis was then used to transfer these cartridges into the *R. capsulatus* chromosome (Scolnik and Haselkorn 1984). Strain SB1003 was used as a GTA recipient. In those cases where the interposons affected *puf* operon function, the plasmid pDAY1 was mated into the strain (see below). pDAY1 contains a fully functional *pufQ* gene, which is necessary for synthesis of Bchl and its Mg-containing precursors (Bauer and Marrs 1988). This served to allow spectral analysis of BchA status uninfluenced by the PufQ status (Bauer et al. 1988). Interposon analysis revealed a region of DNA coding for *bchA*-like functions to extend at least through the *Xma*III (724) site in the *Eco*RI-Q fragment (Table 2; Fig. 1B). Codon preference analysis of the *Eco*RI-Q region (Fig. 2A) suggests the open reading frame, ending at the 821 bp point, to be the *bchA* gene. We show below that there is only one cistron in this region that gives rise to the BchA phenotype.

Table 2. Genetic analysis of the *bchA* gene size

GTA[a] donor plasmid	Resulting construct	Mated pDAY1 plasmid	Bchl phenotype
pDAY1	DE311	–	BchA
pΩNae1	DE333	–	BchA
pDAY39.2	DE320	+	BchA[b]
pDAY58	DE335	+	BchA[b]
p1255exΩNco1	DE331	+	Wild type[b]
pPH1:Δsp502	CB1064	+	Wild type[b]

[a] SB1003 was used as the gene transfer agent (GTA) recipient (see the results)
[b] Without the addition of pDAY1, little or no BchA precursor or bacteriochlorophyll is accumulated (see Fig. 3)

The bchA *gene overlaps the* puf *operon promoters*

It has previously been demonstrated that the oxygen-regulated *puf* operon promotor (P_{puf1}) is located 507 bp from the *Eco*RI-Q/H junction, and the weaker constitutive promotor (P_{puf2}) is located 695 bp from the same reference point (Bauer et al. 1988). The *puf* operon promotors are therefore located within the ORF identified above as the *bchA* gene. Only one base separates the TGA stop codon of the *bchA* gene from the ATG start codon of the *pufQ* gene. The amount of tetrapyrrole production is dependent on the level of *puf* operon (*pufQ*) expression (Bauer and

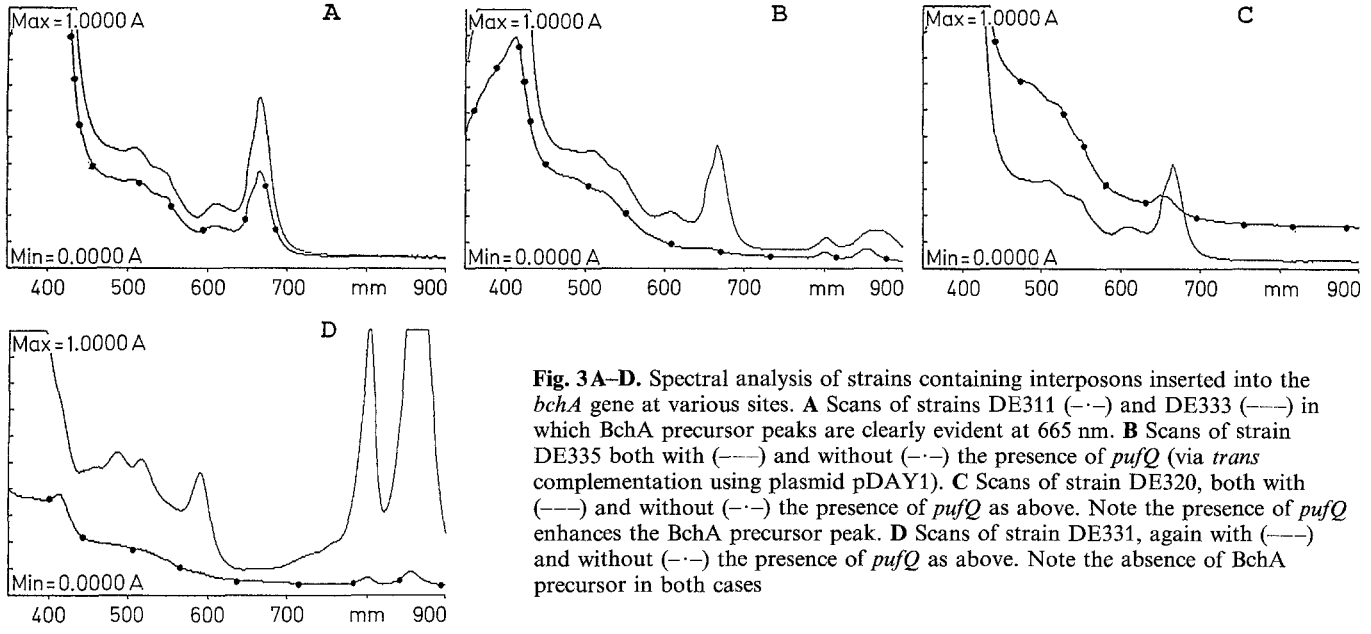

Fig. 3A–D. Spectral analysis of strains containing interposons inserted into the *bchA* gene at various sites. **A** Scans of strains DE311 (–·–) and DE333 (——) in which BchA precursor peaks are clearly evident at 665 nm. **B** Scans of strain DE335 both with (——) and without (–·–) the presence of *pufQ* (via *trans* complementation using plasmid pDAY1). **C** Scans of strain DE320, both with (——) and without (–·–) the presence of *pufQ* as above. Note the presence of *pufQ* enhances the BchA precursor peak. **D** Scans of strain DE331, again with (——) and without (–·–) the presence of *pufQ* as above. Note the absence of BchA precursor in both cases

Marrs 1988). Thus we can determine whether the polar insertion of Ω into the *bchA* gene influences *puf* operon expression by measuring the amount of 2-devinyl,2-hydroxyethyl chlorophyllide *a* (P665, accumulated in BchA mutants) produced from these insertions. The insertion of interposons into the *bchA* gene at either the *Xho*I site located 60 bp or the *Nae*I site located 416 bp from the *Eco*RI-Q/H junction (DE311 and DE333, respectively) results in the accumulation of high levels of 2-devinyl,2-hydroxyethyl chlorophyllide *a* (Fig. 3). In contrast, the insertion of Ω at the *Acc*I site (DE320) located 514 bp from the *Eco*RI-Q/H junction produces significantly reduced amounts of 2-devinyl,2-hydroxyethyl chlorophyllide *a*. This is clearly due to reduced *puf* operon expression, since *trans* addition of the *pufQ* gene (pDAY1) into DE320 results in the return to typical BchA mutant levels of this tetrapyrrole precursor. [Each of the preceding constructions and phenotypic determinations was repeated in a host strain (CB1088) lacking the light-harvesting II antenna complex (LH-II), and analogous results were obtained (data not shown).] Insertion of Ω into the *Xma*III site (DE335) located 724 bp from the *Eco*RI-Q/H junction reveals a BchA tetrapyrrole precursor peak whose presence is dependent upon the presence of PufQ. This insert does however appear to be leaky, based on the trace amount of bacteriochlorophyll synthesized. Finally, insertion of Ω into the *Nco*I site 799 bp from the *Eco*RI-Q/H junction (DE331) causes no discernible accumulation of BchA tetrapyrrole precursor in the presence or absence of plasmid pDAY1, but in its presence normal Bchl is synthesized. This is the phenotype observed for strains with no lesion in the *bchA* gene (Bauer and Marrs 1988). From this analysis, coupled to promoter mapping studies (Bauer et al. 1988) and DNA sequence data, we conclude that both *puf* operon promoters are within the *bchA* structural gene.

Complementation analysis of bchC *and* bchA *mutants*

The preceding data suggest that the *bchA* gene extends across the *Eco*RI site that forms the boundary between the *Eco*RI-H and *Eco*RI-Q fragments, yet past studies reported complementation of *bchA* mutants by the *Eco*RI-H fragment alone (Taylor et al. 1983). To investigate this discrepancy, we repeated the original complementation studies and also tested new plasmid constructs for complementation.

Two hypotheses were initially entertained. It was possible that two adjacent genes existed in this region, either of which could be mutated to give the BchA phenotype. In that case, both the previous complementation data and the current interposon data could be correct, since one of these two hypothetical genes could be completely contained in the *Eco*RI-H fragment. On the other hand, it seemed possible that a high frequency of recombination might have been misinterpreted as complementation in earlier studies. To distinguish between complementation and recombination in the current study, we conducted matings to construct merodiploids, and we then restreaked exconjugants exhibiting the wild-type phenotype (which could be due to either genetic event) without selection for plasmid maintenance. Upon restreaking, recombinant colonies give rise to unsectored colonies, most of which are wild-type and only a few of which are of mutant (BchA) phenotype. In contrast, wild-type exconjugants created by complementation give rise to a majority of sectored colonies and only a few parental or recombinant colonies. The difference between recombination and complementation was easily distinguished by this test.

Two plasmids, pRPSE30 containing only the *Eco*RI-H fragment of the *R. capsulatus* genome, and pDAY26 containing the contiguous *Eco*RI-H and *Eco*RI-Q fragments, were mated into various *bchA* and *bchC* mutants (Fig. 1A). Mutations were selected which spanned the known extent of the respective genes. The results of this test demonstrated that complementation occurred for both *bchC* mutants when either plasmid was present (Table 3). However, the plasmid pRPSE30 failed to complement any *bchA* mutants, whereas pDAY26 complemented all. From these results we conclude that the previous report of complementation of *bchA603* in strain BRP3 by pRSPE30 (Taylor et al. 1983) was wrong.

Table 3. Complementation analysis of *bchC* and *bchA* mutants

Bchl mutation	Recipient strain	Complementation with plasmid	
		pRPSE30	pDAY26
bchC1007	MB1007	+	+
bchC5109	BRY109	+	+
bchA603	BRP3	−	+
bchA617	BRP17	−	+
bchA305	MB10110	−	+
bchA::Kn*1*	DE311	−	+
bchA::*Ω1*	DE319	−	+

The above data clearly dispel the hypothesis of two *bchA* genes with one gene contained completely within the *Eco*RI-H fragment; however, these results do not address the possibility of two *bchA* genes with one gene contained completely within the *Eco*RI-Q fragment. To study this possibility, plasmid pDAY60, which contains the contiguous *Eco*RI-H and *Eco*RI-Q fragments with Ω less than 100 bp from the end of *bchA* in *Xma*III (724), was mated into the *bchA* point mutant MB10110 (*bchA305*). Complementation would indicate two genes with the second completely contained in *Eco*RI-Q. No complementation was found.

These results suggest the presence of only one, rather large, *bchA* gene. The 5′ end of *bchA* is calculated to be no more than 825 bp from the *Bam*HI site in *Eco*RI-H, based on genetic mapping data (Yen and Marrs 1976), while the present data suggest the terminal TGA of *bchA* is in *Eco*RI-Q at position 821, thus giving an estimated total length for *bchA* of approximately 4 kp.

The bchC *and* bchA *genes form an operon*

We used the polar properties of Ω to study the arrangement of *bchA* and *C* genes. The Ω interposon was inserted into the chromosome of SB1003 at a point corresponding to the unique *Bam*HI site of the *Eco*RI-H fragment. This was accomplished by GTA-mediated interposon mutagenesis using SB1003 (pDAY16) as the GTA donor. This interposon, in either orientation, terminates RNA and protein synthesis, thus allowing analysis of transcription and translation units. The resulting spectinomycin resistant strain, DE309, revealed a distinct BchA phenotype: lack of photosynthetic growth, lack of Bchl and the accumulation of 2-devinyl,2-hydroxyethyl chlorophyllide *a* absorbing at 665 nm in all cell free extracts.

The *Bam*HI site of the DNA fragment designated as *Eco*RI-H had been previously determined to be within the boundaries of the *bchC* gene, since BchC point mutants mapping to either side of this restriction site are known (*bchC1007*, Taylor et al. 1983; *bchC5109*, Fig. 1A and this work). Further evidence for the location of the *Bam*HI site within the *bchC* gene was obtained in this study, as demonstrated by the observation that plasmid pRPSE30 complemented the *bchC1007* mutation (Table 3) but pDAY41, which differs from pRPSE30 only in a 4 bp addition at the *Bam*HI site, failed to provide complementation. The *bchA* gene product functions prior to the *bchC* gene product in the biosynthetic pathway of Bchl. Under the above circumstances, the phenotype of DE309 suggests the existence of a single operon transcribed in the direction from *bchC* to *bchA*.

An alternative hypothesis to explain the apparent polarity expressed by the interposon in DE309 is that *bchC* and *bchA* are not encoded by an operon, but that the *bchC* gene product is somehow required for *bchA* gene product synthesis or activity. If a lack of *bchC* gene product produced the BchA phenotype, then supplying the *bchC* gene in *trans* should complement the defect in DE309. However, if the interposon in DE309 caused the BchA phenotype by polarity, then only the *bchCA* combination in *trans* would restore Bchl synthesis to DE309. The results of complementation studies with plasmids pRPSE30 and pDAY26 show the latter to be the case. As was shown above, pRPSE30 carries the entire *bchC* gene and only a portion of the *bchA* gene, whereas pDAY26 carries both genes. DE309(pRPSE30) shows the same BchA phenotype as DE309; DE309(pDAY26) is wild type for Bchl synthesis.

Functional location of the bchCA *promoter*

The complementation results described above do not address the question of the presence or absence of a *bchCA* promoter adjacent to the *bchC* gene, since transcription might have been initiated from a promoter located on the vector. To clarify this situation, plasmids were constructed from the parent plasmid pRPSE30 by the insertion of Ω on the upstream side of the *Eco*RI-H fragment (pDAY27) and on the downstream side (pDAY28). When the above three plasmids were mated into the *bchC* mutant MB1007, each yielded complementation. Since Ω serves to prevent transcription and translation readthrough, complementation using pDAY27 revealed the presence of the *bchCA* operon promoter to be within the *Eco*RI-H fragment.

To facilitate the further localization of the *bchCA* promoter within the *Eco*RI-H fragment, plasmids (pDAY20–22) were constructed in three translational reading frames by the fusion of the 1.5 kb *Eco*RI-*Bam*HI fragment from *Eco*RI-H to the *lacZ* gene using Nigel Minton's *lacZ* fusion vectors pNM480, pNM481, and pNM482 (Minton 1984), respectively. Since the *Bam*HI site is within the *bchC* gene, one of these constructs should yield an in-frame *bchC*::*lacZ* fusion. The Ω fragment was then inserted at the *Eco*RI site of all three plasmids to prevent vector promoter readthrough (pDAY23–25); as expected, only one of these final constructs, pDAY23 (Fig. 4), exhibited β-galactosidase activity and therefore constituted the correct fusion frame for translation of *lacZ*.

Preliminary restriction analysis of the *R. capsulatus* DNA in pDAY23 revealed a unique *Sph*I site approximately midway between the *Eco*RI and *Bam*HI sites. Plasmid pDAY40 (Fig. 4) was constructed from the parent plasmid pDAY20 such that the Ω cartridge was inserted into this blunted *Sph*I site. pDAY40 retained β-galactosidase activity, thus locating the promoter to within the *Sph*I-*Bam*HI region.

Plasmid pDAY31 was constructed from the parent plasmid pDAY23 by removal of the small *Sph*I fragment (one site located in Ω, the second site located in the *R. capsulatus* *Eco*RI-*Bam*HI DNA). The new construct retained the spectinomycin resistance and β-galactosidase activity of the parent. The *Sph*I-*Bam*HI fragment of *R. capsulatus* DNA in plasmid pDAY31 was sequenced by both the Maxam-Gilbert and Sanger methods (Fig. 5). A consensus *R. capsula-*

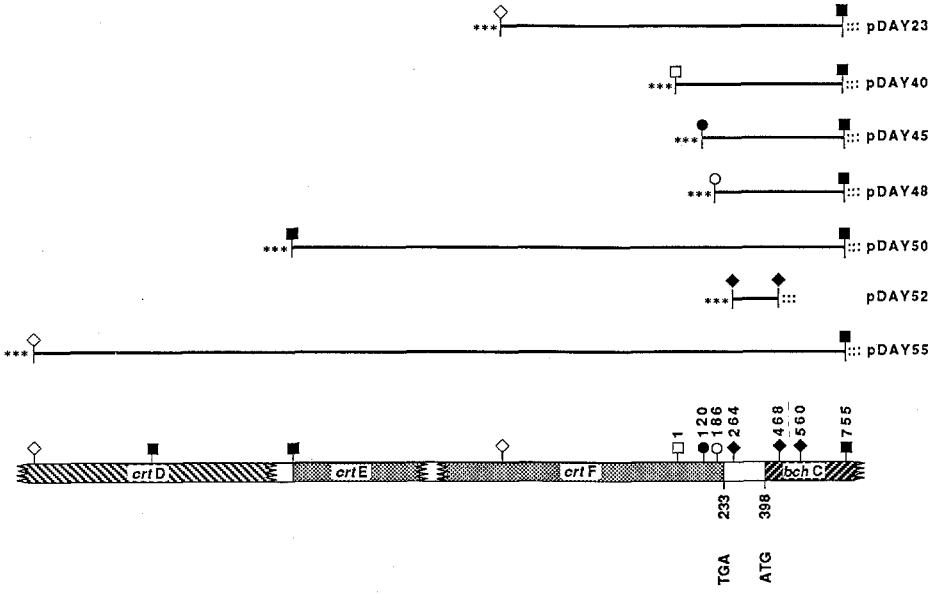

Fig. 4. Plasmid constructs for the analysis of readthrough from *crtEF* into *bchCA* and also the general location of the *bchCA* promoter(s). The *three asterisks* denote the insertion of Ω. ::: denotes the correct translational fusion to the *lacZ* gene. The vector DNA, pNM480, is not indicated. Restriction enzyme sites: *Eco*RI (*open diamond*); *Xma*I (*closed diamond*); *Sph*I (*open box*); *Bam*HI (*closed box*); *Sac*II (*open circle*); and *Mlu*I (*closed circle*)

```
 CC GCC TTG CCG CCG GGC GGG CGT CTG ATC ATC TCG GAG GCG ATG     44
    Arg Leu Ala Pro Gly Gly Arg Leu Ile Ile Ser Glu Ala Met

GCG GGG GGC GCA AAA CCC GAC CGT GCC TGC GAT GTC TAT TTC GCC     89
Ala Gly Gly Ala Lys Pro Asp Arg Ala Cys Asp Val Tyr Phe Ala

TTC TAC ACG ATG GCG ATG AGT TCG GGG CGC ACG CGT TCC CCC GAA   134
Phe Tyr Thr Met Ala Met Ser Ser Gly Arg Thr Arg Ser Pro Glu

GAG ATC AAG CAA ATG CTT GAA AAA GCT GGG TTC ACC AAG GTG TCG   179
Glu Ile Lys Gln Met Leu Glu Lys Ala Gly Phe Thr Lys Val Ser

AAA CCG CGG ACC CTG CGC CCC TTC ATC ACC TCG GTG ATC GAG GCC   240
Lys Pro Arg Thr Leu Arg Pro Phe Ile Thr Ser Val Ile Glu Ala

GAA CGC GGC TGACACGGCTGCGTTCGGACCCGGCTTTGACCCGGGGGTCAGAAAGT  280
Glu Arg Gly End

CGCACATCCGTCTGTCGCAAAAGTGTCTAATCAAATTGACAGTCGGGCGTGTAAGTTCA  337

ATGATACACACAGGCGTGATCAGCCCGACTCTCCGGCCCGATCATACCGGGAGCAAGAA  398

ATG GAA ACG CAA GTC GTC ATA ATG TCC GGG CCC AAG GCC ATC TCG   443
Met Glu Thr Gln Val Val Ile Met Ser Gly Pro Lys Ala Ile Ser

ACG GGC ATC GCC GGT CTG ACC GAC CCC GGG CCG GGG GAC CTC GTC   488
Thr Gly Ile Ala Gly Leu Thr Asp Pro Gly Pro Gly Asp Leu Val

GTG GAT ATC GCC TAT TCC GGC ATT TCG ACT GGC ACC GAG AAA TTG   533
Val Asp Ile Ala Tyr Ser Gly Ile Ser Thr Gly Thr Glu Lys Leu

TTC TGG CTC GGC ACC ATG CCA CCC TTC CCG GGC ATG GGA TAT CCG   578
Phe Trp Leu Gly Thr Met Pro Pro Phe Pro Gly Met Gly Tyr Pro

CTT GTT CCC GGC TAC GAA AGC TTC GGA GAG GTC GTT CAA GCC GCC   623
Leu Val Pro Gly Tyr Glu Ser Phe Gly Glu Val Val Gln Ala Ala

CCC GAC ACC GGC TTC CGA CCG GGC GAT CAC GTC TTC ATT CCC GGC   668
Pro Asp Thr Gly Phe Arg Pro Gly Asp His Val Phe Ile Pro Gly

GCC AAC TGC TTC ACC GGC GGG TTG CGC GGG CTG TTC GGC GGG GCG   713
Ala Asn Cys Phe Thr Gly Gly Leu Arg Gly Leu Phe Gly Gly Ala

TCG AAG CGC CTT GTC ACG GCC GCC TCG CGC GTT TGT CGG CTG GAT   758
Ser Lys Arg Leu Val Thr Ala Ala Ser Arg Val Cys Arg Leu Asp

CC                                                            760
```

Fig. 5. DNA sequence of the junction region of the *crtEF* and *bchCA* operons. The DNA sequence is numbered from the *Sph*I restriction cleavage site (1) in the *crtF* gene through the *Bam*HI restriction cleavage site (760) in the *bchC* gene. Predicted amino acid sequence is given for both gene fragments. The *double solid bar* below the bold DNA sequence denotes the probable Shine-Dalgarno ribosome binding site. The *single solid bar* below the bold DNA sequences indicate potential −35 and −10 promoter sequences

tus Shine-Dalgarno (GGAG) sequence (Youvan and Marrs 1985) was located 6 bp upstream of the ATG start codon of the *bchC* gene. This start site was approximately 400 bp downstream of the *Sph*I site and was also in the reading frame that was proved to be active by the preceding fusion studies. Codon preference analysis supports the interpretation of this open reading frame as the *bchC* gene (Fig. 2B).

Sequence analysis revealed a unique *Mlu*I site 120 bp downstream of the *Sph*I site. Ω was inserted into the *Mlu*I site to produce pDAY45 (Fig. 4). A unique *Sac*II site was located 186 bp downstream of the *Sph*I site. Ω was inserted into the *Sac*II site to produce plasmid pDAY48 (Fig. 4). Both plasmids pDAY45 and pDAY48 express levels of β-galactosidase activity comparable to that of the parent plasmid pDAY23, thus indicating the promoter for the *bchCA* operon to be farther downstream. Two *Xma*I sites (264 bp and 468 bp from *Sph*I, respectively) were present which encompass the ATG start site for the *bchC* gene (398 bp from *Sph*I). This 204 bp *Xma*I fragment was cloned into the *Xma*I cloning site of pNM480 for correct in-frame fusion to the *lacZ* gene. The resulting plasmid construct, pDAY52 (Fig. 4), also expresses β-galactosidase activity. This result indicates the promoter location for the *bchCA* operon to within a 134 bp span (Fig. 4). The oxygen regulation of pDAY52 is approximately comparable to that of the preceding plasmids; however, the overall activity of pDAY52 is about 3-fold greater. This activity difference is most likely to be due to the different fusion sites in the various constructs (Fig. 4).

Location of the crtF *terminus*

Analysis of the codon preference plot (Fig. 2B) suggested a possible gene extending to a point 162 bp upstream of the *bchC* gene start codon. In order to determine whether this additional ORF encodes a gene product, interposon insertion mutagenesis was performed by GTA insertion of Ω into the chromosome of the wild-type host SB1003. Insertion of Ω into the *Sph*I site produced construct DE324; while Ω inserted into the *Mlu*I site produced DE329, and Ω into the *Sac*II site produced DE330 (see Fig. 4). All three strains phenotypically display a muddy coloration characteristic of *crtF* mutations. TLC analysis (Scolnik et al. 1980a) of the carotenoid components of DE324, DE329, DE330, BP503 (*crtF* point mutant), and SB1003 (wild type) confirmed the accumulation of demethylspheroidene and thus the CrtF phenotype of the Ω insertion constructs. We therefore conclude that this ORF is a portion of *crtF*. A search for prokaryotic factor-independent terminators was performed using the "Terminator" program of the Wisconsin Genetics Computer Group Software Package, Version 5 (Brendel and Trifonov 1984a, b). No terminators were discovered in the region shown in Fig. 2B.

Carotenoid transcripts influence bchCA *expression*

Similar to other CrtF mutants, DE324, DE329, and DE330 are capable of photosynthetic growth. Spectral scans under both photosynthetic and low oxygen growth conditions revealed the presence of a peak corresponding to the BchA precursor, 2-devinyl,2-hydroxyethyl chlorophyllide *a*, excreted into the culture media. The amounts of precursor accumulated in the Ω-induced CrtF strains are less by at least a factor of 10 than those seen in BchA strains with mutations located within the *bchCA* operon itself (e.g., DE311). No Bchl precursor was detected in the *crtF* point mutants (PY1291, BP503).

These results suggest that transcripts initiating upstream of the *bchCA* promoter might influence the expression of the *bchCA* operon in vivo. Initial mapping (Scolnik et al. 1980a) had located the *crtE* and *crtF* genes adjacent to the *bchCA* operon. S1 analysis of the carotenoid genes (Giuliano et al. 1988) located two promoters for the *crtE* gene (80 bp and 100 bp, respectively, to the left of the *Bam*HI site in *crtE*; Fig. 4) and no separate promoter was found for the *crtF* gene. Both genes are transcribed in the same directions as the *bchCA* operon (Giuliano et al. 1988).

Two plasmids were constructed to examine the effect of upstream DNA on the transcription of the *bchCA* operon. pDAY50 was generated by adding the intact *crtF* gene and most of *crtE*, minus the two known promoters, to pDAY23 (Fig. 4); while pDAY55 includes all of *crtE* and *crtF* as well as their promoters (Fig. 4). Both new plasmids were mated separately into the *crtF* point mutant BP503. Only plasmid pDAY55 complemented the *crtF* mutant, producing the wild-type phenotype. These data in conjunction with the prior work of Giuliano et al. (1988) support the idea that the *crtE* and *crtF* genes function as an operon.

β-Galactosidase assays of extracts prepared from SB1003 cells containing plasmids pDAY23, pDAY50, and pDAY55 were performed to see if additional upstream DNA influences the expression and/or oxygen regulation of the *bchCA* operon. The results indicate increased expression of the *bchC*::*lacZ* fusion with altered oxygen regulation when the *crtEF* promoters are upstream. Each of the three plasmid-bearing strains was grown under both high oxygen and no oxygen (photosynthetic) conditions and assayed for β-galactosidase activity in seven independent trials. The activity and regulation displayed by plasmids pDAY23 and pDAY50 were comparable to each other but differed from plasmid pDAY55. The functional activity and oxygen regulation for the two *crtEF* promoters was calculated as the difference between values determined for plasmid pDAY55 and those of plasmid pDAY23. Giuliano et al. (1988) using S1 analysis have reported the P_2 promoter to function under aerobic conditions, whereas both P_1

Table 4. Functional strength and oxygen regulation of promoters

Promoter		β-Galactosidase activity[c]		Oxygen regulation
		A[a]	C[b]	C/A
crtEF	P_1**	22.2	63.1	2.8
	P_2**			
bchCA	P_1**	7.5	37.2	5.0
puf	P_1*	60.0	1758.0	29.3
	P_2*	48.0	58.0	1.2

[a] Cells grown in the dark under high oxygen growth conditions as described in the Materials and methods

[b] Cells grown under photosynthetic conditions as described in the Materials and methods

[c] Units are expressed as μmol ONPG hydrolyzed/min per mg protein

* Data from Bauer et al. 1988 (*pufM*::*lacZ* translational fusions)

** Data based on the average of seven independent assays (*bchC*::*lacZ* translational fusions)

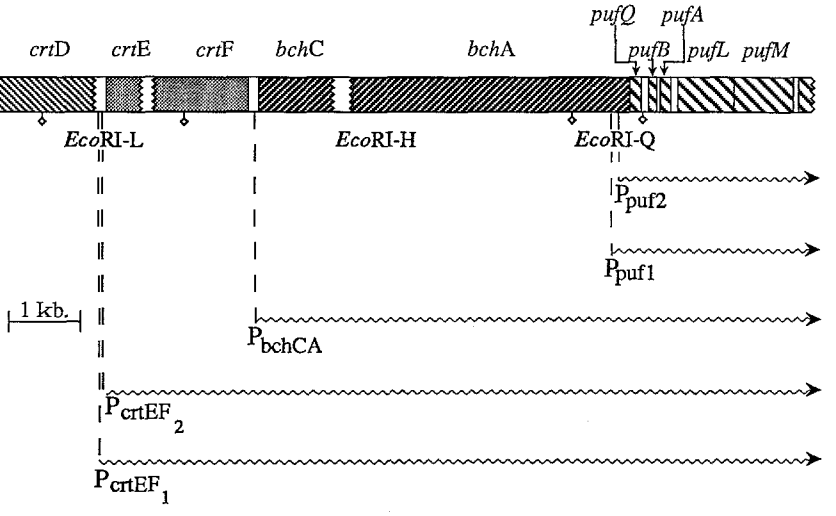

Fig. 6. Genetic map of *Rhodobacter capsulatus* involving superoperonal organization of genes. Genes belonging to the same operon are shaded identically. Restriction site: *Eco*RI (*diamond*); P indicates promoter

and P_2 promoters function under anaerobic conditions. The distinct activity and regulation of each separate carotenoid promoter was not differentiated by our assay method.

The functional activity and oxygen regulation of the combined *crtEF* P_1 and P_2 promoters and the *bchCA* promoter as determined in this work (via the translational fusion of *bchC* to *lacZ*) were compared to the identical parameters calculated for the independently assessed *puf* P_1 and P_2 promoters (via the translational fusion of *pufM* to *lacZ*). These data are brought together in Table 4, although since different β-galactosidase fusion proteins may have different specific activities, we may only compare specific activities among experiments involving the same fusion protein. Each promoter has a distinct response to the presence of oxygen and different promoter strengths are evident.

Discussion

We used DNA sequence analysis, interposon mutagenesis, and complementation studies to examine the interrelationship of three adjacent operons within the *R. capsulatus* chromosomal cluster of genes functioning in the photosynthetic process. All three operons, *crtEF, bchCA*, and *puf* are transcribed in the same direction.

Two genes involved in bacteriochlorophyll biosynthesis, *bchC* and *bchA*, are shown here to comprise an operon with transcription proceeding from the *bchC* gene through the *bchA* gene. Zsebo and Hearst (1984) found that an interposon in *Eco*RI-Q caused the BchA phenotype, and since Taylor et al. (1983) had in effect reported that the entire *bchA* gene was on the adjacent *Eco*RI-H fragment, Zsebo and Hearst proposed that they had disrupted the *bchA* promoter. Since the *bchA* gene in fact extends into *Eco*RI-Q, the observation of Zsebo and Hearst (but not their interpretation) is consistent with the description of *bchCA* presented in Fig. 6. A 134 bp region of DNA upstream of *bchC* was shown to contain the promoter function for this operon.

Since the *bchA* gene appeared to be unusually long (4 kp), we tested the hypothesis that there might be more than one gene in this region that could give rise to the BchA phenotype. Our complementation results (Fig. 2A and Table 4) clearly show that there is only one *bchA*-like gene in this region. The phenotypes of strains with interposons near the end of the *bchA* are complicated and warrant further discussion. Insertion of Ω into the *Nco*I site (799) 24 bp from the 3′ end of this gene does not appear to interfere with the function of the resulting *bchA* gene product, which is expected to be truncated by only 8 amino acids (Fig. 3, panel D). Insertion of Ω at the *Xma*III site (724) on the chromosome results in a phenotype that superficially resembles DE331; addition of *pufQ* to this strain (DE335) does not result in an accumulation of mature Bchl, but rather an accumulation of P665, the precursor characteristic of *bchA* mutants (Fig. 3, panel B). Therefore, we suggest that interruption of *bchA* at this point, about 32 amino acid residues from the carboxy-terminal end, disrupts a region necessary for the *bchA* gene product to respond to the *pufQ* gene product.

One base pair separates the end of the *bchA* gene from the first gene of the following *puf* operon, *pufQ*. We could find no evidence in *R. capsulatus* for the existence of an *R* gene like that proposed by Kiley and Kaplan (1988) for *R. sphaeroides*. We paid special attention to the *bchA* sequences near the *puf* operon, since that region corresponds to the region of *R. sphaeroides* chromosome in which the *R* gene was reported to map. The codon preference plot of this region shows clusters of rare codons in all three reading frames about 150 bases from the *Eco*RI site (Fig. 2A), apparently interrupting the ORF that we have identified as *bchA*. In the absence of other data, this might suggest that the region of good codon usage starting about 175 bp downstream of the *Eco*RI site is an independent gene. The results reported here, however, rule out such an interpretation, since a plasmid carrying all of the *bchA* gene sequences with Ω inserted in this hypothetical gene (pDAY60) fails to complement a point mutation in the portion of *bchA* located proximally to the promoter (*bchA305*).

Both promoters for the *puf* operon are located within the *bchA* gene. This region of DNA thus serves a dual function, both as part of the structural gene coding for the *bchA* polypeptide and also as the initiation sites for transcription of the downstream *puf* operon. We conclude from both sequence data and functional analysis that the transcripts from the *bchCA* operon cannot be terminated before entering the *puf* operon, and therefore these operons "overlap".

Two genes involved in carotenoid biosynthesis, *crtE* and

crtF, are also shown to comprise an operon. A region of 147 bp separates the end of the *crtF* gene from the start of the *bchC* gene, the first gene in the subsequent *bchCA* operon. Interposon mutagenesis of the chromosomal *crtF* gene revealed the accumulation of small amounts of BchA precursor, although all strains remain photosynthetically competent. Addition of the entire *crtEF* operon to plasmid vectors expressing *bchC*::*lacZ* translational fusions produces a clear increase in β-galactosidase activity and a change in the pattern of response to oxygen. These results, in conjunction with the absence of identifiable terminator sequences within the 147 bp junction between these two operons, clearly provide evidence for readthrough from the *crtEF* operon through the *bchCA* operon.

We conclude from this analysis that *crtEF, bchCA*, and *puf* are cotranscribable (Fig. 6). Transcription may begin at the initiation sites in any of these three operons and continue through the downstream operons. This is an unusual gene arrangement for a bacterial chromosome. The promoters servicing this region differ dramatically in sequence structure, regulation by oxygen, and functional strength. The P_{puf1} promoter has sequence homology with the nif-like or σ^{60} promoters of other organisms (Bauer et al. 1988), whereas the promoter region of the *bchCA* operon has no such homology. We find instead a sequence very much like the σ^{70} consensus (see bases 316 through 342, Fig. 5). If P_{bchCA} is indeed recognized by a different polymerase than P_{puf1} and P_{puf2}, it might provide a rationale for the overlapping structure of these operons; i.e., P_{bchCA} could promote a low level expression of the *puf* operon under conditions that would not permit P_{puf1} or P_{puf2} to function. Low level expression of the *puf* operon, via either P_{bchCA} or P_{puf2} might confer a selective advantage to the host during a rapid transition from aerobic to anaerobic illuminated conditions.

Superoperons, or supraoperonic clusters, as described by Wheelis (1975) for *Pseudomonas* sp., have been found among chromosomal genes with catabolic functions. Evolutionary forces cited as contributing to superoperonic clustering include the facilitation of transfer of a complete package of genes necessary to express a new trait (Wheelis 1975) and/or the involvement of physical proximity in the stability or regulation of the genes (Neidle et al. 1987). Many genes functioning in the photosynthetic process in *R. capsulatus* are clustered in one region comprising less than 1.5% of the entire chromosome, and this arrangement is therefore an example of a superoperon for which we propose the name *BPScaps* (*b*acterial *p*hoto*s*ynthesis from R. *caps*ulatus). *BPScaps* is presumed to be sufficient to convey the ability to photosynthesize to a hypothetical nonphotosynthetic recipient, which could both express the information received and in addition interface indigenous cellular systems with the new proteins produced. The genes of *BPScaps* extend tetrapyrrole synthesis from the heme precursor protoporphyrin IX to bacteriochlorophyll, extend isoprenoid metabolism from the polyprenyl alcohol precursors to carotenoids, and encode the structural proteins needed to bind these pigments in a functional photosynthetic unit. Although it is attractive to hypothesize that *BPScaps* could convey the ability to photosynthesize to nonphotosynthetic recipients, such transfer has not to our knowledge been demonstrated. This is despite attempts to do so using promiscuous R-primes, like pRPS404, which carry *BPScaps* (Marrs 1981). These failed attempts do not prove that transfer of photosynthetic ability cannot occur via *BPScaps*, but they do suggest that such transfer is not a high probability event. The results reported in the present work, in contrast, represent a clear demonstration of regulatory interactions between adjacent operons within a superoperonal cluster. While we may not fully understand the utility of the transcriptional linkage of the *crtEF, bchCA*, and *puf* operons, the genetic evidence clearly indicates that significant readthrough occurs and that the superoperonal structure is necessary for such readthrough.

Acknowledgements. We thank J.T. Beatty, C.L. Wellington, Neil Hunter, and Mark Ashby for valuable discussions. We especially thank T. Reed for technical assistance and graphic presentation.

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Communicated by H. Hennecke

Received December 12, 1988