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Transcription of Genes Involved in Bacterio-Opsin Gene Expression in Mutants of a Halophilic Archaebacterium

DIANE LEONG,† HERBERT BOYER, AND MARY BETLACH*

Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94143

Received 29 April 1988/Accepted 22 July 1988

Recent studies on the regulation of the bacterio-opsin (*bop*) gene of the archaebacterium *Halobacterium halobium* suggest that the *brp* and putative *bat* genes are involved in *bop* gene expression or purple membrane assembly. These two genes are located 526 and 1,602 base pairs, respectively, upstream of the *bop* gene and are both transcribed in the opposite orientation to the *bop* gene. Transcription of the *bop*, *brp*, and putative *bat* genes was characterized in the wild type, 11 Bop mutants, and a Bop revertant by using a series of RNA probes. Quantitation of the relative mRNA levels for these three genes in the wild type revealed that the *brp* and *bat* transcripts are present at approximately 2 and 4%, respectively, of *bop* mRNA levels under the growth conditions used. Northern (RNA) blot analysis of Bop mutants indicated that insertions in the *brp* gene affect expression of the putative *bat* gene. In addition, deletion of most of the *bat* gene resulted in virtually undetectable levels of *bop* and *brp* mRNAs. These and other results lead us to propose that (i) *brp* gene expression can affect *bat* gene expression and (ii) the putative *bat* gene is involved in activating *bop* and *brp* gene expression.

The protein bacterio-opsin complexed with retinal functions as a light-driven proton pump in the purple membrane of the archaebacterium *Halobacterium halobium* (19). Purple membrane exists at a basal level under aerobic growth conditions and is present at a four- to fivefold higher level when halobacterial cells are growing under conditions of low oxygen tension in the presence of light (11). Formation of the purple membrane involves the processing of 13 amino-terminal residues and 1 carboxy-terminal residue from a precursor to form the mature bacterio-opsin molecule (6) as well as some level of coordination between bacterio-opsin and retinal syntheses (20, 21).

We have previously described a number of features of bacterio-opsin (*bop*) gene expression. Preliminary experiments suggested that the *bop* gene may be regulated in part at the transcriptional level (2, 14). In addition, another gene which affects *bop* gene expression or purple membrane formation or both has been identified. This gene, called *brp*, is located 526 base pairs (bp) upstream of the *bop* gene and is transcribed in the opposite orientation (1). Transcription initiation sites for both *bop* and *brp* genes are very close to or coincident with the start codons of these genes. DNA homologies up to 120 bp upstream of the transcription initiation sites of the *bop* and *brp* genes (1) appear to be unique to these two genes and may constitute regulatory sequences. Moreover, a study of eight mutants of a Bop revertant (reIV-41) indicated that the integrity of the entire 526 bp separating the two genes is important for *bop* gene expression (14). Whether the *brp* gene exerts a direct or indirect influence on *bop* gene expression is unknown.

The accompanying paper (7) provides evidence for an additional level of regulatory complexity in that another region of DNA appears to be involved in *bop* gene expression. Analysis of three additional Bop mutants (M86, W105, and W109) indicated that the mutations responsible for the Bop$^-$ phenotype in these mutants occurred in a region of DNA up to 3,800 bp upstream of the *bop* gene and immediately downstream of the *brp* gene. Characterization of this region of DNA led us to propose that it contains a putative gene (designated *bat* for bacterio-opsin activator gene) which affects *bop* gene expression (7). The predicted secondary structure of the protein encoded by this gene is indicative of a soluble alpha-beta-type protein, in contrast to the hydrophobic protein structure predicted for the putative *brp* protein. In order to elucidate the functional relationships between this recently discovered gene and the *bop* and *brp* genes, we have determined the transcript sizes and levels expressed from these genes in the wild type, a Bop revertant, the three mutants mentioned above, and eight other Bop mutants. Transcripts have been characterized in cultures grown under aerobic conditions which result in expression of basal levels of purple membrane in the wild type. The results presented here suggest that expression of the *brp* gene does not directly affect *bop* gene expression, whereas expression of the *bat* gene activates expression of the *bop* and *brp* genes.

MATERIALS AND METHODS

Materials. [α-^{32}P]CTP (>400 Ci/mM) was obtained from Amersham Corp., Arlington Heights, Ill. Restriction endonucleases and DNA ligase were obtained from New England BioLabs, Beverly, Mass. The SP6 riboprobe kit was obtained from Promega Biotec, Madison, Wis.

Halobacterial strains and growth conditions. Bop mutants were derived either from strains II-2 and II-7 (16), which are bacterioruberin-deficient derivatives of wild-type *H. halobium* NRC817, or from strain R1, which is a vacuole-deficient derivative of wild-type *H. halobium* NRC34020. Bop mutants are listed and described in Table 1. Halobacterial cells used for RNA isolation were grown in medium described previously (13) and under aerobic conditions of ambient light and oxygen tension. Cultures were typically grown at 41°C with shaking at 100 rpm and with 25 to 50 ml of culture in a 250-ml flask.

* Corresponding author.
† Present address: Advanced Genetic Sciences, Oakland, CA 94608.

TABLE 1. Bop mutants used in this study

Strain	Phenotype	Description	Insertion (location in or near *bop* gene[a])	Reference
II-7	Bop^+	Parent of IV-4	None	14, 16
IV-4	Bop^-	Bop mutant of II-7	ISH24 (3′ end of *brp* gene)	14
reIV-41	Bop^+	Revertant of IV-4	ISH24, "ISH25" (3′ end of *brp* gene)	14
IV-8	Bop^-	Bop mutant of II-2	ISH1 (in *bop* gene)	17
M18[b]	Bop^-	Bop mutant of R1	ISH2 (5′ end of *brp* gene)	1, 15
M86	Bop^-	Bop mutant of R1	Deletion (upstream of *bop* gene)	This study
M89	Bop^-	Bop mutant of R1	ISH2 (5′ end of *brp* gene)	1, 15
M135	Bop^-	Bop mutant of R1	ISH2 (5′ end of *brp* gene)	1, 15
M138	Bop^-	Bop mutant of R1	ISH2 (5′ end of *brp* gene)	1, 15
W1[c]	Bop^-	Bop mutant of reIV-41	ISH2 (between *bop* and *brp* genes)	14
W11	Bop^-	Bop mutant of reIV-41	ISH2 (between *bop* and *brp* genes)	14
W105	Bop^-	Bop mutant of reIV-41	ISH2 (in *Pst*I 1.1-kbp fragment)	This study
W109	Bop^-	Bop mutant of reIV-41	ISH26 (in *Pst*I 1.1-kbp fragment)	This study

[a] The location of ISH elements and "ISH25" in these mutants relative to the *bop* gene and flanking DNA is shown in Fig. 3.
[b] M mutants were the gift of D. Oesterhelt and were isolated as described previously (10).
[c] W mutants also retain insertions of reIV-41.

Construction of RNA probes. Gel-purified restriction fragments were cloned into pSP64 or pSP65 by standard procedures (8). Transformants were screened by restriction analysis of plasmid DNA to identify the appropriate fragment and determine its orientation. Purified supercoiled plasmid DNA from the desired clones was linearized by digestion either at a site in the polylinker beyond the end of the inserted DNA or at a site within the insert. RNA probes were synthesized in vitro by using an SP6 polymerase riboprobe kit (Promega Biotec) (9). The lengths and orientations of the transcribed regions detectable by the RNA probes used in this study are indicated in Fig. 1.

Northern (RNA) blot analysis. Total halobacterial RNAs from the wild type and mutants were isolated by extraction of cell pellets with guanidinium isothiocyanate and ultracentrifugation through a CsCl gradient as described by Chirgwin et al. (3). RNA concentrations were determined spectrophotometrically. Equal amounts of purified RNA from each strain were electrophoresed on vertical 1.5% formaldehyde agarose gels under conditions described previously (1). Two

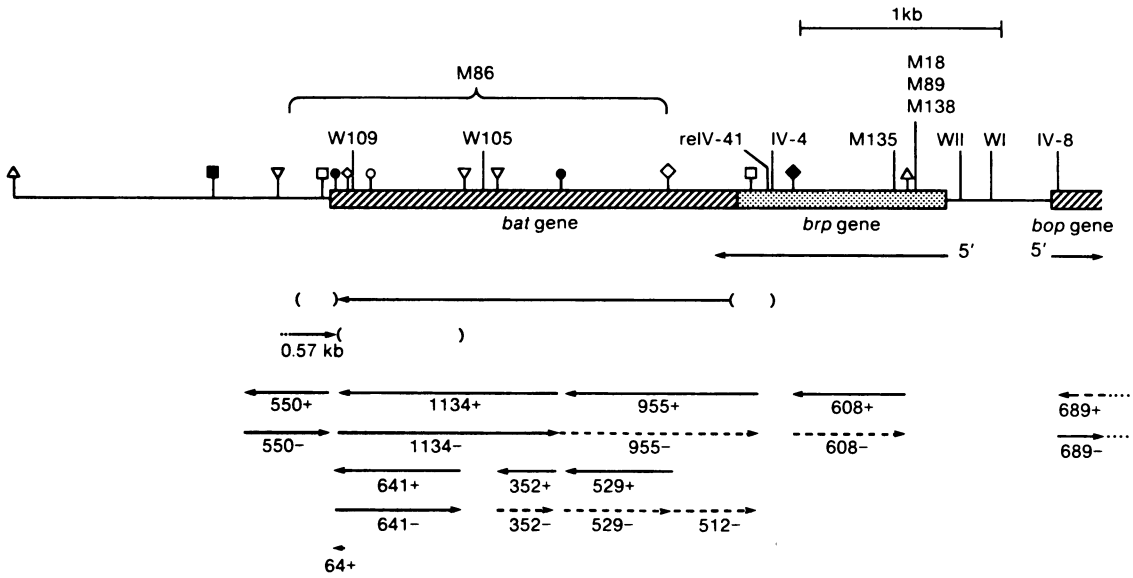


FIG. 1. Restriction map illustrating locations of alterations in Bop mutants, transcripts correlated with the *bop* gene and flanking regions, and RNA probes used for Northern analysis. The *bat* gene, *brp* gene, and part of the *bop* gene are indicated by the hatched and stippled bars. Restriction sites are indicated as follows: □, *Bam*HI; ●, *Pst*I; △, *Nru*I; ▽, *Mlu*I; ○, *Ava*I; ■, *Bgl*I; ◇, *Bss*HII; ◆, *Acc*I. Not all restriction sites are shown. The integration sites of insertions in Bop mutants IV-4, IV-8, M18, M89, M135, M138, revertant reIV-41, and mutants of the revertant W1, W11, W105, and W109 are shown by vertical lines above the map. The deletion in Bop mutant M86 is denoted by a bracket above the map. Arrows below the map indicate the direction and extent of transcription. Termini for the *bat* transcript and the small 0.57-kb transcript near the 3′ terminus of the *bat* gene have not been precisely determined but are probably located within the bracketed region indicated. The dotted line at the 3′ terminus of the small 0.57-kb transcript indicates that a start codon for the corresponding open reading frame has not yet been determined. The array of arrows beneath the map illustrates the lengths and orientations of the transcribed regions detectable by the RNA probes. The numbers below the arrows indicate the sizes of the probes. Probes capable of detecting transcription proceeding in the same (+) and opposite (−) orientations as *brp* gene transcription are indicated. Arrows used to denote the transcribed regions detectable by the *bop* probes are partially dotted because not all of the *bop* gene is shown. Solid arrows indicate probes which detected transcripts, while broken arrows indicate probes which did not detect any transcripts.

RNA ladders consisting of 9.5-, 7.5-, 4.4-, 2.4-, 1.4-, and 0.24-kilobase (kb) RNAs and 1.7-, 1.52-, 1.28-, 0.78-, 0.53-, 0.40-, 0.28-, and 0.16-kb RNAs provided molecular weight standards (Bethesda Research Laboratories, Gaithersburg, Md.). The fractionated RNAs were transferred to nitrocellulose filters (Schleicher & Schuell, Keene, N.H.) which were baked for 2 h and prehybridized at 42°C overnight as described previously (1). The filters were dried and prehybridized at 65°C in the same type of hybridization mix for several hours. The blots were hybridized with 1×10^6 to 5×10^6 cpm of RNA probe at 65°C and washed at 65°C in 0.1× SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate. Autoradiography of the washed blots was done as described previously except without enhancing screens (1). The relative levels of mRNAs from the mutants were determined by scanning the autoradiographs with an Ephortec Joyce Loebl densitometer and comparing the densitometric peaks obtained with those obtained for wild-type RNA present on the same autoradiograph.

RNA quantitation. To obtain an estimate of the relative levels of the *bop*, *brp*, and *bat* mRNAs in the wild type, RNA probes (Fig. 1; 689−, 608+, and 529+, respectively) of approximately equivalent specific activity were synthesized as follows. Equal concentrations of DNA templates of similar size and cytosine content were used in the riboprobe reactions, resulting in comparable levels of incorporation of [α-^{32}P]CTP. The size distribution of the labeled RNAs was examined by electrophoresing a sample of each probe on a 6% acrylamide sequencing gel and autoradiographing the wet gel. Each of the three probes synthesized had a similar size distribution.

Slot blots of twofold dilutions of wild-type total RNA were prepared by using a slot-blotter (Minifold II; Schleicher & Schuell) as follows. Purified RNA in a 7.5% formaldehyde, 6× SSC solution was heated at 65°C for 15 min and then cooled to room temperature. RNA was then filtered onto nitrocellulose (mRNC; Schleicher & Schuell) prewet with 6× SSC and washed with 6× SSC. The filter was dried and baked for 2 h. The conditions used for slot blotting had been tested earlier to confirm that nonspecific hybridization was not significant. The blots were hybridized with equivalent amounts of each RNA probe and washed under the same conditions as described above for Northern blot analysis. The autoradiographs were scanned by using an Ephortec Joyce Loebl densitometer, and the resulting calculations were adjusted for the cytosine content of the probes.

RESULTS

Transcript sizes in the wild type. A series of 17 RNA probes (Fig. 1) was used to determine the sizes of the *bop*, *brp*, and *bat* transcripts in the wild type. Each of the probes was hybridized to Northern blots of total halobacterial RNA isolated from wild-type cells grown as described in Materials and Methods. A Northern blot including wild-type total RNA hybridized with a *bat* gene-specific probe is shown in Fig. 2.

The major wild-type *bop* transcript had a mobility corresponding to 830 nucleotides on Northern blots, in agreement with a previous report (5). The DNA template for the *brp* gene-specific probe was constructed without 286 bp of the 3′ terminus of the *brp* gene, since this region of the *brp* gene may code for an overlapping *bat* transcript. The stop codon of the *brp* gene is only 1 bp out of frame with the start codon of the *bat* gene (7). The *brp* gene-specific probe (Fig. 1, 608+) hybridized to a heterogeneous population of transcripts similar to that seen previously with nick-translated probes (1). The heterogeneity of the *brp* transcript appears to arise at the 3′ terminus, since the 5′ terminus has been determined to be unique by primer extension and cDNA sequencing and is coincident with the ATG start codon of the *brp* gene (1). The largest and major species in the population was determined to be 1.2 kb in size. The location of the *brp* transcript relative to the *brp* gene is shown in Fig. 1.

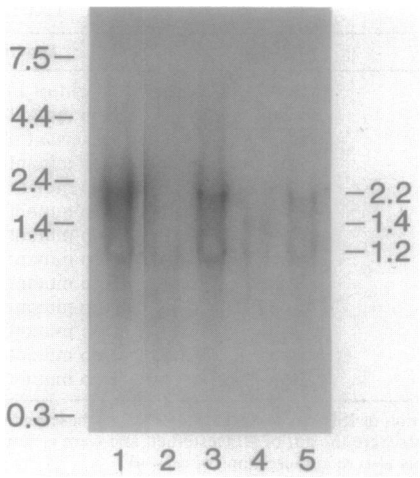
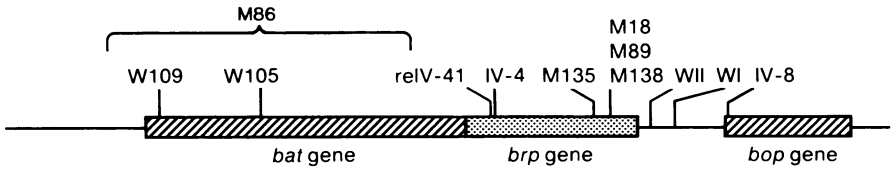


FIG. 2. Northern blot analysis of the *bat* transcript. A Northern blot of total *H. halobium* RNA was hybridized with a *bat* gene-specific probe (1134+). Lanes: 1, II-7 (wild type in the *bop* gene region); 2, IV-4; 3, reIV-41; 4, W105; 5, W109. Lane 1 was exposed for 5 h, whereas the remainder of the lanes were exposed for 3 days, since the *bat* transcript is much more abundant in the wild type than in the other strains shown (see Fig. 3). The 2.2-kb wild-type-sized *bat* transcript, the 1.4-kb truncated *bat* transcript in mutant W105, and the 1.2-kb transcript of unknown origin which hybridizes with *bat* gene-specific probes are indicated on the right. RNA size markers are indicated on the left in kilobases.

The probes for analysis of *bat* gene transcription (Fig. 1) were constructed so as to detect transcription in either direction. In the direction opposite to that of the *brp* gene, the following probes corresponding to the 3′ terminus of the *bat* gene hybridized to a transcript with a mobility of 0.57 kb in Northern blots: the *Pst*I 1134−, the *Mlu*I-*Pst*I 641−, and the *Pst*I-*Bgl*I 550− probes (Fig. 1). Probes corresponding to the 5′ terminus and the central part of the *bat* gene up to bp 1196 did not detect the 0.57-kb transcript. This transcript correlates with an open reading frame for which the start codon has not yet been determined (7). The open reading frame was observed to be at least 381 bp long and does not overlap the *bat* gene.

Transcription specific to the *bat* gene was detected in the same orientation as that from the *brp* gene (Fig. 2, lane 1). For the reason discussed above, some of the *bat* gene probes were designed to lack those regions which might be transcribed as part of both the *brp* and *bat* transcripts (Fig. 1). The *bat* gene-specific RNA probes hybridized to two major RNA transcripts with apparent sizes of 1.2 and 2.2 kb (Fig. 2, lane 1). The pattern of hybridization observed with the *bat* probes remained the same even when the blots were hybridized at 65°C, which is 5 to 10°C higher than the recommended hybridization temperature (9). The 2.2-kb transcript most likely represents the *bat* transcript, since this transcript is truncated in the Bop mutant W105 (Fig. 2, lane 4) by an amount which suggests that the ISH2 copy located within



Strain	Transcripts		Transcripts		Transcripts	
	Size (kb)	Amount[a]	Size (kb)	Amount[a]	Size (kb)	Amount[a]
II-7 (wild-type)	2.2	100%	1.2	100%	.83	100%
IV-4	—	0	—	0	—	0
relV-41	2.2	20%	0.87	75%	.83	100%
IV-8	2.2	100%	1.2	100%	—	ND[b]
M18	2.2	<1%	1.2	<1%	.83	23%
M86	—	0	—	0	.83	<1%
M89	2.2	9%	—	0	.83	5%
M135	2.2	<1%	—	0	.83	6%
M138	—	0	—	0	.83	10%
WI	2.2	+[c]	0.87	12%	.83	<1%
WII	2.2	+[c]	0.87	7%	.83	<1%
W105	1.4	5%	—	0	—	0
W109	2.2	7%	—	0	—	0

[a] % of wild-type

[b] ND, not determined

[c] +, present but not quantitated

FIG. 3. Summary of transcript sizes and amounts in the wild type, revertant relV-41, and various Bop mutants and mutants of the revertant. The map located above the tabulated data illustrates the coding regions of the *bop*, *brp*, and *bat* genes with hatched and stippled bars and the location of the insertion integration sites in various mutants with vertical lines above the map. The deletion in mutant M86 is indicated with a bracket above the map. Sizes and amounts of transcripts are located below the corresponding genes on the map.

the *bat* gene of this mutant contains a transcriptional terminator or RNA-processing signal. The location of the 2.2-kb *bat* transcript relative to the *bat* gene is shown in Fig. 1.

Probes spanning the entire 2,022-bp *bat* gene detected the 1.2-kb transcript, suggesting that homology to the gene encoding the 1.2-kb transcript exists at a number of separate sites in the *bat* gene. The 1.2-kb transcript detected by the *bat* probe does not appear to be the *brp* mRNA, since it is discrete rather than heterogeneous and is not truncated in the revertant relV-41, while the *brp* transcript is shortened to 0.87 kb. No hybridization to other genomic DNA fragments was seen on Southern blots with *bat* gene probes. The origin of this 1.2-kb transcript remains to be determined.

Transcript levels in the wild type. In order to estimate the amounts of *bop*, *brp*, and *bat* transcripts made in the cell, RNA probes of comparable specific activity were synthesized for each gene. Equivalent amounts of each probe were hybridized under identical conditions with slot blots containing dilutions of wild-type total RNA. Densitometry of the resulting autoradiographs and correction for the cytosine content of the probes gave a relative estimate of the levels of each transcript in the wild type. The *brp* mRNA was present at approximately 2% of the level of the *bop* mRNA, while the *bat* transcript was present at approximately 4% of the level of *bop* mRNA.

Transcript sizes and levels in Bop mutants. In order to elucidate the interaction between the *bop*, *brp*, and *bat* genes, we examined the pattern of hybridization to *bop*, *brp*, and *bat* probes in Northern blots of total RNA from the wild type, a Bop revertant, and 11 Bop mutants (Table 1). The sizes and amounts of the three transcripts in these 13 strains are summarized in Fig. 3. In most of the mutants, the regions containing the *brp* gene and the *bat* gene were examined for transcription occurring in both orientations. In the direction opposite to *brp* and *bat* transcription, the only transcript observed was the *bop* mRNA and the 0.57-kb transcript described for the wild type. The levels of the 0.57-kb transcript in the various mutants have yet to be determined.

Mutants M86, M18, M89, M135, and M138 are derived from strain R1, which is wild type in the *bop* gene region. No *brp* or *bat* transcripts and <1% of wild-type levels of *bop* mRNA were detected on Northern blots of mutant M86 total RNA (Fig. 3). Thus, in M86 the 1,883-bp deletion of the *bat* gene is associated with greatly reduced expression of the *bop* and *brp* genes. Mutants M18, M89, M135, and M138 each contain a 520-bp ISH2 element near the 5′ terminus of the *brp* gene (1, 15). All of these mutants, with the exception of M89, contain the ISH2 element in the same orientation in the *brp* gene (15). Northern hybridization revealed little or no *brp* or *bat* transcripts, but *bop* mRNA ranging from 5 to 23% of wild-type levels was detected in these mutants (Fig. 3). Thus, although the initial effect of the insertion is presumably on *brp* gene expression, reduced levels of *bop* and *bat* transcripts are also observed. The low level of *bop* mRNA observed in these mutants and in M86 is apparently not sufficient to confer a Bop^+ phenotype.

Mutant IV-8 is derived from strain II-2, which is wild type in the *bop* gene region. IV-8 contains an ISH1 insertion element within the coding region of the *bop* gene, 7 bp from the start codon (13, 15). This mutant is one of 21 mutants isolated to date that contain an ISH1 element at this specific site in the *bop* gene (12). In agreement with a previous study of similar mutants (18), no *bop* mRNA was detected on Northern blots of IV-8 total RNA (2). Wild-type levels of the *brp* transcript were observed, as published previously (2). Wild-type levels of the *bat* transcript were also observed (Fig. 3).

Mutant IV-4 contains a 3.0-kbp ISH24 insertion element

near the 3′ terminus of the *brp* gene (1, 15). Northern blot analysis of this mutant revealed no observable *bop*, *brp*, or *bat* transcripts (Fig. 2, lane 2 and Fig. 3), suggesting that a single insertion affects the expression of all three genes.

Bop revertant reIV-41, derived from IV-4, contains a 588-bp insertion of DNA (which is not an insertion element) distal to the ISH24 insertion in IV-4 (15). This strain makes wild-type levels of purple membrane (unpublished observations). Wild-type levels of *bop* mRNA were detected in the revertant, while *brp* and *bat* transcripts were detected at approximately 75 and 20% of the level of the wild type, respectively (Fig. 2, lane 3 and Fig. 3). As observed previously (1), the revertant *brp* transcript was truncated and more discrete compared with that of the wild type. This transcript displayed a mobility corresponding to 0.87 kb. The 5′ terminus of the *brp* transcript is the same in both the revertant and the wild type (R. Shand, University of California, San Francisco, unpublished data), implying that the *brp* transcript has a different 3′ terminus in the revertant than in the wild type and most likely terminates within the ISH24 insertion.

Bop mutants W105, W109, W1, and W11 are derived from the revertant rather than from a wild-type strain (7, 14). They each contain three separate insertions: the ISH24 element present in Bop mutant IV-4, the additional 588-bp insertion in the Bop revertant, and a third insertion. The third insertion in mutant W105 is an ISH2 copy located at bp 1239 of the *bat* gene (7). No detectable *bop* mRNA or *brp* transcript were observed on Northern blots for mutant W105 (Fig. 3). The *bat* transcript was truncated with a mobility corresponding to 1.4 kb instead of the 2.2 kb of the parental revertant (Fig. 2, lane 4). Assuming that the *bat* transcript in W105 terminates within the ISH2 element, the observed length of the transcript is consistent with the length of the *bat* DNA sequence.

Mutant W109 has an ISH26 copy at bp 1889 of the *bat* gene (7). As in mutant W105, no *bop* mRNA and a trace of *brp* transcript was observed (Fig. 3). A *bat* transcript of the same size and in an approximately similar amount to that of the parental revertant was detected for mutant W109 (Fig. 2, lane 5). Presumably, the *bat* transcript ends at a site within ISH26 such that it has the same approximate size as the revertant *bat* transcript. In both mutants W105 and W109, insertions in the *bat* gene appear to affect expression of the *bop* and *brp* genes.

In W1 and W11, the insertions are ISH2 elements located in the 526-bp region between the *bop* and *brp* genes. The insertion in W11 is located 449 bp upstream of the *bop* gene and within a 120-bp region containing putative promoter or regulatory sequences or both, upstream of the *brp* gene (14). This 120-bp region is partially homologous to a corresponding region upstream of the *bop* gene (1). The insertion in W1 is located 300 bp upstream of the *bop* gene and is outside both of the 120-bp regions (14). Reduced levels of *bop* and *brp* mRNA relative to the parental revertant were observed for these mutants, whereas levels of the *bat* transcript were unaffected (Fig. 3). At least in W1 and W11, insertions in the region between the *bop* and *brp* genes affect *bop* and *brp* gene expression.

DISCUSSION

As described in the accompanying paper (7), mutations in the *brp* or the *bat* genes result in a Bop$^-$ phenotype. Hence, these two genes are most likely involved in *bop* gene expression or purple membrane assembly or both. Northern blot analysis of the *bop*, *brp*, and *bat* genes in a series of Bop mutants provided information on the interrelated expression of these three genes.

bop gene expression is not required for *brp* or *bat* gene expression, as suggested by the transcriptional pattern of Bop mutant IV-8. The presence of an ISH1 element near the 5′ terminus of the *bop* gene in IV-8 effectively eliminates *bop* mRNA but has no effect on the *brp* and *bat* transcripts.

Analysis of the transcriptional patterns of Bop mutants M18, M89, M135, and M138 suggests that *bat* transcript levels are affected by *brp* transcription. In these mutants, insertion of an ISH2 element in either orientation near the 5′ terminus of the *brp* gene is associated with few or no *brp* transcripts, suggesting that ISH2 contains a transcriptional terminator or RNA-processing signal active in both orientations. Additional evidence for the presence of a transcriptional terminator or RNA-processing signal in ISH2 comes from analysis of mutant W105, in which an ISH2 insertion results in a truncated *bat* transcript. In agreement with these results, DasSarma et al. observed smaller *bop* transcripts in Bop mutants SD12 and L33, which contain ISH2 near the middle of the *bop* gene (4). In mutants M18, M89, M135, and M138, the very low amount of *bat* transcript observed suggests that *brp* gene expression can affect the level of *bat* gene expression. At the same time, the data from these mutants indicated that it is possible to have some level of *bop* mRNA in the absence of *brp* and *bat* gene expression.

The analysis of transcription in mutant IV-4 and revertant reIV-41 provided information relevant to the mechanism by which *brp* gene expression could affect *bat* gene expression. The insertion of an ISH24 element 203 bp upstream of the start of the *bat* gene in mutant IV-4 is correlated with loss of the *bat* transcript as well as of the *bop* and *brp* transcripts. Transcription from the *bat*, *brp*, and *bop* genes is at least partially restored in the revertant by virtue of the additional 588-bp "ISH25" insertion found in the revertant. One possibility is that the *bat* promoter is located somewhere within this region and is disrupted in mutant IV-4. Alternatively, the *brp* and *bat* genes may be cotranscribed to form one long mRNA which is rapidly processed and thus not observed on Northern blots. The ISH24 insertion may cause premature termination of such a *bat-brp* cotranscript. In either case, the "ISH25" insertion in the revertant presumably contains a gratuitous promoter which allows reinitiation of *bat* transcription. Determination of the location and structure of the 5′ terminus of the *bat* transcript would aid in determining how *brp* gene expression affects *bat* gene expression. However, preliminary attempts to determine the 5′ terminus of the *bat* transcript have been unsuccessful due to the low abundance of this transcript.

Analysis of mutants M86, W105, and W109 suggests that the *bat* gene product is involved in activating *bop* and *brp* gene expression. In mutant M86, deletion of most of the *bat* gene (7) results in an absence of *brp* and *bat* transcripts and an extremely low level of *bop* mRNA. The deletion in M86 begins approximately 350 bp beyond the coding region of the *brp* gene and thus is not likely to have a direct effect on *brp* gene expression. Mutants of the revertant, W105 and W109, provide more evidence that the *bat* gene is involved in activating expression of the *bop* and *brp* genes. In both of these mutants, an insertion within the *bat* gene is correlated with an absence of *bop* and *brp* mRNAs.

In the W1 and W11 mutants of the revertant, the presence of an insertion sequence located up to 449 bp upstream of the *bop* gene and up to 226 bp upstream of the *brp* gene appears to affect the expression of the *bop* and *brp* genes but not of

the *bat* gene. The transcriptional pattern of these mutants suggests that the presence of the *bat* transcript is not sufficient to activate *bop* gene expression and that the region between the *bop* and *brp* genes is also involved in *bop* gene expression.

While our data does not rule out the existence of *cis*-acting elements within the *bat* gene, a model which is consistent with the data invokes a *bat* gene product which acts in *trans* to activate *bop* and *brp* gene expression. A *trans*-acting *bat* gene product could affect transcription initiation by binding to the 526-bp region between the *bop* and *brp* genes. Alternatively, the *bat* gene product could be required to stabilize the *bop* and *brp* transcripts. A consequence of this model is that the *bat* gene would be partially autoregulated, since *bat* gene expression would be affected by *brp* gene expression, while the *brp* gene in turn requires the *bat* gene product in order to be expressed.

The *brp* gene was described previously as a gene possibly affecting *bop* gene expression or aiding in assembly of the purple membrane (1, 2). The evidence presented above suggests that mutations in the *brp* gene affect *bop* gene expression indirectly by reducing levels of *bat* transcripts, whereas the *bat* gene product is more directly involved in activating *bop* gene expression. Whether or not the putative *brp* protein has a direct role in purple membrane assembly remains to be determined.

The transcriptional complexity demonstrated by the *bop*, *brp*, and *bat* gene cluster may reflect the complex regulation of purple membrane synthesis in the halobacteria. Purple membrane synthesis involves a coordination between *bop* gene expression and retinal biosynthesis (20, 21) and is regulated by environmental factors such as light and oxygen (11). As discussed in the accompanying paper (7), it is not likely that the *bat* or *brp* genes are involved in mediating the coordination between bacterio-opsin and retinal synthesis. Future experiments using recently established inducing conditions for purple membrane synthesis will determine whether or not the *bat* and *brp* genes are involved in the regulation of the *bop* gene by light and oxygen.

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