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Functional Significance of Overlapping Transcripts of *crtEF*, *bchCA*, and *puf* Photosynthesis Gene Operons in *Rhodobacter capsulatus*

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The *Rhodobacter capsulatus crtEF*, *bchCA*, and *puf* operons each encode products that function together to produce photosynthetic pigment-protein complexes. The *crtEF* operon encodes two enzymes of the carotenoid biosynthetic pathway, the *bchCA* operon encodes two enzymes of the bacteriochlorophyll biosynthetic pathway, and the *puf* operon contains six genes, four of which are known to code for proteins that bind pigment cofactors and position them in the intracytoplasmic membrane. These operons are adjacent on the chromosome and are transcribed in the same direction. Although each of these operons can be expressed from its own promoter, it is possible for transcription to begin at the *crtEF* promoter and continue through the *bchCA* and *puf* operons. We herein present the results of experiments which demonstrate that this overlapping transcriptional arrangement is important for establishment of normal levels of transcripts of the *bchCA* and *puf* operons and that read-through transcription from the *bchCA* operon contributes significantly to the expression of a *pufB'*::*lac'Z* gene fusion. Growth studies of *crtF* and *bchC* interposon mutants show that this read-through transcription is necessary for efficient transition from respiratory to photosynthetic growth modes.

In prokaryotes, groups of genes that are regulated and transcribed together as polycistronic mRNA molecules are called operons. Because operons often encode genes with products that function together, it is generally believed that the arrangement of related genes into operons facilitates the efficient expression of genes whose products are simultaneously needed under certain conditions. Evidence that two or more genes are transcribed as an operon usually consists of genetic experiments that reveal polarity and biochemical experiments that demonstrate the existence of a multicistronic mRNA molecule.

Like many other members of the purple, nonsulfur phototrophic bacteria, *Rhodobacter capsulatus* is capable of several different types of metabolic energy conservation. When grown aerobically, *R. capsulatus* produces ATP by using a respiratory electron transport chain that is similar to that found in mitochondria and several other species of bacteria (15). When grown anaerobically (or under reduced oxygen tension) in the presence of light, *R. capsulatus* produces ATP by cyclic photophosphorylation. Cyclic photophosphorylation is made possible by the presence of integral membrane pigment-protein complexes that are unique to the process of photosynthetic energy transduction, and their synthesis is controlled by oxygen concentration and light intensity. These complexes are the reaction center, which is the site of primary photochemical charge separation, and light-harvesting complexes that function to gather light energy and funnel it to the reaction center (6, 8).

There are a variety of photosynthesis genes in *R. capsulatus* which encode proteins that direct the synthesis and assembly of the components of the photosynthetic complexes. These components include the pigment-binding polypeptides of the reaction center and light-harvesting complexes (encoded by the *puf*, *puh*, and *puc* genes) and the enzymes of the bacteriochlorophyll (Bchl) and carotenoid biosynthetic pathways (encoded by the *bch* and *crt* genes, respectively). With the exception of the *puc* genes, all known *R. capsulatus* photosynthesis genes are arranged in one large cluster in a 50-kb region of the chromosome, where the *puf* and *puh* genes flank the *bch* and *crt* genes (reviewed in reference 13). This pattern of photosynthesis gene clustering has also been found in the closely related species *Rhodobacter sphaeroides*, and a *bchA* gene homolog of *Rhodopseudomonas viridis* appears to lie immediately 5′ of the *puf* operon, just as in *R. capsulatus* and *R. sphaeroides* (5, 21).

Previous studies have shown that the *R. capsulatus crtEF*, *bchCA*, and *puf* operons have an unusual transcriptional relationship. These adjacent operons are transcribed in the same direction, from the *crtEF* operon toward the *puf* operon (Fig. 1). Although each operon can be expressed from its own individually regulated promoter (1–3, 7, 19, 22), transcription initiated at the *crtEF* promoter could extend to the end of the *puf* operon. Similarly, transcription beginning at the *bchCA* promoter may not terminate until the transcriptional terminators at the 3′ end of the *puf* operon are reached. Thus, these three operons may at times be cotranscribed, and the *bchCA* and *puf* genes could be expressed from several overlapping mRNA molecules (19, 20, 22).

It has been speculated that the overlapping transcriptional arrangement of the *crtEF*, *bchCA*, and *puf* operons may be physiologically significant, as a polar mutation in the *crtF* gene resulted in the accumulation of small amounts of a Bchl biosynthetic pigment intermediate, although photosynthetic growth was still obtained (22). In this study, we use interposon mutants to show a decrease in the levels of specific mRNA molecules when transcription initiated at an upstream promoter is prevented from extending into the downstream genes. Additionally, the results of assays of β-galactosidase activities of plasmid-borne *lac'Z* fusions to the *pufB'* gene were consistent with the mRNA experiments, and interposon mutants in which read-through transcription is blocked were found to be impaired in growth during

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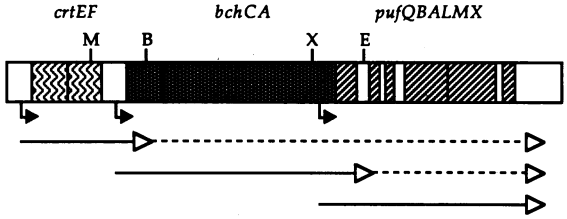

FIG. 1. Representation of the *crtEF*, *bchCA*, and *puf* operons in *R. capsulatus*, showing the proposed overlapping transcripts of this region. Genes are indicated as hatched boxes, promoters as arrows with black heads, and primary transcripts as arrows with white heads. Arrows with solid lines indicate authentic transcripts described previously (20), whereas dashed lines represent mRNA molecules that have not been identified directly but which could exist. Although the *bchA* region is shown as a single gene here and in other figures, it should be noted that the DNA sequence of this region indicates three open reading frames (7a). Relevant restriction endonuclease sites are indicated as M (*Mlu*I), B (*Bam*HI), X (*Xho*II), and E (*Eco*RI).

transition from respiratory to photosynthetic growth. These experiments suggest that interruption of read-through transcription results in a decrease in the levels of mRNAs initiated at downstream promoters and a less effective response to a shift from a dark aerobic to an illuminated anaerobic environment. Thus, we conclude that the overlapping arrangement of these transcripts contributes significantly to oxygen regulation of *R. capsulatus* photosynthesis gene expression and presumably evolved because of the selective advantage that this arrangement would have in natural environments where cells are subjected to changes in the amounts of oxygen.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *R. capsulatus* strains used in this study have been described: the wild-type strains strains B10 (17) and SB1003 (14), the *bchC* interposon mutant strain CW100 (19), and the *crtF* interposon mutant strain DE324 (22).

Plasmids pXCA935 and pXCA935Δ44 have been described previously (1). Plasmids pXCAEHQ and pXCAE HQΔ44 were constructed as described previously (18). Each of these four plasmids contains the same in-frame fusion between the *pufB'* and *lac'Z* genes. Plasmids pXCA935 and pXCA935Δ44 contain an *R. capsulatus* insert that extends from the *Xho*II site in the *bchA* gene to the *pufB'::lac'Z* fusion point and are isogenic except that pXCA935Δ44 contains a 2-nucleotide (nt) change (Δ44) in the *puf* promoter that is known to eliminate approximately 90 to 95% of *puf* promoter activity (1). Plasmids pXCAEHQ and pXCAEH QΔ44 contain *R. capsulatus* DNA extending from the *Eco*RI site in the *crtF* gene to the *pufB'::lac'Z* fusion point. These two plasmids are also identical except that plasmid pXCAE HQ contains wild-type *R. capsulatus* sequences and plasmid pXCAEHQΔ44 contains the 2-nt *puf* promoter mutation.

Growth conditions. *R. capsulatus* was routinely grown in YPS medium (0.3% yeast extract, 0.3% peptone, 2 mM $MgSO_4$, 2 mM $CaCl_2$, pH 6.8) or in a malate minimal medium (RCV; 4) at 34°C; media were supplemented with 0.5 μg of tetracycline-HCl per ml for growth of plasmid-containing strains. High oxygen cultures were grown in Erlenmeyer flasks filled to 8% of their nominal capacity and shaken at 300 rpm in a rotary shaking water bath. Low-oxygen cultures were grown in Erlenmeyer flasks filled to 80% of their nominal volume and shaken at 150 rpm. Photosynthetic liquid cultures in YPS medium were grown in completely filled screw-cap tubes that were held in a glass-sided water bath in front of a bank of Lumiline 60W lamps (General Electric Co.) at an incident light intensity of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. Light intensity was measured with a LI-COR light meter equipped with a quantum sensor (LI-190SB). Cultures were inoculated (from highly aerated precultures in log phase) to an initial turbidity of 10 to 20 photometer units (between 4×10^7 and 8×10^7 CFU/ml), and growth was monitored by measuring the optical density of the culture (at 660 nm) with a Klett-Summerson colorimeter.

Measurement of β-galactosidase specific activity. Plasmids containing *R. capsulatus* in-frame fusions to the *lac'Z* gene were mobilized by conjugation into the desired *R. capsulatus* host strain as described previously (12). Exconjugant cells were grown at 34°C in RCV medium supplemented with 0.1% yeast extract and 8.0 mM potassium phosphate buffer (pH 6.8). Cultures (40 ml) were grown with high or low aeration to a density of ca. 3.5×10^8 CFU/ml, harvested by centrifugation, resuspended in 1 ml of Z buffer (11), and disrupted by sonication. Extracts were cleared of cell debris by centrifugation at 12,000 × *g* for 1 min. The specific activities of β-galactosidase in extracts of *R. capsulatus* cells containing recombinant *lac'Z* fusion plasmids were assayed by as described previously (11) except that the rate of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) cleavage was monitored by a continuous spectrophotometric assay. Values were normalized to the protein content of the extracts as determined by a Lowry protein assay (9), using bovine serum albumin (Sigma Chemical Co.) as a standard.

Isolation of *R. capsulatus* RNA. For the multiple-time-point RNA extractions used in these studies, two 200-ml cultures of *R. capsulatus* cells were grown at 34°C in RCV medium in 2-liter flasks that were shaken at 300 rpm. When the cultures reached a density of 4×10^8 CFU/ml, they were combined into a single 500-ml flask that was shaken at 150 rpm. At the time of the shift and at 15-min intervals for 60 min past the shift, 12-ml samples were removed from the culture and cells were pelleted by centrifugation 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), it was assumed to be under relatively continuous oxygen limitation. Total RNA was extracted from cell pellets with water-saturated unbuffered phenol as described previously (16).

S1 nuclease protection mRNA end mapping and preparation of probes. The bipartite probes (which contained a tail of plasmid vector DNA as well as the *R. capsulatus* sequences) used in these 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 by using standard protocols (10) to a minimal specific activity of 10^6 cpm/μg. Following digestion of the labeled plasmid with a restriction endonuclease that cut in plasmid vector sequences, the probe was purified by gel electrophoresis and electroelution into a dialysis bag.

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 (23) 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. S1-resistant hybrids were ethanol precipitated and resuspended in formamide loading dye prior to electrophoresis in 5% polyacrylamide gels containing 8 M urea. Single-stranded *Hae*III-digested M13 mp11 phage DNA fragments that were radioactively labeled at the 5′ ends served as size markers.

RESULTS

Consequences of interrupting *crtEF* transcriptional read-through on the levels of *bchCA* operon mRNAs. It had previously been reported that an interposon mutation in the *crtF* gene resulted in the accumulation of small amounts of a Bchl biosynthetic intermediate that also is present in cultures of *bchA* mutants, implying that *crtEF* read-through transcription is necessary for optimum expression of the *bchCA* operon (22). Therefore, it was of interest to evaluate the effects of this mutation on the levels of *bchCA* transcripts. In principle, there are two *cis*-active mechanisms whereby *crtF* interposon mutations could cause a shortage of *bchCA* mRNA. If *crtEF* transcripts extend to the end of the *bchA* region, it would be possible to translate the BchC and BchA enzymes from an mRNA molecule initiated at the *crtEF* promoter. Therefore, elimination of the *crtEF* read-through transcripts in *R. capsulatus* DE324 would reduce the amount of mRNA also encoding the *bchCA* operon. A second possibility is that elimination of the *crtEF*/*bchCA* transcriptional overlap in *R. capsulatus* DE324 could impair transcription initiation from the *bchCA* promoter, which would also decrease the steady-state level of *bchCA* transcripts available for translation. These hypotheses are not mutually exclusive.

The possibility that the *crtEF*/*bchCA* transcriptional overlap affected the levels of *bchCA* messages was tested by a quantitative S1 nuclease protection experiment, using a bipartite probe that was 5′ end labeled at the *Bam*HI site in the *bchC* gene and extended to the *Mlu*I site in the *crtF* gene (Fig. 2A), to measure the amounts of 5′ ends of molecules that extended into the *bchC* gene. A molar excess of this probe was hybridized to equal amounts of RNA extracted from wild-type strain SB1003 and the *crtF* interposon mutant DE324 at 15-min intervals following a shift of the cultures from aerobic to anaerobic conditions (Fig. 2B). Comparison of the control lanes (in which the probe was hybridized to *Escherichia coli* tRNA) with the experimental lanes (in which the probe was hybridized to *R. capsulatus* RNA) showed the presence of a prominent band corresponding to 405 nt in length, due to initiation of transcription at the *bchCA* promoter (19, 20). This autoradiogram also showed that the 640- and 550-nt bands were absent in the *R. capsulatus* DE324 samples, indicating that these bands in the SB1003 samples were due to protection of the probe by *crtEF* read-through transcripts. The bands at ca. 350 nt have previously been attributed to processing of primary transcripts or weak transcription initiation (20).

Interestingly, the relative intensities of the 405-nt band at the different time points for *R. capsulatus* DE324 differed from those for the wild-type strain SB1003. Comparison of the 0-min time points showed that the amount of this *bchCA* mRNA in DE324 was approximately 20% of the level observed in SB1003. At 15 min after the shift to inducing conditions, the levels of *bchCA* mRNA appeared to be the same in both strains. At the later time points, the levels of this *bchCA* mRNA 5′ end in SB1003 appeared to be relatively constant, whereas the corresponding levels in DE324

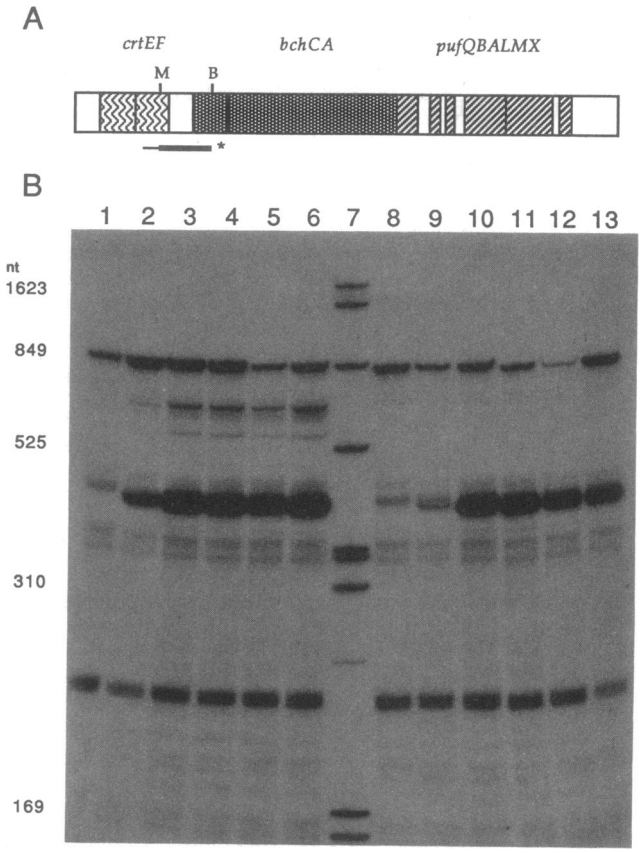

FIG. 2. Comparison of *bchCA* 5′ mRNA ends in *R. capsulatus* SB1003 and DE324. (A) Representation of the 844-bp double-stranded bipartite DNA probe containing *R. capsulatus* DNA (thick line) that was 5′ end labeled (*) at the *Bam*HI (B) site in the *bchC* gene, extended 635 bp to the *Mlu*I (M) site in the *crtF* gene, and included 209 bp of 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. Lanes 1 and 8 contain *E. coli* tRNA. Lanes 2 through 6 contain *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 to low aeration. Lanes 9 through 13 contain *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 a shift of the culture to low aeration. Lane 7 contains *Hae*III-digested M13 mp11 single-stranded DNA size markers, with the sizes of selected bands shown on the left.

gradually decreased over time so that the amount of 60 min was approximately 50% of the maximum observed at 15 min. These results were reproducible and suggested that not only did read-through transcription from the *crtEF* operon seem to increase the activity of the *bchCA* promoter during aerobic growth, but read-through transcription was necessary for high levels of *bchCA* promoter activity in the later stages of this shift of the culture from high to low aeration.

Consequences of interrupting *crtEF* transcriptional read-through on levels of *puf* operon mRNAs. The effects of interrupting *crtEF* read-through transcription were assessed by an S1 nuclease protection experiment designed to test for changes in the levels of *puf* mRNA 5′ ends in DE324 relative to SB1003. The bipartite probe used in this experiment was

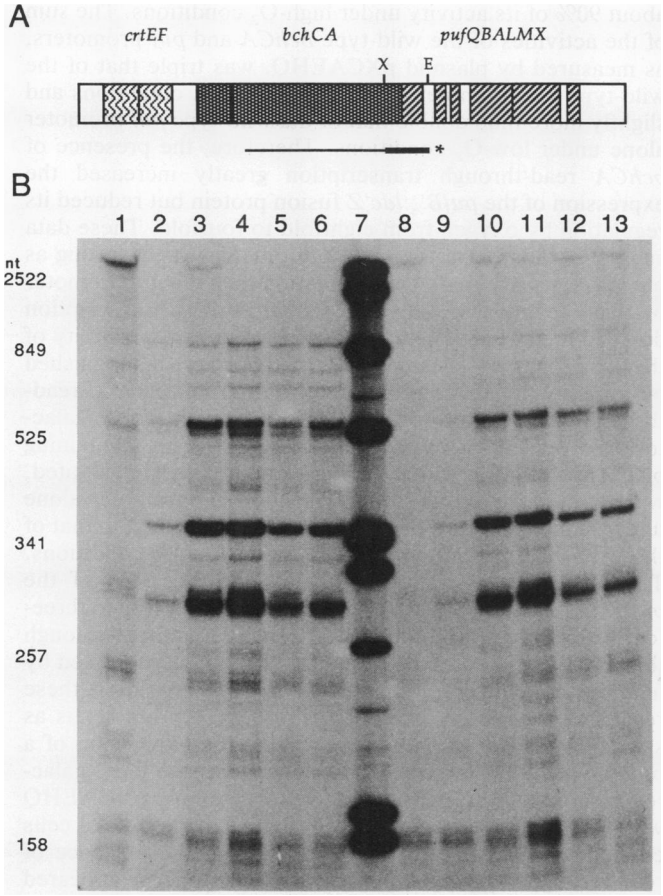

FIG. 3. Comparison of *puf* 5′ mRNA ends in *R. capsulatus* SB1003 and DE324. (A) Representation of the ca. 3.6-kb double-stranded bipartite DNA probe containing *R. capsulatus* DNA (thick line) that was 5′ end labeled (*) at the *Eco*RI (E) site between the *pufQ* and *pufB* genes, extended 885 bp to the *Xho*II (X) site in the *bchA* region, and included 2.7 kb of 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 200-ng sample of probe was hybridized to 10 μg of the indicated RNA sample and digested with 500 U of S1 nuclease. Lanes 1 and 8 contain *E. coli* tRNA. Lanes 2 through 6 contain *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 to low aeration. Lanes 9 through 13 contain *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 a shift of the culture to low aeration. Lane 7 contains *Hae*III-digested M13 mp11 single-stranded DNA size markers, with the sizes of selected bands shown on the left.

5′ end labeled at the *Eco*RI site between the *pufQ* and *pufB* genes and extended to the *Xho*II site in the *bchA* region (Fig. 3A). Samples of RNA extracted from SB1003 and DE324 cultures every 15 min following a shift to low-oxygen conditions were used to protect a molar excess of this probe from digestion with S1 nuclease. An autoradiogram of the resultant gel is shown in Fig. 3B.

In general, bands resulting from protection of the probe by DE324 mRNA were fainter than those resulting from protection of the probe by SB1003 mRNA. The faint bands of 910, 760, and 690 nt are proposed to be due to protection of the probe by *bchCA* read-through transcripts and their processing products (20), whereas the 550-nt band is due to protection of the probe by mRNA initiated at the *puf* promoter. The lower bands are attributed to cleavage of the *puf* primary message (1).

Because the 910-, 760-, and 690-nt bands in this autoradiogram were less intense in the lanes containing DE324 RNA than in those containing SB1003 RNA, interruption of *crtEF* read-through transcription appeared to decrease the number of transcripts that extended from the *bchCA* operon into the *puf* operon. The intensity of the 550-nt band, due to initiation from the *puf* promoter, was reproducibly about 30% lower in lanes containing DE324 RNA than in those containing SB1003 RNA but showed the same pattern of transcription over time.

Consequences of interrupting *crtEF* and *bchCA* transcriptional read-through on *puf* operon transcription. The levels of *puf* operon mRNAs were compared between the *bchC* interposon mutant CW100 and its wild-type parental strain B10. Samples of RNA extracted from CW100 and B10 at 15-min intervals following a shift to low aeration were used to protect a molar excess of the *puf* 5′-end-labeled *Eco*RI probe (Fig. 4A) from digestion with S1 nuclease. An autoradiogram of the resultant S1-protected hybrids (Fig. 4B) showed that, in general, bands resulting from protection of the probe by CW100 mRNA were fainter than those resulting from protection of the probe by B10 mRNA. The bands of particular importance in the interpretation of this experiment are the 910- and 690-nt bands, due to *bchCA* read-through transcription, and the 550-nt band, due to initiation at the *puf* operon promoter (1).

The 910- and 690-nt bands were very faint, but still visible, in lanes containing samples from strain CW100. These residual read-through transcripts may have initiated at either the *crtEF* or *bchCA* promoter or at another, possibly Kn^r cartridge-associated, promoter.

More importantly, the intensity of the 550-nt band, which resulted from initiation of transcription from the *puf* operon promoter, was approximately 40% lower in lanes containing CW100 RNA than in lanes containing B10 RNA. These results suggested that a large reduction in the number of read-through transcripts into the *puf* operon impaired transcription initiation from the *puf* operon promoter.

Contribution of *bchCA* read-through transcription to the expression of *pufB′::lac′Z* fusions. The contribution of *bchCA* read-through transcription to expression of *puf*-encoded polypeptides was assessed by measurement of the β-galactosidase specific activities produced by plasmid-borne *pufB′::lac′Z* fusions in the presence and absence of *bchCA* transcriptional read-through. Each of the four plasmids used in this experiment (Fig. 5) contained the same *pufB′::lac′Z* fusion, but each contained different *R. capsulatus* sequences 5′ to the fusion point. Two of the plasmids contained *R. capsulatus* DNA extending from the *Xho*II site in the *bchA* region to the *pufB′::lac′Z* fusion point; they differed in that one carried the wild-type *puf* promoter (pXCA935) whereas the other contained a 2-nt directed mutation in the *puf* promoter that had previously been shown to eliminate approximately 95% of *puf* promoter activity (pXCA935Δ44; 1). This pair of plasmids was used to measure the activities of either the wild-type or mutant *puf* promoter in the absence of read-through transcription. The other two plasmids contained *R. capsulatus* DNA extending from the *Eco*RI site in the *crtF* gene to the *pufB′::lac′Z* fusion point. One plasmid (pXCAEHQ) was completely wild type in sequence and therefore would measure the sum of the activities of both the *bchCA* and *puf* promoters. The final plasmid (pXCAE

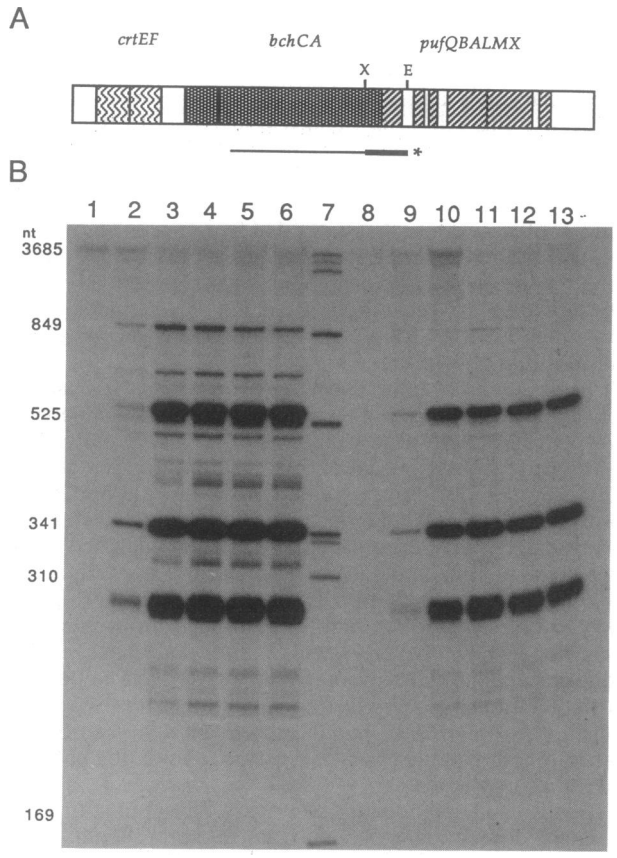

FIG. 4. Comparison of *puf* mRNA 5′ ends in *R. capsulatus* B10 and CW100. (A) Representation of the ca. 3.6-kb double-stranded bipartite DNA probe containing *R. capsulatus* DNA (think line) that was 5′ end labeled (*) at the *Eco*RI (E) site between the *pufQ* and *pufB* genes, extended 885 bp to the *Xho*II (X) site in the *bchA* region, and included 2.7 kb of 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 200-ng sample of probe was hybridized to 10 μg of the indicated RNA sample and digested with 500 U of S1 nuclease. Lanes 1 and 8 contain *E. coli* tRNA. Lanes 2 through 6 contain *R. capsulatus* B10 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 to low aeration. Lanes 9 through 13 contain *R. capsulatus* CW100 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 a shift of the culture to low aeration. Lane 7 contains *Hae*III-digested M13 mp11 single-stranded DNA size markers, with the sizes of selected bands shown on the left.

HQΔ44) contained wild-type *R. capsulatus* sequences except for the 2-nt *puf* promoter mutation and therefore would approximate the ability of the *bchCA* promoter alone to drive expression of the *pufB′::lac′Z* fusion protein.

The β-galactosidase specific activity in extracts of *R. capsulatus* B10 cells containing each of these plasmids was measured (Fig. 5). The activity of the wild-type *puf* promoter alone, as measured by plasmid pXCA935, was used as a baseline value in the interpretation of this experiment. These data showed that transcription of the wild-type *puf* promoter increased approximately eightfold under inducing, or low-O_2, conditions. As expected, the 2-nt mutation in the *puf* promoter, as measured by pXCA935Δ44, eliminated approximately 95% of its activity under low-O_2 conditions and about 90% of its activity under high-O_2 conditions. The sum of the activities of the wild-type *bchCA* and *puf* promoters, as measured by plasmid pXCAEHQ, was triple that of the wild-type *puf* promoter alone under high-O_2 conditions and slightly more than double that of the wild-type *puf* promoter alone under low-O_2 conditions. Therefore, the presence of *bchCA* read-through transcription greatly increased the expression of the *pufB′::lac′Z* fusion protein but reduced its regulation by oxygen from eightfold to fourfold. These data suggested either that the *bchCA* promoter was as strong as but less sensitive to oxygen regulation than the *puf* promoter or that the presence of *bchCA* read-through transcription doubled the strength yet decreased the oxygen sensitivity of the *puf* promoter. These two alternatives were distinguished by directly measuring the contribution of *bchCA* read-through transcription to this sum of activities. The β-galactosidase specific activities in extracts of cells containing pXCAEHQΔ44, in which the *puf* promoter was inactivated, were almost triple that of the wild-type *puf* promoter alone under high-O_2 conditions and were nearly identical to that of the wild-type *puf* promoter alone under low-O_2 conditions. Thus, the degree of oxygen-regulated expression of the *pufB′::lacZ* fusion protein decreased from eightfold to threefold when driven from the *bchCA* promoter alone. Although the activity of the *bchCA* promoter was not as repressed by high oxygen concentrations as was the *puf* promoter, these results clearly demonstrate that the *bchCA* promoter is as strong as the *puf* promoter for driving the expression of a *pufB′::lac′Z* fusion protein. Moreover, because the β-galactosidase activity in extracts of cells containing pXCAEHQ was greater than the sum of the activities in extracts of cells containing pXCA935 plus pXCAEHQΔ44, the presence of read-through transcription from the *bchCA* operon appeared to stimulate *puf* promoter activity, as had been suggested by the RNA studies.

Effects of interrupting read-through transcription on photosynthetic growth. Since the mRNA and *lacZ* gene fusion experiments showed that interposon mutations in the *crtF* and *bchC* genes seemed to affect the expression of downstream photosynthesis genes, it was of interest to test for possible effects on photosynthetic growth of cells.

Cultures of the *crtF* interposon mutant DE324, strain BP503 (which contains a *crtF* point mutation; 22) and the wild-type strain SB1003 were grown aerobically and used as inocula for anaerobic photosynthetic cultures. A representative experiment is shown in Fig. 6A, in which it can be seen that there was a longer lag before photosynthetic growth occurred with the interposon mutant than for the control strains. The length of this lag was variable in different experiments, ranging from 4 to 7 h longer than for the controls. However, once photosynthetic growth began, the exponential growth rates of DE324 cultures were the same as those of the controls.

Analogous experiments were done with the *bchC* interposon mutant CW100. Because expression of *bch* genes is essential for photosynthetic growth, the strains used in these experiments contained plasmid pXCAEHQ, which carries the *bchCA* operon (Fig. 5). Although the plasmid location of the *bchCA* operon complicates interpretation of these experiments, the initiation of photosynthetic growth of *bchCA* interposon mutant cultures (Fig. 6B) was also found to be retarded, although exponential growth rates were nearly equivalent to those of the wild-type controls, consistent with the results obtained with the *crtF* interposon mutant. There were also variations in the lengths of the lag times of the

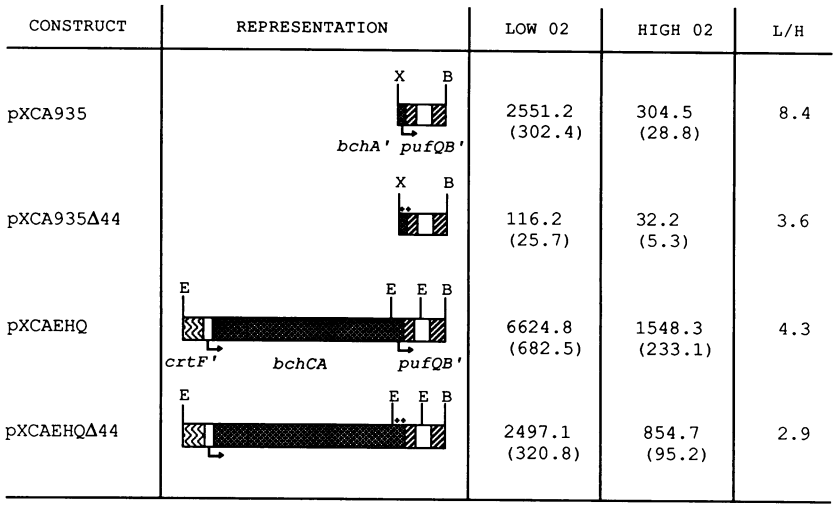

FIG. 5. Contribution of *bchCA* transcription to expression of a *pufB'*::*lac'Z* fusion. Shown are the β-galactosidase specific activities in extracts of cells containing a *pufB'*::*lac'Z* fusion driven as designated by the *puf* and *bchCA* promoters. The *R. capsulatus* segments of DNA fused to the *lac'Z* gene are represented by the hatched boxes, with the structural genes encoded by the *R. capsulatus* inserts shown for each construct. Restriction sites are represented as follows: *Eco*RI (E); *Xho*II (X); and *Bam*HI (B). The 2-nt Δ44 mutation in the *puf* promoter is designated by ++. Arrows indicate the locations of the active promoters in each construct. The β-galactosidase specific activities in extracts of cells grown with high or low aeration are expressed as nanomoles of ONPG cleaved per minute per milligram of protein; the values in parentheses are the standard deviations of at least four independent assays. The ratios of β-galactosidase specific activities obtained from cells grown with low aeration to high aeration are given (L/H).

CW100(pXCAEHQ) cultures, ranging from about 6 to 12 h longer than for the control cultures.

DISCUSSION

Several of the results presented here suggest that the overlapping arrangement of the *R. capsulatus crtEF*, *bchCA*, and *puf* operons contributes to physiologically significant regulation of expression of the *bchCA* and *puf* operons. Although we previously found no evidence for a bottleneck in Bchl synthesis resulting from interposon mutation of the *crtF* gene (in strain DE324) during growth of cultures under low aeration, we did find small amounts of the pigment P668 (indicative of the presence of the Bchl *a* biosynthetic intermediate 2-devinyl-2-hydroxyethyl bacteriochlorophyllide *a*) in cultures grown photosynthetically (18). This contrast with the results reported by Young et al. (22) may have been due to subtle differences in the growth and handling of the cultures.

Nevertheless, the amounts of *bchCA* mRNA 5′ ends due to *crtEF* read-through were undetectable, and initiation of transcription from the *bchCA* promoter was reduced in low-oxygen-grown strain DE324 relative to SB1003 (Fig. 2). This result indicates that elimination of the transcriptional overlap between the *crtEF* and *bchCA* operons did indeed reduce the levels of *bchCA* transcripts such that there were fewer *bchCA* mRNA molecules under high aeration, and there was a failure to maintain high levels of expression during a shift to inducing (low-aeration) conditions. Therefore, a combination of a less active *bchCA* promoter and elimination of the read-through transcripts initiated at the *crtEF* promoter most likely reduces the levels of the BchA protein(s), such that the step catalyzed by this enzyme limits Bchl *a* biosynthesis during photosynthetic growth.

Other experiments showed that the *puf* mRNA 5′ end that results from transcription initiation at the *puf* promoter was present at lower levels in the *crtF* interposon mutant, *R. capsulatus* DE324, than in the wild-type control (Fig. 3). This means that read-through transcription from the *crtEF* and *bchCA* operons appeared to stimulate initiation of transcription from the *puf* promoter. However, it is not possible to conclude from these data how much of this effect was direct and to what extent the *crtF* interposon mutation decreased the activity of the *bchCA* promoter, which in turn decreased the activity of the *puf* promoter.

Similarly, the *puf* promoter was estimated to be approximately 40% less active in the *bchC* interposon mutant, strain CW100, although interruption of read-through transcription did not appear to alter the kinetics of transcription initiation from the *puf* promoter during an aeration shift-down (Fig. 4). It is difficult to compare these different values, because transcripts initiated at the *bchCA* promoter overlap the *puf* operon in strain DE324 but not in CW100. Moreover, the interposon mutant strains used in these experiments originated from different genetic backgrounds. The interposon mutant CW100 was derived from the wild-type strain B10, whereas DE324 was derived from SB1003. Although strain SB1003 is a rifampin-resistant derivative of strain B100, which is a phage-free derivative of strain B10 (14), there are differences between B10 and SB1003 in photosynthetic growth rates and in the amounts of photosynthetic complexes accumulated, which may reflect subtle differences in the regulation of photosynthesis gene expression (18).

In each of the RNA studies described above, S1 nuclease protection experiments were used to generate autoradiograms in which a band due to transcription initiation at a particular promoter could be observed. A decrease in band intensity was interpreted to mean that the particular promoter being studied was less active, assuming that the rate of decay of the mRNA segments detected by the S1 probes in these experiments was the same in the wild-type and mutant cells. However, it could also be argued that the observed decrease in band intensity was due instead to loss of tran-

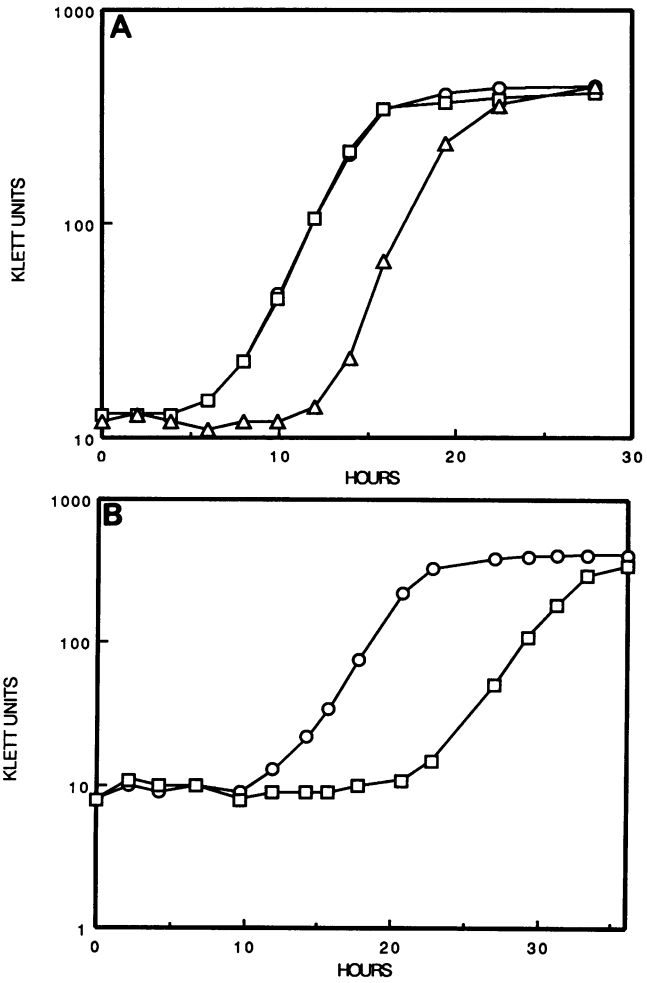

FIG. 6. Kinetics of photosynthetic growth of interposon mutant and wild-type strains. (A) Comparison of *crtF* interposon mutant DE324 (Δ) with wild-type strain SB1003 (○) and *crtF* mutant BP503 (□); (B) comparison of *bchC* interposon mutant CW100 (□) with wild-type strain B10 (○), both containing plasmid pXCAEHQ (see Fig. 5).

scriptional read-through into the region delimited by the probe. For example, if the read-through mRNA were cleaved by an endonuclease that cut at or very near the location of the transcription start site, a processed 5′ end would produce a band of the same length as the 5′ end due to transcription initiation. Although this is conceivable, we believe it is unlikely because such cleavage would have to occur very near both the *bchCA* and *puf* transcription start sites to fit the data.

Because the observed influences on the levels of mRNA 5′ ends that mapped to promoters in these interposon mutant strains relative to the wild-type strains were small, the phenotypic differences attributed to loss of read-through transcription seem likely to be due largely to loss of the overlapping transcript. However, it is noteworthy that the kinetics of *bchCA* transcription initiation during the shift to inducing conditions differed significantly between strains DE324 and SB1003 (Fig. 2), suggesting that read-through transcription from the *crtEF* operon also contributes to regulation of *bchCA* promoter activity in response to lowered oxygen concentrations. This possibility was substantiated by the growth experiments (see below). The precise mechanism by which the apparent stimulation of transcription initiation is accomplished is unknown. It could be due either to the physical proximity of RNA polymerase as it traverses a promoter or perhaps to changes in supercoiling at some distance ahead of an RNA polymerase complex.

The importance of *bchCA* read-through transcription to the production of normal levels of *puf*-encoded polypeptides was shown indirectly by measuring the β-galactosidase specific activities of four plasmids containing the same *pufB′::lacZ* fusion. In these experiments, the relative activity of this fusion was compared in four different contexts: (i) the wild-type *puf* promoter without *bchCA* transcriptional read-through, (ii) the mutant *puf* promoter without *bchCA* transcriptional read-through, (iii) the wild-type *puf* promoter with *bchCA* transcriptional read-through, and (iv) the mutant *puf* promoter with *bchCA* transcriptional read-through (Fig. 5). These results showed that the *bchCA* promoter was equal in strength to the *puf* promoter for driving expression of the *pufB′::lacZ* fusion gene under low-oxygen conditions and was fivefold more active than the *puf* promoter alone under high-oxygen conditions. Therefore, if these plasmid fusions reflect the normal chromosomal context, transcriptional read-through from the *bchCA* operon would result in twice the amount of *puf*-encoded polypeptides that would be expressed from the *puf* promoter alone under inducing conditions and would result in a fivefold increase in levels of aerobically expressed *puf*-encoded polypeptides. An additional contribution to *puf* gene expression from the *crtEF* promoter is indicated by the mRNA experiments shown in Fig. 3.

It is relevant to note that the β-galactosidase specific activity observed when both the *bchCA* and *puf* promoters were active in the same construct (pXCAEHQ; Fig. 5) was approximately 24 to 34% greater than the activity calculated by adding the respective individual contributions from the *bchCA* and *puf* promoters (pXCAEHQΔ44 plus pXCA935; Fig. 5). Therefore, the activity of the *puf* promoter as measured in this way appeared to be stimulated by the presence of transcriptional read-through, in agreement with the S1 nuclease protection results (Fig. 3 and 4).

The biological significance of this overlapping transcriptional arrangement is most clearly revealed by the growth studies (Fig. 6). Although the exponential growth rates of the interposon mutants were not affected at the light intensities used, there were significant deficiencies in the abilities of the interposon mutants to adapt from aerobic respiratory to anaerobic photosynthetic growth conditions. It should be noted that the time scales of the growth experiments were much greater than for the mRNA and *lacZ* fusion experiments, so direct quantitative comparisons of the lengths of lags with levels of mRNAs and β-galactosidase activities cannot be made. Although it would be interesting to determine whether transcription read-through also affects photosynthetic exponential growth rates under very low light intensities, our results imply that the transcriptional organization of these photosynthesis genes evolved in response to natural selection of cells with more efficient coupling of photosynthesis gene expression to changes in oxygen availability.

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