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Bacterio-opsin mRNA in wild-type and bacterio-opsin-deficient *Halobacterium halobium* strains

(*in vitro* capping/mRNA sequence analysis/purple membrane/bacteriorhodopsin/transposable elements)

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ABSTRACT **We have examined transcripts corresponding to the *Halobacterium halobium* bacterio-opsin (*BO*) gene in wild-type and in *BO*-deficient mutant strains containing the insertion elements ISH1 or ISH2 in the *BO* gene. BO mRNA from the wild-type strain was purified by hybrid selection using single-stranded cDNA. Labeling by vaccinia virus capping enzyme and [α-^{32}P]GTP showed that it contains the 5′-terminal nucleotide of the primary transcript. Sequence analysis showed that transcription begins only two nucleotides upstream of the initiator codon for BO. Two species of BO mRNA were found; the major species has a ragged 3′ terminus ≈45 nucleotides downstream from the terminator codon for BO, while the minor species is about 170 nucleotides longer at the 3′ end. Analysis of the transcripts in several *BO* gene mutant strains by RNA gel-transfer hybridization showed that (*i*) mutants with ISH1 insertions within the NH_2-terminal coding region of the gene contain no detectable transcripts, (*ii*) mutants with ISH2 near the middle of the coding region of the gene contain multiple incomplete transcripts, and (*iii*) a mutant that is partially *BO* deficient due to an insertion of ISH2 100 base pairs upstream of the site of initiation of transcription contains a decreased level of BO mRNA.**

Halobacterium halobium contains a specialized membrane, the purple membrane, that carries out light-dependent vectorial proton translocation (1). The purple membrane contains a single protein, bacterio-opsin (BO), that is covalently attached to retinal. The amino acid sequence of BO has been determined by analysis of both the protein and the gene (2–4). It has been shown further that high-frequency spontaneous *BO*-deficient mutants of *H. halobium* are caused by insertion of transposable elements into the *BO* gene (5, 6). Of the 11 mutants characterized, 6 contain a 1.1-kilobase pair insertion element (ISH1) at a single site in the NH_2-terminal coding region of the *BO* gene. The other 5 mutants contain a 520-base pair (bp) insertion element (ISH2) at multiple sites, 4 within and 1 upstream of the coding region of the gene. The latter mutant (SD19) showed partial inactivation of the *BO* gene. A map of the sites of the insertions in the mutants is shown in Fig. 1.

Little is known concerning transcription in *H. halobium*. Previous studies (1–6) on BO, the *BO* gene, and the characterization of *BO*-deficient mutants made it of interest to study the *BO* gene-specific transcripts in the above cells. We have now purified and characterized BO mRNA present in the wild-type *H. halobium* strain. This RNA, which extends only two nucleotides upstream of the initiator codon for BO, contains the 5′-terminal nucleotide of the primary transcript as shown by capping with vaccinia capping enzyme. The major 3′ end is ragged and extends ≈45 nucleotides downstream from the terminator codon for BO. In addition, a minor transcript, which is ≈170 nucleotides longer at the 3′ end, was characterized. We have also studied the *BO* gene transcripts present in five of the *BO*-deficient mutants and find that (*i*) strains with insertions of ISH1 in the NH_2-terminal coding region of the *BO* gene contain no detectable transcripts, (*ii*) strains with insertions of ISH2 near the middle of the gene contain incomplete transcripts, and (*iii*) the strain with ISH2 inserted 100 bp upstream of the site of initiation of transcription (SD19) contains BO mRNA of the same sizes and identical 5′-terminal sequence to those present in the parent strain but in decreased amounts. This paper documents these findings.

MATERIALS AND METHODS

Materials. Vaccinia virus capping enzyme and rabbit globin mRNA were purchased from Bethesda Research Laboratories. Cap analogs G(5′)ppp(5′)N (N represents guanosine, adenosine, cytidine, or uridine) were purchased from P-L Biochemicals. Other enzymes, ^{32}P-labeled nucleotides, and chromatographic materials were obtained as described (5, 7).

***H. halobium* Strains.** The strains used have been described (5, 6). S9 is the parental strain; SD19 is partially deficient in *BO*; and SD10, SD17, SD12, and L33 are completely *BO* deficient.

Probes for Hybridization. The cDNA fragment corresponding to nucleotides 15–76 at the NH_2-terminal coding region of the *BO* gene was used for purification of BO mRNA and as a probe for the *BO* gene in RNA gel-transfer hybridization. The cloning of this cDNA into M13mp7 using *Bam*HI linkers has been described (3, 4). The RNA gel-transfer hybridization probe specific for the 3′-flanking region is a 340-bp *Xor* II/*Bst*EII fragment, 80–420 bp downstream from the *BO* structural gene (Fig. 1). This was cloned into the *Bam*HI site of pBR322 using *Bam*HI linkers (8). The probe used for nuclease S1 mapping of the 3′ end of BO mRNA is a 350-bp *Mst* I/*Bgl* I fragment (Fig. 1) that includes 80 bp from the COOH-terminal coding region of the *BO* gene and 270 bp of 3′-flanking sequence.

Purification of BO mRNA. This was accomplished by using a procedure for hybrid selection developed by C. Guthrie (personal communication). Single-stranded M13mp7 virion DNA containing cloned BO cDNA was bound to a pure grade nitrocellulose filter (50 μg on a 1.3-cm-diameter filter). The filter-bound DNA was hybridized overnight with 0.5 ml of *Escherichia coli* RNA (200 μg/ml)/50% formamide/5× NaCl/Cit (1× NaCl/Cit is 0.15 M NaCl/0.015 M sodium citrate) at 42°C to saturate nonspecific binding sites. The filter was then hybridized overnight under the same conditions with 700 μg of crude *H. halobium* S9 RNA (3, 9) in 0.3 ml. Nonspecifically bound RNA was removed by three washes with 0.2× NaCl/Cit (0.5 ml each) for 1 hr at 42°C. Bound RNA was then eluted with H_2O (0.25 ml) at 90°C for 5 min. Approximately 0.2% RNA applied to the filter was recov-

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Abbreviations: BO, bacterio-opsin; ISH, insertion sequence from *Halobacterium halobium*; bp, base pair(s).

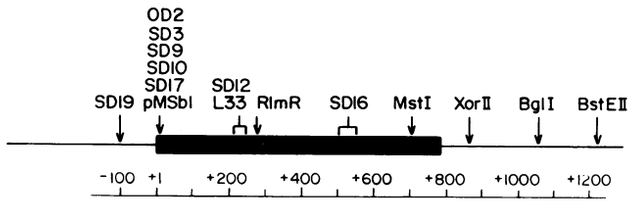

FIG. 1. A partial restriction map of and the sites of insertion of ISH elements into the *BO* gene region (5, 6). Strains SD19, L33, RlmR, and SD16 contain insertions of ISH2 in one orientation; strain SD12 contains ISH2 inserted in the opposite orientation. Strains OD2, SD3, SD9, and SD17 contain ISH1 in one orientation; strain SD10 and the cloned gene in pMSb1 contain ISH1 inserted in the opposite orientation but at the same site. The coding region of the *BO* gene is shown as a black bar. The scale below is in bp.

ered as bound RNA (estimated by ethidium bromide staining on agarose gels using rabbit globin mRNA as standard).

Labeling of RNA at the 5′ End. *Vaccinia virus capping enzyme.* The reaction mixture (25 μl) contained ≈0.1 μg of the purified BO mRNA in 50 mM Tris·HCl, pH 7.5/2.5 mM $MgCl_2$/1 mM dithiothreitol. The mixture was heated at 65°C for 30 sec and quick chilled in ice water. [α-^{32}P]GTP (333 μCi; 450 Ci/mmol; 1 Ci = 37 GBq) and the vaccinia capping enzyme (5 units) was then added (10). Incubation was at 37°C for 15 min.

T4 polynucleotide kinase. The purified BO mRNA (≈0.1 μg) was first treated with 0.005 unit of *E. coli* alkaline phosphatase in 28 μl of 35 mM Tris·HCl (pH 8.0) at 60°C for 30 min. After extraction with phenol and diethyl ether, the solution was adjusted to 50 mM Tris·HCl, pH 7.6/10 mM $MgCl_2$/5 mM dithiothreitol/0.1 mM spermidine/0.1 mM EDTA; 30 μCi of [γ-^{32}P]ATP (500 Ci/mmol) and 8 units of T4 polynucleotide kinase were added (11), and the mixture was incubated at 37°C for 15 min. Unlabeled ATP (200 pmol) was added and the incubation continued for 15 min.

For sequence analysis, the labeled RNA was further purified and recovered by using the following steps: electrophoresis on a 1.5% agarose gel, electroelution, Sephadex G-50 filtration, phenol extraction, and ethanol precipitation.

RNA Sequence Analysis. *5′-End-group analysis.* The end-labeled RNAs were digested to completion with nuclease P1, and the 5′-end groups were identified by either one- or two-dimensional chromatography in the presence of appropriate markers. Analysis of the products from vaccinia capping enzyme-labeled RNA was on a cellulose TLC plate using as the solvents propan-1-ol/concentrated NH_4OH/water, 6:3:1 (vol/vol) in the first dimension and saturated $(NH_4)_2SO_4$/1 M sodium acetate, pH 5.5/propan-2-ol, 40:9:1 (vol/vol) in the second dimension (12) and G(5′)ppp(5′)N as markers. Analysis of products from polynucleotide kinase-labeled RNA was on PEI-cellulose plates using 0.75 M sodium phosphate (pH 3.5) as the solvent and pG, pA, pC, and pU as markers.

5′-Terminal sequence analysis. Two methods were used. (*i*) The end-labeled RNA was partially digested with nuclease P1 and the products were analyzed by two-dimensional homochromatography (7). (*ii*) The RNA was partially digested with base-specific nucleases, and the products were analyzed on a 7 M urea/20% polyacrylamide gel (7).

Nuclease S1 Mapping of the 3′ End of BO mRNA. The 350-bp *Mst* I/*Bgl* I fragment (Fig. 1) was labeled at the *Mst* I generated 3′-dC residue using the Klenow fragment of DNA polymerase I and [α-^{32}P]dCTP (8). The labeled DNA was used for nuclease S1 mapping (13). To generate size markers, the end-labeled DNA was subjected to the Maxam and Gilbert DNA sequencing reactions (14).

RNA Gel Transfer and Hybridization. *H. halobium* RNA prepared by the method of Chirgwin *et al.* (9) was fractionated on a 1.5% agarose/6 M urea gel (15), blotted to diazotized aminothiophenoxycellulose paper (16), hybridized using probes (see above and Fig. 1), and ^{32}P-labeled using the method of O'Farrell as described in Maniatis *et al.* (8).

RESULTS

Purification of BO mRNA. BO cDNA was bound to nitrocellulose and hybridized with total *H. halobium* S9 RNA. After the filter was washed, the bound RNA was eluted with water at 90°C. Agarose gel electrophoresis of this RNA yielded essentially a single band (Fig. 2*A*, lane 1). This RNA hybridizes to the BO cDNA (lane 2) and is the major BO mRNA (see below).

BO mRNA Can Be Labeled at 5′ End with Vaccinia Virus Capping Enzyme. Incubation of the purified BO mRNA with vaccinia capping enzyme and [α-^{32}P]GTP produced a single labeled product (Fig. 2*B*, lane 2). The BO mRNA must, therefore, contain the 5′-terminal nucleotide of the primary *BO* gene transcript. The extent of labeling (≈15% of theoretical after purification) is comparable to that observed for other RNAs (10). The BO mRNA was labeled at the 5′ end also using T4 polynucleotide kinase and [γ-^{32}P]ATP. The extent of labeling was ≈85%. The major product showed the same mobility on electrophoresis (lane 1) as the product labeled by the capping enzyme.

Transcription of BO mRNA Initiates Two Nucleotides Upstream of the *BO* Structural Gene. The 5′-terminal sequence of BO mRNA labeled by the vaccinia capping enzyme was established as follows. (*i*) The 5′ end of the mRNA was shown to be guanosine because complete digestion with nuclease P1 produced G(5′)ppp(5′)G as the only labeled product (Fig. 3*A*). (*ii*) The sequence of eight nucleotides of the 5′ terminus was determined to be 5′-G-C-A-U-G-U-U-G by two-dimensional analysis of a partial digest with nuclease P1 (Fig. 3*B*). (*iii*) Partial digestion with base-specific nucleases followed by analysis of products by polyacrylamide gel electrophoresis (Fig. 3*C*) showed the following sequence at the 5′ end: 5′-G-C-N-N-G-N-N-G-G-A-G-N-N-A-N-N-G-C-C-N-N-C-N-G-C-N-G-N-G-G-N-G-G-G-G-G (N represents either adenosine or uridine).

The sequence of BO mRNA labeled at the 5′ end with ^{32}P using polynucleotide kinase was also determined by two-dimensional analysis of a partial digest with nuclease P1. The major 5′-terminal sequence was again found to be 5′-G-C-A-U-G-U-U-G-G-A (data not shown). This result shows that

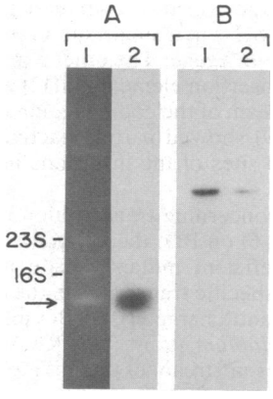

FIG. 2. Purification and labeling of BO mRNA. (*A*) Hybrid-selected BO mRNA was electrophoresed in a 1.5% agarose gel and stained with ethidium bromide (BO mRNA indicated by arrow). After transfer to aminothiophenoxycellulose paper, it was hybridized with the BO cDNA probe (lane 2). (*B*) The BO mRNA, purified for sequence analysis, was electrophoresed on a 7 M urea/3.5% polyacrylamide gel after labeling with either T4 polynucleotide kinase and [γ-^{32}P]ATP (lane 1) or vaccinia capping enzyme and [α-^{32}P]GTP (lane 2).

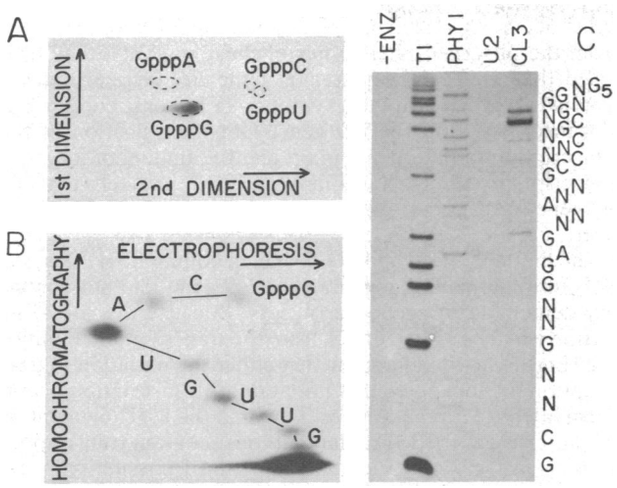

FIG. 3. Sequence analysis at the 5′ end of BO mRNA labeled with vaccinia virus capping enzyme and [α-^{32}P]GTP. (*A*) The 5′-terminal nucleotide was identified by complete digestion with nuclease P1 and analysis by two-dimensional TLC with unlabeled markers. (*B*) The sequence of eight nucleotides at the 5′ terminus was determined by partial digestion with nuclease P1 and two-dimensional analysis. (*C*) Partial sequence of 36 nucleotides at the 5′ terminus was determined by partial digestion with base-specific nucleases and polyacrylamide gel electrophoresis. T1 is guanine specific; Phy I is guanine, adenine, and uracil specific; U2 is adenine specific; and CL3 is cytosine specific. N represents either adenine or uracil.

the bulk of BO mRNA is homogeneous with respect to the 5′ end.

The Major Species of BO mRNA Extends ≈45 Nucleotides Downstream from the Structural Gene. *Nuclease S1 mapping.* A 350-bp *Mst* I/*Bgl* I fragment labeled at the *Mst* I end (Fig. 4*A*) was hybridized to total *H. halobium* S9 RNA and the mixture was treated with nuclease S1. Two sets of fragments (1 and 2 of Fig. 4*B*, lane 3) were protected by S9 RNA. [An additional band ≈78 nucleotides from the BO terminator codon found in both the *E. coli* and *H. halobium* RNA tracks was an artefact produced by nuclease S1 treatment (lanes 2 and 3).] The protected fragments corresponded to approximately 45 and 215 bp downstream from the BO terminator codon. This result suggests that there are two species of BO mRNA, one ≈830 nucleotides long and the other ≈1,000 nucleotides long.

RNA gel transfer-hybridization. Further characterization of the 3′ ends of BO mRNA from strain S9 is shown in Fig. 5. RNA gel-transfer hybridization experiments showed three bands. The major RNA detectable (Fig. 5*A*, band 1) using a BO cDNA probe does not hybridize to the 3′-flanking region probe (Fig. 5*B*). Thus, the 3′ end of the major BO mRNA species is upstream of the *Xor* II site (Fig. 1). A less abundant RNA (band 2) clearly hybridizes to both the cDNA probe and the 3′-flanking region probe. Thus, the ≈830 nucleotide species (band 1) detected by nuclease S1 mapping (Fig. 4) is the major BO mRNA; the ≈1,000 nucleotide species (band 2) is a minor transcript.

The third RNA species that hybridizes to the *BO* gene probe in strain S9 (Fig. 5*A*, band 3), is most likely a conformational isomer of the major BO mRNA species. It does not hybridize to probes derived from either the 3′-flanking region (Fig. 5*B*) or the 5′-flanking region (data not shown). In addition, it is absent in strains containing insertions within the *BO* gene (Fig. 5*A*).

Strain SD19 Contains Decreased Levels of BO mRNA. This strain contains an insertion of ISH2 100 bp upstream of the site of initiation of transcription of the *BO* gene (6). Fig. 5 shows that SD19 contains BO mRNAs identical in size to those in the parent S9 strain, but in lesser amounts. Capping and sequence analysis shows that the major BO mRNA in SD19 has the same sequence at the 5′-end as the S9 BO mRNA (data not shown). Thus, the *BO* gene is faithfully transcribed in SD19 and the signal for initiation of transcription is largely intact within the region immediately upstream of the gene.

Strains SD10 and SD17 Contain No Detectable BO mRNA. These two strains contain ISH1 within the NH_2-terminal coding region of the *BO* gene (5, 6). Fig. 5*A* shows that SD10 and SD17 contain no detectable RNA species hybridizing to the BO cDNA probe.

Strains SD12 and L33 Contain Incomplete *BO* Gene Transcripts. These two strains contain ISH2 inserted near the middle of the *BO* gene (6). Fig. 5*A* shows that SD12 and L33 contain several transcripts hybridizing to the BO cDNA probe. Most of these are smaller than the major S9 BO mRNA.

DISCUSSION

***BO* Gene Transcripts in the Parental Strain.** BO mRNA is the first mRNA to be characterized from *H. halobium* or, indeed, from any archaebacterial species (17). In agreement with our previous observation using nuclease S1 mapping (4), we find that the 5′ terminus of the mRNA extends only

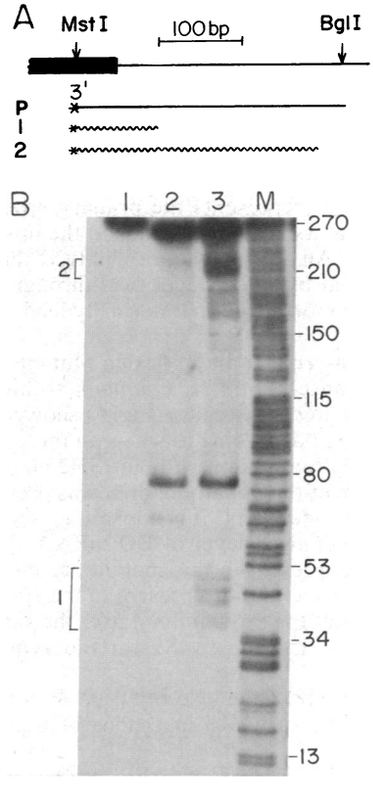

FIG. 4. Mapping of the 3′ end of BO mRNA by nuclease S1 protection. (*A*) Experimental design. The top line is a restriction map of the COOH-terminal coding region of the *BO* gene (black bar) and 3′-flanking region. The *Mst* I/*Bgl* I fragment, end-labeled at the 3′ end of the *Mst* I-generated terminus, was the probe (labeled P). Two sets of fragments (wavy lines) were protected from nuclease S1 digestion (lines 1 and 2). (*B*) Experimental results. The end-labeled *Mst* I/*Bgl* I fragment was hybridized to either *E. coli* RNA (lanes 1 and 2) or *H. halobium* S9 RNA (lane 3) and treated with nuclease S1 (lanes 2 and 3). Markers are shown on the right with the distance from the terminator codon for BO shown in nucleotides. The major BO mRNA protects the fragment until nucleotides 36–47 (labeled 1), while the minor BO mRNA is longer by ≈170 nucleotides at the 3′ end (labeled 2). A strong band in lanes 2 and 3 at about nucleotide 78 is an artefact of nuclease S1 treatment.

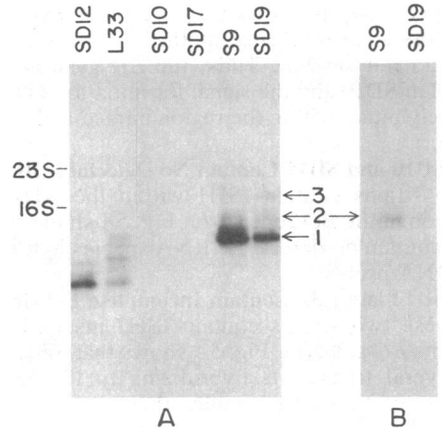

FIG. 5. RNA gel-transfer hybridization for several *H. halobium* strains using the BO cDNA probe (*A*) and the 3'-flanking region probe (*B*). The major BO mRNA is band 1 while the minor transcript is band 2. Band 3 is likely to be a conformational isomer of band 1. The position of the small and large rRNAs are shown.

two nucleotides beyond the initiator codon for BO (Fig. 6*A*). Thus, the BO mRNA has one of the shortest 5'-noncoding leaders found for any mRNA, either eubacterial or eukaryotic. In addition, the finding that the purified BO mRNA is capped by the vaccinia virus capping enzyme shows that the 5' terminus of the BO mRNA is also the start site of transcription of the *BO* gene.

In addition to the above major mRNA species, a minor transcript, ≈170 nucleotides longer at the 3' end than the major species, was observed in strain S9. It is possible that this longer species represents the primary product of transcription and that its processing leads to the observed major mRNA species. An alternative possibility is that the minor species is formed by a low level read-through of the transcription termination signal that normally leads to the major species.

***BO* Gene Transcripts in the Insertion Mutants.** Analysis of the *BO* gene transcripts in *H. halobium* strains containing insertions in the *BO* gene region (Fig. 5) showed results that can be categorized according to where in the gene the insertions occur. SD19, a mutant with an ISH2 element inserted 102 bp upstream of the initiator codon, was previously found to be partially *BO* deficient. It is now found to contain a correspondingly decreased level of BO mRNA. Furthermore, the BO mRNAs present in this mutant are identical in size and 5'-end sequence to those present in the strain S9. These findings show that the SD19 mutant uses the same transcription start site as is used by S9 for the synthesis of BO mRNA.

Betlach *et al.* (18) have also reported on a number of *H. halobium* mutants in which insertions of transposable elements occur 500–1,400 bp upstream of the *BO* coding region. From the observed phenotypes of these mutants, they proposed that the region upstream of the *BO* gene encodes a protein that either regulates synthesis of BO or is coordinately transcribed with the *BO* gene. Our identification of the transcription initiation site in S9 and the finding of identical transcripts in S9 and SD19 make it highly unlikely that the *BO* gene forms a part of such a coordinately transcribed operon.

The mutants SD10 and SD17, which contain ISH1 near the NH_2-terminal coding region of the *BO* gene, did not contain any detectable *BO* gene transcripts. Because the site of insertion is only 17 nucleotides from the transcription initiation site (Fig. 1), it would appear that either the initiation of transcription is blocked or that initiation of transcription occurs normally but termination occurs within the ISH1 element. In the latter case, the ISH1 element must contain transcription stop signals in both orientations. A third possibility is that transcription proceeds through ISH1 but the transcripts are unstable.

Finally, the mutants SD12 and L33, which contain ISH2 near the middle of the *BO* structural gene, produced transcripts that were smaller than the major BO mRNA. There are at least two possible explanations for this result: (*i*) transcription terminates within ISH2 and (*ii*) transcription proceeds through ISH2 but the resulting RNA is cleaved within the ISH2 region because of processing signals. In either case, the mutant transcripts in SD12 and L33 are moderately stable.

Transcription Signals in *H. halobium*. Assuming that the signal for initiation of transcription resides upstream from the site of initiation, the present results with S9 and SD19 show that the bulk of this information is contained in the 100-bp sequence upstream of the 5' end of the BO mRNA. Scrutiny of this sequence shows the absence of any eubacterial or eukaryotic consensus promoter sequences. Because no other transcription initiation site has been identified in *H. halobium*, we are unable to compare the above sequence with similar regions of other genes in this organism. However, we have compared the sequence upstream of the *BO* gene (4) with sequences upstream of the ISH1 long open-reading frame (5), the ISH50 long open-reading frame (19) and the 5' end of a small RNA encoded by a repeated sequence in the related organism, *Halobacterium volcanii* (20). The only homology found is within the region 28–42 nucleotides upstream of translational start codon. It is an A+T-rich hexanucleotide sequence, A-A-G-T-T-A, of which at least five nucleotides are conserved in each case. It should be noted that, except in the case of the *BO* gene, the regions compared have not been shown to contain transcription initiation sites.

Another feature of the DNA sequence upstream of the *BO* gene is the presence of alternating purine–pyrimidine stretches, one of which is 11 nucleotides long (nucleotides

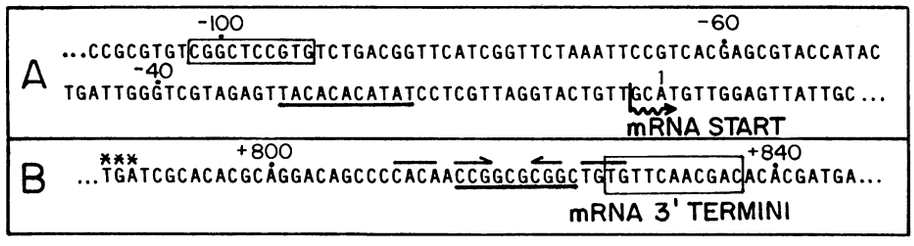

FIG. 6. DNA sequences flanking the BO coding region (4). (*A*) The upstream sequences. The first nucleotide of the translational start codon is indicated by 1 and the mRNA start is indicated by a wavy arrow. The site of ISH2 insertion in SD19 is shown by a solid box around the nucleotides duplicated on insertion. The long alternating purine–pyrimidine stretch is underlined. (*B*) The downstream sequences. The translational stop codon is indicated by asterisks and the ragged 3' end of the major BO mRNA is indicated by the box. An inverted repeat just upstream of the 3' end is shown by arrows and a stretch of 10 G+C bases is underlined.

−20 to −30, underlined in Fig. 6*A*) and another that is 7 nucleotides long (nucleotides −103 to −109 in Fig. 6*A*). Under the high intracellular salt concentration in *H. halobium* (21), these sequences have the potential to adopt Z-DNA structure (22). We do not know whether they play any role in transcription. It is interesting to note, however, that this arrangement is somewhat similar to that of the simian virus 40 enhancer region (23). If this is of functional significance, the decreased level of BO mRNA in strain SD19 might then be caused by the alteration of Z-DNA arrangement in this region by insertion of ISH2 (box in Fig. 6*A*).

The 3′ terminus of the major BO mRNA is ≈45 nucleotides downstream of the terminator codon for BO, whereas a minor species is longer at the 3′ end than the major mRNA by 170 nucleotides. One possibility is that the major mRNA is formed by processing of the larger mRNA. Alternatively, if the mRNA results from independent termination of transcription, the inverted repeat sequence containing a stretch of ten G·C base pairs just upstream of the terminus (Fig. 6*B*) may represent a signal for termination of transcription in *H. halobium*. In *E. coli*, ρ-independent transcriptional termination signals usually contain G+C-rich inverted repeats followed by a stretch of thymines (24). For the *BO* gene, the inverted repeat is followed by the sequence T-T-C-A-A-C-G-A-C, which is somewhat similar to the sequence C-A-A-T-C-A-A, found at several ρ-dependent transcription termination sites in *E. coli* (25).

Further understanding of the transcription signals in *H. halobium* would be aided by the analysis of transcripts of additional genes. It is also most desirable to now develop an *in vitro* transcription system that is capable of accurate initiation and termination. The availability of clones for the wild-type and mutant forms of the *BO* gene (4–6) and the defined start site for transcription should now facilitate such studies. An immediate requirement is the purification of RNA polymerase(s) possessing specificity in transcription. Although a RNA polymerase has been purified from *H. halobium* (26), it lacks the necessary specificity.

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