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Overlapping mRNA Transcripts of Photosynthesis Gene Operons in *Rhodobacter capsulatus*

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The *crtEF*, *bchCA*, and *puf* operons of the facultative phototrophic bacterium *Rhodobacter capsulatus* encode gene products that are necessary for the formation of various components of the photosynthetic apparatus. The *crtEF* operon encodes two enzymes involved in the biosynthesis of carotenoids, the *bchCA* operon codes for two enzymes of the bacteriochlorophyll biosynthetic pathway, and the *puf* operon encodes four pigment-binding polypeptides as well as two polypeptides with less well understood functions. These three operons are adjacent to one another on the chromosome and are transcribed in the same direction. We present the results of RNA blotting and S1 nuclease protection end-mapping experiments which provide direct evidence that the mRNA transcripts of these three operons overlap. Therefore, it is likely that the *crtEF*, *bchCA*, and *puf* operons can be expressed as a single transcriptional unit, although RNA polymerase may initiate transcription at any of several promoters.

Many species of purple, nonsulfur bacteria are able to generate the energy required for growth by either aerobic respiration or anaerobic photosynthesis (see references 13, 21, and 32 for reviews). Photosynthetic growth is dependent on the synthesis of a specialized intracytoplasmic membrane system that is induced in response to a decrease in the external oxygen tension below a threshold level (11). The intracytoplasmic membrane contains all of the components necessary and specific for photosynthetic growth, which in the species *Rhodobacter capsulatus* include two light-harvesting (LH) complexes (LHI [B870] and LHII [B800-850]), which are the primary sites of photon capture, and the reaction center (RC) complex, which is the site of primary photochemical charge separation. Each of these complexes has bound to it the photosynthetic pigments bacteriochlorophyll (bchl) *a* and carotenoids. Although mutants deficient in RCs cannot grow phototrophically, the LH complexes are not required for photosynthetic growth if the incident light intensity is adequate (9, 33).

R. capsulatus cells must coordinate the expression of approximately 30 known genes (1–3, 34, 36, 37, 42, 44, 45) in order to satisfy the pigment-protein stoichiometry required by each complex in the formation of a functional photosynthetic apparatus. Cells also must regulate the expression of these genes in a manner sufficiently flexible to allow for adaptation to transient changes in environmental conditions (22, 27, 30, 31, 46). It is noteworthy that all genes that encode products essential solely for photosynthetic growth have been found to be clustered in a 50-kb region of the *R. capsulatus* chromosome, where genes that code for enzymes of the bchl and carotenoid biosynthetic pathways are flanked by genes that encode the structural polypeptides of the RC and B870 complexes (36).

In this study, we investigated the transcriptional relationships between three adjacent operons of the *R. capsulatus* photosynthesis gene cluster (Fig. 1). The *crtEF* operon encodes two enzymes of the carotenoid biosynthetic pathway (2, 16), and the *bchCA* operon encodes enzymes that catalyze two steps of the bchl biosynthetic pathway (8, 40, 43). Although the *bchA* region seems to contain one cistron (43), recent DNA sequence analysis of the *bchA* region has shown that three open reading frames can be found within the *bchA* region (17a). We have illustrated the *bchA* region as one gene in the figures of this report. The *puf* operon encodes a gene (*pufQ*) whose product is necessary for the biosynthesis of bchl *a* but is itself not a bchl biosynthetic enzyme (3), the α and β polypeptides of the B870 complex, the L and M polypeptides of the RC complex (44), and one gene (*pufX*) that is essential for photosynthetic growth in a minimal medium but whose specific function is presently under investigation (14, 23a). Earlier studies have shown that these three operons are adjacent to one another and are transcribed in the same direction, from *crtEF* toward *pufQBALMX* (2, 16, 36, 40, 42–44).

Recently, Young et al. (43) showed that the level of expression of the *bchCA* operon is influenced by transcription of the *crtEF* operon and demonstrated that the *puf* operon promoter region is located within a *bchA* structural gene. These authors proposed a model in which these three adjacent operons are cotranscribed and suggested that the expression of an individual operon is affected by its chromosomal position. In a previous publication, we reported evidence which suggested that the mRNA transcripts of the *crtEF* operon may extend into the *bchC* coding region (40). In this report, we confirm and extend these previous studies by presenting direct evidence from RNA blotting and end-mapping experiments that transcripts of the *crtEF*, *bchCA*, and *puf* operons overlap and that perturbation of read-through transcription alters the oxygen-dependent accumulation of mRNA molecules.

MATERIALS AND METHODS

Bacterial strains and plasmids. Each of the *R. capsulatus* strains used in this study has been described: the wild-type strain *R. capsulatus* B10 (39), *R. capsulatus* SB1003 (35), the *bchC* interposon mutant strain *R. capsulatus* CW100 (40),

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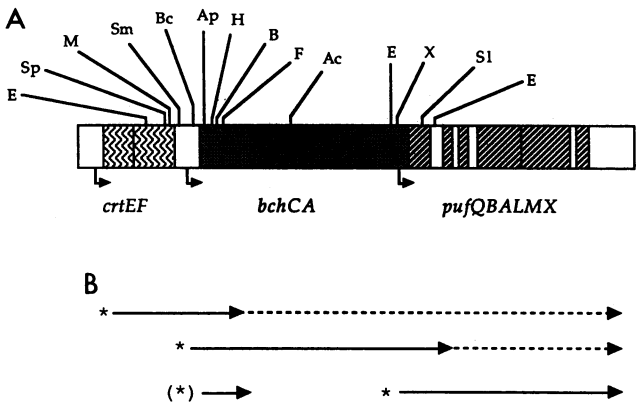

FIG. 1. Restriction site map and model of the overlapping transcripts of the *R. capsulatus crtEF*, *bchCA*, and *puf* operons. (A) *R. capsulatus* chromosomal DNA encoding the *crtEF* operon, the *bchCA* operon, and the *puf* operon, as indicated. Bent arrows designate the locations of and direction of transcription initiated from the promoters of these three operons. Relevant restriction sites: *Eco*RI (E), *Sph*I (Sp), *Mlu*I (M), *Sma*I (Sm), *Bcl*I (Bc), *Apa*I (Ap), *Hin*dIII (H), *Bam*HI (B), *Fsp*I (F), *Acc*I (Ac), *Xho*II (X), and *Sal*I (Sl). The horizontal arrows in panel B represent the mRNA transcripts of these three operons. Solid lines represent authentic transcripts as have been shown by this and other work (see text); dashed lines represent mRNA molecules that have not been identified directly but are likely to exist. The 5′ ends of primary transcripts are indicated by asterisks.

and the *crtF* interposon mutant strain *R. capsulatus* DE324 (43).

The construction of plasmid pCW2 has been described previously (40). Briefly, plasmid pCW2 consists of the 13-kb *Bam*HI-C fragment of the R′ plasmid pRPS404 (36) inserted into the expression plasmid pJAJ9 (20) in an orientation that results in transcription of most of the *bchCA* operon and the entire *puf* operon from the *puf* promoter present on pJAJ9.

Each of plasmids pJP1, pJP100, and pJP101 contains an in-frame fusion of the *bchC* gene to the eighth codon of the *Escherichia coli lac′Z* gene, but each contains a different amount of *R. capsulatus* DNA 5′ to the *bchC* coding region. These plasmids were constructed as follows. The *Eco*RI-H fragment of pRPS404 contains the 3′ terminus of the *crtF* gene, the entire *bchC* gene, and all but the last 821 nucleotides (nt) of the *bchA* gene (2, 43). The 1.5-kb *Eco*RI-to-*Bam*HI segment of the *Eco*RI-H fragment, containing the 3′ terminus of the *crtF* gene and approximately 50% of the *bchC* gene, was purified and inserted into both pUC13 and pBR322, each of which had been digested with *Eco*RI and *Bam*HI, creating plasmids pUC13::EB and pBR322:EB, respectively. To construct pJP1, the entire *R. capsulatus* segment was excised from pBR322:EB as a *Pst*I-to-*Bam*HI fragment, purified by gel electrophoresis, and inserted into the mobilizable promoter fusion vector pXCA601 (1) that had been cut with *Pst*I and *Bam*HI. To construct pJP100, the 0.49-kb *Sma*I-to-*Bam*HI portion of the *R. capsulatus* insert into pUC13::EB was removed, purified, and inserted into pUC13 that had been cut with *Hin*cII and *Bam*HI to create plasmid pUC13::SB. This 0.49-kb *R. capsulatus* segment was then excised from pUC13::SB as a *Pst*I-to-*Bam*HI fragment, purified, and inserted into pXCA601 that had been cut with *Pst*I and *Bam*HI. Similarly, the 0.40-kb *Bcl*I-to-*Bam*HI segment of the *R. capsulatus* insert in pUC13::EB was removed, inserted into pUC13 cut with *Bam*HI to create pUC13::BcB, excised from pUC13::BcB as a *Pst*I-to-*Bam*HI fragment, and inserted into pXCA601 that had been cut with *Pst*I and *Bam*HI, yielding plasmid pJP101. Vectors containing *R. capsulatus* in-frame fusions to the *lac′Z* gene were mobilized by conjugation into *R. capsulatus* as described previously (29).

Growth conditions and measurement of β-galactosidase specific activity. Plasmid-containing cells were grown at 34°C in RCV medium (6) supplemented with 0.1% yeast extract, 8.0 mM potassium phosphate buffer (pH 6.8), and 0.5 μg of tetracycline per ml. The specific activities of β-galactosidase in extracts of *R. capsulatus* cells containing recombinant *lac′Z* fusion plasmids were assayed as previously described (26, 40). Values were normalized to the protein content of the extracts as determined by a Lowry protein assay (24), using bovine serum albumin as a standard. The values presented are the averages of at least four independent assays of each fusion construct.

Isolation of *R. capsulatus* RNA. Two standard conditions were used for growth of cultures of *R. capsulatus* cells for RNA isolation, depending on whether the entire culture was harvested at a single time point or whether samples of the culture were harvested at various time points. For single-time-point RNA isolations, cultures of *R. capsulatus* cells were grown at 34°C in RCV medium with high aeration (40 ml of culture in a 500-ml Erlenmeyer flask shaken at 300 rpm) to a density of 4×10^8 CFU/ml and then shifted to low aeration by transfer of the entire culture to a 50-ml flask that was shaken at 150 rpm to stimulate synthesis of mRNA from photosynthesis genes. Induced cells were collected at 30 min after the shift to low aeration by centrifugation through an ice slurry, and RNA was isolated as previously described (38).

For multiple-time-point RNA extractions, care was taken to ensure that the aeration of the culture did not change appreciably during removal of the samples. This was achieved by growing 400 ml of culture with high aeration (divided equally between two 2-liter flasks that were shaken at 300 rpm) to a density of 4×10^8 CFU/ml and then combining these cultures into a single 500-ml flask that was shaken at 150 rpm. At the time of the shift and at 10- or 15-min intervals for 60 min past the shift, 10- and 12-ml samples, respectively, were removed from the culture and cells were pelleted through an ice slurry. Because the total volume of culture removed from the 500-ml flask during the time course was ≤60 ml (15% of the total volume), the samples were assumed to be under relatively continuous oxygen limitation, although there could be a decrease in oxygen available to cells during the course of the experiment.

RNA electrophoresis, blotting, and preparation of probes. Gel electrophoresis of RNA samples was done as described previously (25). Each lane of the 1% agarose–2.2 M formaldehyde gels used for RNA blots contained 10 μg of *R. capsulatus* RNA. Following electrophoresis, the gels were equilibrated in 0.5× TBE buffer, and the RNA was transferred electrophoretically to a BioTrans (ICN Biomedicals, Inc.) nylon membrane at 30 V for 16 h in 0.5× TBE buffer. RNA size markers (0.3 to 9.5 kb) were purchased from Bethesda Research Laboratories.

Radioactive probes for RNA blots were labeled to an average specific activity of 10^7 cpm/μg by the method of primer extension, using random hexadeoxynucleotide primers as described previously (15). Conditions for the prehybridization, hybridization, and washing of blots have been described previously (5).

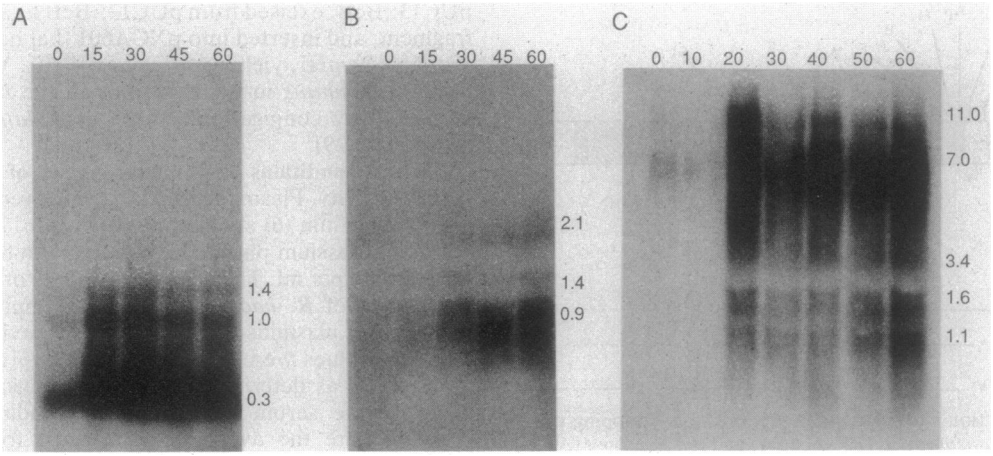

FIG. 2. Blots of *R. capsulatus* B10 and *R. capsulatus* B10(pCW2) RNAs. (A and B) Autoradiograms of blots of *R. capsulatus* B10 RNA isolated at the indicated times in minutes following a shift of the culture to low aeration; (C) autoradiogram of a blot of *R. capsulatus* B10(pCW2) RNA isolated at the indicated times following a shift of the culture from high to low aeration. In panel A, the 0.3-kb *Apa*I-*Bam*HI fragment of the *bchC* gene (Fig. 1A) was uniformly radioactively labeled and used as a probe. In panels B and C, the 2.0-kb *Acc*I-*Eco*RI fragment of the *bchA* region (Fig. 1A) was radioactively labeled and used as a probe. The sizes of apparent bands are given in kilobases on the right and were calculated by comparison with an RNA size ladder.

S1 nuclease protection end mapping and preparation of probes. Most of the S1 nuclease protection end-mapping experiments described in this report made use of bipartite DNA probes consisting of a region of *R. capsulatus* DNA expected to overlap an mRNA end, plus a tail of heterologous (vector) DNA extending from the unlabeled end of the probes. Because this design allows distinction on the basis of size between protection of the entire *R. capsulatus*-derived segment of the probe by an mRNA molecule whose end lies beyond the boundary of the probe and complete protection of the probe by DNA-DNA reannealing, it can be used to detect readthrough mRNA transcripts that result in overlapping mRNA molecules.

The bipartite probes used in most of the S1 nuclease protection experiments were prepared by digestion of a recombinant plasmid containing an *R. capsulatus* fragment of interest at a restriction endonuclease site located within the *R. capsulatus* segment of the plasmid. The ends thus generated were radioactively labeled to a minimal specific activity of 10^6 cpm/μg at either the 5′ or 3′ ends by using standard protocols (25). Following digestion of the labeled plasmid with a restriction endonuclease that cut in the vector rather than at the vector-*R. capsulatus* junction, the probe was purified by gel electrophoresis.

Between 50 and 400 ng of labeled probe was ethanol precipitated with 10 μg of *R. capsulatus* RNA for each sample used in the S1 nuclease protection experiments. Samples were resuspended, denatured, hybridized, and digested as described previously (46) except that hybridizations were performed at 53°C for 3 h. S1 nuclease (Bethesda Research Laboratories) was used at the concentrations specified in the figure legends. Single-stranded *Hae*III-digested M13mp11 phage DNA fragments that were radioactively labeled at the 5′ ends served as size markers.

RESULTS

RNA blot analysis of *bchCA* mRNA transcripts. Although several studies have provided genetic evidence that the *bchC* and *bchA* genes form an operon by analyses of mutations in the *bchC* gene that are polar upon the *bchA* region (8, 40, 43), the existence of an mRNA molecule that encodes these genes has not yet been demonstrated. Recent DNA sequence analyses of the *bchC* gene (40) and the *bchA* region (17a, 43) indicate that the minimum length of such a transcript would be approximately 5.5 kb. To determine whether such an mRNA transcript could be detected in *R. capsulatus* cells and to test for increased levels in response to decreased oxygen concentrations (indicative of transcription initiation at the *bchCA* promoter; see below), RNA was isolated from cultures of *R. capsulatus* B10 at intervals after the cultures were shifted from high to low aeration as outlined in Materials and Methods. Because preliminary experiments with RNA isolated from *R. capsulatus* B10 indicated that *bchCA* mRNA was difficult to detect on an RNA blot (presumably because of its low amounts), we decided to enhance the signal by expression of additional plasmid-borne copies of the *bchCA* region in *R. capsulatus* B10 cells. Plasmid pCW2 (40) contains the *Bam*HI-C fragment (containing the 3′ terminus of the *bchC* gene, the entire *bchA* region, and the entire *puf* operon) of pRPS404 (36) inserted into the mobilizable expression vector pJAJ9. Although the promoter region and 5′ segment of the *bchC* gene are absent in pCW2, transcription of the 3′ segment of the *bchC* gene and the *bchA* region is initiated on the vector by the strong, oxygen-regulated *puf* operon promoter (20).

Equal amounts of RNA isolated at each time point were fractionated by gel electrophoresis and transferred electrophoretically to a nylon membrane. In independent experiments, *R. capsulatus* RNA [from B10 or B10(pCW2) and immobilized separately on membranes] was hybridized with one of two *bchCA* probes. The resultant autoradiograms (Fig. 2) showed that mRNA complementary to these probes was detectable in cells of cultures grown with high aeration, but increased and reached maximum levels approximately 20 min after transcription of the photosynthesis genes was induced by shifting the culture conditions to low aeration.

When B10 RNA was hybridized with a *bchC*-specific probe (consisting of the radioactively labeled 0.3-kb *Apa*I-*Bam*HI fragment of the *bchC* gene; Fig. 1), it detected high levels of an mRNA species approximately 0.33 kb in length

and lower levels of longer transcripts that ranged up to about 1.4 kb.

In other experiments a *bchA*-specific probe, consisting of the radioactively labeled 2.0-kb *Acc*I-*Eco*RI fragment of the *bchA* region (Fig. 1), was used. This probe annealed to mRNA molecules from strain B10 ranging in length from approximately 0.4 to 2.1 kb but not to the predominant 0.33-kb species detected with the *bchC*-specific probe (Fig. 2A and B). The signal obtained by the *bchA*-specific probe on the blot of *R. capsulatus* B10(pCW2) RNA (Fig. 2C) represents the sum of mRNA molecules initiated from the chromosomal *bchCA* promoter and the plasmid-borne *puf* promoter and included sizes as large as approximately 11 kb.

Although the 11-kb mRNA species detected with the *bchA*-specific probe would be long enough to encode the entire *bchCA* operon as well as flanking sequences, it was detected by the technique of RNA blotting only when extra plasmid-borne gene copies were present. Because of the difficulties in determining from these experiments which flanking sequences were present, we next decided to map the ends of the *bchCA* mRNA transcripts relative to those of the *crtEF* and *puf* operons by the more sensitive method of S1 nuclease protection end mapping.

5′ end mapping of *bchCA* mRNA transcripts. Two probes were designed to position the 5′ ends of *bchCA* operon transcripts. One probe was radioactively 5′ end labeled at the *Hin*dIII site in the *bchC* gene, extended 476 bp in the 5′ direction to the *Mlu*I site in the *crtF* gene, and included 181 bp of pUC13 vector DNA as a tail (Fig. 3A). The other probe was 5′ end labeled at the *Bam*HI site in the *bchC* gene, extended 635 bp in the 5′ direction to the *Mlu*I site in the *crtF* gene, and included 209 bp of pUC13 vector DNA as a tail (Fig. 3A). Thus, transcripts that began on the *R. capsulatus* chromosome upstream of the *Mlu*I site and extend into the coding region of the *bchC* gene would produce a band 476 nt in length with the *Hin*dIII probe and 635 nt in length with the *Bam*HI probe. The results of an S1 nuclease protection experiment using these two probes and *R. capsulatus* B10 RNA (Fig. 3B) showed that the 657-nt band for the *Hin*dIII probe and the 844-nt band for the *Bam*HI probe, which were due to reannealing of each bipartite probe, were present both in the control lanes containing yeast tRNA and in the experimental lanes containing *R. capsulatus* RNA. The entire *R. capsulatus*-derived segment of both probes was protected by mRNA originating from upstream of the *Mlu*I site, as was shown by the presence of a band 476 nt in length for the *Hin*dIII probe and a band 635 nt in length for the *Bam*HI probe. Three minor 5′ ends were detected by both probes, positioned approximately 475, 445, and 390 nt upstream of the *Hin*dIII site, corresponding to the minor ends that mapped approximately 640, 620, and 550 nt upstream of the *Bam*HI site (the 640- and 620-nt bands are not well resolved in Fig. 3). These minor 5′ ends, therefore, would be located approximately 280, 255, and 193 nt upstream of the ATG initiation codon of the *bchC* gene (40). The predominant 5′ end, which mapped approximately 250 nt upstream of the *Hin*dIII site and approximately 405 nt upstream of the *Bam*HI site, was identical to the previously positioned 5′ end located 48 nt upstream of the ATG initiation codon of the *bchC* gene (40). Bands indicative of 5′ mRNA ends less than 250 nt from the *Hin*dIII site and less than 405 nt from the *Bam*HI site may have arisen from decay intermediates of mRNA encoding the BchC and BchA enzymes.

Detection of readthrough transcripts capable of protecting the entire *R. capsulatus* segments of both probes suggested that the *bchCA* operon could be transcribed from more than one promoter, at least one of which is located upstream of the *Mlu*I site in the *crtF* gene, and that some *crtEF* transcripts extend into the *bchCA* operon at least as far as the *Bam*HI site in the *bchC* gene.

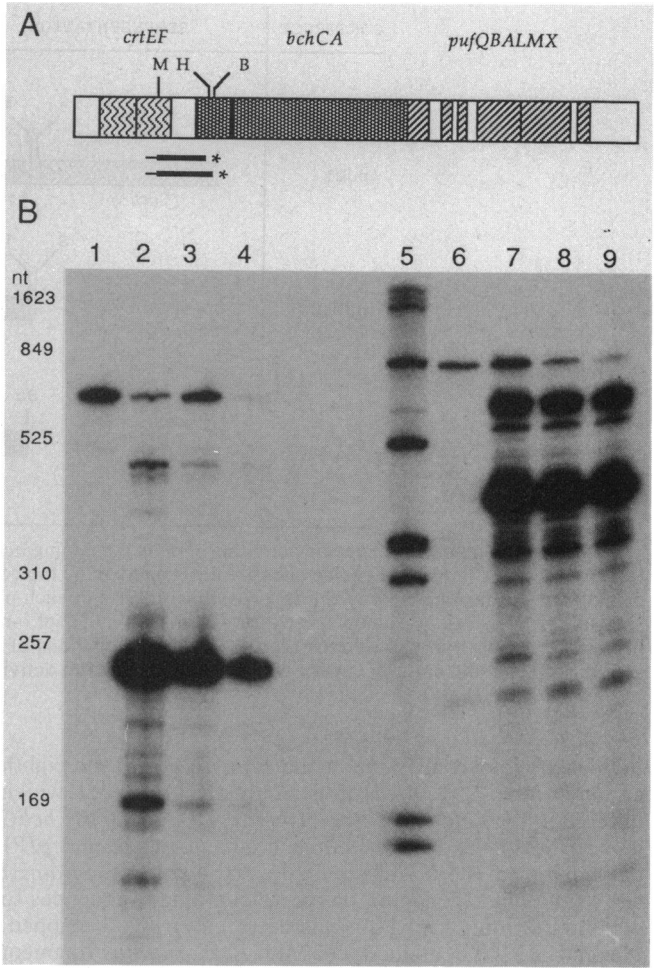

FIG. 3. S1 nuclease protection 5′ end mapping of *bchCA* mRNA transcripts. (A) Representations of the double-stranded bipartite DNA probes that were 5′ end labeled (*) at either the *Hin*dIII (H) or *Bam*HI (B) site in the *bchC* gene. Both probes contain *R. capsulatus* DNA (thick lines) extending to the *Mlu*I (M) site in the *crtF* gene and a tail of pUC13 DNA (thin lines). (B) Autoradiogram of a 5% polyacrylamide gel containing 8 M urea through which denatured, S1 nuclease-resistant hybrids were separated. Lanes: 1 to 4, S1-resistant fragments protected by the *Hin*dIII probe; 5, *Hae*III-digested M13mp11 single-stranded DNA size markers, with the sizes of selected bands shown on the left; 6 to 9, S1-resistant fragments protected by the *Bam*HI probe. Lanes 1 and 6 contain 150 ng of probe hybridized with 10 μg of yeast tRNA and digested with 500 U of S1 nuclease; lanes 2 and 7, 3 and 8, and 4 and 9 contain 150 ng of probe hybridized with 10 μg of *R. capsulatus* B10 RNA isolated 30 min following a shift of the culture to low aeration, which were digested with 500, 1,000, and 1,500 U of S1 nuclease, respectively.

Localization of sequences necessary for *bchCA* promoter activity. Because multiple 5′ mRNA ends were detected upstream of the *bchC* coding region, we wished to determine which of these ends mapped to sequences that were associated with promoter activity. These sequences were determined by analysis of three plasmid-borne *bchC*::*lac′Z* fusions, each of which contained the same 5′ part of the

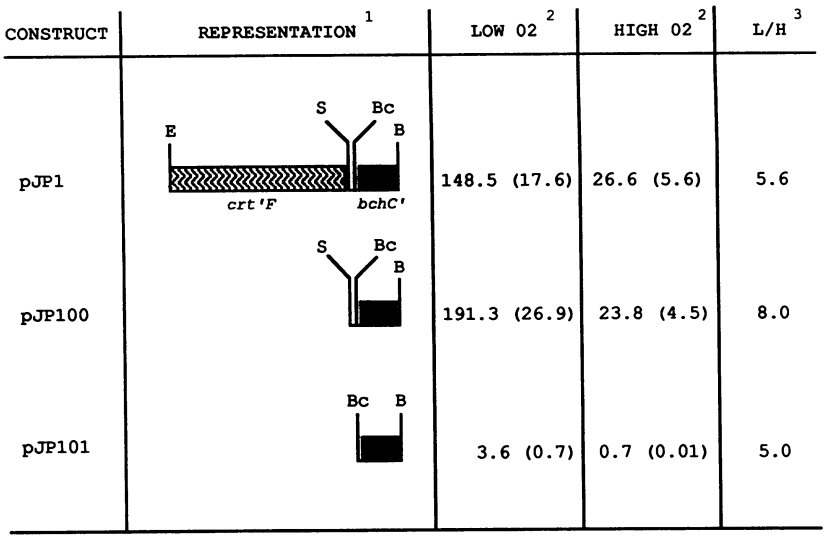

FIG. 4. β-Galactosidase specific activities of cells containing *bchC*::*lac'Z* 5′ deletion constructs. [1]Representation of *R. capsulatus* DNA fused to the *lac'Z* gene. Restriction sites are designated in the top construct as *Eco*RI (E), *Sma*I (S), *Bcl*I (Bc), and *Bam*HI (B). The segments of structural genes encoded by the *R. capsulatus* inserts in each plasmid are shown and are labeled in the top construct. [2]β-Galactosidase specific activities (mean values) are expressed as nanomoles of *o*-nitrophenyl-β-D-galactoside (ONPG) cleaved per minute per milligram of protein. The values in parentheses are the standard deviations of four or more independent assays. [3]The ratio of β-galactosidase specific activities obtained from cells grown with low aeration (L) to activities from cells grown with high aeration (H).

bchC gene joined in frame at the *Bam*HI site to the eighth codon of the *E. coli lac'Z* gene in pXCA601 but which differed in the amount of *R. capsulatus* DNA 5′ to the *bchC* gene. The 1.5-kb *R. capsulatus* fragment in plasmid pJP1 extended from the *Eco*RI site in the *crtF* gene to the *Bam*HI site in the *bchC* gene and therefore contained sequences to which all of the 5′ mRNA ends detected in Fig. 3 mapped. Plasmid pJP100 contained a 0.49-kb *R. capsulatus* fragment that extended from the *Sma*I site between the *crtF* and *bchC* coding regions to the *Bam*HI site in the *bchC* gene and therefore contained sequences to which only the major and shorter minor 5′ ends mapped. Finally, the 0.40-kb *R. capsulatus* segment in plasmid pJP101 extended from the *Bcl*I site between the *crtF* and *bchC* coding regions to the *Bam*HI site in the *bchC* gene and therefore contained only sequences to which the shorter minor 5′ ends mapped. The β-galactosidase specific activities in extracts of *R. capsulatus* cells harboring each of these plasmids were determined. The results (Fig. 4) showed that the β-galactosidase specific activities of pJP1 and pJP100 were similar, which suggested that the *bchCA* promoter is located downstream of the *Sma*I site between the *crtF* and *bchC* genes and that the longer minor 5′ ends observed in Fig. 3 were due to posttranscriptional cleavage of mRNA molecules. Because deletion of the *R. capsulatus* segment between the *Sma*I and *Bcl*I sites to form plasmid pJP101 resulted in a drastic reduction of β-galactosidase specific activity, these sequences must be important for promoter function. The major 5′ end in Fig. 3 was the only 5′ end that mapped between the *Sma*I and *Bcl*I sites in the *crtF-bchC* intergenic region.

3′ end mapping of *crtEF* mRNA transcripts. If transcription from the *crtEF* operon extends into the *bchCA* operon at least as far as the *Bam*HI site in the *bchC* gene (as indicated by the 5′-end-mapping results), then 3′-end-mapping experiments on the *crtEF* operon transcripts should show the existence of molecules that extend past the *Hin*dIII and *Bam*HI sites in the *bchC* gene. The two probes designed to test this hypothesis were similar to the two used for the 5′-end-mapping experiments described above. Both probes were 3′ end labeled at the *Mlu*I site in the *crtF* gene. One probe extended 476 bp in the 3′ direction to the *Hin*dIII site in the *bchC* gene and included 181 bp of pUC13 vector DNA as a tail (Fig. 5A). The other probe extended 635 bp in the 3′ direction to the *Bam*HI site in the *bchC* gene and included 209 bp of pUC13 vector DNA as a tail (Fig. 5A). The results of an S1 nuclease protection experiment using these two probes (Fig. 5B) showed that the 657-nt band for the *Hin*dIII probe and the 844-nt band for the *Bam*HI probe were present in both the control and experimental lanes and were due to reannealing of each bipartite DNA probe. The presence of a band 476 nt in length for the *Hin*dIII probe and 635 nt in length for the *Bam*HI probe indicated that the entire *R. capsulatus* segments of both the *Hin*dIII and *Bam*HI probes were protected by an mRNA molecule. This result verified that mRNA transcripts of the *crtF* gene continued into the *bchCA* operon. Quite unexpectedly, however, the *Bam*HI probe was also protected by an mRNA molecule with a 3′ end that was positioned approximately 525 nt downstream of the *Mlu*I site, and the intensity of this 525-nt band in the autoradiogram was about equal to the intensity of the 635-nt band for the fully protected *R. capsulatus* segment of the probe. This 3′ end, which would map approximately 45 nt downstream of the *Hin*dIII site in the *bchC* gene, suggested that some *crtEF* transcripts either are transcriptionally terminated or are posttranscriptionally processed to give molecules that end between the *Hin*dIII and *Bam*HI sites in the *bchC* gene. We attributed the smaller bands to be due to degradation products of the primary *crtEF* transcript.

Higher resolution end mapping of the 3′ end within the *bchC* gene. The 3′ end located between the *Hin*dIII and *Bam*HI sites in the *bchC* gene was positioned more precisely by using a probe that was 3′ end labeled at the *Hin*dIII site in the *bchC* gene and extended 557 bp in the 3′ direction to the *Fsp*I site in the *bchC* gene (Fig. 6A). Because the probe used in this experiment was not bipartite, the 557-nt band observed in both the control and experimental lanes (Fig. 6B)

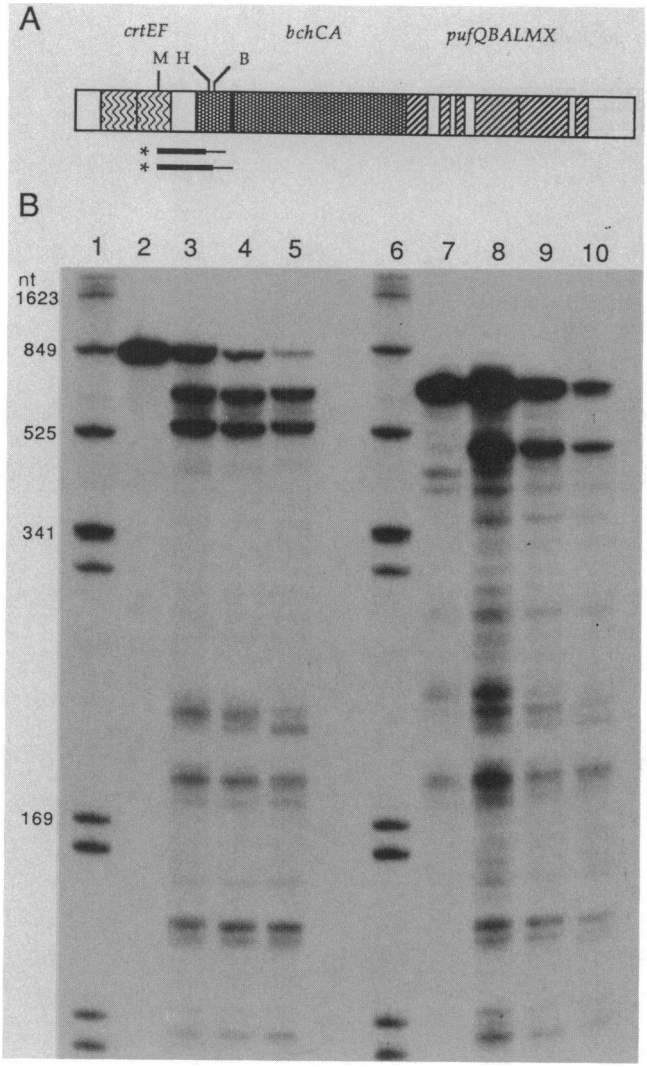

FIG. 5. S1 nuclease protection 3′ end mapping of crtEF mRNA transcripts. (A) Representations of the double-stranded bipartite DNA probes that were 3′ end labeled (*) at the *Mlu*I (M) site in the *crtF* gene, extended to either the *Hin*dIII (H) or *Bam*HI (B) site in the *bchC* gene (thick lines), and included a tail of pUC13 DNA (thin lines). (B) Autoradiogram of a 5% polyacrylamide gel containing 8 M urea through which denatured, S1 nuclease-resistant hybrids were separated. Lanes: 1 and 6, *Hae*III-digested M13mp11 single-stranded DNA size markers, with the sizes of selected bands shown on the left; 2 to 5, S1-resistant fragments protected by the *Bam*HI probe; 7 to 10, S1-resistant fragments protected by the *Hin*dIII probe. Lanes 2 and 7 contain 150 ng of probe hybridized with 10 μg of yeast tRNA and digested with 500 U of S1 nuclease; lanes 3 and 8, 4 and 9, and 5 and 10 contain 150 ng of probe hybridized with 10 μg of *R. capsulatus* B10 RNA isolated 30 min following a shift of the culture to low aeration, which were digested with 500, 1,000, and 1,500 U of S1 nuclease, respectively.

was due to protection of the probe by its complementary DNA strand as well as by mRNA that continued past the 3′-terminal nucleotide of the *R. capsulatus* segment of the probe. Two smaller bands, approximately 57 and 60 nt in length, indicated that there were two 3′ ends that mapped approximately 57 and 60 nt downstream of the *Hin*dIII site. Examination of the DNA sequence of the *bchC* gene (40) showed that two regions of imperfect inverted symmetry could be found between the *Hin*dIII and *Bam*HI sites at approximately the same locations as these two 3′ ends (Fig. 6C). Because the distance from the predominant *bchCA* 5′ end mapped above to these 3′ ends was approximately 350 nt, transcription that began at the *bchCA* promoter and ended at the position of the inverted repeats may have given rise to the ca. 330-nt transcript detected by the *bchC*-specific probe in the RNA blots (Fig. 2A).

However, it was also possible that this short transcript was due instead to degradation of longer *crtEF* readthrough transcripts. That is, if *crtEF* mRNA transcripts that extended to the inverted repeats in the *bchC* coding region were cleaved by an endonuclease that cut at or very near the *bchCA* promoter, a ca. 330-nt degradation product would have been produced. Because this product overlapped the *bchC* gene, it would have been detected by the *bchC*-specific probe used in the RNA blot experiments (Fig. 2A).

Origin of mRNA molecules with 3′ termini within the *bchC* gene. If the *bchC*-internal 3′ ends were exclusively due to termination of *crtEF* readthrough transcripts, then elimination of *crtEF* readthrough transcription should cause these 3′ ends to disappear. Alternatively, if the *bchC*-internal 3′ ends were due to transcription termination or posttranscriptional processing of mRNA molecules derived exclusively from the *bchCA* promoter, then elimination of *crtEF* readthrough transcription should not change the levels of these 3′ ends. If the *bchC*-internal 3′ ends were due to a combination of these models, elimination of *crtEF* readthrough transcription would result in a decrease in the levels of these 3′ ends but would not eliminate them entirely. To test whether the *bchC*-internal 3′ ends were located primarily on the termini of *crtEF* readthrough transcripts, whether they were located primarily on the termini of *bchCA* transcripts, or whether they were located on both mRNA species, we compared the relative quantities of the *bchC*-internal 3′ ends in *R. capsulatus* SB1003 and in the *crtF* interposon mutant *R. capsulatus* DE324, which was derived from *R. capsulatus* SB1003 by the chromosomal insertion of an omega cartridge containing transcription terminators into the *Sph*I site in the *crtF* gene (43). Insertion of this cartridge reduces the amount of readthrough transcription by at least 90% (40a; unpublished data).

The relative quantities of the *bchC*-internal 3′ ends were measured in a S1 nuclease protection experiment by using a bipartite probe that was labeled at the 3′ end at the *Bcl*I site 5 nt downstream of the *bchCA* major 5′ end, which extended 400 nt in the 3′ direction to the *Bam*HI site in the *bchC* gene, and contained 121 bp of pUC13 vector DNA as a tail (Fig. 7A). A molar excess of this probe was used to protect RNA samples from *R. capsulatus* SB1003 and *R. capsulatus* DE324 from digestion with S1 nuclease. The resultant autoradiogram (Fig. 7B) shows the presence of a 521-nt band in all lanes that was due to DNA-DNA reannealing of the probe, a 400-nt band that was due to mRNA that extends beyond the 3′ end of the *R. capsulatus* segment of the probe, and a 295-nt band that was due to mRNA molecules ending at either of the two inverted repeat regions within the *bchC* coding region. Comparison of the relative intensities of the 400- and 295-nt bands on this autoradiogram showed that the intensities of both bands were approximately 40% lower in the lanes containing *R. capsulatus* DE324 RNA than they were in the corresponding lanes containing *R. capsulatus* SB1003 RNA. Because this 295-nt band was still present in the lanes containing *R. capsulatus* DE324 RNA, the short transcript observed on the RNA blots cannot have been solely due to processing of *crtEF* transcripts but must have

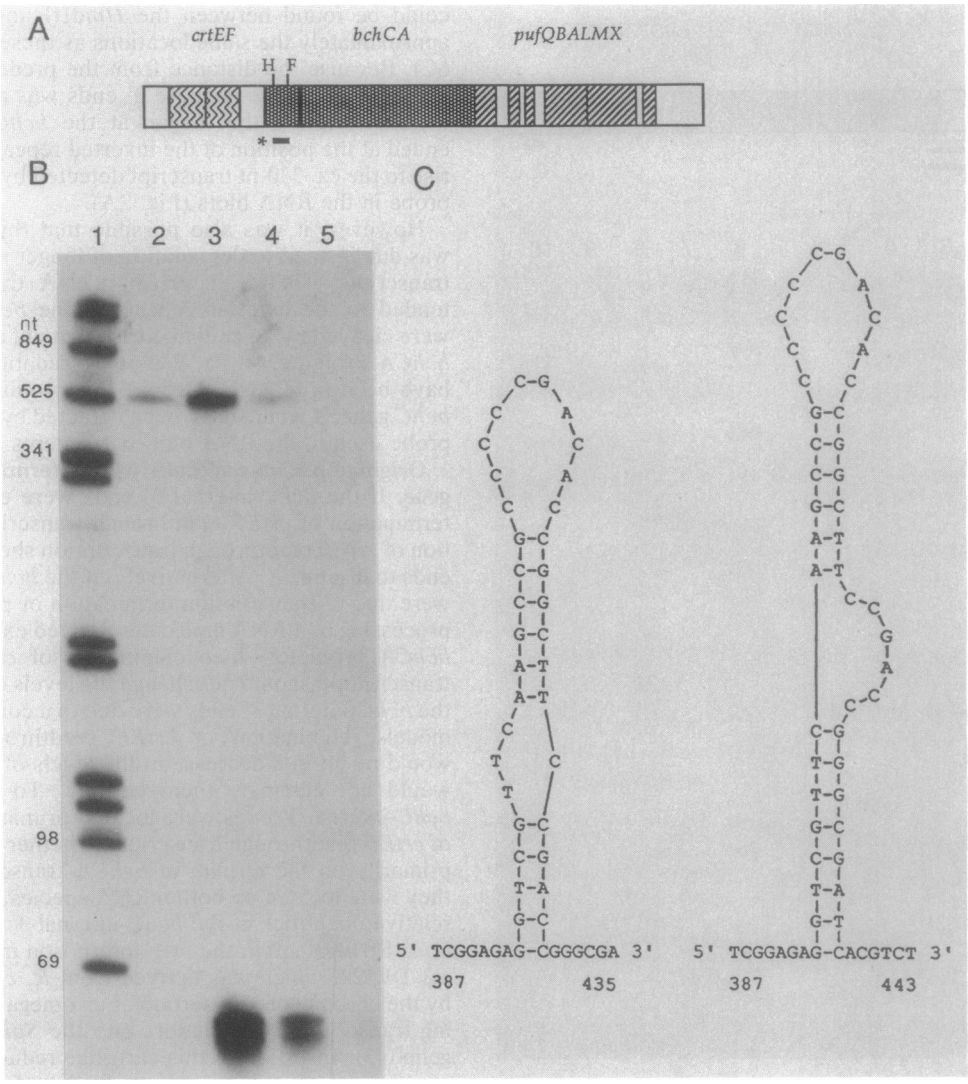

FIG. 6. S1 nuclease protection mapping of the 3′ ends within the *bchC* coding region. (A) Representation of the double-stranded DNA probe that was 3′ end labeled (*) at the *Hin*dIII site in the *bchC* gene and extended to the *Fsp*I site in the *bchC* gene. (B) Autoradiogram of a 5% polyacrylamide gel containing 8 M urea through which denatured, S1 nuclease-resistant hybrids were separated. Lanes: 1, *Hae*III-digested M13mp11 single-stranded DNA size markers, with the sizes of selected bands shown on the left; 2, 100 ng of probe hybridized with 10 μg of yeast tRNA and digested with 500 U of S1 nuclease; 3 to 5, 100 ng of probe hybridized with 10 μg of *R. capsulatus* B10 RNA isolated 30 min following a shift of the culture to low aeration, digested with 500, 1,000, and 1,500 U of S1 nuclease, respectively. (C) Nucleotide sequence of the two inverted repeat sequences within the *bchC* gene. The numbering of nucleotides corresponds to that in Fig. 4 of reference 40.

resulted, in part, from transcription termination or posttranscriptional processing of mRNA molecules initiated at the *bchCA* promoter.

Interestingly, the pattern of expression over time appeared to be slightly different in the two strains. The mRNA transcripts in *R. capsulatus* SB1003 were detectable in highly aerated cells, increased approximately fivefold to reach a maximal level at 30 min after the shift to low aeration, and remained fairly constant in the remaining samples. However, the transcripts in *R. capsulatus* DE324 were present at much lower levels in highly aerated cells (approximately one-fifth of the level observed in *R. capsulatus* SB1003), increased about 10-fold to reach a maximum level at 30 min (to approximately the same level observed in *R. capsulatus* SB1003), and decreased to approximately 30% of the maximum level by 60 min. This pattern was reproduced in three independent experiments and indicates different responses of the *crtEF* and *bchCA* promoters to changes in oxygen concentration.

3′ end mapping of *bchCA* mRNA transcripts. A bipartite probe designed to map the 3′ ends of the *bchCA* operon was radioactively 3′ end labeled at the *Eco*RI site in the *bchA* gene, extended 865 bp in the 3′ direction to the *Sal*I site in the *pufQ* gene, and included 199 bp of pUC13 vector DNA as a tail (Fig. 8A). An autoradiogram of the resultant S1-protected hybrids (Fig. 8B) shows a band approximately 1,060 nt in length in all lanes, due to reannealing of the bipartite DNA probe, and a band approximately 840 nt in length in the lanes containing *R. capsulatus* RNA, which suggested that the probe was protected by an mRNA mole-

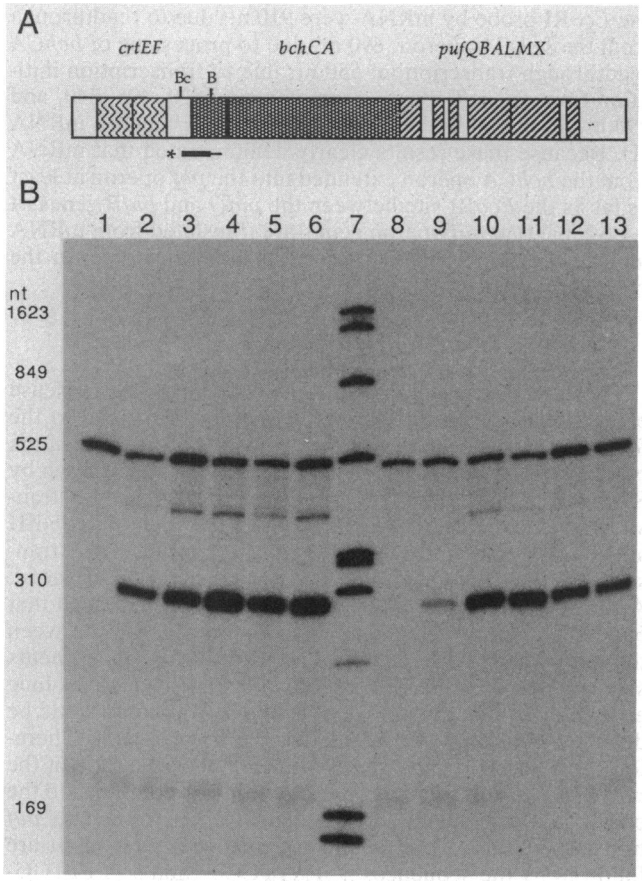

FIG. 7. Comparison of *bchC*-internal 3′ ends in *R. capsulatus* SB1003 and *R. capsulatus* DE324. (A) Representation of the double-stranded bipartite DNA probe that contained *R. capsulatus* DNA (thick line) that was 3′ end labeled (*) at the *Bcl*I (Bc) site between the *crtF* and *bchC* genes, extended to the *Bam*HI (B) site in the *bchC* gene, and included pUC13 DNA as a tail (thin line). (B) Autoradiogram of a 5% polyacrylamide gel containing 8 M urea through which denatured, S1 nuclease-resistant hybrids were separated. A 100-ng sample of probe was hybridized to 10 μg of the indicated RNA sample and digested with 500 U of S1 nuclease. Trimmed hybrids were denatured and separated through a 5% polyacrylamide gel containing 8 M urea. Lanes: 1 and 8, *E. coli* tRNA; 2 through 6, *R. capsulatus* SB1003 RNA isolated at 0 min (lane 2), 15 min (lane 3), 30 min (lane 4), 45 min (lane 5), and 60 min (lane 6) following a shift of the culture from high to low aeration; 7, *Hae*III-digested M13mp11 single-stranded DNA size markers, with the sizes of selected bands shown on the left; 9 through 13, *R. capsulatus* DE324 RNA isolated at 0 min (lane 9), 15 min (lane 10), 30 min (lane 11), 45 min (lane 12), and 60 min (lane 13) following the shift.

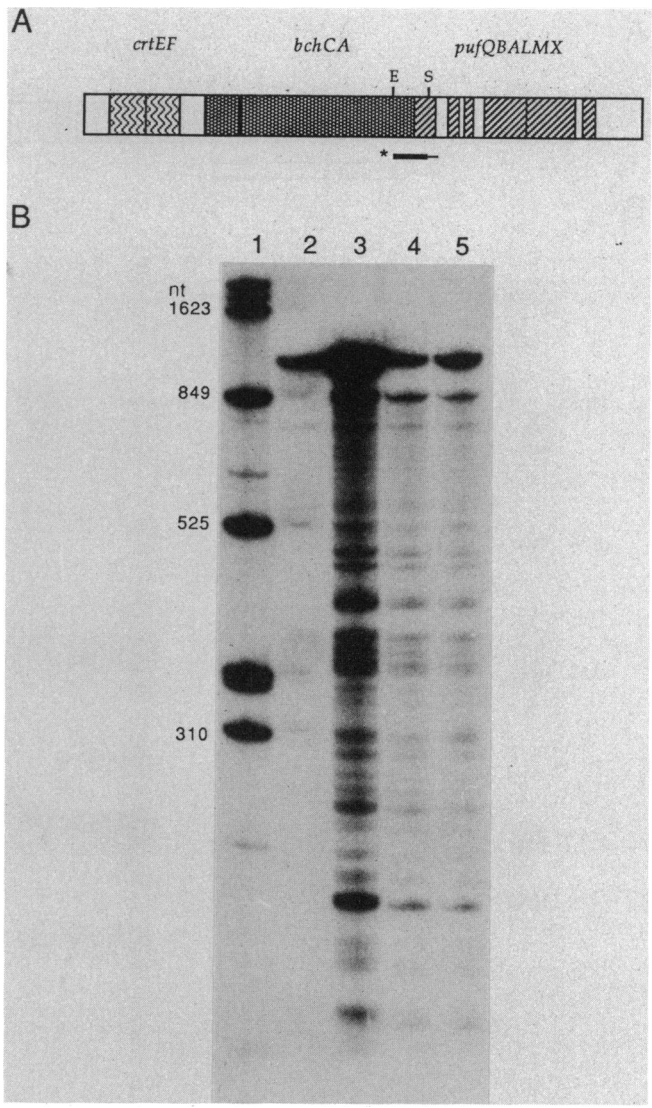

FIG. 8. S1 nuclease protection 3′ end mapping of *bchCA* mRNA transcripts. (A) Representation of the double-stranded bipartite DNA probe containing *R. capsulatus* DNA (thick line) that was 3′ end labeled (*) at the *Eco*RI (E) site in the *bchA* gene, extended to the *Sal*I (S) site in the *pufQ* gene, and contained pUC13 DNA as a tail (thin line). (B) Autoradiogram of a 5% polyacrylamide gel containing 8 M urea through which denatured, S1 nuclease-resistant hybrids were separated. Lanes: 1, *Hae*III-digested M13mp11 single-stranded DNA size markers, with the sizes of selected bands shown on the left; 2, 150 ng of probe hybridized with 10 μg of *E. coli* tRNA and digested with 500 U of S1 nuclease; 3 to 5, 150 ng of probe hybridized with 10 μg of *R. capsulatus* B10 RNA isolated 30 min following a shift of the culture to low aeration, digested with 500, 1,000, and 1,500 U of S1 nuclease, respectively.

cule that extends at least to the *Sal*I site in the *pufQ* gene. The numerous 3′ ends of smaller molecules visible in Fig. 8B presumably arose from degradation products of this unstable *bchCA* mRNA, as noted in the RNA blots.

5′ end mapping of *puf* mRNA transcripts. A 5′-end-mapping experiment was designed to confirm the overlap of *bchCA* and *puf* operon transcripts. Two bipartite probes were prepared. One probe was radioactively 5′ end labeled at the *Sal*I site in the *pufQ* gene, extended 0.69 kb in the 5′ direction to the *Xho*II site in the *bchA* gene, and included 2.7 kb of pUC13 vector DNA as a tail (Fig. 9A). The second probe was 5′ end labeled at the *Eco*RI site between the *pufQ* and *pufB* genes, extended 0.885 kb in the 5′ direction to the *Xho*II site in the *bchA* gene, and included 2.7 kb of pUC13 vector DNA as a tail (Fig. 9A). An autoradiogram of the resultant S1-protected hybrids (Fig. 9B) shows a 3.4-kb band for the *Sal*I probe and a 3.6-kb band for the *Eco*RI probe that were due to reannealing of each bipartite DNA probe. The lengths of bands resulting from protection of the *Sal*I probe by mRNA molecules were 730 nt, due to readthrough from the *bchCA* operon; 475 nt, which we attribute to cleavage of

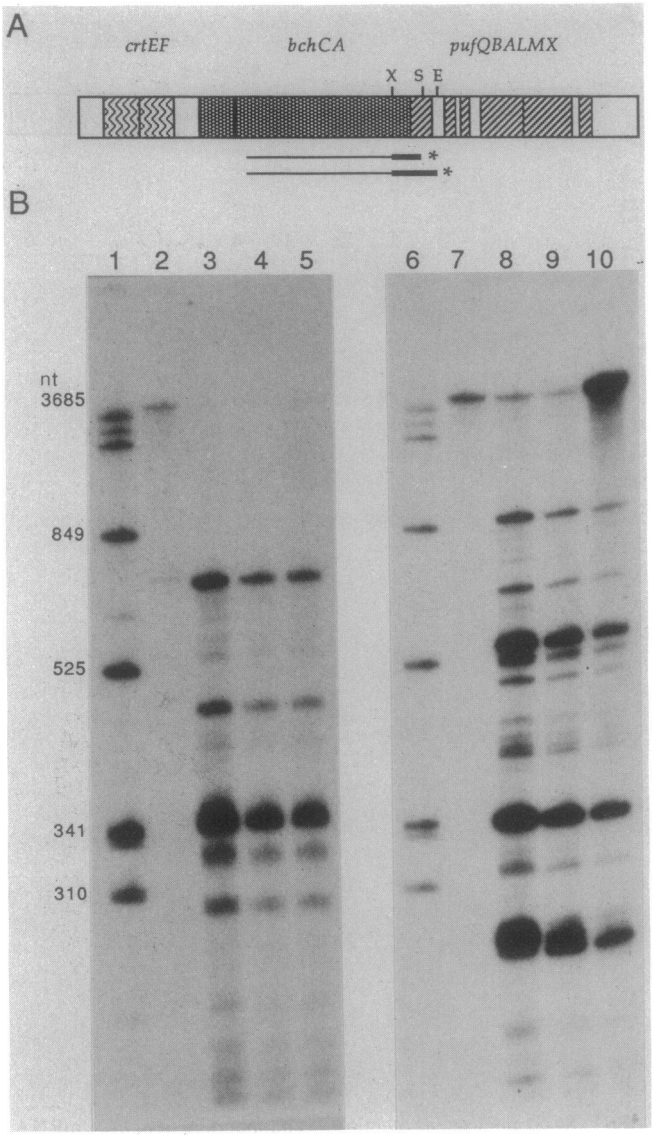

FIG. 9. S1 nuclease protection 5′ end mapping of *puf* mRNA transcripts. (A) Representations of the double-stranded bipartite DNA probes containing *R. capsulatus* DNA (thick lines) that were 5′ end labeled (*) at either the *Sal*I (S) site in the *pufQ* gene or the *Eco*RI (E) site between the *pufQ* and *pufB* genes, extended to the *Xho*II (X) site in the *bchA* gene (thick lines), and included a tail of pUC13 DNA (thin lines). (B) Autoradiogram of a 5% polyacrylamide gel containing 8 M urea through which denatured, S1 nuclease-resistant hybrids were separated. Lanes: 1 and 6, *Hae*III-digested M13mp11 single-stranded DNA size markers, with the sizes of selected bands shown on the left; 2 to 5, S1-resistant fragments protected by the *Sal*I probe; 7 to 10, S1-resistant fragments protected by the *Eco*RI probe. Lanes 2 and 7 contain 200 ng of probe hybridized with 10 μg of yeast tRNA and digested with 500 U of S1 nuclease; lanes 3 and 8, 4 and 9, and 5 and 10 contain 200 ng of probe hybridized with 10 μg of *R. capsulatus* B10 RNA isolated 30 min following a shift of the culture to low aeration, which were digested with 500, 1,000, and 1,500 U of S1 nuclease, respectively.

the *bchCA* readthrough transcript; and 360 nt, due to initiation of transcription from the *puf* operon promoter (1). The 330- and 305-nt bands have been observed previously and have been attributed to processing products of *puf* operon mRNA (1). The lengths of bands resulting from protection of the *Eco*RI probe by mRNA were 910 nt, due to readthrough from the *bchCA* operon; 690 nt, due to processing of *bchCA* readthrough transcription; 550 nt, due to transcription initiation from the *puf* operon promoter; and 520, 495, 340, and 290 nt, all probably due to processing of *puf* operon mRNA (1). Because these results clearly demonstrated that mRNA from the *bchCA* operon extended into the *puf* operon at least as far as the *Eco*RI site between the *pufQ* and *pufB* genes, it is possible that *puf* operon genes are translated from mRNA molecules that initiate at the *bchCA* promoter or even the *crtEF* promoter.

DISCUSSION

The combined results of the RNA blotting and S1 nuclease protection experiments presented in this report lead to the following model of the overlapping mRNA transcripts of the *crtEF*, *bchCA*, and *puf* operons (Fig. 1). We have shown by S1 nuclease protection experiments that some *crtEF* transcripts have 3′ ends that are positioned between the *Hin*dIII and *Bam*HI sites in the *bchC* gene, and other *crtEF* transcripts extend beyond the *Bam*HI site in the *bchC* gene. Analogous S1 nuclease protection experiments showed that *bchCA* transcripts extend beyond the *Eco*RI site between the *pufQ* and *pufB* genes. The RNA blot experiments showed that a transcript 11 kb in length, which is long enough to encode both the *bchCA* and *puf* operons, could be detected with a probe specific for the *bchA* region. Therefore, it is possible that transcription is initiated at any of the *crtEF*, *bchCA*, or *puf* operon promoters and continues to the end of the *puf* operon. The 3′ ends of operon-length *puf* transcripts map to two regions of dyad symmetry that are followed by the sequences 5′-TATTCC-3′ and 5′-TTTTGG-3′, respectively (10, 44). Although it is probable that these regions act as efficient transcriptional terminators, the possibility of readthrough transcription extending beyond the *puf* operon has not been tested. Young et al. (43) have previously proposed a similar "superoperon" model of overlapping *crtEF*, *bchCA*, and *puf* mRNAs primarily on the basis of interposon mutagenesis, gene fusion, and sequence analysis experiments. The RNA studies presented in this report support and extend their model.

Although it is possible that single mRNA molecules encoding the *crtEF*, *bchCA*, and *puf* operons exist in *R. capsulatus*, obtaining direct evidence for such long multioperonic transcripts would be difficult, given the very rapid degradation of mRNA transcribed from this region (1, 7, 10). However, the results of the blotting experiments using *R. capsulatus* B10(pCW2)) RNA (Fig. 2C) provided some evidence for an mRNA species long enough to extend from the *bchCA* promoter to the *puf* operon 3′ termination signals proposed previously (7, 10). Although it could be argued that this 11-kb transcript was an artifact due to transcription of the hybrid *puf-bchCA* mRNA molecule from pCW2, we think that this is unlikely, because the sequence of the hybrid *puf-bchCA* molecule would differ from the sequence of the chromosomally expressed *bchCA* primary transcript only within a 0.5-kb segment at the extreme 5′ end of the *puf-bchCA* hybrid primary transcript. Moreover, the *Bam*HI-C fragment that was inserted into pCW2 contains the relatively well characterized *puf* operon transcriptional terminators (7, 10) as well as an additional 2.25 kb of *R. capsulatus* DNA. Therefore, the 3′ end of a hybrid *puf-bchCA-puf* mRNA transcript would be expected map to the same position as would a chromosomally derived *bchCA-puf* mRNA transcript. The dramatic increase in the amounts of

hybrid *puf-bchCA* mRNA molecules detected (Fig. 2C), compared with the blots in which chromosomally derived *bchCA* RNA was detected (Fig. 2A and B), presumably reflects a copy number increase due to the plasmid-borne sequences and the different sensitivities of the *bchCA* and *puf* promoters to oxygen regulation (1, 11, 40, 43).

Each transcriptional overlap presented in Fig. 1 was confirmed by at least two S1 nuclease protection experiments, the results of which were consistent with one another. One type of experiment was designed to detect 3′ ends plus continuation of transcription into the next downstream operon, whereas the other type of experiment was designed to detect 5′ ends plus the presence of transcripts originating from the adjacent upstream operon. The use of bipartite probes was essential for the direct demonstration of the overlap between the mRNA transcripts of the *crtEF*, *bchCA*, and *puf* operons. We suggest it is likely that other examples of overlapping mRNAs similar to the *R. capsulatus crtEF*, *bchCA*, and *puf* operons will be found as more detailed maps of transcriptional units are assembled.

Interestingly, high levels of a 0.33-kb transcript were detected with a *bchC*-specific probe on the RNA blots (Fig. 2A). The results of S1 nuclease 3′ end-mapping experiments showed that two 3′ ends were found within the *bchC* coding sequence near two regions with the potential to form hairpin loops in the mRNA (Fig. 6C). Therefore, mRNA molecules starting at the *bchCA* promoter and ending at either of the *bchC*-internal inverted repeat regions would be approximately 330 nt in length. We offer two plausible explanations for the origin of this short molecule. One possibility is that the *bchC*-internal areas of inverted symmetry form regions of secondary structure in the mRNA that result in premature termination of *bchCA* transcription to yield the short transcript. An alternative possibility is that these regions of secondary structure are a barrier to mRNA degradation so that the short transcripts are stable remnants of a processed primary transcript, as has been documented previously for the region of secondary structure found between the *pufA* and *pufL* genes in *R. capsulatus* (7, 10). Chen et al. (10) have calculated that at least 75% of RNA polymerase molecules transcribing the *puf* operon read through the *pufA-pufL* intercistronic hairpin, which consists of a G+C-rich region of dyad symmetry followed by the sequence 5′-CATACC-3′ (44). They have also shown that the efficiency of transcription termination at mRNA hairpins in *R. capsulatus* increases as the number of uridine residues immediately following such structures increases. Because the sequences following the two inverted repeat regions in the *bchC* gene are 5′-GGGCGA-3′ and 5′-ACGTCT-3′, it seems unlikely that either inverted repeat effectively terminates transcription. Although we favor the hypothesis that the two inverted repeat regions are more likely to act as decay barriers than transcription terminators, some transcription termination by these secondary structures cannot be excluded.

Although the precise nucleotides that make up the *bchCA* promoter have not yet been identified, we have shown that the *bchCA* promoter and *cis*-active sequences required for oxygen regulation lie within a 91-bp segment of DNA in the *crtF-bchC* intergenic region. This was done by correlating the presence of a major 5′ mRNA end (Fig. 3) with sequences necessary for *bchCA* promoter activity (Fig. 4). These results are in agreement with the previous results of Young et al. (43) and the suggested location of the *bchCA* promoter based on DNA sequence analysis (2, 40). Additionally, this major 5′ end has been positioned to a region of DNA with significant sequence similarity to the previously characterized *R. capsulatus puf* promoter (1, 4, 40), which strengthens our conclusion that this major 5′ end is of a *bchCA* primary transcript. However, the residual activities seen with pJP101 (Fig. 4) indicate that some low-level initiation of transcription may be associated with the minor 5′ ends downstream of the major end shown in Fig. 3.

The effects of interposon blockage of *crtEF* readthrough on regulation of *bchCA* mRNA levels by culture aeration (Fig. 7) indicated that overlapping transcription of these operons is necessary to obtain normal *bchCA* mRNA levels during transitions in oxygen availability. A mechanism whereby the effect is manifested is described elsewhere (40a).

The model of overlapping *crtEF*, *bchCA*, and *puf* transcripts presented here and by Young et al. (43) raises an interesting semantic point. On one hand, the *crtEF*, *bchCA*, and *puf* operons can be viewed as separate but overlapping operons. On the other hand, they can be viewed as one large operon with two internal promoters: one that promotes transcription of the *bchCA* and *puf* genes, and another that promotes transcription of only the *puf* genes. The grouping of cotranscribed genes into units called operons, therefore, is somewhat arbitrary when a model such as this is considered.

Superoperonic clustering was first proposed for *Pseudomonas* species, in which it was found that functionally related genes were tightly clustered on the chromosome (18, 23, 41). However, these conclusions were drawn from classical genetic mapping studies, most of which used cotransduction frequencies to map specific markers. Because little is known about the mRNA transcripts of these gene clusters, the "superoperonic" clustering observed in *Pseudomonas* species may simply mean that the separate operons are located reasonably close to each other on the chromosome but are not transcriptionally linked. Therefore, the *Pseudomonas* "superoperons" may have little in common with the *R. capsulatus crtEF-bchCA-puf* superoperon.

There are, however, a few other examples of true superoperons in the literature. For example, the promoter of the *E. coli ampC* gene (encoding β-lactamase) is found within the coding region of the last gene in the *frdABCD* operon (encoding the fumarate reductase complex), so that the carboxy-terminal 12 codons of the *frdD* gene are also contained on an mRNA transcript initiated from the *ampC* promoter (17). This 12-codon sequence also forms an attenuator that has been proposed to moderate growth-rate-dependent transcription of the *ampC* gene (19). Furthermore, the *ampC* attenuator also acts as a transcription terminator for the *frd* operon (17). A second example is the *E. coli ptsH-ptsI-crr* operon that encodes three proteins of the phosphoenolpyruvate-dependent phosphotransferase system (HPr, enzyme I, and enzyme III^{Glc}, respectively; 12). Transcription of this operon may be initiated at either of two promoters: one (P1) that promotes transcription of the entire operon and another (P2) that is located in the *ptsI* coding region and promotes transcription of only the *crr* gene. Approximately 85% of *crr* mRNA transcripts are due to initiation at P2, which is regulated differently than P1. Whereas P1 is stimulated by growth on glucose (and the cyclic AMP-cyclic AMP receptor protein complex), P2 is unresponsive to this condition and promotes constitutive transcription of *crr*. This region may alternatively be considered to contain two overlapping transcriptional units. One unit, encoding *crr*, is constitutively expressed. However, its expression can be increased under certain conditions by

transcriptional readthrough from the upstream unit encoding *ptsH* and *ptsI*.

Although phage lambda may also be considered to use transcription readthrough, this system is controlled through the N and Q antitermination systems (28). Expression of lambda intermediate and late genes is dependent on the synthesis of the N and Q antiterminator proteins because these genes do not have their own promoters. Therefore, antitermination differs from the overlapping mRNAs described in this report for two major reasons. Each of the *R. capsulatus crtEF*, *bchCA*, and *puf* operons has its own individually regulated promoters (1, 4, 16, 40, 43) and thus can be expressed without transcription readthrough. Also, because there do not seem to be transcription terminators between the *crtEF*, *bchCA*, and *puf* operons equivalent to the lambda arrangement, the production of these overlapping mRNAs may be independent of the synthesis of antiterminator proteins.

The results of these RNA studies complement the results of Young et al. (43) to clearly demonstrate the transcriptional overlap between the *crtEF*, *bchCA*, and *puf* operons. In a separate report (40a), we address the significance of this genetic arrangement. It will be interesting to determine whether overlapping operons in other bacteria are common and whether the organization of functionally related operons into overlapping transcription units plays a broader role in the regulation of gene expression than has heretofore been appreciated.

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